



BEATRICHĖ RAZMIENĖ

**SYNTHESIS AND
INVESTIGATION OF
FUNCTIONALIZED
2*H*-PYRAZOLO[4,3-*c*]
PYRIDINES**

DOCTORAL DISSERTATION

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KAUNAS UNIVERSITY OF TECHNOLOGY

BEATRIČĖ RAZMIENĖ

SYNTHESIS AND INVESTIGATION OF
FUNCTIONALIZED
2H-PYRAZOLO[4,3-*c*]PYRIDINES

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Natural Sciences, Chemistry (N 003)

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KAUNO TECHNOLOGIJOS UNIVERSITETAS

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TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND PHYSICAL UNITS.....	7
INTRODUCTION	10
1. LITERATURE REVIEW.....	13
1.1. Synthesis of condensed pyrazole systems <i>via</i> formation of pyrazole onto a preexisting hetero or aromatic cycle.....	13
1.2. Synthesis of condensed pyrazolo systems by formation of another heterocycle onto a preexisting pyrazole	17
1.3. Synthesis of condensed pyrazole systems by using multicomponent reactions	35
1.4. Progress of condensed pyrazole systems in KTU	45
1.5. Summary	48
2. RESULTS AND DISCUSSION	49
2.1. Synthesis of 2 <i>H</i> -pyrazolo[4,3- <i>c</i>]pyridine core	49
2.1.1. Synthesis of 1-phenyl-3-[(substituted)ethynyl]-1 <i>H</i> -pyrazole-4-carbaldehydes	49
2.1.2. Synthesis of 4-substituted 7-iodo-2,6-diphenyl-2 <i>H</i> -pyrazolo[4,3- <i>c</i>]pyridines	50
2.2. Synthesis, optical and biological investigation of 7-aryl-2,6-diphenyl-2 <i>H</i> -pyrazolo[4,3- <i>c</i>]pyridines	55
2.2.1. Synthesis of 7-aryl-2,6-diphenyl-2 <i>H</i> -pyrazolo[4,3- <i>c</i>]pyridines	55
2.2.2. Optical properties of 7-aryl-2,6-diphenyl-2 <i>H</i> -pyrazolo[4,3- <i>c</i>]pyridines....	59
2.2.3. Biological investigation of 7-aryl-2,6-diphenyl-2 <i>H</i> -pyrazolo[4,3- <i>c</i>]pyridines.....	63
2.3. Synthesis, optical and biological investigation of <i>N</i> -aryl-2,6-diphenyl-2 <i>H</i> -pyrazolo[4,3- <i>c</i>]pyridin-7-amines.....	66
2.3.1. Synthesis of <i>N</i> -aryl-2,6-diphenyl-2 <i>H</i> -pyrazolo[4,3- <i>c</i>]pyridin-7-amines....	66
2.3.2. Optical properties of <i>N</i> -aryl-2,6-diphenyl-2 <i>H</i> -pyrazolo[4,3- <i>c</i>]pyridin-7-amines	69
2.2.3. Biological investigation of <i>N</i> -aryl-2,6-diphenyl-2 <i>H</i> -pyrazolo[4,3- <i>c</i>]pyridin-7-amines	72
2.4. Synthesis, optical and biological investigation of 2,6-diphenyl-7-[(<i>E</i>)-2-phenylethenyl]-2 <i>H</i> -pyrazolo[4,3- <i>c</i>]pyridines and (2 <i>E</i>)-3-(2,6-diphenyl-2 <i>H</i> -pyrazolo[4,3- <i>c</i>]pyridin-7-yl)- <i>N</i> -phenylprop-2-enamides	78

2.4.1. Synthesis of 2,6-diphenyl-7-[(<i>E</i>)-2-phenylethenyl]-2 <i>H</i> -pyrazolo[4,3- <i>c</i>]pyridines.....	78
2.4.2. Synthesis of (<i>2E</i>)-3-(2,6-diphenyl-2 <i>H</i> -pyrazolo[4,3- <i>c</i>]pyridin-7-yl)- <i>N</i> -phenylprop-2-enamides.....	81
2.4.3. Optical properties of 2,6-diphenyl-7-[(<i>E</i>)-2-phenylethenyl]-2 <i>H</i> -pyrazolo[4,3- <i>c</i>]pyridines and (<i>2E</i>)-3-(2,6-diphenyl-2 <i>H</i> -pyrazolo[4,3- <i>c</i>]pyridin-7-yl)- <i>N</i> -phenylprop-2-enamide.....	83
2.4.4. Biological investigation of 2,6-diphenyl-7-[(<i>E</i>)-2-phenylethenyl]-2 <i>H</i> -pyrazolo[4,3- <i>c</i>]pyridine and (<i>2E</i>)-3-(2,6-diphenyl-2 <i>H</i> -pyrazolo[4,3- <i>c</i>]pyridin-7-yl)- <i>N</i> -phenylprop-2-enamide.....	86
3. METHODS AND MATERIALS	88
4. MAIN RESULTS AND CONCLUSIONS	133
5. SANTRAUKA	135
REFERENCES	157
CURRICULUM VITAE.....	175
LIST OF PUBLICATIONS.....	176
ACKNOWLEDGMENTS.....	179

LIST OF ABBREVIATIONS AND PHYSICAL UNITS

Φ_f	quantum yield
δ	chemical shift parameter
ε	extinction coefficient
ν	wavelength in cm^{-1}
λ	wavelength
λ_{ex}	fluorescence excitation wavelength
λ_{em}	fluorescence emission wavelength
^{13}C NMR	carbon (^{13}C) nuclear magnetic resonance
^1H NMR	hydrogen (^1H) nuclear magnetic resonance
Ac	acetyl
ACN	acetonitrile
Aliph	aliphatic
Ar	aromatic
Bn	benzyl
Bu	butyl
BINAP	(<i>R</i>)-(+)-(1,1'-binaphthalene-2,2'-diyl)bis(diphenylphosphine)
Boc	<i>tert</i> -butoxycarbonyl
BODIPY	dipyrrrometheneboron difluoride
BPO	benzoyl peroxide
bpy	2,2'-bipyridine
BrdU	bromodeoxyuridin
CDK	cyclin-dependent kinase
COSY	homonuclear correlation spectroscopy
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	<i>N,N'</i> -dicyclohexylcarbodiimide
DCB	1,2-dichlorobenzene
DCM	dichlormethane
DCE	dichlorethane
DIBAL-H	diisobutylaluminium hydride
DMAP	4-dimethylaminopyridine
DMF	<i>N,N</i> -dimethylformamide
DMSO	dimethyl sulfoxide
DMSO- d_6	deuterated dimethyl sulfoxide
DNA	deoxyribonucleic acid
dppf	1,1'-bis(diphenylphosphino)ferrocene
EC ₅₀	the concentration of a drug that gives half-maximal response
EDC·HCl	ethyl-(<i>N,N'</i> -dimethylamino)propylcarbodiimide hydrochloride
EGFR	epidermal growth factor receptor
equiv	equivalent
ESI	electrospray ionization
Et	ethyl
EtOAc	ethylacetate
FL	fluorescence

FT-IR	Fourier-transform infrared spectroscopy
G2	gap 2 phase
GABAA	γ -aminobutyric acid type A
GI ₅₀	concentration causing 50% cell growth inhibition
h	hour
H2BC	heteronuclear 2 bond correlation
HAT	human African trypanosomiasis
HATU	1-[bis(dimethylamino)methylene]-1 <i>H</i> -1,2,3-triazolo[4,5- <i>b</i>]pyridinium 3-oxid hexafluorophosphate
HMBC	heteronuclear multiple bond correlation
Hex	hexane
HMDS	hexamethyldisilazane
HOSA	hydroxylamine- <i>O</i> -sulfonic acid
HRMS	high resolution mass spectrometry
HIV	human immunodeficiency virus
HSQC	heteronuclear single-quantum correlation
IR	infrared
IC ₅₀	median inhibition concentration
LC3	microtubule-associated protein 1-light chain 3
LDA	lithium diisopropylamide
LED	light-emitting diode
LiHMDS	lithium bis(trimethylsilyl)amide
Me	methyl
Mcl-1	induced myeloid leukemia cell differentiation protein
MS	mass spectrometry
MRC-5	Medical Research Council cell strain 5
MWI	microwave irradiation
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NaOtBu	sodium <i>tert</i> -butoxide
NBS	<i>N</i> -bromosuccinimide
NIS	<i>N</i> -iodosuccinimide
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser effect spectroscopy
OTf	trifluoromethanesulfonate
PARP	poly(ADP-ribose) polymerase
PCNA	proliferating cell nuclear antigen
PDT	photodynamic therapy
Pd ₂ dba ₃	tris(dibenzylideneacetone)dipalladium(0)
PDE5	phosphodiesterase 5
PEG400	polyethylene glycol 400
PE	petroleum ether
PivOH	pivalic acid
Ph	phenyl
ppm	parts per million

Pr	propyl
R _f	retention factor
ROS	reactive oxygen species
rt	room temperature
SAR	structure–activity relationship
SEGPhos	5,5'-bis(diphenylphosphino)-4,4'-bi-1,3-benzodioxole
Sphos	2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl
SS	Stokes shift
SSA	sulfosalicylic acid
t	temperature
t _m	melting temperature
TASK-3	potassium channel subfamily K member 9
TBAB	tetrabutylammonium bromide
THF	tetrahydrofuran
TEA	triethylamine
TEMPO	2,2,6,6-tetramethylpiperidine- <i>N</i> -oxyl
TFA	trifluoroacetic acid
TLC	thin-layer chromatography
TMSN ₃	trimethylsilylazide
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TsOH	<i>p</i> -toluenesulfonic acid
USI	ultrasound irradiation
UV	ultraviolet
UV-vis	ultraviolet-visible light
Xantphos	4,5-bis(diphenylphosphino)-9,9-dimethylxanthene

INTRODUCTION

Over the last decades, pyrazoles and their heteroannulated derivatives have become an important part in organic and medicinal chemistry. The incorporation of a pyrazole nucleus into drug-like molecules has become a common practice to develop new materials featuring anti-cancer, anti-diabetic, anti-viral, anti-inflammatory, anti-bacterial, anti-fungal, antineurodegenerative, anti-tubercular, anthelmintic, antimalarial, and photosensitizing properties [1-5], etc. As a result, numerous officially approved therapeutics involve the pyrazole core [6]. Furthermore, pyrazoles have been documented to possess dyeing and fluorescence properties [7, 8] as well. On top of that, some of them can be used as colorimetric or fluorescent probes for sensing small molecules, ions, or pH [9-12]; thus they may be applied in *in vivo* imaging [13].

Pyrazolopyridines are among the most studied annealed pyrazole systems in organic and pharmaceutical chemistry. For example, 6-(3,5-dimethoxyphenyl)-3-(4-fluorophenyl)-1*H*-pyrazolo[3,4-*b*]pyridine has been researched as a Kit kinase inhibitor [14]. Lately, various 1*H*-pyrazolo[3,4-*b*]pyridine derivatives have been reported as potent CDK8 inhibitors [15], and antimicrobial, anti-quorum-sensing, and anticancer agents [16], while the 3-amino-1*H*-pyrazolo[3,4-*b*]pyridine core has been examined as an innovative scaffold for MELK kinase inhibitors [17]. Furthermore, several 1*H*-pyrazolo[4,3-*b*]pyridin-3-amine derivatives have been detected to act as positive allosteric modulators of the metabotropic glutamate receptor 4 (mGlu4) [18], while 3-phenylpyrazolo[3,4-*c*]pyridines possess antiproliferative activity [19], and 1-(4-methoxybenzyl)-7-(4-methylpiperazin-1-yl)-*N*-[4-(4-methylpiperazin-1-yl)phenyl]-3-phenyl-1*H*-pyrazolo[3,4-*c*]pyridin-5-amine is a potential angiogenesis inhibitor [20]. Only a few examples of biologically active pyrazolo[4,3-*c*]pyridines have been reported, including 3-amino-2-phenyl-2*H*-pyrazolo[4,3-*c*]pyridine-4,6-diol which has shown inhibitory activity against p90 ribosomal S6 kinases 2 (RSK2) [21] and 3-aminopyrazolopyridinone derivatives demonstrating moderate inhibitory potency against CK1d, p38a, and aurora A kinases [22].

Since the pyrazolopyridine system is of great importance to medicinal chemists, it is essential to develop novel paths of synthesis to access their diverse functionalized derivatives. Exploring the synthesis and properties of 2*H*-pyrazolo[4,3-*c*]pyridines is fundamentally significant considering the lack of reported derivatives in literature and the vast potential scope of applications.

The aim of this work:

To prepare a library of functionalized 2*H*-pyrazolo[4,3-*c*]pyridines by employing ring closing and palladium catalyzed cross-coupling reactions and to investigate their properties.

The tasks proposed to achieve the above stated aim were as follows:

1. To synthesize 3-alkynyl-1-phenyl-1*H*-pyrazole-4-carbaldehydes and investigate their transformation to 7-iodo-2*H*-pyrazolo[4,3-*c*]pyridines.
2. To employ the Suzuki-Miyaura cross-coupling reaction to obtain 7-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines, and to investigate and discuss their functional properties.
3. To employ the Buchwald-Hartwig cross-coupling reaction to obtain *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines, and to investigate and discuss their functional properties.
4. To employ the Heck cross-coupling reaction to obtain 2,6-diphenyl-7-[(*E*)-2-phenylethenyl]-2*H*-pyrazolo[4,3-*c*]pyridines and (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)-*N*-phenylprop-2-enamides, and to investigate and discuss their functional properties.

Scientific novelty:

An effective multistep synthesis method to obtain functionalized 2*H*-pyrazolo[4,3-*c*]pyridines from easily accessible starting materials has been developed. Substituents can be introduced to 2-, 3-, 4-, 6- and 7-positions at various stages of synthesis by using various substrates. For instance, 2- and 3- substituents have been derived from the starting material 1,5-substituted-1*H*-pyrazol-3-ol, 6-substituent from various acetylenes as Sonogashira cross-coupling substrates, whereas 7- substituents come from various substrates for palladium catalyzed cross-coupling reactions (Suzuki-Miyaura, Buchwald-Hartwig, Heck). In this work, 2,6-diphenyl-4,7-disubstituted-2*H*-pyrazolo[4,3-*c*]pyridines have been synthesized from 1-phenyl-1*H*-pyrazol-3-ol *via* 1-phenyl-3-(phenylethynyl)-1*H*-pyrazole-4-carbaldehyde which was converted into 4-substituted-7-iodo-2*H*-pyrazolo[4,3-*c*]pyridines during a three-step procedure. Substituents to the 7-position were introduced by using palladium catalyzed cross-coupling reactions to create a versatile library of 4,7-substituted-2*H*-pyrazolo[4,3-*c*]pyridines. All the derivatives exhibited different optical and biological properties with interesting and potent applications: for example, 7-(4-methoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine showed potential fluorescent pH sensing, 4-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)phenol displayed antiproliferative activity against MV4-11, K562 and MCF-7 cancer cells, while *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines and 2,6-diphenyl-7-[(*E*)-2-phenylethenyl]-2*H*-pyrazolo[4,3-*c*]pyridines exhibited photosensitizing properties for photodynamic therapy against G361 melanoma cancer cells.

Main statements to be defended:

1. 1-Phenyl-3-(ethynyl)-1*H*-pyrazole-4-carbaldehydes can be used as precursors to obtain 7-iodo-2*H*-pyrazolo[4,3-*c*]pyridines *via* (1-phenyl-3-(phenylethynyl)-1*H*-pyrazol-4-yl)alcohol and 4-(azidosubstituted)-1-phenyl-3-(phenylethynyl)-1*H*-pyrazole intermediates.

2. 7-Aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines can be obtained by the Suzuki-Miyaura cross-coupling reaction and have a potential as fluorescent pH sensors and anticancer agents.

3. *N*-Aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines can be obtained by the Buchwald-Hartwig cross-coupling reaction and have a potential as photosensitizers for photodynamic therapy against melanoma G361 cells.

4. 2,6-Diphenyl-7-[(*E*)-2-phenylethenyl]-2*H*-pyrazolo[4,3-*c*]pyridines and (*2E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)-*N*-phenylprop-2-enamides can be obtained by the Heck cross-coupling reaction, and they have a potential as photosensitizers for photodynamic therapy against melanoma G361 cells.

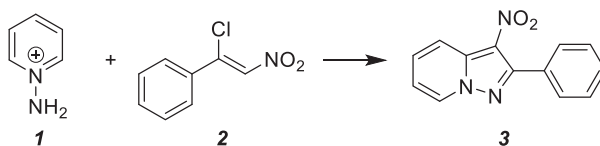
1. LITERATURE REVIEW

The purpose of this literature review is to provide a thorough and systematic overview of the current methods of synthesis and the application of various condensed pyrazole systems. It is divided to three sections designated to different types of condensation reactions: the formation of the pyrazole ring onto a preexisting hetero or aromatic cycle, the formation of another heterocycle onto preexisting pyrazole, and the use of multi-component condensation reactions.

1.1. Synthesis of condensed pyrazole systems *via* formation of pyrazole onto a preexisting hetero or aromatic cycle

Various condensed pyrazole systems can be synthesized starting from a wide range of substituted heterocycles by closing the pyrazole ring.

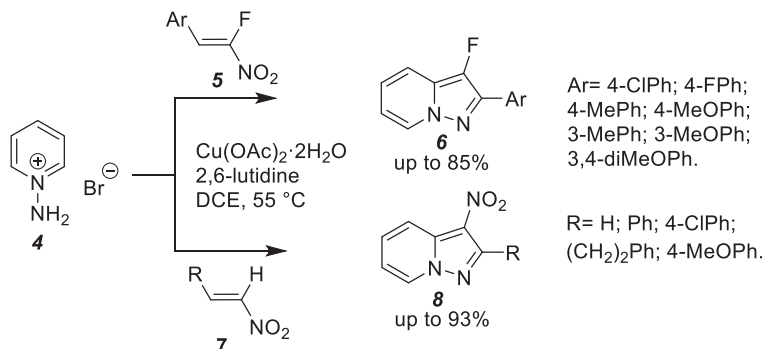
Recently, an efficient protocol for [3+2] cycloaddition of pyridinium ylides and 1-chloro-2-nitrostyrenes has been developed [23]. The authors tested the formation of pyrazolo[1,5-*a*]pyridines in a base assisted interaction between 2-halo-1-nitroalkenes and *N*-aminopyridinium salts (Scheme 1.1). The reaction of *N*-aminopyridinium iodide (**1**) with (1-chloro-2-nitrovinyl)benzene (**2**) was firstly carried out in ethanol in the presence of 1 equiv. of trimethylamine, which resulted in a low yield of the product (27%). Increasing the concentration of the base to 2 or 3 equiv. improved the reaction yield insignificantly (thereby reaching 40% and 45%, appropriately); however, switching to a polar aprotic solvent medium was far more beneficial for the reaction performance, especially in the presence of stronger bases. The best yield (95%) was achieved when performing the reaction in acetonitrile in the presence of DBU. It is noteworthy that, in a less polar solvent, such as DCM, the yields were substantially lower (62%). Most likely, the poor solubility of the starting material caused the decreased reaction yield. All of the reactions were performed at room temperature; they lasted up to 1.5 hours.



Scheme 1.1. Synthesis of pyrazolo[1,5-*a*]pyridines from 2-halo-1-nitroalkenes and *N*-aminopyridinium salts

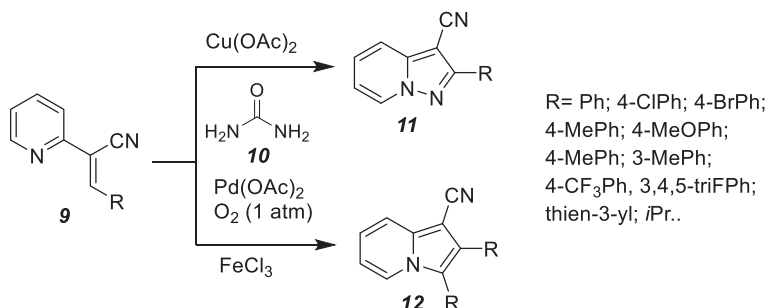
Another attempt to synthesize pyrazolo[1,5-*a*]pyridines was reported by Motornov and colleagues [24]. The reaction between aminopyridinium salts **4** and α -fluoronitroalkenes **5** led to the preparation of 3-fluoro-pyrazolo[1,5-*a*]pyridines **6**, while the reaction with α -unsubstituted nitroalkenes **7** provided access to 3-nitro-pyrazolo[1,5-*a*]pyridines **8** (Scheme 1.2). The oxidative annulation was mediated by

the mild copper diacetate/lutidine system in DCE while heating the mixture at 55 °C temperature.



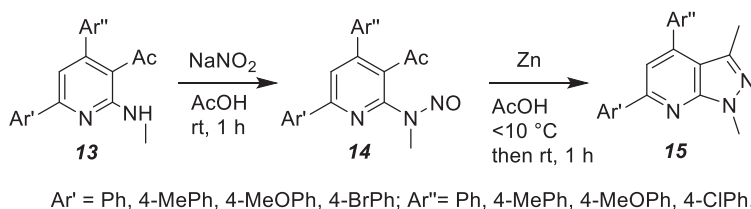
Scheme 1.2. Synthesis of 3-fluoro and 3-nitropyrzolo[1,5-*a*]pyridines

Lu *et al.* presented the results of palladium-catalyzed oxidative formal [4+1] annulation of pyridine-substituted acrylonitriles towards divergent synthesis of fused *N*-heterocycles [25]. The optimization of (pyridin-2-yl)acrylonitriles **9** reaction with urea **10** showed that the best additive to Pd(OAc)₂ to obtain pyrazolo[1,5-*a*]pyridines **11** was Cu(OAc)₂ (Scheme 1.3). The reaction was carried out in DMF at 120 °C. Interestingly, changing the additive to FeCl₃ resulted in the formation of indolizine-1-carbonitrile **12**.



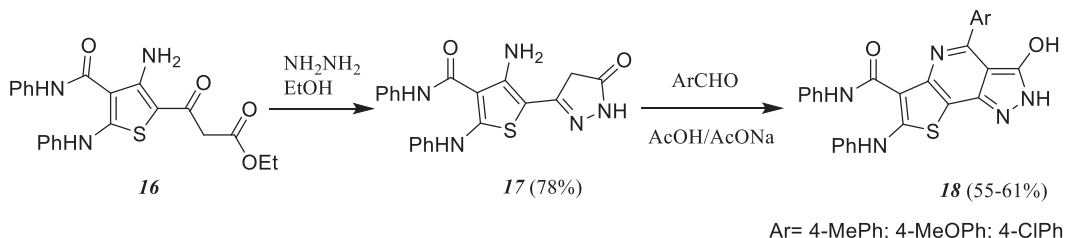
Scheme 1.3. Synthesis of pyrazolo[1,5-*a*]pyridines or indolizine-1-carbonitrile from (pyridin-2-yl)acrylonitriles

Sizova *et al.* reported a novel method for the annulation of the pyrazole moiety to the pyridine ring to access 1*H*-pyrazolo[3,4-*b*]pyridines [26]. The annulation of the pyrazole ring was carried out by *N*-nitrosation of 3-acetyl-4,6-diaryl-2-methylaminopyridines (**13**) with sodium nitrite in acetic acid followed by the reduction of *N*-nitrosopyridine **14** with zinc dust in acetic acid to 2-hydrazinopyridine with the simultaneous intramolecular reaction of hydrazine and acetyl groups (Scheme 1.4). We should note that both steps were completed with high yields.



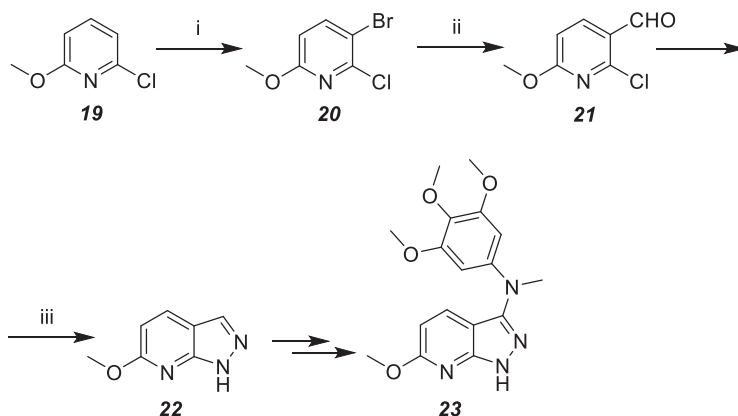
Scheme 1.4. Synthesis of 1*H*-pyrazolo[3,4-*b*]pyridines

The 3-(3-aminothiophen-2-yl)-3-oxopropanoate derivative was reported as a good precursor for the construction of pyrazolo[3,4-*d*]thieno[3,2-*b*]pyridine derivatives [27]. This synthesis strategy involved the intramolecular cyclization between the amino group (at C-3 of thiophene) and the ester function to construct annulated thienopyridine-based compounds (Scheme 1.5). To produce the corresponding pyrazolyl-thiophene derivative **17**, first, β -ketoester motif of the functionalized thiophene **16** reacted with hydrazine hydrate under reflux in ethanol. Then, through heating of pyrazolyl-thiophene derivative (**17**) with different types of substituted benzaldehydes, intramolecular cyclization yielded a tricyclic ring system – pyrazolo[3,4-*d*]thieno[3,2-*b*]pyridines **18**.



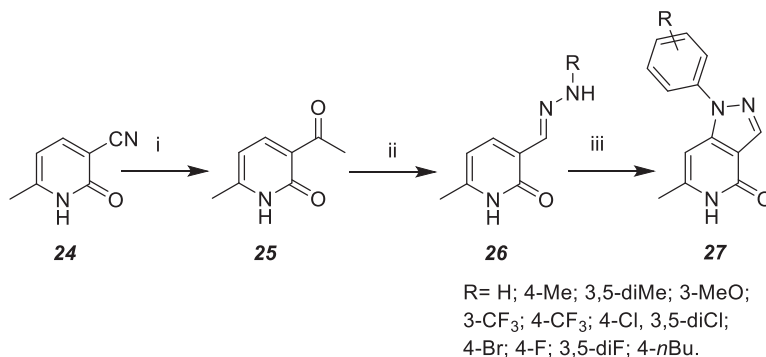
Scheme 1.5. Synthesis of pyrazolo[3,4-*d*]thieno[3,2-*b*]pyridines

Hao *et al.* reported the synthesis of novel *N*-(3,4,5-trimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine derivatives [28]. The 1*H*-pyrazolo[3,4-*b*]pyridine core was obtained in three-step synthesis (Scheme 1.6). Firstly, 2-chloro-6-methoxypyridine (**19**) reacted with *N*-bromosuccinimide (NBS) in the presence of benzoyl peroxide (BPO) to yield 3-bromo-2-chloro-6-methoxypyridine (**20**). The Bouveault reaction of the latter provided 2-chloro-6-methoxynicotinaldehyde (**21**), which then reacted with hydrazine hydrate under refluxing conditions to produce 6-methoxy-1*H*-pyrazolo[3,4-*b*]pyridine (**22**). The most potent derivative 6-methoxy-*N*-methyl-*N*-(3,4,5-trimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (**23**) showed strong anti-tubulin polymerization activity and played the anti-angiogenic role by blocking tubule formation and inhibiting the migration and invasion of human umbilical vein endothelial (HUVEC) cells, and thus it could serve as an effective tubulin polymerization inhibitor.



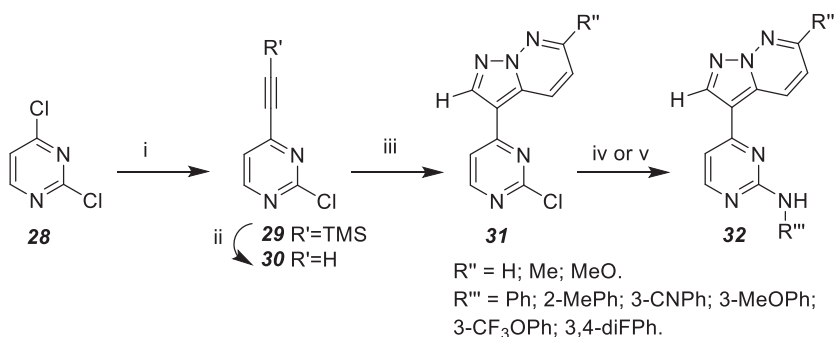
Scheme 1.6. Synthesis of *N*-(3,4,5-trimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine derivatives. *Reagents and conditions:* i: NBS, BPO, CAN, 90 °C, 3 h; ii: DMF, *n*-BuLi, dry THF, -78 °C 1.5 h, then rt 6 h; iii: N₂H₄·H₂O, H₂O, reflux 5 h

Kumar *et al.* published the principles of synthesis of novel pyrazolo[4,3-*c*]pyridin-4-one derivatives (Scheme 1.7) [29]. Firstly, the nitrile group of 6-methyl-2-oxo-1,2-dihydropyridine-3-carbonitrile (**24**), synthesized from 3-ketobutyraldehyde dimethyl acetal and cyanoacetamide, was converted into formyl group by using DIBAL-H to yield compound **25**. The latter was converted into pyrazolo[4,3-*c*]pyridin-4-one derivatives in two steps. In the first step, the formyl group of compound **25** reacted with the substituted phenylhydrazine hydrochlorides to yield intermediate hydrazones **26**, which were then converted into pyrazolo[4,3-*c*]pyridin-4-one derivatives **27** in refluxing nitrobenzene. The synthesized compounds were screened for anti-HIV activity, and one of the derivatives showed high potential as a nucleosidal or nonnucleosidal reverse transcriptase inhibitor.



Scheme 1.7. Synthesis of pyrazolo[4,3-*c*]pyridin-4-one derivatives. *Reagents and conditions:* i: (a) HMDS, toluene, 110 °C, 2 h, (b) DIBAL-H, toluene, 0 °C, 8 h; ii: R-NH₂·HCl, EtOH, rt, 2 h; iii: nitrobenzene, 210 °C, 15 min

Having identified pyrazolo[1,5-*b*]pyridazine as a potent scaffold to treat Human African trypanosomiasis (HAT), commonly referred to as *sleeping sickness*, which is a parasitic disease caused by two subspecies of *Trypanosoma brucei*, Tear *et al.* developed a synthetic strategy which allowed late stage derivatization at three positions (Scheme 1.8) [30]. 2,4-Dichloropyrimidine (**28**) was chosen as the starting material, and Sonogashira coupling was employed with trimethylsilylacetylene while following silyl deprotection with potassium hydroxide to 2-chloro-4-ethynylpyrimidine (**30**). The latter reacted with 1-aminopyridizinium ion generated *in situ* from pyridazine and hydroxylamine-*O*-sulfonic acid (HOSA). The [3+2] cycloaddition reaction led to 3-(2-chloropyrimidin-4-yl)pyrazolo[1,5-*b*]pyridazine intermediate **31**, which subsequently underwent nucleophilic aromatic substitution or palladium-mediated cross-coupling to provide the corresponding products **32**.

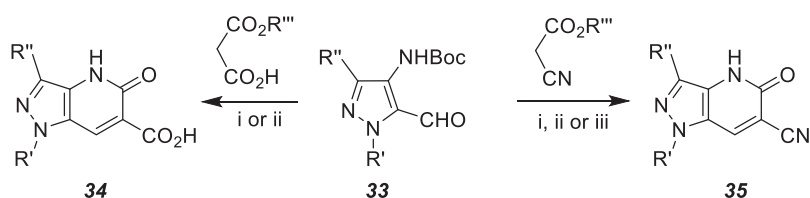


Scheme 1.8. Synthesis of pyrazolo[1,5-*b*]pyridazine analogs. *Reagents and conditions:* i: HCCR', CuI, Pd(PPh₃)₂Cl₂, TEA, THF, 50 °C, 12 h; ii: KOH, MeOH, rt, 30 min; iii: 3-substituted pyridazine, hydroxylamine-*O*-sulfonic acid, KOH, NaHCO₃, H₂O, DCM, 70 °C, then rt, 12 h; iv: R'''NH₂, *n*-BuOH, 150 °C, 2h; v: R'''NH₂, Pd(OAc)₂, BINAP or Xantphos, Cs₂CO₃, dioxane, 160 °C, 1 h

1.2. Synthesis of condensed pyrazole systems by formation of another heterocycle onto a preexisting pyrazole

Numerous condensed pyrazole systems can also be synthesized from various pyrazole derivatives by forming a new heterocycle ring. Multiple reactions presented in the literature use aminopyrazole as the starting material.

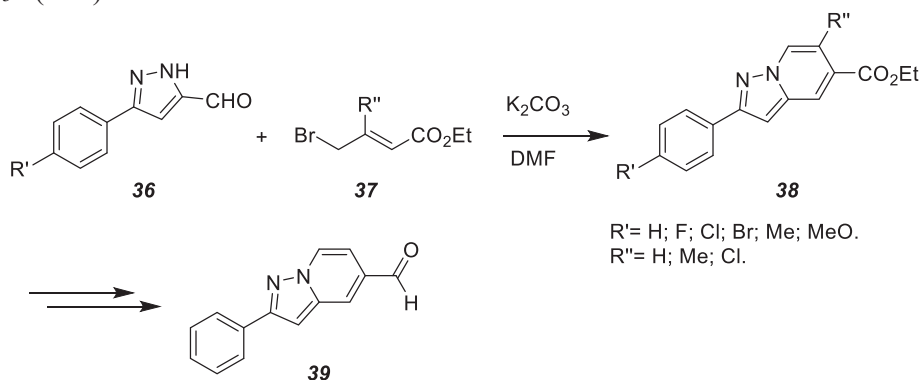
Yakovenko *et al.* presented synthesis of 5-oxopyrazolo[4,3-*b*]pyridine-6-carboxylic acids and their nitriles (Scheme 1.9) [31]. *N*-Boc-protected 5-formyl-1*H*-pyrazol-4-amines **33** reacted with malonic acid in pyridine in the presence of pyrrolidine at 45–50 °C, or with malonic acid monomethyl ether in the presence of pyrrolidine in AcOH under reflux to obtain 5-oxo-4,5-dihydro-1*H*-pyrazolo[4,3-*b*]pyridine-6-carboxylic acids **34**. The production of 5-oxo-4,5-dihydro-1*H*-pyrazolo[4,3-*b*]pyridine-6-carbonitriles **35** was achieved in the same manner by performing the reaction with cyanoacetic acid or methylcyanoacetate. The 5-oxo-4,5-dihydro-1*H*-pyrazolo[4,3-*b*]pyridine-6-carbonitriles **35** were also obtained in ACN containing pyrrolidine and a catalytic amount of proline heated under reflux.



$R' = \text{Me, Et, } t\text{-Bu, Ph, 2-Py}$; $R'' = \text{H, MeO, Ph}$; $R''' = \text{H, Me}$

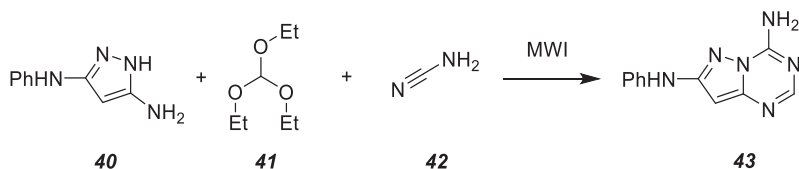
Scheme 1.9. Synthesis of 5-oxopyrazolo[4,3-*b*]pyridine-6-carboxylic acids and nitriles. *Reagents and conditions:* i: pyrrolidine, pyridine, 45–50 °C ($R''' = \text{H}$); ii: pyrrolidine, AcOH, rt, 5–6 h ($R''' = \text{Me}$); iii: pyrrolidine, proline, ACN, rt, 4–6 h ($R''' = \text{Me}$)

Ge *et al.* reported intramolecular condensation of α,β -unsaturated esters with aldehydes to obtain pyrazolo[1,5-*a*]pyridine derivatives [32]. The reactions were carried out with 1*H*-pyrazole-5-carbaldehydes **36** and ethyl (*E*)-4-bromobut-2-enoates **37** in DMF in the presence of potassium carbonate at room temperature (Scheme 1.10). Recently, this synthesis method has been applied to design a new fluorescent probe **39** which could sense sulphite in dry white wine [33]. The probe showed both high sensitivity and selectivity, and it also exhibited a fast response to SO_3^{2-} (30 s).



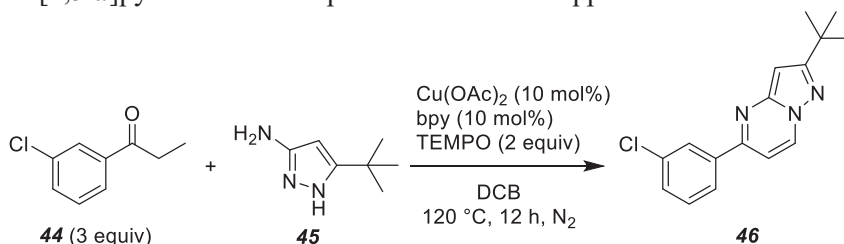
Scheme 1.10. Synthesis of pyrazolo[1,5-*a*]pyridines from α,β -unsaturated esters and 1*H*-pyrazole-5-carbaldehyde

Lin Lim *et al.* developed a one-pot multicomponent reaction to afford 4-amino-7-phenylaminopyrazolo[1,5-*a*][1,3,5]triazine [34]. The reaction was carried out with 5-amino-3-phenylaminopyrazole **40**, triethyl orthoformate **41** and cyanamide **42** in MeOH at 150 °C under microwave irradiation for 25 min, thereby furnishing the product in 60% yield (Scheme 1.11). Attempts to manipulate the ratios of the reagents, the reaction temperature and even different solvents did not lead to any further improvements in the reaction outcome. The authors also proved that the synthesis method is reproducible in various microwave reactors and under microwave-like heating.



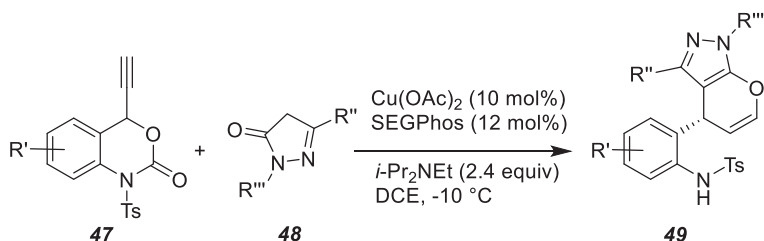
Scheme 1.11. Synthesis of 4-amino-7-phenylaminopyrazolo[1,5-*a*][1,3,5]triazine

A study was published exploring the coupling between *m*-chloropropiophenone **44** and 5-(*tert*-butyl)-1*H*-pyrazol-3-amine **45** (Scheme 1.12) [35]. It was found that the best conditions to obtain [3+3] cycloaddition product pyrazolo[1,5-*a*]pyrimidine **46** was by using excess of *m*-chloropropiophenone, Cu(OAc)₂ as a catalyst, and bpy as a ligand, with TEMPO as mediator and DCB as solvent. The reaction proceeded under N₂ atmosphere at 120 °C for 12 h. A prominently high 96% yield was reported. The reaction conditions tolerated a wide range of ketones and aminopyrazoles with diverse functional groups; thus this method may offer a wide range of applications in the synthesis of novel pyrazolo[1,5-*a*]pyrimidines with potential medicinal applications.



Scheme 1.12. Synthesis of pyrazolo[1,5-*a*]pyrimidines

Copper(II) acetate was also applied in synthesizing 1,4-dihydropyrano[2,3-*c*]pyrazole derivatives **49** from ethynyl benzoxazinanes **47** and pyrazolones **48** (Scheme 1.13) [36]. The high yield and enantioselectivity of asymmetric [3+3] annulation was achieved by using Cu(OAc)₂ as a catalyst, SEGPhos as a ligand, *N,N*-diisopropylethylamine as a base while stirring the reaction mixture in DCE at −10 °C.



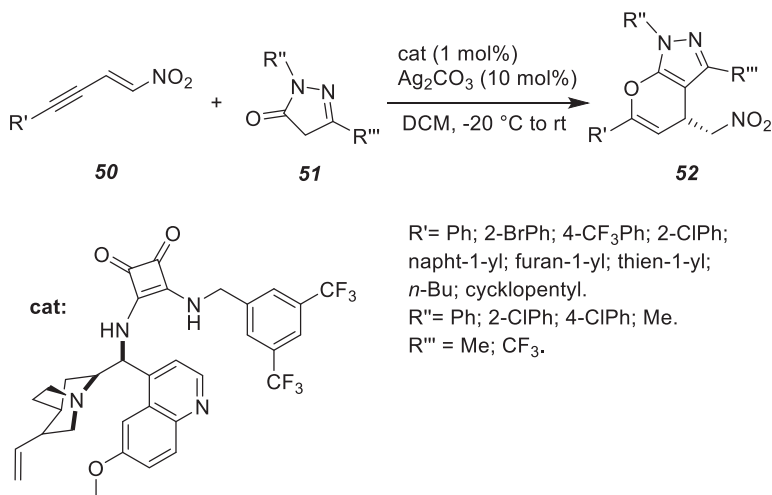
R' = H; 6-Me; 6-F; 7-F; 6-Br.

R'' = Ph; 4-MePh; 4-MeOPh.

R''' = Ph; 2-MePh; 4-MePh; 4-MeOPh; 2-FPh; 4-FPh; 3-ClPh; 4-ClPh; Bn; Me; napht-2-yl.

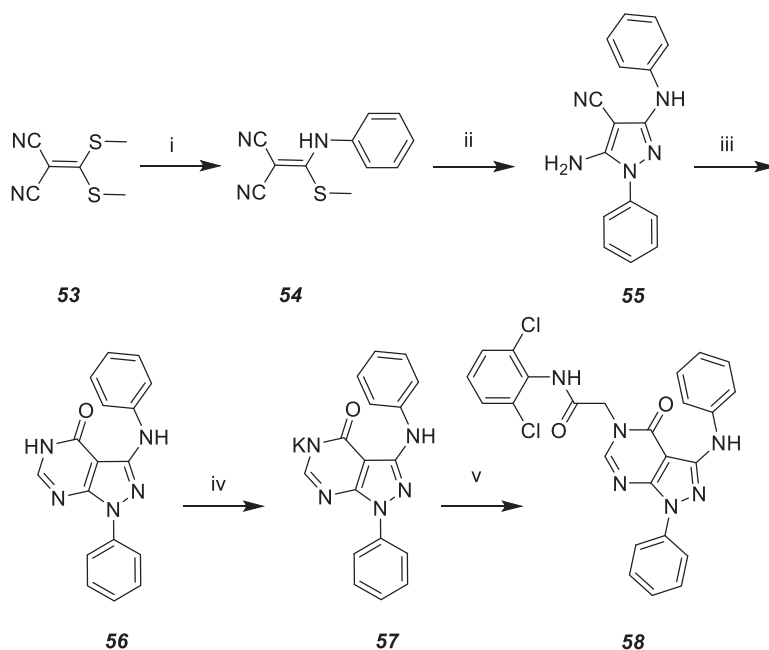
Scheme 1.13. Synthesis of 1,4-dihydropyrano[2,3-*c*]pyrazoles

Hack *et al.* developed a novel synthesis method of chiral pyrano-annulated pyrazoles by the combination of squaramide and silver catalysis [37]. The one-pot two step protocol consists of an asymmetric Michael addition powered by cinchona-derived squaramides followed by hydroalkoxylation catalyzed by Ag_2CO_3 (Scheme 1.14). The reaction proceeded under mild conditions (DCM, -20°C) and with low catalyst loading, yet it gave excellent yields and offered excellent enantioselectivities.



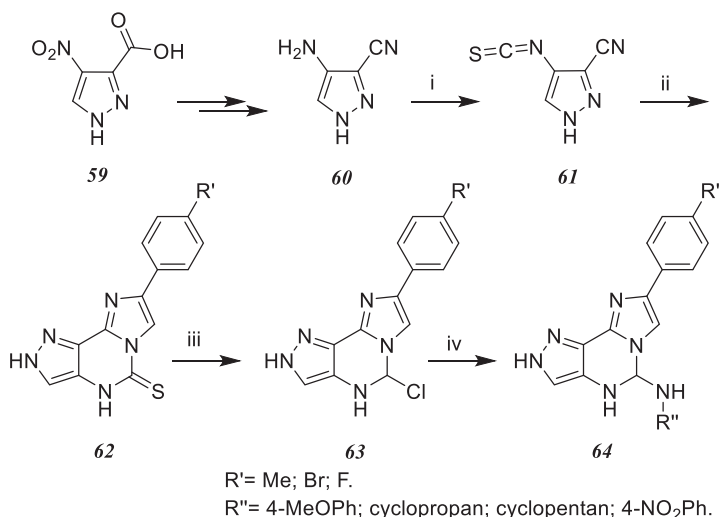
Scheme 1.14. Synthesis of chiral pyrano-annulated pyrazoles

Sherbiny *et al.* published the results of broad-spectrum cytotoxic pyrazolo[3,4-*d*]pyrimidine derivatives as potent EGFR inhibitors and cell apoptosis inducers [38]. The pyrazolo[3,4-*d*]pyrimidine core was obtained *via* a multistep synthesis route (Scheme 1.15). Starting from 2-[bis(methylthio)methylene]malononitrile (53) which reacted with aniline in ethanol followed by the reaction with phenyl hydrazine in the presence of ethanol, 5-amino-1-phenyl-3-(phenylamino)-1*H*-pyrazole-4-carbonitrile (55) was produced. The latter was cyclized by reacting with formic acid to yield 1-phenyl-3-(phenylamino)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (56), that was converted to potassium salt 57. The intermediate was utilized to obtain a wide variety of pyrazolo[3,4-*d*]pyrimidine derivatives. The most potent one 58 was achieved by reacting the potassium salt with 2-chloro-*N*-(2,6-dichlorophenyl)acetamide in DMF at reflux.



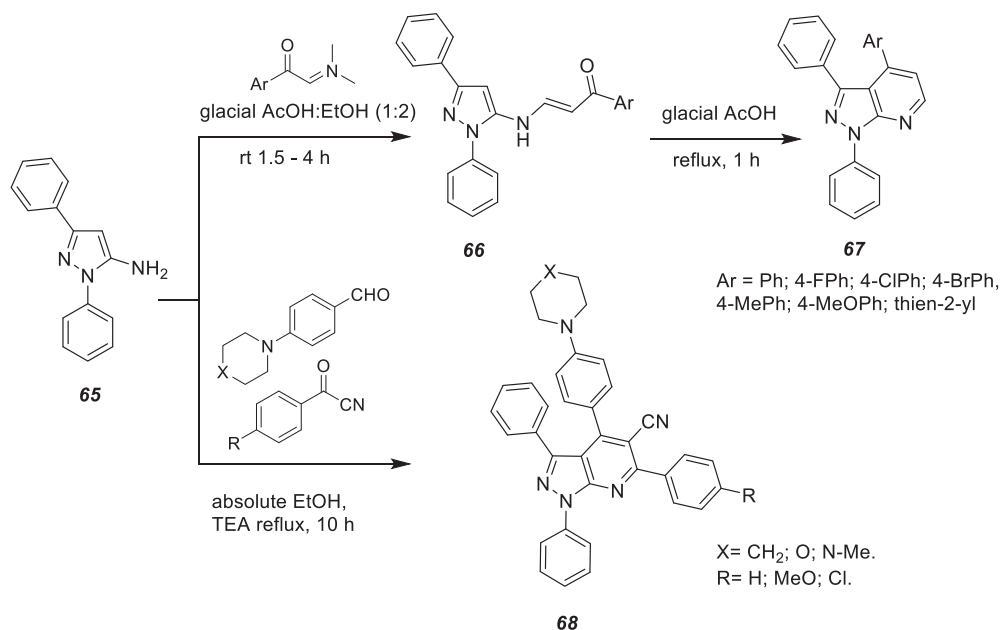
Scheme 1.15. Synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives. *Reagents and conditions:* i: aniline, absolute EtOH, reflux, 6 h; ii: phenyl hydrazine, absolute EtOH, reflux, 12 h; iii: formic acid, reflux, 14 h; iv: alcoholic KOH, stirring 1 h; v: 2,6-dichloro-*N*-phenylacetamide, DMF, reflux, 8 h

Recently, new 2*H*-imidazo[1,2-*c*]pyrazolo[3,4-*e*]pyrimidine derivatives have been designed [39]. Firstly, 4-amino-1*H*-pyrazole-3-carbonitrile **60** was obtained from 4-nitro-1*H*-pyrazole-3-carboxylic acid **59** via the sequence of esterification, ammoniation, von Braun amide dehydration, and reduction reactions. Then, it was treated with thiophosgene, and, after reacting with differently substituted 2-amino-1-phenylethan-1-ones, the 2*H*-imidazo[1,2-*c*]pyrazolo[3,4-*e*]pyrimidine core **62** was obtained (Scheme 1.16). In order to introduce more substituents to 5-position, the thio group was treated with POCl₃, and the halide was then reacted with various amines.



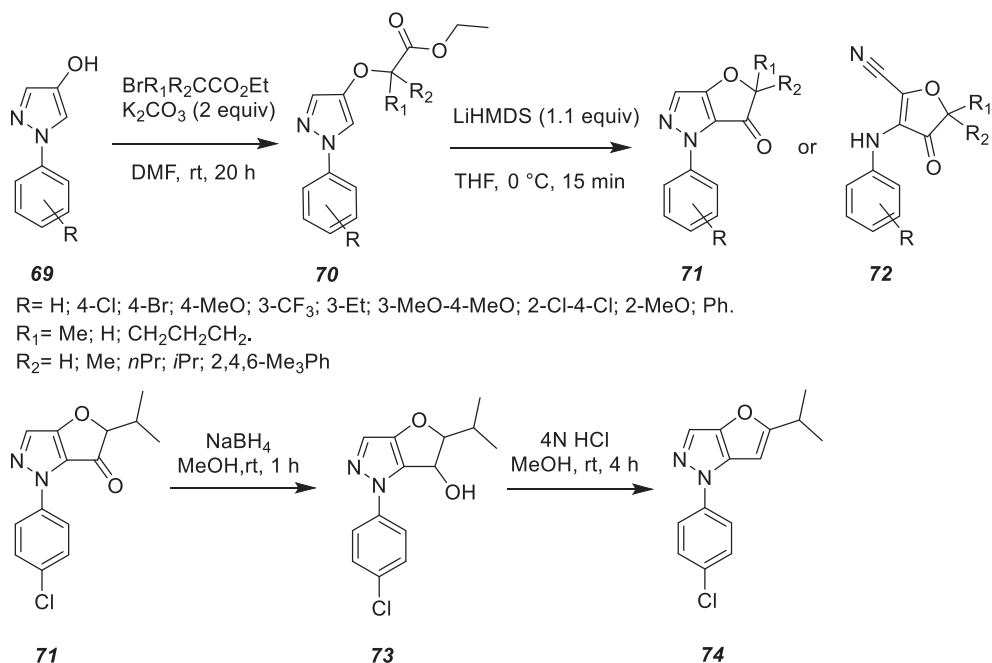
Scheme 1.16. Synthesis of 2*H*-imidazo[1,2-*c*]pyrazolo[3,4-*e*]pyrimidine derivatives.
Reagents and conditions: i: CSeCl₂, DCM; ii: differently substituted 2-amino-1-phenylethan-1-ones, Na₂CO₃, H₂O, DCM, EtOH, iii: POCl₃, DMF, iv: TsOH, *n*-butanol

Hassan *et al.* reported the synthesis of pyrazolo[3,4-*b*]pyridines [40]. To achieve this, 1,3-diphenyl-1*H*-pyrazol-5-amine (**65**) was chosen as the key intermediate which reacted with 3-(dimethylamino)-1-(aryl)prop-2-en-1-one in glacial acetic acid:ethanol (1:2) at an ambient temperature (Scheme 1.17). Intramolecular cyclization was carried out in refluxing glacial acetic acid. To obtain more substituted 1,3-diphenyl-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile derivatives **68**, a simple and efficient one-pot three-components reaction of the 1,3-diphenyl-1*H*-pyrazol-5-amine **65** with the subsequent 3-oxo-3-un/substituted phenylpropanenitriles and the appropriate 4-(un/substituted-1-yl)benzaldehydes, while using a catalytic amount of TEA, in absolute EtOH was performed.



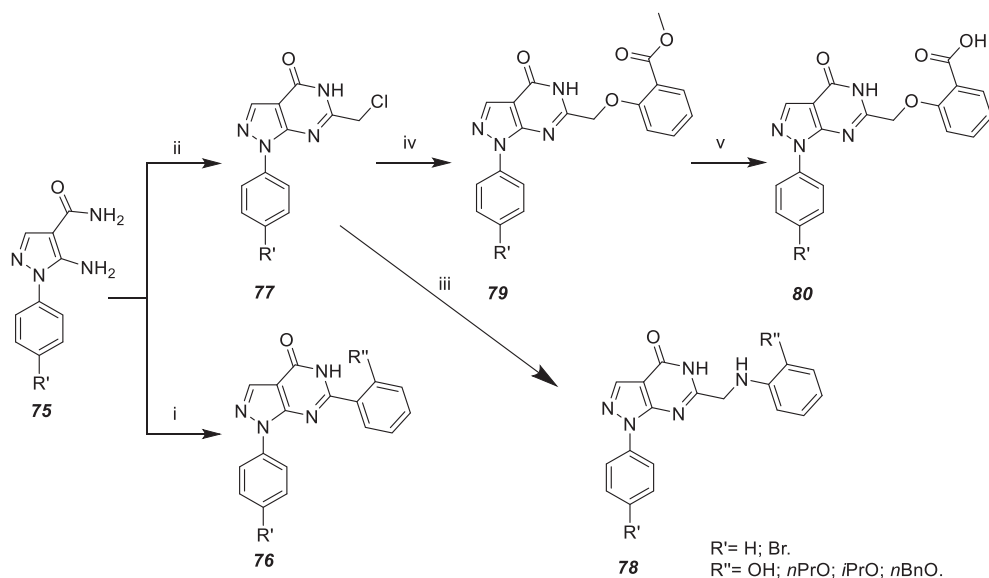
Scheme 1.17. Synthesis of 1,3,4- and 1,3,4,5,6-substituted 1*H*-pyrazolo[3,4-*b*]pyridines

Researchers Jacob and Lamberth reported a method to synthesize furo[3,2-*c*]pyrazoles starting from pyrazole and building blocks instead of using furanes [41]. 1-(4-Chlorophenyl)-4-hydroxypyrazoles **69** were chosen as the starting materials (Scheme 1.18). Since the hydroxyl function in 4-position can be easily alkylated, reacting with ethyl 2-bromoisobutyrate delivered the pyrazole ether **70**. Since the ring closure with both acids and simple bases could not be successfully achieved, the authors tried lithiation of the pyrazole ring by using LiHMDS. It was already known that pyrazoles undergo regioselective hydrogen–lithium exchange in ring position 5, which then leads to the lithiated pyrazole species attack on the ester function resulting in the ring closure. Interestingly, the unsubstituted derivative at α -position of the ester gave no ring closure; meanwhile, derivatives with an *ortho*-substituted phenyl ring yielded 2-cyano-3-anilinofuranones **72**. Subsequently, the fully aromatic heterobicyclic **74** was obtained by applying reduction and dehydration to furo[3,2-*c*]pyrazol-6-one **71**.



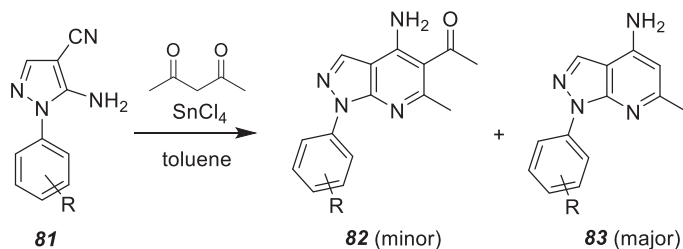
Scheme 1.18. Synthesis of 1*H*-furo[3,2-*c*]pyrazol-6(5*H*)-ones and 1*H*-furo[3,2-*c*]pyrazole

In 2020, Shaaban *et al.* rationally designed pyrazolo[3,4-*d*]pyrimidin-7-one scaffolds *via* consecutive molecular modelling in the course of a study aiming to find phosphodiesterase 5 (PDE5) inhibitors [42]. 1-Substituted-arylpyrazolo[3,4-*d*]pyrimidines **76** were synthesized by the cyclization of 5-amino-1-substituted-phenylpyrazolo-4-carboxamides **75** with 2-substituted benzaldehydes in the presence of iodine in ACN at reflux (Scheme 1.19). Another synthetic pathway was reported by the reaction of **75** with chloroacetyl chloride, which afforded chloromethyl derivative **77**. The latter underwent nucleophilic substitution with an aromatic amine or phenol to give methyl amino derivatives **79** and methoxy derivative **80** which was hydrolyzed with sodium hydroxide to give carboxy acid **81**. Some of the derivatives possessed inhibitory activities against PDE5 that were similar to that of Sildenafil.



Scheme 1.19. Synthesis of 1,6-disubstituted-pyrazolo[3,4-*d*]pyrimidinones. *Reagents and conditions:* i: 2-hydroxybenzaldehyde or 2-alkoxybenzaldehyde, I₂, ACN, reflux, 6 h; ii: chloroacetyl chloride, 6 h; iii: *o*-phenylene diamine or aminophenol, KOH, DMSO, 70 °C, 10 h; iv: methyl salicylate, KOH, DMSO, 70 °C, 5 h; v: NaOH, abs. EtOH, reflux 8 h, AcOH

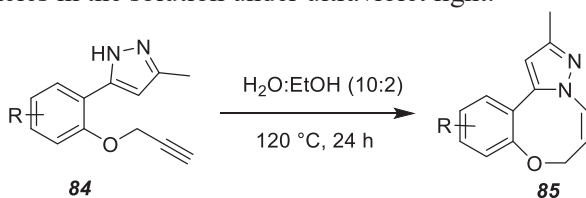
Hu *et al.* reported a series of novel 6-methyl-1-aryl-1*H*-pyrazolo[3,4-*b*]pyridin-4-amines that were prepared by the annulation of 5-amino-1-aryl-1*H*-pyrazole-4-carbonitriles **81** with acetylacetone in the presence of tin(IV) chloride in refluxing toluene (Scheme 1.20) [43]. Although the major products of the annulation were expected to be 1-(4-amino-6-methyl-1-aryl-1*H*-pyrazolo[3,4-*b*]pyridin-5-yl)-ethanones **82**, the work up of the reaction (proceeding while adding saturated sodium carbonate solution) resulted in deacetylation. This proved that ethanones **82** were a minor product of the reaction, and only the precursor of the deacetyl derivatives **83**.



Scheme 1.20. Annulation of *o*-aminonitriles with acetylacetone

Sarkar and Sahoo demonstrated that the heating of propyloxy-phenyl pyrazoles **84** in a solution of H₂O:EtOH (10:2) at 120 °C caused direct 8-*endo dig* ring annulation (Scheme 1.21) [44]. As a result, (*Z*)-2-methyl-7*H*-benzo[*b*]pyrazolo[5,1-

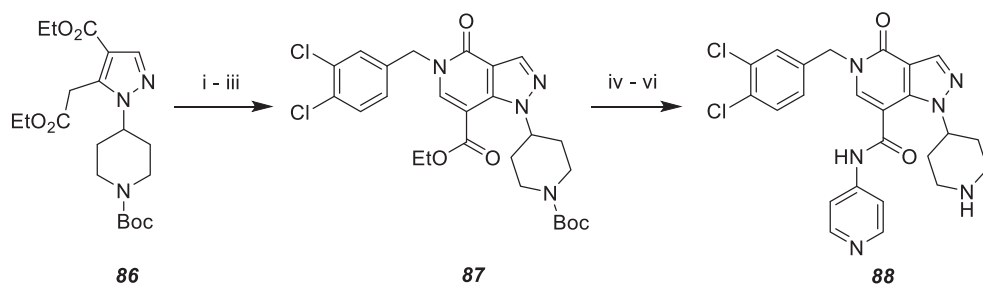
d][1,5]oxazocine derivatives **85** were obtained in good yields. They showed high emissive tendencies in the solution under ultraviolet light.



R= H; 11-Me; 11-Br; 11-Cl; 9-Me; 10-(prop-2-yn-1-yloxy); 9,11-diBr; 9,11-diCl.

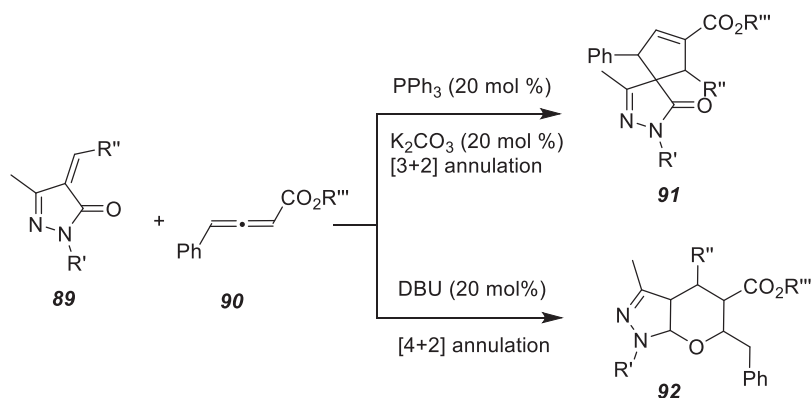
Scheme 1.21. Synthesis of (*Z*)-2-methyl-7*H*-benzo[*b*]pyrazolo[5,1-*d*][1,5]oxazocine derivatives

Francis *et al.* designed novel pyrazolo[4,3-*c*]pyridine derivatives as inhibitors of fascin 1 as potential anti-metastatic agents [45]. Pyrazolopyridinone **87** was obtained in good yield over the three-step synthesis from pyrazole **86** with ethoxycarbonyl substituent at 4-position and 2-ethoxy-2-oxoethyl substituent at 5-position (Scheme 1.22). It was functionalized by refluxing with the Brederick's reagent followed by the addition of dichlorobenzylamine. Finally, it was fully cyclized with sodium ethoxide. The subsequent saponification of the ester, coupling of 4-amino pyridyl, and the removal of the Boc protecting group furnished the final compound that was a potent and functionally active fascin binder.



Scheme 1.22. Synthesis of 1*H*-pyrazolo[4,3-*c*]pyridine derivatives. *Reagents and conditions:* i: Brederick's reagent, toluene, reflux; ii: 3,4-dichlorophenylmethanamine, AcOH, toluene; iii: sodium ethoxide, EtOH; iv: NaOH, EtOH, rt; v: HATU, *N,N*-diisopropylethylamine, DMF, rt; vi: TFA, DCM, rt

The Luo team developed an effective Lewis base catalyst-controlled α -regioselective annulation reaction of γ -substituted allenates with unsaturated pyrazolones [46]. It was found that the use of PPh₃ and K₂CO₃ in toluene afforded [3+2] annulation product – spirocyclopentene-pyrazolones **91** while changing the catalyst to DBU without any additive in DCM led to the formation of [4+2] annulation product pyrano[2,3-*c*]pyrazoles **92** (Scheme 1.23).



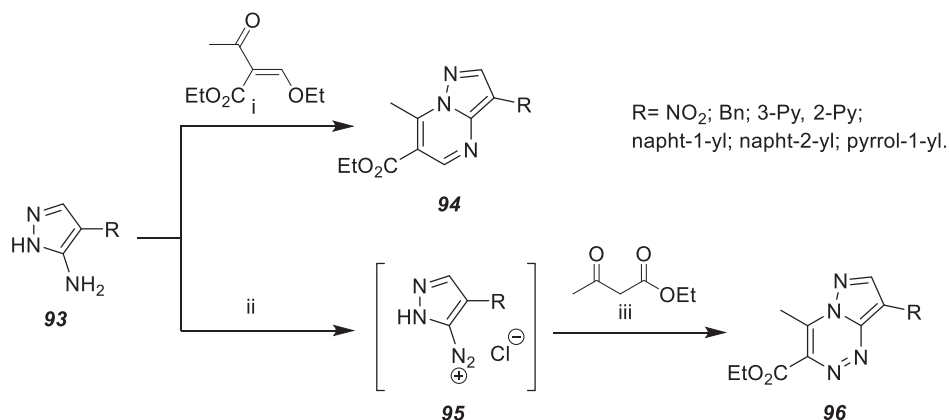
$\text{R}' = \text{Ph}; 4\text{-FPh}; 4\text{-ClPh}; 4\text{-BrPh}; 4\text{-MePh}; 4\text{-}i\text{BuPh}; 4\text{-}t\text{Bu}; 4\text{-MeOPh}; 2\text{-ClPh};$
 $3\text{-MePh}; \text{naph-1-yl}; \text{naph-2-yl}; \text{thien-2-yl}.$

$\text{R}'' = 4\text{-ClPh}; 4\text{-MePh}.$

$\text{R}''' = \text{Bn}; t\text{Bu}.$

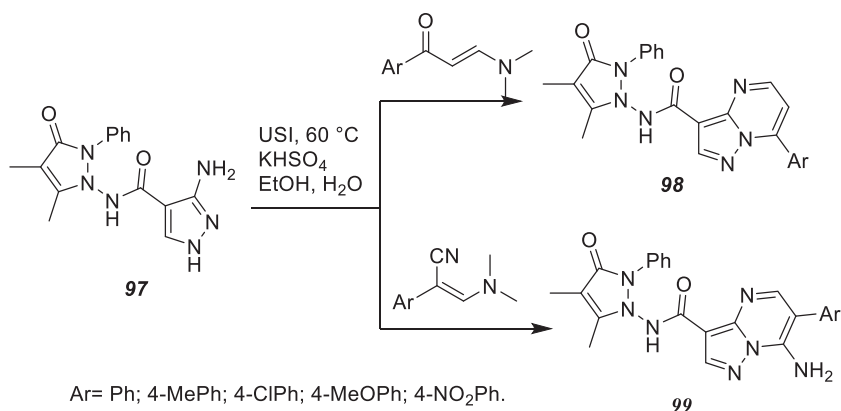
Scheme 1.23. Catalyst-controlled α -regioselective annulation reaction of γ -substituted allenates with unsaturated pyrazolones

In 2018, a team of researchers conducted a study comparing compounds with the pyrazolo[1,5-*a*]pyrimidine structure and the pyrazolo[5,1-*c*][1,2,4]triazine core as ligands of the GABAA-receptor subtype [47]. For the synthesis of both annulated pyrazoles, 3-aminopyrazoles substituted at 4-position **93** were chosen (Scheme 1.24). In order to obtain the core of pyrazolo[1,5-*a*]pyrimidine, **93** reacted with ethyl 2-acetyl-3-ethoxyacrylate, which resulted in the intermediates 3-substituted ethyl 7-methylpyrazolo[1,5-*a*]pyrimidine-6-carboxylates **94**. The latter substances were further derivatized to obtain the desired compounds. The pyrazolo[5,1-*c*][1,2,4]triazine scaffold was obtained *via* two-step synthesis: 4-substituted 3(5)-aminopyrazoles were diazotized and then cyclized with ethyl acetoacetate. This afforded intermediate 8-substituted ethyl 4-methylpyrazolo[5,1-*c*][1,2,4]triazine-3-carboxylate **96** that was later transformed into 1,4-dihydro-3-(10*H*-pyrazolo-3-yl)-8-(substituted)pyrazolo[5,1-*c*][1,2,4]triazin-4-ones.



