KAUNAS UNIVERSITY OF TECHNOLOGY

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SYNTHESIS AND INVESTIGATION OF NOVEL FUNCTIONALIZED PYRAZOLE OR INDOLE RING CONTAINING HETEROCYCLIC COMPOUNDS

Doctoral dissertation
Natural sciences, Chemistry (N 003)

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KAUNO TECHNOLOGIJOS UNIVERSITETAS

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NAUJŲ FUNKCIONALIZUOTŲ PIRAZOLO AR INDOLO ŽIEDĄ TURINČIŲ HETEROCIKLINIŲ JUNGINIŲ SINTEZĖ IR TYRIMAS

Daktaro disertacija
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CONTENTS

List of Abbreviations and Physical Units

1,1-ADEQUATE	correlation between protonated and non-protonated carbons
^{13}C NMR	carbon nuclear magnetic resonance
^{1}H NMR	proton nuclear magnetic resonance
^{15}N NMR	nitrogen nuclear magnetic resonance
ν	wavenumber
ACN	acetonitrile
Ac_2O	acetic anhydride
AcOH	acetic acid
Boc	*tert*-butoxycarbonyl protecting group
BSO	L-buthionine sulfoximine
$CDCl_3$	deuterated chloroform
CDI	1,1-carbonyldiimidazole
d	doublet
dd	doublet of doublets
DCM	dichloromethane
DIAD	diisopropyl azodicarboxylate
DMAC	*N*,*N*-dimethylacetamide
DMAP	4-(dimethylamino)pyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et	ethyl
EtOH	ethanol
FA	Friedreich's ataxia
Fe(III)Cl-Pc	iron (III) phthalocyanine chloride
HMBS	heteronuclear multiple bond coherence
HPLC	high pressure liquid chromatography
HRMS	high-resolution mass spectrometry
HSQC	heteronuclear single quantum coherence spectroscopy
Hz	hertz, frequency unit
IBD	iodobenzenediacetate
IR	infrared spectroscopy
J	coupling constant
LA	*R*-lipoic acid
LC-MS	liquid chromatography – mass spectrometry

LiHMDS	lithium bis(trimethylsilyl)amide
m	multiplet
mp	melting point
Me	methyl
MeOH	methanol
MW	microwave irradiation
NBS	*N*-bromosuccinimide
n.d.	not determined
NOESY	nuclear Overhauser effect spectroscopy
OMs	mesylate
Ph	phenyl
ppm	parts per million
rt	room temperature
R_f	retention factor
ROS	reactive oxygen species
s	singlet
SEM	standard error of the mean
t	triplet
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
Ts	tosyl
UAAs	unnatural amino acids

INTRODUCTION

Over the past several decades, bioactive peptides have been attracting scientific interest due to their ability to interact with receptors or inhibit protein–protein interactions [1]. They can fill the huge target receptor's area which cannot be filled by small molecule drugs [2]. However, most peptides are denoted by low lipophilicity, high molecular weights and charged functional groups; as a result, they are unable to cross the intestinal epithelial barrier [3]. For these reasons, in order to improve the properties of the native peptides, such as their biological activity and metabolic stability, unnatural amino acids (UAAs) are usually used as rigidifying elements [1]. Moreover, combining peptides with other types of natural bioactive or synthetic molecules allows to cover a broader chemical space. This is particularly relevant for drug discovery and development as it allows combining the best of the two worlds, such as the higher target affinity of peptides, and the better membrane permeability of small nonpolar synthetic compounds. These new classes of protein-based drugs are generally referred to as 'biologics' and include such molecules as insulin, growth factors, and engineered antibodies [4].

Unnatural amino acids are important building blocks in the pharmaceutical industry. UAAs exhibit biological activity not only as free acids, but they can also be incorporated into biologically active linear or cyclic peptides. UAAs can be used to analyze the protein structure and dynamics, to study protein interactions, or to modulate the activity of proteins in living cells. UAAs can be obtained by modifying natural amino acids or related compounds [5]. Almost all the structural features of UAAs correspond to the extended Lipinski's rules, including the molecular weight (<500 Da), the number of hydrogen bond acceptors, and the number of rotatable bonds [6].

There is a limited number of synthetic commercially available amino acids, especially heterocyclic ones, which can be applied as building blocks for the synthesis of more complex functional molecules. Moreover, all the obtained libraries become similar if the same commercially available building block collections are used to generate them [6]. In order to obtain a variety of building-blocks with the *N*-heterocycle core, cyclization reactions with hydrazines or their derivatives become important.

Finally, it is of importance to mention that the obtained organic molecules with pharmacophoric heterocyclic rings could be used not only with the objective to generate peptides, but they also may possess interesting biological properties themselves.

The aim of this work:

To prepare a library of new building blocks of amino acids for the synthesis of peptides and to obtain biologically active 1,3,4-oxadiazole derivatives by employing hydrazine cyclization and alkylation reactions.

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

1. To synthesize novel 5-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1*H*-pyrazole-3-carboxylates and evaluate their activity in *N*-alkylation reactions.

2. To synthesize novel amino acid-like building blocks with the pyrazole core and an azetidine unit.

3. To synthesize novel amino acid-like building blocks with the pyrazole core and a piperidine ring.

4. To synthesize pyrazolo[4,3-*c*]pyridine and evaluate its activity in the *N*-alkylation reaction.

5. To synthesize 5-[(1*H*-indol-3-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thiones and evaluate their activity in *S*-alkylation reaction.

6. To discuss the antioxidative properties of the newly developed 2-[(1*H*-indol-3-yl)methyl]-5-(alkylthio)-1,3,4-oxadiazoles.

Scientific novelty:

An effective method for convenient synthesis of new amino acid-like building blocks from easily accessible starting materials has been developed. 5-[1-(*tert*-Butoxycarbonyl)azetidin-3-yl]-1*H*-pyrazole-3-carboxylate was synthesized from commercially available *N*-Boc-protected azetidine 3-carboxylic acid *via* a 5-step synthetic approach. This pyrazole was easily *N*-alkylated with various types of alkyl halogenides with the objective to obtain two separable regioisomers suitable for the building-block library. Easily accessible *N*-Boc-3-iodoazetidine was used as the *N*-alkylating agent in the reaction with commercially available pyrazole and indazole carboxylates so that to extend the variety of synthetic amino acids. Pyrazole and the piperidine ring were easily connected by the *N*-alkylation reaction with mesylate activated nucleophile. Pyrazolo[4,3-*c*]pyridine was synthesized from commercially available *tert*-butyl 4-oxopiperidine-1-carboxylate by using lithium bis(trimethylsilyl)amide as a strong non-nucleophilic base. Later, pyrazolo[4,3-*c*]pyridine was easily *N*-alkylated with various types of alkyl halogenides to obtain two separable regioisomers suitable for the building-block library. The carbonyl group of two selected indole-3-acetic acids was transformed into a 1,3,4-oxadiazole ring. The prepared 1,3,4-oxadiazoles possess antioxidant properties at low concentrations. The structures of the novel compounds and the identification of the formed regioisomers was reliably confirmed by methods of advanced NMR spectroscopy, namely, by the application of the ^{1}H,^{1}H-NOESY, ^{1}H,^{13}C-HMBC and ^{1}H,^{15}N-HMBC experiments.

Main statements to be defended:

1. 5-[1-(*tert*-Butoxycarbonyl)azetidin-3-yl]-1*H*-pyrazole-3-carboxylate and 5-(*tert*-butyl) 3-ethyl 1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate are convenient starting materials for the synthesis of novel unnatural amino acids.

2. *N*-Boc-3-Iodoazetidine and *N*-Boc-4-[(methylsulfonyl)oxy]piperidine are suitable for *N*-alkylation reactions.

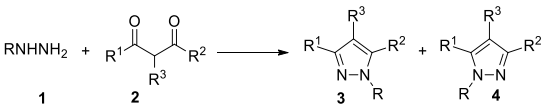
3. Treatment of 5-[(1*H*-indol-3-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thiones with alkyl halides affords biologically active 2-[(1*H*-indol-3-yl)methyl]-5-(alkylthio)-1,3,4-oxadiazoles.

1. LITERATURE REVIEW

The objective of this literature review is to analyze the cyclization reactions of hydrazines and hydrazides to provide pyrazole, indole, oxadiazole and other derivatives.

1.1. Hydrazines cyclization in Knorr pyrazole or pyrazolone synthesis

Hydrazine as well as hydrazine derivatives are one of the components in Knorr pyrazole synthesis which was first reported in 1883 by L. Knorr [7]. The traditional mechanism of the Knorr reaction between hydrazine or substituted hydrazine **1** with 1,3-dicarbonyl compounds **2** provide a pyrazole or a pyrazolone ring system (*Scheme 1.1*) [8].



R = H, Alkyl, Aryl, Het-aryl, Acyl, *etc.*

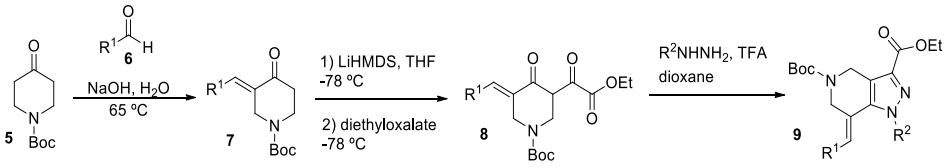
Scheme 1.1 Synthesis of pyrazoles **3** and **4**

Pyrazole derivatives are the most commonly used and tested heterocycles for their wide range of biological activities such as: anti-inflammatory [9], antitumor [10], antibacterial and analgesic properties [11]. There are some pharmaceuticals with the pyrazole core: cyclooxigenase-2 (COX-2) inhibitors celecoxib [12] and mavacoxib [11], cannabinoid receptor-1 (CB1) antagonist rimonabant [13].

1.1.1. Synthesis of pyrazole derivatives under acidic conditions

Knorr also described pyrazole synthesis where hydrazine, or its derivatives, and a 1,3-dicarbonyl compound were converted to pyrazole by using acid as a catalyst [14].

Zhu *et al.* reported that *N*-Boc-4-piperidone **5** reacted with aryl aldehyde **6** in aqueous sodium hydroxide solution to give alkene derivative **7** (*Scheme 1.2*). Treatment of compound **7** with lithium bis(trimethylsilyl)amide at -78 ºC followed by diethyl oxalate led to intermediate **8**. The condensation of compound **8** with aryl hydrazine was achieved in the presence of trifluoroacetic acid (TFA) in dioxane to give the desired products **9** [15].

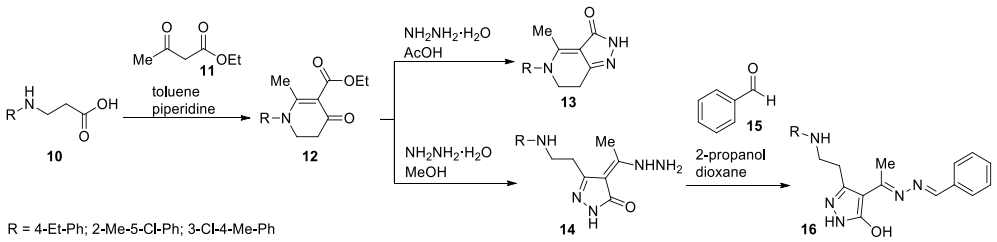


R^1 = 4-Cl-Ph; 4-F-Ph; 4-MeO-Ph
R^2 = 4-Cl-Ph; 2,4-Cl-Ph

Scheme 1.2 Synthesis of compound **9**

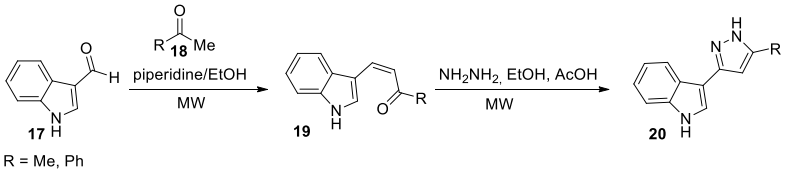
Sapijanskaitė and coauthors showed that the reaction of compound **12** with hydrazine monohydrate can be carried out in acetic acid or methanol (*Scheme 1.3*).

When the reaction was carried out in acetic acid, compound **13** was formed directly without the isolation of intermediate compound **14**. However, intermediate compound **14** was obtained when the reaction was carried out in methanol. After that, pyrazole **14** further reacted with aldehyde **15** to yield the desired product **16**. Intermediate compound **12** can be obtained after the condensation of *N*-aryl-*β*-alanines **10** with ethyl 3-oxobutanoate **11** in the presence of piperidine as a catalyst in toluene [16].



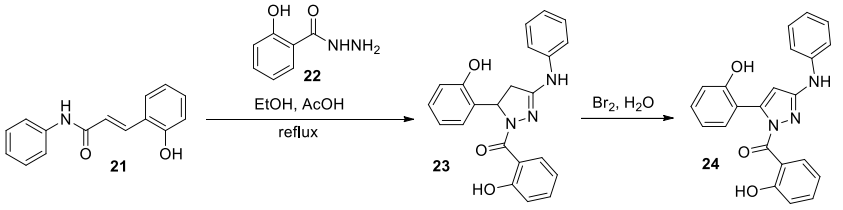
Scheme 1.3 Synthesis of compounds **13** and **16**

Deepak *et al.* presented a novel synthesis method of pyrazole **20**, where chalcone **19** and hydrazine hydrate were cyclized in ethanol and a few drops of acetic acid under microwave irradiation (*Scheme 1.4*). The intermediate compound chalcone **19** was obtained by the condensation reaction of indole aldehyde **17** and ketone **18** in a mixture of piperidine and ethanol [17].



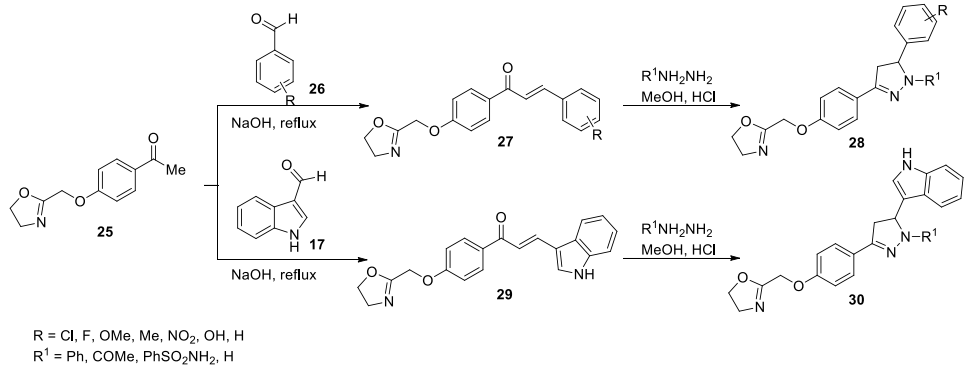
Scheme 1.4 Synthesis of indole derivative **20**

Ghareb and team demonstrated that 2-hydroxybenzohydrazide **22** was condensed with chalcone **21** in a mixture of ethanol and acetic acid, under sonication, to give dihydro pyrazole derivative **23** (*Scheme 1.5*). The desired pyrazole **24** was obtained after the oxidation of pyrazole **23** with bromine water [18].



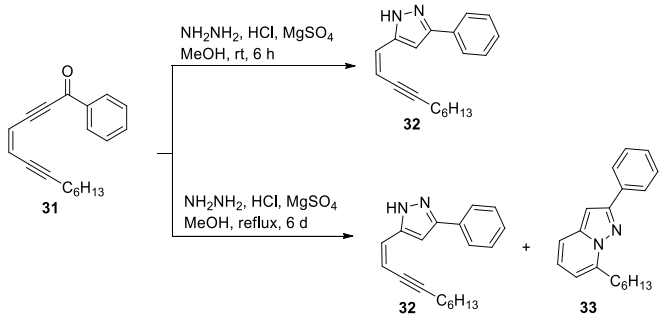
Scheme 1.5 Synthesis of pyrazole derivative **24**

Another method to synthesize pyrazole derivatives **28** and **30** is the cycloaddition of chalcone **27** and **29**, respectively, to hydrazine under hydrochloric acid medium (*Scheme 1.6*). Intermediate compounds **27** or **29** were obtained after the condensation of ketone **25** and aldehyde **26** or **17** under the basic medium [19].



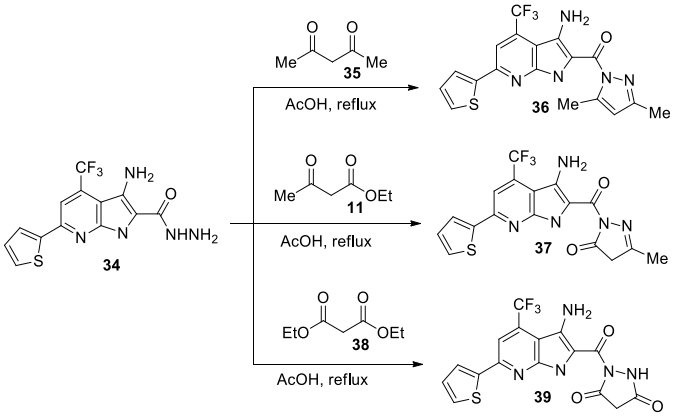
Scheme 1.6 Synthesis of pyrazole derivatives **28** and **30**

Moreover, Wu *et al.* showed that pyrazole **32** could be obtained from alkyne **31** (*Scheme 1.7*). The starting material **31** was treated with hydrazine hydrate under hydrogen chloride and methanol conditions. However, when the reaction mixture was refluxed for 6 days, two products **32** and **33** were isolated, accordingly, in 73% and 14% yields [20].



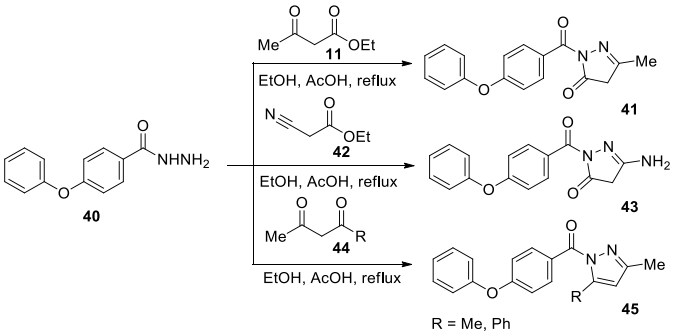
Scheme 1.7 Synthesis of pyrazole derivatives **32** and **33**

Rateb demonstrated that hydrazide **34** reacted with different diketones **35** and **11** in boiling glacial acetic acid to afford pyrazole analogues **36** and **37** (*Scheme 1.8*). Also, the treatment of the starting material **34** with diethylmalonate (**38**) under the same conditions gave pyrazolidine-3,5-dione **39** [21].



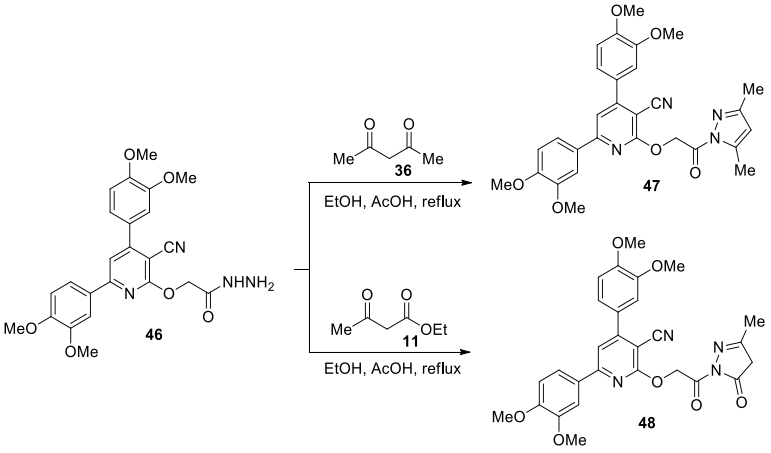
Scheme 1.8.Synthesis of pyrazole derivatives **36**, **37** and **39**

Tarek and coauthors demonstrated another set of hydrazide cyclocondensation reaction conditions (*Scheme 1.9*). Compound **40** was reacted with *β*-dicarbonyl derivatives **11** and **44** in ethanol when using the catalytic amount of acetic acid to yield the corresponding pyrazole derivatives **41** and **45**. When hydrazide **40** was reacted with ethyl cyanoacetate **42**, compound **43** was formed [22].



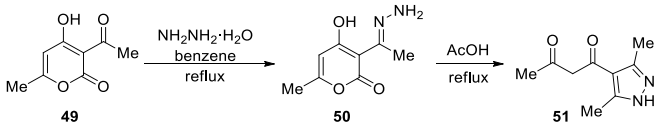
Scheme 1.9 Synthesis of compounds **41**, **43** and **45**

Furthermore, when the condensation reaction of hydrazide **46** with acetylacetone (**35**) or ethyl acetoacetate (**11**) was carried out in an ethanol and glacial acid mixture at a ratio of 2/1, 3,5-substituted pyrazole derivatives **47** or **48** were also obtained (*Scheme 1.10*) [23].



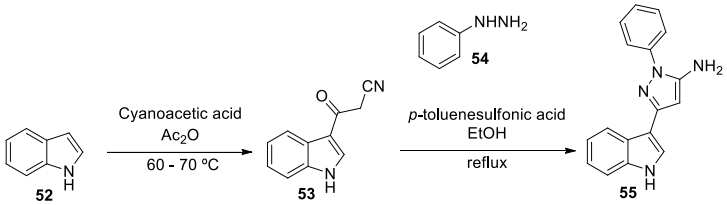
Scheme 1.10 Synthesis of pyrazole derivatives **47** and **48**

Badr and team reported that pyran-2-one **49** reacted with hydrazine hydrate in benzene to give the intermediate hydrazono derivative **50** (*Scheme 1.11*). Diketopyrazole **51** was obtained through the hydrazone rearrangement reaction under the acid medium [24].



Scheme 1.11 Synthesis of pyrazole **51**.

Anand *et al.* presented a novel synthesis method of pyrazole *via* 3-cyanoacetyl indole (**53**) (*Scheme 1.12*). The reaction was initiated when indole **52** was reacted with cyanoacetic acid in acetic anhydride at 60–70 ºC. The previously obtained 3-cyanoacetyl indole (**53**) was treated with phenylhydrazine **54** in the presence of *p*-toluenesulfonic acid to give the desired product **55** [25].



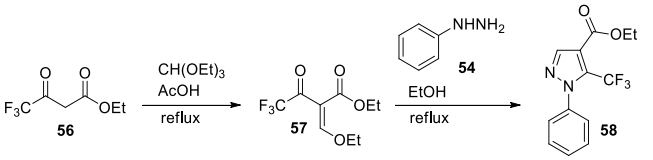
Scheme 1.12 Synthesis of pyrazole-indole derivative **55**

1-Aryl-3-indolyl-5-amino pyrazole **55** can be used as a starting material for the synthesis of an antileishmanial precursor.

1.1.2. Synthesis of pyrazole derivatives in ethanolic conditions

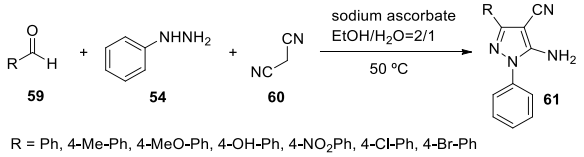
In this path, pyrazole synthesis was carried out only in ethanol or in a mixture of ethanol and a base.

Zhang *et al.* showed that ethyltrifluoroacetoacetate **56** was easily condensed with triethyl orthoformate under the acid medium (*Scheme 1.13*). Later, intermediate **57** was treated with phenylhydrazine **54** in ethanol conditions to provide product **58** [26].



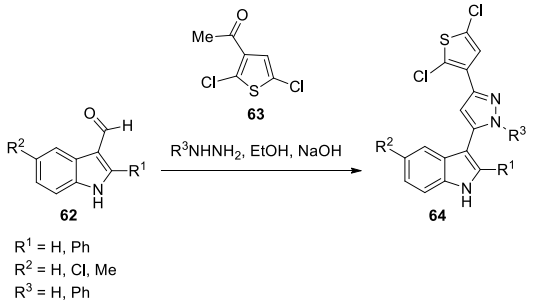
Scheme 1.13 Synthesis of pyrazole **58**

Kiyani and Bandad demonstrated one-pot 'green' reaction for pyrazole synthesis (*Scheme 1.14*). The reaction was initiated by the treatment of benzaldehyde (**59**) with phenylhydrazine (**54**), malononitrile (**60**) and a catalytic amount of sodium ascorbate in a mixture of ethanol-water at a ratio of 2/1. It should be mentioned that pyrazole **61** was obtained in 98% yield after 10 min [27].



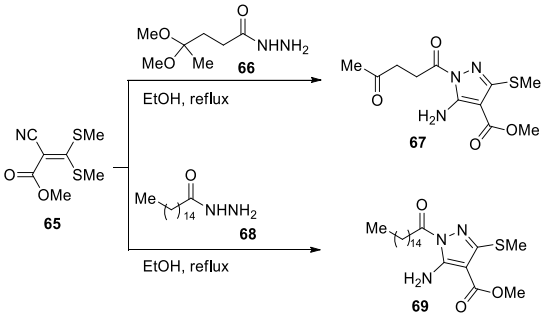
Scheme 1.14 Synthesis of pyrazole derivative **61**

Furthermore, Somappa and team reported another one-pot reaction for pyrazole-appended heterocycles synthesis (*Scheme 1.15*). Pyrazole **64** was obtained after indole-3-carboxaldehyde **62** was reacted with 2,5-dichloro-3-acetyl-thiophene (**63**), and various hydrazines were reacted in a mixture of ethanol and sodium hydroxide [28].



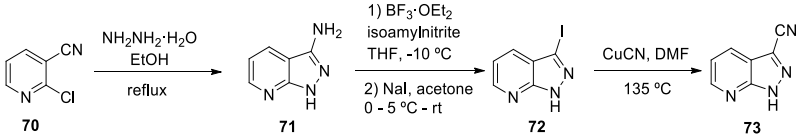
Scheme 1.15 Synthesis of pyrazole-indole derivative **64**

Pereira and his research group demonstrated the cyclization reaction of *α*-oxeketene *S,S*-dimethyl acetal (**65**) and hydrazides **66** and **68** (*Scheme 1.16*). This type of reaction was used for the synthesis of novel pyrazole analogues **67** and **69** [29].



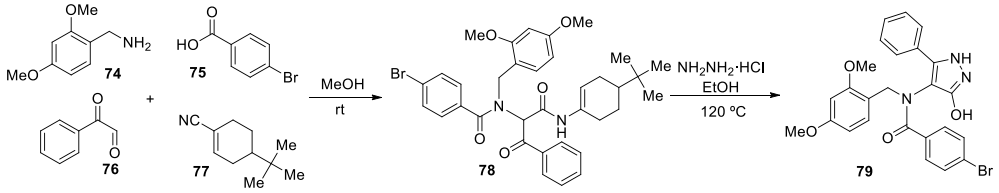
Scheme 1.16 Synthesis of pyrazole derivatives **67** and **69**

Another selected example of pyrazole synthesis is the cyclocondensation reaction between 2-chloro-3-cyanopyridine (**70**) and hydrazine hydrate in ethanol under reflux conditions (*Scheme 1.17*). The amino group of compound **71** was transformed to iodine by the diazotization reaction. Finally, 3-iodo-1*H*-pyrazolo[3,4-*b*]pyridine (**72**) was treated with copper (I) cyanide to furnish the desired product **73**. Regardless of the low yields of the reactions (15–35%), this type of compounds serves as useful building blocks for 3-substituted 1*H*-pyrazolo[3,4-*b*]pyridine synthesis [30].



Scheme 1.17 Synthesis of 3-substituted 1*H*-pyrazolo[3,4-*b*]pyridine **73**

Shaw and team reported a multi-component Ugi reaction involving 2,4-dimethoxybenzylamine **74**, 4-bromobenzoic acid **75**, phenylglyoxal **76** and cyclohexen-1-yl isocyanide **73** (*Scheme 1.18*). The obtained intermediate Ugi product **78** was reacted with hydrazine mono-hydrochloride in ethanol to give 3,4,5-trisubstituted pyrazole **79** [31].

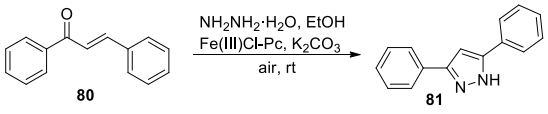


Scheme 1.18 Synthesis of 3,4,5-trisubstituted pyrazole **79**

1.1.3. Pyrazole synthesis with metal catalysis

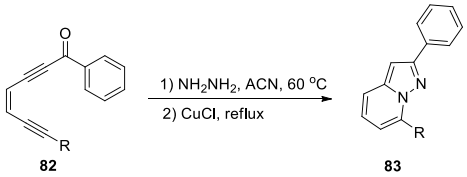
Beside several pyrazole ring synthesis methods, it is important to highlight the reaction of metal irradiation.

Zhao *et al.* reported 3,5-disubstituted pyrazole **81** synthesis under mild reaction conditions (*Scheme 1.19*). Chalcone **80** was condensed with hydrazine hydrate by using iron (III) phthalocyanine chloride. It is of importance to note that the catalyst is recyclable and can be used once again [11].



Scheme 1.19 Synthesis of 3,5-substituted pyrazole **81**

Another selected example of a metal catalysis reaction is the cyclocondensation of 4-en-2,6-diyn-1-one **82** when using copper (I) chloride (*Scheme 1.20*). Pyrazole **83** was isolated in 42–74% yields [20].



R = C_4H_9, C_5H_{11}, C_6H_{13}, Ph, 4-Me-Ph, 4-OMe-Ph

Scheme 1.20 Synthesis of pyrazole derivative **83**

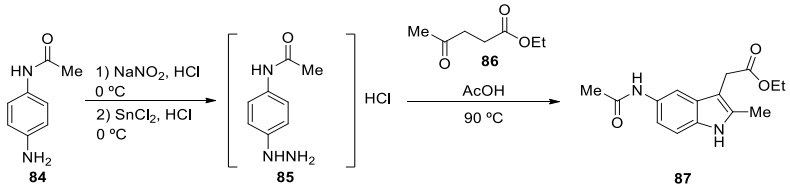
1.2. Hydrazine cyclization for the synthesis of indole derivatives

Another class of the reaction where hydrazine derivatives play an important role is the Fischer indole synthesis. In this type of chemical reactions, aromatic heterocycle indoles are produced from a phenylhydrazine and an aldehyde or ketone under acidic conditions. Traditionally, for the indole ring formation, the stoichiometric amount of Bronsted or Lewis acids (H_2SO_4, HCl, polyphosphoric acid, $ZnCl_2$, $FeCl_3$, $AlCl_3$) was used [32].

Indole and its derivatives are widely used and tested heterocycles. The indole core is found in the structures of tryptophan, neurotransmitters serotonin and melatonin [33], in the plant hormone auxin [34], perfumes [35], and indole alkaloids [36]. Synthetic pharmaceuticals with the indole core are used as antioxidant [37], anticancer [38], and anti-inflammatory [39] agents.

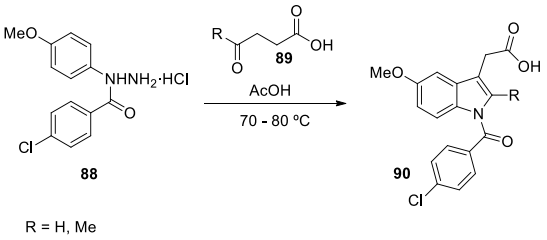
1.2.1. Fischer indole synthesis under acidic medium

One of the methods to obtain the indole ring includes a reaction between hydrazine hydrochloride **85** and ketone **86** in acetic acid at 90 °C under inert atmosphere (*Scheme 1.21*). The intermediate hydrazine hydrochloride **85** can be obtained *via* diazotization of 4-aminoacetanilide (**84**) [40].



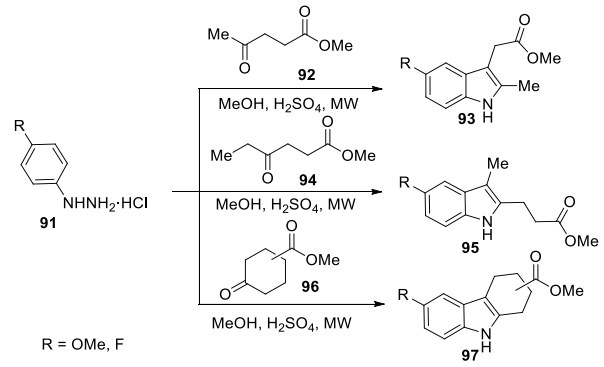
Scheme 1.21 Synthesis of indole **87**

Another example of indole synthesis was reported by Liedtke and team. *N*,*N*-disubstituted hydrazine hydrochloride **88** was treated with ketoacid **89** in the acetic acid medium to give the auxin type product **90** (*Scheme 1.22*) [41].



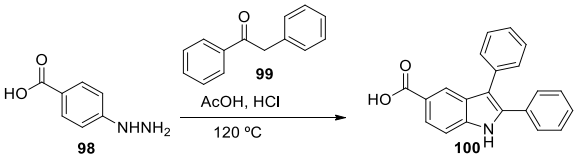
Scheme 1.22 Synthesis of indole derivative **90**

In another publication, the same author replaced acetic acid with a mixture of methanol and H_2SO_4 for better solubility of starting material **91** (*Scheme 1.23*). The reactions were accelerated by microwave irradiation and yielded different types of indoles **93**, **95** and **97** in 45–80% isolated yields [41].



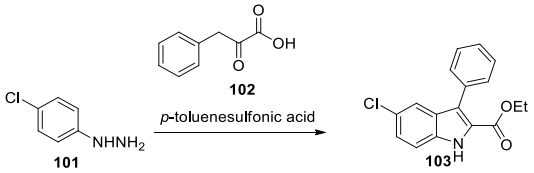
Scheme 1.23 Synthesis of indole derivatives **93**, **95** and **97**

Houck and co-authors reported hydrazine **98** cyclizations with ketone **99** in a mixture of acetic and hydrochloric acids (*Scheme 1.24*). The obtained diphenyl indole **100** can be used in the reaction with triazolinediones [42].



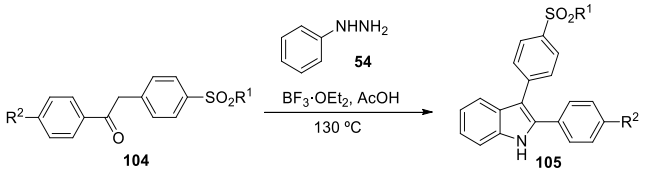
Scheme 1.24 Synthesis of indole **100**

At the same time, Abdelrahman *et al.* demonstrated Fischer indole synthesis in *p*-toluenesulfonic acid (*Scheme 1.25*). The obtained indole **103** can be used as a starting material for further transformation to indole-2-carboxamides which are denoted by anticancer activity [43].



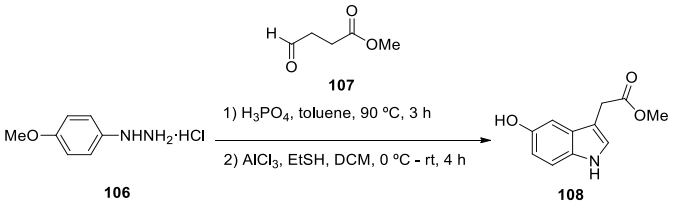
Scheme 1.25 Synthesis of indole **103**

Laube with team reported that compound **104** reacted with phenylhydrazine **54** when using boron trifluoride diethyl etherate as Lewis acid to obtain a product whose yields did not exceed 20% (*Scheme 1.26*) [44].



Scheme 1.26 Synthesis of indole derivative **105**

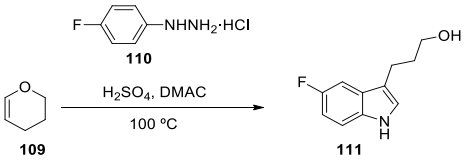
The final example of this part is the synthesis of indole-3-acetic acid derivative **108** (*Scheme 1.27*). The indole ring was obtained when the reaction between hydrazine **106** and aldehyde **107** was carried out in a mixture of phosphoric acid and toluene. The desired 3-indolylacetic acid methyl ester **108** was obtained by the demethylation reaction with $AlCl_3$ and ethanethiol [45].



Scheme 1.27 Synthesis of indole **108**

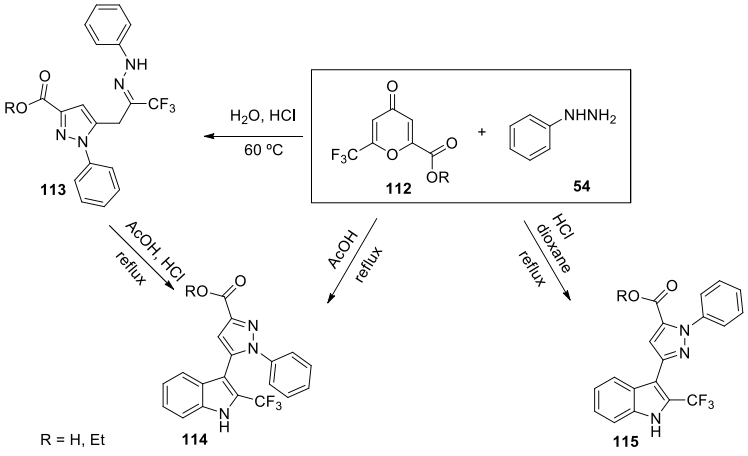
1.2.2. Fischer indole synthesis from pyran and pyrone

Recently, Zheng-Song Gu and team demonstrated a novel Fischer indole synthesis method *(Scheme 1.28*). In this type of reaction, pyran **109** was treated with 4-fluorophenylhydrazine hydrochloride (**110**) and *N*,*N*-dimethylacetamide in sulfuric acid to obtain 3-(5-fluoro-1*H*-indol-3-yl)propan-1-ol (**111**) [46].



