Synthesis of pyridyl substituted pyrazolo[4,3-*c*]pyridines as potential inhibitors of protein kinases

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Dedicated with our best wishes to Prof. Dr. Rosa Maria Claramunt on the occasion of her 65th birthday

Abstract

A synthetic route towards 3-(2-pyridyl)-6-(hetero)aryl-1*H*-pyrazolo[4,3-*c*]pyridines is described. The key step consists of a microwave-assisted multi-component reaction, including a Sonogashira type cross-coupling of appropriate 5-chloropyrazole-4-carbaldehydes with alkynyl-(hetero)arenes followed by pyridine ring formation of the coupling products in the presence of *tert*-butylamine, directly affording the title compounds. A congener without substituent at N-1 was accessed *via* cleavage of a *tert*-butyl protecting group. Detailed NMR spectroscopic studies (^{1}H, ^{13}C and ^{15}N) were undertaken with the obtained compounds. Selected representatives were evaluated for their potential as inhibitors of protein kinases.

Keywords: Pyrazolones; Sonogashira reaction; multi-component reactions; fused pyrazoles, pyridines; cyclin-dependent kinase (CDK) inhibitors; ^{15}N NMR spectroscopy

Introduction

Cyclin-dependent protein kinases (CDKs) are import drug targets with regard to the treatment of proliferative diseases such as cancer and inflammation.[1-3] Thus, in the last years considerable effort has been devoted to the development of small molecule inhibitors of CDKs as potential

drug candidates for oncology.[1,2,4] The purine system became one of the first systematically investigated scaffolds for CDK inhibitors, leading to the discovery of roscovitine (Figure 1), which represents one of the first candidates to enter clinical trials.[5-7] Naturally, the latter compound has inspired further exploration of new CDK inhibitors by variation of the substituents and by repositioning of nitrogen atoms on the purine scaffold. Thus, various congeners containing 2-5 nitrogen atoms in the bicyclic core have been considered. In the course of these investigations, representatives of four classes of bioisosteres revealed improved biological properties, namely pyrazolo[4,3-*d*]pyrimidines (**A**), pyrazolo[1,5-*a*]pyrimidines (**B**), pyrazolo[1,5-*a*]pyridines (**C**) and pyrazolo[1,5-*a*][1,3,5]triazines (**D**) (Figure 1).[8,9] In view of this we became interested in somewhat related compounds of type **E**, characterized by a pyrazolo[4,3-*c*]pyridine core and carrying at least one pyridyl substituent in order to provide a slightly basic side-chain (Figure 1).

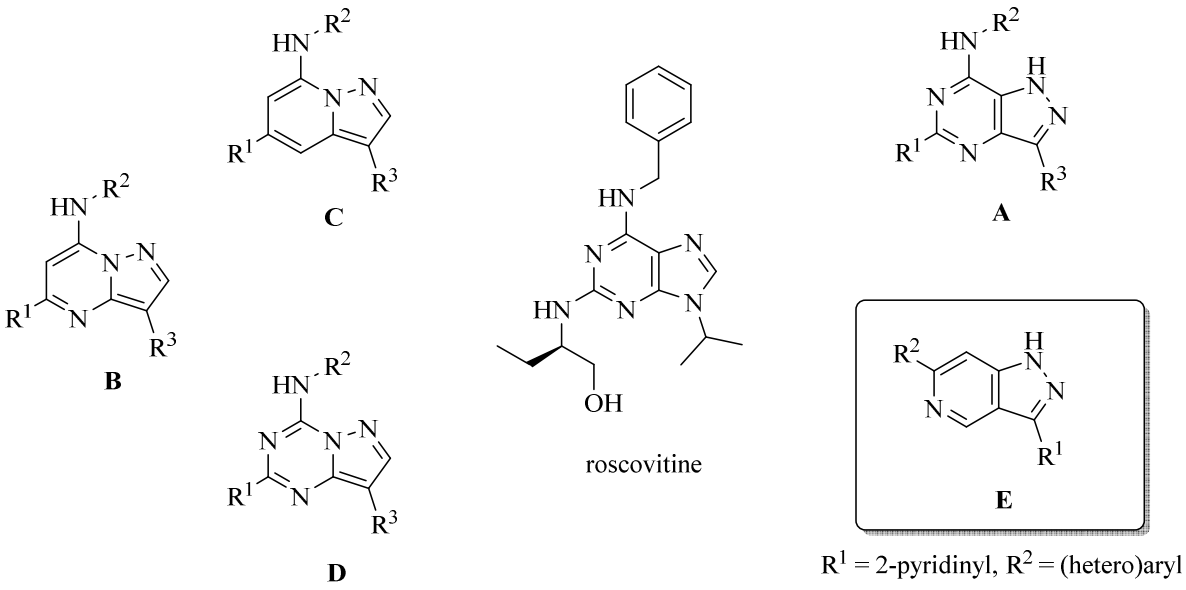


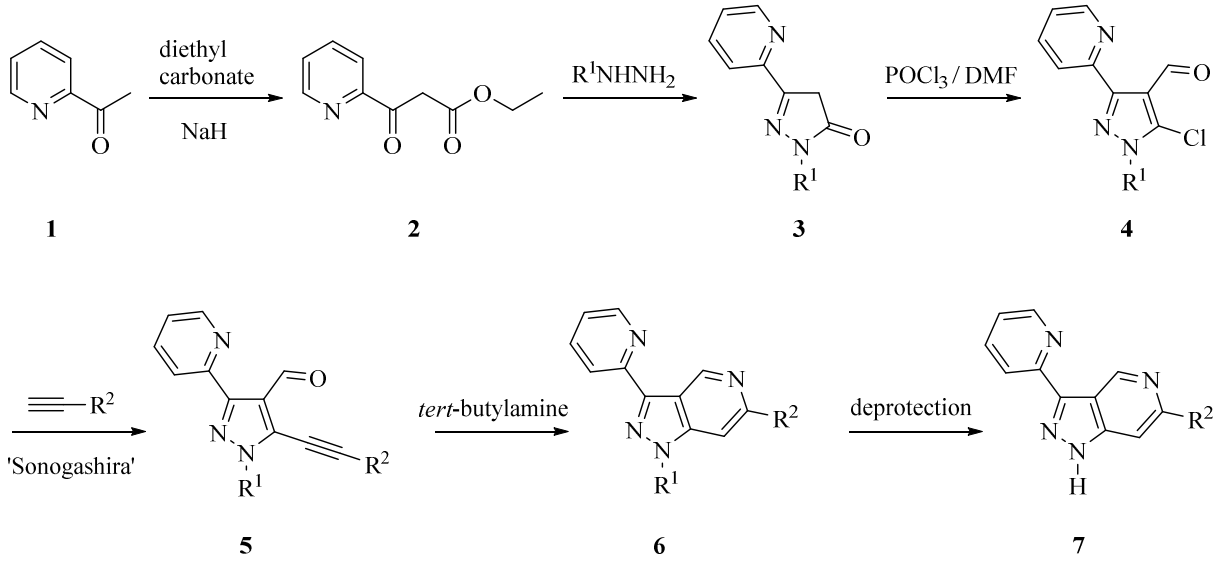
Figure 1. Roscovitine and related systems as (potential) CDK inhibitors.

