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Late-Stage Diversification of Pyrazoles as Antileishmanial Agents

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N-Pyrazolylcarboxamides and *N*-pyrazolylureas represent promising lead compounds for the development of novel antileishmanial drugs. Herein, we report the late-stage diversification of 3-bromopyrazoles **10A/B** and **14A** by Pd-catalyzed Sonogashira and Suzuki-Miyaura cross coupling reactions. The electron-withdrawing properties of the cyano moiety in 4-position of the pyrazole ring limited the acylation of the primary amino moiety in 5-position. A large set of pyrazoles bearing diverse aryl and alkynyl substituents in 3-position was prepared and the antileishmanial and antitrypanosomal activity was recorded. The urea **38** lacking the electron withdrawing cyano moiety in 4-position and containing the large 4-benzylpiperidinoo moiety exhibited a modest antileishmanial (IC_{50} = 19 μM) and antitrypanosomal activity (IC_{50} = 7.9 μM)). However, its considerable toxicity against the PMM and MRC-5 cells indicates low selectivity, *i.e.* a small gap between the desired antiparasitic activity and undesired cytotoxicity of < 2- to 4-fold.

1. Introduction

Leishmaniasis is a neglected tropical disease caused by protozoan parasites of the genus Leishmania, *i.e. Leishmania donovani a*nd *Leishmania infantum*.[1] Leishmaniasis is spread over more than 80 countries, predominantly in Africa, Asia and the Mediterranean area. It is assumed that 350 million people worldwide are potentially affected by this disease.[2] It affects predominantly financially disadvantaged people and is regarded as one of the most neglected tropical diseases.[3] The parasite is transferred by the bite of phlebotomine sand flies (*Phlebotominae*). Therefore, poor housing conditions, lack of hygiene and lack of garbage disposal supporting the spread of the sand fly represent risk factors for the infection.[4]

The human leishmaniasis is differentiated into two forms. The most common form is cutaneous leishmaniasis affecting predominantly the skin leading to skin sores. The parasites live and reproduce in the skin, but do not invade into deeper tissue or organs. Although the disease heals within 6–12 months without treatment, patients often endure a prolonged period of infection and may suffer long term scarring. In order to reduce the discomfort, social stigma associated with cutaneous leihmaniasis and scar formation local treatment with pentavalent antimony compounds, such as sodium stibogluconate (**1**) and *N*-methylglucamine antimonate (meglumin-antimonate, **2**) is possible.[5] (Figure 1)

On the other hand, visceral leishmaniasis (kala-azar, black fever) affects several organs, *e.g.*, spleen, liver and bone marrow. Without diagnosis and proper treatment, it leads to high mortality. Historically visceral leishmaniasis was predominantly treated by *i.v.* application of pentavalent antimony drugs such as sodium stibogluconate (**1**, Figure 1) over a prolonged period of time, sometimes in combination with paromomycin. Despite its proven efficacy in several parts of the world, the use of antimony compounds for the treatment of visceral leishmaniasis has been abandoned in the ISC due to increasing drug resistance. Alternatively, a liposomal preparation of amphotericin B (AmBisome®) is used for the treatment of visceral leishmaniasis, but is complicated to administer, costly, and reliant on a cold chain to preserve potency. In HIV-visceral leishmaniasis co-infected patients, a co-administration of amphotericin B and miltefosine is recommended.

For the proper treatment of patients suffering from leishmaniasis in developing countries, potent drugs without severe side effects, which are cheap and can be applied orally, are urgently required. The increasing number of publications in this field reflects the increasing research activities around discovering novel drugs for the treatment of this fatal disease.[6] In 2015, *N*-pyrazolyl substituted amides of type **3** were reported as promising lead compounds for the treatment of leishmaniasis.[7] (Figure 1) In activity assays against *L. infantum*

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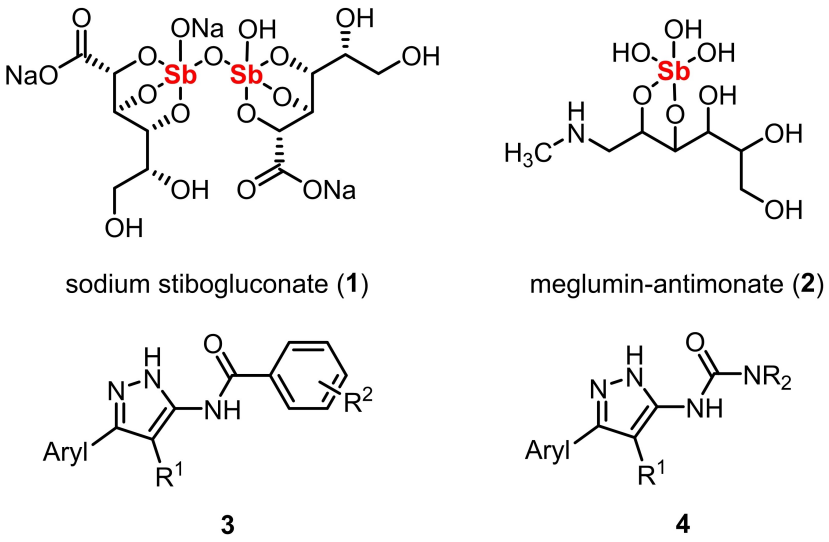


Figure 1. Sodium stibogluconate (**1**), *N*-methylglucamine antimonate (meglumin-antimonate, **2**) the first line drugs for the treatment of leishmaniasis. *N*-pyrazolylamides **3** and *N*-pyrazolylureas **4** showed promising antileishmanial activity.

and *L. donovani*, these *N*-pyrazolylamides **3** show IC_{50}-values below 1 μM.[7] However, the low metabolic stability inhibited *in vivo* studies with the amides **3**. Replacement of the *N*-pyrazolylamides **3** by *N*-pyrazolylureas **4** resulted in drugs with submicromolar activity in *in vitro* studies and reduced cytotoxicity ($CC_{50} > 45$ μM). Finally, the metabolic stability was improved by introduction of cyclic amines such as piperidines (NR_2 = piperidin-1-yl) and piperazines (NR_2 = piperazin-1-yl) as second amino component of the urea **4**.

A common feature of the antileishmanial pyrazoles **3** and **4** is the aryl moiety in the 3-position of the pyrazole ring. The synthesis of arylpyrazoles **3** and **4** started with an appropriate arenecarboxylic acid ester, *i.e.* the aryl moiety is already present in the first reaction step. In order to introduce a novel dimension of diversity and broaden the relationships between the substitution pattern of the pyrazole ring and the antileishmanial activity, a novel strategy should be developed allowing the introduction of different aryl and alkynyl residues at a later stage / at the end of the synthesis (late stage diversification). For this purpose, Pd-catalyzed coupling reactions with bromopyrazoles were envisaged.

As this project was performed within the open synthesis network (OSN) of the Drugs for Neglected Diseases initiative (DNDi), we planned to synthesize novel pyrazoles with various aryl moieties in the 3-position, which are similar to the lead compounds **3** and **4**. In addition to the antileishmanial activity, the antitrypanosomal activity of the novel pyrazoles should be tested.

2. Results and Discussion

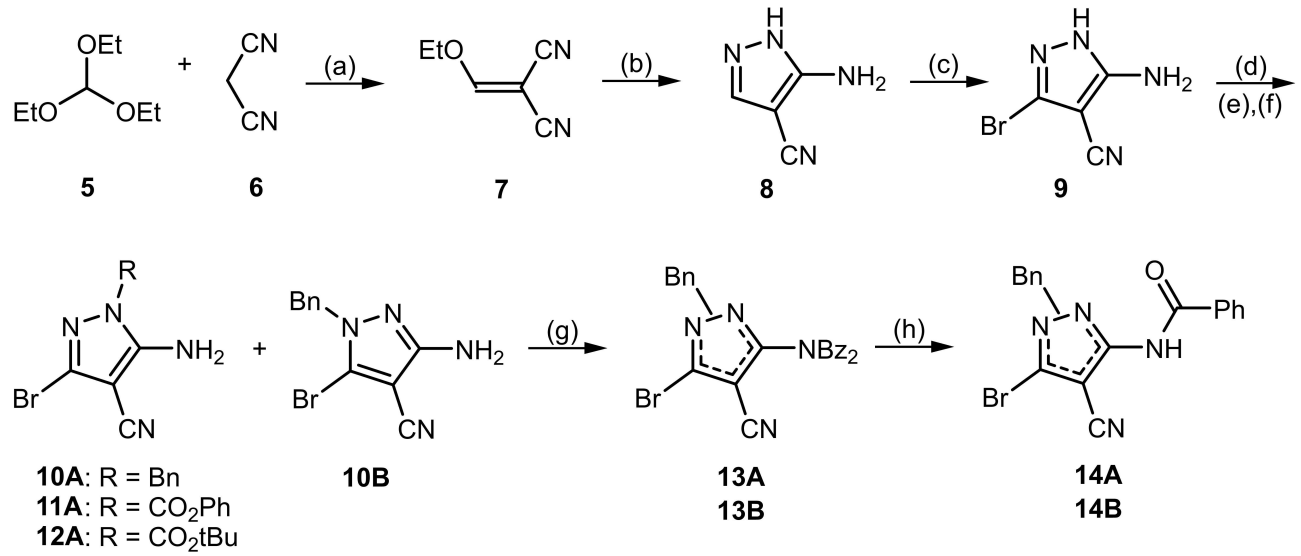
2.1. Synthesis

The synthesis started with the condensation of triethyl orthoformate (**5**) with malononitrile (**6**) leading to (ethoxymethylene)malononitrile (**7**),[8] which reacted with hydrazine to afford the pyrazolecarbonitrile **8**. (Scheme 1) All attempts to transform the primary amine of **8** into amides resulted in acylation of the pyrazole ring. The reaction of **8** with benzoyl chloride, phenyl chloroformate, and $(Boc)_2O$ led to low amounts of mixtures bearing the acyl moiety at either of the pyrazole N-atoms. It is assumed that the electron withdrawing cyano moiety reduces the nucleophilicity of the primary amine as well as those of the pyrazole N-atoms.

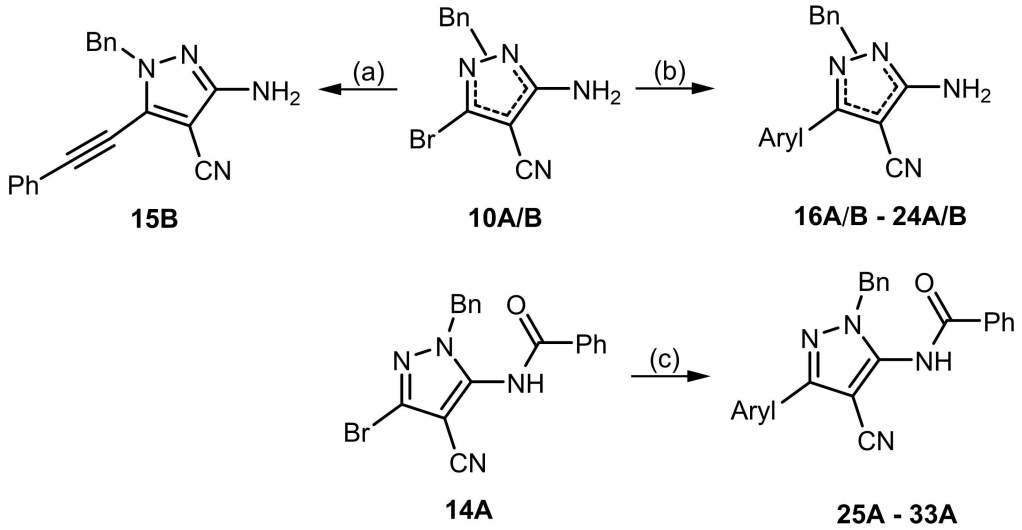
Therefore, the aminopyrazole **8** was brominated with NBS to obtain the 3-bromopyrazole **9** in 55% yield. Since the large Br-atom shielded the 2-position of the pyrazole ring, acylation of **9** occurred regioselectively in 1-position. The 1-phenoxycarbonyl and 1-Boc pyrazoles **11A** and **12A** were isolated in yields of 61% and 82%, respectively. Alkylation of bromopyrazole **9** with benzyl bromide at 80 °C led to benzylated pyrazoles **10A** and **10B** in 78% yield. In this case, the ratio of regioisomeric pyrazoles **10A** and **10B** was 80:20 confirming the preferred reaction in 1-postion of the pyrazole ring. (Scheme 1)

It turned out that subsequent acylation of the primary amino moiety of **10A**/**B** was very difficult. Neither CDI nor phenyl chloroformate nor phenyl isocyanate reacted with **10A**/**B** to give N-acylated products. However, the reaction of **10A**/**B** with an excess of benzoyl chloride in refluxing THF resulted in double acylation. The regioisomeric imides **13A** and **13B** were separated by flash chromatography and, subsequently, one benzoyl moiety was removed with NH_3 to afford the regioisomeric benzamides **14A** and **14B** in 74% and 81% yield, respectively. (Scheme 1)

In the next step, the Br-atom at the pyrazole ring should be exploited to introduce diverse substituents. At first, a Sonogashira reaction of a 75:25 mixture of regioisomers **10A** and **10B** with phenylethyne in the presence of $Pd(PPh_3)_4$/CuI was performed. Unfortunately, only the regioisomer **15B** resulting from the minor regioisomer **10B** could be isolated in 11% yield. (Scheme 2)



Scheme 1. Synthesis of bromopyrazoles **10**–**14**. Reagents and reaction conditions: (a) Ac_2O, 125 °C, 5 h, 98%. (b) H_2NNH_2, EtOH, rt, 5 min, 58%. (c) NBS, DMF, rt, 3 h, 55%. (d) BnBr, THF, K_2CO_3, 80 °C, 6 h, 78% (**10A**:**10B** = 80:20). (e) PhOCOCl, THF, DIPEA, rt, 16 h, 61% (**11A**), (f) Boc_2O, THF, $NaHCO_3$, rt, 14 h, 82%. (**12A**). (g) BzCl, THF, NEt_3, 80 °C, 24 h, 36% (**13A**), 15% (**13B**). (h) NH_3, THF, 0 °C, 3 h, 74% (**14A**), 81% (**14B**).



Scheme 2. Sonogashira and Suzuki-Miyaura coupling of brominated pyrazoles. Reagents and reaction conditions: (a) PhC≡CH, $Pd(PPh_3)_4$, CuI, CH_3CN, 80 °C, 16 h, 11%. (b) ArylB$(OH)_2$, Pd(dppf)Cl_2, Cs_2CO_3, dioxane:H_2O 4:1, microwave irradiation, 120 °C, 1 h, 76–99% (exception: pyrazole-boronic acid, yield 41% (**22A**/**B**)). (c) ArylB$(OH)_2$, Pd(dppf)Cl_2, Cs_2CO_3, dioxane : H_2O 4:1, 120 °C, 1.5 h, 73–97%; for the synthesis of **20A**/**B** and **28A**, pyridine-3-boronic acid pinacol ester was used. For definition of Aryl moieties see Table 1).

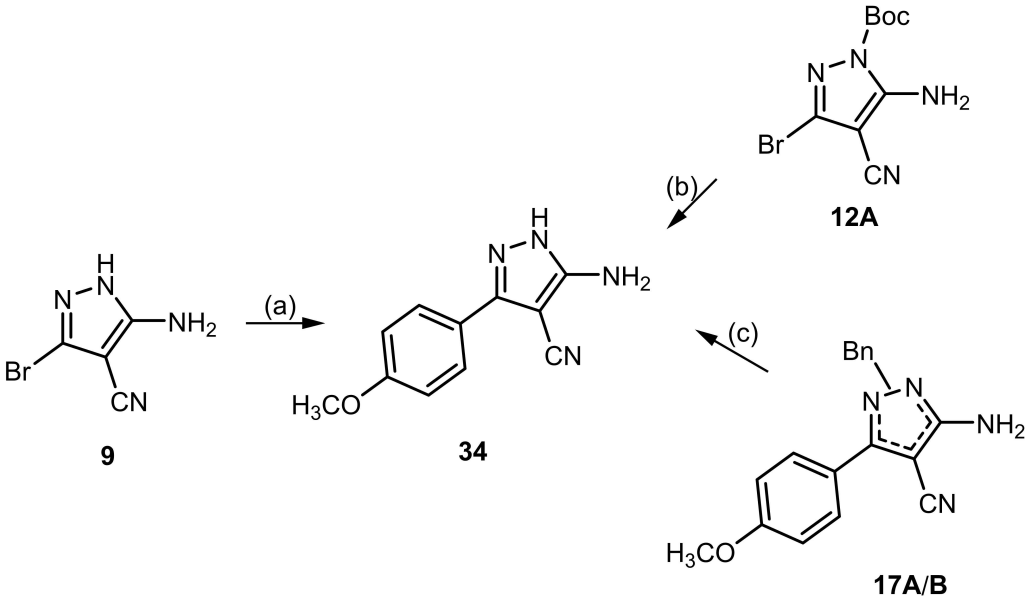
The Suzuki-Miyaura cross coupling of **10A**/**B** was carefully optimized for the reaction with 4-methoxyphenylboronic acid. 8 mol% of Pd(dppf)Cl_2 and two equivalents of Cs_2CO_3 in a 4:1 mixture of dioxane/H_2O at 120 °C under microwave irradiation turned out to give the highest yield of 4-methoxyphenylpyrazole **17A**/**B** (95%). The same reaction conditions were applied for the introduction of diverse aryl moieties into the pyrazole **10A**/**B**. With exception of the bipyrazole **22A**/**B**, the yields of the arylated pyrazoles were very high (76–99%, see Table 1). For the synthesis of the pyridin-3-yl derivative **20A**/**B** the corresponding boronic pinacol ester was employed. Phenyl moieties with various substituents, electron rich and electron deficient heteroaryl rings as well as annulated aromatic systems were successfully introduced into the pyrazole ring. (Scheme 2)

The arylation of bromopyrazole **14A** with an additional benzoyl moiety at the exocyclic amino group, was performed under the same reaction conditions as the arylation of aminopyrazole **10A**/**B**, but without microwave irradiation. The arylated pyrazoles **25A** – **33A** were obtained in 73–97% yield. (Scheme 2, Table 1)