Scheme 1.28 Synthesis of indole **111**

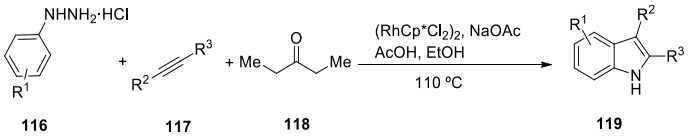
A few years ago, Obydenov with team reported that pyrone **112** is a suitable substrate for indole synthesis (*Scheme 1.29*). When pyrone **112** and phenylhydrazine **54** were heated in aqueous hydrochloric acid, the intermediate compound **113** was obtained which could be further transformed to indole **114** *via* refluxing in a mixture of acetic and hydrochloric acids. However, the targeted indole **114** was obtained immediately under refluxing acetic acid, while when the reaction mixture of the starting material was refluxed in dioxane and in the presence of hydrochloric acid, another isomer **115** was obtained [47].



Scheme 1.29 Synthesis of indole derivatives **114** and **115**

1.2.3. Fischer indole synthesis under other types of conditions

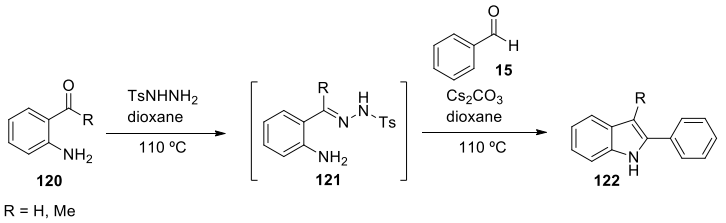
Muralirajan with research group showed a one-pot reaction for indole **119** synthesis (*Scheme 1.30*). The reaction was initiated by the treatment of phenylhydrazine hydrochloride **116** with acetylene **117** and diethyl ketone **118** in the presence of a rhodium complex in acetic acid and ethanol [48].



R^1 = H, Me, OMe, F, Cl, Br, I, CF_3
R^2 = Me, Et, OMe, Cl, Br
R^3 = 4-Me-Ph, 4-OMe-Ph, 4-F-Ph

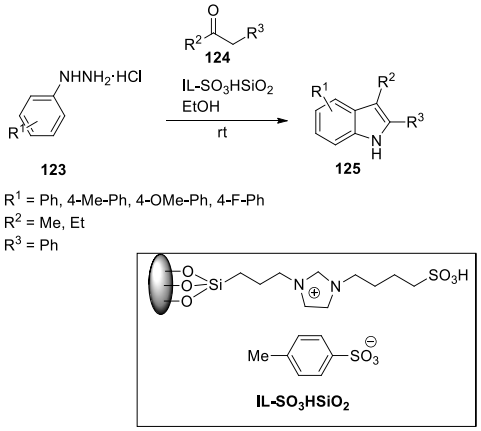
Scheme 1.30 Synthesis of indole derivative **119**

Yan-Dong Wu and coauthors reported that the starting material **120** was condensed with tosylhydrazine to get intermediate tosylate **121** (*Scheme 1.31*). Then, the latter compound **121** was treated with aldehyde **15** and Cs_2CO_3 to form the indole ring. It is important to highlight that Fischer indole synthesis can be carried out under basic conditions [49].



Scheme 1.31 Synthesis of indole **122**

Yu-Li Hu *et al.* presented a novel Fischer indole synthesis method. These authors suggested that a catalyst functionalized with 4-methylbenzenesulfonic acid based on silica gel surface (IL-SO_3HSiO_2) can be used in the reaction between phenylhydrazine **123** and ketone **124** at room temperature to form the indole ring in good yields (*Scheme 1.32*). It is of importance to mention, that the IL-SO_3HSiO_2 catalyst can be easily recovered without losing any catalytic activity [50].



Scheme 1.32 Synthesis of indole **125**

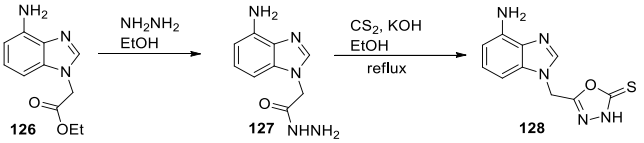
1.3. Other hydrazine cyclization reactions

1.3.1. Oxadiazole synthesis

Hydrazine and its derivatives are useful for oxadiazole synthesis. Oxadiazoles are five-membered heterocyclic compounds [51] which exist in four isomeric forms [52]. Nowadays, 1,3,4-oxadiazole derivatives are of interest in drug design for their antimicrobial, antioxidant [53], antiparasitic [54], anticonvulsant [55] and anticancer [56] activities.

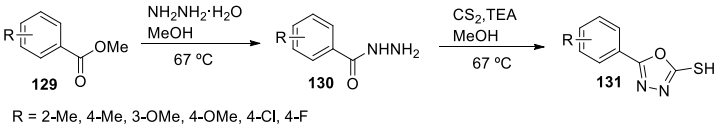
Yildirir and co-authors showed one of the traditional 1,3,4-oxadiazole thione synthesis methods (*Scheme 1.33*). To begin with, ethyl 2-(6-amino-9*H*-purin-9-

yl)acetate (**126**) was treated with hydrazine hydrate in ethanol. The latter hydrazide **127** was reacted with carbon disulfide in the presence of KOH in ethanol to obtain the corresponding oxadiazole **128**, which can be used in the *S*-alkylation reaction [57].



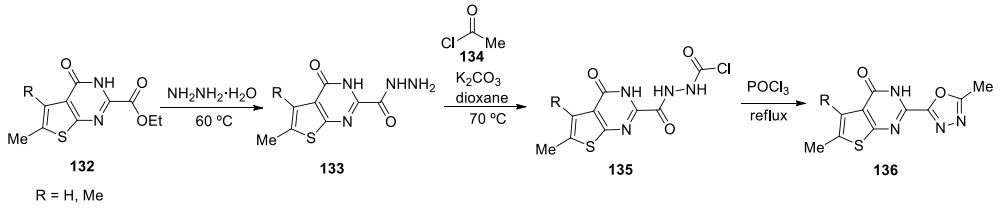
Scheme 1.33 Synthesis of 1,3,4-oxadiazole **128**

At the same time, Kumar *et al.* showed that oxadiazole-3-thiol **131** can be obtained from hydrazide **130** (*Scheme 1.34*). In this reaction, triethylamine was chosen as the base compared to the previously discussed *Scheme 1.33*. The corresponding hydrazide **130** was obtained from carboxylic acid ester **129** and hydrazine hydrate condensation [58].



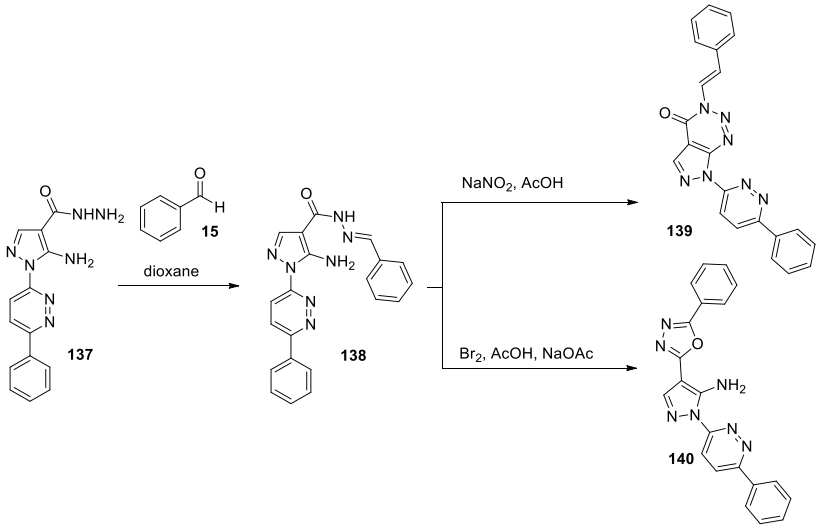
Scheme 1.34 Synthesis of oxadiazole-3-thiol **131**

Other selected examples of the synthesis of 1,3,4-oxadiazole derivatives were presented by Changmok Oh. Condensation of hydrazide **133** with acetyl chloride **134** in 1,4-dioxane yielded intermediate **135** (*Scheme 1.35*). Then desired product **136** was prepared by the cyclization of the previously obtained intermediate **135** under $POCl_3$ conditions [59].



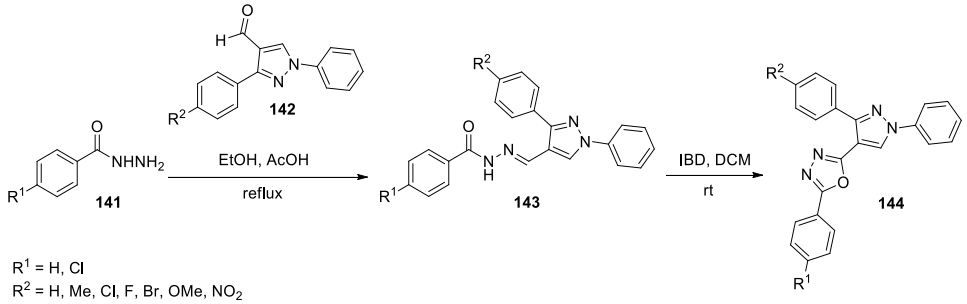
Scheme 1.35 Synthesis of 1,3,4-oxadiazole derivative **136**

One more attempt to synthesize 1,3,4-oxadiazole compounds was reported by Shamroukh and team. To start with, the treatment of hydrazine **137** with aldehyde **15** gave benzylidene hydrazide **138** (*Scheme 1.36*). After that, the intermediate **138** was cyclized with $NaNO_2$ in acetic acid to obtain 1,5-dihydropyrazolo[3,4-*d*][1,2,3]triazin-4-one **139**. However, when cyclization was carried out under bromine water and acetic acid conditions, the desired product **140** was prepared. Finally, the authors mention that pyrazoles showed significant antibacterial activities against *Escherichia coli*, *Bacillus subtilis*, *Candida albicans*, and *Aspergillus niger* [60].



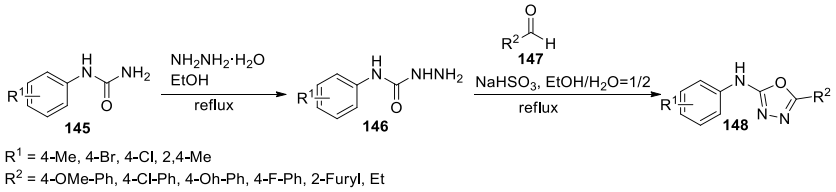
Scheme 1.36 Synthesis of 1,3,4-oxadiazole derivatives **139** and **140**

Bansal *et al.* also showed the reaction between aldehyde **142** and hydrazide **141** to give the intermediate hydrazones **143** under acid conditions (*Scheme 1.37*). After the oxidative cyclization of hydrazones **143** with iodobenzenediacetate (IBD), the title compound **144** was isolated. All the novel 1,3,4-oxadiazole derivatives **144** were tested as COX-2-selective inhibitors. The most active inhibitor was the compound with the electron withdrawing nitro group ($R^2 = NO_2$) [61].



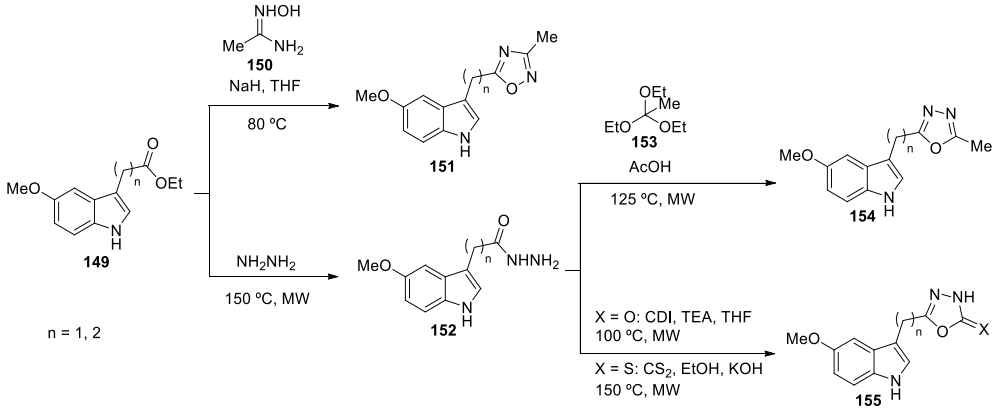
Scheme 1.37 Synthesis of 1,3,4-oxadiazole **144**

Ahsan *et al.* constructed a library of *N*-aryl-5-substituted-1,3,4-oxadiazol-2-amine analogue **148** by preparing phenyl semicarbazides **146** from phenyl ureas **145** and later used them for the cyclization reaction with $NaHSO_3$ in an ethanol/water system (1/2, v/v) (*Scheme 1.38*). All the obtained compounds **148** demonstrated anticancer activity against leukemia, melanoma, the central nervous system, prostate, and breast cancer cell lines [62].



Scheme 1.38 Synthesis of *N*-aryl-5-substituted-1,3,4-oxadiazol-2-amine analogue **148**

Revenga and co-authors demonstrated the synthesis of 1,2,4-oxadiazole **151** and 1,3,4-oxadiazole **152** derivatives under microwave promoted conditions (*Scheme 1.39*). 1,2,4-Oxadiazole **151** can be obtained from the cyclocondensation reaction between ester **149** and acetamidoxine **150** in good yields at a rate of 45–56%. Meanwhile, ethyl 2- or 3-(methoxy-1*H*-indol-3-yl) ester **149** treated with hydrazine gave intermediate hydrazides **152**. Later, these hydrazides **152** can be transformed into 1,3,4-oxadiazoles **154**, 1,3,4-oxadiazol-2-ones **155** (X=O) and 1,3,4-oxadiazol-2-thiones **155** (X=S) under microwave irradiation. Compound **154** (n=2) can be used as a useful candidate for further development in the search for reparative agents for central nervous system diseases [63].

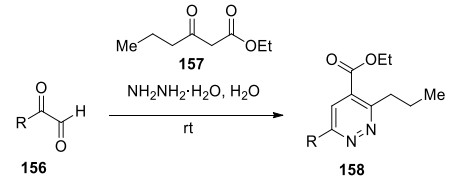


Scheme 1.39 Synthesis of 1,3,4-oxadiazole derivatives **154** and **155**

1.3.2. Hydrazine cyclization for pyridazine synthesis

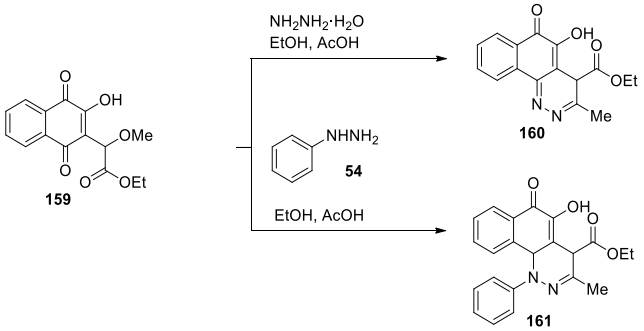
Moreover, hydrazine and its derivatives are used for the synthesis of derivatives of pyridazines. Pyridazines and their derivatives are important heterocycles for their antimicrobial, antifungal [64], anti-inflammatory and antitubercular activities [65]. Derivatives of pyridazines with metallic complexes are denoted by catalytic properties [66].

Khalify with colleagues demonstrated the synthesis of 6-aryl-3-propylpyridazine-4-carboxylate (**158**) (*Scheme 1.40*). The targeted products are obtained by the reaction of arylglyoxal **156** with ethyl butyrylacetate **157** in the presence of hydrazine hydrate at room temperature [66].



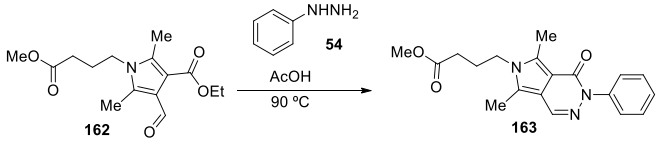
Scheme 1.40 Synthesis of pyridazine carboxylate **158**

Berghot Ma *et al.* Showed the cyclocondensation reaction of the starting material **159** with hydrazine hydrate or phenylhydrazine **54** in the presence of acetic acid to get pyridazine derivatives **160** and **161** (*Scheme 1.41*) [67].



Scheme 1.41 Synthesis of pyridazine derivatives **160** and **161**

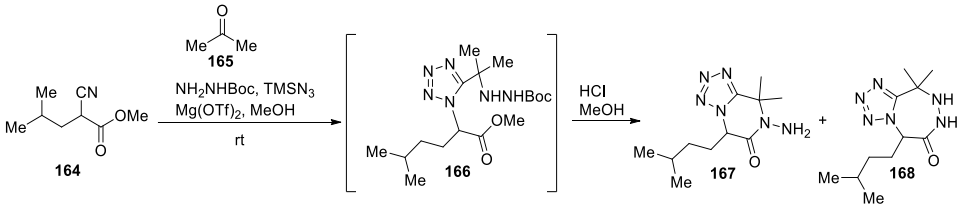
In another selected example, aldehyde **162** was condensed with phenylhydrazine **54** in acetic acid to give pyridazinone **163** (*Scheme 1.42*) [68]. Murarka with colleagues mentioned that pyradazinone **163** could be used as the primary material for pyrrolopyradizinone and pyrazolopyridazinone synthesis as these substances are denoted by anticancer activity.



Scheme 1.42 Synthesis of pyridazinone **163**

1.3.3. Tetrazole synthesis

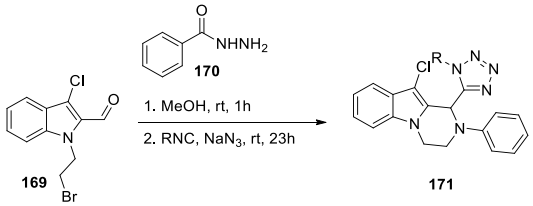
Wang with coworkers demonstrated a one-pot reaction for tetrazole synthesis (*Scheme 1.43*). Ugi compound **166** can be obtained after isocyanide **164** is treated with acetone **165**, *N*-Boc-hydrazine, trimethylsilyl azide and magnesium trifluoromethanesulfonate in methanol at room temperature. The intermediate tetrazole **166** was cyclized under acidic conditions to prepare products **167** and **168** at 36% and 6% yield, respectively [69].



Scheme 1.43 Synthesis of tetrazoles **167** and **168**

It is well-known that Lewis acids could help the activation and formation of the Schiff base, which can be the rate-limiting step in the Ugi-adduct synthesis.

Salahi *et al.* Showed one-pot four-component Ugi-azide reaction (*Scheme 1.44*). Ugi compound **171** can be obtained after carbaldehyde **169** is treated with phenylhydrazide **170** in methanol. After 1 h, sodium azide and isocyanide need to be added to obtain the desired product **171** [70].



R = *tert*-butyl, *tert*-butylCH$_2$CMe$_2$

Scheme 1.44 Synthesis of Ugi compound **171**

1.4. Conclusions

A literature review of the cyclization reaction with hydrazine or hydrazide derivatives has been systematically presented and discussed. This overview demonstrates that hydrazine or hydrazide derivatives play an important role for the synthesis of biologically active *N*-heterocycles such as pyrazole, indole, oxadiazole and pyridazine.

2. RESULTS AND DISCUSSION

2.1. Synthesis of pyrazole-azetidine derivatives

Pyrazole carboxylic acids are widely used as building blocks in organic synthesis for designing agrochemicals and pharmaceuticals [71]. For example, pyrazole carboxylate with indole unit **I** manifest anticancer activity [72], while *N*-substituted pyrazole carboxylate **II** exhibits anticancer activity [73] (*Figure 2.1*).

Azetidine is a four-member aza-heterocycle which has an important role as a building-block in organic chemistry and drug design [6]. The azetidine skeleton was used for the synthesis of the analogues of naturally occurring amino acids such as 4-aminobutanoic acid (*γ*-aminobutyric acid, GABA) and 3-aminopropanoic acid (*β*-alanine) [6, 75]. One of the best-known azetidine-containing pharmaceuticals is the antihypertensive drug azelnidipine **III** [74]. As another example, Faust with colleagues synthesized GABA-uptake inhibitor **IV** (*Figure 2.1*) [75].

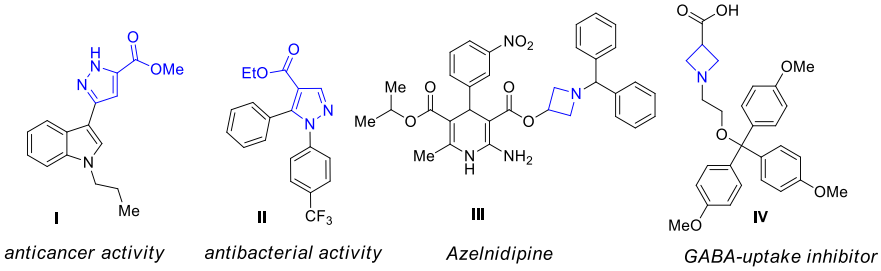


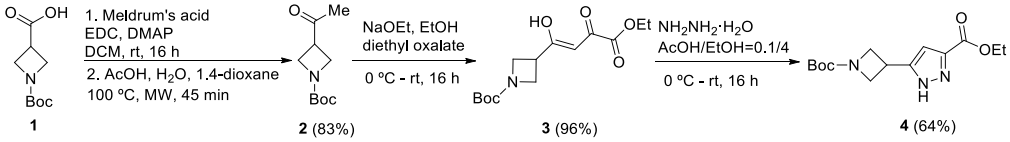
Figure 2.1 Structure of biologically active pyrazole carboxylate and azetidine-containing derivatives

Surprisingly, compounds combining these two privileged scaffolds in one structure are still scarce/elusive in the scientific literature. In order to fill this gap, we ought to prepare pyrazole-azetidine derivatives.

2.1.1. Synthesis of *N*-alkyl-1*H*-pyrazole-5-carboxylates

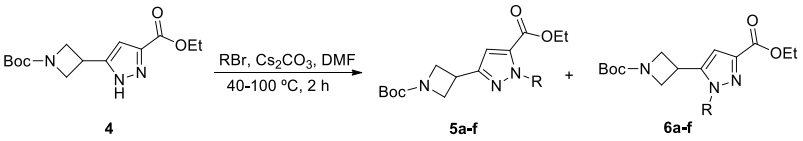
The synthesis strategy for the preparation of pyrazole-azetidine derivatives included the construction of pyrazole carboxylates bearing an azetidin-3-yl substituent at the 3- or 5-position of the pyrazole ring.

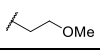
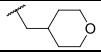
It is known that the Michael reaction between hydrazine and *α,β*-unsaturated ketones is performed to furnish pyrazole carboxylates [76]. For this reason, according to the literature methodology [77], *N*-Boc azetidine 3-carboxylic acid (**1**) was modified to ketone **2** (*Scheme 2.1*). Later, ketone **2** was reacted with NaOEt and diethyl oxalate to obtain the enolic form of compound **3** in 96% yield, which was then cyclized with hydrazine hydrate in an ethanol/acidic system (4/0.1, v/v) [78] in order to obtain the desired pyrazole **4**.



Scheme 2.1 Synthesis of pyrazole carboxylate **4**

With the targeted 5-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1*H*-pyrazole-3-carboxylate (**4**) in our possession, we started the *N*-alkylation reaction with various types of alkyl halogenides (*Scheme 2.2*). Treatment of pyrazole **4** with alkyl halide in the presence of Cs_2CO_3 in DMF afforded a mixture of regioisomers **5a-f** at higher than 60% yields and **6a-f** with yields not exceeding 15%. It is of importance to remark that the major products **5a-f** were formed at N-2 nitrogen of the starting substrate.



	a	**b**	**c**	**d**	**e**	**f**
R						
temperature, °C	90	100	40	40	100	100
5, yield, %	91	77	63	70	78	67
6, yield, %	9	15	14	11	5	14

Scheme 2.2 Synthesis of pyrazole carboxylates **5a-f** and **6a-f**

The difference between targeted regioisomers **5a-f** and **6a-f** retention coefficient distinctly different, for example **5a** R_f = 0.54, while **6a** R_f = 0.14. For this reason, regioisomers were easily separated by column chromatography.

Regioisomers **5a-f** and **6a-f** were identified by using ^{1}H, ^{13}C-HMBC and ^{1}H, ^{15}N-HMBC spectrometry. For example, the ^{1}H, ^{13}C-HMBC spectrum of **5a** showed a strong three-bond connectivity of the isobutyl group CH_2 proton (4.24–4.36 ppm) with the pyrazole C-5 carbon at 151.8 ppm (*Figure 2.2*). Moreover, the CH_2 and CH (2.13–2.23 ppm) protons of the isobutyl group showed correlation with N-1 nitrogen at -167.7 ppm in the ^{1}H, ^{15}N-HMBC spectrum. However, the azetidine proton 3-H in the ^{1}H, ^{13}C-HMBC spectrum showed two-bond connectivity with C-3 carbon at 133.4 ppm and two-bond connectivity in the ^{1}H, ^{15}N-HMBC spectrum with N-2 nitrogen at -63.0 ppm. Meanwhile, the ^{1}H, ^{13}C-HMBC spectrum of compound **6a** showed a strong three-bond connectivity of the isobutyl group CH_2 proton with the pyrazole C-5 carbon at 145.0 ppm. Furthermore, the azetidine proton 3-H in the ^{1}H, ^{13}C-HMBC spectrum also showed two-bond connectivity with the C-5 carbon at 145.0 ppm. The ^{1}H, ^{15}N-HMBC spectrum showed strong three-bond connectivity of N-1 nitrogen at -166.8 ppm with the isobutyl group CH proton and azetidine proton 3-H.

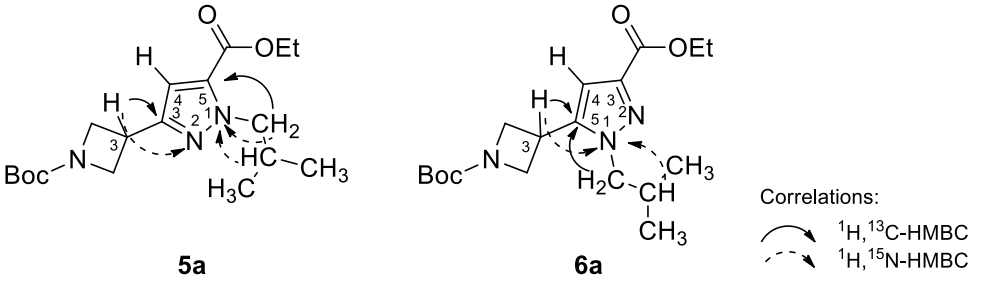


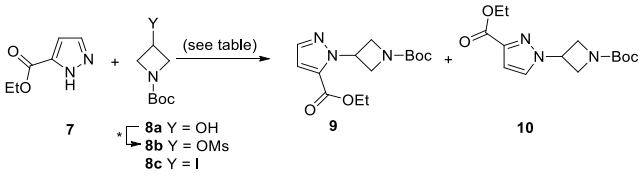
Figure 2.2 Relevant ^{1}H, ^{13}C-HMBC and ^{1}H, ^{15}N-HMBC correlations of compounds **5a** and **6a**

To sum up, analysis of ^{1}H, ^{13}C-HMBC and ^{1}H, ^{15}N-HMBC spectrum revealed that, when the proton of the alkyl group has connectivity with carbon, which, in turn, has connectivity with the carboxyl group (C-5, *Figure 2.2*), **5a-f** compounds are formed. On the other hand, when azetidine 3-H and the proton of the alkyl group have the same carbon interaction, compounds **6a-f** are formed.

2.1.2. *N*-Alkylation reaction of pyrazole and indazole carboxylates with *N*-Boc-3-iodoazetidine

We have demonstrated that a new building block of amino acid could be easily achieved by *N*-alkylation of heterocyclic carboxylates with *N*-Boc-3-iodoazetidine [6]. In this part, we have chosen *N*-Boc-3-hydroxyazetidine, *N*-Boc-3-[(methylsulfonyl)oxy]azetidine and *N*-Boc-3-iodoazetidine as the starting material, which is connected with various commercially available pyrazole and indazole carboxylates.

Our strategy of synthesis started from the optimization of the reaction conditions. First, ethyl pyrazole-5-carboxylate **7** was coupled with *N*-Boc-3-hydroxyzetidine **8a** under the standard Mitsunobu reaction conditions (DIAD, PPh_3, DCM) (*Scheme 2.3*, *Table 2.1*) [79]. The Mitsunobu reaction furnished two regioisomers **9** and **10** at a ratio of 2:1 with the yield of **9** not exceeding 29% (*Table 2.1*, *Entry 1*). Further, it was decided to transform alcohol **8a** into mesylate **8b** under the standard procedure for the mesylation of alcohols [80]. The treatment of compound **7** with active nucleophilic agent **8b** in the presence of Cs_2CO_3 in DMF afforded a mixture of regioisomers **9** and **10**. The yield of the desired product **10** increased to 46%, while the yield of compound **9** decreased to 12% (*Table 2.1*, *Entry 2*). Later, it was decided to replace mesylate **8b** with the commercially available iodide **8c**. The *N*-Alkylation reaction with *N*-Boc-3-iodoazetidine (**8c**) was carried out in the presence of Cs_2CO_3 in DMF to obtain regioisomers **9** and **10** in a ratio of approximately 1:1 with yields higher than 40% for both regioisomers (*Table 2.1*, *Entry 3*). Unfortunately, changing Cs_2CO_3 and replacing the conventional heating did not improve reaction yields (*Table 2.1*, *Entry 4 and 5*).



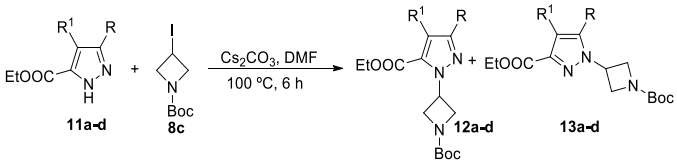
Scheme 2.3 Synthesis of pyrazole carboxylates **9** and **10**

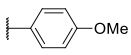
Table 2.1 Optimization of reaction conditions for construction of pyrazole carboxylates **9** and **10**

Entry	Y	Conditions	**9**, yield, %	**10**, yield, %
1.	OH	DIAD, PPh_3, DCM, rt, 48 h	29	12
2.	OMs	Cs_2CO_3, DMF, 100 ºC, 5 h	12	46
3.	I	Cs_2CO_3, DMF, 100 ºC, 6 h	44	40
4.	I	Cs_2CO_3, DMF, MW, 40 ºC, 1 h	31	20
5.	I	K_2CO_3, DMF, 100 ºC, 6 h	34	41

***8a**, mesyl chloride, TEA, DCM, rt, 2 h [80].

With the optimal reaction conditions in hand, the *N*-alkylation reaction was performed with other pyrazole carboxylates **11a-d** (*Scheme 2.4*). Heating iodide **8c** with pyrazole carboxylate **11a-d** in the presence of Cs_2CO_3 in DMF gave a mixture of separable regioisomers **12a-d** and **13a-d**. It is noteworthy that when pyrazole has methyl groups at either C-3 or C-4 carbon, products **12a**, **b** and **13a**, **b** are obtained in similar yields. However, when changing the methyl group at the C-3 carbon to (hetero)aryl substituents, the *N*-alkylation reaction proceeded mainly at the N-1 nitrogen, and major regioisomers **12c** and **12d** were obtained in yields higher than 60%. Meanwhile, the yields of minor regioisomers **13c** and **13d** did not exceed 5%.



	a	**b**	**c**	**d**
R		H		
R^1	H		H	H
12, yield, %	45	30	70	63
13, yield, %	27	26	4	5

Scheme 2.4 Synthesis of pyrazole carboxylates **12a-d** and **13a-d**

Regioisomers **9**, **10**, **12a-d** and **13a-d** were identified by using ^1H, ^{13}C-HMBC and ^1H, ^{15}N-HMBC spectrometry. For example, the azetidine 3-H proton in the ^1H, ^{13}C-HMBC spectrum of compound **12a** has three-bond connectivity with the C-5 carbon at 132.2 ppm, while the methyl group has two-bond correlation with the C-3 carbon at 148.0 ppm (*Figure 2.3*). The pyrazole proton 4-H at 5.63 ppm in the ^1H,

^{13}C-HMBC spectrum has two-bond connectivity with the C-3 and C-5 carbons (148.0 ppm and 132.2 ppm) and three-bond connectivity with the carbon of the methyl group (3-CH_3, 13.6 ppm). The ^{1}H, ^{15}N-HMBC spectrum revealed correlation between the methylene protons of azetidine with the N-1 nitrogen at -169.9 ppm, while the azetidine ring 3-H proton and 3-CH_3 has correlation with the N-2 nitrogen at -68.6 ppm. Meanwhile, the ^{1}H, ^{13}C-HMBC spectrum of compound **13a** showed strong three-bond connectivity of the azetidine ring 3-H at 4.98–5.07 ppm with the pyrazole C-5 carbon at 140.0 ppm. Furthermore, the ^{1}H, ^{15}N-HMBC spectrum revealed connectivity between the methyl group protons (5-CH_3, 2.28 ppm) and the CH_2 group of the azetidine ring at 4.494.58 ppm with the N-1 nitrogen at -162.9 ppm. Finally, the N-2 nitrogen at -71.8 ppm interacts with the azetidine ring 3-H proton at 4.98–5.07 ppm.

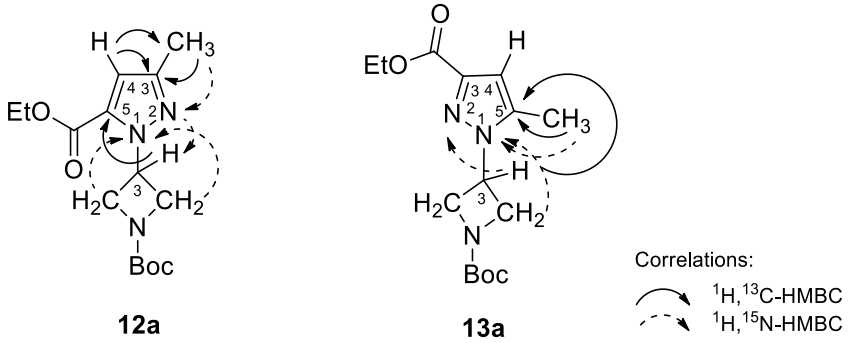
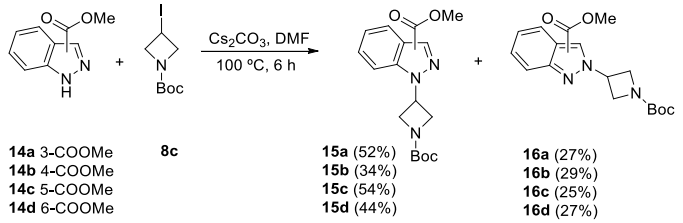


Figure 2.3 Relevant ^{1}H, ^{13}C-HMBC and ^{1}H, ^{15}N-HMBC correlations of compounds **12a** and **13a**

When a variety of pyrazole carboxylates with the azetidine substituent was obtained, it was decided to expand the building-blocks scaffolds/libraries. For this reason, *N*-Boc-3-iodoazetidine (**8c**) was coupled with indazole carboxylates. Indazole carboxylate has fused pyrazole and benzene rings, which could be important for filling an active pocket of the enzyme [81]. It is important to remark that the amino and carboxyl groups could be located at a greater space in the plane of the ring system [6] in the case of the pyrazole carboxylate. Also, it is anticipated that two *N*-substituted regioisomers could be obtained.

For the reasons outlined above, indazole carboxylates **14a-d** were coupled with *N*-Boc-3-iodoazetidine (**8c**) in the presence of Cs_2CO_3 in DMF to obtain the separable desired products **15a-d** and **16a-d** whose yields did not exceed 55% and 30%, respectively (*Scheme 2.5*).



Scheme 2.5 Synthesis of indazole carboxylates **15a-d** and **16a-d**

The regioisomers **15a-d** and **16a-d** were discriminated by using the ^{1}H, ^{13}C-HMBC, ^{1}H, ^{13}C-HSQC and ^{1}H, ^{15}N-HMBC spectrometry. For example, the ^{1}H, ^{13}C-HSQC experiment of **15d** showed that the 3-H proton at 8.13 ppm has one-bond connectivity with the indazole C-3 carbon at 134.4 ppm, and the indazole 7-H proton at 8.18 ppm has the same connectivity with the C-7 carbon at 111.2 ppm (*Figure 2.4*). Moreover, the ^{1}H, ^{13}C-HMBC spectrum showed correlation between the azetidine 3-H proton and the indazole C-7a carbon (139.1 ppm). Finally, the ^{1}H, ^{15}N-HMBC spectrum showed that the azetidine 3-H proton has two-bond interaction with the N-2 nitrogen at -61.8 ppm and three-bond interaction with the N-1 nitrogen at -195.2 ppm. Meanwhile, the ^{1}H, ^{13}C-HMBC spectrum of compound **16d** demonstrated the azetidine 3-H proton (5.30–5.39 ppm) interaction with the indazole C-3 carbon at 122.3 ppm. The ^{1}H, ^{15}N-HMBC experiment showed the azetidine 3-H proton correlation with the N-1 and N-2 nitrogen (-94.8 ppm and -148.5 ppm).