Results and Discussion

Synthetic study

Recently, we have presented a novel and general access to the synthesis of pyrazolo[4,3-*c*]-pyridines *via* Sonogashira cross-coupling of 5-chloropyrazole-4-carbaldehydes and subsequent pyridine ring closure of the resulting 5-alkynylpyrazole-4-carbaldehydes in the presence of *tert*-butylamine as the nitrogen source.[10] According to this approach, the envisaged synthetic pathway to the target compounds of type **6** and **7**, respectively, is presented in Scheme 1. β-Keto ester **2**, obtained *via* condensation of 2-acetylpyridine (**1**) with diethyl carbonate, should be transformed

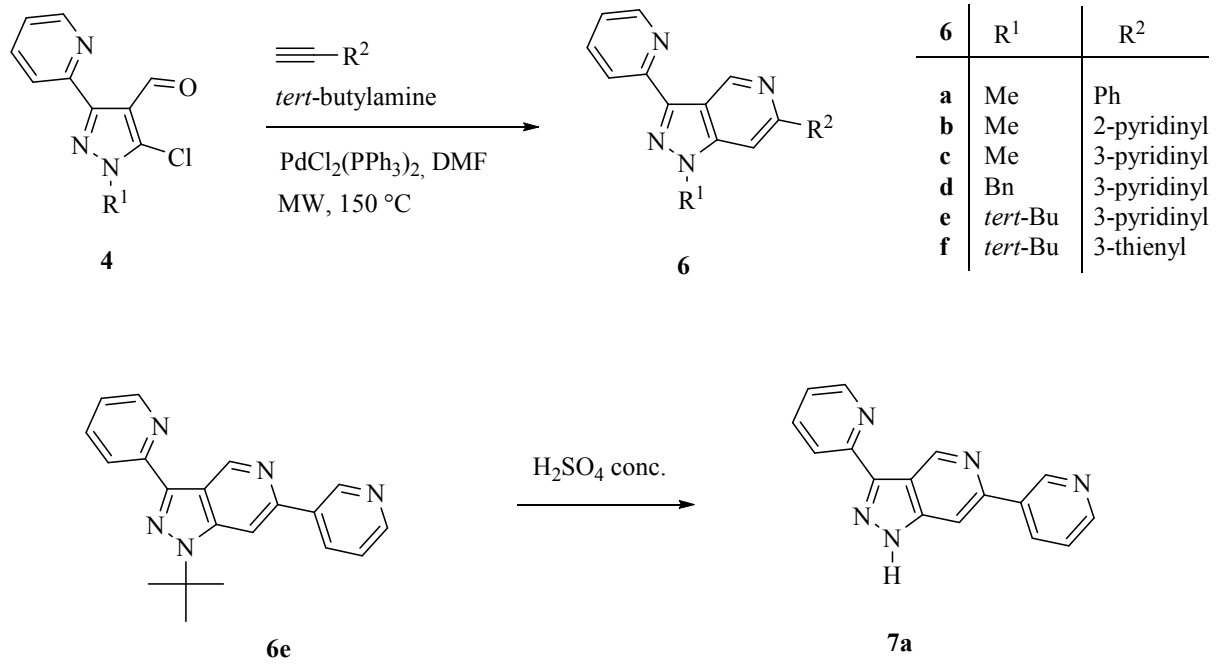
into the appropriate pyrazolone **3** and the latter by Vilsmeier reaction - with concomitant transformation of the OH group into a chloro functionality - into the corresponding *N*-protected 5-chloropyrazole-4-carbaldehyde **4**. Subsequently, Sonogashira coupling reaction with terminal alkynes should generate the respective alkyne intermediate **5**, which in the presence of *tert*-butylamine should undergo ring closure into the respective pyrazolo[4,3-*c*]pyridine **6**. Optional removal of the protecting R^1 should provide congeners of type **7** unsubstituted at pyrazole N-1.



Scheme 1. Envisaged synthetic route to the target compounds **6** and **7**.

Initially, employment of a *para*-methoxybenzyl (PMB) protecting group (R^1 = PMB) was considered for further procedure, because PMB is an approved protecting group for pyrazoles and condensed pyrazoles, and several examples show more or less uncomplicated cleavage by treatment with TFA.[11-13] Unfortunately, an orienting Vilsmeier-Haak formylation[14] applied to 2-(4-methoxybenzyl)-2,4-dihydro-3*H*-pyrazol-3-one demonstrated that the PMB group does not survive the harsh conditions of this reaction. For this reason, the more stable but harder to cleave benzyl group and the even less vulnerable methyl group were considered as possible R^1 protecting groups. The synthesis of pyrazolones **3** (**3a**: R^1 = Me, **3b**. R^1 = $PhCH_2$) was accomplished *via* reaction of 3-oxoester **2** – the latter obtained by condensation of 2-acetyl-pyridine (**1**) with diethyl carbonate – with methylhydrazine and benzylhydrazine, respectively.
The high electron density at position 4 of the pyrazole system of compounds **3a**,**b** permits smooth Vilsmeier-Haack formylation (DMF/$POCl_3$), whereas due to the presence of excessive $POCl_3$ the OH-group (the tautomeric equivalent of pyrazolone C=O) is simultaneously transformed into a chloro function to afford 5-chloropyrazole-4-carbaldehydes **4a**,**b** in good yields.[15] In recent studies we have shown that 1-substituted 5-chloropyrazole-4-carbaldehydes

are suitable reactants in Sonogashira cross-coupling reactions with appropriate alkynes.[10,16] Although the general order of reactivity for substrates is known to be R-I > R-Br ~ R-OTf > R-Cl,[17] the activated chloro atom in the latter turned out to be a good leaving group. Surprisingly, aldehydes **4a**,**b** proved to be fairly inert in cross-couplings with phenylacetylene or 2-ethynylpyridine under standard conditions. Neither did variation of the Pd catalyst and/or solvent, nor applying the reaction under microwave assistance, lead to satisfying results, *i.e*. to the formation of coupling products **5** in acceptable yields. However, a breakthrough was achieved when Sonogashira coupling and subsequent pyridine ring closure to bicycles **6** were carried out as a one-pot multi-component reaction. Thus, microwave heating (800 W, 150 °C, 1h) of aldehydes **4** with an alkyne and excessive *tert*-butylamine in DMF in the presence of $PdCl_2(PPh_3)_2$ afforded pyrazolopyridines **6a-d** in acceptable to good yields (Scheme 2). It should be emphasized that in recent years such multi-component reactions (MCRs) have attracted considerable attention due to their unmatched synthetic efficiency which permits the construction of complex molecules in an elegant and sufficient manner.[18,19]



6	R^1	R^2
a	Me	Ph
b	Me	2-pyridinyl
c	Me	3-pyridinyl
d	Bn	3-pyridinyl
e	*tert*-Bu	3-pyridinyl
f	*tert*-Bu	3-thienyl

Scheme 2. Synthesis of pyrazolo[4,3-*c*]pyridines **6a-f** and **7a**.

