Synthesis and biological evaluation of imidazo[4,5-b]pyridine and 4-heteroaryl-pyrimidine derivatives as anti-cancer agents

ARTICLE *in* EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY · OCTOBER 2012

Impact Factor: 3.45 · DOI: 10.1016/j.ejmech.2012.09.034 · Source: PubMed

European Journal of Medicinal Chemistry 57 (2012) 311–322

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and biological evaluation of imidazo[4,5-*b*]pyridine and 4-heteroaryl-pyrimidine derivatives as anti-cancer agents

Pawel M. Lukasik [a], Sherifa Elabar [a], Frankie Lam [a,b], Hao Shao [a], Xiangrui Liu [a], Abdullah Y. Abbas [a], Shudong Wang [a,b,*]

[a] School of Pharmacy and Centre for Biomolecular Sciences, University of Nottingham, Nottingham NG7 2RD, UK
[b] School of Pharmacy and Medical Sciences, University of South Australia, Adelaide SA 5001, Australia

ARTICLE INFO

Article history:
Received 26 July 2012
Received in revised form
21 September 2012
Accepted 25 September 2012
Available online 3 October 2012

Keywords:
Cyclin-dependent kinase
RNA polymerase II
Anti-tumour activity
Induction of apoptosis
Mcl-1 anti-apoptotic protein
Drug discovery
Cancer therapy

ABSTRACT

A series of *N*-phenyl-imidazo[4,5-*b*]pyridin-2-amines, 4-indazolyl-*N*-phenylpyrimidin-2-amines and *N*-phenyl-4-pyrazolo[3,4-*b*]pyridin-pyrimidin-2-amines have been synthesized. Their anti-proliferative activities were tested in HCT-116 human colon carcinoma and MCF-7 breast carcinoma cell lines. Many exhibited potent anti-proliferative and CDK9 inhibitory activities. A lead compound **18b** demonstrated the ability to reduce the level of Mcl-1 anti-apoptotic protein, to activate caspase 3/7 and induce cancer cell apoptosis.

1. Introduction

Cyclin-dependent kinases (CDKs) are the major regulators of the cell cycle and transcription. The complexes of cyclin D-CDK4/6 and cyclin E-CDK2 facilitate the G1-S phase transition by sequentially phosphorylating the retinoblastoma protein (Rb), while cyclinA-CDK1/2 and cyclinB-CDK1 are essential for S-phase progression and G2-M transition, respectively [1]. Multiple CDKs control the cell cycle in mammals and have been long considered essential for normal proliferation, development and homeostasis. CyclinH-CDK7 and cyclinT1-CDK9 is primarily involved in the regulation of transcription through phosphorylation of the C-terminal domain (CTD) of RNA polymerase-II (RNAP-II) [2]. RNA polymerase II (RNAPII) transcription is highly regulated and involves a sequence of events leading to phosphorylation of the carboxy-terminal domain (CTD) during which the general transcription factor II (TFIIH) complex, containing CDK7, first phosphorylates the Serine-5 residue of the CTD heptapeptide repeats Y1S2P3T4S5P6S7. The positive transcriptional elongation factor b (p-TEFb), consisting of cyclinT-CDK9, then phosphorylates the DRB-sensitive-inducing factor (DSIF) and the negative elongation factor (NELF) followed by Serine-2 of the CTD to facilitate transcriptional elongation [2]. Phosphorylation of these Serine residues provides the stimulus for efficient initiation and elongation of mRNA synthesis by the RNAP-II transcriptional complex.

Deregulation of various components controlling the cell cycle plays an essential role in tumour pathogenesis. This has promoted the development of pharmacological small molecule CDK inhibitors that can be used for cancer therapy. However, the fact that transformed cells depleted of cyclins and CDKs continue to proliferate [3] indicates that the specific targeting of individual cell cycle CDKs may not be an optimal therapeutic strategy due to functional redundancy [2]. The current research in the field suggests that a therapeutic agent targeting transcriptional CDK, particularly CDK9, or targeting a combination of cell-cycle and transcriptional CDKs may lead to superior anti-cancer efficacy. This has been achieved by a pioneer CDK inhibitor flavopiridol. Flavopiridol has demonstrated the marked efficacy in refractory and relapsed

* Corresponding author. School of Pharmacy and Medical Sciences, University of South Australia, Adelaide SA 5001, Australia. Tel.: +61 883022372; fax: +61 883021087.
E-mail address: shudong.wang@unisa.edu.au (S. Wang).

0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2012.09.034

chronic lymphocytic leukaemia (CLL) [4]. Flavopiridol inhibits a number of CDKs and other kinases, but targeting transcriptional CDK kinases, particularly cyclinT-CDK9 which leads to down-regulation of the transcription of anti-apoptotic proteins in CLL cells [5,6], has underlined its pharmacological activity.

Our interest in developing pharmacological CDK inhibitor has resulted in the discovery of several preclinical and clinical candidates for cancer therapy [7–12]. In order to identify further novel CDK inhibitor classes we prepared a series of *N*-phenyl-imidazo[4,5-*b*]pyridine-2-amines, 4-(1-substituted-1*H*-indazol-3-yl)-*N*-phenylpyrimidin-2-amines and 4-pyrazolo-[3,4-*b*]pyridinyl-*N*-phenylpyrimidin-2-amines. Here we report the synthesis, structure–activity relationship and biological characterization of these compounds.

2. Chemistry

The synthetic chemistry employed to prepare *N*-phenyl-imidazo[4,5-*b*]pyridin-2-amine derivatives **9a**–**i** is outlined in Scheme 1. Treatment of *N*-benzylpyridine-2,3-diamine **2**, obtained by alkylation of 2,3-diaminopyridine **1** with benzyldehyde in the presence of sodium borohydride, with isothiocyanatobenzene at 160 °C under microwave irradiation for 30 min resulted in 1-benzyl-1*H*-imidazo[4,5-*b*]pyridine-2(3*H*)-thione **3**. Bromination of the latter followed by reaction between 1-benzyl-2-bromo-1*H*-imidazo[4,5-*b*]pyridine **4** and anilines yielded compounds **9a**–**h**. Preparation of analogues **9i** started from 2-chloro-3-nitropyridine **5** which was reacted with phenylmethanamine followed by catalytic reduction reaction yielded **6**. Treatment of **6** with isothiocyanatobenzene gave **7**, which was converted to 1-benzyl-2-(methylsulfonyl)-1*H*-imidazo[4,5-*b*]pyridine **8** by methylation, followed by oxidation in the presence of mCPBA. Microwave-assisted reactions between **8** and anilines afforded **9i**.

The chemistry for the synthesis of 4-indazol-3-yl-*N*-phenylpyrimidin-2-amines is summarized in Scheme 2. Treatment of 1*H*-indazole-3-carboxylic acid **10** with *N*,*O*-dimethylhydroxylamine yielded *N*-methoxy-*N*-methyl-1*H*-indazole-3-carboxamide **11** [13]. The nucleophilic addition of Grignard reagent to the Weinreb amide **11**, followed by alkylation afforded efficiently the corresponding indazole ketones **12**. Intermediate enaminone **13** was conveniently obtained by treating **12** with DMF-DMA using the method described previously [7]. Finally, preparations of **14a**–**z** were achieved by microwave-aided reaction between **13** and the appropriate phenylguanidines.

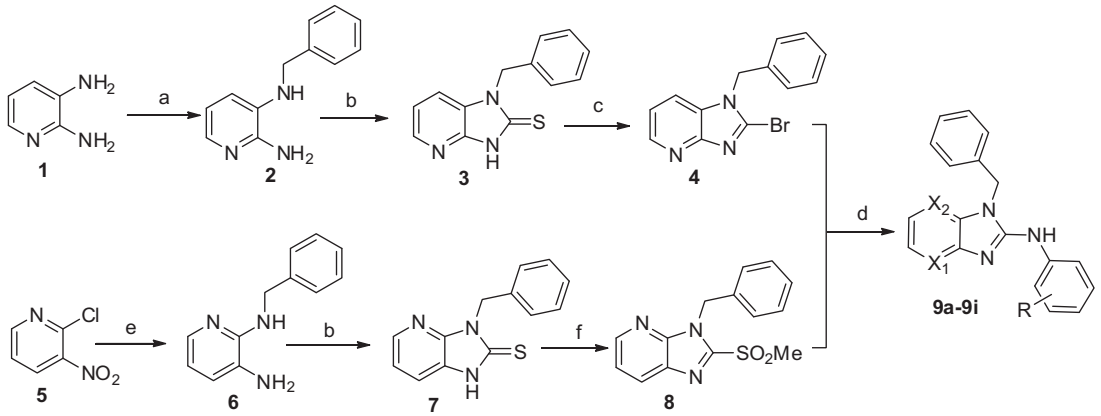
4-(1*H*-Indazol-1-yl)-*N*-phenylpyrimidin-2-amine **18a**,**b** can be effectively synthesized by alkylation reaction between the appropriate anilines and 1-(2-chloropyrimidin-4-yl)-1*H*-indazole **17**, which was obtained by treating 2,4-dichloropyrimidine **16** with 1*H*-indazole **15** (Scheme 3). The synthesis of *N*-phenyl-4-(1*H*-pyrazolo[3,4-*b*]pyridin-1-yl)pyrimidin-2-amine derivatives **25** is outlined in Scheme 4. 2-Chloronicotinic acid **19** was converted to 2-chloropyridine ketones **21** via the Weinreb amide **20** [13]. Treatment of **21** with hydrazine resulted in 1*H*-pyrazolo[3,4-*b*]pyridine **22** which was conveniently converted to 1-(2-(methylthio)pyrimidin-4-yl)-1*H*-pyrazolo[3,4-*b*]pyridine **23** by reacting with 4-chloro-2-(methylthio)pyrimidine. Oxidation of **23** to its methylsulfonyl derivative **24**, followed by nucleophilic substitution reaction of the appropriate anilines afforded **25a**–**f**.

3. Pharmacology

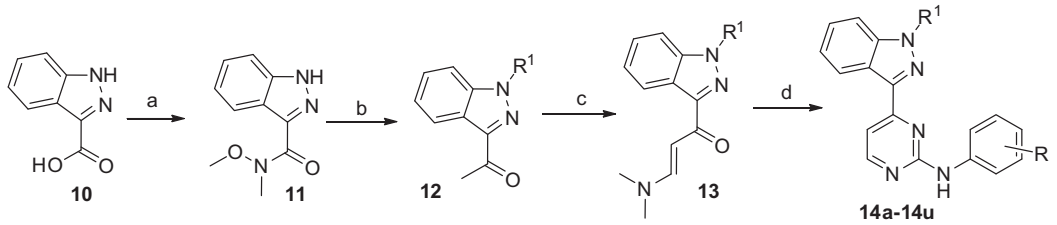
3.1. Cytotoxicity in human tumour cell lines

The anti-proliferative activity of each compound was assessed against HCT-116 colorectal carcinoma and MCF-7 breast cancer cells using a 72h-MTT cytotoxicity assay [11] and the results are summarized in Table 1. *N*-phenyl-imidazo[4,5-*b*]pyridin-2-amine derivatives **9a**–**e** and **9i** showed low activities with $GI_{50} > 40$ μM, irrespective of which substituent the phenyl ring bears; either $X^1 = N$ and $X^2 = CH$ or $X^2 = N$ and $X^1 = CH$. Analogues **9g** (R = *m*-NO_2) and **9h** (R = *m*-NO_2, *p*-Me) exhibited increased potency in MCF-7 breast cancer cells with GI_{50} values of 24.24 μM and 27.84 μM respectively.

The second class of compounds, i.e. 4-indazol-3-yl-*N*-phenylpyrimidin-2-amines, demonstrated overall improved cellular potency. As shown in Table 1, compounds **14a**, **14c**–**o**, **14q**–**u** exhibited sub-μM potency against the tumour cell lines ($GI_{50} < 10$ μM). However a considerable loss in cellular potency was observed with **14b** (R^1 = Me, R = *m*-OH) and **14p** (R^1 = Et, R = *m*-OH) with GI_{50} = 17–37 μM, indicating intolerance of the *m*-OH substituted aniline. The most potent anti-proliferative agents, **14h** (GI_{50} = 0.49–0.58 μM), **14i** (GI_{50} = 0.94–2.28 μM), and **14j** (GI_{50} = 1.98–2.27 μM), were obtained when the respective *m*-NO_2, *m*-SO_2NH_2 and *p*-SO_2NH_2 were introduced to the aniline moiety, when a methyl group was located at R^1 position. However, replacement of the methyl group with a bulkier ethyl, *n*-propyl or 3-methylpyridine, in the context of *m*-nitroaniline, *m*-aminobenzenesulfonamide or *p*-aminobenzenesulfonamide, as shown in **14k**–**u**, did not offer improvement in cellular potency when compared to **14h**–**j**.



Scheme 1. *Reagents and conditions*: (a) *i*. Benzaldehyde, molecular sieves, THF, reflux, 4h; *ii*. $NaBH_4$, EtOH, reflux, 20 h; (b) PhNCS, MeCN, Discovery microwave, 160 °C, 30 min; (c) AcOH, Br_2, 0 °C, 4 h; (d) aniline, MeCN, Discovery microwave, 160 °C, 40 min. (e) *i*. Phenylmethanamine, NEt_3, 80 °C, 2h; *ii*. Fe, HCl, EtOH/H_2O, 80 °C, 4 h; (f) *i*. MeI, KOH, EtOH, 0 °C, 3 h; *ii*. *m*CPBA, DCM, 0 °C-r.t., 15 h.



Scheme 2. *Reagents and conditions*: (a) *N,O*-Dimethylhydroxylamine, THF, pyridine, EDC, 0 °C-r.t., 16 h; (b) *i*. CH_3MgBr, THF, −78 °C, 3 h; *ii*. alkyl iodide, DMF, NaH, 0 °C-r.t, 4h; (c) DMF-DMA, DMF, 140 °C, 16 h; (d) phenylguanidine, MeCN, Discovery microwave, 140 °C, 40 min.

