Supporting Information.

Design, Synthesis, Biological Activity and ADME Properties of Pyrazolo[3,4-*d*]pyrimidines Active in Hypoxic Human Leukaemia Cells: a Lead Optimization Study

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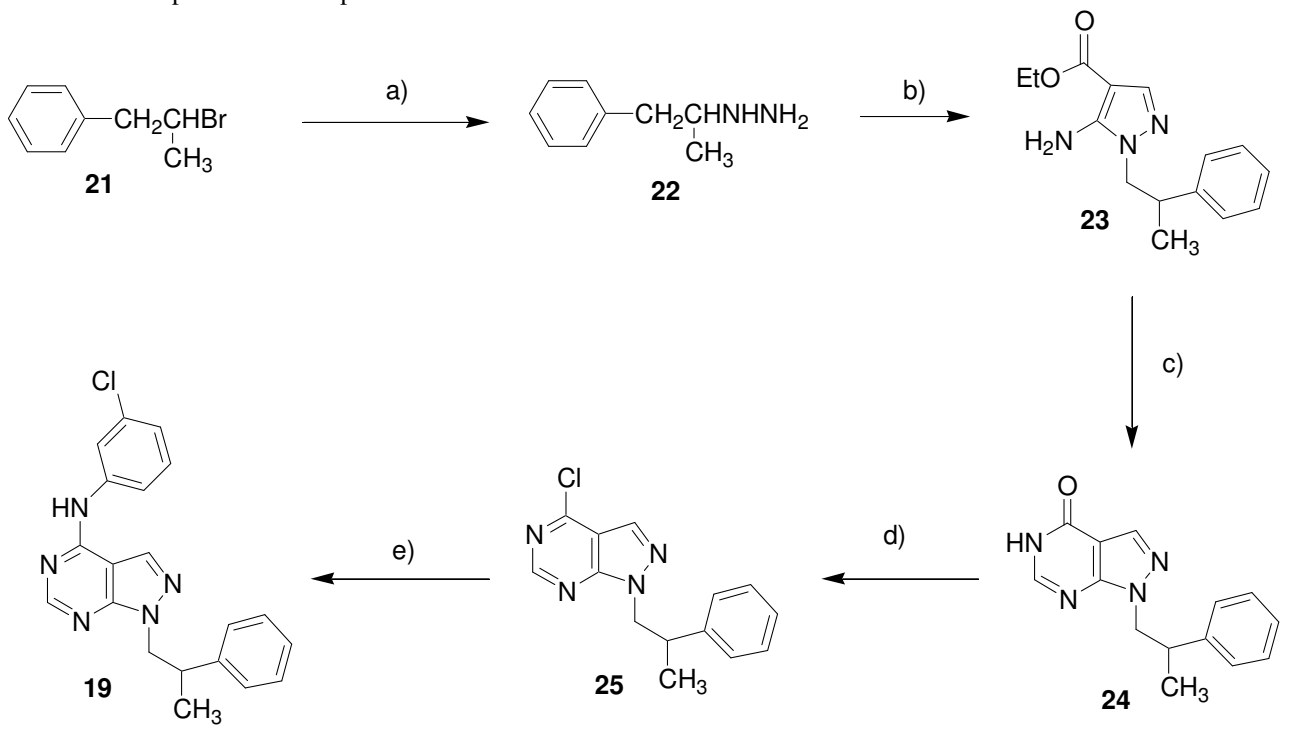
Synthesis of compound **19** is reported in Scheme S1. The (1-methyl-2-phenylethyl)hydrazine **22**, prepared by us starting from 1-bromo-2-phenylpropane **21** and used as crude, was reacted with ethyl(ethoxymethylene)cyanoacetate in absolute ethanol at reflux to afford the ethyl 5-ammino-1-(2-phenylpropyl)-1*H*-pyrazole-4-carboxylate **23**, which, treated with an excess of formamide for 4 h at 190 °C, gave the corresponding pyrazolo[3,4-*d*]pyrimidinone **24** with good yield (60%). Treatment of the latter with an excess of the Vilsmeier complex ($POCl_3$/DMF, 1:1, 10 equiv.) for 8 h at reflux in $CHCl_3$ led to the formation of the halogenated derivative **25**. Finally, the C4 chlorine atom was in turn substituted with 3-chloroaniline in absolute ethanol, to afford the desired compound **19** in good yield (61%).