In order to prepare *N*-unsubstituted congeners, for instance **7a**, which could be used in biological tests, removal of the N-1 protecting group in the corresponding compounds **6** was necessary. It is known from the literature that *N*-methyl groups can be removed from N-1 of pyrazoles or condensed pyrazoles by melting the *N*-methyl derivatives with anhydrous pyridine hydrochloride.[20] However, when compounds **6a-c** were subjected to such reaction conditions (210 °C) in most cases complex reaction mixtures resulting from decomposition reactions were

obtained. Removal of the benzyl group of **6d** was attempted by various hydrogenolytic methods (Pd/C or $Pd(OH)_2$ / formic acid / ammonium formate; Pd/C, H_2, MeOH; Pd/C, 1,4-cyclo-hexadiene, EtOH).[21-23] Either unreacted starting material was obtained or decomposition was observed. As benzylic protecting groups alternatively can be removed by oxidative methods, also the system KO*t*-Bu/DMSO[24] was applied, but no reaction was observed. Furthermore, treatment with TFA, HBr or $AlCl_3$ in toluene was not successful. As an alternative, the *tert*-butyl protecting group was considered; this has been successfully employed in pyrazole chemistry.[25] Thus, pyrazolone **3c** (R^1 = *tert*-butyl) was prepared from **2** and *tert*-butylhydrazine and subsequently converted into aldehyde **4c** (R^1 = *tert*-butyl, Scheme 1), however in low yields. Multi-component reaction with 3-alkynylpyridine or 3-alkynylthiophene, respectively, afforded bicycles **6e** and **6f**. The deprotection of **6e** was tried under different conditions, for instance in TFA, TFA-H_2O (rt and afterwards at 120 °C), as well in HCOOH, HCl, at rt and reflux, where no reaction was observed. Finally, the removal of the protecting group was accomplished by treatment of **6e** with conc. H_2SO_4 affording the target compound **7a** (Scheme 2).

NMR Spectroscopic investigations

Pyrazolones of type **3** are capable of prototropic tautomerism.[26,27] Discounting participation of the pyridine system in the tautomerism, in principle three different tautomeric forms are possible, *i.e.* the OH (**A**), the CH (**B**) and the NH form (**C**) (Figure 2, upper row). The ^1H and the ^{13}C NMR spectra of pyrazolones **3a** (R^1 = Me), **3b** (R^1 = $PhCH_2$) and **3c** (R^1 = *tert*-butyl) clearly show that these compounds are exclusively present as CH-isomers **B** in $CDCl_3$ solution due to the appearance of a CH_2 fragment at position 4 of the pyrazole ring (for instance **3b**: ^1H: 3.81 ppm, 2H; corresponding ^{13}C: 38.2 ppm, CH_2 multiplicity according to APT; Figure 2, lower row, left).

Because pyrazolone **3b** showed limited solubility in $CDCl_3$, spectra were also taken of a DMSO-d_6 solution. In the latter solvent, **3b** turned out to exist as 5-hydroxypyrazole (form **A**), as confirmed by the appearance of pyrazole C-4 as CH-fragment (^1H: 5.98 ppm, ^{13}C: 84.5 ppm), the ^{13}C chemical shift of pyrazole C-5 (153.2 ppm) and particularly by the ^{15}N chemical shift of N-2 (−102.2) ppm (Figure 2, lower row, right), the latter definitely ruling out the NH-form **C**.

Due to the low solubility of **7a** in chloroform the NMR spectra were recorded of a DMSO-d_6 solution. In principle, for **7a** annular prototropic tautomerism at the pyrazole ring is possible (proton attached to N-1 or N-2). Moreover, the flexible proton theoretically could be also located at N-5 or at the pyridine N-atom of the 3-substituent, however the latter possibilities can be definitely ruled out considering the ^{15}N chemical shifts of the concerning nitrogen atoms. Nevertheless, a distinct NOE between the acidic proton (δ 13.89 ppm) and H-7 (δ 8.17 ppm) unequivocally confirms preference for the N1-H form (Figure 3, right). Moreover, the similarity of ^1H, ^{13}C and ^{15}N NMR chemical shifts of **7a** with those of the 'fixed' *N*-methyl congener **6c** (Figure 3, left) strongly confirms this assignment.

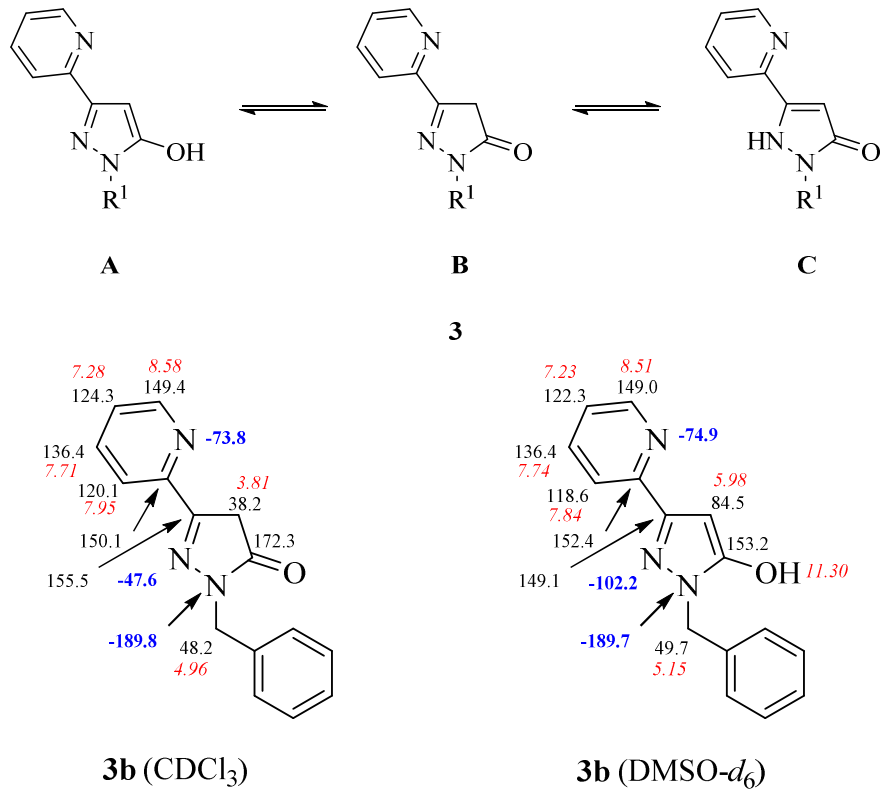


Figure 2. Upper row: tautomeric forms of pyrazolones **3**; lower row: selected ^{1}H (italics, red), ^{13}C and ^{15}N NMR (bold, blue) chemical shifts of **3b** in $CDCl_3$ and DMSO-d_6 solution.