Retaining *m*-benzenesulfonamide or *p*-benzenesulfonamide while introducing an indazole at the pyrimidine 4C-position afforded compounds **18a** (X^1 = CH, R^1 = H, R = *p*-SO_2NH_2) and **18b** (X^1 = CH, R^1 = H, R = *m*-SO_2NH_2), the most promising cytotoxic agents with respective GI_{50} values of 0.59 and 0.75 μM against HCT-116 tumour cells as shown in Table 1. However MCF-7 breast cancer cells seemed resistant to **18a** and **18b** treatment, which resulted in >3- and >7-fold reduced potency respectively. The replacement of the indazol with pyrazolo[3,4-*b*]pyridine ring, resulting in **25a**–**f**, has a detrimental effect on cellular activity compared to **18a**,**b**. Interestingly, compounds **25b**, **25d**, and **25f**, containing benzenesulfonamide at the *para*-position of aniline, were more potent than their respective *meta*-benzenesulfonamide analogues **25a**, **25c** and **25e**, irrespective of the substitution of hydrogen, methyl, or ethyl groups at the 3C-position of pyrazolo[3,4-*b*]pyridine (i.e. R^1 = H, Me or Et).

3.2. CDK inhibitory activity

In the light of potent anti-proliferative activity, the CDK kinase inhibitory activities of **14i**–**l**, **14r**–**u** and **18a**,**b** were evaluated using biochemical assays. As shown in Table 2, these compounds were potent CDK9 inhibitors with a K_i values in a range of 0.017–0.30 μM and all, except **14u**, also inhibited CDK1 with K_i < 0.95 μM. Interestingly, these compounds, except **18a**, exhibited no activity against CDK7 with a K_i value >5 μM. Analogue **14u** was the most selective CDK9 inhibitor, being 14-fold more potent for CDK9 than CDK1, while **18a** appeared the least selective, targeting all three CDKs with the sub-μM K_i values.

3.3. Cellular mechanism of action

As the most potent CDK9 inhibitor, **18b** was further studied for its cellular mechanism of action. We first investigated whether the cytotoxic effect of **18b** was a consequence of activation of cellular apoptosis. Induction of caspase 3/7 activity was measured in HCT-116 cancer cells after treatment with **18b** for 24 h (Fig. 1). **18b** significantly induced caspase 3/7 activity at its GI_{50} μM (the concentration caused cytotoxicity by MTT assay) compared with the untreated cells, with enhanced activity at higher concentrations. We next examined the cell-cycle effects of this compound. HCT-116 cancer cells were treated with **18b** for a period of 24 h and then analysed by flow cytometry. As shown in Fig. 2 no effect on cell-cycle progression following the treatment of HCT-116 cells with GI_{50} and 5 × GI_{50} μM of **18b** was observed, but at the higher concentration, i.e. 10 × GI_{50} μM the treatment resulted in significant pre-G1 cell accumulation indicating cell death. The data suggests that CDK1/cyclinB inhibition of **18b** may not be the primary cellular mechanism of action.

The cellular CDK9 inhibitory activity was confirmed by Western blot analysis. HCT-116 cells were cultured with 5, 25 or 50 μM **18b** for 24 h. As shown in Fig. 3, the levels of phosphorylated Ser-2 of RNAPII CTD were abolished, indicating cellular CDK9 inhibition. The phosphorylation of RNAPII CTD at Ser-5 was not affected; confirming the selectivity for CDK9 over CDK7. The same treatment resulted in down-regulation of the levels of Mcl-1 anti-apoptotic protein, and consequently induction of PARP cleavage indicated apoptosis in HCT-116 cells. These findings are consistent with the CDK9-mediated RNAPII transcriptional inhibition in cancer cells [11].

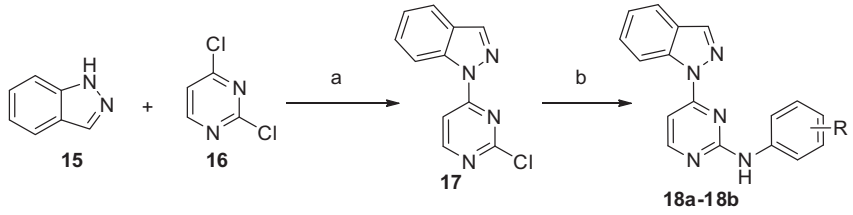
4. Conclusion

In conclusion, a series of derivatives of *N*-phenyl-imidazo[4,5-*b*]pyridin-2-amine, 4-(indazol-3-yl)-*N*-phenylpyrimidin-2-amine, 4-(indazol-1-yl)-*N*-phenylpyrimidin-2-amine and *N*-phenyl-4-(pyrazolo[3,4-*b*]pyridin-1-yl)pyrimidin-2-amine were prepared. The structure and anti-proliferative activity relationship were studied. Many compounds exhibited excellent CDK9 and CDK1 inhibitory activity and potent anti-proliferative activity. The lead compound **18b** inhibited CDK9 activity and was capable of activation of caspase 3/7 activity and inducing apoptosis via down-regulation of Mcl-1 anti-apoptotic protein in cancer cells.

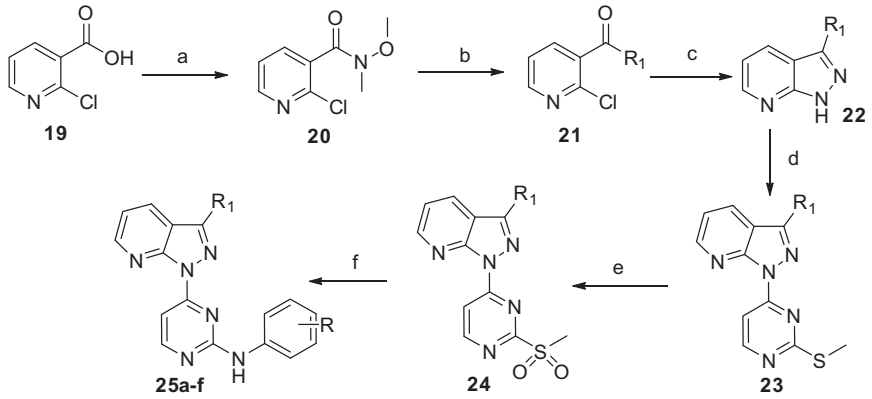
5. Experimental

5.1. Chemistry

Chemical reagents and solvents were obtained from commercial sources. When necessary, solvents were dried and/or purified by standard methods. Melting points (mp) were determined with an Electrothermal 9100 capillary melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were obtained using



Scheme 3. *Reagents and conditions*: (a) NaH, DMF, 0 °C, 6 h; (b) aniline, EtOH, Discovery microwave, 140 °C, 40 min.



Scheme 4. *Reagents and conditions*: (a) *N,O*-Dimethylhydroxylamine, pyridine, EDC, THF, 0 °C – r.t., 24 h; (b) R^1MgBr, THF, −78 °C – r.t., 24 h; (c) hydrazin monohydrate, r.f., 0–90 °C, 24 h; (d) 4-chloro-2-(methylthio)pyrimidine, NaH, MeCN, 0 °C – r.t., 24 h; (e) *m*-CPBA, $CHCl_3$, r.f., 4 h; (f) aniline, *p*-toluenesulfonic acid, isopropanol, Discovery microwave, 150 °C, 20 min.

a Bruker 400 Ultrashield™ spectrometer at 400 MHz and 100 MHz respectively. These were analyzed using the Bruker TOPSPIN 2.1 program. Chemical shifts are reported in parts per million relative to internal tetramethylsilane standard. Coupling constants (*J*) are quoted to the nearest 0.1 Hz. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and br, broad. High resolution mass spectra (HR-MS) were obtained using a Waters 2795 single quadrupole mass spectrometer/micromass LCT platform. Silica gel (EM Kieselgel 60, 0.040–0.063 mm, Merck) or ISOLUTE pre-packed columns were used for flash chromatography.

5.1.1. General method for the synthesis of benzylimidazo[4,5-b] pyridine-2-thiones (**3**, **7**)

A solution of benzylpyridine-2,3-diamine **2** or **6** [14] (200 mg, 1.0 mmol) and phenylisothiocyanate (132 μL, 1.1 mmol) in acetonitrile was heated at 160 °C for 20 min in a microwave reactor. The reaction mixture was purified by chromatography on silica gel (Pet/EtOAc: 1/1). 1-*Benzylimidazo*[4,5-*b*]*pyridine*-2-*thione* (**3**). White crystals (143 mg, 60% yield); mp 196–198 °C; ^{1}H NMR (DMSO-d_6) δ 5.52 (2H, s, CH_2), 7.14 (1H, dd, Ar–H, J = 8.0, 5.2 Hz), 7.24–7.39 (5H, m, Ar–H), 7.61 (1H, dd, Ar–H, J = 8.0, 1.2 Hz), 8.15 (1H, dd, Ar–H, J = 5.2, 1.6 Hz), 13.49 (1H, s, NH); HR-MS (*m*/*z*): calcd for $C_{13}H_{11}N_3S$ 241.0674; found 242.0712 $[M + 1]^+$. 3-*Benzylimidazo*[4,5-*b*]*pyridine*-2-*thione* (**7**). White crystals (213 mg, 88% yield); mp 162–164 °C; ^{1}H NMR (DMSO-d_6) δ 5.46 (2H, s, CH_2), 7.22 (1H, dd, Ar–H, J = 8.0, 5.2 Hz), 7.30 (5H, m, Ar–H), 7.57 (1H, dd, Ar–H, J = 7.6, 1.2 Hz), 8.17 (1H, dd, Ar–H, J = 5.2, 1.2 Hz), 13.11 (lH, s, NH); HR-MS (*m*/*z*): calcd for $C_{13}H_{11}N_3S$ 241.0674; found 242.0666 $[M + 1]^+$.

5.1.2. 1-Benzyl-2-bromoimidazo[4,5-b]pyridine (**4**)

To a mixture of **3** (180 mg, 0.75 mmol) in AcOH (30 mL) and HBr (55 μL, 1.01 mmol, 48% aqueous) cooling in an ice bath was added bromine (0.14 mL, 2.72 mmol) dropwise. The mixture was stirred for 4.5 h, diluted with 20 mL water, and the titled compound was precipitated by addition of NH_4OH aqueous. The precipitate was filtered, washed with water, and dried to afford **4** as pink solid (0.155 g, 72% yield); mp 212–214 °C; ^{1}H NMR (DMSO-d_6) δ 5.57 (2H, s, CH_2), 7.20 (2H, d, Ph–H, J = 8.4 Hz), 8.43 (1H, dd, Ar–H, J = 4.8, 1.6 Hz), 8.08 (1H, dd, Ar–H, J = 8.4, 1.6 Hz), 7.33 (4H, m, Ar–H); HR-MS (*m*/*z*): calcd for $C_{13}H_{10}BrN_3$ 287.0058; found 288.0097 $[M + 1]^+$.

5.1.3. 3-Benzyl-2-(methylsulfonyl)-3H-imidazo[4,5-b]pyridine (**8**)

A mixture of **7** (200 mg, 0.83 mmol) in 2 mL ethanol was treated with potassium hydroxide (125 mg, 2.23 mmol) in water (0.5 mL). After stirring at r.t. for 10 min methyl iodide (55 μL, 0.91 mmol) was added. After stirring for an additional 3 h it was poured into water (10 mL). The precipitate was filtered off, washed and dried. Recrystallisation from ethanol afforded 3-benzyl-2-(methylthio)-3*H*-imidazo[4,5-*b*]pyridine as an off-white solid (178 mg, yield 85%); ^{1}H NMR (DMSO-d_6) δ 2.72 (3H, s, CH_3), 5.38 (2H, s, CH_2), 7.23–7.34 (6H, m, Pyr–H, Ph–H), 7.97 (1H, dd, Pyr–H, J = 8.0, 1.2 Hz), 8.25 (1H, dd, Pyr–H, J = 4.8, 1.2 Hz); HR-MS (*m*/*z*): calcd for $C_{14}H_{13}N_3S$ 255.0830; found 256.0461 $[M + 1]^+$. To a solution of the latter (100 mg, 0.39 mmol) in dichloromethane (3 mL) cooling in an ice bath 3-chloroperoxybenzoic acid (170 mg, 1.05 mmol) was added. The mixture was warmed to room temperature and stirred for a further 12 h. After this time an aqueous solution of saturated sodium hydrogen carbonate (10 mL) was added and the mixture was separated. The aqueous solution was extracted with dichloromethane (3 × 5 mL), the combined organic layers was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography to afford the titled compound as white solid (68 mg, 61% yield); ^{1}H NMR (DMSO-d_6) δ 3.57 (3H, s, CH_3), 5.85 (2H, s, CH_2), 7.31 (5H, m, Ph–H), 7.53 (1H, dd, Pyr–H, J = 8.0, 4.8 Hz), 8.37 (1H, dd, Pyr–H, J = 8.4, 1.6 Hz), 8.63 (1H, dd, Pyr–H, J = 4.4, 1.6 Hz); HR-MS (*m*/*z*): calcd for $C_{14}H_{13}N_3O_2S$ 287.0728; found 287.9989.

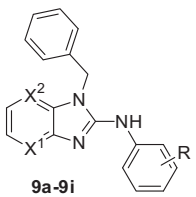
5.1.4. General procedure for the preparation of 1-benzyl-N-phenyl-1H-imidazo[4,5-b]pyridin-2-amines (**9a**–**i**)

A solution of **4** or **8** (50 mg, 0.175 mmol) and the appropriate aniline (0.195 mmol) in acetonitrile was heated at 160 °C for 30 min in a microwave reactor. The mixture was purified by chromatography on silica (EtOAc/MeOH 25/1) to yield desired compounds. 1-*Benzyl*-*N*-*phenyl*-1*H*-*imidazo*[4,5-*b*]*pyridin*-2-*amine* (**9a**). From **4** and aniline as a light brown solid (70% yield); mp 272–275 °C; ^{1}H NMR (DMSO-d_6) δ 5.70 (2H, s, CH_2), 7.19 (1H, t, Ph–H, J = 7.6 Hz), 7.28–7.45 (8H, m, Ph–H, Pyr–H), 7.78 (2H, dd, Ph–H, J = 8.8, 0.8 Hz), 8.08 (1H, d, Pyr–H, J = 6.8 Hz), 8.23 (1H, dd, Pyr–H, J = 6.0, 1.2 Hz), 10.29 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 45.62, 115.39, 121.08, 124.39, 126.92, 127.94, 128.91, 128.93, 130.93, 135.18, 138.01, 150.13, 155.80; Anal. RP-HPLC: t_R 11.27 min (30–100% MeCN over 22 min, purity 100%). HR-MS (*m*/*z*): calcd for $C_{19}H_{16}N_4$ 300.1375; found 301.1244 $[M + 1]^+$.

