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Practical synthesis of novel purine analogues as Hsp90 inhibitors

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ABSTRACT

The development of a straightforward synthesis of 4-amino-6-benzyl-6*H*-pyrrolo[3,4-*d*]pyrimidine and 7-amino-2-benzyl-2*H*-pyrazolo[4,3-*d*]pyrimidine derivatives allowed for the preparation of a small family of potential Hsp90 inhibitors. Some of the newly synthesized compounds showed Hsp90 inhibitory activity in preliminary biological assays.

1. Introduction

Hsp90 belongs to a family of proteins called molecular chaperones that regulate the folding and influence the degradation of key signal transduction proteins, referred to as 'client proteins'. Hsp90 clients include proteins that function as key regulators in cellular growth, differentiation and apoptotic pathways that are directly involved in driving multistep malignancy. As a result, pharmacological blockade of the Hsp90 function should deliver a combinatorial effect on all the hallmark traits of malignancy.[1–3]

Making use of rational drug design, Chiosis et al. were able to design a class of purine scaffold (PU-class) derivatives, such as **1** (Fig. 1), with Hsp90 inhibitory activities.[4–6] Moreover, Kasibhatla et al. have synthesized and evaluated several pyrazolopyrimidines[7] and pyrrolopyrimidines,[8] such as **2** and **3** (Fig. 1), also active against Hsp90.

As a part of our ongoing research into new Hsp90 inhibitors, we became interested in synthesizing the novel deazapurine derivatives **4**, **7**–**12** (Fig. 1, Table 1), characterized by a pyrrolo[3,4-*d*]pyrimidine scaffold, that are isomers of the pyrrolo[2,3-*d*]pyrimidines **3**. This heterocyclic system has not been investigated in detail and, to the best of our knowledge, only one example of 4-amino-6*H*-pyrrolo[3,4-*d*]pyrimidine has been reported in the literature so far.[9] Finally, the two pyrazolo[4,3-*d*]pyrimidine derivatives **13** and **14** were prepared representing isomers of **2** as well as isosteric analogues of the pyrrolo[3,4-*d*]pyrimidines **4**, **7**–**12**.

2. Results and discussion

All the new pyrrolopyrimidines were prepared starting from the common precursors 2-(ethoxymethylene)malononitrile and diethyl 2-aminomalonate, which were condensed to give ethyl 3-amino-4-cyano-1*H*-pyrrole-2-carboxylate (**5**) (Scheme 1).[10] Direct phase transfer catalyzed alkylation using different benzyl halides allowed the decoration of the pyrrole nitrogen of **5** affording the benzyl derivatives **6** with no need for any NH_2 protection. Pyrrole compounds **6** were efficiently converted, by reaction with formamidine acetate, into the deazapurine derivatives **4**, which were used as key intermediates for the preparation of the final compounds **7**–**12**.

In particular, alkaline hydrolysis of the esters **4** gave the carboxylic acids **7** (Scheme 2), which were either transformed into the corresponding amides **8** by reaction with the appropriate amine in the presence of HBTU and HOBt[11] or decarboxylated by heating in the presence of HCl[12] to give compounds **9**, lacking the C7 side chain.

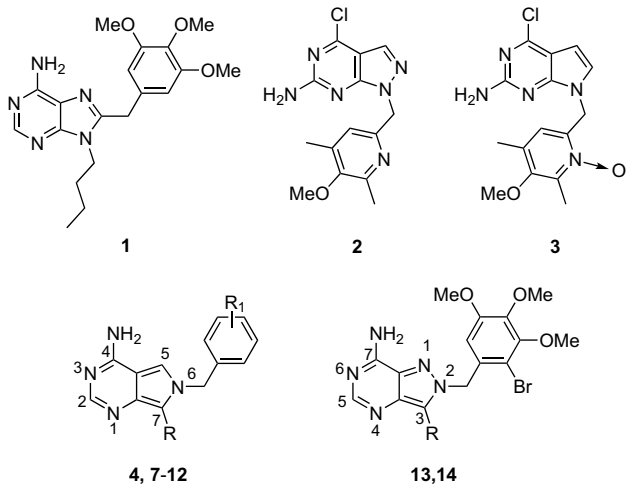

Figure 1. For R, R_1 see Table 1.

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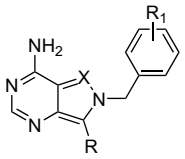
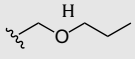
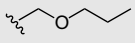
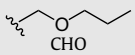
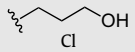
Table 1
Structure and IC_{50} value of compounds **4a–4e**, **7a–7e**, **8a–j**, **9a–d**, **10a–c**, **11–14**

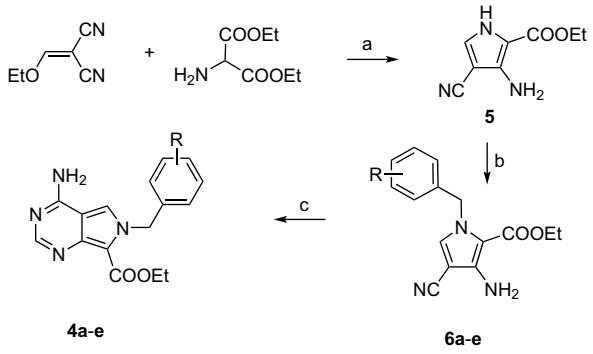


Compound	X	R	R_1	IC_{50}, μM
4a	CH	COOEt	2-Bromo-3,4,5-trimethoxy	n.a.[a]
4b	CH	COOEt	3,5-Dimethoxy	n.t.[b]
4c	CH	COOEt	4-Methoxy	n.t.
4d	CH	COOEt	3,4-Methylenedioxy	n.t.
4e	CH	COOEt	2-Chloro-3,4-methylenedioxy	n.t.
7a	CH	COOH	2-Bromo-3,4,5-trimethoxy	n.t.
7b	CH	COOH	3,5-Dimethoxy	n.t.
7c	CH	COOH	4-Methoxy	n.t.
7d	CH	COOH	3,4-Methylenedioxy	n.t.
7e	CH	COOH	2-Chloro-3,4-methylenedioxy	n.t.
8a	CH	CONH 	4-Methoxy	n.a.
8b	CH	CONH 	4-Methoxy	n.a.
8c	CH	CONH 	4-Methoxy	n.a.
8d	CH	CONH 	3,4-Methylenedioxy	21.0
8e	CH	CONH 	3,4-Methylenedioxy	40.8
8f	CH	CONH 	3,4-Methylenedioxy	n.a.
8g	CH	CONH 	3,4-Methylenedioxy	235.6
8h	CH	CONH 	2-Bromo-3,4,5-trimethoxy	204.8
8i	CH	CONH 	2-Bromo-3,4,5-trimethoxy	n.t.
8j	CH	CONH 	2-Chloro-3,4-methylenedioxy	8.0
9a	CH	H	2-Chloro-3,4-methylenedioxy	60.0
9b	CH	H	3,4-Methylenedioxy	647.0
9c	CH	H	3,5-Dimethoxy	n.a.
9d	CH	H	2-Bromo-3,4,5-trimethoxy	29.2
10a	CH		3,5-Dimethoxy	n.a.
10b	CH		2-Bromo-3,4,5-trimethoxy	147.9
10c	CH		3,4,5-Trimethoxy	n.a.
11	CH	CHO	4-Methoxy	n.t.
12	CH		4-Methoxy	n.a.
13	N	Cl	2-Bromo-3,4,5-trimethoxy	6.0
14	N	H	2-Bromo-3,4,5-trimethoxy	2.0

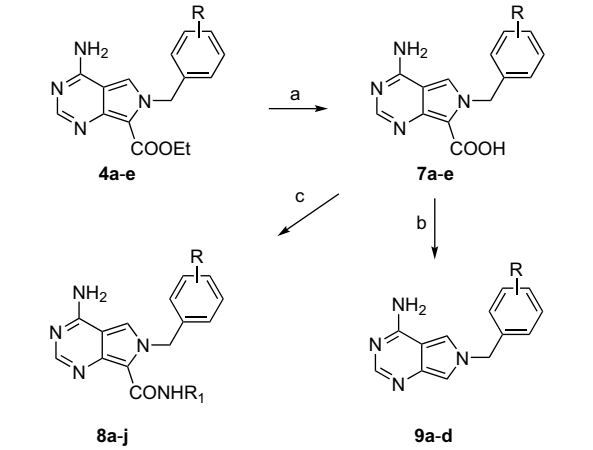
[a] n.a. Not active. Compounds endowed with an IC_{50} value ≥1000 μM have been considered inactive.
[b] n.t. Not tested.