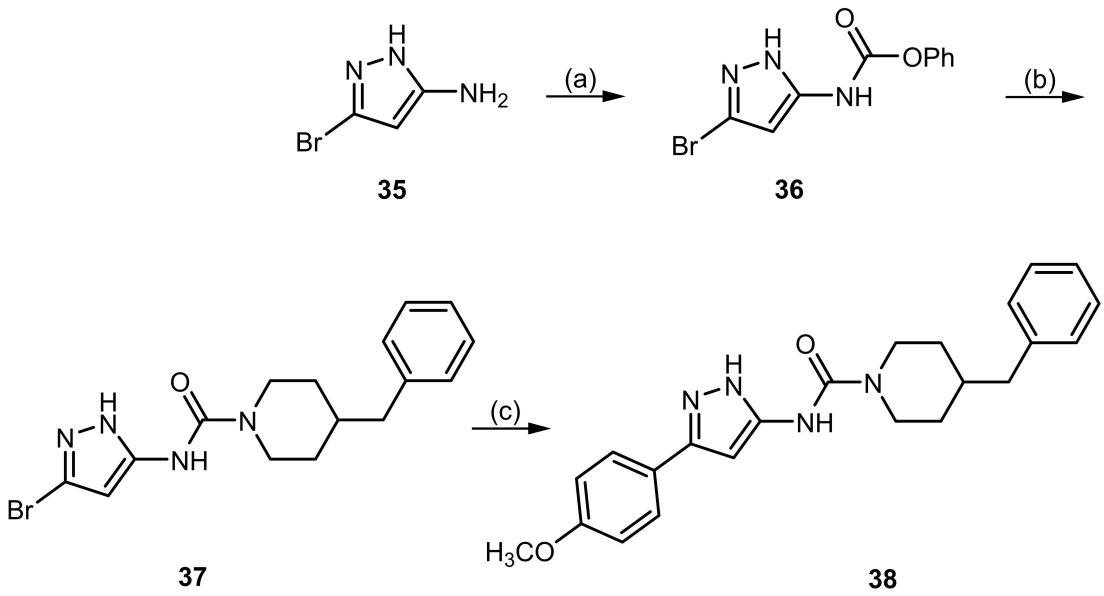
For the synthesis of the aminopyrazole **34** without substituents at the N-atoms, three approaches were pursued. (1) The bromopyrazole **9** without *N*-substituents was coupled with 4-methoxyphenylboronic acid and Pd(dppf)Cl_2 to give the arylated pyrazole **34** in 7% yield. This yield is already the result

of various optimizations. (2) The Boc derivative **12A** was arylated in a Suzuki-Miyaura reaction with 4-methoxyphenylboronic acid and Pd(dppf)Cl_2 to obtain **34** in 13% yield. In the presence of water, the loss of the Boc group of **12A** was catalyzed by Pd. (3) In the third approach, the benzyl moiety of the arylated pyrazole **17A/B** was removed by a transfer hydrogenolysis using NH_4CO_2 as H_2 source and Pd/C as catalyst in refluxing isopropanol. Although the benzyl moiety could be removed under these conditions, the pyrazole **34** was isolated in only 14% yield. (Scheme 3)

In addition to the bromopyrazoles **10** and **14** bearing an electron withdrawing cyano moiety, the bromopyrazole **37** without cyano moiety should be employed in a Suzuki-Miyaura coupling reaction as well. For this purpose, commercially available bromopyrazolamine **35** was acylated with phenyl chloroformate resulting in the carbamate **36**, which reacted with benzylpiperidine to afford the urea derivative **37**. The Suzuki-Miyaura cross coupling reaction at the very end of the synthesis provided the arylated pyrazole **38** in 30% yield. (Scheme 4)



Scheme 3. Various possibilities to synthesize arylated pyrazolamine **34**. Reagents and reaction conditions: (a) 4-$MeOC_6H_4B(OH)_2$, Pd(dppf)Cl_2, dioxane, H_2O, Cs_2CO_3, 120 °C, 16 h, 7%. (b) 4-$MeOC_6H_4B(OH)_2$, Pd(dppf)Cl_2, dioxane, H_2O, Cs_2CO_3, 60 °C, 15 h, 13%. (c) NH_4CO_2, Pd/C, isopropanol, 90 °C, 16 h, 14%.



Scheme 4. Synthesis of urea **38**. Reagents and reaction conditions: (a) PhOCOCl, pyridine, THF, rt, 16 h, 70%. (b) 4-Benyzlpiperidine, NEt_3, toluene, 120 °C, 16 h, 84%. (c) 4-Methoxyphenylboronic acid (4-$MeOC_6H_4B(OH)_2$), Pd XPhos G2, K_2CO_3, EtOH, H_2O, microwave irradiation (max 300 Watt, max. 300 psi), 60 °C, 10 min, 30%.

In conclusion, the developed methods allow the introduction of diverse aryl moieties at the 3-position of the pyrazole core at a late stage of the synthesis sequence. These methods allow diversification of the otherwise difficult to modify aryl moiety.

2.2. Antileishmanial and antitrypanosomal activity of synthesized pyrazoles

The antileishmanial and antitrypanosomal activity of the synthesized arylpyrazoles **16–34** and **38** was determined within the network of DNDi. In brief, each well of a 96-well microtiter plate was charged with 10 μL of the compound dilution and 190 μL of the macrophage/*L. infantum* (MHOM/MA(BE)/67 strain) inoculum. After five days of incubation, the cells were stained with 10% Giemsa solution and the total parasite burdens were microscopically assessed. Miltefosine and amphotericin B were used as the reference drugs for the *L. infantum* assay.[9] In the antitrypanosomal assay, 190 μL of the MRC-5 cell/*T. cruzi* (Tulahuen CL2, β-galactosidase strain) inoculum were incubated with 10 μL of the test compound solution. After incubation for seven days at 37 °C, the substrate chlorophenolred β-*D*-galactopyranoside (CPRG) was added. The intensity of the formed color (540 nm) correlates with the amount of parasites. Benznidazole and nifurtimox were used as reference compounds in the antitrypanosomal assay. In order to evaluate the general toxicity of the new pyrazole derivatives, the toxicity against the MRC-5 human fibroblast cell line and primary peritoneal mouse macrophages (PMM) was investigated. The results are summarized in Table 1.

The antileishmanial activity of the novel pyrazole derivatives is more than three-fold lower (IC_{50} > 30 μM) than the activity of the reference compound miltefosine (IC_{50} = 10 μM). The indolyl derivative **24A/B** (IC_{50} = 24 μM) and the urea **38** (IC_{50} = 19 μM) are the only exceptions. It was hypothesized that a dinuclear aryl moiety such as an indole ring is favorable for the antileishmanial activity. The analogous naphthalen-1-yl derivative **23A/B** (IC_{50} = 34 μM) belongs also to the pyrazole derivatives with higher antileishmanial activity. A similar trend was observed for the benzamides with indolyl (**31A**, IC_{50} = 32 μM)) and naphthalen-1-yl moiety (**30A**, IC_{50} = 32 μM). However, the most promising antileishmanial activity was detected for the urea **38**. Its IC_{50} value (IC_{50} = 19 μM) is close to the IC_{50} value of the reference compound miltefosine. The high antileishmanial activity of **38** was attributed to the urea with the benzylpiperidine substructure and/or the missing cyano moiety in 4-position of the pyrazole ring. Even for the most active antileishmanial pyrazoles **24A/B** and **38** a less than twofold selectivity was shown in the toxicity assays against the MRC-5 and PMM cell lines indicating only a small difference between the desired antileishmanial activity and unwanted cytotoxicity.

In addition to the antileishmanial activity, the antitrypanosomal activity of the novel pyrazole derivatives was tested. (Table 1) While most of the pyrazoles revealed only low antitrypanosomal activity, the fluorophenylpyrazole **19A/B**, the naphthalen-1-yl derivative **24A/B** and the urea **38** displayed low micromolar activity with IC_{50} values of 10 μM, 8.1 and 7.9 μM, respectively. All three pyrazoles showed a 4–6-fold selectivity, when taking the cytotoxicity against the given cell lines into account. The pyrazoles **19A/B** and **24A/B** contain a primary amino and cyano moiety at the pyrazole ring. The urea **38** is lacking the cyano moiety and the primary amino moiety is expanded to a large urea derivative.

3. Conclusions

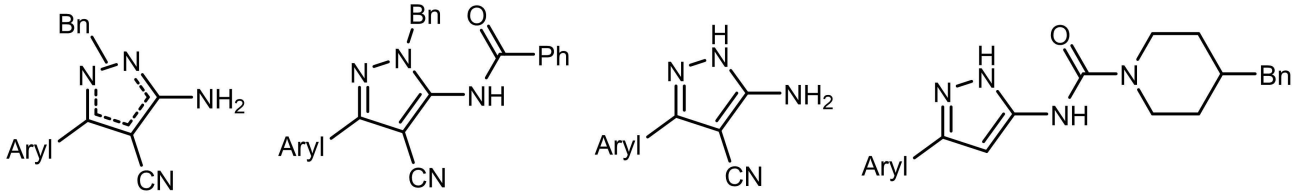
Sonogashira and Suzuki Miyaura cross coupling reactions of bromopyrazoles **10A/B**, **14A**, and **37** allow the introduction of diverse alkynyl and aryl moieties in 3-postion at a rather late stage of the synthesis (late stage diversification strategy). However, the electron withdrawing cyano moiety renders the further modification of the primary amino moiety of **8**, and **13** difficult. The urea **38** with the large 4-benzylpiperidino moiety showed a modest antileishmanial (IC_{50} = 19 μM) and antitrypanosomal activity (IC_{50} = 7.9 μM), but only low selectivity when considering the cytotoxic effects against MRC-5 and PMM cells.

4. Experimental, Chemistry

4.1. General

Oxygen and moisture sensitive reactions were carried out under nitrogen, dried with silica gel with moisture indicator (orange gel, VWR, Darmstadt, Germany) and in dry glassware (Schlenk flask or Schlenk tube). Temperature was controlled with dry ice/acetone (−78 °C), ice/water (0 °C), Cryostat (Julabo TC100E-F, Seelbach, Germany), magnetic stirrer MR 3001 K (Heidolph, Schwalbach, Germany) or RCT CL (IKA, Staufen, Germany), together with temperature controller EKT HeiCon (Heidolph) or VT-5 (VWR) and PEG or silicone bath. All solvents were of analytical or technical grade quality. Demineralized water was used. CH_2Cl_2 was distilled from CaH_2; THF was distilled from sodium/benzophenone; MeOH was distilled from magnesium methanolate. Thin layer chromatography (tlc): tlc silica gel 60 F_{254} on aluminum sheets (VWR). Flash chromatography (fc): Silica gel 60, 40–63 μm (VWR); parentheses include: diameter of the column (∅), length of the stationary phase (h), fraction size (v) and eluent. Automated flash chromatography: Isolera™ Spektra One (Biotage®); parentheses include: cartridge size, eluent, fractions size was always 20 mL. Dry column vacuum chromatography (DCVC) was performed according to Pedersen *et al* using glass funnels with sintered glass disc filters and a height of 11 cm;[10] parentheses include: diameter of the column (∅), length of the compressed stationary phase (h), eluent. Preparative HPLC separations are described in the supplementary information (method 2 and method 3). Melting point: Melting point system MP50 (Mettler Toledo, Gießen, Germany), open capillary, uncorrected. MS: MicroTOFQII mass spectrometer (Bruker Daltonics, Bremen, Germany); deviations of the found exact masses from the calculated exact masses were 5 ppm or less; the data were analyzed with DataAnalysis®

Table 1. Yields, antileishmanial and antitrypanosomal activity as well as cytotoxicity of pyrazoles with diverse aryl moieties in 3-position.



16-24A/B **25-33A** **34** **38**

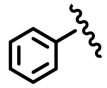
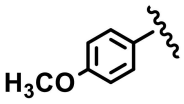
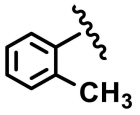
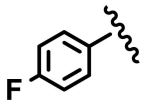
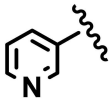
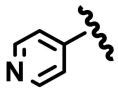
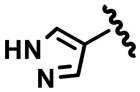
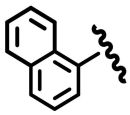
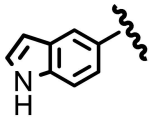
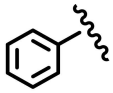
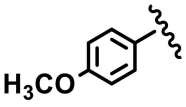
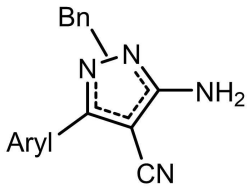
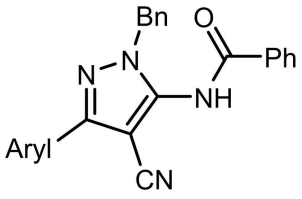
comp.	Aryl	Yield (%)	ratio **A:B**	*L. infantum* IC_{50} [μM][a]	*T. cruzi* IC_{50} [μM][a]	Cytotoxicity PMM CC_{50} [μM][a]	Cytotoxicity MRC-5 CC_{50} [μM][a]
16A/B		99	80:20	57	25	64	35
17A/B		95	80:20	64	64	64	64
18A/B		93	83:17	57	35	64	53
19A/B		75	82:18	51	10	64	64
20A/B		97	85:15	64	64	64	64
21A/B		81	86:14	64	64	64	64
22A/B		41	50:50	64	64	64	30
23A/B		93	95:5	34	8.1	45	39
24A/B		97	78:22	24	31	32	25
25A		91	–	64	64	64	64
26A		89	–	46	64	45	64

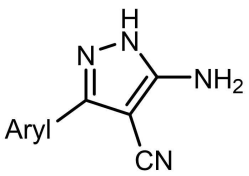
Table 1. continued



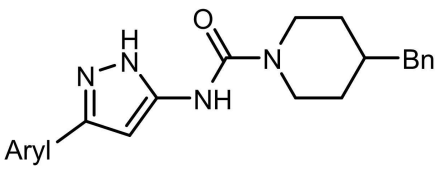
16-24A/B



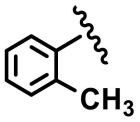
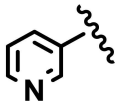
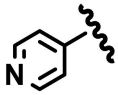
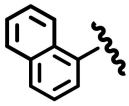
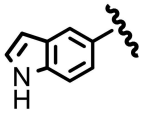
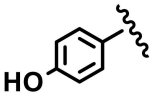
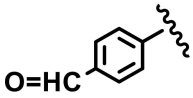
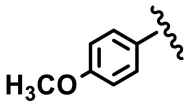
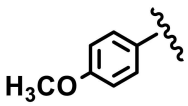
25-33A



34



38

comp.	Aryl	Yield (%)	ratio **A**:**B**	*L. infantum* IC_{50} [μM][a]	*T. cruzi* IC_{50} [μM][a]	Cytotoxicity PMM CC_{50} [μM][a]	Cytotoxicity MRC-5 CC_{50} [μM][a]
27 A		97	–	64	64	64	64
28 A		88	–	33	64	33	64
29 A		93	–	55	58	64	64
30 A		80	–	32	64	64	64
31 A		82	–	32	64	45	64
32 A		73	–	36	64	45	64
33 A[#]		76	–	–	–	–	–
34		14	–	–	–	–	–
38		30	–	19	7.9	32	32
miltefosine		–	–	10	–		
amphotericin B		–	–	1.2	–		
benznidazole		–	–	–	2.9		
nifurtimox		–	–	–	0.91		

[#] Due to the labile aldehyde moiety, **33 A** could not be tested. [a] Geometric mean value of at least two independent tests.

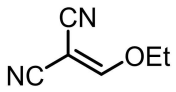
(Bruker Daltonics). NMR: NMR spectra were recorded in deuterated solvents on Agilent DD2 400 MHz and 600 MHz spectrometers (Agilent, Santa Clara CA, USA); chemical shifts (δ) are reported in parts per million (ppm) against the reference substance tetramethylsilane and calculated using the solvent residual peak of the undeuterated solvent; coupling constants are given with 0.5 Hz resolution; assignment of ^{1}H and ^{13}C NMR signals was supported by 2-D NMR techniques where necessary. IR: FT/IR IR Affinity®-1 spectrometer (Shimadzu, Düsseldorf, Germany) using ATR technique.

4.2. HPLC method to determine the purity of products

Purity by HPLC: DIONEX UltiMate 3000; UV detector: VWD-3400RS; autosampler: ACC-3000T; pump: LPG-3400SD; degasser: DG-1210; Method: column: LiChrospher® 60 RP-select B (5 μm), 250–4 mm; flow rate: 1.00 mL/min; injection volume: 5.0 μL; detection at $\lambda = 210$ nm; solvents: A: water with 0.05 % (v/v) trifluoroacetic acid; B: acetonitrile with 0.05 % (v/v) trifluoroacetic acid: gradient elution: (A%): 0–4 min: 90 %, 4 min: 90 %, 4–29 min: gradient from 90 % to 0 %, 29–31 min: 0 %, 31–31.5 min: gradient from 0 % to 90 %, 31.5–40 min: 90 %. Unless otherwise noted, the purity of all test compounds is >95 % according to the HPLC method. Purity by quantitative NMR (qNMR) was performed according to literature using 1,3,5-trimethoxybenzene (Sigma-Aldrich, standard for quantitative NMR, TraceCERT®) as the standard.[11]

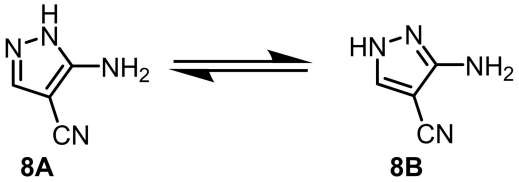
4.3. Synthetic procedures

4.3.1. 2-(Ethoxymethylene)malononitrile (7)[8]



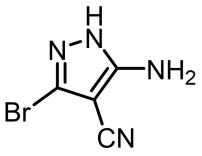
The reaction was performed according to the patent GB 2503523 A. Malononitrile (**6**, 5.0 g, 75.7 mmol, 1.00 eq.) and triethyl orthoformate (**5**, 19 mL, 113 mmol, 1.50 eq.) were dissolved in acetic anhydride (14 mL, 151 mmol, 2.00 eq.) and the mixture was heated to 125 °C for 5 h. The solvent was removed *in vacuo* and the crude product was recrystallized with EtOH. Yellow solid, mp 65–67 °C, yield 7.2 g (83 %), $R_f = 0.65$ (cHex/EtOAc 50 : 50). $C_6H_6N_2O$ (122.1 g/mol). ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) = 1.47 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 4.40 (q, $J = 7.1$ Hz, 2H, OCH_2CH_3), 7.60 (s, 1H, C=*CH*COR). ^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) = 15.3 (CH_2CH_3), 67.4 (*C*=CHOR), 75.1 (OCH_2CH_3), 109.9 (*C*N), 112.1 (*C*N), 173.8 (C=*C*HOR). HRMS (APCI): m/z = 123.0553, calcd. 123.0552 for $C_6H_7N_2O$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3028 (=C–H), 2943 (–C–H), 2225 (C≡N), 1600 (C=C), 1303 (C–O).

4.3.2. 5-Aminopyrazole-4-carbonitrile and 3-aminopyrazole-4-carbonitrile (8)[12]



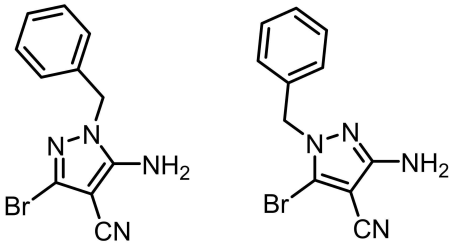
8A **8B**

4.3.3. 5-Amino-3-bromopyrazole-4-carbonitrile (9)[13]



Aminopyrazole **8** (1.0 g, 9.32 mmol, 1.00 eq.) was dissolved in DMF (50 mL) and the solution was cooled down to 0 °C. *N*-Bromosuccinimide (1.8 g, 9.73 mmol, 1.05 eq.) was added in portions. The solution was warmed up to room temperature and stirred for 3 h. The solution was transferred into a saturated Na_2SO_3 solution (50 mL), filtered and extracted with EtOAc (5×30 mL). The combined organic phases were washed with brine, dried (Na_2SO_4) and the solvent was removed *in vacuo*. The crude product was purified by flash column chromatography (Ø = 6 cm, h = 14 cm, CH_2Cl_2/CH_3OH 95 : 5, V = 15 mL). Yellow solid, mp. 196–198 °C, yield 0.96 g, (55 %) (Lit.[13] 97 %), $R_f = 0.20$ (CH_2Cl_2/CH_3OH 95 : 5). $C_4H_3BrN_4$ (187.0 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 6.71 (s, 2H, NH_2), 12.32 (s, 1H, N$H_{pyrazole}$). ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 74.9 (*C*-4), 114.1 (*C*N), 127.3 (*C*-3), 153.9 (*C*-5). HRMS (APCI): m/z = 186.9613, calcd. 186.9614 for $C_4H_4{}^{79}BrN_4$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3167 (N–H), 2230 (C≡N). Purity (HPLC): $t_R = 10.1$ min, purity 99.1 %.