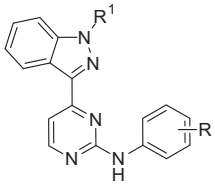
5.1.5. 1-Benzyl-N-(4-hydroxyphenyl)-1H-imidazo[4,5-b]pyridin-2-amine (**9b**)

From **4** and 4-hydroxyaniline as a brown solid (78% yield); mp 266–268 °C; ^{1}H NMR (DMSO-d_6) δ 5.62 (2H, s, CH_2), 6.81 (2H, d, Ph–H, J = 8.8 Hz), 7.22 (1H, dd, CH_2, J = 7.6, 6.0 Hz), 7.26–7.40 (5H,

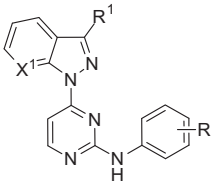
Table 1
Structure and growth inhibitory activity of compounds against human tumour cancer cells.



9a-9i



14a-14u



18a-18b, 25a-25f

Compd	Structure				Anti-proliferation (GI_{50}, μM)[a]	
	X^1	X^2	R^1	R	HCT-116	MCF-7
9a	N	CH	–	H	64.74	52.47
9b	N	CH	–	*p*-OH	72.62	52.49
9c	N	CH	–	*p*-Cl	59.47	48.03
9d	N	CH	–	*m*-Cl	36.33	43.47
9e	N	CH	–	*m*-Br	77.66	78.66
9f	N	CH	–	*p*-OMe	87.33	59.08
9g	N	CH	–	*m*-NO_2	41.71	24.24
9h	N	CH	–	*m*-NO_2, *p*-Me	40.36	27.84
9i	CH	N	–	H	60.21	51.81
14a	–	–	Me	*p*-OH	8.43	8.91
14b	–	–	Me	*m*-OH	17.21	28.16
14c	–	–	Me	*p*-Me	8.64	6.10
14d	–	–	Me	*m*-Me	9.42	9.15
14e	–	–	Me	*p*-Cl	8.73	7.83
14f	–	–	Me	*m*-Cl	6.40	5.97
14g	–	–	Me	*p*-NO_2	8.22	11.84
14h	–	–	Me	*m*-NO_2	0.49	0.58
14i	–	–	Me	*p*-SO_2NH_2	2.28	0.94
14j	–	–	Me	*m*-SO_2NH_2	1.98	2.27
14k	–	–	Et	*p*-SO_2NH_2	2.05	2.28
14l	–	–	Et	*m*-SO_2NH_2	9.01	7.26
14m	–	–	Et	*p*-NO_2	4.06	23.96
14n	–	–	Et	*m*-NO_2	2.82	2.31
14o	–	–	Et	*p*-OH	5.29	5.78
14p	–	–	Et	*m*-OH	36.59	33.50
14q	–	–	n-Pr	*p*-OH	5.45	3.34
14r	–	–	n-Pr	*p*-SO_2NH_2	6.74	0.59
14s	–	–	n-Pr	*m*-SO_2NH_2	7.95	4.26
14t	–	–	3-methylpyridine	*p*-SO_2NH_2	5.13	2.63
14u	–	–	3-methylpyridine	*m*-SO_2NH_2	8.28	5.27
18a	CH	–	H	*p*-SO_2NH_2	0.59	1.87
18b	CH	–	H	*m*-SO_2NH_2	0.75	5.56
25a	N	–	H	*m*-SO_2NH_2	14.63	6.93
25b	N	–	H	*p*-SO_2NH_2	8.04	7.02
25c	N	–	Me	*m*-SO_2NH_2	24.84	19.95
25d	N	–	Me	*p*-SO_2NH_2	8.84	8.64
25e	N	–	Et	*m*-SO_2NH_2	65.14	73.23
25f	N	–	Et	*p*-SO_2NH_2	6.34	0.88

[a] 72h-MTT assay; the data given are mean values derived from at least two replicates.

m, Ph–H), 7.50 (2H, d, Ph–H, J = 8.8 Hz), 7.93 (1H, d, Ph–H, J = 6.8 Hz), 8.15 (1H, dd, Pyr–H, J = 6.0, 1.2 Hz), 9.42 (1H, s, OH), 10.00 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 44.69, 114.73, 115.07, 121.01, 126.43, 126.77, 127.49, 128.73, 131.59, 136.61, 141.58, 152.76, 153.00, 155.85; Anal. RP-HPLC: t_R 9.07 min (30–100% MeCN over 22 min, purity 97%); HR-MS (m/z): calcd for $C_{19}H_{16}N_4O$ 316.1324, found 317.1357 $[M + 1]^+$.

5.1.6. 1-Benzyl-N-(4-chlorophenyl)-1H-imidazo[4,5-b]pyridin-2-amine (**9c**)

From **4** and 4-chloroaniline as a light brown solid (17% yield); mp 270–272 °C; ^{1}H NMR (DMSO-d_6) δ 5.71 (2H, s, CH_2), 7.28–7.39 (6H, m, Ph–H, Pyr–H), 7.51 (2H, d, Ph–H, J = 8.8 Hz), 7.84 (2H, d, Ph–H, J = 8.8 Hz), 8.12 (1H, dd, Pyr–H, J = 7.6, 0.8 Hz), 8.26 (1H, dd, Pyr–H, J = 6.0, 1.2 Hz), 10.43 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 45.61, 114.65, 115.08, 120.98, 123.61, 126.94, 127.91, 128.91, 130.83, 135.16, 152.60, 153.05 156.17; Anal. RP-HPLC: t_R 12.85 min (30–40% MeCN over 22 min, purity 100%); HR-MS (m/z): calcd for $C_{19}H_{15}ClN_4$ 334.0985; found 335.0751 $[M + 1]^+$.

Table 2
CDK inhibitory activity of the selected compounds.

Compound	Kinase inhibition (K_i, μM)[a]		
	CDK1/CyclinB	CDK7/CyclinH	CDK9/CyclinT1
14i	0.096	>5	0.140
14j	0.061	>5	0.098
14k	0.055	>5	0.253
14l	0.471	>5	0.154
14r	0.127	>5	0.176
14s	0.945	>5	0.285
14t	0.287	>5	0.207
14u	4.53	>5	0.314
18a	0.028	0.328	0.091
18b	0.053	>5	0.017

[a] Apparent inhibition constants (K_i) were calculated from IC_{50} values and the appropriate K_m (ATP) values for each kinase. The data given are mean values derived from two replicates.

5.1.7. 1-Benzyl-N-(3-chlorophenyl)-1H-imidazo[4,5-b]pyridin-2-amine (**9d**)

From **4** and 3-chloroaniline as a light brown solid (22% yield); mp 266–268 °C; ^{1}H NMR (DMSO-d_6) δ 5.65 (2H, s, CH_2), 7.16 (1H, dd, Ph–H, J = 8.4, 1.6 Hz), 7.19–7.38 (6H, m, Ph–H, Pyr–H), 7.42 (1H, t, Ph–H, J = 8.0 Hz), 7.42 (1H, t, Ph–H, J = 8.0 Hz), 7.87 (1H, d, Ph–H, J = 7.2 Hz), 8.16 (1H, t, Ph–H, J = 2.0 Hz), 8.22 (1H, dd, Pyr–H, J = 5.6, 1.2 Hz), 10.02 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 46.33, 116.25, 118.37, 119.34, 122.95, 127.30, 128.16, 129.25, 130.96, 133.71, 136.23, 137.84, 143.16, 152.95, 154.21, 157.13, 158.05; Anal. RP-HPLC: t_R 12.67 min (30–100% MeCN over 22 min, purity 100%); HR-MS (m/z): calcd for $C_{19}H_{15}ClN_4$ 334.0985; found 335.0813 $[M + 1]^+$.

5.1.8. 1-Benzyl-N-(3-bromophenyl)-1H-imidazo[4,5-b]pyridin-2-amine (**9e**)

From **4** and 3-bromoaniline as a light brown solid (41% yield); mp 269–271 °C; ^{1}H NMR (DMSO-d_6) δ 5.68 (2H, s, CH_2), 7.25–7.41 (8H, m, Ph–H, Pyr–H), 7.76 (1H, ddd, Pyr–H, J = 8.0, 2.0, 0.8 Hz), 7.99 (1H, d, Ph–H, J = 7.6 Hz), 8.25 (1H, dd, Pyr–H, J = 3.2, 0.8 Hz), 8.26 (1H, s, Ph–H), 10.20 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 45.56, 115.76, 118.89, 121.67, 122.26, 126.09, 126.86, 127.86, 128.88, 130.82,

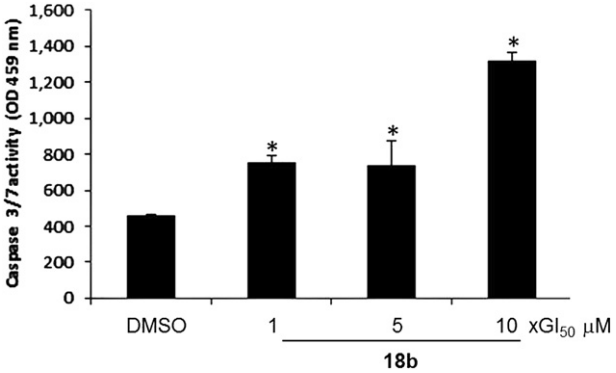


Fig. 1. Induction of caspase-3/7 activity in HCT-116 cells after treatment with **18b** at the GI_{50}, 5 × GI_{50} or 10 × GI_{50} μM for 24 h. Vertical bars represent the mean ± s.d. of two independent experiments. Values significantly ($p < 0.01$) different from DMSO control are marked with an asterisk (*).

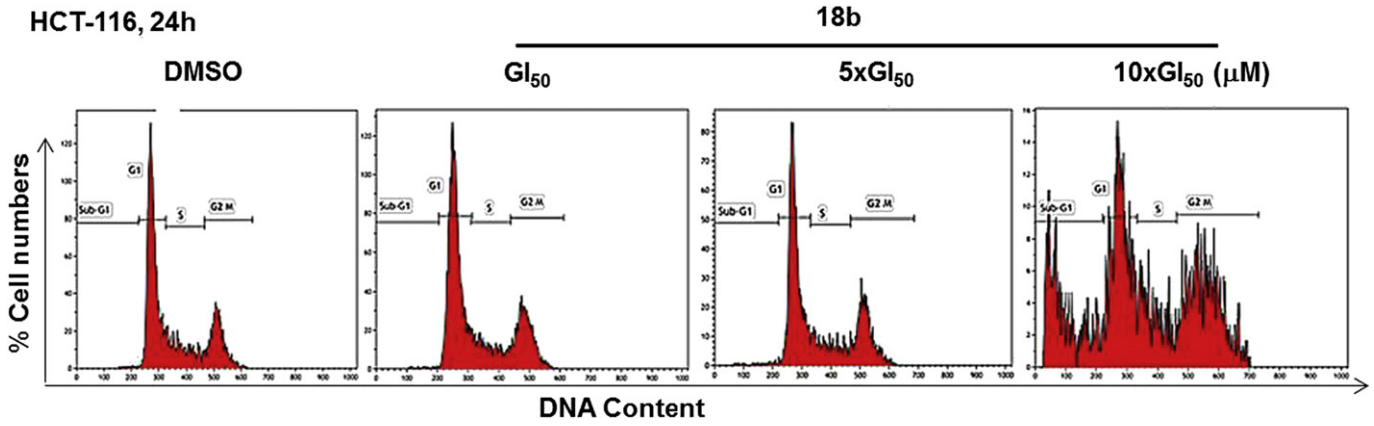


Fig. 2. Cell-cycle analysis of HCT-116 cells following treatment with **18b**. The cells were fixed, stained with propidium iodide and subjected to FACS analysis for the DNA content. **18b** shows a minimal cell cycle effect at the GI_{50} and 5 × GI_{50}, but induced significant cells death at 10 × GI_{50} concentration.

135.39, 140.28, 154.12; Anal. RP-HPLC: t_R 12.97 min (30–100% MeCN over 22 min, purity 100%); HR-MS (*m*/*z*): calcd for $C_{19}H_{15}BrN_4$ 378.0480; found 379.0628 $[M + 1]^+$.

*5.1.9. 1-Benzyl-N-(4-methoxyphenyl)-1H-imidazo[4,5-b]pyridin-2-amine (**9f**)*

From **4** and 4-methoxyaniline as a light brown solid (67% yield); mp 267–269 °C; ^{1}H NMR (DMSO-d_6) δ 3.78 (3H, s, CH_3), 5.67 (2H, s, CH_2), 7.02 (2H, d, Ph–H, J = 9.2 Hz), 7.35 (6H, m, Ph–H, Pyr–H), 7.63 (2H, d, Ph–H, J = 8.8 Hz), 8.05 (1H, d, Pyr–H, J = 7.6 Hz), 8.18 (1H, d, Pyr–H, J = 6.4 Hz), 10.27 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 45.58, 55.35, 114.14, 115.13, 120.79, 123.44, 126.96, 127.95, 128.92, 130.59, 135.14, 156.50; Anal. RP-HPLC: t_R 11.20 min (30–100% MeCN over 22 min, purity 100%); HR-MS (*m*/*z*): calcd for $C_{20}H_{18}N_4O$ 330.1481; found 331.1452 $[M + 1]^+$.

*5.1.10. 1-Benzyl-N-(3-nitrophenyl)-1H-imidazo[4,5-b]pyridin-2-amine (**9g**)*

From **4** and 3-nitroaniline as a light brown solid (9% yield); mp 270–272 °C; ^{1}H NMR (DMSO-d_6) δ 5.68 (2H, s, CH_2), 7.26 (1H, dd, Pyr–H, J = 8.0, 6.8 Hz), 7.27–7.38 (5H, m, Ph–H), 7.70 (1H, t, Ph–H, J = 8.0 Hz), 7.88 (1H, d, Ph–H, J = 7.6 Hz), 7.95 (1H, dd, Pyr–H, J = 8.0, 1.6 Hz), 8.25 (1H, dd, Pyr–H, J = 5.6, 1.2 Hz), 8.28 (1H, dd, Ph–H, J = 8.0, 1.6 Hz), 8.93 (1H, s, Ph–H), 10.29 (1H, s, NH); ^{13}C NMR

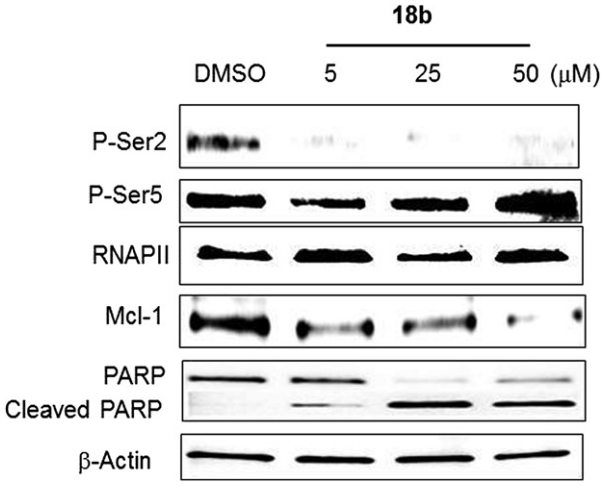


Fig. 3. Cellular mode of action of **18b** by western blotting analysis: HCT-116 cells were treated with **18b** for 24 h at the concentrations shown. β-Actin was used as internal control.