Finally, reduction of the ester function of **4** by $LiAlH_4$, followed by etherification with *n*-propanol in the presence of hydrogen chloride gave compounds **10** characterized by an ethereal function at C7 (Scheme 3). In particular, compounds **10b** and **10c** were obtained as a 1:1 mixture starting from **4a**, which underwent partial debromination on the benzyl group during the reduction step.

Aldehyde **11**, obtained from **4c** by a reduction/oxidation cycle, was initially transformed into the corresponding α,β-unsaturated ester by Wittig reaction in the presence of methyl (triphenylphosphoranylidene)acetate; double bond hydrogenation, followed by reduction of the ester function with $LiAlH_4$ gave the alcohol **12**.

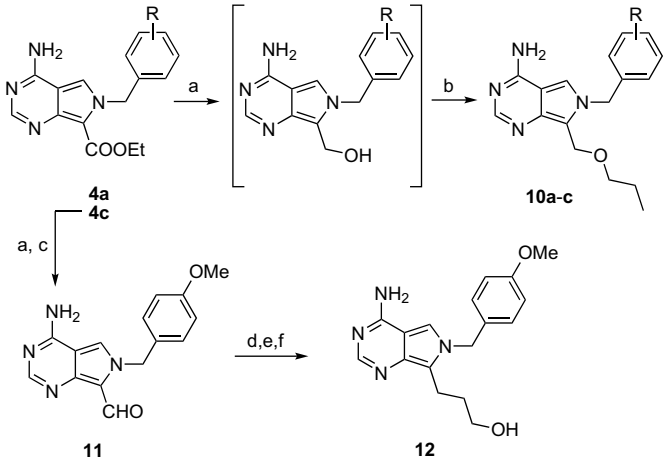


Scheme 1. Reagents and conditions: (a) NaOEt, EtOH, rt, 3 h; (b) benzyl halide, *n*-Bu_4N^+Cl, NaI, 20% NaOH, DCM, rt, 2 h; (c) NH=$CHNH_2$·AcOH, EtOH or 2-methoxyethanol, reflux, 4–12 h. For R see Table 1.

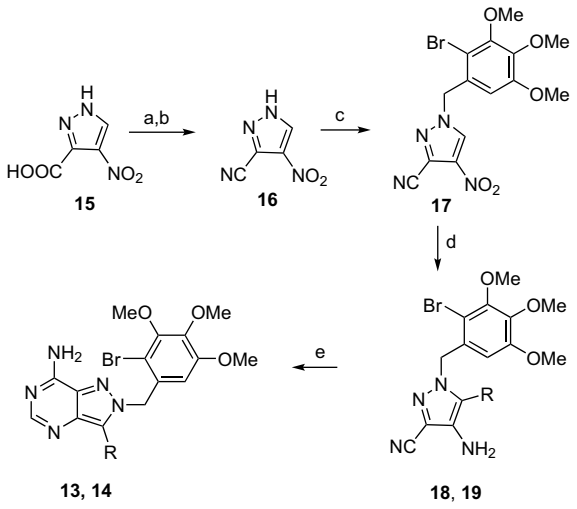


Scheme 2. Reagents and conditions: (a) KOH, MeOH/H_2O, reflux, 3–12 h; (b) 6 M HCl, reflux, 1–4 h; (c) HBTU, HOBt, DIPEA, R_1NH_2, rt, 2–6 h. For R, R_1 see Table 1.

The pyrazolopyrimidines **13** and **14** (Scheme 4) were prepared starting from the commercially available 4-nitro-3-pyrazolecarboxylic acid (**15**), which was first converted into the corresponding nitrile **16**[13] and then alkylated in the presence of 2-bromo-3,4,5-trimethoxybenzyl bromide to give **17**. The regioselectivity of the alkylation reaction was proved by means of NOESY experiments, which showed a spatial proximity between the pyrazole hydrogen and the methylene group.



Scheme 3. Reagents and conditions: (a) $LiAlH_4$, THF, rt, 1–3 h; (b) *n*-propanol, HCl (g), rt, 2–4 h; (c) MnO_2, *t*-BuOH, reflux, 16 h; (d) Ph_3P=CHCOOMe, 2-methoxyethanol, reflux, 16 h; (e) H_2, Pd/C, MeOH, rt, 3 h; (f) $LiAlH_4$, THF, rt, 2 h. For R see Table 1.



Scheme 4. Reagents and conditions: (a) $SOCl_2$, reflux, 3 h; then, NH_4OH, rt, 12 h; (b) triphosgene, pyridine, DCM, rt, 16 h; (c) 2-bromo-3,4,5-trimethoxybenzyl bromide, *n*-$Bu_4N^+Br^-$, NaOH 20%, DCM, rt, 2 h; (d) For **18**: Fe^0, HCl/H_2O/MeOH, reflux, 1 h; for **19**: H_2, Rh/aluminium oxide, AcOEt, rt, 16 h; (e) NH=$CHNH_2$·AcOH, EtOH, reflux, 4 h. For R see Table 1.

The use of Fe^0 in the presence of HCl to accomplish the reduction of the nitro group determined, at the same time, the introduction of a Cl atom at C3[14] (compound **18**); only by catalytic hydrogenation using a Rh/aluminium oxide system, the reduction of the nitro group was obtained without side reactions to give **19** in 87% yield. Condensation of the two intermediates **18** and **19** with formamidine acetate afforded the corresponding pyrazolopyrimidines **13** and **14**.

Most of the new compounds were tested in a competitive assay using fluorescence polarization. The results obtained are reported in Table 1. Three compounds of the pyrrolo[3,4-*d*]pyrimidine series, all characterized by a saturated alkylamido group at C7 and a 3,4-methylenedioxybenzyl or 2-chloro-3,4-methylenedioxybenzyl substitution at N6, showed interesting IC_{50} values ranging from 8 to 40.8 μM. Neverthless, the best results were obtained with the pyrazole derivatives **13** and **14**, with the best compound showing an IC_{50} value lower by one order of magnitude with respect to the corresponding deazaguanine **9d**. This finding underlines the positive effect exerted by the N atom at position 1 of the ligand **14** in reinforcing interactions within the adenine binding site.

3. Conclusion

Considering that only scattered examples of the pyrrolo[3,4-*d*] pyrimidine scaffold are present in the literature, a straightforward approach for the synthesis of 4-amino-6-benzyl-6*H*-pyrrolo[3,4-*d*] pyrimidines has been developed and has been applied to the preparation of a small family of potential Hsp90 inhibitors. Moreover, the first representatives of 7-amino-2-benzyl-2*H*-pyrazolo[4,3-*d*]pyrimidines **13** and **14** have been prepared, since no examples of 2-benzyl substituted derivatives of this family have been reported so far. Among those prepared, a number of derivatives proved to be able to bind to Hsp90 protein with IC_{50} values in the low micromolar range.

4. Experimental section

4.1. Chemistry

4.1.1. General

Reagents were obtained from commercial suppliers and used without further purifications. The solvents were dried according to standard procedures. Anhydrous reactions were run under a positive pressure of dry N_2. IR spectra were recorded on a Perkin–Elmer BX FT-IR system using $CHCl_3$ as the solvent or a Nujol dispersion. TLC was carried out using Merck TLC plates Kieselgel 60 F_{254}. Chromatographic purifications were performed on columns packed with Merck 60 silica gel, 23–400 mesh, for flash technique. Melting points were taken using a Gallenkamp melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 400 MHz on a Brucker Avance DPX400 and at 200 MHz on a Bruker AC200F spectrometer; chemical shifts are reported in δ values, relative to TMS at δ 0.00 ppm. Mass spectral (MS) data were obtained using an Agilent 1100 LC/MSD VL system (G1946C) with a 0.4 mL/min flow rate using a binary solvent system of 95:5 MeOH/water. UV detection was monitored at 254 nm. The following ion source parameters were used: drying gas flow, 9 mL/min; nebulizer pressure, 40 psi g; drying gas temperature, 350 °C.