4.3.4. 5-Amino-1-benzyl-3-bromopyrazole-4-carbonitrile (10A) and 3-Amino-1-benzyl-5-bromopyrazole-4-carbonitrile (10B)

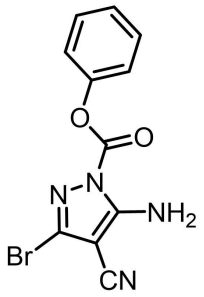


10A **10B**

Aminopyrazole **9** (200 mg, 1.07 mmol, 1.00 eq.) and K_2CO_3 (296 mg, 2.14 mmol, 2.00 eq.) were suspended in THF (20 mL) at room temperature. Benzyl bromide (140 μL, 1.18 μmol, 1.10 eq.) was added and the suspension was stirred at 80 °C for 6 h. H_2O (15 mL) was added to the reaction mixture and the mixture was extracted with EtOAc (4×35 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*.

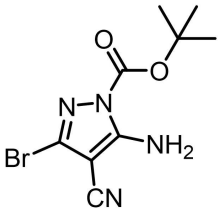
The crude product was purified by flash column chromatography (Ø = 5 cm, h = 20 cm, cHex/EtOAc 67:33, V = 8 mL). The product was further purified by precipitation from EtOAc with cHex. Colorless solid, mp 171–175 °C, yield 232 mg (78 %). (R_f = 0.28 (**10 A**), 0.30 (**10B**) (cHex/EtOAc 67:33)). $C_{11}H_9BrN_4$ (277.1 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.13 (s, 2×0.80H, CH_2), 5.17* (s, 2×0.20H, CH_2), 5.90* (s, 2×0.20H, NH_2), 7.12 (s, 2×0.80H, NH_2), 7.17–7.19 (m, 2×0.80H, 2-H_{Bn}, 6-H_{Bn}), 7.18–7.20* (m, 2×0.20H, 2-H_{Bn}, 6-H_{Bn}), 7.29–7.31 (m, 1×0.80H, 4-H_{Bn}), 7.31–7.33* (m, 1×0.20H, 4-H_{Bn}), 7.34–7.36 (m, 2×0.80H, 3-H_{Bn}, 5-H_{Bn}), 7.35 - 7.37* (m, 2×0.20H, 3-H_{Bn}, 5-H_{Bn}). Signals of the minor regioisomer **10 B** are marked with an asterisk (*). Ratio of **10 A**:**10B** = 80:20. ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 50.3, 53.3* (CH_2), 75.5, 81.2* (C-4$_{pyrazole}$), 113.4*, 113.7 (CN), 118.7* (C-5$_{pyrazole}$), 126.6 (C-3$_{pyrazole}$), 127.3*, 127.4 (2 C, C-2$_{Bn}$, C-6$_{Bn}$), 127.6 (C-4$_{pyrazole}$), 127.9* (C-4$_{Bn}$), 128.6, 128.7* (2 C, C-3$_{Bn}$, C-5$_{Bn}$), 135.7* (C-1$_{Bn}$), 136.1 (C-1$_{Bn}$), 152.7 (C-5), 157.7* (C-3$_{pyrazole}$). Signals of the minor regioisomer **10 B** are marked with an asterisk (*). HRMS: m/z = 277.0090, calcd. 277.0083 for $C_{11}H_{10}{}^{81}BrN_4$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3367 (N–H), 2997 (–C–H), 2298 (C≡N). Purity (HPLC): t_R = 17.5 min, 17.7 min, purity 99.5 %.

4.3.5. Phenyl 5-amino-3-bromo-4-cyanopyrazole-1-carboxylate (11 A)



Under N_2, at 0 °C, a solution of phenyl chloroformate (37 μL, 294 μmol, 1.10 eq.) in THF (1 mL) was added to a solution of aminopyrazole **9** (50 mg, 267 μmol, 1.00 eq.) and DIPEA (91 μL, 555 μmol, 2.00 eq.) in THF (10 mL) over 30 min. The solution was warmed up to room temperature and stirred for 16 h. H_2O (10 mL) was added to the reaction mixture and the aqueous layer was extracted with EtOAc (3×10 mL). The combined organic layers were washed with brine and dried (Na_2SO_4). The solvent was removed *in vacuo* and the crude product was purified by flash chromatography (10 g, Ø = 2.5 cm, CH_2Cl_2/CH_3OH 100:0→95:5), providing the product with minor amounts of phenol and aminopyrazole **9**. Colorless solid, mp 177–179 °C (decomposition), yield 50 mg (61 %), R_f = 0.65 (CH_2Cl_2/CH_3OH 95:5), $C_{11}H_7BrN_4O_2$ (307.1 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 7.35–7.37 (m, 1H, 4-H_{Ph}), 7.36–7.39 (m, 2H, 2-H_{Ph}, 6-H_{Ph}), 7.49–7.51 (m, 2H, 3-H_{Ph}, 5-H_{Ph}). 8.15–8.21 (br, 2H, NH_2). ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 76.0 (C-4$_{pyrazole}$), 115.2 (CN), 121.6 (2 C, C-2$_{Ph}$, C-6$_{Ph}$), 126.9 (C-4$_{Ph}$), 129.8 (2 C, C-3$_{Ph}$, C-5$_{Ph}$), 133.4 (C-3$_{pyrazole}$), 148.2 (C-5$_{pyrazole}$), 149.7 (C-1$_{Ph}$), 155.7 (C-5$_{pyrazole}$). HRMS: m/z = 306.9817, calcd. 306.9825 for $C_{11}H_8{}^{79}BrN_4O_2$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3426 (N-H), 3132 (=C–H), 2985 (–C–H), 2237 (C≡N), 1635 (C=O). Purity (^{1}H NMR): 90 %.

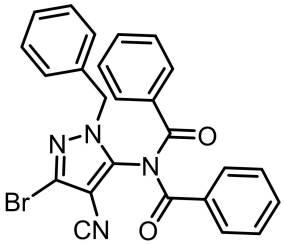
4.3.6. tert-Butyl 5-amino-3-bromo-4-cyanopyrazole-1-carboxylate (12 A)



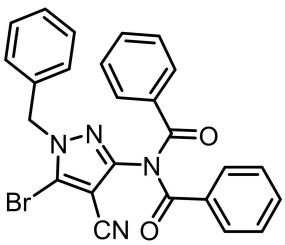
Aminopyrazole **9** (500 mg, 2.71 mmol, 1.00 eq.) and $NaHCO_3$ (450 mg, 5.42 mmol, 2.00 eq.) were dissolved in THF (35 mL) and H_2O (20 mL). Boc_2O (614 μL, 2.71 mmol, 1.00 eq.) dissolved in THF (5 mL) was added over 1.5 h and the mixture was stirred for 14 h at room temperature. To ensure complete conversion, another amount of Boc_2O (190 mg, 0.9 mmol, 0.30 eq.) was added and the solution was stirred for additional 3 h. The solvent was removed *in vacuo* and the crude product was purified by recrystallization with EtOAc.

Colorless solid, mp 127–129 °C (decomposition), yield 664 mg (82 %), R_f = 0.80 (cHex/EtOAc 50:50). $C_9H_{11}BrN_4O_2$ (287.1 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 1.54 (s, 9H, C(CH_3)$_3$), 7.96 (s, 2H, NH_2). ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 30.6 (3 C, C(CH_3)$_3$), 78.9 (C-4), 89.5 (C(CH$_3$)$_3$), 115.8 (CN), 135.2 (C-3), 151.3 (C-5), 158.4 (C=O). HRMS (APCI): m/z = 287.0128, calcd. 287.0138 for $C_9H_{12}{}^{79}BrN_4O_2$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3364 (N–H), 2985 (–C–H), 2226 (C≡N), 1728 (C=O), 1315 (C–O). Purity (HPLC): t_R = 17.9 min, purity 80.3 %.

4.3.7. N-Benzoyl-N-(1-benzyl-3-bromo-4-cyanopyrazol-5-yl)benzamide (13 A) and N-benzoyl-N-(1-benzyl-5-bromo-4-cyanopyrazol-3-yl)benzamide (13B)



13A



13B

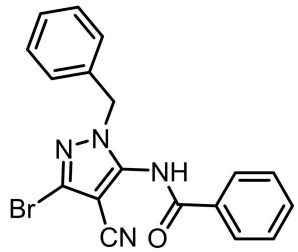
Under N_2, at 0 °C, a solution of benzoyl chloride (755 μL, 6.50 mmol, 2.00 eq.) in THF (5 mL) was added to a solution of a mixture of regioisomeric aminopyrazoles **10 A**/**B** (ratio **10 A**:**10 B** = 75:25), 900 mg, 3.25 mmol, 1.00 eq.) and triethylamine (905 μL, 6.50 mmol, 2.00 eq.) in THF (35 mL). The reaction mixture was stirred for 24 h at 80 °C. After cooling down, H_2O (20 mL) was added and the layer was extracted with EtOAc (3×40 mL). The combined organic layers were washed with HCl solution (1 M, 10 mL) and dried (Na_2SO_4). The organic layer was concentrated *in vacuo* and the crude product was purified by

flash chromatography (100 g, Ø = 4.5 cm, cHex/EtoAc 90:10→65:35) to give the regioisomers **13A** (major) and **13B** (minor). The product **13B** was further purified by recrystallization with EtOAc.

13A: Colorless solid, mp 85–89 °C, yield 568 mg (36%), R_f = 0.72 (cHex/EtOAc 67:33). $C_{25}H_{17}BrN_4O_2$ (485.3 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.51 (s, 2H, CH_2), 7.18–7.22 (m, 2H, 2-H_{Bn}, 6-H_{Bn}), 7.29–7.31 (m, 1H, 4-H_{Bn}), 7.30–7.34 (m, 2H, 3-H_{Bn}, 5-H_{Bn}), 7.43–7.47 (m, 4H, 3-H_{Bz}, 5-H_{Bz}), 7.60 (tt, J = 7.1 / 1.3 Hz, 2H, 4-H_{Bz}), 7.73–7.77 (m, 4H, 2-H_{Bz}, 6-H_{Bz}). ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 53.2 (CH_2), 94.3 (C-4$_{pyrazole}$), 110.5 (CN), 127.8 (C-3$_{pyrazole}$), 128.2 (2 C, C-2$_{Bn}$, C-6$_{Bn}$), 128.4 (C-4$_{Bn}$), 128.6 (2 C, C-3$_{Bn}$, C-5$_{Bn}$), 129.0 (4 C, C-3$_{Bz}$, C-5$_{Bz}$), 129.1 (4 C, C-2$_{Bz}$, C-6$_{Bz}$), 132.1 (2 C, C-1$_{Bz}$), 133.7 (C-1$_{Ph}$), 134.0 (2 C, C-4$_{Bz}$), 142.5 (C-5$_{pyrazole}$), 170.4 (2 C, C=O). HRMS: 485.0614, calcd. 485.0608 for $C_{25}H_{18}{}^{79}BrN_4O_2$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3071 (=C–H), 2978 (–C–H), 2237 (C≡N), 1690 (C=O). Purity (HPLC): t_R = 23.6 min, purity 90.4%.

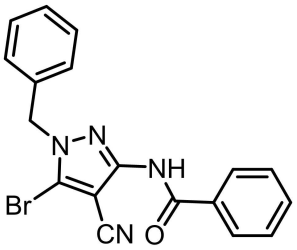
13B: Colorless solid, mp 164–166 °C, yield 255 mg (15%), R_f = 0.55 (cHex/EtOAc 67:33). $C_{25}H_{17}BrN_4O_2$ (485.3 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.37 (s, 2H, CH_2), 6.65–6.69 (m, 2H, 2-H_{Bn}, 6-H_{Bn}), 7.18–7.24 (m, 2H, 3-H_{Bn}, 5-H_{Bn}), 7.26 (tt, J = 7.3 / 1.5 Hz, 1H, 4-H_{Bn}), 7.53 (m, 4H, 3-H_{Bz}, 5-H_{Bz}), 7.67 (tt, J = 7.4 / 1.4 Hz, 2H, 4-H_{Bz}), 7.75–7.81 (m, 4H, 2-H_{Bz}, 6-H_{Bz}). ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 54.5 (CH_2), 92.5 (C-4$_{pyrazole}$), 110.8 (CN), 122.8 (C-5$_{pyrazole}$), 126.5 (2 C, C-2$_{Bn}$, C-6$_{Bn}$), 127.9 (C-4$_{Bn}$), 128.7 (2 C, C-3$_{Bn}$, C-5$_{Bn}$), 128.9 (4 C, C-2$_{Bz}$, C-6$_{Bz}$), 129.2 (4 C, C-3$_{Bz}$, C-5$_{Bz}$), 132.7 (2 C, C-1$_{Bz}$), 133.5 (2 C, C-4$_{Bz}$), 134.5 (C-1$_{Bn}$), 149.3 (C-3$_{pyrazole}$), 171.2 (2 C, C=O). HRMS: m/z = 485.0582, calcd. 485.0608 for $C_{25}H_{18}{}^{79}BrN_4O_2$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 2234 (C≡N), 1694 (C=O). Purity (HPLC): t_R = 22.9 min, purity 96.7%.

4.3.8. N-(1-Benzyl-3-bromo-4-cyanopyrazol-5-yl)benzamide (14A)



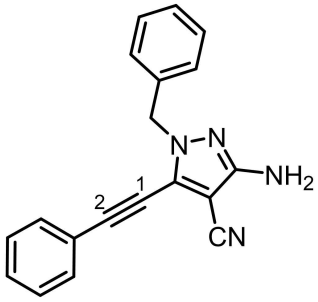
At 0 °C, NH_3 (25%, 20 mL), was added to a solution of imide **13A** (480 mg, 0.99 mmol, 1.00 eq.) in THF (20 mL) and the mixture was stirred for 3 h at 0 °C. HCl (2 M, 20 mL) was added to the reaction mixture and the aqueous layer was extracted with EtOAc (4×50 mL). The combined organic layers were dried (Na_2SO_4) and the organic solvent was removed *in vacuo*. The crude product was purified by flash chromatography (50 g, Ø = 3.5 cm, cHex/EtOAc 90:10→65:35). Colorless solid, mp 184–186 °C, yield 279 mg (74%), R_f = 0.53 (cHex/EtOAc 33:67). $C_{18}H_{13}BrN_4O$ (381.2 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.38 (s, 2H, CH_2), 7.17–7.21 (m, 2H, 2-H_{Bn}, 6-H_{Bn}), 7.30–7.32 (m, 1H, 4-H_{Bn}), 7.32–7.36 (m, 2H, 3-H_{Bn}, 5-H_{Bn}), 7.55–7.61 (m, 2H, 3-H_{Bz}, 5-H_{Bz}), 7.67 (tt, J = 7.4 / 1.5 Hz, 1H, 4-H_{Bz}), 7.93–7.96 (m, 2H, 2-H_{Bz}, 6-H_{Bz}), 11.13 (s, 1H, NH_{amide}). ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 52.8 (CH_2), 91.4 (C-4$_{pyrazole}$), 111.8 (CN), 127.5 (C-3$_{pyrazole}$), 127.8 (2 C, C-2$_{Bn}$, C-6$_{Bn}$), 128.0 (C-4$_{Bn}$), 128.1 (2 C, C-2$_{Bz}$, C-6$_{Bz}$), 128.6 (2 C, C-3$_{Bz}$, C-5$_{Bz}$), 128.7 (2 C, C-3$_{Bn}$, C-5$_{Bn}$), 131.9 (C-1$_{Bz}$), 132.9 (C-4$_{Bz}$), 135.0 (C-1$_{Bn}$), 142.8 (C-5$_{pyrazole}$), 165.8 (C=O). HRMS: m/z = 381.0331, calcd. 381.0346 for $C_{18}H_{14}{}^{79}BrN_4O$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3248 (N–H), 3028 (=C–H), 2993 (–C–H), 2234 (C≡N), 1655 (C=O). Purity (HPLC): t_R = 20.5 min, purity 99.8%.

4.3.9. N-(1-Benzyl-5-bromo-4-cyanopyrazol-3-yl)benzamide (14B)



At 0 °C, NH_3 (25%, 10 mL), was added to a solution of imide **13B** (150 mg, 0.31 mmol, 1.00 eq.) in THF (10 mL) and the mixture was stirred for 3 h at 0 °C. HCl (2 M, 10 mL) was added to the reaction mixture and the layer was extracted with EtOAc (4×25 mL). The combined organic layers were dried (Na_2SO_4) and the organic solvent was removed *in vacuo*. The crude product was purified by flash chromatography (50 g, Ø = 3.5 cm, cHex/EtOAc 90:10→65:35). Colorless solid, mp 185–186 °C, yield 95 mg (81%), R_f = 0.30 (cHex/EtOAc 33:67). $C_{18}H_{13}BrN_4O$ (381.2 g/mol). ^{1}H NMR (400 MHz, DMSO-D_6): δ (ppm) = 5.44 (s, 2H, CH_2), 7.24–7.28 (m, 2H, 2-H_{Bn}, 6-H_{Bn}), 7.34–7.36 (m, 1H, 4-H_{Bn}), 7.38–7.42 (m, 2H, 3-H_{Bn}, 5-H_{Bn}), 7.51–7.55 (m, 2H, 3-H_{Bz}, 5-H_{Bz}), 7.62 (tt, J = 7.4 / 1.5 Hz, 1H, 4-H_{Bz}), 8.00 (m, 2H, 2-H_{Bz}, 6-H_{Bz}), 11.19 (s, 1H, NH_{amide}). ^{13}C NMR (101 MHz, DMSO-D_6): δ (ppm) = 54.2 (CH_2), 91.0 (C-4$_{pyrazole}$), 112.3 (CN), 121.4 (C-5$_{pyrazole}$), 127.5 (2 C, C-2$_{Bn}$, C-6$_{Bn}$), 128.0 (2 C, C-2$_{Bz}$, C-6$_{Bz}$), 128.2 (C-4$_{Bn}$), 128.5 (2 C, C-3$_{Bz}$, C-5$_{Bz}$), 128.8 (2 C, C-3$_{Bn}$, C-5$_{Bn}$), 132.4 (C-1$_{Bz}$), 132.5 (C-4$_{Bz}$), 135.1 (C-1$_{Bn}$), 148.3 (C-5$_{pyrazole}$), 165.4 (C=O). HRMS: m/z = 381.0331, calcd. 381.0346 for $C_{18}H_{14}{}^{79}BrN_4O$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3240 (N-H), 3024 (=C–H), 2973 (–C–H), 2234 (C≡N), 1655 (C=O). Purity (HPLC): t_R = 19.7 min, purity 94.7%.