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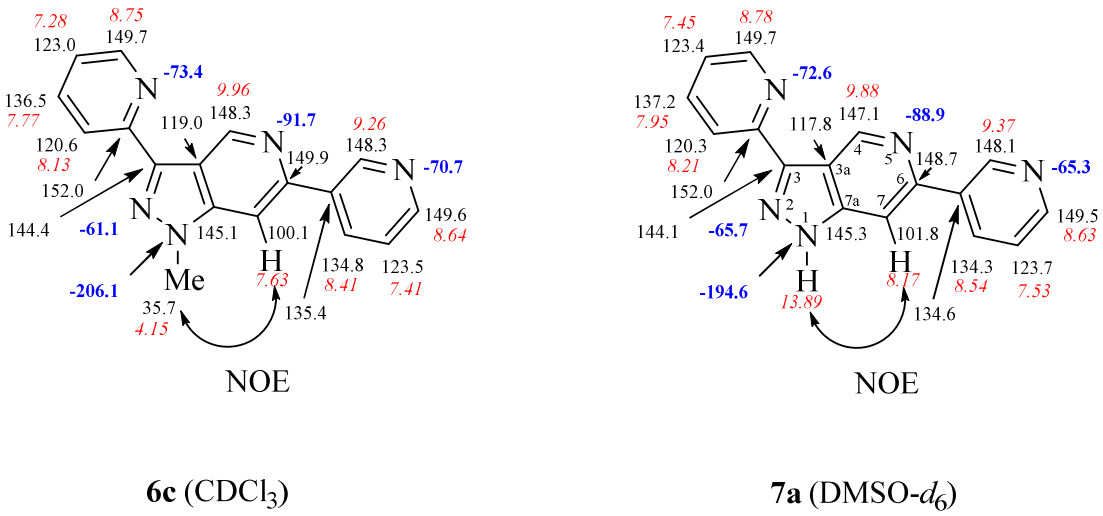

6c ($CDCl_3$) **7a** (DMSO-d_6)

Figure 3. ^{1}H (italics, red), ^{13}C and ^{15}N NMR (bold, blue) chemical shifts for compounds **6c** and **7a**.

Biological tests

Our aim was to prepare a new scaffold of protein kinase inhibitors. Therefore, the biological activity of prepared compounds **6** and **7** was assayed as described previously.[28] The compounds have been tested in kinase inhibition assays for their inhibitory potency towards recombinant CDK2, CDK9, CK2 and c-Abl, and for their cytotoxicity against various cancer cell lines. None of the tested compounds proved biological activity, but we consider these results to be preliminary and further modification of the molecules are necessary to identify first hits amongst our pyrazolo[4,3-*c*]pyridines. Most known kinase inhibitors are heterocyclic organic molecules that act like ATP competitors, interacting through one to three hydrogen bonds with the hinge region of the kinase.[1] Our future work will focus on modifications that would create typical H-bond donor-acceptor motif and on modifications increasing the polarity (solubility).

Experimental Section

General. Melting points were determined with a Reichert–Kofler hot-stage microscope. Mass spectra were obtained on a Shimadzu GP 1000 instrument (EI, 70 eV), a Bruker maXis 4G instrument (ESI-TOF, HRMS) and a Finnigan MAT 8230 instrument (EI, 70 eV, HRMS). ^{1}H, ^{13}C and ^{15}N NMR spectra were recorded with a Bruker Avance III 400 spectrometer at 293 K (400 MHz for ^{1}H, 100 MHz for ^{13}C, 40 MHz for ^{15}N). The center of the solvent signal was used as an internal standard which was related to TMS with δ 7.26 ppm (^{1}H in $CDCl_3$), δ 2.49 ppm (^{1}H in DMSO-d_6), δ 77.0 ppm (^{13}C in $CDCl_3$), δ 39.5 ppm (^{13}C in DMSO-d_6). ^{15}N NMR spectra (gs-HMBC, gs-HSQC) (40.56 MHz) were obtained using a "directly" detecting broadband

observe (BBFO) probe were referenced against neat, external nitromethane. Digital resolutions were 0.25 Hz/data point in the ^{1}H and 0.4 Hz/data point in the ^{1}H-coupled ^{13}C-NMR spectra (gated decoupling). Unequivocal assignments of signals was carried out by the combined application of standard NMR spectroscopic techniques such as ^{1}H coupled ^{13}C-NMR spectra, APT, HMQC, gs-HSQC, gs-HMBC, COSY, TOSCY, NOESY and NOE difference spectroscopy.[29] The elemental analyses were performed at the microanalytical laboratory (Faculty of Chemistry) and were in good agreement (+/- 0.4%) with the calculated values. For the microwave reaction system an Anton Paar Synthos 3000 was employed. Light petroleum refers to the fraction with boiling point 40-65 °C. Yields are not optimized.

Synthesis of 2-methyl-5-(2-pyridyl)-2,4-dihydro-3*H*-pyrazol-3-one (**3a**).[30] To a solution of ethyl 3-oxo-3-(2-pyridyl)propionate (**2**) (15.46 g, 80.00 mmol) in EtOH (40 mL) methylhydrazine (4.3 mL; 80.00 mmol) was added and the mixture was refluxed for 4 h. Upon completion, the solvent was removed under reduced pressure and the residue was washed with light petroleum and dried *in vacuo*. The pyrazolone **3a** was a colorless solid (13.39 g, 96%), mp 135-136 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.43 (s, 3H, CH_3), 3.76 (s, 2H, CH_2), 7.29 (m, 1H, Pyr 5-H), 7.74 (m, 1H, Pyr 4-H), 7.93 (m, 1H, Pyr 3-H), 8.59 (m, 1H, Pyr 6-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 31.5 ($^{1}J_{NCH3}$ 140.1 Hz, CH_3), 38.1 ($^{1}J_{C\text{-}4,4\text{-}H}$ 135.2 Hz,C-4), 120.0 (Pyr C-3), 124.3 (Pyr C-5), 136.5 (Pyr C-4), 149.5 (Pyr C-6), 149.9 (Pyr C-2), 155.2 (C-3), 172.6 (C-5) ppm. ^{15}N NMR (40 MHz, $CDCl_3$): δ −201.8 (N-1), −74.1 (Pyr N-1), −45.8 (N-2) ppm. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.71 (s, 3H, CH_3), 5.96 (s, 1H, CH), 7.35 (m, 1H, Pyr 5-H), 7.87 (m, 1H, Pyr 4-H), 7.98 (m, 1H, Pyr 3-H), 8.58 (m, 1H, Pyr 6-H), 14.40 (s, 1H, OH) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 33.7 ($^{1}J_{NCH3}$ 139.9 Hz, CH_3), 85.7 (C-4), 122.5 (Pyr C-5), 123.3 (Pyr C-3), 138.7 (Pyr C-4), 144.2 (C-3), 146.4 (Pyr C-6), 152.8 (Pyr C-2), 153.3 (C-5) ppm. ^{15}N NMR (40 MHz, DMSO-d_6): δ −202.2 (N-1), −102.2 (Pyr N-1), −111.8 (N-2) ppm. HRMS: Calcd for $(C_9H_9N_3O+H)^+$: 176.0818. Found: 176.0820.

