KAUNAS UNIVERSITY OF TECHNOLOGY

FACULTY OF CHEMICAL TECHNOLOGY

LITHUANIAN UNIVERSITY OF HEALTH SCIENCES

FACULTY OF PHARMACY

Beatričė Razmienė

SYNTHESIS, BIOLOGICAL ACTIVITY AND OPTICAL PROPERTIES OF 2,4,6,7-TETRASUBSTITUTED-2*H*-PYRAZOLO[4,3-*c*]PYRIDINES

Master's Final Degree Project

Supervisor

Dr. Asta Žukauskaitė

KAUNAS, 2018

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Medicinal chemistry (code 628F10001)

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Study programme Medicinal chemistry (code 628F10001)

‘Synthesis, biological activity and optical properties of 2,4,6,7-tetrasubstituted-2*H*-pyrazolo[4,3-*c*]pyridines’

DECLARATION OF ACADEMIC INTEGRITY

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TABLE OF CONTENTS

SUMMARY 1

SANTRAUKA 2

LIST OF ABBREVIATIONS 3

INTRODUCTION 6

1. LITERATURE REVIEW 8

1.1 Biologically active naturally occuring pyrazoles 8

1.2 Biologically active synthetic pyrazole derivatives 8

1.2.1 Pharmaceuticals and other commercial pyrazole derivatives 8

1.2.2 Synthesis of biologically active pyrazole derivatives 9

1.3 Biologically active annelated pyrazole derivatives 11

1.3.1 Pharmaceuticals and other commercially available condensed pyrazole derivatives 11

1.3.2 Synthesis of biologically active annealed pyrazolo derivatives 12

2. MATERIALS AND EXPERIMENTAL METHODS 25

2.1. Methods and devices used for analysis 25

2.2. Compound synthesis and experimental data 25

3. RESULTS AND DISCUSSION 37

3.1 Synthesis of 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine and 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine 37

3.2 7-Iodopyrazolo[4,3-*c*]pyridine as a substrate for Suzuki-Miyaura cross-coupling reactions 42

3.3 7-Iodopyrazolo[4,3-*c*]pyridine as a substrate for Buchwald-Hartwig cross-coupling reactions 45

3.4 Optical properties of 2,4,6,7-tetrasubstituted pyrazolo[4,3-*c*]pyridines 49

3.5 Biological activity of 2,4,6,7-tetrasubstituted pyrazolo[4,3-*c*]pyridines 53

THE MAIN RESULTS AND CONCLUSIONS 58

REFERENCES 59

ACKNOWLEDGEMENTS 66

Beatričė, Razmienė. Synthesis, biological activity and optical properties of 2,4,6,7-tetrasubstituted-2*H*-pyrazolo[4,3-*c*]pyridines. Master's thesis in Chemistry / supervisor dr. Asta Žukauskaitė; Faculty of Chemical Technology, Kaunas University of Technology, Faculty of Pharmacy, Lithuanian university of health sciences.

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SUMMARY

Pyrazole is a common structural moiety in many pharmaceuticals, agrochemicals, optoelectronics and dyes. In particular, design and synthesis of new annealed pyrazole derivatives is an important research field in current medicinal chemistry because they demonstrate a wide spectrum of biological activities.

The aim of this work was to synthesise novel 2,4,6,7-tetrasubstituted-2*H*-pyrazolo[4,3-*c*]pyridines and examine the biological activity and optical properties. 1-Phenyl-3-(2-phenylethynyl)-1*H*-pyrazole-4-carbaldehyde, which was chosen as a starting material, was prepared from 1-phenyl-1*H*-pyrazol-3-ol by consecutive alkylation, formylation and Sonogashira cross-coupling reactions. The pyrazolo[4,3-*c*]pyridine core was obtained *via* a three step route. Firstly, aldehydes were converted to alcohols using either Gringnard reagents or reduction conditions and then transformed into azide-alkynes. The latter were used in electrophilic cyclization reaction to obtain 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine and 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine. The library of 2,4,6,7-tetrasubstituted-2*H*-pyrazolo[4,3-*c*]pyridine derivatives was obtained *via* palladium catalysed cross-coupling reactions.

Subsequently, the optical properties of new derivatives were assessed. 7-(4-Methoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine showed the best result with quantum yield value of 71.77%. Furthermore, evaluation of anticancer activity against myelogenous leukemia and breast adenocarcinoma cells showed that the compounds obtained from Suzuki cross-coupling reactions and bearing various aryl substituents at the 7-position appeared to be generally more cytotoxic than compounds, resulting from Buchwald-Hartwig cross-coupling and bearing various anilines in the same 7-position. Noteworthy, compounds bearing no substituents at the 4-position were generally more cytotoxic compared to their methyl counterparts. The most potent compound 7-(4-methoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine exhibited GI_{50} value of 2.3 μM on leukemia cell line.

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Studijų kryptis ir sritis: Fiziniai mokslai, chemija.

Reikšminiai žodžiai: *pirazolo[4,3-c]piridinai, priešvėžinis aktyvumas, fluorescencija, Suzuki-Miyaura, Buchwald-Hartwig* .

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SANTRAUKA

Pirazolas – dažnas struktūrinis farmacinių junginių, agrochemikalų, optoelektroninių medžiagų ir dažų fragmentas. Naujų kondensuotų pirazolo sistemų kūrimas ir sintetinimas yra ypač svarbus dabartinei medicininei chemijai, nes šie heterociklai pasižymi įvairiomis biologinėmis savybėmis.

Šio darbo tikslas buvo susintetinti naujus 2,4,6,7-tetrapakeistus-2*H*-pirazolo[4,3-*c*]piridinus ir ištirti jų biologines bei optines savybes. Pradine medžiaga pasirinktas 1-fenil-3-(2-feniletinil)-1*H*-pirazol-4-karbaldehidas buvo gautas iš 1-fenil-1*H*-pirazol-3-olio alkinimo, formilinimo ir Sonogashira kryžminio jungimo reakcijų metu. Pirazolo[4,3-*c*]piridino struktūra gauta per tris etapus. Pirmiausia, aldehidai buvo paversti į alkoholius naudojant arba Gringnardo reagentus, arba redukcijos sąlygas, bei vėliau transformuoti į azido-alkinus. Pastarieji buvo panaudoti elektrofilinėje ciklokondensacijoje siekiant gauti 7-jod-4-metil-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridiną ir 7-jod-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridiną. Įvairūs 2,4,6,7-tetrapakeisti-2*H*-pirazolo[4,3-*c*]piridino dariniai gauti paladžio katalizuojamų kryžminių jungimo reakcijų metu.

Išmatavus naujų junginių optines savybes nustatyta, kad 7-(4-metoksifenil)-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridinas pasižymėjo geriausia kvantine išeiga, kuri siekė net 71,77 %. Taip pat atlikus priešvėžinius tyrimus prieš krūtų adenokarcinomos ir lėtinės mielogeninės leukemijos ląsteles nustatyta, kad junginiai, gauti Suzuki kryžminio jungimo metu ir turintys įvarius arilo pakaitus septintoje padėtyje, buvo aktyvesni nei junginiai, gauti Buchwald-Hartwig jungimo metu bei turintys įvairius anilinus septintoje padėtyje. Pažymėtina, kad junginiai neturintys pakaito 4-padėtyje buvo aktyvesni nei junginiai turintys metilo pakaitą. Geriausiu citotoksiškumu pasižymėjo 7-(4-metoksifenil)-2,6-difenil-2*H*-pirazolo[4,3-*c*]piridinas, pasiekęs 2,3 μM GI_{50} vertę prieš leukemijos ląsteles.

LIST OF ABBREVIATIONS

Ar	aryl
APT	attached proton test
AcOH	acetic acid
A549	human lung cancer cell line
$BF_3 \cdot Et_2O$	boron trifluoride diethyl etherate
BGC823	curcumin induced human gastric cancer cell line
B16-F10	mouse melanoma cancer cell line
Boc	*tert*-butoxycarbonyl
BuOH	butanol
$CDCl_3$	deuterated chloroform
COSY	correlation spectroscopy
COX	cyclooxygenase
cm^{-1}	reciprocal centimetre
d	dublet
DCM	dicholmethane
DMF	*N,N'*-dimethylformamide
DNA	deoxyribonucleic acid
DU145	human prostate cancer cell line
ESI	electrospray ionization
EtOAc	ethyl acetate
EtOH	ethanol
FT-IR	Fourier transformation infrared spectroscopy
GABA	γ-aminobutyric acid
HCT116	human colon cancer cell line
HeLa	human cervix epitheloid cancer cell line
HepG2	human liver cancer cell line
Hex	n-hexane
HMBC	heteronuclear multiple bond coherence
HRMS	high resolution mass spectrometry
HSQC	heteronuclear single quantum coherence
HT1080	human fibrosarcoma cell line

J	coupling constant
K562	human myelogenous leukemia cell line
m	multiplet
MIA PaCa-2	human pancreatic cancer cell line
MCF-7	human adenocarcinoma cell line
mp	melting point
MW	microwave irradiation
Me	methyl
MeO	methoxy
MeOH	methanol
MHz	megahertz
MS	mass spectrometry
m/z	ratio of mass and charge
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser effect spectroscopy
PC-3	human prostate adenocarcinoma cell line
PE	petroleum ether
Ph	phenyl
ppm	parts per million
PTSA	*p*-toluenesulfonic acid
$Pd(dba)_2$	bis(dibenzylideneacetone)palladium(0)
rt	room temperature
R_f	retention factor
RNA	ribonucleic acid
s	singlet
SAR	structure activity relationship
SH-SY5Y	human neuroblast cell line from neutral tissue
SKOV3	human ovary cancer cell line
SPhos	2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl
TEA	triethylamine
TFA	trifluoroacetic acid
Tf_2O	trifluoromethanesulfonic anhydride
THF	tetrahydrofuran
TLC	thin layer chromatography
$TMSN_3$	trimethylsilyl azide

TOCSY	total correlation spectroscopy
UV	ultraviolet
v/v	volume to volume ratio
X-Phos	2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl
δ	chemical shift
ν	wavenumber
Δ	reflux
^{13}C NMR	carbon ^{13}C isotope nuclear magnetic resonance
^{1}H NMR	proton nuclear magnetic resonance
^{15}N NMR	nitrogen ^{15}N isotope nuclear magnetic resonance

INTRODUCTION

Pyrazole is a common structural unit in many pharmaceuticals, agrochemicals, optoelectronics and dyes. Thus, it is a central axis of numerous ongoing studies devoted to the synthesis, biological and optical evaluation of novel pyrazole moiety-bearing molecules.

Condensed pyrazole derivatives are widely used in agrochemistry [1], chemosensors [2], dyes [3], light emitting diodes [4] and other optoelectronics[5]. Annelated pyrazoles are of particular interest to medicinal chemistry as they constitute the core of several well-known drugs, such as sildenafil, apixaban, etazolate. Moreover, pyrazolopyridines have been reported with numerous pharmaceutical activities including potent cyclin dependent kinase 1 (CDK1) inhibitors [6], A1 adenosine receptor antagonists [7], p110a-selective PI3 kinase inhibitors [8], Acetyl-CoA carboxylase (ACC) inhibitors [9], guanylate cyclase stimulators [10] etc.

Therefore, the synthesis of new pyrazole derivatives is needed because of the great potential and versatile application. Interestingly, among the vast variety of up to now developed biologically active annelated pyrazole derivatives, synthetically demanding 2*H*-pyrazolo[4,3-*c*]pyridines are relatively understudied constituting an ideal niche for the development of potentially biologically active pyrazoles.

Aim of this work: synthesise novel 2,4,6,7-tetrasubstituted-2*H*-pyrazolo[4,3-*c*]pyridines and examine the biological activity and optical properties.

Tasks of the work:

1. Review pharmacological application of pyrazole derivatives in recently reported literature;
2. Review methods of pyrazolopyridines synthesis and their pharmacological application in recently reported literature;
3. Synthesise 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine and 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine;
4. Perform Pd-catalysed cross-coupling reactions of the new 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine and 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine;
5. Determine structures of the new compounds using methods of NMR and IR spectroscopy, MS and HRMS spectrometry;
6. Investigate optical properties of the new derivatives;
7. Examine anticancer activity of the new derivatives.

Methods of the work:

1. Gathering and analysing of scientific literature related to the subject of the work;
2. Performing synthesis in the laboratory;
3. Structure elucidation of newly prepared compounds using methods of NMR and IR spectroscopy, MS and HRMS spectrometry;
4. Analysis of optical properties data;
5. Analysis of biological activity data.

1. LITERATURE REVIEW

1.1 Biologically active naturally occuring pyrazoles

Pyrazoles are rarely found in nature presumably due to the difficulty to form N-N bond by living organisms [11]. Nevertheless, naturally occurring pyrazoles (Fig. 1.1) display a vast variety of biological activities [12]. For example, L-α-amino-β-(pyrazolyl-N)-propanoic acid **1** is a naturally occurring amino acid extracted from watermelon and has anti-diabetic activity [13]. Pyrazofurin **2** possesses anticancer and antiviral activity against a broad spectrum of DNA and RNA viruses [14]. Furthermore, bicyclic nitrogen rich antibiotics produced by *Pseudomonas fluorescenes* classified as fluviols, namely fluviol C **3** and fluviol E **4**, exhibit a broad spectrum of antimicrobial actions and show antitumor effect on Ehrlich carcinoma [15]. Good antimicrobial activity was also discovered in pyrazole derivative 3-n-nonylpyrazole **5**, which was extracted from piperaceae family plant from tropical Asia [16]. Thus, inspiration for development of novel pyrazole moiety bearing molecules initially came from nature.

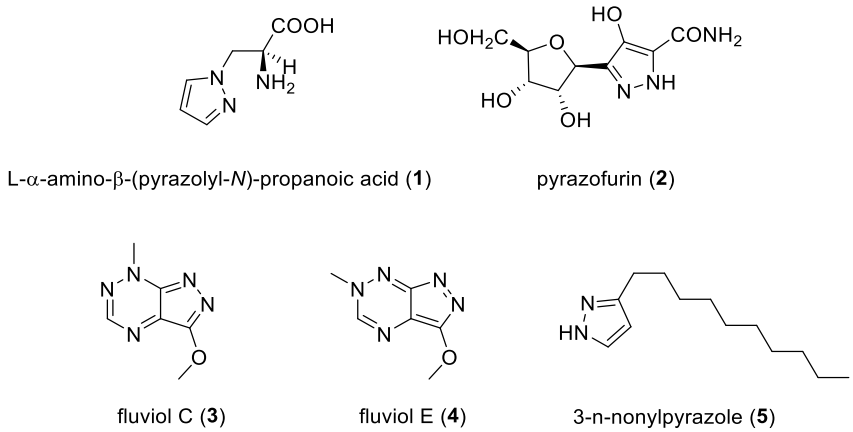


Fig. 1.1 Naturally occurring pyrazoles.

1.2 Biologically active synthetic pyrazole derivatives

1.2.1 Pharmaceuticals and other commercial pyrazole derivatives

In medicinal chemistry, various compounds containing pyrazole rings have showed interesting biological properties, including antimicrobial [17, 18], antifungal [17], leishmanicidal [19, 20, 21], antiviral [18], antichagasic [19], pesticidal [22], antihyperglycemic [23], anti-inflammatory [24] and antitumoral activities [18]. A large number of synthetic pyrazoles (Fig. 1.2) have been developed and accepted for use, such as fipronil **6**, as an insecticide [25]; difenamizole **10**, as an NSAID and analgetic [26]; rimonabant **11**, for the treatment of obesity [27]; and fezolamine **12**, as an antidepressant [28]. Celecoxib **7**, tepoxalin **8**, deracoxib **9** and lonazolac **13** are

pyrazole derivatives used as anti-inflammatory agents [29, 30, 31, 32]. These drugs provide analgesic and antipyretic effects and are commonly used to treat inflammatory diseases, including rheumatoid arthritis. They are inhibitors of cyclooxygenases enzymes COX-1, COX-2, and COX-3, which are responsible for the production of prostaglandins. Therefore, in a view of the biological importance of pyrazole moyiety-bearing molecules, the design and synthesis of new pyrazole derivatives is an important research field in current medicinal chemistry.

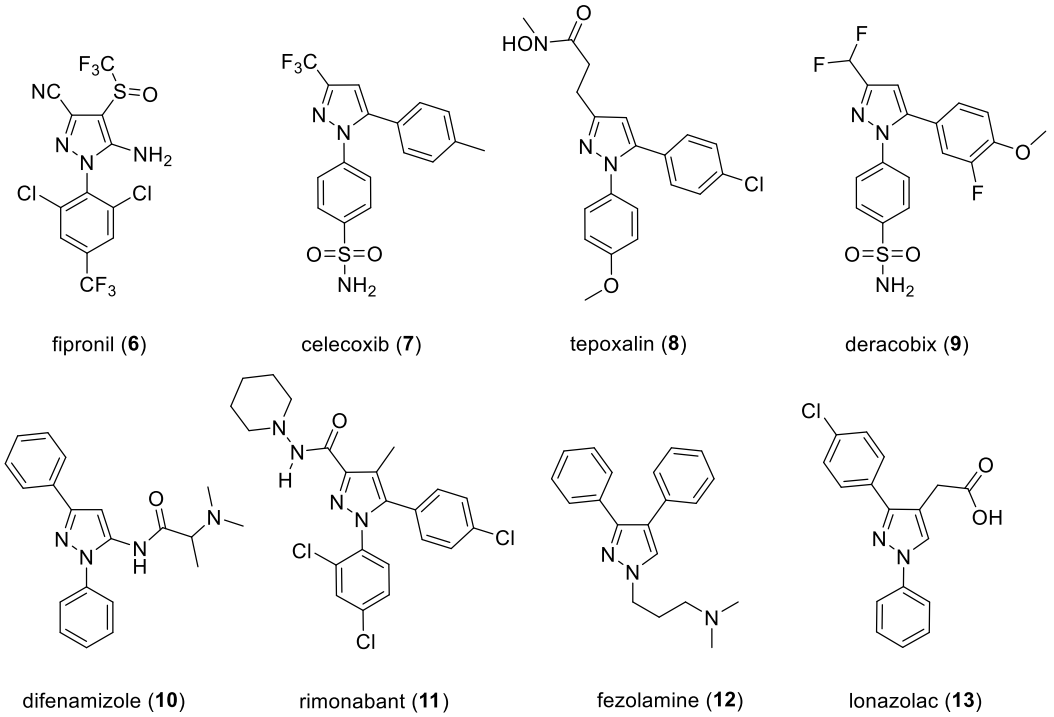


Fig. 1.2. Commercially available pyrazole derivatives.

1.2.2 Synthesis of biologically active pyrazole derivatives

Hassan *et al.* (2014) synthesized celecoxib analogs with benzofuran moieties and evaluated them for COX-2 inhibitory activity *in vitro* [33]. The compounds **14** and **15** (Fig. 1.3) showed the highest anti-inflammatory activity, with IC_{50} values of 0.40 μM and 0.36 μM respectively, compared to 0.28 μM for celecoxib.

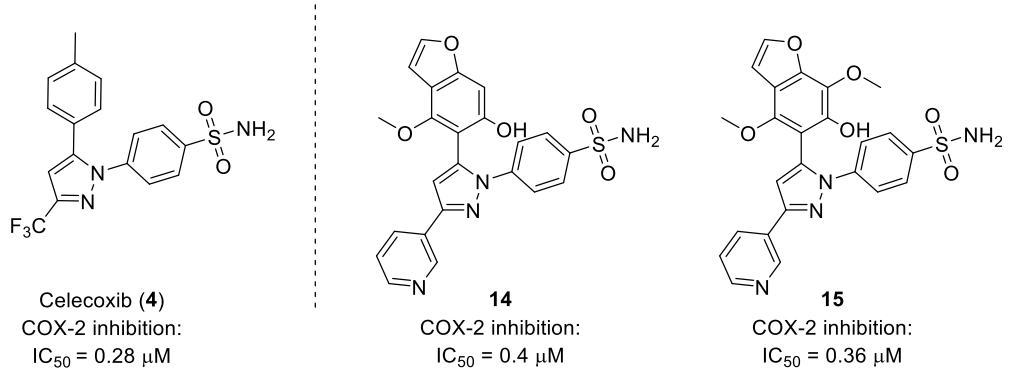


Fig 1.3. Celocoxib and its analogs with benzofuran moieties.

Alegaon *et al*. (2014) synthesized 1,3,4-trisubstituted pyrazole derivatives and tested them for anti-inflammatory activity in a carrageenan-induced rat paw edema model (Fig. 1.4) [34]. The most active pyrazoles were compounds **16** and **17** (>84.2% inhibition) compared to the reference drug diclofenac (86.72%).

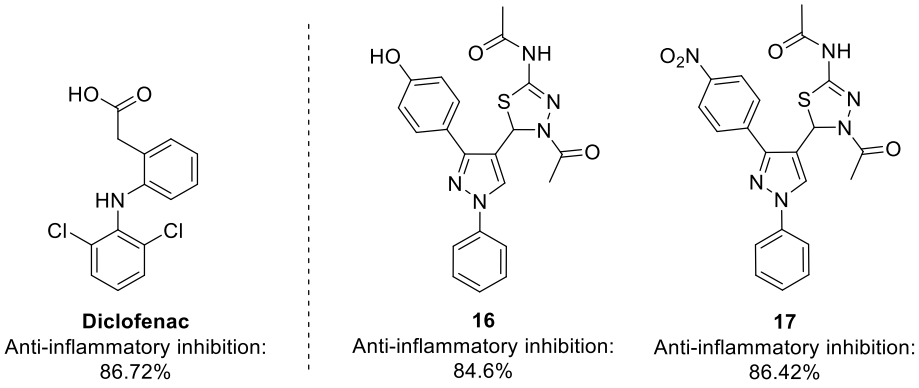


Fig 1.4. Pyrazole derivatives with anti-inflammatory activity.

A series of 1-aryl-3,4-substituted-1*H*-pyrazol-5-ol derivatives were synthesized and assayed against cancer antigen-1 (PCA-1ALKBH3) [35]. The best results were obtained for compound **18** (Fig. 1.5), which showed high inhibition against the proliferation of DU145 (human hormone-independent prostate cancer cells) with no apparent side effects when administered in a xenograft mouse model.

Xing *et al*. (2014) synthesized a series of pyrazoles containing an acylhydrazone group [36]. The compounds were screened for inhibition of MCF-7 (breast cancer) and B16-F10 (melanoma) cancer cell lines. Compound **19** (Fig. 1.5) was the most potent against both cell lines with IC_{50} values of 0.57 μM and 0.49 μM respectively.