*4.1.2. General procedure for the synthesis of **4a–e***

Formamidine acetate (323 mg, 3 mmol) was added to a solution of **6** (1 mmol) and *p*-toluenesulfonic acid (catalytic amount) in EtOH or 2-methoxyethanol (5 mL) and the solution was heated at reflux for 4–12 h. The solvent was evaporated to dryness and the crude product was purified by silica gel column chromatography.

*4.1.2.1. Ethyl 4-amino-6-(2-bromo-3,4,5-trimethoxybenzyl)-6H-pyrrolo[3,4-d]pyrimidine-7-carboxylate (**4a**).* Eluent: DCM/MeOH, 9:1. White solid (61% yield); mp 231–232 °C (EtOAc); ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 8.14 (s, 1H), 7.62 (s, 1H), 7.54 (br, 2H), 6.64 (s, 1H), 5.76 (s, 2H), 4.26 (q, *J*=7.0 Hz, 2H), 3.79 (s, 3H), 3.77 (s, 3H), 3.65 (s, 3H), 1.24 (t, *J*=7.0 Hz, 3H); MS *m*/*z* 465, 466 $(M+H)^+$; IR (Nujol): ν 3419, 1683, 878, 842 cm^{-1}. Anal. Calcd for $C_{19}H_{21}BrN_4O_5$: C, 49.04; H, 4.55; N, 12.04. Found: C, 49.16; H, 4.52; N, 11.95.

*4.1.2.2. Ethyl 4-amino-6-(3,5-dimethoxybenzyl)-6H-pyrrolo[3,4-d]pyrimidine-7-carboxylate (**4b**).* Eluent: DCM/MeOH, 95:5. White solid (85% yield); mp 216–217 °C (EtOAc); ^{1}H NMR (200 MHz, CD_3OD-d_4): δ 8.20 (s, 1H), 7.81 (s, 1H), 6.38 (d, *J*=1.9 Hz, 1H), 6.31 (d, *J*=1.9 Hz, 2H), 5.74 (s, 2H), 4.37 (q, *J*=7.0 Hz, 2H), 3.70 (s, 6H), 1.33 (t, *J*=7.0 Hz, 3H); MS *m*/*z* 357$(M+H)^+$; IR (Nujol): ν 2924, 1676, 1376, 1296, 1153 cm^{-1}. Anal. Calcd for $C_{18}H_{20}N_4O_2$: C, 60.66; H, 5.66; N, 15.72. Found: C, 60.48; H, 5.67; N, 15.76.

*4.1.2.3. Ethyl 4-amino-6-(4-methoxybenzyl)-6H-pyrrolo[3,4-d]pyrimidine-7-carboxylate (**4c**).* Eluent: DCM/MeOH, 10:1. White solid (85% yield); mp 190–191 °C (EtOH); ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 8.42 (br s, 2H), 8.26 (s, 1H), 7.98 (s, 1H), 7.16 (d, *J*=8.7 Hz, 2H), 6.89 (d, *J*=8.7 Hz, 2H), 5.71 (s, 2H), 4.30 (q, *J*=7.1 Hz, 2H), 3.70 (s, 3H), 1.26 (t, *J*=7.1 Hz, 3H); MS *m*/*z* 327 $(M+H)^+$; IR (Nujol): ν 3332, 3146, 2916, 1715, 1682 cm^{-1}. Anal. Calcd for $C_{17}H_{18}N_4O_3$: C, 62.57; H, 5.56; N, 17.17. Found: C, 62.77; H, 5.57; N, 17.05.

*4.1.2.4. Ethyl 4-amino-6-((benzo[d][1,3]dioxol-5-yl)methyl)-6H-pyrrolo[3,4-d]pyrimidine-7-carboxylate (**4d**).* Eluent: DCM/MeOH, 85:15. White solid (68% yield); mp 236–237 °C (EtOH); ^{1}H NMR (200 MHz, CD_3OD-d_4): δ 8.19 (s, 1H), 7.80 (s, 1H), 6.72 (m, 3H), 5.90 (s, 2H), 5.70 (s, 2H), 4.39 (q, *J*=7.0 Hz, 2H), 1.33 (t, *J*=7.0 Hz, 3H); MS *m*/*z* 341 $(M+H)^+$; IR (Nujol): ν 2924, 1674, 1330, 1133, 922 cm^{-1}. Anal. Calcd for $C_{17}H_{16}N_4O_4$: C, 59.99; H, 4.74; N, 16.46. Found: C, 60.14; H, 4.71; N, 16.38.

*4.1.2.5. Ethyl 4-amino-6-((6-chlorobenzo[d][1,3]dioxol-5-yl)methyl)-6H-pyrrolo[3,4-d]pyrimidine-7-carboxylate (**4e**).* Eluent: DCM/MeOH, 85:15. White solid (70% yield); mp 242–243 °C (dec, EtOAc); ^{1}H NMR (200 MHz, CD_3OD-d_4): δ 8.23 (s, 1H), 7.74 (s, 1H), 6.97 (s, 1H), 6.48 (s, 1H), 5.97 (s, 2H), 5.81 (s, 2H), 4.40 (q, *J*=6.9 Hz, 2H), 1.34 (t, *J*=6.9 Hz, 3H); MS *m*/*z* 375 $(M+H)^+$, 397 $(M+Na)^+$, 771

(2M+Na)$^+$; IR (Nujol): ν 3329, 3110, 1682, 931, 839 cm^{-1}. Anal. Calcd for $C_{17}H_{15}ClN_4O_4$: C, 54.48; H, 4.03; N, 14.95. Found: C, 54.61; H, 4.00; N, 14.89.

*4.1.3. Synthesis of ethyl 3-amino-4-cyano-1H-pyrrole-2-carboxylate (**5**)*

A solution of ethoxymethylenemalononitrile (6 g, 49 mmol) and diethyl aminomalonate hydrochloride (12.4 g, 59 mmol) in dry EtOH (50 mL) was slowly added to a stirred solution of EtONa in dry EtOH (4 g in 100 mL, 172 mmol). The reaction mixture was stirred at room temperature for 3 h, then acetic acid was added (until pH 7) and the reaction mixture was concentrated in vacuo. The orange residue was dissolved in EtOAc and washed with water and brine. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure; the crude product was crystallized from toluene affording compound **5** (6.8 g, 77% yield) as a white solid; mp 125.5–127 °C; ^{1}H NMR (200 MHz, Me$_2$SO-d_6): δ 11.68 (br s, 1H), 7.38 (s, 1H), 5.53 (br s, 2H), 4.18 (q, J=7.5 Hz, 2H), 1.23 (t, J=7.5 Hz, 3H); MS m/z 180 (M+H)$^+$; IR ($CHCl_3$): ν 3019, 2223, 1684 cm^{-1}. Anal. Calcd for $C_8H_9N_3O_2$: C, 53.63; H, 5.06; N, 23.45. Found: C, 53.77; H, 5.04; N, 23.40.

*4.1.4. General procedure for synthesis of **6a–e***

To a solution of **5** (540 mg, 3 mmol) in DCM (6 mL) the appropriate benzyl halide (3.6 mmol) was added followed by NaI (225 mg, 1.5 mmol), tetra-*n*-butylammonium chloride (1 g, 3.6 mmol) and 20% NaOH (4 mL). The reaction mixture was stirred at room temperature for 2 h, then the organic layer was separated, the water phase was extracted with DCM and the combined organic phases were washed with brine, dried over Na_2SO_4 and the solvent was removed in vacuo. The crude residue was purified by silica gel column chromatography.