(DMSO-d_6) δ 45.64, 101.44, 114.83, 115.58, 121.45, 124.92, 126.01, 127.63, 127.94, 128.83, 133.38, 135.29, 148.65, 155.14; Anal. RP-HPLC: t_R 11.95 min (30–100% MeCN over 22 min, purity 91%); HR-MS (*m*/*z*): calcd for $C_{19}H_{15}N_5O_2$ 345.1226; found 346.1210 $[M + 1]^+$.

*5.1.11. 1-Benzyl-N-(4-methyl-3-nitrophenyl)-1H-imidazo[4,5-b]pyridin-2-amine (**9h**)*

From **4** and 4-methyl-3-nitroaniline as a light brown solid (49% yield); mp 268–270 °C; ^{1}H NMR (DMSO-d_6) δ 2.53 (3H, s, CH_3), 5.73 (2H, s, CH_2), 7.30–7.41 (6H, m, Ph–H, Pyr–H), 7.58 (1H, d, Ph–H, J = 8.8 Hz), 8.06 (1H, dd, Pyr–H, J = 8.4, 2.4 Hz), 8.14 (1H, d, Ph–H, J = 7.2 Hz), 8.29 (1H, dd, Pyr–H, J = 6.0, 0.8 Hz), 8.61 (1H, d, Ph–H, J = 2.4 Hz), 10.61 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 19.22, 45.66, 101.20, 115.88, 115.98, 121.43, 124.74, 126.87, 127.67, 127.93, 128.92, 133.35, 135.29, 148.65, 155.90; Anal. RP-HPLC: t_R 14.00 min (30–100% MeCN over 22 min, purity 96%); HR-MS (*m*/*z*): calcd for $C_{20}H_{17}N_5O_2$ 359.1382; found 360.1122 $[M + 1]^+$.

*5.1.12. 3-Benzyl-N-phenyl-3H-imidazo[4,5-b]pyridin-2-amine (**9i**)*

From **8** and aniline as pink solid (24% yield). ^{1}H NMR (DMSO-d_6) δ 5.59 (2H, s, CH_2), 6.99 (1H, t, Ph–H, J = 7.6 Hz), 7.10 (1H, dd, Pyr–H, J = 7.6, 4.8 Hz), 7.22 (3H, m, Ph–H), 7.31 (4H, m, Ph–H), 7.71 (1H, d, Pyr–H, J = 7.6 Hz), 7.88 (2H, d, Ph–H, J = 7.6 Hz), 8.01 (1H, d, Pyr–H, J = 4.8 Hz), 9.22 (lH, s, NH); ^{13}C NMR (DMSO-d_6) δ 43.76, 118.07, 119.09, 122.27, 122.83, 127.22, 127.79, 129.08, 129.13, 135.07, 137.55, 140.18, 140.53, 147.51, 151.33; Anal. RP-HPLC: t_R 6.0 min (30–100% MeCN over 22 min, purity 100%); HR-MS (*m*/*z*): calcd for $C_{19}H_{16}N_4$ 300.1375, found 301.1066 $[M + 1]^+$.

*5.1.13. General procedure for the preparation of **12***

A solution of 1-(1*H*-indazol-3-yl)ethanone **11** [13] (5.0 g, 31.24 mmol) in dry dimethylformamide (150 mL) cooling on an ice bath was treated with sodium hydride (1.39 g, 34.40 mmol). The reaction mixture was warmed to room temperature and stirred for 1 h before being cooled to 0 °C, after which alkyl iodide (37.50 mmol) was added dropwise. The mixture was then stirred at r.t. for a further 3 h. Water (10 mL) was added and the mixture was extracted with ethyl acetate (3 × 100 mL), dried with $MgSO_4$ and evaporated. The residue was purified by flash chromatography (Pet/EtOAc 5/1). 1-(1-*Ethyl*-1*H*-*indazol*-3-*yl*)*ethanone* (**12**, R^1 = Et) as bright yellow crystalline solid (3.92 g, 67% yield); ^{1}H NMR (DMSO-d_6) δ 1.47 (3H, td, CH_3, J = 7.2, 2.0 Hz), 2.62 (3H, s, CH_3), 4.57 (2H, dd, CH_2, J = 7.2, 2.0 Hz), 7.35 (1H, t, Ind–H, J = 7.2 Hz), 7.49 (1H, t, Ind–H, J = 8.0 Hz), 7.82 (1H, d, Ind–H, J = 8.0 Hz), 8.18 (1H, d, Ind–H, J = 8.0 Hz); HR-MS (*m*/*z*): calcd for $C_{11}H_{12}N_2O$ 188.0950; found 189.1488 $[M + 1]^+$.

*5.1.14. 1-(1-Propyl-1H-indazol-3-yl)ethanone (**12**, R^1 = n-Pr)*

As bright yellow crystalline solid (4.72 g, 75%); ^{1}H NMR (DMSO-d_6) δ 0.85 (3H, t, CH_3, J = 7.2 Hz), 1.89 (2H, q, CH_2, J = 7.2 Hz), 2.61 (3H, s, CH_3), 4.46 (2H, t, CH_2, J = 7.2 Hz), 7.32 (1H, t, Ind–H, J = 7.6 Hz), 7.46 (1H, t, Ind–H, J = 7.6 Hz), 7.79 (1H, d, Ind–H, J = 8.4 Hz), 8.18 (1H, d, Ind–H, J = 8.0 Hz); HR-MS (m/z): calcd for $C_{12}H_{14}N_2O$ requires 202.1106; found 203.1392 $[M + 1]^+$.

*5.1.15. 1-(1-(Pyridin-3-ylmethyl)-1H-indazol-3-yl)ethanone (**12**, R^1 = 3-methylpyridine)*

As a bright yellow crystalline solid (1.01 g, 64%); ^{1}H NMR (DMSO-d_6) δ 2.63 (3H, s, CH_3), 5.86 (2H, s, CH_2), 7.34 (1H, d, Pyr–H, J = 6.4 Hz), 7.35 (1H, d, Ind–H, J = 7.6 Hz), 7.48 (1H, t, Ind–H, J = 8.0 Hz), 7.68 (1H, dt, Pyr–H, J = 7.6, 1.6 Hz), 7.89 (1H, d, Ind–H, J = 8.4 Hz), 8.20 (1H, d, Ind–H, J = 8.4 Hz), 8.49 (1H, dd, Pyr–H, J = 4.8, 1.6 Hz), 8.64 (1H, d, Pyr–H, J = 2.0 Hz); HR-MS (m/z): calcd for $C_{15}H_{13}N_3O$ 251.1059; found 252.0645 $[M + 1]^+$.

*5.1.16. General procedure for the preparation of (E)-3-(dimethylamino)-1-(1-substituted-1H-indazol-3-yl)prop-2-en-1-ones (**13**)*

A solution of **12** (20.20 mmol) in 25 mL dimethylformamide was treated with dimethylformamide dimethyl acetal (40.40 mmol). The reaction mixture was heated at 140 °C for 16 h. After concentrated the residue was purified by column chromatography (Pet/EtOAc 2/1) to afford desired product. (*E*)-3-(*Dimethylamino*)-1-(1-*methyl*-1*H*-*indazol*-3-*yl*)*prop*-2-*en*-1-*one* (**13**, R^1 = Me). From **12** (R^1 = H); ^{1}H NMR ($CDCl_3$) δ 2.96 (3H, bs, CH_3), 3.17 (3H, bs, CH_3), 5.74 (1H, d, CH, J = 12.4 Hz), 7.18 (1H, t, Ind–H, J = 7.6 Hz), 7.28 (1H, t, Ind–H, J = 7.6 Hz), 7.43 (1H, d, Ind–H, J = 8.4 Hz), 7.84 (1H, d, CH, J = 12.4 Hz), 7.85 (1H, d, Ind–H, J = 8.4 Hz); HR-MS (m/z): calcd for $C_{13}H_{15}N_3O$ 229.1215; found 230.1230 $[M + 1]^+$.

*5.1.17. (E)-3-(Dimethylamino)-1-(1-ethyl-1H-indazol-3-yl)prop-2-en-1-one (**13**, R^1 = Et)*

^{1}H NMR (DMSO-d_6) δ 1.43 (3H, t, CH_3, J = 7.2 Hz), 2.90 (3H, bs, CH_3), 3.14 (3H, bs, CH_3), 4.51 (2H, q, CH_2, J = 7.2 Hz), 6.06 (1H, d, CH, J = 12.8 Hz), 7.24 (1H, t, Ind–H, J = 7.6 Hz), 7.41 (1H, t, Ind–H, J = 7.6 Hz), 7.71 (1H, d, Ind–H, J = 8.0 Hz), 7.75 (1H, d, CH, J = 12.8 Hz), 8.30 (1H, d, Ind–H, J = 8.0 Hz); HR-MS (m/z): calcd for $C_{14}H_{17}N_3O$ 243.1372; found 244.1025 $[M + 1]^+$.

*5.1.18. (E)-3-(Dimethylamino)-1-(1-propyl-1H-indazol-3-yl)prop-2-en-1-one (**13**, R^1 = n-Pr)*

^{1}H NMR (DMSO-d_6) δ 0.85 (3H, t, CH_3, J = 7.2 Hz), 1.88 (2H, sext, CH_2, J = 7.2 Hz), 2.89 (3H, bs, CH_3), 3.14 (3H, bs, CH_3), 4.43 (2H, t, CH_2, J = 7.2 Hz), 6.04 (1H, d, CH, J = 12.8 Hz), 7.23 (1H, t, Ind–H, J = 7.6 Hz), 7.40 (1H, t, Ind–H, J = 7.6 Hz), 7.72 (1H, d, CH, J = 13.2 Hz), 7.73 (1H, d, Ind–H, J = 8.4 Hz), 8.30 (1H, d, Ind–H, J = 8.0 Hz); HR-MS (m/z): calcd for $C_{15}H_{19}N_3O$ 257.1528; found 258.0974 $[M + 1]^+$.

*5.1.19. (E)-3-(Dimethylamino)-1-(1-(pyridin-3-ylmethyl)-1H-indazol-3-yl)prop-2-en-1-one (**13**, R^1 = 3-methylpyrdine)*

^{1}H NMR (DMSO-d_6) δ 2.89 (3H, bs, CH_3), 3.14 (3H, bs, CH_3), 5.81 (2H, s, CH_2), 6.03 (1H, d, CH, J = 12.4 Hz), 7.25 (1H, t, Ind–H, J = 8.0 Hz), 7.34 (1H, dd, Pyr–H, J = 4.8, 3.2 Hz), 7.43 (t, 1H, Ind–H, J = 7.6 Hz), 7.61 (1H, dt, Pyr–H, J = 8.0, 2.0 Hz), 7.78 (2H, m, Ind–H, CH), 8.31 (1H, d, Ind–H, J = 8.0 Hz), 8.48 (1H, dd, Pyr–H, J = 3.2, 1.6 Hz), 8.48 (1H, d, Pyr–H, J = 1.6 Hz); HR-MS (m/z): calcd for $C_{18}H_{18}N_4O$ 306.1481; found 307.0366 $[M + 1]^+$.

*5.1.20. General procedure for the preparation of 4-(1H-indazol-3-yl)-N-phenylpyrimidin-2-amines (**14a**–**u**)*

A solution of **13** (1.0 mmol) and the appropriate phenylguanidines (2.0 mmol) in acetonitrile (3 mL) was heated at 140 °C for 40 min in a microwave reactor. The reaction mixture was purified by chromatography. 4-(4-(1-*Methyl*-1*H*-*indazol*-3-*yl*)*pyrimidin*-2-*ylamino*)*phenol* (**14a**) as a white solid (4% yield); mp 242–244 °C; ^{1}H NMR (DMSO-d_6) δ 4.16 (3H, s, CH_3), 6.75 (2H, d, Ph–H, J = 8.8 Hz), 7.25 (1H, t, Ind–H, J = 8.4 Hz), 7.37 (1H, d, Pyrim–H, J = 5.2 Hz), 7.48 (1H, t, Ind–H, J = 8.0 Hz), 7.52 (2H, d, Ph–H, J = 8.8 Hz), 7.73 (1H, d, Ind–H, J = 8.4 Hz), 8.42 (1H, d, Pyrim–H, J = 5.2 Hz), 8.64 (1H, d, Ind–H, J = 7.6 Hz), 9.09 (1H, s, OH), 9.28 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 35.95, 106.50, 110.17, 114.91, 121.69, 121.88, 121.93, 123.30, 126.52, 131.89, 139.87, 141.23, 152.61, 158.01, 160.11, 160.57; Anal. RP-HPLC: t_R 10.65 min (30–100% MeCN over 22 min, purity 100%); HR-MS (m/z): calcd for $C_{18}H_{15}N_5O$ 317.1277; found 316.1372 $[M - 1]^-$.