Scheme 1.24. Synthesis of the pyrazolo[1,5-*a*]pyrimidine structure and pyrazolo[5,1-*c*][1,2,4]triazines. *Reagents and conditions:* i: ethyl ethoxymethylacetoacetate/EtOH; ii: HCl, NaNO₂/H₂O; iii: sodium acetate, ethyl acetoacetate

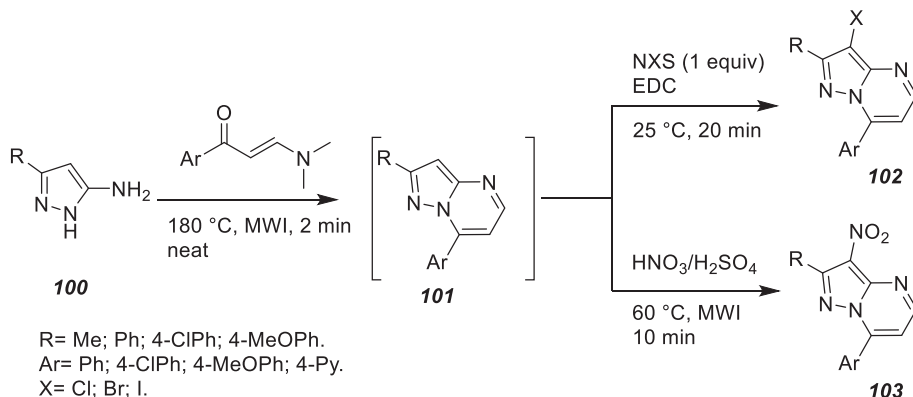
In 2020, Kapin and coworkers developed a method to synthesize antipyrinyl-pyrazolo[1,5-*a*]pyrimidines by using ultrasound irradiation (USI) [48]. The reaction of aminopyrazole **97** with various enaminones, enamionitriles and hetarylen aminones was carried out in a water-ethanol system by using KHSO₄ (Scheme 1.25). Switching to sonication not only shortened the reaction time (from 60 min to 4–6 min), but also significantly increased the yield (from 50% to almost quantitative yields). Another advantage of sonication was that the product precipitated and could be isolated by simple filtration in a virtually pure form.



Scheme 1.25. Synthesis of antipyrinyl-pyrazolo[1,5-*a*]pyrimidines by using USI

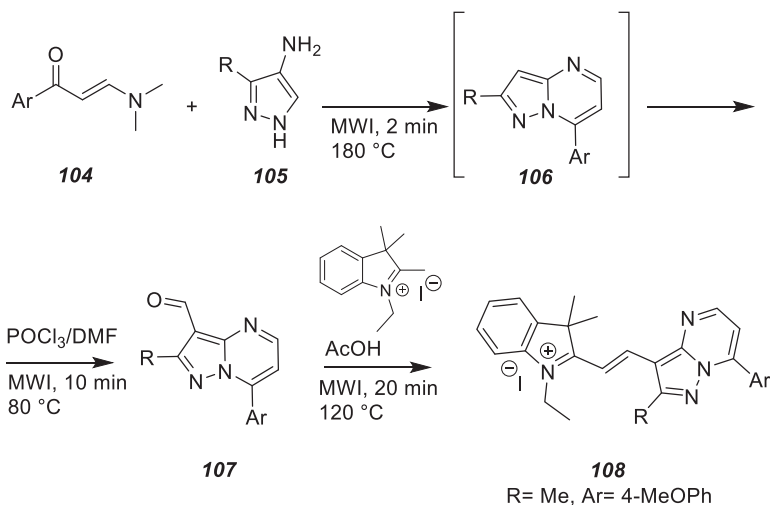
Castillo *et al.* developed a one-pot route to obtain diversely substituted 3-halo and 3-nitropyrazolo[1,5-*a*]pyrimidines [49]. The optimization of the reaction between 1-*H*-pyrazol-5-amines **100** and β -enaminones revealed that solvent-free microwave irradiation at 180 °C was the best protocol which led to a decreased

reaction time (2 min) and an increased yield for achieving pyrazolo[1,5-*a*]pyrimidines **101** (Scheme 1.26). A wide range of differently substituted 1-*H*-pyrazol-5-amines were tested, and virtually no loss of efficiency was observed, which indicated the low electronic influence of the substituents on the reactivity. The authors also used these conditions as a first step in the synthesis of 3-halo and 3-nitro substituted pyrazolo[1,5-*a*]pyrimidines. The second stage involved the use of various *N*-halosuccinimides in 1,2-dichloroethane as a solvent at room temperature for 20 min for 3-halo derivatives **102** and a mixture of HNO₃:H₂SO₄ (2:1) at 60 °C for 10 min under solvent-free conditions for 3-nitro derivative **103**. The reactions were done in the same vessel, thereby proving the advantages of this method: short reaction times, high yields, operational simplicity, a broad substrate scope, and pot-economy.



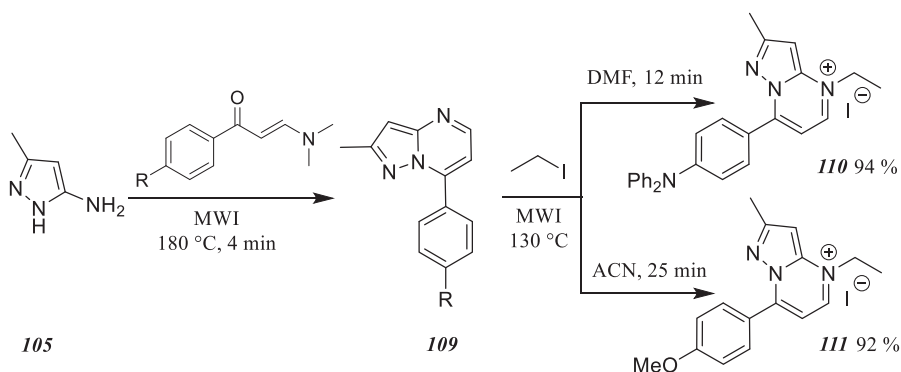
Scheme 1.26. One-pot synthesis of 3-halo and 3-nitropyrazolo[1,5-*a*]pyrimidines

Castillo's team also employed this method to obtain 3-formylpyrazolo[1,5-*a*]pyrimidines and performed the cyclo-condensation–formylation sequence by using MWI (Scheme 1.27) [50]. The formylation was done by using the Vilsmeier–Haack reagent while stirring at 80 °C for 10 minutes. Since the pyrazolo[1,5-*a*]pyrimidine core is a fluorophore, the scientists also explored derivatives that could be used as chemosensors for anion or metal ion detection. They reported microwave assisted synthesis of (*E*)-1-ethyl-2-{2-[7-(4-methoxyphenyl)-2-methylpyrazolo[1,5-*a*]pyrimidin-3-yl]vinyl}-3,3-dimethyl-3*H*-indol-1-ium iodides **108** from 3-formyl-7-(4-methoxyphenyl)-2-methylpyrazolo[1,5-*a*]pyrimidines **107** and 1-ethyl-2,3,3-trimethyl-3*H*-indol-1-ium iodide in acetic acid [51]. It was proven to have high selectivity and sensitivity for cyano ions; thus it can be used as a probe for cyanide detection.



Scheme 1.27. Microwave-assisted synthesis of 3-formylpyrazolo[1,5-*a*]pyrimidines and probe (**108**)

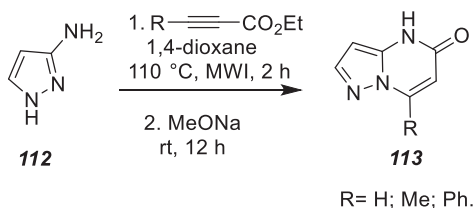
Most recently, Castillo's team analyzed 7-[4-(diphenyl-amino)phenyl]-4-ethyl-2-methylpyrazolo[1,5-*a*]pyrimidin-4-ium iodide (**110**) and 4-ethyl-7-(4-methoxyphenyl)-2-methylpyrazolo[1,5-*a*]pyrimidin-4-ium iodide (**111**) as probes for cyano ions (Scheme 1.28) and found that **111** can be used to detect small amounts of cyanide ions in tap water and also in the solid state (for example, in bitter almonds) [52].



Scheme 1.28. Two-step synthesis under MWI of probes (**110**) and (**111**)

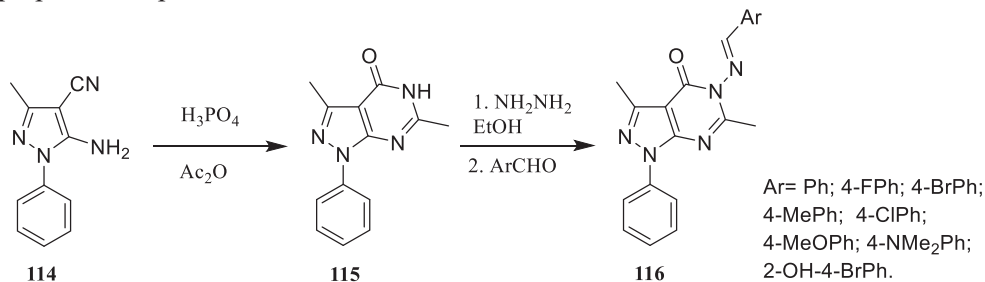
Jismy's team published extensive data regarding the synthesis and functionalization of 7-substituted pyrazolo[1,5-*a*]pyrimidin-5-ones. Firstly, to obtain the pyrazolo[1,5-*a*]pyrimidine core **113**, 3-aminopyrazole **112** was reacted with alkynes in dioxane under heating to reflux in microwave-assisted conditions for 2 h, followed by the addition of sodium methoxide at room temperature for 12 h (Scheme 1.29) [53]. To introduce a different substituent at 5-position, C-O

activation of the amide function with PyBroP as the activating reagent and Pd catalyzed cross-couplings or S_NAr reactions were carried out in the one-pot manner. Thus (het)aryl, alkynyl, amino, and thiol groups were introduced very efficiently. The same protocol was later used to synthesize a library of 7-trifluoromethyl-2,5-disubstituted pyrazolo[1,5-*a*]pyrimidines [54]. 3-Amino-5-substituted-pyrazoles reacted with fluorinated alkynes to provide fluorinated pyrazolo[1,5-*a*]pyrimidin-5-one as the only regio-isomer. Recently, they also reported the synthesis of 3,5-diarylated 7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine derivatives [55]. To introduce an aryl substituent to 3-position of 7-(trifluoromethyl)pyrazolo[1,5-*a*]pyrimidine, NBS-mediated bromination and Suzuki-Miyaura cross-coupling were carried out.



Scheme 1.29. Synthesis of 7-substituted pyrazolo[1,5-*a*]pyrimidin-5-ones

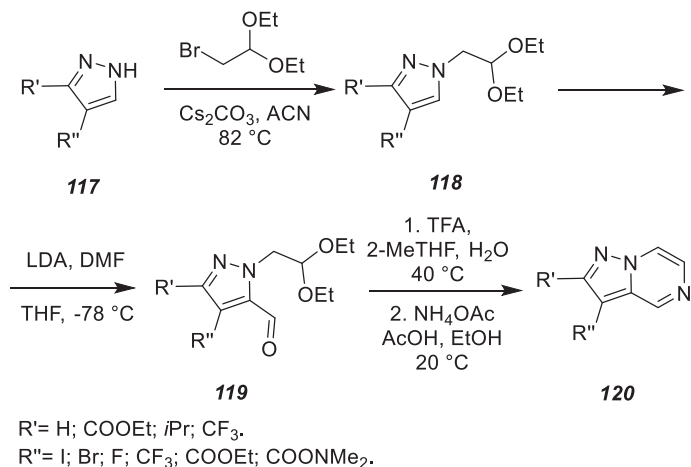
Recently, Horchani *et al.* presented the synthesis of new pyrazolopyrimidinone derivatives possessing antibacterial properties [56]. 5-Amino-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonitrile (**114**) (Scheme 1.30) was chosen as a starting material to reflux in acetic anhydride in the presence of phosphoric acid to afford cyclized product pyrazolo[3,4-*d*]pyrimidinone **115**. The latter was condensed with hydrazine monohydrate and coupled with the appropriate aromatic aldehydes to yield the fused Schiff base-pyrazolopyrimidinones **116**. After *in vitro* screening for their antibacterial activity, the results showed that the derivative with the 4-methylphenyl substituent exhibited the best inhibitory potential against most bacteria among the prepared compounds.



Scheme 1.30. Synthesis of pyrazolo[3,4-*d*]pyrimidinone derivatives

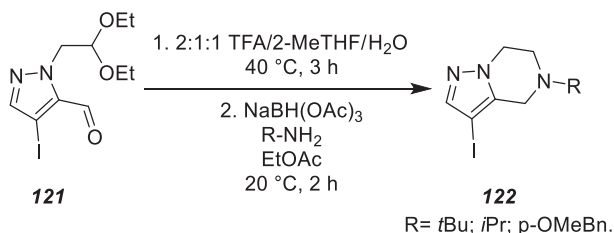
Lindsay-Scott and coworkers investigated the construction of condensed heterocycles from commercially available pyrazoles *via* regioselectively alkylated and formylated intermediates [57]. The first attempt to obtain pyrazolo[1,5-*a*]pyrazines from 4-bromo-1*H*-pyrazole-3-carbaldehyde was unsuccessful because

the major product of alkylation with 2-bromo-1,1-diethoxy-ethane was not the desired regioisomer as the alkylation of 3-substituted and 3,4-disubstituted pyrazoles generally leads to mixtures of regioisomeric products favoring the N-1 alkylated isomer [58]. To overcome these regioselectivity issues, the alkylation–formylation strategy was applied (Scheme 1.31). Alkylation was carried out by using Cs_2CO_3 stirring in acetonitrile at 82 °C for 16 hours. It was observed that 4-substituted pyrazoles were alkylated smoothly without any issues of regioselectivity; however, the alkylation of pyrazoles bearing substituents in the 3-position generated mixtures of regioisomeric products that were readily separable by column chromatography. The formylation step was executed by using lithium diisopropylamide (LDA) at a low temperature to deprotonate pyrazoles followed by the addition of DMF. The pyrazole-aldehydes **119** were formed in a regioselective manner from both 3- and 4-substituted pyrazoles with formylation occurring exclusively at 5-position. Finally, the acetal deprotection of pyrazole-aldehydes with aqueous TFA followed by solvent swapping and the subsequent cyclization with ammonium acetate furnished the desired products **120** without the need to isolate the intermediate.



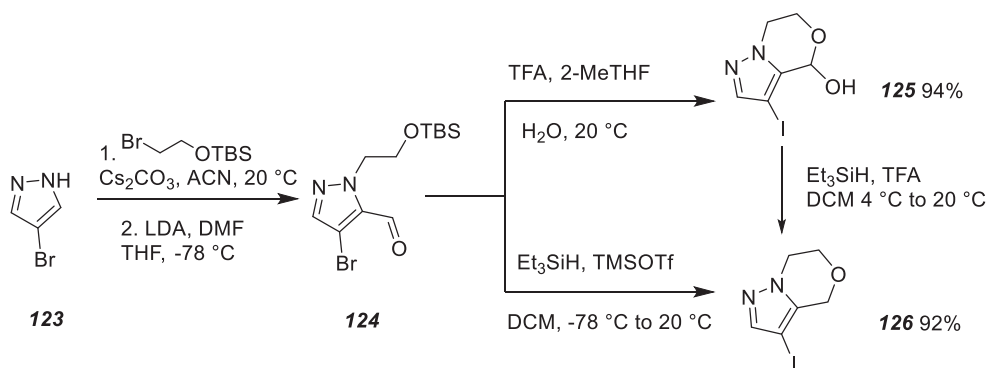
Scheme 1.31. Synthesis of pyrazolo[1,5-*a*]pyrazines *via* alkylation, formylation, deprotection, and cyclization steps

Moreover, it was found that structurally related compounds 4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrazines **122** can also be obtained in a similar manner by using the double-reductive amination approach (Scheme 1.32).



Scheme 1.32. Synthesis of 4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyrazines

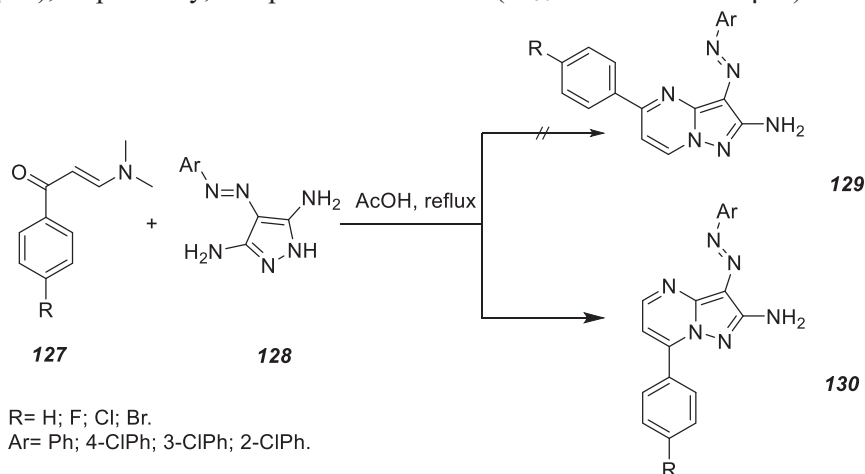
6,7-Dihydro-4*H*-pyrazolo[5,1-*c*][1,4]oxazines were obtained by adapting the same alkylation-formylation approach (Scheme 1.33) [59]. Alkylation was carried out with (2-bromoethoxy)-*tert*-butyldimethylsilane by using the same conditions as described above to get N1-alkylated pyrazoles **124** followed by formylation with LDA and DMF. Finally, the deprotection of TBS groups was achieved by using TFA to obtain pyrazole lactols **125**. The latter products were reduced with Et₃SiH and TFA in DCM to yield the desired 6,7-dihydro-4*H*-pyrazolo[5,1-*c*][1,4]oxazines **126**. It is noteworthy that the deprotection-reduction sequence was also achieved in a one-pot fashion when switching from TFA to TMSOTf, which significantly increased the yield from 62% to 93%.



Scheme 1.33. Synthesis of 6,7-dihydro-4*H*-pyrazolo[5,1-*c*][1,4]oxazines

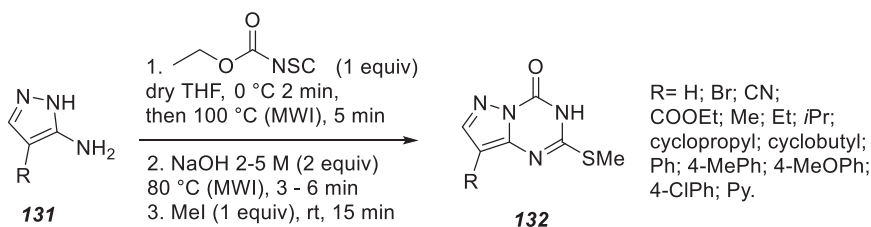
New series of pyrazolo[1,5-*a*]pyrimidines were synthesized by Almekhadi and colleagues [60]. The target molecules were obtained by reacting derivatives of enaminones **127** with 4-arylazo-1*H*-pyrazole-3,5-diamines **128** in glacial acetic acid under reflux for 5 h (Scheme 1.34). It was proven by using NMR that 7-arylpyrazolo[1,5-*a*]pyrimidines **130** were formed instead of 5-arylpyrazolo[1,5-*a*]pyrimidines **129**. All the newly synthesized derivatives were evaluated for their CDK2 inhibitory activity and their anti-leukemia activity against two different leukemia cell lines: acute lymphoblastic leukemia (MOLT-4) and acute myeloid leukemia (HL-60). It was determined that 7-(4-bromo-phenyl)-3-(3-chloro/2-chloro-phenylazo)-pyrazolo[1,5-*a*]pyrimidin-2-ylamines demonstrated the best CDK2 inhibitory activity with a potency (IC₅₀ = 22 and 24 nM, respectively) comparable to that of dinaciclib (IC₅₀ = 18 nM). Moreover, both analogues exhibited potent activities against CDK1, CDK5 and CDK9 at nanomolar concentrations (IC₅₀ = 28–

80 nM). After antileukemia screening of the target compounds, it was found that the compound 7-(4-bromo-phenyl)-3-(3-chloro-phenylazo)-pyrazolo[1,5-*a*]pyrimidin-2-ylamine inhibited MOLT-4 and HL-60 by 1.4- and 2.3-fold more (IC_{50} = 0.93 and 0.80 μ M), respectively, compared to dinaciclib (IC_{50} = 1.30 and 1.84 μ M).



Scheme 1.34. Synthesis of 7-arylpyrazolo[1,5-*a*]pyrimidine derivatives

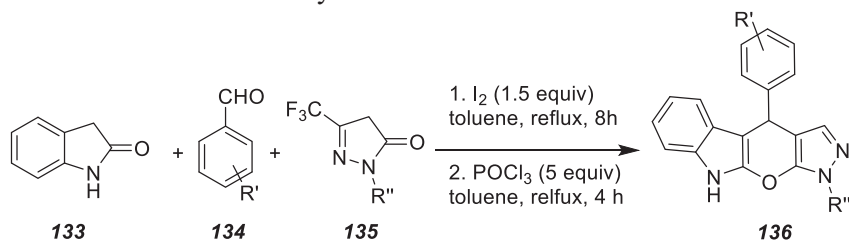
Recently, Elie and colleagues have reported the synthesis of pyrazolo[1,5-*a*][1,3,5]triazine derivatives from 4-substituted 5-aminopyrazoles [61]. Since the protocol to obtain the target molecules required at least three steps, in order to avoid work-up and purification processes of the intermediate compounds, the authors decided to try sequential one-pot microwave-assisted reactions (Scheme 1.35). Firstly, ethoxycarbonyl isothiocyanate was slowly added dropwise to a solution of 5-aminopyrazole **131** in dry THF at 0 °C. Then, the mixture was stirred for 2 min at room temperature, and later under MWI at 100 °C for 5 min. The next step required the addition of 2N NaOH at 50 °C, followed by MWI at 80 °C for 3 min. Finally, methyl iodide was added dropwise to a cooled mixture, and it was further stirred for 15 min at room temperature. The 2-(methylsulfanyl)triazine derivative **132** was isolated without flash chromatography as it was obtained as a pure white solid after the steps of acidification, filtration, washing and drying. The overall yield of this multistep reaction was 77%, which only slightly decreased when the reaction scale-up was attempted. It should be noted that the application of the same sequential one-pot procedures (a sealed vessel, same quantities of reactants, solvent, base, and equal reaction times) with a conventional heating system caused the overall yields to decrease to the 45–50% range.



Scheme 1.35. Microwave-assisted sequential one-pot synthesis of 2-(methylsulfanyl)pyrazolo[1,5-*a*][1,3,5]triazin-4(3*H*)-ones

1.3. Synthesis of condensed pyrazole systems by using multicomponent reactions

In 2018, Zhu and colleagues reported a one-pot, two-step, three-component reaction of 2-indolinone **133**, aromatic aldehydes **134** and 1-aryl-3-trifluoromethyl-5-pyrazolones **135** to obtain trifluoromethylated poly-heterocyclic compounds containing the pyrazolo[4',3':5,6]pyrano[2,3-*b*]indole skeleton **136** (Scheme 1.36) [62]. Molecular iodine was essential in the first conversion because other conventionally used bases or acids failed to afford any product. After treating the three-component reaction mixture with molecular iodine, the intramolecular annulation reaction with POCl_3 proceeded in the one-pot manner without isolation. The investigation of the scope and limitations of this reaction with a variety of aromatic aldehydes **134** and 1-aryl-3-(trifluoromethyl)-1*H*-pyrazol-5(4*H*)-ones **135** showed that aromatic aldehydes with electron-withdrawing groups gave better yields, but the *para*-methoxy group on the phenyl ring of pyrazolone had no significant effect on the reaction yields.



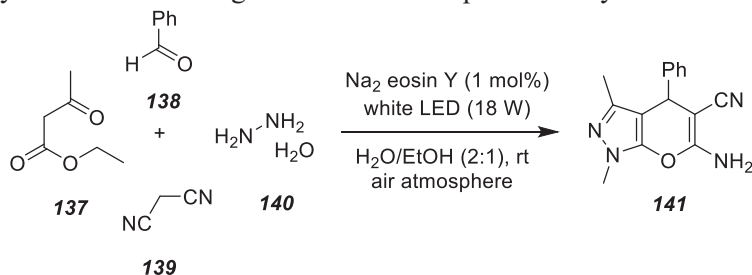
R' = H, 4-MeO, 4-Me; 4-Br; 4- NO_2 ; 3-MeO; 3-Me; 3-Br; 2-MeO.

R'' = Ph; 4-MeOPh.

Scheme 1.36. One-pot two-step synthesis of pyrazolo[4',3':5,6]pyrano[2,3-*b*]indole derivatives

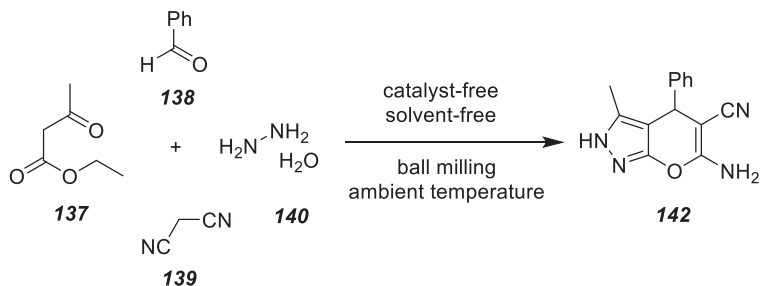
Mohamadpour reported an interesting study about green and eco-safe synthesis of dihydropyrano[2,3-*c*]pyrazole scaffolds [63]. A four-component condensation domino reaction of tandem Knoevenagel-Michael cyclocondensation of ethyl acetoacetate **137**, malononitrile **138**, aryl aldehyde derivatives **139** and hydrazine hydrate **140** was carried out in aqueous ethanol under visible light (white LED) at ambient temperature under air atmosphere (Scheme 1.37). The use of photocatalyst Na_2 eosin Y significantly increased the yield of the reaction from 41% to 93%. It is noteworthy that other catalysts have also been reported to increase the yield of this

cyclocondensation, but Na₂ eosin Y exhibited the prominently highest catalytic ability as only 1 mol% was enough to reach almost quantitative yield.



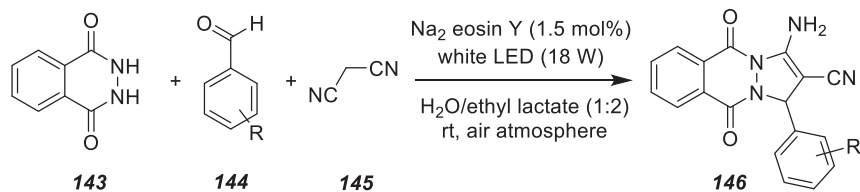
Scheme 1.37. Synthesis of dihydropyrano[2,3-c]pyrazole scaffolds

Another green synthesis approach was described by Dekamin *et al.* where dihydropyrano[2,3-c]pyrazole derivatives were obtained by a catalyst- and solvent-free procedure using the ball milling technique at ambient temperature at quantitative yields (Scheme 1.38) [64].



Scheme 1.38. Synthesis of 6-amino-3-methyl-4-phenyl-2,4-dihydropyrano[2,3-c]pyrazole-5-carbonitrile

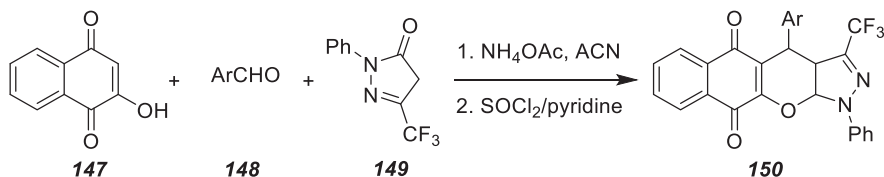
Mohamadpour also reported using photocatalyst Na₂ eosin Y to obtain 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-diones [65]. The reaction between phthalhydrazide **143**, benzaldehydes **144** and malononitrile **145** in ethyl lactate/H₂O at room temperature led to the preparation of compound **146** in 51% yield without a catalyst in comparison to 96% when using Na₂ eosin Y (Scheme 1.39).



R= H; 4-Me; 4-OH; 4-F; 3,4-diMeO; 2-Cl; 4-(thiophen-2-yl); 4-(furan-2-yl); 4-Br; 2-MeO; 4-NO₂; 3-NO₂; 2-NO₂; 2,4-diCl; 2-F.

Scheme 1.39. Synthesis of 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-diones

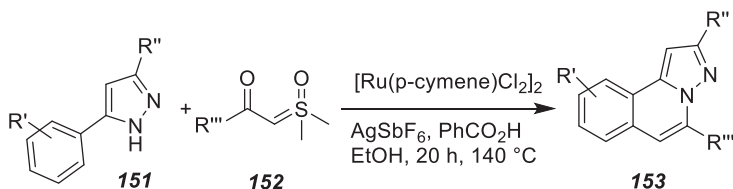
In 2020, Duan *et al.* presented a one-pot, two-step, three-component reaction of 2-hydroxy-1,4-naphthoquinone **147**, aromatic aldehydes **148** and 1-aryl-3-trifluoromethyl-5-pyrazolones **149** to yield trifluoromethylated benzo[6,7]chromeno[2,3-*c*]pyrazole derivatives **150** [66]. The protocol consisted of the condensation of the three components in acetonitrile when using NH₄OAc as a catalyst and then dehydration of the intermediate when using SOCl₂/pyridine as a dehydrating agent (Scheme 1.40).



Ar = Ph; 4-MeOPh; 4-OHPh; 4-ClPh; 4-BrPh; 4-NO₂Ph; 4-CNPh; 3-ClPh; 2-NO₂Ph; 3,4-diOHPh; 2,4-diClPh; 4-MePh; 3-FPh; 2-FPh; 3-MePh; fur-2-yl; *i*Pr.

Scheme 1.40. Synthesis of trifluoromethylated benzo[6,7]chromeno[2,3-*c*]pyrazole derivatives

Chen and colleagues described the synthesis of various pyrazolo-[1,5-*a*]isoquinolines **153** from aryl substituted pyrazoles [67]. It was achieved *via* Ru(II)-catalyzed α -carbonyl sulfoxonium ylide **152** annulation with aryl substituted pyrazoles **151** (Scheme 1.41). This oxidant-free transformation occurred through pyrazole-directed C–H activation, Ru-carbene insertion, and acid-promoted carbonyl condensation processes, thereby enabling the formation of the dual C–C and C–N bond. The optimized conditions involved [Ru(*p*-cymene)Cl₂]₂, AgSbF₆ salts and PhCO₂H additive in EtOH at 140 °C.



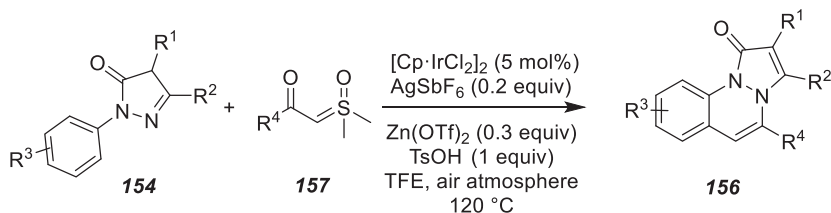
R' = H; 10-MeO; 10-F; 10-Br; 8-Me; 8-MeO; 8-F; 8-Cl; 8-Br; 8-CF₃; 8-NO₂; 10-NO₂.

R'' = H; Me; Ph.

R''' = Ph; 4-MePh; 4-MeOPh; 4-CF₃Ph; 4-FPh; 4-ClPh; 4-BrPh; 3-BrPh; 2-BrPh; 2-MePh; EtPh; thien-2-yl; furan-2-yl; naphthalen-2-yl.

Scheme 1.41. Synthesis of pyrazolo-[1,5-*a*]isoquinolines

A novel approach to access various pyrazolo[1,2-*a*]quinoline derivatives was reported by Liu and colleagues [68]. The synthetic protocol consisted of the annulation of pyrazolones **154** and sulfoxonium ylides **155** by using catalytic amounts of iridium(III) – [IrCp*Cl₂]₂ – and Zn(OTf)₂ in trifluoroethanol at 120 °C (Scheme 1.42). It was observed that the addition of TsOH and AgSbF₆ substantially increased the yield from 26% to 92%, respectively. This reaction can be carried out with variously substituted pyrazolones and sulfoxonium ylides.



R¹= H; Me; benzoyl.

R²= Me; Et, *n*Pr.

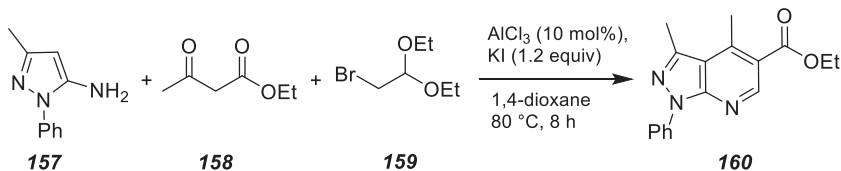
R³= H; 8-Me; 8-MeO; 8-F; 8-Cl; 10-Me; 10-Cl; 11-Cl.

R⁴= Ph; 4-MeOPh; 4-MePh; 4-ClPh; 4-NO₂Ph; 3-MeOPh; 3-ClPh; 2-MePh; 2-ClPh;

Me; fur-2-yl; naphen-2-yl; cyclopentyl.

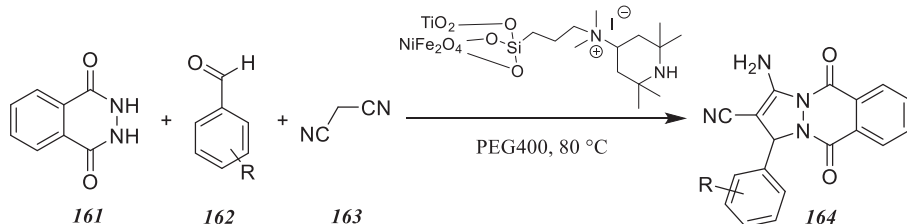
Scheme 1.42. Synthesis of pyrazolo[1,2- α]cinnoline derivatives

Huang *et al.* developed an efficient and practical one-pot multicomponent reaction of (hetero)arylamines with α -bromoacetaldehyde acetal and 1,3-dicarbonyl compounds when using AlCl₃ as a catalyst to synthesize *N*-(hetero)aryl-4,5-unsubstituted pyrroles [69]. They also observed that, concerning the use of the same protocol with additional KI, 3-methyl-1-phenyl-1*H*-pyrazol-5-amine (**157**) reacted smoothly with ethyl 3-oxobutanoate (**158**) and 2-bromo-1,1-diethoxyethane (**159**), and afforded the polysubstituted pyrazolo[3,4-*b*]pyridine **160** in 53% yield (Scheme 1.43).



Scheme 1.43. Synthesis of polysubstituted pyrazolo[3,4-*b*]pyridine

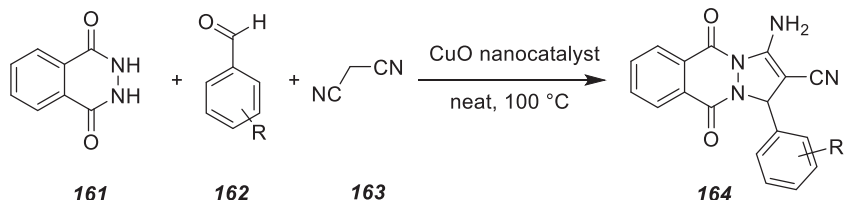
Hamidinasab and colleagues developed a green synthesis method for 1*H*-pyrazolo[1,2-*b*]phthalazine-2-carbonitrile derivatives [70]. They prepared a novel heterogeneous magnetic nanocatalyst by grafting ionic liquid-base *N,N*,2,2,6,6-hexamethyl-*N*-[3-(trimethoxysilyl)propyl]piperidin-4-amonium iodide onto titan-coated NiFe₂O₄ nanoparticles. Subsequently, it was used in one-pot, three-component condensation reaction of phthalhydrazides **161**, 4-chlorobenzaldehydes **162** and malononitrile **163** (Scheme 1.44). The best yield (97%) and the shortest reaction time (10 min) were achieved when using 20 mg of the nanocatalyst, and PEG400 as a solvent at 80 °C. Excellent yields were also achieved when using different aromatic aldehydes with electron-donating and electron-withdrawing substituents. The authors claimed that the high isolated yield of pure products, the good thermal and chemical stability of the nanocatalyst, the easy separation of the hybrid catalyst with an external magnet, the catalyst reusability without considerable loss of its performance (up to 5 times), and the green reaction conditions were the main advantages of their catalyst and synthesis protocol.



R= H; 3-NO₂; 4-NO₂; 3-NO₂-4-OH; 2-Cl; 3-Cl; 4-Cl; 2,4-diCl; 4-Br; 2-MeO; 3-MeO; 4-MeO; 3,4-diMeO.

Scheme 1.44. Synthesis of 1*H*-pyrazolo[1,2-*b*]phthalazine-2-carbonitrile derivatives

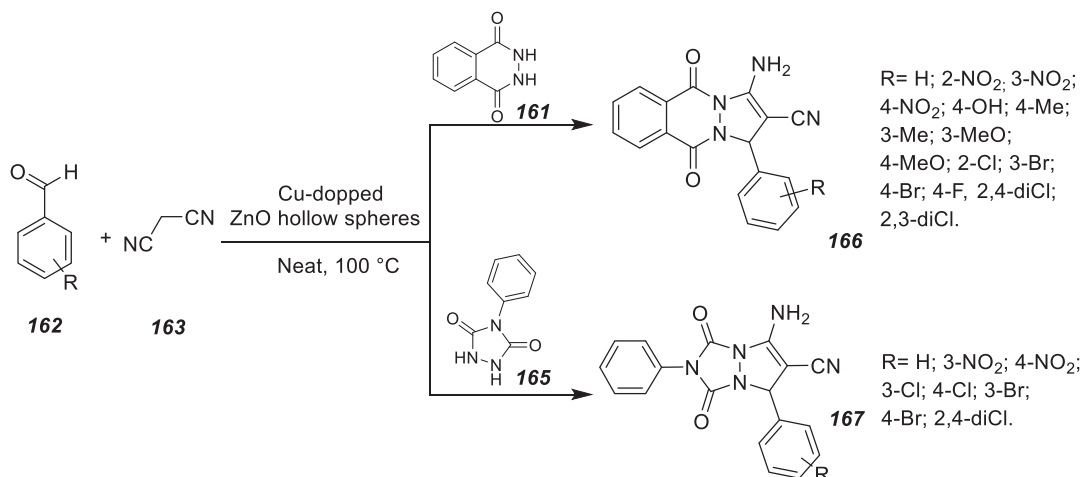
Patil and colleagues also investigated solvent-free synthesis of 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-diones [71]. Nanostructured CuO was chosen as a catalyst (Scheme 1.45), and it was observed that the reactions are very tolerant towards various functional groups present on the aromatic ring of aldehydes with higher yields than in the case of using Cu-ZnO hollow sphere nanostructures (86–94%). Moreover, the CuO nanocatalyst exhibits good reusability over five catalytic cycles with the yield dropping by 6% at the fifth cycle.



R= H; 4-Me; 4-CN; 2-NO₂; 3-NO₂; 4-NO₂; 2-Cl; 4-Cl; 2,4-diCl; 4-F; 2-MeO; 4-MeO; 3,4-diMeO, pyridine; 2-thiophene; 1*H*-pyrrole.

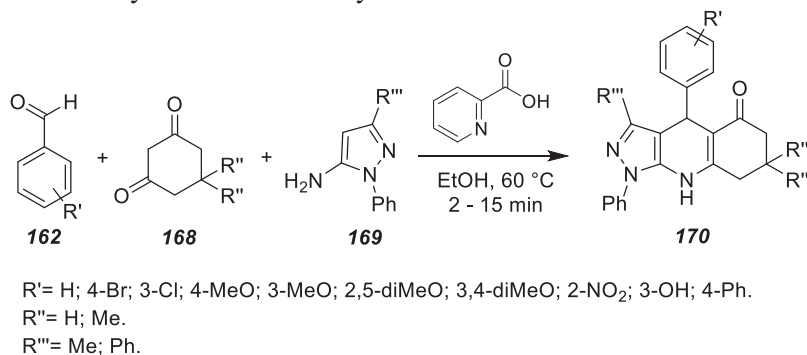
Scheme 1.45. Synthesis of 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-diones when using CuO nanocatalyst

Another similar method was proposed by Maleki *et al.* in order to synthesize 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-diones and pyrazolo[1,2-*a*][1,2,4]triazole-1,3-diones when using Cu-ZnO hollow sphere nanostructures as a catalyst [72]. The reactions were carried out at 100 °C under solvent-free conditions with 0.02 g of the catalyst for 1 mmol of each starting material (Scheme 1.46). 1*H*-Pyrazolo[1,2-*b*]phthalazine-5,10-diones (**166**) were obtained in relatively short reaction times in good yields (81–93%). Pyrazolo[1,2-*a*][1,2,4]triazole-1,3-diones (**167**) were obtained analogously at 80 °C. The main advantage of this method is the ease of separation and the reusability of the catalyst.



Scheme 1.46. Synthesis of 1*H*-pyrazolo[1,2-*b*]phthalazine-5,10-diones and pyrazolo[1,2-*a*][1,2,4]triazole-1,3-diones

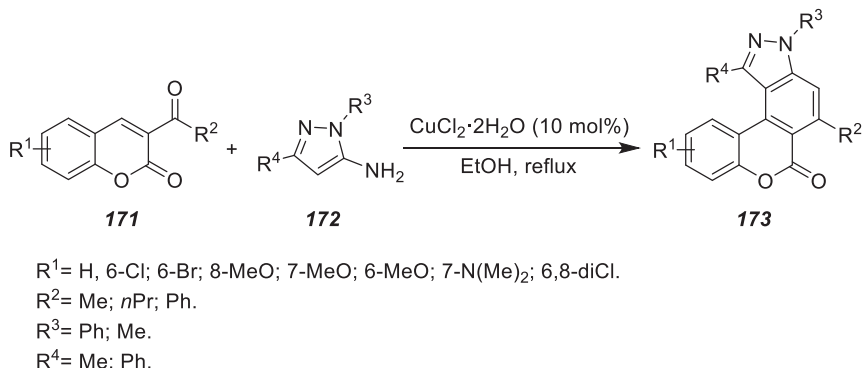
Sharma *et al.* examined pyridine-2-carboxylic acid as a green and efficient catalyst in a multi-component reaction to produce pyrazolo[3,4-*b*]quinolinones [73]. They determined that the reaction between benzaldehydes **162**, dimedones **168**, and 5-amino-3-methyl-1-phenyl-1*H*-pyrazoles **169** can be carried out in quantitative yield when using 10 mol% of pyridine-2-carboxylic acid in EtOH at 60 °C in as little as 5 minutes (Scheme 1.47). The reaction time varied depending on the electronic effect of the substituents on the aromatic ring. The synthetic protocol was proven to be eco-compatible and cost-effective because the catalyst can be reused up to 4 times with only minor loss in the yields.



Scheme 1.47. Synthesis of pyrazolo[3,4-*b*]quinolinone derivatives

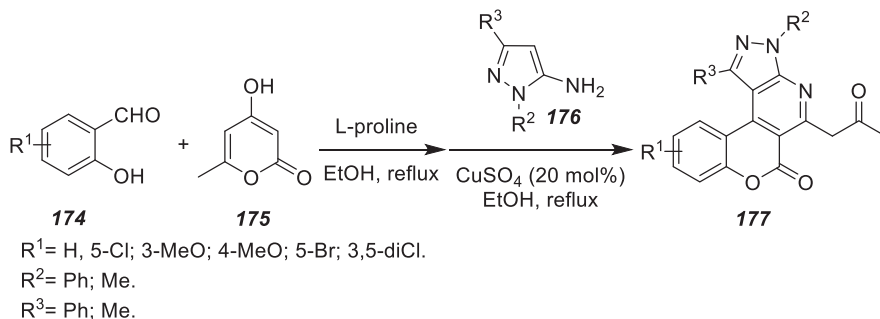
In recent years, a team of scientists researched the synthesis of coumarin-fused pyrazolo[3,4-*b*]pyridine derivatives [74]. One path of the tested synthesis was refluxing an equimolar mixture of 3-acetyl-2*H*-chromen-2-one **171** and 3-methyl-1-phenyl-1*H*-pyrazol-5-amine **172** in EtOH when using 10 mol% of CuCl₂·2H₂O as a catalyst (Scheme 1.48). The reaction time as well as the yields highly depended on the substituents and varied from 2 to 24 h and 38–96%, accordingly. The emission

wavelength of the new compounds appeared in the visible light range (434.4–498.5 nm), and they exhibited high quantum yields, which showed their value as possible luminescence or fluorescence probes.



Scheme 1.48. Synthesis of chromeno[4,3-*d*]pyrazolo[3,4-*b*]pyridine-6(3*H*)-ones

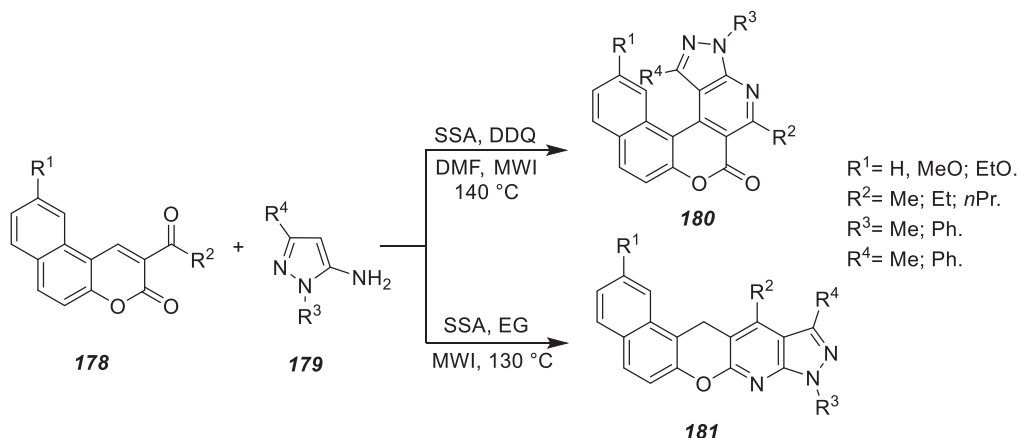
Afterwards, a novel one-pot three-component reaction was proposed to construct functionalized chromeno[4,3-*d*]pyrazolo[3,4-*b*]pyridine derivatives [75]. It consisted of easily accessible starting materials, specifically, salicylaldehydes **174**, 4-hydroxy-6-methyl-2*H*-pyran-2-one (**175**) and pyrazol-5-amines **176** refluxing in EtOH catalyzed by L-proline and CuSO₄ (Scheme 1.49).



Scheme 1.49. Synthesis of functionalized chromeno[4,3-*d*]pyrazolo[3,4-*b*]pyridine derivatives

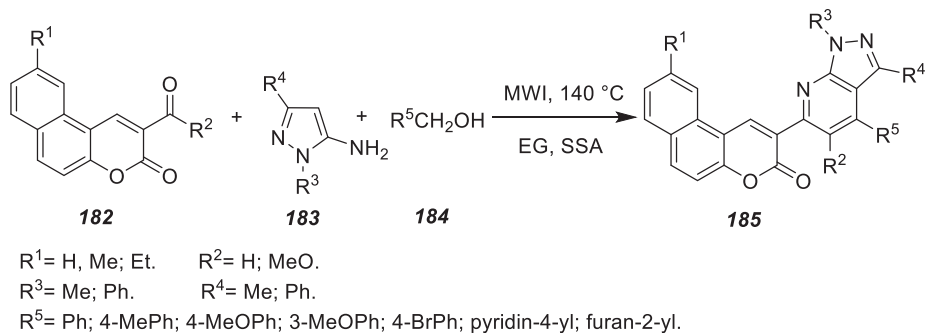
However, the same catalyst did not work as anticipated when 2-butyryl-3*H*-benzo[*f*]chromen-3-ones **178** and 3-methyl-1-phenyl-1*H*-pyrazol-5-amines **179** were attempted as the starting materials. Thus, new conditions had to be optimized to obtain benzo[5,6]chromeno[4,3-*d*]pyrazolo[3,4-*b*]pyridin-3(13*H*)-one derivatives [76]. The best yield was achieved when using silica sulfuric acid (SSA) as a catalyst, and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone as an oxidant additive in DMF at 140 °C under microwave irradiation (Scheme 1.50.). Interestingly, the oxidant additive was needed because 1,2-dihydrobenzo[5,6]chromeno[4,3-*d*]pyrazolo[3,4-*b*]pyridin-3(13*H*)-one formed under microwave irradiation while conventional heating led straight to the formation of benzo[5,6]chromeno[4,3-*d*]pyrazolo[3,4-*b*]pyridin-3(13*H*)-ones **180**. Moreover, it was found that the reaction product

depended on the solvent as the change from ethanol to ethylene glycol caused the formation of benzo[5,6]chromeno[2,3-*b*]pyrazolo[4,3-*e*]pyridines **181**.



Scheme 1.50. Synthesis of benzo[5,6]chromeno[4,3-*d*]pyrazolo[3,4-*b*]pyridin-3(13*H*)-one and benzo[5,6]chromeno[2,3-*b*]pyrazolo[4,3-*e*]pyridine derivatives

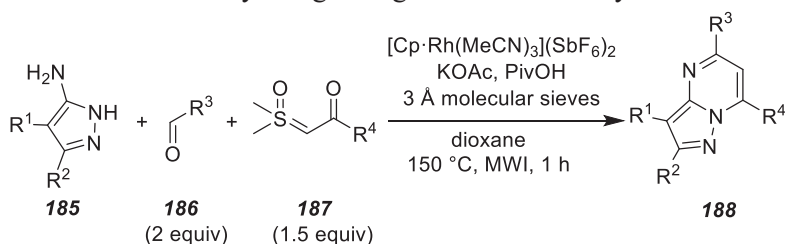
Finally, it was found that new coumarin-fused pyrazolo[3,4-*b*]pyridine derivatives were formed using ethanol and ethylene glycol as a mixture of solvents, which indicates that ethanol also participates in the reaction [77]. Thus, a new one-pot three-component domino reaction using arylbenzo[*f*]chromen-3-one **182**, enaminone **183**, and alkyl or aryl alcohol **184** with SSA as a catalyst under microwave irradiation was presented (Scheme 1.51). The reaction can be carried out when using either alkyl or aryl alcohols; however, when aminopyrazole had methyl substituents, the desired products could not be obtained at all.



Scheme 1.51. Synthesis of coumarin-fused pyrazolo[3,4-*b*]pyridine derivatives

In 2018, Hoang and colleagues reported the synthesis of pyrazolo[1,5-*a*]pyrimidines from readily available aldehydes, 3-aminopyrazoles and sulfoxonium ylides [78]. The three-component coupling was carried *via* rhodium(III)-catalyzed imido C–H activation (Scheme 1.52). After a variety of

reaction parameters were examined, the best yield (85%) was achieved when using the commercially available and air-stable cationic catalyst $[\text{Cp}\cdot\text{Rh}(\text{MeCN})_3](\text{SbF}_6)_2$ with potassium acetate (KOAc), pivalic acid (PivOH), and 3 Å molecular sieves as additives in dioxane (0.4 M) under microwave irradiation at 150 °C for 1 h. It is noteworthy that, in order to achieve a similar yield when using conventional heating in oil bath, the reaction had to be stirred at 100 °C for 16 h. After exploring the scope of the starting materials used, it was found that good yields can be obtained for many substituted aminopyrazoles and a very large variety of aromatic and heteroaromatic aldehydes of a wide variety including electron-withdrawing, electron-donating, basic nitrogen, halide and acidic functionality. Only ionizable aldehydes provided the desired pyrazolopyrimidines in poor yields. In addition, it was demonstrated that aryl, heteroaryl, and branched and unbranched alkyl substituents can be introduced by using a range of sulfoxonium ylides.



$\text{R}^1 = \text{H, Me; Ph.}$

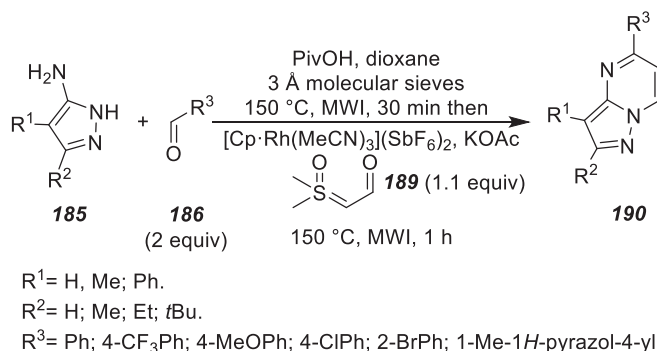
$\text{R}^2 = \text{H; Me; Et; } t\text{Bu; } i\text{Pr; cyclopropyl; Ph; CF}_3.$

$\text{R}^3 = \text{Ph; 4-CF}_3\text{Ph; 4-MeOPh; 2-ClPh; 3-BrPh; 4-COOHPh; 4-COOMePh; 4-NHCOMePh; pyridine-4-yl; 6-Cl-pyridin-3-yl; furan-2-yl; thiophen-3-yl; 1-Me-1H-pyrazol-4-yl; Me.}$

$\text{R}^4 = \text{Me; } i\text{Pr; } t\text{Bu; 4-MeOPh; 4-CF}_3\text{Ph; thien-2-yl.}$

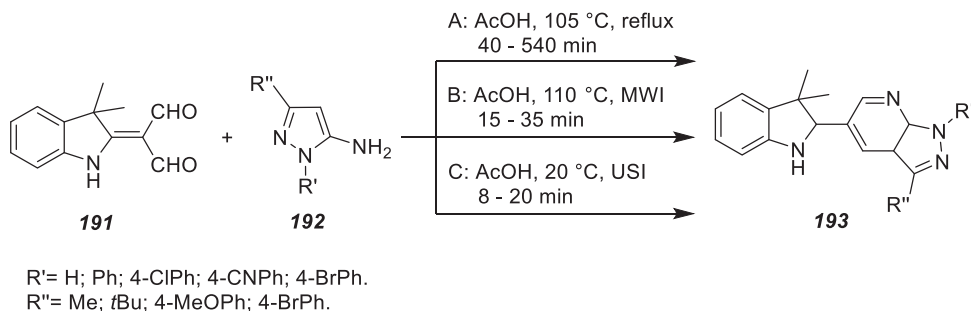
Scheme 1.52. Synthesis of 2,3,5,7-tetrasubstituted pyrazolo[1,5-*a*]pyrimidine derivatives

In order to obtain pyrazolopyrimidines with only one pyrimidine ring substituent from the aldehyde, researchers explored formyl sulfoxonium ylide as a possible reactant (Scheme 1.53). In an effort to prevent competitive condensation of the formyl group with the aminopyrazole, a two-step, one-pot sequence was implemented instead of the original one-step conditions. Firstly, the aminopyrazoles **185** and aldehydes **186** were condensed by microwave heating at 150 °C for 30 min in the presence of molecular sieves and PivOH, which was needed to catalyze the imine formation. Subsequently, formyl ylide **189**, KOAc, and $[\text{Cp}\cdot\text{Rh}(\text{MeCN})_3](\text{SbF}_6)_2$ were added, and the mixture was heated at 150 °C for 1 h under microwave irradiation. The best yield was observed with diluted concentration of 0.1 M. Moreover, it was found that ester and methoxy functionalities can also be directly installed on the pyrimidine ring by employing ethyl glyoxylate and trimethyl orthoformate in place of the aldehyde, respectively, by using the two-step procedure the same way as with formyl ylide.



Scheme 1.53. Synthesis of 2,3,5,-trisubstituted pyrazolo[1,5-*a*]pyrimidine derivatives

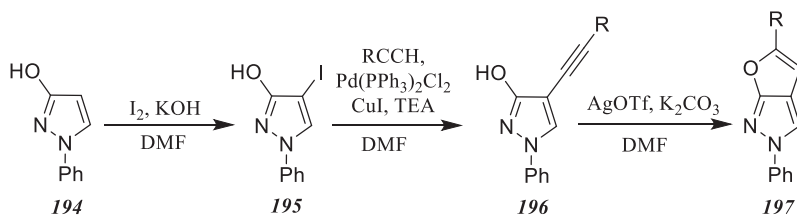
Recently, Ramirez *et al.* has designed and synthesized 5-(indol-2-yl)pyrazolo[3,4-*b*]pyridines as potent TASK-3 channel blockers [79]. Since TASK-3 is expressed in different cancer cell types and neurons, and the inhibition of these channels leads to membrane depolarization, the discovery of novel TASK-3 inhibitors is a highly appealing opportunity to explore new cancer and neurological therapies. Seeing that the cyclocondensation reaction between 2-(3,3-dimethylindolin-2-ylidene)malonaldehyde **191** and 5-aminopyrazoles **192** was unsuccessful under reflux conditions when using ethanol as a solvent or under basic conditions when using TEA, it was decided to examine three different methodologies under acidic conditions (Scheme 1.54.). All the tested methods involved the use of acetic acid (AcOH), but under different energy sources. Method A: conventional heating/reflux; method B: MWI at 110 °C and 200 W; method C: ultrasound irradiation (USI) at 20 KHz/500 W at 20 °C. The highest yield and the shortest reaction time were observed when using USI as an energy source at room temperature (Method C). Conventional heating led to the longest reaction times but fairly good yields, while MWI offered a shortened time, but also cut the yields as a lot of byproducts were formed. However, in all the cases, regioisomers **193** were selectively obtained as the main products.



Scheme 1.54. Synthesis of 5-(indol-2-yl)pyrazolo[3,4-*b*]pyridines

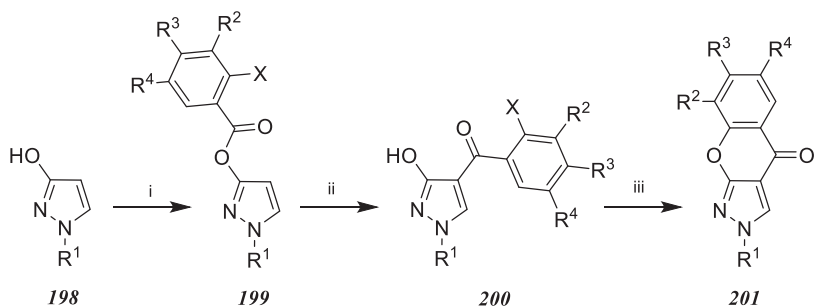
1.4. Progress of condensed pyrazole systems in KTU

In recent years, researchers from prof. Šackus group at Kaunas University of Technology have extensively explored fused pyrazoles and their biological potency and application. The 2*H*-Furo[2,3-*c*]pyrazole ring was obtained *via* the silver(I) ion-mediated ring-closure reaction (Scheme 1.55) [80]. 1-Phenylpyrazole **196** containing adjacent ethyne and hydroxy groups was chosen for this cyclization. It was easily attained from 1-phenyl-1*H*-pyrazol-3-ol (**194**) in two steps: iodination and Sonogashira coupling. After investigating a variety of conditions for the cyclization reactions, the best yields were achieved when using AgOTf as a catalyst and K₂CO₃ as a base in DMF at 120 °C for 14 h.



Scheme 1.55. Synthesis of 2,5-disubstituted 2*H*-furo[2,3-*c*]pyrazoles

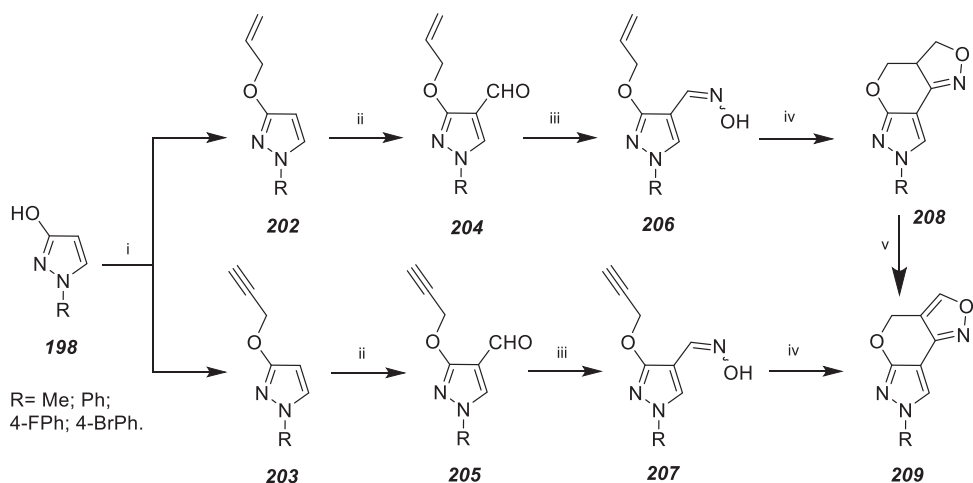
1-Substituted-1*H*-pyrazol-3-ols **198** were used as the starting material in the multistep synthesis of benzopyrano[2,3-*c*]pyrazol-4(2*H*)-one derivatives (Scheme 1.56) [5]. Firstly, acylation was performed by using either 2-halogen-substituted aroyl chlorides with TEA as the base in chloroform, or various benzoic and nicotinic acids with the DCC/DMAP activation system in DCM. Subsequently, *O*-acylated pyrazoles **199** underwent Fries rearrangement while using excess AlCl₃ in carbon disulfide. Finally, benzopyrano[2,3-*c*]pyrazol-4(2*H*)-ones **201** were obtained by using K₂CO₃ in DMF at 120 °C. The new derivatives were evaluated for their anthelmintic activity on *Caenorhabditis elegans*. Five compounds, namely, 2-phenyl[1]benzopyrano[2,3-*c*]pyrazol-4(2*H*)-one and its 7-fluoro-, 7-chloro-, 7-bromo- and 8-fluoro-analogues, blocked the development of *C. elegans* at L1 stage with IC₅₀ as low as 1–3 μM.



$R^1 = \text{Me; Ph. } R^2 = \text{H; F. } R^3 = \text{H; F; Cl; Br; Me; NO}_2. R^4 = \text{H; F. } X = \text{Cl; Br; F.}$

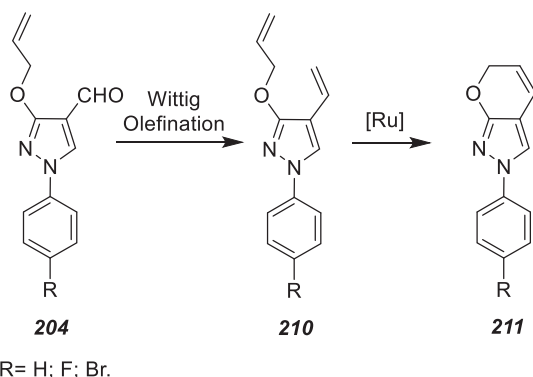
Scheme 1.56. Synthesis of 2,6,7,8-substituted benzopyrano[2,3-*c*]pyrazol-4(2*H*)-ones. *Reagents and conditions:* i: $R^2\text{COCl}$, TEA, CHCl_3 , rt, 30 min or $R^2\text{COOH}$, DCC, DMAP, DCM, $0\text{ }^\circ\text{C}$ – rt, 15 h; ii: AlCl_3 , CS_2 , $50\text{ }^\circ\text{C}$, 3 – 12 h; iii: K_2CO_3 , DMF, $120\text{ }^\circ\text{C}$, 18 h

1-Substituted-1*H*-pyrazol-3-ols **198** were employed in constructing the pyrazolo[4',3':5,6]pyrano[4,3-*c*][1,2]oxazole ring (Scheme 1.57) [81]. Firstly, 3-(prop-2-en-1-yloxy)-**204** or 3-(prop-2-yn-1-yloxy)-1*H*-pyrazole-4-carbaldehyde **205** were obtained from **198** after *O*-alkylation and formylation by using the Vilsmeier–Haack reaction procedure. Subsequently, the carbaldehydes **204**, **205** were transformed to the corresponding aldoximes **206**, **207** after treatment with hydroxylamine hydrochloride in the presence of sodium acetate. The intramolecular cyclization reaction was carried out by oxidation with sodium hypochlorite. Moreover, the obtained 3a,4-dihydro-3*H*,7*H*-pyrazolo[4',3':5,6]pyrano[4,3-*c*][1,2]oxazole system **208** was further oxidized by using activated MnO_2 as an oxidant in toluene in a Dean–Stark apparatus for 4 h at reflux temperature.



Scheme 1.57. Synthetic route for the 4*H*,7*H*-pyrazolo[4',3':5,6]pyrano[4,3-*c*][1,2]oxazole ring system. *Reagents and conditions:* (i) NaH, DMF, 0 °C, 15 min, allylbromide (for **202**) or propargylbromide (for **203**), 60 °C, 1 h; (ii) DMF, POCl₃, -10 °C, 15 min, 70 °C, 1 h; (iii) NH₂OH·HCl, NaOAc, EtOH, reflux, 15 min; (iv) aq. NaOCl, DCM, rt, 1 h; (v) MnO₂, toluene, reflux, 4 h

Moreover, 3-(prop-2-en-1-yloxy)-1*H*-pyrazole-4-carbaldehyde **204** was also used to prepare 2,6-dihydropyrano[2,3-*c*]pyrazole [82] (Scheme 1.58). Carbaldehyde **204** was converted to 4-ethenylpyrazole **210** by using the Wittig reaction conditions: methyltriphenylphosphonium iodide and KO*t*Bu in toluene. Finally, ring-closing metathesis was employed to obtain 2,6-dihydropyrano[2,3-*c*]pyrazole **211**. Specifically, 4-ethenylpyrazole **210** was heated with Grubbs' first-generation catalyst in THF in 42% yield. Interestingly, no reaction occurred when DCM was used as solvent. It was also found that microwave heating shortened the reaction time from 48 h to 3 h; however, the isolated yield decreased to the level of 34%.



Scheme 1.58. Synthesis of 2,6-dihydropyrano[2,3-*c*]pyrazole ring system

1.5. Summary

A wide array of synthetic methods used for preparing various annulated pyrazoles has been analyzed and classified into three main methodologies: the formation of a heterocycle ring into an existing pyrazole ring, the formation of the pyrazole ring into a preexisting heterocycle and multicomponent reactions. Recent studies at KTU focus on the synthesis paths that form new cycles onto the pyridine ring to obtain dihydropyrano[2,3-*c*]pyrazole, 4*H*,7*H*-pyrazolo[4',3':5,6]pyrano[4,3-*c*][1,2]oxazole, benzopyrano[2,3-*c*]pyrazol-4(2*H*)-ones and 2*H*-furo[2,3-*c*]pyrazoles ring systems. However, a thorough review of the recent publications revealed that, amongst a variety of new methodologies to synthesize different condensed pyrazolo systems with various biological and optical applications, only scarce examples of procedures to obtain 2*H*-pyrazolo[4,3-*c*]pyridines can be found.