Yao *et al*. (2014) described a series of 1,3-disubstituted pyrazoles with activity against various cancer cell lines, such as MCF-7, BGC823 (stomach cancer), K562 (myeloid leukemia), HT1080 (sarcoma) and A549 (lung cancer) [37]. Compound **20** (Fig. 1.5), containing a pyrazole with biphenyl group, was able to inhibit histone deacetylase enzymes (iHDAC) with higher potency (IC_{50} = 0.033 μM) than the drug suberoylanilide hydroxamic acid (SAHA) (IC_{50} = 0.131 μM). These iHDAC inhibitors have emerged as a new class of antitumor agents that demonstrate activity against various types of cancer and have notable effects on the proliferation, programmed cell death, differentiation and angiogenesis of tumor cells *in vitro* and *in vivo*.

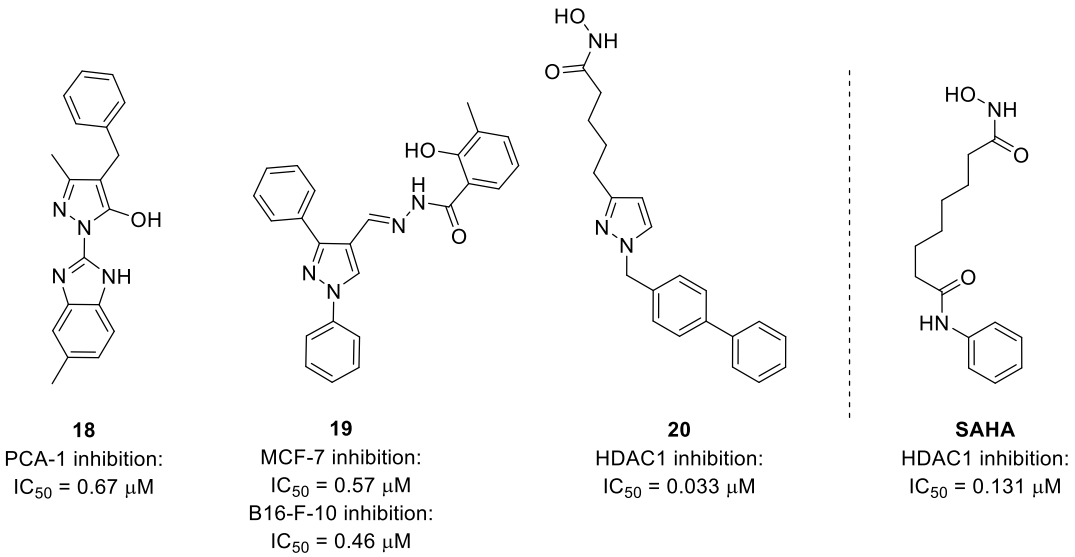


Fig 1.5. Pyrazole derivatives with anti-cancer activity.

1.3 Biologically active annelated pyrazole derivatives

1.3.1 Pharmaceuticals and other commercially available condensed pyrazole derivatives

Condensed pyrazole derivatives are important heterocyclic compounds due to their excellent biological activities and have been widely applied in pharmaceutical and agromedical fields. In recent years, numerous condensed pyrazole derivatives have been synthesized and advanced to clinical studies with various biological activities. Many of them are approved pharmaceuticals (Fig. 1.6), for example sildenafil **21** is used to treat erectile dysfunction and pulmonary arterial hypertension [38]. Apixaban **22** is an anticoagulant for the treatment of venous thromboembolic events [39]. Pyrazolopirimidine class drugs indiplon **23** and zaleplon **24** are sedatives that enhance the action of the inhibitory neurotransmitter GABA [40, 41], while divaplon **25** is an anxiolytic and anticonvulsant drug [42].

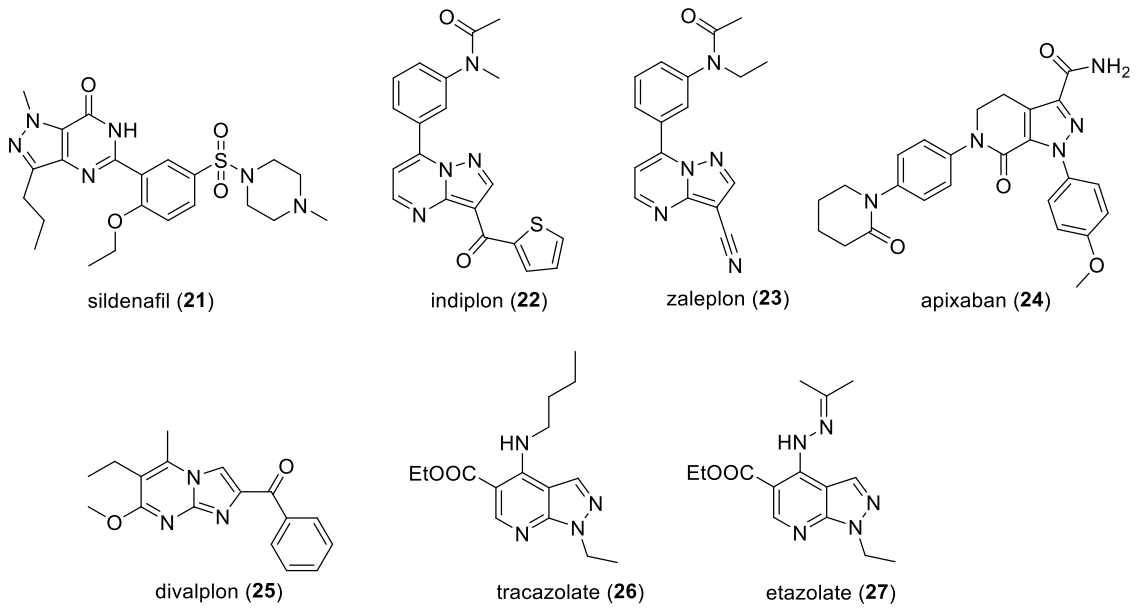


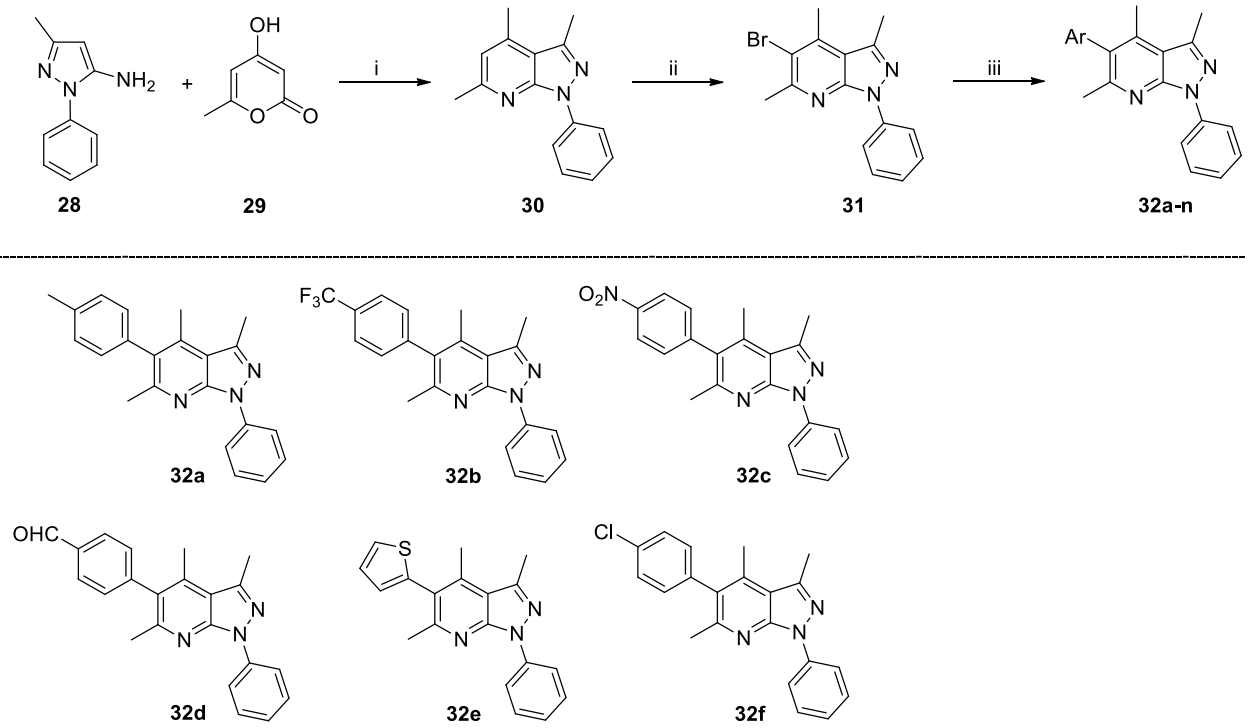
Fig. 1.6. Commercially used annealed pyrazole derivatives.

One of the most researched condensed pyrazole systems in organic and pharmaceutical chemistry are pyrazolopyridines. This heterocyclic system is found in a number of molecules that possess biological and/or pharmacological properties. They exhibit antimicrobial [43, 44], antimalarial [45], antibacterial [46, 47], anti-cancer [48], anti-inflammatory [49] and anti-viral [50] activities. Moreover, it is known that 1*H*-pyrazolo[3,4-*b*]pyridine derivatives are selective inhibitors of cyclin-dependent kinase (CDK) [51]. Tracazolate **26** and etazolate **27** have been approved for the treatment of anxiety disorders associated with neuronal inhibition induced by GABA (Fig. 1.6) [52, 53, 54].

1.3.2 Synthesis of biologically active annealed pyrazolo derivatives

1.3.2.1 Pyrazolo[3,4-*b*]pyridines

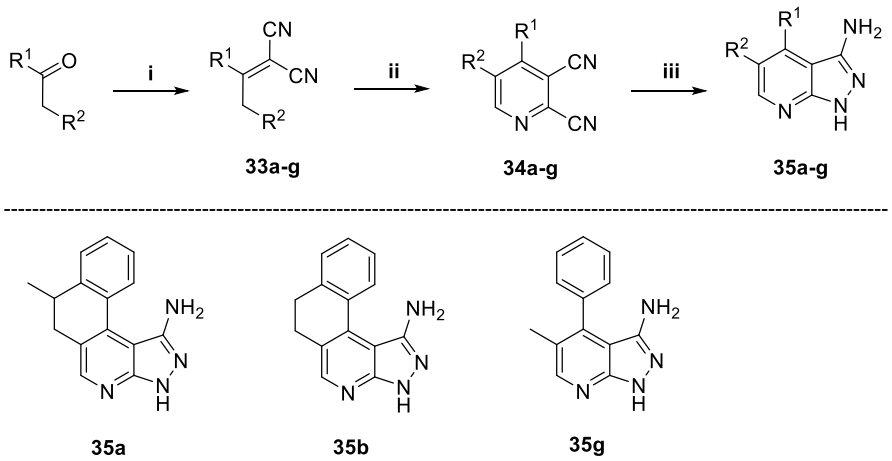
Recently, Jouha *et al.* (2017) reported synthesis of novel pyrazolopyridine derivatives as potential neuroprotective agents [55]. Microwave assisted condensation of 5-amino-1-phenyl-3-methylpyrazole **28** and 2-pyrone **29** in BuOH, using PTSA as a catalyst, efficiently afforded 3,4,6-trimethyl-1-phenyl-*1H*-pyrazolo[3,4-*b*]pyridine **30** in 98% yield. Subsequently, regioselective introduction of bromine atom at 5-position was achieved with an equimolar amount of *N*-bromosuccinimide (NBS) giving rise to synthetically versatile scaffold **31** in 81% yield. The latter was used in Pd-catalyzed Suzuki–Miyaura cross-coupling reactions (Scheme 1.1) to obtain fourteen new derivatives in 71-98% yield.



Scheme 1.1. Synthesis of pyrazolopyridine derivatives and most potent compounds. Reagents and conditions: i: BuOH, PTSA, MW, 180 °C, 3 h; ii: NBS, THF, rt, 3 h; iii: $ArB(OH)_2$, $Pd(PPh_3)_4$, $NaHCO_3$, toluene/EtOH (2/1), 110 °C, 12 h.

Subsequently, the role of the new compounds **32a-n** in MPP^+-induced neurodegeneration in SH-SY5Y (human neuroblastoma) cell line was evaluated. Interestingly, at 5 μM concentration all compounds led to 20-30% neuroprotection against MPP^+-induced neurotoxicity in SH-SY5Y cells. Moreover, neuroprotection by compounds **32a-f** in *in vitro* Parkinson's disease model was confirmed by measuring pro- and anti-apoptotic protein levels. The significant changes in these proteins suggested that these selected compounds may have a significant role in cell death mechanisms.

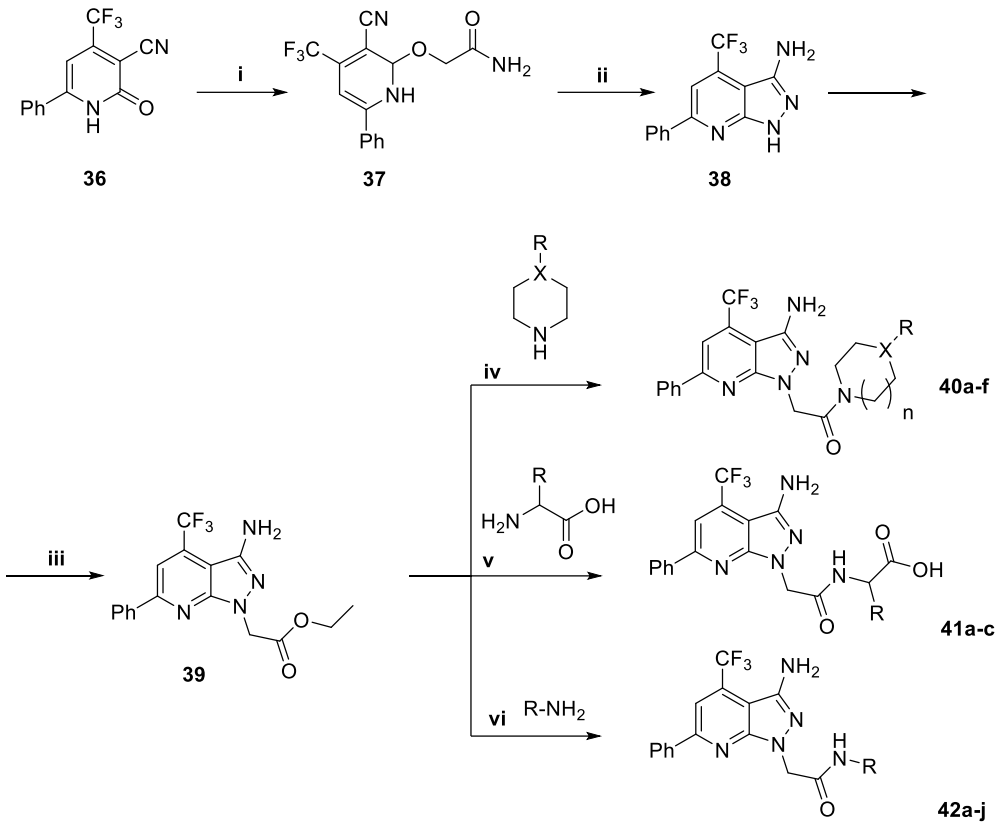
Orlikova *et al*. (2014) investigated the synthesis and biological activity of novel amino-pyrazolopyridines [56]. The alkylidene-malonodinitriles **33a-g** were prepared by Knoevenagel reaction from α-methylene ketones and after treatment by Vilsmeier-Haack reagent at 70–80 °C, furnishing 2-chloro-3-cyanopyridines **34a-g**, substituted at 4- and 5-positions. Subsequently, cyclization of the 2-chloro-3-cyanopyridines **34a-g** with hydrazine hydrate led to the formation of the previously unknown amino-pyrazolopyridines **35a-g** (Scheme 1.2).



Scheme 1.2. Synthesis and chemical structures of amino-pyrazolopyridines**.** Reagents and conditions: i: $CH_2(CN)_2$, NH_4Ac, CH_3COOH, toluene, Δ, 24 h; ii: $POCl_3$, DMF, 70–80 °C, 3 h; iii: $NH_2NH_2{\cdot}H_2O$, Δ, 2–3 h.

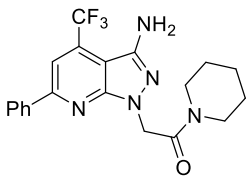
Amino-pyrazolopyridines **35a**, **35b** and **35g** induced apoptotic cell death in K562 cancer cells with IC_{50} values of 36.5, 27.6 and 35.0 μM respectively, after 72 h. In addition, compounds **35a**, **35b** and **35g** exerted NF-kB inhibition activity with IC_{50} values of 4.7, 6.9 and 39.8 μM, respectively, after 8 h in K562 cells activated with TNFα. Furthermore, compounds **35b** and **35g** showed selective cytotoxicity towards K562 cancer cells compared to cells from healthy donors as the viability of peripheral blood mononuclear cells (PBMCs) remained largely unaffected by these compounds.

Chavva *et al*. (2013) had demonstrated synthesis of a number of novel *N*-alkyl amide functionalized trifluoromethyl substituted pyrazolo[3,4-*b*]pyridine derivatives [57]. The reaction of 2(1*H*)-pyridone **36** with 2-chloroacetamide in refluxing acetone led to formation of 2-*O*-acetamido-3-cyano-4-trifluoromethyl-6-phenyl pyridine **37**, which was then stirred with excess hydrazine monohydrate at 100 °C to afford 6-phenyl-4-(trifluoromethyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine **38**. Subsequently, *N*-alkyl ester pyrazolo[3,4-*b*]pyridin-3-amine **39** was obtained from compound **38** in 90% yield by reacting it with bromo ethyl acetate in acetone. The latter was further used to synthesize *N*-alkyl acetamide pyrazolo[3,4-*b*]pyridines **40a-f**, secondary amine ethanone tagged pyrazolo[3,4-*b*]pyridines **41a-c** and α-amino acid functionalised pyrazolo[3,4-*b*]pyridines **42a-j** (Scheme 1.3).



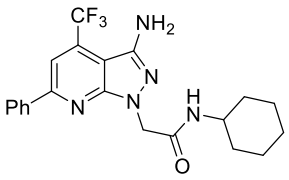
Scheme 1.3. Synthesis of novel *N*-alkyl amide functionalized trifluoromethyl substituted pyrazolo[3,4-*b*]pyridine derivatives. Reagents and conditions: i: 2-chloroacetamide, NaI, K_2CO_3, acetone, Δ, 24 h; ii: $NH_2NH_2{\cdot}H_2O$, 100 °C, 6 h; iii: ethyl 2-bromoacetate, K_2CO_3, NaI, acetone, rt, 24 h; iv: K_2CO_3, 100 °C, 10-12 h; v: K_2CO_3, DMSO, 100 °C, 10–12 h; vi: 80-90 °C, 10-12 h.

The new derivatives were screened *in vitro* for anticancer activity against four cancer cell lines: A549, MCF7, DU145 and HeLa (cervical cancer, CCL-2). All compound showed cytotoxicity at micromolar concentration. Two most active derivatives were identified (Table 1.1) and further SAR studies confirmed that cytotoxicity against all four cancer cell lines was promoted by cyclohexyl group on primary amine or cyclic secondary amine.



40a

A549 inhibition:
IC_{50} = 1.2 μM
MCF7 inhibition:
IC_{50} = 4.1 μM
DU145 inhibition:
IC_{50} = 3.2 μM
HeLa inhibition:
IC_{50} = 2.9 μM

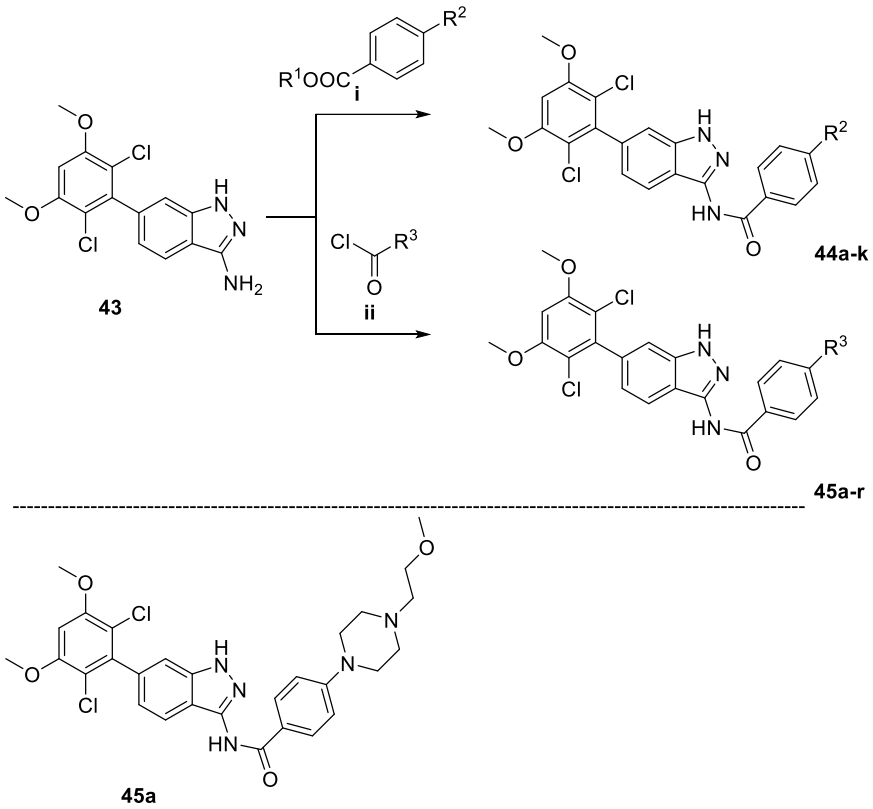


42a

A549 inhibition:
IC_{50} = 2.3 μM
MCF7 inhibition:
IC_{50} = 5.2 μM
DU145 inhibition:
IC_{50} = 3.1 μM
HeLa inhibition:
IC_{50} = 3.9 μM

Fig. 1.7. IC_{50} values of most active pyrazolo[4,3-*b*]derivatives.

Zhao *et al.* (2016) reported synthesis of novel 1*H*-pyrazolo[3,4-*b*]pyridine derivatives with chlorines at the 2- and 6-positions of the phenyl ring [58]. The target compounds were prepared from intermediate **43** by employing two synthetic routes (Scheme 1.4). At first compounds **44a-k** were obtained by condensation of the amine **43** and various substituted benzoic acid esters in the presence of trimethylaluminium. Due to the poor yields (4–28%), derivatives **45a-r** were alternatively synthesized reacting amine with various acyl chlorides in the presence of *N*,*N*-diisopropylethylamine. In this case the compounds were afforded in more satisfactory 18–68% yields.