4.3.10. 3-Amino-1-benzyl-5-(phenylethynyl)pyrazole-4-carbonitrile (15B)

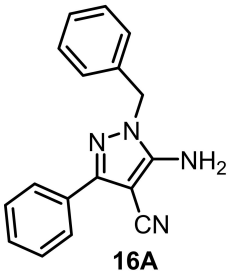


Under N_2, a mixture of regioisomeric aminopyrazoles **10A/B** (ratio **10A**:**10B** = 75:25, 50 mg, 180 μmol, 1.00 eq.), CuI (5.5 mg, 28 μmol, 0.16 eq.) and $Pd(PPh_3)_4$ (17 mg, 14 μmol, 0.08 eq.) were dissolved in acetonitrile (2.5 mL) and triethylamine (2.5 mL). Phenylacetylene (40 μL, 360 μmol, 2.00 eq.) was added and the mixture was stirred at 80 °C for 16 h. After cooling down, the reaction mixture was filtered through Celite® and eluted with EtOAc (10 mL). H_2O was added to the filtrate and the aqueous layer was extracted with EtOAc (3×20 mL). the combined organic layers were dried (Na_2SO_4) and the organic solvent removed *in vacuo*. The crude product was purified by flash chromatography (25 g, Ø = 3.5 cm, cHex/EtOAc 95:5→80:20). Only regioisomer **15B** could be isolated. Colorless solid, mp 156–158 °C, yield 6 mg (11%), R_f = 0.30 (cHex/EtOAc 67:33). $C_{19}H_{14}N_4$ (298.4 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.29 (s, 2H, CH_2), 5.93 (s, 2H, NH_2), 7.28–7.32 (m, 2H, 2-H_{Bn}, 6-H_{Bn}), 7.30–7.32 (m, 1H, 4-H_{Bn}), 7.35–7.39 (m, 2H, 3-H_{Bn}, 5-H_{Bn}), 7.47–7.51 (m, 2H, 3-H_{Ph}, 5-H_{Ph}), 7.53 (tt, J = 7.1 / 1.4 Hz, 1H, 4-H_{Ph}), 7.61–7.65 (m, 2H, 2-H_{Bn}, 6-H_{Bn}). ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 53.7 (*C*H_2), 74.8 (*C*-1_{ethyne}), 80.3 (*C*-$4_{pyrazole}$), 100.2 (*C*-2_{ethyne}), 113.6 (*C*N), 120.0 (*C*-1_{Ph}), 127.7 (2 C, *C*-2_{Bn}, *C*-6_{Bn}), 128.0 (*C*-4_{Bn}), 128.5 (*C*-$5_{pyrazole}$), 128.7 (2 C, *C*-3_{Bn}, *C*-5_{Bn}), 129.0 (2 C, *C*-3_{Ph}, *C*-5_{Ph}), 130.4 (*C*-4_{Ph}), 131,7 (2 C, *C*-2_{Ph}, *C*-6_{Ph}), 136.2 (1 C, *C*-4_{Bn}), 157.0 (*C*-$3_{pyrazole}$). HRMS: m/z = 299.1393, calcd. 299.1291 for $C_{19}H_{15}N_4$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3406 (N–H), 3059 (= C–H), 2928 (−C–H), 2222 (C≡N). Purity (HPLC): t_R = 21.1 min, purity 96.9%.

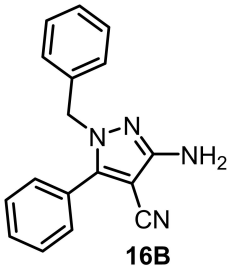
4.3.11. General method A: Suzuki-Miyaura coupling of bromopyrazole 10A/B using microwave irradiation

A mixture of regioisomeric aminopyrazoles **10A/B** (ratio **10A**:**10B** = 75:25–90:10, 30 mg, 108 μmol, 1.00 eq.), Cs_2CO_3 (70 mg, 216 μmol, 2.00 eq.), Pd(dppf)Cl_2 (6.3 mg, 8.6 μmol, 0.08 eq.) and the respective boronic acid or boronic ester (173 μmol, 1.60 eq.) were filled into a microwave tube. 1,4-Dioxane (1.6 mL) and H_2O (0.4 mL) were added, the microwave tube was flushed with N_2 and sealed. The vial was inserted into the microwave reactor and the solution was stirred at 120 °C for 1 h under microwave irradiation (variable power, max. 300 W, max. 300 psi). After cooling down, the reaction mixture was filtered through Celite® and eluted with EtOAc (20 mL). H_2O (20 mL) was added to the filtrate and the aqueous layer was extracted with EtOAc (3×25 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. The residue was mixed with silica and purified by flash chromatography (cHex/EtOAc 95:5→60:40 or CH_2Cl_2/CH_3OH 99:1→95:5).

4.3.12. 5-Amino-1-benzyl-3-phenylpyrazole-4-carbonitrile (16A) and 3-amino-1-benzyl-5-phenylpyrazole-4-carbonitrile (16B)



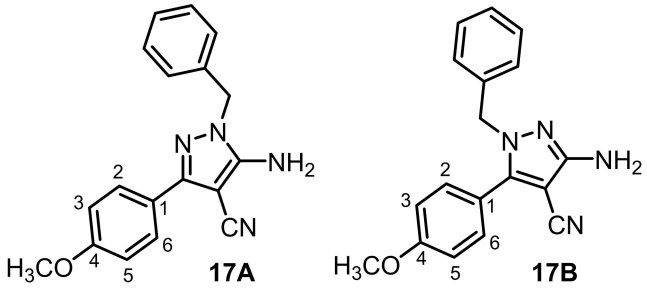
16A



16B

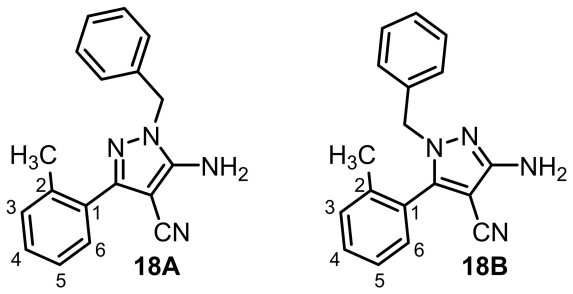
The compound was synthesized according to the **General method A** using phenylboronic acid (21 mg, 173 μmol, 1.60 eq.). The product was purified by flash chromatography (25 g, Ø = 3.5 cm, EtOAc/CH 5:95→40:60). Colorless solid, mp 152–155 °C, yield 29 mg (99%), R_f = 0.28 (**16A**), 0.30 (**16B**) (cHex/EtOAc 67:33). $C_{17}H_{14}N_4$ (274.3 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.08* (s, 2×0.20H, CH_2), 5.23 (s, 2×0.80H, CH_2), 5.73* (s, 2×0.20H, NH_2), 6.86 (s, 2×0.80H, NH_2), 7.02–7.04* (m, 2×0.20H, 2-H_{Bn}, 6-H_{Bn}), 7.22–7.24 (m, 2×0.80H, 2-H_{Bn}, 2-H_{Bn}), 7.26–7.28* (m, 1×0.20H, 4-H_{Bn}), 7.28–7.30* (m, 2×0.20H, 3-H_{Bn}, 5-H_{Bn}), 7.29–7.31 (m, 1×0.80H, 4-H_{Bn}), 7.33–7.36 (m, 2×0.80H, 3-H_{Bn}, 5-H_{Bn}), 7.40 (tt, J = 7.4 / 1.4 Hz, 1×0.80H, 4-H_{Ph}), 7.43–7.47 (m, 2×0.80H, 3-H_{Ph}, 5-H_{Ph}), 7.48–7.50* (m, 2×0.20H, 2-H_{Ph}, 6-H_{Ph}), 7.54–7.56* (m, 1×0.20H, 4-H_{Ph}), 7.55–7.57* (m, 2×0.20H, 3-H_{Ph}, 5-H_{Ph}), 7.77–7.81 (m, 2×0.80H, 2-H_{Ph}, 6-H_{Ph}). Signals of the minor regioisomer **16B** are marked with an asterisk (*). Ratio of **16A**:**16B** = 80:20. ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 50.1, 52.3* (*C*H_2), 70.0, 78.3* (*C*-$4_{pyrazole}$), 114.8, 116.0* (*C*N), 125.7, 126.9* (2 C, *C*-2_{Bn}, *C*-6_{Bn}), 127.1* (*C*-1_{Ph}), 127.3 (2 C, *C*-2_{Bn}, *C*-6_{Bn}), 127.5* (2 C, *C*-3_{Bn}, *C*-5_{Bn}), 127.5* (*C*-4_{Bn}), 128.5 (2 C, *C*-3_{Bn}, *C*-5_{Bn}), 128.5 (*C*-4_{Bn}), 128.7 (2 C, *C*-3_{Ph}, *C*-5_{Ph}), 128.7 (*C*-4_{Ph}), 128.8* (2 C, *C*-2_{Ph}, *C*-6_{Ph}), 129.2* (2 C, *C*-3_{Ph}, *C*-5_{Ph}), 130.1* (*C*-4_{Ph}), 131.5 (*C*-1_{Ph}), 136.6, 136.7* (*C*-1_{Bn}), 147.8* (*C*-$5_{pyrazole}$), 149.3 (*C*-$3_{pyrazole}$), 153.4 (*C*-$5_{pyrazole}$), 157.1* (*C*-$3_{pyrazole}$). Signals of the minor regioisomer **16B** are marked with an asterisk (*). HRMS: m/z = 275.1286, calcd. 275.1291 for $C_{17}H_{15}N_4$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3375 (N–H), 2924 (−C–H), 2207 (C≡N). Purity (HPLC): t_R = 18.9 min, 19.4 min, purity 99.7%.

4.3.13. 5-Amino-1-benzyl-3-(4-methoxyphenyl)pyrazole-4-carbonitrile (17A) and 3-amino-1-benzyl-5-(4-methoxyphenyl)pyrazole-4-carbonitrile (17B)



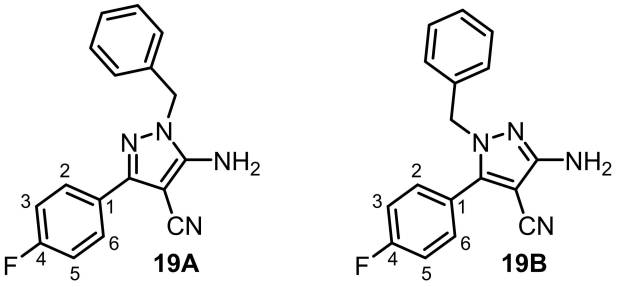
The compound was synthesized according to the **General method A** using 4-methoxyphenylboronic acid (26 mg, 173 μmol, 1.60 eq.). The product was purified by flash chromatography (25 g, Ø = 3.5 cm, cHex/EtOAc 95 : 5→60 : 40). Colorless solid, mp 160 −162 °C, yield 31 mg (95 %), R_f = 0.25 (**17A**), 0.29 (**17B**) (cHex/EtOAc 67 : 33). $C_{18}H_{16}N_4O$ (304.4 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 3.78 (s, 3×0.80H, OCH_3), 3.81* (s, 3×0.20H, OCH_3), 5.07* (s, 2×0.20H, CH_2), 5.20 (s, 2×0.80H, CH_2), 5.67* (s, 2×0.20H, NH_2), 6.80 (s, 2×0.80H, NH_2), 7.00–7.02 (m, 2×0.80H, 3-H_{PhOMe}, 5-H_{PhOMe}), 7.03–7.05* (m, 2×0.20H, 2-H_{Bn}, 6-H_{Bn}), 7.10–7.12* (m, 2×0.20H, 3-H_{PhOMe}, 5-H_{PhOMe}), 7.20–7.22 (m, 2×0.80H, 2-H_{Bn}, 6-H_{Bn}), 7.25–7.27* (m, 1×0.20H, 4-H_{Bn}), 7.27–7.29 (m, 1×0.80H, 4-H_{Bn}), 7.29–7.31* (m, 2×0.20H, 3-H_{Bn}, 5-H_{Bn}), 7.33–7.35 (m, 2×0.80H, 3-H_{Bn}, 5-H_{Bn}), 7.40–7.42* (m, 2×0.20H, 2-H_{PhOMe}, 6-H_{PhOMe}), 7.71–7.73 (m, 2×0.80H, 2-H_{PhOMe}, 6-H_{PhOMe}). Signals of the minor regioisomer **17B** are marked with an asterisk (*). Ratio of **17A**:**17B** = 80 : 20. ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 50.0, 52.2* (CH_2), 55.2, 55.3* (OCH_3), 69.6, 78.0* (C-$4_{pyrazole}$), 114.1, 114.7* (2 C, C-3_{PhOMe}, C-5_{PhOMe}), 115.0, 116.1 (CN), 119.2*, 124.1 (C-1_{PhOMe}), 126.8* (2 C, C-2_{Bn}, C-6_{Bn}), 127.0 (2 C, C-2_{PhOMe}, C-6_{PhOMe}), 127.3 (C-2_{Bn}, C-6_{Bn}), 127.4, 127.5* (C-4_{Bn}), 128.5, 128.5* (2 C, C-3_{Bn}, C-5_{Bn}), 130.2* (2 C, C-2_{PhOMe}, C-6_{PhOMe}), 136.7, 136.8* (C-1_{Bn}), 147.8* (C-$5_{pyrazole}$), 149.2 (C-$3_{pyrazole}$), 153.2 (C-$5_{pyrazole}$), 157.0* (C-$3_{pyrazole}$), 159.6, 160.4* (C-4_{PhOMe}). Signals of the minor regioisomer **17B** are marked with an asterisk (*). HRMS: m/z = 305.1398, calcd. 305.1397 for $C_{18}H_{17}N_4O$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3395 (N–H), 3005 (=C–H), 2214 (C≡N), 1250 (C–O). Purity (HPLC): t_R = 19.2 min, 19.5 min, purity 99.9 %.

4.3.14. 5-Amino-1-benzyl-3-(o–tolyl)pyrazole-4-carbonitrile (18A) and 3-amino-1-benzyl-5-(o–tolyl)pyrazole-4-carbonitrile (18B)



The compound was synthesized according to the **General method A** using 2-methylphenylboronic acid (30 mg, 173 μmol, 1.60 eq.). The product was purified by flash chromatography (25 g, Ø = 3.5 cm, cHex/EtOAc 95 : 5→60 : 40). Colorless solid, mp 107–110 °C, yield 29 mg (93 %), R_f = 0.35 (**18A**), 0.32 (**18B**) (cHex/EtOAc 67 : 33). $C_{18}H_{16}N_4$ (288.4 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 2.08* (s, 3×0.17H, CH_3), 2.32 (s, 3×0.83H, CH_3), 4.75–4.90* (m, 2×0.17H, CH_2), 5.22 (s, 2×0.83H, CH_2), 5.72* (s, 2×0.17H, NH_2), 6.81 (s, 2×0.83H, NH_2), 6.95–6.97* (m, 2×0.17H, 2-H_{Bn}, 6-H_{Bn}), 7.21–7.25 (m, 2×0.83H, 2-H_{Bn}, 6-H_{Bn}), 7.34–7.36 (m, 1×0.83H, 5-H_{tolyl}), 7.26–7.28* (m, 1×0.17H, 4-H_{Bn}), 7.27–7.29 (m, 1×0.83H, 4-H_{Bn}), 7.28–7.32* (m, 2×0.17H, 3-H_{Bn}, 5-H_{Bn}), 7.29–7.31 (m, 1×0.83H, 3-H_{tolyl}), 7.30–7.31 (m, 1×0.83H, 4-H_{tolyl}), 7.30–7.32* (m, 1×0.17H, 6-H_{tolyl}), 7.33–7.35* (m, 1×0.17H, 5-H_{tolyl}), 7.33–7.36 (m, 1×0.83H, 6-H_{tolyl}), 7.34–7.38 (m, 2×0.83H, 3-H_{Bn}, 5-H_{Bn}), 7.37–7.39* (m, 1×0.17H, 3-H_{tolyl}), 7.45–7.47* (m, 1×0.17H, 4-H_{tolyl}). Signals of the minor regioisomer **18B** are marked with an asterisk (*). Ratio of **18A**:**18B** = 83 : 17. ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 18.9*, 20.0 (CH_3), 50.00, 52.3* (CH_2), 72.0*, 72.7 (C-$4_{pyrazole}$), 114.6*, 115.5 (CN), 125.7, 126.3* (C-5_{tolyl}), 126.8* (C-2_{tolyl}), 127.2, 127.3* (2 C, C-2_{Bn}, C-6_{Bn}), 127.4, 127.6* (C-4_{Bn}), 128.4*, 128.5 (2 C, C-3_{Bn}, C-5_{Bn}), 128.6 (C-4_{tolyl}), 129.3, 129.9* (C-6_{tolyl}), 130.4* (C-4_{tolyl}), 130.6, 130.6* (C-3_{tolyl}), 131.1 (C-2_{tolyl}), 136.2 (C-1_{tolyl}), 136.3*, 136.7 (C-1_{Ph}), 137.1* (C-1_{tolyl}), 147.1* (C-$5_{pyrazole}$), 151.2 (C-$3_{pyrazole}$), 152.4 (C-$5_{pyrazole}$), 156.9* (C-$3_{pyrazole}$). Signals of the minor regioisomer **18B** are marked with an asterisk (*). HRMS: m/z = 289.1455, calcd. 289.1448 for $C_{18}H_{17}N_4$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3337 (N-H), 3067 (=C–H), 2924 (−C–H), 2210 (C≡N). Purity (HPLC): t_R = 19.6 min, 19.7 min, purity 99.1 %.