2. RESULTS AND DISCUSSION

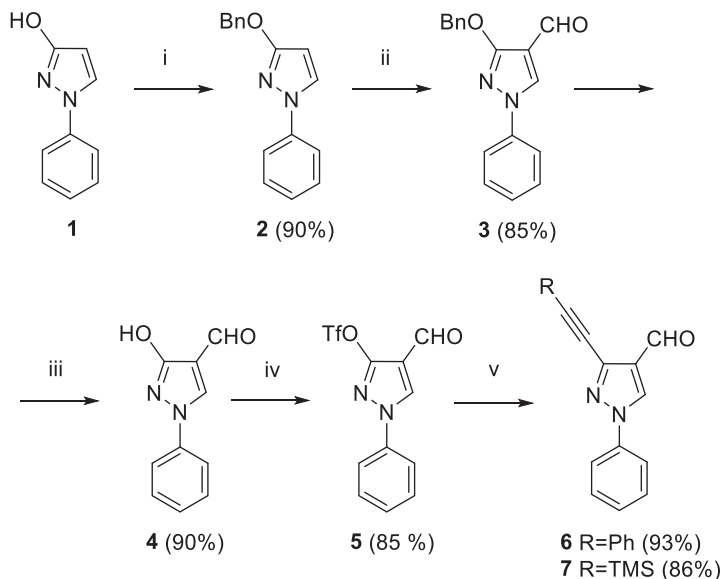
2.1. Synthesis of 2*H*-pyrazolo[4,3-*c*]pyridine core

Although a vast variety of biologically active annealed pyrazole derivatives is already known [83, 84], the more synthetically demanding 2*H*-pyrazolo[4,3-*c*]pyridines are not so common. Synthesis of 2*H*-pyrazolo[4,3-*c*]pyridines can be accomplished by the treatment of 4-chloro or 4-iodopyridines possessing a carbonyl moiety at the 3-position with various hydrazines [85, 86]. Blench *et al.* obtained 2-phenyl-2*H*-pyrazolo[4,3-*c*]pyridines after heating mixtures of (*E*)-1-(4-azidopyridin-3-yl)-*N*-phenylmethanimines in toluene at 105 °C [87]. It was also reported that 2*H*-pyrazolo[4,3-*c*]pyridines can be easily accessed from 3-hydroxy-1-phenyl-1*H*-pyrazole-4-carbaldehydes and the corresponding ethanones *via* a multistep synthesis path which includes the Sonogashira cross-coupling and cyclization reaction induced by dry ammonia [88]. This method was successfully used to obtain a variety of 2,4,6-trisubstituted-2*H*-pyrazolo[4,3-*c*]pyridines which displayed good anticancer activity *in vitro* through arresting the cell cycle in mitosis and the induction of apoptosis [89].

2.1.1. Synthesis of 1-phenyl-3-[(substituted)ethynyl]-1*H*-pyrazole-4-carbaldehydes

As reported above, 1-substituted-1*H*-pyrazol-3-ol is a valuable starting material to access condensed pyrazole systems [5, 80, 81]. Moreover, pyrazole-4-carbaldehydes carrying an alkynyl function adjacent to the formyl moiety were reported as important intermediates in the synthesis of annealed pyrazole systems, such as 2*H*- and 3*H*-pyrazolo[4',3':3,4]pyrido[1,2-*a*]benzimidazoles, 3*H*-pyrazolo[4,3-*c*]imidazo[1,2-*a*:5,4-*b'*]dipyridines and 13,13a-dihydro-3*H*-pyrazolo[4',3':3,4]pyrido[1,2-*a*]pyrimidines [90], dipyrazolo[1,5-*a*:4,3-*c*]pyridines [91] and 2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines [92-94]. Thus, 1-phenyl-3-(2-phenylethynyl)-1*H*-pyrazole-4-carbaldehyde (**6**) and 1-phenyl-3-[(trimethylsilyl)ethynyl]-1*H*-pyrazole-4-carbaldehyde (**7**) were chosen as the starting materials for this study. They were prepared from 1-phenyl-1*H*-pyrazol-3-ol (**1**) *via* a multistep synthetic route in accordance with a previously published procedure (Scheme 2.1) [88]. First of all, *O*-alkylation was applied to protect the hydroxy group of 1-phenyl-1*H*-pyrazol-3-ol (**1**) with a benzyl group [88], followed by formylation using the Vilsmeier–Haack reaction conditions [95] to introduce a formyl group at the 4-position of the pyrazole ring. The cleavage of the benzyl group was performed in acidic conditions with TFA, and later the hydroxy group was substituted with triflate [96]. Since this pseudohalogenide is a convenient leaving group for Pd-catalyzed cross-coupling reactions, compound **5** was exposed to the standard Sonogashira reaction conditions, i.e. phenylacetylene or trimethylsilylacetylene, TEA as a base, CuI as a co-catalyst, and Pd(PPh₃)₂Cl₂ as a catalyst [88, 89] to obtain 1-phenyl-3-(2-phenylethynyl)-1*H*-pyrazole-4-

carbaldehyde (**6**) and 1-phenyl-3-[(trimethylsilyl)ethynyl]-1*H*-pyrazole-4-carbaldehyde (**7**) in 93% and 86% yields, respectively.

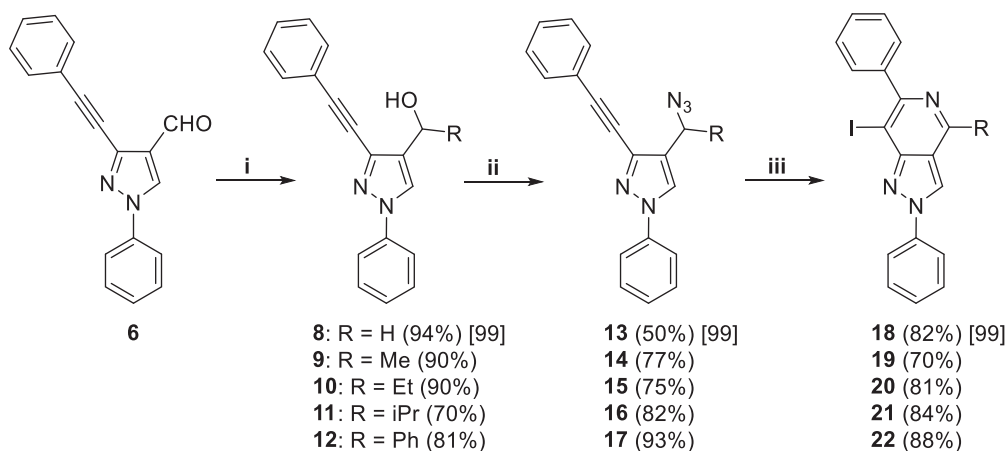


Scheme 2.1. Synthesis of 1-phenyl-3-[(substituted)ethynyl]-1*H*-pyrazole-4-carbaldehydes **6** and **7**. *Reagents and conditions:* i: BnCl, NaH, DMF, argon atmosphere 60 °C, 1 h; ii: POCl₃, DMF, 0–60 °C; iii: TFA, toluene, rt, 15 h; iv: Tf₂O, TEA, DCM, rt, 1 h

2.1.2. Synthesis of 4-substituted 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines

4-Substituted 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines **18–22** were obtained in a three-step route as depicted in Scheme 2.2. The preparation of 1-[1-phenyl-3-(phenylethynyl)-1*H*-pyrazol-4-yl]alcohol was carried out by using two different approaches depending on the type of alcohol. The reduction of the aldehyde group was performed to obtain the primary alcohol **8** by using sodium borohydride as a reducing agent [97]. The reaction was carried out in MeOH at 0 °C under an argon atmosphere. After the full consumption of carbaldehyde **6** as determined by TLC, the reaction mixture was protonated with an aqueous ammonium chloride solution to give primary alcohol **8** from the intermediate complex. For the synthesis of secondary alcohols **9–12**, carbaldehyde **6** was dissolved in THF and reacted with the appropriate alkyl or arylmagnesium halogenides at room temperature when applying the previously reported procedure [98, 99]. We should note that – as Grignard reagents are sensitive to air and moisture exposure – the reactions were carried out under an argon atmosphere while using a dry solvent [100]. Although it has been reported that this kind of secondary alcohols might be unstable [101], all of them were successfully purified by column chromatography, and their structures were determined with spectroscopic data.

Moreover, the formation of alcohols can be conveniently confirmed by FT-IR spectra where a broad absorption peak of the alcohol group can be found at around 3300–3400 cm^{-1} , while the aldehyde group peak ($\sim 1710\text{--}1685\text{ cm}^{-1}$) is absent.



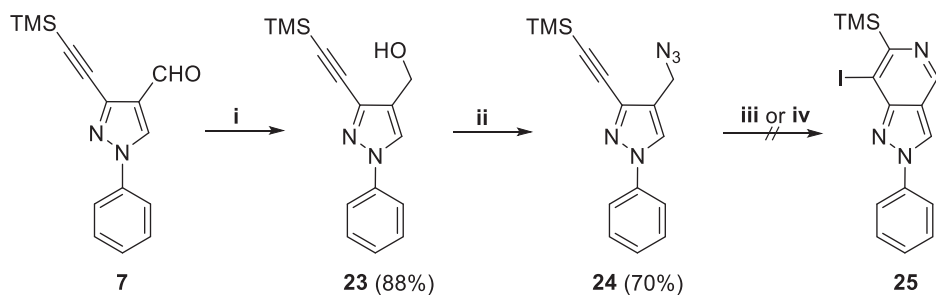
Scheme 2.2. Synthesis of 4-substituted 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines **18–22**. *Reagents and conditions*: i: NaBH_4 , MeOH, 0 $^\circ\text{C}$, 30 min (for **8**); MeMgBr , EtMgBr , PrMgCl , or PhMgBr and THF (abs.), rt, 10 min (for **9**), (**10**), (**11**) and (**12**); ii: TMSN_3 , $\text{BF}_3 \cdot \text{Et}_2\text{O}$, DCM, rt, 10–30 min; iii: K_3PO_4 (for **18**) and (**22**) or NaHCO_3 (for **19**), (**20**) and (**21**)), I_2 , DCM, rt, 12 h

The next step in the synthetic sequence was the preparation of azides **13–17**. Numerous methods have been reported for alcohol transformation to azide, for example, Mitsunobu-type displacements [102, 103], two-step strategies involving a halogenated [104] or mesylated intermediate [105], the one-pot halogenation-azidation of alcohols [106], reactions with phosphitine intermediates [107], *N*-methyl-2-pyrrolidone hydrosulphate, and trimethylsilylazide (TMSN_3) [101]. Due to the easy execution and short reaction times, TMSN_3 was chosen for the conversion, and the reactions were performed in DCM with a catalytic amount of boron trifluoride diethyl etherate ($\text{BF}_3 \cdot \text{Et}_2\text{O}$). The reactions were carried out at room temperature under an argon atmosphere and with a dry solvent in order to protect both reagents from moisture. Transformation was completed in 30 minutes, and the reaction products **14–17** were furnished in good-to-excellent yields, except for azidomethylpyrazole **13** which only reached 50% yield. The main reason for the modest yield was the incomplete conversion of the primary alcohol (**8**) which was regenerated during the purification of azide. The structures were confirmed by NMR, IR spectroscopy, and MS and HRMS spectrometry. Similarly as in the case of alcohols, FT-IR spectra can also be used for quick verification of the transformation: the characteristic absorption band of the azide group can be found at 2102–2087 cm^{-1} , while the absorption band of the alcohol group is not registered.

As the final step, the pyrazolo[4,3-*c*]pyridine core with iodine in the 7-position was obtained from the newly synthesized azides **13–17** by adopting the electrophilic

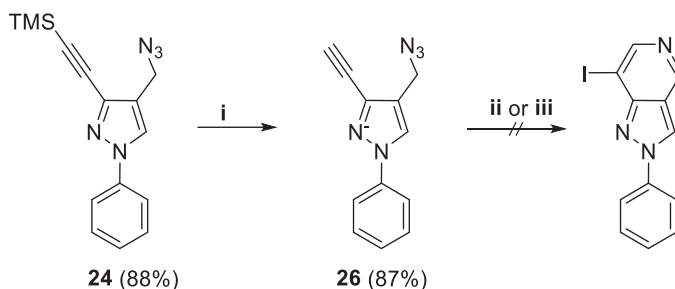
substitution reaction conditions that were previously used to synthesize 1,3,4-trisubstituted isoquinolines from 2-alkynyl benzyl azides [108]. In order for cyclization to occur, azides **13–17** had to be treated with iodine and a proper base. In the case of the primary azides **13** and **17**, five equivalents of K_3PO_4 were used; meanwhile, a weaker base was required for the secondary azides **14–16**, specifically, one equivalent of $NaHCO_3$. The reactions were carried out in DCM at room temperature in the dark for 12 h, and ultimately furnishing compounds **18–22** in 70–88% yields. An attempt to apply the weaker base $NaHCO_3$ for the reaction with the primary azide **13** caused the formation of the dehalogenated side product 2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine, which resulted in a troublesome purification and a lower yield of the target product.

However, the preparation of 7-iodo-2-phenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**25**) from 1-phenyl-3-[(trimethylsilyl)ethynyl]-1*H*-pyrazole-4-carbaldehyde (**7**) was unsuccessful. The first two steps of the synthesis sequence (Scheme 2.3) proceeded smoothly as described previously. Alcohol was obtained by using sodium borohydride, and then treated with $TMSN_3$ to form azide. We should highlight that the yield of this transformation was better than that of its phenyl counterpart (**13**). Unfortunately, condensation when using K_3PO_4 and I_2 was ineffective as no traces of 7-iodo-2-phenyl-2*H*-pyrazolo[4,3-*c*]pyridine were observed. Alternatively, a different source of iodine – *N*-iodosuccinimide (NIS) – was used. Nonetheless, cyclization did not occur, either.



Scheme 2.3. Synthesis of 7-iodo-2-phenyl-6-(trimethylsilyl)-2*H*-pyrazolo[4,3-*c*]pyridine (**25**). *Reagents and conditions:* i: $NaBH_4$, MeOH, 0 °C, 30 min; ii: $TMSN_3$, $BF_3 \cdot Et_2O$, DCM, rt, 10 min; iii: K_3PO_4 , I_2 , DCM, rt, 12 h; iv: $NaHCO_3$, NIS, DCE, 50 °C, over 2 days

Finally, it was decided to attempt cyclization by using azide with terminal acetylene (Scheme 2.4). Although the TMS group can be cleaved by using HF-based reagents, the use of tetrabutylammonium fluoride (Bu_4NF) in THF [109] was found to be ineffective. Thus 4-(azidomethyl)-3-ethynyl-1-phenyl-1*H*-pyrazole (**26**) was prepared by using anhydrous K_2CO_3 in dry MeOH while adapting the previously reported conditions [110]. Unfortunately, condensation was unsuccessful when using either of the iodine sources.



Scheme 2.4. Synthesis of 7-iodo-2-phenyl-2H-pyrazolo[4,3-c]pyridine. *Reagents and conditions:* i: K_2CO_3 (anhydrous), MeOH (dry); ii: K_3PO_4 I_2 , DCM, rt, 12 h; iii: $NaHCO_3$, NIS, DCE, 50 °C, over 2 days

The structures of 4-substituted 7-iodo-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridines **18-22** were confirmed by NMR and IR spectroscopy, as well as MS and HRMS spectrometry. The formation of the cyclisation product can be illustrated by the comparison of ^1H NMR spectra of azide **17** and pyrazolopyridine **22** (Fig. 2.1). The singlet of the proton next to the azide group at 5.90 ppm is no longer observed, and the singlet of the pyrazole proton goes downfield from 7.82 ppm to 8.90 ppm. Moreover, in ^{13}C , the NMR spectra signals of acetylenic bond carbons (80.0 and 94.3 ppm) and aliphatic carbon are no longer observed.

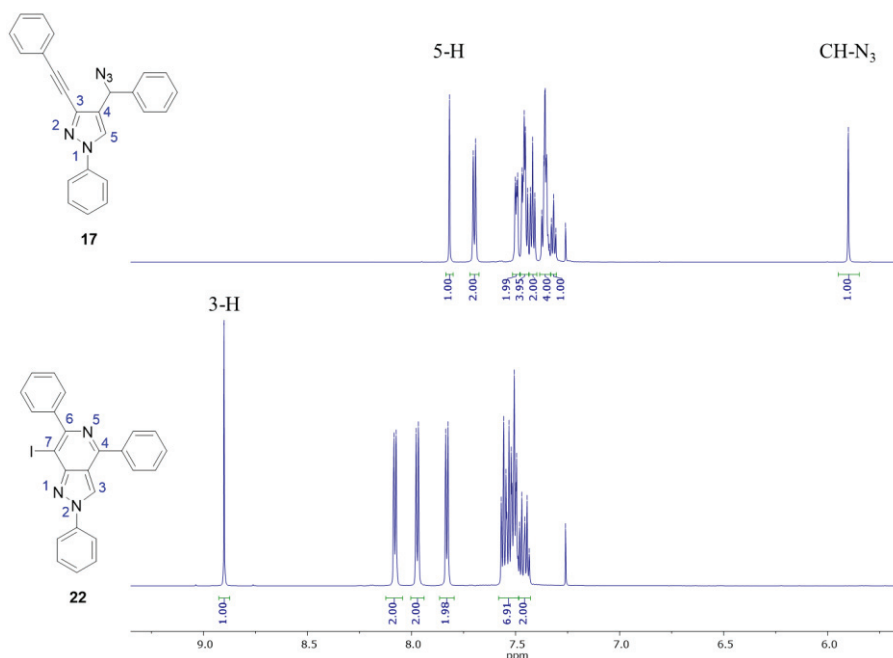


Figure. 2.1. Comparison of the ^1H NMR spectra of compounds **17** and **22**

Detailed NMR spectroscopic investigations employing a combination of different NMR-techniques (^1H -NMR, ^{13}C -NMR, COSY, NOESY, HSQC, HMBC) permitted full and unambiguous assignment of all ^1H - and ^{13}C -NMR resonances. The structure of pyrazolo[4,3-*c*]pyridine can be verified by using the ^1H - ^{15}N HMBC spectrum (Figure 2.2), where three nitrogen atom signals and their interaction with the corresponding protons is displayed. More specifically, in the case of 4-ethyl-7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**20**), a distinct correlation between the nitrogen atom (N-5) from the pyridine moiety with CH_2 protons is observed with the signal of this nitrogen at -82.2 ppm. Meanwhile, the nitrogen atoms N-2 and N-1 both interact with the 3-H proton with the peaks at -148.2 and -89.9 ppm, respectively. The interactions between nitrogen atom N-2 and the protons from the phenyl group are helpful in assigning their signal positions. The signal at 7.93–7.98 ppm can be assigned to 2,6- protons of the phenyl group because the interaction signal is stronger with the protons being closer to nitrogen. Meanwhile, the protons at 7.54–7.58 ppm can be assigned to 3,5- protons of the phenyl group due to the weaker signal of the interaction.

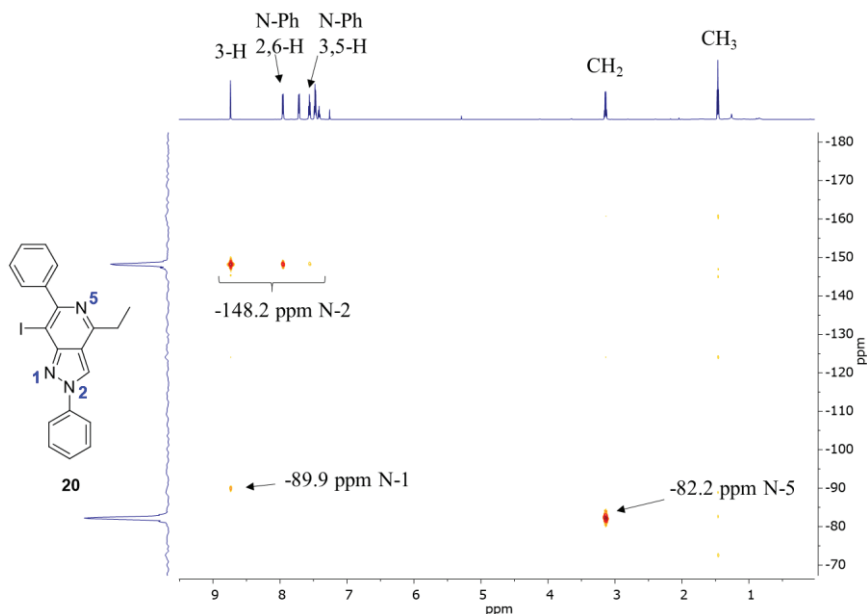


Figure. 2.2. ^1H - ^{15}N HMBC spectrum of compound **20**

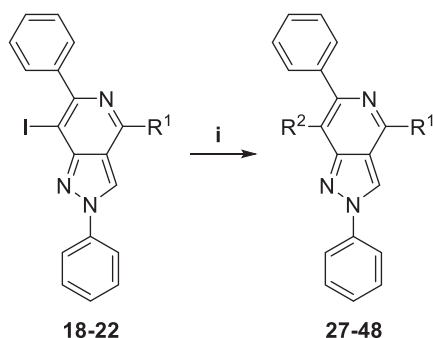
Having successfully prepared 4-substituted 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines, their ability to participate in the Suzuki-Miyaura, Buchwald-Hartwig and Heck coupling reactions was examined.

2.2. Synthesis, optical and biological investigation of 7-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines

2.2.1. Synthesis of 7-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines

The Suzuki–Miyaura cross-coupling reaction has exhibited extensive applications in the natural product synthesis, as well as the synthesis of medically important pharmacophores and organic materials. It has been applied in making diverse libraries of substituted annealed heterocycles. For example, Crozet and colleagues reported a microwave-mediated Suzuki coupling reaction of 2-(arylsulfonylmethyl)-6-bromo-3-nitroimidazo[1,2-*a*]pyridines and arylboronic acids in water when using $\text{Pd}(\text{PPh}_3)_4$, Na_2CO_3 as a base and tetrabutylammonium bromide [111]. Similarly, 4-chloropyrazolo[3,4-*b*]pyridine reacted with various arylboronic acids when using the same Pd-catalyst and a base, but in a different solvent system – toluene/*t*BuOH/water [112]. Recently, Krajcovicova also employed the conditions of Suzuki–Miyaura cross-coupling to introduce substituents to the imidazo[4,5-*b*]pyridine 5-position by using either $\text{Pd}(\text{PPh}_3)_4$, 2M aq. K_3PO_4 , toluene/ethanol, or SPhos Pd G2, K_3PO_4 , and a dioxane/water system [113]. Sequential arylation of 6-chloro-3-iodo-1-(4-methoxybenzyl)-1*H*-pyrazolo[3,4-*b*]pyridine was achieved when using $\text{Pd}(\text{OAc})_2$ as a catalyst, dppf as a ligand, Cs_2CO_3 as a base, and a dioxane:water (3:1) solvent system [114]. Firstly, C-3 arylation occurred at 60 °C, and C-6 arylation took place at 100 °C. This chemoselective sequential arylation strategy can be also performed in one pot without loss of efficiency when compared to the corresponding stepwise synthesis. Another catalyst – $\text{Pd}_2(\text{dba})_3$ with xantphos, K_3PO_4 in DMF/water – was used to introduce the aryl substituent in the 3-position of pyrazolo[3,4-*b*]pyridines [115]. Regioselective arylation of 5-bromo-3-iodo-1*H*-pyrazolo[3,4-*b*]pyridine was firstly performed at the C-3 position, and then at the C-5 position by using $\text{Pd}(\text{dppf})\text{Cl}_2 \cdot \text{CH}_2\text{Cl}_2$ and 2M K_2CO_3 in ACN [116]. Martinez-Gualda *et al.* employed a different palladium-catalyzed reaction to obtain a wide range of 3,5- and 3,6-disubstituted isothiazolo[4,3-*b*]pyridines [117]. Aryl groups were conveniently introduced by Suzuki cross-couplings when using the classical reaction conditions: an appropriate arylboronic acid, $\text{Pd}(\text{PPh}_3)_4$ as a catalyst, and K_2CO_3 as a base in a mixture of dioxane/water.

Thus, in order to obtain 7-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines, palladium-catalyzed Suzuki–Miyaura cross-coupling conditions were employed. Specifically, aromatic boronic acids were reacted with 7-iodo-2*H*-pyrazolo[4,3-*c*]pyridines **18–22** when using $\text{Pd}(\text{OAc})_2$ as a catalyst, Cs_2CO_3 as a base, and aqueous EtOH as a solvent under argon atmosphere (Scheme 2.5) [118]. To ensure a short reaction time, arylations were carried out under microwave irradiation, thus giving rise to compounds **27–48** in 0.5–1 h. Compounds **27** and **29–48** (Table 2.1) were obtained in moderate to excellent yields (48–96%), but the full cross-coupling conversion of **18** when using 2-methoxyphenyl boronic acid could not be achieved, which resulted in a lower yield of compound **28**.



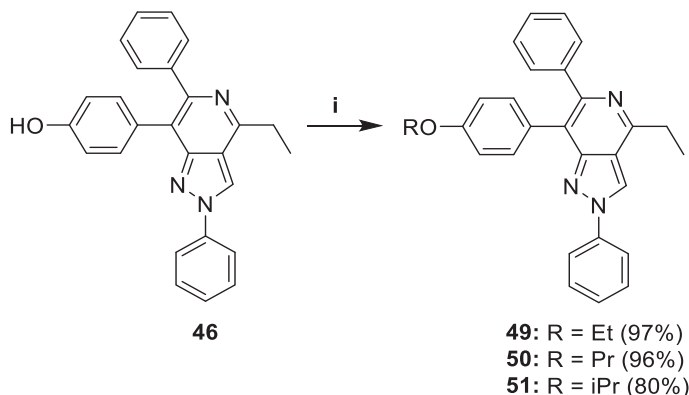
Scheme 2.5. Synthesis of 7-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines **27–48**. *Reagents and conditions:* i: arylboronic acid, Pd(OAc)₂, Cs₂CO₃, EtOH/H₂O 3/1, MWI, 100 °C, 0.5–1 h

Both electron accepting and donating groups were well tolerated except in the case of hydroxyl substituted phenyl boronic acid – all the yields were significantly lower (50–67%), probably due to the lower solubility of the obtained compounds in hexane. When comparing the influence of the substituent on the 4-position of the pyridine ring, it is obvious that the bulky phenyl group caused a decrease in the reaction efficiency.

Table 2.1. Yields of new 7-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines **27–48**

Compound	R ¹	R ²	Yield, %
27	H	H	96
28	H	2-MeO-Ph	40
29	H	3-MeO-Ph	78
30	H	4-MeO-Ph	78
31	H	3,4-diMeO-Ph	72
32	H	4-HO-Ph	60
33	Me	H	94 [99]
34	Me	2-MeO-Ph	80
35	Me	3-MeO-Ph	80
36	Me	4-MeO-Ph	89 [99]
37	Me	3,4-diMeO-Ph	67
38	Me	4-HO-Ph	50
39	Et	H	81
40	Et	4-MeO-Ph	82
41	Et	2,4-diMeO-Ph	48
42	Et	4-Me-Ph	81
43	Et	4-CF ₃ -Ph	92
44	Et	4- CF ₃ O-Ph	85
45	Et	4-Cl-Ph	83
46	Et	4-HO-Ph	67
47	iPr	4-MeO-Ph	83
48	Ph	4-MeO-Ph	62

Subsequently, 4-ethyl-7-(4-hydroxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**46**) was subjected to hydroxyl group alkylation with ethyl, propyl, and isopropyl iodides when using NaH as a base in DMF while stirring for 1 h at 70 °C. As a result, 7-(4-alkoxyphenyl)-4-ethyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines **49–51** were obtained in high yields (80–97%) (Scheme 2.6).



Scheme 2.6. Synthesis of compounds **49–51**. *Reagents and conditions:* i: NaH, RI, DMF, 70 °C, 1 h

The structures of the new compounds **27–51** were confirmed by NMR and IR spectroscopy, as well as MS and HRMS spectrometry. In comparison to the starting materials **18–22**, new aromatic signals are observed in the ^1H NMR spectra of the new compounds. Additionally, in the ^1H NMR spectra of compounds **28–31**, **34–37**, **40–41** and **47–48**, new methoxy group singlets with a typical chemical shift within a 3.37–3.90 ppm range are observed. The formation of derivatives with a hydroxyl substituent at the phenyl ring and their alkylation can be quickly confirmed by FT-IR spectra, in which, broad absorption peaks of alcohol groups are observed and then not registered after alkoxyphenyl groups have been formed.

Detailed NMR spectroscopic investigations employing a combination of various NMR-techniques (^1H -NMR, ^{13}C -NMR, COSY, NOESY, HSQC, HMBC) permitted full and unambiguous assignment of all ^1H - and ^{13}C -NMR resonances. For instance, in the case of **41** (Fig. 2.3), N-1 ‘pyridine-like’ (δ –96.2 ppm) and N-2 ‘pyrrole-like’ (δ –147.9 ppm) nitrogen atoms were easily distinguished as they exhibited long-range HSQMBC correlations with the neighboring 3-H proton (singlet, δ 8.58 ppm), while the pyridine N-5 (–84.6 ppm) nitrogen atom exhibited correlation with CH_2 protons (quartet, δ 3.21 ppm), as in the previous example. The identification of different phenyl groups was achieved by using the ^1H - ^1H NOESY spectrum as it exhibited NOEs between the N-phenyl group 2,6-H protons and pyridine 3-H proton and C6-phenyl group 2,6-H protons, and CH_2 protons. Also, correlations between the C7-phenyl 3-H proton (multiplet, δ 6.41 ppm) and both methoxy singlets and the 5-H proton (multiplet, δ 6.56 ppm) sole correlation with the 4-methoxy group singlet led to the characterization of the aforementioned protons. Subsequently, the H2BC spectrum helped determine the remaining phenyl protons as 3,5-H protons as they had correlations with both 2,6-H and 4-H proton

multiplets. Finally, the quarterly carbons were assigned by using the HMBC spectrum. For example, the 3-H proton had correlations with both C-3a (119.0 ppm) and C-7a (152.4 ppm), while CH₂ protons only had correlations with C-3a. Three-bond connectivities between C-6 (149.9 ppm) and C-7 (116.8 ppm) with 2,6-H phenyl protons allowed to confirm the structure of pyrazole[4,3-*c*]pyridine **41**.

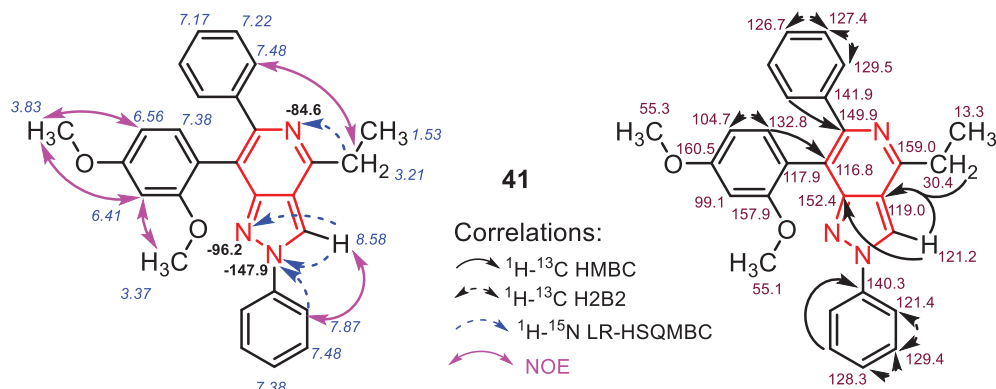


Figure 2.3. Relevant ¹H-¹³C HMBC, ¹H-¹³C H2BC, ¹H-¹⁵N HMBC and ¹H-¹H NOESY correlations and ¹H NMR (italics), ¹³C NMR and ¹⁵N NMR (bold) chemical shifts of compound **41**

It was also observed that 2,6,7-triphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-5-ium **27H** can be obtained in an acidic environment. The formation of the ion was confirmed via NMR spectroscopy (Fig. 2.4). Namely, a big change in the shift is observed in the ¹H-¹⁵N NMR spectrum where the peak of the pyridine's nitrogen is registered in much weaker fields (the value changes from -80.5 ppm to -177.5 ppm). The chemical shifts of the protons of pyrazolopyridinium are also recorded downfield in comparison to the shifts of the protons of pyrazolopyridine.

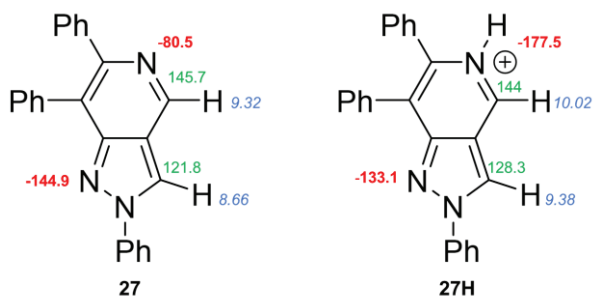


Figure. 2.4. Comparison of pyrazolopyridine and pyrazolopyridinium NMR spectrum chemical shifts: ¹⁵N NMR (bold), ¹H NMR (italic) and ¹³C NMR

2.2.2. Optical properties of 7-aryl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridines

The fluorescence properties of 7-aryl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridines **27–51** were first investigated in THF, with the excitation wavelength λ_{ex} being set to 350 nm (Table 2.2). The emission maxima λ_{em} of all the compounds were located within the 437–487 nm range, which corresponds to the blue part of the visible light spectrum. A polar 4-hydroxyphenyl substituent at the 7-position bearing compounds **32**, **38**, and **46**, as well as derivatives **40–41**, **47**, **49–51** (all of which bear 4-alkoxyphenyl substituents at the 7-position, and ethyl or isopropyl substituents at the 4-position) exhibited the longest bathochromic shift and were denoted by the most pronounced fluorescence properties. Particularly, the Stokes shifts for these compounds were within the 199–205 nm range, and the quantum yield reached approximately 60–85%. Interestingly, the quantum yield increased slightly with the elongation of the 4-alkyl chain, but decreased significantly when the phenyl group was introduced.

Table 2.2. Fundamental absorption (UV-vis spectra of 10^{-4} mol solutions) and fluorescence (spectra of 10^{-5} mol solutions) characteristics of compounds **27–51** in THF ($\lambda_{\text{ex}} = 350$ nm)

Compound	λ_{abs} (nm)	$\varepsilon \times 10^3$ (dm ³ mol ⁻¹ cm ⁻¹)	λ_{em} (nm)	SS (nm)	Φ_f (%)
27	260	30.38	442	130	18.9
	312	13.23			
28	261	34.67	447	136	26.2
	311	16.86			
29	260	35.55	437	126	53.2
	311	16.29			
30	263	31.95	461	151	71.8
	310	15.80			
	351 shoulder	8.07			
31	267	32.04	478	167	56.8
	311	16.09			
	357 shoulder	7.74			
32	265	28.94	467	202	72.7
33	261	36.46	449	138	17.6
	311	17.34			
34	261	29.68	449	139	30.0
	310	15.11			
35	263	38.05	450	140	48.7
	310	17.48			
36	265	31.58	466	117	72.2
	317	14.93			
	349 shoulder	9.31			

Continued Table 2.2.

Compound	λ_{abs} (nm)	$\varepsilon \times 10^3$ (dm ³ mol ⁻¹ cm ⁻¹)	λ_{em} (nm)	SS (nm)	Φ_f (%)
37	266	30.31	481	125	62.8
	316	13.14			
	356 shoulder	7.28			
38	264	24.96	465	201	74.8
39	262	32.79	449	137	21.3
	312	14.13			
40	265	35.93	464	199	73.1
41	264	39.45	465	201	64.7
42	263	35.01	449	141	52.2
	308	14.49			
43	260	21.84	453	140	0.9
	313	10.61			
44	260	45.49	445	133	7.0
	312	21.11			
45	262	37.32	455	141	14.2
	314	16.56			
46	266	36.30	470	204	61.7
47	265	41.18	467	202	68.9
48	269	29.03	487	118	39.7
	369	9.84			
49	265	48.93	469	204	83.5
50	265	36.45	467	202	69.9
51	265	42.38	470	205	84.6

Intracellular pH is of crucial significance in many biological processes since its changes from normal to abnormal levels can lead to cellular dysfunction, various diseases, and a decrease in physical performance [119]. The development of pH-sensitive fluorescent indicators permitted accurate measurement of intracellular pH, thus enabling to consequently supply valuable information about the ongoing physiological and pathological processes at the cellular and sub-cellular levels [120]. Multiple pH probes based on the typical fluorescent chromophores, such as fluorescein, coumarin, naphthalimide, BODIPY, and rhodamine, have been reported [120-122]. Recently, a pyrazolo[1,5-*a*]pyridine derivative has been developed to detect the pH value in acidic conditions, and it exhibited a high quantum yield (64%) when the pH value was 2.4 [123]. Thus, in order to assess whether the fluorescence properties of the 7-arylsusbstituted pyrazolo[4,3-*c*]pyridine derivatives exhibit pH-dependence, they were all analyzed in pH 5, 7, and 9 buffers with the

excitation wavelength λ_{ex} set to 360 nm (Table 2.3). The measurements were done by Mgr. Radek Ostruszka and Doc. Martin Kubala at the Department of Experimental Physics, Faculty of Science, Palacký University in the Czech Republic.

Table 2.3 Fundamental absorption* and fluorescence characteristics of compounds **27–51** (2 μM solutions) in the Britton–Robinson buffer at pH 5, 7 and 9 ($\lambda_{\text{ex}} = 360 \text{ nm}$)

Compound	pH = 5			pH = 7			pH = 9		
	λ_{abs} (nm)	λ_{em} (nm)	Φ_f (%)	λ_{abs} (nm)	λ_{em} (nm)	Φ_f (%)	λ_{abs} (nm)	λ_{em} (nm)	Φ_f (%)
27	259	447	40.4	254	439	16.0	253	436	22.1
28	258	455	4.4	253	459	33.4	253	458	34.3
29	261	444	2.0	255	440	6.5	255	439	5.7
30	259	497	12.2	256	458	37.2	256	458	59.9
31	257	507	0.4	256	511	4.3	256	495	7.1
32	259	445	0.1	255	461	0.1	255	469	0.1
33	261	443	81.3	254	442	17.7	254	435	17.7
34	259	486	2.0	253	456	18.2	252	455	43.1
35	262	446	0.4	257	446	6.1	256	440	6.6
36	264	509	17.0	257	459	22.0	258	458	24.0
37	257	499	0.2	256	504	9.6	255	495	14.2
38	262	446	0.3	255	466	0.2	254	454	0.2
39	261	444	77.5	260	438	14.0	262	436	15.3
40	262	511	18.2	269	457	35.3	267	457	37.8
41	257	497	1.3	255	491	13.5	255	485	14.9
42	263	459	35.7	265	449	17.9	266	448	17.4
43	264	438	8.6	266	439	8.7	264	440	10.1
44	267	438	10.1	269	440	9.9	267	438	11.1
45	265	443	15.3	266	441	14.1	265	443	17.6
46	264	443	0.4	254	495	0.1	255	475	0.2
47	269	457	28.9	270	458	35.6	272	458	36.0
48	285	479	26.6	280	481	31.5	277	480	34.3
49	266	493	10.1	269	481	15.0	268	482	17.4
50	271	474	11.6	272	472	17.0	270	470	19.1
51	269	458	18.5	271	459	33.6	270	458	37.5

*In aqueous solution, the absorption bands above 300 nm become extremely broad, and they are manifested as an extended shoulder, for which it is impossible to identify the precise maximum position. Nevertheless, for all the species, it was possible to use 360 nm as an excitation wavelength in fluorescence experiments within the examined pH range.

Generally, all the compounds exhibited lower quantum yields in aqueous solutions than THF. It was observed that the quantum yield of compounds **27**, **33**, and **39** bearing a phenyl substituent at the 7-position increased from approximately 2- to 5-fold at acidic pH without substantial changes in the emission maxima which were found to be within the 435–447 nm range. Moreover, further analysis of compound **27** in a range of pH 2–11 buffers demonstrated a gradual decrease in the fluorescence intensity with an increase of pH (Fig. 2.5).

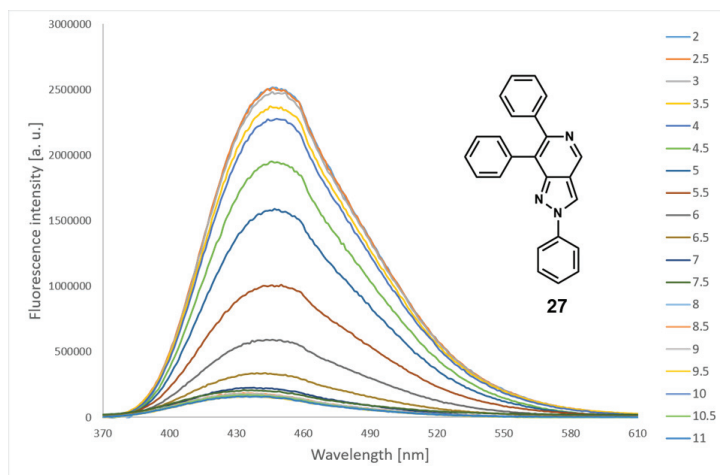


Figure. 2.5. Fluorescence spectra ($\lambda_{\text{ex}} = 360$ nm) and titration profiles for compound **27** in Britton–Robinson buffer at pH 2–11

In contrast, the quantum yield of 2-methoxyphenyl or 4-methoxyphenyl substituent at the 7-position possessing compounds **28**, **30**, **34**, **36**, and **40** was higher in the basic media. Additionally, in the case of 4-methoxyphenyl substituent at the 7-position bearing compounds **30**, **36**, and **40**, an acidic medium caused the red shift of the emission maxima changing to the green part of the visible spectrum. For example, in the case of compound **30**, the emission spectrum was found to be composed of two partly overlapping bands (Fig. 2.6). The short-wavelength part is pH-sensitive. It is dominant in the basic environment, and a decrease of the pH value from 11 to 6 caused a decrease in the fluorescence intensity without a shift in the emission maxima which was maintained at 458 nm. After a further decrease of pH, it was the long-wavelength band that became more dominant, which was manifested as a gradual shift of the emission maxima to 519 nm. Any other 4-alkoxyphenyl substituent at the 7-position bearing compounds only exhibited a drop of the quantum yield at acidic pH without a shift of the emission maxima.

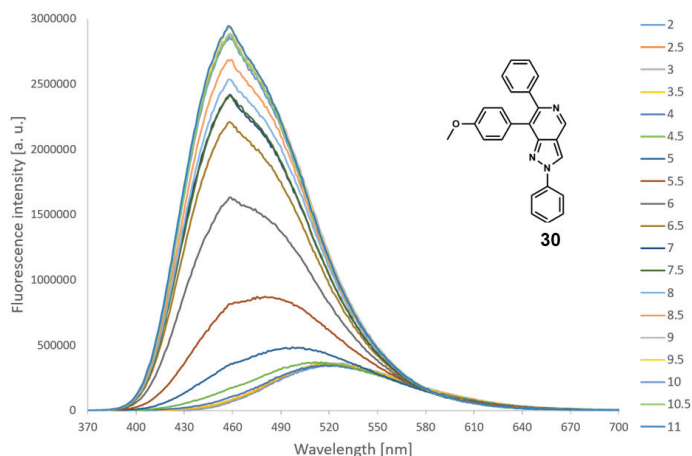


Figure 2.6. Fluorescence spectra ($\lambda_{\text{ex}} = 360$ nm) and titration profiles for compound **30** in Britton–Robinson buffer at pH 2–11

Interestingly, a polar 4-hydroxyphenyl substituent at the 7-position bearing compounds **32**, **38**, and **46** which exhibits the highest quantum yields in THF, also has the lowest quantum yields of up to 0.3% in an aqueous solution. It is well known that hydroxyphenyl groups are sensitive to photochemical reactions [124]. Typically, their pK_{a} drops from ~ 10 in the ground state to ~ 3 in the excited state, and excited-state proton transfer reactions are common in aqueous solutions. For our molecules, these reactions resulted in fluorescence quenching.

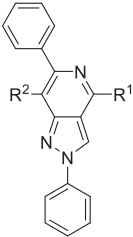
Essentially, preliminary observations suggest that most of the compounds, except for the polar derivatives **32**, **38**, and **46**, could be of potential interest as pH indicators. Considering the approximately 5-fold quantum yield increase and 40 nm blue shift of the emission spectrum maximum when moving from pH 5 to 9, compound **30** seems to be the best pH indicator from the set of the examined molecules as it enables both fluorescence-intensity-based and ratiometric pH sensing.

2.2.3. Biological investigation of 7-aryl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridines

All biological investigations discussed in this chapter were performed by Dr. Eva Řezníčková and the technical staff from Prof. Vladimír Kryštof research group at the Department of Experimental Biology, Faculty of Science, Palacký University in the Czech Republic. The synthesized 2H-pyrazolo[4,3-c]pyridines **27–51** were evaluated for their antiproliferative activity against three human cancer cell lines, i.e., MV4-11 (biphenotypic B myelomonocytic leukaemia cells), K562 (chronic myeloid leukaemia cells), and MCF-7 (human breast cancer cells) (Table 2.4). As observed previously [92], an increase of the bulkiness of the substituent at the 4-position gradually reduced or completely abolished the activity of 2H-pyrazolo[4,3-c]pyridine derivatives. Namely, while methyl-substituted compounds **33–38**

possessed lower antiproliferative values than their unsubstituted counterparts **27–32**, isopropyl-, ethyl-, and phenyl-substituted compounds **39–51** were mostly non-active up to the tested 10 μM concentration. Overall, a polar 4-hydroxyphenyl substituent at the 7-position bearing the 4-unsubstituted derivative **32** proved to be the most potent candidate. Furthermore, both derivative **32** and its 4-methyl counterpart **38** were more potent than the previously reported 7-unsubstituted 2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines **A** and **B** [92].

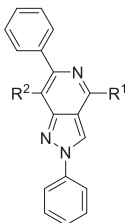
Table 2.4. *In vitro* antiproliferative activity of 2*H*-pyrazolo[4,3-*c*]pyridine derivatives **27–51**

Structure	Compound	R ¹	R ²	GI ₅₀ $\pm$ SD, μM^*		
				MV4-11	K562	MCF-7
	27	H	Ph	7.7 $\pm$ 2.6	>10	>10
	28	H	2-MeO-Ph	6.5 $\pm$ 1.3	7.1 $\pm$ 2.9	6.3 $\pm$ 2.2
	29	H	3-MeO-Ph	5.0 $\pm$ 1.8	>10	>10
	30	H	4-MeO-Ph	3.5 $\pm$ 1.2	4.8 $\pm$ 2.5	7.3 $\pm$ 0.2
	31	H	3,4-di-MeO-Ph	2.4 $\pm$ 1.3	6.0 $\pm$ 3.8	4.2 $\pm$ 0.9
	32	H	4-OH-Ph	1.5 $\pm$ 0.7	2.4 $\pm$ 1.0	1.6 $\pm$ 0.2
	33	Me	Ph	>10	>10	>10
	34	Me	2-MeO-Ph	>10	>10	>10
	35	Me	3-MeO-Ph	>10	>10	>10
	36	Me	4-MeO-Ph	>10	>10	8.4 $\pm$ 2.1
	37	Me	3,4-di-MeO-Ph	7.6 $\pm$ 3.0	>10	>10
	38	Me	4-OH-Ph	4.7 $\pm$ 2.7	3.9 $\pm$ 0.3	4.1 $\pm$ 0.4
	39	Et	Ph	>10	>10	>10
	40	Et	4-MeO-Ph	>10	>10	>10
	41	Et	2,4-di-MeO-Ph	>10	>10	>10
	42	Et	4-Me-Ph	>10	>10	>10
	43	Et	4-CF ₃ -Ph	>10	>10	>10
	44	Et	4-CF ₃ O-Ph	>10	>10	>10
	45	Et	4-Cl-Ph	>10	>10	>10
	46	Et	4-OH-Ph	8.0 $\pm$ 3.1	>10	3.9 $\pm$ 0.4
	47	iPr	4-MeO-Ph	5.7 $\pm$ 1.4	>10	>10
	48	Ph	4-MeO-Ph	>10	>10	7.9 $\pm$ 3.8
	49	Et	4-EtO-Ph	>10	>10	>10
	50	Et	4-PrO-Ph	>10	>10	>10
	51	Et	4-iPrO-Ph	>10	>10	>10
	A [92]	H	H		3.4	4.8
	B [92]	Me	H		6.3	11.0
	Flavopiridol			0.2 $\pm$ 0.03	0.8 $\pm$ 0.1	0.2 $\pm$ 0.03

* All data are means of at least three independent measurements

Moreover, a study to determine the toxicity on non-cancer cells was carried out on human retinal pigment epithelial-1 (RPE-1) and fibroblast MRC-5 cells. It was found that the elongation of the alkyl group at the 4-position reduced the toxicity as the GI₅₀ values increased (Table 2.5).

Table 2.5. *In vitro* toxicity of 2*H*-pyrazolo[4,3-*c*]pyridine derivatives **30**, **32**, **36**, **38**, **40** and **46** on normal cells

General structure	Compound	R ¹	R ²	Normal cells (GI ₅₀ , μM)	
				RPE-1	MRC-5
	30	H	4-MeO-Ph	7.5	7.8
	32	H	4-OH-Ph	1.3	3.0
	36	Me	4-MeO-Ph	>10	>10
	38	Me	4-OH-Ph	8.9	9.6
	40	Et	4-MeO-Ph	>10	>10
	46	Et	4-OH-Ph	>10	>10

* All data are means of at least three independent measurements

Consequently, the effects of the most active compound **32**, its less active 4-substituted analogues **38** and **46**, and derivative **30** were studied on K562 leukemic cells. Asynchronously growing K562 cells were treated with 10 μM concentrations of the selected compounds for 24, 48, and 72 h and analyzed by using immunoblotting and flow cytometry.

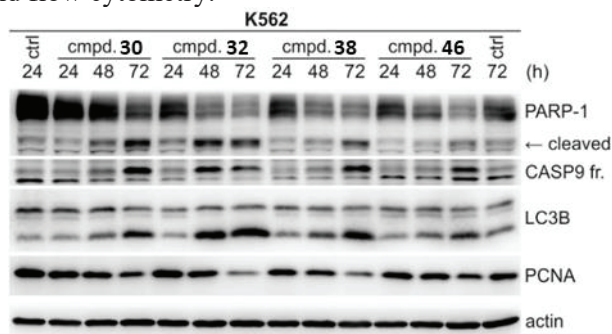


Figure 2.7. Immunoblotting of the selected markers of cell death and proliferation. K562 cell line was treated with compounds **30**, **32**, **38**, and **46** (10 μM) for 24, 48, and 72 h. The actin level was detected to verify equal protein loading

Immunoblotting (Fig. 2.7) revealed that 48 h treatment with the most potent compound **32** was plentiful for the induction of poly(ADP-ribose) polymerase 1 (PARP-1) cleavage [125] and the activation of the initiator enzyme of apoptotic cascade caspase 9 [126]. Interestingly, in addition to the clear pro-apoptotic effects, the time-dependent fragmentation of the microtubule-associated protein 1-light chain 3 (LC3), which appears during autophagy [127], was observed. Similar findings with lower efficiencies were reported in all the tested compounds. Beside

cell-death-related proteins, the expression levels of proliferating cell nuclear antigen (PCNA), which plays a key role in the DNA replication [128], were analyzed. The results showed that all the studied compounds exhibited time-dependent reduction of the levels of PCNA, with the most pronounced effect being observed for compounds **32** and **38**. In order to independently support this observation, immunoblotting was complemented with the flow cytometric analysis of bromodeoxyuridine (BrdU) incorporation, which allowed to detect replicating BrdU-positive cells in the population [129] (Fig. 2.8). In the control samples, the number of proliferating cells came up to 40%, but 10 μ M treatment with the tested compounds **30**, **32**, **38**, and **46** reduced the proportion of actively proliferating BrdU-positive cells by up to approximately 10% for the most active compounds **32** and **38**.

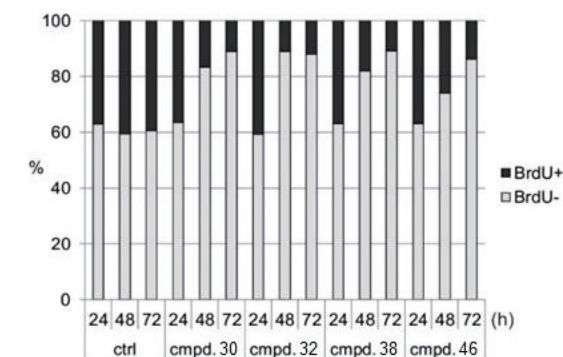


Figure 2.8. Analysis of BrdU incorporation. Results are representative of two independent experiments. K562 cell line was treated with compounds **30**, **32**, **38**, and **46** (10 μ M) for 24, 48, and 72 h

Overall, the obtained results suggest the complex action of the compounds as they combine antiproliferative effects with the induction of cell death.

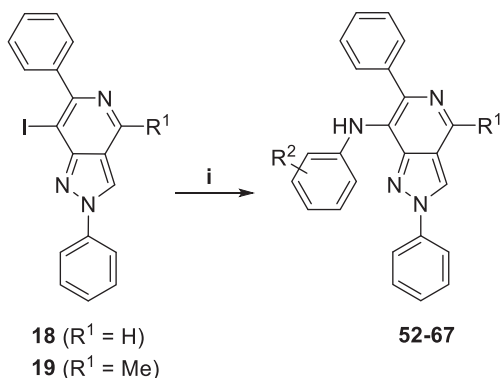
2.3. Synthesis, optical and biological investigation of *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines

2.3.1. Synthesis of *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines

The Buchwald-Hartwig reaction is applied in synthetic organic chemistry for the formation of C-N bonds through Pd-mediated cross-coupling of amines and aryl halides. It is widely employed in the synthesis of new materials, heterocyclic systems, pharmaceuticals, and is an indispensable tool in the total synthesis of several natural products exhibiting diverse biological activities [130-132]. Park *et al.* adopted the conditions of Buchwald-Hartwig cross-coupling to introduce the amine group in the 5-position of the 1*H*-pyrazolo[3,4-*b*]pyridine core. Specifically, Pd₂dba₃ was chosen as a catalyst, Xantphos as a ligand, and Cs₂CO₃ as a base while stirring in 1,4-dioxane [116]. Recently, these conditions have also been implemented in the synthesis of *N*-(3,4,5-trimethoxyphenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amines,

however, by using a different catalyst – Pd(OAc)₂ [133]. The new compounds acted as tubulin polymerization inhibitors and displayed anti-proliferation activity on tumor cells. Moreover, palladium-catalyzed cross-coupling was applied in the reaction between amide and halogenated pyrazolopyridine to obtain a selective phosphatidylinositol-3-kinase inhibitor that can be used for the treatment of autoimmune diseases and leukocyte malignancies [134]. Martinez-Gualda *et al.* achieved coupling of various anilines at position 6 of the isothiazolo[4,3-*b*]pyridine scaffold by using Pd(dba)₂ as a catalyst, SPhos as a ligand, *t*BuOK as a base and toluene as a solvent, which yielded compounds that were tested as potent antiviral agents targeting cyclin G-associated kinase [117]. Bazin and colleagues investigated the functionalization of the 2-chloro-3-nitroimidazo[1,2-*a*]pyridine scaffold [135]. In order to introduce derivatives to the 3-position, a reduction of the nitro group was performed, which was followed by amination with various haloarenes in the presence of Pd₂(dba)₃, BINAP, *t*BuONa and toluene in a sealed tube. Buchwald amination was also carried out in the 2-position by adapting the same conditions. It is noteworthy that the reaction time was dramatically reduced by replacing classical heating with MWI (from 20 h to 1 h, respectively). Buchwald-Hartwig cross-coupling was also utilized to obtain C2-substituted imidazo[4,5-*b*]pyridine analogues while using enolizable heterocycles (i.e. pyridone), and Xantphos and Pd(OAc)₂ were found to be most effective in dry dioxane when using Cs₂CO₃ as a base [136].

Thus, in order to introduce an amine group, the prepared 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) and 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**19**) were used in the palladium catalyzed Buchwald-Hartwig cross-coupling reaction adopting the previously described reaction conditions [99, 137]. 7-Iodopyrazolo[4,3-*c*]pyridines **18** and **19** were treated with an appropriate aniline, Pd(OAc)₂ as a catalyst, SPhos as a ligand, and sodium *tert*-butoxide as a base in dioxane (Scheme 2.7). In order to ensure short reaction times, cross-couplings were performed under microwave irradiation, and compounds **52–67** were obtained in moderate to excellent 48–95% yields (Table 2.5). We should emphasize that the reactions were carried out under argon atmosphere and in a dry solvent due to the sensitivity of the ligand and the catalyst to air and moisture.



Scheme 2.7. Synthesis of *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines **52–67**.
Reagents and conditions: (i) R^2PhNH_2 , $Pd(OAc)_2$, SPhos, Na^tBuO , dioxane, MW, $120\text{ }^\circ C$, 280 W, 1 h

There was no clear pattern regarding the electronic influence of the substituents on their reactivity, and the yields were mostly dependent on the success of purification *via* column chromatography. Some of the reaction mixtures had a lot of residual anilines which were complicated to dispose of.

Table 2.5. Yields of *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines **52–67**

Structure	Compound	R^1	R^2	Yield, %
	52	H	H	92
	53	H	4-MeO	48
	54	H	3,4-diMeO	86
	55	H	3,5-diMeO	60
	56	H	2,4-diMeO	63
	57	H	3,4,5-triMeO	95
	58	H	2-CF ₃ O	56
	59	H	3-CF ₃ O	56
	60	Me	H	65 [99]
	61	Me	4-MeO	77
	62	Me	3,4-diMeO	60
	63	Me	3,5-diMeO	49
	64	Me	2,4-diMeO	73
	65	Me	3,4,5-triMeO	63
	66	Me	2-CF ₃ O	62
	67	Me	3-CF ₃ O	82

The structures of compounds **52–67** were confirmed by NMR and IR spectroscopy, as well as MS and HRMS spectrometry. The formation of amine can be quickly confirmed by the IR spectrum in which the peaks of the amine group can be found at $3204\text{--}3412\text{ cm}^{-1}$. Also, in comparison to the starting materials **18** and **19**, the ^1H NMR spectra of all the new derivatives feature a broad NH singlet in the

range of 5.89–6.46 ppm, and the appropriate signals of the N-aryl protons at 5.97–7.21 ppm, while compounds **53–57** and **61–65** additionally have singlets of the methoxy groups protons in the area of 3.65–3.83 ppm.

Detailed NMR spectroscopic investigations employing a combination of various NMR-techniques (^1H -NMR, ^{13}C -NMR, COSY, NOESY, HSQC, HMBC) permitted full and unambiguous assignment of all ^1H - and ^{13}C -NMR resonances. For example, signals of the amine proton and protons of the phenyl ring next to it were assigned by using the ^1H - ^{15}N HMBC spectrum (Fig. 2.9). It showed a correlation between the proton singlet at 6.17 ppm and nitrogen at -304.0 ppm, thus assigning them to NH while other correlations with multiplets in the interval of 6.81–7.16 ppm were assigned to phenyl protons. Signals of phenyl protons were also assigned by using the HMBC spectrum, where the signal of the amine proton had one three-bond correlation with the signal of carbon at 117.5 ppm. Also, a correlation with the multiplet of protons at 6.81–6.88 ppm is observed, thus assigning it to NH-Ph 2,6-*H*.

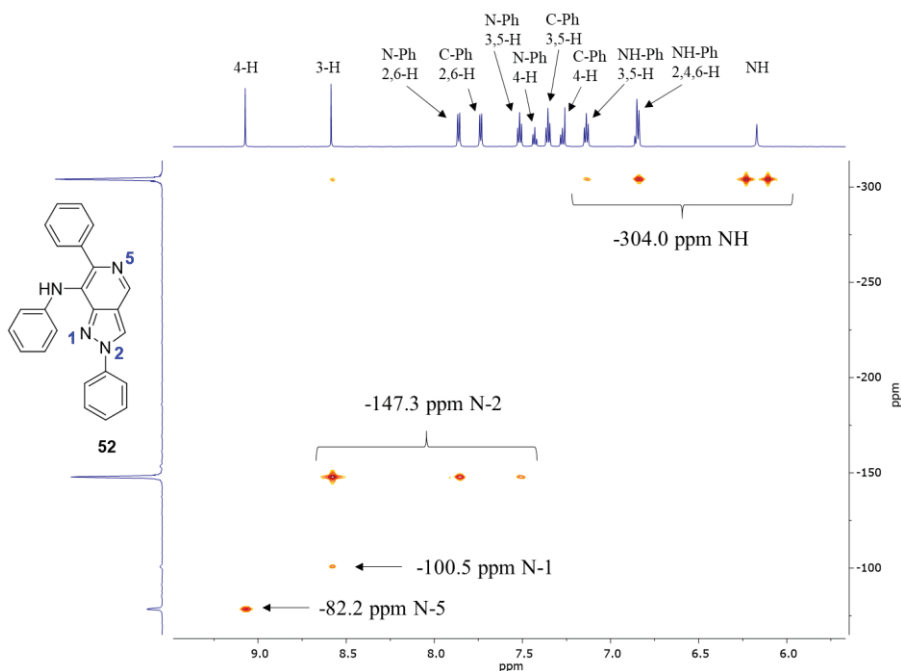


Figure 2.9. ^1H - ^{15}N HMBC spectrum of compound **52**

2.3.2. Optical properties of *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines

As outlined above, the fluorescence properties of *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines **52–67** were first investigated in THF, with the excitation wavelength λ_{ex} being set to 350 nm (Table 2.6). The emission maxima λ_{em} of most compounds were located in the 453–486 nm range, which corresponds to the blue part of the visible light spectrum, except for compounds **52**, **55**, **60** and **63** with phenyl or 3,5-dimethoxyphenyl substituents which exhibited emission maxima in

the green part (506–520 nm) of the spectrum. In comparison with 7-aryl pyrazolopyridines, the addition of the amine group led to a significant decrease both in the Stokes shift and in the quantum yield. Methoxy substituents almost completely eliminated photon emission, with the exception of 3,5-dimethoxy substituted *N*-arylamines. Replacing protons with fluoro atoms provided derivatives with the best fluorescence properties and the quantum yield reaching approximately 37–45%

Table 2.6. Fundamental absorption (UV-vis spectra of 10^{-4} mol solutions) and fluorescence (spectra of 10^{-5} mol solutions) characteristics of compounds **52–67** in THF ($\lambda_{\text{ex}} = 350$ nm)

Compound	λ_{abs} (nm)	$\varepsilon \times 10^3$ ($\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$)	λ_{em} (nm)	SS (nm)	Φ_f (%)
52	249	25.42	506	123	20.47
	282	30.75			
	383	5.12			
53	252	26.56	483	90	0.15
	284	29.22			
	393	4.55			
54	254	29.25	464	67	<0.1
	284	32.94			
	397	5.18			
55	224	32.03	520	137	14.55
	250	28.35			
	278	31.40			
	383	5.09			
56	253	31.45	479	79	<0.1
	288	34.15			
	400	5.61			
57	256	26.86	453	59	0.37
	281	30.28			
	394	4.93			
58	250	27.72	482	118	32.46
	274	34.04			
	364	5.35			
59	249	24.30	479	105	37.27
	277	31.93			
	374	5.21			
60	253	27.03	506	124	38.09
	285	30.89			
	382	5.09			
61	254	29.52	486 590	196	0.41
	288	31.36			
	394	4.83			

Continued Table 2.6

Compound	λ_{abs} (nm)	$\varepsilon \times 10^3$ ($\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$)	λ_{em} (nm)	SS (nm)	Φ_f (%)
62	256	28.38	455	59	0.22
	285	30.0			
	396	4.64			
63	223	29.26	512	129	18.35
	252	24.46			
	276	26.21			
	383	4.09			
64	253	27.54	458	58	<0.1
	290	27.95			
	400	4.49			
65	257	27.78	457	64	<0.1
	280	29.75			
	393	4.78			
66	246	26.65	484	121	34.74
	274	31.90			
	363	5.12			
67	254	27.26	486	113	44.58
	277	33.62			
	373	5.72			

The fluorescence properties of the final compounds **52–67** were also briefly assessed in pH 7 Britton–Robinson buffer (Fig. 2.10). The measurements were done by Doc. Martin Kubala at the Department of Experimental Physics, Faculty of Science, Palacký University in the Czech Republic. The excitation wavelength λ_{ex} was set to 380 nm. With an exception of 2,4-dimethoxyphenyl and 3,4,5-trimethoxyphenyl substituents at 7-position possessing compounds **56**, **57**, **64** and **65** which had an extremely low fluorescence intensity, the emission maxima λ_{em} of the remaining compounds were located in the 450–550 nm range. Even though these compounds absorbed both UV and visible blue light, in order to minimize the negative effects of UV and near-UV light to the cells [138], a 414 nm emitting LED source was used for further photodynamic experiments [139].

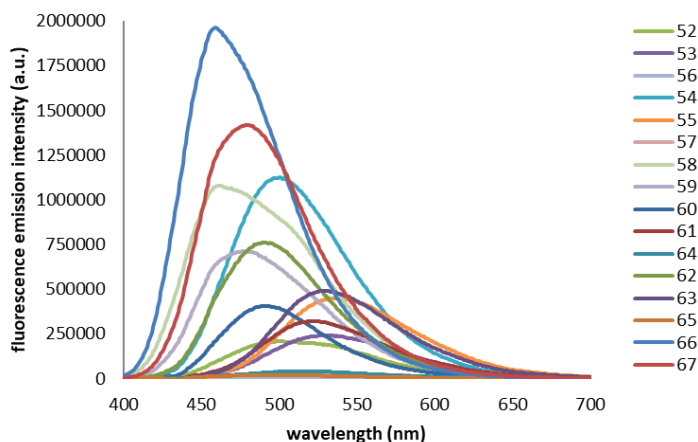


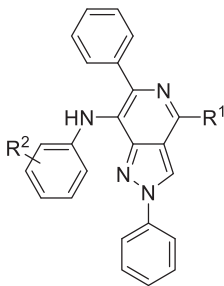
Figure 2.10. Fluorescence emission spectra ($\lambda_{\text{ex}} = 380$ nm) of compounds **52–67** ($2\mu\text{M}$ solutions) in Britton–Robinson buffer at pH 7

2.2.3. Biological investigation of *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines

All biological investigations discussed in this chapter were performed by Dr. Eva Řezníčková and Mgr. Veronika Vojáčková representing Prof. Vladimír Kryštof's research group at the Department of Experimental Biology, Faculty of Science, Palacký University in the Czech Republic and by Mgr. Lukáš Malina representing Prof. Hana Kolářová's research group at the Department of Medical Biophysics, Faculty of Medicine and Dentistry, Palacký University in the Czech Republic.

As already discussed in this thesis, synthesized *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines **52–67** were evaluated for their antiproliferative activity against two human cancer cell lines, specifically, K562 and MCF-7 (Table 2.7). It is noteworthy that the incorporation of the amine group led to a decrease in the biological activity in comparison to the derivatives obtained *via* Suzuki coupling. Out of *N*-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines **52–60**, derivative **56** bearing *N*-2,4-dimethoxyphenylamine substituent at 7-position exhibited the best results with a moderate GI_{50} value of $7.1\ \mu\text{M}$ against K562 cells. The methyl counterparts **60–67** were mostly inactive, apart from the derivatives with phenyl, 4-methoxyphenyl and trifluoromethoxyphenyl moieties which displayed moderate activity.