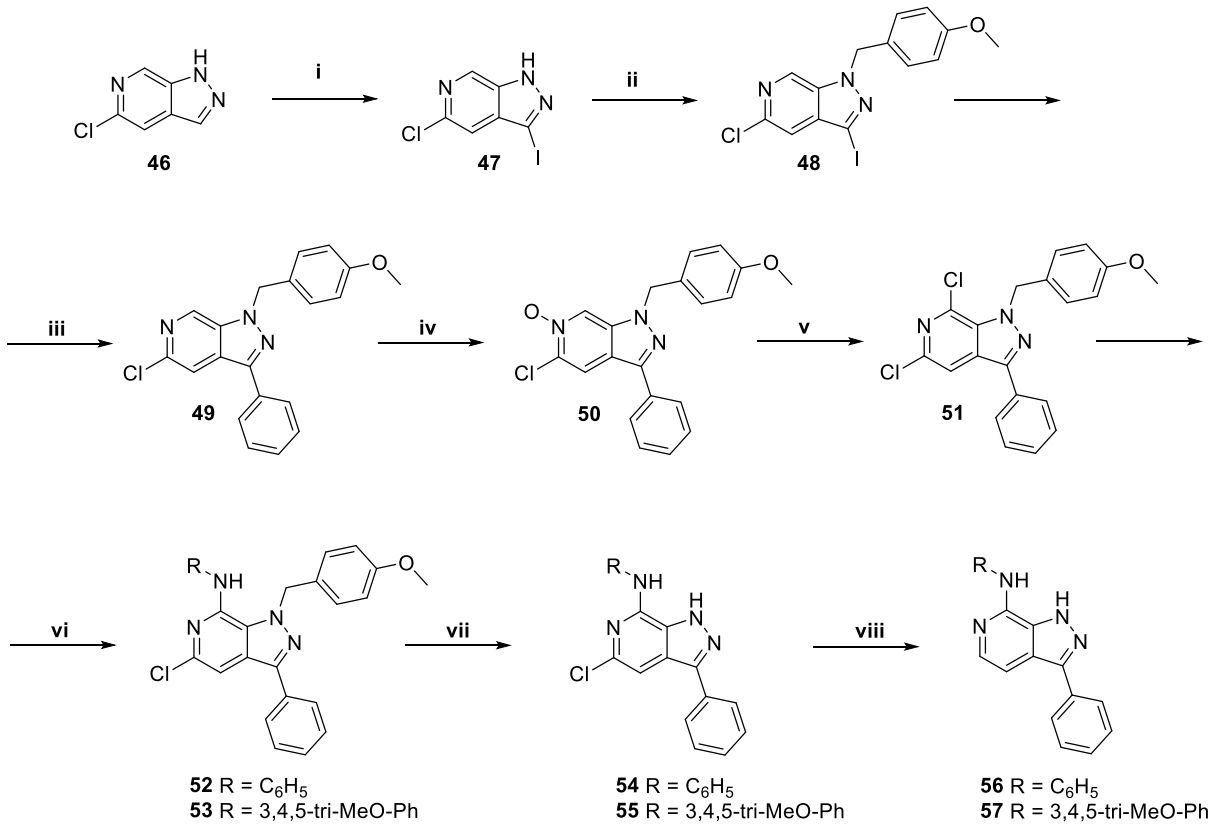
Scheme 1.4. Synthesis of novel pyrazolo[3,4-*b*]pyridine derivatives with chlorines at the 2- and 6-positions of the phenyl ring and the structure of the most active obtained derivative. Reagents and conditions: i: $AlMe_3$, toluene, rt to 60 °C; ii: DIPEA, THF, 0 °C to rt.

Subsequently, the final products were screened for their inhibitory activity against FGFR1 and the proliferation of FGFR1-amplified H1581 cells. The results of SAR studies showed that the 2,3-unsubstituted phenyl ring was optimal for good activity against FGFR1, as both chlorine and methoxy groups incorporated at 2- or 3-positions reduced enzymatic and cellular potencies. Additionally, heterocyclic bioisosteres, that replaced phenyl ring, showed weaker potencies as well. Moreover, after testing the influence of substituents at the 4-position of phenyl ring, the most potent compound was **45a**, with IC_{50} values of 0.3 nM and 0.7 nM against FGFR1 and H1581 cells, respectively. Additionally, evaluation of **45a** selectivity against 71 protein kinases indicated that it

is a selective FGFR inhibitor, as IC_{50} values for all other kinases were more than 400 nM. Finally, the *in vitro* antitumor efficacy in H1581 xenograft model study showed that **45a** could repress tumor growth with tumor growth inhibition rate of 54% and 100.4% at the doses of 12.5 mg/kg and 50 mg/kg, respectively. These results suggested that compound **45a** could be a potent lead for further anticancer drug development.

1.3.2.2 Pyrazolo[3,4-*c*]pyridines

Giannouli *et al.* (2016) designed and synthesized a number of new 3,7-disubstituted pyrazolo[3,4-*c*]pyridines [59]. The multi-step synthesis for the preparation of the 3-phenyl derivatives is summarized in Scheme 1.5. The intermediate 5-chloropyrazolo[3,4-*c*]pyridine **46** was iodinated upon treatment with NIS to obtain 3-iodide **47**, which was selectively protected at N1, using 4-methoxybenzylchloride. The iodide **48** was converted to the 3-phenyl derivative **49** by Suzuki coupling using phenylboronic acid in the presence of $Pd(PPh_3)_4$. The latter was converted to *N*-oxide **50**, using *m*-CPBA as an oxidizing agent. After treatment with phosphorous oxychloride, *N*-oxide **50** rearranged to 5,7-dichloropyrazolopyridine **51**, which was reacted with aniline or 3,4,5-trimethoxyaniline to provide compounds **52-53**. Subsequently, these derivatives were deprotected and the analogues **54-55** were dehalogenated resulting in target compounds **56-57**.

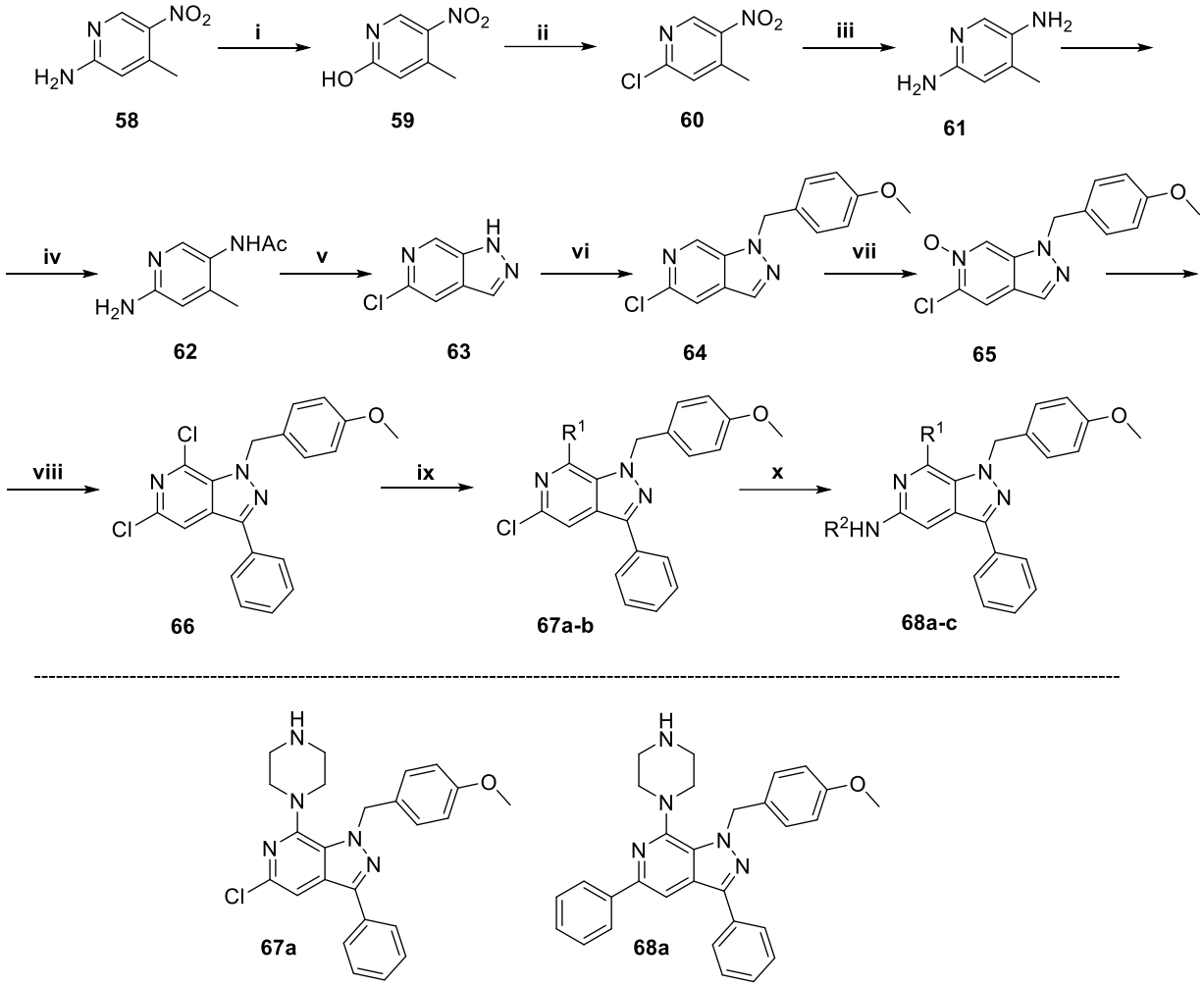


Scheme 1.5. Synthesis of new 3,7-disubstituted pyrazolo[3,4-*c*]pyridines. Reagents and conditions. i: NIS, MeOH, rt; ii: (a) NaH, DMF, rt, (b) 4-methoxybenzyl chloride, DMF, rt; iii: phenylboronic acid,

$Pd(PPh_3)_4$, $NaHCO_3$, PhMe/EtOH/H_2O (10/1/0.2), Δ; iv: *m*-CPBA, $CHCl_3$, rt; v: $POCl_3$, THF, rt; vi: NaH, aniline (for **52**) or 3,4,5-trimethoxyaniline (for **53**), DMF, 100 °C; vii: TFA, rt; viii: Pd/C, H_2, AcOK, EtOH, 50 psi, rt.

Subsequently, the cytotoxic activity of the new compounds was tested against two cancer cell lines: MIA PaCa-2 (pancreatic) and SVOV3 (ovarian), as well as against normal human fibroblasts (WI-38). The SAR studies revealed that derivatives with only one substituent at 7-position are inactive against both cancer cell lines. It was also noticed that 3-phenylpyrazolopyridines show uniformly strong to good cytotoxicity and are selective to cancer cells compared to normal cells. Compound **57**, which bears the 7-(3,4,5-trimethoxyphenyl)amino substituent, was the most active with IC_{50} values of 3.8 μmol and 0.73 μmol against MIA PaCa-2 and SVOV3 cell lines, respectively. Furthermore, compound **57** was tested against DU145, A2058 (melanoma) and PC-3 (prostate cancer) cell lines, for which it exhibited high antiproliferative activity with IC_{50} values of 0.92 μM, 0.89 μM and 0.38 μM, respectively. After further investigations it was confirmed that this compound blocks the cell cycle at the G0/G1 phase and induces apoptosis.

Michailidou *et al.* (2016) reported synthesis of novel pyrazolo[3,4-*c*]pyridine derivatives as potential angiogenesis inhibitors [60]. 2-Amino-5-nitro-4-picoline **58** was used as a starting material. After diazotisation the resulting pyridinone **59** was treated with phosphorus oxychloride in order to obtain chloropicoline **60**. Subsequently, the nitroderivative **60** was reduced using stannous chloride to give the aminopyridine **61**, which was acetylated to the acetamide **62**. The latter was heated at reflux in benzene with isoamyl nitrite and acetyl groups were easily cleaved upon treatment with methanolic ammonia affording pyrazolo[3,4-*c*]pyridine **63**. The derivatives were obtained using similar multi-step synthesis as described above. In this case, however, instead of dehalogenation, the chlorides **67a-b** were coupled with aniline or 4-(4-methylpiperazin-1-yl)aniline, in the presence of caesium carbonate, using $Pd(dba)_2$ as a catalyst and X-Phos as a ligand to form derivatives **68a-c**.

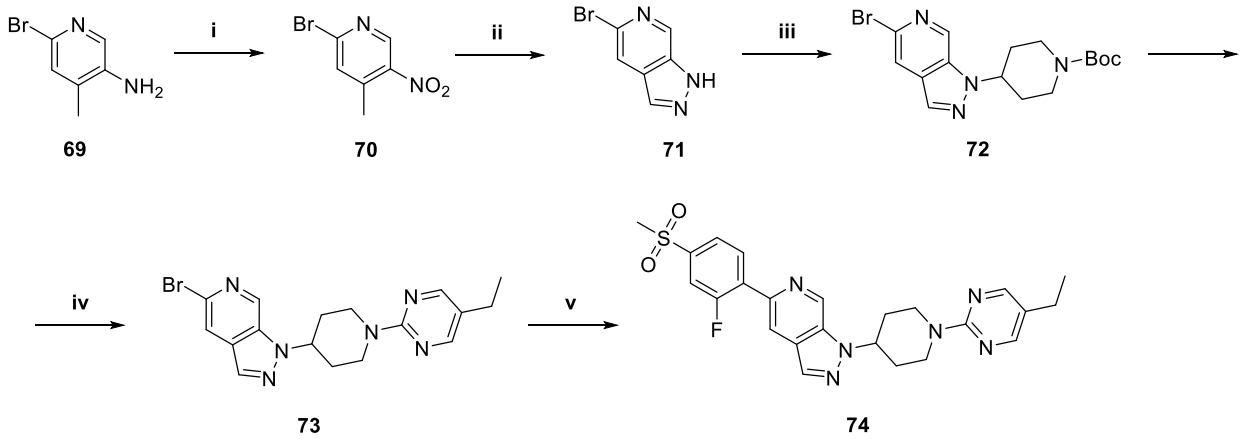


Scheme 1.6. Synthesis of novel pyrazolo[3,4-*c*]pyridines and structures of the most active derivatives. Reagents and conditions: i: $NaNO_2$, H_2SO_4, H_2O; ii: $POCl_3$, 110 °C; iii: $SnCl_2 \cdot 2H_2O$, conc. HCl, 55 °C; iv: Ac_2O, CH_2Cl_2, rt; v: (a) AcOK, Ac_2O, isoamyl nitrite, benzene, Δ, (b) NH_3 (g.), MeOH, rt.; vi: (a) NaH, DMF, rt., (b) 4-methoxybenzyl chloride, DMF, rt.; vii: m-CPBA, $CHCl_3$, rt.; viii:: $POCl_3$, THF, rt.; ix: piperazine (for **67a**) or *N*-methylpiperazine (for **67b**), MW, 300W, 160 °C; vii: aniline (for **68a**) or 4-(4-methylpiperazin-1-yl)aniline (for **70b**, **70c**), X-Phos, $Pd(dba)_2$, Cs_2CO_3, toluene, Δ.

Interestingly, only the 3-phenylsubstituted derivatives **67a** and **68a-c** significantly inhibited both the endothelial cells growth and migration in the applied *in vitro* assays. This indicated that 3-phenyl group substitution is required for antiangiogenic activity. IC_{50} values for cytostatic activity were in low micromolar range, i.e. 4.3 and 7.2 μM for the most active derivatives **67a** and **68a**, respectively. Moreover, these derivatives *in vivo* exhibited a significant regression of Lewis lung carcinoma growth and a prominent reduction of tumor microvessel density, without obvious side effects.

Matsuda *et al.* (2016) designed pyrazolo[3,4-*c*]pyridine derivatives as GPR119 agonists that can be used to treat type 2 diabetus mellitus [61]. The synthesis started from 2-bromo-4-methyl-5-nitropyridine **69**, which was reduced with hydrogen using Reney-Ni as a catalyst and then cyclized

under acidic conditions to obtain 5-bromo-1*H*-pyrazolo[3,4-*c*]pyridine **71** (Scheme 1.7). The latter was treated with *tert*-butyl 4-(methylsulfonyloxy)piperidine-1-carboxylate and the intermediate **72** was used in different couplings, i.e., Suzuki and copper-mediated Ullmann-type, in order to synthesize a variety of derivatives.



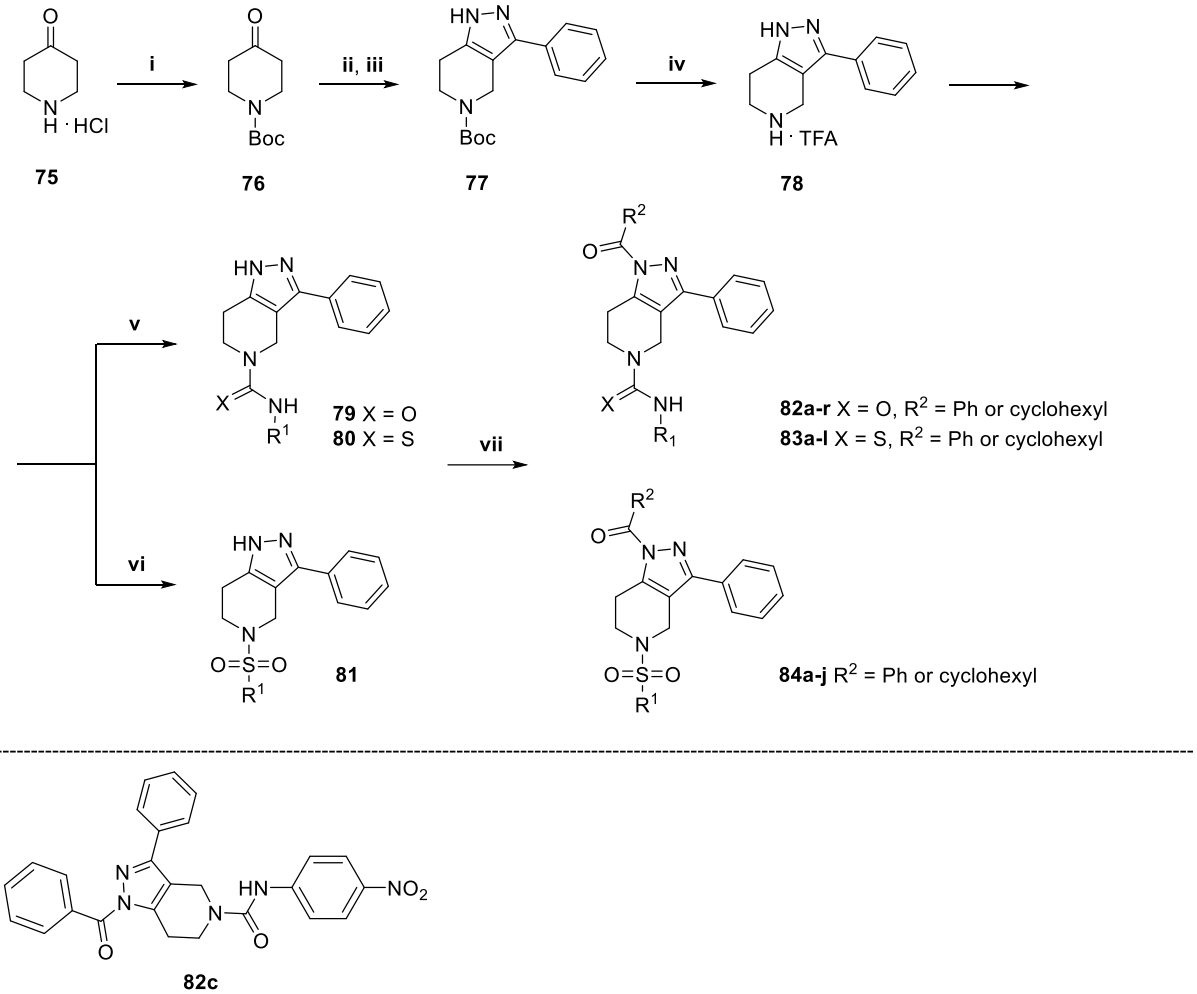
Scheme 1.7. Synthesis of GPR119 agonist. Reagents and conditions i: H_2, Raney-Ni, THF, rt; ii: $NaNO_2$, AcOH, rt; iii: Cs_2CO_3, *tert*-butyl-4-(methylsulfonyloxy)piperidine-1-carboxylate, DMF, 80 °C; iv: (a) HCl, MeOH, 1,4-dioxane, rt; (b) isopropyl chloroformate, DIPEA, $CHCl_3$, rt; v: $ArB(OH)_2$, $PdCl_2(dppf)$, Na_2CO_3, H_2O, CH_2Cl_2, DMF, 100 °C.

Subsequently, the GPR119 agonist potency of the new compounds was evaluated by using a cAMP assay in a human GPR119 cell line. The results of SAR study on the effects of substituent at the 5-position of the pyrazolopyridine ring and the piperidine *N*-substituent on the agonist potency led to the discovery of the lead compound **74** containing 4-methylsulfonyl-2-fluorophenyl at 5-position and 5-ethylpyrimidin-2-yl at 1-position. It exhibited agonist potency with EC_{50} value reaching 4 nM. Additionally, pharmacokinetics study in SD rats was conducted. Compound **74** had low systemic clearance, low volume of distribution and poor oral bioavailability. As derivative **74** had a very low aqueous solubility, further optimization of the 1*H*-pyrazolo[3,4-*c*]pyridine derivatives is needed.