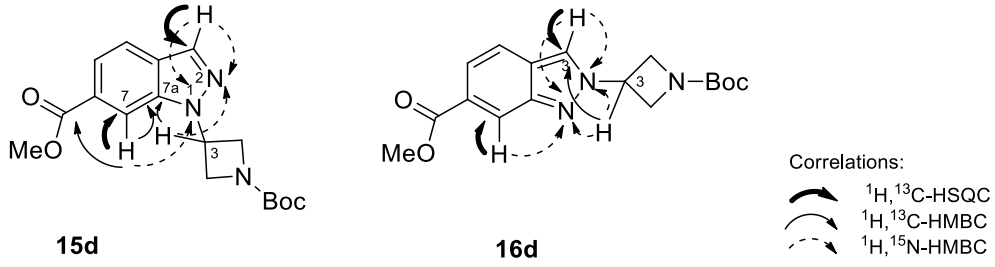
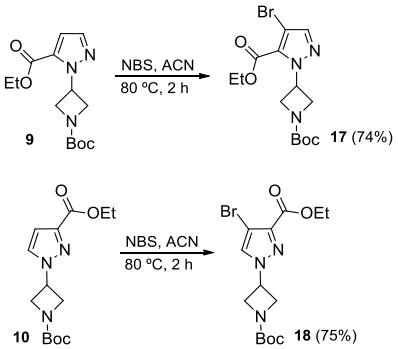


Figure 2.4 Relevant ^{1}H, ^{13}C-HSQC, ^{1}H, ^{13}C-HMBC and ^{1}H, ^{15}N-HMBC correlations of compounds **15d** and **16d**

Another way to expand the building-blocks scaffolds/libraries could be the Suzuki-Miyaura cross-coupling reaction with the halogenated substrate and (hetero)aryl boronic acid. For this reason, *N*-alkylated products **9** and **10** were chosen as the substrates to explore the halogenation reaction (*Scheme 2.6*). A few authors have suggested bromination of the pyrazole ring. Pyrazole could be treated with Br_2 in water [82] or NBS in organic solvents such as $CHCl_3$, acetone, CCl_4 [83], ethyl acetate and THF [84]. In this work, *N*-(azetidine-3-yl)pyrazole carboxylates **9** or **10** were brominated with NBS in acetonitrile via an adapted literature procedure [85], and, regardless of the presence of the substituents at 3- and 5-positions, 4-brominated desired products **17** and **18** were obtained. It is worth noting that there was no decomposition of the *N*-Boc protecting group during the reaction.



Scheme 2.6 The bromination reaction of compounds **9** and **10**

The brominated product **17** was identified by using the 1,1-ADEQUATE and ^{1}H, ^{13}C-HSQC spectrometry. The ^{1}H, ^{13}C-HSQC experiment showed that the 3-H proton at 7.61 ppm has one-bond connectivity of with the C-3 carbon at 141.0 ppm (*Figure 2.5*). Moreover, the 1,1-ADEQUATE experiment, which exclusively shows the correlation between the protonated and non-protonated carbons [86], revealed only one correlation of the protonated C-3 carbon (141.0 ppm) with the non-protonated C-4 carbon at 100.3 ppm. However, if product **Y** had formed, we would have seen two pairs of correlation C-4 with C-3 and C-4 with C-5 in the 1,1-ADEQUATE experiment.

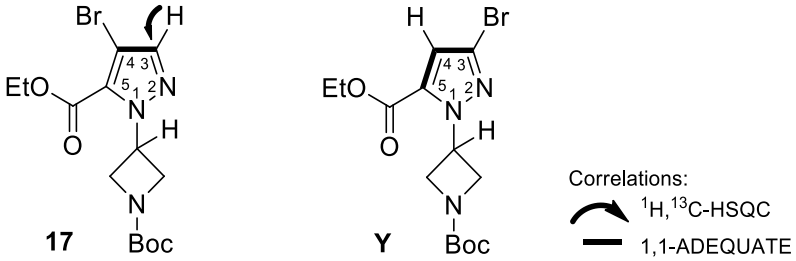


Figure 2.5 Relevant ^{1}H, ^{13}C-HSQC and 1,1-ADEQUATE correlations of compound **17**

The brominated product **18** was identified by using the ^{1}H, ^{1}H-NOESY and ^{1}H, ^{13}C-HMBC spectrometry. The ^{1}H, ^{1}H-NOESY spectrum demonstrated NOEs among the pyrazole 5-H proton and the azetidine H_a and 3-H protons (*Figure 2.6*). Besides, the 5-H proton has tree-bond correlation with the azetidine C-3 carbon in the ^{1}H, ^{13}C-HMBC spectrum. Meanwhile, if the product **Z** had been obtained, the 4-H proton would only have had one NOE with the azetidine 3-H proton [87].

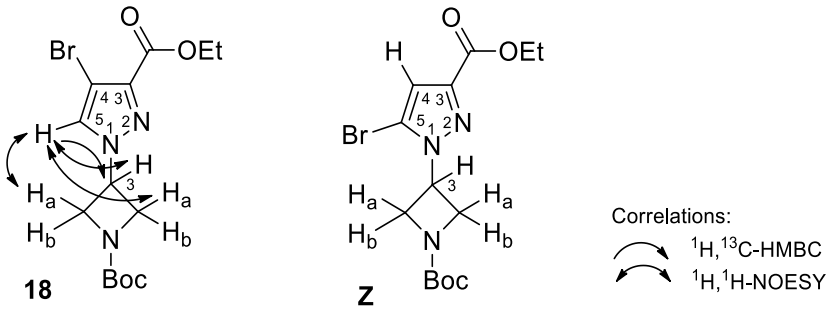
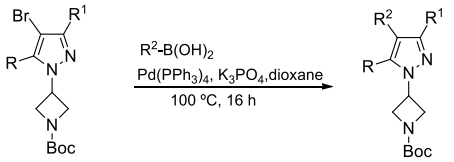


Figure 2.6 Relevant ^{1}H, ^{13}C-HMBC and ^{1}H, ^{1}H-NOESY correlations of compound **18**

With the *N*-(azetidin-3-yl)-4-bromopyrazolecarboxylate **17** or **18** in hand, Pd-cross coupling reactions with some organoboronic acids were conducted (*Scheme 2.7*). Substrate **17** was coupled with phenylboronic acid in the presence of K_3PO_4, $Pd(PPh_3)_4$ in dioxane to obtain product **19a** in 93% yield. Further, the coupling was completed with thiophen-3-ylboronic acid and 3,6-dihydro-2*H*-pyran-4-boronic acid to get the desired products **19b** and **19c** in good yields of 48% and 86%, respectively. When the cross-coupling reaction was completed with substrate **18** under the same reaction conditions as used previously, the yields of products **20a-c** were lower.



17 R = COOEt, R^1 = H
18 R = H, R^1 = COOEt

19a-c R = COOEt, R^1 = H
20a-c R = H, R^1 = COOEt

	a	**b**	**c**
R^2			
19 yield, %	93	48	86
20 yield, %	29	35	52

Scheme 2.7 The Suzuki-Miyaura cross-coupling reaction

To conclude, pyrazole- or indazolecarboxylates were easily *N*-alkylated with 3-iodoazetidine to obtain two separable regioisomers. The identification of regioisomers was carried out by NMR spectroscopy techniques, such as the ^{1}H, ^{1}H-NOESY, ^{1}H, ^{13}C-HMBC and ^{1}H, ^{15}N-HMBC experiments.

2.2. Synthesis of pyrazole-piperidine derivatives

The six-membered piperidine ring is an important building-block in organic synthesis. The piperidine core is a part of naturally occurring alkaloids which possess a broad range of biological activities including insecticidal, anti-HIV, antibacterial and antifungal properties [88]. Piperidine is one of the most frequently used ring systems in small molecule drugs [89]. It is especially important to note several cases

when the structural unit of piperidine connected to a pyrazole ring nitrogen atom is present in small molecular weight biologically active molecules. For example, Xalkori™ (crizotinib, PF-02341066) **V** is used as a first-line treatment for anaplastic lymphoma kinase-positive lung cancer patients [90], ethyl 5-phenyl-1-(piperidin-4-yl)-1*H*-pyrazole-4-carboxylate **VI** shows significant antibacterial activity against *S. Aureus, B. Subtilis, E. Coli* and *P. Aeruginosa* bacterial strains [91] (*Figure 2.7*).

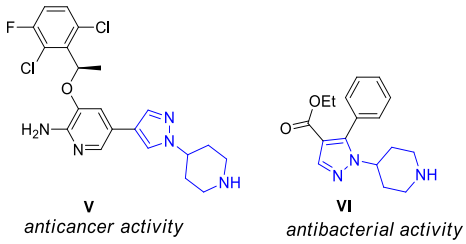
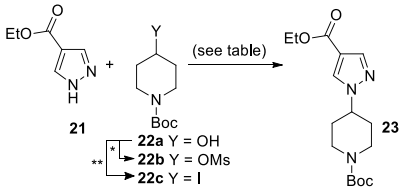


Figure 2.7 Structure of biologically active pyrazole-piperidine derivatives crizotinib **III** and ethyl 5-phenyl-1-(piperidin-4-yl)-1*H*-pyrazole-4-carboxylate (**IV**)

2.2.1. Synthesis and structure determination of alkyl *N*-(1-Boc-piperidin-4-yl)pyrazole carboxylates

Taking into account previous Subsection 2.1.2., it was decided to construct novel pyrazole carboxylate building-blocks scaffolds/libraries where the azetidine ring would be changed to the six-member piperidine core.

To begin with, the synthesis of *N*-(1-Boc-piperidin-4-yl)pyrazole carboxylate **23** was started from the optimization of the reaction conditions (*Scheme 2.8*, *Table 2.2*). The initial attempt was the Mitsunobu reaction where pyrazole-4-carboxylate **21** was coupled with *N*-Boc-4-hydroxypiperidine **22a** in the presence of DIAD, PPh_3 in DCM, and the desired product **23** was obtained at 35% yield (*Table 2.2*, *Entry 1*). Another way to synthesize pyrazole **23** is to transform the OH-functional group to mesylate by using the above outlined procedure [80]. When treating compound **21** with active nucleophilic agent **22b** in the presence of Cs_2CO_3 in DMF, it afforded compound **23** with a yield higher than 40% (*Table 2.2*, *Entry 2*). Later, alcohol **22a** was transformed to iodide **22c** via a procedure described in literature [92]. Surprisingly, the *N*-alkylation reaction with *N*-Boc-4-iodopiperidine **22c** and pyrazole **21** did not give the desired product **23** (*Table 2.2*, *Entry 3*). We can assume that iodide **22c** was unstable under a higher reaction temperature (100 °C). Therefore, further *N*-alkylation reactions were carried out with mesylate **22b**.



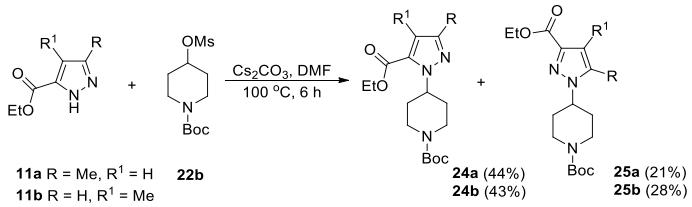
Scheme 2.8 Synthesis of pyrazole carboxylate **23**

Table 2.2. Optimization of reaction conditions for synthesis of pyrazole carboxylates **23**.

Entry	Y	Conditions	**23**, yield, %
1.	OH	DIAD, PPh_3, DCM, rt, 48 h	35
2.	OMs	Cs_2CO_3, DMF, 100 ºC, 6 h	43
3.	I	Cs_2CO_3, DMF, 100 ºC, 6 h	-

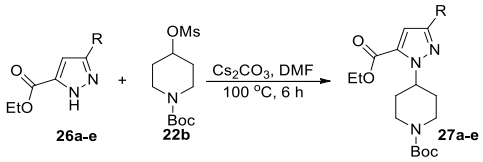
***22a**, mesyl chloride, TEA, DCM, rt, 2 h [80].
****22b**, imidazole, PPh_3, I_2, toluene, 100 ºC, 1 h [92].

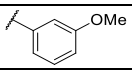
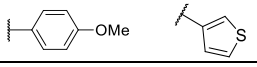
With mesylate **22b** in our possession, *N*-alkylating reactions with other pyrazole carboxylates **11a**, **b** were performed (*Scheme 2.9*). Heating mesylate **22b** with pyrazole carboxylate **11a**, **b** in the presence of Cs_2CO_3 in DMF gave a mixture of major regioisomers **24a**, **b** and minor regioisomers **25a**, **b**. The major product **24a**, **b** was formed at N-1 nitrogen, and their yields were higher than 40%, while the yields of minor products **25a**, **b** did not exceed 28%.



Scheme 2.9 Synthesis of pyrazole carboxylates **24a**, **b** and **25a**, **b**

When the methyl group at the C-3 carbon was changed to (hetero)aryl substituents, the *N*-alkylation reaction was carried out at the N-1 nitrogen, and only one type of major products **27a-e** was obtained in yields higher than 37% (*Scheme 2.10*). The second type of regioisomers was either not formed, or only formed at very small amounts, and, for this reason, the separation was aggravated by chromatography techniques.



	a	**b**	**c**	**d**	**e**
R					
27, yield, %	39	51	38	45	57

Scheme 2.10 Synthesis of pyrazole carboxylates **27a-e**

Regioisomers **24a**, **b**, **25a, b** and **27a-d** were discriminated by using the ^1H, ^{13}C-HMBC, ^1H, ^1H-NOESY or ^1H, ^{15}N-HMBC spectrometry. For example, the ^1H, ^{13}C-HMBC experiment of **24a** showed that the piperidine 4-H proton (5.13–5.26 ppm) has three-bond connectivity with the pyrazole C-5 carbon at 129.1 ppm (*Figure 2.8*). The

^{1}H, ^{15}N-HMBC spectrum showed that the piperidine 4-H proton and pyrazole 3-H proton have interactions with the N-1 nitrogen at -156.8 ppm and the N-2 nitrogen at -69.9 ppm. Meanwhile, the pyrazole 5-H proton at 7.23 ppm of compound **25a** has NOEs with protons of the methyl group at 2.27 ppm and piperidine 4-H at 4.18–4.42 ppm. Meanwhile, the ^{1}H, ^{13}C-HMBC experiment revealed three-bond correlation of the pyrazole C-5 carbon at 127.2 ppm and the piperidine 4-H proton.

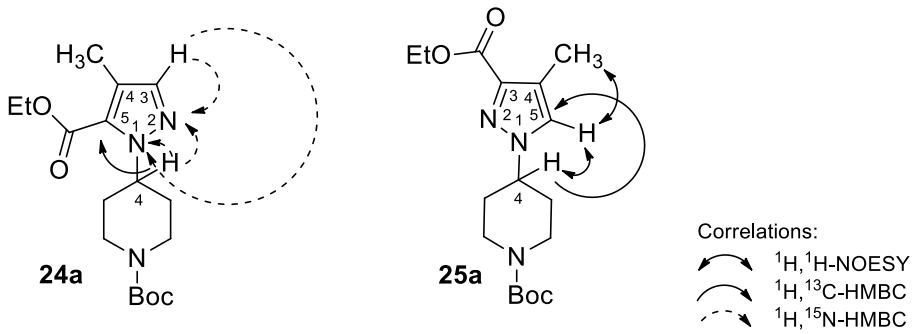
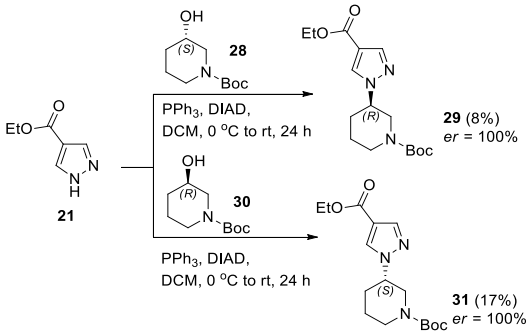


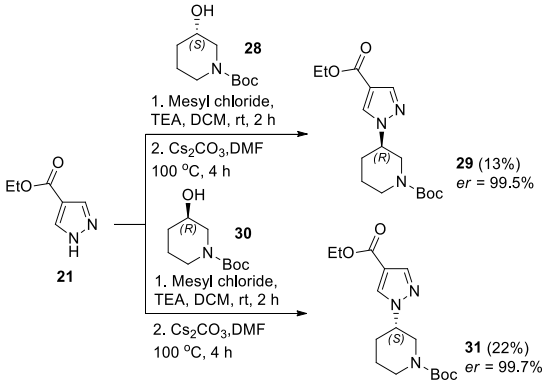
Figure 2.8 Relevant ^{1}H, ^{13}C-HMBC, ^{1}H, ^{1}H-NOESY and ^{1}H, ^{15}N-HMBC correlations of compounds **24a** and **25a**

As some reactions with chiral piperidines are known in literature [93], for this aspect, it was decided to explore the coupling reaction with pyrazole carboxylate and (*S*)- or (*R*)-1-Boc-3-hydroxypiperidine (*Scheme 2.11*). The Mitsunobu reaction mechanism showed that the coupling of chiral alcohol with nucleophile leads to the inversion of the stereochemical configuration [94]. Pyrazole-4-carboxylate **21** was coupled with (*S*)- or (*R*)-1-Boc-3-hydroxypiperidine **28** or **30** in the presence of DIAD, PPh_3 at DCM, and the desired products **29** and **31** were obtained in low yields.



Scheme 2.11 Mitsunobu reaction

Later, for better yields of compounds **29** and **31**, it was decided to explore the pyrazole **21** *N*-alkylation reaction with mesylate (*Scheme 2.12*). Firstly, alcohols **28** and **30** were transformed to the corresponding mesylates by a well-known procedure [80] in short, pyrazole **21** was coupled with mesylate in the presence of Cs_2CO_3 at DMF. The yield of products **29** and **31** was increased by 5 percentage points.



Scheme 2.12 *N*-Alkylation reaction with mesylate

The purity and chirality of enantiomers were tested by chiral column chromatography by colleague Dr. Miglė Dagilienė (Institute of Synthetic Chemistry, Kaunas University of Technology). After the Mitsunobu reaction, products **29** and **31** are pure enantiomers; when the coupling was obtained with mesylate, the purity of enantiomers decreased by 0.5% (*Figure 2.9*). Enantiomers interact differently and selectively with a chiral stationary phase (CSP); for that, they exhibit a distinct retention time [95]. Figure 2.9 shows that (*R*) enantiomer **29** (black color) which was obtained during the Mitsunobu reaction (*Scheme 2.11*), and enantiomer **29** (green color) which was obtained by coupling with mesylate (*Scheme 2.12*) exhibit the same retention time. However, (*S*) enantiomer **31** (pink color) exhibits a distinct retention time.

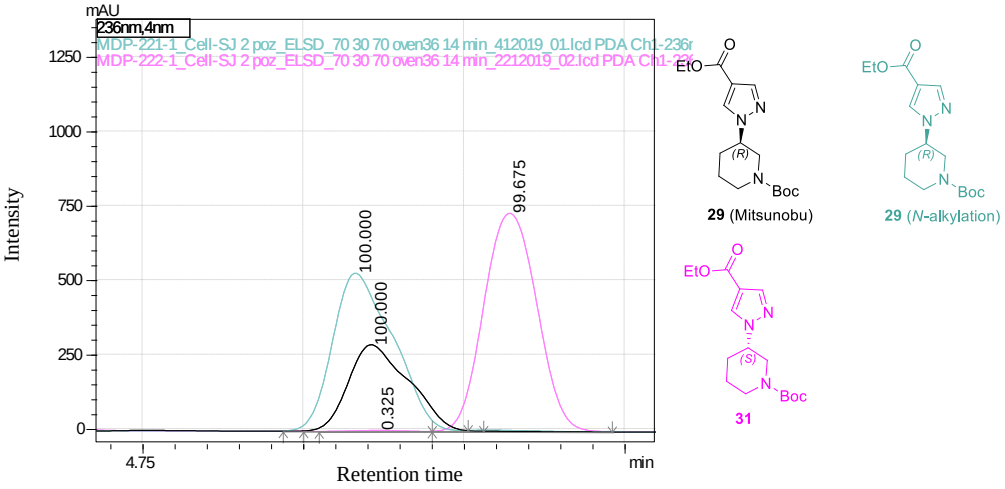


Figure 2.9 Enantioseparation (*R*)- and (*S*)-*N*-Boc-3-[4-(ethoxycarbonyl)-1*H*-pyrazol-1-yl]piperidine-1-carboxylate (**31**) and **29** by using chiral column chromatography

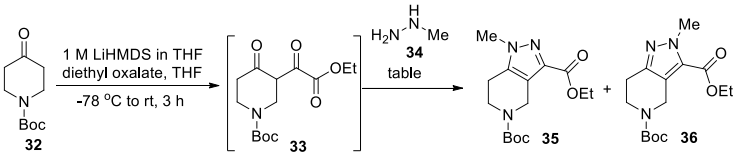
To sum up, pyrazoles were easily coupled with *N*-Boc-4-mesylpiperidine, and we obtained one or two regioisomers **24a**, **b**, **25a**, **b** and **27a-e** separable by chromatography. The enantioseparation with chiral column chromatography confirmed that the *N*-alkylating reaction with (*S*)- and (*R*)-*N*-Boc-3-mesylpiperidine

leads the inversion of the stereochemical configuration from (*S*)- to (*R*)- and from (*R*)- to (*S*)-. The identification of regioisomers can be carried out by NMR spectroscopy techniques, such as the ^{1}H, ^{1}H-NOESY and ^{1}H, ^{13}C-HMBC experiments.

2.2.2. Synthesis of *N*-alkyl-2,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylates

Recently, Dawidowski with colleagues demonstrated novel pyrazolo[4,3-*c*]pyridine derivatives for healing human African trypanosomiasis (the so-called sleeping sickness) and Chagas disease (American trypanosomiasis), which are the deadliest parasitic diseases among poor communities. These diseases were caused by *Trypanosoma* protists which are transmitted by insects [96]. For this reason, we have decided to create a small building-blocks library of pyrazolo[4,3-*c*]pyridine which could be used for further transformation in the future.

The synthesis started from pyrazolo[4,3-*c*]pyridine reaction condition optimization. Firstly, mixed-Claisen condensation product **33** [96] was obtained via a procedure described in literature [97] from the starting material *tert*-butyl 4-oxopiperidine-1-carboxylate (**32**) (*Scheme 2.13*). Later, intermediate **33** without further purification was cyclized with methyl hydrazine in various solvents (*Table 2.3*). When the reaction mixture was heated in acetic acid, the desired regioisomers **35** and **36** did not form (*Table 2.3*, *Entry 1*). When acetic acid was replaced with ethanol, two separable regioisomers **36** and **37** were formed at a ratio of approximately 1:1.5 (*Table 2.3*, *Entry 2*). Combining ethanol with acetic acid did not increase the yields (*Table 2.3*, *Entry 3*).

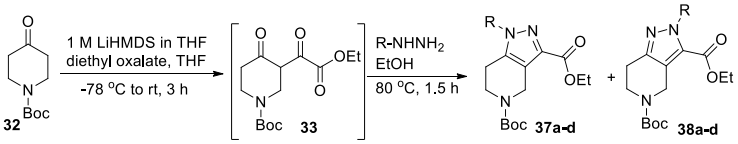


Scheme 2.13 Synthesis of pyrazolo[4,3-*c*]pyridines **35** and **36**

Table 2.3. Optimization of reaction conditions for construction of pyrazolo[4,3-*c*]pyridines **35** and **36**

Entry	Conditions	**35**, yield, %	**36**, yield, %
1.	AcOH, 80 ºC, 1.5 h	-	-
2.	EtOH, 80 ºC, 1.5 h	31	49
3.	EtOH/AcOH = 4/0.1, 80 ºC, 1.5 h	28	43

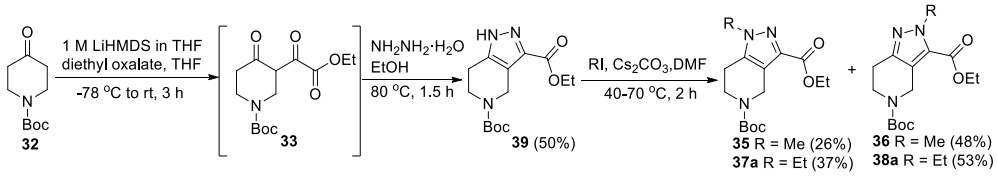
With the optimal cyclization condition being available, further pyrazolo[4,3-*c*]pyridines **37a-d** and **38a-d** were formed (*Scheme 2.14*). When cyclization was carried out with cyclohexylhydrazine, only one product **37c** was obtained at 15% yield. In this case, the expected regioisomers **37a-d** and **38a**, **b**, **d** were obtained at much lower yields. Only products **37a** and **38d** were isolated at 23% yield, while the values of other products ranged from 2% to 15%.



	a	b	c	d
R				
37, yield, %	23	4	15	15
38, yield, %	2	6	-	23

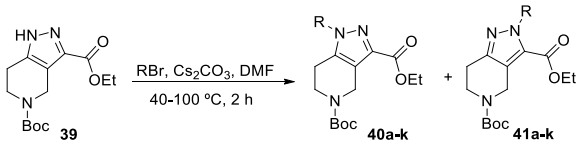
Scheme 2.14 Synthesis of pyrazolo[4,3-*c*]pyridines **37a-d** and **38a**, **b**, **d**

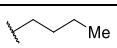
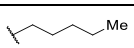
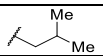
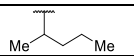
It was therefore decided to change the synthesis strategy: first of all, to synthesize 5-(*tert*-butyl) 3-ethyl 1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (**39**), and then perform *N*-alkylation reactions with methyl and ethyl iodides (*Scheme 2.15*). The synthesis of the common intermediate **39** started with mixed-Claisen condensation affording **33** and the subsequent pyrazole ring formation with hydrazine hydrate in EtOH. Later, the treatment of pyrazolo[4,3-*c*]pyridine **39** with methyl and ethyl iodides in the presence of Cs_2CO_3 in DMF [98] afforded a mixture of separable regioisomers **35** and **36** or **37a** and **38a**. The yields of major regioisomers **36** and **38a** were higher than 48%, while the yields of minor products **35** and **37a** did not exceed 37%. The synthesis of four-step pyrazolo[4,3-*c*]pyridines gave a better result than the three-step synthesis of pyrazole employing hydrazide cyclization.



Scheme 2.15 Synthesis of pyrazolo[4,3-*c*]pyridines **35**, **36**, **37a** and **38a**

With the targeted 5-(*tert*-butyl) 3-ethyl 1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (**39**) being available, we started *N*-alkylation reactions with a different type of alkyl halogenides (*Scheme 2.16*). The treatment of pyrazolo[4,3-*c*]pyridine **39** with alkyl halide in the presence of Cs_2CO_3 in DMF afforded a mixture of two separable regioisomers **40a-j** and **41a-j** at a ratio of approximately 1:2, except for **40**, **41k** which exhibited a ratio of 1:1. The major products **41a-k** were formed at N-2 nitrogen of the starting substrate.



	a	**b**	**c**	**d**	**e**	**f**
R						
temperature, °C	70	100	100	40	90	100
40, yield, %	19	30	30	23	28	20
41, yield, %	49	57	48	46	44	53
	g	**h**	**i**	**j**	**k**	
R						
temperature, °C	100	100	100	100	80	
40, yield, %	29	31	29	31	26	
41, yield, %	50	43	53	44	28	

Scheme 2.16 Synthesis of pyrazolo[4,3-*c*]pyridines **40a-k** and **41a-k**

Regioisomers **35**, **36**, **37a-d**, **38a**, **b**, **d**, **40a-k** and **41a-k** were identified by using the ^{1}H,^{1}H-NOESY, ^{1}H, ^{13}C-HMBC or ^{1}H, ^{15}N-HMBC spectrometry. For example, the protons of the methyl group of compound **35** at 3.83 ppm have NOE with the pyrazolo[4,3-*c*]pyridine ring methylene proton 7-H$_a$ at 2.68 ppm (*Figure 2.10*). Moreover, the ^{1}H, ^{13}C-HMBC experiment revealed that the aforementioned protons of the methyl group have three-bond correlation with the pyrazolo[4,3-*c*]pyridine C-7a carbon at 138.8 ppm. Meanwhile, the ^{1}H, ^{13}C-HMBC spectrum of compound **36** demonstrated pyrazolo[4,3-*c*]pyridine C-3 carbon three-bond interaction with the protons of the methyl group at 4.09 ppm and four-bond interaction with the protons of the ethyl group at 4.31 ppm. The ^{1}H, ^{15}N-HMBC spectrum showed strong three-bond connectivity of the methyl group protons with the N-1 nitrogen at -72.0 ppm and the N-2 nitrogen at -181.8 ppm.

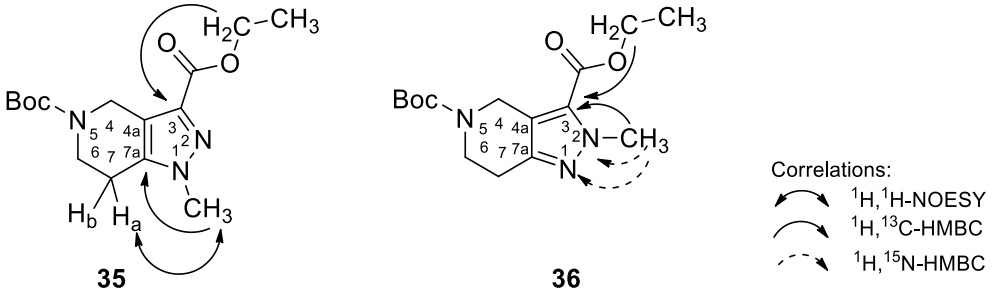


Figure 2.10 Relevant ^{1}H, ^{1}H-NOESY, ^{1}H, ^{13}C-HMBC and ^{1}H, ^{15}N-HMBC correlations of compounds **35** and **36**

All the obtained regioisomers are easily separable by column chromatography. The identification of regioisomers was carried out by such NMR spectroscopy

techniques as the ^{1}H, ^{1}H-NOESY, ^{1}H, ^{13}C-HMBC and the ^{1}H, ^{15}N-HMBC experiments.

2.3. Synthesis of indole derivatives and investigation of biological activity

Zhang with colleagues demonstrated that combining the indole core with 1,3,4-oxadiazole can yield 5-alkyl- or 5-aryl-substituted indole-1,3,4-oxadiazole **VII** which manifest antifungal activity (*Figure 2.11*) [99]. Meanwhile, Song *et al*. obtained amino-bridge 1,3,4-oxadiazoles derivatives **VIII** which exhibit anticonvulsant activity [100]. The biological activity of the derivatives with a sulfur-bridge are interesting, but they have not been sufficiently tested yet. For example, compound **IX** showed antifungal activity [101], whereas compound **X** delivers antitumor activity [102].

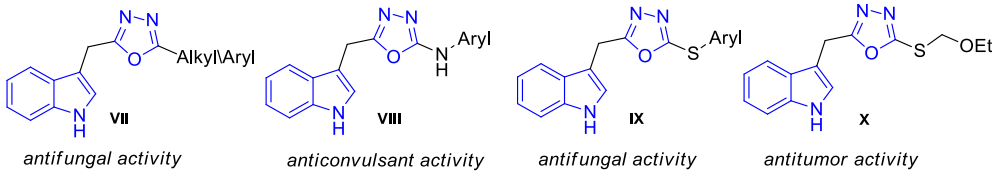
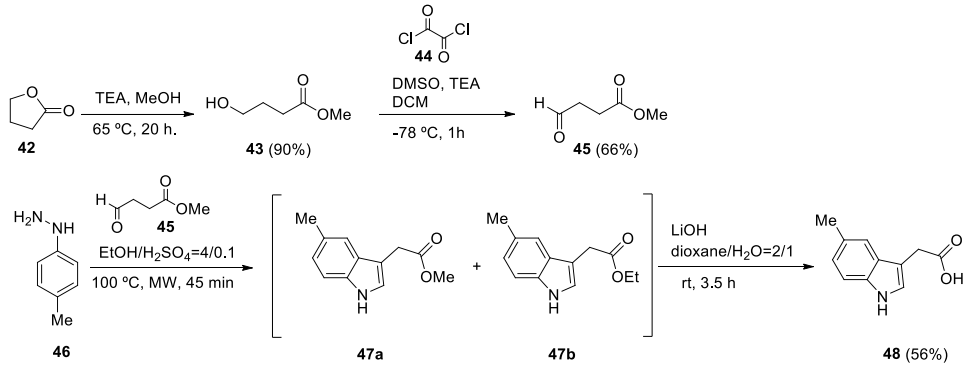


Figure 2.11 Biologically active compounds with indole and 1,3,4-oxadiazole fragments.

2.3.1. Synthesis of 2-[(1*H*-indol-3-yl)methyl]-5-(alkylthio)-1,3,4-oxadiazoles

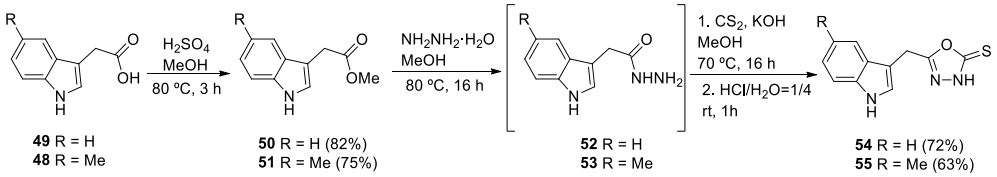
By taking one part of my Master's thesis, it was decided to transform plant hormone 3-indoleacetic acid [103] and its 5-methyl derivative to 2-[(1*H*-indol-3-yl)methyl]-5-(alkylthio)-1,3,4-oxadiazoles as these compounds may offer protective activity against oxidative stress [52].

The synthesis was started from the preparation of 5-methyl-3-indoleacetic acid **48** (*Scheme 2.17*). According to literature, *γ*-butyrolactone **42** ring was opened by using methanol and TEA [104]. The previously obtained alcohol **43** was used in the Swern oxidation reaction where the primary or secondary alcohol was transformed to aldehyde by using oxalyl chloride and absolute DMSO [105]. Aldoester **45** was used immediately for the Fischer indole synthesis, during which, 4-methylphenyl hydrazine **46** was condensed with aldoester **45** in a mixture of ethanol and H_2SO_4 (4/0.1, v/v) under microwave irradiation to get a mixture of methyl and ethyl esters **47a** and **47b**. Later, a mixture of esters **47a** and **47b** was hydrolyzed with LiOH in a mixture of dioxane and water (2/1, v/v), and the obtained desired product **48** was purified with column chromatography.



Scheme 2.17 Synthesis of 5-methyl-3-indoleacetic acid **48**

The previously obtained 5-methyl-3-indoleacetic acid **48** and 3-indolyl acetic acid **49** were used as starting materials to obtain 5-[(1*H*-indol-3-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thiones **54** and **55** (*Scheme 2.18*). The synthesis started from 3-indolyl acetic acid **48** and **49** transformations to esters **50** and **51**, respectively, by heating in a mixture of H_2SO_4 and methanol. Hydrazide could be obtained by heating ester with hydrazine hydrate in methanol [106] or ethanol [107]. In this work, acetates **50** and **51** were reacted with hydrazine hydrate in methanol to get intermediates **52** and **53** which were used in the subsequent reaction without purification. Later, hydrazides **52** and **53**, *via* an adapted procedure described in literature [101] were reacted with carbon disulfide in the presence of KOH in methanol, and, after 16 hours, the reaction mixture was acidified by a mixture of hydrochloric acid and water to obtain the corresponding 1,3,4-oxadiazoles **54** and **55** in good yields.



Scheme 2.18 Synthesis of 1,3,4-oxadiazole-2(3*H*)-thiones **54** and **55**

It is known that the 1,3,4-oxadiazole derivative has two possible tautomers – thione (**T1**) and a relatively stable thiol (**T2**) (*Figure 2.12*) [108]. Usually, one of the tautomer forms is major [109]. In this work, the predominant tautomer is thione-tautomer (**T1**). This statement was confirmed by NMR and IR spectroscopy. The ^{13}C NMR spectrum of compound **54** showed the C=S carbon signal at 177.8 ppm, while the ^{13}C NMR spectrum of compound **56a** exhibited the shift of the 1,3,4-oxadiazole ring C-5 carbon at 167.0 ppm. Moreover, the IR absorption spectrum of compound **54** exhibited the amino group (NH) absorption band at 3393 cm^{-1}, while the thiol group **T2** (SH) absorption band was not detected at all.

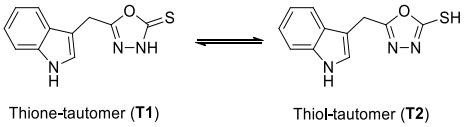


Figure 2.12 Two possible tautomer structures of compound **55**

1,3,4-Oxadiazole derivatives can be used in the *S*-alkylation reaction [101] and the reaction with the Mannich base [110]. The *S*-Alkylation reaction can be carried out with some bases, such as NaH [111], TEA [101] and K_2CO_3 [112]. 1,3,4-oxadiazole-2(3*H*)-thiones **54** and **55** were coupled with alkyl halide in the presence of K_2CO_3 in DMF at ambient temperature to get the corresponding products **56a-i** and **57a-e**, respectively, in high yields (63–91%) (*Scheme 2.19*).