*5.1.21. 3-(4-(1-Methyl-1H-indazol-3-yl)pyrimidin-2-ylamino) phenol (**14b**)*

As a white solid (5% yield); mp 230–232 °C; ^{1}H NMR (DMSO-d_6) δ 4.17 (3H, s, CH_3), 6.42 (1H, dd, Ph–H, J = 8.0, 1.2 Hz), 7.10 (1H, t, Ph–H, J = 8.0 Hz), 7.23 (1H, d, Ph–H, J = 8.0 Hz), 7.29 (1H, t, Ind–H, J = 7.2 Hz), 7.35 (1H, t, Ph–H, J = 2.0 Hz), 7.46 (1H, d, Pyrim–H, J = 5.2 Hz), 7.50 (1H, t, Ind–H, J = 7.6 Hz), 7.73 (1H, d, Ind–H, J = 8.4 Hz), 8.50 (1H, d, Pyrim–H, J = 5.2 Hz), 8.76 (1H, d, Ind–H, J = 8.0 Hz), 9.27 (1H, s, NH), 9.51 (1H, s, OH); ^{13}C NMR (DMSO-d_6) δ 36.02, 106.61, 107.31, 108.86, 110.20, 110.41, 121.74, 122.09, 123.41, 126.60, 129.00, 139.76, 141.29, 141.55, 157.62, 157.94, 160.22; Anal. RP-HPLC: t_R 12.42 min (30–100% MeCN over 22 min, purity 100%); HR-MS (m/z): calcd for $C_{18}H_{15}N_5O$ 317.1277; found 317.9983 $[M + 1]^+$

*5.1.22. 4-(1-Methyl-1H-indazol-3-yl)-N-p-tolylpyrimidin-2-amine (**14c**)*

As an off-white solid (15% yield); mp 198–200 °C; ^{1}H NMR (DMSO-d_6) δ 4.17 (3H, s, CH_3), 7.15 (2H, d, Ph–H, J = 8.0 Hz), 7.29 (1H, t, Ind–H, J = 8.0 Hz), 7.44 (1H, d, Pyrim–H, J = 5.2 Hz), 7.49 (1H, t, Ind–H, J = 8.0 Hz), 7.69 (2H, d, Ph–H, J = 8.4 Hz), 7.74 (1H, d, Ind–H, J = 8.8 Hz), 8.49 (1H, d, Pyrim–H, J = 4.8 Hz), 8.70 (1H, d, Ind–H, J = 8.4 Hz), 9.52 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 20.41, 36.00, 107.11, 110.25, 119.55, 121.69, 122.03, 123.22, 126.57, 128.86, 130.45, 137.90, 139.78, 141.27, 158.02, 160.18, 160.26; Anal. RP-HPLC: t_R 16.08 min (30–100% MeCN over 22 min, purity 100%); HR-MS (m/z): calcd for $C_{19}H_{17}N_5$ 315.1484; found 316.0171 $[M + 1]^+$.

*5.1.23. 4-(1-Methyl-1H-indazol-3-yl)-N-m-tolylpyrimidin-2-amine (**14d**)*

As an off-white solid (18% yield); mp 183–185 °C; ^{1}H NMR (DMSO-d_6) δ 2.33 (3H, s, CH_3), 4.17 (3H, s, CH_3), 6.83 (1H, d, Ph–H, J = 7.2 Hz), 7.21 (1H, t, Ph–H, J = 8.0 Hz), 7.29 (1H, t, Ind–H, J = 7.6 Hz), 7.46 (1H, d, Pyrim–H, J = 5.2 Hz), 7.51 (2H, m, Ph–H, Ind–H), 7.74 (1H, d, Ind–H, J = 6.4 Hz), 7.75 (1H, s, Ph–H), 8.51 (1H, d, Pyrim–H, J = 5.2 Hz), 8.69 (1H, d, Ind–H, J = 8.0 Hz), 9.56 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 21.29, 36.03, 107.34, 110.31, 116.71, 119.96, 121.68, 122.06, 122.40, 123.09, 126.61, 128.32, 137.56, 139.80, 140.37, 141.32, 158.16, 160.10, 160.23; Anal. RP-HPLC: t_R 15.90 min (30–100% MeCN over 22 min, purity 100%); HR-MS (m/z): calcd for $C_{19}H_{17}N_5$ requires 315.1484; found 316.0157 $[M + 1]^+$.

*5.1.24. N-(4-Chlorophenyl)-4-(1-methyl-1H-indazol-3-yl) pyrimidin-2-amine (**14e**)*

As an off-white solid (25% yield); mp 194–197 °C; ^{1}H NMR (DMSO-d_6) δ 4.18 (3H, s, CH_3), 7.32 (1H, t, Ind–H, J = 8.0 Hz), 7.38 (2H, d, Ph–H, J = 8.8 Hz), 7.49 (1H, d, Pyrim–H, J = 5.2 Hz), 7.51 (1H, td, Ind–H, J = 7.6, 1.2 Hz), 7.76 (1H, d, Ind–H, J = 8.8 Hz), 7.86 (2H, d, Ph–H, J = 8.8 Hz), 8.53 (1H, d, Pyrim–H, J = 5.2 Hz), 8.69 (1H, d, Ind–H, J = 8.0 Hz), 9.78 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 36.05,

107.82, 110.34, 120.63, 121.70, 122.19, 123.08, 124.97, 126.64, 128.32, 139.54, 139.59, 141.31, 158.02, 159.91, 160.33; Anal. RP-HPLC: t_R 18.17 min (30–100% MeCN over 22 min, purity 96%); HRMS (*m*/*z*): calcd for $C_{18}H_{14}ClN_5$ requires 335.0938; found 335.9240 $[M + 1]^+$.

5.1.25. N-(3-Chlorophenyl)-4-(1-methyl-1H-indazol-3-yl) pyrimidin-2-amine (**14f**)

As an off-white crystalline solid (19% yield); mp 177–180°; ^{1}H NMR (DMSO-d_6) δ 4.18 (3H, s, CH_3), 7.04 (1H, ddd, Ph–H, J = 7.6, 2.0, 0.8 Hz), 7.32 (1H, t, Ind–H, J = 8.0 Hz), 7.35 (1H, t, Ph–H, J = 8.0 Hz), 7.51 (1H, t, Ind–H, J = 8.0 Hz), 7.53 (1H, d, Pyrim–H, J = 5.2 Hz), 7.64 (1H, dd, Ph–H, J = 8.4, 1.2 Hz), 7.76 (1H, d, Ph–H, J = 8.4 Hz), 8.16 (1H, t, Ph–H, J = 2.0 Hz), 8.56 (1H, d, Pyrim–H, J = 5.2 Hz), 8.70 (1H, d, Ind–H, J = 8.0 Hz), 9.87 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 36.07, 108.11, 110.37, 117.51, 118.34, 121.01, 121.64, 122.26, 122.94, 126.66, 130.11, 133.06, 139.57, 141.34, 142.08, 158.15, 159.82, 160.25; Anal. RP-HPLC: t_R 18.55 min (30–100% MeCN over 22 min, purity 98%); HR-MS (*m*/*z*): calcd for $C_{18}H_{14}ClN_5$ 335.0938; found 335.9348 $[M + 1]^+$.

5.1.26. 4-(1-Methyl-1H-indazol-3-yl)-N-(4-nitrophenyl)pyrimidin-2-amine (**14g**)

As a bright yellow solid (48% yield); mp 266–269 °C; ^{1}H NMR (DMSO-d_6) δ 4.19 (3H, s, CH_3), 7.36 (1H, t, Ind–H, J = 8.0 Hz), 7.52 (1H, t, Ind–H, J = 8.0 Hz), 7.64 (1H, d, Pyrim–H, J = 5.2 Hz), 7.78 (1H, d, Ind–H, J = 8.8 Hz), 8.11 (2H, d, Ph–H, J = 9.6 Hz), 8.25 (2H, d, Ph–H, J = 9.6 Hz), 8.65 (1H, d, Pyrim–H, J = 5.2 Hz), 8.73 (1H, d, Ind–H, J = 8.0 Hz), 10.44 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 36.10, 109.31, 110.42, 117.81, 121.72, 122.38, 122.96, 124.97, 126.72, 139.28, 140.40, 141.34, 147.22, 158.04, 159.30, 160.55; Anal. RP-HPLC: t_R 18.15 min (30–100% MeCN over 22 min, purity 98%); HR-MS (*m*/*z*): calcd for $C_{18}H_{14}N_6O_2$ 346.1178; found 346.9664 $[M + 1]^+$.

5.1.27. 4-(1-Methyl-1H-indazol-3-yl)-N-(3-nitrophenyl)pyrimidin-2-amine (**14h**)

As a bright yellow solid (68% yield); mp 248–251 °C; ^{1}H NMR (DMSO-d_6) δ 4.19 (3H, s, CH_3), 7.30 (1H, t, Ind–H, J = 7.6 Hz), 7.52 (1H, t, Ind–H, J = 8.0 Hz), 7.59 (1H, d, Pyrim–H, J = 5.2 Hz), 7.63 (1H, t, Ph–H, J = 8.0 Hz), 7.77 (1H, d, Ind–H, J = 8.4 Hz), 7.84 (1H, ddd, Ph–H, J = 8.0, 2.4, 0.8 Hz), 8.17 (1H, ddd, Ph–H, J = 8.4, 2.0, 0.8 Hz), 8.62 (1H, d, Pyrim–H, J = 5.2 Hz), 8.70 (1H, d, Ind–H, J = 8.0 Hz), 8.90 (1H, t, Ph–H, J = 2.0 Hz), 10.17 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 36.09, 108.60, 110.36, 112.68, 115.76, 121.68, 122.26, 123.00, 124.92, 126.68, 129.81, 139.41, 141.34, 141.88, 148.17, 158.13, 159.68, 160.45; Anal. RP-HPLC: t_R 17.32 min (30–100% MeCN over 22 min, purity 100%); HR-MS (*m*/*z*): calcd for $C_{18}H_{14}N_6O_2$ requires 346.1178; found 346.9461 $[M + 1]^+$.

5.1.28. 4-(4-(1-Methyl-1H-indazol-3-yl)pyrimidin-2-ylamino) benzenesulfonamide (**14i**)

As a white solid (43% yield); mp 240–242 °C; ^{1}H NMR (DMSO-d_6) δ 4.19 (3H, s, CH_3), 7.20 (2H, s, NH_2), 7.34 (1H, t, Ind–H, J = 7.6 Hz), 7.52 (1H, t, Ind–H, J = 7.6 Hz), 7.56 (1H, d, Pyrim–H, J = 5.2 Hz), 7.77 (3H, m, Ph–H, Ind–H), 8.00 (1H, d, Ph–H, J = 8.8 Hz), 8.59 (1H, d, Pyrim–H, J = 5.2 Hz), 8.72 (1H, d, Ind–H, J = 8.4 Hz), 10.06 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 36.08, 108.44, 110.40, 118.15, 121.71, 122.24, 123.04, 126.53, 126.68, 136.28, 139.50, 141.34, 143.67, 158.09, 159.72, 160.39; Anal. RP-HPLC: t_R 12.32 min (30–100% MeCN over 22 min, purity 100%); HR-MS (*m*/*z*): calcd for $C_{18}H_{16}N_6O_2S$ 380.1055; found 380.8972 $[M + 1]^+$.

5.1.29. 3-(4-(1-Methyl-1H-indazol-3-yl)pyrimidin-2-ylamino) benzenesulfonamide (**14j**)

As a white solid (13% yield); mp 231–234 °C; ^{1}H NMR (DMSO-d_6) δ 4.18 (3H, s, CH_3), 7.32 (3H, m, NH_2, Ind–H), 7.47 (1H, td, Ind–H, J = 7.6, 1.2 Hz), 7.51–7.55 (3H, m, Ph–H, Pyrim–H), 7.75 (1H, d, Ind–H, J = 8.8 Hz), 8.06 (1H, d, Ph–H, J = 8.0 Hz), 8.32 (1H, s, Ph–H), 8.56 (1H, d, Pyrim–H, J = 5.2 Hz), 8.74 (1H, d, Ind–H, J = 8.4 Hz), 9.97 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 36.06, 108.07, 110.24, 115.91, 118.51, 121.74, 121.92, 122.32, 123.34, 126.64, 129.12, 139.49, 140.97, 141.30, 144.55, 157.98, 159.92, 160.47; Anal. RP-HPLC: t_R 12.52 min (30–100% MeCN over 22 min, purity 100%); HR-MS (*m*/*z*): calcd for $C_{18}H_{16}N_6O_2S$ 380.1055; found 379.0844 $[M - 1]^-$.

5.1.30. 4-(4-(1-Ethyl-1H-indazol-3-yl)pyrimidin-2-ylamino) benzenesulfonamide (**14k**)

As a white solid (50% yield); mp 229–232 °C; ^{1}H NMR (DMSO-d_6) δ 1.49 (3H, t, CH_3, J = 7.2 Hz), 4.58 (2H, q, CH_2, J = 7.2 Hz), 7.20 (2H, s, NH_2), 7.33 (1H, td, Ind–H, J = 8.0, 0.8 Hz), 7.51 (1H, td, Ind–H, J = 7.2, 0.8 Hz), 7.58 (1H, d, Pyrim–H, J = 5.2 Hz), 7.78 (2H, d, Ph–H, J = 8.8 Hz), 7.81 (1H, d, Ind–H, J = 8.8 Hz), 8.01 (2H, d, Ph–H, J = 8.8 Hz), 8.59 (1H, d, Pyrim–H, J = 5.2 Hz), 8.72 (1H, d, Ind–H, J = 8.0 Hz), 10.06 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 14.80, 43.82, 108.51, 110.30, 118.13, 121.81, 122.24, 123.13, 126.52, 126.61, 136.26, 139.61, 140.45, 143.67, 158.07, 159.71, 160.42; Anal. RP-HPLC: t_R 13.48 min (30–100% MeCN over 22 min, purity 100%); HR-MS (*m*/*z*): calcd for $C_{19}H_{18}N_6O_2S$ 394.1212; found 394.8860 $[M + 1]^+$.

5.1.31. 3-(4-(1-Ethyl-1H-indazol-3-yl)pyrimidin-2-ylamino) benzenesulfonamide (**14l**)

As a white crystalline solid (16% yield); mp 219–222 °C; ^{1}H NMR (DMSO-d_6) δ 1.48 (3H, t, CH_3, J = 7.2 Hz), 4.57 (2H, q, CH_2, J = 7.2 Hz), 7.32 (3H, m, NH_2, Ind–H), 7.47 (2H, t, Ind–H, Ph–H, J = 8.0 Hz), 7.52 (1H, d, Ph–H, J = 8.0 Hz), 7.55 (1H, d, Pyrim–H, J = 5.2 Hz), 7.79 (1H, d, Ind–H, J = 8.8 Hz), 8.07 (1H, d, Ph–H, J = 8.4 Hz), 8.33 (1H, s, Ph–H), 8.56 (1H, d, Pyrim–H, J = 5.2 Hz), 8.74 (1H, d, Ind–H, J = 8.0 Hz), 9.98 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 14.81, 43.81, 108.16, 110.14, 115.90, 118.50, 121.86, 121.91, 122.34, 123.45, 126.59, 129.12, 139.62, 140.42, 140.98, 144.55, 157.96, 159.92, 160.52; Anal. RP-HPLC: t_R 13.35 min (30–100% MeCN over 22 min, purity 100%); HR-MS (*m*/*z*): calcd for $C_{19}H_{18}N_6O_2S$ requires 394.1212; found 394.8616 $[M + 1]^+$.