1.3.2.3 Pyrazolo[4,3-*c*]pyridines

Samala *et al.* (2013) reported the synthesis of novel 3-phenyl-4,5,6,7-tetrahydro-1*H*-pyrazolo[4,3-*c*]pyridine derivatives [62]. The starting material, 4-piperidone hydrochloride salt **75**, was protected by *tert*-butyloxycarbonyl (Boc) protecting group and the obtained 4-*N*-Boc-piperidone **76** was reacted with morpholine, using benzoyl chloride and *p*-toluenesulfonic acid as a catalyst to produce 1,3-dicarbonyl intermediate which was subsequently treated with hydrazine hydrate to form pyrazolopyridine core **77**. The Boc-protecting group was cleaved using TFA and

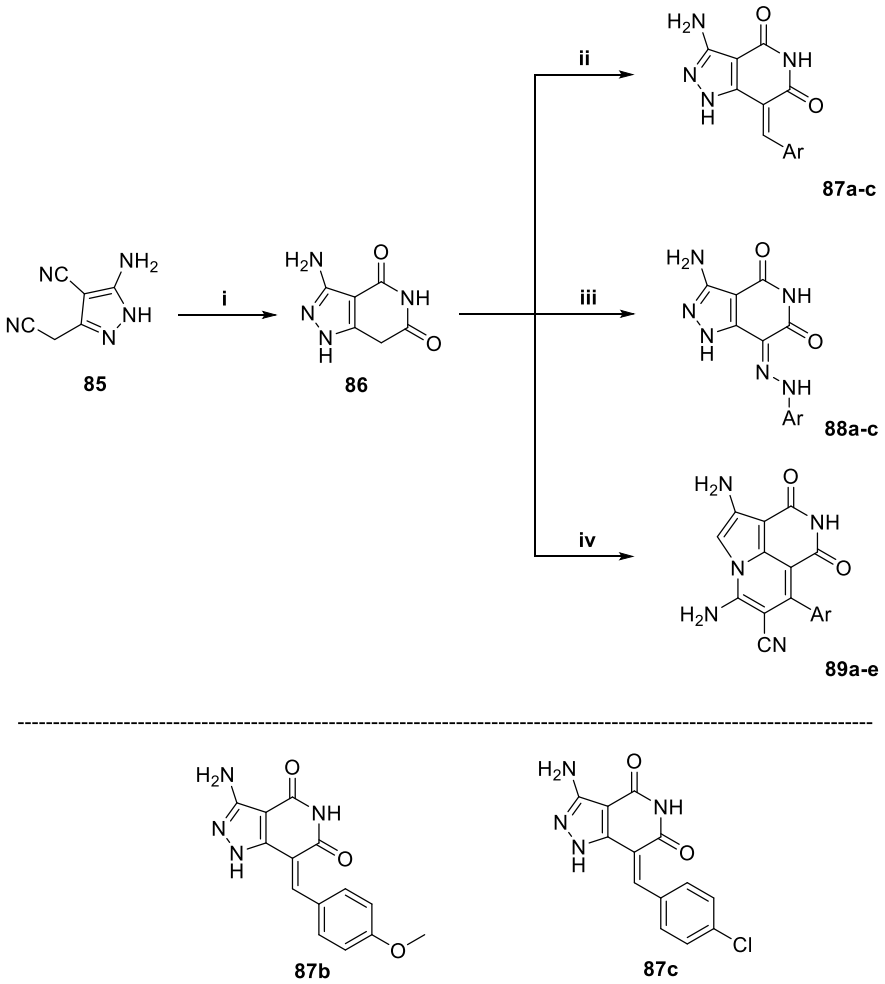
the resulting intermediate was selectively reacted with different substituted isocyanates, isothiocyanates and arylsulfonyl halides using DIPEA as a base and DMF as a solvent at room temperature to yield corresponding urea **79**, thiourea **80** and sulphonamides **81**, respectively. The target compounds **82-84** were obtained after treating intermediates with benzoyl or cyclohexanecarbonyl chloride using DIPEA as a base (Scheme 1.8).



Scheme 1.8. Synthesis of novel pyrazolo[4,3-*c*]pyridine derivatives and the most active compound. Reagents and conditions: i: $(Boc)_2O$, Et_3N, CH_2Cl_2, MeOH, rt, 12 h; ii: morpholine, PTSA, benzoyl chloride, toulene, Δ, 16 h; iii: $N_2H_4 \cdot H_2O$, EtOH, rt, 7 h; iv: TFA, CH_2Cl_2, rt, 2 h; v: R^1NCO for **81**, R^1NCS for **82**, Et_3N, DMF, rt, 6 h; vi: R^1SO_2Cl, Et_3N, DMF, rt, 6 h; vii: R^2COCl, DIPEA, DMF.

Subsequently, new derivatives were screened for *Mycobacterium tuberculosis* pantothenate synthetase enzyme inhibition. It was found that benzoyl group substituted compounds showed better activity than cyclohexanecarbonyl derivatives. Moreover, results showed that urea derivatives were the most, while thiourea – the least active. Derivative **82c** was the most potent compound with IC_{50} value reaching 21.8 μM. It also inhibited the growth of MTB with minimum inhibitory concentration of 26.7 μM.

Metwally *et al.* (2018) reported synthesis of pyrazolo[4,3-*c*]pyridine derivatives and their anticancer activity [63]. Acidic hydrolysis of 3-cyanomethyl-4-cyano-5-amino-1*H*-pyrazole **85** efficiently afforded the main intermediate 3-aminopyrazolo[4,3-*c*]pyridine-4,6-dione **86**. The latter was used in three types of reactions (Scheme 1.9). It was reacted with aromatic aldehydes in refluxing DMF to form derivatives **87a-c**. Additionally, coupling reaction of the arenediazonium chlorides with dione **86** in DMF at 0-5 °C yielded the corresponding arylhydrazo derivatives **88a-c**. Finally, it was also reacted with cinammonitriles in refluxing absolute EtOH in the presence of piperidine to obtain compounds **89a-e**.

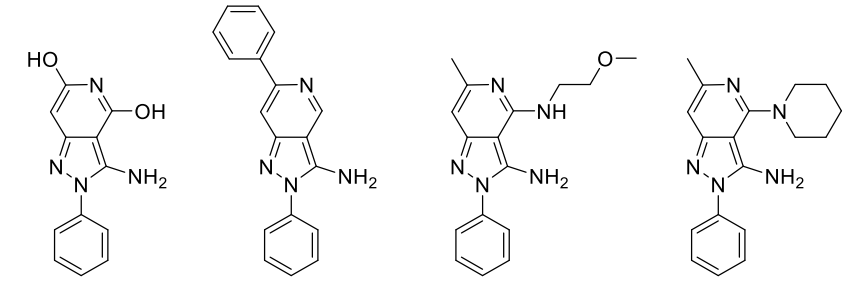


Scheme 1.9. Synthesis of novel cytotoxic pyrazolo[3,4-*c*]pyridine derivatives. Reagents and conditions: i: conc. HCl, Δ, 15 min; ii: DMF/piperidine, ArCHO, Δ, 5 h; iii: ArN=NCl, DMF, 0–5 °C, 1 h; iv: cinnamonitrile, EtOH, piperidine, Δ, 6 h.

Then, novel compounds were tested for anticancer activity against MCF7, HepG2 (liver) and HCT116 (colon) cancer cell lines. The results showed that introduction of a substituted aryl group at 5-position in starting compound **86** is crucial for better activity against these cell lines as IC_{50} values for **86** are much lower than for other derivatives. Moreover, compound **87b** was the most active against MCF-7 and HepG2 cell lines with promising IC_{50} values of 1.937 and 3.695 μg/mL,

respectively. Derivative **87c**, bearing chloro group, showed lower activity against these cell lines, however it was more potent against cell line HCT116 with IC_{50} value of 2.914 μg/mL.

Moreover, few examples of 2-phenyl-2*H*-pyrazolo[4,3-*c*]pyridines were reported as kinases inhibitors (Figure 1.7). For example, 3-amino-2-phenyl-2*H*-pyrazolo[4,3-*c*]pyridine-4,6-diol **90** showed inhibitory activity against p90 ribosomal S6 kinases 2 (RSK2) and could potentially play an important role in preventing cancer initiation and metastasis [64, 65]. Also, Smyth *et al.* (2011) evaluated 3-aminopyrazolopyridinone derivatives against a wide range of protein kinases from which 2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-3-amine **91**, N^4-(2-methoxyethyl)-6-methyl-2-phenyl-2*H*-pyrazolo[4,3-*c*]pyridine-3,4-diamine **92** and 6-methyl-2-phenyl-4-(piperidin-1-yl)-2*H*-pyrazolo[4,3-*c*]pyridin-3-amine **93** exhibited moderate inhibitory potency against p38a, aurora A and CK1d, respectively [66].



90
RSK2 inhibition:
IC_{50} = 14.45 μM

91
p38a inhibition:
55% at 30 μM

92
AurA inhibition:
32% at 30 μM

93
CK1d inhibition:
24% at 30 μM

Fig. 1.7. Biologically active 2-phenyl-2*H*-pyrazolo[4,3-*c*]pyridine derivatives.

Recently, a library of 2,4-, 2,6-disubstituted and 2,4,6-trisubstituted-2*H*-pyrazolo[4,3-*c*]pyridines was synthesized [67]. These compounds were evaluated for their cytotoxicity against K562 and MCF-7 cancer cell lines. It was noticed that the most potent derivatives bear a phenyl moiety at the 2-position, with no substituent at the 4-position and either an aryl or alkyl at the 6-position (Fig. 1.8). The tested compounds exhibited anticancer activity *in vitro* through arresting cell cycle in mitosis and induction of apoptosis.

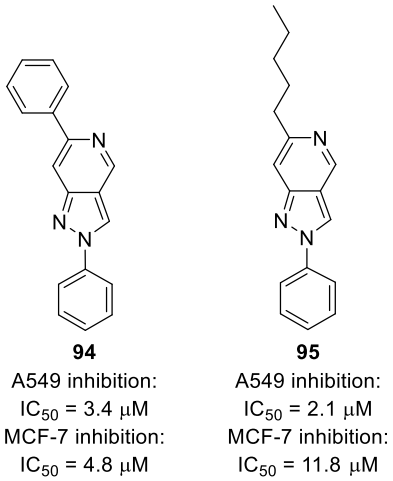


Figure 1.8. The most active 2,6-trisubstituted-2*H*-pyrazolo[4,3-*c*]pyridines from reported 2,4,6-trisubstituted-2*H*-pyrazolo[4,3-*c*]pyridines library.

In conclusion, pyrazolopyridine derivatives demonstrate a wide spectrum of biological activities. Because of their cytotoxic potential the interest in developing and synthesizing novel pyrazolopyridines is increasing. Due to the rapidly growing needs for medicines the design and evaluation of pyrazolo[4,3-*c*]pyridine derivatives with structural diversity is needed.

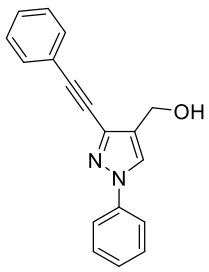
2. MATERIALS AND EXPERIMENTAL METHODS

2.1. Methods and devices used for analysis

All starting materials were obtained from commercial suppliers and used without further purification. Microwave irradiation was carried out with Discover LabMate or Discover SP microwave reactor. Melting points were determined on a Büchi M-565 melting point apparatus and were uncorrected. Mass spectra were obtained on a Shimadzu LCMS 2020 Single Quadrupole Liquid Chromatograph Mass Spectrometer and Q-TOF MICRO spectrometer. IR spectra were recorded on a Bruker Vertex 70v spectrometer and are reported in frequency of absorption (cm^{-1}). High-resolution ESI-TOF mass spectra were measured on a Bruker maXis spectrometer. ^{1}H NMR, ^{13}C NMR and ^{15}N NMR spectra were recorded from $CDCl_3$ solutions at 25 °C on either a Bruker Avance III 400 instrument (400 MHz for ^{1}H, 100 MHz for ^{13}C, 40 MHz for ^{15}N) using a directly detecting BBFO probe or on a Bruker Avance III 700 instrument (700 MHz for ^{1}H, 176 MHz for ^{13}C) equipped with a 5 mm TCI ^{1}H-^{13}C/^{15}N/D *z*-gradient cryoprobe or on Jeol 500 JNM-ECA spectrometer (500 MHz for ^{1}H, 125 MHz for ^{13}C) at room temperature. The solvent (residual) signals were used as internal standards and were related to TMS, with δ 7.26 ppm (^{1}H) and δ 77.00 ppm (^{13}C). ^{15}N NMR spectra were referenced against neat, external nitromethane. The full and unambiguous assignments of ^{1}H, ^{13}C and ^{15}N NMR resonances were achieved using combined applications of standard NMR spectroscopic techniques such as APT, COSY, TOCSY, NOESY, HSQC and HMBC. The UV–vis spectra were recorded on a Perkin Elmer Lambda 35 UV/vis spectrometer. The fluorescence spectra were recorded on an FL920 fluorescence spectrometer from Edinburgh Instruments. The PL quantum yields were measured from dilute THF solutions by an absolute method using the Edinburgh Instruments integrating sphere excited with a Xe lamp. All optical measurements were performed at room temperature under ambient conditions. For chromatographic separations, silica gel 60 (230-400 mesh, Merck) was used.

2.2. Compound synthesis and experimental data

[1-Phenyl-3-(phenylethynyl)-1*H*-pyrazol-4-yl]methanol **6**



1-Phenyl-3-(2-phenylethynyl)-1*H*-pyrazole-4-carbaldehyde **2** (560 mg, 2.06 mmol) was dissolved in MeOH (12 mL) and the solution was cooled to 0 °C temperature. Subsequently, $NaBH_4$ (156 mg, 4.12 mmol) 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 NH_4Cl solution (20 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over Na_2SO_4,

filtered and evaporated under reduced pressure. The residue was purified by column chromatography (EtOAc/Hex, 1:3 v/v).

Yield: 530 mg (94%), white crystals, mp = 114 – 115 °C, R_f = 0.18 (EtOAc/Hex, 1:3).

^{1}H NMR (700 MHz, $CDCl_3$): δ 1.99 (1H, s, OH), 4.77 (2H, d, *J* = 7.0 Hz, C*H*$_2$OH), 7.28-7.32 (1H, m, N-Ph, 4-H), 7.33-7.38 (3H, m, C-Ph, 3,4,5), 7.42-7.47 (2H, m, N-Ph, 3,5-H), 7.54-7.61 (2H, m, C-Ph, 2,6-H), 7.66-7.72 (2H, m, N-Ph, 2,6-H), 7.96 (1H, s, 5-H).

^{13}C NMR (126 MHz, $CDCl_3$): 55.9 (CH_2OH), 80.3 (*C*≡CPh), 93.7 (C≡*C*Ph), 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).

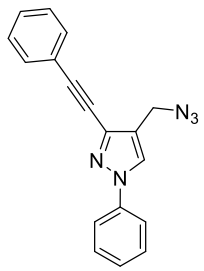
^{15}N BMR (71 MHz, $CDCl_3$): *δ* -163.5 (N-1), N-2 not found.

IR (ν, 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 (CH_2-OH), 749, 686 (CH=CH of mono- and disubstituted benzenes).

MS (ES^+): *m/z* (%): 275 ($[M+H]^+$, 100).

HRMS (ESI) for $C_{18}H_{14}N_2ONa$ ($[M+Na]^+$): calcd 297.0998, found 297.0988.

4-(Azidomethyl)-1-phenyl-3-(phenylethynyl)-1*H*-pyrazole **8**



(1-Phenyl-3-(2-phenylethynyl)-1*H*-pyrazol-4-yl)methanol **4** (100 mg, 0.36 mmol) was dissolved in dry DCM (1.5 mL). Subsequently, $TMSN_3$ (0.07 mL, 0.55 mmol) and $BF_3{\cdot}Et_2O$ (0.01 mL, 0.07 mmol) were added dropwise. The reaction was stirred for 10 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 × 25 mL). The combined organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (EtOAc:Hex, 1:8 v/v).

Yield: 54 mg (50%), light yellow liquid, R_f = 0.71 (EtOAc/Hex, 1:3).

^{1}H NMR (700 MHz, $CDCl_3$): *δ* 4.45 (2H, s, CH_2N_3), 7.33-7.38 (1H, m, N-Ph 4-H), 7.36-7.41 (3H, m, C-Ph 3,4,5-H), 7.44-7.51 (2H, m, N-Ph 3,5-H), 7.58-7.63 (2H, m, C-Ph 2,6-H), 7.69-7.75 (2H, m, N-Ph 2,6-H), 7.97 (1H, s, 5-H).

^{13}C NMR (126 MHz, $CDCl_3$): *δ* 44.8 (CH_2N_3), 79.9 (*C*≡CPh), 94.1 (C≡*C*Ph), 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).

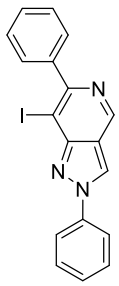
^{15}N BMR (71 MHz, $CDCl_3$): *δ* -162.2 (N-1), -306.6 and -132.9 (N_3, one not found), N-2 not found.

IR (ν, cm^{-1}): 3050 (CH_{arom}), 2921 (CH_{aliph}), 2087 (N_3), 1595, 1501, 1331, 1250 (C=C, C=N, C–N), 753, 688 (CH=CH of mono- and disubstituted benzenes).

MS (ES^+): *m/z* (%): 300 ($[M+H]^+$, 99).

HRMS (ESI) for $C_{18}H_{14}N_5$ ($[M+H]^+$): calcd 300.1242, found 300.1244; for $C_{18}H_{13}N_5Na$ ($[M+Na]^+$): calcd 322.1065, found 322.1063.

7-Iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine **10**



4-(Azidomethyl)-1-phenyl-3-(2-phenylethynyl)-1*H*-pyrazole **6** (276 mg, 0.92 mmol) was dissolved in DCM (9.8 mL). Subsequently, K_3PO_4 (978 mg, 4.6 mmol) and I_2 (1.472 g, 4.6 mmol) 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 Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified 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).

^{1}H NMR (700 MHz, $CDCl_3$): δ 7.39-7.46 (1H, m, C-Ph 4-H), 7.46-7.53 (3H, m, C-Ph 3,5-H; N-Ph 4-H), 7.53-7.63 (2H, m, N-Ph 3,5-H), 7.68-7.74 (2H, m, C-Ph 2,6-H), 7.92-8.00 (2H, m, N-Ph 2,6-H), 8.77 (1H, s, 3-H), 9.13 (1H, s, 4-H).

^{13}C NMR (126 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 BMR (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).

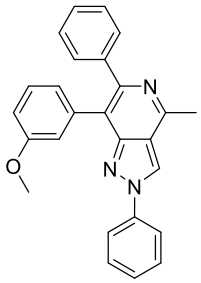
HRMS (ESI) for $C_{18}H_{13}N_3I$ ($[M+H]^+$): calcd 398.0149, found 398.0149.

General procedure for the synthesis of 7-substituted 4-methyl-pyrazolo[4,3-*c*]pyridine derivatives by Suzuki-Miyaura cross-coupling with boronic acids

7-Iodo-4-methyl-2,6-diphenyl-2*H*-pyrazol[4,3-*c*]pyridine **9** (50 mg, 0.12 mmol) was dissolved in the mixture of EtOH (0.72 mL) and water (0.34 mL). Subsequently, respective boronic acid (0.145 mmol), Cs_2CO_3 (79 mg, 0.24 mmol) and $Pd(OAc)_2$ (1.9 mg, 0.008 mmol) 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 to 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). 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 Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (EtOAc/PE, 1:4 to 1:2, v/v).

7-(3-Methoxyphenyl)-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine **11a**



Yield: 38 mg (80%), light yellow crystals, mp = 190 – 191 °C, R_f = 0.18 (EtOAc/Hex, 1:3).

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.97 (3H, s, Me), 3.65 (3H, s, MeO), 6.78-6.87 (1H, m, 7C-Ph, 4-H), 6.96-7.03 (1H, m, 7C-Ph, 2-H), 7.05-7.12 (1H, m, 7C-Ph, 6-H), 7.22-7.29 (4H, m, 7C-Ph, 5-H, 6C-Ph, 3,4,5-H), 7.41-7.48 (1H, m, N-Ph, 4-H), 7.49-7.46 (4H, m, N-Ph, 3,5-H, C-Ph, 2,6-H), 7.87-7.96 (2H, m, N-Ph, 2,6-H), 8.67 (1H, s, 3-H).

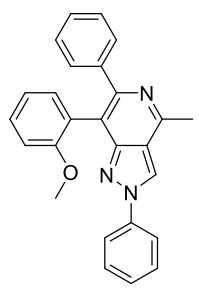
^{13}C NMR (126 MHz, $CDCl_3$): δ 23.1 (Me), 55.2 (MeO), 113.4 (7C-Ph, C-4), 116.6 (7C-Ph, C-2), 120.1 (C-3a), 120.8 (C-7), 121.3 (N-Ph, C-2,6), 121.9 (C-3), 123.9 (7C-Ph, C-6), 127.2 (6C-Ph, C-4), 127.9 (6C-Ph, C-3,5), 128.6 (N-Ph, C-4), 129.0 (7C-Ph, C-5), 129.7 (N-Ph, C-3,5), 130.6 (6C-Ph, C-2,6), 137.2 (7C-Ph, C-1), 140.1 (N-Ph, C-1), 140.8 (6C-Ph, C-1), 149.3 (C-6), 151.4 (C-7a), 154.6 (C-4), 159.2 (7C-Ph, C-3).

IR (ν, cm^{-1}): 3067, 3002 (CH_{arom}), 2954, 2923, 2852 (CH_{aliph}), 1599, 1589, 1575, 1547, 1488, 1443, 1284, 1211 (C=C, C=N, C–N), 1160, 1049 (C-O-C), 753, 700, 686 (CH=CH of mono- and disubstituted benzenes).

MS (ES^+): *m/z* (%): 392 ($[M+H]^+$, 100).

HRMS (ESI) for $C_{26}H_{22}N_3O$ ($[M+H]^+$): calcd 392.1757, found 392.1757.

7-(2-Methoxyphenyl)-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine **11b**



Yield: 38 mg (80%), light yellow crystals, mp = 159 – 160 °C, R_f = 0.13 (EtOAc/Hex, 1:3).

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.95 (3H, s, Me), 3.42 (3H, s, MeO), 6.79-6.85 (1H, m, Ph), 6.95-7.03 (1H, m, Ph), 7.14-7.23 (3H, m, Ph), 7.27-7.33 (1H, m,

Ph), 7.38-7.46 (4H, m, Ph), 7.46-7.53 (2H, m, Ph), 7.83-7.91 (2H, m, Ph), 8.62 (1H, s, 3-H).

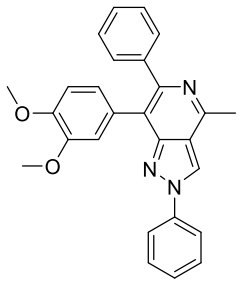
^{13}C NMR (126 MHz, $CDCl_3$): δ 22.9 (Me), 55.4 (MeO), 111.5, 117.8, 120.1, 120.6, 121.6, 122.2, 125.0, 127.2, 127.6, 128.7, 129.2, 129.7, 132.6, 140.3, 140.8, 149.8, 151.8, 154.8, 157.1.

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 $C_{26}H_{22}N_3O$ ($[M+H]^+$): calcd 392.1758, found 392.1757.

7-(3,4-Dimethoxyphenyl)-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine **11c**



Yield: 41 mg (67%), light yellow crystals, mp = 180 – 181 °C, R_f = 0.12 (EtOAc/Hex, 1:3).