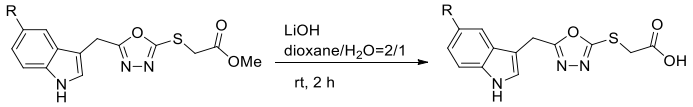
54 R = H
55 R = Me

56a-i R = H
57a-e R = Me

	a	**b**	**c**	**d**	**e**
R^1	Me	Me	OH	OMe	
56, yield, %	88	65	79	91	90
57, yield, %	90	85	68	83	64
	f	**g**	**h**	**i**	
R^1	Me	Me	OMe	F	
56, yield, %	71	66	77	63	

Scheme 2.19 Synthesis of 1,3,4-oxadiazole derivatives **56a-i** and **57a-e**

Finally, the polarity of compounds **56d** and **57d** was increased by simple alkaline conditions (*Scheme 2.20*). Esters **56d** and **57d** were hydrolized with LiOH in a mixture of dioxane and water (2/1, v/v) to obtain carboxylic acids **58** and **59**, respectively.



56d R = H
57d R = Me

58 R = H (73%)
59 R = Me (47%)

Scheme 2.20 Synthesis of carboxylic acids **58** and **59**

To conclude, in this work, hydrazides **52** and **53** were transformed to 1,3,4-oxadiazole-2(3*H*)-thiones **54** and **55**. 1,3,4-oxadiazole-2(3*H*)-thiones **54** and **55** could be used in the *S*-alkylation reaction to make a small library of 2-[(1*H*-indol-3-yl)methyl]-5-(alkylthio)-1,3,4-oxadiazoles **56a-i** and **57a-e**.

2.3.2. Investigation of the antioxidant properties of prepared 1,3,4-oxadiazole derivatives

The antioxidant properties and, subsequently, the cytotoxicity of the prepared 5-[(1*H*-indol-3-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thiones **54**, **55**, and 2-[(1*H*-indol-3-yl)methyl]-5-(alkylthio)-1,3,4-oxadiazoles **56a-i**, **57a-e**, **58** and **59** were studied by our collaborators, Dr. Alena Kadlecová and Dr. Jiří Voller, at Palacký University Olomouc, the Czech Republic.

Oxidative stress is a result of an imbalance between the production and accumulation of reactive oxygen species (ROS) in a biological system [113]. Large amounts of ROS cause cell aging which is related with various diseases, such as cancer, diabetes, neurodegenerative disorders, etc. [114]. ROS removal is typically achieved by either natural detoxification mechanisms or antioxidants [115]. As the natural detoxification mechanisms are not always able to cope with excess ROS and as natural antioxidants are giving somewhat disappointing clinical results [116], new types of synthetic antioxidants with improved properties are in high demand. Oxadiazoles are well known for their antioxidant properties [117]. For instance, 2-aryl-5-(arylselenylmethylthio)-1,3,4-oxadiazole/thiadiazoles **XI** [118], 5-(thiomorpholin-4-ylmethyl)-1,3,4-oxadiazole-2(3*H*)-thione **XII** [119] and 6-bromo-3-(5-phenyl-1,3,4-oxadiazol-2-yl)-2*H*-chromen-2-one (**XIII**) [120] showed antioxidant activity against 2,2-diphenyl-2-picrylhydrazyl (DPPH) (*Figure 2.13*).

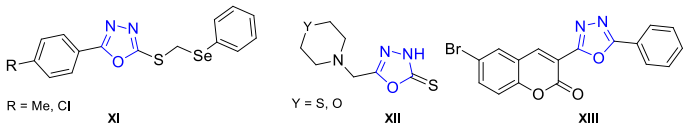
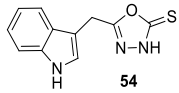
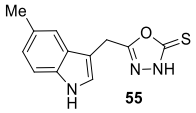
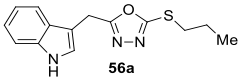
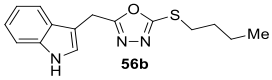
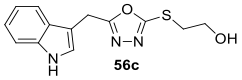
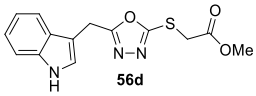
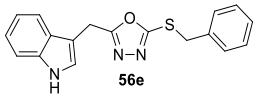
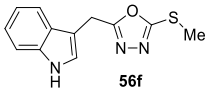
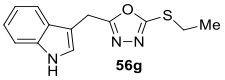
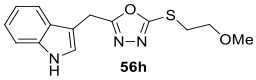
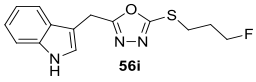
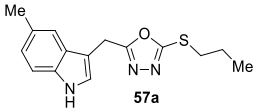
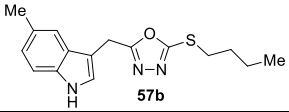
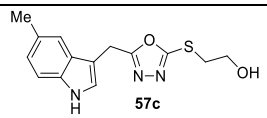
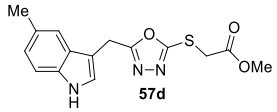
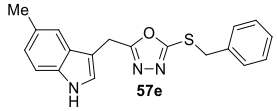
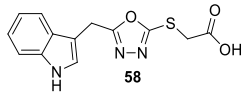
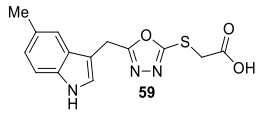


Figure 2.13 Biologically active compounds with 1,3,4-oxadiazole motifs

First of all, the antioxidant properties of the compounds were studied on nematode *Caenorhabditis elegans* which is denoted by high homology with human genes [121] and is therefore a useful model organism to study various aspects of human diseases [122]. 1,3,4-Oxadiazole derivatives **54**, **55**, **56a-i**, **57a-e**, **58** and **59** were tested for the ability to counteract oxidative stress induced by 500 µM concentration of 5-hydroxy-1,4-naphthoquinone (juglone). The effect of the compounds was evaluated at 5 µM and 50 µM concentrations, while curcumin was chosen as the positive control and was used at 100 µM concentration [123]. Three days-old nematodes were treated with a mixture of DMSO and, respectively, compounds **54**, **55**, **56a-i**, **57a-e**, **58** and **59** (5 µM or 50 µM concentration) or curcumin at 100 µM concentration and left to grow for further 3 days. After 3 days, the nematodes were treated with the reactive oxygen species generator 5-hydroxy-1,4-naphthoquinone (juglone) [124], and *C. elegans* motility was monitored by using wMicroTracker. At 5 µM concentration, six 1,3,4-oxadiazoles, namely, **56b**, **56f**, **56h**, **56i** and **57b**, **e**, were able to increase *C. elegans* survival by more than 5%, thereby confirming the antioxidant properties of the compounds (*Table 2.4*).

Table 2.4. Effect of compounds **54**, **55**, **56a-i**, **57a-e**, **58** and **59** on the motility of *C. elegans* populations subjected to 500 µM concentration of 5-hydroxy-1,4-naphthoquinone (juglone) induced oxidative stress (measured by wMicroTracker). The values represent mean ± SEM from 3 independent experiments

Compound	% mean change in motility ± SEM		
	5 µM	50 µM	100 µM
 **54**	4.5 ± 2.7	1.5 ± 2.2	n.d.
 **55**	0.9 ± 2.0	1.5 ± 3.1	n.d.
 **56a**	6.5 ± 4.4	9.6 ± 4.7	n.d.
 **56b**	8.8 ± 3.7	7.5 ± 3.8	n.d.
 **56c**	3.6 ± 3.8	-0.4 ± 1.8	n.d.
 **56d**	3.4 ± 3.0	0.0 ± 0.8	n.d.
 **56e**	5.5 ± 4.2	13.7 ± 4.4	n.d.
 **56f**	11.6 ± 0.7	5.7 ± 0.7	n.d.
 **56g**	2.9 ± 2.9	9.4 ± 2.6	n.d.
 **56h**	8.5 ± 0.5	11.7 ± 0.9	n.d.
 **56i**	9.6 ± 0.6	8.8 ± 1.1	n.d.
 **57a**	5.9 ± 2.0	7.3 ± 2.6	n.d.
 **57b**	9.3 ± 1.0	9.4 ± 3.0	n.d.

 **57c**	7.2 ± 2.9	-0.2 ± 1.9	n.d.
 **57d**	1.1 ± 1.2	4.2 ± 1.0	n.d.
 **57e**	11.5 ± 2.5	-1.8 ± 3.5	n.d.
 **58**	1.9 ± 1.0	-0.2 ± 1.2	n.d.
 **59**	3.8 ± 1.6	2.5 ± 0.6	n.d.
curcumin	n.d.	n.d.	11.7 ± 1.6

n.d. – not determined

Interestingly, while, for some derivatives, the antioxidant properties at 50 μM concentration increased, some of them suffered a decline in the antioxidant effect. It was therefore concluded that the investigated compounds could feature antioxidant properties at low concentrations, while, at higher concentrations, they can be toxic by contributing to the oxidative stress themselves.

Oxidative damage and mitochondrial dysfunction are some of the main causes of neurodegenerative diseases, such as Alzheimer and Parkinson diseases, as well as the lesser-known Friedreich ataxia [125]. Friedreich ataxia, which is caused by a mutation in the first intron of the frataxin gene [126], causes progressive degeneration of the central and peripheral nervous systems, cardiomyopathy, and increased incidence of diabetes mellitus, which results in walking difficulties over time, loss of sensation in the arms and legs, and impaired speech [127]. Friedreich ataxia is currently untreatable, and all the therapies are only limited to the treatment of the symptoms [128].

As the prepared 1,3,4-oxadiazole derivatives showed promising antioxidant properties in *C. elegans*, they were also screened for the cytoprotective effect in human cells against the oxidative stress induced by glutathione synthesis inhibitor L-buthionine sulfoximine (BSO) at 100 μM concentration. Skin fibroblasts GM03816 and GM04078 derived from Friedreich ataxia patients were treated with compounds **54**, **55**, **56a-i**, **57a-e**, **58** and **59** (at 10 μM or 50 μM concentrations) for 8 hours. Subsequently, the samples were exposed to BSO at 100 μM concentration for 24 hours. The resazurin test showed protective effects of compounds **57a**, **b** and **57e** which were further screened across a wider concentration range (*Figure 2.14*). Among the three, 1,3,4-oxadiazole **57e** showed protective activity similar or superior to that of a known antioxidant *R*-lipoic acid (LA).

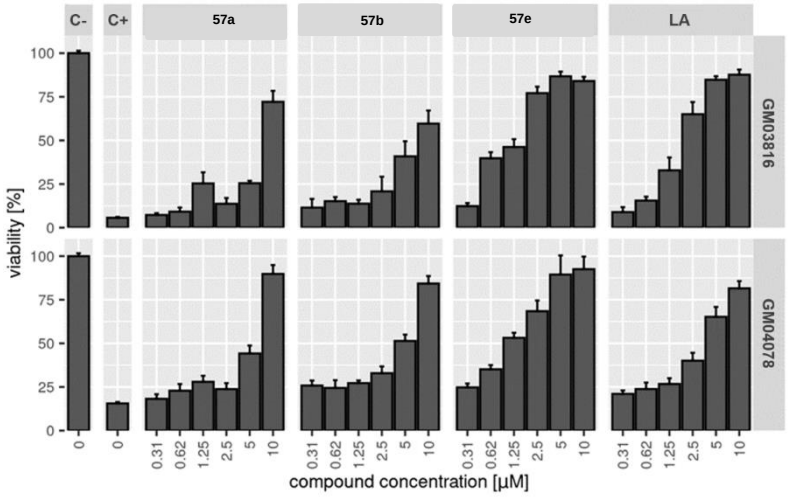


Figure 2.14 Protective effects evaluation of 1,3,4-oxadiazoles **57a**, **b** and **57e** against L-buthionine sulfoximine (BSO) in FA fibroblasts. Concentrations of the compounds during 6-hour pre-treatment are shown. In the following 24-hour BSO treatment, compound concentrations were two thirds of the initial value due to dilution after BSO addition. C-control FA fibroblasts without BSO. C+ control. FA fibroblasts with BSO. LA – *R*-lipoic acid. Error bars are SEM

It is noteworthy that active compounds **57a**, **b** and **57e** were proven to be generally nontoxic to FA fibroblasts and other noncancer human cell lines by the resazurin test (*Figure 2.15*). Only in some cases was the cell viability reduced to 80% at 60 µM concentration.

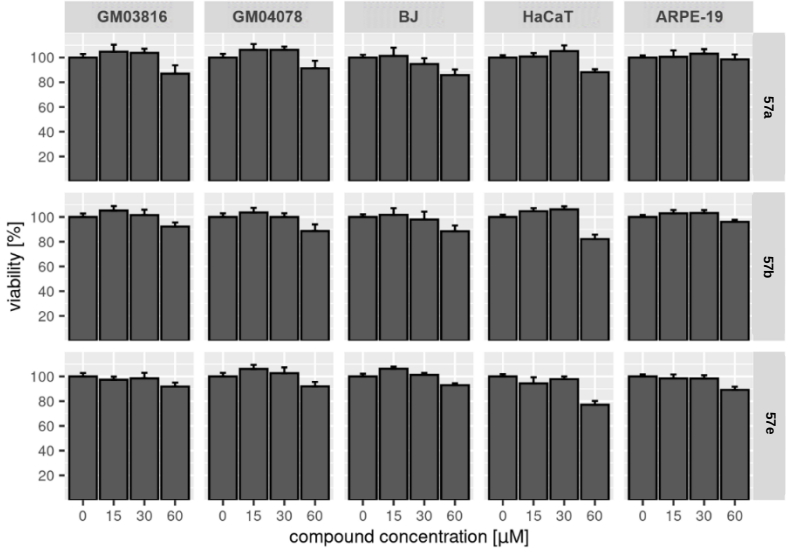


Figure 2.15 Toxicity evaluation of compounds **57a**, **b** and **57e** after 48-hour exposure. The error bars are SEM

3. EXPERIMENTAL PART

3.1. Instrumentation

The microwave reactions were conducted by using a *CEM Discover Synthesis Unit* (*CEM Corp*., Matthews, NC) and performed in glass vessels (capacity: 10 ml) sealed with a septum. The pressure was controlled by a load cell connected to the vessel. The temperature of the contents of the vessel was monitored by using a calibrated infrared temperature control mounted under the reaction vessel. All the experiments were performed with stirring. The melting points were determined on a *Büchi M-565* melting point apparatus and were left uncorrected. The mass spectra were obtained on a *Shimadzu LCMS 2020 Single Quadrupole Liquid Chromatograph Mass Spectrometer*. The IR spectra were recorded on a *Bruker Tensor 27* spectrometer and are reported in frequency of absorption (cm^{-1}) or on a *Bruker Vertex v70* FTIR spectrometer equipped with a diamond ATR accessory. The HRMS spectra were recorded with a *Bruker micrOTOF-QIII* spectrometer. The ^{1}H NMR, ^{13}C NMR and ^{15}N NMR spectra were recorded from $CDCl_3$ and DMSO-d_6 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) when using a directly detecting BBFO probe. The solvent (residual) signals were used as internal standards and were related to TMS, $CDCl_3$ at δ 7.26 ppm (^{1}H) and δ 77.00 ppm (^{13}C) and DMSO-d_6 at δ 2.50 ppm (^{1}H) and δ 39.52 ppm (^{13}C). The ^{15}N NMR spectra were referenced against neat, external nitromethane. The full and unambiguous assignments of the ^{1}H, ^{13}C and ^{15}N NMR resonances were achieved by using combined applications of standard NMR spectroscopic techniques, such as COSY, NOESY, gs-HSQC and gs-HMBC. The purification of the reaction mixtures was performed by using flash chromatography on a glass column with silica gel (high-purity grade, *Merck* grade 9385, pore size 60 Å, 230–400 mesh particle size). For thin layer chromatography (TLC), *Merck* pre-coated TLC plates (Silica gel 60 F254) were employed.

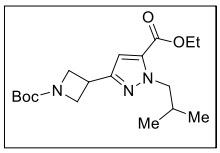
3.2. Materials

All the chemicals were bought and used as received without further purification from *Sigma-Aldrich*, *Fluorochem* and *Combi-Blocks*. Organic solvents were purified and dried by the standard methods.

3.2.1 General procedure for alkylation of ethyl 5-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1*H*-pyrazole-3-carboxylate (4) with haloalkanes

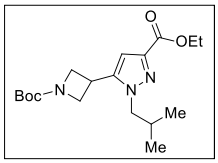
To a solution of compound **4** (885 mg, 3 mmol) in dimethylformamide (6 mL), the appropriate haloalkane (3.3 mmol) and Cs_2CO_3 (1300 mg, 3.99 mmol) were added, and the reaction mixture was heated at the desired temperature (40–100 ºC) for 2 h. After cooling to rt, the reaction mixture was poured into water (20 mL), and the solution was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography while using an appropriate eluent.

Ethyl 3-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1-isobutyl-1*H*-pyrazole-5-carboxylate (5a)



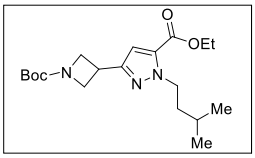
1-Bromo-2-methylpropane (452 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 90 ºC. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 958 mg (91%), colorless oil. R_f = 0.54 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 0.87 (d, J = 6.7 Hz, 6H, 2 × CH_3), 1.37 (t, J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.45 (s, 9H, $C(CH_3)_3$), 2.13–2.23 (m, 1H, CH), 3.74–3.82 (m, 1H, Az 3-H), 3.99 (dd, J = 8.4, 6.1 Hz, 2H, Az 2,4-H_a), 4.24–4.36 (m, 6H, $OC\underline{H}_2CH_3$, CH_2, Az 2,4-H_b), 6.80 (s, 1H, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 ($OCH_2\underline{C}H_3$), 19.9 (2 × CH_3), 27.4 (Az C-3), 28.6 ($C(\underline{C}H_3)_3$), 30.1 (CH), 55.7 (Az C-2,4), 58.5 (CH_2), 61.1 ($O\underline{C}H_2CH_3$), 79.6 ($\underline{C}(CH_3)_3$), 108.6 (C-4), 133.4 (C-5), 151.8 (C-3), 156.5 (Boc C=O), 159.8 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −310.1 (Az N-1), −167.7 (N-1), −63.0 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1700 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 374.2051. $[C_{18}H_{29}N_3O_4+Na]^+$ requires 374.2050.

Ethyl 5-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1-isobutyl-1*H*-pyrazole-3-carboxylate (6a)



Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (1/1, v/v). Yield 214 mg (9%), colorless oil. R_f = 0.14 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 0.86 (d, J = 6.7 Hz, 6H, 2 × CH_3), 1.38 (t, J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.45 (s, 9H, $C(CH_3)_3$), 2.16–2.26 (m, 1H, CH), 3.72–3.84 (m, 3H, Az 3-H, CH_2), 3.95 (dd, J = 8.2, 6.4 Hz, 2H, Az 2,4-H_a), 4.31 (t, J = 8.5, 2H, Az 2,4-H_b), 4.39 (q, J = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 6.80 (s, 1H, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.6 ($OCH_2\underline{C}H_3$), 20.0 (2 × CH_3), 24.9 (Az C-3), 28.5 ($C(\underline{C}H_3)_3$), 29.7 (CH), 55.4 (Az C-2,4), 57.3 (CH_2), 61.1 ($O\underline{C}H_2CH_3$), 80.2 ($\underline{C}(CH_3)_3$), 106.3 (C-4), 143.0 (C-3), 145.0 (C-5), 156.2 (Boc C=O), 162.4 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −312.3 (Az N-1), −166.8 (N-1), −68.2 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1693 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 374.2050. $[C_{18}H_{29}N_3O_4+Na]^+$ requires 374.2050.

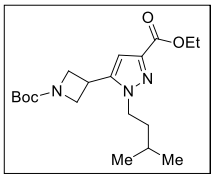
Ethyl 3-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1-isopentyl-1*H*-pyrazole-5-carboxylate (5b)



1-Bromo-3-methylbutane (498 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 100 ºC. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 843 mg (77%), colorless oil. R_f = 0.63 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 0.95 (d, J = 6.5 Hz, 6H, 2 × CH_3), 1.38 (t, J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.45 (s, 9H, $C(CH_3)_3$), 1.58–1.75 (m, 3H, CH, CH_2), 3.72–3.83 (m, 1H, Az 3-H), 3.99 (dd, J = 8.4, 6.1 Hz, 2H, Az 2,4-H_a), 4.28 (t, J = 8.6 Hz, 2H, Az 2,4-H_b), 4.31–4.38 (m, 2H, $OC\underline{H}_2CH_3$), 4.50 (dd, J = 8.7, 6.8 Hz, 2H, CH_2), 6.80 (s, 1H, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 ($OCH_2\underline{C}H_3$), 22.6 (2 × CH_3), 26.1 (CH), 27.4 (Az C-3), 28.6 ($C(\underline{C}H_3)_3$), 39.6 (CH_2), 50.5 (CH_2), 55.7 (Az C-

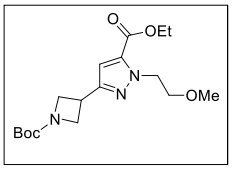
2,4), 61.1 (O<u>C</u>H$_2$CH$_3$), 79.6 (<u>C</u>(CH$_3$)$_3$), 108.6 (C-4), 133.0 (C-5), 151.8 (C-3), 156.5 (Boc C=O), 159.7 (C=O). ^{15}N NMR (41 MHz, CDCl$_3$): δ_N ppm −310.9 (Az N-1), −166.7 (N-1), −64.5 (N-2). IR (neat, ῡ, cm^{-1}): 1701 (C=O). HRMS (ESI TOF): [M+H]$^+$, found 366.2368. [C$_{19}$H$_{31}$N$_3$O$_4$+H]$^+$ requires 366.2387.

Ethyl 5-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1-isopentyl-1*H*-pyrazole-3-carboxylate (6b*)*



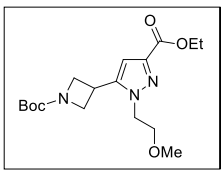
Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (2/1, v/v). Yield 169 mg (15%), colorless oil. R_f = 0.24 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, CDCl$_3$): δ_H ppm 0.93 (d, *J* = 6.3 Hz, 6H, 2 × CH$_3$), 1.37 (t, *J* = 7.1 Hz, 3H, OCH$_2$C<u>H</u>$_3$), 1.44 (s, 9H, C(CH$_3$)$_3$), 1.55–1.69 (m, 3H, CH, CH$_2$), 3.71–3.81 (m, 1H, Az 3-H), 3.99 (t, *J* = 7.2 Hz, 2H, Az 2,4-H$_a$), 3.99–4.06 (m, 2H, CH$_2$), 4.32 (t, *J* = 8.5 Hz, 2H, Az 2,4-H$_b$), 4.38 (q, *J* = 7.1 Hz, 2H, OC<u>H</u>$_2$CH$_3$), 6.77 (s, 1H, 4-H). ^{13}C NMR (100 MHz, CDCl$_3$): δ_C ppm 14.5 (OCH$_2$<u>C</u>H$_3$), 22.5 (2 × CH$_3$), 24.8 (Az C-3), 26.1 (CH), 28.5 (C(<u>C</u>H$_3$)$_3$), 39.4 (CH$_2$), 49.0 (CH$_2$), 55.1 (Az C-2,4), 61.0 (O<u>C</u>H$_2$CH$_3$), 80.2 (<u>C</u>(CH$_3$)$_3$), 106.6 (C-4), 142.9 (C-3), 144.2 (C-5), 156.2 (Boc C=O), 162.4 (C=O). ^{15}N NMR (41 MHz, CDCl$_3$): δ_N ppm −311.1 (Az N-1), −165.1 (N-1), −68.5 (N-2). IR (neat, ῡ, cm^{-1}): 1701 (C=O). HRMS (ESI TOF): [M+H]$^+$, found 366.2368. [C$_{19}$H$_{31}$N$_3$O$_4$+H]$^+$ requires 366.2387.

Ethyl 3-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1-(2-methoxyethyl)-1*H*-pyrazole-5-carboxylate (5c)



1-Bromo-2-methoxyethane (459 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 40 ºC. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (2/1, v/v). Yield 666 mg (63%), colorless oil. R_f = 0.20 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, CDCl$_3$): δ_H ppm 1.37 (t, *J* = 7.1 Hz, 3H, OCH$_2$C<u>H</u>$_3$), 1.45 (s, 9H, C(CH$_3$)$_3$), 3.31 (s, 3H, OCH$_3$), 3.72–3.85 (m, 3H, Az 3-H, CH$_2$), 3.99 (dd, *J* = 8.3, 6.2 Hz, 2H, Az 2,4-H$_a$), 4.23–4.38 (m, 4H, OC<u>H</u>$_2$CH$_3$, Az 2,4-H$_b$), 4.70 (t, *J* = 5.7 Hz, 2H, CH$_2$), 6.81 (s, 1H, 4-H). ^{13}C NMR (100 MHz, CDCl$_3$): δ_C ppm 14.4 (OCH$_2$<u>C</u>H$_3$), 27.4 (Az C-3), 28.5 (C(<u>C</u>H$_3$)$_3$), 50.9 (CH$_2$), 55.7 (Az C-2,4), 59.0 (OCH$_3$), 61.2 (O<u>C</u>H$_2$CH$_3$), 71.5 (CH$_2$), 79.6 (<u>C</u>(CH$_3$)$_3$), 108.7 (C-4), 133.8 (C-5), 152.3 (C-3), 156.5 (Boc C=O), 159.8 (C=O). ^{15}N NMR (41 MHz, CDCl$_3$): δ_N ppm −310.5 (Az N-1), −173.6 (N-1), −64.2 (N-2). IR (neat, ῡ, cm^{-1}): 1694 (C=O). HRMS (ESI TOF): [M+H]$^+$, found 354.2015. [C$_{17}$H$_{27}$N$_3$O$_5$+H]$^+$ requires 354.2023.

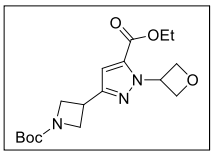
Ethyl 5-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1-(2-methoxyethyl)-1*H*-pyrazole-3-carboxylate (6c)



Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (2/1, v/v). Yield 150 mg (14%), yellow oil. R_f = 0.02 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, CDCl$_3$): δ_H ppm 1.38 (t, *J* = 7.1 Hz, 3H, OCH$_2$C<u>H</u>$_3$), 1.45 (s, 9H, C(CH$_3$)$_3$), 3.23 (s, 3H, OCH$_3$), 3.67 (t, *J* = 4.9 Hz, 2H, CH$_2$), 3.83–3.96 (m, 3H, Az 3-H, Az 2,4-H$_a$), 4.20 (t, *J* = 4.9 Hz, 2H,

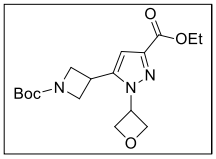
OC<u>H</u>$_2$CH$_3$), 4.28 (t, J = 8.2 Hz, 2H, Az 2,4-H$_b$), 4.39 (q, J = 7.1 Hz, 2H, CH$_2$), 6.79 (s, 1H, 4-H). ^{13}C NMR (100 MHz, CDCl$_3$): δ_C ppm 14.6 (OCH$_2$<u>C</u>H$_3$), 25.0 (Az C-3), 28.5 (C(<u>C</u>H$_3$)$_3$), 50.6 (CH$_2$), 55.5 (Az C-2,4), 59.2 (OCH$_3$), 61.1 (O<u>C</u>H$_2$CH$_3$), 71.6 (CH$_2$), 80.0 (<u>C</u>(CH$_3$)$_3$), 106.2 (C-4), 143.4 (C-3), 146.5 (C-5), 156.3 (Boc C=O), 162.4 (C=O). ^{15}N NMR (41 MHz, CDCl$_3$): δ_N ppm −311.8 (Az N-1), −170.1 (N-1), −70.4 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1698 (C=O). HRMS (ESI TOF): [M+H]$^+$, found 354.2018. [C$_{17}$H$_{27}$N$_3$O$_5$+H]$^+$ requires 354.2023.

Ethyl 3-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1-(oxetan-3-yl)-1*H*-pyrazole-5-carboxylate (5d)



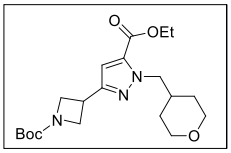
3-Bromooxetane (452 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 40 ºC. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 732 mg (70%), white solid, mp 76–77 ºC (ethyl acetate). R_f = 0.50 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, CDCl$_3$): δ_H ppm 1.36 (t, J = 7.1 Hz, 3H, OCH$_2$C<u>H</u>$_3$), 1.46 (s, 9H, C(CH$_3$)$_3$), 3.79–3.88 (m, 1H, Az 3-H), 4.03 (dd, J = 8.4, 6.1 Hz, 2H, Az 2,4-H$_a$), 4.28–4.34 (m, 4H, OC<u>H</u>$_2$CH$_3$, Az 2,4-H$_b$), 5.00 (t, J = 7.2 Hz, 2H, CH$_2$), 5.14 (t, J = 6.5 Hz, 2H, CH$_2$), 6.08–6.18 (m, 1H, CH), 6.85 (s, 1H, 4-H). ^{13}C NMR (100 MHz, CDCl$_3$): δ_C ppm 14.3 (OCH$_2$<u>C</u>H$_3$), 27.5 (Az C-3), 28.5 (C(<u>C</u>H$_3$)$_3$), 54.2 (CH), 55.6 (Az C-2,4), 61.4 (O<u>C</u>H$_2$CH$_3$), 76.7 (2 × CH$_2$), 79.7 (<u>C</u>(CH$_3$)$_3$), 109.4 (C-4), 133.4 (C-5), 152.6 (C-3), 156.5 (Boc C=O), 159.6 (C=O). ^{15}N NMR (41 MHz, CDCl$_3$): δ_N ppm −310.5 (Az N-1), −169.3 (N-1), −69.9 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1694 (C=O). HRMS (ESI TOF): [M+Na]$^+$, found 374.1686. [C$_{17}$H$_{25}$N$_3$O$_5$+Na]$^+$ requires 374.1686.

Ethyl 5-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1-(oxetan-3-yl)-1*H*-pyrazole-3-carboxylate (6d)



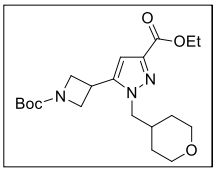
Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (1/1, v/v). Yield 112 mg (11%), colorless oil. R_f = 0.05 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, CDCl$_3$): δ_H ppm 1.40 (t, J = 7.1 Hz, 3H, OCH$_2$C<u>H</u>$_3$), 1.44 (s, 9H, C(CH$_3$)$_3$), 3.66–3.76 (m, 1H, Az 3-H), 3.92 (dd, J = 8.2, 6.2 Hz, 2H, Az 2,4-H$_a$), 4.32 (t, J = 8.5 Hz, 2H, Az 2,4-H$_b$), 4.40 (q, J = 7.1 Hz, 2H, OC<u>H</u>$_2$CH$_3$), 4.92 (t, J = 7.0 Hz, 2H, CH$_2$), 5.51 (t, J = 6.5 Hz, 2H, CH$_2$), 5.25–5.33 (m, 1H, CH), 6.80 (s, 1H, 4-H). ^{13}C NMR (100 MHz, CDCl$_3$): δ_C ppm 14.5 (OCH$_2$<u>C</u>H$_3$), 24.5 (Az C-3), 28.5 (C(<u>C</u>H$_3$)$_3$), 52.8 (CH), 54.7 (Az C-2,4), 61.3 (O<u>C</u>H$_2$CH$_3$), 76.6 (2 × CH$_2$), 80.4 (<u>C</u>(CH$_3$)$_3$), 107.3 (C-4), 143.9 (C-3), 144.7 (C-5), 156.1 (Boc C=O), 162.2 (C=O). ^{15}N NMR (41 MHz, CDCl$_3$): δ_N ppm −312.5 (Az N-1), −167.9 (N-1), −72.1 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1694 (C=O). HRMS (ESI TOF): [M+Na]$^+$, found 374.1686. [C$_{17}$H$_{25}$N$_3$O$_5$+Na]$^+$ requires 374.1686.

Ethyl 3-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1-[(tetrahydro-2*H*-pyran-4-yl)methyl]-1*H*-pyrazole-5-carboxylate (5e)



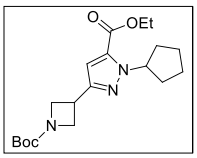
4-(Bromomethyl)tetrahydropyran (591 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 100 ºC. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (2/1, v/v).Yield 920 mg (78%), colorless solid, mp 67–68 ºC (ethyl acetate). R_f = 0.20 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.37 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.39–1.43 (m, 4H, 2 × CH_2), 1.44 (s, 9H, $C(CH_3)_3$), 2.06–2.17 (m, 1H, CH), 3.32 (td, *J* = 11.2, 4.1 Hz, 2H, CH_2), 3.72–3.80 (m, 1H, Az 3-H), 3.89–4.00 (m, 4H, CH_2, Az 2,4-H_a), 4.25–4.35 (m, 4H, $OC\underline{H}_2CH_3$, Az 2,4-H_b), 4.40 (d, *J* = 7.2 Hz, 2H, CH_2), 6.80 (s, 1H, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.3 ($OCH_2\underline{C}H_3$), 27.3 (Az C-3), 28.5 ($C(\underline{C}H_3)_3$), 30.4 (2 × CH_2), 36.6 (CH), 55.5 (Az C-2,4), 56.7 (CH_2), 61.2 ($O\underline{C}H_2CH_3$), 67.6 (CH_2), 79.6 ($\underline{C}(CH_3)_3$), 108.6 (C-4), 133.5 (C-5), 152.0 (C-3), 156.5 (Boc C=O), 159.8 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −310.5 (Az N-1), −170.7 (N-1), −63.2 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1697 (C=O). HRMS (ESI TOF): $[M+H]^+$, found 394.2321. $[C_{20}H_{31}N_3O_5+H]^+$ requires 394.2336.

Ethyl 5-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1-[(tetrahydro-2*H*-pyran-4-yl)methyl]-1*H*-pyrazole-3-carboxylate (6e)



Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (1/1, v/v).Yield 59 mg (5%), colorless oil. R_f = 0.03 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.18–1.35 (m, 7H, 2 × CH_2, $OCH_2C\underline{H}_3$), 1.39 (s, 9H, $C(CH_3)_3$), 2.09–2.23 (m, 1H, CH), 3.26 (t, *J* = 11.5 Hz, 2H, CH_2), 3.69–3.76 (m, 1H, Az 3-H), 3.79–3.92 (m, 6H, 2 × CH_2, Az 2,4-H_a), 4.21–4.40 (m, 4H, $OC\underline{H}_2CH_3$, Az 2,4-H_b), 6.74 (s, 1H, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 ($OCH_2\underline{C}H_3$), 24.7 (Az C-3), 28.4 ($C(\underline{C}H_3)_3$), 30.4 (2 × CH_2), 36.1 (CH), 55.2 (Az C-2,4), 55.3 (CH_2), 61.0 ($O\underline{C}H_2CH_3$), 67.3 (CH_2), 80.1 ($\underline{C}(CH_3)_3$), 106.2 (C-4), 143.2 (C-3), 145.2 (C-5), 156.1 (Boc C=O), 162.2 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1698 (C=O). HRMS (ESI TOF): $[M+H]^+$, found 394.2327. $[C_{20}H_{31}N_3O_5+H]^+$ requires 394.2336.

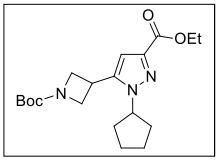
Ethyl 3-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1-cyclopentyl-1*H*-pyrazole-5-carboxylate (5f)



Bromocyclopentane (492 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 100 ºC. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 729 mg (67%), colorless oil. R_f = 0.70 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.37 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.45 (s, 9H, $C(CH_3)_3$), 1.63–1.70 (m, 2H, CH_2), 1.83–1.95 (m, 2H, CH_2), 1.97–2.12 (m, 4H, 2 × CH_2), 3.72–3.84 (m, 1H, Az 3-H), 3.99 (dd, *J* = 8.3, 6.1 Hz, 2H, Az 2,4-H_a), 4.25–4.35 (m, 4H, $OC\underline{H}_2CH_3$, Az 2,4-H_b), 5.57 (p, *J* = 7.4 Hz, 1H, CH), 6.78 (s, 1H, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 ($OCH_2\underline{C}H_3$), 24.7 (2 × CH_2), 27.5 (Az C-3), 28.6 ($C(\underline{C}H_3)_3$),

33.0 (2 × CH_2), 55.8 (Az C-2,4), 61.0 (O<u>C</u>H_2CH_3), 61.2 (CH), 79.9 (<u>C</u>$(CH_3)_3$), 108.5 (C-4), 133.2 (C-5), 151.5 (C-3), 156.6 (Boc C=O), 160.0 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −310.6 (Az N-1), −158.7 (N-1), −69.3 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1701 (C=O). HRMS (ESI TOF): $[M+H]^+$, found 364.2223. $[C_{19}H_{29}N_3O_4+H]^+$ requires 364.2231.