Table 2.7. *In vitro* antiproliferative activity of *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines **52–67**

Structure	Compound	R ¹	R ²	GI ₅₀ , μM*	
				K562	MCF-7
	52	H	H	>12.5	>12.5
	53	H	4-MeO	100	33
	54	H	3,4-diMeO	30.5	>50
	55	H	3,5-diMeO	42.1	>50
	56	H	2,4-diMeO	7.1	32.4
	57	H	3,4,5-triMeO	36.1	32.4
	58	H	2-CF ₃ O	19	44
	59	H	3-CF ₃ O	22	32
	60	Me	H	25	31
	61	Me	4-MeO	63	>100
	62	Me	3,4-diMeO	>100	>100
	63	Me	3,5-diMeO	>100	>100
	64	Me	2,4-diMeO	>100	>100
	65	Me	3,4,5-triMeO	30	>100
	66	Me	2-CF ₃ O	55	44
	67	Me	3-CF ₃ O	>100	31

* The data is means of at least three independent measurements

It was observed afterwards that the antiproliferative activity of the compounds increased upon light irradiation. It was hypothesized that the derivatives could possess photosensitizing properties which can further be used in photodynamic therapy (PDT). The main mechanism of PDT involves activating a photosensitizer by a specific light source within the target cells to produce reactive oxygen species (ROS) which damage such biomolecules as lipids, proteins and nucleic acids, thereby causing the cell destruction [140]. The main advantages of PDT over the traditional treatments include low systemic toxicity, spatiotemporal selectivity, and the possibility of undergoing repeated treatments without the development of resistance [141, 142]. It is thus a promising treatment for various, especially oncological, diseases [143], including but not limited to skin basal cell carcinoma [144, 145], lung [146], breast [147], prostate [148], cervical [149], head and neck [150] cancers. Moreover, PDT can be used for treating macular degeneration [151], acne [152] and atherosclerosis [153]. Although derivatives of porphyrins [154] are the most commonly used and studied photosensitizers, several fused pyrazoles were also reported to be potential agents for PDT. For example, a number of pyrazolo[3,4-*h*]quinolines were found to possess sub-micromolar phototoxicity and to photoinduce cell death without DNA damage in cancer cells [155]. Pereira *et al.* synthesised dihydroxymethyl-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridine-fused meso-tetraphenylchlorin which exhibited nanomolar activity against melanoma cells which are typically resistant to photodynamic therapy [156]. Researchers of Kaunas University of Technology also recently reported on the synthesis of a pyrazole–indole hybrid which exhibited a strong photodynamic effect in human skin

melanoma cells G361 by inducing cell death through the production of ROS and extensive DNA damage [4].

Thus, in order to assess the photodynamic potency of the prepared compounds **52–67**, G361 cells were treated with the compounds for 4 h and then exposed to the blue light (LED source, 414 nm) which was selected *a priori* based on the optical properties of the compounds at a total irradiation dose of 10 J/cm². The viability of the cells was quantified at 72 h after irradiation by an MTT assay which revealed that most of the compounds possess photodynamic properties with 4-unsubstituted derivatives **52–59** being typically more active than their 4-methyl counterparts **60–67**. Compounds **53** and **58** were found to be the most potent photosensitizers with the lowest EC₅₀ (1.1 μM), and, with an exception of compound **56**, no dark toxicity was observed up to the tested concentration (10 μM) (Table 2.8).

Table 2.8. EC₅₀ values of compounds **52–67** kept in dark and light-irradiated (414 nm, 10 J/cm²) G361 cells

Compound	EC ₅₀ ± SD (μM)	
	dark	light energy (10 J/cm ²)
52	>10	2.7 ± 0.5
53	>10	1.1 ± 0.5
54	>10	1.3 ± 0.3
55	>10	8.0 ± 1.7
56	9.6 ± 0.2	3.1 ± 0.1
57	>10	>10
58	>10	1.1 ± 0.0
59	>10	1.8 ± 0.8
60	>10	2.1 ± 0.7
61	>10	8.8 ± 1.7
62	>10	7.1 ± 0.5
63	>10	4.5 ± 1.8
64	>10	9.9 ± 0.2
65	>10	>10
66	>10	5.3 ± 2.3
67	>10	2.0 ± 0.5

Subsequently, the viability of G361 cells upon treatment with one of the most potent compounds (**58**) was evaluated across a range of irradiation doses (1, 5, and 10 J/cm²). As predicted, the photoactivation depended on the light exposure. Namely, a decrease of the irradiation dose to 5 and 1 J/cm² increased the EC₅₀ value to 1.6 and 3.5 μM, respectively. Conducting the experiment on human keratinocyte HaCaT cells revealed that these non-cancerous cells are approximately 2 times less sensitive to the treatment, thereby confirming the potential of compound **58** as a photodynamic agent (Table 2.9).

Table 2.9. Sensitivity of G361 and HaCaT cells to compound **58** in the dark and after exposure to various doses of light (414 nm)

Light energy (J/cm ²)	EC ₅₀ ± SD (μM)	
	G361	HaCaT
0	>10	>10
1	3.5 ± 0.3	5.7 ± 2.2
5	1.6 ± 0.4	1.9 ± 0.1
10	0.9 ± 0.3	2.0 ± 0.1

The main mechanism by which PDT causes localized cell death is the generation of ROS [56]. Thus, in order to further investigate the phototoxic activity of compound **58**, the production of ROS in treated G361 cells was quantified by using 2',7'-dichlorodihydrofluorescein diacetate. As anticipated, no ROS production was observed in untreated and/or irradiated cells, while the combination of compound **58** throughout the range of tested concentrations (1, 1.1, 3.3 and 10 μM) and irradiation doses (1, 5 and 10 J/cm²) resulted in the production of ROS (Fig. 2.11).

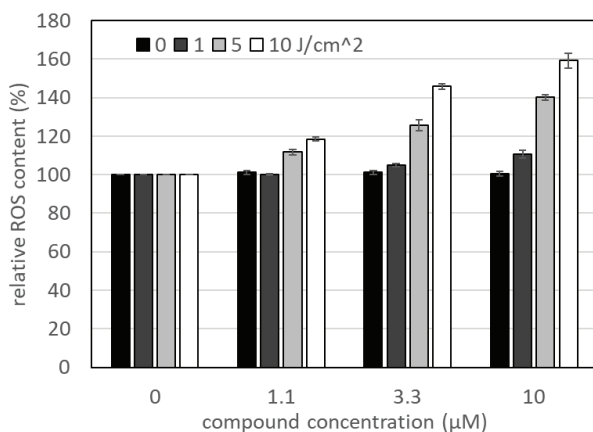


Figure 2.11. Production of reactive oxygen species in G361 cells treated with compound **58** (1.1, 3.3 or 10 μM) in combination with light irradiation (414 nm)

The subsequent analysis of the cellular DNA content of compound **58** treated and irradiated G361 cells by flow cytometry revealed increased subdiploid populations in both concentration in a light irradiation-dependent manner (Table 2.10), which indicated that apoptosis may be the cause of cell death. It should be noted that a mild increase of subdiploid populations was also detected in compound-untreated cells at higher irradiation doses. This was anticipated as high fluences irradiation with the blue light (412–426 nm) is known to exert toxic effects in human skin cells [157]. Moreover, slightly increased subdiploid populations in the cell cultures kept in the dark indicated that compound **58** exhibits some toxic effect by itself; however, this effect is low, as documented by EC₅₀ values > 10 μM.

Table 2.10. Quantification (%) of subdiploid population of G361 cells treated with compound **58** in combination with light irradiation (414 nm)

Concentration (μM)	Light energy (J/cm^2)			
	0	1	5	10
0	3.4	5.0	4.4	6.1
1.1	4.6	5.4	4.5	9.7
3.3	5.0	5.0	15.0	70.0
10	5.9	5.3	63.3	88.3

To further confirm apoptosis as a mechanism of cell death induced by irradiation in cells treated by compound **58**, the activity of pro-apoptotic caspases 3 and 7, which are involved in mediating of cell death *via* signaling transduction, was assessed (Fig. 2.12). The obtained results confirmed that apoptosis is induced by compound **58** in both concentration- and light irradiation-dependent manner. Interestingly, a combination of 10 μM concentration and 10 J/cm^2 irradiation led to severe cell death and thus no caspase 3/7 activity could be observed.

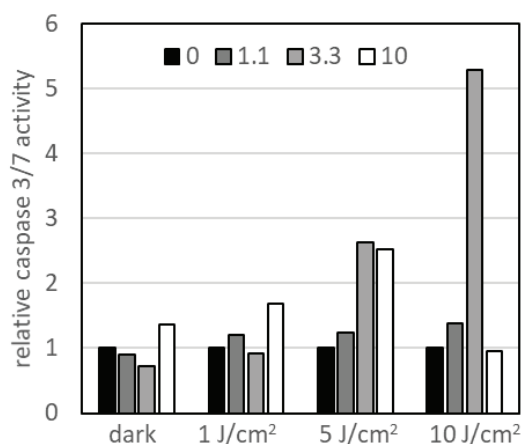


Figure 2.12. Relative caspase 3/7 activity in G361 cells treated with compound **58** (1.1, 3.3 or 10 μM) in combination with light irradiation (414 nm). The cells were treated with compound **58** for 4 h, irradiated (414 nm) and cultivated for further 24 h. After this period, the cells were harvested, and the lysates were assayed for caspase 3/7 activity

As demonstrated above, the compounds can kill cancer cells by inducing apoptotic cell death, which then causes extensive DNA damage. In order to determine the extent of DNA fragmentation in G361 cells exposed to a combination of compound **58** and irradiation, comet assay was performed. The percentual DNA content in the comet heads (nuclei) and tails were quantified. As anticipated and in compliance with other performed experiments, compound **58** caused significant DNA fragmentation, which was dependent both on the compound dose and the light energy (Fig. 2.13). Importantly, no marks of DNA damage were exhibited in the

control cells (i.e. in the cells treated by **58** and kept in the dark or irradiated cells untreated with the compound).

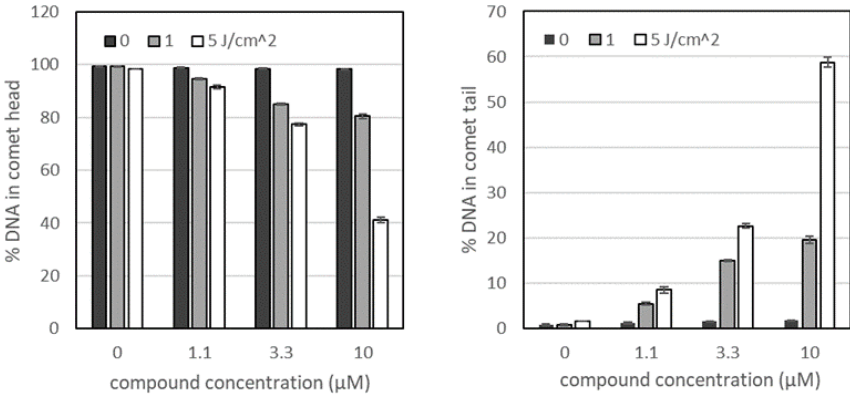


Figure 2.13. Percentual DNA content in comet heads (nuclei) and tails of G361 cells treated with compound **58** (1.1, 3.3 or 10 μM) in combination with light irradiation (414 nm)

Finally, DNA damage and apoptotic cell death were independently confirmed by the means of immunoblotting analysis. G361 cells were treated with compound **58** at 1, 3 and 10 μM concentrations for 4 h, and then either kept in the dark or exposed to light with a total irradiation dose of 1, 5 or 10 J/cm², followed by the subsequent cultivation for 24 h. A combination of the higher concentrations of compound **58** and irradiation doses increased the levels of phosphorylated histone H2A.X at Ser-139, which is a marker of DNA damage (Fig. 2.14.). This effect was most apparent at 10 μM concentration and 5 J/cm² irradiation, or at 3 μM concentration and 10 J/cm² irradiation. Analogously, PARP, an enzyme involved in DNA repair, which gets cut and inactivated by caspases during apoptotic cell death, was cleaved when combining said concentrations and irradiation doses. Finally, the activation of caspase 7 and downregulation of Mcl-1 further confirmed apoptosis. It is of importance that none of the above mentioned changes was observed for the cells kept either in the dark or without compound **58**. This confirms that all of the observed effects are in fact photodynamic.

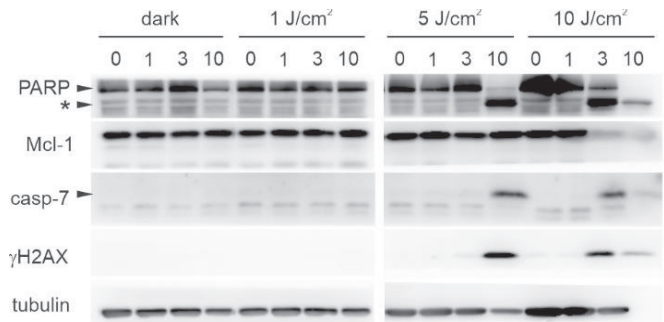


Figure 2.14. Light irradiation (414 nm) increases the sensitivity of G361 cells to compound **58**. The cells were treated with 1, 3 or 10 μM compound for 4 h, irradiated and harvested 24 h after irradiation. Asterisk indicates 89 kDa cleavage fragment of PARP

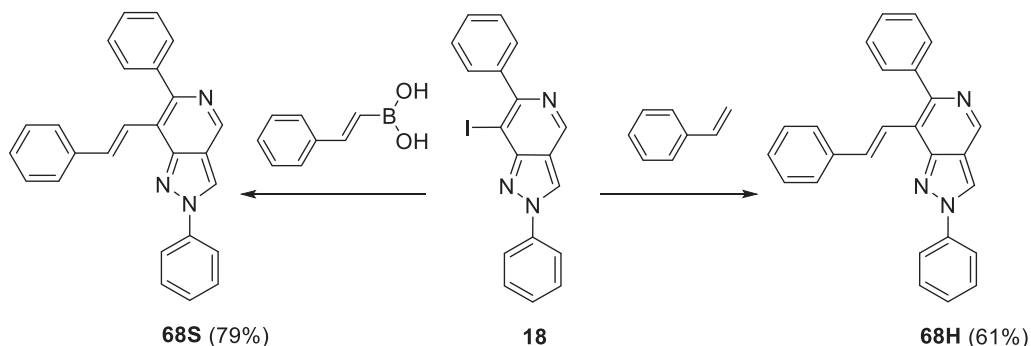
2.4. Synthesis, optical and biological investigation of 2,6-diphenyl-7-[(*E*)-2-phenylethenyl]-2*H*-pyrazolo[4,3-*c*]pyridines and (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)-*N*-phenylprop-2-enamides

2.4.1. Synthesis of 2,6-diphenyl-7-[(*E*)-2-phenylethenyl]-2*H*-pyrazolo[4,3-*c*]pyridines

The Heck reaction, the cross-coupling of organohalides and olefins to form new alkene derivatives, represents one of the most important carbon-carbon forming reactions. It has been extensively applied in organic synthesis [158, 159]. Recently, Han *et al.* applied the conditions of Heck cross-coupling to obtain a variety of quinolin-2(1*H*)-one derivatives [160]. Pd₂(dba)₃ was chosen as the catalyst, and the reaction proceeded very effectively overnight with *t*BuXPhos as the ligand and TEA as the base and the solvent. Martinez-Gualda *et al.* reported the synthesis of 6-(*trans*-styryl)-3-(*cis*-2,6-dimethylmorpholino)isothiazolo[4,3-*b*]pyridine *via* palladium-catalyzed cross-coupling between styrene and isothiazolo[4,3-*b*]pyridine when applying the conditions of the Heck reaction, namely, when using Pd(OAc)₂ as the catalyst, *N*-phenylurea as the ligand, and K₂CO₃ as the base [117]. Ducray *et al.* employed the conditions of Heck cross-coupling to develop intermediates for the synthesis of substituted 3-(pyrimidin-4-yl)-imidazo[1,2-*a*]pyridines and 3-(pyrimidin-4-yl)-pyrazolo[1,5-*a*]pyridines; thus 2,4,5-trichloropyrimidine reacted with 1-(vinylloxy)butane in the presence of Pd(OAc)₂, TEA in polyethylene glycol to obtain (*E*)-4-(2-butoxyvinyl)-2,5-dichloropyrimidine in 53% yield [161]. Davies and colleagues reported a reaction between 4-bromo-1*H*-indazole and acrylamide when using Pd(dtbpf)Cl₂, tetrabutylammonium chloride, *N*-cyclohexyl-*N*-methylcyclohexanamine in ACN under MWI at 130 °C [162]. Heck cross-coupling can also be applied in intramolecular cyclization. The synthesis of benzo[4,5]furo[3,2-*c*]pyridines was achieved from 4-(2-bromophenoxy)pyridines by using Pd(OAc)₂, 1,3-bis-(2,6-diisopropylphenyl)imidazolium chloride (IPr-HCl) and K₂CO₃ in DME at 130 °C (a sealed tube) to obtain the desired product in 95% isolated yield. Another example was reported by Madaiah and colleagues [163], namely, (*E*)-*tert*-butyl 3,3-difluoro-4-(phenylimino)piperidine-1-carboxylate derivatives underwent intramolecular cyclization to form difluoropyridoindole in the presence of Pd(PPh₃)₂Cl₂ and pyridine with diisopropylethylamine.

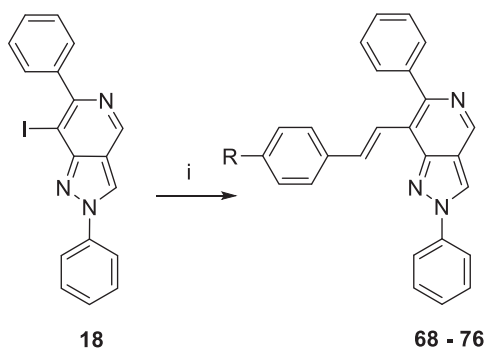
To introduce a vinyl moiety to pyrazolo[4,3-*c*]pyridine, two routes were examined (Scheme 2.8). Firstly, 7-iodopyrazolo[4,3-*c*]pyridine **18** was treated with styrene by applying the Heck cross-coupling conditions when using Pd(PPh₃)Cl₂ as the catalyst, TEA as base stirring in DMF at reflux. Since the reaction was not completed in more than 6 hours *via* conventional heating, microwave irradiation was implemented to ensure a short reaction time. In fact, the coupling finished in 1 h at 120 °C, thereby yielding 2,6-diphenyl-7-[(*E*)-2-phenylethenyl]-2*H*-pyrazolo[4,3-*c*]pyridine (**68H**) in moderate yield. It should be noted that the reaction was carried out under argon atmosphere and dry solvent due to the high catalyst sensitivity to air and moisture. Afterwards, the previously applied Suzuki-Miyaura conditions were

employed, and 7-iodopyrazolo[4,3-*c*]pyridine **18** reacted with *trans*-2-phenylvinylboronic acid in the presence of Pd(OAc)₂, Cs₂CO₃ in aqueous EtOH under MWI. The reaction was completed in 1 h, thereby furnishing compound **68S** in good yield.



Scheme 2.8. Comparison of 2,6-diphenyl-7-[(*E*)-2-phenylethenyl]-2*H*-pyrazolo[4,3-*c*]pyridine synthesis when using various palladium catalyzed cross-coupling reactions

Although Suzuki-Miyaura cross-coupling provided higher yields than Heck cross-coupling (79% and 61%, respectively), due to the wider scope and the lower price of styrene derivatives, it was decided to proceed with Heck cross-coupling to obtain a library of 7-[(*E*)-2-(4-substitutedphenyl)ethenyl]-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines. All of the reactions proceeded smoothly in the 1 h to 3 h timeframe, and compounds **69–76** were obtained in fair to very good 40–84% yields (Table 2.11).



Scheme 2.9. Synthesis of 2,6-diphenyl-7-[(*E*)-2-phenylethenyl]-2*H*-pyrazolo[4,3-*c*]pyridines **68–76**. *Reagents and conditions:* i: appropriate styrene, TEA, Pd(PPh₃)Cl₂, DMF, MWI, 120 °C, 100 W, 1–3 h

It was observed that *para*-substituted styrenes possessing electron donating groups (specifically, compounds **69**, **72**) furnished products in better yields comparing to their counterparts with electron withdrawing groups (compounds **71**, **73–76**), except for the case of nitrile which had the same yield as methyl. Bromo substituted styrene reacted poorly in comparison with fluoro and chloro substituted

styrenes due to its strong reactivity in palladium catalyzed cross-coupling which could have caused a competing coupling cycle with the alkene group of the styrene.

Table 2.11. Yields of 2,6-diphenyl-7-[(*E*)-2-phenylethenyl]-2*H*-pyrazolo[4,3-*c*]pyridines **68–76**

Compound	R	Yield, %
68	H	61
69	OMe	81
70	CN	84
71	COOMe	52
72	Me	84
73	CF ₃	65
74	F	76
75	Br	40
76	Cl	77

The structures of the newly produced compounds **68–76** were confirmed by NMR and IR spectroscopy, as well as MS spectrometry. In comparison to the starting material **18**, additional aromatic signals and doublets of ethenylic protons are observed in the ¹H NMR spectra of the new compounds (Fig. 2.15). The chemical shifts of the protons next to phenyl H_A are downfield (8.47–8.56 ppm) in comparison to those of the protons next to pyridine H_B (7.27–7.55 ppm) in all the derivatives. It is of importance that the signals of pyrazolo[4,3-*c*]pyridine protons also changed their place as 3-H goes slightly upfield, while 4-H goes downfield.

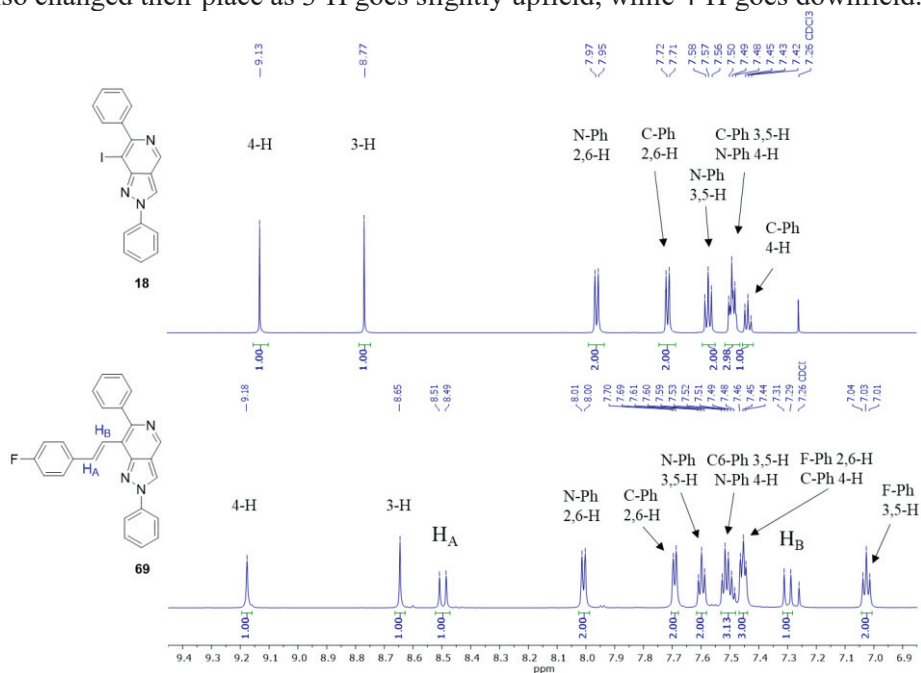


Figure 2.15. Comparison of ¹H NMR spectra of compounds **18** and **69**

Detailed NMR spectroscopic investigations employing a combination of various NMR-techniques (^1H -NMR, ^{13}C -NMR, COSY, NOESY, HSQC, HMBC) permitted full and unambiguous assignment of all ^1H - and ^{13}C -NMR resonances. For example, in the case of compound (**72**), the distinction between ethenylic protons was made by using ^1H - ^{13}C HMBC spectrum where proton H_B had distinct long-range correlations with the quarterly carbons of pyridine, while proton H_A had correlations with 2- and 6-carbons of the phenyl groups (Fig. 2.16). The ^1H - ^1H NOESY spectrum further elucidated the connectivities based on through-space correlations. In this case, distinct NOEs were exhibited between olefinic proton H_A and N-phenyl protons 2,6-H, while olefinic proton H_B displayed a correlation with C6-phenyl protons 2,6-H, thus further confirming the structure of (**72**). Finally, ^1H - ^{13}C HSQC spectral data was applied to identify the chemical shifts of ethenylic group carbons which also exhibited the same pattern as protons: C_B peaks are found upfield (121.7–127.4 ppm) in comparison to C_A peaks (133.3–135.6 ppm).

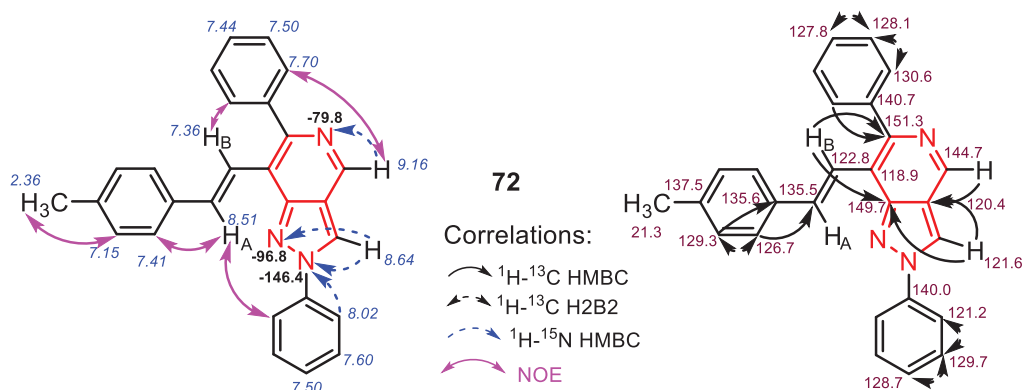
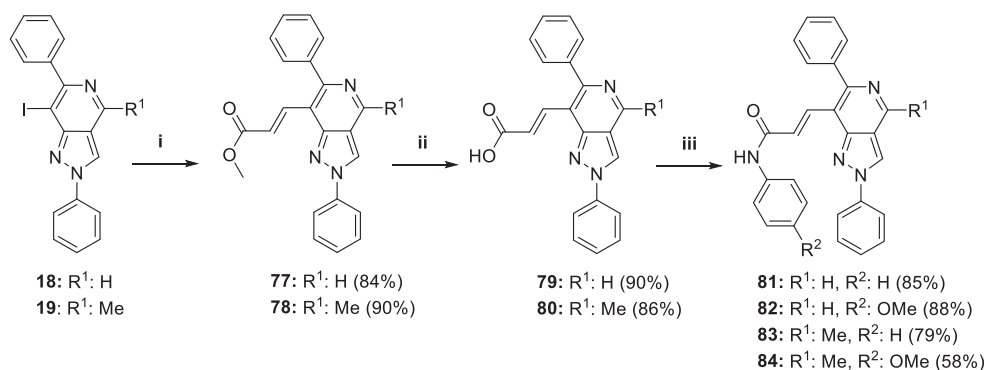


Figure 2.16. Relevant ^1H - ^{13}C HMBC, ^1H - ^{13}C H2BC, ^1H - ^{15}N HMBC and ^1H - ^1H NOESY correlations and ^1H NMR (italics), ^{13}C NMR and ^{15}N NMR (bold) chemical shifts of compound **72**

2.4.2. Synthesis of (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)-*N*-phenylprop-2-enamides

In order to further functionalize the pyrazolopyridine core, Heck coupling was performed by using methyl acrylate (Scheme 2.10). The same conditions as with styrenes were employed, however, the reaction was completed in a shorter timeframe, and the yields of the coupling products were better: 84% for methyl (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)prop-2-enoate (**77**) and 90% for methyl (2*E*)-3-(4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)prop-2-enoate (**78**). Then, esters were hydrolyzed by using NaOH in refluxing MeOH. The reactions finished in 3 h, and the conversion could be sighted by the change of the solution clarity as the esters were poorly soluble in MeOH, while acids dissolved completely. The products could be directly used in the next step after acidic work up without the need for purification on silica gel. Since amide is a key functional group in organic chemistry for its widespread occurrence in peptide and non-peptide natural products and therapeutic small molecules [130, 164, 165], we decided to

perform the condensation of acrylic acids **79** and **80** with either aniline or 4-methoxyaniline. To form the amide bond, ethyl-(*N,N'*-dimethylamino)propylcarbodiimide hydrochloride (EDC·HCl) was chosen as the carboxyl activating agent. Both carbodiimide and its urea byproduct are water soluble; therefore, the byproduct and any excess reagent can be removed by aqueous extraction. Thus, EDC·HCl was used together with DMAP acting as the catalyst. The mixture was stirred in room temperature DCM for 20 h. It is noteworthy that 4-unsubstituted pyrazolo[4,3-*c*]pyridinyl-*N*-phenylacrylamides **81**, **82** were obtained in better yields than their 4-methyl pyrazolo[4,3-*c*]pyridinyl-*N*-phenylacrylamides **83**, **84**.



Scheme 2.10. Synthesis of compounds (**81–84**). *Reagents and conditions:* i: methyl acrylate, TEA, Pd(PPh₃)Cl₂, DMF, MW, 120 °C, 100 W, 30 min; ii: NaOH, MeOH, 65 °C, 3 h; iii: aniline (**81** and **83**) or 4-methoxyaniline (**82** and **84**), DMAP, EDC·HCl, DCM, rt, 20 h

The structures of the new compounds **77–84** were confirmed by NMR and IR spectroscopy, as well as MS spectrometry. The replacement of iodine with methyl acrylate can be quickly proven by IR spectroscopy. In the case of 4-unsubstituted derivatives, the intense absorption band of the ester carbonyl group is observed at 1670 cm⁻¹ which, after hydrolysis, is no longer observed as the absorption band of the acid carbonyl group and the new hydroxy group absorption band are observed at 1709 cm⁻¹ and 3400 cm⁻¹, respectively. In comparison to the starting materials **18** and **19**, the ¹H NMR spectra of the new compounds display new doublets of ethenylic protons, while acrylamides **81–84** also exhibit new aromatic multiplets and NH singlets. The chemical shifts of ethenylic protons next to pyridine H_B are observed downfield (7.74–8.01 ppm) in comparison to those of protons next to carbonyl group H_A (7.65–7.82 ppm) in acrylate and acrylic acid derivatives **77–80**. The ethenylic proton signals give an AB pattern (Fig. 2.17), but the proton doublets can appear within multiplets of other aromatic protons. It is of note that the ethenylic protons of acrylamides **81–84** exhibit different patterns: in the case of 4-unsubstituted pyrazolo[4,3-*c*]pyridinyl-*N*-phenylacrylamides **81**, **82**, the chemical shifts are the same as outlined above (H_B are downfield in comparison to H_A); meanwhile, the chemical shifts of 4-methylpyrazolo[4,3-*c*]pyridinyl-*N*-

phenylacrylamides **83**, **84** switches and olefinic proton H_B are upfield in comparison to proton H_A . As outlined above, the ethenylic proton signals give an AB pattern, and proton doublets are mostly separated from other aromatic signals.

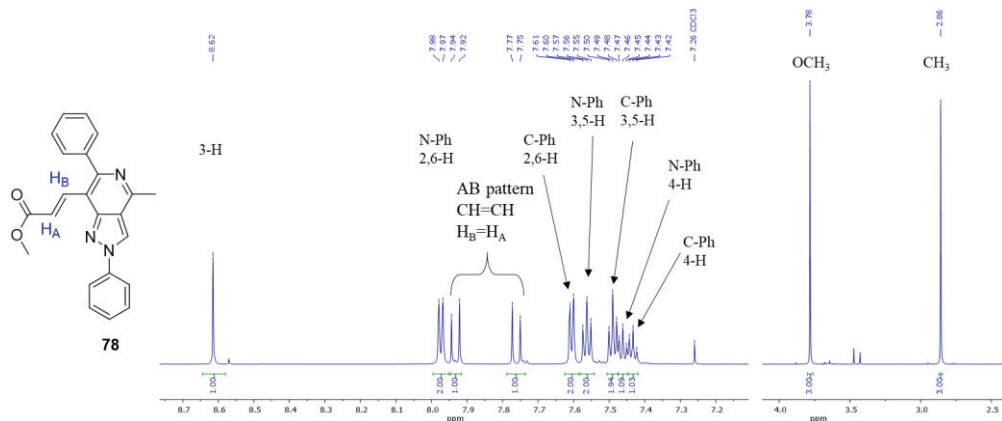


Figure 2.17. ^1H NMR spectrum of compound **78**

Detailed NMR spectroscopic investigations employing a combination of various NMR-techniques (^1H -NMR, ^{13}C -NMR, COSY, NOESY, HSQC, HMBC) permitted full and unambiguous assignment of all ^1H - and ^{13}C -NMR resonances. For example, the distinction between ethenylic protons was made by using HMBC spectrum as explained above. The chemical shifts of ethenylic group carbons also exhibited the same pattern as protons: C_B peaks are found downfield (134.7–139.75 ppm), while C_A is located upfield (122.8–127.3 ppm), i.e., differently from the case of the phenylethenyl derivatives described above.

2.4.3. Optical properties of 2,6-diphenyl-7-[(*E*)-2-phenylethenyl]-2*H*-pyrazolo[4,3-*c*]pyridines and (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)-*N*-phenylprop-2-enamide

The photophysical properties of 7-phenylethenyl-2*H*-pyrazolo[4,3-*c*]pyridines **68**–**76** were investigated in THF and 0.5% aqueous solution of DMSO, with the excitation wavelength λ_{ex} being set to 360 nm and 380 nm, accordingly (Table 2.12). The emission maxima λ_{em} of all the compounds in THF were located within the 458–485 nm range, while a bathochromic shift was observed in aqueous solution with λ_{em} being in the 491–524 nm range.

Moreover, 7-phenylethenyl-2*H*-pyrazolo[4,3-*c*]pyridine **69** with the electron donating methoxy group exhibited the longest bathochromic shift and the highest quantum yield. Notably, methyl benzoate **71** and methyl derivative **72** possess a similar shorter Stokes shift and lower quantum yields despite their different electron effect. Other compounds with electron withdrawing groups have shorter red shifts and lower quantum yields, with 4-bromophenylethenyl substituent **75** possessing the lowest quantum yield.

Table 2.12. Fundamental absorption (UV-vis spectra of 10^{-4} mol solutions) and fluorescence (spectra of 10^{-5} mol solutions) characteristics compounds **68–76** in THF ($\lambda_{\text{ex}} = 360$ nm) (white) and 0.5% DMSO aqueous solution ($\lambda_{\text{ex}} = 380$ nm) (grey)

Compound	λ_{abs} (nm)	$\varepsilon \times 10^3$ ($\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$)	λ_{em} (nm)	SS (nm)	Φ_f (%)
68	367	12.69	459	92	36.4
	273	29.06			
	208	35.36			
	386	11.38	494	108	
	289	14.36			
	218	23.03			
69	380	16.12	485	105	55.37
	278	34.18			
	207	43.77			
	396	20.24	524	128	
	278	31.95			
	219	41.58			
70	371	17.49	474	103	39.53
	267	31.05			
	207	35.38			
	387	25.76	496	109	
	278	38.25			
	219	52.96			
71	374	26.46	460	86	44.36
	270	39.19			
	207	53.72			
	insoluble		499		
72	370	18.87	470	100	41.24
	275	41.99			
	207	53.21			
	391	22.15	497	106	
	289	31.28			
	219	43			
73	366	17.95	459	93	38.1
	320	17.33			
	270	33.93			
	207	41.92			
	376	22.46	491	115	
	280	35.28			
	218	44.20			
74	365	11.87	458	93	36.94
	272	28.17			
	208	33.92			
	392	20.98	502	110	
	285	26.89			
	226	23.94			

Continued Table 2.12

Compound	λ_{abs} (nm)	$\varepsilon \times 10^3$ ($\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$)	λ_{em} (nm)	SS (nm)	Φ_f (%)
75	367	19.67	462	95	25.83
	272	40.23			
	207	53.74			
	380	26.72	497	117	
	282	42.89			
	219	44.2			
76	367	21.64	462	95	37.69
	272	44.53			
	207	57.55			
	380	17.43	494	114	
	282	30.37			
	219	35.17			

It should be noted that no fluorescence was observed when the amide bond was incorporated into 7-position of 2*H*-pyrazolo[4,3-*c*]pyridine. The electronic spectra of (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)-*N*-phenylprop-2-enamides **81–84** (Table 2.13) possessed a near ultraviolet band with an absorption maximum at range of 326–363 nm in all cases. The methyl substituents attached at 4-positions of the heterocyclic ring system and the methoxy substituent attached at *N*-phenyl led to a slight bathochromic shift of the absorption bands.

Table 2.13. Fundamental absorption characteristics of compounds **81–84** in THF (10^{-4} mol solutions)

Compound	λ_{abs} (nm)	$\varepsilon \times 10^3$ ($\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$)
81	326	17.77
	261	34.51
	206	43.30
82	360	19.69
	326	20.30
	263	42.24
83	357	18.78
	262	35.78
	207	47.15
84	363	15.16
	264	29.8
	207	36.49

2.4.4. Biological investigation of 2,6-diphenyl-7-[(*E*)-2-phenylethenyl]-2*H*-pyrazolo[4,3-*c*]pyridine and (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)-*N*-phenylprop-2-enamide

All biological investigations discussed in this chapter were performed by Dr. Eva Řezníčková and Mgr. Veronika Vojáčková representing Prof. Vladimír Kryštof's research group at the Department of Experimental Biology, Faculty of Science, Palacký University in the Czech Republic. 2,6-diphenyl-7-[(*E*)-2-phenylethenyl]-2*H*-pyrazolo[4,3-*c*]pyridines **68–76** and (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)-*N*-phenylprop-2-enamides **81–84** were assessed for their photodynamic potency. G361 cells were treated with compounds for 4 h and then exposed to the blue light (LED source, 414 nm) which was selected prior based on the optical properties of the compounds, at a total irradiation dose of 10 J/cm². The viability of the cells was quantified at 72 h after irradiation by an MTT assay, which revealed that styryl derivatives **68–76** possess excellent photodynamic properties with IC₅₀ as low as 0.043 μM (Table 2.14). Compounds **69**, **72** and **76** were found to be the most potent photosensitizer with similar IC₅₀; however only compound **76** exhibited no dark toxicity up to the tested concentration (10 μM), thus being most suitable for PDT. Most importantly, 2,6-diphenyl-7-[(*E*)-2-phenylethenyl]-2*H*-pyrazolo[4,3-*c*]pyridines **68–76** show up to 25-fold superior cytotoxicity in comparison to *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines **52–67**.

Moreover, methyl (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)prop-2-enoate (**77**) and (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)prop-2-enoic acid (**79**) did not possess any photodynamical potency; however, compound **77** exhibited moderate dark cytotoxicity. Generally, 2*H*-pyrazolo[4,3-*c*]pyridine-*N*-phenylprop-2-enamides **81–84** display moderate or no cytotoxicity both in the dark and after light irradiation.

Table 2.14. IC₅₀ values of compounds (**68–84**) kept in dark and irradiated with light (414 nm, 10 J/cm²) G361 cells

Compound	G361 - IC ₅₀ , μM	
	dark	10 J/cm ²
68	8.125	0.14995
69	6.04	0.047
70	>10	0.205
71	>10	0.346
72	9.15	0.043
73	>10	0.1515
74	>10	0.1775
75	>10	0.158
76	>10	0.0465
77	3.325	>10
79	>10	>10

Continued Table 2.14

Compound	G361 – IC ₅₀ , μM	
	dark	10 J/cm ²
81	>10	9.11
82	>10	>10
83	7.64	9.36
84	>10	>10

Further experiments are ongoing to evaluate the mechanism of action causing cell death.

3. METHODS AND MATERIALS

3.1 General methods

All chemicals and solvents were purchased from commercial suppliers and used without further purification unless otherwise specified. The ^1H , ^{13}C and ^{15}N NMR spectra were recorded in CDCl_3 or $\text{DMSO}-d_6$ solutions at 25 °C on either a *Bruker Avance III 700* (700 MHz for ^1H , 176 MHz for ^{13}C , 71 MHz for ^{15}N) spectrometer equipped with a 5 mm TCI ^1H - $^{13}\text{C}/^{15}\text{N}/\text{D}$ z-gradient cryoprobe or on *Jeol ECA-500* (500 MHz for ^1H , 126 MHz for ^{13}C) spectrometer equipped with a 5 mm *Royal* probe. The chemical shifts, expressed in ppm, were relative to tetramethylsilane (TMS). The ^{15}N NMR spectra were referenced to neat, external nitromethane (coaxial capillary). The FT-IR spectra were collected by using the ATR method on a *Bruker Vertex 70v* spectrometer with an integrated *Platinum ATR* accessory or on a *Bruker Tensor 27* spectrometer in KBr pellets. The melting points of crystalline compounds were determined in open capillary tubes with a *Buchi M – 565* apparatus (temperature gradient – 2 °C/min) and are uncorrected. The mass spectra were recorded on a *Q-TOF MICRO* spectrometer (*Waters*), analyses were performed in the positive (ESI^+) mode, and molecular ions were recorded in $[\text{M}+\text{H}]^+$ forms. The high-resolution mass spectrometry (HRMS) spectra were obtained in the ESI mode on a *Bruker MicrOTOF-Q III* spectrometer. All reactions were performed in oven-dried flasks under argon atmosphere with magnetic stirring. The reaction progress was monitored by TLC analysis on *Macherey-Nagel*TM *ALUGRAM® Xtra SIL G/UV254* plates. TLC plates were visualized with UV light (wavelengths 254 and 365 nm) or iodine vapor. The UV-vis spectra of 10^{-4} mol solutions were recorded on a *Perkin Elmer Lambda 35* UV-vis spectrometer. The fluorescence spectra of 10^{-5} mol solutions were recorded on an FL920 fluorescence spectrometer designed by *Edinburgh Instruments*. The PL quantum yields were measured from dilute THF solutions by the absolute method while using the *Edinburgh Instruments* integrating sphere excited with a Xe lamp. The optical densities of the sample solutions were ensured to be below 0.1 to avoid reabsorption effects. All optical measurements were performed at room temperature under ambient conditions. The compounds were purified by flash chromatography in a glass column (stationary phase – silica gel, high-purity grade 9385, pore size 60 Å, particle size 230–400 mesh, supplier *Sigma-Aldrich*).

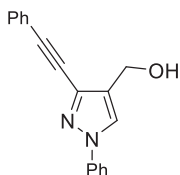
3.2. Synthesis

General procedure (A) for the synthesis of alcohols 8 and 23

Appropriate 1-phenyl-3-(substitutedethynyl)-1*H*-pyrazole-4-carbaldehyde **6** or **7** (1 equiv) was dissolved in MeOH, and the solution was cooled down to 0 °C. Subsequently, NaBH_4 (2 equiv) was added under argon atmosphere, and the mixture was stirred for 30 min. Upon completion (monitored by TLC), the reaction mixture

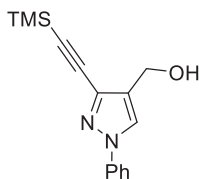
was diluted with saturated aqueous NH_4Cl solution (20 mL) and extracted with EtOAc (3×30 mL). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered and evaporated under reduced pressure. The residue was purified by column chromatography (EtOAc/Hex, 1:3 v/v).

[1-Phenyl-3-(phenylethynyl)-1*H*-pyrazol-4-yl]methanol (**8**)



Prepared in accordance to general procedure (A) from 1-phenyl-3-(2-phenylethynyl)-1*H*-pyrazole-4-carbaldehyde (**6**) (560 mg, 2.06 mmol), NaBH_4 (156 mg, 4.12 mmol) in MeOH (12 mL). Yield: 530 mg (94%), white crystals, mp = 114–115 °C, R_f = 0.18 (EtOAc/Hex, 1:3 v/v). ^1H NMR (700 MHz, CDCl_3): δ 2.12 (1H, s, OH), 4.77 (2H, d, J = 7.0 Hz, CH_2OH), 7.28–7.32 (1H, m, N-Ph 4-H), 7.33–7.38 (3H, m, C-Ph 3,4,5), 7.42–7.46 (2H, m, N-Ph 3,5-H), 7.55–7.59 (2H, m, C-Ph 2,6-H), 7.66–7.72 (2H, m, N-Ph 2,6-H), 7.95 (1H, s, 5-H). ^{13}C NMR (176 MHz, CDCl_3): δ 55.9 (CH_2OH), 80.3 ($\text{C}\equiv\text{CPh}$), 93.7 ($\text{C}\equiv\text{CPh}$), 119.4 (N-Ph C-2,6), 122.5 (C-4), 126.4 (C-5 and C-Ph C-1), 127.1 (N-Ph C-4), 128.5 (C-Ph C-3,5), 128.9 (C-Ph C-4), 129.6 (N-Ph C-3,5), 131.9 (C-Ph C-2,6), 135.6 (C-3), 139.7 (N-Ph C-1). ^{15}N NMR (71 MHz, CDCl_3): δ -163.5 (N-1), N-2 not found. IR (v, cm^{-1}): 3373 (OH), 3126, 3066, 3056 (CH_{arom}), 2920, 2864 (CH_{aliph}), 1599, 1502, 1335, 1217 (C=C, C=N, C-N), 1063, 1014 ($\text{CH}_2\text{-OH}$), 749, 686 (CH=CH of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 275 ($[\text{M}+\text{H}]^+$, 100). HRMS (ESI) for $\text{C}_{18}\text{H}_{14}\text{N}_2\text{ONa}$ ($[\text{M}+\text{Na}]^+$): calcd 297.0998, found 297.0988.

{1-Phenyl-3-[(trimethylsilyl)ethynyl]-1*H*-pyrazol-4-yl}methanol (**23**)

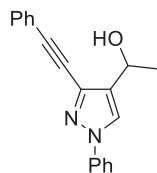


Prepared in accordance to general procedure (A) from 1-phenyl-3-[(trimethylsilyl)ethynyl]-1*H*-pyrazole-4-carbaldehyde (**7**) (400 mg, 1.49 mmol), NaBH_4 (113 mg, 2.98 mmol) in MeOH (12 mL). Yield: 354 mg (88%), a yellowish liquid, R_f = 0.21 (EtOAc/Hex, 1:4 v/v). ^1H NMR (700 MHz, CDCl_3): δ 0.27 (9H, s, TMS), 4.70 (2H, s, CH_2OH), 7.26–7.32 (1H, m, N-Ph 4-H), 7.40–7.44 (2H, m, N-Ph 3,5), 7.62–7.69 (2H, m, N-Ph 2,6-H), 7.90 (1H, s, 5-H). ^{13}C NMR (176 MHz, CDCl_3): δ -0.1 (TMS), 55.9 (CH_2OH), 95.6 ($\text{C}\equiv\text{C-TMS}$), 99.9 ($\text{C}\equiv\text{C-TMS}$), 119.4 (N-Ph C-2,6), 126.1 (C-5), 126.7 (C-4), 127.1 (N-Ph C-4), 129.6 (N-Ph C-3,5), 135.3 (C-3), 139.6 (N-Ph C-1). ^{15}N NMR (71 MHz, CDCl_3): δ -163.7 (N-1), N-2 not found. MS (ES^+): m/z (%): 271 ($[\text{M}+\text{H}]^+$, 100).

General procedure (B) for the synthesis of alcohols 9–12

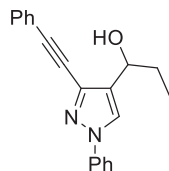
1-Phenyl-3-(2-phenylethynyl)-1*H*-pyrazole-4-carbaldehyde (**6**) (1 equiv) was dissolved in dry THF under argon atmosphere. Subsequently, the appropriate Grignard reagent (1.2 equiv) was added, and the mixture was stirred at room temperature for 10 min. Upon completion (monitored by TLC), the reaction mixture was diluted with water (20 mL) and extracted with EtOAc (3×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered and evaporated under reduced pressure. The residue was purified by column chromatography.

1-[1-Phenyl-3-(phenylethynyl)-1*H*-pyrazol-4-yl]ethanol-1-ol (9)



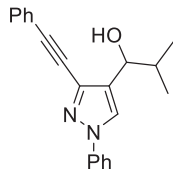
Prepared in accordance to general procedure (B) from 1-phenyl-3-(2-phenylethynyl)-1*H*-pyrazole-4-carbaldehyde (**6**) (350 mg, 1.287 mmol) and methylMgCl (0.52 mL, 1.56 mmol) in THF (4 mL). The desired compound was purified by column chromatography (EtOAc/Hex, 1:4 v/v). Yield: 286 mg (70%), a colorless liquid, R_f = 0.23 (EtOAc/Hex, 1:5 v/v). ^1H NMR (700 MHz, CDCl_3): δ 1.64 (3H, d, J = 6.5 Hz, CH_3), 2.18 (1H, s, OH), 5.13 (1H, q, J = 6.5 Hz, CH), 7.29–7.1 (1H, m, N-Ph 4-H), 7.34–7.38 (3H, m, C-Ph 3,4,5-H), 7.43–7.47 (2H, m, N-Ph 3,5-H), 7.55–7.59 (2H, m, C-Ph 2,6-H), 7.67–7.72 (2H, m, N-Ph, 2,6-H). ^{13}C NMR (176 MHz, CDCl_3): δ 23.8 (CH_3), 62.7 (CH) 80.6 ($\text{C}\equiv\text{CPh}$), 93.8 ($\text{C}\equiv\text{CPh}$), 119.1 (N-Ph C-2,6), 122.4 (C-Ph C-1), 124.4 (C-5), 126.9 (N-Ph C-4), 128.4 (C-Ph C-3,5), 128.7 (C-Ph C-4), 129.4 (N-Ph C-3,5), 131.5 (C-1), 134.2 (C-2), 139.6 (N-Ph C-1). ^{15}N NMR (71 MHz, CDCl_3): δ -75.4 (N-2), -164.4 (N-1). MS (ES^+): m/z (%): 275 ($[\text{M}+\text{H}]^+$, 96).

1-[1-Phenyl-3-(phenylethynyl)-1*H*-pyrazol-4-yl]propan-1-ol (10)



Prepared in accordance to general procedure (B) from 1-phenyl-3-(2-phenylethynyl)-1*H*-pyrazole-4-carbaldehyde (**2**) (100 mg, 0.37 mmol), ethylMgBr (0.15 mL, 0.44 mmol) in THF (2 mL). The desired compound was purified by column chromatography (EtOAc/Hex, 1:3 v/v). Yield: 100 mg (90%), yellowish crystals, mp = 87–88 °C, R_f = 0.28 (EtOAc/Hex, 1:3 v/v). ^1H NMR (700 MHz, CDCl_3): δ 1.03 (3H, t, J = 7.7 Hz, CH_3), 1.93–1.99 (2H, m, CH_2), 4.87 (1H, t, J = 6.5 Hz, CH), 5.29 (1H, s, OH), 7.29–7.32 (1H, m, N-Ph 4-H), 7.34–7.38 (3H, m, C-Ph 3,4,5-H), 7.43–7.46 (2H, m, N-Ph 3,5-H), 7.55–7.59 (2H, m, C-Ph 2,6-H), 7.68–7.72 (2H, m, N-Ph 2,6-H), 7.91 (1H, s, 5-H). ^{13}C NMR (176 MHz, CDCl_3): δ 10.0 (CH_3), 30.8 (CH_2), 68.1 (CHOH), 80.7 ($\text{C}\equiv\text{CPh}$), 93.6 ($\text{C}\equiv\text{CPh}$), 119.1 (N-Ph C-2,6), 122.5 (C-Ph C-4), 124.8 (C-5), 126.9 (N-Ph C-4), 128.4 (C-Ph C-3,5), 128.7 (C-Ph C-4), 129.4 (N-Ph C-3,5), 130.2 (C-4), 131.6 (C-Ph C-2,6), 134.5 (C-3), 139.6 (N-Ph C-1). ^{15}N NMR (71 MHz, CDCl_3): δ -75.8 (N-2), -164.1 (N-1). IR (KBr, ν , cm^{-1}): 3441 (OH), 3056 (CH_{arom}), 2960, 2874 (CH_{aliph}), 1596, 1502, 1335, 1212 (C=C, C=N, C-N), 1063, 109 ($\text{CH}_2\text{-OH}$), 755, 691 ($\text{CH}=\text{CH}$ of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 303 ($[\text{M}+\text{H}]^+$, 98). HRMS (ESI) for $\text{C}_{20}\text{H}_{18}\text{N}_2\text{ONa}$ ($[\text{M}+\text{Na}]^+$): calcd 325.1311, found 325.1311.

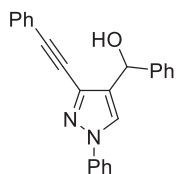
2-Methyl-1-[1-phenyl-3-(phenylethynyl)-1*H*-pyrazol-4-yl]propan-1-ol (11)



Prepared in accordance to general procedure (B) from 1-phenyl-3-(2-phenylethynyl)-1*H*-pyrazole-4-carbaldehyde (**6**) (350 mg, 1.287 mmol) and isopropylMgCl (0.97 mL, 1.93 mmol) in THF (6 mL). The desired compound was purified by column chromatography (EtOAc/Hex, 1:4 v/v). Yield: 286 mg (70%),

yellow crystals, mp = 78–79 °C, R_f = 0.23 (EtOAc/Hex, 1:5 v/v). ^1H NMR (700 MHz, CDCl_3): δ 0.98 (3H, d, J = 6.8 Hz, CH_3), 1.05 (3H, d, J = 6.8 Hz, CH_3), 2.12 (1H, s, OH), 2.15–2.21 (1H, m, $\text{CH}-(\text{CH}_3)_2$), 4.70 (1H, d, J = 6.3 Hz, CH-OH), 7.26–7.32 (1H, m, N-Ph, 4-H), 7.34–7.39 (3H, m, C-Ph 3,4,5-H), 7.43–7.47 (2H, m, N-Ph 3,4-H), 7.55–7.59 (2H, m, C-Ph 2,6-H), 7.67–7.76 (2H, m, N-Ph 2,6-H), 7.91 (1H, s, 5-H). ^{13}C NMR (176 MHz, CDCl_3): δ 18.1 (CH_3), 18.8 (CH_3), 34.9 ($\text{CH}-(\text{CH}_3)_2$), 72.2 (CH-OH), 81.1 ($\text{C}\equiv\text{CPh}$), 93.7 ($\text{C}\equiv\text{CPh}$), 119.2 (N-Ph C-2,6), 122.7 (C-Ph C-1), 125.3 (C-5), 127.0 (N-Ph C-4), 128.5 (C-Ph C-3,5), 128.8 (C-Ph C-4), 129.4 (C-4), 129.6 (N-Ph C-3,5), 131.8 (C-Ph C-2,6), 134.9 (C-3), 139.7 (N-Ph C-1). ^{15}N NMR (71 MHz, CDCl_3): δ -163.8 (N-1), N-2 not found. IR (v, cm^{-1}): 3383 (OH), 3054 (CH_{arom}), 2959, 2872 (CH_{aliph}), 1596, 1501, 1458, 1376, 1331, 1219 ($\text{C}=\text{C}$, $\text{C}=\text{N}$, $\text{C}-\text{N}$), 1056, 1033 (CH-OH), 963, 752, 688 ($\text{CH}=\text{CH}$ of monosubstituted benzenes). MS (ES^+): m/z (%): 317 ($[\text{M}+\text{H}]^+$, 99). HRMS (ESI) for $\text{C}_{18}\text{H}_{14}\text{N}_2\text{ONa}$ ($[\text{M}+\text{Na}]^+$): calcd 339.1468, found 339.1467.

Phenyl[1-phenyl-3-(phenylethynyl)-1*H*-pyrazol-4-yl]methanol (12)

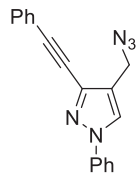


Prepared in accordance to general procedure (B) from 1-phenyl-3-(2-phenylethynyl)-1*H*-pyrazole-4-carbaldehyde (**6**) (100 mg, 0.37 mmol) and phenylMgBr (0.15 mL, 0.44 mmol) in DCM (2 mL). The desired compound was purified by column chromatography (EtOAc/Hex, 1:7 v/v). Yield: 105 mg (81%), a colorless liquid, R_f = 0.35 (EtOAc/Hex, 1:3 v/v). ^1H NMR (700 MHz, CDCl_3): δ 6.06 (1H, s, CH), 7.27–7.31 (1H, m, N-Ph 4-H), 7.31–7.33 (1H, m, C4-Ph 4-H), 7.33–7.36 (3H, m, C3-Ph 3,4,5-H), 7.37–7.40 (2H, m, C4-Ph 3,5-H), 7.41–7.45 (2H, m, N-Ph 3,5-H), 7.48–7.51 (2H, m, C3-Ph 2,6-H), 7.52–7.54 (2H, m, C4-Ph 2,6-H), 7.65–7.70 (2H, m, N-Ph 2,6-H), 7.80 (1H, s, 5-H). ^{13}C NMR (176 MHz, CDCl_3): δ 68.8 (CH), 80.5 ($\text{C}\equiv\text{CPh}$), 94.0 ($\text{C}\equiv\text{CPh}$), 119.2 (N-Ph C-2,6), 122.4 (C3-Ph C-1), 125.5 (C-5), 126.4 (C4-Ph C-2,6), 126.9 (N-Ph C-4), 127.9 (C4-Ph C-1), 128.3 C3-Ph C-3,5), 128.5 (C4-Ph C-3,5), 128.7 (C3-Ph C-4), 129.4 (N-Ph C-3,5), 130.2 (C-4), 131.7 (C3-Ph C-2,6), 134.8 (C-3), 139.5 (N-Ph C-1), 142.6 (C4-Ph C-1). ^{15}N NMR (71 MHz, CDCl_3): δ -163.9 (N-1). MS (ES^+): m/z (%): 351 ($[\text{M}+\text{H}]^+$, 95). HRMS (ESI) for $\text{C}_{24}\text{H}_{18}\text{N}_2\text{ONa}$ ($[\text{M}+\text{Na}]^+$): calcd 373.1311, found 373.1311.

General procedure (C) for the synthesis of azide-alkynes **13–17**, **24**, **26**

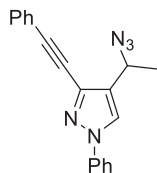
Suitable pyrazole alcohol **8–12** or **23** (1 equiv) was dissolved in dry DCM. Subsequently, TMSN_3 (1.5 equiv) and $\text{BF}_3\cdot\text{Et}_2\text{O}$ (0.2 equiv) were added dropwise. The reaction was stirred for 10 to 60 min under argon atmosphere at room temperature. Upon completion (monitored by TLC), the reaction mixture was diluted with aqueous NaHCO_3 solution (10 mL) and extracted with DCM (3 $\times$ 25 mL). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered and evaporated under reduced pressure. The residue was purified by column chromatography.

4-(Azidomethyl)-1-phenyl-3-(phenylethynyl)-1H-pyrazole (13)



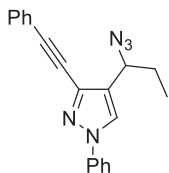
Prepared in accordance to general procedure (C) from [1-phenyl-3-(2-phenylethynyl)-1H-pyrazol-4-yl]methanol (**8**) (100 mg, 0.36 mmol), TMSN₃ (0.07 mL, 0.55 mmol) and BF₃·Et₂O (0.01 mL, 0.07 mmol) in DCM (1.5 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:8 v/v). Yield: 54 mg (50%), a light yellow liquid, *R*_f = 0.71 (EtOAc/Hex, 1:3 v/v). ¹H NMR (700 MHz, CDCl₃): δ 4.44 (2H, s, CH₂N₃), 7.31–7.35 (1H, m, N-Ph 4-H), 7.35–7.40 (3H, m, C-Ph 3,4,5-H), 7.45–7.49 (2H, m, N-Ph 3,5-H), 7.58–7.63 (2H, m, C-Ph 2,6-H), 7.69–7.74 (2H, m, N-Ph 2,6-H), 7.96 (1H, s, 5-H). ¹³C NMR (176 MHz, CDCl₃): δ 44.8 (CH₂N₃), 79.9 (C≡CPh), 94.1 (C≡CPh), 119.5 (N-Ph C-2,6), 120.9 (C-4), 122.4 (C-Ph C-1), 126.7 (C-5), 127.4 (N-Ph C-4), 128.5 (C-Ph C-3,5), 129.0 (C-Ph C-4), 129.7 (N-Ph C-3,5), 132.0 (C-Ph C-2,6), 136.5 (C-3), 139.6 (N-Ph C-1). ¹⁵N NMR (71 MHz, CDCl₃): δ -162.2 (N-1), -306.6 and -132.9 (N₃, third not found), N-2 not found. IR (ν, cm⁻¹): 3050 (CH_{arom}), 2921 (CH_{aliph}), 2087 (N₃), 1595, 1501, 1331, 1250 (C=C, C=N, C-N), 753, 688 (CH=CH of monosubstituted benzenes). MS (ES⁺): *m/z* (%): 300 ([M+H]⁺, 99). HRMS (ESI) for C₁₈H₁₄N₅ ([M+H]⁺): calcd 300.1242, found 300.1244; for C₁₈H₁₃N₅Na ([M+Na]⁺): calcd 322.1065, found 322.1063.

4-(1-Azidoethyl)-1-phenyl-3-(phenylethynyl)-1H-pyrazole (14)



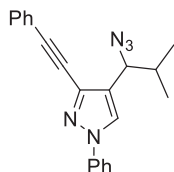
Prepared in accordance to general procedure (C) from 1-[1-phenyl-3-(phenylethynyl)-1H-pyrazol-4-yl]ethan-1-ol (**9**) (205 mg, 0.71 mmol), TMSN₃ (0.14 mL, 1.07 mmol) and BF₃·Et₂O (0.02 mL, 0.14 mmol) in DCM (2 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:4 v/v). Yield: 160 mg (72%), a colorless oil, *R*_f = 0.72 (EtOAc/Hex, 1:3 v/v). ¹H NMR (700 MHz, CDCl₃): δ 1.70 (3H, d, *J* = 7.0 Hz, CH₃), 4.87 (1H, q, *J* = 7.0 Hz, CHN₃), 7.34–7.36 (1H, m, N-Ph 4-H), 7.39–7.40 (3H, m, C-Ph 3,4,5-H), 7.48–7.51 (2H, m, N-Ph 3,5-H), 7.62–7.63 (2H, m, C-Ph 2,6-H), 7.74–7.75 (2H, m, N-Ph 2,6-H), 7.94 (1H, s, 5-H). ¹³C NMR (176 MHz, CDCl₃): δ 20.7 (CH₃), 52.7 (CHN₃), 80.2 (C≡CPh), 94.0 (C≡CPh), 119.3 (N-Ph C-2,6), 122.4 (C-Ph C-1), 124.9 (C-5), 126.7 (C-4), 127.2 (N-Ph C-4), 128.4 (C-Ph C-3,5), 128.9 (C-Ph C-4), 129.5 (N-Ph C-3,5), 131.8 (C-Ph C-2,6), 135.1 (C-3), 139.5 (N-Ph C-1). ¹⁵N NMR (71 MHz, CDCl₃): δ -294.3 (N₃), -163.3 (N-1), -133.9 (N₃), -73.6 (N-2). IR (KBr, ν, cm⁻¹): 3146 (C≡CH), 3055 (CH_{arom}), 2985, 2936 (CH_{aliph}), 2102 (N₃), 1597, 1549, 1502, 1216 (C=C, C-N), 820, 756, 688 (CH=CH of monosubstituted benzenes). MS (ES⁺): *m/z* (%): 314 ([M+H]⁺, 100). HRMS (ESI) for C₁₉H₁₆N₅ ([M+H]⁺): calcd 314.1400, found 314.1395.

4-(1-Azidopropyl)-1-phenyl-3-(phenylethynyl)-1*H*-pyrazole (15)



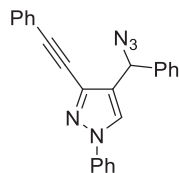
Prepared in accordance to general procedure (C) from 1-[1-phenyl-3-(phenylethynyl)-1*H*-pyrazol-4-yl]propan-1-ol (**10**) (100 mg, 0.33 mmol), TMSN₃ (0.07 mL, 0.5 mmol) and BF₃·Et₂O (0.01 mL, 0.07 mmol) in DCM (1 mL). The desired compound was obtained after purification by column chromatography (EtOAc:Hex, 1:12 v/v). Yield: 81 mg (75%), yellowish crystals, mp = 74–75 °C, *R*_f = 0.73 (EtOAc/Hex, 1:3 v/v). ¹H NMR (700 MHz, CDCl₃): δ 1.06 (3H, t, *J* = 7.3 Hz, CH₃), 1.99 (2H, p, *J* = 7.2 Hz, CH₂), 4.63 (1H, t, *J* = 7.0 Hz, CH-N₃), 7.31–7.35 (1H, m, N-Ph 4-H), 7.36–7.40 (3H, m, C-Ph 3,4,5-H), 7.44–7.50 (2H, m, N-Ph 3,5-H), 7.57–7.63 (2H, m, C-Ph 2,6-H), 7.69–7.77 (2H, m, N-Ph 2,6-H), 7.91 (1H, s, 5-H). ¹³C NMR (176 MHz, CDCl₃): δ 10.7 (CH₃), 28.4 (CH₂), 58.9 (CH), 80.2 (C≡CPh), 93.8 (C≡CPh), 119.2 (N-Ph C-2,6), 122.3 (C-Ph C-1), 125.1 (C-5), 125.4 (C-4), 127.1 (N-Ph C-4), 128.4 (C-Ph C-3,5), 128.8 (C-Ph C-4), 129.5 (N-Ph C-3,5), 131.7 (C-Ph C-2,6), 135.4 (C-3), 139.4 (N-Ph C-1). ¹⁵N NMR (71 MHz, CDCl₃): δ -163.4 (N-1), -134.5 (CH-N=N=N), -116.4 (CH-N=N=N), -74.4 (N-2). IR (KBr, ν, cm⁻¹): 3147 (CH_{arom}), 2967, 2934, 2870 (CH_{aliph}), 2092 (N₃), 1598, 1502, 1328, 1215 (C=C, C=N, C-N), 959, 757, 689 (CH=CH of monosubstituted benzenes). MS (ES⁺): *m/z* (%): 328 ([M+H]⁺, 99). HRMS (ESI) for C₂₀H₁₇N₅Na ([M+Na]⁺): calcd 350.1376, found 350.1376.

4-(Azido-2-methylpropyl)-1-phenyl-3-(phenylethynyl)-1*H*-pyrazole (16)



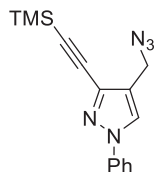
Prepared in accordance to general procedure (C) from 2-methyl-1-[1-phenyl-3-(phenylethynyl)-1*H*-pyrazol-4-yl]propan-1-ol (**11**) (200 mg, 0.63 mmol), TMSN₃ (0.1 mL, 0.76 mmol) and BF₃·Et₂O (0.02 mL, 0.13 mmol) in DCM (2 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:10 v/v). Yield: 177 mg (82%), a colorless oil, *R*_f = 0.68 (EtOAc/Hex, 1:4 v/v). ¹H NMR (700 MHz, CDCl₃): δ 1.00 (3H, d, *J* = 6.8 Hz, CH₃), 1.06 (3H, d, *J* = 6.7 Hz, CH₃), 2.18–2.25 (1H, m, CHCH₃)₂, 4.52 (1H, d, *J* = 7.0 Hz, CH-N₃), 7.31–7.35 (1H, m, N-Ph 4-H), 7.37–7.40 (3H, m, C-Ph 3,4,5-H), 7.45–7.50 (2H, m, N-Ph 3,5-H), 7.56–7.62 (2H, m, C-Ph 2,6-H), 7.71–7.79 (2H, m, N-Ph 2,6-H), 7.91 (1H, s, 5-H). ¹³C NMR (176 MHz, CDCl₃): δ 19.0 (CH₃), 19.4 (CH₃), 33.7 (CH- (CH₃)₂), 64.2 (CH-N₃), 80.5 (C≡CPh), 93.9 (C≡CPh), 119.4 (N-Ph C-2,6), 122.5 (C-Ph C-1), 124.5 (C-4), 125.6 (C-5), 127.3 (N-Ph C-4), 128.5 (C-Ph C-3,5), 129.0 (C-Ph C-4), 129.6 (N-Ph C-3,5), 131.9 (C-Ph C-2,6), 135.9 (C-3), 139.6 (N-Ph C-1). ¹⁵N NMR (71 MHz, CDCl₃): δ -299.2 (N₃), -163.1 (N-1), -134.1 (N₃). IR (ν, cm⁻¹): 3060 (CH_{arom}), 2963, 2873 (CH_{aliph}), 2093 (N₃), 1598, 1502, 1329, 1244 (C=C, C=N, C-N), 753, 687 (CH=CH of monosubstituted benzenes). MS (ES⁺): *m/z* (%): ([M+H]⁺, 99). HRMS (ESI) for C₁₈H₁₄N₅ ([M+Na]⁺): calcd 364.1533, found 364.1533.