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.91 (3H, s, Me), 3.60 (3H, s, 3-MeO), 3.89 (3H, s, 4-MeO), 6.83-6.91 (2H, m, 7C-Ph, 2,5-H), 7.19-7.24 (2H, m, 7C-Ph, 6-H, 6C-Ph, 4-H), 7.24-7.29 (2H, m, 6C-Ph, 3,5-H), 7.39-7.44 (1H, m, N-Ph, 4-H), 7.44-7.47 (2H, m, 6C-Ph, 2,6-H), 7.48-7.56 (2H, m, N-Ph, 3,5-H), 7.88-7.96 (2H, m, N-Ph, 2,6-H), 8.62 (1H, s, 3-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 23.2 (Me), 55.7 (3-MeO), 55.9 (4-MeO), 110.8 (7C-Ph, C-5), 114.9 (7C-Ph, C-2), 120.3 (C-3a), 120.6 (C-7), 121.4 (N-Ph, C-2,6), 121.8 (C-3), 123.9 (7C-Ph, C-6), 127.1 (6C-Ph, C-4), 128.1 (6C-Ph, C-3,5), 128.3 (7C-Ph, C-1), 128.6 (N-Ph, C-4), 129.7 (N-Ph, C-3,5), 130.6 (6C-Ph, C-2,6), 140.3 (N-Ph, C-1), 141.3 (C-Ph, C-1), 148.2 (7C-Ph, C-4), 148.3 (7C-Ph, C-3), 149.3 (C-6), 151.6 (C-7a), 154.2 (C-4).

^{15}N BMR (71 MHz, $CDCl_3$): 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).

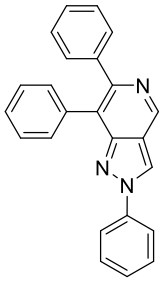
HRMS (ESI) for $C_{27}H_{24}N_3O_2$ ($[M+H]^+$): calcd 422.1863, found 422.1863.

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

7-Iodo-2,6-diphenyl-2*H*-pyrazol[4,3-*c*]pyridine **10** (60 mg, 0.15 mmol) was dissolved in the mixture of EtOH (0.9 mL) and water (0.3 mL). Subsequently, respective boronic acid (0.18 mmol),

Cs_2CO_3 (98 mg, 0.3 mmol) and $Pd(OAc)_2$ (2.4 mg, 0.01 mmol) were added to the solution under argon atmosphere. The mixture was stirred at 100 °C under microwave irradiation (100 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). 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 Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (EtOAc:Hex, 1:4 to 1:2, v/v).

2,6,7-Triphenyl-2*H*-pyrazolo[4,3-*c*]pyridine **12a**



Yield: 50 mg (96%), brown crystals, mp = 151 – 152 °C, R_f = 0.08 (EtOAc/Hex, 1:3).

^{1}H NMR (700 MHz, $CDCl_3$): δ 7.29-7.33 (2H, m, Ph), 7.34-7.40 (6H, m, Ph), 7.40-7.45 (2H, m, Ph), 7.51-7.55 (1H, m, Ph), 7.56-7.61 (2H, m, Ph), 7.94-8.04 (2H, m N-Ph 2,6-H), 9.35 (1H, s, 3-H), 10.02 (1H, s, 4-H).

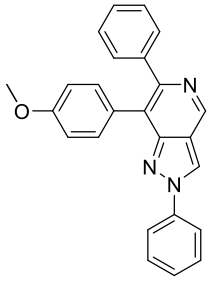
^{13}C NMR (126 MHz, $CDCl_3$): δ 118.7, 121.8, 126.9, 128.1, 128.5, 128.7, 128.9, 129.8, 130.1, 130.4, 130.5, 130.9, 132.0, 132.2, 138.9, 141.8, 143.9, 150.8.

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 $C_{24}H_{18}N_3$ ($[M+H]^+$): calcd 348.1495, found 348.1495.

7-(4-Methoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine **12b**



Yield: 44 mg (78%), white crystals, mp = 192 – 193 °C, R_f = 0.08 (EtOAc/Hex, 1:3).

^{1}H NMR (500 MHz, $CDCl_3$): δ 3.82 (3H, s, MeO), 6.76-6.94 (2H, m, 7C-Ph, 3,5-H), 7.21-7.25 (1H, m, 6C-Ph, 4-H), 7.25-7.3 (2H, m, 6C-Ph, 3,5-H), 7.41-7.48 (5H, m, N-Ph 4-H, 6C-Ph 2,6-H, 7C-Ph 2,6-H), 7.51-7.54 (2H, m, N-Ph, 3,5-H), 7.85-7.96 (2H, m, N-Ph, 2,6-H), 8.63 (1H, s, 3-H), 9.28 (1H, s, 4-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 55.2 (MeO), 113.6 (7C-Ph, C-3,5), 120.1 (C-3a), 121.4 (N-Ph, C-2,6), 121.8 (C-3), 122.7 (C-7), 127.0 (6C-Ph, C-4), 127.8 (7C-Ph, C-1), 127.9 (6C-Ph, C-3,5), 128.7 (N-Ph, C-4), 129.6 (N-Ph, C-3,5), 130.5 (6C-Ph, C-2,6), 132.4 (7C-Ph, C-2,6), 140.1 (N-Ph, C-1), 140.9 (6C-Ph, C-1), 145.3 (C-4), 149.2 (C-6), 151.4 (C-7a), 158.9 (7C-Ph, C-4).

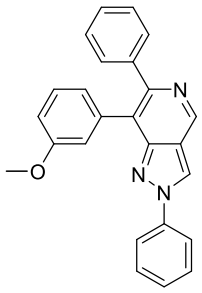
^{15}N BMR (71 MHz, $CDCl_3$): δ -145.4 (N-2), -97.0 (N-1), -79.1 (N-5).

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 (CH=CH of mono- and disubstituted benzenes).

MS (ES^+): *m/z* (%): 378 ($[M+H]^+$,100).

HRMS (ESI) for $C_{25}H_{20}N_3O$ ($[M+H]^+$): calcd 378.1603, found 378.1601.

7-(3-Methoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine **12c**



Yield: 44 mg (78%), white crystals, mp = 71 – 72°C, R_f = 0.08 (EtOAc/Hex, 1:3).

^{1}H NMR (500 MHz, $CDCl_3$): δ 3.66 (3H, s, MeO), 6.80-6.88 (1H, m, 7C-Ph, 4-H), 7.03-7.07 (1H, m, 7C-Ph, 2-H), 7.09-7.16 (1H, m, 7C-Ph, 6-H), 7.20-7.29 (4H, m, 7C-Ph, 5-H, 6C-Ph, 3,4,5-C), 7.39-7.44 (1H, m, N-Ph, 4-H), 7.45-7.48 (2H, m, 6C-Ph, 2,6-H), 7.48-7.54 (2H, m, N-Ph, 3,5-H), 7.85-7.95 (2H, m, N-Ph, 2,6-H), 8.64 (1H, s, 3-H), 9.30 (1H, s, 4-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 55.3 (MeO), 113.7 (7C-Ph, C-4), 116.6 (7C-Ph, C-2), 120.2 (C-3a), 121.5 (N-Ph, C-2,6), 121.9 (C-3), 123.0 (C-7), 123.9 (7C-Ph, C-6), 127.3 (6C-Ph, C-4), 128.0 (6C-Ph, C-3,5), 128.8 (N-Ph, C-4), 129.1 (7C-Ph, C-5), 129.8 (N-Ph, C-3,5), 130.6 (6C-Ph, C-2,6), 137.0 (7C-Ph, C-1), 140.2 (N-Ph, C-1), 140.9 (6C-Ph, C-1), 145.9 (C-4), 149.6 (C-6), 151.2 (C-7a), 159.3 (7C-Ph, C-3).

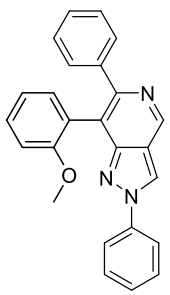
^{15}N BMR (71 MHz, $CDCl_3$): δ -145.2 (N-2), -96.9 (N-1), -79.4 (N-5).

IR (ν, cm^{-1}): 3394, 3058, 3011 (CH_{arom}), 2920, 2849 (CH_{aliph}), 1592, 1575, 1507, 1464, 1367, 1317, 1286, 1212 (C=C, C=N, C–N), 1150, 1051 (C-O-C), 764, 756, 699, 689 (CH=CH of mono- and disubstituted benzenes).

MS (ES^+): *m/z* (%): 378 ($[M+H]^+$, 99).

HRMS (ESI) for $C_{25}H_{20}N_3O$ ($[M+H]^+$): calcd 378.1601, found 378.1601.

7-(2-Methoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine **12d**



Yield: 14 mg (24%), light yellow crystals, mp = 169 – 170 °C, R_f = 0.08 (EtOAc/Hex, 1:3).

^{1}H NMR (500 MHz, $CDCl_3$): δ 3.42 (3H, s, MeO), 6.82-6.87 (1H, m, 7C-Ph), 6.97-7.04 (1H, m, 7C-Ph), 7.17-7.21 (1H, m, 7C-Ph), 7.21-7.25 (2H, m, Ph), 7.30-

7.35 (1H, m, Ph,), 7.38-7.42 (1H, m, Ph), 7.43-7.47 (2H, m, Ph), 7.46-7.52 (3H, m, Ph), 7.79-7.93 (2H, m, N-Ph, 2,6-H), 8.61 (s, 1H, 3-H), 9.34 (s, 1H, 4-H).

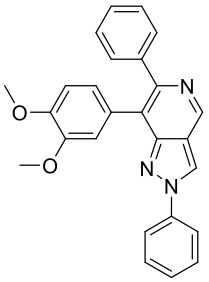
^{13}C NMR (126 MHz, $CDCl_3$): δ 55.3, 111.6, 119.7, 120.1, 120.7, 121.6, 121.8, 125.1, 127.1, 127.6, 128.7, 129.3, 129.6, 129.7, 132.5, 140.3, 141.5, 145.9, 150.4, 151.6, 157.0.

IR (ν, cm^{-1}): 3062, 3019 (CH_{arom}), 2922, 2852 (CH_{aliph}), 1600, 1590, 1501, 1478, 1435, 1242, 1233, 1203 (C=C, C=N, C–N), 1112, 1043, 1021 (C-O-C), 763, 750, 697, 686 (CH=CH of mono- and disubstituted benzenes).

MS (ES$^+$): *m/z* (%): 378 ([M+H]$^+$, 99).

HRMS (ESI) for $C_{25}H_{20}N_3O$ ([M+H]$^+$): calcd 378.1601, found 378.1601.

7-(3,4-Dimethoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine **12e**



Yield: 44 mg (72%), white crystals, mp = 163 – 164 °C, R_f = 0.08 (EtOAc/Hex, 1:3).

^{1}H NMR (500 MHz, $CDCl_3$): δ 3.61 (3H, s, 3-MeO), 3.90 (3H, s, 4-MeO), 6.86-6.90 (1H, m, 7C-Ph, 5-H), 6.91-6.94 (1H, m, 7C-Ph, 2-H), 7.21-7.30 (4H, m, 6C-Ph, 3,4,5-H, 7C-Ph, 6-H), 7.42-7.45 (1H, m, N-Ph, 4H), 7.45-7.48 (2H, m, N-Ph, 2,6-H), 7.50-7.57 (2H, m, N-Ph, 3,5-H), 7.86-7.98 (2H, m, N-Ph, 2,6-H), 8.65 (1H, s, 3-H), 9.28 (1H, s, 4-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 55.7 (3-MeO), 55.9 (4-MeO), 110.9 (7C-Ph, C-5), 114.9 (7C-Ph, C-2), 120.3 (C-3a), 121.5 (N-Ph, C-2,6), 121.9 (C-3), 122.8 (C-7), 124.0 (7C-Ph, C-6), 127.2 (6C-Ph, C-4), 128.0 (7C-Ph, C-1), 128.1 (6C-Ph, C-3,5), 128.8 (N-Ph, C-4), 129.8 (N-Ph, C-3,5), 130.5 (6C-Ph, C-2,6), 140.2 (N-Ph, C-1), 141.2 (6C-Ph, C-1), 145.4 (C-4), 148.4 (7C-Ph, C-3,4), 149.4 (C-6), 151.4 (C-7a).

^{15}N BMR (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 (CH=CH of mono- and disubstituted benzenes).

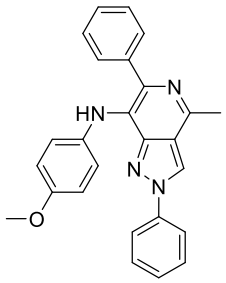
MS (ES$^+$): *m/z* (%): 408 ([M+H]$^+$, 100).

HRMS (ESI) for $C_{26}H_{22}N_3O_2$ ([M+H]$^+$): calcd 408.1707, found 408.1707.

General procedure for the synthesis of 7-substituted 4-methyl-pyrazolo[4,3-*c*]pyridine derivatives by Buchwald-Hartwig cross-coupling with anilines

7-Iodo-4-methyl-2,6-diphenyl-2*H*-pyrazol[4,3-*c*]pyridine **9** (50 mg, 0.12 mmol) was dissolved in dry dioxane (4 mL). Subsequently, respective aniline (0.18 mmol), NaO*t*Bu (18.7 mg, 0.19 mmol), SPhos (15 mg, 0.036 mmol) and $Pd(OAc)_2$ (1.7 mg, 0.012mmol) 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). 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 Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (EtOAc/PE, 1:4 to 1:2, v/v).

N-4-Methoxyphenyl-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine **13a**



Yield: 38 mg (77%), light yellow crystals, mp = 89 – 90 °C, R_f = 0.11 (EtOAc/Hex, 1:3).

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.86 (3H, s, Me), 3.74 (3H, s, MeO), 5.89 (1H, s, NH), 6.66-6.75 (2H, m, NH-Ph, 3,5-H), 6.76-6.83 (2H, m, NH-Ph, 2,6-H), 7.23-7.30 (1H, m, C-Ph, 4-H), 7.31-7.39 (2H, m, C-Ph, 3,5-H), 7.39-7.45 (1H, m, N-Ph, 4-H), 7.46-7.56 (2H, m, N-Ph, 3,5-H), 7.64-7.76 (2H, m, C-Ph, 2,6-H), 7.80-7.89 (2H, m, N-Ph, 2,6-H), 8,55 (1H, s, 3-H).

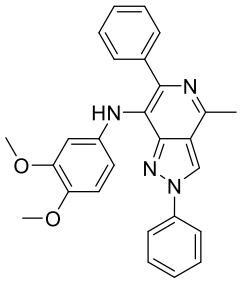
^{13}C NMR (126 MHz, $CDCl_3$): δ 22.8 (Me), 55.7 (MeO), 114.0 (NH-Ph, C-2,6), 119.1 (NH-Ph, C-3,5), 121.1 (N-Ph, C-2,6) , 121.2 (C-3a), 121.7 (C-3), 123.4 (C-7), 127.6 (C-Ph, C-4), 128.5 (C-Ph, C-3,5), 128.6 (N-Ph, C-4), 129.0 (C-Ph, C-2,6), 129.7 (N-Ph, C-3,5), 138.0 (NH-Ph, C-1), 139.3 (C-Ph, C-1), 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 (ν, 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 **13b**



Yield: 32 mg (60%), brown crystals, mp = 202 – 203 °C, R_f = 0.08 (EtOAc/Hex, 1:3).

^{1}H NMR (500 MHz, $CDCl_3$): δ 2.88 (3H, s, Me), 3.67 (3H, s, 3-MeO), 3.81 (3H, s, 4-MeO), 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), 6.65-6.71 (1H, m, NH-Ph, 5-H), 7.22-7.30 (1H, m, C-Ph, 4-H), 7.31-7.38 (2H, m, C-Ph, 3,5-H), 7.39-7.48 (1H, m, N-Ph, 4-H), 7.47-7.57 (2H, m, N-Ph, 3,5-H), 7.69-7.77 (2H, m, C-Ph, 2,6-H), 7.81-7.91 (2H, m, N-Ph, 2,6-H), 8.57 (1H, s, 3-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 22.5 (Me), 55.8 (3-MeO), 56.4 (4-MeO), 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 (ν, 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).

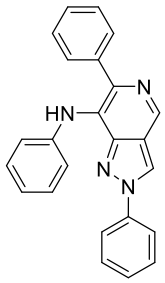
MS (ES^+): *m/z* (%): 437 ($[M+H]^+$, 100).

HRMS (ESI) for $C_{27}H_{25}N_4O_2$ ($[M+H]^+$): calcd 437.1972, found 437.1972.

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

7-Iodo-2,6-diphenyl-2*H*-pyrazol[4,3-*c*]pyridine **9** (60 mg, 0.15 mmol) was dissolved in dry dioxane (4,5 mL). Then appropriate aniline (0.16 mmol), NaO*t*Bu (23 mg, 0.24 mmol), SPhos (18.6 mg, 0.045 mmol) and $Pd(OAc)_2$ (3.4 mg, 0.015mmol) 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). 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 Na_2SO_4 and evaporated under reduced pressure. The residue was purified by column chromatography (EtOAc/Hex, 1:3 to 1:2, v/v).

N,2,6-Triphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine **14a**



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

^{1}H NMR (500 MHz, $CDCl_3$): δ 6.17 (1H, s, NH), 6.81-6.88 (3H, m, NH-Ph, 2,4,6-H), 7.10-7.16 (2H, m, NH-Ph, 3,5-H), 7.24-7.31 (1H, m, C-Ph, 4-H), 7.33-7.38 (2H, m, C-Ph, 3,5-H), 7.41-7.46 (1H, m, N-Ph, 4-H), 7.48-7.57 (2H, m, N-Ph, 3,5-H), 7.68-7.77 (2H, m, C-Ph, 2,6-H), 7.82-7.89 (2H, m, N-Ph, 2,6-H), 8.59 (1H, s, 3-H), 9.07 (1H, s, 4-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 117.5 (NH-Ph, C-2,6), 120.6 (NH-Ph, C-4), 121.2 (C-3a), 121.3 (N-Ph, C-2,6), 121.8 (C-3), 124.5 (C-7), 127.8 (C-Ph, C-4), 128.6 (C-Ph, C-3,5), 128.7 (NH-Ph, C-3,5), 128.8 (N-Ph, C-4), 129.0 (C-Ph, C-2,6), 129.8 (N-Ph, C-3,5), 139.4 (C-6), 140.0 (C-Ph, C-1), 141.0 (N-Ph, C-1), 141.2 (C-4), 143.7 (NH-Ph, C-1), 147.6 (C-7a).

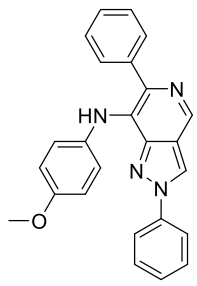
^{15}N NMR (71 MHz, $CDCl_3$): δ -304.0 (NH), -147.3 (N-2), -100.5 (N-1), -78.1 (N-5).

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 $C_{24}H_{19}N_4$ ($[M+H]^+$): calcd 363.1606, found 363.1604.

N-(4-Methoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine **14b**



Yield: 29 mg (48%), red crystals, mp = 135 – 136 °C, R_f = 0.08 (EtOAc/Hex, 1:3).

^{1}H NMR (500 MHz, $CDCl_3$): δ 3.74 (3H, s, MeO) , 6.18 (1H, s, NH), 6.65-6.73 (2H, m, NH-Ph, 3,5-H), 6.78-6.86 (2H, m, NH-Ph, 2,6-H), 7.25-7.28 (1H, m, C-Ph, 4-H), 7.30-7.38 (2H, m, C-Ph, 3,5-H), 7.40-7.46 (1H, m, N-Ph, 4-H), 7.48-7.56 (2H, m, N-Ph, 3,5-H), 7.64-7.73 (2H, m, C-Ph, 2,6-H), 7.80-7.88 (2H, m, N-Ph, 2,6-H), 8.62 (1H, s, 3-H), 9.04 (1H, s, 4-H).

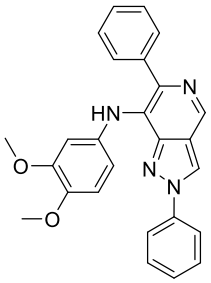
^{13}C NMR (126 MHz, $CDCl_3$): δ 55.7 (MeO), 113.9 (NH-Ph, C-3,5), 120.3 (NH-Ph, C-2,6), 120.8 (C-3a), 121.2 (N-Ph, C-2,6), 122.2 (C-3), 126.2 (C-7), 127.8 (C-Ph, C-4), 128.6 (C-Ph, C-3,5), 128.8 (C-Ph, C-2,6), 128.9 (N-Ph, C-4), 129.8 (N-Ph, C-3,5), 136.5 (NH-Ph, C-1), 137.7 (C-6), 138.4 (C-Ph, C-1), 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 (C=C, C=N, C–N), 1033 (C-O-C), 755, 699, 688 (CH=CH of mono- and disubstituted benzenes).

MS (ES^+): *m/z* (%): 393 ($[M+H]^+$, 100).

HRMS (ESI) for $C_{25}H_{21}N_4O$ ($[M+H]^+$) calcd 393.1710, found 393.1710.

N-(3,4-Dimethoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine **14c**



Yield: 55 mg (86%), orange crystals, mp = 93 – 94 °C, R_f = 0.20 (EtOAc/Hex, 1:1).

^{1}H NMR (500 MHz, $CDCl_3$): δ 3.66 (3H, s, 3-MeO), 3.80 (3H, s, 4-MeO), 6.21 (1H, s, NH), 6.37-6.51 (2H, m, NH-Ph, 2,6-H), 6.61-6.73 (1H, m, NH-Ph, 3-H), 7.20-7.27 (1H, m, C-Ph, 4-H), 7.30-7.38 (2H, m, C-Ph, 3,5-H), 7.38-7.46 (1H, m, N-Ph, 4-H), 7.46-7.57 (2H, m, N-Ph, 3,5-H), 7.68-7.78 (2H, m, C-Ph, 2,6-H), 7.83-7.92 (2H, m, N-Ph, 2,6-H), 8.58 (1H, s, 3-H), 9.01 (1H, s, 4-H).

^{13}C NMR (126 MHz, $CDCl_3$): δ 55.8 (3-MeO), 56.4 (4-MeO), 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_{alif}), 1596, 1509, 1439, 1228 (C=C, C=N, C–N), 1024 (C-O-C), 756, 696 (CH=CH of mono- and disubstituted benzenes).

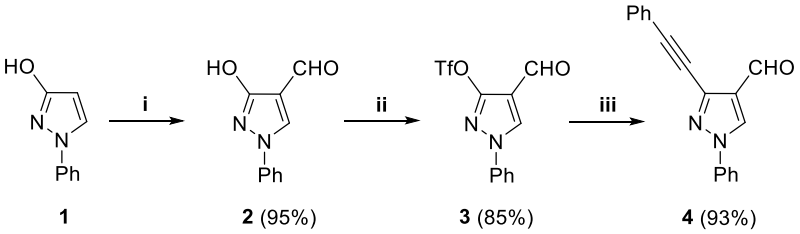
MS (ES^+): *m/z* (%): 423 ($[M+H]^+$, 100).