Ethyl 5-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1-cyclopentyl-1*H*-pyrazole-3-carboxylate (6f)

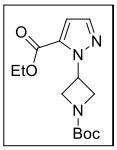


Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (2/1, v/v). Yield 152 mg (14%), yellow oil. R_f = 0.38 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.37 (t, J = 7.1 Hz, 3H, OCH_2C<u>H</u>$_3$), 1.45 (s, 9H, C$(CH_3)_3$), 1.60–1.70 (m, 2H, CH_2), 1.90–2.06 (m, 4H, 2 × CH_2), 2.08–2.18 (m, 2H, CH_2), 3.78–3.87 (m, 1H, Az 3-H), 3.98 (dd, J = 8.1, 6.4 Hz, 2H, Az 2,4-H$_a$), 4.30–4.40 (m, 5H, CH, OC<u>H</u>$_2$CH_3, Az 2,4-H$_b$), 6.73 (s, 1H, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.5 (OCH_2<u>C</u>H_3), 24.5 (2 × CH_2), 24.9 (Az C-3), 28.5 (C(<u>C</u>$H_3)_3$), 32.9 (2 × CH_2), 54.8 (Az C-2,4), 60.5 (O<u>C</u>H_2CH_3), 60.9 (CH), 80.1 (<u>C</u>$(CH_3)_3$), 106.5 (C-4), 142.6 (C-3), 144.2 (C-5), 156.2 (Boc C=O), 162.6 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −311.8 (Az N-1), −157.6 (N-1), −72.6 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1700 (C=O). HRMS (ESI TOF): $[M+H]^+$, found 364.2231. $[C_{19}H_{29}N_3O_4+H]^+$ requires 364.2231.

3.2.2 General procedure for alkylation of pyrazole carboxylates with *N*-Boc-3-iodomethylazetidine 8c

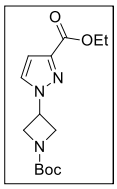
To a solution of pyrazole carboxylate (3 mmol) in dimethylformamide (6 mL), *N*-Boc-3-iodoazetidine (**8c**) (849 mg, 3 mmol), and Cs_2CO_3 (1300 mg, 3.99 mmol) were added, and the reaction mixture was heated at 100 ºC for 6 h. After cooling to rt, the reaction mixture was poured into water (20 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography by using an appropriate eluent.

Ethyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1*H*-pyrazole-5-carboxylate (9)



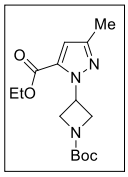
Ethyl 1*H*-pyrazole-3-carboxylate (**7**) (420 mg) was used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 385 mg (44%), colorless oil. R_f = 0.64 (*n*-hexane/ethylacetae 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.37 (t, J = 7.1 Hz, 3H, OCH_2C<u>H</u>$_3$), 1.45 (s, 9H, C$(CH_3)_3$), 4.29–4.38 (m, 4H, OC<u>H</u>$_2$CH_3, Az 2,4-H$_a$), 4.42 (dd, J = 9.3, 5.5 Hz, 2H, Az 2,4-H$_b$), 5.86–5.94 (m, 1H, Az 3-H), 6.87 (d, J = 1.9 Hz, 1H, 4-H), 7.58 (d, J = 1.9 Hz, 1H, 3-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.3 (OCH_2<u>C</u>H_3), 28.5 (C(<u>C</u>$H_3)_3$), 48.9 (Az C-3), 55.9 (Az C-2,4), 61.4 (O<u>C</u>H_2CH_3), 79.9 (<u>C</u>$(CH_3)_3$), 112.2 (C-4), 132.8 (C-5), 138.8 (C-3), 156.4 (Boc C=O), 159.8 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −313.7 (Az N-1), −165.6 (N-1), −66.7 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1700 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 296.1605. $[C_{14}H_{21}N_3O_4+H]^+$ requires 296.1605.

Ethyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1*H*-pyrazole-3-carboxylate (10)



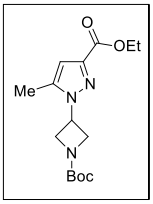
Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 345 mg (40%), colorless oil. R_f = 0.93 (*n*-hexane/ethylacetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.38 (t, J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.45 (s, 9H, $C(CH_3)_3$), 4.29 (dd, J = 9.5, 5.4 Hz, 2H, Az 2,4-H_a), 4.35–4.43 (m, 4H, $OC\underline{H}_2CH_3$, Az 2,4-H_b), 5.09–5.21 (m, 1H, Az 3-H), 6.86 (d, J = 2.3 Hz, 1H, 4-H), 7.61 (d, J = 2.4 Hz, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.5 ($OCH_2\underline{C}H_3$), 28.4 ($C(\underline{C}H_3)_3$), 51.0 (Az C-3), 56.5 (Az C-2,4), 61.2 ($O\underline{C}H_2CH_3$), 80.4 ($\underline{C}(CH_3)_3$), 109.9 (C-4), 129.2 (C-5), 144.5 (C-3), 156.1 (Boc C=O), 162.2 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −315.2 (Az N-1), −161.6 (N-1), −70.4 (N-2). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1695 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 310.1768. $[C_{15}H_{23}N_3O_4+H]^+$ requires 310.1761.

Ethyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-3-methyl-1*H*-pyrazole-5-carboxylate (12a)



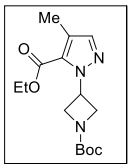
Ethyl 3-methyl-1*H*-prazole-5-carboxylate (**11a**) (462 mg) was used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 405 mg (45%), colorless oil. R_f = 0.57 (n-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.35 (t, J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.45 (s, 9H, $C(CH_3)_3$), 2.29 (s, 3H, 3-CH_3), 4.27–4.35 (m, 4H, $OC\underline{H}_2CH_3$, Az 2,4-H_a), 4.40 (dd, J = 9.3, 5.6 Hz, 2H, Az 2,4-H_b), 5.79–5.88 (m, 1H, Az 3-H), 6.63 (s, 1H, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 13.6 (3-CH_3), 14.3 ($OCH_2\underline{C}H_3$), 28.5 ($C(\underline{C}H_3)_3$), 48.4 (Az C-3), 55.9 (Az C-2,4), 61.2 ($O\underline{C}H_2CH_3$), 79.8 ($\underline{C}(CH_3)_3$), 111.6 (C-4), 133.2 (C-5), 148.0 (C-3), 156.4 (Boc C=O), 159.9 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −312.6 (Az N-1), −169.9 (N-1), −68.6 (N-2). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1701 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 310.1760. $[C_{15}H_{23}N_3O_4+H]^+$ requires 310.1761.

Ethyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-5-methyl-1*H*-pyrazole-3-carboxylate (13a)



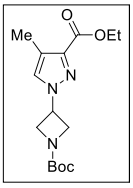
Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 243 mg (27%), white solid, mp 138–139 ºC (ethyl acetate). R_f = 0.95 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.38 (t, J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.45 (s, 9H, $C(CH_3)_3$), 2.28 (s, 3H, 5-CH_3), 4.30 (t, J = 8.5 Hz, 2H, Az 2,4-H_a), 4.36 (d, J = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 4.49–4.58 (m, 2H, Az 2,4-H_b), 4.98–5.07 (m, 1H, Az 3-H), 6.57 (s, 1H, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 11.2 (5-CH_3), 14.5 ($OCH_2\underline{C}H_3$), 28.5 ($C(\underline{C}H_3)_3$), 47.3 (Az C-3), 55.6 (Az C-2,4), 61.1 ($O\underline{C}H_2CH_3$), 79.7 ($\underline{C}(CH_3)_3$), 109.2 (C-4), 140.0 (C-5), 143.4 (C-3), 156.1 (Boc C=O), 162.6 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −311.9 (Az N-1), −162.9 (N-1), −71.8 (N-2). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1693 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 310.1761. $[C_{15}H_{24}N_3O_4+H]^+$, requires 310.1760.

Ethyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-4-methyl-1*H*-pyrazole-5-carboxylate (12b)



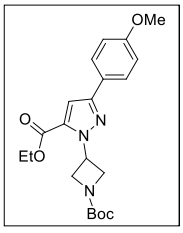
Ethyl 3-methyl-1*H*-prazole-5-carboxylate (**11b**) (462 mg) was used for the *N*-alkylation reaction. Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 273 mg (30%), colorless oil. R_f = 0.77 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.38 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.44 (s, 9H, $C(CH_3)_3$), 2.25 (s, 3H, 4-CH_3), 4.29–4.36 (m, 4H, $OC\underline{H}_2CH_3$, Az 2,4-H_a), 4.36–4.43 (m, 2H, Az 2,4-H_b), 5.73–5.83 (m, 1H, Az 3-H), 7.41 (s, 1H, 3-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 10.9 (4-CH_3), 14.4 ($OCH_2\underline{C}H_3$), 28.5 ($C(\underline{C}H_3)_3$), 49.3 (Az C-3), 55.7 (Az C-2,4), 61.1 ($O\underline{C}H_2CH_3$), 79.8 ($\underline{C}(CH_3)_3$), 123.7 (C-4), 129.9 (C-5), 140.1 (C-3), 156.4 (Boc C=O), 160.6 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −310.9 (Az N-1), −164.9 (N-1), −66.8 (N-2).IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1700 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 310.1764. $[C_{15}H_{23}N_3O_4+H]^+$ requires 310.1761.

Ethyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-4-methyl-1*H*-pyrazole-3-carboxylate (13b)



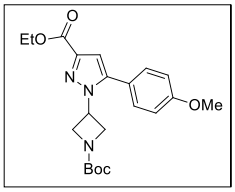
Yield 236 mg (26%), colorless oil. R_f = 0.10 (*n*-hexane/ethyl acetate 4/1, v/v).^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.40 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.45 (s, 9H, $C(CH_3)_3$), 2.30 (s, 3H, 4-CH_3), 4.23 (dd, *J* = 9.5, 5.2 Hz, 2H, Az 2,4-H_a), 4.35–4.44 (m, 4H, $OC\underline{H}_2CH_3$, Az 2,4-H_b), 5.06–5.15 (m, 1H, Az 3-H), 7.45 (s, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 10.0 (4-CH_3), 14.5 ($OCH_2\underline{C}H_3$), 28.5 ($C(\underline{C}H_3)_3$), 51.0 (Az C-3), 56.5 (Az C-2,4), 60.9 ($O\underline{C}H_2CH_3$), 80.4 ($\underline{C}(CH_3)_3$), 122.0 (C-4), 128.1 (C-5), 141.5 (C-3), 156.1 (Boc C=O), 162.9 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −314.9 (Az N-1), −165.7 (N-1), −69.8 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1694 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 310.1762. $[C_{15}H_{23}N_3O_4+H]^+$ requires 310.1761.

Ethyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-3-(4-methoxyphenyl)-1*H*-pyrazole-5-carboxylate (12c)



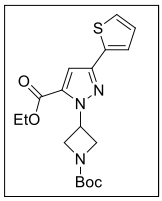
Ethyl 3-(4-methoxyphenyl)-1*H*-pyrazole-5-carboxylate(**11c**) (738 mg) was used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 825 mg (70%), white solid, mp 95–96 ºC (ethyl acetate). R_f = 0.64 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.40 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.47 (s, 9H, $C(CH_3)_3$), 3.85 (s, 3H, OCH_3), 4.32–4.40 (m, 4H, $OC\underline{H}_2CH_3$, Az 2,4-H_a), 4.50–4.56 (m, 2H, Az 2,4-H_b), 5.86–5.94 (m, 1H, Az 3-H), 6.95 (d, *J* = 8.8 Hz, 2H, Ph), 7.09 (s, 1H, 4-H), 7.77 (d, *J* = 8.8 Hz, 2H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 ($OCH_2\underline{C}H_3$), 28.5 ($C(\underline{C}H_3)_3$), 49.0 (Az C-3), 55.5 (OCH_3), 55.9 (Az C-2,4), 61.4 ($O\underline{C}H_2CH_3$), 79.9 ($\underline{C}(CH_3)_3$), 108.3 (C-4), 114.3 (Ph 2 × C), 125.3 (C-3), 127.1 (Ph 2 × C), 133.8 (C-5), 150.4 (Ph C-1), 156.5 (Boc C=O), 159,8 (C=O), 159.9 (Ph C-4). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −312.9 (Az N-1), −168.8 (N-1), −73.6 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1693 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 402.2037. $[C_{21}H_{27}N_3O_5+H]^+$ requires 402.2037**Ethyl 1-[1-(*tert*-**

butoxycarbonyl)azetidin-3-yl]-5-(4-methoxyphenyl)-1*H*-pyrazole-3-carboxylate (13c)



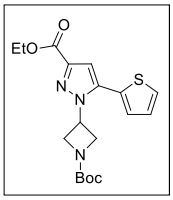
Yield 47 mg (4%), colorless oil. R_f = 0.89 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.40 (t, *J* = 7.2 Hz, 3H, $OCH_2C\underline{H}_3$), 1.43 (s, 9H, $C(CH_3)_3$), 3.85 (s, 3H, OCH_3), 4.20 (t, *J* = 8.4 Hz, 2H, Az 2,4-H_a), 4.41 (q, *J* = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 4.49–4.57 (m, 2H, Az 2,4-H_b), 5.05–5.14 (m, 1H, Az 3-H), 6.77 (s, 1H, 4-H), 6.98 (d, *J* = 8.6 Hz, 2H, Ph), 7.19 (d, *J* = 8.6 Hz, 2H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.5 ($OCH_2\underline{C}H_3$), 28.5 ($C(\underline{C}H_3)_3$), 47.7 (Az C-3), 55.5 (OCH_3), 56.0 (Az C-2,4), 61.2 ($O\underline{C}H_2CH_3$), 80.0 ($\underline{C}(CH_3)_3$), 109.1 (C-4), 114.6 (Ph 2 × C), 121.4 (Ph C-1), 130.3 (Ph 2 × C), 143.7 (C-3), 145.3 (C-5), 156.0 (Boc C=O), 160.5 (Ph C-4),162.5 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −314.4 (Az N-1), −167.2 (N-1), −75.1 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1698 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 402.2023. $[C_{21}H_{27}N_3O_5+H]^+$ requires 402.2023.

Ethyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-3-(thiophen-2-yl)-1*H*-pyrazole-5-carboxylate (12d)



5-(Thiophen-2-yl)-1*H*-pyrazole-3-carboxylate (**11d**) (711 mg) was used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with n-hexane/ethyl acetate (8/1, v/v). Yield 696 mg (63%), yellowish solid, mp 79–80 ºC (ethyl acetate). R_f = 0.78 (*n*-hexane/ethyl acetate 6/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.39 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.47 (s, 9H, $C(CH_3)_3$), 4.31–4.40 (m, 4H, $OC\underline{H}_2CH_3$, Az 2,4-H_a), 4.51 (dd, *J* = 9.3, 5.6 Hz, 2H, Az 2,4-H_b), 5.81–5.90 (m, 1H, Az 3-H), 7.04 (s, 1H, 4-H), 7.06 (dd, *J* = 5.0, 3.6 Hz, 1H, Th), 7.28 (dd, *J* = 5.0, 1.0 Hz, 1H, Th), 7.37 (dd, *J* = 3.6, 1.0 Hz, 1H, Th). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 ($OCH_2\underline{C}H_3$), 28.5 ($C(\underline{C}H_3)_3$), 49.2 (Az C-3), 55.8 (Az C-2,4), 61.5 ($O\underline{C}H_2CH_3$), 80.0 ($\underline{C}(CH_3)_3$), 108.8 (C-4), 124.5 (Th), 125.4 (Th), 127.7 (Th), 133.9 (C-5), 135.6 (Th C-3), 146.0 (C-3), 156.4 (Boc C=O), 159.6 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −313.5 (Az N-1), −169.0 (N-1), −74.3 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1689 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 378.1488. $[C_{18}H_{23}N_3O_4S+H]^+$ requires 378.1482.

Ethyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-5-(thiophen-2-yl)-1*H*-pyrazole-3-carboxylate (13d)



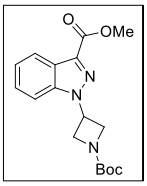
Yield 55 mg (5%), yellowish solid, mp 123–124 ºC (ethyl acetate). R_f = 0.93 (*n*-hexane/ethyl acetate 6/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.39 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.43 (s, 9H, $C(CH_3)_3$), 4.26 (t, *J* = 8.5 Hz, 2H, Az 2,4-H_a), 4.41 (q, *J* = 7.2 Hz, 2H, $OC\underline{H}_2CH_3$), 4.51–4.59 (m, 2H, Az 2,4-H_b), 5.22–5.30 (m, 1H, Az 3-H), 6.89 (s, 1H, 4-H), 7.07 (dd, *J* = 3.5, 0.9 Hz, 1H, Th), 7.13 (dd, *J* = 5.0, 3.7 Hz, 1H, Th), 7.47 (dd, *J* = 5.1, 0.9 Hz, 1H, Th). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.5 ($OCH_2\underline{C}H_3$), 28.46 ($C(\underline{C}H_3)_3$), 48.0 (Az C-3), 55.8 (Az C-2,4), 61.3 ($O\underline{C}H_2CH_3$), 80.1 ($\underline{C}(CH_3)_3$), 110.5 (C-4), 128.0 (Th), 128.2 (Th), 128.3 (Th), 129.1 (Th C-3), 138.2 (C-5), 143.7 (C-3), 156.0 (Boc C=O), 162.2

(C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −313.3 (Az N-1), −165.1 (N-1), −70.3 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1700 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 378.1488. $[C_{18}H_{23}N_3O_4S+H]^+$ requires 378.1482.

3.2.3 General procedure for alkylation of indazole carboxylates 14a-d with *N*-Boc-3-iodomethylazetidine 8c

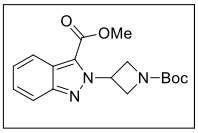
To a solution of indazole carboxylates **14a-d** (528 mg, 3 mmol) in dimethylformamide (6 mL), *N*-Boc-3-iodoazetidine (**8c**) (849 mg, 3 mmol), and Cs_2CO_3 (1300 mg, 3.99 mmol) were added, and the reaction mixture was heated at 100 ºC for 6 h. After cooling to rt, the reaction mixture was poured into water (20 ml), and the solution was extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, washed with brine (20 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography by using an appropriate eluent.

Methyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1*H*-indazole-3-carboxylate (15a)



Methyl 1*H*-indazole-3-carboxylate (**14a**) was used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v).Yield 516 mg (52%), white solid, mp 160–161 ºC (ethyl acetate). R_f = 0.85 (*n*-hexane/ethyl acetate 6/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.49 (s, 9H, $C(CH_3)_3$), 4.05 (s, 3H, OCH_3), 4.49 (t, J = 8.8 Hz, 2H, Az 2,4-H_a), 4.60 (d, J = 5.5 Hz, 2H, Az 2,4-H_b), 5.44–5.56 (m, 1H, Az 3-H), 7.36 (t, J = 7.5 Hz, 1H, 5-H), 7.48 (t, J = 7.7 Hz, 1H, 6-H), 7.56 (d, J = 8.5 Hz, 1H, 4-H), 8.26 (d, J = 8.2 Hz, 1H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 28.5 ($C(\underline{C}H_3)_3$), 48.8 (Az C-3), 52.3 (OCH_3), 55.6 (Az C-2,4), 80.4 ($\underline{C}(CH_3)_3$), 109.6 (C-4), 122.8 (C-7), 123.8 (C-5), 124.4 (C-3a), 127.5 (C-6), 135.7 (C-3), 140.2 (C-7a), 156.2 (Boc C=O), 163.0 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −314.4 (Az N-1), −189.3 (N-1), −58.3 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1691 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 354.1424. $[C_{17}H_{21}N_3O_4+Na]^+$ requires 354.1424.

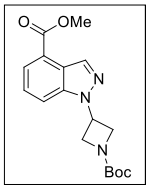
Methyl 2-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-2*H*-indazole-3-carboxylate (16a)



Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (10/1, v/v).Yield 270 mg (27%), yellowish solid, mp 130–131 ºC (ethyl acetate). R_f = 0.63 (*n*-hexane/ethyl acetate 6/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.47 (s, 9H, $C(CH_3)_3$), 4.02 (s, 3H, OCH_3), 4.49 (t, J = 8.7 Hz, 2H, Az 2,4-H_a), 4.59 (dd, J = 9.4, 5.4 Hz, 2H, Az 2,4-H_b), 6.20–6.30 (m, 1H, Az 3-H), 7.28–7.34 (m, 1H, 6-H), 7.35–7.41 (m, 1H, 5-H), 7.83 (d, J = 8.6 Hz, 1H, 7-H), 8.00 (d, J = 8.4 Hz, 1H, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 28.4 ($C(\underline{C}H_3)_3$), 50.6 (Az C-3), 52.1 (OCH_3), 55.7 (Az C-2,4), 79.9 ($\underline{C}(CH_3)_3$), 118.6 (C-7), 121.3 (C-4), 123.8 (C-7a), 124.0 (C-3), 125.6 (C-6), 126.8 (C-5), 147.5 (C-3a), 156.2 (Boc C=O), 160.7 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −314.8 (Az N-1), −152.2 (N-2), −82.5 (N-1). IR

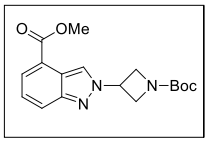
(neat, ῡ, cm^{-1}): 1703 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 354.1424. $[C_{17}H_{21}N_3O_4+Na]^+$ requires 354.1424.

Methyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1*H*-indazole-4-carboxylate (15b)



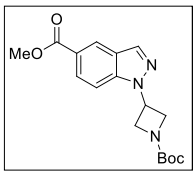
Methyl 1*H*-indazole-4-carboxylate (**14b**) was used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 338 mg (34%), orange solid, mp 129–130 ºC (ethyl acetate). R_f = 0.74 (*n*-hexane/ethyl acetate 2/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.48 (s, 9H, $C(CH_3)_3$), 4.02 (s, 3H, OCH_3), 4.46 (t, *J* = 8.6 Hz, 2H, Az 2,4-H_a), 4.54 (dd, *J* = 8.9, 5.7 Hz, 2H, Az 2,4-H_b), 5.37–5.46 (m, 1H, Az 3-H), 7.46 (dd, *J* = 8.4, 7.3 Hz, 1H, 6-H), 7.66 (d, *J* = 8.5 Hz, 1H, 7-H), 7.94 (d, *J* = 7.2 Hz, 1H, 5-H), 8.58 (s, 1H, 3-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 28.5 ($C(\underline{C}H_3)_3$), 47.5 (Az C-3), 52.4 (OCH_3), 56.2 (Az C-2,4), 80.2 ($\underline{C}(CH_3)_3$), 113.7 (C-7), 123.2 (C-3a), 123.5 (C-4), 124.8 (C-5), 126.1 (C-6), 135.2 (C-3), 139.9 (C-7a), 156.4 (Boc C=O), 166.7 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −313.5 (Az N-1), −193.1 (N-1), −64.6 (N-2). IR (neat, ῡ, cm^{-1}): 1696 (C=O). HRMS (ESI), m/z: calculated for $C_{17}H_{21}N_3O_4Na^+$ 354.1424 $[M + Na]^+$, found 354.1424.

Methyl 2-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-2*H*-indazole-4-carboxylate (16b)



Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 288 mg (29%), orange solid, mp 91–92 ºC (ethyl acetate). R_f = 0.81 (*n*-hexane/ethyl acetate 2/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.48 (s, 9H, $C(CH_3)_3$), 3.98 (s, 3H, OCH_3), 4.51 (d, *J* = 6.7 Hz, 4H, Az 2,4-H), 5.34–5.41 (m, 1H, Az 3-H), 7.37 (dd, *J* = 8.6, 7.1 Hz, 1H, 6-H), 7.93 (d, *J* = 7.0 Hz, 1H, 5-H), 7.96 (d, *J* 8.7 Hz, 1H, 7-H), 8.58 (s, 1H, 3-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 28.5 ($C(\underline{C}H_3)_3$), 51.7 (Az C-3), 52.2 (OCH_3), 56.7 (Az C-2,4), 80.3 ($\underline{C}(CH_3)_3$), 120.2 (C-7a), 122.5 (C-4), 123.5 (C-5), 124.1 (C-3), 125.7 (C-6), 126.8 (C-7), 149.5 (C-3a), 156.2 (Boc C=O), 166.8 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −314.8 (Az N-1), −150.4 (N-2), −99.6 (N-1). IR (neat, ῡ, cm^{-1}): 1686 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 354.1424. $[C_{17}H_{21}N_3O_4+Na]^+$ requires 354.1424.

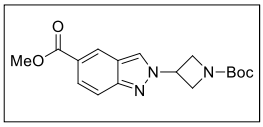
Methyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1*H*-indazole-5-carboxylate (15c)



Methyl 1*H*-indazole-5-carboxylate (**14c**) was used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 536 mg (54%), yellow solid, mp 99–100 ºC (ethyl acetate). R_f = 0.62 (*n*-hexane/ethyl acetate 2/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.48 (s, 9H, $C(CH_3)_3$), 3.95 (s, 3H, OCH_3), 4.46 (t, *J* = 8.6 Hz, 2H, Az 2,4-H_a), 4.53 (dd, *J* = 9.2, 5.5 Hz, 2H, Az 2,4-H_b), 5.34–5.46 (m, 1H, Az 3-H), 7.45 (d, *J* = 8.9 Hz, 1H, 7-H), 8.08 (d, *J* = 10.2 Hz, 1H, 6-H), 8.17 (s, 1H, 3-H), 8.52 (s, 1H, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 28.5 ($C(\underline{C}H_3)_3$), 47.5 (Az C-3), 52.3 (OCH_3), 56.1 (Az C-2,4), 80.3 ($\underline{C}(CH_3)_3$), 108.6 (C-7), 123.7 (C-5), 124.4 (C-

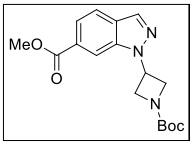
3a), 125.0 (C-3), 127.7 (C-6), 135.9 (C-4), 141.2 (C-7a), 156.4 (Boc C=O), 167.2 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −313.8 (Az N-1), −192.3 (N-1), −64.2 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1695 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 354.1424. $[C_{17}H_{21}N_3O_4+Na]^+$ requires 354.1424.

Methyl 2-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-2*H*-indazole-5-carboxylate (16c)



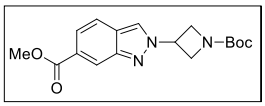
Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 248 mg (25%), yellow solid, mp 126–127 ºC (ethyl acetate). R_f = 0.76 (*n*-hexane/ethyl acetate 2/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.47 (s, 9H, $C(CH_3)_3$), 3.93 (s, 3H, OCH_3), 4.49 (d, *J* = 6.6 Hz, 4H, Az 2,4-H), 5.30–5.37 (m, 1H, Az 3-H), 7.73 (d, *J* = 9.2 Hz, 1H, 7-H), 7.92 (d, *J* = 10.7 Hz, 1H, 6-H), 8.20 (s, 1H, 3-H), 8.49 (s, 1H, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 28.5 ($C(\underline{C}H_3)_3$), 51.8 (Az C-3), 52.2 (OCH_3), 56.6 (Az C-2,4), 80.5 ($\underline{C}(CH_3)_3$), 117.6 (C-7), 121.3 (C-7a), 124.5 (C-5), 124.7 (C-3), 125.0 (C-4), 126.4 (C-6), 150.5 (C-3a), 156.2 (Boc C=O), 167.4 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −315.8 (Az N-1), −150.6 (N-2), −100.5 (N-1). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1682 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 354.1425. $[C_{17}H_{21}N_3O_4+Na]^+$ requires 354.1424.

Methyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1*H*-indazole-6-carboxylate (15d)



Methyl 1*H*-indazole-5-carboxylate (**14d**) was used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 437 mg (44%), yellowish solid, mp 115–116 ºC (ethyl acetate). R_f = 0.60 (*n*-hexane/ethyl acetate 2/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.48 (s, 9H, $C(CH_3)_3$), 3.97 (s, 3H, OCH_3), 4.47 (t, *J* = 8.6 Hz, 2H, Az 2,4-H_a), 4.54 (dd, *J* = 9.1, 5.6 Hz, 2H, Az 2,4-H_b), 5.40–5.50 (m, 1H, Az 3-H), 7.78 (d, *J* = 8.5 Hz, 1H, 4-H), 7.84 (d, *J* = 8.5 Hz, 1H, 5-H), 8.13 (s, 1H, 3-H), 8.18 (s, 1H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 28.5 ($C(\underline{C}H_3)_3$), 47.2 (Az C-3), 52.6 (OCH_3), 56.2 (Az C-2,4), 80.2 ($\underline{C}(CH_3)_3$), 111.2 (C-7), 121.4 (C-4), 121.8 (C-5), 127.2 (C-3a), 128.5 (C-6), 134.4 (C-3), 139.1 (C-7a), 156.4 (Boc C=O), 167.2 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −314.3 (Az N-1), −195.2 (N-1), −61.8 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1685 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 354.1424. $[C_{17}H_{21}N_3O_4+Na]^+$ requires 354.1423.

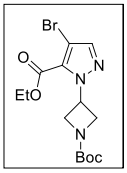
Methyl 2-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-2*H*-indazole-6-carboxylate (16d)



Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 268 mg (27%), yellow solid, mp 141–142 ºC (ethyl acetate). R_f = 0.79 (*n*-hexane/ethyl acetate 2/1). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.48 (s, 9H, $C(CH_3)_3$), 3.95 (s, 3H, OCH_3), 4.45–4.54 (m, 4H, Az 2,4-H), 5.30–5.39 (m, 1H, Az 3-H), 7.67 (d, *J* = 8.8 Hz, 1H, 4-H), 7.72 (d, *J* = 8.8 Hz, 1H, 5-H), 8.10 (s, 1H, 3-H), 8.52 (s, 1H, 7-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 28.5 ($C(\underline{C}H_3)_3$), 51.9 (Az C-3), 52.4 (OCH_3), 56.7 (Az C-2,4), 80.5 ($\underline{C}(CH_3)_3$), 120.3 (C-

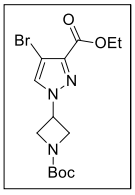
4), 122.3 (C-3), 122.0 (C-5), 121.6 (C-7), 124.1 (C-7a), 128.5 (C-6), 148.5 (C-3a), 156.2 (Boc C=O), 167.5 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −317.7 (Az N-1), −148.5 (N-2), −94.8 (N-1). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1682 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 354.1424. $[C_{17}H_{21}N_3O_4+Na]^+$ requires 354.1424.

3.2.4 Synthesis of ethyl 4-bromo-1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1*H*-pyrazole-5-carboxylate (17)



To a solution of compound **9** (885 mg, 3 mmol) in acetonitrile (12 mL), *N*-bromosuccinimide (1068 mg, 6 mmol) was added, and the reaction mixture was heated at 80 ºC for 2 h. After cooling to rt, the reaction mixture was poured into water (20 mL), and the solution was extracted with ethyl acetate (2 × 20 mL). The organic layers were combined, washed with brine (20 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (*n*-hexane/ethyl acetate 6/1, v/v). Yield 828 mg (74%), colorless oil. R_f = 0.69 (*n*-hexane/ethyl acetate 6/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.42 (t, J = 7.2 Hz, 3H, $OCH_2C\underline{H}_3$), 1.44 (s, J = 4.4 Hz, 9H, $C(CH_3)_3$), 4.32–4.42 (m, 6H, $OC\underline{H}_2CH_3$, Az 2,4-H), 5.71–5.81 (m, 1H, Az 3-H), 7.61 (s, 1H, 3-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.2 ($OCH_2\underline{C}H_3$), 28.5 ($C(\underline{C}H_3)_3$), 50.2 (Az C-3), 55.6 (Az C-2,4), 62.0 ($O\underline{C}H_2CH_3$), 80.1 ($\underline{C}(CH_3)_3$), 100.3 (C-4), 130.7 (C-5), 141.0 (C-3), 156.3 (Boc C=O), 159.1 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −312.5 (Az N-1), −162.4 (N-1), −65.9 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1699 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 374.0709. $[C_{14}H_{20}N_3O_4Br+H]^+$ requires 374.0710.

3.2.5 Synthesis of ethyl 4-bromo-1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1*H*-pyrazole-3-carboxylate (18)

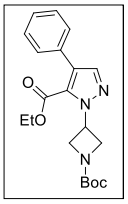


To a solution of compound **10** (885 mg, 3 mmol) in acetonitrile (12 mL), *N*-bromosuccinimide (1068 mg, 6 mmol) was added, and the reaction mixture was heated at 80 ºC for 2 h. After cooling to rt, the reaction mixture was poured into water (20 mL), and the solution was extracted with ethyl acetate (2 × 20 mL). The organic layers were combined, washed with brine (20 ml), dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (*n*-hexane/ethyl acetate 4/1, v/v). Yield 839 mg (75%), colorless oil. R_f = 0.90(*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.41 (t, J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.45 (s, 9H, $C(CH_3)_3$), 4.25 (dd, J = 9.7, 5.2 Hz, 2H, Az 2,4-H_a), 4.37–4.45 (m, 4H, $OC\underline{H}_2CH_3$, Az 2,4-H_b), 5.07–5.17 (m, 1H, Az 3-H), 7.72 (s, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 ($OCH_2\underline{C}H_3$), 28.4 ($C(\underline{C}H_3)_3$), 51.7 (Az C-3), 56.3 (Az C-2,4), 61.6 ($O\underline{C}H_2CH_3$), 80.7 ($\underline{C}(CH_3)_3$), 97.4 (C-4), 130.6 (C-5), 141.2 (C-3), 156.0 (Boc C=O), 161.0 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −315.0 (Az N-1), −162.6 (N-1), −67.4 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1697 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 374.0709. $[C_{14}H_{20}N_3O_4Br+H]^+$ requires 374.0710.

3.2.6 General procedure for Suzuki-Miyaura cross-coupling

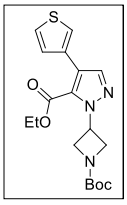
To a mixture of *N*-(azetidin-3-yl)-4-bromo-pyrazole carboxylates (**17** or **18**, 746 mg, 2 mmol), the given amount of an appropriate boronic acid (2.6 mmol), $Pd(PPh_3)_4$ (115 mg, 0.1 mmol) and K_3PO_4 (1272 mg, 6 mmol) in absolute dioxane (6 mL) were added, and the reaction mixture was heated under Ar atmosphere at 100 °C for 16 h. After cooling to rt, the reaction mixture was filtered over Celite®, and the filter cake was washed with ethyl acetate (2 mL). The filtrate was diluted with ethyl acetate (20 mL), the solution was washed with brine (20 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography by using an appropriate eluent.

Ethyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-4-phenyl-1*H*-pyrazole-5-carboxylate (19a)



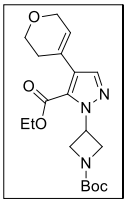
Phenylboronic acid (317 mg) was used for coupling. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 690 mg (93%), colorless oil. R_f = 0.62 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.13 (t, J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.47 (s, 9H, $C(CH_3)_3$), 4.21 (q, J = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 4.38 (t, J = 8.7 Hz, 2H, Az 2,4-H_a), 4.49 (dd, J = 9.1, 5.5 Hz, 2H, Az 2,4-H_b), 5.74–5.82 (m, 1H, Az 3-H), 7.32–7.38 (m, 5H, Ph), 7.62 (s, 1H, 3-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 13.8 ($OCH_2\underline{C}H_3$), 28.5 ($C(\underline{C}H_3)_3$), 49.5 (Az C-3), 55.9 (Az C-2,4), 61.4 ($O\underline{C}H_2CH_3$), 80.0 ($\underline{C}(CH_3)_3$), 127.7 (Ph C-4), 128.0 (Ph 2 × C), 128.3 (Ph C-1), 129.4 (C-4), 129.6 (Ph 2 × C), 132.4 (C-5), 139.5 (C-3), 156.4 (Boc C=O), 160.4 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −311.5 (Az N-1), −163.3 (N-1), −67.8 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1689 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 372.1921. $[C_{20}H_{25}N_3O_4+H]^+$ requires 372.1918.

Ethyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-4-(thiophen-3-yl)-1*H*-pyrazole-5-carboxylate (19b)



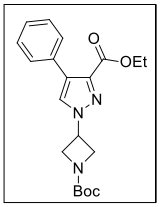
3-Thienylboronic acid (333 mg) was used for coupling. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 362 mg (48%), brown oil. R_f = 0.73 (*n*-hexane/ethyl acetate 6/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.26 (t, J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.46 (s, 9H, $C(CH_3)_3$), 4.29 (q, J = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 4.37 (t, J = 8.5 Hz, 2H, Az 2,4-H_a), 4.47 (dd, J = 9.0, 5.5 Hz, 2H, Az 2,4-H_b), 5.71–5.79 (m, 1H, Az 3-H), 7.19 (dd, J = 5.0, 1.1 Hz, 1H, Th), 7.32 (dd, J = 5.0, 3.0 Hz, 1H, Th), 7.39 (dd, J = 2.9, 1.1 Hz, 1H, Th), 7.66 (s, 1H, 3-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.0 ($OCH_2\underline{C}H_3$), 28.5 ($C(\underline{C}H_3)_3$), 49.7 (Az C-3), 55.8 (Az C-2,4), 61.6 ($O\underline{C}H_2CH_3$), 80.0 ($\underline{C}(CH_3)_3$), 123.0 (C-4), 123.8 (Th), 124.8 (Th), 129.1 (Th), 129.2 (C-5), 132.1 (Th C-3), 139.5 (C-3), 156.4 (Boc C=O), 160.2 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −314.3 (Az N-1), −166.3 (N-1), −70.9 (N-2). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1697 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 378.1485. $[C_{18}H_{23}N_3O_4S+H]^+$ requires 378.1482.

Ethyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-4-(3,6-dihydro-2*H*-pyran-4-yl)-1H-pyrazole-5-carboxylate (19c)



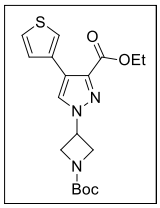
3,6-Dihydro-2*H*-pyran-4-boronic acid pinacol ester (546 mg) was used for coupling. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 648 mg (86%), yellowish solid, mp 77–78 ºC (ethyl acetate). R_f = 0.93 (*n*-hexane/ethyl acetate 6/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.36 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.44 (s, 9H, $C(CH_3)_3$), 2.34–2.37 (m, 2H, Pyran 5-CH_2), 3.87 (t, *J* = 5.4 Hz, 2H, Pyran 2-CH_2), 4.25 (q, *J* = 2.7 Hz, 2H, Pyran 6-CH_2), 4.30–4.35 (m, 4H, $OC\underline{H}_2CH_3$, Az 2,4-H_a), 4.38–4.46 (m, 2H, Az 2,4-H_b), 5.73–5.65 (m, 1H, Az 3-H), 5.74–5.78 (m, 1H, Pyran 3-H), 7.45 (s, 1H, 3-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.3 ($OCH_2\underline{C}H_3$), 28.5 ($C(\underline{C}H_3)_3$), 29.7 (Pyran C-5), 49.5 (Az C-3), 55.7 (Az C-2,4), 61.6 ($O\underline{C}H_2CH_3$), 64.5 (Pyran C-2), 65.7 (Pyran C-6), 80.0 ($\underline{C}(CH_3)_3$), 125.7 (Pyran C-3), 127.5 (C-4), 128.6 (Pyran C-4), 129.2 (C-5), 138.6 (C-3), 156.4 (Boc C=O), 160.25 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −312.1 (Az N-1), −164.8 (N-1), −69.4 (N-2). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1684 (C=O), 1127 (C-O), 773, 680 (C=CH). HRMS (ESI TOF): $[M + H]^+$, found 378.2024. $[C_{19}H_{27}N_3O_5+H]^+$ requires 378.2023.

Ethyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-4-phenyl-1*H*-pyrazole-3-carboxylate (20a)



Phenylboronic acid (317 mg) was used for coupling. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 215 mg (29%), white solid, mp 156–157 ºC (ethyl acetate). R_f = 0.93 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.29 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.46 (s, 9H, $C(CH_3)_3$), 4.29–4.37 (m, 4H, $OC\underline{H}_2CH_3$, Az 2,4-H_a), 4.44 (t, *J* = 8.7 Hz, 2H, Az 2,4-H_b), 5.15–5.22 (m, 1H, Az 3-H), 7.30–7.41 (m, 3H, Ph), 7.44–7.46 (m, 2H, Ph), 7.68 (s, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.3 ($OCH_2\underline{C}H_3$), 28.5 ($C(\underline{C}H_3)_3$), 51.2 (Az C-3), 56.5 (Az C-2,4), 61.2 ($O\underline{C}H_2CH_3$), 80.5 ($\underline{C}(CH_3)_3$), 127.2 (C-4), 127.7 (Ph C-4), 128.1 (Ph 2 × C), 128.4 (C-5), 129.4 (Ph 2 × C), 131.5 (Ph C-1), 140.4 (C-3), 156.1 (Boc C=O), 162.4 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −314.9 (Az N-1), −164.3 (N-1), −69.2 (N-2). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1688 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 372.1913. $[C_{20}H_{25}N_3O_4+H]^+$ requires 372.1918.

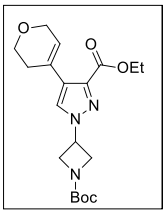
Ethyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-4-(thiophen-3-yl)-1*H*-pyrazole-3-carboxylate (20b)



3-Thienylboronic acid (333 mg) was used for coupling. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 264 mg (35%), brownish solid, mp 142–143 ºC (ethyl acetate). R_f = 0.93 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.36 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.46 (s, 9H, $C(CH_3)_3$), 4.30 (dd, *J* = 9.7, 5.3 Hz, 2H, Az 2,4-H_a), 4.36–4.46 (m, 4H, $OC\underline{H}_2CH_3$, Az 2,4-H_b), 5.13–5.22 (m, 1H, Az 3-H), 7.27 (dd, *J* = 5.3, 1.5 Hz, 1H, Th), 7.32 (dd, *J* = 5.0, 3.0 Hz, 1H, Th), 7.61 (dd, *J* = 2.9, 1.2 Hz,

1H, Th), 7.76 (s, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 ($OCH_2\underline{C}H_3$), 28.5 ($C(\underline{C}H_3)_3$), 51.2 (Az H-3), 56.5 (Az H-2,4), 61.3 ($O\underline{C}H_2CH_3$), 80.6 ($\underline{C}(CH_3)_3$), 122.0 (C-4), 123.7 (Th), 125.1 (Th), 128.0 (C-5), 128.7 (Th), 131.2 (Th C-3), 140.10 (C-3), 156.1 (Boc C=O), 162.4 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N −315.0 (Az N-1), −164.7 (N-1), −69.2 (N-2). IR (neat, ῡ, cm^{-1}): 1686 (C=O). HRMS (ESI TOF): $[M + H]^+$, found 378.1482. $[C_{18}H_{23}N_3O_4S+H]^+$ requires 378.1482.

Ethyl 1-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-4-(3,6-dihydro-2*H*-pyran-4-yl)-1H-pyrazole-3-carboxylate (20c)

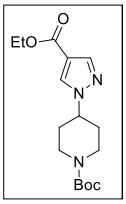


3,6-Dihydro-2*H*-pyran-4-boronic acid (546 mg) was used for coupling. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 392 mg (52%), yellowish solid, mp 129–130 ºC (ethyl acetate). R_f = 0.92 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.38 (t, J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.45 (s, 9H, $C(CH_3)_3$), 2.34–2.47 (m, 2H, Pyran 5-CH_2), 3.89 (t, J = 5.1 Hz, 2H, Pyran 2-CH_2), 4.23–4.27 (m, 4H, Pyran 6-CH_2, Az 2,4-H_a), 4.31–4.46 (m, 4H, $OC\underline{H}_2CH_3$, Az 2,4-H_b), 5.06–5.20 (m, 1H, Az 3-H), 5.93–5.94 (m, 1H, Pyran 3-H), 7.52 (s, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.5 ($OCH_2\underline{C}H_3$), 28.4 ($C(\underline{C}H_3)_3$), 29.3 (Pyran C-5), 51.1 (Az C-3), 56.5 (Az C-2,4), 61.3 ($O\underline{C}H_2CH_3$), 64.5 (Pyran C-2), 65.6 (Pyran C-6), 80.5 ($\underline{C}(CH_3)_3$), 125.5 (Pyran C-3), 126.7 (Pyran C-4), 127.3 (C-5), 127.5 (C-3), 140.4 (C-3), 156.1 (Boc C=O), 162.4 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −315.2 (Az N-1), −165.6 (N-1), −69.4 (N-2). IR (neat, ῡ, cm^{-1}): 1685 (C=O), 1128 (C-O), 771, 659 (C=CH). HRMS (ESI TOF): $[M + H]^+$, found 378.2019. $[C_{19}H_{27}N_3O_5+H]^+$ requires 378.2023.

3.2.7 General procedure for alkylation of pyrazole carboxylates with *tert*-butyl 4-[(methylsulfonyl)oxy]piperidine-1-carboxylate (22b)

To a solution of pyrazole carboxylate (3 mmol) in dimethylformamide (6 mL), *N*-Boc-piperidin-4-yl methanesulfonate **22b** (921 mg, 3.3 mmol), and Cs_2CO_3 (1467 mg, 4.5 mmol) were added, and the reaction mixture was heated at 100 ºC for 6 h. After cooling to rt, the reaction mixture was poured into water (20 mL), and the solution was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over sodium sulphate, and filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography by using an appropriate eluent.

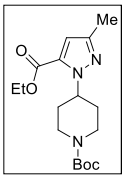
***tert*-Butyl 4-[4-(ethoxycarbonyl)-1*H*-pyrazol-1-yl]piperidine-1-carboxylate (23)**



Ethyl pyrazole-4-carboxylate (**21**) (420 mg) was used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 411 mg (43%), white solid, mp 102–103 ºC (ethyl acetate). R_f = 0.13 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.33 (t, J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.46 (s, 9H, $C(CH_3)_3$), 1.89 (qd, J = 12.4, 4.4 Hz, 2H, Pip CH_2), 2.13 (d, J = 12.0 Hz, 2H, Pip CH_2), 2.88 (t, J = 12.5 Hz, 2H, Pip

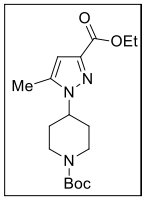
CH_2), 4.19–4.31 (m, 5H, Pip 4-H, Pip CH_2, OC$\underline{H_2}CH_3$), 7.25 (d, J = 4.0 Hz, 2H, 3-H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.5 (OCH$_2\underline{C}H_3$), 28.5 (C($\underline{C}H_3)_3$), 32.3 (Pip 2 × CH_2), 42.7 (Pip 2 × CH_2), 59.9 (O$\underline{C}H_2CH_3$), 60.3 (Pip C-4), 80.2 ($\underline{C}(CH_3)_3$), 115.1 (C-4), 130.3 (C-5), 140.9 (C-3), 154.6 (Boc C=O), 163.2 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1692, 1705 (C=O). HRMS (ESI TOF): $[M+H]^+$, found 324.1905. $[C_{16}H_{25}N_3O_4+H]^+$ requires 324.1918.

***tert*-Butyl 4-[5-(ethoxycarbonyl)-3-methyl-1*H*-pyrazol-1-yl]piperidine-1-carboxylate (24a)**



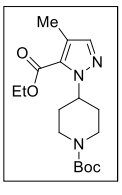
Ethyl 3-methyl-1*H*-prazole-5-carboxylate (**11a**) (462 mg) was used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 445 mg (44%), colorless solid, mp 60–61 ºC (ethyl acetate). R_f = 0.38 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.37 (t, J = 7.1 Hz, 3H, OCH$_2$C$\underline{H_3}$), 1.45 (s, 9H, C($CH_3)_3$), 1.91 (d, J = 11.2 Hz, 2H, Pip CH_2), 2.08 (d, J = 11.1 Hz, 2H, Pip CH_2), 2.26 (s, 3H, 3-CH_3), 2.85 (s, 2H, Pip CH_2), 4.14–4.35 (m, 4H, Pip CH_2, OC$\underline{H_2}CH_3$), 5.15–5.23 (m, 1H, Pip 4-H), 6.60 (s, 1H, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 13.6 (3-CH_3), 14.4 (OCH$_2\underline{C}H_3$), 28.6 (C($\underline{C}H_3)_3$), 32.3 (Pip 2 × CH_2), 43.1 (Pip 2 × CH_2), 57.3 (Pip C-4), 61.0 (O$\underline{C}H_2CH_3$), 79.7 ($\underline{C}(CH_3)_3$), 110.8 (C-4), 132.2 (C-5), 147.2 (C-3), 154.6 (Boc C=O), 160.2 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1685, 1719 (C=O). HRMS (ESI TOF): $[M+H]^+$, found 338.2067. $[C_{17}H_{27}N_3O_4+H]^+$ requires 338.2067.

***tert*-Butyl 4-[3-(ethoxycarbonyl)-5-methyl-1*H*-pyrazol-1-yl]piperidine-1-carboxylate (25a)**



Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 212 mg (21%), colorless solid, mp 63–64 ºC (ethyl acetate). R_f = 0.08 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.36 (t, J = 7.1 Hz, 3H, OCH$_2$C$\underline{H_3}$), 1.46 (s, 9H, C($CH_3)_3$), 1.86 (d, J = 11.2 Hz, 2H, Pip CH_2), 2.15–2.28 (m, 2H, Pip CH_2), 2.32 (s, 3H, 5-CH_3), 2.83 (t, J = 12.0 Hz 2H, Pip CH_2), 4.13–4.20 (m, 1H, Pip 4-H), 4.24–4.42 (m, 4H, Pip CH_2, OC$\underline{H_2}CH_3$), 6.54 (s, 1H, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 11.3 (5-CH_3), 14.5 (OCH$_2\underline{C}H_3$), 28.6 (C($\underline{C}H_3)_3$), 31.7 (Pip 2 × CH_2), 42.9 (Pip 2 × CH_2), 56.9 (Pip C-4), 60.9 (O$\underline{C}H_2CH_3$), 80.0 ($\underline{C}(CH_3)_3$), 108.5 (C-4), 138.8 (C-5), 142.7 (C-3), 154.5 (Boc C=O), 162.8 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1687, 1722 (C=O). HRMS (ESI TOF): $[M+H]^+$, found 338.2056. $[C_{17}H_{27}N_3O_4+H]^+$ requires 338.2074.

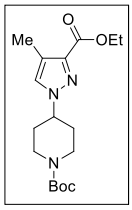
***tert*-Butyl 4-[5-(ethoxycarbonyl)-4-methyl-1*H*-pyrazol-1-yl]piperidine-1-carboxylate (24b)**



Ethyl 3-methyl-1*H*-prazole-5-carboxylate (**11b**) (462 mg) was used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 435 mg (43%), white solid, mp 82–83 ºC (ethyl acetate). R_f = 0.50 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.39 (t, J = 7.1 Hz, 3H, OCH$_2$C$\underline{H_3}$), 1.46 (s, 9H, C($CH_3)_3$), 1.93 (d, J = 11.7 Hz, 2H, Pip CH_2), 2.06 (qd, J = 11.5, 3.7 Hz, 2H, Pip CH_2), 2.24 (s, 3H, 4-CH_3), 2.86 (t, J = 12.1

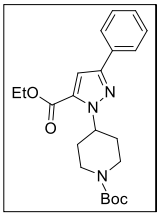
Hz, 2H, Pip CH_2), 4.23–4.24 (m, 2H, Pip CH_2), 4.35 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3), 5.13–5.26 (m, 1H, Pip 4-H), 7.33 (s, 1H, 3-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 11.1 (4-CH_3), 14.4 (OCH_2CH_3), 28.6 ($C(CH_3)_3$), 32.3 (Pip 2 × CH_2), 43.4 (Pip 2 × CH_2), 57.9 (Pip C-4), 60.9 (OCH_2CH_3), 79.8 ($C(CH_3)_3$), 122.8 (C-4), 129.1 (C-5), 139.5 (C-3), 154.7 (Boc C=O), 161.0 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1686, 1702 (C=O). HRMS (ESI TOF): $[M+H]^+$, found 338.2065. $[C_{17}H_{27}N_3O_4+H]^+$ requires 338.2074.

***tert*-Butyl 4-[3-(ethoxycarbonyl)-4-methyl-1*H*-pyrazol-1-yl]piperidine-1-carboxylate (25b)**



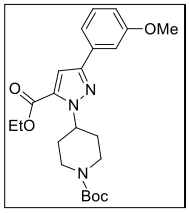
Purified by column chromatography on silica gel with dichloromethane/methanol (100/2, v/v). Yield 283 mg (28%), colorless gum. R_f = 0.10 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.39 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3), 1.46 (s, 9H, $C(CH_3)_3$), 1.85 (qd, *J* = 12.5, 4.4 Hz, 2H, Pip CH_2), 2.07–2.15 (m, 2H, Pip CH_2), 2.27 (s, 3H, 4-CH_3), 2.78–2.89 (m, 2H, Pip CH_2), 4.18–4.42 (m, 5H, Pip 4-H, Pip CH_2, OCH_2CH_3), 7.23 (s, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 10.0 (4-CH_3), 14.6 (OCH_2CH_3), 28.5 ($C(CH_3)_3$), 32.5 (Pip 2 × CH_2), 43.0 (Pip 2 × CH_2), 60.3 (Pip C-4), 60.7 (OCH_2CH_3), 80.1 ($C(CH_3)_3$), 121.0 (C-4), 127.2 (C-5), 140.6 (C-3), 154.7 (Boc C=O), 163.2 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1680, 1709 (C=O). HRMS (ESI TOF): $[M+H]^+$, found 338.2065. $[C_{17}H_{27}N_3O_4+H]^+$ requires 338.2074.

***tert*-Butyl 4-[5-(ethoxycarbonyl)-3-phenyl-1*H*-pyrazol-1-yl]piperidine-1-carboxylate (27a)**



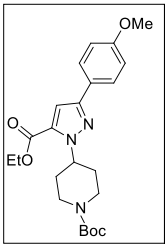
Ethyl 3-phenyl-1*H*-pyrazole-5-carboxylate (**26a**) (648 mg) was used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 467 mg (39%), white solid, mp 98–99 ºC (ethyl acetate). R_f = 0.30 (*n*-hexane/ethyl acetate 6/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.41 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3), 1.49 (s, 9H, $C(CH_3)_3$), 2.01 (d, *J* = 10.6 Hz, 2H, Pip CH_2), 2.15–2.27 (m, 2H, Pip CH_2), 2.93 (t, *J* = 12.4 Hz, 2H, Pip CH_2), 4.22–4.42 (m, 4H, Pip CH_2, OCH_2CH_3), 5.25–5.33 (m, 1H, Pip 4-H), 7.13 (s, 1H, 4-H), 7.31 (t, *J* = 7.4 Hz, 1H, Ph), 7.38–7.42 (m, 2H, Ph), 7.78–7.83 (m, 2H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 (OCH_2CH_3), 28.6 ($C(CH_3)_3$), 32.2 (Pip 2 × CH_2), 43.2 (Pip 2 × CH_2), 57.8 (Pip C-4), 61.2 (OCH_2CH_3), 79.8 ($C(CH_3)_3$), 108.2 (C-4), 125.7 (Ph 2 × CH), 128.1 (Ph C-4), 128.8 (Ph 2 × CH), 132.8 (Ph C-1), 132.9 (C-5), 149.9 (C-3), 154.8 (Boc C=O), 160.1 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1688, 1710 (C=O). HRMS (ESI TOF): $[M+H]^+$, found 400.2220. $[C_{22}H_{29}N_3O_4+H]^+$ requires 400.2231.

***tert*-Butyl 4-[5-(ethoxycarbonyl)-3-(3-methoxyphenyl)-1*H*-pyrazol-1-yl]piperidine-1-carboxylate (27b)**



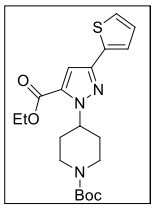
Ethyl 5-(3-methoxyphenyl)-1*H*-pyrazole-3-carboxylate (**26b**) (738 mg) was used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 656 mg (51%), white solid, mp 94–95 ºC (ethyl acetate). R_f = 0.30 (*n*-hexane/ethyl acetate 6/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.41 (t, *J* = 6.6 Hz, 3H, $OCH_2C\underline{H}_3$), 1.49 (s, 9H, $C(CH_3)_3$), 2.00 (d, *J* = 10.7 Hz, 2H, Pip CH_2), 2.17–2.26 (m, 2H, Pip CH_2), 2.93 (t, *J* = 12.4 Hz, 2H, Pip CH_2), 3.87 (s, 3H, OCH_3), 4.26–4.29 (m, 2H, Pip CH_2), 4.37 (q, *J* = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 5.23–5.34 (m, 1H, Pip 4-H), 6.85–6.88 (m, 1H, Ph), 7.11 (s, 1H, 4-H), 7.31 (t, *J* = 8.1 Hz, 1H, Ph),7.36–7.40 (m, 2H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 ($OCH_2\underline{C}H_3$), 28.6 ($C(\underline{C}H_3)_3$), 32.2 (Pip 2 × CH_2), 43.1 (Pip 2 × CH_2), 55.5 (OCH_3), 57.9 (Pip C-4), 61.2 ($O\underline{C}H_2CH_3$), 79.8 ($\underline{C}(CH_3)_3$), 108.3 (C-4), 111.0 (Ph), 113.9 (Ph), 118.2 (Ph), 129.8 (Ph), 132.9 (C-5), 134.2 (Ph C-1), 149.7 (C-3), 154.8 (Boc C=O), 160.0 (Ph C-3), 160.1 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1686, 1711 (C=O). HRMS (ESI TOF): $[M+H]^+$, found 430.2333. $[C_{23}H_{31}N_3O_5+H]^+$ requires 430.2336.

tert*-Butyl 4-[5-(ethoxycarbonyl)-3-(4-methoxyphenyl)-1*H*-pyrazol-1-yl]piperidine-1-carboxylate (27c*)



Ethyl 5-(4-methoxyphenyl)-1*H*-pyrazole-3-carboxylate (**26c**) (738 mg) was used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 489 mg (38%), white solid, mp 103–104 ºC (ethyl acetate). R_f = 0.47 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.40 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.49 (s, 9H, $C(CH_3)_3$), 1.99 (d, *J* = 11.0 Hz, 2H, Pip CH_2), 2.13–2.26 (m, 2H, Pip CH_2), 2.92 (d, *J* = 12.2 Hz, 2H, Pip CH_2), 3.83 (OCH_3), 4.20–4.40 (m, 4H, Pip CH_2, $OC\underline{H}_2CH_3$), 5.21–5.34 (m, 1H, Pip 4-H), 6.93 (d, *J* = 8.8 Hz, 2H, Ph), 7.05 (s, 1H, H-4), 7.73 (d, *J* = 8.8 Hz, 2H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 ($OCH_2\underline{C}H_3$), 28.6 ($C(\underline{C}H_3)_3$), 32.2 (Pip 2 × CH_2), 43.1 (Pip 2 × CH_2), 55.5 (OCH_3), 57.7 (Pip C-4), 61.1 ($O\underline{C}H_2CH_3$), 79.8 ($\underline{C}(CH_3)_3$), 107.6 (C-4), 114.2 (Ph 2 × CH), 125.7 (Ph C-1), 127.0 (Ph 2 × CH), 132.8 (C-5), 149.8 (C-3), 154.8 (Boc C=O), 159.7 (Ph C-4), 160.1 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1687, 1708 (C=O). HRMS (ESI TOF): $[M+H]^+$, found 430.2338. $[C_{23}H_{31}N_3O_5+H]^+$ requires 430.2336.

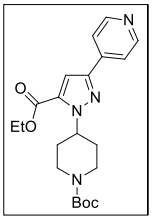
***tert*-Butyl 4-[5-(ethoxycarbonyl)-3-(thiophen-2-yl)-1*H*-pyrazol-1-yl]piperidine-1-carboxylate (27d)**



5-(Thiophen-2-yl)-1*H*-pyrazole-3-carboxylate (**26d**) (711 mg) was used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 545 mg (45%), yellow solid, mp 107–108 ºC (ethyl acetate). R_f = 0.29 (*n*-hexane/ethyl acetate 6/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.40 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.48 (s, 9H, $C(CH_3)_3$),

1.98 (d, J = 12.4 Hz, 2H, Pip CH_2), 2.13–2.27 (m, 2H, Pip CH_2), 2.91 (t, J = 12.2 Hz, 2H, Pip CH_2), 4.21–4.41 (m, 4H, Pip CH_2, O$\underline{C}H_2CH_3$), 5.21–5.31 (m, 1H, Pip 4-H), 7.00 (s, 1H, 4-H), 7.04 (dd, J = 5.0, 3.6 Hz, 1H, Th), 7.25 (dd, J = 5.1, 1.0 Hz, 1H, Th), 7.32 (dd, J = 3.5, 1.0 Hz, 1H, Th). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 (OCH$_2\underline{C}H_3$), 28.6 (C($\underline{C}H_3)_3$), 32.2 (Pip 2 × CH_2), 43.1 (Pip 2 × CH_2), 57.9 (Pip C-4), 61.3 (O$\underline{C}H_2CH_3$), 79.8 ($\underline{C}(CH_3)_3$), 108.1 (C-4), 124.0 (Th), 124.9 (Th), 127.6 (Th), 132.9 (C-5), 136.1 (Th C-2), 145.4 (C-3), 154.8 (Boc C=O), 159.9 (C=O). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1682, 1715 (C=O). HRMS (ESI TOF): $[M+H]^+$, found 406.1788. $[C_{20}H_{27}N_3O_4+H]^+$ requires 406.1795.

***tert*-Butyl 4-[5-(ethoxycarbonyl)-3-(pyridin-4-yl)-1*H*-pyrazol-1-yl]piperidine-1-carboxylate (27e)**



5-(4-Pyridinyl)-1*H*-pyrazole-3-carboxylate (**26e**) (651 mg) was used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (2/1, v/v). Yield 684 mg (57%), colorless gum. R_f = 0.13 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.41 (t, J = 7.1 Hz, 3H, OCH$_2$C$\underline{H}_3$), 1.48 (s, 9H, C($CH_3)_3$), 1.97–2.04 (m, 2H, Pip CH_2), 2.12–2.23 (m, 2H, Pip CH_2), 2.85–3.00 (m, 2H, Pip CH_2), 4.20–4.42 (m, 4H, Pip CH_2, O$\underline{C}H_2CH_3$), 5.25–5.36 (m, 1H, Pip 4-H), 7.22 (s, 1H, 4-H), 7.69 (d, J = 5.8 Hz, 2H, Pyr), 8.62 (d, J = 5.7 Hz, 2H, Pyr). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.3 (OCH$_2\underline{C}H_3$), 28.5 (C($\underline{C}H_3)_3$), 32.1 (Pip 2 × CH_2), 43.0 (Pip 2 × CH_2), 58.1 (Pip C-4), 61.3 (O$\underline{C}H_2CH_3$), 79.6 ($\underline{C}(CH_3)_3$), 109.0 (C-4), 119.9 (Pyr 2 × CH), 133.4 (C-5), 140.2 (Pyr C-1), 147.1 (C-3), 150.1 (Pyr 2 × CH), 154.6 (Boc C=O), 159.5 (C=O). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1683, 1710 (C=O). HRMS (ESI TOF): $[M+H]^+$, found 401.2183. $[C_{21}H_{28}N_4O_4+H]^+$ requires 401.2183.

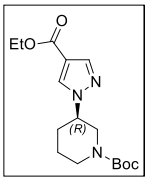
3.2.8 General procedures for the synthesis of (*R*) or (*S*) pyrazole piperidine carboxylates 29 and 31

Method A: To a solution of pyrazole carboxylate **21** (420 mg, 3 mmol) in absolute dichloromethane (10 mL), (*S*)- or (*R*)-1-Boc-3-hydroxypiperidine (603 mg, 3 mmol), PPh_3 (524 mg, 6 mmol) were added, and the reaction mixture was stirred at 0 °C under argon atmosphere. After 10 min, DIAD (0.94 mL, 4.8 mmol) was added dropwise to the stirred solution. The reaction mixture was stirred at room temperature for 24 h under argon atmosphere. The resulting solution was diluted with DCM (20 mL), and washed with water (3 × 20 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (*n*-hexane/acetone 4/1) to yield compounds **29** (77 mg, 8%) and **31** (164 mg, 17%).

Method B: To a solution of pyrazole carboxylate **21** (420 mg, 3 mmol) in dimethylformamide (6 mL), (*S*)- or (*R*)-1-Boc-piperidin-3-yl methanesulfonate (921 mg, 3.3 mmol), and Cs_2CO_3 (1467 mg, 4.5 mmol) were added, and the reaction mixture was heated at 80 ºC for 4 h. After cooling to rt, the reaction mixture was poured into water (20 mL), and the solution was extracted with ethyl acetate (3 × 20

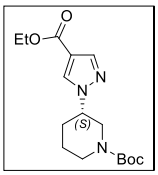
mL). The combined organic layers were washed with brine (20 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (*n*-hexane/acetone 4/1) to yield compounds **29** (126 mg, 13%) and **31** (213 mg, 22%).

(*R*)-*tert*-Butyl 3-[4-(ethoxycarbonyl)-1*H*-pyrazol-1-yl]piperidine-1-carboxylate (29)



Colorless oil. R_f = 0.31 (*n*-hexane/acetone 3/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.33 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H_3}$), 1.47 (s, 9H, $C(CH_3)_3$), 1.58–1.65 (m, 1H, Pip H_a), 1.72–1.79 (m, 1H, Pip H_b), 2.06–2.19 (m, 2H, Pip CH_2), 2.97–3.04 (m, 1H, Pip H_a), 3.35 (s, 1H, Pip H_a), 3.81–3.89 (m, 1H, Pip H_b), 4.16–4.24 (m, 2H, Pip H_b, Pip 3-H), 4.28 (q, *J* = 7.1 Hz, 2H, $OC\underline{H_2}CH_3$), 7.92 (s, 1H, 3-H), 7.98 (s, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.5 ($OCH_2\underline{C}H_3$), 23.5 (Pip CH_2), 28.5 ($C(\underline{C}H_3)_3$), 30.6 (Pip CH_2), 43.2 (Pip CH_2), 49.0 (Pip CH_2), 58.9 (Pip C-3), 60.3 ($O\underline{C}H_2CH_3$), 80.5 ($\underline{C}(CH_3)_3$), 115.0 (C-4), 131.4 (C-5), 141.1 (C-3), 154.6 (Boc C=O), 163.1 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −295.8 (Pip N-1), −159.4 (N-1), −76.5 (N-2). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1690 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 346.1737. $[C_{16}H_{25}N_3O_4+Na]^+$ requires 346.1737.

(*S*)-*tert*-Butyl 3-[4-(ethoxycarbonyl)-1*H*-pyrazol-1-yl]piperidine-1-carboxylate (31)



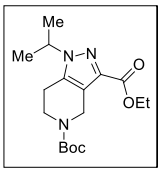
Colorless oil. R_f = 0.31 (*n*-hexane/acetone 3/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.33 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H_3}$), 1.46 (s, 9H, $C(CH_3)_3$), 1.57–1.69 (m, 1H, Pip H_a), 1.72–1.79 (m, 1H, Pip H_b), 2.04–2.21 (m, 2H, Pip CH_2), 2.96–3.06 (m, 1H, Pip H_a), 3.35 (s, 1H, Pip H_a), 3.84–3.89 (m, 1H, Pip H_b), 4.17–4.24 (m, 2H, Pip H_b, Pip 3-H), 4.28 (q, *J* = 7.1 Hz, 2H, $OC\underline{H_2}CH_3$), 7.92 (s, 1H, 3-H), 7.97 (s, 1H, 5-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.5 ($OCH_2\underline{C}H_3$), 23.6 (Pip CH_2), 28.5 ($C(\underline{C}H_3)_3$), 30.6 (Pip CH_2), 43.7 (Pip CH_2), 49.0 (Pip CH_2), 57.9 (Pip C-3), 60.3 ($O\underline{C}H_2CH_3$), 80.5 ($\underline{C}(CH_3)_3$), 115.0 (C-4), 131.4 (C-5), 141.1 (C-3), 154.6 (Boc C=O), 163.1 (C=O). ^{15}N NMR (41 MHz, $CDCl_3$): δ_N ppm −295.7 (Pip N-1), −159.6 (N-1), −76.7 (N-2). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1690 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 346.1737. $[C_{16}H_{25}N_3O_4+Na]^+$ requires 346.1737.

3.2.9 General procedure for the cyclization of hydrazine to obtain pyrazolo[3,4-*c*]pyridines 35, 36, 37a-d and 38a-d

To LiHMDS (1 M in THF, 6.5 mL, 6.5 mmol), a solution of *tert*-butyl 4-oxopiperidine-1-carboxylate **32** (995 mg, 5 mmol) in THF (10 mL) was added dropwise at -78 ºC under Ar atmosphere. The reaction mixture was stirred at -78 ºC for 30 minutes under Ar atmosphere. Then, diethyl oxalate (0.88 mL, 6.5 mmol) was added dropwise. After addition, the reaction mixture was warmed to rt and stirred for 2.5 h. The reaction mixture was quenched with saturated aqueous solution of NH_4Cl (20 mL), acidity with $KHSO_4$ (5%), the solution was extracted with ethyl acetate (2 × 50 mL). The combined organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was used directly for the next step.

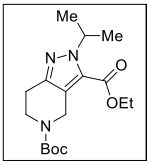
To a solution of yellow oil in ethanol (10 mL), an appropriate hydrazine (7.5 mmol) was added. After addition, the reaction mixture was heated at 80 ºC for 2 h. After cooling to rt, the reaction mixture was concentrated.

5-(*tert*-Butyl) 3-ethyl 1-isopropyl-1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (37b), *previously reported by Andrews et al*. [129]



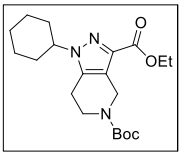
Isopropylhydrazine (555 mg) was used for the cyclization reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 84 mg (5%), yellow solid, mp 82–83 ºC (ethyl acetate). R_f = 0.26 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.37 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.45–1.51 (m, 15H, $C(CH_3)_3$, 2 × CH_3), 2.70 (s, 2H, Pip 7-CH_2), 3.70 (s, 2H, Pip 6-CH_2), 4.32–4.47 (m, 3H, $OC\underline{H}_2CH_3$, CH), 4.59 (s, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 ($OCH_2\underline{C}H_3$), 22.2 (2 × CH_3), 22.7 (Pip 7-CH_2), 28.4 ($C(\underline{C}H_3)_3$), 40.0 (Pip 6-CH_2), 41.7 (Pip 4-CH_2), 51.7 (CH), 60.6 ($O\underline{C}H_2CH_3$), 80.1 ($\underline{C}(CH_3)_3$), 117.4, 137.1, 137.7, 155.0 (Boc C=O), 162.7 (C=O). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1698, 1724 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 360.1894. $[C_{17}H_{27}N_3O_4+Na]^+$ requires 360.1894.

5-(*tert*-Butyl) 3-ethyl 2-isopropyl-2,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (38b), *previously reported by Andrews et al*. [129]



Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 101 mg (6%), white solid, mp 85–86 ºC (ethyl acetate). R_f = 0.64 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.38 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.43–1.50 (m, 15H, $C(CH_3)_3$, 2 × CH_3), 2.74 (s, 2H, Pip 7-CH_2), 3.67 (s, 2H, Pip 6-CH_2), 4.32 (q, *J* = 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), 4.59 (s, 2H, Pip 4-CH_2), 5.47–5.53 (m, 1H, CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 ($OCH_2\underline{C}H_3$), 22.9 (2 × CH_3), 23.8 (Pip 7-CH_2), 28.5 ($C(\underline{C}H_3)_3$), 41.7 (Pip 6-CH_2), 42.3 (Pip 4-CH_2), 51.8 (CH), 60.9 ($O\underline{C}H_2CH_3$), 80.0 ($\underline{C}(CH_3)_3$), 119.0, 126.8, 145.7, 155.2 (Boc C=O), 160.0 (C=O). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1694, 1727 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 360.1894. $[C_{17}H_{27}N_3O_4+Na]^+$ requires 360.1894.

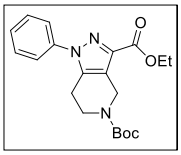
5-(*tert*-Butyl) 3-ethyl 1-cyclohexyl-1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (37c)



Cyclohexylhydrazine (855 mg) was used for the cyclization reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 170 mg (15%), yellow oil. R_f = 0.27 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.35–1.38 (m, 5H, CH_2, $OCH_2C\underline{H}_3$), 1.47 (s, 9H, $C(CH_3)_3$), 1.68–1.71 (m, 1H, CH), 1.88–2.03 (m, 7H, CH, 3 × CH_2), 2.70 (s, 2H, Pip 7-CH_2), 3.70 (s, 2H, Pip 6-CH_2), 3.93–4.03 (m, 1H, CH), 4.37 (q, *J* = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 4.59 (s, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.5 ($OCH_2\underline{C}H_3$), 22.4 (Pip 7-CH_2), 25.1 (CH_2), 25.7 (2 × CH_2), 28.5 ($C(\underline{C}H_3)_3$), 32.5 (2 × CH_2), 40.1 (Pip 6-CH_2), 41.8 (Pip 4-CH_2), 59.6 (CH), 60.7 ($O\underline{C}H_2CH_3$), 80.2 ($\underline{C}(CH_3)_3$), 117.4, 137.3, 137.8, 155.2 (Boc C=O), 162.8 (C=O). IR (neat, $\bar{\upsilon}$, cm^{-1}):

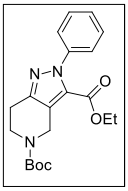
1693, 1730 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 400.2207. $[C_{20}H_{31}N_3O_4+Na]^+$ requires 400.2207.

5-(*tert*-Butyl) 3-ethyl 1-phenyl-1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (37d)



Phenylhydrazine (810 mg) was used for the cyclization reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 167 mg (15%), yellow solid, mp 100–101 ºC (ethyl acetate). R_f = 0.16 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.41 (t, J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.50 (s, 9H, $C(CH_3)_3$), 2.81 (t, J = 5.1 Hz, 2H, Pip 7-CH_2), 3.69 (s, 2H, Pip 6-CH_2), 4.42 (q, J = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 4.70 (s, 2H, Pip 4-CH_2), 7.39 (t, J = 7.0 Hz, 1H, Ph CH), 7.44–7.52 (m, 4H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.5 ($OCH_2\underline{C}H_3$), 24.2 (Pip 7-CH_2), 28.6 ($C(\underline{C}H_3)_3$), 40.3 (Pip 6-CH_2), 41.7 (Pip 4-CH_2), 61.2 ($O\underline{C}H_2CH_3$), 80.4 ($\underline{C}(CH_3)_3$), 118.8, 123.8 (3 × CH), 128.3, 129.4 (2 × CH), 138.9, 155.2 (Boc C=O), 162.6 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1681, 1726 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 394.1737. $[C_{20}H_{25}N_3O_4+Na]^+$ requires 394.1737.