5.1.32. 4-(1-Ethyl-1H-indazol-3-yl)-N-(4-nitrophenyl)pyrimidin-2-amine (**14m**)

As a bright yellow solid (37% yield); mp 227–229 °C; ^{1}H NMR (DMSO-d_6) δ 1.49 (3H, t, CH_3, J = 7.2 Hz), 4.58 (2H, q, CH_2, J = 7.2 Hz), 7.35 (1H, td, Ind–H, J = 8.0, 0.8 Hz), 7.51 (1H, td, Ind–H, J = 8.0, 0.8 Hz), 7.64 (1H, d, Pyrim–H, J = 5.2 Hz), 7.81 (1H, d, Ind–H, J = 8.4 Hz), 8.10 (2H, d, Ph–H, J = 9.6 Hz), 8.23 (2H, d, Ph–H, J = 9.2 Hz), 8.63 (1H, d, Pyrim–H, J = 5.2 Hz), 8.72 (1H, d, Ind–H, J = 8.0 Hz), 10.43 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 14.78, 43.85, 109.38, 110.31, 117.79, 121.83, 122.38, 123.06, 124.95, 126.65, 139.40, 140.38, 140.46, 147.22, 157.99, 159.29, 160.58; Anal. RP-HPLC: t_R 19.40 min (30–100% MeCN over 22 min, purity 100%); HR-MS (*m*/*z*): calcd for $C_{19}H_{16}N_6O_2$ requires 360.1335; found 359.1286 $[M - 1]^-$.

5.1.33. 4-(1-Ethyl-1H-indazol-3-yl)-N-(3-nitrophenyl)pyrimidin-2-amine (**14n**)

As a bright yellow solid (67% yield); mp 217–220 °C; ^{1}H NMR (DMSO-d_6) δ 1.49 (3H, t, CH_3, J = 7.2 Hz), 4.58 (2H, q, CH_2, J = 7.2 Hz), 7.27 (1H, td, Ind–H, J = 8.0, 0.8 Hz), 7.50 (1H, td, Ind–H, J = 8.0, 1.2 Hz), 7.59 (1H, d, Pyrim–H, J = 5.2 Hz), 7.63 (1H, d, Ph–H, J = 8.4 Hz), 7.81 (1H, d, Ind–H, J = 8.4 Hz), 7.84 (1H, ddd, Ph–H, J = 8.0, 2.4, 0.8 Hz), 8.17 (1H, ddd, Ph–H, J = 8.0, 2.4, 0.8 Hz), 8.61 (1H, d, Pyrim–H, J = 5.2 Hz), 8.70 (1H, d, Ind–H, J = 8.0 Hz), 8.91 (1H, t, Ph–H, J = 2.0 Hz), 10.17 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 14.80, 43.83, 108.68, 110.26, 112.67, 115.74, 121.79, 122.28, 123.09, 124.91, 126.62, 129.80, 139.54, 140.46, 141.89, 148.17, 158.11, 159.68, 160.49; Anal. RP-HPLC: t_R 18.53 min (30–100% MeCN over 22 min, purity 100%); HR-MS (*m*/*z*): calcd for $C_{19}H_{16}N_6O_2$ requires 360.1335; found 360.9510 $[M + 1]^+$.

*5.1.34. 4-(4-(1-Ethyl-1H-indazol-3-yl)pyrimidin-2-ylamino) phenol (**14o**)*

As a white solid (30% yield); mp 222–225 °C; ^{1}H NMR (DMSO-d_6) δ 1.47 (3H, t, CH_3, J = 7.2 Hz), 4.55 (2H, q, CH_2, J = 7.2 Hz), 6.76 (2H, d, Ph–H, J = 8.8 Hz), 7.24 (1H, t, Ind–H, J = 7.6 Hz), 7.38 (1H, d, Pyrim–H, J = 5.2 Hz), 7.47 (1H, td, Ind–H, J = 8.0, 0.8 Hz), 7.52 (2H, d, Ph–H, J = 8.8 Hz), 7.77 (1H, d, Ind–H, J = 8.8 Hz), 8.42 (1H, d, Pyrim–H, J = 5.2 Hz), 8.63 (1H, d, Ind–H, J = 8.0 Hz), 9.09 (1H, s, OH), 9.28 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 14.80, 43.72, 106.61, 110.09, 114.93, 121.84, 121.91, 123.43, 126.48, 131.93, 140.01, 140.37, 152.62, 158.01, 160.18, 160.59; Anal. RP-HPLC: t_R 11.34 min (30–100% MeCN over 22 min, purity 100%); HR-MS (m/z): calcd for $C_{19}H_{17}N_5O$ requires 331.1433; found 332.0010 $[M + 1]^+$.

*5.1.35. 3-(4-(1-Ethyl-1H-indazol-3-yl)pyrimidin-2-ylamino) phenol (**14p**)*

As a white solid (23% yield); mp 214–216 °C; ^{1}H NMR (DMSO-d_6) δ 1.47 (3H, t, CH_3, J = 7.2 Hz), 4.56 (2H, q, CH_2, J = 7.2 Hz), 6.43 (1H, ddd, Ph–H, J = 8.0, 2.4, 0.8 Hz), 7.10 (1H, t, Ph–H, J = 8.0 Hz), 7.23 (1H, d, Ph–H, J = 8.0 Hz), 7.28 (1H, td, Ind–H, J = 7.2, 0.8 Hz), 7.36 (1H, t, Ph–H, J = 2.0 Hz), 7.46 (1H, d, Pyrim–H, J = 5.2 Hz), 7.48 (1H, td, Ind–H, J = 7.6, 1.2 Hz), 7.77 (1H, d, Ind–H, J = 8.4 Hz), 8.50 (1H, d, Pyrim–H, J = 5.2 Hz), 8.76 (1H, d, Ind–H, J = 8.4 Hz), 9.27 (1H, s, OH), 9.52 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 14.81, 43.76, 106.58, 107.38, 108.84, 110.09, 110.38, 121.85, 122.10, 123.51, 126.54, 128.99, 139.87, 140.41, 141.56, 157.62, 157.91, 160.21, 160.27; Anal. RP-HPLC: t_R 13.19 min (30–100% MeCN over 22 min, purity 100%); HR-MS (m/z): calcd for $C_{19}H_{17}N_5O$ 331.1433; found 331.9904 $[M + 1]^+$.

*5.1.36. 4-(4-(1-Propyl-1H-indazol-3-yl)pyrimidin-2-ylamino) phenol (**14q**)*

As a white solid (19% yield); mp 203–206 °C; ^{1}H NMR (DMSO-d_6) δ 0.87 (3H, t, CH_3, J = 7.2 Hz), 1.91 (2H, sext, CH_2, J = 7.2 Hz), 4.48 (2H, t, CH_2, J = 7.2 Hz), 6.76 (2H, d, Ph–H, J = 8.8 Hz), 7.24 (1H, t, Ind–H, J = 7.6 Hz), 7.38 (1H, d, Pyrim–H, J = 5.2 Hz), 7.46 (1H, td, Ind–H, J = 8.0, 0.8 Hz), 7.52 (2H, d, Ph–H, J = 8.8 Hz), 7.77 (1H, d, Ind–H, J = 8.8 Hz), 8.42 (1H, d, Pyrim–H, J = 5.2 Hz), 8.64 (1H, d, Ind–H, J = 8.0 Hz), 9.09 (1H, s, OH), 9.28 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 11.09, 22.80, 50.16, 106.61, 110.18, 114.91, 121.67, 121.86, 121.92, 123.38, 126.48, 131.92, 140.03, 140.99, 152.62, 158.01, 160.18, 160.58; Anal. RP-HPLC: t_R 15.10 min (30–100% MeCN over 22 min, purity 96%); HR-MS (m/z): calcd for $C_{20}H_{19}N_5O$ requires 345.1590; found 345.9887 $[M + 1]^+$.

*5.1.37. 4-(4-(1-Propyl-1H-indazol-3-yl)pyrimidin-2-ylamino) benzenesulfonamide (**14r**)*

As a white solid (55% yield); mp 190–192 °C; ^{1}H NMR (DMSO-d_6) δ 0.89 (3H, t, CH_3, J = 7.2 Hz), 1.93 (2H, sext, CH_2, J = 7.2 Hz), 4.51 (2H, t, CH_2, J = 6.8 Hz), 7.20 (2H, s, NH_2), 7.33 (1H, td, Ind–H, J = 7.6, 0.8 Hz), 7.50 (1H, td, Ind–H, J = 7.6, 0.8 Hz), 7.57 (1H, d, Pyrim–H, J = 5.2 Hz), 7.78 (2H, d, Ph–H, J = 8.8 Hz), 7.82 (1H, d, Ind–H, J = 8.4 Hz), 8.01 (2H, d, Ph–H, J = 9.2 Hz), 8.59 (1H, d, Pyrim–H, J = 5.2 Hz), 8.72 (1H, d, Ind–H, J = 8.4 Hz), 10.05 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 11.08, 22.89, 50.28, 108.59, 110.43, 118.17, 121.65, 122.23, 123.15, 126.54, 136.27, 139.65, 141.15, 143.72, 158.14, 159.69, 160.51; Anal. RP-HPLC: t_R 14.90 min (30–100% MeCN over 22 min, purity 100%); HR-MS (m/z): calcd for $C_{20}H_{20}N_6O_2S$ requires 408.1368; found 408.8520 $[M + 1]^+$.

*5.1.38. 3-(4-(1-Propyl-1H-indazol-3-yl)pyrimidin-2-ylamino) benzenesulfonamide (**14s**)*

As a white solid (37% yield); mp 172–174 °C; ^{1}H NMR (DMSO-d_6) δ 0.88 (3H, t, CH_3, J = 7.2 Hz), 1.93 (2H, sext, CH_2, J = 7.2 Hz), 4.50 (2H, t, CH_2, J = 7.2 Hz), 7.32 (3H, m, NH_2, Ind–H), 7.45–7.51 (3H, m, Ind–H, Ph–H), 7.55 (1H, d, Pyrim–H, J = 5.2 Hz), 7.80 (1H, d, Ind–H, J = 8.4 Hz), 8.07 (1H, ddd, Ph–H, J = 8.0, 2.4, 1.2 Hz), 8.33 (1H, t, Ph–H, J = 2.0 Hz), 8.56 (1H, d, Pyrim–H, J = 5.2 Hz), 8.74 (1H, d, Ind–H, J = 8.0 Hz), 9.97 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 11.08, 22.79, 50.22, 108.14, 110.20, 115.88, 118.47, 121.68, 121.89, 122.28, 123.39, 126.57, 129.09, 139.61, 140.95, 141.02, 144.52, 157.95, 159.89, 160.50; Anal. RP-HPLC: t_R 14.97 min (30–100% MeCN over 22 min, purity 100%); HR-MS (m/z): calcd for $C_{20}H_{20}N_6O_2S$ requires 408.1368; found 408.8440 $[M + 1]^+$.

*5.1.39. 4-(4-(1-(Pyridin-3-ylmethyl)-1H-indazol-3-yl)pyrimidin-2-ylamino)benzene-sulfonamide (**14t**)*

As a white solid (46% yield); mp 247–250 °C; ^{1}H NMR (DMSO-d_6) δ 5.87 (2H, s, CH_2), 7.20 (2H, s, NH_2), 7.33–7.37 (2H, m, Ind–H, Pyr–H), 7.53 (1H, t, Ind–H, J = 8.0 Hz), 7.58 (1H, d, Pyrim–H, J = 5.2 Hz), 7.70 (1H, d, Pyr–H, J = 7.6 Hz), 7.78 (2H, d, Ph–H, J = 8.8 Hz), 7.92 (1H, d, Ind–H, J = 8.4 Hz), 8.00 (2H, d, Ph–H, J = 8.8 Hz), 8.50 (1H, d, Pyr–H, J = 4.8 Hz), 8.60 (1H, d, Pyrim–H, J = 5.2 Hz), 8.64 (1H, d, Pyr–H, J = 2.0 Hz), 8.73 (1H, d, Ind–H, J = 8.0 Hz), 10.08 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 50.40, 109.19, 110.95, 118.69, 122.41, 123.04, 123.76, 124.28, 127.05, 127.64, 132.96, 135.85, 136.82, 141.10, 141.55, 144.08, 149.36, 149.59, 158.77, 160.22, 160.67; Anal. RP-HPLC: t_R 11.67 min (30–100% MeCN over 22 min, purity 100%); HRMS (m/z): calcd for $C_{23}H_{19}N_7O_2S$ 457.1321; found 457.7601 $[M + 1]^+$.

*5.1.40. 3-(4-(1-(Pyridin-3-ylmethyl)-1H-indazol-3-yl)pyrimidin-2-ylamino)benzene-sulfonamide (**14u**)*

As a white solid (43%); mp 226–229 °C; ^{1}H NMR (DMSO-d_6) δ 5.86 (2H, s, CH_2), 7.33–7.37 (4H, m, NH_2, Ind–H, Pyr–H), 7.45–7.55 (3H, m, Ind–H, Ph–H), 7.56 (1H, d, Pyrim–H, J = 5.2 Hz), 7.69 (1H, dt, Pyr–H, J = 8.4, 2.0, 1.6 Hz), 7.89 (1H, d, Ind–H, J = 8.4 Hz), 8.06 (1H, ddd, Ph–H, J = 8.0, 2.0, 1.2 Hz), 8.33 (1H, t, Ph–H, J = 2.0 Hz), 8.49 (1H, dd, Pyr–H, J = 4.8, 1.6 Hz), 8.57 (1H, d, Pyrim–H, J = 5.2 Hz), 8.64 (1H, d, Pyr–H, J = 2.0 Hz), 8.76 (1H, d, Ind–H, J = 8.0 Hz), 9.99 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 49.89, 108.33, 110.27, 115.94, 118.57, 121.98, 122.65, 123.57, 123.79, 127.12, 129.14, 132.50, 135.34, 140.62, 140.93, 141.00, 144.56, 148.83, 149.07, 158.16, 159.93, 160.24; Anal. RP-HPLC: t_R 11.78 min (30–100% MeCN over 22 min, purity 100%); HR-MS (m/z): calcd for $C_{23}H_{19}N_7O_2S$ requires 457.1321; found 456.1268 $[M - 1]^-$.