HRMS (ESI) for $C_{26}H_{23}N_4O_2$ ($[M+H]^+$) calcd 423.1816, found 423.1816.

3. RESULTS AND DISCUSSION

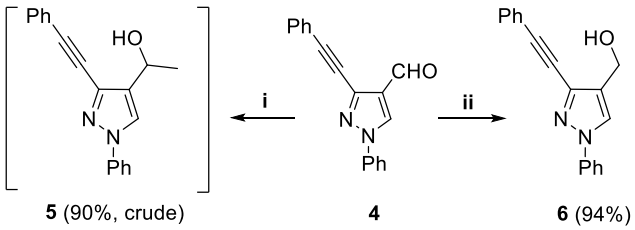
3.1 Synthesis of 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine and 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine

1-Phenyl-3-(2-phenylethynyl)-1*H*-pyrazole-4-carbaldehyde (**4**), which was chosen as a starting material for this study, was prepared from 1-phenyl-1*H*-pyrazol-3-ol (**1**) *via* a multistep synthetic route (Scheme 3.1). Firstly, the hydroxy group of 1-phenyl-1*H*-pyrazol-3-ol **1** was blocked with a protecting benzyl group [68] and a formyl group at the 4-position of the pyrazole ring was introduced by the Vilsmeier–Haack reaction as reported previously [69]. After deprotection of the alcohol using TFA, the hydroxy group was substituted with triflate [70], a pseudohalogenide which is a convenient leaving group for Pd-catalysed cross-coupling reactions. Subsequently, using standard Sonogashira reaction conditions, i.e. phenylacetylene, TEA as a base, CuI as co-catalyst and $Pd(PPh_3)_2Cl_2$ as catalyst [68], compound **4** was obtained in high yield (93%).



Scheme 3.1. Synthesis of 1-phenyl-3-(2-phenylethynyl)-1*H*-pyrazole-4-carbaldehyde **4.** Reagents and conditions: i: (a) NaH, BnCl, DMF, 60 ºC, 50 min.; (b) $POCl_3$, DMF, 0 ºC for 10 min to 60 ºC for 30 min; (c) TFA, toluene, rt, 24 h. ii: Tf_2O, TEA, DCM, rt, 60 min. iii: Phenylacetylene, TEA, CuI, $Pd(PPh_3)_2Cl_2$, DMF, 65 ºC, 45 min.

Subsequently, 1-phenyl-3-(2-phenylethynyl)-1*H*-pyrazole-4-carbaldehyde (**4**) was further used as a substrate to synthesise 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**7**) and 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine (**8**) in a three-step route.



Scheme 3.2. Synthesis of [1-phenyl-3-(phenylethynyl)-1*H*-pyrazol-4-yl] alcohols **5** and **6.** Reagents and conditions: i: MeMgBr, THF (abs.), rt, 10 min. ii $NaBH_4$, MeOH, 0 °C, 30 min.

Two different approaches were applied when obtaining alcohols **5** and **6** (Scheme 3.2). For the synthesis of secondary alcohol **5**, carbaldehyde **4** was dissolved in THF and then reacted with methylmagnesium bromide at room temperature, as described previously [71]. Noteworthy, reaction was carried out under argon atmosphere and dry solvent due to the sensitivity of Grignard reagents to air and moisture [72]. Furthermore, it is known that this kind of secondary alcohol is unstable [73], therefore it was used in the next step without purification. On the other hand, primary alcohol **6** was obtained by the reduction of an aldehyde group [74]. Sodium borohydride was chosen as a reducing agent and the reaction was carried out in methanol at 0 °C under argon atmosphere. The reaction mixture was protonated with aqueous ammonium chloride solution to create primary alcohol **6** from the intermediate complex. Contrary to compound **5**, [1-phenyl-3-(2-phenylethynyl)-1*H*-pyrazol-4-yl]methanol **6** appeared to be stable and therefore could be purified on a silica gel. The formation of alcohol can be quickly confirmed by FT-IR spectra in which broad absorption peak of alcohol group can be found at 3373 cm^{-1} while aldehyde group peak (~1710-1685) is absent (Figure 3.1).

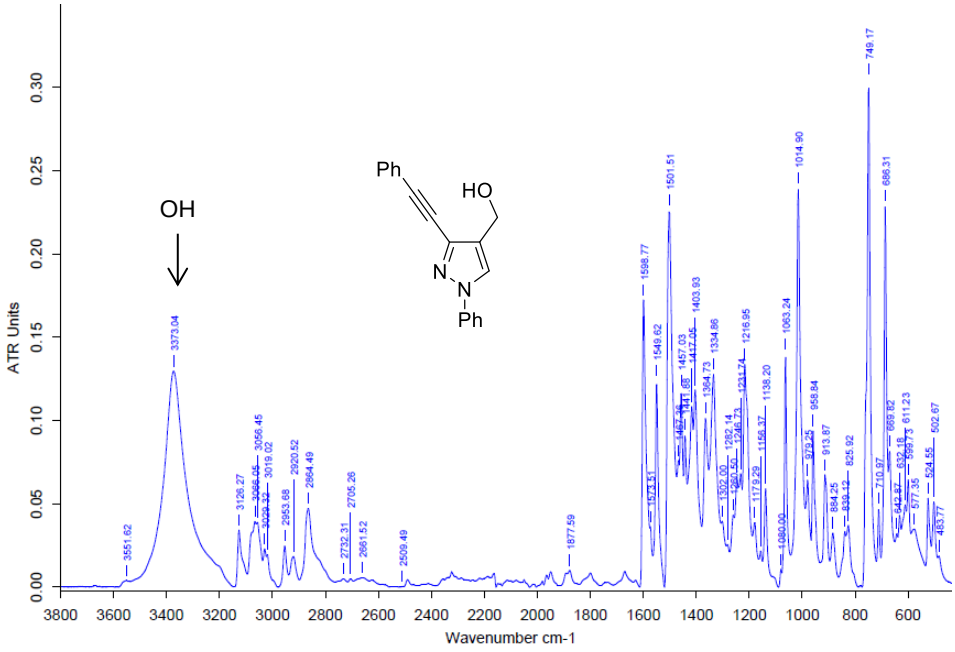
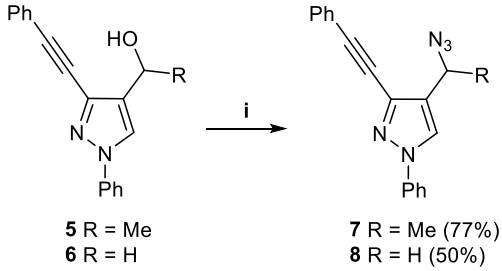


Fig. 3.1. FT-IR spectra of compound **6**.



5 R = Me
6 R = H

7 R = Me (77%)
8 R = H (50%)

Scheme 3.3. Synthesis of azides**.** Reagents and conditions: i: $TMSN_3$, $BF_3{\cdot}Et_2O$, DCM, rt, 10 min.

The obtained alcohols **5** and **6** were further converted into azides **7** and **8**, respectively. Many methods were developed for such transformation, including Mitsunobu-type displacements [75, 76], two-step procedures that involve a halogenated intermediate [77], one-pot halogenation-azidation of alcohols [78], reactions with phosphitine intermediates [79], *N*-methyl-2-pyrolidone hydrosulphate [80] and $TMSN_3$ [73]. The latter method was chosen for the synthesis and the reactions were performed in DCM with catalytic amount of boron trifluoride diethyl etherate. The reactions were carried out at room temperature under argon atmosphere and in dry solvent in order to protect both boron trifluoride and $TMSN_3$ from moisture (Scheme 3.3). Conversion was completed in 10 to 30 minutes and the reaction products **7** and **8** were furnished in 72% and 50% yield, respectively. The structures were confirmed by NMR, IR spectroscopy and mass spectrometry. The substitution of alcohol group with azide can be proved by increased masses of compounds **7** and **8**, with peaks at the positive mode of mass spectra being at 314 and 300, respectively. Furthermore, formation of an azide can be confirmed by the emerged characteristic peak in FT-IR spectra. In case of compounds **7** and **8** absorption peaks of their respective azide groups can be observed at 2120 and 2087 cm^{-1}, respectively, while the peaks of alcohol groups are absent (Figure 3.2).

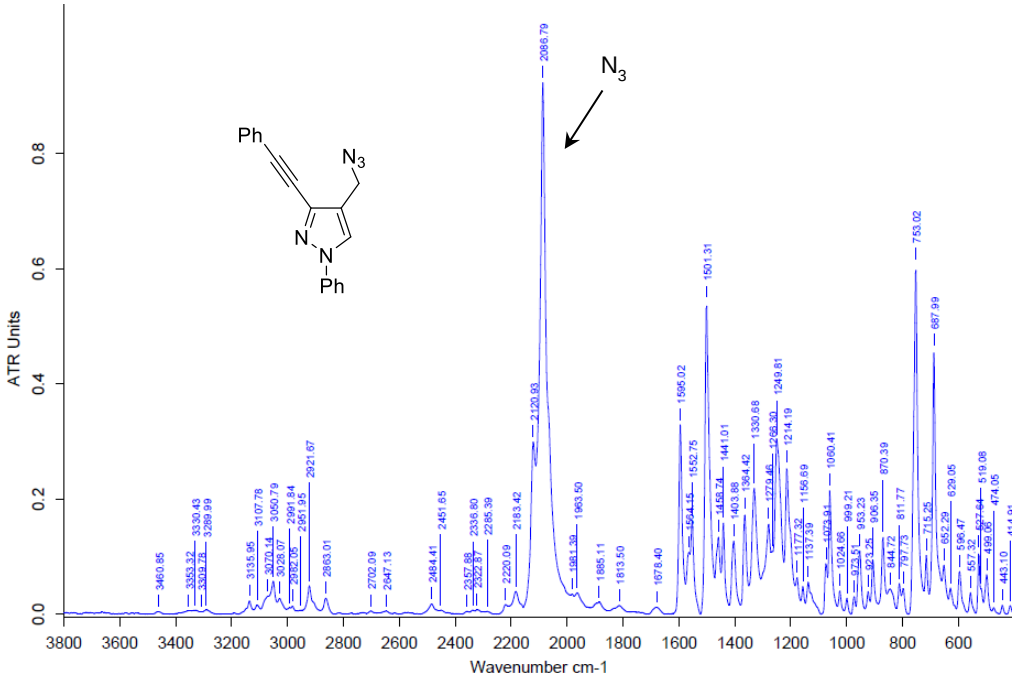
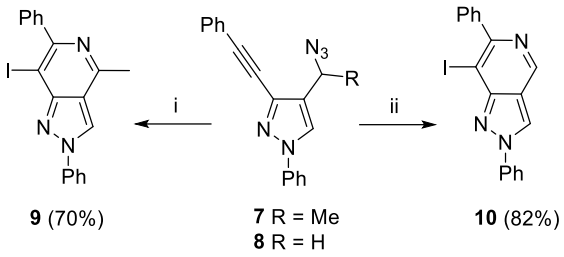


Fig. 3.2. FT-IR spectra of compound **8**.

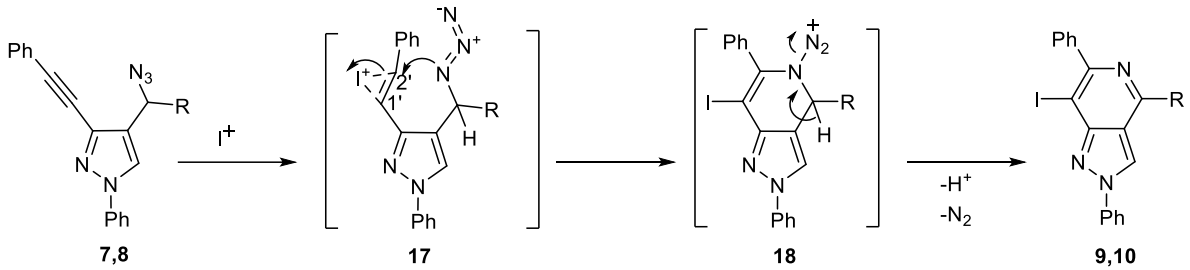
The new azides **7** and **8** were further used to form the pyrazolo[4,3-*c*]pyridine core with iodine in 7-position, adopting electrophilic substitution reaction conditions that were previously used to obtain 1,3,4-trisubstituted isoquinolines from 2-alkynyl benzyl azides [81]. Namely, azides **7** and **8** were dissolved in DCM and treated with iodine and a proper base (Scheme 3.4). Five equivalents of K_3PO_4 were used in case of the primary azide **8**, while one equivalent of $NaHCO_3$

was used for the secondary azide **7**. The reactions were carried out at room temperature in the dark for 12 h. Both compounds were obtained in high yields, although yield of pyrazolo[4,3-*c*]pyridine without substituent at 4-position **10** was higher (82%), while compound **9** with methyl substituent was obtained in a slightly lower yield (70%). Noteworthy, attempt to make use of a weaker base $NaHCO_3$ for the reaction with the primary azide **8** led to the formation of the dehalogenation side product 2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine, which resulted in a troublesome purification and lower yield of the target product.



Scheme 3.4. Synthesis of 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine and 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-c]pyridine. Reagents and conditions: i: $NaHCO_3$, I_2, DCM, rt, overnight. ii: K_3PO_4, I_2, DCM, rt, overnight.

The reaction mechanism was not investigated but in accordance to Fischer *et al*. (2008) [81] it can be proposed to work as shown in scheme 3.5. At first, the electrophilic I^+ ion coordinates at the alkyne and activates the triple bond towards the nucleophilic ring forming intermediate **17**. Subsequently, the azide closes at the C2' affording the core of pyrazolo[4,3-*c*]pyridine **18**. Finally, elimination of a proton and a nitrogen molecule results in the formation of compounds **9** and **10**.



Scheme 3.5. Proposed reaction mechanism of electrophilic cyclisation reaction from azide to pyrazolo[4,3-*c*]pyridine.

The structures of compounds **9** and **10** were confirmed by NMR and IR spectroscopy, MS and HRMS spectrometry. The difference in mass proves the addition of iodine, with peaks in the positive mode of mass spectra being at 412 and 398 for compounds **9** and **10**, respectively. The structure of the cyclisation product can be illustrated by the comparison of ^{1}H NMR spectra of azide **8** and pyrazolopyridine **10** (Fig. 3.3). The absence of a singlet peak at 4.45 ppm and the emergence of new singlets at 8.77 and 9.13 ppm collectively confirm that the cyclisation was successful.

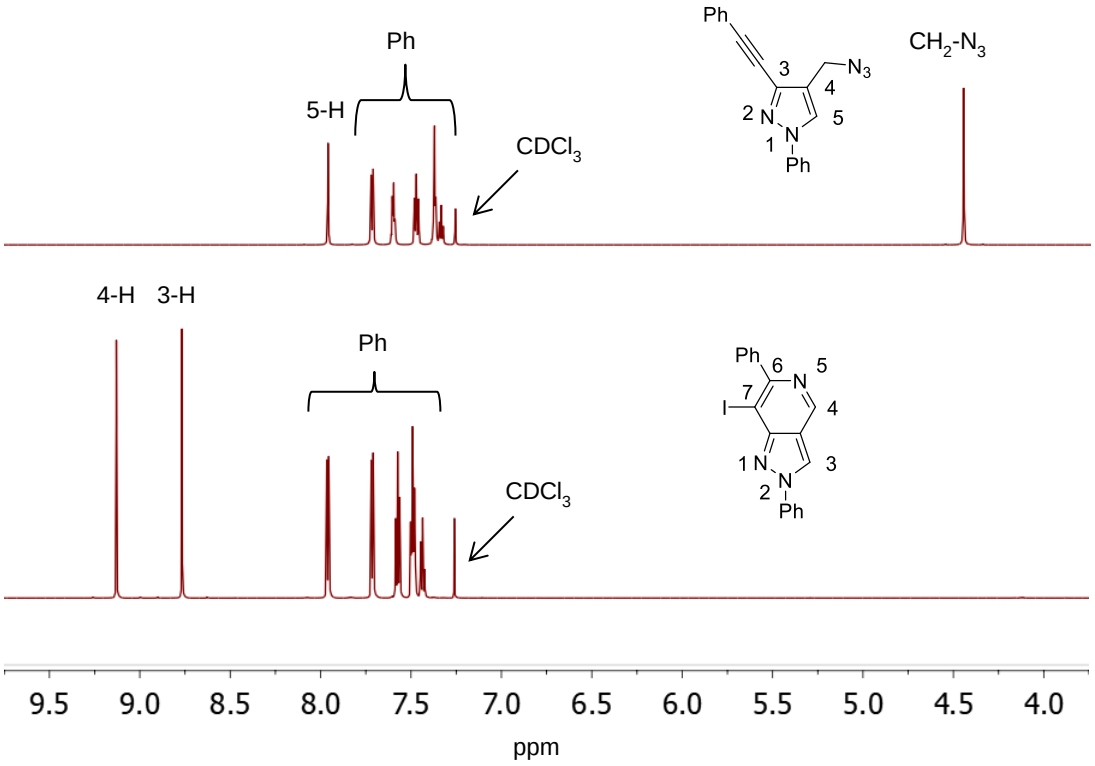


Fig. 3.3. ^{1}H NMR spectra of compounds **8** and **10**.

Furthermore, the structure of 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine **10** can be verified by ^{1}H-^{15}N HMBC spectrum (Figure 3.4), where three nitrogen atom signals and their interaction with adequate protons is observed. The nitrogen atom (N-5) from pyridine moiety has a strong interaction with 4-H proton. The signal of this nitrogen is observed at -78.4 ppm. Meanwhile, the nitrogen atoms N-2 and N-1 from pyrazole moiety both interact with 3-H proton with peaks at -146.1 and -90.6 ppm, respectively. Noteworthy, the N-2 interaction is stronger than that of N-1. Nitrogen atom N-2 also interacts with protons from phenyl group and helps to determine their signal positions. The interaction is stronger with the protons closer to nitrogen, thus signal at 7.92-8.00 ppm can be assigned to 2,6- protons of phenyl group, while protons at 7.53-7.63 ppm can be assigned to 3,5- protons of phenyl group because the latter interaction is weaker.

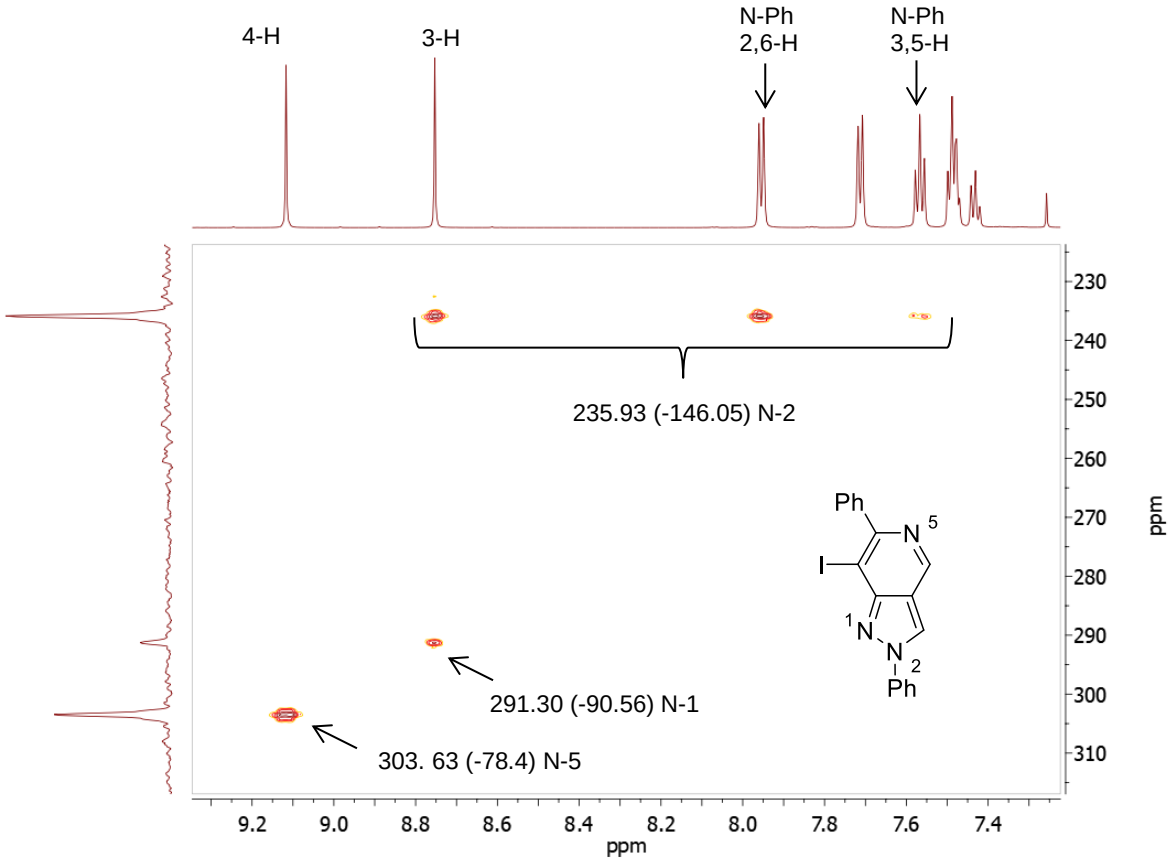
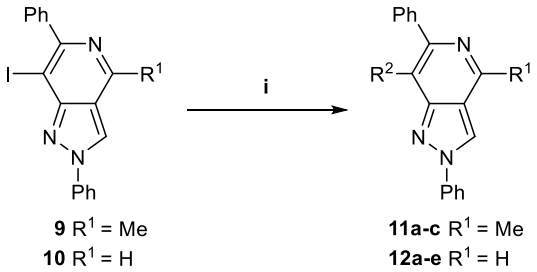


Fig. 3.4. ^{1}H-^{15}N HMBC spectrum of compound **10**.

3.2 7-Iodopyrazolo[4,3-*c*]pyridine as a substrate for Suzuki-Miyaura cross-coupling reactions

Previously reported 2*H*-pyrazolo[4,3-*c*]pyridines varying by the substituents at the 2-, 4- and 6-positions had displayed good anticancer activity *in vitro* through arresting cell cycle in mitosis and induction of apoptosis [67]. Thus, to further extend the library of variously substituted 2*H*-pyrazolo[4,3-*c*]pyridines and examine how substituents at 7-position influence biological and optical activities of these compounds, 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine **9** and 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine **10** were further used in palladium catalysed Suzuki-Miyaura cross-coupling reaction (Scheme 3.6). The standard substrates and conditions for this type of reaction are aromatic or aliphatic halogenide, organic boronic acid, Pd catalyst and a base. In this case, aromatic boronic acids were reacted with compounds **9** and **10** using palladium acetate as a catalyst and caesium carbonate as a base in an aqueous ethanol solution (EtOH:H_2O, 3:1 v/v) under argon atmosphere. To reduce reaction time cross-coupling reactions were carried out under microwave irradiation giving rise to compounds **11a-c** and **12a-e**. While compounds **11a-c** and **12a-c** and **12e** (Table 3.1) were obtained in fair to excellent yields (67-96%), full cross-coupling conversion using 2-methoxyphenyl boronic acid could not be achieved, presumably due to

the steric hindrance between methoxy group and phenyl groups at 2,6-positions, resulting in a low yield of compound **12d**.