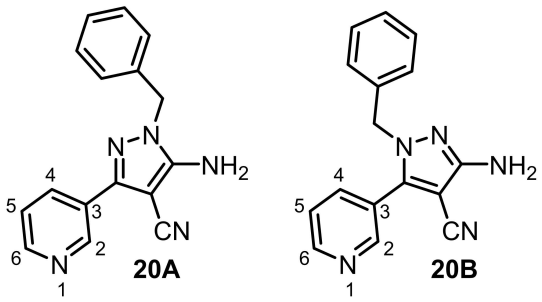
4.3.15. 5-Amino-1-benzyl-3-(4-fluorophenyl)pyrazole-4-carbonitrile (19A) and 3-amino-1-benzyl-5-(4-fluorophenyl)pyrazole-4-carbonitrile (19B)



The compound was synthesized according to the **General method A** using 4-fluorophenylboronic acid (24 mg, 173 μmol, 1.60 eq.). The product was purified by flash chromatography (25 g, Ø = 3.5 cm, cHex/EtOAc 95 : 5→60 : 40). Colorless solid, mp 130–132 °C, yield 24 mg (76 %), R_f = 0.28 (**19A**), 0.30 (**19B**) (cHex/EtOAc 67 : 33). $C_{17}H_{13}FN_4$ (292.3 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.07* (s, 2×0.18H, CH_2), 5.22 (s, 2×0.82H, CH_2), 5.74* (s, 2×0.18H, NH_2), 6.89 (s, 2×0.82H, NH_2), 7.02–7.04* (m, 2×0.18H, 2-H_{Bn}, 6-H_{Bn}), 7.21–7.25 (m, 2×0.82H, 2-H_{Bn}, 6-H_{Bn}), 7.28–7.30* (m, 1×0.18H, 4-H_{Bn}), 7.28–7.32 (m, 2×0.82H, 3-H_{PhF}, 5-H_{PhF}), 7.28–7.32 (m, 1×0.82H, 4-H_{Bn}), 7.27–7.29* (m, 2×0.18H, 3-H_{Bn}, 5-H_{Bn}), 7.33–7.37 (m, 2×0.82H, 3-H_{Bn}, 5-H_{Bn}), 7.40–7.42* (m, 2×0.18H, 3-H_{PhF}, 5-H_{PhF}), 7.54–7.56* (m, 2×0.18H, 2-H_{PhF}, 6-H_{PhF}),

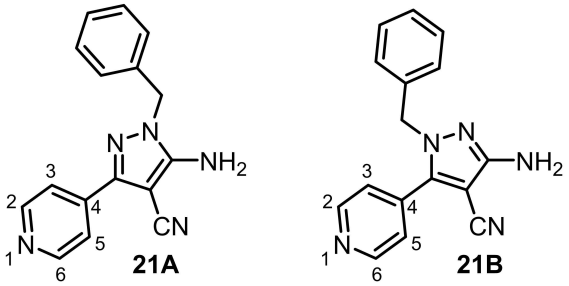
7.80–7.84 (m, 2×0.82H, 2-H_{PhF}, 6-H_{PhF}). Signals of the minor regioisomer **19B** are marked with an asterisk (*). Ratio ov **19A**:**19B** = 82:18. ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 50.1, 52.4* (CH_2), 69.9, 78.5* (*C*-4$_{pyrazole}$), 114.7* (*C*N), 115.8 (d, J_{CF} = 22.3 Hz, 2 C, *C*-3$_{PhF}$, *C*-5$_{PhF}$), 115.9 (*C*N), 116.4* (d, J_{CF} = 22.0 Hz, 2 C, *C*-3$_{PhF}$, *C*-5$_{PhF}$), 123.6* (d, J_{CF} = 3.2 Hz, *C*-1$_{PhF}$), 126.9*, 127.3 (2 C, *C*-2$_{Bn}$, *C*-6$_{Bn}$), 127.5* (2 C, *C*-3$_{Bn}$, *C*-5$_{Bn}$), 127.6* (*C*-4$_{Bn}$), 127.8 (d, J_{CF} = 8.3 Hz, 2 C, *C*-2$_{PhF}$, *C*-6$_{PhF}$), 128.1 (d, J_{CF} = 3.0 Hz, *C*-1$_{PhF}$), 128.6 (2 C, *C*-3$_{Bn}$, *C*-5$_{Bn}$), 128.6 (*C*-4$_{Bn}$), 131.3* (d, J_{CF} = 9.0 Hz, 2 C, *C*-2$_{PhF}$, *C*-6$_{PhF}$), 136.5, 136.6* (*C*-1$_{Bn}$), 146.8* (*C*-5$_{pyrazole}$), 148.4 (*C*-3$_{pyrazole}$), 153.4 (*C*-5$_{pyrazole}$), 157.0* (*C*-3$_{pyrazole}$), 162.3 (d, J_{CF} = 245.8 Hz, *C*-4$_{PhF}$), 162.9* (d, J_{CF} = 241 Hz, *C*-4$_{PhF}$). Signals of the minor regioisomer **19B** are marked with an asterisk (*). ^{19}F NMR (376 MHz, DMSO-D_6 + CCl_3F): δ (ppm) = −109.7–−110.0* (m, 1F, 4-F_{PhF}), −112.0–−112.4 (m, 1F, 4-F_{PhF}). Signals of the minor regioisomer **19B** are marked with an asterisk (*). HRMS: m/z = 293.1188, calcd. 293.1197 for $C_{17}H_{14}FN_4$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3452 (N–H), 3194 (=C–H), 2951 (−C–H), 2214 (C≡N). Purity (HPLC): t_R = 19.3 min, 20.0 min, purity 99.9 %.

4.3.16. 5-Amino-1-benzyl-3-(pyridin-3-yl)pyrazole-4-carbonitrile (20A) and 3-amino-1-benzyl-5-(pyridin-3-yl)pyrazole-4-carbonitrile (20B)



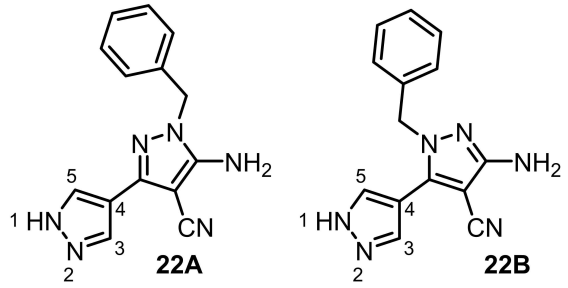
The compound was synthesized according to the **General method A** using pinacol ester 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (36 mg, 172 μmol, 1.60 eq.). The product was purified by flash chromatography (25 g, Ø = 3.5 cm, CH_2Cl_2/CH_3OH 99:1→95:5). Colorless solid, mp 167–169 °C, yield 29 mg (97 %), R_f = 0.50 (**20A**), 0.45 (**20B**) (CH_2Cl_2/CH_3OH 95:5. $C_{16}H_{13}N_5$ (275.3 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.11* (s, 2×0.15H, CH_2), 5.25 (s, 2×0.85H, CH_2), 5.82* (s, 2×0.15H, NH_2), 6.98 (s, 2×0.85H, NH_2), 7.02–7.04* (m, 2×0.15H, 2-H_{Bn}, 6-H_{Bn}), 7.23–7.25 (m, 2×0.85H, 2-H_{Bn}, 6-H_{Bn}), 7.28–7.30 (m, 1×0.85H, 4-H_{Bn}), 7.29–7.31* (m, 2×0.15H, 3-H_{Bn}, 5-H_{Bn}), 7.33–7.35* (m, 1×0.15H, 4-H_{Bn}), 7.34–7.36 (m, 2×0.85H, 3-H_{Bn}, 5-H_{Bn}), 7.50 (td, J = 4.0 / 1.0 Hz, 1H, 5-$H_{pyridyl}$), 7.58* (m, 1×0.15H, 5-$H_{pyridyl}$), 7.94* (m, 1×0.15H, 4-$H_{pyridyl}$), 8.11 (ddd, J = 4.0 / 1.8 / 1.1 Hz, 1×0.85H, 4-$H_{pyridyl}$), 8.60 (dd, J = 4.0 / 1.1 Hz, 1H, 6-$H_{pyridyl}$), 8.67–8.69* (m, 1×0.15H, 2-$H_{pyridyl}$), 8.71–8.73* (m, 1×0.15H, 6-$H_{pyridyl}$), 8.95–8.96 (m, 1×0.85H, 2-$H_{pyridyl}$). Signals of the minor regioisomer **20B** are marked with an asterisk (*). Ratio of **20A**:**20B** = 85:15. ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 50.2, 52.6* (CH_2), 70.2, 78.9* (*C*-4$_{pyrazole}$), 114.5*, 115.6 (*C*N), 123.5* (*C*-3$_{pyridyl}$), 123.9 (*C*-5$_{pyridyl}$), 124.1* (*C*-5$_{pyridyl}$), 126.9*, 127.3 (2 C, *C*-2$_{Bn}$, *C*-6$_{Bn}$), 127.4 (*C*-3$_{pyridyl}$), 127.6, 127.6* (*C*-4$_{Bn}$), 128.6, 128.6* (2 C, *C*-3$_{Bn}$, *C*-5$_{Bn}$), 133.1 (*C*-4$_{pyridyl}$), 136.4, 136.5* (*C*-1$_{Bn}$), 136.6* (*C*-4$_{pyridyl}$), 144.6* (*C*-5$_{pyrazole}$), 146.5 (*C*-2$_{pyridyl}$), 146.8 (*C*-3$_{pyrazole}$), 148.9* (*C*-2$_{pyridyl}$), 149.7, 151.0* (*C*-6$_{pyridyl}$), 153.4 (*C*-5$_{pyrazole}$), 157* (*C*-3$_{pyrazole}$). Signals of the minor regioisomer **20B** are marked with an asterisk (*). HRMS: m/z = 276.1244, calcd. 276.1244 for $C_{16}H_{14}N_5$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3441 (N-H), 3063 (=C–H), 2987 (−C–H), 2207 (CN). Purity (HPLC): t_R = 13.7 min, 14.1 min, purity 97.4 %.

4.3.17. 5-Amino-1-benzyl-3-(pyridin-4-yl)pyrazole-4-carbonitrile (21A) and 3-amino-1-benzyl-5-(pyridin-4-yl)pyrazole-4-carbonitrile (21B)



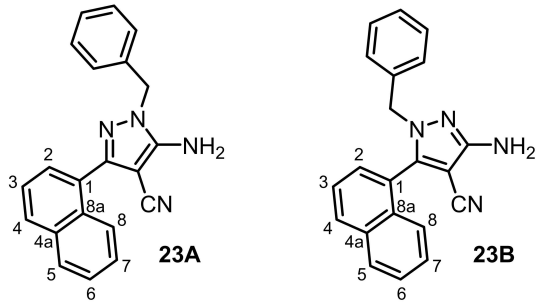
The compound was synthesized according to the **General method A** using pyridine-4-ylboronic acid (21 mg, 173 μmol, 1.60 eq.). The product was purified by flash chromatography (25 g, Ø = 3.5 cm, CH_2Cl_2/CH_3OH 99:1→95:5). Colorless solid, mp 246 °C, yield 24 mg (81 %), R_f = 0.45 (**21A**), 0.40 (**21B**) (CH_2Cl_2/CH_3OH 95:5). $C_{16}H_{13}N_5$ (275.3 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.15* (s, 2×0.14H, CH_2), 5.27 (s, 2×0.86H, CH_2), 5.86* (s, 2×0.14H, NH_2), 7.02 (s, 2×0.86H, NH_2), 7.03–7.05* (m, 2×0.14H, 2-H_{Bn}, 6-H_{Bn}), 7.21–7.25 (m, 2×0.86H, 2-H_{Bn}, 6-H_{Bn}), 7.27–7.29* (m, 2×0.14H, 3-H_{Bn}, 5-H_{Bn}), 7.28–7.30 (m, 1×0.86H, 4-H_{Bn}), 7.33–7.37 (m, 2×0.86H, 3-H_{Bn}, 5-H_{Bn}), 7.34–7.36* (m, 1×0.14H, 4-H_{Bn}), 7.51–7.53* (m, 2×0.14H, 3-$H_{pyridyl}$, 5-$H_{pyridyl}$), 7.70–7.76 (m, 2×0.86H, 3-$H_{pyridyl}$, 5-$H_{pyridyl}$), 8.62–8.67 (m, 2×0.86H, 2-$H_{pyridyl}$, 6-$H_{pyridyl}$), 8.75–8.77* (m, 2×0.14H, 2-$H_{pyridyl}$, 6-$H_{pyridyl}$). Signals of the minor regioisomer **21B** are marked with an asterisk (*). Ratio of **21A**:**21B** = 86:14. ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 50.4, 52.8* (CH_2), 70.3, 78.6* (*C*-4$_{pyrazole}$), 114.3*, 115.4 (*C*N), 119.7, 123.1* (2 C, *C*-3$_{pyridyl}$, *C*-5$_{pyridyl}$), 127.0*, 127.3 (2 C, *C*-2$_{Bn}$, *C*-6$_{Bn}$), 127.6, 127.7* (*C*-4$_{Bn}$), 128.6, 128.6* (2 C, *C*-3$_{Bn}$, *C*-5$_{Bn}$), 134.6* (*C*-4$_{pyridyl}$), 136.2, 136.4* (*C*-1$_{Bn}$), 138.4 (*C*-4$_{pyridyl}$), 144.9* (*C*-5$_{pyrazole}$), 146.7 (*C*-3$_{pyrazole}$), 150.4, 150.6* (2 C, *C*-2$_{pyridyl}$, *C*-6$_{pyridyl}$), 153.7 (*C*-5$_{pyrazole}$), 157.4* (*C*-3$_{pyrazole}$). Signals of the minor regioisomer **21B** are marked with an asterisk (*). HRMS: m/z = 276.1245, calcd. 276.1244 for $C_{16}H_{14}N_5$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3363 (N–H), 3086 (=C–H), 2988 (−C-H), 2214 (C≡N). Purity (HPLC): t_R = 13.1 min, 13.6 min, purity 95.3 %.

4.3.18. 5-Amino-1-benzyl-[3,4′-bipyrazole]-4-carbonitrile (22 A) and 3-amino-1-benzyl-[5,4′-bipyrazole]-4-carbonitrile (22 B)



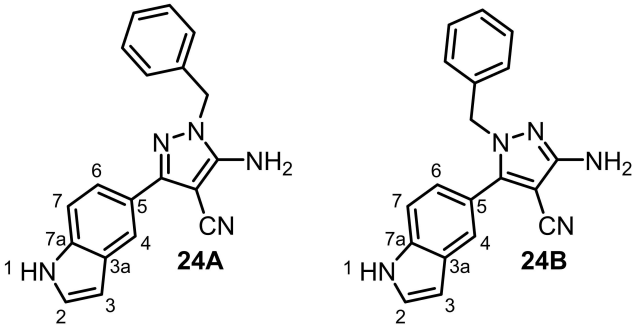
The compound was synthesized according to the **General method A** using pyrazol-4-ylboronic acid (19 mg, 173 μmol, 1.60 eq.). The product was purified by flash chromatography (25 g, Ø = 3.5 cm, CH_2Cl_2/CH_3OH 99 : 1→95 : 5). Yellow solid, mp 127–129 °C, yield 12 mg (41 %), R_f = 0.35 (**22 A**), 0.30 (**22 B**) (CH_2Cl_2/CH_3OH 95 : 5). $C_{14}H_{12}N_6$ (264.3 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.17, 5.20* (s, 2×0.50H, CH_2), 5.64*, 6.78 (s, 2×0.50H, NH_2), 7.04–7.08*, 7.16–7.20 (m, 2×0.50H, 2-H_{Bn}, 6-H_{Bn}), 7.25–7.27* (m, 1×0.50H, 4-H_{Bn}), 7.27 (tt, J = 7.0 / 1.3 Hz, 1×0.50H, 4-H_{Bn}), 7.29–7.33*, 7.32–7.36 (m, 2×0.50H, 3-H_{Bn}, 5-H_{Bn}), 7.71–7.75*, 7.77–7.81 (br, 1×0.50H, 5-$H_{pyrazole}$), 8.00–8.04, 8.08–8.12* (br, 1×0.50H, 3-$H_{pyrazole}$), 13.06–13.11, 13.40–13.45* (br, 1×0.50H, N$H_{pyrazole}$). Signals of the regioisomer **22 B** are marked with an asterisk (*). Ratio of **22 A** : **22 B** = 50 : 50. ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 49.9 (CH_2), 52.5* (CH_2), 69.8 (C-$4_{pyrazoleCN}$), 77.0* (C-$4_{pyrazoleCN}$), 107.1* (C-$4_{pyrazoleH}$), 112.9 (C-$4_{pyrazoleH}$), 115.3* (CN), 115.9 (CN), 125.8 (C-$3_{pyrazoleH}$), 126.7* (2 C, C-2_{Bn}, C-6_{Bn}), 127.1 (2 C, C-2_{Bn}, C-6_{Bn}), 127.4 (C-4_{Bn}), 127.5* (C-4_{Bn}), 128.5 (2 C, C-3_{Bn}, C-5_{Bn}), 128.6* (2 C, C-3_{Bn}, C-5_{Bn}), 136.1 (C-$5_{pyrazoleH}$), 136.8 (C-1_{Bn}), 136.8* (C-1_{Bn}), 140.6* (C-$5_{pyrazoleCN}$), 144.5 (C-$3_{pyrazoleCN}$), 152.4 (C-$5_{pyrazoleCN}$), 157.0* (C-$3_{pyrazoleCN}$). Signals for C-$5_{pyrazoleH}$(**22 B**) and C-$3_{pyrazoleH}$(**22 B**) are missing. Signals of the regioisomer **22 B** are marked with an asterisk (*). HRMS: m/z = 265.1201, calcd. 265.1196 for $C_{14}H_{13}N_6$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3302 (N–H), 3178 (N–H), 3052 (=C–H), 2920 (–C–H), 2210 (CN). Purity (HPLC): t_R = 13.9 min, 14.5 min, purity 93.5 %.

4.3.19. 5-Amino-1-benzyl-3-(naphthalen-1-yl)pyrazole-4-carbonitrile (23 A) and 3-amino-1-benzyl-5-(naphthalen-1-yl)pyrazole-4-carbonitrile (23 B)



The compound was synthesized according to the **General method A** using naphthalen-1-ylboronic acid (30 mg, 173 μmol, 1.60 eq.). The product was purified by flash chromatography (25 g, Ø = 3.5 cm, cHex/EtOAc 95 : 5→60 : 40). Colorless solid, mp 177–179 °C, yield 33 mg (93 %), R_f = 0.30 (**23 A**), 0.32 (**23B**) (cHex/EtOAc 67 : 33). $C_{21}H_{16}N_4$ (324.4 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.31 (s, 2H, CH_2), 6.92 (s, 2H, NH_2), 7.27–7.31 (m, 2H, 2-H_{Bn}, 6-H_{Bn}), 7.30–7.32 (m, 1H, 4-H_{Bn}), 7.36–7.40 (m, 2H, 3-H_{Bn}, 5-H_{Bn}), 7.52–7.54 (m, 1H, 7-$H_{naphthyl}$), 7.54–7.58 (m, 1H, 6-$H_{naphthyl}$), 7.59–7.61 (m, 1H, 3-$H_{naphthyl}$), 7.60–7.62 (m, 1H, 2-$H_{naphthyl}$), 7.97–8.01 (m, 1H, 4-$H_{naphthyl}$), 7.98–8.02 (m, 1H, 5-$H_{naphthyl}$), 8.22–8.24 (m, 1H, 8-$H_{naphthyl}$). Ratio of **23 A** : **23 B** = 95 : 5. Only the signals of the major isomer **23 A** are characterized. ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 50.2 (CH_2), 73.4 (C-$4_{pyrazole}$), 115.4 (CN), 125.3 (C-$3_{naphthyl}$), 125.6 (C-$8_{naphthyl}$), 126.1 (C-$6_{naphthyl}$), 126.6 (C-$7_{naphthyl}$), 127.3 (C-$2_{naphthyl}$), 127.3 (2 C, C-2_{Bn}, C-6_{Bn}), 127.5 (C-4_{Bn}), 128.3 (C-$5_{naphthyl}$), 128.6 (2 C, C-3_{Bn}, C-5_{Bn}), 128.8 (C-$4a_{naphthyl}$), 129.1 (C-$4_{naphthyl}$), 130.6 (C-$8a_{naphthyl}$), 133.4 (C-$1_{naphthyl}$), 136.7 (C-1_{Bn}), 150.2 (C-$3_{pyrazole}$), 152.6 (C-$5_{pyrazole}$). Only the signals of the major isomer **23 A** are characterized. HRMS: m/z = 325.1458, calcd. 325.1448 for $C_{21}H_{17}N_4$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3333 (N–H), 3078 (=C–H), 2987 (–C–H), 2218 (C≡N). Purity (HPLC): t_R = 20.4 min, 20.5 min, purity 99.5 %.