5-(*tert*-Butyl) 3-ethyl 2-phenyl-2,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (38d)

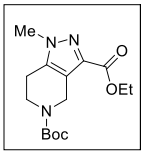


Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 256 mg (23%), brown solid, mp 92–93 ºC (ethyl acetate). R_f = 0.26 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.26 (t, J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.51 (s, 9H, $C(CH_3)_3$), 2.84 (s, 2H, Pip 7-CH_2), 3.75 (s, 2H, Pip 6-CH_2), 4.24 (q, J = 7.2 Hz, 2H, $OC\underline{H}_2CH_3$), 4.71 (s, 2H, Pip 4-CH_2), 7.37–7.44 (m, 5H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.2 ($OCH_2\underline{C}H_3$), 23.8 (Pip 7-CH_2), 28.6 ($C(\underline{C}H_3)_3$), 41.6 (Pip 6-CH_2), 42.1 (Pip 4-CH_2), 61.2 ($O\underline{C}H_2CH_3$), 80.3 ($\underline{C}(CH_3)_3$), 120.7, 126.2 (2 × CH), 128.7 (2 × CH), 140.5 (CH), 148.0, 155.1 (Boc C=O), 159.2 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1686, 1732 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 394.1737. $[C_{20}H_{25}N_3O_4+Na]^+$ requires 394.1737.

3.2.10 General procedure for the alkylation of 5-(*tert*-butyl) 3-ethyl 1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate 39 with haloalkanes

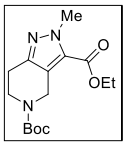
To a solution of pyrazolo[3,4-*c*]pyridine **39** (885 mg, 3 mmol) in dimethylformamide (6 mL), an appropriate haloalkane (3.3 mmol) and Cs_2CO_3 (1300 mg, 3.99 mmol) were added, and the reaction mixture was heated at the desired temperature (40–100 ºC) for 2 h. After cooling to rt, the reaction mixture was poured into water (20 mL), and the solution was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography by using an appropriate eluent.

5-(*tert*-Butyl) 3-ethyl 1-methyl-1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (35), *previously reported by Sivagurunathan et al*. [130]



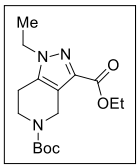
Iodomethane (469 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 40 ºC. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (2/1, v/v). Yield 241 mg (26%), yellow solid, mp 121–123 ºC (ethyl acetate). R_f = 0.09 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.40 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H_3}$), 1.49 (s, 9H, $C(CH_3)_3$), 2.68 (s, 2H, Pip 7-CH_2), 3.72 (s, 2H, Pip 6-CH_2), 3.83 (s, 3H, CH_3), 4.39 (q, *J* = 7.0 Hz, 2H, $OC\underline{H_2}CH_3$), 4.61 (s, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm 14.5 ($OCH_2\underline{C}H_3$), 22.0 (Pip 7-CH_2), 28.5 ($C(\underline{C}H_3)_3$), 36.6 (CH_3), 40.0 (Pip 6-CH_2), 41.6 (Pip 4-CH_2), 60.9 ($O\underline{C}H_2CH_3$), 80.3 ($\underline{C}(CH_3)_3$), 117.8, 138.0, 138.8, 155.2 (Boc C=O), 162.5 (C=O). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1695, 1724 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 332.1581. $[C_{15}H_{23}N_3O_4+Na]^+$ requires 332.1581.

5-(*tert*-Butyl) 3-ethyl 2-methyl-2,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (36), *previously reported by Dawidowski et al*. [96]



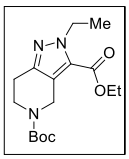
Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 445 mg (48%), yellowish oil. R_f = 0.24 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.35 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H_3}$), 1.45 (s, 9H, $C(CH_3)_3$), 2.70 (s, 2H, Pip 7-CH_2), 3.65 (s, 2H, Pip 6-CH_2), 4.09 (s, 3H, CH_3), 4.31 (q, *J* = 7.0 Hz, 2H, $OC\underline{H_2}CH_3$), 4.57 (s, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.3 ($OCH_2\underline{C}H_3$), 23.5 (Pip 7-CH_2), 28.5 ($C(\underline{C}H_3)_3$), 39.5 (CH_3), 41.5 (Pip 6-CH_2), 42.2 (Pip 4-CH_2), 61.0 ($O\underline{C}H_2CH_3$), 80.0 ($\underline{C}(CH_3)_3$), 119.1, 127.8, 145.8, 155.1 (Boc C=O), 160.0 (C=O). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1683, 1724 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 332.1580. $[C_{15}H_{23}N_3O_4+Na]^+$ requires 332.1581.

5-(*tert*-Butyl) 3-ethyl 1-ethyl-1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (37a)



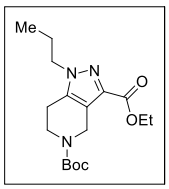
Iodoethane (515 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 70 ºC. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (2/1, v/v). Yield 359 mg (37%), yellow solid, mp 117–118 ºC (ethyl acetate). R_f = 0.10 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.39 (dt, *J* = 14.4 Hz, 6H, $OCH_2C\underline{H_3}$, CH_3), 1.47 (s, 9H, $C(CH_3)_3$), 2.67 (s, 2H, Pip 7-CH_2), 3.71 (s, 2H, Pip 6-CH_2), 4.12 (q, *J* = 7.3 Hz, 2H, CH_2), 4.37 (q, *J* = 7.0 Hz, 2H, $OC\underline{H_2}CH_3$), 4.59 (s, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm 14.5 ($OCH_2\underline{C}H_3$), 15.5 (CH_3), 22.2 (Pip 7-CH_2), 28.5 ($C(\underline{C}H_3)_3$), 40.1 (Pip 6-CH_2), 41.7 (Pip 4-CH_2), 44.9 (CH_2), 60.8 ($O\underline{C}H_2CH_3$), 80.3 ($\underline{C}(CH_3)_3$), 117.7, 137.6, 137.8, 155.2 (Boc C=O), 162.6 (C=O). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1693, 1722 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 346.1737. $[C_{16}H_{25}N_3O_4+Na]^+$ requires 346.1737.

5-(*tert*-Butyl) 3-ethyl 2-ethyl-2,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (38a)



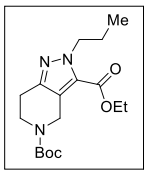
Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 514 mg (53%), colorless oil. R_f = 0.48 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.39 (q, *J* = 7.3 Hz, 6H, $OCH_2C\underline{H}_3$, CH_3), 1.47 (s, 9H, $C(CH_3)_3$), 2.73 (s, 2H, Pip 7-CH_2), 3.67 (s, 2H, Pip 6-CH_2), 4.33 (q, *J* = 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), 4.54 (q, *J* = 7.2 Hz, 2H, CH_2), 4.60 (s, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C ppm 14.4 ($OCH_2\underline{C}H_3$), 16.1 (CH_3), 23.6 (Pip 7-CH_2), 28.6 ($C(\underline{C}H_3)_3$), 41.6 (Pip 6-CH_2), 42.0 (Pip 4-CH_2), 47.0 (CH_2), 61.0 ($O\underline{C}H_2CH_3$), 80.1 ($\underline{C}(CH_3)_3$), 119.3, 127.0, 145.9, 155.2 (Boc C=O), 159.8 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1698, 1720 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 346.1737. $[C_{16}H_{25}N_3O_4+Na]^+$ requires 346.1737.

5-(*tert*-Butyl) 3-ethyl 1-propyl-1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (40a)



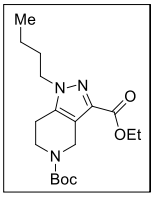
1-Bromopropane (403 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 70 ºC. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (2/1, v/v). Yield 192 mg (19%), yellowish solid, mp 59–60 ºC (ethyl acetate). R_f = 0.24 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 0.89 (t, *J* = 7.4 Hz, 3H, CH_3), 1.37 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.47 (s, 9H, $C(CH_3)_3$), 1.83 (dt, *J* = 14.7, 7.4 Hz, 2H, CH_2), 2.67 (s, 2H, Pip 7-CH_2), 3.70 (s, 2H, Pip 6-CH_2), 4.01 (t, *J* = 7.3 Hz, 2H, CH_2), 4.37 (q, *J* = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 4.59 (m, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 11.2 (CH_3), 14.5 ($OCH_2\underline{C}H_3$), 22.1 (Pip 7-CH_2), 23.6 (CH_2), 28.5 ($C(\underline{C}H_3)_3$), 40.1 (Pip 6-CH_2), 41.7 (Pip 4-CH_2), 51.5 (CH_2), 60.8 ($O\underline{C}H_2CH_3$), 80.3 ($\underline{C}(CH_3)_3$), 117.5, 138.1, 138.2, 155.2 (Boc C=O), 162.7 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1694, 1715 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 360.1894. $[C_{17}H_{27}N_3O_4+Na]^+$ requires 360.1894.

5-(*tert*-Butyl) 3-ethyl 2-propyl-2,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (41a)



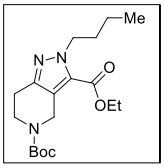
Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 495 mg (49%), yellowish solid, mp 59–60 ºC (ethyl acetate). R_f = 0.73 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 0.91 (t, *J* = 7.3 Hz, 3H, CH_3), 1.38 (t, *J* = 6.9 Hz, 3H, $OCH_2C\underline{H}_3$), 1.48 (s, 9H, $C(CH_3)_3$), 1.82 (dd, *J* = 14.5, 7.2 Hz, 2H, CH_2), 2.73 (s, 2H, Pip 7-CH_2), 3.67 (s, 2H, Pip 6-CH_2), 4.32 (q, *J* = 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), 4.45 (t, *J* = 7.2 Hz, 2H, CH_2), 4.60 (m, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 11.2 (CH_3), 14.3 ($OCH_2\underline{C}H_3$), 23.7 (Pip 7-CH_2), 24.3 (CH_2), 28.6 ($C(\underline{C}H_3)_3$), 41.0 (Pip 6-CH_2), 42.3 (Pip 4-CH_2), 53.4 (CH_2), 61.0 ($O\underline{C}H_2CH_3$), 80.1 ($\underline{C}(CH_3)_3$), 119.2, 127.4, 145.9, 155.2 (Boc C=O), 159.9 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1686, 1722 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 360.1894. $[C_{17}H_{27}N_3O_4+Na]^+$ requires 360.1894.

5-(*tert*-Butyl) 3-ethyl 1-butyl-1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (40b)



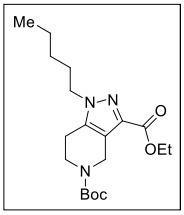
1-Bromobutane (449 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 100 ºC. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (2/1, v/v). Yield 316 mg (30%), yellow oil. R_f = 0.24 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 0.92 (t, J = 7.4 Hz, 3H, CH_3), 1.28–1.39 (m, 5H, $OCH_2C\underline{H}_3$, CH_2), 1.47 (s, 9H, $C(CH_3)_3$), 1.75–1.85 (m, 2H, CH_2), 2.67 (s, 2H, Pip 7-CH_2), 3.70 (s, 2H, Pip 6-CH_2), 4.05 (t, J = 7.4 Hz, 2H, CH_2), 4.49 (q, J = 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), 4.59 (s, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 13.8 (CH_3), 14.6 ($OCH_2\underline{C}H_3$), 20.0 (CH_2), 22.1 (Pip 7-CH_2), 28.5 ($C(\underline{C}H_3)_3$), 32.3 (CH_2), 40.2 (Pip 6-CH_2), 41.8 (Pip 4-CH_2), 49.8 (CH_2), 60.8 ($O\underline{C}H_2CH_3$), 80.3 ($\underline{C}(CH_3)_3$), 117.5, 138.0, 138.1, 155.2 (Boc C=O), 162.7 (C=O). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1684, 1728 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 374.2050. $[C_{18}H_{29}N_3O_4+Na]^+$ requires 374.2050.

5-(*tert*-Butyl) 3-ethyl 2-butyl-2,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (41b)



Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 600 mg (57%), colorless oil. R_f = 0.67 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 0.92 (t, J = 7.4 Hz, 3H, CH_3), 1.31–1.40 (m, 5H, $OCH_2C\underline{H}_3$, CH_2), 1.48 (s, 9H, $C(CH_3)_3$), 1.72–1.82 (m, 2H, CH_2), 2.73 (s, 2H, Pip 7-CH_2), 3.67 (s, 2H, Pip 6-CH_2), 4.33 (q, J = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 4.49 (t, J = 7.4 Hz, 2H, CH_2), 4.60 (s, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 13.8 (CH_3), 14.4 ($OCH_2\underline{C}H_3$), 20.0 (CH_2), 23.7 (Pip 7-CH_2), 28.6 ($C(\underline{C}H_3)_3$), 33.1 (CH_2), 41.6 (Pip 6-CH_2), 42.2 (Pip 4-CH_2), 51.7 (CH_2), 61.0 ($O\underline{C}H_2CH_3$), 80.1 ($\underline{C}(CH_3)_3$), 119.2, 127.1, 145.8, 155.2 (Boc C=O), 159.9 (C=O). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1696, 1722 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 374.2050. $[C_{18}H_{29}N_3O_4+Na]^+$ requires 374.2050.

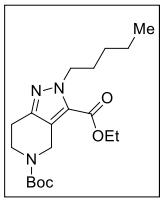
5-(*tert*-Butyl) 3-ethyl 1-pentyl-1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (40c)



1-Bromopentane (495 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 100 ºC. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (2/1, v/v). Yield 328 mg (30%), yellow oil. R_f = 0.35 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 0.87 (t, J = 7.0 Hz, 3H, CH_3), 1.20–1.40 (m, 7H, $OCH_2C\underline{H}_3$, 2 × CH_2), 1.47 (s, 9H, $C(CH_3)_3$), 1.74–1.86 (m, 2H, CH_2), 2.66 (s, 2H, Pip 7-CH_2), 3.70 (s, 2H, Pip 6-CH_2), 4.03 (t, J = 7.4 Hz, 2H, CH_2), 4.37 (q, J = 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), 4.59 (s, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.0 (CH_3), 14.5 ($OCH_2\underline{C}H_3$), 22.2 (Pip 7-CH_2), 22.3 (CH_2), 28.5 ($C(\underline{C}H_3)_3$), 28.8 (CH_2), 30.0 (CH_2), 40.1 (Pip 6-CH_2), 41.6 (Pip 4-CH_2), 50.0 (CH_2), 60.8 ($O\underline{C}H_2CH_3$), 80.3 ($\underline{C}(CH_3)_3$), 117.5, 138.0, 138.1, 155.1 (Boc C=O), 162.7

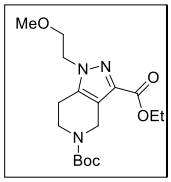
(C=O). IR (neat, ῡ, cm^{-1}): 1684, 1728 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 388.2207. $[C_{19}H_{31}N_3O_4+Na]^+$ requires 388.2207.

5-(*tert*-Butyl) 3-ethyl 2-pentyl-2,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (41c)



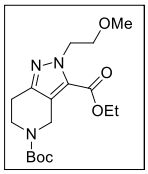
Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 523 mg (48%), colorless oil. R_f = 0.67 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 0.88 (t, *J* = 6.8 Hz, 3H, CH_3), 1.27–1.39 (m, 7H, $OCH_2C\underline{H}_3$, 2 × CH_2), 1.48 (s, 9H, $C(CH_3)_3$), 1.74–1.83 (m, 2H, CH_2), 2.73 (s, 2H, Pip 7-CH_2), 3.67 (s, 2H, Pip 6-CH_2), 4.33 (q, *J* = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 4.48 (t, *J* = 7.5 Hz, 2H, CH_2), 4.60 (s, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.1 (CH_3), 14.4 ($OCH_2\underline{C}H_3$), 22.4 (CH_2), 23.6 (Pip 7-CH_2), 28.5 ($C(\underline{C}H_3)_3$), 28.9 (CH_2), 30.7 (CH_2), 41.6 (Pip 6-CH_2), 42.2 (Pip 4-CH_2), 52.0 (CH_2), 61.0 ($O\underline{C}H_2CH_3$), 80.0 ($\underline{C}(CH_3)_3$), 119.1, 127.4, 145.9, 155.2 (Boc C=O), 159.9 (C=O). IR (neat, ῡ, cm^{-1}): 1699, 1721 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 388.2206. $[C_{19}H_{31}N_3O_4+Na]^+$ requires 388.2206.

5-(*tert*-Butyl) 3-ethyl 1-(2-methoxyethyl)-1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (40d)



1-Bromo-2-methoxyethane (455 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 40 ºC. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (2/1, v/v). Yield 244 mg (23%), yellowish oil. R_f = 0.20 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.37 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.48 (s, 9H, $C(CH_3)_3$), 1.61 (s, 2H, CH_2), 2.73 (s, 2H, Pip 7-CH_2), 3.27 (s, 3H, OCH_3), 3.70–3.74 (m, 4H, Pip 6-CH_2, CH_2), 4.23 (t, *J* = 5.1 Hz, 2H, CH_2), 4.39 (q, *J* = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 4.60 (s, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.6 ($OCH_2\underline{C}H_3$), 22.2 (Pip 7-CH_2), 28.6 ($C(\underline{C}H_3)_3$), 40.1 (Pip 6-CH_2), 41.7 (Pip 4-CH_2), 50.0 (CH_2), 59.2 (OCH_3), 60.1 ($OC\underline{H}_2CH_3$), 71.6 (CH_2), 80.3 ($\underline{C}(CH_3)_3$), 117.3, 139.7, 139.8, 155.2 (Boc C=O), 162.7 (C=O). IR (neat, ῡ, cm^{-1}): 1689, 1717 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 376.1844. $[C_{17}H_{27}N_3O_5+Na]^+$ requires 376.1843.

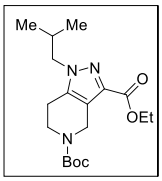
5-(*tert*-Butyl) 3-ethyl 2-(2-methoxyethyl)-2,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (41d)



Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 487 mg (46%), colorless oil. R_f = 0.23 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.37 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.47 (s, 9H, $C(CH_3)_3$), 2.74 (s, 2H, Pip 7-CH_2), 3.32 (s, 3H, OCH_3), 3.66–3.75 (m, 4H, Pip 6-CH_2, CH_2), 4.32 (q, *J* = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 4.60 (s, 2H, Pip 4-CH_2), 4.71 (t, *J* = 5.6 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 ($OCH_2\underline{C}H_3$), 23.7 (Pip 7-CH_2), 28.5 ($C(\underline{C}H_3)_3$), 40.9 (Pip 6-CH_2), 41.6 (Pip 4-CH_2), 50.9 (OCH_3), 59.0 (CH_2), 61.1 ($O\underline{C}H_2CH_3$), 71.7 (CH_2), 80.1 ($\underline{C}(CH_3)_3$), 119.4, 127.8, 146.4, 155.1

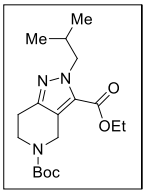
(Boc C=O), 160.0 (C=O). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1696, 1722 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 376.1842. $[C_{17}H_{27}N_3O_5+Na]^+$ requires 376.1843.

5-(*tert*-Butyl) 3-ethyl 1-isobutyl-1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (40e)



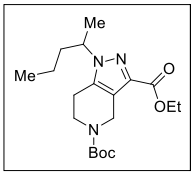
1-Bromo-2-mehylpropane (449 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 90 ºC. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 295 mg (28%), yellow solid, mp 95–96 ºC (ethyl acetate). R_f = 0.23 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 0.90 (d, J = 6.7 Hz, 6H, 2 × CH_3) 1.39 (t, J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.49 (s, 9H, $C(CH_3)_3$), 2.22–2.31 (m, 1H, CH), 2.68 (s, 2H, Pip 7-CH_2), 3.71 (s, 2H, Pip 6-CH_2), 3.86 (d, J = 7.5 Hz, 2H, CH_2), 4.39 (q, J = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 4.61 (s, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 ($OCH_2\underline{C}H_3$), 19.9 (2 × CH_3), 22.3 (Pip 7-CH_2), 28.4 ($C(\underline{C}H_3)_3$), 29.6 (CH), 40.2 (Pip 6-CH_2), 41.6 (Pip 4-CH_2), 56.9 (CH_2), 60.7 ($O\underline{C}H_2CH_3$), 80.2 ($\underline{C}(CH_3)_3$), 117.2, 138.1, 138.5, 155.1 (Boc C=O), 162.6 (C=O). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1682, 1705 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 374.2050. $[C_{18}H_{29}N_3O_4+Na]^+$ requires 374.2050.

5-(*tert*-Butyl) 3-ethyl 2-isobutyl-1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (41e)



Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 463 mg (44%), white solid, mp 53–55 ºC (ethyl acetate). R_f = 0.63 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 0.89 (d, J = 6.7 Hz, 6H, 2 × CH_3) 1.38 (t, J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.48 (s, 9H, $C(CH_3)_3$), 2.13–2.21 (m, 1H, CH), 2.74 (s, 2H, Pip 7-CH_2), 3.68 (s, 2H, Pip 6-CH_2), 4.28–4.38 (m, 4H, $OC\underline{H}_2CH_3$, CH_2), 4.61 (s, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 ($OCH_2\underline{C}H_3$), 20.0 (2 × CH_3), 23.7 (Pip 7-CH_2), 28.5 ($C(\underline{C}H_3)_3$), 30.1 (CH), 41.7 (Pip 6-CH_2), 42.3 (Pip 4-CH_2), 58.6 (CH_2), 61.0 ($O\underline{C}H_2CH_3$), 80.1 ($\underline{C}(CH_3)_3$), 119.2, 127.6, 145.9, 155.2 (Boc C=O), 160.0 (C=O). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1689 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 374.2050. $[C_{18}H_{29}N_3O_4+Na]^+$ requires 374.2050.

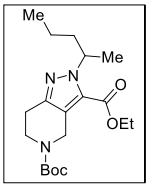
5-(*tert*-Butyl) 3-ethyl 1-(pentan-2-yl)-1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (40f)



2-Bromopentane (495 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 100 ºC. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 219 mg (20%), colorless oil. R_f = 0.50 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 0.86 (t, J = 7.4 Hz, 3H, CH_3), 1.04–1.14 (m, 1H, CH), 1.18–1.22 (m, 1H, CH), 1.37 (t, J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.43 (d, J = 6.6 Hz, 3H, CH_3), 1.48 (s, 12H, $C(CH_3)_3$, CH_3), 1.68–1.73 (m, 1H, CH), 1.95–2.03 (m, 1H, CH), 2.68 (s, 2H, Pip 7-CH_2), 3.70 (s, 2H, Pip 6-CH_2), 4.20–4.26 (m, 1H, CH), 4.38

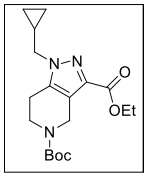
(q, J = 7.1 Hz, 2H, OC<u>H</u>$_2$CH$_3$), 4.51–4.65 (m, 2H, Pip 4-CH$_2$). ^{13}C NMR (100 MHz, CDCl$_3$): δ_C ppm 13.8 (CH$_3$), 14.6 (OCH$_2$<u>C</u>H$_3$),19.8 (2 × CH), 21.0 (CH$_3$), 22.4 (Pip 7-CH$_2$), 28.6 (C(<u>C</u>H$_3$)$_3$), 38.5 (2 × CH), 40.1 (Pip 6-CH$_2$), 41.8 (Pip 4-CH$_2$), 56.1 (CH), 60.7 (O<u>C</u>H$_2$CH$_3$), 80.3 (<u>C</u>(CH$_3$)$_3$), 117.1, 137.6, 137.8, 155.3 (Boc C=O), 162.8 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1691, 1732 (C=O). HRMS (ESI TOF): [M+Na]$^+$, found 388.2207. [C$_{19}$H$_{31}$N$_3$O$_4$+Na]$^+$ requires 388.2207.

5-(*tert*-Butyl) 3-ethyl 2-(pentan-2-yl)-2,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (41f)



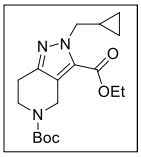
Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 576 mg (53%), colorless oil. R_f = 0.77 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, CDCl$_3$): δ_H ppm 0.86 (t, J = 7.4 Hz, 3H, CH$_3$), 1.04–1.17 (m, 1H, CH), 1.18–1.25 (m, 1H, CH), 1.38 (t, J = 7.1 Hz, 3H, OCH$_2$C<u>H</u>$_3$), 1.43 (d, J = 6.6 Hz, 3H, CH$_3$), 1.48 (s, 9H, C(CH$_3$)$_3$), 1.64–1.70 (m, 1H, CH), 1.90–1.99 (m, 1H, CH), 2.75 (s, 2H, Pip 7-CH$_2$), 3.68 (s, 2H, Pip 6-CH$_2$), 4.32 (q, J = 7.0 Hz, 2H, OC<u>H</u>$_2$CH$_3$), 4.60 (s, 2H, Pip 4-CH$_2$), 5.39–5.44 (m, 1H, CH). ^{13}C NMR (100 MHz, CDCl$_3$): δ_C ppm 13.9 (CH$_3$), 14.4 (OCH$_2$<u>C</u>H$_3$),19.6 (2 × CH), 21.5 (CH$_3$), 23.9 (Pip 7-CH$_2$), 28.6 (C(<u>C</u>H$_3$)$_3$), 39.2 (2 × CH), 41.8 (Pip 6-CH$_2$), 42.3 (Pip 4-CH$_2$), 55.5 (CH), 60.9 (O<u>C</u>H$_2$CH$_3$), 80.0 (<u>C</u>(CH$_3$)$_3$), 118.6, 127.5, 145.9, 155.2 (Boc C=O), 160.1 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1695, 1719 (C=O). HRMS (ESI TOF): [M+Na]$^+$, found 388.2207. [C$_{19}$H$_{31}$N$_3$O$_4$+Na]$^+$ requires 388.2207.

5-(*tert*-Butyl) 3-ethyl 1-(cyclopropylmethyl)-1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (40g)



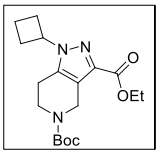
(Bromomethyl)cyclopropane (442 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 100 ºC. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 304 mg (29%), yellowish solid, mp 100–101 ºC (ethyl acetate). R_f = 0.2. (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, CDCl$_3$): δ_H ppm 0.33–0.37 (m, 2H, CH$_2$), 0.54–0.61 (m, 2H, CH$_2$), 1.19–1.28 (m, 1H, CH), 1.37 (t, J = 7.1 Hz, 3H, OCH$_2$C<u>H</u>$_3$), 1.47 (s, 9H, C(CH$_3$)$_3$), 2.70 (s, 2H, Pip 7-CH$_2$), 3.71 (s, 2H, Pip 6-CH$_2$), 3.96 (d, J = 7.0 Hz, 2H, CH$_2$), 4.38 (q, J = 7.1 Hz, 2H, OC<u>H</u>$_2$CH$_3$), 4.60 (s, 2H, Pip 4-CH$_2$). ^{13}C NMR (100 MHz, CDCl$_3$): δ_C ppm 4.11 (2 × CH$_2$), 11.4 (CH), 14.5 (OCH$_2$<u>C</u>H$_3$), 22.6 (Pip 7-CH$_2$), 28.5 (C(<u>C</u>H$_3$)$_3$), 40.1 (Pip 6-CH$_2$), 41.7 (Pip 4-CH$_2$), 54.7 (CH$_2$), 60.8 (O<u>C</u>H$_2$CH$_3$), 80.3 (<u>C</u>(CH$_3$)$_3$), 117.7, 137.9, 138.0, 155.1 (Boc C=O), 162.7 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1695 (C=O). HRMS (ESI TOF): [M+Na]$^+$, found 372.1894. [C$_{18}$H$_{27}$N$_3$O$_4$+Na]$^+$ requires 372.1894.

5-(*tert*-Butyl) 3-ethyl 2-(cyclopropylmethyl)-2,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (41g)



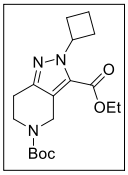
Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 524 mg (50%), colorless oil. R_f = 0.72 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 0.39–0.41 (m, 2H, CH_2), 0.47–0.52 (m, 2H, CH_2), 1.30–1.40 (m, 4H, CH, $OCH_2C\underline{H}_3$), 1.48 (s, 9H, $C(CH_3)_3$), 2.74 (s, 2H, Pip 7-CH_2), 3.68 (s, 2H, Pip 6-CH_2), 4.26–4.39 (m, 4H, $OC\underline{H}_2CH_3$, CH_2), 4.61 (s, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 3.74 (2 × CH_2), 12.1 (CH), 14.4 ($OCH_2\underline{C}H_3$), 23.7 (Pip 7-CH_2), 28.6 ($C(\underline{C}H_3)_3$), 41.7 (Pip 6-CH_2), 42.2 (Pip 4-CH_2), 56.1 (CH_2), 61.0 ($O\underline{C}H_2CH_3$), 80.1 ($\underline{C}(CH_3)_3$), 119.3, 127.2, 145.9, 155.1 (Boc C=O), 160.0 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1688, 1726 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 372.1893. $[C_{18}H_{27}N_3O_4+Na]^+$ requires 372.1894.

5-(*tert*-Butyl) 3-ethyl 1-cyclobutyl-1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (40h)



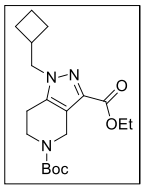
Bromocyclobutane (442 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 100 ºC. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 325 mg (31%), colorless oil. R_f = 0.40 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.38 (t, J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.47 (s, 9H, $C(CH_3)_3$), 1.78–1.92 (m, 2H, CH_2), 2.37 (q, J = 10.1 Hz, 2H, CH_2), 2.66 (s, 2H, Pip 7-CH_2), 2.69–2.81 (m, 2H, CH_2), 3.69 (s, 2H, Pip 6-CH_2), 4.38 (q, J = 7.0 Hz, 2H, $OC\underline{H}_2CH_3$), 4.55–7.70 (m, 3H, CH, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.5 ($OCH_2\underline{C}H_3$), 15.0 (CH_2), 22.1 (Pip 7-CH_2), 28.5 ($C(\underline{C}H_3)_3$), 29.8 (2 × CH_2), 40.0 (Pip 6-CH_2), 41.7 (Pip 4-CH_2), 53.2 (CH), 60.8 ($O\underline{C}H_2CH_3$), 80.2 ($\underline{C}(CH_3)_3$), 117.6, 137.6, 137.7, 155.1 (Boc C=O), 162.7 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1688, 1708 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 372.1894. $[C_{18}H_{27}N_3O_4+Na]^+$ requires 372.1894.

5-(*tert*-Butyl) 3-ethyl 2-cyclobutyl-2,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (41h)



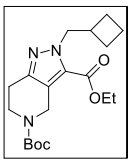
Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 450 mg (43%), white solid, mp 89–90 ºC (ethyl acetate). R_f = 0.29 (*n*-hexane/ethyl acetate 4/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.37 (t, J = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.47 (s, 9H, $C(CH_3)_3$), 1.74–1.89 (m, 2H, CH_2), 2.34–2.45 (m, 2H, CH_2), 2.57–2.68 (m, 2H, CH_2), 2.77 (s, 2H, Pip 7-CH_2), 3.68 (s, 2H, Pip 6-CH_2), 4.32 (q, J = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 4.59 (s, 2H, Pip 4-CH_2), 5.66 (p, J = 8.2 Hz, 1H, CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 ($OCH_2\underline{C}H_3$), 14.6 (CH_2), 23.9 (Pip 7-CH_2), 28.6 ($C(\underline{C}H_3)_3$), 30.2 (2 × CH_2), 41.1 (Pip 6-CH_2), 42.3 (Pip 4-CH_2), 54.0 (CH), 61.0 ($O\underline{C}H_2CH_3$), 80.0 ($\underline{C}(CH_3)_3$), 119.3, 127.2, 145.9, 155.2 (Boc C=O), 160.0 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1686, 1720 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 372.1893. $[C_{18}H_{27}N_3O_4+Na]^+$ requires 372.1894.

5-(*tert*-Butyl) 3-ethyl 1-(cyclobutylmethyl)-1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (40i)



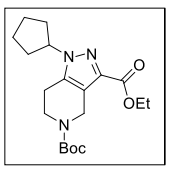
(Bromomethyl)cyclobutane (488 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 100 ºC. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 315 mg, (29%), yellowish solid, mp 58–59 ºC (ethyl acetate). R_f = 0.24 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.37 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.47 (s, 9H, $C(CH_3)_3$), 1.71–1.79 (m, 2H, CH_2), 1.81–1.90 (m, 2H, CH_2), 1.96–2.05 (m, 2H, CH_2), 2.67 (s, 2H, Pip 7-CH_2), 2.78–2.85 (m, 1H, CH), 3.69 (s, 2H, Pip 6-CH_2), 4.07 (d, *J* = 7.2 Hz, 2H, CH_2), 4.34 (q, *J* = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 4.59 (s, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.5 ($OCH_2\underline{C}H_3$), 18.3 (CH_2), 22.3 (Pip 7-CH_2), 26.0 (CH_2), 28.5 ($C(\underline{C}H_3)_3$), 35.9 (CH), 40.0 (Pip 6-CH_2), 41.7 (Pip 4-CH_2), 54.8 (CH_2), 60.8 ($O\underline{C}H_2CH_3$), 80.3 ($\underline{C}(CH_3)_3$), 117.4, 137.9, 138.1, 155.2 (Boc C=O), 162.7 (C=O). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1696 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 386.2050. $[C_{19}H_{29}N_3O_4+Na]^+$ requires 386.2050.

5-(*tert*-Butyl) 3-ethyl 2-(cyclobutylmethyl)-2,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (41i)



Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 577 mg (53%), colorless oil. R_f = 0.62 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.39 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.49 (s, 9H, $C(CH_3)_3$), 1.77–1.91 (m, 4H, 2 × CH_2), 1.96–2.03 (m, 2H, CH_2), 2.70–2.85 (m, 3H, CH, Pip 7-CH_2), 3.68 (s, 2H, Pip 6-CH_2), 4.34 (q, *J* = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 4.55 (d, *J* = 7.3 Hz, 2H, CH_2), 4.61 (s, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 ($OCH_2\underline{C}H_3$), 18.4 (CH_2), 23.6 (Pip 7-CH_2), 25.8 (CH_2), 28.6 ($C(\underline{C}H_3)_3$), 36.4 (CH), 41.7 (Pip 6-CH_2), 42.2 (Pip 4-CH_2), 56.2 (CH_2), 61.0 ($O\underline{C}H_2CH_3$), 80.1 ($\underline{C}(CH_3)_3$), 119.1, 127.4, 145.7, 155.2 (Boc C=O), 160.0 (C=O). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1698, 1720 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 386.2050. $[C_{19}H_{29}N_3O_4+Na]^+$ requires 386.2050.

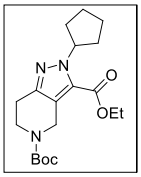
5-(*tert*-Butyl) 3-ethyl 1-cyclopentyl-1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (40j)



Bromocyclopentane (488 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 100 ºC. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (6/1, v/v). Yield 338 mg (31%), colorless solid, mp 86–88 ºC (ethyl acetate). R_f = 0.28 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.37 (t, *J* = 7.1 Hz, 3H, $OCH_2C\underline{H}_3$), 1.48 (s, 9H, $C(CH_3)_3$), 1.63–1.67 (m, 2H, CH_2), 1.88–1.98 (m, 2H, CH_2), 2.01–2.20 (m, 4H, 2 × CH_2), 2.70 (s, 2H, Pip 7-CH_2), 3.71 (s, 2H, Pip 6-CH_2), 4.36 (q, *J* = 7.1 Hz, 2H, $OC\underline{H}_2CH_3$), 4.45–4.56 (m, 1H, CH), 4.59 (s, 2H, Pip 4-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.5 ($OCH_2\underline{C}H_3$), 22.3 (Pip 7-CH_2), 24.4 (2 × CH_2), 28.5 ($C(\underline{C}H_3)_3$), 32.4 (2 × CH_2), 40.1 (Pip 6-CH_2), 41.9 (Pip 4-CH_2), 60.6 ($O\underline{C}H_2CH_3$), 60.7 (CH), 80.2

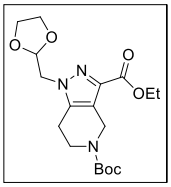
(C(CH$_3$)$_3$), 117.5, 137.7, 137.9, 155.1 (Boc C=O), 162.8 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1687, 1705 (C=O). HRMS (ESI TOF): [M+Na]$^+$, found 386.2050. [$C_{19}H_{29}N_3O_4$+Na]$^+$ requires 386.2050.

5-(*tert*-Butyl) 3-ethyl 2-cyclopentyl-2,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (41j)



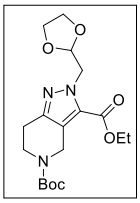
Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (15/1, v/v). Yield 479 mg (44%), colorless oil. R_f = 0.74 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.37 (t, J = 7.1 Hz, 3H, OCH$_2$CH$_3$), 1.47 (s, 9H, C(CH$_3$)$_3$), 1.60–1.67 (m, 2H, CH$_2$), 1.63–1.91 (m, 2H, CH$_2$), 1.99–2.10 (m, 4H, 2 × CH$_2$), 2.73 (s, 2H, Pip 7-CH$_2$), 3.67 (s, 2H, Pip 6-CH$_2$), 4.32 (q, J = 7.0 Hz, 2H, OCH$_2$CH$_3$), 4.59 (s, 2H, Pip 4-CH$_2$), 5.60 (p, J = 7.4 Hz, 1H, CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 (OCH$_2$CH$_3$), 23.9 (Pip 7-CH$_2$), 24.6 (2 × CH$_2$), 28.5 (C(CH$_3$)$_3$), 33.0 (2 × CH$_2$), 41.0 (Pip 6-CH$_2$), 42.3 (Pip 4-CH$_2$), 60.9 (OCH$_2$CH$_3$), 61.0 (CH), 80.0 (C(CH$_3$)$_3$), 119.1, 127.5, 145.6, 155.1 (Boc C=O), 160.1 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1695, 1720 (C=O). HRMS (ESI TOF): [M+Na]$^+$, found 386.2050. [$C_{19}H_{29}N_3O_4$+Na]$^+$ requires 386.2050.