9 R^1 = Me
10 R^1 = H

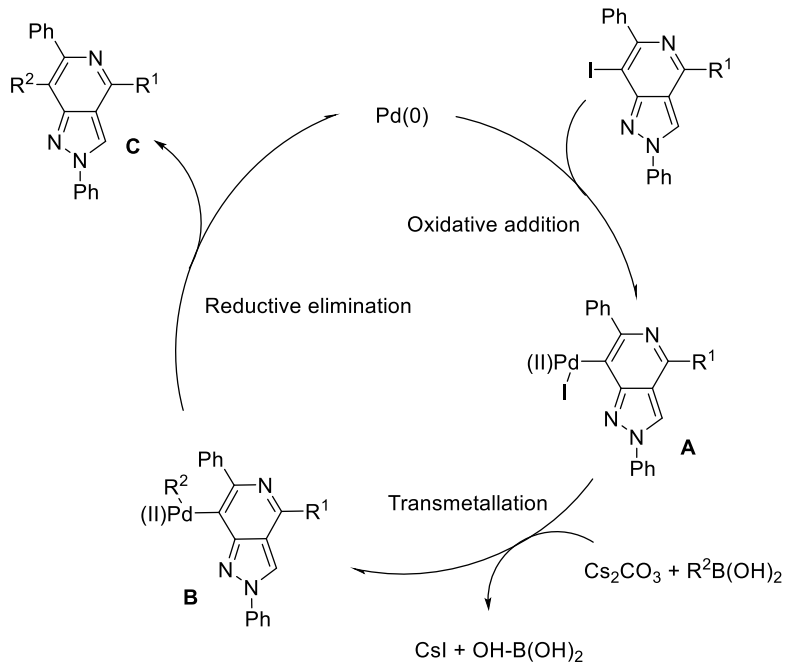
11a-c R^1 = Me
12a-e R^1 = H

Scheme 3.6. Synthesis of compounds **11a-c** and **12a-e**. Reagents and conditions: i: $R^2B(OH)_2$, $Pd(OAc)_2$, Cs_2CO_3, EtOH/H_2O 3/1, MW, 100 °C, 100 W, 0.5-1 h.

Table 3.1. Yields of new compounds **11a-b** and **12a-d**.

Compound	R^1	R^2	Yield, %
11a	Me	3-MeO-Ph	80
11b	Me	2-MeO-Ph	80
11c	Me	3,4-di-MeO-Ph	67
12a	H	Ph	96
12b	H	4-MeO-Ph	78
12c	H	3-MeO-Ph	78
12d	H	2-MeO-Ph	24
12e	H	3,4-di-MeO-Ph	72

The final products were formed *via* Suzuki-Miyaura reaction mechanism, as illustrated in scheme 3.7. The mechanism begins with oxidative addition of the organohalide 7-iodopyrazolo[4,3-*c*]pyridine **7** or **8** to the Pd(0) to form a Pd(II) complex **A**. Subsequently, follows transmetalation step with the appropriate boronic acid, in which R group replaces the halide anion on the palladium complex forming a new complex **B**. Finally, reductive elimination gives the final 2,4,6,7-tetrasubstituted-2*H*-pyrazolo[4,3-*c*]pyridine **C**, regenerates the palladium catalyst, and the catalytic cycle begins again.



Scheme 3.7. Reaction mechanism of Suzuki-Miyaura cross-coupling.

The structures of compounds **11a-c** and **12a-e** were confirmed by NMR and IR spectroscopy, MS and HRMS spectrometry. The adequate decrease in mass proves iodine replacement with phenyl or methoxyphenyl substituent, with peaks in a positive mode of the mass spectra being at 391 and 377 for compounds **11a** and **12b**, respectively. In comparison to starting materials **9** and **10**, the ^{1}H NMR spectrum of **12a** has new aromatic signals, while **11a-c**, **12b-e** additionally have new methoxy group singlets with a typical chemical shift in a 3.42-3.90 ppm range.

The full analysis and determination of structures was done using different NMR methods. For example, signals of phenyl ring at 2-position (four protons at 7.48-7.54 ppm and 7.85-7.95 ppm) of 7-(3-methoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine **12c** were assigned using ^{1}H-^{15}N HMBC spectrum as explained above. Then, signals of phenyl ring at 7-position were determined using NOESY spectrum, where 3-methoxy group singlet had strong interactions with two protons at 6.80-6.88 and 7.03-7.07 ppm and weak interactions with two protons at 7.09-7.16 and 7.20-7.29 ppm. Subsequently, the HSQC spectrum was used to determine proton – carbon interactions (Fig. 3.5). H2BC experiment correlates carbons and protons that are two bonds away by using one-bond carbon-proton and proton-proton couplings. Thus, two protons at 7.85-7.95 ppm were assigned to N-Ph 2,6-H because they only had one interaction with 129.8 ppm signal in ^{13}C spectrum which was assigned to N-Ph C-3,5 as it had second interaction with one proton at 7.39-7.44 ppm appointed to N-Ph 4-H. The signals of 7-phenyl protons were assigned in a similar manner. Singlet at 7.03-7.07 ppm had no interaction, thus it was assigned to A. Protons at 6.80-6.88 ppm and 7.09-7.16 ppm both had one interaction with neighbouring carbons but because 6.80-6.88 ppm had strong NOESY interaction with methoxy group it was appointed to B, while proton at 7.09-7.16 ppm to D.

Lastly, 6-phenyl signals were determined. The two protons at 7.45-7.48 ppm were appointed to 2,6-H as it only had one interaction with carbon at 128.0 ppm. The four protons at 7.20-7.29 ppm were the most difficult to assign as it had three carbon-proton interactions in HSQC spectrum, namely, at 127.3, 128.0 and 129.1 ppm. One proton with carbon signal at 129.1 was already appointed to C because of carbon interaction with B protons. Thus, the rest of the protons were assigned to 6C-Ph, 3,4,5-H. However, because 6C-Ph, 2,6-H had interaction with carbon at 128.0 ppm, this signal was appointed to 6C-Ph, C-3,5. The remaining signal at 127.3 ppm was assigned to 6C-Ph, C-4. This completed determination of aromatic tertiary carbon signals with appropriate protons. The quarterly carbons were then assigned using HMBC spectrum.

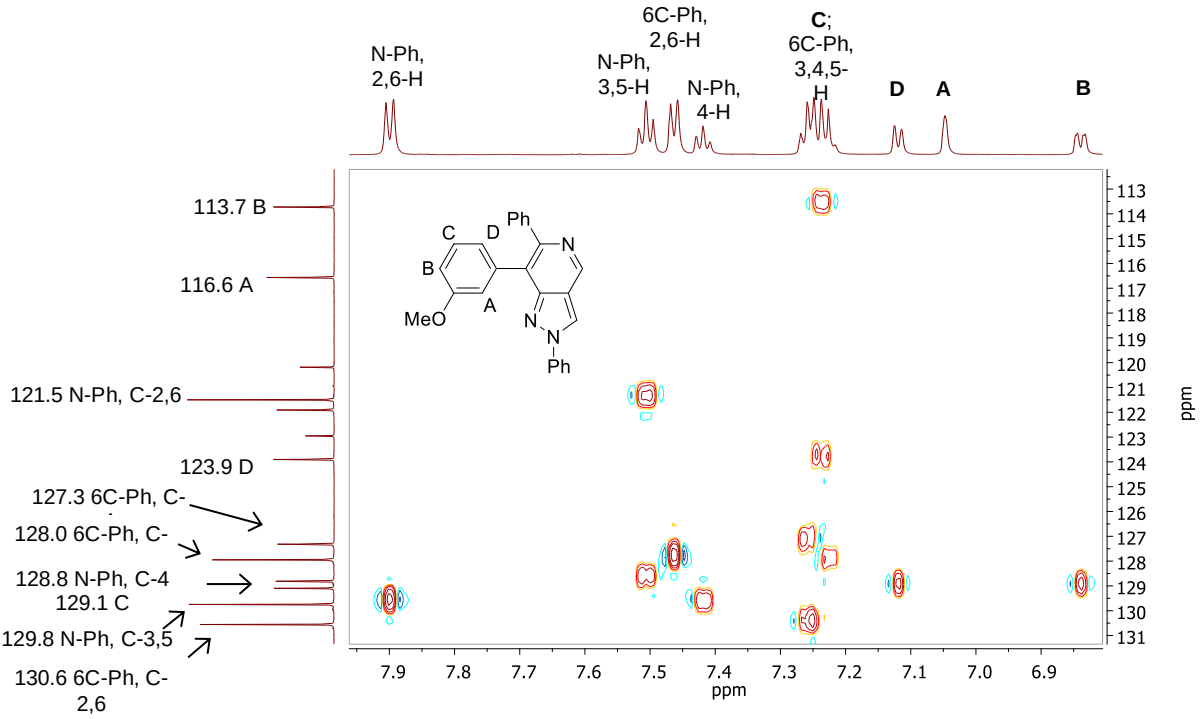
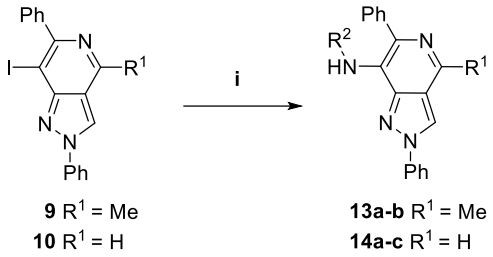


Fig. 3.5. Aromatic part of H2BC spectrum of compound **12c.**

3.3 7-Iodopyrazolo[4,3-*c*]pyridine as a substrate for Buchwald-Hartwig cross-coupling reactions

Subsequently, to introduce various amine groups to 7-position 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine **9** and 7-iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine **10** were used in palladium catalysed Buchwald-Hartwig cross-coupling reaction (Scheme 3.8) adopting reaction conditions described previously [82]. 7-Iodopyrazolo[4,3-*c*]pyridines **9** or **10** were treated with an appropriate aniline, using palladium acetate as a catalyst, 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl as a ligand and sodium *tert*-butoxide as a base in dioxane. Noteworthy, reactions were carried out under argon atmosphere and dry solvent due to the sensitivity of ligand

and catalyst to air and moisture. To reduce reaction time cross-couplings were performed under microwave irradiation and the compounds **13a-b** and **13a-c** (Table 3.2) were obtained in fair to excellent yields (48-96%).



9 R^1 = Me
10 R^1 = H

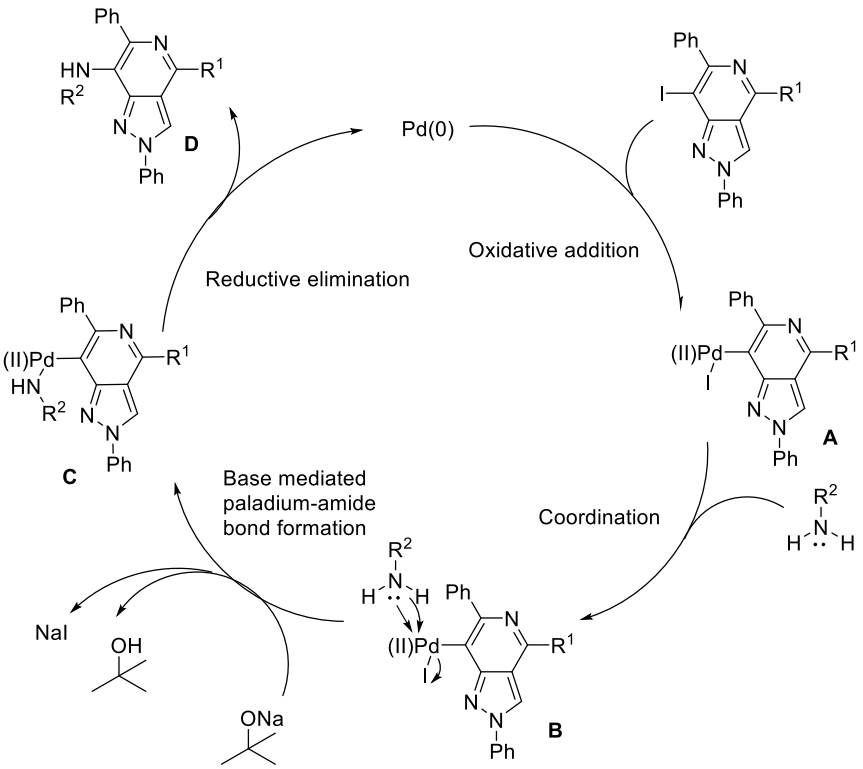
13a-b R^1 = Me
14a-c R^1 = H

Scheme 3.8. Synthesis of compounds **13a-b** and **14a-c**. Reagents and conditions: i: R^2NH_2, $Pd(OAc)_2$, SPhos, Na*t*BuO, dioxane, MW, 120 °C, 280 W, 1 h.

Table 3.2. Yields of new compounds **13a-b** and **14a-c**.

Compound	R^1	R^2	Yield, %
13a	Me	4-MeO-Ph	77
13b	Me	3,4-diMeO-Ph	60
14a	H	Ph	92
14b	H	4-MeO-Ph	48
14c	H	3,4-diMeO-Ph	86

The Buchwald-Hartwig amination was used to introduce various anilines at 7-position. The reaction mechanism (Scheme 3.9) begins by oxidative addition of the aryl halide **9** or **10** to the palladium which is followed by coordination of the amine to the palladium. Then, the strong base abstracts a proton from the amine, forming an amide, which attacks the palladium and replaces the halide. Then reductive elimination produces the final product and regenerates the catalyst.



Scheme 3.9. Reaction mechanism of Buchwald-Hartwig cross-coupling.

The structures of compounds **13a-b** and **14a-c** were confirmed by NMR and IR spectroscopy, MS and HRMS spectrometry. The formation of amine can be quickly confirmed by IR spectrum in which peaks of amine group can be found at 3375, 3299, 3376 cm^{-1} for compounds **13**, **14a** and **14b**, respectively. Also, in comparison to starting materials **8** and **10**, the ^{1}H NMR spectra of all new derivatives have a broad amine group singlet, new aromatic signals, while **13b** and **14b-c** additionally have new methoxy group singlets. Figure 3.6 shows the ^{1}H NMR spectrum of *N*-4-methoxyphenyl-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amine **13**. Presence of singlet signals at 3.74 ppm which was assigned to MeO group and 5.89 ppm assigned to NH group, as well as multiplets at 6.66-6.75 and 6.76-6.83 ppm, assigned to the new phenyl group, proves the formation of desired product.

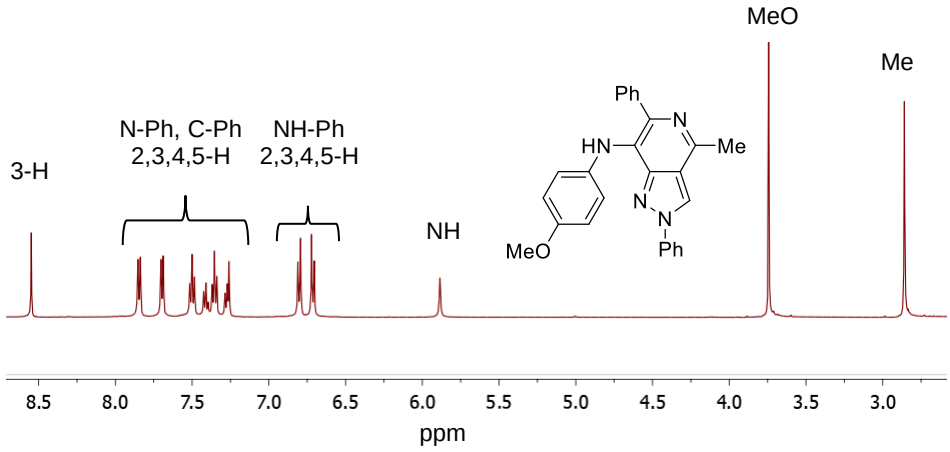


Fig. 3.6. ^{1}H NMR spectrum of compound **13**.

Moreover, ^{1}H-^{15}N HMBC spectra were used to confirm structures of new amines and to determine which signals belong to which phenyl group (Fig. 3.7). For example, compound **14a** has similar signals for pyrazolopyridine core nitrogens as substrate **10**. The signal at -78.1 ppm can be assigned to pyridine N-5 atom because of the strong interaction with 4-H. As described previously, N-2 atom at -147.3 ppm interacts with phenyl 2,3,5,6 proton multiplets and 3-H singlet. Also, the emergence of new signal at -304.0 ppm proves the substitution of iodine with amine. The strongest interaction can be observed with singlet at 6.17 ppm indicating the signal of NH group proton. Other interactions can be assigned to phenyl group protons 2,4,6 and 3,5 with signals at 6.81-6.88 and 7.10-7.16 ppm, respectively.

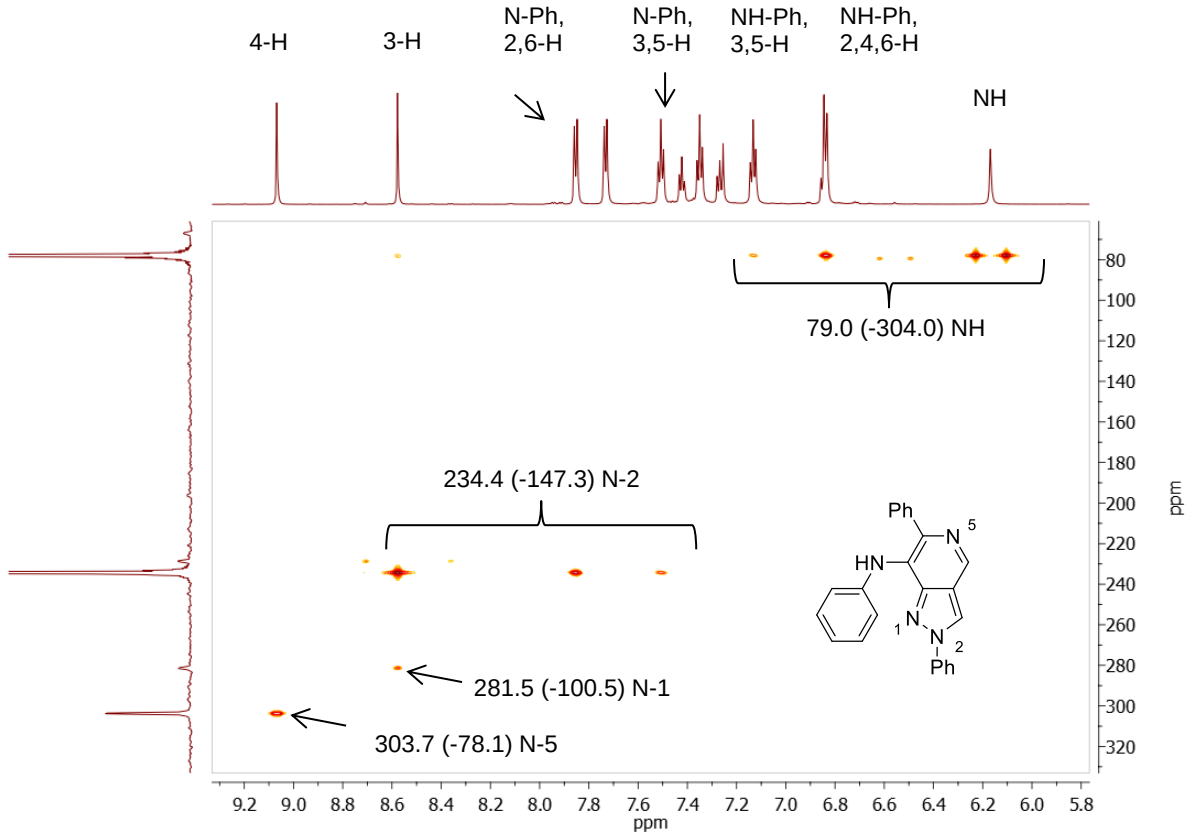


Fig. 3.7. ^{1}H-^{15}N HMBC spectrum of compound **14a**.

3.4 Optical properties of 2,4,6,7-tetrasubstituted pyrazolo[4,3-*c*]pyridines

It is known that annealed pyrazole systems can exhibit fluorescence and thus can be applied in light-emitting diodes. Such examples are dipyrazolo[3,4-*b*:3′,4′-*d*]pyridines [83], pyrazole[3,4-*b*]thieno[2,3-*e*]pyridine derivatives [84], pyrazolo[4′,3′:3,4]pyrido[1,2-*a*]benzimidazoles [85] and others. Thus, upon noticing that the new pyrazalo[3,4-*c*]pyridine derivatives are fluorescing under UV light, investigation of their optical properties was carried out. This included measuring UV absorption, calculating extinction coefficient, measuring fluorescence, calculating Stokes shifts and quantum yields (Table 3.3). The measurements and calculations were done by doctoral student Aurimas Bieliauskas, Kaunas University of Tehchnology, Department of Organic Chemistry.

Table 3.3. The absorption and fluorescence of new and previously synthesised [67, 86] pyrazolo[4,3-*c*]pyridine derivatives solutions in THF.