*4.1.4.1. Ethyl3-amino-1-(2-bromo-3,4,5-trimethoxybenzyl)-4-cyano-1H-pyrrole-2-carboxylate (**6a**).* Eluent: PE/AcOEt, 2:1. White solid (60% yield); mp 163–164 °C (Et_2O); ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.93 (s, 1H), 6.18 (s, 1H), 5.37 (s, 2H), 4.95 (br s, 2H), 4.24 (q, J=7.0 Hz, 2H), 3.88 (s, 3H), 3.85 (s, 3H), 3.70 (s, 3H), 1.23 (t, J=7.0 Hz, 3H); MS m/z 438, 440; IR ($CHCl_3$): ν 3496, 3024, 2940, 2221, 1689, 1607, 1485, 1398, 1207, 1110, 1007 cm^{-1}. Anal. Calcd for $C_{18}H_{20}BrN_3O_5$: C, 49.33; H, 4.60; N, 9.59. Found: C, 49.45; H, 4.58; N, 9.50.

*4.1.4.2. Ethyl 3-amino-4-cyano-1-(3,5-dimethoxybenzyl)-1H-pyrrole-2-carboxylate (**6b**).* Eluent: PE/AcOEt, 1.5:1. Pale pink solid (58% yield); mp 146–147 °C (Et_2O); ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.93 (s, 1H), 6.29 (d, J=1.9 Hz, 1H), 6.16 (d, J=1.9 Hz, 2H), 5.23 (s, 2H), 4.91 (br s, 2H), 4.04 (q, J=7.4 Hz, 2H), 3.68 (s, 6H), 1.18 (t, J=7.4 Hz, 3H); MS m/z 330 (M+H)$^+$; IR ($CHCl_3$): ν 3025, 2222, 1685, 1606, 1483, 1289 cm^{-1}. Anal. Calcd for $C_{17}H_{19}N_3O_4$: C, 62.00; H, 5.81; N, 12.76. Found: C, 62.16; H, 5.79; N, 12.70.

*4.1.4.3. Ethyl 3-amino-4-cyano-1-(4-methoxybenzyl)-1H-pyrrole-2-carboxylate (**6c**).* Eluent: PE/AcOEt, 1:1. White solid (70% yield); mp 112–113 °C (Et_2O); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.05 (d, J=8.5 Hz, 2H), 6.89 (s, 1H), 6.84 (d, J=8.5 Hz, 2H), 5.28 (s, 2H), 4.87 (br s, 2H), 4.27 (q, J=7.1 Hz, 2H), 3.78 (s, 3H), 1.29 (t, J=7.1 Hz, 3H); MS m/z 322 (M+Na)$^+$; IR ($CHCl_3$): ν 3357, 2935, 2218, 1690 cm^{-1}. Anal. Calcd for $C_{16}H_{17}N_3O_3$: C, 64.20; H, 5.72; N, 14.04. Found: C, 64.06; H, 5.73; N, 14.09.

*4.1.4.4. Ethyl 3-amino-1-(benzo[d][1,3]dioxol-5-ylmethyl)-4-cyano-1H-pyrrole-2-carboxylate (**6d**).* Eluent: PE/AcOEt, 2.5:1. Yellow solid (66% yield); mp 107–108 °C (Et_2O); ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.92 (s, 1H), 6.73 (d, J=8.8 Hz, 1H), 6.59 (dd, J_1=8.8 Hz, J_2=1.7 Hz, 1H), 6.57 (d, J=1.7 Hz, 1H), 5.92 (s, 2H), 4.88 (br s, 2H), 4.27 (q, J=6.9 Hz, 2H), 1.29 (t, J=6.9 Hz, 3H); MS m/z 336 (M+Na)$^+$; IR ($CHCl_3$): ν 3452, 3341, 3127, 2986, 2222, 1659, 1614, 1556, 1503, 1413, 1306 cm^{-1}. Anal. Calcd for $C_{16}H_{15}N_3O_4$: C, 61.34; H, 4.83; N, 13.41. Found: C, 61.49; H, 4.81; N, 13.37.

*4.1.4.5. Ethyl 3-amino-1-((6-chlorobenzo[d][1,3]dioxol-5-yl)methyl)-4-cyano-1H-pyrrole-2-carboxylate (**6e**).* Eluent: PE/AcOEt, 2:1. White solid (76% yield); mp 177–178 °C (DCM); ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.93 (s, 1H), 6.84 (s, 1H), 6.30 (s, 1H), 5.95 (s, 2H), 5.34 (s, 2H), 4.90 (br s, 2H), 4.25 (q, J=7.1 Hz, 2H), 1.25 (t, J=7.1 Hz, 3H); MS m/z 370 (M+Na)$^+$; IR ($CHCl_3$): ν 2928, 2222, 1686, 1607, 1483, 1288, 1248, 1040 cm^{-1}. Anal. Calcd for $C_{16}H_{14}ClN_3O_4$: C, 55.26; H, 4.06; N, 12.08. Found: C, 55.06; H, 4.07; N, 11.98.

*4.1.5. General procedure for the synthesis of **7a–e***

A suspension of the ester **4** (1 equiv) in a solution of KOH (4 equiv) in MeOH/H_2O (3:1) was heated under reflux for 3–12 h. After cooling to room temperature, the reaction mixture was acidified using 12 M HCl. The resulting precipitate was filtered and washed with water and PE to give the desired acid **7**.

*4.1.5.1. 4-Amino-6-(2-bromo-3,4,5-trimethoxybenzyl)-6H-pyrrolo[3,4-d]pyrimidine-7-carboxylic acid (**7a**).* White solid (92% yield); mp 233–234 °C (dec, DMF); ^{1}H NMR (200 MHz, Me$_2$SO-d_6): δ 9.45 (br s, 1H), 9.25 (br s, 2H), 8.33 (s, 1H), 7.84 (s, 1H), 6.88 (s, 1H), 5.80 (s, 2H), 3.79 (s, 3H), 3.78 (s, 3H), 3.71 (s, 3H); MS m/z 436 (M−H)$^-$; IR (Nujol): ν 3283, 1693, 919 cm^{-1}. Anal. Calcd for $C_{17}H_{17}BrN_4O_5$: C, 46.70; H, 3.92; N, 12.81. Found: C, 46.56; H, 3.95; N, 12.92.

*4.1.5.2. 4-Amino-6-(3,5-dimethoxybenzyl)-6H-pyrrolo[3,4-d]pyrimidine-7-carboxylic acid (**7b**).* White solid (79% yield); mp 199–200 °C (dec, DMF); ^{1}H NMR (200 MHz, Me$_2$SO-d_6): δ 9.75 (br s, 1H), 9.40 (br s, 2H), 8.34 (s, 1H), 8.33 (s, 1H), 6.43 (d, J=1.9 Hz, 1H), 6.38 (d, J=1.9 Hz, 2H), 5.75 (s, 2H), 3.67 (s, 6H); MS m/z 327 (M−H)$^-$; IR (Nujol): ν 3382, 3213, 1660, 840 cm^{-1}. Anal. Calcd for $C_{16}H_{16}N_4O_4$: C, 58.53; H, 4.91; N, 17.06. Found: C, 58.37; H, 4.93; N, 17.19.

*4.1.5.3. 4-Amino-6-(4-methoxybenzyl)-6H-pyrrolo[3,4-d]pyrimidine-7-carboxylic acid (**7c**).* White solid (82% yield); mp 186–187 °C (DMF); ^{1}H NMR (200 MHz, Me$_2$SO-d_6+D_2O): δ 8.17 (s, 1H), 7.81 (s, 1H), 7.22 (d, J=8.6 Hz, 2H), 6.89 (d, J=8.6 Hz, 2H), 5.76 (s, 2H), 3.69 (s, 3H); MS m/z 297 (M−H)$^-$; IR (Nujol): ν 3493, 2929, 1682, 1634, 1513, 1364, 1012 cm^{-1}. Anal. Calcd for $C_{15}H_{14}N_4O_3$: C, 60.40; H, 4.73; N, 18.78. Found: C, 60.56; H, 4.71; N, 18.66.

*4.1.5.4. 4-Amino-6-((benzo[d][1,3]dioxol-5-yl)methyl)-6H-pyrrolo[3,4-d]pyrimidine-7-carboxylic acid (**7d**).* White solid (58% yield); mp 174–175 °C (DMF); ^{1}H NMR (200 MHz, Me$_2$SO-d_6): δ 8.50 (br, 2H), 8.22 (s, 1H), 7.86 (s, 1H), 6.82 (m, 3H), 5.97 (s, 2H), 5.72 (s, 2H); MS m/z 311 (M−H)$^-$; IR (Nujol): ν 3597, 3300, 1675, 930 cm^{-1}. Anal. Calcd for $C_{15}H_{12}N_4O_4$: C, 57.69; H, 3.87; N, 17.94. Found: C, 57.83; H, 3.88; N, 17.83.