4-[Azido(phenyl)methyl]-1-phenyl-3-(phenylethynyl)-1H-pyrazole (17)



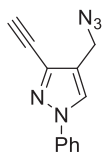
Prepared in accordance to general procedure (C) from phenyl[1-phenyl-3-(phenylethynyl)-1H-pyrazol-4-yl]methanol (**12**) (105 mg, 0.3 mmol), TMSN₃ (0.16 mL, 0.45 mmol) and BF₃·Et₂O (0.01 mL, 0.06 mmol) in DCM (2 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:7 v/v). Yield: 105 mg (93%), white crystals, mp = 86–87 °C, *R*_f = 0.73 (EtOAc/Hex, 1:3 v/v). ¹H NMR (700 MHz, CDCl₃): δ 5.90 (1H, s, CH), 7.30–7.33 (1H, m, N-Ph 4-H), 7.33–7.39 (4H, m, C4-Ph 4-H and C3-Ph 3,4,5-H), 7.40–7.44 (2H, m, C4-Ph 3,5-H), 7.44–7.48 (4H, m, C4-Ph 2,6-H, N-Ph 3,5-H), 7.48–7.52 (2H, m, C3-Ph 2,6-H), 7.68–7.72 (2H, m, N-Ph 2,6-H), 7.82 (1H, s, 5-H). ¹³C NMR (176 MHz, CDCl₃): δ 60.6 (CH), 80.0 (C≡CPh), 94.3 (C≡CPh), 119.2 (N-Ph C-2,6), 122.3 (C3-Ph C-1), 125.97 (C-5), 126.0 (C-4), 127.1 (N-Ph C-4), 127.2 (C4-Ph C-2,6), 128.3 (C3-Ph C-3,5), 128.4 (C4-Ph C-4), 128.78 (C3-Ph C-4), 128.79 (C4-Ph C-3,5), 129.4 (N-Ph C-3,5), 131.7 (C3-Ph C-2,6), 135.4 (C-3), 138.5 (C4-Ph C-1), 139.4 (N-Ph C-1). ¹⁵N NMR (71 MHz, CDCl₃): δ –163.4 (N-1), –134.7 (N₃). IR (KBr, ν, cm^{−1}): 3058 (CH_{arom}), 2097 (N₃), 1597, 1502, 1303, 1227 (C=C, C=N, C–N), 958, 751, 686 (CH=CH of monosubstituted benzenes). MS (ES⁺): *m/z* (%): 376 ([M+H]⁺, 99). HRMS (ESI) for C₂₄H₁₇N₅Na ([M+Na]⁺): calcd 398.1376, found 398.1376.

4-[Azidomethyl]-1-phenyl-3-((trimethylsilyl)ethynyl)-1H-pyrazole (24)



Prepared in accordance to general procedure (C) from phenyl[1-phenyl-3-((trimethylsilyl)ethynyl)-1H-pyrazol-4-yl]methanol (**23**) (100 mg, 0.37 mmol), TMSN₃ (0.075 mL, 0.56 mmol) and BF₃·Et₂O (0.01 mL, 0.06 mmol) in DCM (2 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:8 v/v). Yield: 96 mg (88%), a colorless liquid, *R*_f = 0.67 (EtOAc/Hex, 1:4 v/v). ¹H NMR (700 MHz, CDCl₃): δ 0.28 (9H, s, TMS), 4.37 (2H, s, CH₂N₃), 7.29–7.34 (1H, m, N-Ph 4-H), 7.43–7.47 (2H, m, N-Ph 3,5-H), 7.66–7.70 (2H, m, N-Ph 2,6-H), 7.90 (1H, s, 5-H). ¹³C NMR (176 MHz, CDCl₃): δ –0.1 (TMS), 44.7 (CH), 94.9 (C≡C-TMS), 100.4 (C≡C-TMS), 119.5 (N-Ph C-2,6), 121.3 (C-4), 126.4 (C-5), 127.4 (N-Ph C-4), 129.6 (N-Ph C-3,5), 136.4 (C-3), 139.5 (N-Ph C-1). ¹⁵N NMR (71 MHz, CDCl₃): δ –163.4 (N-1), –306.4, –134.7 (N₃).

4-[Azidomethyl]-1-phenyl-3-(ethynyl)-1H-pyrazole (26)



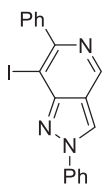
4-[Azidomethyl]-1-phenyl-3-((trimethylsilyl)ethynyl)-1H-pyrazole (**24**) (120 mg, 0.41 mmol) was dissolved in dry MeOH (2 mL). Subsequently, anhydrous K₂CO₃ was added, and the reaction mixture was stirred at rt overnight. Upon completion (monitored by TLC), the solvent was evaporated under reduced pressure, and the residue was purified by

column chromatography (1:15 EtOAc: Hex) to obtain a yellow-to-orange oil. Yield: 84 mg (92%).

General procedure (D) for the synthesis of 7-iodo-2*H*-pyrazolo[4,3-*c*]pyridines 18–22

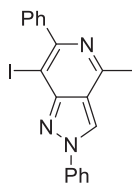
Suitable azide-alkyne **13–17** (1 equiv) was dissolved in DCM. Subsequently, the appropriate base K_3PO_4 (5 equiv) or $NaHCO_3$ (1 equiv) and I_2 (5 equiv) were added to the solution. The reaction mixture was stirred at room temperature for 12 h. Upon completion (monitored by TLC), the reaction mixture was diluted with aqueous $Na_2S_2O_4$ solution (20 mL) and extracted with EtOAc (3×25 mL). The combined organic layers were dried over anhydrous Na_2SO_4 , filtered and evaporated under reduced pressure. The residue was purified by column chromatography.

7-Iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**)



Prepared in accordance to general procedure (D) from 4-(azidomethyl)-1-phenyl-3-(2-phenylethynyl)-1*H*-pyrazole (**13**) (276 mg, 0.92 mmol), K_3PO_4 (978 mg, 4.6 mmol) and I_2 (1.472 g, 4.6 mmol) in DCM (9.8 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:2 v/v). Yield: 299 mg (82%), light yellow crystals, mp = 110–111 °C, R_f = 0.13 (EtOAc/Hex, 1:3 v/v). ^1H NMR (700 MHz, $CDCl_3$): δ 7.42–7.45 (1H, m, C-Ph 4-H), 7.46–7.52 (3H, m, C-Ph 3,5-H and N-Ph 4-H), 7.55–7.59 (2H, m, N-Ph 3,5-H), 7.68–7.74 (2H, m, C-Ph 2,6-H), 7.93–7.99 (2H, m, N-Ph 2,6-H), 8.77 (1H, s, 3-H), 9.13 (1H, s, 4-H). ^{13}C NMR (176 MHz, $CDCl_3$): δ 82.1 (C-7), 118.5 (C-3a), 121.8 (N-Ph, C-2,6), 123.3 (C-3), 128.1 (C-Ph, C-3,5), 128.4 (C-Ph, C-4), 129.3 (N-Ph, C-4), 129.9 (N-Ph, C-3,5), 130.1 (C-Ph, C-2,6), 139.9 (N-Ph, C-1), 142.2 (C-Ph, C-1), 146.1 (C-4), 153.5 (C-7a), 155.7 (C-6). ^{15}N NMR (71 MHz, $CDCl_3$): δ -146.1 (N-2), -90.6 (N-1), -78.4 (N-5). IR (ν , cm^{-1}): 3044, 3035 (CH_{arom}), 1604, 1590, 1505, 1465, 1202 (C=C, C=N, C-N), 741, 700, 679 ($CH=CH$ of monosubstituted benzenes). MS (ES^+): m/z (%): 398 ($[M+H]^+$, 100). HRMS (ESI) for $C_{18}H_{13}N_3I$ ($[M+H]^+$): calcd 398.0149, found 398.0149.

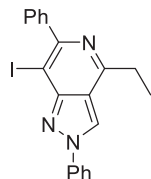
7-Iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**19**)



Prepared in accordance to general procedure (D) from 4-(1-azidoethyl)-1-phenyl-3-(phenylethynyl)-1*H*-pyrazole (**14**) (255 mg, 0.79 mmol), $NaHCO_3$ (69 mg, 0.82 mmol) and I_2 (1034 mg, 4.07 mmol) in DCM (8.1 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:2 v/v). Yield: 238 mg (72%), light yellow crystals, mp = 186–189 °C, R_f = 0.43 (EtOAc/Hex, 1:2 v/v). ^1H NMR (700 MHz, $CDCl_3$): δ 2.83 (s, 3H, CH_3), 7.40–7.43 (m, 1H, C-Ph 4-H), 7.46–7.49 (m, 3H, C-Ph 3,5-H; N-Ph 4-H), 7.55–7.57 (m, 2H, N-Ph 3,5-H), 7.67–7.69 (m, 2H, C-Ph 2,6-H), 7.95–7.96 (m, 2H, N-Ph 2,6-H), 8.72 (s, 1H, 3-H). ^{13}C NMR (176 MHz, $CDCl_3$): δ 22.5 (CH_3), 79.0 (C-7), 119.0 (C-3a),

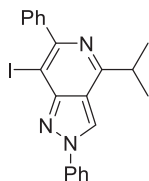
121.6 (N-Ph C-2,6), 123.0 (C-3), 128.0 (C-Ph C-3,5), 128.3 (C-Ph C-4), 129.0 (N-Ph C-4), 129.8 (N-Ph C-3,5), 130.0 (C-Ph C-2,6), 140.0 (N-Ph C-1), 142.5 (C-Ph C-1), 153.5 (C-7a), 155.3 (C-4), 155.6 (C-6). ^{15}N NMR (71 MHz, CDCl_3): δ -147.5 (N-2), -88.5 (N-1), -80.5 (N-5). IR (KBr, ν , cm^{-1}): 3131, 3107 (CH_{arom}), 2956 (CH_{aliph}), 1586, 1504, 1370, 1205 (C=C, C=N, C-N), 798, 768, 750, 696 ($\text{CH}=\text{CH}$ of monosubstituted benzenes). MS (ES^+): m/z (%): 412 ($[\text{M}+\text{H}]^+$, 100). HRMS (ESI) $\text{C}_{19}\text{H}_{15}\text{IN}_3$ ($[\text{M}+\text{H}]^+$): calcd 412.0305, found 412.0304.

4-Ethyl-7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (20)



Prepared in accordance to general procedure (D) from 4-(1-azidopropyl)-1-phenyl-3-(phenylethynyl)-1*H*-pyrazole (**15**) (329 mg, 1.01 mmol), NaHCO_3 (85 mg, 1.01 mmol) and I_2 (1278 mg, 5.03 mmol) in DCM (10 mL) the desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:6 v/v). Yield: 348 mg (81%), orange crystals, $\text{mp} = 145\text{--}146\text{ }^\circ\text{C}$, $R_f = 0.39$ (EtOAc/Hex, 1:3 v/v). ^1H NMR (700 MHz, CDCl_3): δ 1.46 (3H, t, $J = 7.6$ Hz, CH_3), 3.14 (2H, q, $J = 7.6$ Hz, CH_2), 7.40–7.43 (1H, m, C-Ph 4-H), 7.45–7.50 (3H, m, C-Ph 3,5-H; N-Ph 4-H), 7.54–7.58 (2H, m, N-Ph 3,5-H), 7.69–7.75 (2H, m, C-Ph 2,6-H), 7.93–7.98 (2H, m, N-Ph 2,6-H), 8.74 (1H, s, 3-H). ^{13}C NMR (176 MHz, CDCl_3): δ 13.5 (CH_3), 29.7 (CH_2), 78.8 (C-7), 117.8 (C-3a), 121.5 (N-Ph C-2,6), 122.6 (C-3), 127.8 (C-Ph C-3,5), 128.1 (C-Ph C-4), 128.9 (N-Ph C-4), 129.7 (N-Ph C-3,5), 130.0 (C-Ph, C-2,6), 139.8 (N-Ph C-1), 142.4 (C-Ph C-1), 153.8 (C-7a), 155.2 (C-6), 160.2 (C-4). ^{15}N NMR (71 MHz, CDCl_3): δ -148.2 (N-2), -89.9 (N-1), -82.2 (N-5). IR (KBr, ν , cm^{-1}): 3059 (CH_{arom}), 2969, 2929 (CH_{aliph}), 1584, 1504, 1464, 1374, 1273, 1201 (C=C, C=N, C-N), 905, 768, 698 ($\text{CH}=\text{CH}$ of monosubstituted benzenes). MS (ES^+): m/z (%): 425 ($[\text{M}+\text{H}]^+$, 99). HRMS (ESI) $\text{C}_{20}\text{H}_{17}\text{IN}_3$ ($[\text{M}+\text{H}]^+$): calcd 426.0462, found 426.0462.

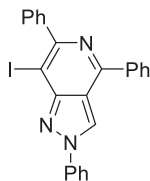
7-Iodo-4-isopropyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (21)



Prepared in accordance to general procedure (D) from 4-(azido-2-methylpropyl)-1-phenyl-3-(phenylethynyl)-1*H*-pyrazole (**16**) (177 mg, 0.52 mmol), NaHCO_3 (44 mg, 0.52 mmol) and I_2 (659 mg, 2.6 mmol) in DCM (5.2 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:6 v/v). Yield: 191 mg (84%), white crystals, $\text{mp} = 134\text{--}135\text{ }^\circ\text{C}$, $R_f = 0.50$ (EtOAc/Hex, 1:5 v/v). ^1H NMR (700 MHz, CDCl_3): δ 1.49 (6H, d, $J = 7.0$ Hz, $\text{CH}-(\text{CH}_3)_2$), 3.47 (1H, hept, $J = 7.0$ Hz, $\text{CH}-(\text{CH}_3)_2$), 7.39–7.44 (1H, m, C-Ph 4-H), 7.45–7.51 (3H, m, C-Ph 3,5-H and N-Ph 4-H), 7.54–7.59 (2H, m, N-Ph 3,5-H), 7.74–7.82 (2H, m, C-Ph 2,6-H), 7.93–7.99 (2H, m, N-Ph 2,6-H), 8.76 (1H, s, 3-H). ^{13}C NMR (176 MHz, CDCl_3): δ 22.1 ($\text{CH}-(\text{CH}_3)_2$), 35.6 ($\text{CH}-(\text{CH}_3)_2$), 78.8 (C-7), 117.0 (C-3a), 121.7 (N-Ph C-2,6), 122.5 (C-3), 127.8 (C-Ph C-3,5), 128.2 (C-Ph C-4), 129.0 (N-Ph C-4), 129.8 (N-Ph C-3,5), 130.4 (C-Ph C-2,6), 140.1 (N-Ph C-1),

142.5 (C-Ph C-1), 154.3 (C-7a), 154.9 (C-6), 163.8 (C-4). ^{15}N NMR (71 MHz, CDCl_3): δ -158.9 (N-2), -90.3 (N-1), -82.7 (N-5). IR (ν , cm^{-1}): 3114, 3083, 3062 (CH_{arom}), 2970, 2928, 2868 (CH_{aliph}), 1584, 1507, 1468, 1391, 1212 ($\text{C}=\text{C}$, $\text{C}=\text{N}$, $\text{C}-\text{N}$), 1106, 1023, 915, 763, 697 ($\text{CH}=\text{CH}$ of monosubstituted benzenes). MS (ES^+): m/z (%): 440 ($[\text{M}+\text{H}]^+$, 97.7). HRMS (ESI) for $\text{C}_{18}\text{H}_{13}\text{N}_3\text{I}$ ($[\text{M}+\text{H}]^+$): calcd 440.0618, found 440.0618.

7-Iodo-2,4,6-triphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (22)

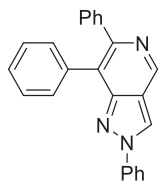


Prepared in accordance to general procedure (D) from 4-[azido(phenyl)methyl]-1-phenyl-3-(phenylethynyl)-1*H*-pyrazole (**17**) (62 mg, 0.165 mmol), K_3PO_4 (210 mg, 0.83 mmol) and I_2 (175 mg, 0.83 mmol) in DCM (1.7 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:8 v/v). Yield: 69 mg (88%), white crystals, mp = 93–94 °C, R_f = 0.59 (EtOAc/Hex, 1:3 v/v). ^1H NMR (700 MHz, CDCl_3): δ 7.43–7.49 (2H, m, C6-Ph 4-H and N-Ph 4-H), 7.49–7.58 (7H, m, C6-Ph, N-Ph and C4-Ph 3,5-H; C4-Ph 4-H), 7.80–7.87 (2H, m, C6-Ph 2,6-H), 7.94–8.00 (2H, m, N-Ph 2,6-H), 8.04–8.12 (2H, m, C4-Ph 2,6-H), 8.90 (1H, s, 3-H). ^{13}C NMR (176 MHz, CDCl_3): δ 80.3 (C-7), 116.7 (C-3a), 121.5 (N-Ph C-2,6), 123.7 (C-3), 127.8 (C6-Ph C-3,5), 128.2 (C6-Ph C-4), 128.4 (C4-Ph C-2,6), 128.9 (C4-Ph C-3,5), 129.0 (N-Ph C-4), 129.7 (N-Ph C-3,5), 129.9 (C4-Ph C-4), 130.2 (C6-Ph C-2,6), 138.4 (C4-Ph C-1), 139.7 (N-Ph C-1), 142.3 C6-Ph C-1), 154.5 (C-7a), 154.7 (C-4), 155.4 (C-6). ^{15}N NMR (71 MHz, CDCl_3): δ -146.4 (N-2), -90.3 (N-1), N-5 not found. IR (KBr, ν , cm^{-1}): 3058 (CH_{arom}), 2922 (CH_{aliph}), 1570, 1505, 1464, 1371, 1212 ($\text{C}=\text{C}$, $\text{C}=\text{N}$, $\text{C}-\text{N}$), 969, 750, 698 ($\text{CH}=\text{CH}$ of monosubstituted benzenes). MS (ES^+): m/z (%): 474 ($[\text{M}+\text{H}]^+$, 99). HRMS (ESI) $\text{C}_{24}\text{H}_{17}\text{IN}_3$ ($[\text{M}+\text{H}]^+$): calcd 474.0462, found 474.0462.

General procedure (E) for the synthesis of 7-substituted pyrazolo[4,3-*c*]pyridine derivatives 27–48 by Suzuki-Miyaura cross-coupling with boronic acids

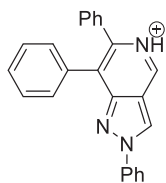
Appropriate 7-iodo-2*H*-pyrazolo[4,3-*c*]pyridine **18–22** (1 equiv) was dissolved in the mixture of EtOH and water (3:1, v/v). Subsequently, the respective boronic acid (1.2 equiv), Cs_2CO_3 (2 equiv) and $\text{Pd}(\text{OAc})_2$ (0.07 equiv) were added to the solution under argon atmosphere. The mixture was stirred at 100 °C under microwave irradiation (100 W, 300 Pa) for 0.5–1 h. Upon completion (monitored by TLC), the reaction mixture was cooled to room temperature, filtered through a pad of Celite, and the filter cake was washed with EtOAc (20 mL). The filtrate was diluted with water (20 mL) and extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4 , filtered and evaporated under reduced pressure. The residue was purified by column chromatography.

2,6,7-Triphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (27)



Prepared in accordance to general procedure (E) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (60 mg, 0.15 mmol), phenylboronic acid (22 mg, 0.18 mmol), Cs₂CO₃ (98 mg, 0.3 mmol) and Pd(OAc)₂ (2.4 mg, 0.01 mmol), EtOH (0.9 mL) and water (0.3 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:4 to 1:2 v/v). Yield: 50 mg (96%), brown crystals, mp = 151–152 °C, *R*_f = 0.08 (EtOAc/Hex, 1:3 v/v). ¹H NMR (700 MHz, CDCl₃): δ 7.21–7.26 (3H, m, C6-Ph 3,4,5-H), 7.28–7.31 (1H, m, C7-Ph 4-H), 7.31–7.35 (2H, m, C7-Ph 3,5-H), 7.42–7.46 (3H, m, C6-Ph 2,6-H and N-Ph 4-H), 7.49–7.55 (4H, m, N-Ph, 3,5-H and C7-Ph 2,6-H), 7.89–7.94 (2H, m N-Ph 2,6-H), 8.66 (1H, s, 3-H), 9.32 (1H, s, 4-H). ¹³C NMR (176 MHz, CDCl₃): δ 120 (C-3a), 121.4 (N-Ph C-2,6), 121.8 (C-3), 123.1 (C-6), 127.2 (C6-Ph C-4), 127.3 (C7-Ph C-4), 127.8 (C6-Ph C-2,6), 128.0 (C7-Ph C-3,5), 128.7 (N-Ph C-4), 129.6 (N-Ph C-3,5), 130.5 (C7-Ph C-2,6), 131.1 (C7-Ph C-2,6), 135.6 (C7-Ph C-1), 140.0 (N-Ph C-1), 140.6 (C6-Ph C-1), 145.7 (C-4), 149.3 (C-4), 151.2 (C-7a). ¹⁵N NMR (71 MHz, CDCl₃): δ -144.9 (N-2), -96.8 (N-1), -80.5 (N-5). IR (ν, cm⁻¹): 3502, 3081 (CH_{arom}), 1663, 1610, 1592, 1504, 1203, 1180, 1127 (C=C, C=N, C-N), 761, 697, 688 (CH=CH of monosubstituted benzenes). MS (ES⁺): *m/z* (%): 348 ([M+H]⁺, 98). HRMS (ESI) for C₂₄H₁₈N₃ ([M+H]⁺): calcd 348.1495, found 348.1495.

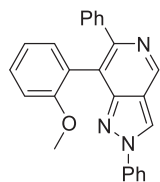
2,6,7-Triphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-5-ium (27H)



Obtained from **23** in acidic environment.

¹H NMR (700 MHz, CDCl₃): δ 7.29–7.32 (2H, m, C6-Ph 3,5-H), 7.33–7.40 (6H, m, C6-Ph 2,4,6-H and C7-Ph 3,4,5-H), 7.41–7.45 (2H, m, C7-Ph 2,6-H), 7.51–7.55 (1H, m, N-Ph, 4-H), 7.56–7.60 (2H, m, N-Ph, 3,5-H), 7.94–8.03 (2H, m N-Ph 2,6-H), 9.38 (1H, s, 3-H), 10.02 (1H, s, 4-H). ¹³C NMR (126 MHz, CDCl₃): δ 118.8 (C-3a), 121.9 (N-Ph C-2,6), 127.0 (C-7), 128.3 (C-3), 128.6 (C7-Ph C-3,5), 128.8 (C6-Ph C-3,5), 129.0 (C7-Ph C-4), 129.9 (C6-Ph C-4), 130.2 (N-Ph C-3,5), 130.5 (C6-Ph C-2,6), 130.6 (N-Ph C-4), 131.0 (C7-Ph C-2,6), 132.2 (C6-Ph C-1), 132.4 (C7-Ph C-1), 139.0 (N-Ph C-1), 141.9 (C-6), 144.0 (C-4), 150.9 (C-7a). ¹⁵N BMR (71 MHz, CDCl₃): δ -133.1 (N-2), -177.5 (N-5).

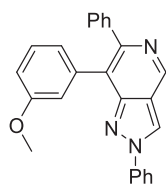
7-(2-Methoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (28)



Prepared in accordance to general procedure (E) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (60 mg, 0.15 mmol), (2-methoxyphenyl)boronic acid (27 mg, 0.18 mmol), Cs₂CO₃ (98 mg, 0.3 mmol) and Pd(OAc)₂ (2.4 mg, 0.01 mmol), EtOH (0.9 mL) and water (0.3 mL). The reaction was stopped after 1 h. The desired compound was obtained after purification by column chromatography (EtOAc/Hex,

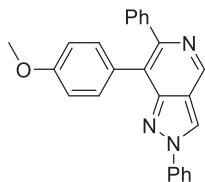
1:3 v/v). Yield: 23 mg (40%), light yellow crystals, mp = 169–170 °C, R_f = 0.08 (EtOAc/Hex, 1:3 v/v). ^1H NMR (700 MHz, CDCl_3): δ 3.42 (3H, s, OCH_3), 6.82–6.87 (1H, m, C7-Ph), 7.00–7.03 (1H, m, C7-Ph), 7.17–7.21 (1H, m, C7-Ph), 7.21–7.24 (2H, m, Ph), 7.30–7.35 (1H, m, Ph), 7.39–7.42 (1H, m, Ph), 7.43–7.46 (2H, m, Ph), 7.47–7.51 (3H, m, Ph), 7.81–7.92 (2H, m, N-Ph, 2,6-H), 8.61 (s, 1H, 3-H), 9.34 (s, 1H, 4-H). ^{13}C NMR (176 MHz, CDCl_3): δ 55.3, 111.6, 119.7, 120.1, 120.7, 121.6, 121.8, 125.1, 127.1, 127.6, 128.7, 129.3, 129.6, 129.7, 132.5, 140.3, 141.5, 145.9, 150.4, 151.6, 157.0. IR (ν , cm^{-1}): 3062, 3019 (CH_{arom}), 2922, 2852 (CH_{aliph}), 1600, 1590, 1501, 1478, 1435, 1242, 1233, 1203 ($\text{C}=\text{C}$, $\text{C}=\text{N}$, $\text{C}-\text{N}$), 1112, 1043, 1021 ($\text{C}-\text{O}-\text{C}$), 763, 750, 697, 686 ($\text{CH}=\text{CH}$ of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 378 ($[\text{M}+\text{H}]^+$, 99). HRMS (ESI) for $\text{C}_{25}\text{H}_{20}\text{N}_3\text{O}$ ($[\text{M}+\text{H}]^+$): calcd 378.1601, found 378.1601.

7-(3-Methoxyphenyl)-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine (29)



Prepared in accordance to general procedure (E) from 7-iodo-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine (**18**) (60 mg, 0.15 mmol), (3-methoxyphenyl)boronic acid (27 mg, 0.18 mmol), Cs_2CO_3 (98 mg, 0.3 mmol) and $\text{Pd}(\text{OAc})_2$ (2.4 mg, 0.01 mmol), EtOH (0.9 mL) and water (0.3 mL). The reaction was finished after 1 h. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:3 v/v). Yield: 44 mg (78%), white crystals, mp = 71–72 °C, R_f = 0.08 (EtOAc/Hex, 1:3 v/v). ^1H NMR (700 MHz, CDCl_3): δ 3.66 (3H, s, OCH_3), 6.81–6.87 (1H, m, C7-Ph 4-H), 7.02–7.08 (1H, m, C7-Ph 2-H), 7.10–7.14 (1H, m, C7-Ph 6-H), 7.21–7.29 (4H, m, C7-Ph 5-H, C6-Ph 3,4,5-C), 7.40–7.44 (1H, m, N-Ph 4-H), 7.44–7.48 (2H, m, C6-Ph 2,6-H), 7.49–7.54 (2H, m, N-Ph 3,5-H), 7.86–7.94 (2H, m, N-Ph 2,6-H), 8.64 (1H, s, 3-H), 9.30 (1H, s, 4-H). ^{13}C NMR (176 MHz, CDCl_3): δ 55.3 (OCH_3), 113.7 (C7-Ph C-4), 116.6 (C7-Ph C-2), 120.2 (C-3a), 121.5 (N-Ph C-2,6), 121.9 (C-3), 123.0 (C-7), 123.9 (C7-Ph C-6), 127.3 (C6-Ph C-4), 128.0 (C6-Ph C-3,5), 128.8 (N-Ph C-4), 129.1 (C7-Ph C-5), 129.8 (N-Ph C-3,5), 130.6 (C6-Ph C-2,6), 137.0 (C7-Ph C-1), 140.2 (N-Ph C-1), 140.9 (C6-Ph C-1), 145.9 (C-4), 149.6 (C-6), 151.2 (C-7a), 159.3 (C7-Ph C-3). ^{15}N NMR (71 MHz, CDCl_3): δ -145.2 (N-2), -96.9 (N-1), -79.4 (N-5). IR (ν , cm^{-1}): 3394, 3058, 3011 (CH_{arom}), 2920, 2849 (CH_{aliph}), 1592, 1575, 1507, 1464, 1367, 1317, 1286, 1212 ($\text{C}=\text{C}$, $\text{C}=\text{N}$, $\text{C}-\text{N}$), 1150, 1051 ($\text{C}-\text{O}-\text{C}$), 764, 756, 699, 689 ($\text{CH}=\text{CH}$ of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 378 ($[\text{M}+\text{H}]^+$, 99). HRMS (ESI) for $\text{C}_{25}\text{H}_{20}\text{N}_3\text{O}$ ($[\text{M}+\text{H}]^+$): calcd 378.1601, found 378.1601.

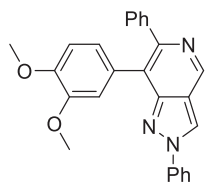
7-(4-Methoxyphenyl)-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine (30)



Prepared in accordance to general procedure (E) from 7-iodo-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine (**18**) (60 mg, 0.15 mmol), (4-methoxyphenyl)boronic acid (27 mg, 0.18 mmol), Cs_2CO_3 (98 mg, 0.3 mmol) and $\text{Pd}(\text{OAc})_2$ (2.4 mg, 0.01 mmol), EtOH (0.9 mL) and water (0.3 mL). The reaction was finished

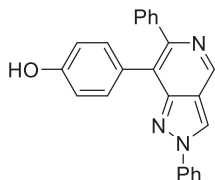
after 1 h. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:3 v/v). Yield: 44 mg (78%), white crystals, mp = 192–193 °C, R_f = 0.08 (EtOAc/Hex, 1:3 v/v). ^1H NMR (700 MHz, CDCl_3): δ 3.82 (3H, s, OCH_3), 6.82–6.93 (2H, m, C7-Ph 3,5-H), 7.21–7.25 (1H, m, C6-Ph 4-H), 7.25–7.30 (2H, m, C6-Ph 3,5-H), 7.41–7.49 (5H, m, N-Ph 4-H, C6-Ph 2,6-H, C7-Ph 2,6-H), 7.50–7.56 (2H, m, N-Ph 3,5-H), 7.84–7.97 (2H, m, N-Ph 2,6-H), 8.63 (1H, s, 3-H), 9.28 (1H, s, 4-H). ^{13}C NMR (176 MHz, CDCl_3): δ 55.2 (OCH_3), 113.5 (C7-Ph C-3,5), 120.1 (C-3a), 121.4 (N-Ph C-2,6), 121.7 (C-3), 122.6 (C-7), 127.0 (C6-Ph C-4), 127.8 (C7-Ph C-1), 127.9 (C6-Ph C-3,5), 128.7 (N-Ph C-4), 129.6 (N-Ph C-3,5), 130.5 (C6-Ph C-2,6), 132.4 (C7-Ph C-2,6), 140.1 (N-Ph C-1), 140.9 (C6-Ph C-1), 145.3 (C-4), 149.2 (C-6), 151.4 (C-7a), 158.9 (C7-Ph C-4). ^{15}N NMR (71 MHz, CDCl_3): δ -145.4 (N-2), -97.0 (N-1), -79.1 (N-5). IR (ν , cm^{-1}): 3135, 3062, 3020 (CH_{arom}), 2927, 2837 (CH_{aliph}), 1589, 1503, 1438, 1290, 1252 (C=C, C=N, C-N), 1178, 1031 (C-O-C), 763, 758, 689 ($\text{CH}=\text{CH}$ of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 378 ($[\text{M}+\text{H}]^+$, 100). HRMS (ESI) for $\text{C}_{25}\text{H}_{20}\text{N}_3\text{O}$ ($[\text{M}+\text{H}]^+$): calcd 378.1603, found 378.1601.

7-(3,4-Dimethoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (31)



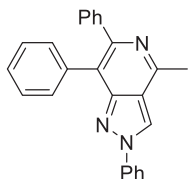
Prepared in accordance to general procedure (E) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (60 mg, 0.15 mmol), (3,4-dimethoxyphenyl)boronic acid (33 mg, 0.18 mmol), Cs_2CO_3 (98 mg, 0.3 mmol) and $\text{Pd}(\text{OAc})_2$ (2.4 mg, 0.01 mmol), EtOH (0.9 mL) and water (0.3 mL). The reaction was finished after 1 h. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:3 v/v). Yield: 44 mg (72%), white crystals, mp = 163–164 °C, R_f = 0.08 (EtOAc/Hex, 1:3 v/v). ^1H NMR (700 MHz, CDCl_3): δ 3.61 (3H, s, 3- OCH_3), 3.90 (3H, s, 4- OCH_3), 6.85–6.90 (1H, m, C7-Ph 5-H), 6.91–6.95 (1H, m, C7-Ph 2-H), 7.21–7.30 (4H, m, C6-Ph 3,4,5-H, C7-Ph 6-H), 7.42–7.45 (1H, m, N-Ph 4H), 7.45–7.49 (2H, m, N-Ph 2,6-H), 7.50–7.56 (2H, m, N-Ph 3,5-H), 7.87–7.98 (2H, m, N-Ph 2,6-H), 8.65 (1H, s, 3-H), 9.28 (1H, s, 4-H). ^{13}C NMR (176 MHz, CDCl_3): δ 55.7 (3- OCH_3), 55.9 (4- OCH_3), 110.9 (C7-Ph C-5), 114.9 (C7-Ph C-2), 120.3 (C-3a), 121.5 (N-Ph C-2,6), 121.9 (C-3), 122.8 (C-7), 124.0 (C7-Ph C-6), 127.2 (C6-Ph C-4), 128.0 (C7-Ph C-1), 128.1 (C6-Ph C-3,5), 128.8 (N-Ph C-4), 129.8 (N-Ph C-3,5), 130.5 (C6-Ph C-2,6), 140.2 (N-Ph C-1), 141.2 (C6-Ph C-1), 145.4 (C-4), 148.4 (C7-Ph C-3,4), 149.4 (C-6), 151.4 (C-7a). ^{15}N NMR (71 MHz, CDCl_3): δ -145.4 (N-2), -97.1 (N-1), -79.2 (N-5). IR (ν , cm^{-1}): 3042, 3019 (CH_{arom}), 2967, 2919, 2850 (CH_{aliph}), 1592, 1507, 1468, 1253, 1225 (C=C, C=N, C-N), 1141, 1014 (C-O-C), 753, 699, 688 ($\text{CH}=\text{CH}$ of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 408 ($[\text{M}+\text{H}]^+$, 100). HRMS (ESI) for $\text{C}_{26}\text{H}_{22}\text{N}_3\text{O}_2$ ($[\text{M}+\text{H}]^+$): calcd 408.1707, found 408.1707.

4-(2,6-Diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)phenol (32)



Prepared in accordance to general procedure (E) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (80 mg, 0.2 mmol), (4-hydroxyphenyl)boronic acid (33 mg, 0.24 mmol), Cs₂CO₃ (131 mg, 0.4 mmol) and Pd(OAc)₂ (3 mg, 0.014 mmol), EtOH (1.2 mL) and water (0.4 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:2 to 2:1 v/v). Yield: 44 mg (60%), yellowish crystals, mp = 307–308 °C, *R*_f = 0.18 (EtOAc/Hex, 1:2 v/v). ¹H NMR (700 MHz, DMSO-*d*₆): δ 6.70–6.75 (2H, m, C7-Ph 3,5-H), 7.20–7.28 (5H, m, C7-Ph 2,6-H; C6-Ph 3,4,5-H), 7.37–7.40 (2H, m, C6-Ph 2,6-H), 7.49–7.52 (1H, m, N-Ph 4-H), 7.59–7.63 (2H, m, N-Ph 3,5-H), 8.04–8.09 (2H, m, N-Ph 2,6-H), 9.29 (1H, s, 4-H), 9.46 (1H, s, 3-H), 9.50 (1H, s, OH). ¹³C NMR (176 MHz, DMSO-*d*₆): δ 115.0 (C7-Ph C-3,5), 119.7 (C-3a), 121.0 (N-Ph C-2,6), 122.3 (C-7), 124.2 (C-3), 125.8 (C7-Ph C-1), 127.0 (C6-Ph C-4), 127.6 (C6-Ph C-3,5), 128.7 (N-Ph C-4), 129.8 (N-Ph C-3,5), 130.3 (C6-Ph C-2,6), 132.0 (C7-Ph C-2,6), 139.5 (N-Ph C-1), 140.6 (C6-Ph C-1), 145.5 (C-4), 147.8 (C-6), 150.5 (C-7a), 156.7 (C7-Ph C-4). ¹⁵N NMR (71 MHz, DMSO-*d*₆): δ –144.8 (N-2), N-1 and N-5 not found. IR (KBr, ν, cm^{–1}): 3449 (OH), 2924 (CH_{arom}), 1607, 1592, 1505, 1440, 1273, 1172 (C=C, C=N, C–N), 767, 695 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): *m/z* (%): 364 ([M+H]⁺, 96). HRMS (ESI) for C₂₄H₁₈N₃O ([M+H]⁺): calcd 364.1444, found 364.1446.

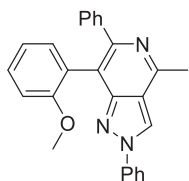
4-Methyl-2,6,7-triphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (33)



Prepared in accordance to general procedure (E) from 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**19**) (50 mg, 0.12 mmol), phenylboronic acid (18 mg, 0.145 mmol), Cs₂CO₃ (79 mg, 0.24 mmol) and Pd(OAc)₂ (1.9 mg, 0.008 mmol), EtOH (0.9 mL) and water (0.3 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:4 to 1:2 v/v). Yield: 116 mg (94%), white crystals, mp = 202–205 °C, *R*_f = 0.17 (EtOAc/Hex, 1:3 v/v). ¹H NMR (700 MHz, CDCl₃): δ 2.93 (3H, s, CH₃), 7.19–7.24 (3H, m, C7-Ph), 7.25–7.29 (1H, m, C6-Ph 4-H), 7.29–7.33 (2H, m, C6-Ph 3,5-H), 7.41–7.48 (5H, m, C6-Ph 2,6-H, N-Ph 4-H and C7-Ph), 7.49–7.54 (2H, m, N-Ph 3,5-H), 7.89–7.91 (2H, m, N-Ph 2,6-H), 8.61 (1H, s, 3-H). ¹³C NMR (176 MHz, CDCl₃): δ 22.8 (CH₃), 120.0 (C-3a), 121.1 (C-7), 121.3 (N-Ph C-2,6), 121.9 (C-3), 127.1 (C7-Ph C-4), 127.2 (C6-Ph C-4), 127.6 (C7-Ph C-1), 127.8 (C7-Ph), 127.9 (C6-Ph C-3,5), 128.6 (N-Ph C-4), 129.6 (N-Ph C-3,5), 130.6 (C7-Ph), 131.2 (C6-Ph C-2,6), 135.7 (C6-Ph C-1), 140.0 (N-Ph C-1), 148.8 (C-6), 151.5 (C-7a), 154.5 (C-4). ¹⁵N NMR (71 MHz, CDCl₃): δ –145.9 (N-2), –95.0 (N-1), –88.2 (N-5). IR (KBr, ν, cm^{–1}): 3137, 3061 (CH_{arom}), 2916 (CH_{aliph}), 1588, 1545, 1505, 1476, 1371 (C=C, C=N, C–N), 762, 700, 661

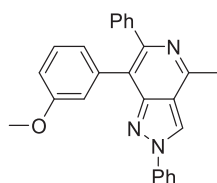
(CH=CH monosubstituted benzenes). MS (ES⁺): *m/z* (%): 362 ([M+H]⁺, 100). HRMS (ESI) C₂₅H₂₀N₃ ([M+H]⁺): calcd 362.1652, found 362.1650.

7-(2-Methoxyphenyl)-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (34)



Prepared in accordance to general procedure (E) from 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**19**) (50 mg, 0.12 mmol), (2-methoxyphenyl)boronic acid (22 mg, 0.145 mmol), Cs₂CO₃ (79 mg, 0.24 mmol) and Pd(OAc)₂ (1.9 mg, 0.008 mmol), EtOH (0.9 mL) and water (0.3 mL). The reaction was finished after 1 h. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:3 v/v). Yield: 38 mg (80%), light yellow crystals, mp = 159–160 °C, *R*_f = 0.13 (EtOAc/Hex, 1:3 v/v). ¹H NMR (500 MHz, CDCl₃): δ 2.95 (3H, s, CH₃), 3.42 (3H, s, OCH₃), 6.81–6.85 (1H, m, C7-Ph 3-H), 6.97–7.01 (1H, m, C6-Ph 4-H), 7.17–7.23 (3H, m, N-Ph 3,5-H and C6-Ph 4-H), 7.28–7.32 (1H, m, C7-Ph 4-H), 7.39–7.45 (4H, m, N-Ph 4-H, C6-Ph 2,6-H and C7-Ph 5-H), 7.47–7.51 (2H, m, C6-Ph 3,5-H), 7.85–7.89 (2H, m, N-Ph 2,6-H), 8.62 (1H, s, 3-H). ¹³C NMR (126 MHz, CDCl₃): δ 22.9 (CH₃), 55.4 (OCH₃), 111.5 (C7-Ph C-3), 117.8 (C-7), 120.1 (C-3a), 120.6 (C7-Ph C-5), 121.6 (N-Ph C-2,6), 122.2 (C-3), 125.0 (C7-Ph C-1), 127.2 (C6-Ph C-4), 127.6 (N-Ph C-3,5), 128.7 (N-Ph C-4), 129.2 (C7-Ph C-4), 129.7 (C6-Ph C-2,3,5,6), 132.6 (C7-Ph C-5), 140.3 (N-Ph C-1), 140.8 (C6-Ph C-1), 149.8 (C-6), 151.8 (C-7a), 154.8 (C-4), 157.1 (C7-Ph C-2). IR (ν, cm⁻¹): 3143, 3069, 3019 (CH_{arom}), 2955, 2922, 2853 (CH_{aliph}), 1599, 1586, 1578, 1552, 1504, 1495, 1479, 1434, 1372, 1240, 1217 (C=C, C=N, C-N), 1046, 1022 (C-O-C), 750, 698, 685 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): *m/z* (%): 392 ([M+H]⁺, 100). HRMS (ESI) for C₂₆H₂₂N₃O ([M+H]⁺): calcd 392.1758, found 392.1757.

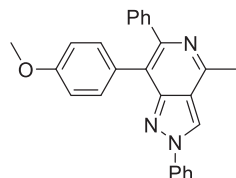
7-(3-Methoxyphenyl)-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (35)



Prepared in accordance to general procedure (E) from 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**19**) (50 mg, 0.12 mmol), (3-methoxyphenyl)boronic acid (22 mg, 0.145 mmol), Cs₂CO₃ (79 mg, 0.24 mmol) and Pd(OAc)₂ (1.9 mg, 0.008 mmol), EtOH (0.9 mL) and water (0.3 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:3 v/v). Yield: 38 mg (80%), light yellow crystals, mp = 190–191 °C, *R*_f = 0.18 (EtOAc/Hex, 1:3 v/v). ¹H NMR (500 MHz, CDCl₃): δ 2.97 (3H, s, CH₃), 3.65 (3H, s, OCH₃), 6.79–6.85 (1H, m, C7-Ph 4-H), 6.96–7.03 (1H, m, C7-Ph 2-H), 7.06–7.12 (1H, m, C7-Ph 6-H), 7.17–7.32 (4H, m, C7-Ph 5-H, C6-Ph 3,4,5-H), 7.41–7.47 (1H, m, N-Ph 4-H), 7.50–7.55 (4H, m, N-Ph 3,5-H, C6-Ph 2,6-H), 7.86–7.95 (2H, m, N-Ph 2,6-H), 8.67 (1H, s, 3-H). ¹³C NMR (126 MHz, CDCl₃): δ 23.1 (CH₃), 55.2 (OCH₃), 113.4 (C7-Ph C-4), 116.6 (C7-Ph C-2), 120.1 (C-3a), 120.8 (C-7), 121.3 (N-Ph C-2,6), 121.9 (C-3), 123.9 (C7-Ph C-6), 127.2 (C6-Ph C-4), 127.9 (C6-Ph C-3,5), 128.6 (N-Ph, C-

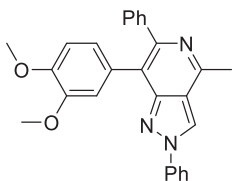
4), 129.0 (C7-Ph C-5), 129.7 (N-Ph C-3,5), 130.6 (C6-Ph C-2,6), 137.2 (C7-Ph C-1), 140.1 (N-Ph C-1), 140.8 (C6-Ph C-1), 149.3 (C-6), 151.4 (C-7a), 154.6 (C-4), 159.2 (C7-Ph C-3). IR (ν , cm^{-1}): 3067, 3002 (CH_{arom}), 2954, 2923, 2852 (CH_{aliph}), 1599, 1589, 1575, 1547, 1488, 1443, 1284, 1211 ($\text{C}=\text{C}$, $\text{C}=\text{N}$, $\text{C}-\text{N}$), 1160, 1049 ($\text{C}-\text{O}-\text{C}$), 753, 700, 686 ($\text{CH}=\text{CH}$ of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 392 ($[\text{M}+\text{H}]^+$, 100). HRMS (ESI) for $\text{C}_{26}\text{H}_{22}\text{N}_3\text{O}$ ($[\text{M}+\text{H}]^+$): calcd 392.1757, found 392.1757.

7-(4-Methoxyphenyl)-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (36)



Prepared in accordance to general procedure (E) from 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**19**) (50 mg, 0.12 mmol), (4-methoxyphenyl)boronic acid (22 mg, 0.145 mmol), Cs_2CO_3 (79 mg, 0.24 mmol) and $\text{Pd}(\text{OAc})_2$ (1.9 mg, 0.008 mmol), EtOH (0.9 mL) and water (0.3 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:3 v/v). Yield: 118 mg (89%), light yellow crystals, $\text{mp} = 157\text{--}161\text{ }^\circ\text{C}$, $R_f = 0.15$ (EtOAc/Hex, 1:3 v/v). ^1H NMR (700 MHz, CDCl_3): δ 2.92 (3H, s, CH_3), 3.84 (3H, s, OCH_3), 6.88–6.89 (2H, m, C7-Ph 3,5-H), 7.23–7.25 (1H, m, C6-Ph 4-H), 7.27–7.30 (2H, m, C6-Ph 3,5-H), 7.42–7.45 (3H, m, N-Ph 4-H and C7-Ph 2,6-H), 7.49–7.50 (2H, m, C6-Ph 2,6-H), 7.52–7.54 (2H, m, N-Ph 3,5-H), 7.92–7.93 (2H, m, N-Ph 2,6-H), 8.61 (1H, s, 3-H). ^{13}C NMR (176 MHz, CDCl_3): δ 23.2 (CH_3), 55.3 (OCH_3), 113.6 (C7-Ph C-3,5), 120.2 (C-3a), 120.5 (C-7), 121.4 (N-Ph C-2,6), 121.7 (C-3), 127.0 (C6-Ph C-4), 127.9 (C6-Ph C-3,5), 128.3 (N-Ph C-4), 128.5 (C7-Ph C-1), 129.7 (N-Ph C-3,5), 130.7 (C6-Ph C-2,6), 132.5 (C7-Ph C-2,6), 140.3 (N-Ph C-1), 141.3 (C6-Ph C-1), 149.2 (C-6), 151.8 (C-7a), 154.0 (C-4), 158.7 (C7-Ph C-4). ^{15}N NMR (71 MHz, CDCl_3): δ -146.8 (N-2), -95.7 (N-1), -81.6 (N-5). IR (KBr, ν , cm^{-1}): 3056, 3012 (CH_{arom}), 2951, 2834, 2903, 2831 (CH_{aliph}), 1607, 1598, 1588, 1507, 1247 ($\text{C}=\text{C}$, $\text{C}=\text{N}$, $\text{C}-\text{N}$), 1075, 1038 ($\text{C}-\text{O}$), 755, 728, 701, 685 ($\text{CH}=\text{CH}$ of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 392 ($[\text{M}+\text{H}]^+$, 100). HRMS (ESI) $\text{C}_{26}\text{H}_{22}\text{N}_3\text{O}$ ($[\text{M}+\text{H}]^+$): calcd 392.1757, found 392.1759.

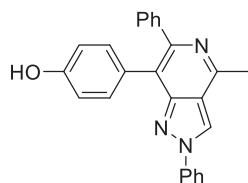
7-(3,4-Dimethoxyphenyl)-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (37)



Prepared in accordance to general procedure (E) from 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**19**) (50 mg, 0.12 mmol), (3,4-dimethoxyphenyl)boronic acid (26 mg, 0.145 mmol), Cs_2CO_3 (79 mg, 0.24 mmol) and $\text{Pd}(\text{OAc})_2$ (1.9 mg, 0.008 mmol), EtOH (0.9 mL) and water (0.3 mL). The reaction was finished after 1 h. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:3 v/v). Yield: 41 mg (67%), light yellow crystals, $\text{mp} = 180\text{--}181\text{ }^\circ\text{C}$, $R_f = 0.12$ (EtOAc/Hex, 1:3 v/v). ^1H NMR (700 MHz, CDCl_3): δ 2.91 (3H, s, CH_3), 3.60 (3H, s, 3- OCH_3),

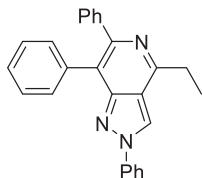
3.89 (3H, s, 4-OCH₃), 6.85–6.90 (2H, m, C7-Ph 2,5-H), 7.20–7.24 (2H, m, C7-Ph 6-H, C6-Ph 4-H), 7.24–7.28 (2H, m, C6-Ph 3,5-H), 7.41–7.44 (1H, m, N-Ph 4-H), 7.44–7.47 (2H, m, C6-Ph 2,6-H), 7.50–7.54 (2H, m, N-Ph 3,5-H), 7.90–7.95 (2H, m, N-Ph 2,6-H), 8.62 (1H, s, 3-H). ¹³C NMR (176 MHz, CDCl₃): δ 23.2 (CH₃), 55.7 (3-OCH₃), 55.9 (4-OCH₃), 110.8 (C7-Ph C-5), 114.9 (C7-Ph C-2), 120.3 (C-3a), 120.6 (C-7), 121.4 (N-Ph C-2,6), 121.8 (C-3), 123.9 (C7-Ph C-6), 127.1 (C6-Ph C-4), 128.1 (C6-Ph C-3,5), 128.3 (C7-Ph C-1), 128.6 (N-Ph C-4), 129.7 (N-Ph C-3,5), 130.6 (C6-Ph C-2,6), 140.3 (N-Ph C-1), 141.3 (C-Ph C-1), 148.2 (C7-Ph C-4), 148.3 (C7-Ph C-3), 149.3 (C-6), 151.6 (C-7a), 154.2 (C-4). ¹⁵N NMR (71 MHz, CDCl₃): δ –147.2 (N-2), –96.4 (N-1), –82.9 (N-5). IR (ν, cm^{–1}): 3121, 3049, 2999 (CH_{arom}), 2987, 2949, 2937, 2832 (CH_{aliph}), 1589, 1519, 1508, 1481, 1465, 1256, 1231 (C=C, C=N, C–N), 1164, 1138, 1023 (C–O–C), 759, 728, 701, 686, 667 (CH=CH of mono- and trisubstituted benzenes). MS (ES⁺): *m/z* (%): 422 ([M+H]⁺, 98). HRMS (ESI) for C₂₇H₂₄N₃O₂ ([M+H]⁺): calcd 422.1863, found 422.1863.

4-(4-Methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)phenol (38)



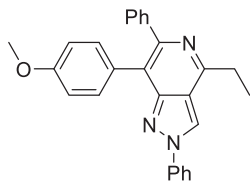
Prepared in accordance to general procedure (E) from 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (19) (80 mg, 0.2 mmol), (4-hydroxyphenyl)boronic acid (32 mg, 0.23 mmol), Cs₂CO₃ (127 mg, 0.39 mmol) and Pd(OAc)₂ (3 mg, 0.014 mmol), EtOH (1.2 mL) and water (0.4 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:4 to 1:2 v/v). Yield: 37 mg (50%), yellowish crystals, mp = 269–270 °C, R_f = 0.20 (EtOAc/Hex, 1:2 v/v). ¹H NMR (700 MHz, DMSO-*d*₆): δ 2.80 (3H, s, CH₃), 6.68–6.73 (2H, m, C7-Ph 3,5-H), 7.16–7.19 (2H, m, C7-Ph 2,6-H), 7.20–7.22 (2H, m, C6-Ph 4-H), 7.22–7.26 (2H, m, C6-Ph 3,5-H), 7.34–7.38 (2H, m, C6-Ph 2,6-H), 7.46–7.49 (1H, m, N-Ph 4-H), 7.57–7.63 (2H, m, N-Ph 3,5-H), 8.04–8.08 (2H, m, N-Ph 2,6-H), 9.44 (1H, s, OH), 9.51 (1H, s, 3-H). ¹³C NMR (176 MHz, DMSO-*d*₆): δ 22.6 (CH₃), 114.9 (C7-Ph C-3,5), 120.0 (C-7), 120.6 (N-Ph C-2,6), 123.9 (C-3), 126.3 (C7-Ph C-1), 126.7 (C6-Ph C-4), 127.4 (C6-Ph C-3,5), 128.4 (N-Ph C-4), 129.7 (N-Ph C-3,5), 130.3 (C6-Ph C-2,6), 132.0 (C7-Ph C-2,6), 139.6 (N-Ph C-1), 141.1 (C6-Ph C-1), 148.0 (C-6), 150.9 (C-7a), 153.9 (C-4), 156.4 (C7-Ph C-4). ¹⁵N NMR (71 MHz, DMSO-*d*₆): δ –147.2 (N-2), –80.5 (N-5), N-1 not found. IR (KBr, ν, cm^{–1}): 3455 (OH), 3149 (CH_{arom}), 2920 (CH_{aliph}), 1609, 1591, 1509, 1397, 1264 (C=C, C=N, C–N), 828, 757, 698 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): *m/z* (%): 378 ([M+H]⁺, 97). HRMS (ESI) for C₂₅H₂₀N₃O ([M+H]⁺): calcd 378.1601, found 378.1602.

4-Ethyl-2,6,7-triphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (39)



Prepared in accordance to general procedure (E) from 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**20**) (80 mg, 0.19 mmol), phenylboronic acid (31 mg, 0.23 mmol), Cs₂CO₃ (123 mg, 0.38 mmol) and Pd(OAc)₂ (3 mg, 0.013 mmol), EtOH (1.2 mL) and water (0.4 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:5 v/v). Yield: 58 mg (81%), yellowish crystals, mp = 179–180 °C, *R*_f = 0.39 (EtOAc/Hex, 1:3 v/v). ¹H NMR (700 MHz, CDCl₃): δ 1.54 (3H, t, *J* = 7.6 Hz, CH₃), 3.23 (2H, q, *J* = 7.6 Hz, CH₂), 7.19–7.23 (2H, m, C6-Ph 4-H), 7.23–7.26 (2H, m, C6-Ph 3,5-H), 7.26–7.29 (1H, m, C7-Ph 4-H), 7.29–7.34 (2H, m, C7-Ph 3,5-H), 7.40–7.44 (1H, m, N-Ph 4-H), 7.46–7.50 (4H, m, C-Ph 2,6-H), 7.50–7.53 (2H, m, N-Ph 3,5-H), 7.87–7.95 (2H, m, N-Ph 2,6-H), 8.62 (1H, s, 3-H). ¹³C NMR (176 MHz, CDCl₃): δ 13.3 (CH₃), 30.4 (CH₂), 119.0 (C-3a), 120.7 (C-7), 121.2 (C-4), 121.3 (N-Ph C-2,6), 127.0 (C6-Ph and C7-Ph C-4), 127.7 (C6-Ph C-3,5), 127.9 (C7-Ph C-3,5), 128.4 (N-Ph C-4), 129.5 (N-Ph C-3,5), 130.7 (C6-Ph C-2,6), 131.2 (C7-Ph C-2,6), 136.2 (C7-Ph C-1), 140.2 (N-Ph C-1), 141.0 (C6-Ph C-1), 149.1 (C-6), 151.9 (C-7a), 159.3 (C-4). ¹⁵N NMR (71 MHz, CDCl₃): δ -147.3 (N-2), N-1 not found, -83.4 (N-5). IR (KBr, ν, cm⁻¹): 3059 (CH_{arom}), 2970, 2930 (CH_{aliph}), 1587, 1547, 1476, 1371, 1213 (C=C, C=N, C-N), 753, 697, 686 (CH=CH monosubstituted benzenes). MS (ES⁺): *m/z* (%): 375 ([M+H]⁺, 99). HRMS (ESI) for C₂₆H₂₂N₃ ([M+H]⁺): calcd 376.1808, found 376.1808.

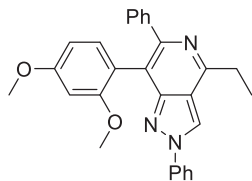
4-Ethyl-7-(4-methoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (40)



Prepared in accordance to general procedure (E) from 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**20**) (80 mg, 0.19 mmol), (4-methoxyphenyl)boronic acid (34 mg, 0.23 mmol), Cs₂CO₃ (123 mg, 0.38 mmol) and Pd(OAc)₂ (3 mg, 0.013 mmol), EtOH (1.2 mL) and water (0.4 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:5 v/v). Yield: 63 mg (82%), yellowish crystals, mp = 161–162 °C, *R*_f = 0.34 (EtOAc/Hex, 1:3 v/v). ¹H NMR (700 MHz, CDCl₃): δ 1.53 (3H, t, *J* = 7.6 Hz, CH₃), 3.21 (2H, q, *J* = 7.6 Hz, CH₂), 3.82 (3H, s, OCH₃), 6.83–6.90 (2H, m, C7-Ph 3,5-H), 7.19–7.23 (1H, m, C6-Ph 4-H), 7.24–7.27 (2H, m, C6-Ph 3,5-H), 7.40–7.44 (3H, m, C7-Ph 2,6-H and N-Ph 4-H), 7.47–7.50 (2H, m, C6-Ph 2,6-H), 7.50–7.53 (2H, m, N-Ph 3,5-H), 7.86–7.97 (2H, m, N-Ph 2,6-H), 8.61 (1H, s, 3-H). ¹³C NMR (176 MHz, CDCl₃): δ 13.3 (CH₃), 30.4 (CH₂), 55.1 (OCH₃), 113.5 (C7-Ph C-3,5), 119.0 (C-3a), 120.3 (C-7), 121.2 (C-4), 121.3 (N-Ph C-2,6), 126.9 (C6-Ph C-4), 127.7 (C6-Ph C-3,5), 128.4 (N-Ph C-4), 129.5 (N-Ph C-3,5), 130.6 (C6-Ph C-2,6), 132.3 (C7-Ph C-2,6), 140.2 (N-Ph C-1), 141.2 (C6-Ph C-1), 148.9 (C-6), 152.1 (C-7a), 158.6 (C7-Ph C-4), 158.9 (C-4). ¹⁵N NMR (71 MHz, CDCl₃): δ -147.6 (N-2), N-1 not found, -83.2 (N-5). IR

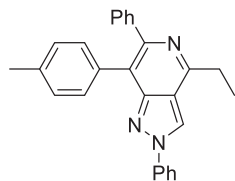
(KBr, ν , cm^{-1}): 3058, 3016 (CH_{arom}), 2986, 2934, 2889 (CH_{aliph}), 1607, 1507, 1462, 1376, 1290, 1248, 1176 ($\text{C}=\text{C}$, $\text{C}=\text{N}$, $\text{C}-\text{N}$), 1045 ($\text{C}-\text{O}$), 830, 753, 699, 686 ($\text{CH}=\text{CH}$ of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 405 ($[\text{M}+\text{H}]^+$, 99). HRMS (ESI) for $\text{C}_{27}\text{H}_{24}\text{N}_3\text{O}$ ($[\text{M}+\text{H}]^+$): calcd 406.1914, found 406.1914.

7-(2,4-Dimethoxyphenyl)-4-ethyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine (41)



Prepared in accordance to general procedure (E) from 7-iodo-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine (**20**) (80 mg, 0.19 mmol), (2,4-dimethoxyphenyl)boronic acid (41 mg, 0.23 mmol), Cs_2CO_3 (123 mg, 0.38 mmol) and $\text{Pd}(\text{OAc})_2$ (3 mg, 0.013 mmol), EtOH (1.2 mL) and water (0.4 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:5 v/v). Yield: 40 mg (48%), yellow crystals, mp = 202–203 °C, R_f = 0.24 (EtOAc/Hex, 1:3 v/v). ^1H NMR (700 MHz, CDCl_3): δ 1.53 (3H, t, J = 7.6 Hz, CH_3), 3.21 (2H, q, J = 7.6 Hz, CH_2), 3.37 (3H, s, 2-OCH₃), 3.83 (3H, s, 4-OCH₃), 6.38–6.44 (1H, m, C7-Ph 3-H), 6.53–6.59 (1H, m, C7-Ph 5-H), 7.15–7.19 (1H, m, C6-Ph 4-H), 7.19–7.25 (2H, m, C6-Ph 3,5-H), 7.35–7.41 (2H, m, N-Ph 4-H and C7-Ph 6-H), 7.45–7.51 (4H, m, C6-Ph 2,6-H and N-Ph 3,5-H), 7.84–7.90 (2H, m, N-Ph 2,6-H), 8.58 (1H, s, 3-H). ^{13}C NMR (176 MHz, CDCl_3): δ 13.3 (CH_3), 30.4 (CH_2), 55.1 (2-OCH₃), 55.3 (4-OCH₃), 99.1 (C7-Ph C-3), 104.6 (C7-Ph C-5), 116.8 (C-7), 117.9 (C7-Ph C-1), 119.0 (C-3a), 121.2 (C-3), 121.4 (N-Ph C-2,6), 126.7 (C6-Ph C-4), 127.4 (C6-Ph C-3,5), 128.3 (N-Ph C-4), 129.5 (N-Ph C-3,5 and C6-Ph C-2,6), 132.8 (C7-Ph C-6), 140.3 (N-Ph C-1), 141.9 (C6-Ph C-1), 149.9 (C-6), 152.4 (C-7a), 157.9 (C7-Ph C-2), 159.0 (C-4), 160.5 (C7-Ph C-4). ^{15}N NMR (71 MHz, CDCl_3): δ -147.9 (N-2), -96.4 (N-1), -84.6 (N-5). IR (KBr, ν , cm^{-1}): 3134, 3058 (CH_{arom}), 2966, 2930, 2836 (CH_{aliph}), 1606, 1588, 1547, 1505, 1462, 1305, 1204 ($\text{C}=\text{C}$, $\text{C}=\text{N}$, $\text{C}-\text{N}$), 1027 ($\text{C}-\text{O}$), 829, 764, 705, 691 ($\text{CH}=\text{CH}$ of mono- and trisubstituted benzenes). MS (ES^+): m/z (%): 435 ($[\text{M}+\text{H}]^+$, 100). HRMS (ESI) for $\text{C}_{28}\text{H}_{26}\text{N}_3\text{O}_2$ ($[\text{M}+\text{H}]^+$): calcd 436.2020, found 436.2020.

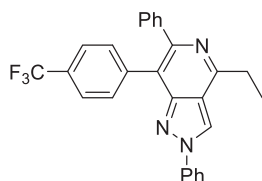
4-Ethyl-2,6-diphenyl-7-(4-methylphenyl)-2H-pyrazolo[4,3-c]pyridine (42)



Prepared in accordance to general procedure (E) from 7-iodo-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine (**20**) (80 mg, 0.19 mmol), (4-methylphenyl)boronic acid (31 mg, 0.23 mmol), Cs_2CO_3 (123 mg, 0.38 mmol) and $\text{Pd}(\text{OAc})_2$ (3 mg, 0.013 mmol), EtOH (1.2 mL) and water (0.4 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:8 v/v). Yield: 60 mg (81%), yellowish crystals, mp = 179–180 °C, R_f = 0.41 (EtOAc/Hex, 1:3 v/v). ^1H NMR (700 MHz, CDCl_3): δ 1.52 (3H, t, J = 7.6 Hz, CH_2CH_3), 2.34 (3H, s, $\text{Ph}-\text{CH}_3$), 3.19 (2H, q, J = 7.6 Hz, CH_2CH_3), 7.09–7.14 (2H, m, C7-Ph 3,5-H), 7.19–7.22 (1H, m, C6-Ph 4-H), 7.23–7.25 (2H, m, C6-Ph 3,5-H), 7.34–7.38 (2H, m, C6-Ph 2,6-H), 7.38–7.41

(1H, m, N-Ph 4-H), 7.46–7.51 (4H, m, N-Ph 3,5-H and C7-Ph 2,6-H), 7.86–7.91 (2H, m, N-Ph 2,6-H), 8.59 (1H, s, 3-H). ¹³C NMR (176 MHz, CDCl₃): δ 13.3 (CH₂CH₃), 21.3 (Ph-CH₃), 30.4 (CH₂CH₃), 119.0 (C-3a), 120.7 (C-7), 121.2 (C-4), 121.3 (N-Ph C-2,6), 126.9 (C6-Ph C-4), 127.7 (C6-Ph C-3,5), 128.3 (N-Ph C-4), 129.5 (N-Ph C-3,5), 130.6 (C7-Ph C-2,6), 131.0 (C6-Ph C-2,6), 136.5 (C7-Ph C-4), 140.2 (N-Ph C-1), 141.2 (C6-Ph C-1), 148.9 (C-6), 152.0 (C-7a), 159.0 (C-4). ¹⁵N NMR (71 MHz, CDCl₃): δ -147.5 (N-2), -96.1 (N-1), -83.3 (N-5). IR (KBr, ν, cm⁻¹): 3028 (CH_{arom}), 2990, 2939 (CH_{aliph}), 1587, 1505, 1463, 1377, 1211, 1045 (C=C, C=N, C-N), 822, 753, 699, 686 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): *m/z* (%): 389 ([M+H]⁺, 98). HRMS (ESI) for C₂₇H₂₄N₃ ([M+H]⁺): calcd 390.1965, found 390.1965.