Scheme S1. Preparation of compound **19**.



Conditions: a) Hydrazine monohydrate, abs. ethanol, reflux, 24 h; b) Ethyl (ethoxymethylene)cyanoacetate, abs. ethanol, reflux, 5 h; c) formamide, 190 °C, 4 h; d) $POCl_3$/DMF, $CHCl_3$, reflux, 8 h; e) 3-chloroaniline, abs. EtOH, reflux, 5 h.

Synthesis of (1-methyl-2-phenylethyl)hydrazine (22).
A solution of 1-bromo-2-phenylpropane **21** (1.99 g, 10 mmol) and hydrazine monohydrate (4.00 g, 80 mmol) in absolute ethanol (20 mL) was refluxed for 24 h. The solvent was evaporated under reduced pressure and the crude was treated with anhydrous K_2CO_3, then extracted with $CHCl_3$ (3 x 20 mL). The organic solution was dried ($MgSO_4$), and concentrated under reduced pressure. The crude was purified by bulb to bulb distillation to obtain the pure product **22** as pale yellow oil.

Synthesis of ethyl 5-amino-1-(2-phenylpropyl)-1*H*-pyrazole-4-carboxylate (23).
A solution of (1-methyl-2-phenylethyl)hydrazine **22** (1.50 g, 10 mmol) and ethyl(ethoxymethylene)cyanoacetate (1.69 g, 10 mmol) in absolute ethanol was refluxed for 5 h. The solvent was evaporated under reduced pressure and the crude was purified by column chromatography (Florisil 100-200 mesh) using diethyl ether as the eluent, to afford the pure product **23** as yellow oil (1.65 g, 60%). ^{1}H NMR: δ 1.06-1.20 (m, 6H, $2CH_3$), 3.24-3.26 (m, 1H, CH), 3.78-3.83 and 3.85-3.90 (2m, 2H, CH_2N), 4.10 (q, J = 7.0, 2H, CH_2O), 4.79 (br s, 2H, NH_2 disappears with D_2O), 7.00-7.16 (m, 5H Ar), 7.55 (s, 1H, H-3). IR cm^{-1}: 3398, 3291, 3215, 3161 (NH_2 + OH), 1681 (CO). MS: m/z 273 $[M+1]^+$. Anal.($C_{15}H_{19}N_3O_2$) C, H, N.

Synthesis of 1-(2-phenylpropyl)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (24).
A suspension of ethyl 5-amino-1-(2-phenylpropyl)-1*H*-pyrazole-4-carboxylate **23** (2.73 g, 10 mmol) in formamide (10 g, 333 mmol) was heated at 190 °C for 5 h and then poured in H_2O (300 mL). The crude product was filtered and purified by dissolving in 2 M NaOH (100 mL) and boiling with charcoal, followed by precipitation with glacial acetic acid. The solid was filtered and recrystallized from absolute ethanol to give compound **24** as white solid (1.52 g, 60%). Mp: 216-217 °C. ^{1}H NMR: δ 1.19-1.21 (m, 3H, CH_3), 3.45-3.47 (m, 1H, $\underline{CH}CH_3$), 4.40-4.44 (m, 2H, CH_2N), 7.11-7.20 (m, 5H Ar), 7.84 (s, 1H, H-3), 8.03 (s, 1H, H-6), 11.99 (br s, 1H, NH disappears with D_2O). IR cm^{-1}: 3250-3000 (NH +OH), 1688 (CO). MS: m/z 254 $[M+1]^+$. Anal.($C_{14}H_{14}N_4O$) C, H, N.

Synthesis of 4-chloro-1-(2-phenylpropyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (25).

The Vilsmeier complex, previously prepared from $POCl_3$ (1.53 g, 10 mmol) and anhydrous dimethylformamide (DMF) (0.73 g, 10 mmol) was added to a suspension of 1-(2-phenylpropyl)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one **24** (0.25 g, 1 mmol) in $CHCl_3$ (10 mL). The mixture was refluxed for 8 h. The solution was washed with H_2O (20 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The crude was purified by column chromatography (Florisil 100-200 mesh) using diethyl ether as the eluent, to afford the pure product **25** as a yellow oil, which crystallized standing in a refrigerator by a mixture 1/1 of diethyl ether/petroleum ether (bp 40-60 °C) as white solid (0.24 g, 88%). Mp: 59-60 °C. ^{1}H NMR: δ 1.25-1.29 (m, 3H, CH_3), 3.46-3.67 (m, 1H, $\underline{CH}CH_3$), 4.52-4.69 (m, 2H, CH_2N), 7.06-7.28 (m, 5H Ar), 8.11 (s, 1H, H-3), 8.67 (s, 1H, H-6). MS: m/z 273 $[M+1]^+$. Anal.($C_{14}H_{13}N_4Cl$) C, H, N.