*4.1.5.5. 4-Amino-6-((6-chlorobenzo[d][1,3]dioxol-5-yl)methyl)-6H-pyrrolo[3,4-d]pyrimidine-7-carboxylic acid (**7e**).* White solid (82% yield); mp 191–193 °C (DMF); ^{1}H NMR (200 MHz, Me$_2$SO-d_6): δ 8.85 (br s, 2H), 8.29 (s, 1H), 7.86 (s, 1H), 7.18 (s, 1H), 6.78 (s, 1H), 6.07 (s, 2H), 5.79 (s, 2H); MS m/z 345 (M−H)$^-$, 381 (M+Cl)$^-$; IR (Nujol): ν 3375, 3193, 1675, 936, 840 cm^{-1}. Anal. Calcd for $C_{15}H_{11}ClN_4O_4$: C, 51.96; H, 3.20; N, 16.16. Found: C, 52.06; H, 3.17; N, 16.01.

*4.1.6. General procedure for the synthesis of **8a–j***

To a solution of **7** (0.19 mmol) in DMF, HBTU (146 mg, 0.38 mmol) was added followed by HOBt (26 mg, 0.19 mmol) and DIPEA (50 μL, 0.285 mmol). The mixture was stirred at room

temperature under N_2 for 1 h and the appropriate amine (0.023 mmol) and DIPEA (50 μL, 0.285 mmol) were then added. The reaction mixture was stirred at room temperature for 2–6 h, then the solvent was removed in vacuo, the residue was dissolved in EtOAc and the organic layer was washed with H_2O, aqueous HCl, aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated to dryness. The crude residue was purified by column chromatography to give **8** as pure compounds.

*4.1.6.1. 4-Amino-6-(4-methoxybenzyl)-N-propyl-6H-pyrrolo[3,4-d]-pyrimidine-7-carboxamide (**8a**).* Eluent: DCM/MeOH, 9:1. White solid (28% yield); mp 178–179 °C (DCM); ^{1}H NMR (200 MHz, CD_3OD-d_4): δ 8.12 (s, 1H), 7.59 (s, 1H), 7.20 (d, *J*=8.4 Hz, 2H), 6.86 (d, *J*=8.4 Hz, 2H), 5.87 (s, 2H), 3.74 (s, 3H), 3.36 (t, *J*=6.9 Hz, 2H), 1.67 (m, 2H), 0.99 (t, *J*=7.0 Hz, 3H); MS *m*/*z* 340 $(M+H)^+$; IR (Nujol): ν 3304, 2968, 1672, 1609 cm^{-1}. Anal. Calcd for $C_{18}H_{21}N_5O_2$: C, 63.70; H, 6.24; N, 20.64. Found: C, 63.90; H, 6.20; N, 20.49.

*4.1.6.2. 4-Amino-6-(4-methoxybenzyl)-N-allyl-6H-pyrrolo[3,4-d]pyrimidine-7-carboxamide (**8b**).* Eluent: DCM/MeOH, 11:1. Pale yellow solid (20% yield); mp 206–207 °C (EtOAc); ^{1}H NMR (200 MHz, CD_3OD-d_4): δ 8.11 (s, 1H), 7.60 (s, 1H), 7.20 (d, *J*=8.6 Hz, 2H), 6.85 (d, *J*=8.6 Hz, 2H), 6.01 (m, 1H), 5.86 (s, 2H), 5.26 (dd, J_1=5.0 Hz, J_2=1.7 Hz, 2H), 4.02 (d, *J*=5.0 Hz, 2H), 3.74 (s, 3H); MS *m*/*z* 338 $(M+H)^+$; IR (Nujol): ν 3432, 3208, 2360, 1632 cm^{-1}. Anal. Calcd for $C_{18}H_{19}N_5O_2$: C, 64.08; H, 5.68; N, 20.76. Found: C, 63.90; H, 5.70; N, 20.87.

*4.1.6.3. 4-Amino-6-(4-methoxybenzyl)-N-(2-propynyl)-6H-pyrrolo-[3,4-d]pyrimidine-7-carboxamide (**8c**).* Eluent: DCM/MeOH, 9:1. Pale yellow solid (16%); mp 213–215 °C (EtOAc); ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 8.17 (s, 1H), 7.69 (s, 1H), 7.60 (br s, 2H), 7.18 (d, *J*=8.7 Hz, 2H), 6.87 (d, *J*=8.7 Hz, 2H), 5.85 (s, 2H), 4.13 (m, 2H), 3.69 (s, 1H), 2.49 (m, 1H); MS *m*/*z* 336 $(M+H)^+$; IR (Nujol): ν 3402, 3298, 1668, 956 cm^{-1}. Anal. Calcd for $C_{18}H_{17}N_5O_2$: C, 64.47; H, 5.11; N, 20.88. Found: C, 64.18; H, 5.13; N, 20.97.

*4.1.6.4. 4-Amino-6-((benzo[d][1,3]dioxol-5-yl)methyl)-N-propyl-6H-pyrrolo[3,4-d]pyrimidine-7-carboxamide (**8d**).* Yellow solid (98% yield); mp 196–197 °C (EtOAc); ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.11 (t, *J*=5.0 Hz, 1H), 8.24 (s, 1H), 7.18 (s, 1H), 6.63 (m, 3H), 5.82 (s, 2H), 5.79 (s, 2H), 3.41(m, 2H), 1.63 (m, 2H), 0.96 (t, *J*=7.0 Hz, 3H); MS *m*/*z* 354 $(M+H)^+$; IR (Nujol): ν 3448, 3202, 1657, 930 cm^{-1}. Anal. Calcd for $C_{18}H_{19}N_5O_{32}$: C, 61.18; H, 5.42; N, 19.82. Found: C, 61.33; H, 5.40; N, 19.73.

*4.1.6.5. 4-Amino-6-((benzo[d][1,3]dioxol-5-yl)methyl)-N-(cyclopropylmethyl)-6H-pyrrolo[3,4-d]pyrimidine-7-carboxamide (**8e**).* Eluent: DCM/MeOH, 98:2. Pale yellow solid (72% yield); mp 171–173 °C (cyclohexane); ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.16 (t, *J*=5.0 Hz, 1H), 8.24 (s, 1H), 7.23 (s, 1H), 6.62 (s, 1H), 6.58 (s, 2H), 5.79 (s, 2H), 5.75 (s, 2H), 3.30 (t, *J*=6.1 Hz, 2H), 1.07 (m, 1H), 0.49 (m, 2H), 0.24 (m, 2H); MS *m*/*z* 366 $(M+H)^+$; IR (neat): ν 3207, 1629, 1530, 1248, 1038 cm^{-1}. Anal. Calcd for $C_{19}H_{19}N_5O_3$: C, 62.46; H, 5.24; N, 19.17. Found: C, 62.59; H, 5.22; N, 19.04.

*4.1.6.6. 4-Amino-6-((benzo[d][1,3]dioxol-5-yl)methyl)-N-isobutyl-6H-pyrrolo[3,4-d]pyrimidine-7-carboxamide (**8f**).* White solid (98% yield); mp 163–165 °C (toluene); ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.17 (br s, 1H), 8.31 (s, 1H), 7.10 (s, 1H), 6.76 (m, 3H), 5.91 (s, 4H), 3.31 (t, *J*=6.3 Hz, 2H), 1.94 (m, 1H), 1.00 (d, *J*=6.9 Hz, 6H); MS *m*/*z* 368 $(M+H)^+$; IR (neat): ν 3389, 1631, 1248, 1156, 1039 cm^{-1}. Anal. Calcd for $C_{19}H_{21}N_5O_3$: C, 62.11; H, 5.76; N, 19.06. Found: C, 62.31; H, 5.73; N, 18.98.