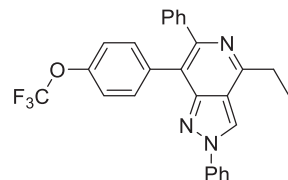
4-Ethyl-2,6-diphenyl-7-(4-(trifluoromethyl)phenyl)-2H-pyrazolo[4,3-*c*]pyridine (43)



Prepared in accordance to general procedure (E) from 7-iodo-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-*c*]pyridine (**20**) (80 mg, 0.19 mmol), 4-(trifluoromethyl)phenylboronic acid (43 mg, 0.23 mmol), Cs₂CO₃ (123 mg, 0.38 mmol) and Pd(OAc)₂ (3 mg, 0.013 mmol), EtOH (1.2 mL) and water (0.4 mL). The reaction was finished after 30 min. The

desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:8 v/v). Yield: 77 mg (92%), yellowish crystals, mp = 203–204 °C, *R_f* = 0.39 (EtOAc/Hex, 1:3 v/v). ¹H NMR (700 MHz, CDCl₃): δ 1.54 (3H, t, *J* = 7.6 Hz, CH₃), 3.19 (2H, q, *J* = 7.6 Hz, CH₂), 7.22–7.28 (3H, m, C6-Ph 3,4,5-H), 7.41–7.46 (3H, m, C6-Ph 2,6-H, N-Ph 4-H), 7.50–7.54 (2H, m, N-Ph 3,5-H), 7.55–7.58 (2H, m, C7-Ph 3,5-H), 7.59–7.63 (2H, m, C7-Ph 2,6-H), 7.88–7.92 (2H, m, N-Ph 2,6-H), 8.64 (1H, s, 3-H). ¹³C NMR (176 MHz, CDCl₃): δ 13.3 (CH₃), 30.4 (CH₂), 119.0 (C-3a), 119.2 (C-7), 121.3 (N-Ph C-2,6), 121.5 (C-4), 124.3 (CF₃, *J* = 201.6 Hz), 124.8 (C7-Ph C-3,5, *J* = 2.52 Hz), 127.4 (C6-Ph C-4), 127.9 (C6-Ph C-3,5), 128.6 (N-Ph C-4), 128.8 (C7-Ph C-4, *J* = 25.2 Hz), 129.6 (N-Ph C-3,5), 130.7 (C6-Ph C-2,6), 131.5 (C7-Ph C-2,6), 140.0 (N-Ph C-1), 140.1 (C7-Ph C-1), 140.4 (C6-Ph C-1), 149.6 (C-6), 151.5 (C-7a), 160.1 (C-4). ¹⁵N NMR (71 MHz, CDCl₃): δ -146.9 (N-2), -97.4 (N-1), -83.5 (N-5). ¹⁹F NMR (376 MHz, CDCl₃): δ -65.59 (3F, s, CF₃). IR (KBr, ν, cm⁻¹): 3053 (CH_{arom}), 2971 (CH_{aliph}), 1585, 1484, 1327, 1130 (C=C, C=N, C-N, C-F), 759, 696 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): *m/z* (%): 443 ([M+H]⁺, 99). HRMS (ESI) for C₂₇H₂₁F₃N₃ ([M+H]⁺): calcd 444.1682, found 444.1682.

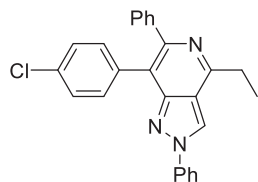
4-Ethyl-2,6-diphenyl-7-[4-(trifluoromethoxy)phenyl]-2H-pyrazolo[4,3-*c*]pyridine (44)



Prepared in accordance to general procedure (E) from 7-iodo-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-*c*]pyridine (**20**) (80 mg, 0.19 mmol), 4-(trifluoromethoxy)phenylboronic acid (47 mg, 0.23 mmol),

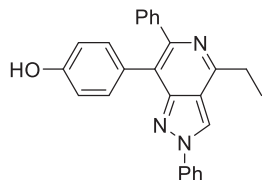
Cs₂CO₃ (123 mg, 0.38 mmol) and Pd(OAc)₂ (3 mg, 0.013 mmol), EtOH (1.2 mL) and water (0.4 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:8 v/v). Yield: 74 mg (85%), white crystals, mp = 153–154 °C, *R*_f = 0.41 (EtOAc/Hex, 1:3 v/v). ¹H NMR (700 MHz, CDCl₃): δ 1.54 (3H, t, *J* = 7.6 Hz, CH₃), 3.22 (2H, q, *J* = 7.6 Hz, CH₂), 7.12–7.19 (2H, m, C7-Ph 3,5-H), 7.22–7.28 (3H, m, C6-Ph 3,4,5-H), 7.40–7.48 (3H, m, C6-Ph 2,6-H, N-Ph 4-H), 7.49–7.56 (4H, m, N-Ph 3,5-H and C7-Ph C-2,6), 7.87–7.94 (2H, m, N-Ph 2,6-H), 8.63 (1H, s, 3-H). ¹³C NMR (176 MHz, CDCl₃): δ 13.3 (CH₃), 30.4 (CH₂), 119.0 (C-3a), 119.2 (C-7), 120.3 (C7-Ph C-3,5), 120.5 (CF₃, *J* = 176.4 Hz), 121.3 (N-Ph C-2,6), 121.5 (C-4), 127.2 (C6-Ph C-4), 127.8 (C6-Ph C-3,5), 128.6 (N-Ph C-4), 129.6 (N-Ph C-3,5), 130.6 (C6-Ph C-2,6), 132.6 (C7-Ph C-2,6), 134.8 (C7-Ph C-4), 140.1 (N-Ph C-1), 140.6 (C6-Ph C-1), 148.2 (C7-Ph C-1), 149.4 (C-6), 151.6 (C-7a), 159.8 (C-4). ¹⁵N NMR (71 MHz, CDCl₃): δ -147.1 (N-2), -97.4 (N-1), -83.5 (N-5). ¹⁹F NMR (376 MHz, CDCl₃): δ -60.82 (3F, s, CF₃). IR (KBr, ν, cm⁻¹): 3049 (CH_{arom}), 2969, 2934 (CH_{aliph}), 1586, 1507, 1367, 1259, 1225, 1168 (C=C, C=N, C-N, C-F), 757, 699, 687 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): *m/z* (%): 459 ([M+H]⁺, 99). HRMS (ESI) for C₂₇H₂₁F₃N₃O ([M+H]⁺): calcd 460.1630, found 460.1631.

7-(4-Chlorophenyl)-4-ethyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (45)



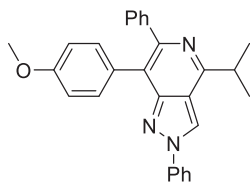
Prepared in accordance to general procedure (E) from 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**20**) (80 mg, 0.19 mmol), 4-chlorophenylboronic acid (36 mg, 0.23 mmol), Cs₂CO₃ (123 mg, 0.38 mmol) and Pd(OAc)₂ (3 mg, 0.013 mmol), EtOH (1.2 mL) and water (0.4 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:8 v/v). Yield: 65 mg (83%), yellow crystals, mp = 226–227 °C, *R*_f = 0.49 (EtOAc/Hex, 1:3 v/v). ¹H NMR (700 MHz, CDCl₃): δ 1.52 (3H, t, *J* = 7.6 Hz, CH₃), 3.21 (2H, q, *J* = 7.6 Hz, CH₂), 7.22–7.30 (5H, m, C6-Ph 3,4,5-H; C7-Ph 3,5-H), 7.40–7.44 (3H, m, C7-Ph 2,6-H; N-Ph 4-H), 7.44–7.47 (2H, m, C6-Ph 2,6-H), 7.50–7.53 (2H, m, N-Ph 3,5-H), 7.86–7.93 (2H, m, N-Ph 2,6-H), 8.61 (1H, s, 3-H). ¹³C NMR (176 MHz, CDCl₃): δ 13.3 (CH₃), 30.4 (CH₂), 119.0 (C-3a), 119.4 (C-7), 121.3 (N-Ph C-2,6), 121.4 (C-3), 127.2 (C6-Ph C-4), 127.9 (C6-Ph C-3,5), 128.2 (C7-Ph C-3,5), 128.5 (N-Ph C-4), 129.6 (N-Ph C-3,5), 130.6 (C6-Ph C-2,6), 132.5 (C7-Ph C-2,6), 132.8 (C7-Ph C-4), 134.6 (C7-Ph C-1), 140.1 (N-Ph C-1), 140.6 (C6-Ph C-1), 149.3 (C-6), 151.6 (C-7a), 159.7 (C-4). ¹⁵N NMR (71 MHz, CDCl₃): δ -147.3 (N-2), -97.0 (N-1), -83.3 (N-5). IR (KBr, ν, cm⁻¹): 3057 (CH_{arom}), 2991, 2939 (CH_{aliph}), 1691, 1587, 1501, 1463, 1213, 1092 (C=C, C=N, C-N), 825, 756, 698, 688 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): *m/z* (%): 409 ([M+H]⁺, 96). HRMS (ESI) for C₂₆H₂₁ClN₃ ([M+H]⁺): calcd 410.1419, found 410.1419.

4-(4-Ethyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)phenol (46)



Prepared in accordance to general procedure (E) from 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**20**) (80 mg, 0.19 mmol), (4-hydroxyphenyl)boronic acid (31 mg, 0.23 mmol), Cs₂CO₃ (123 mg, 0.38 mmol) and Pd(OAc)₂ (3 mg, 0.013 mmol), EtOH (1.2 mL) and water (0.4 mL). The reaction was finished after 30 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:8 v/v). Yield: 50 mg (67%), yellow-brown crystals, mp = 199–200 °C, *R*_f = 0.17 (EtOAc/Hex, 1:3 v/v). ¹H NMR (700 MHz, CDCl₃): δ 1.50 (3H, t, *J* = 7.6 Hz, CH₃), 2.05 (1H, s, OH), 3.21 (2H, q, *J* = 7.6 Hz, CH₂), 6.58–6.64 (2H, m, C7-Ph 3,5-H), 7.15–7.18 (1H, m, C6-Ph C-4), 7.19–7.24 (4H, m, C6-Ph 3,5-H and C7-Ph 2,6-H), 7.40–7.45 (3H, m, C6-Ph 2,6-H and N-Ph 4-H), 7.49–7.53 (2H, m, N-Ph 3,5-H), 7.85–7.90 (2H, m, N-Ph 2,6-H), 8.60 (1H, s, 3-H). ¹³C NMR (176 MHz, CDCl₃): δ 13.6 (CH₃), 30.1 (CH₂), 115.3 (C7-Ph C-3,5), 118.9 (C-3a), 120.7 (C-7), 121.6 (N-Ph C-2,6), 122.0 (C-3), 126.9 (N-Ph C-3,5), 127.3 (C7-Ph C-1), 127.7 (C6-Ph C-3,5), 128.6 (N-Ph C-4), 130.6 (C6-Ph C-2,6), 132.1 (C7-Ph C-2,6), 140.0 (N-Ph C-1), 140.8 (C7-Ph C-1), 148.9 (C-6), 152.1 (C-7a), 155.3 (C7-Ph C-4), 159.0 (C-4). ¹⁵N NMR (71 MHz, CDCl₃): δ -147.5 (N-2), -98.5 (N-1), -85.0 (N-5). IR (KBr, ν, cm⁻¹): 3147 (OH), 3064 (CH_{arom}), 2963, 2932, 2873 (CH_{aliph}), 1613, 1586, 1507, 1481, 1267, 1209 (C=C, C=N, C-N), 1040 (C-O), 816, 760, 695 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): *m/z* (%): 391 ([M+H]⁺, 98). HRMS (ESI) for C₂₆H₂₂N₃O ([M+H]⁺): calcd 392.1757, found 392.1757.

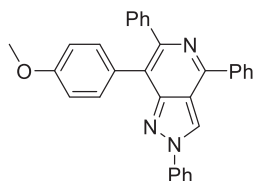
4-Isopropyl-7-(4-methoxyphenyl)-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (47)



Prepared in accordance to general procedure (E) from 7-iodo-4-isopropyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**21**) (100 mg, 0.23 mmol), 4-methoxyphenylboronic acid (42 mg, 0.27 mmol), Cs₂CO₃ (148 mg, 0.46 mmol) and Pd(OAc)₂ (4 mg, 0.015 mmol), EtOH (1.5 mL) and water (0.5 mL). The reaction was finished after 1 h. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:8 v/v). Yield: 80 mg (83%), white crystals, mp = 159–160 °C, *R*_f = 0.54 (EtOAc/Hex, 1:3 v/v). ¹H NMR (700 MHz, CDCl₃): δ 1.55 (6H, d, *J* = 7.0 Hz, CH-(CH₃)₂), 3.53 (1H, p, *J* = 7.0 Hz, CH-(CH₃)₂), 3.83 (3H, s, OCH₃), 6.86–6.91 (2H, m, C7-Ph 3,5-H), 7.20–7.24 (1H, m, C6-Ph 4-H), 7.24–7.28 (2H, m, C6-Ph 3,5-H), 7.39–7.45 (3H, m, C7-Ph 2,6-H and N-Ph 4-H), 7.50–7.55 (4H, m, C6-Ph 2,6-H and N-Ph 3,5-H), 7.89–7.94 (2H, m, N-Ph 2,6-H), 8.63 (1H, s, 3-H). ¹³C NMR (176 MHz, CDCl₃): δ 22.0 (CH-(CH₃)₂), 36.2 (CH-(CH₃)₂), 55.3 (OCH₃), 113.7 (C7-Ph C-3,5), 118.1 (C-3a), 120.3 (C-7), 121.2 (C-3), 121.5 (N-Ph C-2,6), 127.0 (C6-Ph C-4), 127.8 (C6-Ph C-3,5), 128.5 (N-Ph C-4), 128.6 (C7-Ph C-1), 129.7 (N-Ph C-3,5), 131.0 (C6-Ph C-2,6), 132.4 (C7-Ph C-2,6), 140.4 (N-Ph C-1), 141.4 (C6-Ph C-1),

148.5 (C-6), 152.6 (C-7a), 158.7 (C7-Ph C-4), 162.5 (C-4). ^{15}N NMR (71 MHz, CDCl_3): δ -147.9 (N-2), -96.8 (N-1), -83.9 (N-5). IR (ν , cm^{-1}): 3136, 3041 (CH_{arom}), 2961, 2926, 2869 (CH_{aliph}), 1587, 1547, 1506, 1482, 1464, 1379, 1288, 1242 (C=C, C=N, C-N), 1212, 1177, 1031 (C-O-C), 763, 758, 689 ($\text{CH}=\text{CH}$ of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 421 ($[\text{M}+2\text{H}]^+$, 97.4). HRMS (ESI) for ($[\text{M}+\text{H}]^+$): calcd 420.2070, found 420.2070.

7-(4-Methoxyphenyl)-2,4,6-triphenyl-2H-pyrazolo[4,3-c]pyridine (48)

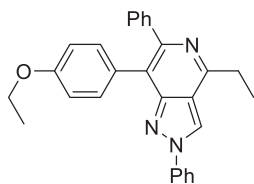


Prepared in accordance to general procedure (E) from 7-iodo-2,4,6-triphenyl-2H-pyrazolo[4,3-c]pyridine (**22**) (64 mg, 0.135 mmol), (4-methoxyphenyl)boronic acid (25 mg, 0.16 mmol), Cs_2CO_3 (88 mg, 0.27 mmol) and $\text{Pd}(\text{OAc})_2$ (2 mg, 0.009 mmol), EtOH (0.9 mL) and water (0.3 mL). The reaction was finished after 45 min. The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:10 v/v). Yield: 38 mg (62%), yellow crystals, mp = 246–247 °C, R_f = 0.54 (EtOAc/Hex, 1:3 v/v). ^1H NMR (700 MHz, CDCl_3): δ 3.84 (3H, s, CH_3), 6.88–6.92 (2H, m, C7-Ph C-3,5), 7.23–7.26 (1H, m, C6-Ph 4-H), 7.26–7.31 (2H, m, 3,5-H), 7.41–7.44 (1H, m, N-Ph 4-H), 7.48–7.54 (5H, m, C4-Ph 4-H; C7-Ph 2,6-H; N-Ph 3,5-H), 7.55–7.62 (4H, m, C6-Ph 2,6-H; C4-Ph 3,5-H), 7.91–7.96 (2H, m, N-Ph 2,6-H), 8.14–8.20 (2H, m, C4-Ph 2,6-H), 8.80 (1H, s, 3-H). ^{13}C NMR (176 MHz, CDCl_3): δ 55.2 (CH_3), 113.5 (C7-Ph C-3,5), 118.1 (C-3a), 121.2 (C-7), 121.4 (N-Ph C-2,6), 122.3 (C-4), 127.0 (C6-Ph C-4), 128.1 (C7-Ph C-1), 128.3 (C4-Ph C-2,6), 128.6 (N-Ph C-4), 128.8 (C4-Ph C-3,5), 129.4 (C4-Ph C-4), 129.6 (N-Ph C-3,5), 130.6 (C6-Ph C-2,6), 132.4 (C7-Ph C-2,6), 139.7 (C4-Ph C-1), 140.1 (N-Ph C-1), 141.1 (C6-Ph C-1), 149.2 (C-6), 152.8 (C-7a), 153.4 (C-4), 158.8 (C7-Ph C-4). ^{15}N NMR (71 MHz, CDCl_3): δ -145.6 (N-2), -96.6 (N-1), N-5 not found. IR (KBr, ν , cm^{-1}): 3056 (CH_{arom}), 2924, 2833 (CH_{aliph}), 1609, 1504, 1463, 1353, 1249, 1175 (C=C, C=N, C-N), 1038 (C-O), 838, 754, 695, 687 ($\text{CH}=\text{CH}$ of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 454 ($[\text{M}+\text{H}]^+$, 96). HRMS (ESI) for $\text{C}_{31}\text{H}_{24}\text{N}_3\text{O}$ ($[\text{M}+\text{H}]^+$): calcd 454.1914, found 454.1914.

General procedure (F) of 4-(4-ethyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-yl)phenol alkylation 49-51

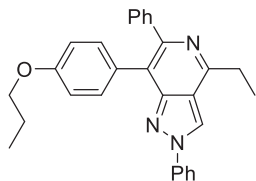
4-(4-Ethyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-yl)phenol (**46**) (1 equiv) was dissolved in DMF. Then NaH (60% in mineral oil) (1.1 equiv) was added at room temperature. Then, the appropriate iodide (1.1 equiv) was added at 70 °C, and the mixture was stirred for 1 h. Upon completion (monitored by TLC), the reaction mixture was cooled to room temperature and was diluted with water (20 mL) and extracted with EtOAc (3 $\times$ 25 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4 , filtered and evaporated under reduced pressure. The residue was purified by column chromatography.

7-(4-Ethoxyphenyl)-4-ethyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (49)



Prepared in accordance to general procedure (F) from (4-ethyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)phenol (**46**) (60 mg, 0.15 mmol), NaH (60%) (7 mg, 0.17 mmol), ethyl iodide (0.014 mL, 0.17 mmol) and DMF (2 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:4 v/v). Yield: 62 mg (97%), yellow crystals, mp = 140–141 °C, R_f = 0.38 (EtOAc/Hex, 1:3 v/v). ^1H NMR (700 MHz, CDCl_3): δ 1.42 (3H, t, J = 7.0 Hz, OCH_2CH_3), 1.52 (3H, t, J = 7.6 Hz, CH_2CH_3), 3.20 (2H, q, J = 7.6 Hz, CH_2CH_3), 4.04 (2H, q, OCH_2CH_3), 6.82–6.88 (2H, m, C7-Ph 3,5-H), 7.19–7.23 (1H, m, C6-Ph 4-H), 7.23–7.27 (2H, m, C6-Ph 3,5-H), 7.37–7.43 (3H, m, N-Ph 4-H and C6-Ph 2,6-H), 7.47–7.52 (4H, m, N-Ph 3,5-H and C7-Ph 2,6-H), 7.88–7.93 (2H, m, N-Ph 2,6-H), 8.60 (1H, s, 3-H). ^{13}C NMR (176 MHz, CDCl_3): δ 13.4 (CH_2CH_3), 14.9 (OCH_2CH_3), 30.3 (CH_2CH_3), 63.2 (OCH_2CH_3), 114.0 (C7-Ph C-3,5), 119.0 (C-3a), 120.4 (C-7), 121.2 (C-3), 121.3 (N-Ph C-2,6), 126.8 (C6-Ph C-4), 127.7 (C6-Ph C-3,5), 128.1 (C7-Ph C-1), 128.4 (N-Ph C-4), 129.5 (N-Ph C-3,5), 130.6 (C6-Ph C-2,6), 132.3 (C7-Ph C-2,6), 140.1 (N-Ph C-1), 141.2 (C6-Ph C-1), 148.8 (C-6), 152.0 (C-7a), 158.0 (C7-Ph C-4), 158.8 (C-4). ^{15}N NMR (71 MHz, CDCl_3): δ -147.6 (N-2), -96.5 (N-1), -83.8 (N-5). IR (KBr, ν , cm^{-1}): 3059 (CH_{arom}), 2983, 2930 (CH_{aliph}), 1587, 1506, 1479, 1375, 1244, 1179 (C=C, C=N, C-N), 1047 (C-O), 826, 754, 670, 686 (CH=CH of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 419 ($[\text{M}+\text{H}]^+$, 97). HRMS (ESI) for $\text{C}_{28}\text{H}_{26}\text{N}_3\text{O}$ ($[\text{M}+\text{H}]^+$): calcd 420.2070, found 420.2070.

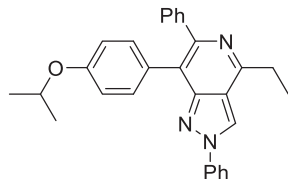
4-Ethyl-2,6-diphenyl-7-(4-propoxyphenyl)-2*H*-pyrazolo[4,3-*c*]pyridine (50)



Prepared in accordance to general procedure (F) from (4-ethyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)phenol (**46**) (60 mg, 0.15 mmol), NaH (60%) (7 mg, 0.17 mmol), 1-iodo propane (0.016 mL, 0.17 mmol) and DMF (2 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:6 v/v). Yield: 64 mg (96%), yellow crystals, mp = 117–118 °C, R_f = 0.41 (EtOAc/Hex, 1:3 v/v). ^1H NMR (700 MHz, CDCl_3): δ 1.04 (3H, t, J = 7.4 Hz, $\text{OCH}_2\text{CH}_2\text{CH}_3$), 1.52 (3H, t, J = 7.6 Hz, CH_2CH_3), 1.81 (2H, hept, J = 7.1 Hz, $\text{OCH}_2\text{CH}_2\text{CH}_3$), 3.20 (2H, q, J = 7.6 Hz, CH_2CH_3), 3.92 (2H, t, J = 6.6 Hz, $\text{OCH}_2\text{CH}_2\text{CH}_3$), 6.83–6.87 (2H, m, C7-Ph 3,5-H), 7.19–7.22 (1H, m, C6-Ph 4-H), 7.23–7.27 (2H, m, C6-Ph 3,5-H), 7.37–7.42 (3H, m, C7-Ph 2,6-H and N-Ph 4-H), 7.47–7.51 (4H, m, N-Ph 3,5-H and C6-Ph 2,6-H), 7.88–7.92 (2H, m, N-Ph 2,6-H), 8.59 (1H, s, 3-H). ^{13}C NMR (176 MHz, CDCl_3): δ 10.5 ($\text{OCH}_2\text{CH}_2\text{CH}_3$), 13.4 (CH_2CH_3), 22.6 ($\text{OCH}_2\text{CH}_2\text{CH}_3$), 30.3 (CH_2CH_3), 69.3 ($\text{OCH}_2\text{CH}_2\text{CH}_3$), 114.0 (C7-Ph C-3,5), 119.0 (C-3a), 120.4 (C-7), 121.2 (C-3), 121.3 (N-Ph C-2,6), 126.8 (C6-Ph C-4), 127.7 (C6-Ph C-3,5), 128.0 (C7-Ph C-1), 128.3 (N-Ph C-4), 129.5 (N-Ph C-3,5), 130.6 (C6-Ph C-2,6), 132.3 (C7-Ph C-2,6), 140.1 (N-Ph C-1), 141.2 (C6-Ph C-1), 148.8 (C-6), 152.0 (C-7a), 158.2 (C7-Ph C-4), 158.8

(C-4). ^{15}N NMR (71 MHz, CDCl_3): δ -147.5 (N-2), -96.2 (N-1), -83.5 (N-5). IR (KBr, ν , cm^{-1}): 3045 (CH_{arom}), 2970, 2931, 2872 (CH_{aliph}), 1609, 1588, 1508, 1250, 1242, 1176 ($\text{C}=\text{C}$, $\text{C}=\text{N}$, $\text{C}-\text{N}$), 1041 ($\text{C}-\text{O}$), 758, 697, 687 ($\text{CH}=\text{CH}$ of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 433 ($[\text{M}+\text{H}]^+$, 95). HRMS (ESI) for $\text{C}_{29}\text{H}_{28}\text{N}_3\text{O}$ ($[\text{M}+\text{H}]^+$): calcd 434.2227, found 434.2227.

4-Ethyl-7-(4-isopropoxyphenyl)-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine (51)



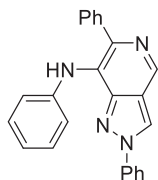
Prepared in accordance to general procedure (F) from (4-ethyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-yl)phenol (**46**) (60 mg, 0.15 mmol), NaH (60%) (7 mg, 0.17 mmol), 2-iodo propane (0.016 mL, 0.17 mmol) and DMF (2 mL). The desired compound was obtained after purification by column chromatography (EtOAc/Hex, 1:6 v/v). Yield: 52 mg (80%), yellow crystals, mp = 139–140 °C, R_f = 0.41 (EtOAc/Hex, 1:3 v/v). ^1H NMR (700 MHz, CDCl_3): δ 1.35 (6H, d, J = 6.1 Hz, $\text{CH}(\text{CH}_3)_2$), 1.53 (3H, t, J = 7.6 Hz, CH_2CH_3), 3.21 (2H, q, J = 7.6 Hz, CH_2CH_3), 4.56 (1H, hept, J = 6.1 Hz, CH), 6.82–6.86 (2H, m, C7-Ph 3,5-H), 7.20–7.23 (1H, m, C6-Ph 4-H), 7.24–7.27 (2H, m, C6-Ph 3,5-H), 7.38–7.43 (3H, m, N-Ph 4-H and C6-Ph 2,6-H), 7.48–7.53 (4H, m, C6-Ph 2,6-H and N-Ph 3,5-H), 7.89–7.94 (2H, m, N-Ph 2,6-H), 8.61 (1H, s, 3-H). ^{13}C NMR (176 MHz, CDCl_3): δ 13.4 (CH_2CH_3), 22.1 ($\text{CH}(\text{CH}_3)_2$), 30.3 (CH_2CH_3), 69.7 (OCH_2), 115.3 (C7-Ph C-3,5), 119.0 (C-3a), 120.4 (C-7), 121.25 (C-3), 121.29 (N-Ph C-2,6), 126.8 (C6-Ph C-4), 127.7 (C6-Ph C-3,5), 127.9 (C7-Ph C-1), 128.4 (N-Ph C-4), 129.5 (N-Ph C-3,5), 130.6 (C6-Ph C-2,6), 132.3 (C7-Ph C-2,6), 140.2 (N-Ph C-1), 141.2 (C6-Ph C-1), 148.8 (C-6), 152.0 (C-7a), 156.9 (C7-Ph C-4), 158.8 (C-4). ^{15}N NMR (71 MHz, CDCl_3): δ -147.5 (N-2), -96.2 (N-1), -83.6 (N-5). IR (KBr, ν , cm^{-1}): 3124, 3063 (CH_{arom}), 2971, 2933 (CH_{aliph}), 1608, 1588, 1507, 1280, 1238, 1182 ($\text{C}=\text{C}$, $\text{C}=\text{N}$, $\text{C}-\text{N}$), 1036 ($\text{C}-\text{O}$), 765, 702, 694 ($\text{CH}=\text{CH}$ of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 433 ($[\text{M}+\text{H}]^+$, 97). HRMS (ESI) for $\text{C}_{29}\text{H}_{28}\text{N}_3\text{O}$ ($[\text{M}+\text{H}]^+$): calcd 434.2227, found 434.2227.

General procedure (G) for the synthesis of 7-substituted pyrazolo[4,3-c]pyridine derivatives (**52**–**67**) by Buchwald-Hartwig cross-coupling with anilines

Appropriate 7-iodo-2H-pyrazolo[4,3-c]pyridine (**18**) or (**19**) (1 eq) was dissolved in dry dioxane. Then appropriate aniline (1.1 eq), NaOtBu (1.6 eq), SPhos (0.3 eq) and $\text{Pd}(\text{OAc})_2$ (0.1 eq) were added to the solution under argon atmosphere. The mixture was stirred at 120 °C under microwave irradiation (280 W, 300 Pa) for 1 h. Upon completion (monitored by TLC), the reaction mixture was cooled to room temperature, filtered through a pad of Celite, and the filter cake was washed with EtOAc (20 mL). The filtrate was diluted with water (20 mL) and extracted with EtOAc (3 $\times$ 25 mL). The combined organic layers were washed with brine (10 mL),

dried over Na₂SO₄ and evaporated under reduced pressure. The residue was purified by column chromatography.

***N*,2,6-Triphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine (52)**

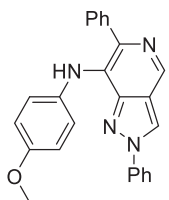


Prepared in accordance to general procedure (G) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (60 mg, 0.15 mmol), aniline (0.015 mL, 0.16 mmol), NaOtBu (23 mg, 0.24 mmol), SPhos (18.6 mg, 0.045 mmol) and Pd(OAc)₂ (3.4 mg, 0.015 mmol) and dioxane (4.5 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:3 to 1:2, v/v).

Yield: 50 mg (92%), orange crystals, mp = 234–235 °C, *R*_f = 0.11 (EtOAc/Hex, 1:3).

¹H NMR (700 MHz, CDCl₃): δ 6.17 (1H, s, NH), 6.80–6.89 (3H, m, NH-Ph 2,4,6-H), 7.10–7.17 (2H, m, NH-Ph 3,5-H), 7.24–7.29 (1H, m, C-Ph 4-H), 7.33–7.38 (2H, m, C-Ph 3,5-H), 7.41–7.45 (1H, m, N-Ph 4-H), 7.48–7.54 (2H, m, N-Ph 3,5-H), 7.69–7.77 (2H, m, C-Ph 2,6-H), 7.82–7.90 (2H, m, N-Ph 2,6-H), 8.59 (1H, s, 3-H), 9.07 (1H, s, 4-H). ¹³C NMR (176 MHz, CDCl₃): δ 117.4 (NH-Ph C-2,6), 120.5 (NH-Ph C-4), 121.1 (C-3a), 121.2 (N-Ph C-2,6), 121.6 (C-3), 124.3 (C-7), 127.6 (C-Ph C-4), 128.4 (C-Ph C-3,5), 128.5 (NH-Ph C-3,5), 128.7 (N-Ph C-4), 128.8 (C-Ph C-2,6), 129.7 (N-Ph C-3,5), 139.2 (C-6), 139.9 (C-Ph C-1), 140.8 (N-Ph C-1), 141.1 (C-4), 143.5 (NH-Ph C-1), 147.5 (C-7a). ¹⁵N NMR (71 MHz, CDCl₃): δ –304.0 (NH), –147.3 (N-2), –100.5 (N-1), –78.1 (N-5). IR (ν, cm^{–1}): 3299 (NH), 3059, 3020 (CH_{arom}), 1601, 1590, 1497, 1426, 1346, 1202 (C=C, C=N, C–N), 751, 692, 684 (CH=CH of monosubstituted benzenes). MS (ES⁺): *m/z* (%): 363 ([M+H]⁺, 100). HRMS (ESI) for C₂₄H₁₉N₄ ([M+H]⁺): calcd 363.1606, found 363.1604.

***N*-(4-Methoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine (53)**

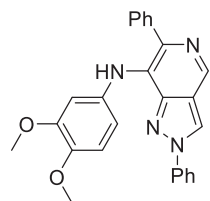


Prepared in accordance to general procedure (G) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (60 mg, 0.15 mmol), 4-methoxyaniline (20 mg, 0.16 mmol), NaOtBu (23 mg, 0.24 mmol), SPhos (18.6 mg, 0.045 mmol) and Pd(OAc)₂ (3.4 mg, 0.015 mmol) and dioxane (4.5 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:3 to 1:2, v/v).

Yield: 29 mg (48%), red crystals, mp = 135–136 °C, *R*_f = 0.08 (EtOAc/Hex, 1:3). ¹H NMR (500 MHz, CDCl₃): δ 3.74 (3H, s, OCH₃), 6.19 (1H, s, NH), 6.68–6.72 (2H, m, NH-Ph 3,5-H), 6.81–6.85 (2H, m, NH-Ph 2,6-H), 7.24–7.29 (1H, m, C-Ph 4-H), 7.33–7.37 (2H, m, C-Ph 3,5-H), 7.42–7.45 (1H, m, N-Ph 4-H), 7.50–7.54 (2H, m, N-Ph 3,5-H), 7.67–7.71 (2H, m, C-Ph 2,6-H), 7.83–7.87 (2H, m, N-Ph 2,6-H), 8.63 (1H, s, 3-H), 9.05 (1H, s, 4-H). ¹³C NMR (126 MHz, CDCl₃): δ 55.7 (OCH₃), 113.9 (NH-Ph C-3,5), 120.3 (NH-Ph C-2,6), 120.8 (C-3a), 121.2 (N-Ph C-2,6), 122.2 (C-3), 126.2 (C-7), 127.8 (C-Ph C-4), 128.6 (C-Ph C-3,5), 128.8 (C-Ph C-2,6), 128.9 (N-Ph C-4), 129.8 (N-Ph C-3,5), 136.5 (NH-Ph C-1), 137.7 (C-6), 138.4 (C-Ph C-1), 139.3 (C-4), 139.9 (N-Ph C-1), 146.9 (C-7a), 154.8 (NH-Ph C-4).

IR (ν , cm^{-1}): 3376 (NH), 3056, 3025 (CH_{arom}), 2923, 2852 (CH_{aliph}), 1595, 1505, 1464, 1234, 1212 ($\text{C}=\text{C}$, $\text{C}=\text{N}$, $\text{C}-\text{N}$), 1033 ($\text{C}-\text{O}-\text{C}$), 755, 699, 688 ($\text{CH}=\text{CH}$ of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 393 ($[\text{M}+\text{H}]^+$, 100). HRMS (ESI) for $\text{C}_{25}\text{H}_{21}\text{N}_4\text{O}$ ($[\text{M}+\text{H}]^+$): calcd 393.1710, found 393.1710.

***N*-(3,4-Dimethoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine (54)**

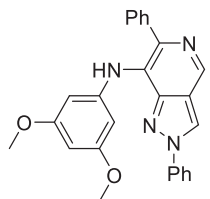


Prepared in accordance to general procedure (G) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (60 mg, 0.15 mmol), 4-methoxyaniline (20 mg, 0.16 mmol), NaOtBu (23 mg, 0.24 mmol), SPhos (18.6 mg, 0.045 mmol) and $\text{Pd}(\text{OAc})_2$ (3.4 mg, 0.015 mmol) and dioxane (4.5 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:3 to 1:2,

v/v).

Yield: 55 mg (86%), orange crystals, mp = 93–94 °C, R_f = 0.20 (EtOAc/Hex, 1:1). ^1H NMR (700 MHz, CDCl_3): δ 3.66 (3H, s, 3- OCH_3), 3.80 (3H, s, 4- OCH_3), 6.21 (1H, s, NH), 6.41–6.48 (2H, m, NH-Ph 2,6-H), 6.65–6.70 (1H, m, NH-Ph 3-H), 7.22–7.28 (1H, m, C-Ph 4-H), 7.31–7.36 (2H, m, C-Ph 3,5-H), 7.41–7.45 (1H, m, N-Ph 4-H), 7.49–7.54 (2H, m, N-Ph 3,5-H), 7.71–7.77 (2H, m, C-Ph 2,6-H), 7.83–7.90 (2H, m, N-Ph 2,6-H), 8.57 (1H, s, 3-H), 9.02 (1H, s, 4-H). ^{13}C NMR (176 MHz, CDCl_3): δ 55.8 (3- OCH_3), 56.4 (4- OCH_3), 103.4 (NH-Ph C-2), 110.2 (NH-Ph C-6), 111.8 (NH-Ph C-5), 121.0 (C-3a), 121.2 (N-Ph C-2,6), 121.7 (C-3), 125.3 (C-7), 127.6 (C-Ph C-4), 128.5 (C-Ph C-3,5), 128.7 (N-Ph C-4), 128.8 (N-Ph C-2,6), 129.8 (N-Ph C-3,5), 137.3 (NH-Ph C-1), 139.0 (C-6), 139.6 (N-Ph C-1), 140.0 (N-Ph C-1), 140.1 (C-4), 143.8 (NH-Ph C-4), 147.3 (C-7a), 149.0 (NH-Ph C-3). ^{15}N NMR (71 MHz, CDCl_3): δ -306.4 (NH), -148.2 (N-2), -101.4 (N-1), -77.3 (N-5). IR (ν , cm^{-1}): 3363 (NH), 3055, 2995 (CH_{arom}), 2929, 2831 (CH_{aliph}), 1596, 1509, 1439, 1228 ($\text{C}=\text{C}$, $\text{C}=\text{N}$, $\text{C}-\text{N}$), 1024 ($\text{C}-\text{O}-\text{C}$), 756, 696 ($\text{CH}=\text{CH}$ of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 423 ($[\text{M}+\text{H}]^+$, 100). HRMS (ESI) for $\text{C}_{26}\text{H}_{23}\text{N}_4\text{O}_2$ ($[\text{M}+\text{H}]^+$): calcd 423.1816, found 423.1816.

***N*-(3,5-Dimethoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine (55)**



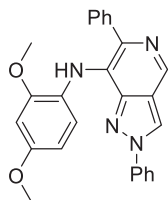
Prepared in accordance to general procedure (G) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (60 mg, 0.15 mmol), 3,5-dimethoxyaniline (25 mg, 0.16 mmol), NaOtBu (23 mg, 0.24 mmol), SPhos (18.6 mg, 0.045 mmol) and $\text{Pd}(\text{OAc})_2$ (3.4 mg, 0.015 mmol) and dioxane (4.5 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:3 to 1:2,

v/v).

Yield: 38 mg (60%), brown crystals, mp = 140–141 °C, R_f = 0.28 (Hex:EtOAc 1:1). ^1H NMR (700 MHz, CDCl_3): δ 3.64 (3H, s, OCH_3), 5.98–6.01 (1H, m, NH-Ph 4-H), 6.01–6.05 (2H, m, NH-Ph 2,6-H), 6.17 (1H, s, NH), 7.27–7.30 (1H, m, C-Ph 4-H), 7.35–7.39 (2H, m, C-Ph 3,5-H), 7.42–7.45 (1H, m, N-Ph 4-H), 7.50–7.54 (2H, m, N-Ph 3,5-H), 7.74–7.78 (2H, m, C-Ph 2,6-H), 7.85–7.91 (2H, m, N-Ph 2,6-H),

8.58 (1H, s, 3-H), 9.08 (1H, s, 4-H). ^{13}C NMR (176 MHz, CDCl_3): δ 55.2 (OCH_3), 93.2 (NH-Ph C-4), 95.7 (NH-Ph C-2,6), 121.0 (C-3a), 121.2 (N-Ph C-2,6), 121.8 (C-4), 124.0 (C-7), 127.7 (C-Ph C-4), 128.4 (C-Ph C-3,5), 128.70 (N-Ph C-4), 128.74 (C-Ph C-2,6), 129.7 (N-Ph C-3,5), 139.2 (C-Ph C-1), 139.9 (N-Ph C-1), 141.3 (C-6), 141.5 (C-4), 145.6 (NH-Ph C-1), 147.6 (C-7a), 161.1 (NH-Ph C-3,5). ^{15}N NMR (71 MHz, CDCl_3): -302.6 (NH), -146.9 (N-2), -101.7 (N-1), -79.3 (N-5). IR (ν , cm^{-1}): 3376 (NH), 3057, 2998 (CH_{arom}), 2933, 2837 (CH_{aliph}), 159, 1480, 1461, (C=C, C=N, C-N), 1199, 1148, 1054 (C-O-C), 756, 696, 686 (CH=CH of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 423 ($[\text{M}+\text{H}]^+$, 99,7). HRMS (ESI) $\text{C}_{26}\text{H}_{23}\text{N}_4\text{O}_2$ ($[\text{M}+1]^+$): calcd 423.1816, found 423.1816.

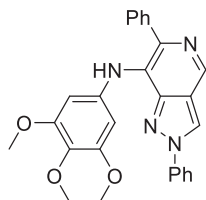
***N*-(2,4-Dimethoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine (56)**



Prepared in accordance to general procedure (G) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (60 mg, 0.15 mmol), 2,4-dimethoxyaniline (25 mg, 0.16 mmol), NaOtBu (23 mg, 0.24 mmol), SPhos (18.6 mg, 0.045 mmol) and $\text{Pd}(\text{OAc})_2$ (3.4 mg, 0.015 mmol) and dioxane (4.5 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:3 to 1:2, v/v).

Yield: 40 mg (63%), brown crystals, mp = 108–110 °C, R_f = 0.28 (Hex:EtOAc 1:1). ^1H NMR (700 MHz, CDCl_3): δ 3.74 (3H, s, OCH_3), 3.83 (3H, s, OCH_3), 6.18–6.24 (1H, m, NH-Ph 4-H), 6.32 (1H, s, NH), 6.44–6.51 (1H, m, NH-Ph 3-H), 6.65–6.73 (1H, m, NH-Ph 6-H), 7.24–7.28 (1H, m, C-Ph 4-H), 7.32–7.37 (2H, m, C-Ph 3,5-H), 7.38–7.43 (1H, m, N-Ph 4-H), 7.47–7.53 (2H, m, N-Ph 3,5-H), 7.68–7.76 (2H, m, C-Ph 2,6-H), 7.83–7.91 (2H, m, N-Ph 2,6-H), 8.55 (1H, s, 3-H), 9.00 (1H, s, 4-H). ^{13}C NMR (176 MHz, CDCl_3): δ 55.7 (2,4- OCH_3), 98.9 (NH-Ph C-3), 103.3 (NH-Ph C-5), 117.3 (NH-Ph C-6), 121.1 (C-3a), 121.2 (N-Ph C-2,6), 121.5 (C-3), 125.4 (C-7), 126.7 (NH-Ph C-1), 127.5 (C-Ph C-4), 128.4 (C-Ph C-3,5), 128.6 (N-Ph C-4), 128.9 (C-Ph C-2,6), 129.7 (N-Ph C-3,5), 139.6 (C-Ph C-1), 139.8 (C-6), 140.1 (C-4 and N-Ph C-1), 147.5 (C-7a), 150.1 (NH-Ph C-2), 154.4 (NH-Ph C-4). IR (ν , cm^{-1}): 3381 (NH), 3056, 2999 (CH_{arom}), 2932, 2832 (CH_{aliph}), 1595, 1512, 1463, 1283, 1203 (C=C, C=N, C-N), 1155, 1030 (C-O-C), 755, 699, 689 (CH=CH of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 423 ($[\text{M}+\text{H}]^+$, 100). HRMS (ESI) $\text{C}_{26}\text{H}_{23}\text{N}_4\text{O}_2$ ($[\text{M}+1]^+$): calcd 423.1816, found 423.1816.

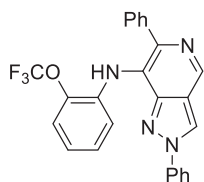
***N*-(3,4,5-Trimethoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine (57)**



Prepared in accordance to general procedure (G) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (60 mg, 0.15 mmol), 3,4,5-trimethoxyaniline (29 mg, 0.16 mmol), NaOtBu (23 mg, 0.24 mmol), SPhos (18.6 mg, 0.045 mmol) and $\text{Pd}(\text{OAc})_2$ (3.4 mg, 0.015 mmol) and dioxane (4.5 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:3 to 1:2, v/v).

Yield: 65 mg (95%), orange crystals, mp = 90–91 °C, R_f = 0.21 (Hex:EtOAc 1:1). ^1H NMR (700 MHz, CDCl_3): δ 3.65 (6H, s, 3,5- OCH_3), 3.75 (3H s, 4- OCH_3), 6.02–6.12 (2H, m, NH-Ph 2,6-H), 6.27 (1H, s, NH), 7.23–7.27 (1H, m, C-Ph 4-H), 7.32–7.37 (2H, m, C-Ph 3,5-H), 7.42–7.45 (1H, m, N-Ph 4-H), 7.50–7.55 (2H, m, N-Ph C-3,5), 7.72–7.79 (2H, m, C-Ph 2,6-H), 7.83–7.91 (2H, m, N-Ph 2,6-H), 8.57 (1H, s, 3-H), 9.04 (1H, s, 4-H). ^{13}C NMR (176 MHz, CDCl_3): δ 56.0 (3,5- OCH_3), 61.1 (OCH_3), 95.6 (NH-Ph C-2,6), 121.0 (C-3a), 121.2 (N-Ph C-2,6), 121.9 (C-3), 124.6 (C-7), 127.7 (C-Ph C-4), 128.5 (C-Ph C-3,5), 128.8 (C-Ph C-2,6), 128.9 (N-Ph C-4), 129.9 (N-Ph C-3,5), 132.4 (NH-Ph C-4), 139.4 (NH-Ph C-1), 139.6 (C-6 and C-Ph C-1), 140.0 (N-Ph C-1), 140.7 (C-4), 147.6 (C-7a), 153.2 (NH-Ph C-3,5). ^{15}N NMR (71 MHz, CDCl_3): δ -304.0 (NH), -147.3 (N-2), -101.7 (N-1), -76.9 (N-5). IR (ν , cm^{-1}): 3345 (NH), 3120, 3057 (CH_{arom}), 2930, 2838 (CH_{aliph}), 1595, 1501, 1463, 1409, 1231 (C=C, C=N, C-N), 1122, 1006 (C-O-C), 804, 756, 697, 689 (CH=CH of mono- and trisubstituted benzenes). MS (ES^+): m/z (%): 453 ($[\text{M}+\text{H}]^+$, 98). HRMS (ESI) $\text{C}_{27}\text{H}_{25}\text{N}_4\text{O}_3$ ($[\text{M}+1]^+$): calcd 453.1921, found 453.1921.

2,6-Diphenyl-*N*-[2-(trifluoromethoxy)phenyl]-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine (58)

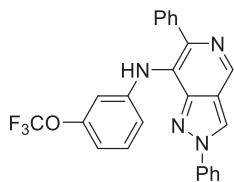


Prepared in accordance to general procedure (G) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (60 mg, 0.15 mmol), 2-(trifluoromethoxy)aniline (28 mg, 0.16 mmol), NaOtBu (23 mg, 0.24 mmol), SPhos (18.6 mg, 0.045 mmol) and $\text{Pd}(\text{OAc})_2$ (3.4 mg, 0.015 mmol) and dioxane (4.5 mL). The residue was purified by column chromatography (EtOAc/Hex,

1:3 to 1:2, v/v).

Yield: 38 mg (56%), yellow crystals, mp = 66–68 °C, R_f = 0.38 (Hex:EtOAc 1:1). ^1H NMR (500 MHz, CDCl_3): δ 6.46 (1H, s, NH), 6.67–6.73 (1H, m, NH-Ph 6-H), 6.75–6.79 (1H, m, NH-Ph 4-H), 6.86–6.91 (1H, m, NH-Ph 5-H), 7.17–7.21 (1H, m, NH-Ph 3-H), 7.24–7.28 (1H, m, C-Ph 4-H), 7.31–7.35 (2H, m, C-Ph 3,5-H), 7.44–7.48 (1H, m, N-Ph 4-H), 7.52–7.57 (2H, m, N-Ph 3,5-H), 7.72–7.76 (2H, m, C-Ph 2,6-H), 7.87–7.92 (2H, m, N-Ph 2,6-H), 8.68 (1H, s, 3-H), 9.18 (1H, s, 4-H). ^{13}C NMR (126 MHz, CDCl_3): δ 117.7 (NH-Ph C-6), 119.9, 120.9, 122.0 (CF_3 , J = 126 Hz), 120.2 (NH-Ph C-4), 120.8 (NH-Ph C-3), 121.3 (N-Ph C-2,6), 122.5 (C-3), 123.5 (C-7), 126.6 (NH-Ph C-5), 128.2 (C-Ph C-4), 128.6 (C-Ph C-3,5), 128.7 (C-Ph C-2,6), 129.1 (N-Ph C-4), 129.9 (N-Ph C-3,5), 135.7 (NH-Ph C-1), 138.3 (C-Ph C-1 and NH-Ph C-2), 139.9 (N-Ph C-1), 140.9 (C-6), 141.7 (C-4), 147.8 (C-7a). IR (ν , cm^{-1}): 3404 (NH), 3060, 3031 (CH_{arom}), 1606, 1501, 1484, 1244, 1213, 1165 (C=C, C=N, C-N, C-F), 751, 697, 687 (CH=CH of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 447 ($[\text{M}+\text{H}]^+$, 97.8). HRMS (ESI) $\text{C}_{25}\text{H}_{18}\text{F}_3\text{N}_4\text{O}$ ($[\text{M}+1]^+$): calcd 447.1427, found 447.1427.

2,6-Diphenyl-*N*-[3-(trifluoromethoxy)phenyl]-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine (59)

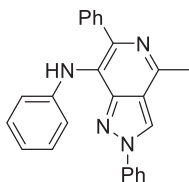


Prepared in accordance to general procedure (G) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (60 mg, 0.15 mmol), 3-(trifluoromethoxy)aniline (28 mg, 0.16 mmol), NaOtBu (23 mg, 0.24 mmol), SPhos (18.6 mg, 0.045 mmol) and Pd(OAc)₂ (3.4 mg, 0.015 mmol) and dioxane (4.5 mL). The residue was purified by column chromatography

(EtOAc/Hex, 1:3 to 1:2, v/v).

Yield: 38 mg (56%) orange crystals, mp = 77–78 °C, *R*_f = 0.38 (Hex:EtOAc 1:1). ¹H NMR (500 MHz, CDCl₃): δ 6.22 (1H, s, NH), 6.65–6.71 (2H, m, NH-Ph 4-H, 2-H or 6-H), 6.72–6.76 (1H, m, NH-Ph 2-H or 6-H), 7.09–7.16 (1H, m, NH-Ph 5-H), 7.28–7.32 (1H, m, C-Ph 4-H), 7.36–7.40 (2H, m, C-Ph 3,5-H), 7.43–7.48 (1H, m, N-Ph 4-H), 7.51–7.56 (2H, m, N-Ph 3,5-H), 7.68–7.75 (2H, m, C-Ph 2,6-H), 7.84–7.90 (2H, m, N-Ph 2,6-H), 8.64 (1H, s, 3-H), 9.14 (1H, s, 4-H). ¹³C NMR (126 MHz, CDCl₃): δ 109.7 (NH-Ph C-2 or C-6), 112.5 (NH-Ph C-5), 115.5 (NH-Ph C-2 or C-6), 119.5 (CF₃), 121.1 (C-3a), 121.4 (N-Ph C-2,6), 122.4 (C-3), 123.5 (C-7), 128.1 (C-Ph C-4), 128.7 (C-Ph C-3,5), 128.9 (C-Ph C-2,6), 129.0 (N-Ph C-4), 129.6 (NH-Ph C-5), 129.8 (N-Ph C-3,5), 138.6 (C-Ph C-1), 139.9 (N-Ph C-1), 141.6 (C-6), 142.0 (C-4), 145.2 (NH-Ph C-1), 147.5 (C-7a), 149.8 (NH-Ph C-3). IR (ν, cm⁻¹): 3204 (NH), 3061, 3048 (CH_{arom}), 1610, 1596, 1490, 1486, 1249, 1213, 1153 (C=C, C=N, C-N, C-F), 756, 695 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): *m/z* (%): 447 ([M+H]⁺, 100). HRMS (ESI) C₂₅H₁₈F₃N₄O ([M+1]⁺): calcd 447.1427, found 447.1427.

4-Methyl-*N*-2,6-triphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine (60)

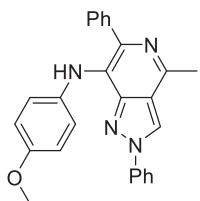


Prepared in accordance to general procedure (G) from 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**19**) (50 mg, 0.12 mmol), aniline (0.012 mL, 0.13 mmol), NaOtBu (18.7 mg, 0.19 mmol), SPhos (15 mg, 0.036 mmol) and Pd(OAc)₂ (1.7 mg, 0.012 mmol) and dioxane (4 mL). The residue was purified by column chromatography (EtOAc/PE, 1:4 to 1:2, v/v).

Yield: 31 mg (65%), light yellow crystals, mp = 165–169 °C. ¹H NMR (700 MHz, CDCl₃): δ 2.87 (3H, s, CH₃), 5.94 (1H, s, NH), 6.81–6.83 (3H, m, NH-Ph 2,4,6-H), 7.13–7.15 (2H, m, NH-Ph 3,5-H), 7.26–7.29 (1H, m, C-Ph 4-H), 7.35–7.37 (2H, m, C-Ph 3,5-H), 7.40–7.42 (1H, m, N-Ph 4-H), 7.48–7.51 (2H, m, N-Ph 3,5-H), 7.72–7.73 (2H, m, C-Ph 2,6-H), 7.85–7.87 (2H, m, N-Ph 2,6-H), 8.57 (s, 1H, 3-H). ¹³C NMR (176 MHz, CDCl₃): δ 22.8 (CH₃), 117.0 (NH-Ph C-2,6), 120.1 (NH-Ph C-4), 121.20 (C-3a), 121.23 (N-Ph C-2,6), 122.0 (C-3), 122.4 (C-7), 127.8 (C-Ph C-4), 128.6 (C-Ph C-3,5), 128.65 (N-Ph C-4), 128.7 (NH-Ph C-3,5), 129.1 (C-Ph C-2,6), 129.7 (N-Ph C-3,5), 139.1 (C-6), 140.0 (N-Ph C-1), 141.6 (C-Ph C-1), 144.5 (NH-Ph C-1), 148.0 (C-7a), 150.4 (C-4). ¹⁵N NMR (71 MHz, CDCl₃): δ -285.3 (NH), -160.2 (N-1), -148.3 (N-2), -82.2 (N-5). IR (KBr, ν, cm⁻¹): 3387 (NH), 3031, 3051 (CH_{arom}), 2985, 2914, 2850 (CH_{aliph}), 1598, 1511, 1499,

1310 (C=C, C=N, C-N), 760, 747, 696 (CH=CH of mono- and disubstituted benzenes). MS m/z (%): 377 ($[M+H]^+$, 100). HRMS (ESI) $C_{25}H_{21}N_4$ ($[M+H]^+$): calcd 377.1761, found 377.1763.

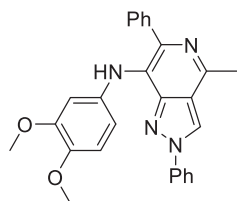
***N*-4-Methoxyphenyl-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine (61)**



Prepared in accordance to general procedure (G) from 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**19**) (50 mg, 0.12 mmol), 4-methoxyaniline (16 mg, 0.13 mmol), NaOtBu (18.7 mg, 0.19 mmol), SPhos (15 mg, 0.036 mmol) and Pd(OAc)₂ (1.7 mg, 0.012 mmol) and dioxane (4 mL). The residue was purified by column chromatography (EtOAc/PE, 1:4 to 1:2, v/v).

Yield: 38 mg (77%), light yellow crystals, mp = 89–90 °C, R_f = 0.11 (EtOAc/Hex, 1:3). ¹H NMR (500 MHz, CDCl₃): δ 2.86 (3H, s, CH₃), 3.74 (3H, s, OCH₃), 5.89 (1H, s, NH), 6.67–6.75 (2H, m, NH-Ph 3,5-H), 6.76–6.85 (2H, m, NH-Ph 2,6-H), 7.26–7.29 (1H, m, C-Ph 4-H), 7.32–7.38 (2H, m, C-Ph 3,5-H), 7.39–7.43 (1H, m, N-Ph 4-H), 7.48–7.53 (2H, m, N-Ph 3,5-H), 7.66–7.73 (2H, m, C-Ph 2,6-H), 7.81–7.88 (2H, m, N-Ph 2,6-H), 8.55 (1H, s, 3-H). ¹³C NMR (126 MHz, CDCl₃): δ 22.8 (CH₃), 55.7 (OCH₃), 114.0 (NH-Ph C-2,6), 119.1 (NH-Ph C-3,5), 121.1 (N-Ph C-2,6), 121.2 (C-3a), 121.7 (C-3), 123.4 (C-7), 127.6 (C-Ph C-4), 128.5 (C-Ph C-3,5), 128.6 (N-Ph C-4), 129.0 (C-Ph C-2,6), 129.7 (N-Ph C-3,5), 138.0 (NH-Ph C-1), 139.3 (C-Ph C-1), 140.0 (N-Ph C-1), 140.3 (C-6), 147.6 (C-7a), 149.3 (C-4), 154.1 (NH-Ph C-4). IR (v, cm⁻¹): 3375 (NH), 3053, 2994 (CH_{arom}), 2947, 2929, 2831 (CH_{aliph}), 1596, 1506, 1488, 1410, 1234, 1212 (C=C, C=N, C-N), 1029 (C-O-C), 756, 696, 689 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): m/z (%): 407 ($[M+H]^+$, 100). HRMS (ESI) for $C_{26}H_{23}N_4O$ ($[M+H]^+$): calcd 407.1866, found 407.1866.

***N*-(3,4-Dimethoxyphenyl)-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine (62)**

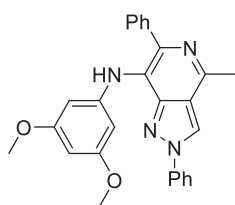


Prepared in accordance to general procedure (G) from 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**19**) (50 mg, 0.12 mmol), 3,4-dimethoxyaniline (20 mg, 0.13 mmol), NaOtBu (18.7 mg, 0.19 mmol), SPhos (15 mg, 0.036 mmol) and Pd(OAc)₂ (1.7 mg, 0.012 mmol) and dioxane (4 mL). The residue was purified by column chromatography (EtOAc/PE, 1:4 to 1:2, v/v).

Yield: 32 mg (60%), brown crystals, mp = 202–203 °C, R_f = 0.08 (EtOAc/Hex, 1:3). ¹H NMR (500 MHz, CDCl₃): δ 2.88 (3H, s, CH₃), 3.67 (3H, s, 3-OCH₃), 3.81 (3H, s, 4-OCH₃), 5.98 (1H, s, NH), 6.38–6.43 (1H, m, NH-Ph 6-H), 6.43–6.46 (1H, m, NH-Ph 2-H), 6.65–6.72 (1H, m, NH-Ph 5-H), 7.23–7.29 (1H, m,

C-Ph 4-H), 7.32–7.37 (2H, m, C-Ph 3,5-H), 7.41–7.45 (1H, m, N-Ph 4-H), 7.49–7.54 (2H, m N-Ph, 3,5-H), 7.70–7.75 (2H, m, C-Ph 2,6-H), 7.84–7.90 (2H, m, N-Ph 2,6-H), 8.57 (1H, s, 3-H). ^{13}C NMR (126 MHz, CDCl_3): δ 22.5 (CH_3), 55.8 (3- OCH_3), 56.4 (4- OCH_3), 103.0 (NH-Ph C-2), 109.6 (NH-Ph C-6), 111.8 (NH-Ph C-5), 121.0 (C-3a), 121.1 (N-Ph C-2,6), 122.0 (C-3), 123.5 (C-7), 127.8 (C-Ph C-4), 128.6 (C-Ph C-3,5), 128.7 (N-Ph C-4), 129.1 (C-Ph C-2,6), 129.8 (N-Ph C-3,5), 138.0 (NH-Ph C-1), 138.8 (N-Ph C-1), 139.4 (C-6), 139.9 (N-Ph C-1), 143.6 (NH-Ph C-4), 147.6 (C-7a), 149.1 (NH-Ph C-3), 149.3 (C-4). IR (v, cm^{-1}): 3335 (NH), 3124, 3000 (CH_{arom}), 2951, 2931, 2830 (CH_{aliph}), 1595, 1526, 1507 1464, 1405, 1220, 1207 (C=C, C=N, C–N), 1153, 1027 (C–O–C), 759, 691 (CH=CH of mono- and trisubstituted benzenes). MS (ES^+): m/z (%): 437 ($[\text{M}+\text{H}]^+$, 100). HRMS (ESI) for $\text{C}_{27}\text{H}_{25}\text{N}_4\text{O}_2$ ($[\text{M}+\text{H}]^+$): calcd 437.1972, found 437.1972.

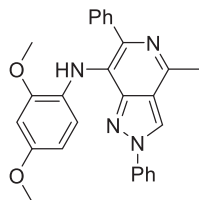
***N*-(3,5-Dimethoxyphenyl)-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine (63)**



Prepared in accordance to general procedure (G) from 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**19**) (50 mg, 0.12 mmol), 3,5-dimethoxyaniline (20 mg, 0.13 mmol), NaOtBu (18.7 mg, 0.19 mmol), SPhos (15 mg, 0.036 mmol) and $\text{Pd}(\text{OAc})_2$ (1.7 mg, 0.012 mmol) and dioxane (4 mL). The residue was purified by column chromatography (EtOAc/PE , 1:4 to 1:2, v/v).

Yield: 31 mg (49%), brown crystals, mp = 157–159 °C, R_f = 0.49 (Hex:EtOAc 1:1). ^1H NMR (500 MHz, CDCl_3): δ 2.89 (3H, s, CH_3), 3.65 (6H, s, OCH_3), 5.95 (1H, s, NH), 5.97–6.02 (3H, m, NH-Ph 2,5,6-H), 7.27–7.32 (1H, m, C-Ph 4-H), 7.35–7.40 (2H, m, C-Ph 3,5-H), 7.40–7.45 (1H, m, N-Ph 4-H), 7.49–7.54 (2H, m, N-Ph 3,5-H), 7.72–7.78 (2H, m, C-Ph 2,6-H), 7.85–7.91 (2H, m, N-Ph 2,6-H), 8.58 (1H, s, 3-H). ^{13}C NMR (126 MHz, CDCl_3): δ 22.7 (CH_3), 55.3 (OCH_3), 92.9 (NH-Ph C-4), 95.4 (NH-Ph C-2,6), 121.1 (C-3a), 121.3 (N-Ph C-2,6), 122.3 (C-3a and C-7), 128.0 (C-Ph C-4), 128.6 (C-Ph C-3,5), 128.8 (N-Ph C-4), 129.1 (C-Ph C-2,6), 129.8 (N-Ph C-3,5), 138.8 (C-Ph C-1), 140.0 (N-Ph C-1), 141.9 (C-6), 146.5 (NH-Ph C-1), 148.3 (C-7a), 151.0 (C-3), 161.2 (NH-Ph C-3,5). IR (v, cm^{-1}): 3394, 3310 (NH), 3058, 3000 (CH_{arom}), 2920, 2851 (CH_{aliph}), 1595, 1516, 1459, 1416, 1378 (C=C, C=N, C–N), 1197, 1144, 1068, 1050 (C–O–C), 818, 763, 753, 688 (CH=CH of mono- and trisubstituted benzenes). MS (ES^+): m/z (%): 437 ($[\text{M}+\text{H}]^+$, 97.3). HRMS (ESI) $\text{C}_{27}\text{H}_{25}\text{N}_4\text{O}_2$ ($[\text{M}+\text{H}]^+$): calcd 437.1972, found 437.1972.

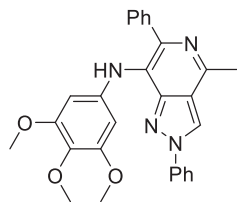
***N*-(2,4-Dimethoxyphenyl)-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine (64)**



Prepared in accordance to general procedure (G) from 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**19**) (50 mg, 0.12 mmol), 3,5-dimethoxyaniline (20 mg, 0.13 mmol), NaOtBu (18.7 mg, 0.19 mmol), SPhos (15 mg, 0.036 mmol) and Pd(OAc)₂ (1.7 mg, 0.012 mmol) and dioxane (4 mL). The residue was purified by column chromatography (EtOAc/PE, 1:4 to 1:2, v/v).

Yield: 39 mg (73%), orange crystals, mp = 92–95 °C, *R*_f = 0.44 (Hex:EtOAc 1:1). ¹H NMR (500 MHz, CDCl₃): δ 2.89 (3H, s, CH₃), 3.74 (3H, s, 4-OCH₃), 3.80 (3H, s, 3-OCH₃), 6.14 (1H, s, NH), 6.18–6.26 (1H, m, NH-Ph 5-H), 6.42–6.51 (1H, m, NH-Ph 3-H), 6.61–6.73 (1H, m, NH-Ph 6-H), 7.24–7.29 (1H, m, C-Ph 4-H), 7.31–7.37 (2H, m, C-Ph 3,5-H), 7.39–7.44 (1H, m, N-Ph 4-H), 7.48–7.54 (2H, m, N-Ph 3,5-H), 7.64–7.74 (2H, m, C-Ph 2,6-H), 7.83–7.94 (2H, m, N-Ph 2,6-H), 8.57 (1H, s, 3-H). ¹³C NMR (126 MHz, CDCl₃): δ 22.8 (Me), 55.66 (4-MeO), 55.69 (2-MeO), 98.9 (NH-Ph C-3), 103.4 (NH-Ph C-5), 116.1 (NH-Ph C-6), 121.1 (C-3a), 121.2 (N-Ph C-2,6), 121.6 (C-3), 123.4 (C-7), 127.6 (C-Ph C-4), 127.8 (NH-Ph C-1), 128.4 (C-Ph C-3,5), 128.5 (N-Ph C-4), 129.0 (C-Ph C-2,6), 129.7 (N-Ph C-3,5), 139.4 (C-Ph C-1), 140.2 (N-Ph C-1), 140.9 (C-6), 148.1 (C-7a), 149.4 (C-4), 149.6 (NH-Ph C-2), 154.0 (NH-Ph C-4). ¹⁵N NMR (51 MHz, CDCl₃): δ -316.2 (NH), -149.2 (N-2), -98.8 (N-5), -83.4 (N-1). IR (ν, cm⁻¹): 3382 (NH), 3053, 2997 (CH_{arom}), 2933, 2832 (CH_{aliph}), 1595, 1511, 1463, 1283, 1204 (C=C, C=N, C-N), 1155, 1029 (C-O-C), 829, 756, 696, 689 (CH=CH of mono- and trisubstituted benzenes). MS (ES⁺): *m/z* (%): 437 ([M+H]⁺, 98,7). HRMS (ESI) C₂₇H₂₅N₄O₂ ([M+1]⁺): calcd 437.1972, found 437.1972.

***N*-(3,4,5-Trimethoxyphenyl)-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine (65)**

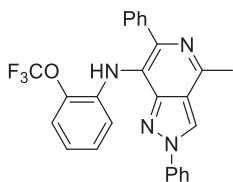


Prepared in accordance to general procedure (G) from 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**19**) (50 mg, 0.12 mmol), 3,4,5-trimethoxyaniline (24 mg, 0.13 mmol), NaOtBu (18.7 mg, 0.19 mmol), SPhos (15 mg, 0.036 mmol) and Pd(OAc)₂ (1.7 mg, 0.012 mmol) and dioxane (4 mL). The residue was purified by column chromatography (EtOAc/PE, 1:4 to 1:2, v/v).

Yield: 36 mg (63%), reddish brown crystals, mp = 107–108 °C, *R*_f = 0.31 (Hex:EtOAc 1:1). ¹H NMR (500 MHz, CDCl₃): δ 2.93 (3H, s, CH₃), 3.66 (6H, s, 3,5-OCH₃), 3.75 (3H, s, 4-OCH₃), 6.06 (2H, s, NH-Ph 2,6-H), 6.07–6.09 (1H, br s, NH), 7.26–7.30 (1H, m, C-Ph 4-H), 7.34–7.38 (2H, m, C-Ph 3,5-H), 7.43–7.47 (1H, m, N-Ph 4-H), 7.21–7.56 (2H, m, N-Ph 3,5-H), 7.73–7.77 (2H, m, C-Ph 2,6-H), 7.87–7.90 (2H, m, N-Ph 2,6-H), 8.62 (1H, s, 3-H). ¹³C NMR (126 MHz, CDCl₃): δ 22.8 (CH₃), 56.0 (3,5-OCH₃), 61.1 (4-OCH₃), 95.0 (NH-Ph C-2,6), 121.1 (C-3a),

121.2 (N-Ph C-2,6), 122.0 (C-7), 122.6 (C-3), 127.8 (C-Ph C-4), 128.5 (C-Ph C-3,5), 128.7 (N-Ph C-4), 129.0 (C-Ph C-2,6), 129.8 (N-Ph C-3,5), 132.1 (NH-Ph C-4), 139.4 (NH-Ph C-1), 140.0, 140.3 and 140.5 (C-6, C-Ph C-1 or N-Ph C-1), 148.0 (C-7a), 150.0 (C-4), 153.3 (NH-Ph C-4,5). IR (ν , cm^{-1}): 3346, 3314 (NH), 3112, 3057 (CH_{arom}), 2923, 2852 (CH_{aliph}), 1596, 1502, 1463, 1412, 1232 (C=C, C=N, C-N), 1125, 1006 (C-O-C), 804, 757, 690 ($\text{CH}=\text{CH}$ of mono- and tetrasubstituted benzenes). MS (ES^+): m/z (%): 467 ($[\text{M}+\text{H}]^+$, 100). HRMS (ESI) $\text{C}_{28}\text{H}_{27}\text{N}_4\text{O}_3$ ($[\text{M}+1]^+$): calcd 467.2078, found 467.2078.