*5.1.41. 1-(2-Chloropyrimidin-4-yl)-1H-indazole (**17**)*

A solution of 1*H*-indazole **15** (590.5 mg, 5.0 mmol) in dry DMF (15 mL) cooled to 0 °C was treated with NaH (200 mg, 5.0 mmol, 60% in mineral oil). After stirring at r.t. for 2 h the mixture was cooled to 0 °C and added 2,4-dichloropyrimidine **16** (740 mg, 5.0 mmol). After stirring for 4 h the mixture was quenched with water and extracted with ethyl acetate, dried over $MgSO_4$ and evaporated. The residue was purified by column chromatography (Pet/EtOAc 20/1) to afford the product as a white solid 250 mg, 26% yield; ^{1}H NMR ($CDCl_3$) δ 7.39 (1H, t, Ind–H, J = 8.0 Hz), 7.62 (1H, t, Ind–H, J = 8.0 Hz), 7.79 (1H, t, Ind–H, J = 8.0, 0.8 Hz), 7.92 (1H, d, Pyrim–H, J = 5.6 Hz), 8.27 (1H, s, Ind–H), 8.57 (1H, d, Pyrim–H, J = 5.6 Hz), 8.81 (1H, dd, Ind–H, J = 8.4, 0.8 Hz); HR-MS (m/z): calcd for $C_{11}H_7ClN_4$ 230.0359; found 231.0358 $[M + 1]^+$.

*5.1.42. General procedure for the preparation of 4-(1H-indazol-1-yl)-N-phenylpyrimidin-2-amines (**18a**,**b**)*

A mixture of **17** (0.43 mmol) and appropriate aniline (0.47 mmol) in ethanol (3 mL) was reacted at 140 °C for 40 min in a microwave reactor The reaction mixture was filtered and the crude product was washed with methanol. The pure compound was obtained by recrystallisation from a mixture of DMF and acetone. 4-(4-(1*H*-*Indazol*-1-*yl*)*pyrimidin*-2-*ylamino*)*benzenesulfonamide* (**18a**) as a white crystalline solid (16% yield); mp 304–307 °C; ^{1}H NMR

(DMSO-d_6) δ 7.24 (2H, bs, NH_2), 7.41 (1H, t, Ind–H, J = 7.2 Hz), 7.46 (1H, d, Pyrim–H, J = 5.6 Hz), 7.62 (1H, t, Ind–H, J = 7.2 Hz), 7.81 (2H, d, Ph–H, J = 8.8 Hz), 7.94 (1H, d, Ind–H, J = 8.0 Hz), 7.97 (2H, d, Ph–H, J = 8.8 Hz), 8.57 (1H, s, Ind–H), 8.59 (1H, d, Pyrim–H, J = 5.6 Hz), 8.93 (1H, d, Ind–H, J = 8.4 Hz), 10.33 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 100.82, 115.99, 118.84, 121.59, 123.67, 126.27, 126.60, 128.67, 136.93, 138.24, 139.69, 143.15, 158.84, 158.92, 159.55; Anal. RP-HPLC: t_R 13.37 min (30–100% MeCN over 22 min, purity 100%); HR-MS (m/z): calcd for $C_{17}H_{14}N_6O_2S$ requires 366.0899; found 366.8807 $[M + 1]^+$.

*5.1.43. 3-(4-(1H-Indazol-1-yl)pyrimidin-2-ylamino) benzenesulfonamide (**18b**)*

As a white solid (17% yield); mp 295–297 °C; ^{1}H NMR (DMSO-d_6) δ 7.40 (2H, bs, NH_2), 7.40 (1H, t, Ind–H, J = 7.6 Hz), 7.44 (1H, d, Pyrim–H, J = 5.6 Hz), 7.56 (3H, m, Ind–H, Ph–H), 7.93 (1H, d, Ph–H, J = 7.6 Hz), 8.03 (1H, d, Ind–H, J = 8.0 Hz), 8.29 (1H, s, Ph–H), 8.56 (1H, s, Ind–H), 8.56 (1H, d, Pyrim–H, J = 5.6 Hz), 8.95 (1H, d, Ind–H, J = 8.4 Hz), 10.19 (1H, s, NH); ^{13}C NMR (DMSO-d_6) δ 100.50, 116.11, 116.91, 119.52, 121.57, 122.97, 123.79, 126.30, 128.90, 129.37, 138.25, 139.98, 140.11, 144.70, 157.89, 158.51, 159.78; Anal. RP-HPLC: t_R 13.19 min (30–100% MeCN over 22 min, purity 100%); HR-MS (m/z): calcd for $C_{17}H_{14}N_6O_2S$ 366.0899; found 336.8809 $[M + 1]^+$.

*5.1.44. 2-Chloro-N-methoxy-N-methylnicotinamide (**20**) [13]*

White solid (93% yield); mp 92–93 °C; ^{1}H NMR (DMSO-d_6) δ 8.49 (d, 1H, J = 2.8 Hz, Pyr–H), 8.00 (dd, 1H, J = 6 Hz, Pyr–H), 7.53 (q, 1H, Pyr–H), 3.47 (s, 3H, $NOCH_3$), 3.30 (s, 3H, NCH_3). ^{13}C NMR (DMSO-d_6) δ 169.8, 151.3, 148.2, 138.4, 132.5, 122.4, 61.1, 31.4. HR-MS (m/z): calcd for $C_8H_9ClN_2O_2$ 201.0353; found 201.0418.

*5.1.45. 1-(2-Chloropyridin-3-yl)ethanone (**21**, R^1 = Me) [13] as an orange liquid (99% yield)*

^{1}H NMR (DMSO-d_6) δ 8.54 (dd, 1H, J = 2.8 Hz, Pyr–H), 8.17 (dd, 1H, J = 5.6 Hz, Pyr–H), 7.57 (q, 1H, Pyr–H), 2.62 (s, 3H, $COCH_3$). ^{13}C NMR (DMSO-d_6) δ 200.1, 151.3, 145.5, 139.2, 135.4, 122.4, 30.1. HR-MS (m/z): calcd for C_7H_6ClNO 156.0138, found 156.0248.

*5.1.46. 1-(2-chloropyridin-3-yl)propan-1-one (**21**, R^1 = Et) as a yellow liquid (99% yield)*

^{1}H NMR (DMSO-d_6) δ 8.53 (dd, 1H, J = 2.8 Hz, Pyr–H), 8.20 (dd, 1H, J = 5.6 Hz, Pyr–H), 7.58 (q, 1H, Pyr–H), 3.11 (q, 2H, J = 7.6 Hz, $COCH_2$), 1.50 (t, 3H, J = 7.6 Hz, CH_3). ^{13}C NMR (DMSO-d_6) δ 200.3, 152.1, 148.4, 139.4, 135.5, 122.8, 30.3, 7.1. HR-MS (m/z): calcd for C_8H_8ClNO 170.0294; found 170.1243.

*5.1.47. 3-Methyl-1H-pyrazolo[3,4-b]pyridine (**22**, R^1 = Me) as a white solid (98% yield)*

Mp 160–161 °C; ^{1}H NMR (DMSO-d_6) δ 13.18 (bs, 1H, NH), 8.48 (dd, 1H, J = 2.8 Hz, Pyridine–H), 8.19 (dd, 1H, J = 7.2 Hz, Pyridine–H), 7.14 (q, 1H, Pyridine–H), 2.50 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 152.5, 149.4, 142.3, 129.3, 115.2, 113.1, 13.3. HR-MS (m/z): calcd for $C_7H_7N_3$134.0640; found 134.0680.

*5.1.48. 3-Ethyl-1H-pyrazolo[3,4-b]pyridine (**22**, R^1 = Et) as an orange liquid (98% yield)*

^{1}H NMR (DMSO-d_6) δ 13.21 (bs, 1H, NH), 8.46 (dd, 1H, J = 2.8 Hz, Pyr–H), 8.17 (dd, 1H, J = 6.4 Hz, Pyr–H), 7.09 (q, 1H, Pyr–H), 2.89 (q, 2H, J = 7.6 Hz, CH_2), 1.28 (t, 3H, J = 7.6 Hz, CH_3). ^{13}C NMR (DMSO-d_6) δ 153.4, 152.3, 148.1, 130.2, 116.3, 114.5, 21.6, 14.7. HR-MS (m/z): calcd for $C_8H_9N_3$148.0796 $[M+H]^+$, found 148.0885.

*5.1.49. 1H-pyrazolo[3,4-b]pyridine (**22**, R^1 = H) as a dark orange solid (98% yield)*

Mp 110–111 °C; ^{1}H NMR (DMSO-d_6) δ 13.65 (bs, 1H, NH), 8.51 (dd, 1H, J = 2.8 Hz, Pyr–H), 8.22 (dd, 1H, J = 6.4 Hz, Pyr–H), 8.14 (bs, 1H, Pyr–H), 7.16 (q, 1H, Pyr–H). ^{13}C NMR (DMSO-d_6) δ 152.5, 148.4, 131.4, 130.2, 117.3, 114.5. HR-MS (m/z): calcd for $C_6H_5N_3$ 120.0483, found 120.0439.

*5.1.50. 1-(2-(Methylthio)pyrimidin-4-yl)-1H-pyrazolo[3,4-b] pyridine (**23**, R^1 = H) as a yellow solid (9.6% yield)*

Mp 118–119 °C; ^{1}H NMR (DMSO-d_6) δ 8.77 (dd, 1H, J = 3.6 Hz, Pyridine–H), 8.76 (d, 1H, J = 5.2 Hz, Pyr–H), 8.63 (bs, 1H, Pyr–H), 8.45 (dd, 1H, J = 6.4 Hz, Pyr–H), 8. 27 (d, 1H, J = 5.6 Hz, Pyr–H), 7.49 (q, 1H, Pyr–H), 2.63 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 172.5, 172.1, 160.2, 158.4, 156.6, 149.7, 132.5, 127.9, 119.3, 108.3, 15.3. HR-MS (m/z): calcd for $C_{11}H_9N_5S$ 244.0578; found 244.0623.

*5.1.51. 3-Methyl-1-(2-(methylthio)pyrimidin-4-yl)-1H-pyrazolo [3,4-b]pyridine (**23**, R^1 = Me) as a yellow solid (26% yield)*

Mp 139–140 °C; ^{1}H NMR (DMSO-d_6) δ 8.74 (dd, 1H, J = 3.2 Hz, Pyridine–H), 8.71 (d, 1H, J = 5.6 Hz, Pyr–H), 8.42 (dd, 1H, J = 6 Hz, Pyr–H), 8.23 (d, 1H, J = 5.6 Hz, Pyr–H), 7.47 (q, 1H, Pyr–H), 2.63 (d, 6H, J = 2.8 Hz, CH_3, SCH_3). ^{13}C NMR (DMSO-d_6) δ 172.7, 173.4, 158.6, 157.5, 148.4, 131.3, 129.2, 119.5, 118.3, 105.3, 15.1, 14.2. HR-MS (m/z): calcd for $C_{12}H_{11}N_5S$ 258.0735; found 258.0770.

*5.1.52. 3-Ethyl-1-(2-(methylthio)pyrimidin-4-yl)-1H-pyrazolo[3,4-b]pyridine (**23**, R^1 = Et) as a yellow solid (38% yield)*

Mp 92–93 °C; ^{1}H NMR (DMSO-d_6) δ 8.72 (dd, 1H, J = 1.6 Hz, Pyr–H), 8.71 (d, 1H, J = 5.6 Hz, Pyr–H), 8.45 (dd, 1H, J = 6.4 Hz, Pyr–H), 8.21 (d, 1H, J = 6 Hz, Pyr–H), 7.45 (q, 1H, Pyr–H), 3.05 (q, 2H, J = 7.6 Hz, CH_2), 2.62 (s, 3H, SCH_3), 1.38 (t, 3H, J = 7.6 Hz, CH_3). ^{13}C NMR (DMSO-d_6) δ 172.5, 172.4, 158.3, 157.2, 149.4, 143.2, 130.1, 118.2, 116.3, 105.3, 20.1, 14.3, 13.2. HR-MS (m/z): calcd for $C_{13}H_{13}N_5S$ 272.0892; found 272.0887.

*5.1.53. 1-(2-(Methylsulfonyl)pyrimidin-4-yl)-1H-pyrazolo[3,4-b] pyridine (**24**, R^1 = H) as a yellow solid (84% yield)*

Mp 170–171 °C; ^{1}H NMR (DMSO-d_6) δ 9.16 (d, 1H, J = 6, Pyr–H), 8.89 (d, 1H, J = 5.6 Hz, Pyr–H), 8.75 (dd, 1H, J = 2.8 Hz, Pyr–H), 8.61 (bs, 1H, Pyr–H), 8.51 (dd, 1H, J = 6.4 Hz, Pyr–H), 7.48 (q, 1H, Pyr–H), 3.51 (s, 3H, SO_2CH_3). ^{13}C NMR (DMSO-d_6) δ 174.5, 168.8, 159.3, 156.4, 149.1, 132.6, 127.2, 117.3, 115.2, 107.4, 43.3. HR-MS (m/z): calcd for $C_{11}H_9N_5O_2S$ 276.0477; found 276.0522.

*5.1.54. 3-Methyl-1-(2-(methylsulfonyl)pyrimidin-4-yl)-1H-pyrazolo[3,4-b]pyridine (**24**, R^1 = Me) as a yellow solid (99% yield)*

Mp 187–188 °C; ^{1}H NMR (DMSO-d_6) δ 9.11 (d, 1H, J = 6 Hz, Pyr–H), 8.84 (d, 1H, J = 5.6 Hz, Pyr–H), 8.79 (dd, 1H, J = 1.6 Hz, Pyr–H), 8.48 (dd, 1H, J = 6.4 Hz, Pyr–H), 7.53 (q, 1H, Pyr–H), 3.33 (s, 3H, SO_2CH_3), 2.67 (s, 3H, CH_3). ^{13}C NMR (DMSO-d_6) δ 174.5, 168.3, 159.4, 158.4, 148.3, 132.2, 130.1, 116.3, 115.4, 106.5, 43.3, 14.2. HR-MS (m/z): calcd for $C_{12}H_{11}N_5O_2S$ 290.0633; found 290.0628.

*5.1.55. 3-Ethyl-1-(2-(methylsulfonyl)pyrimidin-4-yl)-1H-pyrazolo [3,4-b]pyridine (**24**, R^1 = Et) as an off-white solid (98% yield)*

Mp 170–171 °C; ^{1}H NMR (DMSO-d_6) δ 9.11 (d, 1H, J = 5.6 Hz, Pyr–H), 8.82 (d, 1H, J = 6 Hz, Pyr–H), 8.79 (dd, 1H, J = 3.2 Hz, Pyr–H), 8.51 (dd, 1H, J = 6.4 Hz, Pyr–H), 7.52 (q, 1H, Pyr–H), 3.33 (s, 3H, SO_2CH_3), 3.01 (q, 2H, J = 7.6 Hz, CH_2), 1.41 (t, 1H, J = 7.6 Hz, CH_3). ^{13}C NMR (DMSO-d_6) δ 173.5, 168.6, 158.6, 157.4, 148.3, 143.2, 130.1, 118.2, 116.3, 111.1, 43.4, 22.4, 13.3. HR-MS (m/z): calcd for $C_{13}H_{13}N_5O_2S$ 304.0790; found 304.0709.