*4.1.6.7. Ethyl 2-(4-amino-6-((benzo[d][1,3]dioxol-5-yl)methyl)-6H-pyrrolo[3,4-d]pyrimidine-7-carboxamido)acetate (**8g**).* Pale yellow solid (98% yield); mp 218–220 °C (EtOAc); ^{1}H NMR (200 MHz, CD_3OD-d_4): δ 8.13 (s, 1H), 7.64 (s, 1H), 6.76 (m, 3H), 5.88 (s, 2H), 5.81 (s, 2H), 4.18 (m, 4H), 1.25 (t, *J*=7.0 Hz, 3H); MS *m*/*z* 398 $(M+H)^+$; IR (Nujol): ν 3423, 3322, 3222, 1726, 1641, 923 cm^{-1}. Anal. Calcd for $C_{19}H_{19}N_5O_5$: C, 57.43; H, 4.82; N, 17.62. Found: C, 57.28; H, 4.85; N, 17.75.

*4.1.6.8. 4-Amino-6-(2-bromo-3,4,5-trimethoxybenzyl)-N-allyl-6H-pyrrolo[3,4-d]pyrimidine-7-carboxamide (**8h**).* Eluent: DCM/MeOH, 9:1. Pale yellow solid (74% yield); mp 174–175 °C (EtOAc); ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.23 (br s, 1H), 8.21 (s, 1H), 7.30 (s, 1H), 6.52 (s, 1H), 6.37 (br s, 2H), 6.00 (s, 2H), 5.96–5.82 (m, 1H), 5.16 (dd, J_1=10.1 Hz, J_2=1.6 Hz, 2H), 4.06 (br s, 2H), 3.81 (s, 3H), 3.78 (s, 3H), 3.61 (s, 3H); MS *m*/*z* 478 $(M+H)^+$; IR ($CHCl_3$): ν 3406, 3291, 3106, 1667, 925 cm^{-1}. Anal. Calcd for $C_{20}H_{22}BrN_5O_4$: C, 50.43; H, 4.66; N, 14.70. Found: C, 50.56; H, 4.63; N, 14.61.

*4.1.6.9. 4-Amino-6-(2-bromo-3,4,5-trimethoxybenzyl)-N-propyl-6H-pyrrolo[3,4-d]pyrimidine-7-carboxamide (**8i**).* Eluent: DCM/MeOH, 92:8. Yellow solid (68% yield); mp 204–205 °C (EtOAc/*n*-hexane); ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.14 (t, *J*=5.6 Hz, 1H), 8.25 (s, 1H), 7.27 (s, 1H), 6.57 (s, 1H), 6.15 (br s, 2H), 6.05 (s, 2H), 3.84 (s, 3H), 3.81 (s, 3H), 3.63 (s, 3H), 3.41 (q, *J*=6.5 Hz, 2H), 1.64 (m, 2H), 0.97 (t, *J*=7.3 Hz, 3H); MS *m*/*z* 479.9$(M+H)^+$; IR ($CHCl_3$): ν 3412, 3293, 3004, 1624, 1169, 1006 cm^{-1}. Anal. Calcd for $C_{20}H_{24}BrN_5O_4$: C, 50.22; H, 5.06; N, 16.70. Found: C, 50.07; H, 5.07; N, 16.81.

*4.1.6.10. 4-Amino-6-((6-chlorobenzo[d][1,3]dioxol-5-yl)methyl)-N-propyl-6H-pyrrolo[3,4-d]pyrimidine-7-carboxamide (**8j**).* Brown solid (82% yield); mp 218.5–219 °C (EtOAc/*n*-hexane); ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.11 (t, *J*=5.4 Hz, 1H), 8.28 (s, 1H), 7.21 (s, 1H), 6.78 (s, 1H), 6.56 (s, 1H), 6.00 (s, 2H), 5.88 (s, 2H), 3.42 (q, *J*=6.6 Hz, 2H), 1.66 (m, 2H), 0.99 (t, *J*=7.4 Hz, 3H); MS *m*/*z* 389 $(M+H)^+$; IR ($CHCl_3$): ν 3412, 3001, 2964, 1636, 1249, 1120, 1040 cm^{-1}. Anal. Calcd for $C_{18}H_{18}ClN_5O_3$: C, 55.75; H, 4.68; N, 18.06. Found: C, 55.89; H, 4.67; N, 17.98.

*4.1.7. General procedure for the synthesis of **9a–d***

A suspension of **7** (0.23 mmol) in 6 M HCl (10 mL) was heated at reflux for 1–4 h (until the suspension became a solution). After cooling to room temperature, the solution was neutralized using Na_2CO_3 and the resulting precipitate was extracted with EtOAc. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by flash chromatography or recrystallized to give the desired compounds in a pure form.

*4.1.7.1. 6-((6-Chlorobenzo[d][1,3]dioxol-5-yl)methyl)-6H-pyrrolo[3,4-d]pyrimidin-4-amine (**9a**).* Pale yellow solid (80% yield); mp 237–238 °C (EtOAc); ^{1}H NMR (200 MHz, CD_3OD-d_4): δ 7.93 (s, 1H), 7.46 (s, 1H), 7.15 (s, 1H), 6.93 (s, 1H), 6.73 (s, 1H), 5.97 (s, 2H), 5.38 (s, 2H); MS *m*/*z* 305 $(M+H)^+$; IR (Nujol): ν 3322, 927, 874 cm^{-1}. Anal. Calcd for $C_{14}H_{11}ClN_4O_2$: C, 55.55; H, 3.66; N, 18.51. Found: C, 55.72; H, 3.67; N, 18.33.

*4.1.7.2. 6-((Benzo[d][1,3]dioxol-5-yl)methyl)-6H-pyrrolo[3,4-d]pyrimidin-4-amine (**9b**).* Eluent: DCM/MeOH, 88:12. Pale brown solid (36% yield); mp 233–234 °C (dec, MeOH); ^{1}H NMR (400 MHz, CD_3OD-d_4): δ 7.93 (s, 1H), 7.44 (s, 1H), 7.14 (s, 1H), 6.78 (s, 2H), 6.75 (s, 1H), 5.91 (s, 2H), 5.24 (s, 2H); MS *m*/*z* 269 $(M+H)^+$; IR (Nujol): ν 3316, 920, 826 cm^{-1}. Anal. Calcd for $C_{14}H_{12}N_4O_2$: C, 62.68; H, 4.51; N, 20.88. Found: C, 62.46; H, 4.54; N, 20.97.

*4.1.7.3. 6-(3,5-Dimethoxybenzyl)-6H-pyrrolo[3,4-d]pyrimidin-4-amine (**9c**).* Eluent: DCM/MeOH, 85:15. White solid (42% yield); mp 191–192 °C (EtOAc); ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 7.88 (s, 1H), 7.38 (s, 1H), 7.20 (s, 1H), 7.09 (br s, 2H), 6.42 (m, 3H), 5.25 (s, 2H),

3.68 (s, 6H); MS *m/z* 285 $(M+H)^+$; IR (Nujol): ν 3113, 908 cm^{-1}. Anal. Calcd for $C_{15}H_{16}N_4O_2$: C, 63.37; H, 5.67; N, 19.71. Found: C, 63.16; H, 5.70; N, 19.87.

*4.1.7.4. 6-(2-Bromo-3,4,5-trimethoxybenzyl)-6H-pyrrolo[3,4-d]pyrimidin-4-amine (**9d**).* Eluent: DCM/MeOH, 8:2. White solid (83% yield); mp 218–220 °C (dec, EtOAc); ^1H NMR (200 MHz, Me_2SO-d_6): δ 7.81 (s, 1H), 7.33 (s, 1H), 7.16 (s, 1H), 7.06 (br s, 2H), 6.89 (s, 1H), 5.31 (s, 2H), 3.70 (s, 3H), 3.69 (s, 3H), 3.78 (s, 3H); MS *m/z* 395 $(M+H)^+$; IR (Nujol): ν 3324, 920 cm^{-1}. Anal. Calcd for $C_{16}H_{17}BrN_4O_3$: C, 48.87; H, 4.36; N, 14.25. Found: C, 49.02; H, 4.39; N, 14.39.