Synthesis of 2-(phenylmethyl)-5-(2-pyridyl)-2,4-dihydro-3*H*-pyrazol-3-one (**3b**). Under stirring at 0 °C, benzylhydrazine (1.22 g, 10.00 mmol) was added dropwise to ethyl 3-oxo-3-(2-pyridyl)propionate (**2**) (1.93 g, 10.00 mmol) and the mixture was stirred for 30 min at 0 °C. The product **3b** was filtered off, washed with cold Et_2O and dried *in vacuo,* forming a colorless solid (2.01 g 79%), mp 184-185 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.81 (s, 2H, CH_2), 4.96 (s, 2H, NCH_2), 7.28 (m, 1H, Pyr 5-H), 7.29 (m, 1H, Ph 4-H), 7.35 (m, 2H, Ph 3,5-H), 7.40 (m, 2H, Ph 2,6-H), 7.71 (m, 1H, Pyr 4-H), 7.95 (m, 1H, Pyr 3-H), 8.58 (m, 1H, Pyr 6-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 38.2 (C-4), 48.2 (CH_2), 120.1 (Pyr C-3), 124.3 (Pyr C-5), 127.8 (Ph C-4), 128.2 (Ph C-2,6), 128.6 (Ph C-3,5), 136.4 (Pyr C-4), 136.5 (Ph C-1), 149.4 (Pyr C-6), 150.1 (Pyr C-2), 155.5 (C-3), 172.3 (C-5) ppm. ^{15}N NMR (40 MHz, $CDCl_3$): δ −189.8 (N-1), −73.8 (Pyr N-1), −47.6 (N-2) ppm. ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.15 (s, 2H, CH_2), 5.98 (s, 1H, 4-H), 7.21 (m, 2H, Ph 2,6-H), 7.23 (m, 1H, Pyr 5-H), 7.25 (m, 1H, Ph 4-H), 7.32 (m, 2H, Ph 3,5-H), 7.74 (m, 1H, Pyr 4-H), 7.84 (m, 1H, Pyr 3-H), 8.51 (m, 1H, Pyr 6-H), 11.30 (br s, 1H, OH) ppm. ^{13}C NMR (125 MHz, DMSO-d_6): δ 49.7 (CH_2), 84.5 (C-4), 118.6 (Pyr C-3), 122.3 (Pyr C-5),

127.1 (Ph C-2,6), 127.2 (Ph C-4), 128.4 (Ph C-3,5), 136.4 (Pyr C-4), 137.7 (Ph C-1), 149.0 (Pyr C-6), 149.1 (C-3), 152.4 (Pyr C-2), 153.2 (C-5) ppm. ^{15}N NMR (50 MHz, DMSO-d_6): δ −189.7 (N-1), −102.2 (N-2), −74.9 (Pyr N-1) ppm. MS: *m/z* (%): 251 (M^+, 40), 104 (42), 91 (100), 78 (29). Anal. Calcd for $C_{15}H_{13}N_3O$ (251.29): C, 71.70; H, 5.21; N, 16.72. Found: C, 71.35; H, 5.08; N, 16.40.

Synthesis of 2-(1,1-dimethylethyl)-5-(2-pyridyl)-2,4-dihydro-3*H*-pyrazol-3-one (**3c**). *tert*-Butylhydrazine hydrochloride (7.85 g, 0.061 mol) was dissolved in acetic acid (20 mL) and ethyl 3-oxo-3-(2-pyridyl)propionate (**2**) (9.8 g, 0.05 mol) was added. The mixture was stirred at 110 °C for 3 h. The reaction mixture was cooled to rt and neutralized with aqueous sodium hydrogen carbonate solution and then exhaustively extracted with ethyl acetate. The combined organic layers were washed with brine and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography using ethyl acetate/light petroleum (2:1 v/v) as the eluent. The pyrazolone **3c** was isolated as a pale yellow solid (7.10 g, 65%), mp 179-180 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.57 (s, 9H, 3 × CH_3), 3.75 (s, 2H, CH_2), 7.25 (ddd, *J* 7.5, 4.9, 1.2 Hz, 1H, Pyr 5-H), 7.71 (ddd, *J* 8.0, 7.5, 1.7 Hz, 1H, Pyr 4-H), 7.96 (ddd, *J* 8.0, 1.2, 1.0 Hz, 1H, Pyr 3-H), 8.56 (ddd, *J* 4.9, 1.7, 1.0 Hz, 1H, Pyr 6-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.3 (CH_3), 40.0 (C-4), 58.1 (*C*(CH_3)$_3$), 119.7 (Pyr C-3), 123.9 (Pyr C-5), 136.3 (Pyr C-4), 149.3 (Pyr C-6), 150.6 (Pyr C-2), 153.2 (C-3), 172.8 (C-5) ppm. ^{15}N NMR (40 MHz, $CDCl_3$): δ −176.5 (N-1), −75.0 (Pyr N-1), −44.3 (N-2) ppm. MS *m/z* (%): 217 (34) $[M]^+$, 202 (48), 161 (100), 105 (25), 104 (95), 78 (30), 57 (40), 56 (28), 51 (24), 41 (40). HRMS: Calcd for $(C_{12}H_{15}N_3O+H)^+$: 218.1288. Found: 218.1291.

Synthesis of 5-chloro-3-(2-pyridyl)-1*H*-pyrazole-4-carbaldehydes (**4**)**. General procedure.** Under anhydrous conditions $POCl_3$ (2.60 mL, 4.29 g, 28.00 mmol) was carefully added dropwise to dry DMF (0.98 mL, 936 mg, 12.80 mmol) under cooling. Then the appropriate 5-(2-pyridyl)-2,4-dihydro-3*H*-pyrazol-3-one (**3**) (4.00 mmol) was added and the mixture was heated to reflux for 2 h. The reaction mixture was then cooled to room temperature and the dark coloured solution was poured into ice water with stirring. The reaction mixture was neutralized with 1N NaOH until a precipitate formed and then extracted with ethyl acetate (3 × 10 mL). The combined organic phases were dried (Na_2SO_4), the solvent was removed under reduced pressure, and the residue was purified by column chromatography using ethyl acetate/light petroleum; 1:4 v/v.

5-Chloro-1-methyl-3-(2-pyridyl)-1*H*-pyrazole-4-carbaldehyde (**4a**). Yellow solid (628 mg, 70%), mp 137-138 °C (lit.[31] mp 135-136 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.94 (s, 3H, CH_3), 7.30 (ddd, *J* 7.5, 4.9, 1.2 Hz, 1H, Pyr 5-H), 7.78 (ddd, *J* 8.0, 7.5, 1.8 Hz, 1H, Pyr 4-H), 7.99 (ddd, *J* 8.0, 1.2, 1.0 Hz, 1H, Pyr 3-H), 8.65 (ddd, *J* 4.9, 1.8, 1.0 Hz, 1H, Pyr 6-H), 10.64 (s, 1H, C*H*O). ^{13}C NMR (100 MHz, $CDCl_3$): δ 36.4 (CH_3), 116.9 (C-4), 121.8 (Pyr C-3), 123.6 (Pyr C-5), 131.8 (C-5), 136.7 (Pyr C-4), 149.4 (Pyr C-6), 151.1 (Pyr C-2), 151.7 (C-3), 186.9 (*C*HO). ^{15}N NMR (40 MHz, $CDCl_3$): δ −179.5 (N-1), −77.7 (N-2), −71.7 (Pyr N-1). HRMS: Calcd for $(C_{10}H_8ClN_3O+H)^+$: 222.0429. Found: 222.0430.