4.3.20. 5-Amino-1-benzyl-3-(1H-indol-5-yl)pyrazole-4-carbonitrile (24 A) and 3-amino-1-benzyl-5-(1H-indol-5-yl)pyrazole-4-carbonitrile (24 B)



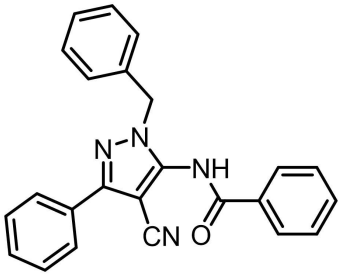
The compound was synthesized according to the **General method A** using indol-5-ylboronic acid (28 mg, 173 μmol, 1.60 eq.). The product was purified by flash chromatography (25 g, Ø = 3.5 cm, cHex/EtOAc 95 : 5→60 : 40). Colorless solid, mp 106 -108 °C, yield 33 mg (97 %), R_f = 0.15 (**24 A**), 0.13 (**24B**) (cHex/EtOAc 67 : 33). $C_{19}H_{15}N_5$ (313.4 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.10* (s, 2×0.22H, CH_2), 5.22 (s, 2×0.78H, CH_2), 5.64* (s, 2×0.22H, NH_2), 6.48 –6.50 (m, 1×0.78H, 3-$H_{indolyl}$), 6.52–6.54* (m, 1×0.22H, 3-$H_{indolyl}$), 6.76 (s, 2×0.78H, NH_2), 7.03–7.07* (m, 2×0.78H, 2-H_{Bn}, 6-H_{Bn}), 7.15–7.17* (m, 1×0.22H, 6-$H_{indolyl}$), 7.23–7.27 (m, 2×0.78H, 2-H_{Bn}, 6-H_{Bn}), 7.27–7.29 (m, 1×0.78H, 4-H_{Bn}), 7.28–7.30* (m, 2×0.22H, 3-H_{Bn}, 5-H_{Bn}), 7.29–7.31* (m, 1×0.22H, 4-H_{Bn}), 7.33–7.37 (m, 2×0.78H, 3-H_{Bn}, 5-H_{Bn}), 7.36–7.38 (m, 1×0.78H, 2-$H_{indolyl}$), 7.41–7.45 (m, 1×0.78H, 7-$H_{indolyl}$), 7.47–7.49* (m, 1×0.22H, 2-$H_{indolyl}$), 7.53–7.57 (m, 1×0.78H, 6-$H_{indolyl}$), 7.54–7.56* (m, 1×0.22H, 7-$H_{indolyl}$), 7.67–7.70* (m, 1×0.22H, 4-$H_{indolyl}$), 7.99–8.01 (m, 1×0.78H, 4-$H_{indolyl}$), 11.21 (s, 1×0.78H, N$H_{indolyl}$), 11.42* (s, 1×0.22H, N$H_{indolyl}$). Signals of the minor regioisomer **24 B** are marked with an asterisk (*).Ratio of **24 A** :; **24B** = 78 : 22. ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 50.0, 52.1* (CH_2), 69.8, 78.1* (C-$4_{pyrazole}$), 101.7, 101.8* (C-$3_{indolyl}$), 111.6, 112.2* (C-$7_{indolyl}$), 115.3*, 116.5 (CN), 117.6*, 117.7 (C-

$4_{indolyl}$), 119.4, 120.9* (*C*-$6_{indolyl}$), 121.4*, 122.7 (*C*-$5_{indolyl}$), 126.2 (*C*-$2_{indolyl}$), 126.9*, 127.3 (2 C, *C*-2_{Bn}, *C*-6_{Bn}), 127.4 (*C*-4_{Bn}), 127.5 (*C*-$3a_{indolyl}$), 127.7* (*C*-$2_{indolyl}$), 128.5, 128.5* (2 C, *C*-3_{Bn}, *C*-5_{Bn}), 128.5* (*C*-4_{Bn}), 128.5* (*C*-$3a_{indolyl}$), 136.0 (*C*-$7a_{indolyl}$), 136.3* (*C*-$7a_{indolyl}$), 136.8, 137.0* (*C*-1_{Bn}), 149.8* (*C*-$5_{pyrazole}$), 150.9 (*C*-$3_{pyrazole}$), 153.2 (*C*-$5_{pyrazole}$), 157.0* (*C*-$3_{pyrazole}$). Signals of the minor regioisomer **24 B** are marked with an asterisk (*). HRMS: m/z = 314.1402, calcd. 314.1400 for $C_{19}H_{16}N_5$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3329 (N–H), 3109 (= C–H), 2928 (– C–H), 2207 (C≡N). Purity (HPLC): t_R = 18.4 min, 18.5 min, purity 98.4 %.

4.3.21. General method B: *Suzuki Miyaura* coupling of 14 A

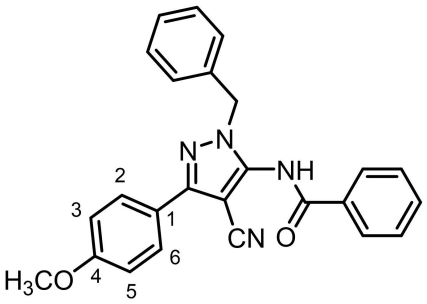
Under N_2, aminopyrazole **14 A** (41 mg, 108 µmol, 1.00 eq.), Cs_2CO_3 (70 mg, 215 µmol, 2.00 eq.), Pd(dppf)Cl_2 (6.3 mg, 8.6 µmol, 0.08 eq.) and the respective boronic acid or boronic ester (172 µmol, 1.60 eq.) were filled into a SCHLENK tube. The tube was evacuated and backfilled with N_2. 1,4-Dioxane (1.6 mL) and H_2O (0.4 mL) were added and the reaction mixture was stirred at 120 °C for 1.5 h. After cooling down, the reaction mixture was filtered through Celite® and eluted with EtOAc (20 mL). H_2O (20 mL) was added to the filtrate and the aqueous layer was extracted with EtOAc (3×25 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. The residue was mixed with silica and purified by flash chromatography (cHex/EtOAc 95 : 5→60 : 40 or CH_2Cl_2/CH_3OH 99 : 1→95 : 5).

4.3.22. N-(1-Benzyl-4-cyano-3-phenylpyrazol-5-yl)benzamide (25 A)



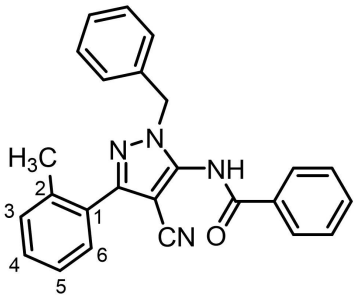
The compound was synthesized according to the **General method B** using phenylboronic acid (21 mg, 172 µmol, 1.60 eq.). The product was purified by flash chromatography (10 g, Ø = 2.5 cm, cHex/EtOAc 95 : 5→60 : 40) and subsequent recrystallization with EtOH. Colorless solid, mp 203–206 °C, yield 37 mg (91 %), R_f = 0.52 (cHex/EtOAc 67 : 33). $C_{24}H_{18}N_4O$ (378.4 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.42 (s, 2H, CH_2), 7.22–7.28 (m, 2H, 2-H_{Bn}, 6-H_{Bn}), 7.29–7.31 (m, 1H, 4-H_{Bn}), 7.31–7.35 (m, 2H, 3-H_{Bn}, 5-H_{Bn}), 7.49 (tt, *J* = 7.6 / 1.6 Hz, 1H, 4-H_{Ph}), 7.51–7.54 (m, 2H, 3-H_{Ph}, 5-H_{Ph}), 7.57–7.62 (m, 2H, 3-H_{Bz}, 5-H_{Bz}), 7.68 (tt, *J* = 7.3 / 1.3 Hz, 1H, 4-H_{Bz}), 7.86–7.90 (m, 2H, 2-H_{Ph}, 6-H_{Ph}), 7.96–8.00 (m, 2H, 2-H_{Bz}, 6-H_{Bz}), 11.04 (s, 1H, NH_{amide}). ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 52.6 (CH_2), 86.0 (*C*-$4_{pyrazole}$), 113.7 (CN), 125.9 (2 C, *C*-2_{Ph}, *C*-6_{Ph}), 127.6 (2 C, *C*-2_{Bn}, *C*-6_{Bn}), 127.9 (*C*-4_{Bn}), 128.0 (2 C, *C*-2_{Bz}, *C*-6_{Bz}), 128.6 (2 C, *C*-3_{Bn}, *C*-5_{Bn}), 128.7 (2 C, *C*-3_{Bz}, *C*-5_{Bz}), 129.1 (2 C, *C*-3_{Ph}, *C*-5_{Ph}), 129.5 (*C*-4_{Ph}), 130.5 (*C*-1_{Ph}), 132.2 (*C*-1_{Bz}), 132.8 (*C*-4_{Bz}), 135.5 (*C*-1_{Bn}), 143.1 (*C*-$5_{pyrazole}$), 150.6 (*C*-$3_{pyrazole}$), 165.9 (*C* = O). HRMS: m/z = 379.1570, calcd. 379.1553 for $C_{24}H_{19}N_4O$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3252 (N–H), 3032 (= C–H), 2222 (C≡N), 1659 (C=O). Purity (HPLC): t_R = 21.5 min, purity 94.6 %.

4.3.23. N-[1-Benzyl-4-cyano-3-(4-methoxyphenyl)-pyrazol-5-yl]benzamide (26 A)



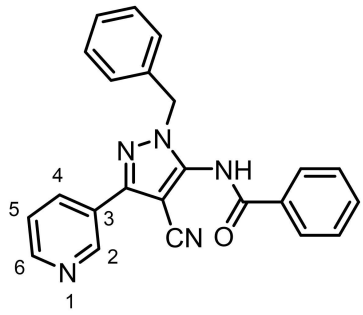
The compound was synthesized according to the **General method B** using 4-methoxyphenylboronic acid (26 mg, 172 µmol, 1.60 eq.) and the reaction mixture was stirred for 1 h. The product was purified by flash chromatography (25 g, Ø = 3.5 cm, cHex/EtOAc 95 : 5→60 : 40). Colorless solid, mp 220–223 °C, yield 39 mg (89 %), R_f = 0.45 (cHex/EtOAc 67 : 33). $C_{25}H_{20}N_4O_2$ (408.5 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 3.81 (s, 3H, OCH_3), 5.39 (s, 2H, CH_2), 7.08–7.10 (m, 2H, 3-H_{Ph}, 5-H_{Ph}), 7.22–7.24 (m, 2H, 2-H_{Bn}, 6-H_{Bn}), 7.30 (tt, *J* = 7.3 / 1.5 Hz, 1H, 4-H_{Bn}), 7.30–7.33 (m, 2H, 3-H_{Bn}, 5-H_{Bn}), 7.57–7.61 (m, 2H, 3-H_{Bz}, 5-H_{Bz}), 7.68 (tt, *J* = 7.4 / 1.4 Hz, 1H, 4-H_{Bz}), 7.80–7.82 (m, 2H, 2-H_{Ph}, 6-H_{Ph}), 7.95–7.99 (m, 2H, 2-H_{Bz}, 6-H_{Bz}), 11.00 (s, 1H, NH_{amide}). ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 52.5 (CH_2), 55.3 (OCH_3), 85.5 (*C*-$4_{pyrazole}$), 114.0 (CN), 114.5 (2 C, *C*-3_{Ph}, *C*-5_{Ph}), 123.1 (*C*-1_{Ph}), 127.4 (2 C, *C*-2_{Ph}, *C*-6_{Ph}), 127.6 (2 C, *C*-2_{Bn}, *C*-6_{Bn}), 127.9 (*C*-4_{Bn}), 128.1 (2 C, *C*-2_{Bz}, *C*-6_{Bz}), 128.6 (2 C, *C*-3_{Bn}, *C*-5_{Bn}), 128.7 (2 C, *C*-3_{Bz}, *C*-5_{Bz}), 132.2 (*C*-1_{Bz}), 132.8 (*C*-4_{Bz}), 135.6 (*C*-1_{Bn}), 142.9 (*C*-$5_{pyrazole}$), 150.5 (*C*-$3_{pyrazole}$), 160.2 (*C*-4_{Ph}), 166.0 (*C* = O). HRMS: m/z = 409.1664, calcd. 409.1659 for $C_{25}H_{21}N_4O_2$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3264 (N–H), 3005 (= C–H), 2931 (– C–H), 2222 (C≡N), 1659 (C=O), 1246 (C–O). Purity (HPLC): t_R = 21.5 min, purity 96.1 %.

4.3.24. N-[1-Benzyl-4-cyano-3-(o-tolyl)pyrazol-5-yl]benzamide (27 A)



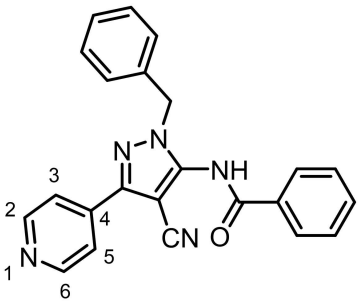
The compound was synthesized according to the **General method B** using *2*-methylphenylboronic acid (23 mg, 172 μmol, 1.60 eq.). The product was purified by flash chromatography (10 g, Ø = 3.5 cm, cHex/EtOAc 95:5→60:40) and subsequent recrystallization with EtOH. Colorless solid, mp 176–178 °C, yield 41 mg (97 %), R_f = 0.53 (cHex/EtOAc 67:33). $C_{25}H_{20}N_4O$ (392.5 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 2.35 (s, 3H, CH_3), 5.42 (s, 2H, CH_2), 7.21–7.23 (m, 2H, 2-H_{Bn}, 6-H_{Bn}), 7.28–7.30 (m, 1H, 4-H_{Bn}), 7.31–7.35 (m, 2H, 3-H_{Bn}, 5-H_{Bn}), 7.32–7.36 (m, 1H, 5-H_{tolyl}), 7.37–7.39 (m, 1H, 3-H_{tolyl}), 7.36–7.39 (m, 1H, 4-H_{tolyl}), 7.42–7.44 (m, 1H, 6-H_{tolyl}), 7.57–7.61 (m, 2H, 3-H_{Bz}, 5-H_{Bz}), 7.67 (tt, *J* = 7.4 / 1.3 Hz, 1H, 4-H_{Bz}), 7.95–7.99 (m, 2H, 2-H_{Bz}, 6-H_{Bz}), 11.02 (s, 1H, NH_{amide}). ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 19.9 (*C*H_3), 52.5 (*C*H_2), 88.4 (*C*-$4_{pyrazole}$), 113.4 (*C*N), 126.0 (*C*-5_{tolyl}), 127.6 (2 C, *C*-2_{Bn}, *C*-6_{Bn}), 127.8 (*C*-4_{Bn}), 128.0 (2 C, *C*-2_{Bz}, *C*-6_{Bz}), 128.6 (2 C, *C*-3_{Bn}, *C*-5_{Bn}), 128.7 (2 C, *C*-3_{Bz}, *C*-5_{Bz}), 129.3 (*C*-4_{tolyl}), 129.5 (*C*-6_{tolyl}), 130.0 (*C*-2_{tolyl}), 130.8 (*C*-3_{tolyl}), 132.2 (*C*-1_{Bz}), 132.8 (*C*-4_{Bz}), 135.6 (*C*-1_{Bn}), 136.3 (*C*-1_{tolyl}), 141.9 (*C*-$5_{pyrazole}$), 152.2 (*C*-$3_{pyrazole}$), 165.8 (C=O). HRMS: m/z = 393.1712, calcd. 393.1710 for $C_{25}H_{21}N_4O$ [M + H]$^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3237 (N–H), 3028 (=C–H), 2970 (−C–H), 2226 (C≡N), 1651 (C=O). Purity (HPLC): t_R = 21.8 min, purity 95.7 %.

4.3.25. N-[1-Benzyl-4-cyano-3-(pyridin-3-yl)-pyrazol-5-yl]benzamide (28 A)



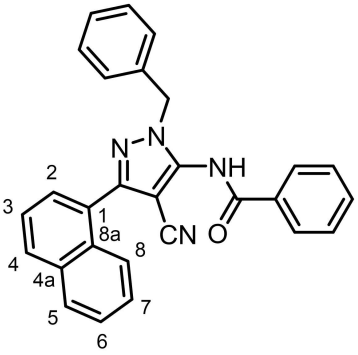
The compound was synthesized according to the **General method B** using pinacol ester 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (35 mg, 172 μmol, 1.60 eq.). The product was purified by flash chromatography (25 g, Ø = 3.5 cm, CH_2Cl_2/CH_3OH 99:1→95:5). Colorless solid, mp 212–214 °C, yield 36 mg (88 %), R_f = 0.40 (CH_2Cl_2/CH_3OH 95:5). $C_{23}H_{17}N_5O$ (379.4 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.46 (s, 2H, CH_2), 7.22–7.26 (m, 2H, 2-H_{Bn}, 6-H_{Bn}), 7.29 (tt, *J* = 7.3 / 1.7 Hz, 1H, 4-H_{Bn}), 7.31–7.36 (m, 2H, 3-H_{Bn}, 5-H_{Bn}), 7.57–7.61 (m, 2H, 3-H_{Bz}, 5-H_{Bz}), 7.58–7.60 (m, 1H, 5-$H_{pyridyl}$), 7.68 (tt, *J* = 7.2 / 1.4 Hz, 1H, 4-H_{Bz}), 7.96–8.00 (m, 2H, 2-H_{Bz}, 6-H_{Bz}), 8.22 (dt, *J* = 8.0 / 2.0 Hz, 1H, 4-$H_{pyridyl}$), 8.67–8.69 (m, 1H, 6-$H_{pyridyl}$), 9.04–9.06 (m, 1H, 2-$H_{pyridyl}$), 11.10 (s, 1H, NH_{amide}). ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 52.8 (*C*H_2), 86.4 (*C*-$4_{pyrazole}$), 113.4 (*C*N), 124.2 (*C*-$5_{pyridyl}$), 126.6 (*C*-$3_{pyridyl}$), 127.7 (2 C, *C*-2_{Bn}, *C*-6_{Bn}), 128.0 (*C*-4_{Bn}), 128.1 (2 C, *C*-2_{Bz}, *C*-6_{Bz}), 128.6 (2 C, *C*-3_{Bn}, *C*-5_{Bn}), 128.7 (2 C, *C*-3_{Bz}, *C*-5_{Bz}), 132.2 (*C*-1_{Bz}), 132.8 (*C*-4_{Bz}), 133.5 (*C*-$4_{pyridyl}$), 135.4 (*C*-1_{Bn}), 143.3 (*C*-$5_{pyrazole}$), 146.7 (*C*-$2_{pyridyl}$), 148.2 (*C*-$3_{pyrazole}$), 150.4 (*C*-$6_{pyridyl}$), 165.9 (*C*=O). HRMS: m/z = 380.1508, calcd. 380.1506 for $C_{23}H_{18}N_5O$ [M + H]$^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3244 (N–H), 3055 (=C–H), (2997 (−C–H), 2226 (C≡N), 1659 (C=O). Purity (HPLC): t_R = 17.1 min, purity 97.2 %.