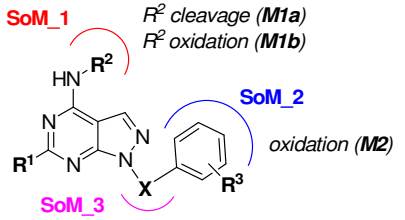
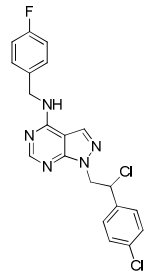
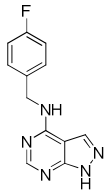
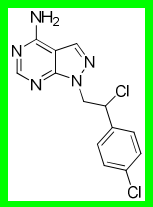
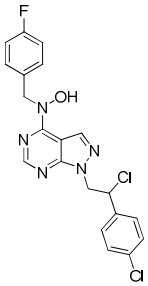
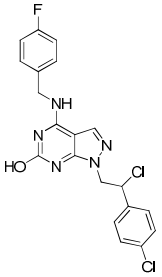
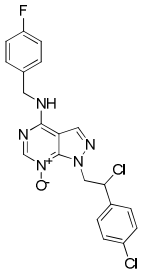
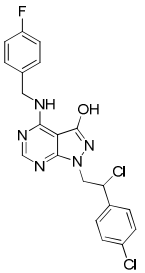
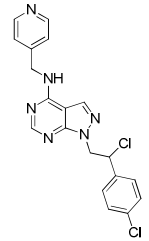
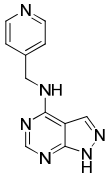
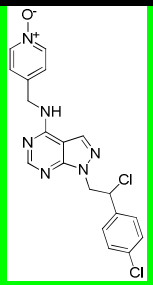
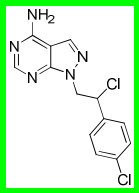
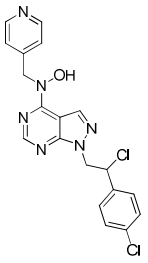
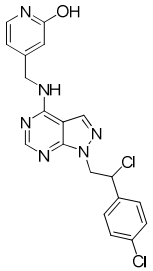
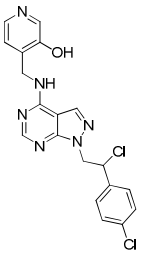
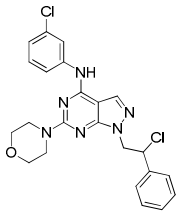
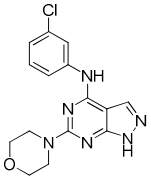
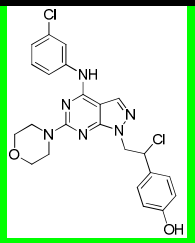
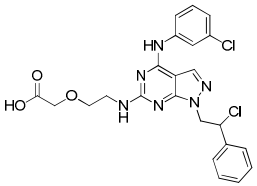
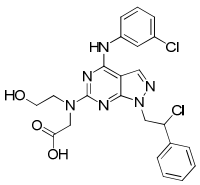
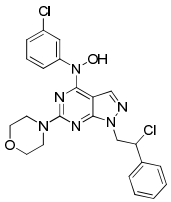
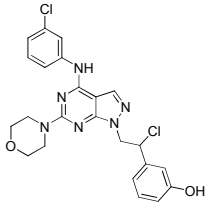
Synthesis of *N*-(3-chlorophenyl)-1-(2-phenylpropyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (19).