5-Chloro-1-(phenylmethyl)-3-(2-pyridyl)-1*H*-pyrazole-4-carbaldehyde (**4b**). Colorless solid (726 mg, 61%), mp 122-123 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.43 (s, 2H, CH_2), 7.29 (ddd, *J* 7.6, 4.8, 1.2 Hz, 1H, Pyr 5-H), 7.30 (m, 2H, Ph 2,6-H), 7.32 (m, 1H, Ph 4-H), 7.33 (m, 2H, Ph 3,5-H), 7.77 (ddd, *J* 8.0, 7.6, 1.8 Hz, 1H, Pyr 4-H), 8.03 (ddd, *J* 8.0, 1.2, 0.9 Hz, 1H, Pyr 3-H), 8.64 (ddd, *J* 4.8, 1.8, 0.9 Hz, 1H, Pyr 6-H), 10.71 (s, 1H, C*H*O) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 53.1 (CH_2), 117.2 ($^2J_{C\text{-}4,CHO}$ = 26.0 Hz, C-4), 121.9 (Pyr C-3), 123.5 (Pyr C-5), 127.7 (Ph C-2,6), 128.4 (Ph C-4), 128.9 (Ph C-3,5), 131.3 ($^3J_{C\text{-}5,CHO}$ = 5.9 Hz, $^3J_{C\text{-}5,CH2}$ = 3.2 Hz, C-5), 134.5 (Ph C-1), 136.7 (Pyr C-4), 149.3 (Pyr C-6), 151.2 (Pyr C-2), 152.1 (C-3), 187.2 ($^1J_{CHO}$ = 186.9 Hz, *C*HO) ppm. ^{15}N NMR (40 MHz, $CDCl_3$): δ −169.0 (N-1), −77.7 (N-2), −71.2 (Pyr N-1) ppm. MS *m/z* (%): 298 (M^+, 8), 234 (34), 91 (100), 65 (31). Anal. Calcd for $C_{16}H_{12}ClN_3O$ (297.74): C, 64.54; H, 4.06; N, 14.11. Found: C, 64.27; H, 3.85; N, 13.98 %.

5-Chloro-1-(1,1-dimethylethyl)-3-(2-pyridyl)-1*H*-pyrazole-4-carbaldehyde (**4c**). Pale yellow solid (105 mg, 10%), mp 91-92 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.79 (s, 9H, 3 × CH_3), 7.27 (ddd, *J* 7.5, 4.9, 1.2 Hz, 1H, Pyr 5-H), 7.76 (ddd, *J* 8.0, 7.5, 1.8 Hz, 1H, Pyr 4-H), 7.99 (ddd, *J* 8.0, 1.2, 1.0 Hz, 1H, Pyr 3-H), 8.63 (ddd, *J* 4.9, 1.8, 1.0 Hz, 1H, Pyr 6-H), 10.71 (s, 1H, C*H*O). ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.1 (CH_3), 63.2 (*C*(CH_3)$_3$), 118.3 (C-4), 121.8 (Pyr C-3), 123.2 (Pyr C-5), 129.7 (C-5), 136.5 (Pyr C-4), 149.2 (Pyr C-6), 149.6 (C-3), 151.7 (Pyr C-2), 187.7 (*C*HO). MS *m/z* (%): 263 (1) $[M]^+$, 206 (32), 181 (35), 179 (100), 78 (25). HRMS: Calcd for $(C_{13}H_{14}ClN_3O+H)^+$: 264.0898. Found: 264.0899.

Synthesis of 3-(2-pyridyl)-1*H*-pyrazolo[4,3-*c*]pyridines (**6**). G**eneral procedure.** A mixture of 3-(2-pyridyl)-1*H*-pyrazole-4-carbaldehyde (**4**) (1.00 mmol), $PdCl_2(PPh_3)_2$ (40 mg, 0.06 mmol), DMF (12 mL), phenylacetylene (151 mg, 1.50 mmol) and *tert*-butylamine (0793 g, 10.00 mmol) was irradiated in a microwave oven at 800 W for 1h at 150 °C in a sealed vessel. The reaction mixture was cooled to rt and the solvents were removed under reduced pressure. The residue was dissolved in ethyl acetate (25 mL) and H_2O (28 mL) was added. The organic phase was separated and the aqueous phase was extracted with ethyl acetate (3 × 25 mL). The organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography.

1-Methyl-6-phenyl-3-(2-pyridyl)-1*H*-pyrazolo[4,3-*c*]pyridine (**6a**). Compound **6a** was purified by column chromatography using ethyl acetate/light petroleum, 1:3 v/v. Yellow solid (78 mg, 26%), mp 145-146 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.15 (s, 3H, CH_3), 7.29 (m, 1H, Pyr 5-H), 7.43 (m, 1H, Ph 4-H), 7.51 (m, 2H, Ph 3,5-H), 7.64 (d, *J* 1.2 Hz, 1H, 7-H), 7.79 (m, 1H, Pyr 4-H), 8.09 (m, 2H, Ph 2,6-H), 8.15 (m, 1H, Pyr 3-H), 8.77 (m, 1H, Pyr 6-H), 9.96 (d, *J* 1.2 Hz, 1H, 4-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ 35.6 (CH_3), 99.8 ($^1J_{C7,7H}$ = 163.5 Hz, $^4J_{C7,4H}$ = 1.7 Hz, C-7), 118.8 (C-3a), 120.7 (Pyr C-3), 122.9 (Pyr C-5), 127.3 (Ph C-2,6), 128.69 (Ph C-4), 128.73 (Ph C-3,5), 136.4 (Pyr C-4), 139.9 (Ph C-1), 144.3 (C-3), 145.5 (C-7a), 147.8 ($^1J_{C4,4H}$ = 186.6 Hz, $^4J_{C4,7H}$ = 0.6 Hz, C-4), 149.7 (Pyr C-6), 152.2 (Pyr C-2), 152.9 (C-6) ppm. ^{15}N NMR (50 MHz, $CDCl_3$): δ −206.8 (N-1), −91.3 (N-5), −73.3 (Pyr N-1), −62.2 (N-2). MS *m/z* (%): 287 (21), 286 (M^+, 100), 285 (65), 209 (49), 105 (28), 91 (31), 79 (26), 78 (40), 71 (20), 57 (30), 51 (20), 43 (43) ppm. HRMS: Calcd for $(C_{18}H_{14}N_4+H)^+$: 287.1291. Found: 287.1292.

1-Methyl-3,6-di-(2-pyridyl)-1*H*-pyrazolo[4,3-*c*]pyridine (**6b**). Compound **6b** was purified by column chromatography using ethyl acetate → dichloromethane / MeOH, 9:1 v/v. Colorless solid (232 mg, 81%), mp 157-158 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.17 (s, 3H, CH_3), 7.26 (m, 1H, C-3-Pyr 5-H), 7.30 (m, 1H, C-6-Pyr 5-H), 7.77 (m, 1H, C-3-Pyr 4-H), 7.83 (m, 1H, C-6-Pyr 4-H), 8.14 (m, 1H, C-3-Pyr 3-H), 8.43 (s, 1H, 7-H), 8.56 (m, 1H, C-6-Pyr 3-H), 8.68 (m, 1H, C-6-Pyr 6-H), 8.75 (m, 1H, C-3-Pyr 6-H), 9.93 (s, 1H, 4-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 35.8 (CH_3), 106.6 (C-7), 119.6 (C-3a), 120.6 (C-3-Pyr C-3), 121.8 (C-6-Pyr C-3), 122.9 (C-3-Pyr C-5), 123.5 (C-6-Pyr C-5), 136.4 (C-3-Pyr C-4), 137.0 (C-6-Pyr C-4), 144.3 (C-3), 145.5 (C-7a), 147.4 (C-4), 149.0 (C-6-Pyr C-6), 149.6 (C-3-Pyr C-6), 151.2 (C-6), 152.2 (C-3-Pyr C-2), 156.3 (C-6-Pyr C-2) ppm. ^{15}N NMR (40 MHz, $CDCl_3$): δ −205.1 (N-1), −93.7 (N-5), −79.3 (C-6-Pyr N-1), −73.6 (C-3-Pyr N-1), −60.9 (N-2) ppm. MS m/z (%): 287 (M^+, 33), 278 (38), 277 (100), 204 (31), 77 (23), 57 (20), 51 (25). Anal. Calcd for $C_{17}H_{13}N_5$ (287.33)•0.3 H_2O: C, 69.75; H, 4.68; N, 23.92. Found: C, 69.78; H, 4.30; N, 23.84 %.