4.3.26. N-[1-Benzyl-4-cyano-3-(pyridin-4-yl)-pyrazol-5-yl]benzamide (29 A)



The compound was synthesized according to the **General method B** using pyridin-4-ylboronic acid (21 mg, 172 μmol, 1.60 eq.). The product was purified by flash chromatography (25 g, Ø = 3.5 cm, CH_2Cl_2/CH_3OH 99:1→95:5). Colorless solid, mp 203–205 °C, yield 38 mg (93 %), R_f = 0.38 (CH_2Cl_2/CH_3OH 95:5). $C_{23}H_{17}N_5O$ (379.4 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.47 (s, 2H, CH_2), 7.22–7.26 (m, 2H, 2-H_{Bn}, 6-H_{Bn}), 7.29–7.31 (m, 1H, 4-H_{Bn}), 7.31–7.35 (m, 2H, 3-H_{Bn}, 5-H_{Bn}), 7.57–7.61 (m, 2H, 3-H_{Bz}, 5-H_{Bz}), 7.68 (tt, *J* = 7.4 / 2.1 Hz, 1H, 4-H_{Bz}), 7.80–7.86 (m, 2H, 3-$H_{pyridyl}$, 5-$H_{pyridyl}$), 7.96–8.00 (m, 2H, 2-H_{Bz}, 6-H_{Bz}), 8.72–8.78 (m, 2H, 2-$H_{pyridyl}$, 6-$H_{pyridyl}$), 11.11 (s, 1H, NH_{amide}). ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 52.8 (*C*H_2), 86.6 (*C*-$4_{pyrazole}$), 113.2 (*C*N), 119.9 (2 C, *C*-$3_{pyridyl}$, *C*-$5_{pyridyl}$), 127.7 (2 C, *C*-2_{Bn}, *C*-6_{Bn}), 128.0 (*C*-4_{Bn}), 128.1 (2 C, *C*-2_{Bz}, *C*-6_{Bz}), 128.6 (2 C, *C*-3_{Bn}, *C*-5_{Bn}), 128.7 (*C*-3_{Bz}, *C*-5_{Bz}), 132.2 (*C*-1_{Bz}), 132.8 (*C*-4_{Bz}), 135.2 (*C*-1_{Bn}), 137.5 (*C*-$4_{pyridyl}$), 143.8 (*C*-$5_{pyrazole}$), 148.0 (*C*-$3_{pyrazole}$), 150.6 (*C*-$2_{pyridyl}$, *C*-$6_{pyridyl}$), 165.9 (*C*=O). HRMS: m/z = 380.1506, calcd. 380.1506 for $C_{23}H_{18}N_5O$ [M + H]$^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3237 (N-H), 3032 (=C–H), 2924 (−C–H), 2226 (C≡N), 1655 (C=O). Purity (HPLC): t_R = 17.1 min, purity 92.6 %.

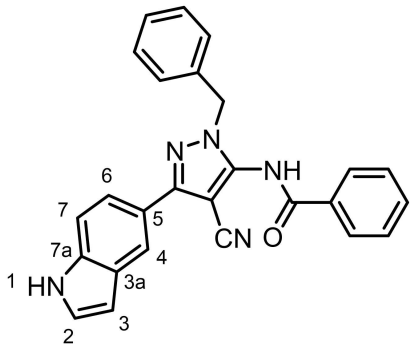
4.3.27. N-[1-Benzyl-4-cyano-3-(naphthalen-1-yl)pyrazol-5-yl]benzamide (30 A)



The compound was synthesized according to the **General method B** using naphthalen-1-ylboronic acid (30 mg, 172 μmol, 1.60 eq.). The product was purified by flash chromatography (25 g, Ø = 3.5 cm, cHex/EtOAc 95:5→60:40). Colorless solid, mp 168–170 °C, yield 37 mg (80 %), R_f = 0.52 (cHex/EtOAc 67:33).

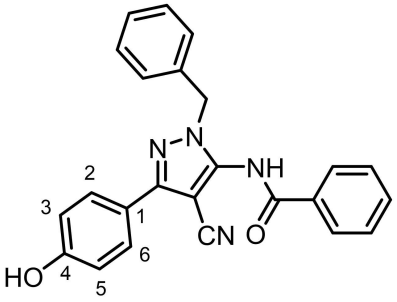
$C_{28}H_{20}N_4O$ (428.5 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.51 (s, 2H, CH_2), 7.27–7.31 (m, 2H, 2-H_{Bn}, 6-H_{Bn}), 7.30–7.32 (m, 1H, 4-H_{Bn}), 7.34–7.38 (m, 2H, 3-H_{Bn}, 5-H_{Bn}), 7.57–7.59 (m, 1H, 7-$H_{naphthyl}$), 7.57–7.63 (m, 2H, 3-H_{Bz}, 5-H_{Bz}), 7.64–7.68 (m, 1H, 6-$H_{naphthyl}$), 7.68 (m, 1H, 3-$H_{naphthyl}$), 7.70–7.72 (m, 1H, 4-H_{Bz}), 7.70–7.72 (m, 1H, 2-$H_{naphthyl}$), 7.98–8.03 (m, 2H, 2-H_{Bz}, 6-H_{Bz}), 8.04–8.06 (m, 1H, 5-$H_{naphthyl}$), 8.07–8.10 (m, 1H, 4-$H_{naphthyl}$), 8.14–8.16 (m, 1H, 8-$H_{naphthyl}$), 11.11 (s, 1H, NH_{amide}). ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 52.7 (CH_2), 89.1 (*C*-$4_{pyrazole}$), 113.2 (*C*N), 125.1 (*C*-$8_{naphthyl}$), 125.4 (*C*-$6_{naphthyl}$), 126.4 (*C*-$7_{naphthyl}$), 127.0 (*C*-$4a_{naphthyl}$), 127.7 (*C*-$2_{naphthyl}$), 127.7 (2 C, *C*-2_{Bn}, *C*-6_{Bn}), 127.8 (*C*-4_{Bz}), 127.9 (*C*-4_{Bn}), 128.1 (2 C, *C*-2_{Bz}, *C*-6_{Bz}), 128.5 (*C*-$5_{naphthyl}$), 128.6 (2 C, *C*-3_{Bn}, *C*-5_{Bn}), 128.7 (2 C, *C*-3_{Bz}, *C*-5_{Bz}), 129.8 (*C*-$4_{naphthyl}$), 130.4 (*C*-$8a_{naphthyl}$), 132.3 (*C*-1_{Bz}), 132.8 (*C*-$3_{naphthyl}$), 133.4 (*C*-$1_{naphthyl}$), 135.6 (*C*-1_{Bn}), 142.3 (*C*-$5_{pyrazole}$), 151.3 (*C*-$3_{pyrazole}$), 165.9 (*C*=O). HRMS: m/z = 429.1694, calcd. 429.1710 for $C_{28}H_{21}N_4O$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3217 (N–H), 3051 (=C–H), 2230 (C≡N), 1659 (C=O). Purity (HPLC): t_R = 22.4 min, purity 98.2 %.

4.3.28. N-[1-Benzyl-4-cyano-3-(indol-5-yl)pyrazol-5-yl]benzamide (31 A)



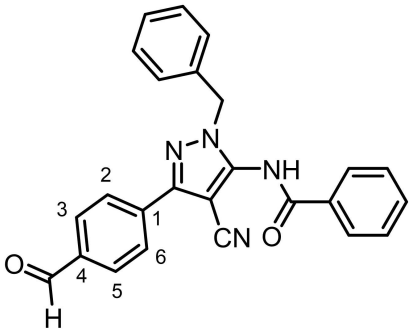
The compound was synthesized according to the **General method B** using indol-5-ylboronic acid (28 mg, 172 μmol, 1.60 eq.). The product was purified by flash chromatography (10 g, Ø = 2.5 cm, cHex/EtOAc 95 : 5→60 : 40). Colorless solid, mp 169–171 °C, yield 37 mg (82 %), R_f = 0.35 (cHex/EtOAc 67 : 33). $C_{26}H_{19}N_5O$ (417.5 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.39 (s, 2H, CH_2), 6.54–6.66 (m, 1H, 3-$H_{indolyl}$), 7.24–7.28 (m, 2H, 2-H_{Bn}, 6-H_{Bn}), 7.29 (tt, *J* = 7.4 / 1.5 Hz, 1H, 4-H_{Bn}), 7.32–7.36 (m, 2H, 3-H_{Bn}, 5-H_{Bn}), 7.43 (m, 1H, 2-$H_{indolyl}$), 7.50–7.53 (m, 1H, 7-$H_{indolyl}$), 7.58–7.61 (m, 2H, 3-H_{Bz}, 5-H_{Bz}), 7.63 (dd, 1H, *J* = 8.5 / 1.7 Hz, 6-$H_{indolyl}$), 7.68 (tt, *J* = 7.4 / 1.4 Hz, 1H, 4-H_{Bz}), 7.97–8.01 (m, 2H, 2-H_{Bz}, 6-H_{Bz}), 8.08–8.10 (m, 1H, 4-$H_{indolyl}$), 10.99 (s, 1H, NH_{amide}), 11.32 (s, 1H, N$H_{indolyl}$). ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 52.4 (CH_2), 85.5 (*C*-$4_{pyrazole}$), 101.8 (*C*-$3_{indolyl}$), 112.0 (*C*-$7_{indolyl}$), 114.3 (*C*N), 118.0 (*C*-$4_{indolyl}$), 119.3 (*C*-$6_{indolyl}$), 121.6 (*C*-$5_{indolyl}$), 126.6 (*C*-$2_{indolyl}$), 127.7 (2 C, *C*-2_{Bn}, *C*-6_{Bn}), 127.7 (*C*-$3a_{indolyl}$), 127.8 (*C*-4_{Bn}), 128.1 (2 C, *C*-2_{Bz}, *C*-6_{Bz}), 128.6 (2 C, *C*-3_{Bn}, *C*-5_{Bn}), 128.7 (2 C, *C*-3_{Bz}, *C*-5_{Bz}), 132.3 (*C*-1_{Bz}), 132.8 (*C*-4_{Bz}), 135.7 (*C*-1_{Bn}), 136.3 (*C*-$7a_{indolyl}$), 142.7 (*C*-$5_{pyrazole}$), 152.3 (*C*-$3_{pyrazole}$), 166.0 (*C*=O). HRMS: m/z = 418.1658, calcd. 418.1662 for $C_{26}H_{20}N_5O$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3375 (N–H), 3023 (=C–H), 2928 (–C–H), 2218 (C≡N), 1666 (C=O). Purity (HPLC): t_R = 20.8 min, purity 92.5 %.

4.3.29. N-[1-Benzyl-4-cyano-3-(4-hydroxyphenyl)pyrazol-5-yl]benzamide (32 A)



The compound was synthesized according to the **General method B** using 4-hydroxyphenylboronic acid (24 mg, 172 μmol, 1.60 eq.). The product was purified by flash chromatography (10 g, Ø = 2.5 cm, cHex/EtOAc 95 : 5→50 : 50). Colorless solid, mp 248–250 °C, yield 31 mg (73 %), R_f = 0.15 (cHex/EtOAc 67 : 33). $C_{24}H_{18}N_4O_2$ (394.4 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.37 (s, 2H, CH_2), 6.87–6.91 (m, 2H, 3-H_{PhOMe}, 5-H_{PhOMe}), 7.20–7.24 (m, 2H, 2-H_{Bn}, 6-H_{Bn}), 7.31–7.33 (m, 1H, 4-H_{Bn}), 7.31–7.35 (m, 2H, 3-H_{Bn}, 5-H_{Bn}), 7.56–7.59 (m, 2H, 3-H_{Bz}, 5-H_{Bz}), 7.67–7.69 (m, 1H, 4-H_{Bz}), 7.67–7.71 (m, 2H, 2-H_{PhOMe}, 6-H_{PhOMe}), 7.95–7.99 (m, 2H, 2-H_{Bz}, 6-H_{Bz}), 9.85 (s, 1H, O*H*), 10.97 (s, 1H, NH_{amide}). ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 52.4 (CH_2), 85.2 (*C*-$4_{pyrazole}$), 114.1 (*C*N), 115.8 (2 C, *C*-3_{PhOMe}, *C*-5_{PhOMe}), 121.5 (*C*-1_{PhOMe}), 127.4 (2 C, *C*-2_{PhOMe}, *C*-6_{PhOMe}), 127.6 (2 C, *C*-2_{Bn}, *C*-6_{Bn}), 127.8 (*C*-4_{Bn}), 128.0 (2 C, *C*-2_{Bz}, *C*-6_{Bz}), 128.6 (2 C, *C*-3_{Bn}, *C*-5_{Bn}), 128.7 (2 C, *C*-3_{Bz}, *C*-5_{Bz}), 132.2 (*C*-1_{Bz}), 132.8 (*C*-4_{Bz}), 135.6 (*C*-1_{Bn}), 142.8 (*C*-$5_{pyrazole}$), 150.9 (*C*-$3_{pyrazole}$), 158.6 (*C*-4_{PhOMe}), 165.9 (*C*=O). HRMS: m/z = 395.1536, calcd. 395.1543 for $C_{24}H_{19}N_4O_2$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3360 (O–H), 3244 (N–H), 3035 (=C–H), 2984 (–C–H), 2234 (C≡N), 1658 (C=O). Purity (HPLC): t_R = 19.4 min, purity 93.7 %.

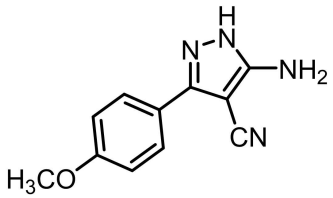
4.3.30. N-[1-Benzyl-4-cyano-3-(4-formylphenyl)pyrazol-5-yl]benzamide (33 A)



The compound was synthesized according to the **General method B** using 4-formylphenylboronic acid (26 mg, 172 μmol, 1.60 eq.). The product was purified by flash chromatography (10 g, Ø = 2.5 cm, cHex/EtOAc 95 : 5→60 : 40). Colorless solid, mp 260–263 °C, yield 33 mg (76 %), R_f = 0.45 (cHex/EtOAc 67 : 33). $C_{25}H_{18}N_4O_2$ (406.5 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 5.46 (s, 2H, CH_2), 7.23–7.27 (m, 2H, 2-H_{Bn}, 6-H_{Bn}), 7.32 (m, 1H, 4-H_{Bn}), 7.32–7.38 (m, 2H, 3-H_{Bn}, 5-H_{Bn}), 7.59–7.61 (m, 2H, 3-

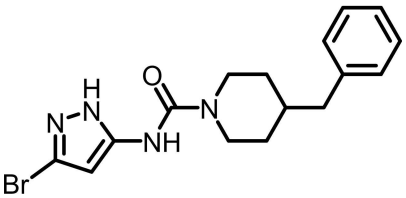
H_{Bz}, 5-H_{Bz}), 7.68 (tt, J = 7.0 / 1.5 Hz, 1H, 4-H_{Bz}), 7.97–7.99 (m, 2H, 2-H_{Bz}, 6-H_{Bz}), 8.06–8.08 (m, 2H, 2-H_{FoPh}, 6-H_{FoPh}), 8.07–8.10 (m, 2H, 3-H_{FoPh}, 5-H_{FoPh}, 10.07 (s, 1H, C*H*=O), 11.10 (s, 1H, NH_{amide}). ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 52.8 (*C*H_2), 86.7 (*C*-4$_{pyrazole}$), 113.5 (*C*N), 126.5 (2 C, *C*-3$_{FoPh}$, *C*-5$_{FoPh}$), 127.7 (2 C, *C*-2$_{Bn}$, *C*-6$_{Bn}$), 128.0 (*C*-4$_{Bn}$), 128.1 (2 C, *C*-2$_{Bz}$, *C*-6$_{Bz}$), 128.6 (2 C, *C*-3$_{Bn}$, *C*-5$_{Bn}$), 128.7 (2 C, *C*-3$_{Bz}$, *C*-5$_{Bz}$), 130.3 (2 C, *C*-2$_{FoPh}$, *C*-6$_{FoPh}$), 132.2 (*C*-1$_{Bz}$), 132.9 (*C*-4$_{Bz}$), 135.4 (*C*-1$_{Bn}$), 135.8 (*C*-3$_{pyrazole}$), 136.4 (*C*-4$_{FoPh}$), 143.6 (*C*-5$_{pyrazole}$), 149.3 (*C*-1$_{FoPh}$), 166.0 (*C*=O$_{amide}$), 192.7 (*C*H=O). HRMS: m/z = 407.1501, calcd. 40.1503 for $C_{25}H_{19}N_4O_2$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3252 (N–H), 3002 (=C–H), 2994 (–C–H), 2224 (C≡N), 1684 (C=O). Purity (HPLC): t_R = 21.1 min, purity 93.6 %.

4.3.31. 5-Amino-3-(4-methoxyphenyl)pyrazole-4-carbonitrile (34)



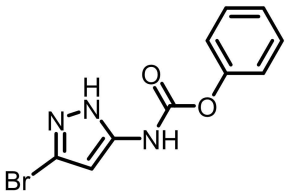
Aminopyrazole **17 A**/**B** (15 mg, 49 μmol, 1.00 eq.), ammonium formate (16 mg, 246 μmol, 5.00 eq.) and Pd/C (10 % (w/w), 4.2 mg) were filled into a SCHLENK tube and suspended in propan-2-ol (2 mL). The suspension was stirred at 90 °C for 16 h. After cooling down, the reaction mixture was filtered through Celite® and eluted with EtOAc (10 mL) and CH_2Cl_2 (10 mL). The organic solvent was removed *in vacuo* and the crude product was purified by flash chromatography (10 g, Ø = 2.5 cm, CH_2Cl_2/CH_3OH 99:1→95 5). Colorless solid, mp 183–185 °C, yield 1.5 mg (14 %), R_f = 0.30 (CH_2Cl_2/CH_3OH 95:5). $C_{11}H_{10}N_4O$ (214.2 g/mol). ^{1}H NMR (600 MHz, DMSO-D_6): δ (ppm) = 3.79 (s, 3H, O*CH*$_3$), 6.18–6.30 (br, 2H, N*H*$_2$), 7.01–7.05 (m, 2H, 3-H_{Ph}, 5-H_{Ph}), 7.70–7.74 (m, 2H, 2-H_{Ph}, 6-H_{Ph}), 12.26–12.38 (br, 1H, N*H*). ^{13}C NMR (151 MHz, DMSO-D_6): δ (ppm) = 55.2 (O*C*H$_3$), 114.2 (2 C, *C*-3$_{Ph}$, *C*-5$_{Ph}$), 116.4 (*C*N), 121.9 (*C*-1$_{Ph}$), 127.1 (2 C, *C*-2$_{Ph}$, *C*-6$_{Ph}$), 159.6 (*C*-4$_{Ph}$). Signals for *C*-3$_{pyrazole}$, *C*-4$_{pyrazole}$ and *C*-5$_{pyrazole}$ are missing. HRMS: m/z = 215.0919, calcd. 215.0927 for $C_{11}H_{11}N_4O$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3343 (N–H), 3216 (N–H), 3085 (=C–H), 2224 (C≡N). 1225 (C–O). Purity (HPLC): t_R = 14.4 min, purity 95.6 %.

4.3.32. Phenyl (3-bromopyrazol-5-yl)carbamate (36)



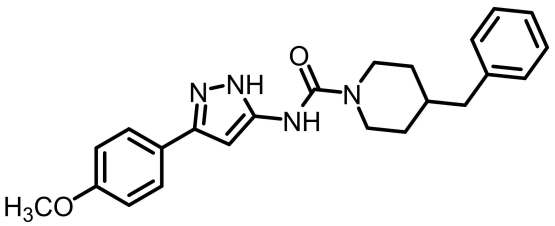
Under N_2 at 0 °C, phenyl chloroformate (1.64 mL, 13.0 mmol, 1.05 eq.) was slowly added to a solution of 3-bromopyrazol-5-amine (**35**, 2.00 g, 12.4 mmol, 1.00 eq.) in dry pyridine (15 mL) and dry THF (15 mL). The solution was stirred for 16 h while warming up to rt. H_2O (10 mL) was added and the aqueous phase was extracted with EtOAc (3×50 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by flash chromatography (50 g, Ø = 4.5 cm, CH_2Cl_2/CH_3OH 99:1→95:5). The crude product was directly used for the next step. Yellow solid, R_f = 0.48 (CH_2Cl_2/CH_3OH 95:5), yield 2.45 g (70 %). $C_{10}H_8BrN_3O_2$ (282.1 g/mol). HRMS: m/z = 281.9870, calcd. 281.9873 for $C_{10}H_9{}^{79}BrN_3O_2{}^+$ $[M+H]^+$.