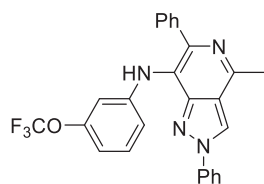
4-Methyl-2,6-diphenyl-*N*-[2-(trifluoromethoxy)phenyl]-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine (66)



Prepared in accordance to general procedure (G) from 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**19**) (50 mg, 0.12 mmol), 2-(trifluoromethoxy)aniline (23 mg, 0.13 mmol), NaOtBu (18.7 mg, 0.19 mmol), SPhos (15 mg, 0.036 mmol) and $\text{Pd}(\text{OAc})_2$ (1.7 mg, 0.012 mmol) and dioxane (4 mL). The residue was purified by column chromatography (EtOAc/PE , 1:4 to 1:2, v/v).

Yield: 46 mg (82%), yellowish crystals, mp = 113–114 °C, R_f = 0.62 (Hex:EtOAc 1:1). ^1H NMR (500 MHz, CDCl_3): δ 2.87 (3H, s, CH_3), 6.28 (1H, s, NH), 6.68–6.77 (2H, m, NH-Ph 4,6-H), 6.87–6.93 (1H, m, NH-Ph 3-H or 5-H), 7.16–7.21 (1H, m, NH-P, 3-H or 5-H), 7.24–7.29 (1H, m, C-P, 4-H), 7.32–7.36 (2H, m, C-Ph 3,5-H), 7.40–7.44 (1H, m, N-Ph 4-H), 7.49–7.53 (2H, m, N-Ph 3,5-H), 7.73–7.79 (2H, m, C-Ph 2,6-H), 7.85–7.89 (2H, m, N-Ph 2,6-H), 8.56 (1H, s, 3-H). ^{13}C NMR (126 MHz, CDCl_3): δ 23.0 (CH_3) 116.7 (NH-Ph C-6), 117.9, 119.2, 124.0 (CF_3 J = 252 Hz), 120.7 (NH-Ph C-3 or C-5), 120.8 (C-7), 121.1 (N-Ph C-2,6), 121.2 (C-3a), 122.0 (C-3), 126.7 (NH-Ph C-3 or C-5), 127.9 (C-Ph C-4), 128.5 (C-Ph C-3,5), 128.7 (N-Ph C-4), 128.8 (C-Ph C-2,6), 129.7 (N-Ph C-3,5), 136.8 (NH-Ph C-1), 137.7 (NH-Ph C-2), 139.1 (C-Ph C-1), 140.0 (N-Ph C-1), 143.1 (C-6), 148.3 (C-7a), 151.5 (C-4). IR (ν , cm^{-1}): 3412, 3396 (NH), 3133, 3056 (CH_{arom}), 2920, 2852 (CH_{aliph}), 1600, 1511, 1485, 1434, 1248, 1213, 1167 (C=C, C=N, C-N, C-F), 755, 697, 688 ($\text{CH}=\text{CH}$ of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 461 ($[\text{M}+\text{H}]^+$, 100). HRMS (ESI) $\text{C}_{26}\text{H}_{20}\text{F}_3\text{N}_4\text{O}$ ($[\text{M}+1]^+$): calcd 461.1584, found 461.1584.

4-Methyl-2,6-diphenyl-*N*-[3-(trifluoromethoxy)phenyl]-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine (67)



Prepared in accordance to general procedure (G) from 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**19**) (50 mg, 0.12 mmol), 3-(trifluoromethoxy)aniline (23 mg, 0.13 mmol), NaOtBu (18.7 mg, 0.19 mmol), SPhos (15 mg, 0.036 mmol) and $\text{Pd}(\text{OAc})_2$ (1.7 mg, 0.012 mmol) and dioxane (4 mL). The residue was purified by column

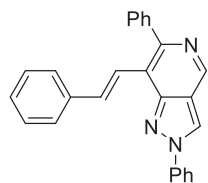
chromatography (EtOAc/PE, 1:4 to 1:2, v/v).

Yield: 35mg (62%), yellowish crystals, mp = 76–78 °C, R_f = 0.59 (Hex:EtOAc 1:1). ^1H NMR (700 MHz, CDCl_3): δ 2.87 (3H, s, CH_3), 5.99 (1H, s, NH), 6.63–6.65 (1H, m, NH-Ph 2-H), 6.65–6.67 (1H, m, NH-Ph 4-H), 6.68–6.72 (1H, m, NH-Ph 6-H), 7.10–7.14 (1H, m, NH-Ph 5-H), 7.28–7.31 (1H, m, C-Ph 4-H), 7.36–7.39 (2H, m, C-Ph 3,5-H), 7.42–7.45 (1H, m, N-Ph 4-H), 7.50–7.53 (2H, m, N-Ph 3,5-H), 7.69–7.72 (2H, m, C-Ph 2,6-H), 7.84–7.88 (2H, m, N-Ph 2,6-H), 8.57 (1H, s, 3-H). ^{13}C NMR (176 MHz, CDCl_3): δ 22.9 (CH_3), 108.8 (NH-Ph C-2), 111.7 (NH-Ph C-4), 114.7 (NH-Ph C-6), 119.7 (OCF_3), 121.0 (C-7), 121.1 (C-3a), 121.2 (N-Ph C-2,6), 122.0 (C-3), 127.8 (C-Ph C-4), 128.5 (C-Ph C-3,5), 128.6 (N-Ph C-4), 128.9 (C-Ph C-2,6), 129.5 (NH-Ph C-5), 129.6 (N-Ph C-3,5), 138.8 (C-Ph C-1), 139.9 (N-Ph C-1), 142.9 (C-6), 146.1 (NH-Ph C-1 or C-3), 147.9 (C-7a), 149.8 (NH-Ph C-1 or C-3), 151.4 (C-4). ^{15}N NMR (71 MHz, CDCl_3): δ -304.5 (NH), -148.3 (N-2), -82.2 (N-5). IR (ν , cm^{-1}): 3384 (NH), 3059 (CH_{arom}), 2923, 2853 (CH_{aliph}), 1610, 1598, 1519, 1487, 1249, 1213, 1178, 1151 (C=C, C=N, C-N, C-F), 756, 697, 689 ($\text{CH}=\text{CH}$ of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 461 ($[\text{M}+\text{H}]^+$, 100). HRMS (ESI) $\text{C}_{26}\text{H}_{20}\text{F}_3\text{N}_4\text{O}$ ($[\text{M}+1]^+$): calcd 461.1583, found 461.1584.

General procedure (H) for the synthesis of 7-substituted pyrazolo[4,3-*c*]pyridine derivatives 68-76 by Heck cross-coupling

7-Iodo-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (1 eq) was dissolved in dry DMF. Then, TEA (1.5 eq), the appropriate vinylbenzene (2 eq) and $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (0.1 eq) were added to the solution under argon atmosphere. The mixture was stirred at 120 °C under microwave irradiation (100 W, 300 Pa) for 30 min to 3 hours. Upon completion (monitored by TLC), the reaction mixture was cooled to room temperature, diluted with water (20 mL), and extracted with EtOAc (3×25 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography.

2,6-Diphenyl-7-[(*E*)-2-phenylethenyl]-2*H*-pyrazolo[4,3-*c*]pyridine (**68**)

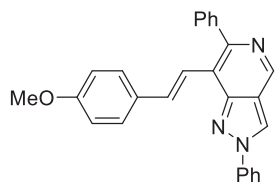


Prepared in accordance to general procedure (H) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (80 mg, 0.2 mmol), TEA (0.04 mL, 0.3 mmol), styrene (0.05 mL, 0.4 mmol) and $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (14 mg, 0.02 mmol) and DMF (1 mL). The residue was purified by column chromatography (EtOAc/toluene, 1:10, v/v).

Yield: 49 mg (65%), yellowish crystals, mp = 203–204 °C, R_f = 0.32 (Hex:EtOAc 1:1). ^1H NMR (700 MHz, CDCl_3): δ 7.24–7.26 (1H, m, C7-Ph 4-H), 7.32–7.36 (2H, m, C6-Ph 3,5-H), 7.41 (1H, d, J = 16.8 Hz, Ph-CH=CH), 7.44–7.47 (1H, m, C6-Ph 4-H), 7.48–7.54 (5H, m, N-Ph 4-H, C6-Ph 3,5-H and C7-Ph 2,6-H), 7.59–7.63 (2H, m, N-Ph 3,5-H), 7.63–7.73 (2H, m, C-Ph 2,6-H), 8.00–8.05 (2H, m, N-Ph 2,6-H), 8.54 (1H, d, J = 16.1 Hz, Ph-CH=CH), 8.66 (1H, s, 3-H), 9.18 (1H, s, 4-H). ^{13}C

NMR (176 MHz, CDCl₃): δ 118.7 (C-7), 120.4 (C-3a), 121.4 (N-Ph C-2,6), 121.7 (C-3), 123.8 (Ph-CH=CH), 126.8 (C7-Ph C-2,6), 127.6 (C7-Ph C-4), 127.9 (C-Ph C-4), 128.1 (C6-Ph C-3,5), 128.6 (C7-Ph C-3,5), 128.7 (N-Ph C-4), 129.8 (N-Ph C-3,5), 130.6 (C6-Ph C-2,6), 135.6 (Ph-CH=CH), 138.4 (C7-Ph C-1), 140.0 (N-Ph C-1), 140.6 (C6-Ph C-1), 144.9 (C-4), 149.7 (C-7a), 151.6 (C-6). ¹⁵N NMR (71 MHz, CDCl₃): δ -146.3 (N-2), -96.5 (N-1), -79.7 (N-5). IR (ν , cm⁻¹): 3059, 3044 (CH_{arom}), 2923 (CH_{aliph}), 1598, 1587, 1506, 1449, 1326, 1200, 1054 (C=C, C=N, C-N), 755, 749, 695, 688 (CH=CH of monosubstituted benzenes). MS (ES⁺): m/z (%): 374 ([M+H]⁺, 100).

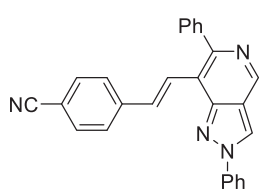
7-[(*E*)-2-(4-methoxyphenyl)ethenyl]-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (69)



Prepared in accordance to general procedure (H) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (80 mg, 0.2 mmol), TEA (0.04 mL, 0.3 mmol), 1-methoxy-4-vinylbenzene (0.054 mL, 0.4 mmol) and Pd(PPh₃)₂Cl₂ (14 mg, 0.02 mmol) and DMF (1 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:3, v/v).

Yield: 66 mg (81%), yellow crystals, mp = 80–81 °C, R_f = 0.29 (Hex:EtOAc 1:1). ¹H NMR (700 MHz, CDCl₃): δ 3.82 (3H, s, OMe), 6.87–6.90 (2H, m, MeO-Ph 3,5-H), 7.27 (1H, d, J = 16.1 Hz, Ph-CH=CH), 7.43–7.46 (3H, m, C-Ph 4-H and MeO-Ph 2,6-H), 7.48–7.52 (3H, m, C-Ph 3,5-H and N-Ph 4-H), 7.58–7.62 (2H, m, N-Ph 3,5-H), 7.69–7.71 (2H, m, C-Ph 2,6-H), 8.00–8.04 (2H, m, N-Ph 2,6-H), 8.50 (1H, d, J = 16.1 Hz, Ph-CH=CH), 8.64 (1H, s, 3-H), 9.15 (1H, s, 4-H). ¹³C NMR (176 MHz, CDCl₃): δ 55.3 (OMe), 114.0 (MeO-Ph C-3,5), 119.0 (C-7), 120.4 (C-3a), 121.2 (N-Ph C-2,6), 121.6 (C-3), 121.7 (Ph-CH=CH), 127.8 (C-Ph C-4), 128.0 (MeO-Ph C-2,6), 128.1 (C-Ph C-3,5), 128.7 (N-Ph C-4), 129.7 (N-Ph C-3,5), 130.6 (C-Ph C-2,6), 131.2 (MeO-Ph C-1), 135.1 (Ph-CH=CH), 140.0 (N-Ph C-1), 140.7 (C-Ph C-1), 144.5 (C-4), 149.7 (C-7a), 151.0 (C-6), 159.3 (OMe). ¹⁵N NMR (71 MHz, CDCl₃): δ -146.5 (N-2), -96.7 (N-1), -80.1 (N-5). IR (ν , cm⁻¹): 3049, 3002 (CH_{arom}), 2931 (CH_{aliph}), 1602, 1591, 1509, 1463, 1248, 1240, 1173, 1031 (C=C, C=N, C-N, C-O), 822, 756, 704, 689 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): m/z (%): 404 ([M+H]⁺, 98).

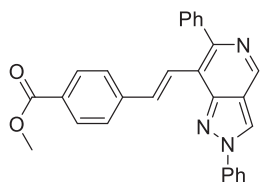
7-[(*E*)-2-(4-isocyanophenyl)ethenyl]-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (70)



Prepared in accordance to general procedure (H) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (80 mg, 0.2 mmol), TEA (0.04 mL, 0.3 mmol), 4-vinylbenzonitrile (52 mg, 0.4 mmol) and Pd(PPh₃)₂Cl₂ (14 mg, 0.02 mmol) and DMF (1 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:2, v/v).

Yield: 70 mg (84%), yellow crystals, mp = 196–197 °C, R_f = 0.29 (Hex:EtOAc 1:1). ^1H NMR (700 MHz, CDCl_3): δ 7.46–7.50 (2H, m, Ph-CH=CH and C-Ph 4-H), 7.50–7.55 (5H, m, CN-Ph 2,6-H, C-Ph 3,5-H and N-Ph 4-H), 7.58–7.63 (4H, m, CN-Ph 3,5-H and N-Ph 3,5-H), 7.66–7.69 (2H, m, C-Ph 2,6-H), 7.96–8.04 (2H, m, N-Ph 2,6-H), 8.52 (1H, d, J = 16.1 Hz, Ph-CH=CH), 8.67 (1H, s, 3-H), 9.21 (1H, s, 4-H). ^{13}C NMR (176 MHz, CDCl_3): δ 110.3 (CN-Ph C-1), 117.9 (C-7), 119.1 (CN), 120.3 (C-3a), 121.2 (N-Ph C-2,6), 122.1 (C-3), 127.0 (CN-Ph C-2,6), 127.4 (Ph-CH=CH), 128.19 (C-Ph C-3,5), 128.21 (C-Ph C-4), 128.9 (N-Ph C-4), 129.8 (N-Ph C-3,5), 130.6 (C-Ph C-2,6), 132.4 (CN-Ph C-3,5), 133.3 (Ph-CH=CH), 139.9 (N-Ph C-1), 140.2 (C-Ph C-1), 142.8 (CN-Ph C-1), 145.8 (C-4), 149.5 (C-7a), 152.7 (C-6). ^{15}N NMR (71 MHz, CDCl_3): δ -146.8 (N-2), -97.1 (N-1), -80.1 (N-5), CN not found. IR (v, cm^{-1}): 3125, 3036 (CH_{arom}), 2924 (CH_{aliph}), 2221 (CN), 1596, 1508, 1476, 1330, 1210, 1048, 937 (C=C, C=N, C-N), 754, 689, 538 (CH=CH of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 399 ($[\text{M}+\text{H}]^+$, 97).

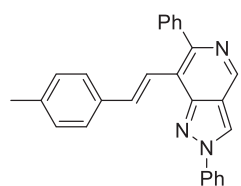
Methyl 4-[(*E*)-2-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)ethenyl]benzoate (71)



Prepared in accordance to general procedure (H) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (80 mg, 0.2 mmol), TEA (0.04 mL, 0.3 mmol), methyl 4-vinylbenzoate (65 mg, 0.4 mmol) and $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (14 mg, 0.02 mmol) and DMF (1 mL). The residue was purified by column chromatography EtOAc/Hex, 1:3, v/v).

Yield: 56 mg (55%), yellow crystals, mp = 183–184 °C, R_f = 0.29 (Hex:EtOAc 1:1). ^1H NMR (700 MHz, CDCl_3): δ 3.91 (3H, s, OMe), 7.45–7.48 (1H, m, C-Ph 4-H), 7.48–7.55 (6H, m, Ph-CH=CH, N-Ph 4-H and O=C-Ph 2,6-H and C-Ph 3,5-H), 7.60–7.64 (2H, m, N-Ph 3,5-H), 7.68–7.71 (2H, m, C-Ph 2,6-H), 7.98–8.01 (2H, m, O=C-Ph 3,5-H), 8.01–8.05 (2H, m, N-Ph 2,6-H), 8.56 (1H, d, J = 16.8 Hz, Ph-CH=CH), 8.67 (1H, s, 3-H), 9.21 (1H, s, 4-H). ^{13}C NMR (176 MHz, CDCl_3): δ 52.1 (OMe), 118.3 (C-7), 120.4 (C-3a), 121.2 (N-Ph C-2,6), 122.0 (C-3), 126.4 (Ph-CH=CH), 126.6 (O=C-Ph C-2,6), 128.1 (C-Ph C-4), 128.2 (C-Ph C-3,5), 128.8 (O=C-Ph C-4), 128.9 (N-Ph C-4), 129.8 (N-Ph C-3,5), 129.9 (O=C-Ph C-3,5), 130.6 (C-Ph C-2,6), 134.3 (Ph-CH=CH), 140.0 (N-Ph C-1), 140.4 (C-Ph C-1), 142.9 (O=C-Ph C-1), 145.5 (C-4), 149.6 (C-7a), 152.3 (C-6), 166.9 (O=C). ^{15}N NMR (71 MHz, CDCl_3): δ -145.9 (N-2), -96.9 (N-1), -80.1 (N-5). IR (v, cm^{-1}): 3233, 3126 (CH_{arom}), 2951 (CH_{aliph}), 1695 (C=O ester), 1600, 1588, 1509, 1433, 1292, 1102 (C=C, C=N, C-N, C-O), 757, 707, 684 (CH=CH of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 432 ($[\text{M}+\text{H}]^+$, 95).

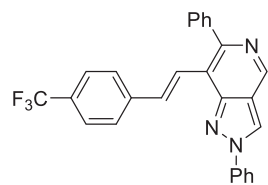
7-[(*E*)-2-(4-methylphenyl)ethenyl]-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (72)



Prepared in accordance to general procedure (H) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (80 mg, 0.2 mmol), TEA (0.04 mL, 0.3 mmol), 1-methyl-4-vinylbenzene (0.053 mL, 0.4 mmol) and Pd(PPh₃)₂Cl₂ (14 mg, 0.02 mmol) and DMF (1 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:2, v/v).

Yield: 66 mg (84%), yellowish crystals, mp = 85–86 °C, *R*_f = 0.32 (Hex:EtOAc 1:1). ¹H NMR (700 MHz, CDCl₃): δ 2.36 (3H, s, Me), 7.13–7.17 (2H, m, Me-Ph 3,5-H), 7.36 (1H, d, *J* = 16.1 Hz, Ph-CH=CH), 7.39–7.42 (2H, m, Me-Ph 2,6-H), 7.43–7.46 (1H, m, C-Ph 4-H), 7.48–7.53 (3H, m, N-Ph 4-H and C-Ph 3,5-H), 7.58–7.62 (2H, m, N-Ph 3,5-H), 7.69–7.72 (2H, m, C-Ph 2,6-H), 8.00–8.04 (2H, m, N-Ph 2,6-H), 8.51 (1H, d, *J* = 16.1 Hz, Ph-CH=CH), 8.64 (1H, s, 3-H), 9.16 (1H, s, 4-H). ¹³C NMR (176 MHz, CDCl₃): δ 21.3 (Me), 118.9 (C-7), 120.4 (C-3a), 121.2 (N-Ph C-2,6), 121.6 (C-3), 122.8 (Ph-CH=CH), 126.7 (Me-Ph C-2,6), 127.8 (C-Ph C-4), 128.1 (C-Ph C-3,5), 128.7 (N-Ph C-4), 129.3 (Me-Ph C-3,5), 129.7 (N-Ph C-3,5), 130.6 (C-Ph C-2,6), 135.5 (Ph-CH=CH), 135.6 (Me-Ph C-1), 137.5 (Me-Ph C-4), 140.0 (N-Ph C-1), 140.7 (C-Ph C-1), 144.7 (C-4), 149.7 (C-7a), 151.3 (C-6). ¹⁵N NMR (71 MHz, CDCl₃): δ -146.4 (N-2), -96.8 (N-1), -79.8 (N-5). IR (ν, cm⁻¹): 3112, 3018 (CH_{arom}), 2915, 2851 (CH_{aliph}), 1590, 1507, 1463, 1325, 1206 (C=C, C=N, C-N), 958, 768, 756, 688 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): *m/z* (%): 388 ([M+H]⁺, 100).

2,6-Diphenyl-7-[(*E*)-2-[4-(trifluoromethyl)phenyl]ethenyl]-2*H*-pyrazolo[4,3-*c*]pyridine (73)

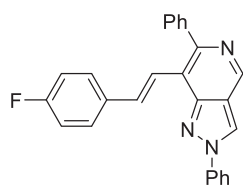


Prepared in accordance to general procedure (H) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (80 mg, 0.2 mmol), TEA (0.04 mL, 0.3 mmol), 1-(trifluoromethyl)-4-vinylbenzene (0.06 mL, 0.4 mmol) and Pd(PPh₃)₂Cl₂ (14 mg, 0.02 mmol) and DMF (1 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:2, v/v).

Yield: 58 mg (65%), white crystals, mp = 108–109 °C, *R*_f = 0.32 (Hex:EtOAc 1:1). ¹H NMR (700 MHz, CDCl₃): δ 7.45–7.48 (2H, m, C-Ph 4-H and Ph-CH=CH), 7.50–7.54 (3H, m, N-Ph 4-H and C-Ph 3,5-H), 7.56–7.60 (4H, m, CF₃-Ph 2,3,5,6-H), 7.60–7.64 (2H, m, N-Ph 3,5-H), 7.67–7.71 (2H, m, C-Ph 2,6-H), 8.00–8.05 (2H, m, N-Ph 2,6-H), 8.55 (1H, d, *J* = 16.1 Hz, Ph-CH=CH), 8.68 (1H, s, 3-H), 9.22 (1H, s, 4-H). ¹³C NMR (176 MHz, CDCl₃): δ 118.2 (C-7), 120.4 (C-3a), 121.3 (N-Ph C-2,6), 122.0 (C-3), 124.2 (*J* = 272.8 Hz CF₃), 125.5 (*J* = 3.52 Hz CF₃-Ph C-3,5), 126.3 (Ph-CH=CH), 126.8 (CF₃-Ph C-2,6), 128.1 (C-Ph C-4), 128.2 (C-Ph C-3,5), 128.9 (N-Ph C-4), 129.1 (*J* = 31.7 Hz, CF₃-Ph C-4), 129.8 (N-Ph C-3,5), 130.6 (C-Ph C-2,6), 133.8 (Ph-CH=CH), 140.0 (N-Ph C-1), 140.4 (C-Ph C-1), 141.9 (CF₃-Ph C-1), 145.6 (C-4), 149.6 (C-7a), 152.3 (C-6). ¹⁵N NMR (71 MHz, CDCl₃): δ -145.9 (N-2), -97.3 (N-1), -79.9 (N-5). IR (ν, cm⁻¹): 3049 (CH_{arom}), 1611, 1590, 1325,

1114, 1066 (C=C, C=N, C–N, C–F), 754, 699, 689 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): *m/z* (%): 442 ([M+H]⁺, 100).

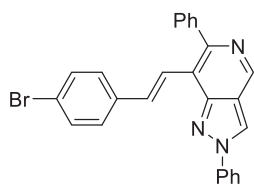
7-[(*E*)-2-(4-fluorophenyl)ethenyl]-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (74)



Prepared in accordance to general procedure (H) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (80 mg, 0.2 mmol), TEA (0.04 mL, 0.3 mmol), 1-fluor-4-vinylbenzene (0.05 mL, 0.4 mmol) and Pd(PPh₃)₂Cl₂ (14 mg, 0.02 mmol) and DMF (1 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:2, v/v).

Yield: 60 mg (76%), yellow-brown crystals, mp = 185–186 °C, *R*_f = 0.32 (Hex:EtOAc 1:1). ¹H NMR (700 MHz, CDCl₃): δ 7.01–7.04 (2H, m, F-Ph 3,5-H), 7.30 (1H, d, *J* = 16.8 Hz, Ph-CH=CH), 7.44–7.47 (3H, m, F-Ph 2,6-H and C-Ph 4-H), 7.48–7.53 (3H, m, C6-Ph 3,5-H and N-Ph 4-H), 7.58–7.62 (2H, m, N-Ph 3,5-H), 7.68–7.70 (2H, m, C-Ph 2,6-H), 7.99–8.02 (2H, m, N-Ph 2,6-H), 8.50 (1H, d, *J* = 16.1 Hz, Ph-CH=CH), 8.65 (1H, s, 3-H), 9.18 (1H, s, 4-H). ¹³C NMR (176 MHz, CDCl₃): δ 115.5 (*J* = 22.9 Hz, F-Ph C-3,5), 118.7 (C-7), 120.3 (C-3a), 121.2 (N-Ph C-2,6), 121.8 (C-3), 123.5 (Ph-CH=CH), 127.9 (C-Ph C-4), 128.1 (C-Ph C-2,6), 128.1 (C-Ph C-3,5), 128.2 (*J* = 8.8 Hz, F-Ph C-2,6), 128.8 (N-Ph C-4), 129.8 (N-Ph C-3,5), 130.5 (C-Ph C-2,6), 134.3 (Ph-CH=CH), 134.5 (*J* = 3.5 Hz, F-Ph C-1), 140.0 (N-Ph C-1), 140.5 (C-Ph C-1), 144.9 (C-4), 149.6 (C-7a), 151.5 (C-6), 162.3 (*J* = 248.2 Hz, F-Ph C-4). ¹⁵N NMR (71 MHz, CDCl₃): δ –146.1 (N-2), –96.8 (N-1), –81.0 (N-5). IR (ν, cm^{–1}): 3064 (CH_{arom}), 2963, 2850 (CH_{aliph}), 1598, 1505, 1262, 1222, 1096, 1151, 1021 (C=C, C=N, C–N, C–F), 802, 751, 700, 687 (CH=CH of mono- and disubstituted benzenes). MS (ES⁺): *m/z* (%): 392 ([M+H]⁺, 96).

7-[(*E*)-2-(4-bromophenyl)ethenyl]-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (75)

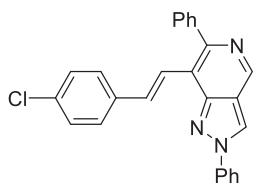


Prepared in accordance to general procedure (H) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (80 mg, 0.2 mmol), TEA (0.04 mL, 0.3 mmol), 1-bromo-4-vinylbenzene (0.053 mL, 0.4 mmol) and Pd(PPh₃)₂Cl₂ (14 mg, 0.02 mmol) and DMF (1 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:2, v/v).

Yield: 36 mg (40%), yellowish crystals, mp = 86–87 °C, *R*_f = 0.32 (Hex:EtOAc 1:1). ¹H NMR (700 MHz, CDCl₃): δ 7.34–7.39 (3H, m, Br-Ph 2,6-H and Ph-CH=CH), 7.44–7.47 (3H, m, C-Ph 4-H and Br-Ph 3,5-H), 7.49–7.53 (3H, m, N-Ph 4-H and C-Ph 3,5-H), 7.59–7.62 (2H, m, N-Ph 3,5-H), 7.67–7.70 (2H, m, C-Ph 2,6-H), 7.99–8.03 (2H, m, N-Ph 2,6-H), 8.47 (1H, d, *J* = 16.8 Hz, Ph-CH=CH), 8.65 (1H, s, 3-H), 9.19 (1H, s, 4-H). ¹³C NMR (176 MHz, CDCl₃): δ 118.4 (C-7), 120.4 (C-3a), 121.2 (N-Ph C-2,6), 121.3 (Br-Ph C-4), 121.9 (C-3), 124.5 (Ph-CH=CH), 128.0 (C-Ph C-4), 128.1 (C-Ph C-3,5), 128.2 (Br-Ph C-2,6), 128.8 (N-Ph C-4), 129.8 (N-Ph C-3,5), 130.6 (C-Ph C-2,6), 131.7 (Br-Ph C-3,5), 134.2 (Ph-CH=CH), 137.3 (Br-Ph C-1),

140.0 (N-Ph C-1), 140.5 (C-Ph C-1), 145.2 (C-4), 149.6 (C-7a), 151.8 (C-6). ^{15}N NMR (71 MHz, CDCl_3): δ -146.1 (N-2), -96.6 (N-1), -79.8 (N-5). IR (ν , cm^{-1}): 3048 (CH_{arom}), 2917 (CH_{aliph}), 1590, 1507, 1324, 1200, 1007 (C=C, C=N, C-N), 754, 701, 686 (CH=CH of mono- and disubstituted benzenes), 501 (C-Br). MS (ES^+): m/z (%): 454 ($[\text{M}+\text{H}]^+$, 100).

7-[(*E*)-2-(4-chlorophenyl)ethenyl]-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (76)



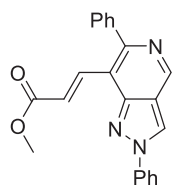
Prepared in accordance to general procedure (H) from 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) (80 mg, 0.2 mmol), TEA (0.04 mL, 0.3 mmol), 1-chloro-4-vinylbenzene (0.051 mL, 0.4 mmol) and $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (14 mg, 0.02 mmol) and DMF (1 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:2, v/v).

Yield: 63 mg (77%), yellowish crystals, mp = 184–185 °C, R_f = 0.30 (Hex:EtOAc 1:1). ^1H NMR (700 MHz, CDCl_3): δ 7.28–7.31 (2H, m, Cl-Ph 3,5-H), 7.35 (1H, d, J = 16.1 Hz, Ph-CH=CH), 7.39–7.42 (2H, m, Cl-Ph 2,6-H), 7.45–7.53 (4H, m, N-Ph 4-H and C-Ph 3,4,5-H), 7.57–7.61 (2H, m, N-Ph 3,5-H), 7.68–7.70 (2H, m, C-Ph 2,6-H), 7.97–8.01 (2H, m, N-Ph 2,6-H), 8.47 (1H, d, J = 16.1 Hz, Ph-CH=CH), 8.62 (1H, s, 3-H), 9.16 (1H, s, 4-H). ^{13}C NMR (176 MHz, CDCl_3): δ 118.4 (C-7), 120.3 (C-3a), 121.1 (N-Ph C-2,6), 121.8 (C-3), 124.3 (Ph-CH=CH), 127.86 (Cl-Ph C-2,6), 127.94 (C-Ph C-4), 128.1 (C-Ph C-3,5), 128.69 (Cl-Ph C-3,5), 128.73 (N-Ph C-4), 129.7 (N-Ph C-3,5), 130.5 (C-Ph C-2,6), 133.1 (Cl-Ph C-4), 134.1 (Ph-CH=CH), 136.7 (Cl-Ph C-1), 139.9 (N-Ph C-1), 140.5 (C-Ph C-1), 145.1 (C-4), 149.5 (C-7a), 151.7 (C-6). ^{15}N NMR (71 MHz, CDCl_3): δ -146.2 (N-2), -96.8 (N-1), -79.9 (N-5). IR (ν , cm^{-1}): 3048 (CH_{arom}), 2922 (CH_{aliph}), 1590, 1507, 1489, 1324, 1190, 1120 (C=C, C=N, C-N), 754 (C-Cl), 960, 700, 688 (CH=CH of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 408 ($[\text{M}+\text{H}]^+$, 95).

General procedure (I) for the synthesis of methyl (pyrazolo[4,3-*c*]pyridin-7-yl)prop-2-enoates (**77–78**) derivatives by Heck cross-coupling

Appropriate 7-iodo-2*H*-pyrazolo[4,3-*c*]pyridine (**18**) or (**19**) (1 eq) was dissolved in dry DMF. Then, TEA (1.5 eq), methyl acrylate (2 eq) and $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (0.1 eq) were added to the solution under argon atmosphere. The mixture was stirred at 120 °C under microwave irradiation (100 W, 300 Pa). Upon completion (monitored by TLC), the reaction mixture was cooled to room temperature, diluted with water (20 mL) and extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography.

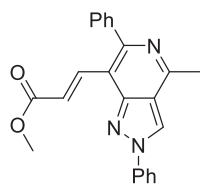
Methyl (2E)-3-(2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-yl)prop-2-enoate (77)



Prepared in accordance to general procedure (I) from 7-iodo-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine (**18**) (100 mg, 0.25 mmol), TEA (0.05 mL, 0.38 mmol), methyl acrylate (0.045 mL, 0.5 mmol) and Pd(PPh₃)₂Cl₂ (18 mg, 0.025 mmol) and DMF (1 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:2, v/v)

Yield: 75 mg (84%), yellowish-brown crystals, mp = 136–137°C, *R*_f = 0.49 (Hex:EtOAc 1:1). ¹H NMR (700 MHz, CDCl₃): δ 3.80 (3H, s, OCH₃), 7.44–7.47 (1H, m, C-Ph 4-H), 7.48–7.52 (3H, m, C-Ph 3,5-H and N-Ph 4-H), 7.57–7.61 (2H, m, N-Ph 3,5-H), 7.61–7.65 (2H, m, C-Ph 2,6-H), 7.82 (1H, d, *J* = 15.4 Hz, O=C-CH=CH), 7.95–8.01 (3H, m, N-Ph 2,6-H and O=C-CH=CH), 8.67 (1H, s, 3-H), 9.26 (1H, s, 4-H). ¹³C NMR (176 MHz, CDCl₃): δ 51.6 (OCH₃), 116.2 (C-7), 120.3 (C-3a), 121.2 (N-Ph C-2,6), 122.2 (C-3), 124.1 (O=C-CH=CH), 128.2 (C-Ph C-3,5), 128.5 (C-Ph C-4), 129.0 (N-Ph C-4), 129.8 (N-Ph C-3,5), 130.6 (C-Ph C-2,6), 139.4 (O=C-CH=CH), 139.6 (C-Ph C-1), 139.7 (N-Ph C-1), 147.4 (C-4), 149.5 (C-7a), 154.9 (C-6), 168.1 (C=O). ¹⁵N NMR (71 MHz, CDCl₃): δ -145.1 (N-2), -96.7 (N-1), -80.2 (N-5). IR (ν, cm⁻¹): 3059 (CH_{arom}), 2969, 2815, 2554 (CH_{aliph}), 1684, 1670 (C=O ester), 1619, 1504, 1325, 1292, 1244, 1199 (C=C, C=N, C-N, C-O), 756, 707 (CH=CH of monosubstituted benzenes). MS (ES⁺): *m/z* (%): 356 ([M+H]⁺, 100).

Methyl (2E)-3-(4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-yl)prop-2-enoate (78)



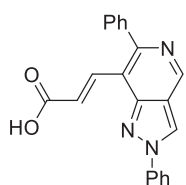
Prepared in accordance to general procedure (H) from 7-iodo-4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridine (**19**) (100 mg, 0.24 mmol), TEA (0.05 mL, 0.36 mmol), methyl acrylate (0.044 mL, 0.49 mmol) and Pd(PPh₃)₂Cl₂ (17 mg, 0.024 mmol) and DMF (1 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:2, v/v).

Yield: 80 mg (89%), white crystals, *R*_f = 0.69 (Hex:EtOAc 1:2). ¹H NMR (700 MHz, CDCl₃): δ 2.86 (3H, s, CH₃), 3.78 (3H, s, OCH₃), 7.42–7.45 (1H, m, C-Ph 4-H), 7.45–7.48 (1H, m, N-Ph 4-H), 7.48–7.51 (2H, m, C-Ph 3,5-H), 7.54–7.58 (2H, m, N-Ph 3,5-H), 7.59–7.63 (2H, m, C-Ph 2,6-H), 7.76 (1H, d, *J* = 16.1 Hz, O=C-CH=CH), 7.93 (1H, d, *J* = 16.1 Hz, O=C-CH=CH), 7.95–8.00 (2H, m, N-Ph 2,6-H), 8.62 (1H, s, 3-H). ¹³C NMR (176 MHz, CDCl₃): δ 22.3 (CH₃), 51.5 (OCH₃), 114.1 (C-7), 120.3 (C-3a), 121.0 (N-Ph C-2,6), 121.9 (C-3), 122.8 (O=CH=CH), 128.2 (C-Ph C-3,5), 128.3 (C-Ph C-4), 128.7 (N-Ph C-4), 129.7 (N-Ph C-3,5), 130.5 (O=C-CH=CH), 139.7 (C-Ph C-1 and N-Ph C-1), 149.7 (C-7a), 155.2 (C-6), 156.8 (C-4), 168.2 (C=O). ¹⁵N NMR (71 MHz, CDCl₃): δ -147.0 (N-2), -96.0 (N-1), -84.1 (N-5). MS (ES⁺): *m/z* (%): 370 ([M+H]⁺, 100).

General procedure (J) for methyl (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)prop-2-enoates **77** and **78** hydrolysis

Appropriate methyl (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)prop-2-enoates **77** or **78** (1 eq) were dissolved in methanol, and 2M NaOH (4 eq) was added to the solution. The mixture was stirred at 65 °C for 3 hours. Upon completion (monitored by TLC), methanol was evaporated under reduced pressure, and the residue was diluted with water (20 mL) and neutralized with 6M HCl (aq). Then, the product was extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with brine (10 mL), dried over Na₂SO₄ and evaporated under reduced pressure. The residue was purified by column chromatography or used in the next step without purification.

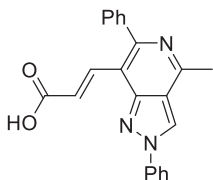
(2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)prop-2-enoic acid (**79**)



Prepared in accordance to general procedure (J) from methyl (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)prop-2-enoate (**77**) (100 mg, 0.28 mmol), NaOH (2M, 0.56 mL, 1.13 mmol) and MeOH (2.8 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:1, v/v)

Yield: 86 mg (90%), yellowish-brown crystals, R_f = 0.39 (Hex:EtOAc 1:1). ¹H NMR (700 MHz, DMSO-*d*₆): δ 7.49–7.52 (1H, m, C-Ph 4-H), 7.53–7.56 (3H, m, C-Ph 3,5-H and N-Ph 4-H), 7.57–7.60 (2H, m, C-Ph 2,6-H), 7.65–7.69 (3H, m, O=C-CH=CH and N-Ph 3,5-H), 7.74 (1H, d, J = 15.4 Hz, O=C-CH=CH), 8.15–8.19 (2H, m, N-h 2,6-H), 9.31 (1H, s, 4-H), 9.54 (1H, s, 3-H), 12.44 (1H, br s, COOH). ¹³C NMR (176 MHz, DMSO-*d*₆): δ 114.9 (C-7), 120.1 (C-3a), 120.9 (N-Ph C-2,6), 124.9 (C-3), 125.0 (O=C-CH=CH), 128.1 (C-Ph C-3,5), 128.4 (C-Ph C-4), 129.0 (N-Ph C-4), 129.9 (N-Ph C-3,5), 130.5 (C-Ph C-2,6), 138.4 (O=C-CH=CH), 139.3 (N-Ph C-1), 139.5 (C-Ph C-1), 148.3 (C-4), 148.5 (C-7a), 154.4 (C-6), 167.9 (COOH). ¹⁵N NMR (71 MHz, DMSO-*d*₆): δ -144.8 (N-2), -99.9 (N-1), -77.9 (N-5). IR (v, cm⁻¹): 3400 (OH), 3112 (CH_{arom}), 2947 (CH_{aliph}), 1731, 1709 (C=O acid), 1600, 1589, 1509, 1431, 1277, 1194, 1176 (C=C, C=N, C-N, C-O), 750, 705, 684 (CH=CH of monosubstituted benzenes). MS (ES⁺): m/z (%): 442 ([M+H]⁺, 100).

(2*E*)-3-(4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)prop-2-enoic acid (**80**)



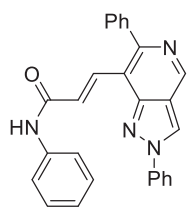
Prepared in accordance to general procedure (J) from methyl (2*E*)-3-(4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)prop-2-enoate (**78**) (100 mg, 0.27 mmol), NaOH (2M, 0.54 mL, 1.08 mmol) and MeOH (2.7 mL). The residue was used in the next step without purification.

Yield: 83 mg (86%) (according to MS purity of the crude product).

General procedure (K) for synthesis of prop-2-enamides 81–84

The appropriate (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)prop-2-enoic acid (**79**) or (**80**) (1 eq) and DMAP (0.1 eq) were dissolved in DCM. The solution was cooled down to -5 °C, and EDC·HCl (1.1 eq) was added dropwise. Finally, the appropriate aniline (1 eq) was added, and the mixture was stirred at room temperature for 24 hours. Upon completion (monitored by TLC), the reaction mixture was diluted with water (20 mL) and extracted with DCM (3 × 25 mL). The combined organic layers were washed with brine (10 mL), dried over Na₂SO₄ and evaporated under reduced pressure. The residue was purified by column chromatography.

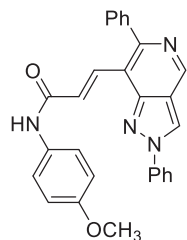
(2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)-*N*-phenylprop-2-enamide (**81**)



Prepared in accordance to general procedure (K) from methyl (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)prop-2-enoic acid (**79**) (80 mg, 0.24 mmol), DMAP (3 mg, 0.02 mmol), EDC·HCl (50 mg, 0.26 mmol), aniline (0.02 mL, 0.24 mmol) and DCM (2.4 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:1 to 2:1, v/v).

Yield: 83 mg (85%), yellow crystals, mp = 229–230 °C, *R*_f = 0.15 (Hex:EtOAc 1:1). ¹H NMR (700 MHz, CDCl₃): δ 7.02–7.12 (1H, m, NH-Ph 4-H), 7.26–7.32 (2H, m, NH-Ph 3,5-H), 7.38–7.41 (1H, m, C-Ph 4-H), 7.43–7.47 (3H, N-Ph 4-H and C-Ph 3,5-H), 7.50–7.54 (2H, m, N-Ph 3,5-H), 7.57–7.63 (4H, m, NH-Ph 2,6-H and C-Ph 2,6-H), 7.83–7.96 (4H, m, NH, N-Ph 2,6-H, O=CH=CH), 8.02 (1H, m, *J* = 15.4 Hz, O=CH=CH), 8.57 (1H, s, 3-H), 9.20 (1H, s, 4-H). ¹³C NMR (176 MHz, CDCl₃): δ 116.3 (C-7), 119.7 (NH-Ph C-2,6), 120.1 (C-3a), 121.5 (N-Ph C-2,6), 122.6 (C-3), 124.0 (1H, s, NH-Ph 4-H), 127.2 (O=CH=CH), 128.2 (C-Ph C-3,5), 128.5 (C-Ph C-4), 128.9 (NH-Ph C-3,5), 129.0 (N-Ph C-4), 129.7 (N-Ph C-3,5), 130.5 (C-Ph C-2,6), 136.7 (O=CH=CH), 138.3 (NH-Ph C-1), 139.4 (C-Ph C-1), 139.7 (N-Ph C-1), 147.0 (C-4), 149.6 (C-7a), 154.8 (C-6), 164.7 (C=O). ¹⁵N NMR (71 MHz, CDCl₃): δ -248.0 (NH), -145.4 (N-2), -97.1 (N-1) -80.3 (N-5). IR (ν, cm⁻¹): 3253 (NH), 3058 (CH_{arom}), 2927, 2852 (CH_{aliph}), 1659 (C=O amide), 1599, 1543, 1441, 1327, 1244, 1178 (C=C, C=N, C-N), 755, 700, 690 (CH=CH of monosubstituted benzenes). MS (ES⁺): *m/z* (%): 417 ([M+H]⁺, 100).

(2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)-*N*-(4-methoxyphenyl)prop-2-enamide (**82**)

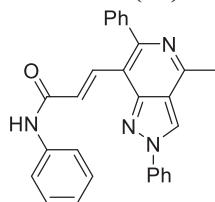


Prepared in accordance to general procedure (K) from methyl (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)prop-2-enoic acid (**79**) (80 mg, 0.24 mmol), DMAP (3 mg, 0.02 mmol), EDC·HCl (50 mg, 0.26 mmol), 4-methoxyaniline (29 mg, 0.24 mmol) and DCM (2.4 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:1 v/v).

Yield: 92 mg (88%), yellow crystals, mp = 223–224 °C, *R*_f = 0.17

(Hex:EtOAc 1:1). ^1H NMR (700 MHz, CDCl_3): δ 3.77 (3H, s, OCH_3), 6.76–6.90 (2H, m, NH-Ph 3,5-H), 7.39–7.42 (1H, m, C-Ph 4-H), 7.44–7.49 (3H, m, N-Ph 4-H and C-Ph 3,5-H), 7.50–7.53 (2H, NH-Ph 2,6-H), 7.53–7.58 (2H, m, N-Ph 3,5-H), 7.59–7.63 (2H, m, C-Ph 2,6-H), 7.69 (1H, s, NH), 7.87–7.95 (3H, m, N-Ph 2,6-H and $\text{O}=\text{CH}=\text{CH}$), 8.01 (1H, d, $J = 15.4$ Hz, $\text{O}=\text{CH}=\text{CH}$), 8.60 (1H, s, 3-H), 9.22 (1H, s, 4-H). ^{13}C NMR (176 MHz, CDCl_3): δ 55.4 (OCH_3), 114.0 (NH-Ph C-3,5), 116.4 (C-7), 120.2 (C-3a), 121.4 (NH-Ph C-2,6), 121.6 (N-Ph C-2,6), 122.6 (C-3), 127.3 ($\text{O}=\text{CH}=\text{CH}$), 128.3 (C-Ph C-3,5), 128.4 (C-Ph C-4), 129.0 (N-Ph C-4), 129.8 (N-Ph C-3,5), 130.5 (C-Ph C-2,6), 131.5 (NH-Ph C-1), 136.4 ($\text{O}=\text{CH}=\text{CH}$), 139.5 (C-Ph C-1), 139.8 (N-Ph C-1), 146.9 (C-4), 149.7 (C-7a), 154.8 (C-6), 156.2 (NH-Ph C-4), 164.4 (C=O). ^{15}N NMR (71 MHz, CDCl_3): δ -250.2 (NH), -145.5 (N-2), -96.9 (N-1) N-5 not found. IR (ν , cm^{-1}): 3282 (NH), 3122, 3008 (CH_{arom}), 2931, 2834 (CH_{aliph}), 1650 (C=O amide), 1599, 1528, 1510, 1326, 1243, 1169 (C=C, C=N, C-N, C-O), 749, 699, 687 ($\text{CH}=\text{CH}$ of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 447 ($[\text{M}+\text{H}]^+$, 100).

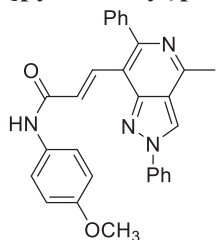
(2E)-3-(4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-yl)-N-phenylprop-2-enamide (83)



Prepared in accordance to general procedure (K) from methyl (2E)-3-(4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-yl) prop-2-enoic acid (**80**) (80 mg, 0.23 mmol), DMAP (3 mg, 0.02 mmol), EDC·HCl (48 mg, 0.25 mmol), aniline (0.02 mL, 0.23 mmol) and DCM (2.3 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:2 to 2:1, v/v).

Yield: 78 mg (80%), yellow crystals, mp = 249–250 °C, R_f = 0.30 (Hex:EtOAc 1:1). ^1H NMR (700 MHz, acetonitrile- d_3): δ 2.79 (3H, s, CH_3), 7.07–7.11 (1H, m, NH-Ph 4-H), 7.30–7.35 (2H, m, NH-Ph 3,5-H), 7.46–7.53 (4H, m, N-Ph 4-H and C-Ph 3,4,5), 7.55–7.59 (2H, m, C-Ph 2,6-H), 7.61–7.65 (2H, m, N-Ph 3,5-H), 7.66–7.73 (2H, m, NH-Ph 2,6-H), 7.79 (1H, d, $J = 15.4$ Hz, $\text{O}=\text{C}-\text{CH}=\text{CH}$), 7.91 (1H, d, $J = 15.4$ Hz, $\text{O}=\text{C}-\text{CH}=\text{CH}$), 8.13–8.22 (2H, m, N-Ph 2,6-H), 8.70 (1H, br s, NH), 9.01 (1H, s, 3-H). ^{13}C NMR (176 MHz, acetonitrile- d_3): δ 23.2 (CH_3), 114.9 (C-7), 120.5 (NH-Ph C-2,6), 121.1 (C-3a), 121.9 (N-Ph C-2,6), 124.5 (NH-Ph 4-H), 124.7 (C-3), 127.1 ($\text{O}=\text{C}-\text{CH}=\text{CH}$), 128.9 (C-Ph C-3,5), 129.1 (C-Ph C-4), 129.6 (N-Ph C-4), 129.7 (NH-Ph C-3,5), 130.6 (N-Ph C-3,5), 131.4 (C-Ph C-2,6), 136.8 ($\text{O}=\text{C}-\text{CH}=\text{CH}$), 140.2 (NH-Ph C-1), 140.8 (N-Ph C-1), 141.3 (C-Ph C-1), 150.3 (C-7a), 155.6 (C-6), 157.9 (C-4), 165.6 (C=O). ^{15}N NMR (71 MHz, acetonitrile- d_3): δ -248.1 (NH), -147.2 (N-2), -98.1 (N-1), -82.0 (N-5). IR (ν , cm^{-1}): 3279, 3244 (NH), 3190, 3077 (CH_{arom}), 2921 (CH_{aliph}), 1654 ($\text{O}=\text{C}$ amide), 1610, 1583, 1544, 1499, 1441, 1220, 1005 (C=C, C=N, C-N), 756, 704, 693 ($\text{CH}=\text{CH}$ of monosubstituted benzenes). MS (ES^+): m/z (%): 431 ($[\text{M}+\text{H}]^+$, 100).

(2E)-N-(4-methoxyphenyl)-3-(4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-yl)prop-2-enamide (84)



Prepared in accordance to general procedure (K) from methyl (2E)-3-(4-methyl-2,6-diphenyl-2H-pyrazolo[4,3-c]pyridin-7-yl)prop-2-enoic acid (**80**) (80 mg, 0.23 mmol), DMAP (3 mg, 0.02 mmol), EDC·HCl (48 mg, 0.25 mmol), 4-methoxyaniline (43 mg, 0.23 mmol) and DCM (2.3 mL). The residue was purified by column chromatography (EtOAc/Hex, 1:2 to 2:1, v/v).

Yield: 62 mg (60%), yellow crystals, mp = 245–246 °C, R_f = 0.29 (Hex:EtOAc 1:1). ^1H NMR (700 MHz, CDCl_3): δ 2.84

(3H, s, CH_3), 3.74 (3H, s, OCH_3), 6.88–6.94 (2H, m, NH-Ph 3,5-H), 7.49–7.52 (1H, m, C-Ph 4-H), 7.52–7.55 (2H, m, C-Ph 3,5), 7.55–7.58 (3H, m, C-Ph 2,6-H and N-Ph 4-H), 7.63–7.67 (2H, m, NH-Ph 2,6-H), 7.68–7.72 (2H, m, N-Ph 3,5-H), 7.74 (1H, d, J = 15.4 Hz, $\text{O}=\text{C}-\text{CH}=\text{CH}$), 7.95 (1H, d, J = 15.4 Hz, $\text{O}=\text{C}-\text{CH}=\text{CH}$), 8.33–8.39 (2H, m, N-Ph 2,6-H), 9.71 (1H, s, 3-H), 10.26 (1H, br s, NH). ^{13}C NMR (176 MHz, CDCl_3): δ 22.8 (CH_3), 55.1 (OCH_3), 113.7 (C-7), 113.8 (NH-Ph C-3,5), 120.0 (C-3a), 120.7 (N-Ph C-2,6), 120.9 (NH-Ph C-2,6), 124.7 (C-3), 126.9 ($\text{O}=\text{C}-\text{CH}=\text{CH}$), 128.0 (C-Ph C-3,5), 128.1 (C-Ph C-4), 128.7 (N-Ph C-4), 129.8 (N-Ph C-3,5), 130.4 (C-Ph C-2,6), 132.6 (NH-Ph C-1), 134.7 ($\text{O}=\text{C}-\text{CH}=\text{CH}$), 139.4 (N-Ph C-1), 140.0 (C-Ph C-1), 148.8 (C-7a), 153.9 (C-6), 155.2 (NH-Ph C-4), 156.6 (C-4), 163.9 (C=O). ^{15}N NMR (71 MHz, CDCl_3): δ -247.6 (NH), -146.9 (N-2), -98.9 (N-1), -81.4 (N-5). IR (v, cm^{-1}): 3282 (NH), 3038 (CH_{arom}), 2916, 2834 (CH_{aliph}), 1649 (C=O amide), 1620, 1598, 1543, 1528, 1250, 1216, 1034 (C=C, C=N, C-N, C-O), 748, 700, 687 ($\text{CH}=\text{CH}$ of mono- and disubstituted benzenes). MS (ES^+): m/z (%): 461 ($[\text{M}+\text{H}]^+$, 100).

4. MAIN RESULTS AND CONCLUSIONS

1. 1-Phenyl-3-(phenylethynyl)-1*H*-pyrazole-4-carbaldehyde is a suitable precursor to obtain 7-iodo-2*H*-pyrazolo[4,3-*c*]pyridines *via* alcohol and azide intermediates.

2. A library of 7-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines can be easily obtained from 7-iodo-2*H*-pyrazolo[4,3-*c*]pyridines by employing the Suzuki-Miyaura cross-coupling reaction. After investigating the properties of 7-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines, it was determined that:

- 7-(4-Alkoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines possess best fluorescence with a quantum yield going up to 85%.

- 7-(4-Methoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine is the most suitable indicator for pH enabling both fluorescence-intensity-based and ratiometric pH sensing.

- Derivatives exhibit anticancer activity against MV4-11, K562 and MCF-7 cancer cell lines *in vitro* by a combination of antiproliferative effects and induction of cell death. 4-(2,6-Diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)phenol is the most potent agent with GI₅₀ concentrations as low as 1.5 μ M.

3. A library of *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines can be synthesized from 7-iodo-2*H*-pyrazolo[4,3-*c*]pyridines by employing the Buchwald-Hartwig cross-coupling reaction. After investigating properties of *N*-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines, it was determined that:

- The addition of an amine group significantly decreases fluorescence as both the Stokes shift and the quantum yield was lower than the corresponding values of other 7-substituted-2*H*-pyrazolo[4,3-*c*]pyridine derivatives.

- *N*-(4-Methoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine and 2,6-diphenyl-*N*-[2-(trifluoromethoxy)phenyl]-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine are the most prospective photosensitizers with the EC₅₀ as low as 1.1 μ M in light irradiated melanoma G361 cells.

- *N*-(4-Methoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine causes cell death by producing ROS and damaging DNA.

4. A library of 2,6-diphenyl-7-[(*E*)-2-phenylethenyl]-2*H*-pyrazolo[4,3-*c*]pyridines and (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)-*N*-phenylprop-2-enamides can be synthesized from 7-iodo-2*H*-pyrazolo[4,3-*c*]pyridines by employing the Heck cross-coupling reaction. After investigating properties of 2,6-diphenyl-7-[(*E*)-2-phenylethenyl]-2*H*-pyrazolo[4,3-*c*]pyridines and (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)-*N*-phenylprop-2-enamides, it was determined that:

- 2,6-diphenyl-7-[(*E*)-2-phenylethenyl]-2*H*-pyrazolo[4,3-*c*]pyridines exhibit the biggest bathochromic emission shift and offer a moderate quantum yield while (2*E*)-3-(2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-yl)-*N*-phenylprop-2-enamides do not exhibit fluorescence.

- The primary investigation shows that methyl 7-[(*E*)-2-(4-methylphenyl)ethenyl]-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine is a promising photosensitizer for photodynamic therapy with IC₅₀ value as low as 0.043 μM in light irradiated melanoma G361 cells.

5. SANTRAUKA

IVADAS

Paskutiniais dešimtmečiais pirazolai ir jų heterokondensuoti dariniai tapo įdomia ir svarbia junginių klase organinėje ir medicininėje chemijoje. Pirazolo žiedo inkorporavimas yra dažnai naudojamas naujų vaistinių molekulių kūrimo praktikoje. Šios molekulės pasižymi antivėžinėmis, antidiabetinėmis, antivirusinėmis, uždegimą slopinančiomis, antigrybelinėmis, antineurodegeneracinėmis, antihelmintinėmis, antimaliarinėmis ir fotoaktyvinančiomis savybėmis [1–5]. Taip pat nemaža dalis jau patvirtintų vaistinių medžiagų turi pirazolo žiedą [6]. Atrasta, kad pirazolai pasižymi dažų ir fluorescencijos savybėmis [7, 8], dalis jų gali būti naudojami kaip kolorimetriniai ar fluorescenciniai žymekliai mažoms molekulėms, jonams ar pH pokyčiams stebėti [9–12], taip pritaikant *in vivo* diagnostiniame vaizdavime [13].

Pirazolopiridinai yra vieni labiausiai studijuojamų kondensuotų pirazolo sistemų organinėje ir medicininėje chemijoje. Pavyzdžiui, atrasta, kad 6-(3,5-dimetoksifenil)-3-(4-fluorofenil)-1*H*-pirazolo[3,4-*b*]piridinas yra Kit kinasės inhibitorius [14]. Pastaruoju metu įvairūs 1*H*-pirazolo[3,4-*b*]piridino dariniai aprašyti kaip potencialūs CDK8 inhibitoriai [15] bei antimikrobiniai ir antivėžiniai agentai [16]. Be to, keletas 1*H*-pirazolo[4,3-*b*]piridin-3-amino junginių veikia kaip teigiami alosteriniai mGlu4 modulatoriai [18], o 3-fenilpirazolo[3,4-*c*]piridinai pasižymi antiproliferaciniu aktyvumu [19]. Tik keli biologiškai aktyvių pirazolo[4,3-*c*]piridinų pavyzdžiai yra aprašyti, tarp jų 3-amino-2-fenil-2*H*-pirazolo[4,3-*c*]piridin-4,6-diolis, pasižymėjęs inhibiciniu aktyvumu prieš p90 ribosominę S6 kinazę 2 (RSK2) [21], ir 3-aminopirazolopiridinono dariniai, pasižymintys vidutiniu CK1d, p38 ir aurora A kinazių inhibicijos potencialu [22].

Kadangi pirazolopiridino sistema yra svarbi kuriant vaistines molekules, būtina sukurti naujas sintezės schemas skirtingiems funkcionalizuotiems dariniams gauti. Dėl mažo junginių kiekio, aprašyto literatūroje, ir galimų plataus pritaikymo galimybių buvo nuspręsta prioritetą suteikti 2*H*-pirazolo[4,3-*c*]piridinų sintezei.

Pagrindinis darbo tikslas:

Susintetinti funkcionalizuotų 2*H*-pirazolo[4,3-*c*]piridinų biblioteką, pritaikant žiedo uždarymo ir paladžio katalizuojamas kryžminio jungimo reakcijas, ir ištirti darinių savybes.

Tiksliui pasiekti buvo iškelti šie uždaviniai:

1. Susintetinti 3-alkinil-1-fenil-1*H*-pirazol-4-karbaldehidus ir ištirti jų transformaciją į 7-jodo-2*H*-pirazolo[4,3-*c*]piridinus.
2. 7-Aril-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridinų sintezei pritaikyti Suzuki-Miyaura kryžminio jungimo reakciją ir ištirti bei aptarti jų funkcines savybes.
3. *N*-Aril-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-aminų sintezei pritaikyti Buchwald-Hartwig kryžminio jungimo reakciją ir ištirti bei aptarti jų funkcines savybes.

4. 2,6-Difenil-7-[(*E*)-2-feniletetil]-2*H*-pirazolo[4,3-*c*]piridinų ir (2*E*)-3-(2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-il)-*N*-fenilprop-2-enamidų sintezei pritaikyti Heck kryžminio jungimo reakciją ir ištirti bei aptarti jų funkcines savybes.

Darbo mokslinis naujumas ir praktinė reikšmė

Sukurtas efektyvus daugiapakopis sintezės metodas funkcionalizuotiems 2*H*-pirazolo[4,3-*c*]piridinams gauti iš lengvai prieinamų pradinių junginių. Pakaitai į 2, 3, 4, 6 ir 7 pozicijas gali būti įvedami skirtingose sintezės stadijose: į 2 ir 3 pasirenkant pradinį junginį 1,5-dipakeistą-1*H*-pirazol-3-olį, į 6 – naudojant skirtingus acetilenus kaip Sonogashiros kryžminio jungimo substratus ir į 7 su skirtingais substratais įvairių paladžio katalizuojamų kryžminių jungimų (Suzuki-Miyaura, Buchwald-Hartwig, Heck) metu. Šiame darbe aptariami 2,6-difenil-4,7-dipakeisti-2*H*-pirazolo[4,3-*c*]piridinai, susintetinti iš 1-fenil-1*H*-pirazol-3-olio per tarpinį 1-fenil-3-(feniletetil)-1*H*-pirazol-4-karbaldehidą, kuris trijų stadijų metu buvo paverstas į 4-pakeistą-7-jodo-2*H*-pirazolo[4,3-*c*]piridiną. Pakaitas į septintą padėtį įvestas naudojant paladžio katalizuojamų kryžminių jungimų reakcijų sąlygas, taip sukuriant didelę 4,7-pakeistų-2*H*-pirazolo[4,3-*c*]piridinų biblioteką. Visi dariniai pasižymi skirtingomis optinėmis ir biologinėmis savybėmis su įdomiais potencialiais pritaikymais, pavyzdžiui, 7-(4-metoksifenil)-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridinas tinkamas kaip fluorescencinis pH sensorius, 4-(2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-il)fenolis pasižymi antiproliferacinėmis savybėmis prieš MV4-11, K562 ir MCF-7 vėžines ląsteles, o *N*-aril-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-aminai ir 2,6-difenil-7-[(*E*)-2-feniletetil]-2*H*-pirazolo[4,3-*c*]piridinai yra galimi fotodinaminės terapijos fotosenzitatoriai prieš melanomos vėžines ląsteles.

Ginamieji teiginiai:

1. 1-Fenil-3-(etilpakeistas)-1*H*-pirazolo-4-karbaldehidas yra tinkamas kaip tarpinis junginys 7-jodo-2*H*-pirazolo[4,3-*c*]piridinui gauti per tarpinės atitinkamo alkoholio ir azido gavimo stadijas.

2. 7-Aril-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridinai gali būti gaunami Suzuki-Miyaura kryžminio jungimo metu, jie yra potencialūs fluorescenciniai pH sensoriai ir antivėžiniai agentai.

3. *N*-Aril-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-aminai gali būti gaunami Buchwald-Hartwig kryžminio jungimo metu, jie yra potencialūs fotodinaminės terapijos agentai prieš melanomos G361 ląsteles.

4. 2,6-Difenil-7-[(*E*)-2-feniletetil]-2*H*-pirazolo[4,3-*c*]piridinais ir (2*E*)-3-(2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-il)-*N*-fenilprop-2-enamidais gali būti gaunami Heck kryžminio jungimo metu, jie yra perspektyvūs fotoaktyvatoriai fotodinaminėje terapijoje prieš melanomos G361 ląsteles.

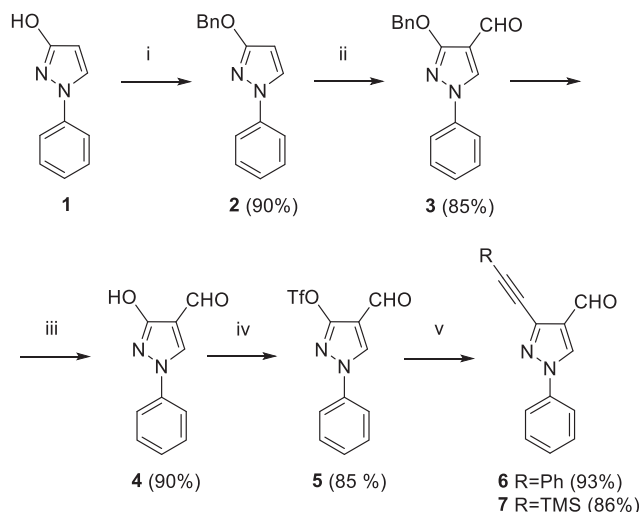
TYRIMŲ REZULTAI IR JŲ APTARIMAS

1. 2*H*-Pirazolo[4,3-*c*]piridino sintezė

Nors yra žinoma daug biologiškai aktyvių kondensuotų pirazolo darinių [83, 84], sudėtingiau susintetinami 2*H*-pirazolo[4,3-*c*]piridiniai nėra dažnai aptinkami. Vienas iš aprašytų 2*H*-pirazolo[4,3-*c*]piridinių sintezės metodų – 4-chlor ar 4-jodpiridinių, turinčių karbonilgrupę 3 pozicijoje, veikimas įvairiais hidraziniais [85, 86]. Taip pat aprašytas 2*H*-pirazolo[4,3-*c*]piridinių nesudėtingas gavimas iš 1-fenil-3-hidroksi-1*H*-pirazol-4-karbaldehido ir atitinkamo etanono daugiapakopės sintezės metu, kai vykdomas Sonogashiros kryžminis jungimas ir sauso amoniako sukelta ciklizacija [88]. Šis metodas sėkmingai pritaikytas įvairiems 2,4,6-tripakeistiams-2*H*-pirazolo[4,3-*c*]piridinams, pasižymintiems geru antivėžiniu aktyvumu *in vitro* stabdant ląstelės ciklą mitozės stadijoje ir sukeliant jos apoptozę, gauti [89].