1-Methyl-3-(2-pyridyl)-6-(3-pyridyl)-1*H*-pyrazolo[4,3-*c*]pyridine (**6c**). Compound **6c** was purified by column chromatography using ethyl acetate. Pale yellow solid (192 mg, 67%), mp 170-172 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.15 (s, 3H, CH_3), 7.28 (ddd, *J* 7.5, 4.8, 1.1 Hz, 1H, 2-Pyr 5-H), 7.41 (ddd, *J* 8.0, 4.8, 0.8 Hz, 1H, 3-Pyr 5-H), 7.63 (d, *J* 1.1 Hz, 1H, 7-H), 7.77 (ddd, *J* 7.6, 7.6, 1.7 Hz, 1H, 2-Pyr 4-H), 8.13 (ddd, *J* 8.0, 1.1, 1.1 Hz, 1H, 2-Pyr 3-H), 8.41 (ddd, *J* 8.0, 2.2, 1.7 Hz, 1H, 3-Pyr 4-H), 8.64 (dd, *J* 4.8, 1.5 Hz, 1H, 3-Pyr 6-H), 8.75 (ddd, *J* 4.8, 1.7, 0.9 Hz, 1H, 2-Pyr 6-H), 9.26 (d, *J* 2.0 Hz, 1H, 3-Pyr 2-H), 9.96 (d, *J* 1.1 Hz, 1H, 4-H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 35.7 (CH_3), 100.1 (C-7), 119.0 (C-3a), 120.6 (2-Pyr C-3), 123.0 (2-Pyr C-5), 123.5 (3-Pyr C-5), 134.8 (3-Pyr C-4), 135.4 (3-Pyr C-3), 136.5 (2-Pyr C-4), 144.4 (C-3), 145.1 (C-7a), 148.29 (C-4), 148.33 (3-Pyr C-2), 149.6 (3-Pyr C-6), 149.7 (2-Pyr C-6), 149.9 (C-6), 152.0 (2-Pyr C-2) ppm. ^{15}N NMR (40 MHz, $CDCl_3$): δ −206.1 (N-1), −91.7 (N-5), −73.4 (2-Pyr N-1), −70.7 (3-Pyr N-1), −61.1 (N-2) ppm. MS *m/z* (%): 288 (20), 287 (M^+, 100), 286 (62), 209 (35), 78 (27). Anal. Calcd for $C_{17}H_{13}N_5$ (287.33)·0.15 H_2O: C, 70.40; H, 4.62; N, 24.15. Found: C, 70.41; H, 4.34; N, 23.83 %.

1-(Phenylmethyl)-3-(2-pyridyl)-6-(3-pyridyl)-1*H*-pyrazolo[4,3-*c*]pyridine (**6d**). Compound **6d** was purified by column chromatography using ethyl acetate. Pale yellow solid (211 mg, 58%), mp 179-180 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.68 (s, 2H, CH_2), 7.27 (m, 1H, Ph 2-H), 7.29 (m, 2H, Ph 4-H, 2-Pyr 5-H), 7.33 (m, 2H, Ph 3,5-H), 7.38 (m, 1H, 3-Pyr 5-H), 7.55 (d, *J* 1.2 Hz, 1H, 7-H), 7.78 (ddd, *J* 8.0, 7.5, 1.8 Hz, 1H, 2-Pyr 4-H), 8.20 (ddd, *J* 8.0, 1.0, 1.0 Hz, 1H, 2-Pyr 3-H), 8.35 (m, 1H, 3-Pyr 4-H), 8.62 (m, 1H, 3-Pyr 6-H), 8.77 (ddd, *J* 4.8, 1.7, 0.9 Hz, 1H, 2-Pyr 6-H), 9.16 (dd, *J* 2.3, 0.8 Hz, 1H, 3-Pyr 2-H), 10.02 (d, *J* 1.2 Hz, 1H, 4-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ 53.3 ($^{1}J_{CH2}$ 139.7 Hz, CH_2), 100.5 ($^{1}J_{C\text{-}7,7\text{-}H}$ 164.1 Hz, $^{4}J_{C\text{-}7,4\text{-}H}$ 1.7 Hz, C-7), 119.5 (C-3a), 120.9 (2-Pyr C-3), 123.1 (2-Pyr C-5), 123.5 (3-Pyr C-5), 127.2 (Ph C-2,6), 128.2 (Ph C-4), 128.9 (Ph C-3,5), 134.8 (3-Pyr C-4), 135.3 (3-Pyr C-3), 135.7 (Ph C-1), 136.4 (2-Pyr C-4), 144.8 (C-3), 144.9 (C-7a), 148.4 (3-Pyr C-2), 148.5 ($^{1}J_{C\text{-}4,4\text{-}H}$ 187.4 Hz, C-4), 149.58 (3-Pyr C-6), 149.64 (2-Pyr C-6), 150.1 (C-6), 152.1 (2-Pyr C-2) ppm. ^{15}N NMR (50 MHz, $CDCl_3$): δ −195.4 (N-1), −90.9 (N-5), −73.2 (2-Pyr N-1), −70.3 (3-Pyr N-1), −61.0 (N-2) ppm. MS *m/z*

(%): 363 (M^+, 60), 362 (33), 287 (39), 286 (92), 285 (46), 209 (34), 95 (21), 91 (100), 78 (24), 69 (68), 57 (29), 43 (22), 41 (24). Anal. Calcd for $C_{23}H_{17}N_5$ (363.43)•0.1 H_2O: C, 75.64; H, 4.75; N, 19.18. Found: C, 75.53; H, 4.48; N, 18.87 %.

1-(1,1-Dimethylethyl)-3-(2-pyridyl)-6-(3-pyridyl)-1*H*-pyrazolo[4,3-*c*]pyridine (**6e**). Compound **6e** was synthesized in analogy to compound **6a** from **4c** (205 mg, 0.75 mmol) except that 3-ethynylpyridine (116 mg, 1.125 mmol) was used. Purification was achieved by column chromatography using ethyl acetate/light petroleum, 1:5, v/v. Pale yellow solid (225 mg, 91%), mp 172-174 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.87 (s, 9H, 3 × CH_3), 7.27 (m, 1H, 2-Pyr 5-H), 7.43 (m, 1H, 3-Pyr 5-H), 7.78 (m, 1H, 2-Pyr 4-H), 7.93 (d, *J* 1.1 Hz, 1H, 7-H), 8.20 (m, 1H, 2-Pyr 3-H), 8.41 (m, 1H, 3-Pyr 4-H), 8.65 (m, 1H, 3-Pyr 6-H), 8.75 (m, 1H, 2-Pyr 6-H), 9.24 (m, 1H, 3-Pyr 2-H), 10.07 (d, *J* 1.1 Hz, 1H, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.8 (CH_3), 60.9 ($C(CH_3)_3$), 103.3 (C-7), 120.4 (C-3a), 120.8 (2-Pyr C-3), 122.7 (2-Pyr C-5), 123.6 (3-Pyr C-5), 135.0 (3-Pyr C-4), 135.9 (3-Pyr C-3), 136.3 (2-Pyr C-4), 142.6 (C-3), 143.4 (C-7a), 148.4 (3-Pyr C-2), 148.8 (C-4), 149.1 (C-6), 149.4 (3-Pyr C-6), 149.5 (2-Pyr C-6), 152.6 (2-Pyr C-2). ^{15}N NMR (40 MHz, $CDCl_3$): δ −175.1 (N-1), −92.7 (N-5), −73.8 (2-Pyr N-1), −70.5 (3-Pyr N-1), −58.0 (N-2). MS *m/z* (%): 329 (45) $[M]^+$, 274 (20), 273 (100), 272 (47), 78 (41), 69 (25), 57 (52), 41 (30). HRMS: Calcd for $(C_{20}H_{19}N_5+H)^+$: 330.1713. Found: 330.1716.