*5.1.56. General procedure for synthesis of N-phenyl-4-(pyrazolo [3,4-b]pyridin-1-yl)pyrimidin-2-amine derivatives (**25a–f**)*

A mixture of **24** (0.3 mmol), aniline (1.0 mmol) and *p*-toluenesulphonic acid (0.6 mmol) in *i*-PrOH (4 mL) was heated at 150 °C for 20 min in a microwave reactor. The mixture was then treated with

$NaHCO_3$ and extracted with ethyl acetate. The organic layers were combined, dried over magnesium sulphate, filtered and concentrated *in vacuo*. The crude product was purified by flash chromatography. 3-(4-(1*H*-*pyrazolo*[3,4-*b*]*pyridin*-1-*yl*)*pyrimidin*-2-*ylamino*) *benzenesulfonamide* (**25a**) as a pale brown solid (9% yield). ^{1}H NMR (DMSO-d_6) δ 10.24 (bs, 1H, NH), 8.85 (dd, 1H, J = 3.2 Hz, Pyr–H), 8.69 (d, 1H, J = 5.6 Hz, Pyr–H), 8.62 (bs, 1H, Pyr–H), 8.45 (dd, 1H, J = 6.4 Hz, Pyr–H), 8.29 (d, 1H, J = 8.8 Hz, Ph–H), 7.91 (d, 1H, J = 5.6 Hz, Pyr–H), 7.56 (t, 1H, J = 8 Hz, Ph–H), 7.49 (q, 1H, Pyr–H), 7.44 (m, 2H, Ph–H), 7.32 (bs, 2H, NH_2); LC-MS, Gemini-NX 3u-110A, 50 × 2 mm, single peak = 369.3 at 2.40 min, Luna 3u (PFP2) 110A, 50 × 2 mm, single peak at 2.50 min; HR-MS (m/z): calcd for $C_{16}H_{13}N_7O_2S$ 368.0851; found 368.0756.

*5.1.57. 4-(4-(1-Indazol-1-yl)pyrimidin-2-ylamino) benzenesulfonamide (**25b**) as a pale brown solid (9% yield)*

^{1}H NMR (DMSO-d_6) δ 10.32 (bs, 1H, NH), 8.86 (dd, 1H, J = 3.2 Hz, Pyr–H), 8.70 (d, 1H, J = 5.6 Hz, Pyr–H), 8.66 (bs, 1H, Pyr–H), 8.47 (dd, 1H, J = 6.4 Hz, Pyr–H), 8.37 (d, 2H, J = 8.8 Hz, Ph–H), 7.86 (d, 1H, J = 5.6 Hz, Pyr–H), 7.79 (d, 2H, J = 8.8 Hz, Ph–H), 7.52 (q, 1H, Pyr–H), 7.19 (bs, 2H, NH_2); LC-MS, Gemini-NX 3u-110A, 50 × 2 mm, single peak = 368.3 at 2.41, Luna 3u (PFP2) 110A, 50 × 2 mm, single peak at 2.47 min; HR-MS (m/z): calcd for $C_{16}H_{13}N_7O_2S$ 368.0851; found 368.0724.

*5.1.58. 3-(4-(3-Methyl-1H-pyrazolo[3,4-b]pyridin-1-yl)pyrimidin-2-ylamino)benzenesulfonamide (**25c**) as a pale brown solid (10% yield)*

^{1}H NMR ($(CD_3)_2CO$-d_6) δ 9.18 (bs, 1H, NH), 8.84 (dd, 1H, J = 2.8 Hz, Pyr–H), 8.66 (m, 2H, Ph–H), 8.59 (d, 1H, J = 5.6 Hz, Pyr–H), 8.36 (dd, 1H, J = 6.4 Hz, Pyr–H), 7.99 (d, 1H, J = 5.2 Hz, Pyr–H), 7.55 (m, 2H, Ph–H), 7.45 (q, 1H, Pyr–H), 6.51 (bs, 2H, NH_2), 2.87 (s, 3H, CH_3); LC-MS, Gemini-NX 3u-110A, 50 × 2 mm, single peak = 382.2 at 2.50 min, Luna 3u (PFP2) 110A, 50 × 2 mm, single peak at 2.56 min; HR-MS (m/z): calcd for $C_{17}H_{15}N_7O_2S$ 382.1008; found 382.0883.

*5.1.59. 4-(4-(3-Methyl-1H-pyrazolo[3,4-b]pyridin-1-yl)pyrimidin-2-ylamino)benzenesulfonamide (**25d**) as a brown solid (10% yield)*

^{1}H NMR (DMSO-d_6) δ 10.28 (bs, 1H, NH), 8.85 (d, 1H, J = 3.2 Hz, Pyr–H), 8.64 (d, 1H, J = 5.6 Hz, Pyr–H), 8.45 (d, 1H, J = 6.4 Hz, Pyr–H), 7.84 (d, 1H, J = 5.6 Hz, Pyr–H), 7.79 (d, 2H, J = 8 Hz, Ph–H), 7.50 (q, 1H, Pyr–H), 7.18 (bs, 2H, NH_2), 2.66 (s, 3H, CH_3); LC-MS, Gemini-NX 3u-110A, 50 × 2 mm, single peak = 382.4 at 2.49 min, Luna 3u (PFP2) 110A, 50 × 2 mm, single peak at 2.55 min; HR-MS (m/z): Calcd for $C_{17}H_{15}N_7O_2S$ 382.1008; found 382.0956.

*5.1.60. 3-(4-(3-Ethyl-1H-pyrazolo[3,4-b]pyridin-1-yl)pyrimidin-2-ylamino)benzenesulfonamide (**25e**) as a brown solid (10% yield)*

^{1}H NMR (CD_3OD-d_4) δ 8.77 (dd, 1H, J = 3.2 Hz, Pyr–H), 8.55 (d, 1H, J = 5.6 Hz, Pyr–H), 8.48 (m, 1H, Ph–H), 8.39 (dd, 1H, J = 6.4 Hz, Pyr–H), 8.09 (d, 1H, J = 7.6 Hz, Ph–H), 7.76 (d, 1H, J = 5.6 Hz, Pyr–H), 7.54 (m, 2H, Ph–H), 7.45 (q, 1H, Pyr–H), 3.13 (q, 2H, J = 7.6 Hz, CH_2), 1.49 (t, 3H, J = 7.6 Hz, CH_3). HR-MS (m/z): calcd for $C_{18}H_{17}N_7O_2S$ 395.1164, found 396.0969.

*5.1.61. 4-(4-(3-Ethyl-1H-pyrazolo[3,4-b]pyridin-1-yl)pyrimidin-2-ylamino)benzenesulfonamide (**25f**) as a white solid (33% yield)*

^{1}H NMR (CD_3OD-d_4) δ 8.80 (dd, 1H, J = 2.8 Hz, Pyr–H), 8.58 (d, 1H, J = 5.6 Hz, Pyr–H), 8.38 (dd, 1H, J = 6.4 Hz, Pyr–H), 8.13 (d, 2H, J = 8.8 Hz, Ph–H), 7.89 (d, 2H, J = 9.2 Hz, Ph–H), 7.75 (d, 1H, J = 5.6 Hz, Pyr–H), 7.46 (q, 1H, Pyr–H), 3.13 (q, 2H, J = 7.6 Hz, CH_2), 1.50 (t, 3H, J = 7.6 Hz, CH_3); LC-MS, Gemini-NX 3u-110A, 50 × 2 mm, single peak = 396.4 at 2.63 min, Luna 3u (PFP2) 110A, 50 × 2 mm, single peak at 2.68 min; HR-MS (m/z): calcd for $C_{18}H_{17}N_7O_2S$ 396.1164; found 396.1054.

5.2. Biological assays

5.2.1. Proliferation assays

The cancer cell lines were obtained from the cell bank at the Centre for Biomolecular Sciences, University of Nottingham, UK, and were maintained in RPMI-1640 with 10% FBS. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma) assays were performed as reported previously [7]. Compound concentrations required to inhibit 50% of cell growth (GI_{50}) were calculated using non-linear regression analysis.

5.2.2. Caspase-3/7 assay

Activity of caspase 3/7 was measured using the Apo-ONE Homogeneous Caspase-3/7 kit (Promega G7790) [11].

5.2.3. Cell cycle analysis

Cell cycle analyses were performed using a Beckman Coulter Gallios flow cytometer. Data were evaluated using Kaluza V1.2 software.

5.2.4. Kinase assay

Inhibition of CDKs was measured by radiometric assay using the Millipore KinaseProfiler services. Half-maximal inhibition (IC_{50}) values were calculated from 10-point dose–response curves and apparent inhibition constants (K_i) were calculated from the IC_{50} values and appropriate K_m (ATP) values for the kinases in question [11].

5.2.5. Western blots

Western blotting was performed as described in Ref. [5]. Antibodies used were as follows: total RNAP-II (8WG16), phosphorylated RNAP-II Ser-2 and Ser-5 (Covance), β-actin (Sigma–Aldrich) and Mcl-1, PARP (Cell Signalling Technologies). Both anti-mouse and anti-rabbit immunoglobulin G (IgG) horse-radish peroxidase-conjugated antibodies were obtained from Dako.

References

[1] S. Lapenna, A. Giordano, Cell cycle kinases as therapeutic targets for cancer, Nat. Rev. Drug Discov. 8 (2009) 547–566.

[2] S. Wang, P.M. Fischer, Cyclin-dependent kinase 9: a key transcriptional regulator and potential drug target in oncology, virology and cardiology, Trends Pharmacol. Sci. 6 (2008) 302–313.

[3] C. Barriere, D. Santamaria, A. Cerqueira, J. Galan, A. Martin, S. Ortega, M. Malumbres, P. Dubus, M. Barbacid, Mice thrive without Cdk4 and Cdk2, Mol. Oncol. 1 (2007) 72–83.

[4] J.C. Byrd, T.S. Lin, J.T. Dalton, D. Wu, M.A. Phelps, B. Fischer, M. Moran, K.A. Blum, B. Rovin, M. Brooker-McEldowney, S. Broering, L.J. Schaaf, A.J. Johnson, D.M. Lucas, N.A. Heerema, G. Lozanski, D.C. Young, J.R. Suarez, A.D. Colevas, M.R. Grever, Flavopiridol administered using a pharmacologically derived schedule is associated with marked clinical efficacy in refractory, genetically high-risk chronic lymphocytic leukemia, Blood 109 (2007) 399–404.

[5] R. Chen, M.J. Keating, V. Gandhi, W. Plunkett, Transcription inhibition by flavopiridol: mechanism of chronic lymphocytic leukemia cell death, Blood 106 (2005) 2513–2519.

[6] R. Chen, W.G. Wierda, S. Chubb, R.E. Hawtin, J.A. Fox, M.J. Keating, V. Gandhi, W. Plunkett, Mechanism of action of SNS-032, a novel cyclin-dependent kinase inhibitor, in chronic lymphocytic leukemia, Blood 113 (2009) 4637–4645.

[7] S. Wang, C. Meades, G. Wood, A. Osnowski, S. Anderson, R. Yuill, M. Thomas, M. Mezna, W. Jackson, C. Midgley, G. Griffiths, I. Fleming, S. Green, I. McNae, S.Y. Wu, C. McInnes, D. Zheleva, M.D. Walkinshaw, P.M. Fischer, 2-Anilino-4-(thiazol-5-yl)pyrimidine CDK inhibitors: synthesis, SAR analysis, X-ray crystallography, and biological activity, J. Med. Chem. 47 (2004) 1662–1675.

[8] S. Wang, G. Wood, C. Meades, G. Griffiths, C. Midgley, I. McNae, C. McInnes, S. Anderson, W. Jackson, M. Mezna, R. Yuill, M. Walkinshaw, P.M. Fischer, Synthesis and biological activity of 2-anilino-4-(1*H*-pyrrol-3-yl) pyrimidine CDK inhibitors, Bioorg. Med. Chem. Lett. 14 (2004) 4237–4240.

[9] S. Wang, G. Griffiths, C.A. Midgley, A.L. Barnett, M. Cooper, J. Grabarek, L. Ingram, W. Jackson, G. Kontopidis, S.J. McClue, C. McInnes, J. McLachlan, C. Meades, M. Mezna, I. Stuart, M.P. Thomas, D.I. Zheleva, D.P. Lane, R.C. Jackson, D.M. Glover, D.G. Blake, P.M. Fischer, Discovery and

characterization of 2-anilino-4- (thiazol-5-yl)pyrimidine transcriptional CDK inhibitors as anticancer agents, Chem. Biol. 17 (2010) 1111–1121.
[10] S. Wang, C.A. Midgley, F. Scaerou, J.B. Grabarek, G. Griffiths, W. Jackson, G. Kontopidis, S.J. McClue, C. McInnes, C. Meades, M. Mezna, A. Plater, I. Stuart, M.P. Thomas, G. Wood, R.G. Clarke, D.G. Blake, D.I. Zheleva, D.P. Lane, R.C. Jackson, D.M. Glover, P.M. Fischer, Discovery of *N*-phenyl-4-(thiazol-5-yl)pyrimidin-2-amine aurora kinase inhibitors, J. Med. Chem. 53 (2010) 4367–4378.
[11] X. Liu, S. Shi, F. Lam, C. Pepper, P.M. Fischer, S. Wang, CDKI-71, a novel CDK9 inhibitor, is preferentially cytotoxic to cancer cells compared to flavopiridol, Int. J. Cancer 130 (2012) 1216–1226.
[12] X. Liu, F. Lam, S. Shi, P.M. Fischer, S. Wang, In vitro antitumor mechanism of a novel cyclin-dependent kinase inhibitor CDKI-83, Invest. New Drugs 30 (2012) 889–897.
[13] F. Crestey, S. Stiebing, R. Legay, V. Collot, S. Rault, Design and synthesis of a new indazole library: direct conversion of *N*-methoxy-*N*-methylamides (Weinreb amides) to 3-keto and 3-formylindazoles, Tetrahedron 63 (2007) 419–428.
[14] I.K. Khanna, R.M. Weier, K.T. Lentz, L. Swenton, D.C. Lankin, Facile, regioselective syntheses of *N*-alkylated 2,3-diaminopyridines and imidazo[4,5-*b*] pyridines, J. Org. Chem. 60 (1995) 960–965.