Compound	**Absorbtion, λ_{abs} (nm)**	**Extinction coefficient $\varepsilon \times 10^3$ ($dm^3mol^{-1}cm^{-1}$)**	**Emision λ_{em} (nm) ($\lambda_{ex\ 350nm}$)**	**Stokes shift (nm)**	**Quantum yield* Φ_f (%)**
11a	263 310	38.05 17.48	450	140	48.67
11b	261 310	29.68 15.11	449	139	30.03
11c	266 316 356	30.31 13.14 7.28	481	125	62.84
11d [86]	261 311	36.46 17.34	449	138	17.65
11e [86]	265 317 349	31.58 14.93 9.31	466	117	72.21
12a	260 312	30.38 13.23	442	130	18.91
12b	263 310 351	31.95 15.80 8.07	461	151	71.77
12c	260 311	35.55 16.29	437	126	53.21
12d	261 311	34.67 16.86	447	136	26.15
12e	267 311 357	32.04 16.09 7.74	478	167	56.84
13a	254 288 394	29.52 31.36 4.83	486 590	196	0.41
13b	256 285 396	28.38 30.0 4.64	455	59	0.22
13c [86]	253 285 382	27.03 30.89 5.09	506	124	38.09
14a	249 282 383	25.42 30.75 5.12	506	123	20.47
14b	252 284 393	26.56 29.22 4.55	483	90	0.15
14c	254 284 397	29.25 32.94 5.18	464	67	<0.1
15 [67]	265 319	37.26 16.60	373 385	66	11.33

Compound	Absorbtion, λ_{abs} (nm)	Extinction coefficient $\varepsilon \times 10^3$ ($dm^3mol^{-1}cm^{-1}$)	Emision λ_{em} (nm) ($\lambda_{ex\ 350nm}$)	Stokes shift (nm)	Quantum yield* Φ_f (%)
16 [67]	266 318	33.45 14.51	370 382	64	6.18

*average of 3 measurements

The fluorescence emission profile of all newly synthesized fused heterocycles was obtained in THF and revealed a strong influence of the molecular structure on the character of the fluorescence emission. In general, the fluorescence maximums of compounds **11-16** were observed at the wavelength interval of 370 – 590 nm. Attaching a substituent at 7- position had a significant effect on fluorescence maximum. Namely, compounds **15** and **16** were in ultraviolet wavelength range, while compounds **11a**, **11c**, **11e**, **12b**, **12e**, **13a**, **13b**, **14c**, **14b** emitted blue, **11b**, **11d**, **12a**, **12d** – violet, **13c**, **14a** – green light [87].

As shown in figure 3.8, bathochromic shift was observed for 7-aryl-2,6-diphenyl-4-methyl-2*H*-pyrazolo[4,3-*c*]pyridines **11a-e** in comparison to compound **15**, lacking a substituent at 7-position. Solutions of compounds bearing phenyl, 2-methoxy-phenyl and 3-methoxy-phenyl moieties had a similar emission maximum at 449 nm (**11b** and **11d**) and 450 nm (**11a**), while 4-methoxy-phenyl moiety (**11e**) led to emission maximum of 466 nm. Attachment of another electron donating methyl group caused the biggest bathochromic shift, thus compound bearing 3,4-dimethoxy-phenyl substituent exhibited emission maximum at 481 nm.

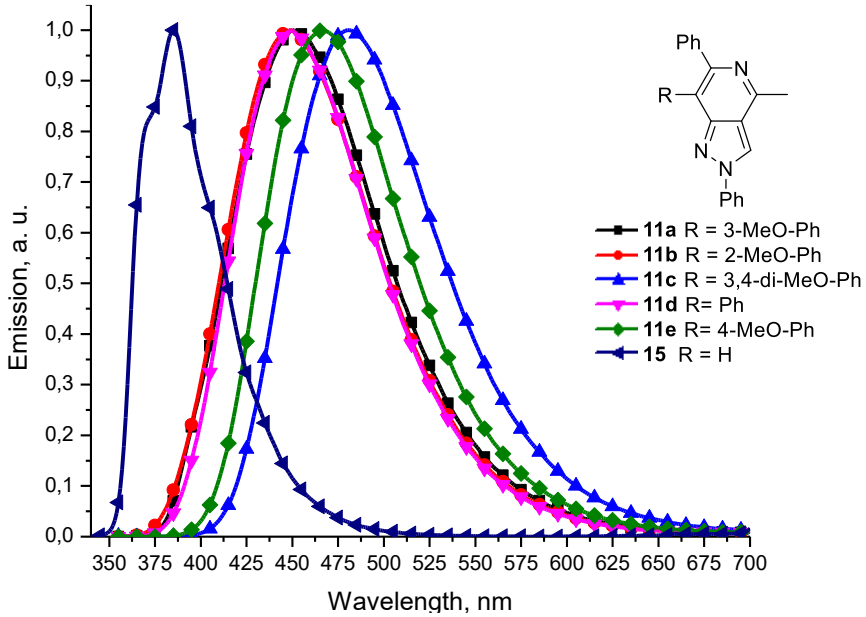


Fig. 3.8. The fluorescence emission maxima of compounds **11a-e** and **15**.

Substitution of proton at 7-position with an aryl group also led to better quantum yields. For 2,6-diphenyl-4-methyl-2*H*-pyrazolo[4,3-*c*]pyridine (**15**), the observed quantum yield value was only 11.33%, whereas attaching a phenyl group at 7-position increased quantum yield value to 17.65%. Even better results were observed with methoxy-phenyl substituents, as the quantum yield values were in the range of 30.03–72.21%. Noteworthy, the position of methoxy group on the phenyl ring had a significant influence on the value of the quantum yield. The derivative with 2-methoxy-phenyl group **11b** possessed the lowest value of 30.03%, while derivative with 4-methoxy-phenyl group **11e** exhibited the highest 72.21% quantum yield.

As anticipated, the 7-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines **12a-e** showed similar relationship between the optical properties and the structure. Only a moderate decline in their optical properties could be observed in contract to their methyl substituent at 4-position bearing counterparts **11**. Namely, the emission maximums of compound **12a-e** and **16** shown in table 3.9 are in the range of 370–461 nm. They displayed a slight hypsochromic effect when comparing to 7-aryl-2,6-diphenyl-4-methyl-2*H*-pyrazolo[4,3-*c*]pyridines. This could be explained by the loss of electron donating methyl group which caused emission maximum shifts to shorter wavelengths. Same tendency was discovered for the values of quantum yields. The best value was observed for compound **12b** with 4-methoxy-phenyl substituent at 7-position of 71.77% which was a little lower than for corresponding derivative **11e** with methyl substituent at 4-position.

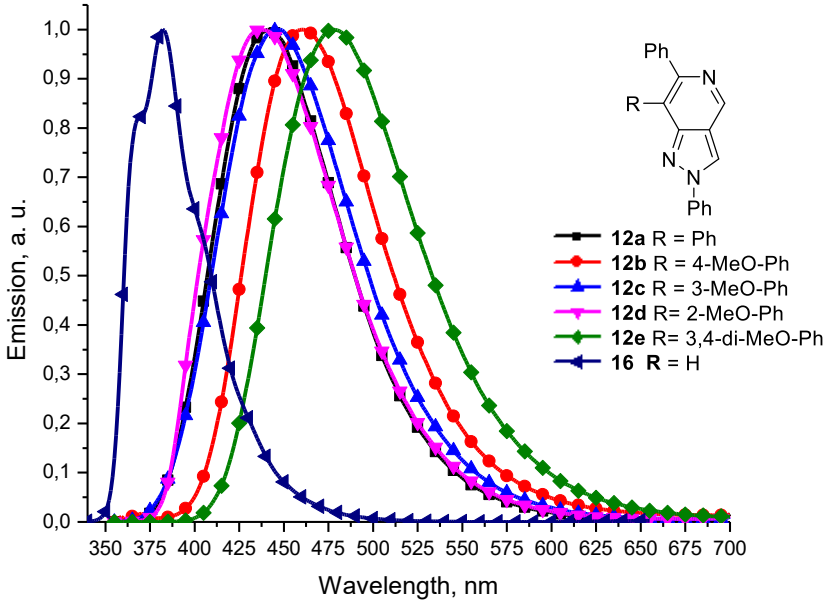


Fig. 3.9. The fluorescence emission maximums of compounds **12a-e** and **16**.

The addition of amine group to the structure of pyrazolo[4,3-*c*]pyridines caused an even bigger bathochromic shift to longer wavelengths (Figure 3.10). The fluorescence maximums of compounds **13-14** were observed at the wavelength interval of 455–590 nm. Unfortunately, the

values of quantum yields were not as good as for other derivatives as they mostly did not reach 1%, with the exception of compounds **13c** and **14a** bearing an aniline moiety, for which quantum yields reached 38.09 and 20.47 %, respectively.

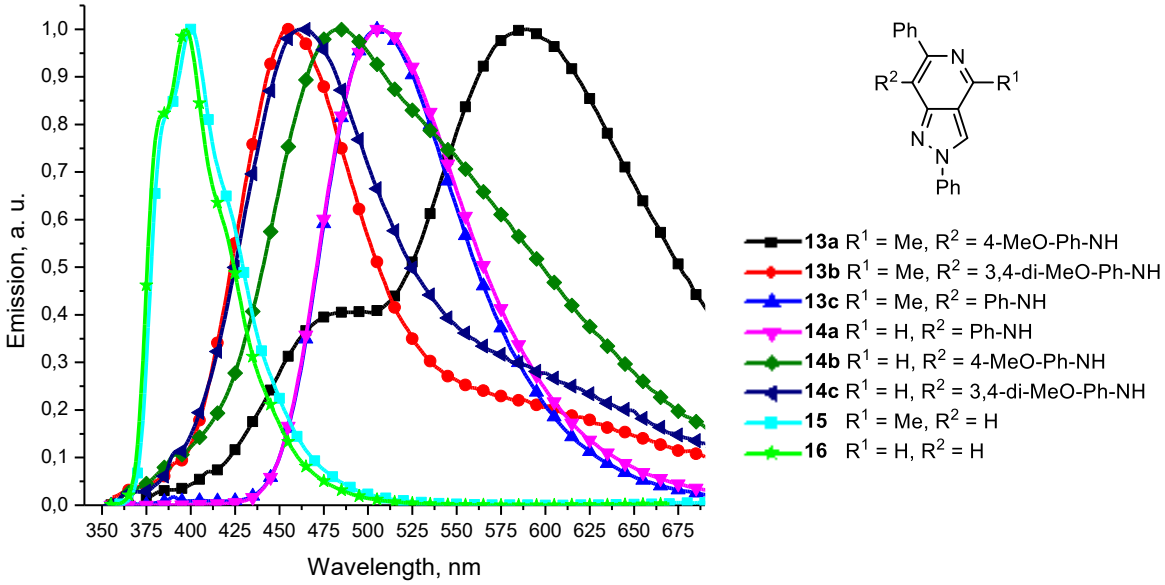


Fig. 3.10. The fluorescence emission maximums of compounds **13 - 16**.

Stokes shift, i.e. the distance between the excitation and emission wavelengths, is an important key aspect in the detection of the emitted fluorescence in biological applications. Fluorophores with large Stokes shifts are easy to distinguish because of the large separation between the excitation and emission wavelengths. Most of the compounds exhibited high Stokes shift in the range of 117–196 nm and only pyrazolo[4,3-*c*]pyrini-7-amines **14b-c**, **13b** and pyrazolo[4,3-*c*]pyridines without substituent at 7-position had Stokes shifts lower than 100 nm.

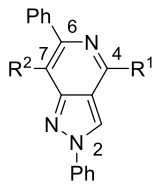
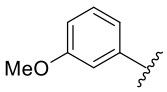
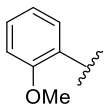
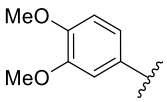
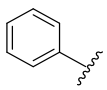
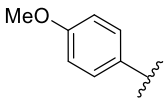
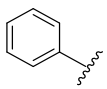
3.5 Biological activity of 2,4,6,7-tetrasubstituted pyrazolo[4,3-*c*]pyridines

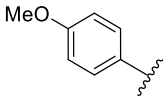
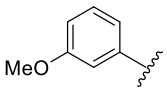
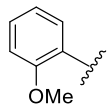
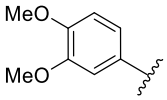
Prepared compounds **11-14** were evaluated for their cytotoxicity against two human cancer cell lines: K562 (chronic myeloid leukemia cells) and MCF-7 (breast cancer cells). The experiments were carried out in the group of Dr. Vladimír Kryštof (Laboratory of growth regulators, Faculty of Science, Palacký University in Olomouc, Czech Republic) using a standard Calcein AM method [88]. In general, most tested compounds exhibited moderate cytotoxicity, with GI_{50} values, i.e. concentration of the anti-cancer drug that inhibits the proliferation of cancer cells by 50%, in the micromolar range (Tables 3.4 and 3.5). Type of the substituent at the 7-position proved to play an important role in the cytotoxicity of the compounds, i.e., compounds **11-12**, resulting from Suzuki cross-coupling reactions and bearing various aryl substituents at the 7-position appeared to be generally more cytotoxic (Table 3.4) than compounds **13-14**, resulting from Buchwald-Hartwig cross-coupling and bearing various anilines in the same 7-position (Table 3.5). Moreover, in

agreement with the recent data on the cytotoxicity of 2,4,6-trisubstituted-2*H*-pyrazolo[4,3-*c*]pyridine [67], which pointed out to negative correlation between the increasing size of the substituents at the 4-position and gradual decrease in the cytotoxicity of the compounds, the same tendency was noticed for 2,4,6,7-tetrasubstituted-2*H*-pyrazolo[4,3-*c*]pyridines **11-14** prepared in this study. Namely, compounds lacking a substituent at the 4-position proved to be more cytotoxic than their 4-methyl counterparts (Table 3.4).

Among 7-aryl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridines **12a-e** (Table 3.4), mono- and di-methoxy-substituted derivatives **12b**, **12c**, **12e** and **12d** exibited good cytotoxicity, with GI_{50} values reaching 2.3-4.5 μM for K562 cells, while GI_{50} value for derivative **12a**, lacking methoxy-substituents on the phenyl ring at the 7-position, only reached 10.2 μM. Among 7-aryl-2,6-diphenyl-4-methyl-2*H*-pyrazolo[4,3-*c*]pyridines **11a-e** only derivative **11c**, bearing 3,4-dimethoxy-phenyl substituent at 7-position exhibited 8.6 μM GI_{50} value for K562 cells. Noteworthy, 7-aryl-2*H*-pyrazolo[4,3-*c*]pyridines were less active against MCF-7 cells. The most active derivative **12e**, bearing 3,4-dimethoxy-phenyl substituent at 7-position and a proton at 4-position, reached GI_{50} value of 9.4 μM.

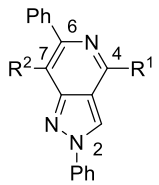
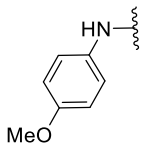
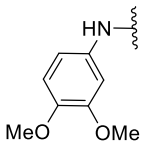
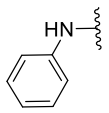
Table 3.4. *In vitro* cytotoxicity of previously [67, 86] and newly synthesized 2*H*-pyrazolo[4,3-*c*]pyridine derivatives *via* Suzuki-Miyaura coupling **9-12** and **15-18**.

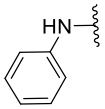
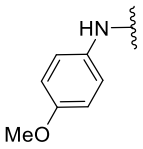
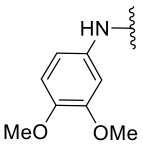
General structure	Compound	R^1	R^2	GI_{50}, μM	
				K562	MCF-7
	**9**	Me	I	16	32
	10	H	I	12.1	22.1
	11a	Me		41	89
	11b	Me		>100	>100
	11c	Me		8.6	18.2
	11d [86]	Me		>50	>50
	11e [86]	Me		17	18
	12a	H		10.2	>12.5

General structure	Compound	R^1	R^2	GI_{50}, μM	
				K562	MCF-7
	12b	H		2.3	>12.5
	12c	H		4	17.5
	12d	H		4.5	17.5
	12e	H		3.9	9.4
	15 [67]	Me	H	6.3	11.0
	16 [67]	H	H	3.4	4.8

Contrary to anticipation, incorporation of amine group resulted in decrease of biological activity (Table 3.5). Among *N*-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines **13a-c**, the most potent compound was derivative **13c** with aminophenyl moiety, which exhibited moderate activity against K562 and MCF-7 cells with GI_{50} values of 25 and 31 μM, respectively. Other derivatives **13a-b** with mono- and di-methoxy-substituted phenyl moieties were mostly inactive. Among *N*-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines **14a-c**, the best results were obtained from derivative **14c**, bearing *N*-3,4-dimethoxy-phenyl-amine substituent at 7-position, with moderate GI_{50} value of 30.5 μM against K562 cells.

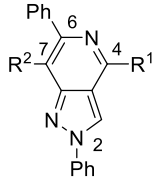
Table 3.5. *In vitro* cytotoxicity of previously [67, 86] and newly synthesized 2*H*-pyrazolo[4,3-*c*]pyridine derivatives *via* Buchwald-Hartwig coupling **13-16**.

General structure	Compound	R^1	R^2	GI_{50}, μM	
				K562	MCF-7
	**13a**	Me		63	>100
	13b	Me		>100	>100
	13c [86]	Me		25	31

General structure	Compound	R^1	R^2	GI_{50}, μM	
				K562	MCF-7
	14a	H		>12.5	>12.5
	14b	H		100	33
	14c	H		30.5	>50
	15 [67]	Me	H	6.3	11.0
	16 [67]	H	H	3.4	4.8

Interestingly, derivatives with iodine substituent at 7- positions **7** and **8** in most cases were more active than derivatives obtained after Buchwald-Hartwig cross-coupling reactions (Table 3.6). However, iodinated compounds are not common in medicinal chemistry as only 1 % of all FDA approved halogenated drugs have iodine mostly because of instability of C-I bond [89]. Nevertheless, because of good anticancer activity of iodopyrazolopyridines, attempts will be made to substitute iodine with an ethynyl moiety, which was proven to be a suitable bioisostere for iodine [90]. Also fluoride could be introduced in 4- or 7-positions as hydrogen bioisostere because derivatives without substituents at 4- or 7-positions were mostly more active than compounds with different substituents. The substitution of hydrogen by fluorine is one of the more commonly employed monovalent isosteric replacements. The pharmacological differences can be attributed to the influence of the electron-withdrawing effect that the fluorine substitution causes on the interaction with either a biological receptor or enzyme, as well as its effect on the metabolic fate of the drug [91].

Table 3.6. *In vitro* cytotoxicity of previously [67, 86] and newly synthesized 2*H*-pyrazolo[4,3-*c*]pyridine derivatives **9-10** and **15-16**.

General structure	Compound	R^1	R^2	GI_{50}, μM	
				K562	MCF-7
	**9**	Me	I	16	32
	10	H	I	12.1	22.1
	15 [67]	Me	H	6.3	11.0
	16 [67]	H	H	3.4	4.8

Moreover, it was found out that 2,4,6,7-tetrasubstituted pyrazolo[4,3-c]pyridines exhibit strong fluorescence in cancer cells *in vitro* as well. As shown in figure 3.11, the intensity of 7-(3,4-dimethoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine **12e** fluorescence is concentration-dependent. The fluorescence of pyrazolopyridine derivatives could be useful when investigating the mechanism of cellular action.

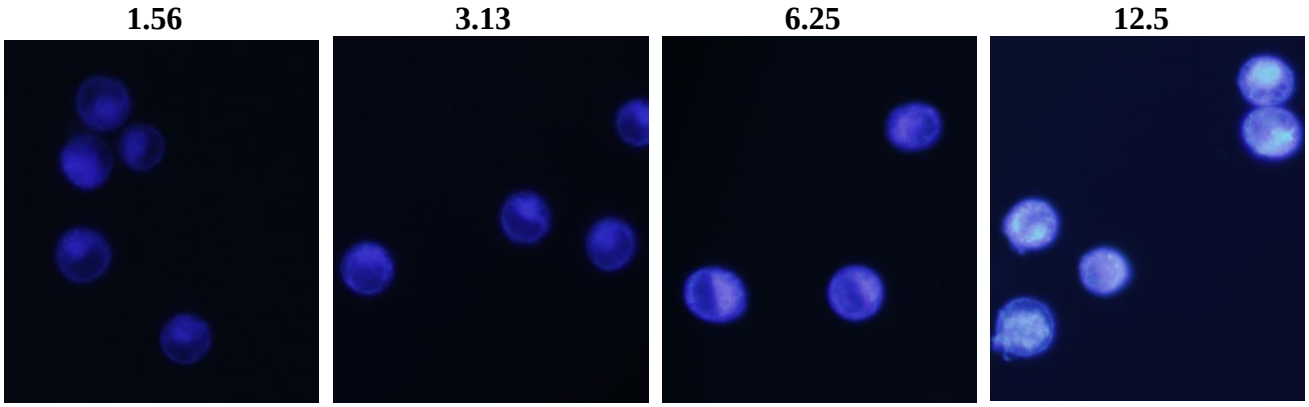


Fig. 3.11. Intensity of compound **12e** fluorescence (μM/24 h) in K562 cancer cells.

The biological evaluation of 2,4,6,7-tetrasubstituted pyrazolo[4,3-*c*]pyridines revealed that methyl substituent at 3-position and amine group at 7-position caused decline in cytotoxicity, as compared to **14** and **15**. Among all the new derivatives the most active compound **12b** bearing 4-methoxy-phenyl substituent at 7-position and a proton at 3-position exhibited high potency with GI_{50} value of 2.3 μM. Other 2,6-diphenylpyrazolo[4,3-*c*]pyridine derivatives with methoxyphenyl moieties at 7-position also showed promising results with GI_{50} values of 3.9-4.5 μM. To fully understand the mode of action of these compounds, further cell-cycle investigation to gain necessary preliminary information for further studies is needed. In a pursuit of compounds with better optical properties and lower cytotoxic values, the library of 2,4,6,7-tetrasubstituted pyrazolo[4,3-*c*]pyridines could be expanded by varying di- and tri- methoxy-phenyl, trifluormethyl and trifluoromethoxy-phenyl substituents.

THE MAIN RESULTS AND CONCLUSIONS

1. 7-Iodo-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine and 7-iodo-4-methyl-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine can be obtained from corresponding azide-alkynes *via* electrophilic cyclization reaction.

2. 7-Aryl-2*H*-pyrazolo[4,3-*c*]pyridines and *N*-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines can be formed from 7-iodo-2*H*-pyrazolo[4,3-*c*]pyridines *via* palladium catalysed Suzuki-Miyaura and Buchwald-Hartwig cross-coupling reactions, respectively. The reactions proceed quickly under microwave irradiation.

3. The fluorescence emission maximum of the new derivatives is in the range of 437-590 nm. 7-Aryl-2*H*-pyrazolo[4,3-*c*]pyridines exhibit better quantum yields while *N*-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines have fluorescence emission maximums in the longer wavelengths. Overall, 7-(4-methoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine shows the best result with quantum yield value of 71.77%.

4. The new derivatives are more potent against myelogenous leukemia cells than breast cancer cells. 7-Aryl-2*H*-pyrazolo[4,3-*c*]pyridines are generally more cytotoxic than 7-iodo-2*H*-pyrazolo[4,3-*c*]pyridines while *N*-2*H*-pyrazolo[4,3-*c*]pyridin-7-amines are the least cytotoxic. Moreover, compounds without a substituent at the 4-position are more potent than their 4-methyl counterparts. Overall, 7-(4-methoxyphenyl)-2,6-diphenyl-2*H*-pyrazolo[4,3-*c*]pyridine is the most active compound with GI_{50} value of 2.3 μM against myelogenous leukemia cells.

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