*4.1.8. General procedure for the synthesis of **10a–c***

$LiAlH_4$ (45 mg, 1.16 mmol) was added to a suspension of **4** (0.39 mmol) in dry THF (15 mL) cooled in an ice bath. The reaction mixture was then warmed to room temperature and stirred for 1–3 h. Water was slowly added to quench $LiAlH_4$, the mixture was concentrated in vacuo and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated to dryness to give the corresponding alcohol. Acetyl chloride (5 mL) was added dropwise to dry propanol (10 mL) and the solution thus obtained was added, after 1 h, to the alcohol. The reaction mixture was stirred at room temperature for 2–4 h then aq $NaHCO_3$ was added until pH 7 and the mixture was concentrated in vacuo and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated to dryness. The residue was purified by flash chromatography to give the desired products in a pure form.

*4.1.8.1. 6-(3,5-Dimethoxybenzyl)-7-(propoxymethyl)-6H-pyrrolo[3,4-d]pyrimidin-4-amine (**10a**).* Eluent: DCM/MeOH, 8:2. Yellow solid (33% yield); mp 146–147 °C (EtOAc); ^1H NMR (200 MHz, CD_3OD-d_4): δ 8.16 (s, 1H), 7.06 (s, 1H), 6.33 (d, *J*=1.6 Hz, 1H), 6.20 (d, *J*=1.6 Hz, 2H), 5.65 (s, 2H), 4.74 (s, 2H), 3.68 (s, 6H), 3.36 (q, *J*=6.9 Hz, 2H), 1.64 (m, 2H), 0.83 (t, *J*=7.3 Hz, 3H); MS *m/z* 357 $(M+H)^+$; IR (Nujol): ν 3012, 952 cm^{-1}. Anal. Calcd for $C_{19}H_{24}N_4O_3$: C, 64.03; H, 6.79; N, 15.72. Found: C, 63.88; H, 6.81; N, 15.80.

*4.1.8.2. 6-(2-Bromo-3,4,5-trimethoxybenzyl)-7-(propoxymethyl)-6H-pyrrolo[3,4-d]pyrimidin-4-amine (**10b**) and 6-(3,4,5-trimethoxybenzyl)-7-(propoxymethyl)-6H-pyrrolo[3,4-d]pyrimidin-4-amine (**10c**).* Compound **10b**. Eluent: DCM/MeOH, 9:1 (R_f 0.54). Orange oil (17% yield); ^1H NMR (200 MHz, $CDCl_3$): δ 8.20 (s, 1H), 7.03 (s, 1H), 6.09 (s, 1H), 5.43 (s, 2H), 4.78 (s, 2H), 3.89 (s, 3H), 3.84 (s, 3H), 3.58 (s, 3H), 3.39 (t, *J*=6.9 Hz, 2H), 1.49 (m, 2H), 0.82 (t, *J*=7.2 Hz, 3H); MS *m/z* $(M+H)^+$; IR ($CHCl_3$): ν 3411, 2939, 1341, 1109 cm^{-1}. Anal. Calcd for $C_{20}H_{25}BrN_4O_4$: C, 51.62; H, 5.42; N, 12.04. Found: C, 51.74; H, 5.39; N, 11.96. Compound **10c**. Obtained by further elution of the above column (R_f 0.43). White solid (16% yield); mp >165 °C (MeOH); ^1H NMR (200 MHz, CD_3OD-d_4): δ 8.03 (s, 1H), 7.69 (s, 1H), 6.54 (s, 2H), 5.41 (s, 2H), 4.80 (s, 2H), 3.82 (s, 6H), 3.73 (s, 3H), 3.43 (t, *J*=6.9 Hz, 2H), 1.53 (m, 2H), 0.88 (t, *J*=7.2 Hz, 3H); MS *m/z* 387 $(M+H)^+$; IR (Nujol): ν 3104, 2972, 1464, 1130 cm^{-1}. Anal. Calcd for $C_{20}H_{26}N_4O_4$: C, 62.16; H, 6.78; N, 14.50. Found: C, 61.99; H, 6.80; N, 14.57.

*4.1.9. Synthesis of 4-amino-6-(4-methoxybenzyl)-6H-pyrrolo[3,4-d]pyrimidine-7-carbaldehyde (**11**)*

$LiAlH_4$ (30 mg, 0.77 mmol) was added to a suspension of **4a** (100 mg, 0.3 mmol) in dry THF (4 mL) cooled in an ice bath. The reaction mixture was warmed to room temperature and stirred for 2 h. Water was slowly added to quench $LiAlH_4$, then the mixture was concentrated in vacuo and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated to dryness to obtain the corresponding alcohol. MnO_2 (250 mg, 3.7 mmol) was added to a solution of the alcohol in dry *tert*-BuOH (10 mL) kept at reflux temperature and after 16 h the mixture was cooled to room temperature, filtered through a pad of Celite®, and the solvent was evaporated. The residue was purified by flash chromatography (eluent: DCM/MeOH, 95:5) to give the desired product as a white solid (57% yield); mp 213–214 °C (EtOH); ^1H NMR (200 MHz, CD_3OD-d_4): δ 10.01 (s, 1H), 8.18 (s, 1H), 7.86 (s, 1H), 7.23 (d, *J*=8.6 Hz, 2H), 6.88 (d, *J*=8.6 Hz, 2H), 5.72 (s, 2H), 3.74 (s, 3H); MS *m/z* 283 $(M+H)^+$; IR (Nujol): ν 3309, 3097, 2784, 1660, 1650, 1595, 1515, 1282, 1255 cm^{-1}. Anal. Calcd for $C_{15}H_{14}N_4O_2$: C, 63.82; H, 5.00; N, 19.85. Found: C, 63.66; H, 5.02; N, 19.79.

*4.1.10. Synthesis of 3-(4-amino-6-(4-methoxybenzyl)-6H-pyrrolo[3,4-d]pyrimidin-7-yl)propan-1-ol (**12**)*

To a solution of **11** (35 mg, 0.12 mmol) in dry 2-methoxyethanol (4 mL), methyl triphenylphosphoranylideneacetate (165 mg, 0.5 mmol) was added and the mixture was heated at reflux for 16 h, then the solvent was evaporated to dryness and the residue was purified by flash chromatography (eluent: DCM/MeOH, 95:5) to give the product as a mixture of cis/trans isomers. The mixture was dissolved in MeOH (10 mL), 10% Pd/C was added and the resulting mixture was stirred at room temperature for 3 h under H_2 atmosphere. The mixture was filtered through a pad of Celite®, the solvent was removed to dryness and the residue was dissolved in dry THF (5 mL). $LiAlH_4$ (5 mg, 0.12 mmol) was added to the solution thus obtained and the mixture was stirred at room temperature for 2 h. Water was slowly added to quench $LiAlH_4$, the mixture was concentrated in vacuo, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated to obtain the desired product as a yellow solid after trituration with PE (30% yield); mp 177–178 °C (triturated with PE); ^1H NMR (200 MHz, CD_3OD-d_4): δ 7.89 (s, 1H), 7.33 (s, 1H), 7.05 (d, *J*=8.6 Hz, 2H), 6.88 (d, *J*=8.6 Hz, 2H), 5.33 (s, 2H), 3.74 (s, 3H), 3.48 (t, *J*=6.5 Hz, 2H), 2.95 (t, *J*=7.6 Hz, 2H), 1.69 (m, 2H); MS *m/z* 313 $(M+H)^+$; IR ($CHCl_3$): ν 3411, 3019, 1513, 1251 cm^{-1}. Anal. Calcd for $C_{17}H_{20}N_4O_2$: C, 65.37; H, 6.45; N, 17.94. Found: C, 65.53; H, 6.44; N, 17.88.