5-(*tert*-Butyl) 3-ethyl 1-[(1,3-dioxolan-2-yl)methyl]-1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (40k)



2-(Bromomethyl)-1,2-dioxolane (548 mg) was used for the *N*-alkylation reaction, and the reaction mixture was heated at 80 ºC. It was purified by column chromatography on silica gel with *n*-hexane/acetone (4/1, v/v). Yield 297 mg (26%), yellowish solid, mp 94–95 ºC (ethyl acetate). R_f = 0.16 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.36 (t, J = 7.1 Hz, 3H, OCH$_2$CH$_3$), 1.47 (s, 9H, C(CH$_3$)$_3$), 2.74 (s, 2H, Pip 7-CH$_2$), 3.69 (s, 2H, Pip 6-CH$_2$), 3.82–3.95 (m, 4H, 2 × CH$_2$), 4.22 (d, J = 4.3 Hz, 2H, CH$_2$), 4.37 (q, J = 7.0 Hz, 2H, OCH$_2$CH$_3$), 4.59 (s, 2H, Pip 4-CH$_2$), 5.21 (t, J = 4.3 Hz, 1H, CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.5 (OCH$_2$CH$_3$), 22.3 (Pip 7-CH$_2$), 28.5 (C(CH$_3$)$_3$), 40.0 (Pip 6-CH$_2$), 41.6 (Pip 4-CH$_2$), 52.7 (CH$_2$), 60.9 (OCH$_2$CH$_3$), 65.3 (2 × CH$_2$), 80.2 (C(CH$_3$)$_3$), 102.2 (CH), 117.6, 138.8, 139.9, 155.1 (Boc C=O), 162.5 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1687, 1731 (C=O). HRMS (ESI TOF): [M+Na]$^+$, found 404.1792. [$C_{18}H_{27}N_3O_6$+Na]$^+$ requires 404.1792.

5-(*tert*-Butyl) 3-ethyl 2-[(1,3-dioxolan-2-yl)methyl]-2,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (41k)



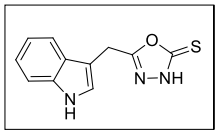
Purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (2/1, v/v). Yield 320 mg (28%), colorless oil. R_f = 0.42 (*n*-hexane/ethyl acetate 2/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H ppm 1.37 (t, J = 7.1 Hz, 3H, OCH$_2$CH$_3$), 1.47 (s, 9H, C(CH$_3$)$_3$), 2.75 (s, 2H, Pip 7-CH$_2$), 3.67 (s, 2H, Pip 6-CH$_2$), 3.81–3.98 (m, 4H, 2 × CH$_2$), 4.33 (q, J = 7.1 Hz, 2H, OCH$_2$CH$_3$), 4.60 (s, 2H, Pip 4-CH$_2$), 4.69 (d, J = 4.5 Hz, 2H, CH$_2$), 5.28 (s, 1H, CH). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C ppm 14.4 (OCH$_2$CH$_3$), 23.7 (Pip 7-CH$_2$), 28.5 (C(CH$_3$)$_3$), 40.9 (Pip 6-CH$_2$), 42.2 (Pip

4-CH_2), 53.6 (CH_2), 61.2 (O<u>C</u>H_2CH_3), 65.3 (2 × CH_2), 80.1 (<u>C</u>$(CH_3)_3$), 102.3 (CH), 119.6, 128.1, 146.8, 155.1 (Boc C=O), 160.0 (C=O). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1695 (C=O). HRMS (ESI TOF): $[M+Na]^+$, found 404.1794. $[C_{18}H_{27}N_3O_6+Na]^+$ requires 404.1792.

3.2.11 General procedure for the synthesis of 1,3,4-oxadiazole-2(3*H*)-thiones 54 and 55

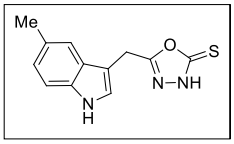
To a solution of **50** or **51** (4 mmol) in methanol (8 mL), aqueous hydrazine hydrate (4 mL) was added, and the reaction mixture was stirred at reflux for 16 h. After cooling to rt, the reaction mixture was poured into water (40 mL), and the solution was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure. The isolated hydrazide **52** or **53** was used for the next step without purification. To a solution of intermediate **52** or **53** in methanol (10 mL), KOH (381 mg, 6.8 mmol) in methanol (2 mL), and CS_2 (0.55 mL, 9.2 mmol) were added, and the reaction mixture was refluxed for 16 h. After cooling to rt, aqueous HCl solution (HCl/H_2O 1/4, v/v (14 mL)) was added to the reaction mixture and left at rt for 1 h. Then, the reaction mixture was filtered, and the filter cake was washed with water (2 mL). The solid was dissolved in ethyl acetate (3 × 20 mL), washed with water (20 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with dichloromethane/methanol (100/1, v/v).

5-[(1*H*-Indol-3-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione (54), *previously reported by Song et al*. [101]



Methyl 2-(1*H*-indol-3-yl)acetate (**50**) (756 mg) was used for the reaction with hydrazine hydrate. Yield 665 mg (72%), yellow solid. R_f = 0.43 (dichloromethane/methanol 20/1, (v/v)). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H ppm 4.21 (s, 2H, CH_2), 7.01 (t, J = 7.5 Hz, 1H, CH), 7.11 (t, J = 7.5 Hz, 1H, CH), 7.35 (d, J = 1.5 Hz, 1H, CH), 7.38 (d, J = 8.1 Hz, 1H, CH), 7.51 (d, J = 7.9 Hz, 1H, CH), 11.08 (br s, 1H, NH), 14.33 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C ppm 21.9 (CH_2), 107.1, 112.1 (CH), 118.7 (CH), 119.2 (CH), 121.8 (CH), 124.7 (CH), 127.1, 136.7, 164.6 (oxadiazole C-2), 177.8 (C=S). IR (neat, $\bar{\upsilon}$, cm^{-1}): 3393 (NH). HRMS (ESI TOF): $[M+Na]^+$, found 254.0359. $[C_{11}H_9N_3OS+Na]^+$ requires 254.0359.

5-[(5-Methyl-1*H*-indol-3-yl)methyl]-1,3,4-oxadiazole-2(3*H*)-thione (55)



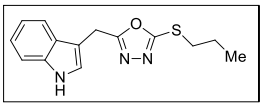
Methyl 2-(5-methyl-1*H*-indol-3-yl)acetate (**51**) (812 mg) was used for reaction with hydrazine hydrate. Yield 617 mg (63%), yellowish solid, mp 188–189 ºC (methanol). R_f = 0.26 (dichloromethane/methanol 20/1, v/v). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H ppm 2.36 (s, 3H, CH_3), 4.17 (s, 2H, CH_2), 6.93 (d, J = 8.3 Hz, 1H, CH), 7.25–7.28 (m, 2H, 2 × CH), 10.94 (br s, 1H, NH), 14.33 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C ppm 21.3 (5-CH_3), 21.7 (CH_2), 105.2 (CH), 117.6 (CH), 123.0 (CH), 124.5 (CH), 126.8, 127.3,

134.5, 163.5 (oxadiazole C-2), 177.8 (C=S). IR (neat, $\bar{\upsilon}$, cm^{-1}): 3359 (NH). HRMS (ESI TOF): $[M+Na]^+$, found 268.0515. $[C_{12}H_{11}N_3OS+Na]^+$ requires 268.0515.

3.2.12 General procedure for the alkylation of 1,3,4-oxadiazole-2-thione 54 and 55 with haloalkanes

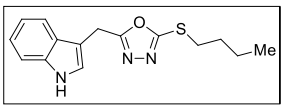
To a solution of compound **54** or **55** (3 mmol) in dimethylformamide (6 mL), an appropriate haloalkane (3.3 mmol), and K_2CO_3 (497 mg, 3.6 mmol) were added, and the reaction mixture was left at rt for 16 h. Upon completion, the reaction mixture was poured into water (20 mL), and the solution was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (20 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography by using an appropriate eluent.

2-[(1*H*-Indol-3-yl)methyl]-5-(propylthio)-1,3,4-oxadiazole (56a)



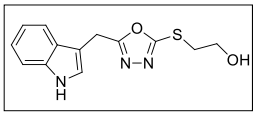
1,3,4-Oxadiazole-2(3*H*)-thione **54** (693 mg) and 1-bromopropane (403 mg) were used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with dichloromethane/methanol (100/1, v/v). Yield 721 mg (88%), brownish solid, mp 73–74 ºC (methanol). R_f = 0.20 (dichloromethane/methanol 50/1, v/v). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H ppm 0.92 (t, J = 7.3 Hz, 3H, $CH_2C\underline{H}_3$), 1.61–1.72 (m, 2H, $C\underline{H}_2CH_3$), 3.14 (t, J = 7.1 Hz, 2H, SCH_2), 4.33 (s, 2H, CH_2), 7.00 (t, J = 7.4 Hz, 1H, CH), 7.09 (t, J = 7.5 Hz, 1H, CH), 7.33 (d, J = 2.0 Hz, 1H, CH), 7.37 (d, J = 8.1 Hz, 1H, CH), 7.50 (d, J = 7.9 Hz, 1H, CH), 11.04 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C ppm 12.8 ($CH_2\underline{C}H_3$), 21.6 (CH_2), 22.4 (CH_2), 33.8 (SCH_2), 106.7, 111.6 (CH), 118.2 (CH), 118.8 (CH), 121.3 (CH), 124.2 (CH), 126.6, 136.2, 163.2 (oxodiazole C-2), 167.0 (oxodiazole C-5). IR (neat, $\bar{\upsilon}$, cm^{-1}): 3316 (NH). HRMS (ESI TOF): $[M+Na]^+$, found 296.0828. $[C_{14}H_{15}N_3OS+Na]^+$ requires 296.0828.

5-(Butylthio)-2-[(1*H*-indol-3-yl)methyl]-1,3,4-oxadiazole (56b), *previously reported by Song et al*. [101]



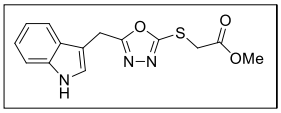
1,3,4-Oxadiazole-2(3*H*)-thione **54** (693 mg) and 1-bromobutane (449 mg) were used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/acetone (4/1, (v/v)). Yield 559 mg (65%), brownish solid, mp 78–80 ºC (acetone). R_f = 0.38 (*n*-hexane/acetone 3/1, (v/v)). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H ppm 0.84 (t, J = 7.4 Hz, 3H, $CH_2C\underline{H}_3$), 1.19–1.42 (m, 2H, CH_2), 1.55–1.83 (m, 2H, CH_2), 3.16 (t, J = 7.3 Hz, 2H, SCH_2), 4.34 (s, 2H, CH_2), 7.00 (t, J = 7.4 Hz, 1H, CH), 7.10 (t, J = 7.5 Hz, 1H, CH), 7.34 (d, J = 1.3 Hz, 1H, CH), 7.38 (d, J = 8.1 Hz, 1H, CH), 7.51(d, J = 7.9 Hz, 1H, CH), 11.05 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C ppm 13.8 ($CH_2\underline{C}H_3$), 21.5 (CH_2), 21.9 (CH_2), 31.5 (CH_2), 32.1 (SCH_2), 107.1, 112.1 (CH), 118.6 (CH), 119.2 (CH), 121.8 (CH), 124.7 (CH), 127.1, 136.7, 163.8 (oxodiazole C-2), 167.5 (oxodiazole C-5). IR (neat, $\bar{\upsilon}$, cm^{-1}): 3281 (NH). HRMS (ESI TOF): $[M+Na]^+$, found 310.0985. $[C_{15}H_{17}N_3OS+Na]^+$ requires 310.0984.

2-({5-[(1*H*-Indol-3-yl)methyl]-1,3,4-oxadiazol-2-yl}thio)ethanol (56c)



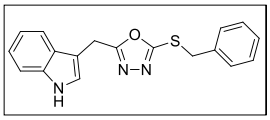
1,3,4-Oxadiazole-2(3*H*)-thione **54** (693 mg) and 2-bromoethanol (409 mg) were used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with dichloromethane/methanol (100/1, v/v). Yield 652 mg (79%), brown gum. R_f = 0.28 (dichloromethane/methanol 20/1, v/v). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H ppm 3.27 (t, J = 6.2 Hz, 2H, SCH_2), 3.66 (q, J = 6.0 Hz, 2H, OCH_2), 4.32 (s, 2H, CH_2), 5.11 (t, J = 5.5 Hz, 2H, OH), 7.00 (t, J = 7.4 Hz, 1H, CH), 7.10 (t, J = 7.5 Hz, 1H, CH), 7.33 (d, J = 2.1 Hz, 1H, CH), 7.38 (d, J = 8.1 Hz, 1H, CH), 7.50 (d, J = 7.9 Hz, 1H, CH), 11.04 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C ppm 21.5 (CH_2), 34.9 (SCH_2), 59.3 (OCH_2), 106.7, 111.6 (CH), 118.2 (CH), 118.8 (CH), 121.4 (CH), 124.2 (CH), 126.6, 136.2, 163.5 (oxodiazole C-2), 167.0 (oxodiazole C-5). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 3276 (NH, OH). HRMS (ESI TOF): $[M+H]^+$, found 276.0801. $[C_{13}H_{13}N_3O_2S+H]^+$ requires 276.0801.

Methyl 2-({5-[(1*H*-indol-3-yl)methyl]-1,3,4-oxadiazol-2-yl}thio)acetate (56d)



1,3,4-Oxadiazole-2(3*H*)-thione **54** (693 mg) and methyl 2-bromoacetate (502 mg) were used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with dichloromethane/methanol (100/1, v/v). Yield 827 mg (91%), white solid, mp 106–107 ºC (methanol). R_f = 0.65 (dichloromethane/methanol 20/1, v/v). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H ppm 3.62 (s, 3H, OCH_3), 4.17 (s, 2H, CH_2), 4.33 (s, 2H, SCH_2), 7.00 (t, J = 7.4 Hz, 1H, CH), 7.10 (t, J = 7.5 Hz, 1H, CH), 7.33 (d, J = 2.1 Hz, 1H, CH), 7.37 (d, J = 8.1 Hz, 1H, CH), 7.50 (d, J = 7.9 Hz, 1H, CH), 11.05 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C ppm 21.4 (CH_2), 33.5 (SCH_2), 52.6 (OCH_3), 106.5, 111.6 (CH), 118.2 (CH), 118.8 (CH), 121.4 (CH), 124.2 (CH), 126.6, 136.2, 162.4 (oxodiazole C-2), 167.3 (oxodiazole C-5), 168.1 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1731 (C=O), 3293 (NH). HRMS (ESI TOF): $[M+H]^+$, found 304.0750. $[C_{14}H_{13}N_3O_3S+H]^+$ requires 304.0750.

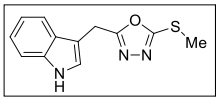
2-[(1*H*-Indol-3-yl)methyl]-5-(benzylthio)-1,3,4-oxadiazole (56e), *previously reported by Song et al*. [101]



1,3,4-Oxadiazole-2(3*H*)-thione **54** (693 mg) and (bromomethyl)benzene (561 mg) were used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with dichloromethane/methanol (100/1, v/v). Yield 867 mg (90%), brownish solid, mp 121–122 ºC (methanol). R_f = 0.16 (dichloromethane/methanol 100/2, v/v). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H ppm 4.33 (s, 2H, CH_2), 4.40 (s, 2H, CH_2), 7.00 (t, J = 7.4 Hz, 1H, CH), 7.10 (t, J = 7.2 Hz, 1H, CH), 7.19–7.24 (m, 3H, 3 × CH), 7.26–7.28 (m, 2H, 2 × CH), 7.32 (d, J = 2.2 Hz, 1H, CH), 7.38 (d, J = 8.1 Hz, 1H, CH), 7.49 (d, J = 7.9 Hz, 1H, CH), 11.05 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C ppm 21.5 (CH_2), 35.8 (CH_2), 106.6, 111.6 (CH), 118.2 (CH), 118.8 (CH), 121.3 (CH), 124.2 (CH), 126.6, 127.7 (CH), 128.5 (2 × CH), 128.9 (2 × CH), 136.2, 136.5, 162.7

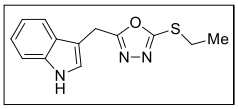
(oxodiazole C-2), 167.3 (oxodiazole C-5). IR (neat, $\bar{\upsilon}$, cm^{-1}): 3274 (NH). HRMS (ESI TOF): $[M+Na]^+$, found 344.0827. $[C_{18}H_{15}N_3OS+Na]^+$ requires 344.0828.

2-[(1*H*-Indol-3-yl)methyl]-5-(methylthio)-1,3,4-oxadiazole (56f)



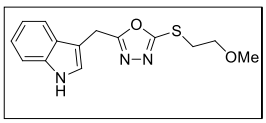
1,3,4-Oxadiazole-2(3*H*)-thione **54** (693 mg) and iodomethane (469 mg) were used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, (v/v)). Yield 522 mg (71%), yellowish solid, mp 114–115 ºC (ethyl acetate). R_f = 0.26 (*n*-hexane/ ethyl acetate 2/1, (v/v)). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H ppm 2.65 (s, 3H, CH_3), 4.34 (s, 2H, CH_2), 7.01 (t, *J* = 7.4 Hz, 1H, CH), 7.11 (t, *J* = 7.5 Hz, 1H, CH), 7.35 (d, *J* = 1.9 Hz, 1H, CH), 7.38 (d, *J* = 8.1 Hz, 1H, CH), 7.51 (d, *J* = 7.9 Hz, 1H, CH), 11.06 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C ppm 14.7 (CH_3), 21.9 (CH_2), 107.1, 112.1 (CH), 118.7 (CH), 119.2 (CH), 121.8 (CH), 124.7 (CH), 127.1, 136.7, 164.6 (oxodiazole C-2), 167.5 (oxodiazole C-5). IR (neat, $\bar{\upsilon}$, cm^{-1}): 3277 (NH). HRMS (ESI TOF): $[M+Na]^+$, found 268.0515. $[C_{12}H_{11}N_3OS+Na]^+$ requires 268.0515.

5-(Ethylthio)-2-[(1*H*-indol-3-yl)methyl]-1,3,4-oxadiazole (56g)



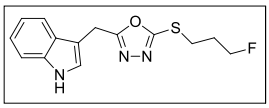
1,3,4-Oxadiazole-2(3*H*)-thione **54** (693 mg) and iodoethane (515 mg) were used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with dichloromethane. Yield 513 mg (66%), brownish gum. R_f = 0.26 (dichloromethane/methanol 20/1, (v/v)). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H ppm 1.32 (t, *J* = 7.3 Hz, 3H, $SCH_2C\underline{H}_3$), 3.17 (q, *J* = 7.3 Hz, 2H, $SC\underline{H}_2CH_3$), 4.33 (s, 2H, CH_2), 7.00 (t, *J* = 7.4 Hz, 1H, CH), 7.10 (t, *J* = 7.5 Hz, 1H, CH), 7.33 (d, *J* = 2.0 Hz, 1H, CH), 7.37 (d, *J* = 8.1 Hz, 1H, CH), 7.50 (d, *J* = 7.9 Hz, 1H, CH), 11.04 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C ppm 14.8 ($SCH_2\underline{C}H_3$), 21.5 (CH_2), 26.5 ($S\underline{C}H_2CH_3$), 106.7, 111.6 (CH), 118.2 (CH), 118.8 (CH), 121.4 (CH), 124.2 (CH), 126.6, 136.2, 163.2 (oxodiazole C-2), 167.1 (oxodiazole C-5). IR (neat, $\bar{\upsilon}$, cm^{-1}): 3267 (NH). HRMS (ESI TOF): $[M+Na]^+$, found 282.0672. $[C_{13}H_{13}N_3OS+Na]^+$ requires 282.0672.

2-[(1*H*-Indol-3-yl)methyl]-5-[(2-methoxyethyl)thio]-1,3,4-oxadiazole (56h)



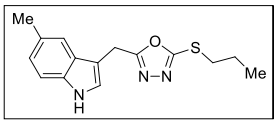
1,3,4-Oxadiazole-2(3*H*)-thione **54** (693 mg) and 1-bromo-2-methoxyethane (455 mg) were used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/acetone (3/1, (v/v)). Yield 667 mg (77%), brownish solid, mp 83–84 ºC (acetone). R_f = 0.27 (*n*-hexane/acetone 3/1, (v/v)). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H ppm 3.21 (s, 3H, OCH_3), 3.37 (t, *J* = 6.0 Hz, 2H, SCH_2), 3.60 (t, *J* = 6.0 Hz, 2H, OCH_2), 4.34 (s, 2H, CH_2), 7.01 (t, *J* = 7.4 Hz, 1H, CH), 7.11 (t, *J* = 7.5 Hz, 1H, CH), 7.34 (s, 1H, CH), 7.38 (d, *J* = 8.1 Hz, 1H, CH), 7.51 (d, *J* = 7.9 Hz, 1H, CH), 11.06 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C ppm 21.9 (CH_2), 32.1 (SCH_2), 58.3 (OCH_3), 70.2 (OCH_2), 107.1, 112.1 (CH), 118.6 (CH), 119.2 (CH), 121.8 (CH), 124.7 (CH), 127.1, 136.7, 163.7 (oxodiazole C-2), 167.6 (oxodiazole C-5). IR (neat, $\bar{\upsilon}$, cm^{-1}): 3266 (NH). HRMS (ESI TOF): $[M+Na]^+$, found 312.0777. $[C_{14}H_{15}N_3O_2S+Na]^+$ requires 312.0777.

5-[(3-Fluoropropyl)thio]-2-[(1*H*-indol-3-yl)methyl]-1,3,4-oxadiazole (56i)



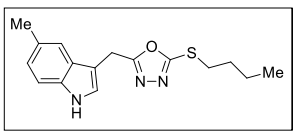
1,3,4-Oxadiazole-2(3*H*)-thione **54** (693 mg) and 1-bromo-3-fluoropropane (462 mg) were used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/acetone (3/1, (v/v)). Yield 550 mg (63%), brown solid, mp 59–61 ºC (acetone). R_f = 0.29 (*n*-hexane/acetone 3/1, (v/v)). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H ppm 1.90–2.20 (m, 2H, $SCH_2C\underline{H_2}$), 3.27 (t, J = 7.2 Hz, 2H, $SC\underline{H_2}CH_2$), 4.34 (s, 2H, CH_2), 4.45 (t, J = 5.7 Hz, 1H, FCH_aH_b), 4.45 (t, J = 5.7 Hz, 1H, FCH_aH_b), 7.01 (t, J = 7.4 Hz, 1H, CH), 7.11 (t, J = 7.5 Hz, 1H, CH), 7.35 (s, 1H, CH), 7.39 (d, J = 8.1 Hz, 1H, CH), 7.51 (d, J = 7.8 Hz, 1H, CH), 11.06 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C ppm 21.9 (CH_2), 28.5 (d, ^3J (C,F) = 5.2 Hz, SCH_2CH_2), 30.3 (d, ^2J (C,F) = 19.8 Hz, SCH_2), 82.6 (d, ^1J (C,F) = 162.9 Hz, FCH_2), 107.1, 112.1 (CH), 118.6 (CH), 119.2 (CH), 121.8 (CH), 124.7 (CH), 127.1, 136.7, 163.5 (oxodiazole C-2), 167.6 (oxodiazole C-5). IR (neat, $\bar{\upsilon}$, cm^{-1}): 3286 (NH). HRMS (ESI TOF): $[M+Na]^+$, found 314.0734. $[C_{14}H_{14}FN_3OS+Na]^+$ requires 314.0734.

2-[(5-Methyl-1*H*-indol-3-yl)methyl]-5-(propylthio)-1,3,4-oxadiazole (57a)



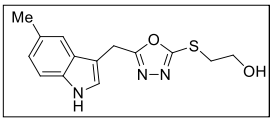
1,3,4-Oxadiazole-2(3*H*)-thione **55** (735 mg) and 1-bromopropane (403 mg) were used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with dichloromethane/methanol (20/1, v/v). Yield 408 mg (90%), brown solid, mp 77–78 ºC (methanol). R_f = 0.29 (dichloromethane/methanol 100/1, v/v). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H ppm 0.92 (t, J = 7.3 Hz, 3H, $CH_2C\underline{H_3}$), 1.62–1.73 (m, 2H, $C\underline{H_2}CH_3$), 2.35 (s, 3H, 5-CH_3), 3.14 (t, J = 7.3 Hz, 2H, SCH_2), 4.28 (s, 2H, CH_2), 6.92 (d, J = 8.2 Hz, 1H, CH), 7.23–7.29 (m, 3H, 3 × CH), 10.90 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C ppm 12.7 (5-CH_3), 21.3 ($CH_2\underline{C}H_3$), 21.4 (CH_2), 22.4 (CH_2), 33.8 (SCH_2), 106.1, 111.3 (CH), 117.7 (CH), 123.0 (CH), 124.2 (CH), 126.8, 127.2, 134.6, 163.3 (oxodiazole C-2), 167.1 (oxodiazole C-5). IR (neat, $\bar{\upsilon}$, cm^{-1}): 3267 (NH). HRMS (ESI TOF): $[M+Na]^+$, found 310.0985. $[C_{15}H_{17}N_3OS+Na]^+$ requires 310.0985.

2-(Butylthio)-5-((5-methyl-1*H*-indol-3-yl)methyl)-1,3,4-oxadiazole (57b)



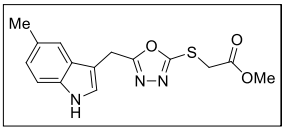
1,3,4-Oxadiazole-2(3*H*)-thione **55** (735 mg) and 1-bromobutane (449 mg) were used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with DCM. Yield 128 mg (85%), brown gum. R_f = 0.50 (DCM/MeOH 20/1, (v/v)). ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.84 (t, J = 7.4 Hz, 3H, $CH_2C\underline{H_3}$), 1.24–1.41 (m, 2H, CH_2), 1.57–1.67 (m, 2H, CH_2), 2.35 (s, 3H, 5-CH_3), 3.16 (t, J = 7.3 Hz, 2H, SCH_2), 4.28 (s, 2H, CH_2), 6.92 (dd, J = 8.3, 0.9 Hz, 1H, CH), 7.23–7.29 (m, 3H, 3 × CH), 10.90 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ 13.3 (5-CH_3), 21.0 ($CH_2\underline{C}H_3$), 21.3 (CH_2), 21.4 (CH_2), 31.0 (CH_2), 31.6 (SCH_2), 106.1, 111.3 (CH), 117.7 (CH), 123.0 (CH), 124.2 (CH), 126.8, 127.2, 134.6, 163.3 (oxodiazole C-2), 167.1 (oxodiazole C-5). IR (neat, $\bar{\upsilon}$, cm^{-1}): 3265 (NH). HRMS (ESI TOF): $[M+Na]^+$, found 324.1141. $[C_{16}H_{19}N_3OS+Na]^+$ requires 324.1141.

2-({5-[(5-Methyl-1*H*-indol-3-yl)methyl]-1,3,4-oxadiazol-2-yl}thio)ethanol (57c)



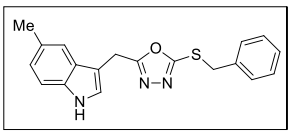
1,3,4-Oxadiazole-2(3*H*)-thione **55** (735 mg) and 2-bromoethanol (409 mg) were used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with dichloromethane/methanol (100/1, v/v). Yield 590 mg (68%), brownish solid, mp 90–91 ºC (methanol). R_f = 0.13 (dichloromethane/methanol 20/1, v/v). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H ppm 2.36 (s, 3H, 5-CH_3), 3.27 (t, J = 6.2 Hz, 2H, SCH_2), 3.66 (q, J = 5.9 Hz, 2H, OCH_2), 4.28 (s, 2H, CH_2), 5.10 (t, J = 5.0 Hz, 1H, OH), 6.92 (d, J = 8.3 Hz, 1H, CH), 7.24–7.28 (m, 3H, 3 × CH), 10.90 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C ppm 21.3 (5-CH_3), 21.4 (CH_2), 34.9 (SCH_2), 59.3 (OCH_2), 106.1, 111.3 (CH), 117.7 (CH), 123.0 (CH), 124.2 (CH), 126.8, 127.2, 134.6, 163.4 (oxodiazole C-2), 167.0 (oxodiazole C-5). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 3244 (NH, OH). HRMS (ESI TOF): $[M+Na]^+$, found 312.0777. $[C_{14}H_{15}N_3O_2S+Na]^+$ requires 312.0777.

Methyl 2-({5-[(5-methyl-1*H*-indol-3-yl)methyl]-1,3,4-oxadiazol-2-yl}thio)acetate (57d)



1,3,4-Oxadiazole-2(3*H*)-thione **55** (735 mg) and methyl 2-bromoacetate (502 mg) were used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (2/1, v/v). Yield 783 mg (83%), brownish solid, mp 85–86 ºC (ethyl acetate). R_f = 0.48 (dichloromethane/methanol 20/1, v/v). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H ppm 2.36 (s, 3H, 5-CH_3), 3.62 (s, 3H, OCH_3), 4.17 (s, 2H, CH_2), 4.29 (s, 2H, SCH_2), 6.92 (d, J = 8.3 Hz, 1H, CH), 7.23–7.30 (m, 3H, 3 × CH), 10.91 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C ppm 21.7 (5-CH_3), 21.9 (CH_2), 34.0 (SCH_2), 53.1 (OCH_3), 106.4, 111.8 (CH), 118.2 (CH), 123.4 (CH), 124.7 (CH), 127.3, 127.7, 135.0, 162.9 (oxodiazole C-2), 167.8 (oxodiazole C-5), 168.6 (C=O). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 1732 (C=O), 3311 (NH). HRMS (ESI TOF): $[M+Na]^+$, found 340.0726. $[C_{15}H_{15}N_3O_3S+Na]^+$ requires 340.0726.

2-(Benzylthio)-5-[(5-methyl-1*H*-indol-3-yl)methyl]-1,3,4-oxadiazole (57e)

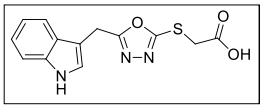


1,3,4-Oxadiazole-2(3*H*)-thione **55** (735 mg) and (bromomethyl)benzene (561 mg) were used for the *N*-alkylation reaction. It was purified by column chromatography on silica gel with *n*-hexane/ethyl acetate (4/1, v/v). Yield 643 mg (64%), brown resin. R_f = 0.43 (dichloromethane/methanol 20/1, v/v). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H ppm 2.35 (s, 3H, 5-CH_3), 4.29 (s, 2H, CH_2), 4.40 (s, 2H, SCH_2), 6.91–6.96 (m, 1H, CH), 7.19–7.24 (m, 3H, 3 × CH), 7.24–7.30 (m, 5H, 5 × CH), 10.92 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C ppm 21.3 (5-CH_3), 21.4 (CH_2), 35.8 (SCH_2), 106.0, 111.3 (CH), 117.7 (CH), 123.0 (CH), 124.3 (CH), 126.8, 127.2, 127.7 (CH), 128.5 (2 × CH), 128.9 (2 × CH), 134.6, 135.5, 162.6 (oxodiazole C-2), 167.3 (oxodiazole C-5). IR (neat, $\tilde{\upsilon}$, cm^{-1}): 3265 (NH). HRMS (ESI TOF): $[M+Na]^+$, found 358.0984. $[C_{19}H_{17}N_3OS+Na]^+$ requires 358.0985.

3.2.13 General procedure for hydrolysis of 1,3,4-oxadiazol-2-yl-thioacetate 56c and 57c

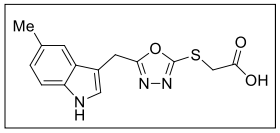
To a solution of compound **56c** or **57c** (3 mmol) in dioxane (8 mL) and water (2 mL), LiOH (216 mg, 3 mmol) was added, and the reaction mixture was left at rt for 2 h. The reaction mixture was diluted with ethyl acetate (20 mL), and the solution was acidified with 5% $KHSO_4$ (20 mL). The combined organic layer was washed with brine (20 mL), dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography by using an appropriate eluent.

2-({5-[(1*H*-Indol-3-yl)methyl]-1,3,4-oxadiazol-2-yl}thio)acetic acid (58)



1,3,4-Oxadiazol-2-thio-acetate **56c** (909 mg) was used for hydrolysis. It was purified by column chromatography on silica gel with dichloromethane/methanol (10/1, v/v). Yield 633 mg (73%), brown solid, mp 119–120 ºC (methanol). R_f = 0.01 (dichloromethane/methanol 10/1, v/v). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H ppm 4.09 (s, 2H, CH_2), 4.33 (s, 2H, SCH_2), 7.00 (t, J = 7.5 Hz, 1H, CH), 7.10 (t, J = 7.5 Hz, 1H, CH), 7.32 (d, J = 1.6 Hz, 1H, CH), 7.37 (d, J = 8.1 Hz, 1H, CH), 7.50 (d, J = 7.9 Hz, 1H, CH), 11.04 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C ppm 21.9 (CH_2), 34.6 (SCH_2), 107.0, 112.1 (CH), 118.7 (CH), 119.3 (CH), 121.8 (CH), 124.6 (CH), 127.0, 136.6, 163.2 (oxodiazole C-2), 167.6 (oxodiazole C-5), 169.3 (C=O). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1700 (C=O), 3298 (NH, OH). HRMS (ESI TOF): $[M+Na]^+$, found 312.0413. $[C_{13}H_{11}N_3O_3S+Na]^+$ requires 312.0413.

2-({5-[(5-Methyl-1*H*-indol-3-yl)methyl]-1,3,4-oxadiazol-2-yl}thio)acetic acid (59)



1,3,4-Oxadiazol-2-thio-acetate **57c** (951 mg) was used for hydrolysis. It was purified by column chromatography on silica gel with dichloromethane/methanol (10/1, v/v). Yield 427 mg (47%), brown solid, mp 68–69 ºC (methanol). R_f = 0.03 (dichloromethane/methanol 20/1, v/v). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H ppm 2.36 (s, 3H, 5-CH_3), 3.97 (s, 2H, CH_2), 4.26 (s, 2H, SCH_2), 6.92 (d, J = 8.2 Hz, 1H, CH), 7.24–7.28 (m, 3H, 3 × CH), 10.92 (br s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C ppm 21.3 (5-CH_3), 21.4 (CH_2), 36.6 (SCH_2), 106.1, 111.4 (CH), 117.7 (CH), 123.0 (CH), 124.2 (CH), 126.9, 127.2, 134.6, 163.6 (oxodiazole C-2), 166.8 (oxodiazole C-5), 170.0 (C=O). IR (neat, $\bar{\upsilon}$, cm^{-1}): 1714 (C=O), 3398 (NH). HRMS (ESI TOF): $[M+H]^+$, found 304.0750. $[C_{14}H_{13}N_3O_3S+H]^+$ requires 304.0750.

MAIN RESULTS AND CONCLUSIONS

1. *N*-Alkylation reaction of 5-[1-(*tert*-butoxycarbonyl)azetidin-3-yl]-1*H*-pyrazole-3-carboxylate (**4**) primarily proceeds at N-2 nitrogen, and it yields derivatives **5a-f** as the major products, while N-1 nitrogen substituted derivatives **6a-f** are formed as the minor products.

2. Pyrazole (**11a-d**) or indazole (**14a-d**) carboxylates can be *N*-alkylated with *N*-Boc-3-iodoazetidine (**8c**) to obtain two separable regioisomers.

3. *N*-Alkylation reaction of ethyl 4-pyrazolecarboxylate (**21**) with (*S*)- and (*R*)-*N*-Boc-3-mesylpiperidines leads to the inversion of the stereochemical configuration.

4. The optimal synthesis path towards *N*-substituted pyrazolo[4,3-*c*]pyridines proceeds *via* the formation of 5-(*tert*-butyl) 3-ethyl 1,4,6,7-tetrahydro-5*H*-pyrazolo[4,3-*c*]pyridine-3,5-dicarboxylate (**39**) and the subsequent *N*-alkylation reactions.

5. 2-[(1*H*-Indol-3-yl)methyl]-5-(alkylthio)-1,3,4-oxadiazoles **56a-i** and **57a-e** can be obtained from hydrazides **52** and **53** upon their transformation to 1,3,4-oxadiazole-2(3*H*)-thiones **54** and **55** followed by *S*-alkylation reactions.

6. 2-[(1*H*-Indol-3-yl)methyl]-1,3,4-oxadiazoles **54**, **55**, **56a-i**, **57a-e**, **58** and **59** have antioxidant properties at low concentrations, while, at higher concentrations, they can be toxic, presumably by contributing to the oxidative stress themselves.

7. 2-(Benzylthio)-5-[(5-methyl-1*H*-indol-3-yl)methyl]-1,3,4-oxadiazole (**57e**) shows protective activity against L-buthionine sulfoximine (BSO) induced oxidative stress in Friedreich's ataxia fibroblasts similar or superior to that of a known antioxidant *R*-lipoic acid (LA).

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