1-(1,1-Dimethylethyl)-3-(2-pyridyl)-6-(3-thienyl)-1*H*-pyrazolo[4,3-*c*]pyridine (**6f**). Compound **6f** was purified by column chromatography using ethyl acetate/light petroleum, 1:5 v/v. Pale yellow solid (280 mg, 84%), mp 193-194 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.86 (s, 9H, 3 × CH_3), 7.26 (ddd, *J* 7.5, 4.9, 1.2 Hz, 1H, Pyr 5-H), 7.43 (dd, *J* 5.1, 3.1 Hz, 1H, Th 5-H), 7.71 (dd, *J* 5.1, 1.3 Hz, 1H, Th 4-H), 7.77 (ddd, *J* 8.0, 7.5, 1.8 Hz, 1H, Pyr 4-H), 7.81 (d, *J* 1.2 Hz, 1H, 7-H), 8.00 (dd, *J* 3.1, 1.3 Hz, 1H, Th 2-H), 8.19 (ddd, *J* 8.0, 1.2, 1.0 Hz, 1H, Pyr 3-H), 8.74 (ddd, *J* 4.8, 1.8, 1.0 Hz, 1H, Pyr 6-H), 9.99 (d, *J* 1.2 Hz, 1H, 4-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.7 (CH_3), 60.7 ($C(CH_3)_3$), 102.4 (C-7), 120.0 (C-3a), 120.8 (Pyr C-3), 122.6 (Pyr C-5), 123.4 (Th C-2), 126.2 (Th C-5), 126.3 (Th C-4), 136.3 (Pyr C-4), 142.6 (C-3), 142.8 (Th C-3), 143.5 (C-7a), 147.9 (C-6), 148.4 (C-4), 149.5 (Pyr C-6), 152.8 (Pyr C-2). ^{15}N NMR (40 MHz, $CDCl_3$): δ −175.8 (N-1), −93.7 (N-5), −73.7 (Pyr N-1), N-2 not found. MS *m/z* (%): 334 (46) $[M]^+$, 279 (21), 278 (100), 277 (56). HRMS: Calcd for $(C_{19}H_{18}N_4S+H)^+$: 335.1325. Found: 335.1328.

Synthesis of 3-(2-pyridyl)-6-(3-pyridyl)-1*H*-pyrazolo[4,3-*c*]pyridine (**7a**). Compound **6e** (130 mg, 0.40 mmol) was dissolved in conc. sulfuric acid (10 mL) and the solution was stirred for 3 h. The reaction mixture was cooled and neutralized with 1N NaOH and then extracted with ethyl acetate (3 × 50 mL). The organic layers were combined, washed with brine and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography using dichloromethane/MeOH, 9:1, v/v to give **7a**. Yield: 60 mg (55%); pale yellow solid; mp 324-326 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45 (m, 1H, 2-Pyr 5-H), 7.53 (m, 1H, 3-Pyr 5-H), 7.95 (m, 1H, 2-Pyr 4-H), 8.17 (d, *J* 1.1 Hz, 1H, 7-H), 8.21 (m, 1H, 2-Pyr 3-H), 8.54 (m, 1H, 3-Pyr 4-H), 8.63 (m, 1H, 3-Pyr 6-H), 8.78 (m, 1H, 2-Pyr 6-H), 9.37 (m, 1H, 3-Pyr 2-H), 9.88 (s, 1H, 4-H), 13.89 (s, 1H, N*H*) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 101.8 (C-7), 117.8 (C-3a), 120.3 (2-Pyr C-3), 123.4 (2-Pyr C-5), 123.7 (3-Pyr C-5),

134.3 (3-Pyr C-4), 134.6 (3-Pyr C-3), 137.2 (2-Pyr C-4), 144.1 (C-3), 145.3 (C-7a), 147.1 (C-4), 148.1 (3-Pyr C-2), 148.7 (C-6), 149.5 (3-Pyr C-6), 149.7 (2-Pyr C-6), 152.0 (2-Pyr C-2) ppm. ^{15}N NMR (40 MHz, $CDCl_3$): δ −194.6 (N-1), −88.9 (N-5), −72.6 (2-Pyr N-1), −65.7 (N-2), −65.3 (3-Pyr N-1) ppm. MS *m/z* (%): 273 (100) $[M]^+$, 272 (56), 78 (37). HRMS: Calcd for $(C_{16}H_{11}N_5+H)^+$: 274.1087. Found: 274.1089.

Biological tests

Kinase inhibition assays. CDK2/Cyclin E kinase was produced in Sf9 insect cells via baculoviral infection and purified on a NiNTA column (Qiagen). CDK5/p35, CDK7Cyclin H/MAT1 and CDK9/Cyclin T1 were purchased from ProQinase GmbH. The kinase reactions were assayed with 1 mg/mL histone H1 (for CDK2 and CDK5) or $(YSPTSPS)_2KK$ peptide (for CDK7 and CDK9) in the presence of 15/0.15/1.5/1.5 μM ATP (for CDK2/CDK5/CDK7CDK9), 0.05 μCi [γ-^{33}P]ATP and of the test compound in a final volume of 10 μL, all in a reaction buffer (60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 μM Na-orthovanadate, 1.2 mM DTT, 2.5 μg / 50 μl $PEG_{20.000}$). The reactions were stopped by adding 5 μL of 3 % aq. H_3PO_4. Aliquots were spotted onto P-81 phosphocellulose (Whatman), washed 3× with 0.5 % aq. H_3PO_4 and finally air-dried. Kinase inhibition was quantified using a FLA-7000 digital image analyzer (Fujifilm). The concentration of the test compounds required to decrease the CDK activity by 50 % was determined from dose-response curves and designated as IC_{50}.[28]

Cell maintenance and cytotoxicity assays. The cytotoxicity of the studied compounds was determined using cell lines of different histological origin as described earlier.[28] Briefly, the cells were assayed with compounds using three-fold dilutions in triplicate. Treatment lasted for 72 h, followed by addition of Calcein AM solution, and measurement of the fluorescence of live cells at 485 nm/538 nm (ex/em) with a Fluoroskan Ascent microplate reader (Labsystems). IC_{50} (the drug concentration that reduced the number of viable cells to 50 %) values were determined from the dose-response curves.

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