*4.1.11. Synthesis of 4-nitro-1H-pyrazole-3-carbonitrile (**16**)*

A suspension of **15** (1 g, 6.4 mmol) in $SOCl_2$ (3.5 mL) was heated at reflux for 3 h. Excess $SOCl_2$ was removed by evaporation under reduced pressure and the resulting slurry was dissolved in THF and added slowly to a cooled (0–5 °C) solution of 30% aqueous NH_4OH (15 mL). The mixture was warmed to room temperature and stirred for 12 h. Then the precipitate was collected by filtration and washed with water and PE to give the corresponding 4-nitro-1*H*-pyrazole-3-carboxamide as a white solid (650 mg, 65% yield). A suspension of the amide (410 mg, 2.6 mmol) in DCM (20 mL) and pyridine (1.65 mL) was treated with a solution of triphosgene (780 mg, 2.6 mmol) in DCM (20 mL). The mixture was stirred for 16 h at room temperature then water (3 mL) was slowly added to the mixture, followed by 6 N HCl (6 mL) and brine (6 mL). The mixture was extracted with DCM and EtOAc. The organic solutions were combined, concentrated and washed with 1 M HCl and brine. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to yield the title compound as a pale yellow solid (99% yield); mp 161–162 °C (EtOAc); ^1H NMR (200 MHz, Me_2SO-d_6): δ 14.96 (br s, 1H), 9.14 (s, 1H); MS *m/z* 137 $(M-H)^-$; IR ($CHCl_3$): ν 3269, 2934, 2253 cm^{-1}. Anal. Calcd for $C_4H_2N_4O_2$: C, 34.79; H, 1.46; N, 40.57. Found: C, 34.90; H, 1.45; N, 40.46.

*4.1.12. Synthesis of 1-(2-bromo-3,4,5-trimethoxybenzyl)-4-nitro-1H-pyrazole-3-carbonitrile (**17**)*

To a solution of **16** (360 mg, 2.6 mmol) in DCM (20 mL), benzyl bromide (975 mg, 2.9 mmol) was added followed by tetra-*n*-

butylammonium bromide (420 mg, 1.3 mmol) and 20% NaOH (10 mL). The mixture was stirred at room temperature for 2 h, then the organic layer was separated, and the water solution was extracted with DCM; the combined organic phases were washed with brine, dried over Na_2SO_4 and the solvent removed in vacuo. The crude residue was purified by silica gel column chromatography (eluent, PE/EtOAc, 1:1) to give the desired product (87% yield) as a white solid; mp 150–151 °C (EtOAc/*n*-hexane); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.22 (s, 1H), 6.80 (s, 1H), 5.43 (s, 2H), 3.89 (s, 6H), 3.87 (s, 3H); MS *m*/*z* 420 $(M+Na)^+$; IR ($CHCl_3$): ν 3144, 3029, 2250, 1220, 1006 cm^{-1}. Anal. Calcd for $C_{14}H_{13}BrN_4O_5$: C, 42.34; H, 3.30; N, 14.11. Found: C, 42.48; H, 3.28; N, 14.01.

*4.1.13. Synthesis of 4-amino-5-chloro-1-(2-bromo-3,4,5-trimethoxybenzyl)-1H-pyrazole-3-carbonitrile (**18**)*

To a suspension of **17** (260 mg, 0.7 mmol) in MeOH (8 mL) and H_2O (0.5 mL), 12 M HCl (0.5 mL) was added followed by Fe powder (73 mg, 1.3 mmol). The mixture was heated at reflux for 1.5 h. Then, the mixture was cooled to room temperature and filtered through a Celite® pad. The solvent was removed under reduced pressure and the slurry thus obtained was extracted with EtOAc. The organic layer was washed with aqueous $NaHCO_3$, water, and brine, dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel column chromatography (eluent, PE/EtOAc, 1:1) to give **19** (58% yield) as a white solid; mp 103–104 °C (EtOAc); ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.26 (s, 1H), 5.37 (s, 2H), 3.89 (s, 3H), 3.86 (s, 3H), 3.74 (s, 3H); MS *m*/*z* 424 $(M+Na)^+$; IR ($CHCl_3$): ν 3379, 3011, 2236, 1215, 1109 cm^{-1}. Anal. Calcd for $C_{14}H_{14}BrClN_4O_3$: C, 41.87; H, 3.51; N, 13.95. Found: C, 42.01; H, 3.48; N, 13.91.

*4.1.14. Synthesis of 4-amino-1-(2-bromo-3,4,5-trimethoxybenzyl)-1H-pyrazole-3-carbonitrile (**19**)*

A solution of **17** (200 mg, 0.5 mmol) in EtOAc (15 mL) was treated with 5% Rh on aluminium oxide (40 mg) and hydrogenated at 1 atm for 16 h. The catalyst was removed by filtration through a Celite® pad, and the solution was evaporated to dryness and the residue was purified by silica gel column chromatography (eluent, PE/AcOEt, 1:1) to give **19** as a yellow oil (87% yield); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.34 (s, 1H), 6.67 (br s, 2H); 6.58 (s, 1H), 5.31 (s, 2H), 3.87 (s, 3H), 3.85 (s, 3H), 3.79 (s, 3H); MS *m*/*z* 389 $(M+Na)^+$; IR ($CHCl_3$): ν 3034, 2234, 1336, 1218, 1109 cm^{-1}. Anal. Calcd for $C_{14}H_{15}BrN_4O_3$: C, 45.79; H, 4.12; N, 15.26. Found: C, 45.64; H, 4.15; N, 15.34.

*4.1.15. General procedure for the synthesis of **13** and **14***

Formamidine acetate (60 mg, 0.57 mmol) was added to a solution of the appropriate pyrazole derivative (0.19 mmol) in EtOH (5 mL) and the solution was heated under reflux for 4 h. The solvent was evaporated and the crude residue was purified by silica gel column chromatography.

*4.1.15.1. 2-(2-Bromo-3,4,5-trimethoxybenzyl)-3-chloro-2H-pyrazolo-[4,3-d]pyrimidin-7-amine (**13**).* Eluent: DCM/MeOH, 9:1. White solid, 80% yield; mp 195–196 °C (EtOAc); ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 8.09 (s, 1H), 7.70 (br s, 2H), 6.54 (s, 1H), 5.65 (s, 2H), 3.77 (s, 3H), 3.75 (s, 3H), 3.64 (s, 3H); MS *m*/*z* 429 $(M+H)^+$; IR ($CHCl_3$): ν 3407, 3019, 1335, 1109 cm^{-1}. Anal. Calcd for $C_{15}H_{15}BrClN_5O_3$: C, 42.03; H, 3.53; N, 16.34. Found: C, 42.18; H, 3.50; N, 16.22.

*4.1.15.2. 2-(2-Bromo-3,4,5-trimethoxybenzyl)-2H-pyrazolo[4,3-d]pyrimidin-7-amine (**14**).* Eluent: DCM/MeOH, 9:1. White solid, 47% yield; mp 188–189 °C (EtOAc); ^{1}H NMR (200 MHz, Me_2SO-d_6): δ 8.29 (s, 1H), 8.04 (s, 1H), 7.49 (br s, 2H), 6.77 (s, 1H), 5.62 (s, 2H), 3.75 (s, 6H), 3.69 (s, 3H); MS *m*/*z* 396 $(M+H)^+$; IR (Nujol): ν 3454, 3001, 1395, 1201, 1107 cm^{-1}. Anal. Calcd $C_{15}H_{16}BrN_5O_3$: C, 45.70; H, 4.09; N, 17.76. Found: C, 45.84; H, 4.07; N, 17.68.

4.2. Biology

The human N-terminal Hsp90 domain, isoform beta, was recombinantly produced in *Escherichia coli* and the protein was purified via affinity tag chromatography. Geldanamycin was fluorescence labelled at the 17-position with the rhodamine fluorescence dye Tamra (λ_{ex} 543 nm, λ_{em} 595 nm). To determine the equilibrium binding constant K_d for the interaction of Hsp90 NTD with geldanamycin a 5 nM solution of the fluorescent conjugate was incubated with increasing concentrations of Hsp90 NTD, followed by incubation for 60 min at room temperature and 2d-FIDA anisotropy measurements on a confocal fluorescence reader (Insight™; Perkin Elmer). The K_d was determined to be 80 nM and this concentration of Hsp90 NTD was subsequently used throughout all compound tests. The assay sensitivity was confirmed by using unlabelled geldanamycin and radicicol as competitors, which produced K_i values of 60 and 4 nM, respectively. Project compounds were dissolved in dimethylsulfoxide (DMSO) to 10 mM stock concentration and subsequently diluted in the organic solvent for IC_{50}/K_i determinations. All compounds were transferred without aqueous predilution into assay buffer containing Hsp90 NTD, not exceeding a 5% residual DMSO concentration. The fluorescent ligand was added as a last step, followed by incubation to equilibrium. The IC_{50} values were calculated using the Graph Pad Prism software.

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