1.1. 1-Fenil-3-[(pakeisto)etininil]-1*H*-pirazol-4-karbaldehido sintezė

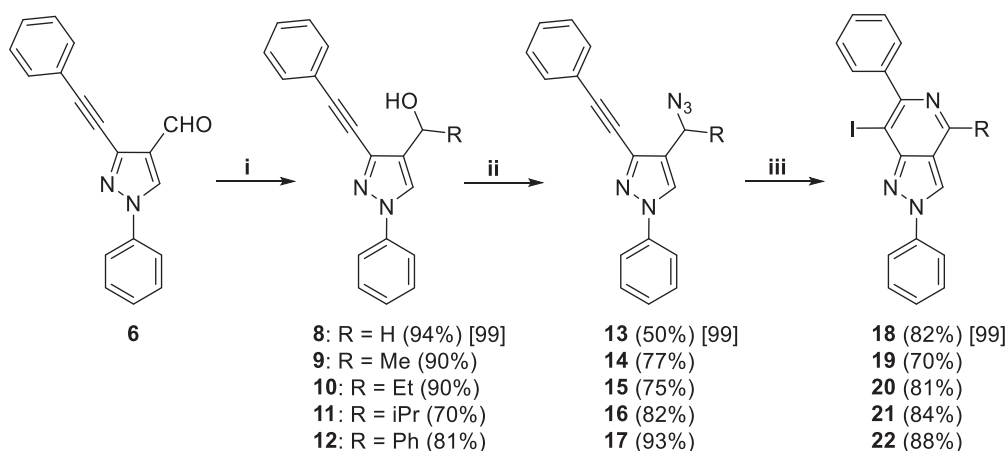
1-Pakeistas-1*H*-pirazol-3-olis yra vertingas pradinis junginys kondensuotoms pirazolo sistemoms gauti [5, 80, 81]. Ypač svarbus tarpinis produktas yra pirazol-4-karbaldehidai, turintys alkinilfunkcinę grupę šalia formilgrupės, iš jo gautos tokios sistemos, kaip 2*H*- ir 3*H*-pirazolo[4',3':3,4]pirido[1,2-*a*]benzimidazolai ir 2,6-difenil-2*H*-pirazolo[4,3-*c*]piridiniai [92-94]. Taigi 1-fenil-3-(2-feniletininil)-1*H*-pirazol-4-karbaldehidai (**6**) ir 1-fenil-3-[(trimetilsilil)etininil]-1*H*-pirazol-4-karbaldehidai (**7**) buvo pasirinkti kaip pradiniai šio tyrimo junginiai. Jie buvo gauti iš 1-fenil-1*H*-pirazol-3-olio (**1**), pasitelkiant daugiapakopę sintezės kelią pagal anksčiau publikuotą protokolą (1 schema) [88]. Pirmiausia pritaikytas *O*-alkilinimas apsaugant hidroksigrupę benzilgrupe [88], vėliau atliktas formilinimas naudojant Vilsmeierio ir Hacko reakcijos sąlygas [95]. Benzilgrupės pašalinimas atliktas rūgštinėje terpėje naudojant TFA rūgštį, tuomet hidroksigrupė pakeista triflato grupe [96]. Šis pseudohalogenidas yra tinkamas Pd-katalizuojamiems kryžminiems jungimams, todėl **5** junginys buvo veikiamas Sonogashiros reakcijos sąlygomis, t. y. fenilacetileno ar trimetilsililacetileno, TEA kaip bazės, CuI kaip ko-katalizatoriaus ir Pd(PPh₃)₂Cl₂ kaip katalizatoriaus [88, 89], taip gauti junginiai **6** ir **7**, atitinkamai 93% ir 86% išeigomis.



1.1 schema. 1-Fenil-3-[(pakeisto)etinin]-1*H*-pirazol-4-karbaldehidų sintezė. *Reagentai ir sąlygos:* i: BnCl, NaH, DMF, argono atmosfera 60 °C, 1 val; ii: POCl₃, DMF, 0-60 °C; iii: TFA, toluene, kamb t., 15 h; iv: Tf₂O, TEA, DCM, kamb t, 1 val.

1.2. 4-Pakeistų 2,6-difenil-7-jod-2*H*-pirazolo[4,3-*c*]piridinų sintezė

4-Pakeisti 7-jod-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridinai **18-22** buvo gauti trijų sintezės etapų, apibūdintų 2 schemeje, metu. 1-[1-Fenil-3-(feniletinin)-1*H*-pirazol-4-il]alkoholiui gauti buvo pasirinkti du metodai, priklausomai nuo galutinio alkoholio tipo. Formilinės grupės redukcija naudojant NaBH₄ buvo atlikta siekiant gauti pirminį alkoholį **8** [97]. Reakcija buvo atlikta MeOH, esant 0 °C temperatūrai argono atmosferoje. Antrinių alkoholių **9-12** sintezei karbaldehidas **6** buvo ištirpintas THF, kur kambario temperatūroje reagavo su atitinkamu alkil ar aril magnio halogenidu pagal anksčiau aprašytą metodologiją [98, 99]. Svarbu pažymėti, kad dėl Grinjaro reagentų nestabilumo ore ir esant drėgnoms sąlygoms reakcija buvo atlikta sausame tirpiklyje argono atmosferoje [100]. Alkoholio susiformavimą galima greitai patvirtinti IR spektru, kuriame platus alkoholio grupės virpesių signalas matomas ties 3300–3400 cm⁻¹, o aldehydinės grupės C=O ryšio virpesių signalas (~1710–1685) nebefiksuojamas.



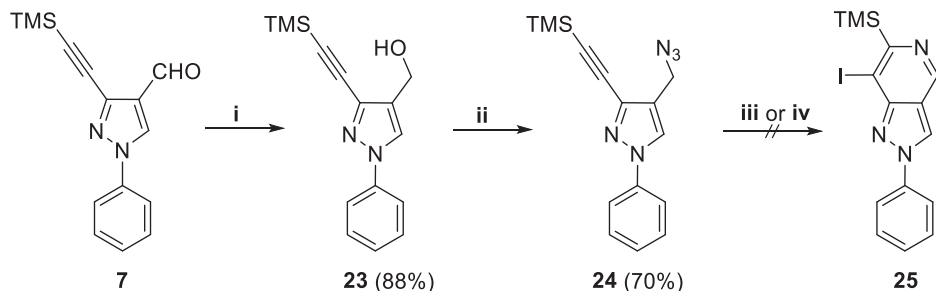
1.2 schema. 4-Pakeistų 7-jod-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridinų **17–22** sintezė.

Reagentai ir sąlygos: i: NaBH₄, MeOH, 0 °C, 30 min (**8**); MeMgBr, EtMgBr, PrMgCl arba PhMgBr, THF (abs.), rt, 10 min (**9**, **10**, **11** ir **12**); ii: TMSN₃, BF₃·Et₂O, DCM, kamb t, 10–30 min.; iii: K₃PO₄ (**18** ir **22**) ar NaHCO₃ (**19**, **20** **21**), I₂, DCM, kamb t, 12 val.

Tolesnis sintezės etapas – azidų **13–17** gavimas. Dėl lengvos ir greitos reakcijos eigos buvo pasirinktas TMSN₃ [101] ir reakcijos atliktos DCM su katalitiniu BF₃·Et₂O kiekiu. Reakcijos vyko kambario temperatūroje sausame tirpiklyje argono atmosferoje, siekiant apsaugoti reagentus nuo drėgmės. Transformacija įvyko per 30 minučių, o produktai **14–17** gauti geromis išeigomis, išskyrus azidometilpirazolą **13**, kurio išeiga siekė tik 50%. Struktūros buvo patvirtintos BMR, IR spektroskopijos ir MS bei HRMS spektrometrijos metodais. Panašiai kaip alkoholių atveju, IR spektras gali būti panaudotas norint greitai įrodyti transformaciją: spektre nebepastebimi alkoholio, o fiksuojami charakteringi azido grupės signalai.

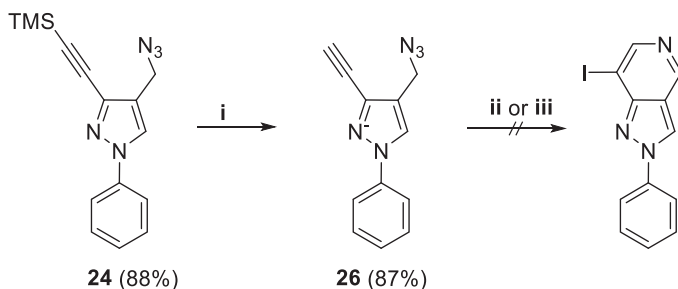
Paskutiniame sintezės etape pritaikytos elektrofilinės pakaitų reakcijos sąlygos, naudotos 1,3,4-tripakeistų izochinolinų sintezei iš 2-alkinilbenzilazidų [108]. Tam, kad įvyktų ciklizacija, azidai **13–17** turėjo būti veikiami jodu ir tinkama baze. Pirminių azidų **13** ir **17** atveju buvo panaudoti 5 ekvivalentai K₃PO₄, o 1 ekvivalentas silpnesnės NaHCO₃ bazės buvo reikalingas antriniams azidams **14–16**. Reakcijos vykdytos DCM kambario temperatūroje 12 val. tamsoje, taip gauti junginiai **18–22** 70–88% išeigomis. Bandymas panaudoti silpnesnę bazę reakcijoje su pirminiu azidu **13** sukėlė dehalogenizuoto papildomo produkto 2,6-difenil-2*H*-pirazolo[4,3-*c*]piridino susiformavimą, dėl kurio sumažėjo tikslinio produkto išeiga ir komplikavosi gryninimas.

Deja, 2-fenil-7-jodo-2*H*-pirazolo[4,3-*c*]piridino (**25**) iš 1-fenil-3-[(trimetilsilil)etininil]-1*H*-pirazol-4-karbaldehido (**7**) gauti nepavyko. Pirmieji du sintezės etapai (3 schema) įvyko sklandžiai, kaip apibūdinta prieš tai. Kondensacija naudojant K₃PO₄ ir I₂ buvo nesėkminga, nesusidarė net 7-jodo-2-fenil-2*H*-pirazolo[4,3-*c*]piridino pėdsakų. Alternatyviai buvo panaudotas kitas jodo šaltinis – *N*-jodosukcinimidas (NIS). Nepaisant to, ciklizacija neįvyko.



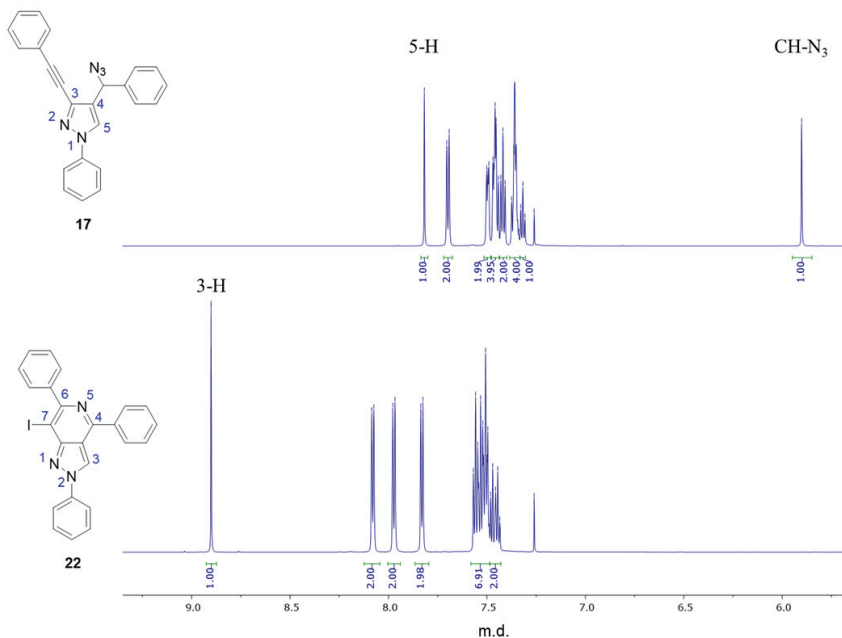
1.3 schema. 2-Fenil-7-jod-6-(trimetilsilil)-2H-pirazolo[4,3-c]piridino (**25**) sintezė. *Reagentai ir sąlygos:* i: NaBH₄, MeOH, 0 °C, 30 min; ii: TMSN₃, BF₃·Et₂O, DCM, kamb t, 10 min; iii: K₃PO₄ I₂, DCM, rt, 12 h; iv: NaHCO₃, NIS, DCE, 50 °C, 2 dienos

Galiausiai nuspręsta panaudoti azidą be pakaito prie trigubojo ryšio (4 schema). Junginys **26** buvo gautas naudojant bevandenį K₂CO₃ sausame MeOH, taip pritaikant anksčiau aprašytas sąlygas [110]. Deja, bet kondensacija nevyko su nei vienu jodo šaltiniu.



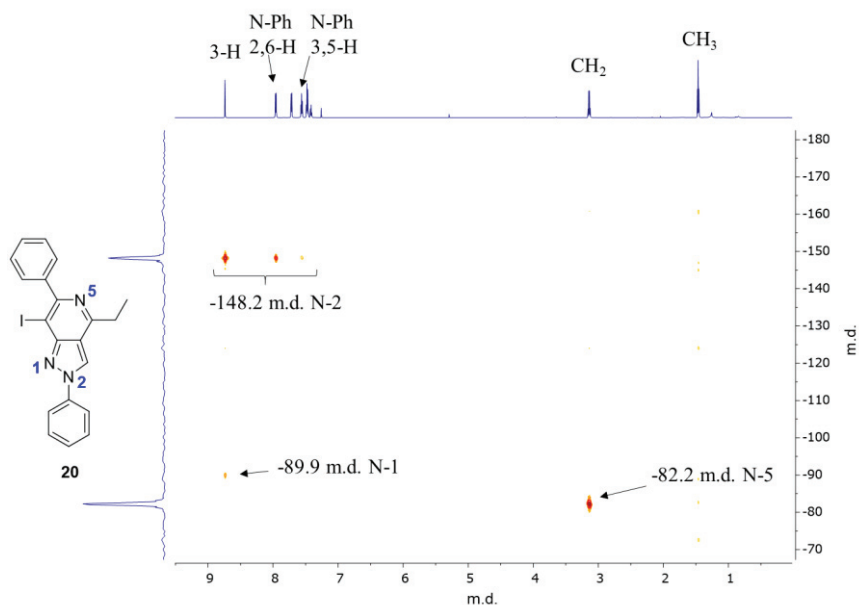
1.4 schema. 2-Fenil-7-jod-2H-pirazolo[4,3-c]piridino sintezė. *Reagentai ir sąlygos:* i: K₂CO₃ (bevandenis), MeOH (sausas); ii: K₃PO₄, I₂, DCM, kamb t, 12 val; iii: NaHCO₃, NIS, DCE, 50 °C, 2 dienos

4-Pakeistų 2,6-difenil-7-jod-2H-pirazolo[4,3-c]piridinų **18-22** struktūros buvo įrodytos BMR, IR spektroskopijos ir MS bei HRMS spektrometrijos metodais. Ciklizacijos produkto struktūros susidarymą galima iliustruoti palyginant azido **17** ir pirazolopiridino **22** ¹H BMR spektrus (1.1 pav.). Spektre matomas pirazolo žiedo protono signalo pasislinkimas į silpnesnius laukus (iš 7.82 m.d. į 8.90 m.d.) ir nematomas charakteringas protonų šalia azido grupės signalas. Taip pat pirazolpiridino **22** ¹³C BMR spektre neberegistruojami acetileninio ryšio ir alifatinių anglių signalai.



1.1 pav. Junginių **17** ir **22** ^1H BMR spektrų palyginimas

Detalus BMR spektroskopinis tyrimas kombinuojant įvairias BMR technikas leido atlikti pilną ir nedviprasmišką visų ^1H - ir ^{13}C -BMR poslinkių priskyrimą. Pirazolo[4,3-*c*]piridino struktūra gali būti įrodyta naudojantis ^1H - ^{15}N HMBC spektru (1.2 pav.), kuriame matoma trijų azoto atomo signalų sąveika su atitinkamais protonais. Konkrečiu junginio **20** atveju matoma piridino azoto (N-5) sąveika su CH_2 protonais, azoto signalui esant ties -82.2 m.d. O pirazolo azotų N-2 ir N-1 sąveikos signalai su 3-H protonais matomos ties atitinkamai -148.2 ir -89.9 m.d.. Sąveikos tarp azoto N-2 ir protonų iš N-fenil grupės yra naudingos priskiriant jų protonų poslinkius. Multipletas ties 7.93-7.98 m.d. priskiriamas N-Ph 2,6 protonams, kadangi jų sąveikos signalas yra stipresnis. Atitinkamai dėl silpnescio sąveikos signalo multipletas ties 7.54-7.58 m.d. priskiriamas N-Ph 3,5-H protonams.



1.2 pav. Junginio 20 ^1H - ^{15}N HMBC spektras

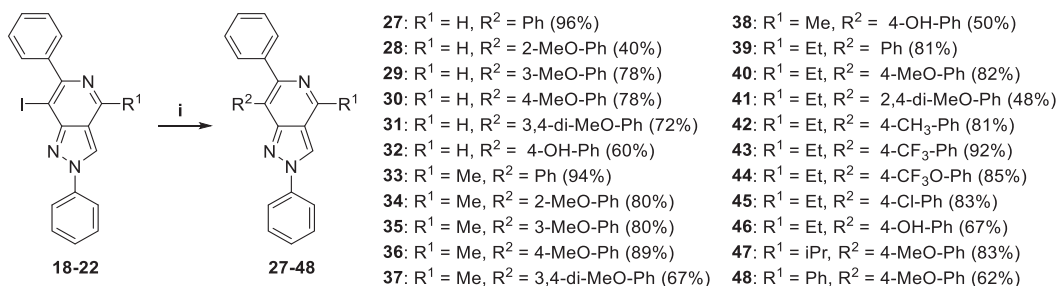
Sėkmingai paruošus 4-pakeistus 2,6-difenil-7-jod-2*H*-pirazolo[4,3-*c*]piridinus, išbandytos jų galimybės dalyvauti Suzuki-Miyaura, Buchwald-Hartwig ir Heck kryžminio jungimo reakcijose.

2. 7-Aril-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridinų sintezė ir jų optinio ir biologinio aktyvumo tyrimas

2.1. 7-Aril-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridinų sintezė

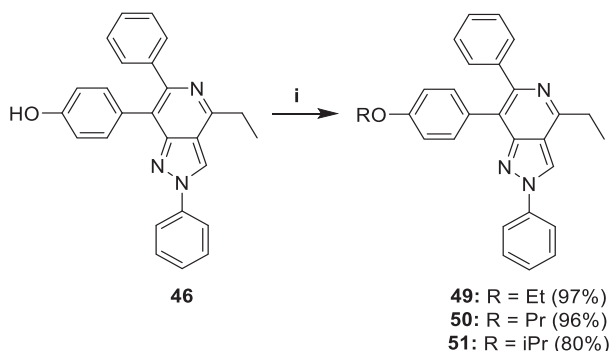
Suzuki-Miyaura kryžminio jungimo reakcija dažnai naudojama medicininėje chemijoje svarbių farmakoforų ir organinių medžiagų sintezėje. Ji pritaikyta gaunant skirtingas pakeistų kondensuotų heterociklų sistemas [111, 113, 115].

Taigi, siekiant gauti 7-aril-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridinus, buvo pritaikytos Pd-katalizuojamo Suzuki-Miyaura kryžminio jungimo sąlygos. Konkrečiau, aromatinė boro rūgštis reagavo su 7-jod-2*H*-pirazolo[4,3-*c*]piridinais **18–22** naudojant $\text{Pd}(\text{OAc})_2$ kaip katalizatorių, Cs_2CO_3 kaip bazę ir vandeninį EtOH kaip tirpiklį esant argono atmosferai (2.1 schema) [118]. Norint užtikrinti trumpą reakcijos laiką, arilimas buvo atliekamas mikrobangų reaktoriuje, taip junginiai **27–48** susidarė per 1 valandą. Junginiai **27** ir **29–48** gauti geromis išeigomis (48–96%), deja, nepavyko įvykdyti visiškos junginio **18** konversijos naudojant 2-metoksifenilboroninę rūgštį, tai nulėmė mažą junginio **28** išeigą.



2.1 schema. Junginių 27–48 sintezė ir išeigos. *Reagentai ir sąlygos:* i: arilboroninė rūgštis, Pd(OAc)₂, Cs₂CO₃, EtOH/H₂O 3/1 MWI 100 °C 0,5–1 val.

Vėliau 4-etil-7-(4-hidroksifenil)-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridinas **46** dalyvavo hidroksigrupės alkiliniame su etil-, propil- ir izopropiljodidais naudojant NaH kaip bazę maišant 1 val. DMF 70 °C temperatūroje. 7-(4-alkoksifenil)-4-etil-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridinai **49–51** gauti didelėmis išeigomis (80–97%) (2.2 schema).

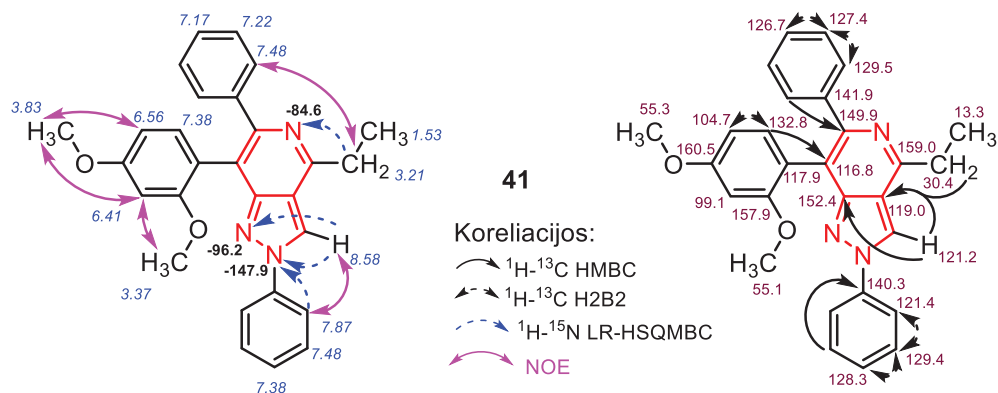


2.2 schema. Junginių 49–51 sintezė. *Reagentai ir sąlygos:* i: NaH, RI, DMF, 70 °C, 1 val.

Naujų junginių 27–51 struktūros buvo įrodytos BMR, IR spektroskopijos ir MS bei HRMS spektrometrijos metodais. Palyginti su pradiniais junginiais 18–22, naujų junginių ¹H BMR spektrai turi naujus aromatinčius signalus, o 28–31, 34–37, 40–41 ir 47–51 taip pat turi naujus metoksigrupės singletus su poslinkiais 3.37–3.90 m.d. srityje. Darinių su hidroksigrupe fenilžiede struktūros ir jų alkilinimas gali būti greitai įrodomas FT-IR spektru, kuriame platus OH ryšio virpesių signalas aptinkamas ir vėliau nebefiksuojamas susiformavus alkoksifenilgrupei.

Detalus BMR spektroskopinis tyrimas leido atlikti pilną ir nedviprasmišką visų ¹H- ir ¹³C-BMR poslinkių priskyrimą. Pavyzdžiui, **41** junginio atveju (2.1 pav.) pirazolo N-1 (δ –96.2 m.d.) ir N-2 (δ –147.9 m.d.) azoto atomai lengvai atskiriami, nes jie pasižymi ilgo nuotolio HSQMB koreliacijomis su kaimyniniu 3-H protonu (singletas, δ 8.58 m.d.), o piridino N-5 (–84.6 m.d.) azoto atomas sąveikauja su CH₂ protonais (kvartetą, δ 3.21 m.d.). Skirtingų fenilgrupių protono prisiskyrimas atliktas naudojantis ¹H-¹H NOESY spektru, kadangi jame buvo matomos erdvinės sąveikos tarp N-fenilgrupės 2,6-H protono ir piridino 3-H atomo ir C6-fenilgrupės 2,6-H protonų ir CH₂ protonų. Taip pat koreliacijos tarp C7-fenilo 3-H protonų (multipletas, δ 6.41 m.d.) ir abiejų metoksigrupės singletų bei 5-H protono

(multipletas, δ 6.56 m.d.) vienetinė sąveika su 4-metoksigrupės singletu leido charakterizuoti šiuos protonus. Vėliau H2BC spektras leido priskirti likusius fenilgrupės protonus, kadangi 3,5-H protonas sąveikavo su abiem 2,6-H ir 4-H protonų multipletais. Galiausiai, ketvirtinės anglis priskirtos pasitelkiant HMBC spektrą. Šiuo atveju 3-H protonas koreliavo tiek su C-3a (119.0 m.d.), tiek su C-7a (152.4 m.d.), o CH₂ protonai tik su C-3a. Trijų ryšių sąveika tarp C-6 (149.9 m.d.) ir C-7 (116.8 m.d.) su 2,6-H fenilgrupės protonais iki galo patvirtino pirazolo[4,3-*c*]piridino **41** struktūrą.



2.1 pav. Aktualios ¹H-¹³C HMBC, ¹H-¹³C H2BC, ¹H-¹⁵N HMBC ir ¹H-¹H NOESY koreliacijos ir ¹H BMR (pasviręs), ¹³C BMR ir ¹⁵N BMR (ryškus) cheminiai junginio **41** poslinkiai

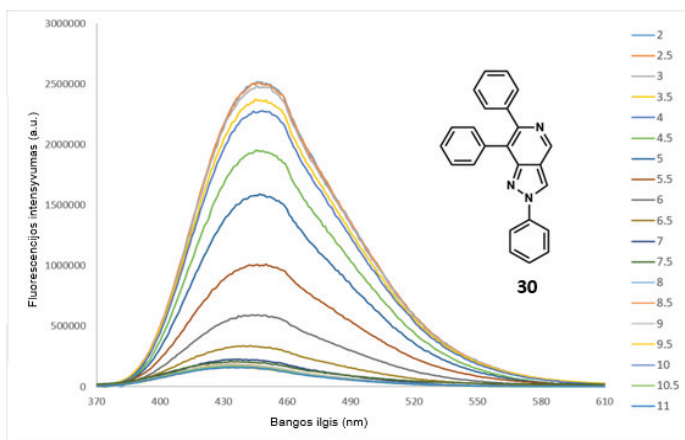
2.2. Optinės 7-aryl-2,6-difenil-2H-pirazolo[4,3-*c*]piridinių savybės

7-Aril-2,6-difenil-2H-pirazolo[4,3-*c*]piridinių **27–51** fluorescencijos savybės pirmiausia buvo įvertintos THF, pasirenkant sužadinimo bangos ilgį ties 350 nm. Visų junginių emisijos maksimumas fiksuotas 437–487 nm srityje, atitinkančioje mėlynąją matomo spektro dalį. Polinį 4-hidroksifenilpakaitą 7-oje pozicijoje turintys junginiai **32**, **38**, ir **46** bei jų dariniai **40–41**, **47**, **49–51** pasižymėjo didžiausiu batochrominiu poslinkiu ir labiausiai išreikštomis fluorescencinėmis savybėmis. Šių junginių Stokso poslinkiai yra 199–205 nm srityje, o kvantinė išeiga siekė apie 60–85%. Įdomu tai, kad kvantinė išeiga šiek tiek padidėja ilgėjant 4-alkilgrandinei, bet gerokai sumenksta esant fenilgrupei (40%).

Intramolekulinis pH svarbus daugelyje biologinių procesų, kadangi jo pokytis gali lemti ląstelės disfunkciją ir įvairias ligas [119]. Tad pH-jautrių fluorescuojančių indikatorių sukūrimas leidžia tiksliai išmatuoti tarpląstelinį pH, taip suteikiant galimybę stebėti vykstančius fiziologinius ir patologinius procesus ląstelių ir subląstelių lygmenimis [120]. Taigi siekiant įvertinti, ar 7-arilpakeistų pirazolo[4,3-*c*]piridinių fluorescencijos savybės yra priklausomos nuo pH, visi junginiai tirti pH 5, 7 ir 9 buferuose, esant 360 nm sužadinimo bangai. Matavimai atlikti mgr. Radeko Ostruszkos ir doc. Martino Kubalos Eksperimentinės fizikos katedroje, Palackio universitete.

Bendrai visų junginių kvantinė išeiga vandeniniuose tirpaluose buvo mažesnė nei THF. Pastebėta, kad junginių **27**, **33** ir **39** su 7-fenilpakaitu kvantinė išeiga padidėjo 2–5 kartus rūgštiniame pH be ryškaus emisijos maksimumo (435–447 nm srityje) pokyčio. Tolimesnis junginio **27** tyrimas pH 2–11 buferiuose parodė, kad, didėjant pH vertei, fluorescencijos intensyvumas mažėja.

Kita vertus, kvantinė 2-metoksifenil- ar 4-metoksifenilpakaitą 7-oje padėtyje turinčių junginių **28**, **30**, **34**, **36** ir **40** išeiga buvo didesnė baziniame pH. Be to, 4-metoksifenilpakaitus turinčių junginių **30**, **36** ir **40** atveju rūgštinis pH sukėlė emisijos maksimumo raudoną poslinkį į žalią matomo spektro dalį. Nustatyta, kad junginio **30** emisijos spektras susidaro iš dviejų persidengiančių dalių (2.2 pav.). Trumpųjų bangų dalis yra jautri pH, ji dominuoja bazinėje terpėje, tad pH mažėjimas nuo 11 iki 6 sukėlė fluorescencijos intensyvumo pokytį, tačiau nepakeitė emisijos maksimumo (išliko ties 458 nm). Toliau mažėjant pH, dominuojanti tapo ilgųjų bangų dalis, kuri pasireiškė laipsnišku emisijos maksimumo poslinkiu iki 519 nm. Kitų 4-alkoksifenilpakaitų atveju buvo matomastik kvantinės išeigos mažėjimas rūgštinėje terpėje, nesikeičiant emisijos maksimumo poslinkiui.



2.2 pav. Junginio **30** fluorescencijos spektrai ($\lambda_{\text{ex}} = 360$ nm) Britton–Robinson pH 2–11 buferiuose

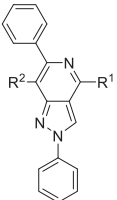
Taigi, pirminiai pastebėjimai rodo, kad dauguma junginių, išskyrus polinius **32**, **38** ir **46**, gali būti įdomūs kaip pH indikatoriai. Atsižvelgiant į 5 kartų kvantinės išeigos padidėjimą ir 40 nm mėlyną emisijos maksimumo poslinkį pH keičiantis nuo 5 iki 9, junginys **30** yra geriausias pH indikatorius iš visų ištirtų molekulių, leidžiantis tiek nuo fluorescencijos intensyvumo priklausomą, tiek ratiometrinį pH nustatymą.

2.3. Biologinis 7-aril-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridinų tyrimas

Susintetintų 2*H*-pirazolo[4,3-*c*]piridinų **27–51** antiproliferacinio poveikio 3 žmogaus vėžinėms ląstelių linijoms, t. y. MV4-11 (bifenotipo B mielomonocitinės leukemijos ląstelės), K562 (chroninės mieloidinės leukemijos ląstelės) ir MCF-7 (krūties vėžio ląstelės), tyrimas buvo atliktas Eksperimentinės biologijos katedroje,

Palackio universitete (2.1 lentelė). Kaip pastebėta anksčiau [92], didėjant pakaitui 4-oje pozicijoje, laipsniškai mažėja ar visai dingsta 2*H*-pirazolo[4,3-*c*]piridino darinių aktyvumas. Šiuo atveju metilpakeisti junginiai **36–38** pasižymi mažesnėmis antiproliferacijos vertėmis nei jų nepakeisti analogai **27–32**, o izopropil-, etil- ir fenilpakeisti junginiai **46–48** buvo daugiausiai neaktyvūs iki tikrintos 10 μM koncentracijos. Bendrai polinis 4-hidroksifenilpakaitas 7-pozicijoje, be pakaito 4-oje darinys **32** buvo pats aktyviausias. Įdomu tai, kad tiek junginys **32**, tiek jo 4-metilanalogs **38** buvo aktyvesni už anksčiau tirtus 7-nepakeistus 2,6-difenil-2*H*-pirazolo[4,3-*c*]piridinus **A** ir **B** [92].

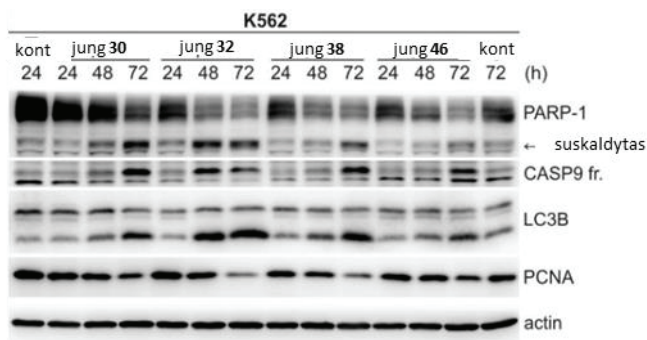
2.1 lentelė. *In vitro* antiproliferacinis 2*H*-pirazolo[4,3-*c*]piridino darinių **27–48** aktyvumas.

Struktūra	Junginys	R ¹	R ²	GI ₅₀ $\pm$ SD, μM *		
				MV4-11	K562	MCF-7
	27	H	Ph	7,7 $\pm$ 2,6	>10	>10
	28	H	2-MeO-Ph	6,5 $\pm$ 1,3	7,1 $\pm$ 2,9	6,3 $\pm$ 2,2
	29	H	3-MeO-Ph	5,0 $\pm$ 1,8	>10	>10
	30	H	4-MeO-Ph	3,5 $\pm$ 1,2	4,8 $\pm$ 2,5	7,3 $\pm$ 0,2
	31	H	3,4-di-MeO-Ph	2,4 $\pm$ 1,3	6,0 $\pm$ 3,8	4,2 $\pm$ 0,9
	32	H	4-OH-Ph	1,5 $\pm$ 0,7	2,4 $\pm$ 1,0	1,6 $\pm$ 0,2
	36	Me	4-MeO-Ph	>10	>10	8,4 $\pm$ 2,1
	37	Me	3,4-di-MeO-Ph	7,6 $\pm$ 3,0	>10	>10
	38	Me	4-OH-Ph	4,7 $\pm$ 2,7	3,9 $\pm$ 0,3	4,1 $\pm$ 0,4
	46	Et	4-OH-Ph	8,0 $\pm$ 3,1	>10	3,9 $\pm$ 0,4
	47	iPr	4-MeO-Ph	5,7 $\pm$ 1,4	>10	>10
	48	Ph	4-MeO-Ph	>10	>10	7,9 $\pm$ 3,8
	A [92]	H	H		3,4	4,8
	B [92]	Me	H		6,3	11,0

* Duomenys yra bent trijų nepriklausomų tyrimų rezultatų vidurkis

Taip pat buvo atliktas tyrimas siekiant nustatyti junginių toksiškumą nevėžinėms ląstelėms, t. y. žmogaus tinklainės epitelio (RPE-1) ir fibroplastų MRC-5 ląstelėms. Nustatyta, kad, ilgėjant alkil grandinei 4-oje padėtyje, toksiškumas mažėja.

Be to, atliktas aktyviausio junginio **32**, bei 4-pakeistų analogų **38** ir **46** bei darinio **30** tyrimas K562 leukemijos ląstelėse. Asinchroniškai augančios K562 ląstelės buvo veikiamos 10 μM pasirinktų junginių koncentracija 24, 48 ir 72 val. ir analizuotos naudojantis imunoblotingo ir tėkmės citometrijos metodais.



2.3 pav. Pasirinktų ląstelės mirties ir proliferacijos žymenų imunoblotingo analizė

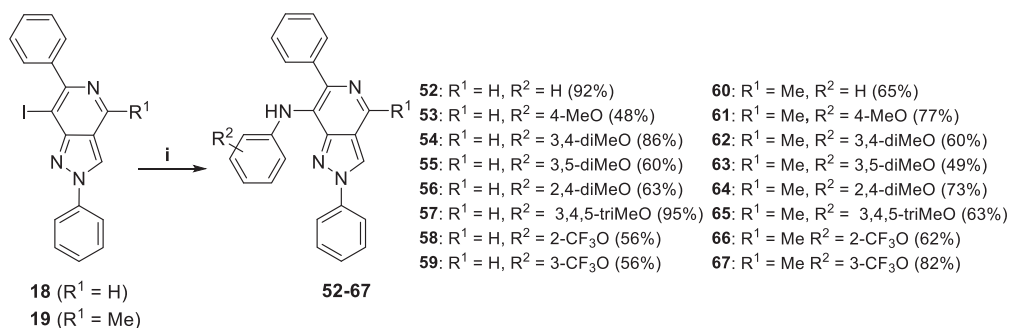
Imunoblotingas (2.3 pav.) parodė, kad užtenka 48 val. veikimo junginių **32**, siekiant paskatinti poli(ADP-ribozės) polimerazės 1 (PARP-1) skilimą [125] ir aktyvuoti apoptotinės kaspazės 9 kaskados iniciatorių [126]. Taip pat pastebėta nuo laiko priklausoma baltymo LC3 fragmentacija, kuri vyksta esant autofagijai [127]. Panašūs rezultatai nustatyti visuose bandiniuose. Toliau ištirta proliferuojančios ląstelės branduolio antigeno (PCNA) ekspresija, kuri labai svarbi DNR dauginimuisi [128]. Nustatyta, kad visi junginiai pasižymi nuo laiko priklausoma PCNA redukcija, kuri labiausiai pasireiškė junginiuose **32** ir **38**.

Bendrai gauti rezultatai rodo, kad junginių veikimo mechanizmas yra kompleksinis ir apima tiek antiproliferacinius, tiek ląstelės mirtį skatinančius efektus.

2.3. *N*-Aril-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-aminų sintezė ir jų optinio ir biologinio aktyvumo tyrimas

2.3.1. *N*-Aril-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-aminų sintezė

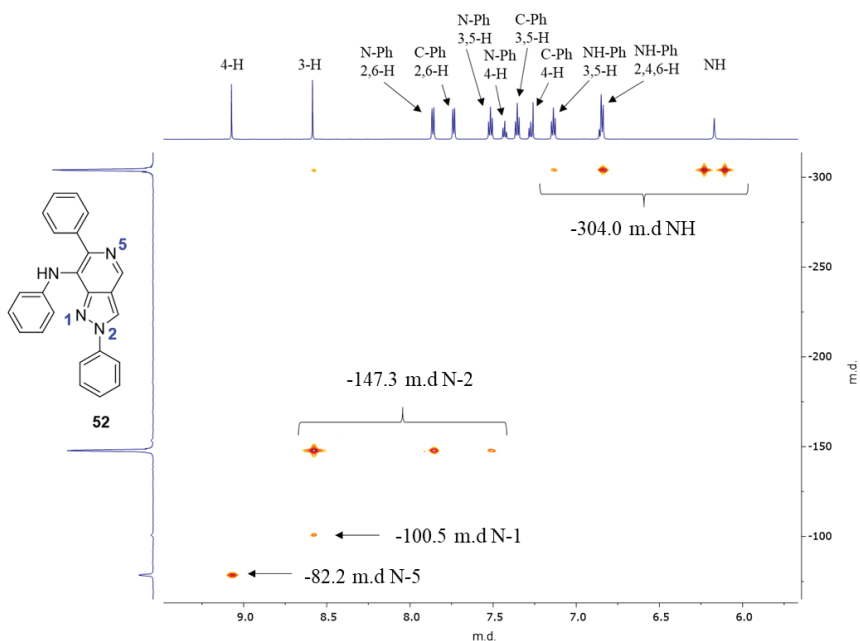
Buchwald-Hartwig reakcija taikoma organinėje chemijoje siekiant sudaryti C-N ryšį tarp aminų ir arilhalidų. Ji plačiai naudojama naujų heterociklinių sistemų, farmacinių medžiagų sintezėje [130–132]. Tad, siekiant prijungti amino grupę, paruošti 7-jod-2*H*-pirazolo[4,3-*c*]piridinai **18** ir **19** buvo paveikti anksčiau aprašytų Buchwald-Hartwig kryžminio jungimo sąlygų [99, 137]. Tiksliau, **18** ir **19** junginiai dioksane reagavo su atitinkamu anilinu, Pd(OAc)₂ veikė kaip katalizatorius, SPhos kaip ligandas ir Na^tOBu kaip bazė (2.3 schema). Siekiant užtikrinti trumpą reakcijos laiką, jos vykdytos mikrobangų reaktoriuje ir junginiai **52–67** gauti 48–95% išeigomis. Svarbu pažymėti, kad reakcijos dėl katalizatoriaus ir ligando jautrumo orui ir drėgmei vykdytos sausame tirpiklyje esant argono atmosferai.



2.3 schema. Junginių **52-67** sintezė ir išeigos. *Reagentai ir sąlygos:* (i) R^2PhNH_2 , $Pd(OAc)_2$, SPhos, Na^tBuO , dioksanas, MWI, 120 °C, 280 W, 1 val.

Junginių **52-67** struktūros buvo įrodytos BMR, IR spektroskopijos ir MS bei HRMS spektrometrijos metodais. Amino susidarymą patvirtina IR spektras, kuriame randami amino grupės virpesių signalai ties 3204–3412 cm^{-1} . Taip pat, palyginti su pradinėmis medžiagomis **18** ir **19**, naujų junginių ^1H BMR spektruose atsiranda platus NH singletas ties 5.89–6.46 m.d., nauji N-arilprotonų signalai ties 5.97–7.21 m.d., o junginiai **53–57** ir **61–65** papildomai turi metoksigrupės protonų singletus ties 3.65–3.83 m.d.

Detalus BMR spektroskopinis tyrimas leido atlikti pilną ir nedviprasmišką visų ^1H - ir ^{13}C -BMR poslinkių priskyrimą. Pavyzdžiui, amino grupės protonas ir prie jo prisijungusio fenilo žiedo protonai buvo priskirti naudojantis ^1H - ^{15}N HMBC spektru (2.4 pav.). Spektre matyti azoto NH sąveika su 6.17 singletu bei multipletais ties 6.81–7.16 m.d., kurie priskirti fenilgrupės protonams. Tikslūs šių protonų signalai priskirti naudojantis ^1H - ^{13}C HMBC spektru, kuriame amino grupės protonas turėjo vieną sąveiką su 117.5 m.d. anglimi, kuri koreliuoja su 6.81–6.88 m.d. protonų multipletu, taip jį priskiriant NH-Ph 2,6-H.



2.4 pav. Junginio **52** ^1H - ^{15}N HMBC spektras

2.3.2. Optinės *N*-aril-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-aminų savybės

Kaip ir ankstesnių junginių, *N*-aril-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-aminų **52–67** fluorescencijos savybės pirmiausia buvo ištirtos THF, sužadinimo bangos ilgį nustačius ties 350 nm. Emisijos maksimumai buvo užfiksuoti 453–486 nm srityje, kuri atitinka mėlynąją matomo spektro dalį, išskyrus junginius **52**, **55**, **60** ir **63** su fenil- ar 3,5-dimetoksifenilpakaitais, kurių emisijos maksimumai užfiksuoti žaliojoje dalyje (506–520 nm). Palyginti su 7-arilpirazolpiridinais, amino grupės atsiradimas nulėmė tiek Stokso poslinkio, tiek kvantinės išeigos sumažėjimą. Geriausiomis savybėmis pasižymėjo trifluorometoksifenilpakaitus turintys junginiai **58–59** ir **66–67**, jų kvantinė išeiga siekė apie 37–45%.

2.2.3. Biologinis *N*-aril-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-aminų tyrimas

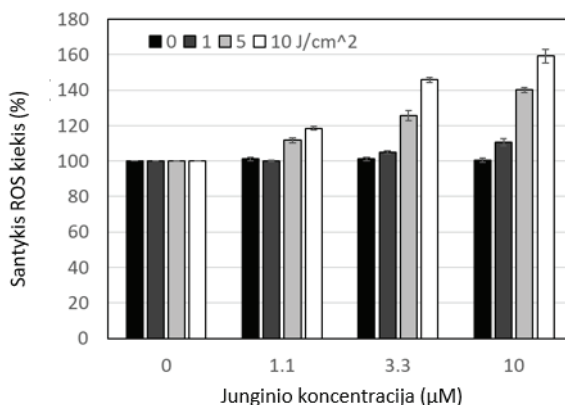
Biologinės *N*-aril-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-aminų savybės buvo ištirtos Eksperimentinės biologijos ir Medicinos biofizikos katedroje, Palackio universitete. Pirmiausia buvo įvertintas susintetintų *N*-aril-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-aminų **52–67** antiproliferacinis aktyvumas veikiant K562 ir MCF-7 vėžines ląsteles. Deja, amino grupės atsiradimas nulėmė biologinio aktyvumo sumažėjimą, palyginti su dariniais, gautais Suzuki reakcijos metu. Geriausias rezultatas buvo junginio **56**, jo GI_{50} vertė siekė 7,1 μM prieš K562 ląsteles.

Vėliau buvo pastebėta, kad aktyvumas padidėja esant didesnei apšvitai. Iškelta hipotezė, kad junginiai pasižymi fotosentizuojančiomis savybėmis, kurios vėliau pritaikomos fotodinaminėje (PDT) terapijoje. Jos metu šviesos aktyvuotas fotosensitizatorius – netoksiškas junginys – tikslinėse molekulėse pradeda gaminti

reaktingus deguonies tipus (ROS), kurie ardo biomolekules (riebalus, baltymus, nukleorūgtįs), taip sukeldami ląstelės suirimą [140]. Taigi, siekiant įvertinti junginių **52–67** fotodinaminį potencialą, melanomos G361 ląstelės 4 val. buvo veikiamos junginiais ir tuomet mėlyna šviesa (LED šaltinis, 414 nm), esant 10 J/cm² apšvitos dozei. Ląstelių gyvybingumas buvo įvertintas 72 val. po apšvitos taikant MTT analizę, kuri parodė, kad dauguma junginių pasižymi fotodinaminiu aktyvumu. 4-Nepakeisti dariniai **52–59** buvo aktyvesni nei jų 4-metilanalagai **60–67**. Junginiai **53** ir **58** yra geriausi fotosensibilizatoriai, jų EC₅₀ koncentracija siekė 1,1 μM. Visi junginiai tikrintose koncentracijose (iki 10 μM), išskyrus **56**, tamsoje buvo neaktyvūs.

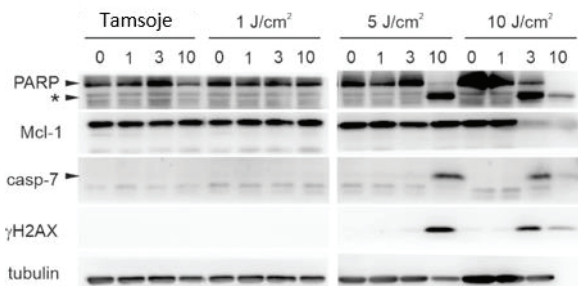
Taip pat nustatyta, kad fotoaktyvacija yra priklausoma nuo apšvitos dydžio. Sumažinant šviesos intensyvumą iki 5 ir 1 J/cm², junginio **58** EC₅₀ koncentracija padidėjo atitinkamai iki 1,6 ir 3,5 μM. Atlikus tokį patį tyrimą ant keratinocitų HaCaT ląstelių, įrodyta, kad šios nevėžinės ląstelės yra 2 kartus mažiau jautrios veikimui junginiu **58**.

Kadangi pagrindinis PDT ląstelių mirties mechanizmas yra ROS generavimas [56], siekiant plačiau ištirti junginio **58** fototoksinį aktyvumą, ROS gamyba paveiktose ląstelėse buvo išmatuota naudojant 2',7'-dichlordihidrofluoresceino diacetatą. Kaip tikėtasi, ROS nebuvo gaminamas nepaveiktose ir / ar neapšviestose ląstelėse, o veikimas įvairia junginio **58** koncentracija (1; 1,1; 3,3 ir 10 μM) bei apšvitos doze (1, 5 ir 10 J/cm²) sukėlė ROS gamybą (2.5 pav.).



2.5 pav. ROS gamyba G361 ląstelėse paveiktose junginiu **58** (1.1, 3.3 or 10 μM) bei apšvita (414 nm)

DNR pažeida bei apoptotinė ląstelių mirtis buvo patvirtintos imunoblotingo analizės. Didesnė junginio **58** koncentracija ir apšvitos dozė lėmė padidėjusį H2A.X, DNR pažeidos žymens, lygį (2.6 pav.). Analogiškai PARP, fermento dalyvaujančio DNR taisyme, koncentracija didėjant junginio dozei ir apšvitai sumažėjo. Galiausiai, kaspazės 7 aktyvacija ir Mcl-1 deaktyvacija patvirtino apoptozę. Svarbu tai, kad šių pokyčių nebuvo ląstelėse be junginio **58** ir / ar tamsoje. Tai patvirtina, kad visi stebimi efektai yra fotodinaminiai.



2.6 pav. G361 ląstelių, paveiktų junginiu **58**, imunoblotingo analizė

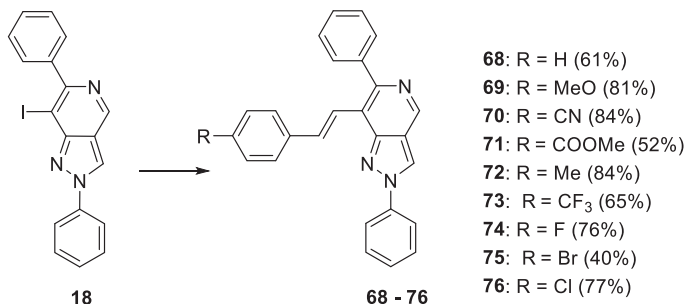
2,4, 2,6-Difenil-7-[(*E*)-2-feniletetil]-2*H*-pirazolo[4,3-*c*]piridinų ir (2*E*)-3-(2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-il)-*N*-fenilprop-2-enamidų sintezė ir jų optinio ir biologinio aktyvumo tyrimas

2.4.1. 2,6-Difenil-7-[(*E*)-2-feniletetil]-2*H*-pirazolo[4,3-*c*]piridinų sintezė

Heck reakcija, kryžminis organohalidų ir olefinų jungimas sukuriant naujus alkenų darinius, priklauso svarbiai reakcijų, sukuriančių C-C ryšį, klasei ir yra plačiai taikoma organinėje sintezėje [158, 159].

Siekiant prie pirazolo[4,3-*c*]piridino prijungti vinil- pakaitą buvo išbandyti 2 sintezės tipai (8 schema). Pirmiausia 7-jodpirazolo[4,3-*c*]piridinas **18** buvo veikiamas Heck kryžminio jungimo sąlygų, t. y. Pd(PPh₃)Cl₂ kaip katalizatoriaus, TEA kaip bazės ir DMF kaip tirpiklio. Reakcija įvyko per 1 val vykdamą j mikrobangų reaktoriuje 120 °C ir 61% išeiga gautas 2,6-difenil-7-[(*E*)-2-feniletetil]-2*H*-pirazolo[4,3-*c*]piridinas **68 H**. Toliau bandytos anksčiau taikytos Suzuki-Miyaura sąlygos. Reakcija taip pat truko 1 val., pasiekta 79% junginio **68S** išeiga.

Nors Suzuki-Miyaura reakcijos išeiga buvo didesnė, dėl didesnio stireno darinių pasirinkimo ir mažesnės kainos buvo nuspręsta tęsti naudojant Heck reakcijos sąlygas (2.4 schema). Visos reakcijos vidutiniškai truko apie 1–3 val., o junginiai **69–76** gauti 40–84% išeigomis.

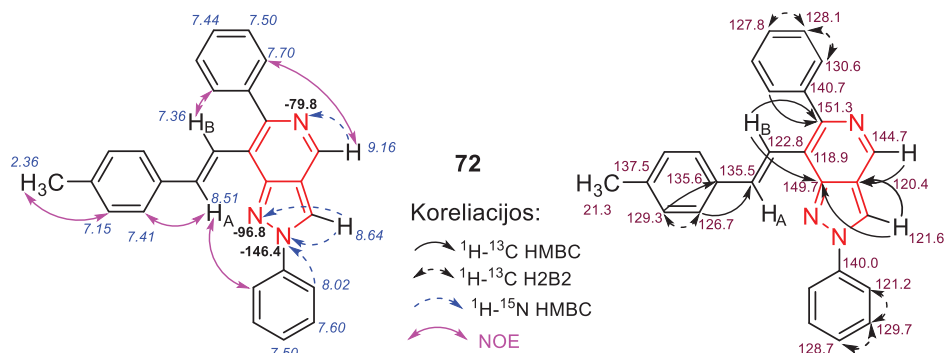


2.4 schema. Junginių **68-76** sintezė. *Reagentai ir sąlygos*: atitinkamas stirenas, TEA, Pd(PPh₃)Cl₂, DMF, MWI, 120 °C, 100 W, 1–3 val.

Pastebėta, kad para pakeisti stirenai su elektrondonorinėmis grupėmis (junginiai **69, 72**) lėmė geresnės išeigas nei elektroakceptorinėmis grupėmis

(junginiai **71**, **73**), išskyrus nitrilo atvejų. Bromstirenas reagavo prasčiau nei fluor- ar chlorstirenas, kadangi dėl didelio reaktingumo kryžminiame jungime jis taip pat galėjo reaguoti su stireno alkeno grupe.

Naujų junginių **68–76** struktūros buvo įrodytos BMR, IR spektroskopijos ir MS bei HRMS spektrometrijos metodais. Palyginti su pradiniu junginiu **18**, naujų darinių ^1H BMR spektrai turi naujus aromatinčius signalus ir etenilinės grupės dubletus (2.7 pav.). Visų darinių etileno protonų, esančių šalia fenilo, signalai H_A yra silpnėsiuose laukuose (8.47–8.56 m.d.), palyginti su protonais šalia piridino (7.27–7.55 m.d.). Detalus BMR spektroskopinis tyrimas leido atlikti pilną ir nedviprasmišką visų ^1H - ir ^{13}C -BMR poslinkių priskyrimą. Pavyzdžiui, etenilinių protonų atskyrimui buvo panaudotas HMBC spektras, kuriame protonas H_B sąveikavo su ketvirtinėmis piridino anglimis, o H_A sąveikavo su 2 ir 6 fenilgrupės anglimi. Priskyrimą patvirtino ^1H - ^1H NOESY spektras, kuriame matomos erdvinės sąveikos tarp olefininio protono H_A ir N-fenil protonų 2,6-H, o protonas H_B sąveikavo su C6-fenil protonais 2,6-H. Galiausiai, ^1H - ^{13}C HSQC spektras buvo panaudotas etenilinės grupės anglių cheminiams poslinkiams nustatyti. Jie pasižymėjo tokiu pat modeliu, kaip protonai: C_B pikai randami stipresniuose laukuose (121.7–127.4 m.d.) nei C_A pikai (133.3–135.6 m.d.).

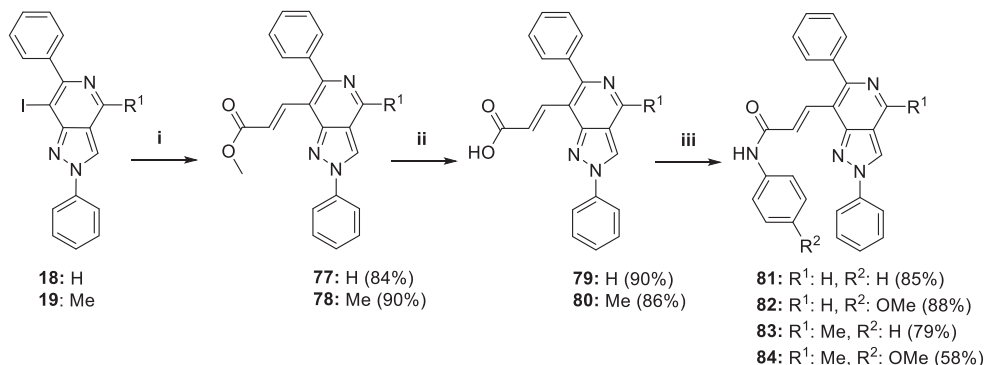


2.7 pav. Aktualios ^1H - ^{13}C HMBC, ^1H - ^{13}C H2BC, ^1H - ^{15}N HMBC ir ^1H - ^1H NOESY koreliacijos bei ^1H BMR (pasvirę), ^{13}C BMR ir ^{15}N BMR (ryškūs) cheminiai **72** junginio poslinkiai

2.4.2. (2E)-3-(2,6-Difenil-2H-pirazolo[4,3-c]piridin-7-il)-N-fenilprop-2-enamidų sintezė

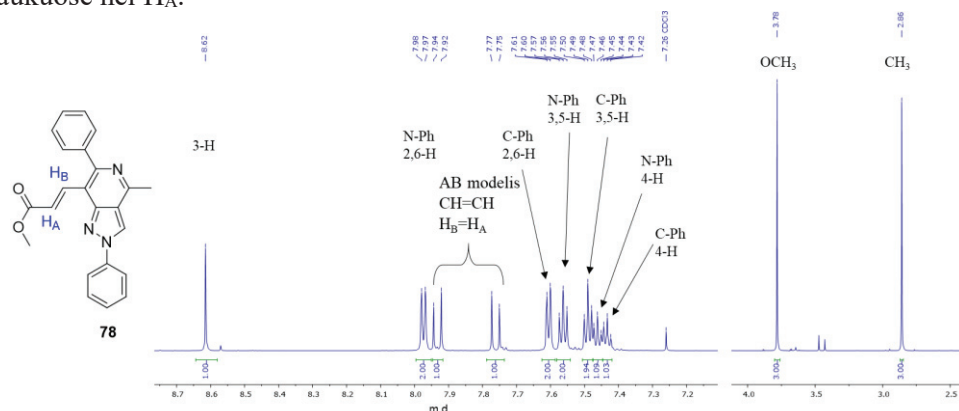
Siekiant prijungti dar daugiau funkcinių grupių, Heck kryžminis jungimas atliktas su metil akrilatu (2.5 schema). Pritaikant tas pačias reakcijos sąlygas kaip stirenų atveju, reakcijos įvyko per trumpesnę laiką, ir produktų išeigos buvo geresnės. Tuomet esteris hidrolizuotas naudojant NaOH verdančiame MeOH. Reakcijos truko apie 3 val., jų pabaigą galima pastebėti pagal tirpiklio skaidrumo pokytį – esteriai buvo prastai tirpūs metanolyje, o rūgštys jame ištirpo visiškai. Reakcijos produktas tolesniame sintezės etape buvo naudojamas tiek be, tiek su grynimu silikageliu. Galiausiai atlikta enoinių rūgščių **79** ir **80** kondensacija su

anilinu arba 4-metoksianilinu. Norint sudaryti amidinį ryšį, kaip karboksilo grupės aktyvatorius buvo pasirinktas EDC·HCl bei katalizatorius DMAP, ir reakcija DCM vyko 20 val. Svarbu pažymėti, kad 4-nepakeisti pirazolo[4,3-*c*]piridinil-*N*-fenilprop-2-enamidai **81–82** buvo gauti geresnėmis išeigomis nei jų 4-metilanalagai **83–84**.



2.5 schema. Junginių **81–84** sintezė. *Reagentai ir sąlygos:* i: metil akrilatas, TEA, Pd(PPh₃)Cl₂, DMF, MW, 120 °C, 100 W, 30 min., ii: NaOH, MeOH, 65 °C, 3 val, iii: anilinas (**81** ir **83**) arba 4-metoksianilinas (**82** ir **84**), DMAP, EDC·HCl, DCM, kamb t, 20 val.

Naujų junginių **77–84** struktūros buvo įrodytos BMR, IR spektroskopijos ir MS spektrometrijos metodais. Palyginti su pradinėmis medžiagomis **18** ir **19**, naujų junginių ¹H BMR spektrai turi naujus etenilinės grupės dubletus, o prop-2-enamidai **81–84** papildomai turi naujus aromatinčius multiplėtus bei NH singletus. Etenilinių protonų cheminiai poslinkiai šalia piridino H_B yra silpnėsiuose laukuose (7.74–8.01 m.d.) nei protonų šalia karbonilinės grupės H_A (7.65–7.82 m.d.) enoatuose ir enoinės rūgšties dariniuose **77–80** (2.8 pav.). Įdomu tai, kad enamidų **81–84** eteniliniai protonai pasižymi kita struktūra: 4-nepakeistų pirazolo[4,3-*c*]piridinil-*N*-fenilprop-2-enamidų **81**, **82** atveju poslinkiai yra tokie patys, kaip aprašyta anksčiau (H_B silpnėsiuose laukuose nei H_A), o 4-metilpirazolo[4,3-*c*]piridinil-*N*-fenilprop-2-enamidų **83**, **84** atveju poslinkiai apsieičia vietomis, ir H_B atsiranda stipresniuose laukuose nei H_A.



2.8 pav. Junginio **78** ¹H BMR spektras

2.4.3. Optinės 2,6-difenil-7-[(*E*)-2-feniletetil]-2*H*-pirazolo[4,3-*c*]piridinų ir (2*E*)-3-(2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-il)-*N*-fenilprop-2-enamidų savybės

7-[(*E*)-2-Feniletetil]-2*H*-pirazolo[4,3-*c*]piridinų **68–76** fluorescencijos savybės ištirtos THF ir 0,5% DMSO vandeniniame tirpaluose, nustatant sužadinimo bangos ilgus atitinkamai ties 360 nm ir 380 nm. Junginių emisijos maksimumai THF fiksuoti 458–485 nm srityje, o vandeniniame tirpale įvyko bathochrominis poslinkis į 491–524 nm sritį. Be to, 7-stiril-2*H*-pirazolo[4,3-*c*]piridinas **69** su elektrondonorine metoksigrupe pasižymėjo ilgiausiu Stokso poslinkiu ir didžiausia kvantine išeiga (55 % THF). Įdomu tai, kad metilbenzoato **71** ir metil **72** dariniai turi panašų Stokso poslinkį ir mažesnes kvantines išeigas, nepaisant skirtingo elektroninio efekto. Kiti junginiai su elektronakceptorinės grupėmis turi trumpesnius raudonuosius poslinkius ir mažesnę kvantinę išeigą, o 4-bromostiril darinio **75** kvantinė išeiga pati mažiausia (26 %).

Įdomu tai, kad, įvedus amidinę grupę į 7-tą 2*H*-pirazolo[4,3-*c*]piridino padėtį, fluorescencija dingsta.

2.4.4. Biologinis 2,6-difenil-7-[(*E*)-2-feniletetil]-2*H*-pirazolo[4,3-*c*]piridinų ir (2*E*)-3-(2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-il)-*N*-fenilprop-2-enamidų tyrimas

Galiausiai Eksperimentinės biologijos katedroje, Palackio universitete, buvo įvertintas 2,6-difenil-7-[(*E*)-2-feniletetil]-2*H*-pirazolo[4,3-*c*]piridinų **68–76** ir (2*E*)-3-(2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-il)-*N*-fenilprop-2-enamidų **81–84** fotodinaminis potencialas. Melanomos G361 ląstelės 4 val. buvo veikiamos junginiais ir tuomet mėlyna šviesa esant 10 J/cm² apšvitos dozei. Ląstelių gyvybingumas buvo įvertintas 72 val. po apšvitos taikant MTT analizę, kuri parodė, kad feniletetildariniai **68–76** pasižymi puikiomis fototoksinėmis savybėmis, IC₅₀ vertėms siekiant vos 0,043 μM (2 lentelė). Junginių **69**, **72**, **76** IC₅₀ vertės buvo panašios, ir tik **76** nebuvo toksiškas tamsoje iki tikrintų koncentracijų (10 μM), todėl jis labiau tinkamas PDT.

Metil (2*E*)-3-(2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-il)prop-2-enoatas **77** ir (2*E*)-3-(2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-il)prop-2-enoinė rūgštis **79** nebuvo fototoksiški, tačiau **77** pasižymėjo vidutiniu toksiškumu neapšviestose ląstelėse. Bendrai 2*H*-pirazolo[4,3-*c*]piridin-*N*-fenilprop-2-enamidai **81–84** pasižymėjo vidutiniu arba nepasižymėjo citoksiškumu tiek apšviestose, tiek neapšviestose ląstelėse.

2.2 lentelė. Junginių 68-84 IC₅₀ vertės tamsoje ir apšviestose G361 ląstelėse

Junginys	G361 - IC ₅₀ , μM	
	tamsoje	10 J/m ²
68	8,125	0,14995
69	6,04	0,047
70	>10	0,205
71	>10	0,346
72	9,15	0,043
73	>10	0,1515
74	>10	0,1775
75	>10	0,158
76	>10	0,0465
77	3,325	>10
79	>10	>10
81	>10	9,11
82	>10	>10
83	7,64	9,36
84	>10	>10

Atliekami tolimesni eksperimentai, siekiant nustatyti junginių veikimo mechanizmą.

3. PAGRINDINIAI REZULTATAI IR IŠVADOS

1. 1-Fenil-3-(feniletinil)-1*H*-pirazol-4-karbaldehidą yra tinkamas pradinis junginys 7-jod-2*H*-pirazolo[4,3-*c*]piridinams gauti per tarpinės transformacijas į atitinkamus alkoholius ir azidus.

2. 7-Aril-2,6-difenil -2*H*-pirazolo[4,3-*c*]piridinų biblioteka gali būti sėkmingai susintetinama iš 7-jodo-2*H*-pirazolo[4,3-*c*]piridinų pritaikant Suzuki-Miyaura kryžminių jungimų reakciją. Ištyrus junginių savybes nustatyta, kad:

- 7-(4-Alkoksifenil)-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridinai pasižymi geriausiomis fluorescencijos savybėmis, kvantinėms išeigoms siekiant iki 85%.

- 7-(4-Metoksifenil)-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridinas yra tinkamiausias pH indikatorius, tinkamas tiek nuo fluorescencijos intensyvumo priklausomam, tiek ratiometriniam pH fiksavimui.

- Sukeldami antiproliferacinius efektus bei skatindami ląstelės mirtį, junginiai yra potencialūs antivėžiniai agentai, veikiantys MV4-11, K562 ir MCF-7 vėžines ląsteles *in vitro*. 4-(2,6-Difenil-2*H*-pirazolo[4,3-*c*]piridin-7-il)fenolis pasižymi žemiausia GI₅₀ koncentracijos verte, siekiančia 1,5 μM.

3. *N*-Aril-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-aminų biblioteka gali būti sėkmingai susintetinama iš 7-jodo-2*H*-pirazolo[4,3-*c*]piridinų pritaikant Buchwald-Hartwig kryžminių jungimų reakciją. Ištyrus junginių savybes rasta, kad:

- Amino grupės atsiradimas sistemoje labai sumažina fluorescenciją, nes tiek Stokso poslinkis, tiek kvantinė išeiga yra mažesni nei kitų 7-pakeistų-2*H*-pirazolo[4,3-*c*]piridino darinių.

- *N*-(4-Metoksifenil)-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-aminas ir 2,6-difenil-*N*-[2-(trifluorometoksi)fenil]-2*H*-pirazolo[4,3-*c*]piridin-7-aminas yra potencialūs fotodinaminės terapijos agentai, EC₅₀ koncentracijai esant 1,1 μM apšviestose melanomos G361 ląstelėse.

- *N*-(4-Metoksifenil)-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-aminas sukelia ląstelės mirtį gamindamas reakingas deguonies rūšis (ROS) ir sukeldamas DNR pažeidimus.

4. 2,6-Difenil-7-[(*E*)-2-feniletetil]-2*H*-pirazolo[4,3-*c*]piridinų ir (2*E*)-3-(2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-il)-*N*-fenilprop-2-enamidų biblioteka gali būti sėkmingai susintetinama iš 7-jod-2*H*-pirazolo[4,3-*c*]piridinų pritaikant Heck kryžminio jungimo reakciją. Ištyrus junginių savybes nustatyta, kad:

- 2,6-Difenil-7-[(*E*)-2-feniletetil]-2*H*-pirazolo[4,3-*c*]piridinai pasižymi didžiausiu batochrominiu emisijos poslinkiu ir vidutine kvantine išeiga, o (2*E*)-3-(2,6-difenil-2*H*-pirazolo[4,3-*c*]piridin-7-il)-*N*-fenilprop-2-enamidai fluorescencija nepasižymi.

- Pirminis tyrimas parodė, kad 7-[(*E*)-2-(4-metilfenil)etenil]-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridinas yra perspektyvus fotodinaminės terapijos agentas, IC₅₀ koncentracija siekia vos 0,043 μM apšviestose melanomos G361 ląstelėse.

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List of Publications

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