4.3.33. 4-Benzyl-N-(3-bromopyrazol-5-yl)piperidine-1-carboxamide (37)



NEt_3 (687 μL, 4.96 mmol, 2.00 eq.) was added to a mixture of **36** (700 mg, 2.48 mmol, 1.00 eq.), 4-benzylpiperidine (610 μL, 3.47 mmol, 1.40 eq.) and dry toluene (50 mL). The suspension was stirred at 120 °C for 16 h. The solvent was removed *in vacuo* and the residue was purified by flash chromatography (50 g, Ø = 4.5 cm, CH_2Cl_2/CH_3OH 99:1→94:6). Colorless solid, mp 80–85 °C (decomposition), R_f = 0.44 (CH_2Cl_2/CH_3OH 95:5), yield 755 mg (84 %).$C_{16}H_{19}BrN_4O$ (363.3 g/mol). ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 0.97–1.15 (m, 2H, 3-C*H*$_{2(ax,\ piperidine)}$, 5-C*H*$_{2(ax,\ piperidine)}$), 1.52–1.56 (m, 2H, 3-C*H*$_{2(eq,\ piperidine)}$, 5-C*H*$_{2(eq,\ piperidine)}$), 1.64–1.72 (m, 1H, 4-C*H*$_{(piperidine)}$), 2.49 (d, 2H, *CH*$_2$Ph), 2.64–2.76 (m, 2H, 2-C*H*$_{2(ax,\ piperidine)}$, 6-C*H*$_{2(ax,\ piperidine)}$), 3.93–4.07 (m, 2H, 2-C*H*$_{2(eq,\ piperidine)}$, 6-C*H*$_{2(eq,\ piperidine)}$), 5.85 (s, 1H, 4-$H_{(pyrazolyl)}$), 7.13–7.20 (m, 3H, 2-$H_{(benzyl)}$, 4-$H_{(benzyl)}$, 6-$H_{(benzyl)}$), 7.22–7.29 (m, 2H, 3-$H_{(benzyl)}$, 5-$H_{(benzyl)}$), 9.23 (s, 1H, N*H*$_{(urea)}$), 12.33 (s, 1H, N*H*$_{(pyrazolyl)}$). ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 34.6 (2 C, *C*-3$_{(piperidine)}$, *C*-5$_{(piperidine)}$), 40.4 (*C*-4$_{(piperidine)}$, 45.2 (*C*H$_2$Ph), 46.9 (2 C, *C*-2$_{(piperidine)}$, *C*-6$_{(piperidine)}$), 98.0 (*C*-4$_{(pyrazolyl)}$), 127.6 (*C*-5$_{(pyrazolyl)}$), 128.9 (*C*-4$_{(benzyl)}$), 131.3 (2 C, *C*-3$_{(benzyl)}$, *C*-5$_{(benzyl)}$), 132.1 (2 C, *C*-2$_{(benzyl)}$, *C*-6$_{(benzyl)}$), 143.1 (*C*-1$_{(benzyl)}$), 144.6 (*C*-3$_{(pyrazolyl)}$), 156.6 (*C*=O). HRMS: m/z = 363.0799, calcd. 363.0815 for $C_{16}H_{19}BrN_4O^+$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3260 (N–H), 2913 (C-H$_{aryl}$), 1643 (C=O). Purity (HPLC): t_R = 19.7 min, purity 95.4 %

4.3.34. 4-Benzyl-N-[3-(4-methoxyphenyl)pyrazol-5-yl]piperidine-1-carboxamide (38)



Bromopyrazole **37** (100 mg, 275 μmol, 1.00 eq.), 4-methoxyphenylboronic acid (83.7 mg, 551 μmol, 2.00 eq.), Xphos Pd G2 (21.7 mg, 27.5 μmol, 0.10 eq.), XPhos (13.1 mg, 27 .5 μmol, 0.10 eq.), K_2CO_3 (76.1 mg, 551 μmol, 2.00 eq.) and a mixture of EtOH (3.2 mL) and H_2O (0.8 mL) were transferred into a microwave tube. The mixture was flushed with N_2 and sealed. The vial was inserted into the microwave reactor and the solution was stirred at 60 °C for 10 min under microwave irradiation (variable power, max 300 W, max 300 psi). After cooling down, the reaction mixture was filtered and the filter was washed with EtOAc. H_2O was added and the aqueous layer was extracted with EtOAc (3×30 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by automated flash chromatography (Biotage®, 10 g, Ø = 2.5 cm, cHex/EtOAc: 85:15→0:100). Colorless solid, mp 181–183 °C, R_f = 0.38 (CH_2Cl_2/CH_3OH 95:5), yield 32.1 mg (30 %).$C_{23}H_{26}N_4O_2$ (390.5 g/mol). ^{1}H NMR (600 MHz, DMSO-d_6): δ (ppm) = 1.00–1.12 (m, 2H, 3-C$H_{2(ax,\ piperidine)}$, 5-C$H_{2(ax,\ piperidine)}$), 1.46–1.57 (m, 2H, 3-C$H_{2(eq,\ piperidine)}$, 5-C$H_{2(eq,\ piperidine)}$), 1.64–1.72 (m, 1H, 4-C$H_{(piperidine)}$), 2.50 (d, *J* = 7.2 Hz, 2H, CH_2Ph), 2.63–2.73 (m, 2H, 2-C$H_{2(ax,\ piperidine)}$, 6-C$H_{2(ax,\ piperidine)}$), 3.75 (s, 3H, OCH_3), 4.03–4.12 (m, 2H, 2-C$H_{2(eq,\ piperidine)}$, 6-C$H_{2(eq,\ piperidine)}$), 6.17–6.71 (br, 1H, 4-$H_{(pyrazolyl)}$), 6.96 (d, *J* = 8.3 Hz, 2H, 3-$H_{(4\text{-methoxyphenyl})}$, 5-$H_{(4\text{-methoxyphenyl})}$), 7.08–7.21 (m, 3H, 2-$H_{(benzyl)}$, 4-$H_{(benzyl)}$, 6-$H_{(benzyl)}$), 7.22–7.32 (m, 2H, 3-$H_{(benzyl)}$, 5-$H_{(benzyl)}$), 7.60 (d, *J* = 8.6 Hz, 2H, 2-$H_{(4\text{-methoxyphenyl})}$, 6-$H_{(4\text{-methoxyphenyl})}$), 8.70–9.00 (br, 1H, N$H_{(urea)}$), 11.99–12.61 (br, 1H, N$H_{(pyrazolyl)}$). ^{13}C NMR (151 MHz, DMSO-d_6): δ (ppm) = 34.7 (2 C, *C*-3$_{(piperidine)}$, *C*-5$_{(piperidine)}$), 40.6 (*C*-4$_{(piperidine)}$), 45.3 (*C*H$_2$Ph), 47.0 (2 C, *C*-2$_{(piperidine)}$, *C*-6$_{(piperidine)}$), 58.3 (O*C*H$_3$), 96.5 (*C*-5$_{(pyrazolyl)}$), 117.4 (2 C, *C*-3$_{(4\text{-methoxyphenyl})}$, *C*-5$_{(4\text{-methoxyphenyl})}$), 125.4 (*C*-1$_{(4\text{-methoxyphenyl})}$), 128.9 (*C*-4$_{(benzyl)}$), 129.2 (2 C, *C*-2$_{(4\text{-methoxyphenyl})}$, *C*-6$_{(4\text{-methoxyphenyl})}$), 131.3 (2 C, *C*-3$_{(benzyl)}$, *C*-5$_{(benzyl)}$), 132.1 (2 C, *C*-2$_{(benzyl)}$, *C*-6$_{(benzyl)}$), 143.2 (*C*-1$_{(benzyl)}$), 144.4 (*C*-3$_{(pyrazolyl)}$), 157.5 (*C* = O), 162.0 (*C*-4$_{(4\text{-methoxyphenyl})}$). A signal for *C*-4$_{pyrazolyl}$ is not observed in the spectrum. HRMS: m/z = 391.2123, calcd. 391.2129 for $C_{23}H_{27}N_4O_2^+$ $[M+H]^+$. IR (neat): $\tilde{\nu}$ [cm^{-1}] = 3202 (N–H), 2913 (C-H_{alkyl}), 1613 (C=O). Purity (HPLC): t_R = 20.0 min, purity 94.0 %

4.4. Biological activity

4.4.1. Antileishmanial activity

4.4.1.1. Parasite and cell cultures

Two *Leishmania species* (*L. infantum* MHOM/MA(BE)/67 and *L. donovani* MHOM/ET/67/L82) are used. The strains are maintained in the Golden Hamster (*Mesocricetus auratus*). Amastigotes are collected from the spleen of an infected donor hamster using three centrifugation purification steps (300 rpm, keeping the supernatants, 2,200 rpm, keeping the supernatants and 3,500 rpm, keeping the pellet) and spleen parasite burdens are assessed using the Stauber technique. Primary peritoneal mouse macrophages are used as host cells and are collected 2 days after peritoneal stimulation with a 2 % potato starch suspension. All cultures and assays are conducted at 37 °C under an atmosphere of 5 % CO_2.

4.4.1.2. Compound solutions/dilutions

Compound stock solutions are prepared in 100 % DMSO at 20 mM. The compounds are serially pre-diluted (2-fold or 4-fold) in DMSO followed by a further (intermediate) dilution in demineralized water to assure a final in-test DMSO concentration of < 1 %.

4.4.1.3. Drug sensitivity assays

Assays are performed in 96-well microtiter plates, each well containing 10 μL of the compound dilutions together with 190 μL of macrophage/parasite inoculum (3×10^4 cells + 4.5×10^5 parasites/well). The inoculum is prepared in RPMI-1640 medium, supplemented with 2 mM L-glutamine, 16.5 mM $NaHCO_3$, and 5 % inactivated fetal calf serum. The macrophages are infected after 48 hours. The compounds are added after 2 hours of infection. Parasite multiplication is compared to untreated-infected controls (100 % growth) and uninfected controls (0 % growth). After 5 days incubation, parasite burdens (mean number of amastigotes/macrophage) are microscopically assessed after staining the cells with a 10 % Giemsa solution. The results are expressed as % reduction in parasite burden compared to untreated control wells and an IC_{50} and an IC_{90} (50 % and 90 % inhibitory concentrations) are calculated.

4.4.1.4. Primary screen

L. infantum MHOM/MA(BE)/67 strain is used. The compounds are tested at 5 or 10 concentrations depending upon the number of compounds (4-fold compound dilutions) (64 – 16 – 4 – 1 – 0.25 – 0.0625 – 0.015625 – 0.0039 – 0.000975 and 0.00024 μM). Amphotericin B and miltefosine are included as the reference drugs.

4.4.1.5. Secondary screen

L. infantum MHOM/MA(BE)/67 and *L. donovani* MHOM/ET/67/L82 strains are used and the IC_{50}-values are determined using an extended dose range (2-fold compound dilutions). Amphotericin B or miltefosine are included as reference drugs.

4.4.2. Antitrypanosomal activity

4.4.2.1. Parasite and cell cultures

Trypanosoma cruzi, Tulahuen CL2, β-galactosidase strain (nifurtimox-sensitive) is used.[14] The strain is maintained on MRC-5SV2 (human lung fibroblast) cells in MEM medium, supplemented with 2 mM L- glutamine, 16.5 mM $NaHCO_3$, and 5 % inactivated fetal calf serum. All cultures and assays are conducted at 37 °C under an atmosphere of 5 % CO_2.

4.4.2.2. Compound solutions/dilutions

Compound stock solutions are prepared in 100% DMSO at 20 mM. The compounds are serially pre-diluted (2-fold or 4-fold) in DMSO followed by a further (intermediate) dilution in demineralized water to assure a final in-test DMSO concentration of $<1\%$.

4.4.2.3. Drug sensitivity assays

Assays are performed in sterile 96-well microtiter plates, each well containing 10 μL of the aqueous compound dilutions together with 190 μL of MRC-5 cell/parasite inoculum (4×10^3 cells/well + 4×10^4 parasites/well). Parasite growth is compared to untreated-infected controls (100% growth) and non- infected controls (0% growth) after 7 days incubation at 37 °C and 5% CO_2. Parasite burdens are assessed after adding the substrate CPRG (chlorophenolred ß-D-galactopyranoside): 50 μL/well of a stock solution containing 15.2 mg CPRG + 250 μL Nonidet in 100 mL PBS. The change in color is measured spectrophotometrically at 540 nm after 4 hours incubation at 37 °C. The results are expressed as % reduction in parasite burdens compared to control wells and IC_{50} and IC_{90} values are calculated.

4.4.2.4. Primary screen

T. cruzi β-galactosidase strain is used. Compounds are tested at 5 or 10 concentrations depending upon the number of compounds (4-fold compound dilutions) (64 – 16 – 4 – 1–0.25 – 0.0625 – 0.015625 – 0.0039 – 0.000975 and 0.00024 μM or μg/mL). Nifurtimox or benznidazole are included as the reference drugs.

4.4.2.5. Secondary screen

T. cruzi β-galactosidase strain is used and IC_{50}-values are determined using an extended dose range (2- fold compound dilutions). Nifurtimox or benznidazole are included as reference drugs.

4.4.3. Toxicity against MRC-5 und PMM cells

4.4.3.1. Parasite and cell cultures

MRC-5SV2 cells are cultured in MEM + Earl's salts-medium, supplemented with L-glutamine, $NaHCO_3$ and 5% inactivated fetal calf serum. All cultures and assays are conducted at 37 °C under an atmosphere of 5% CO_2.

4.4.3.2. Compound solutions/dilutions

Compound stock solutions are prepared in 100% DMSO at 20 mM. The compounds are serially pre-diluted (2-fold or 4-fold) in DMSO followed by a further (intermediate) dilution in demineralized water to assure a final in-test DMSO concentration of $<1\%$.

4.4.3.3. Drug sensitivity assays

Assays are performed in sterile 96-well microtiter plates, each well containing 10 μL of the aqueous compound dilutions together with 190 μL of MRC-5 SV2 inoculum (1.5×10^5 cells/mL). Cell growth is compared to untreated-control wells (100% cell growth) and medium-control wells (0% cell growth). After 3 days incubation, cell viability is assessed fluorimetrically after addition of 50 μL resazurin per well[3]. After 4 hours at 37 °C, fluorescence is measured (λex 550 nm, λem 590 nm). The results are expressed as % reduction in cell growth/viability compared to control wells and IC_{50} and an IC_{90} values are determined.

4.4.3.4. Primary screen

The MRC-5SV2 cell-line is used. The compounds are tested at 5 or 10 concentrations depending upon the number of compounds (4-fold compound dilutions) (64 – 16 – 4–1 – 0.25 – 0.0625 – 0.015625 – 0.0039 – 0.000975 and 0.00024 μM or μg/mL). Cytotoxic reference compounds used are tamoxifen or niclosamide.

4.4.3.5. Secondary screen

The IC_{50} values are determined using an extended dose range (2-fold compound dilutions) still with a highest concentration of 64 μM.

Supporting Information

The Supporting Information contains ^{1}H NMR spectra and HPLC chromatograms of all prepared compounds.

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Conflict of Interests

The authors have no conflict of interests to declare.

Data Availability Statement

Data available on request from the authors.

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[1] World Health Organization, *World Health Organ. Tech. Rep. Ser.* **2010** 1–18.
[2] a) C. Maia, et al., *PLoS neglected tropical diseases* **2023**, *17*, e0011497; b) P. Desjeux, *Clin. Dermatol.* **1996**, *14*, 417–423.
[3] I. R. Ávila, G. R. de Araújo, D. S. Barbosa, J. M. T. Bezerra, *Acta tropica* **2023**, *237*, 106707.
[4] A. Ranjan, D. Sur, V. P. Singh, N. A. Siddique, B. Manna, C. S. Lal, P. K. Sinha, K. Kishore, S. K. Bhattacharya, *Am. J. Trop. Med. Hyg.* **2005**, *73*, 74–78.
[5] Lai A Fat, Esther J. S. K., M. A. Vrede, R. M. Soetosenojo, R. F. M. Lai A Fat, *Int. J. Dermatol.* **2002**, *41*, 796–800.
[6] a) C. Galiana-Roselló, P. Bilbao-Ramos, M. A. Dea-Ayuela, M. Rolón, C. Vega, F. Bolás-Fernández, E. García-España, J. Alfonso, C. Coronel, M. E. González-Rosende, *J. Med. Chem.* **2013**, *56*, 8984–8998; b) J. A. Hutton, et al., *J. Med. Chem.* **2014**, *57*, 8664–8670; c) K. S. van Horn, et al., *J. Med. Chem.* **2014**, *57*, 5141–5156.
[7] C. E. Mowbray, et al., *J. Med. Chem.* **2015**, *58*, 9615–9624.
[8] S. S. Palimkar, J. D. Pawar, B. Sankar, P. Bose, S. R. Kadam, R. M. Hindupur, V. M. Prabhu, H. N. Pati, V. G. Suphala, A. S. Mane, GB 2503523 A, **2012**.
[9] L. Maes, D. Vanden Berghe, N. Germonprez, L. Quirijnen, P. Cos, N. de Kimpe, L. van Puyvelde, *Antimicrobial agents and chemotherapy* **2004**, *48*, 130–136.
[10] D. Pedersen, C. Rosenbohm, *Synthesis* **2001**, 2431–2434.
[11] G. F. Pauli, *Phytochem. Anal.* **2001**, *12*, 28–42.
[12] A. C. Shaikh, C. Chen, *J. Label. Compd. Radiopharm.* **2008**, *51*, 72–76.
[13] X. Chen, Y. Gao, N. X. Kong (BEIJING INNOCARE PHARMA TECH CO LTD [CN]), US2019210997 (A1), **2019**.
[14] F. S. Buckner, C. L. Verlinde, A. C. La Flamme, W. C. van Voorhis, *Antimicrobial agents and chemotherapy* **1996**, *40*, 2592–2597.

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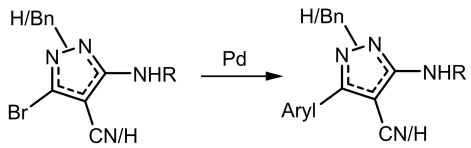
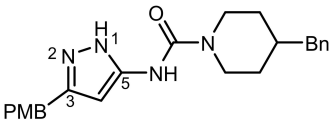


late stage diversification



anti-*L. infantum*: IC_{50} = 19 μM
anti-*T. cruzi*: IC_{50} = 7.9 μM

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Within the framework of "drugs for neglected disease initiative (DNDi)", Pd-catalyzed Sonogashira and Suzuki-Miyaura cross-coupling reactions were used to introduce diverse substituents in 3-position of pyrazoles. The 5-position of pyrazoles was supplied with amino, acylamino and ureido moieties. The pyrazole with *p*-methoxybenzyl moiety in 3-position and 4-benzylpiperidino urea in 5-position showed modest antileishmanial and antitrypanosomal activity, but also considerable unspecific cytotoxicity.