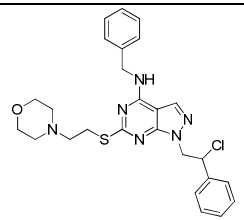
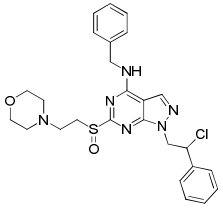
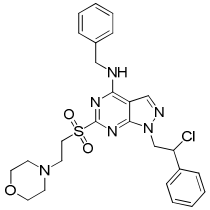
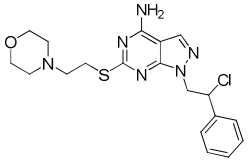
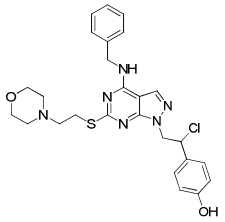
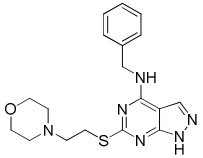
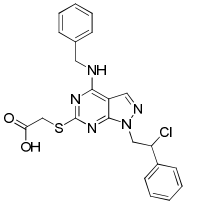
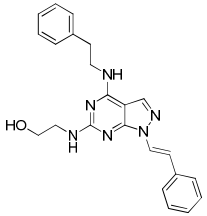
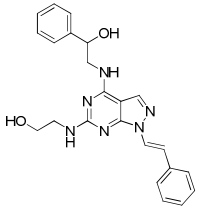
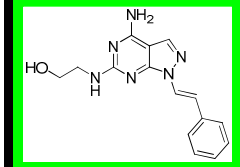
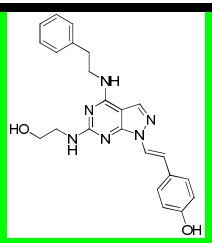
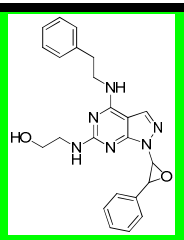
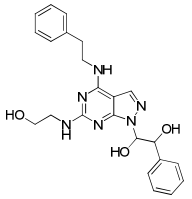
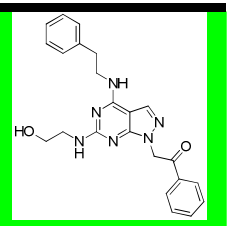
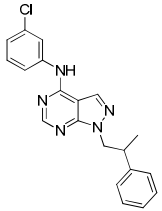
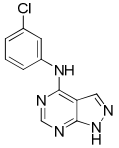
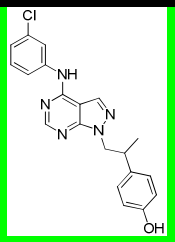
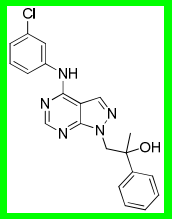
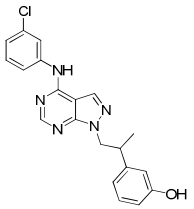
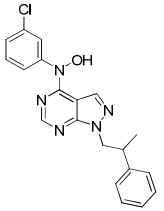
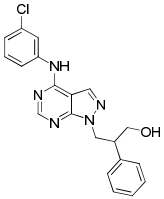
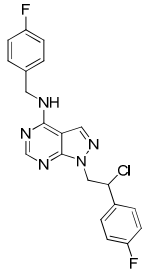
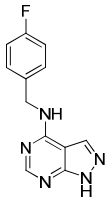
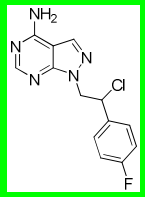
A solution of 4-chloro-1-(2-phenylpropyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine **25** (0.27 g, 1 mmol) and 3-chloroaniline (0.32 g, 2.5 mmol) in absolute ethanol was refluxed for 5 h. After cooling, the solvent was evaporated under reduced pressure and the crude was treated with H_2O (20 mL), then extracted with $CHCl_3$ (20 mL); the organic phase was washed with H_2O (20 mL), dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The oil obtained was purified by column chromatography (Silicagel 0.06-0200 mm, 40 Å) using diethyl ether as the eluent, to afford the pure product **19** as a yellow oil, which crystallized standing in a refrigerator by adding petroleum ether (bp 40-60 °C) as white solid (0.22 g, 61%). Mp: 142-143 °C. ^{1}H NMR: δ 1.49-1.51 (m, 3H, CH_3), 3.69-3.78 (m, 1H, $\underline{CH}CH_3$), 4.70-4.81 (m, 2H, CH_2N), 6.90-7.60 (m, 10H, 9 Ar + H-3), 8.51 (s, 1H, H-6), 11.99 (br s, 1H, NH disappears with D_2O). IR cm^{-1}: 3280 (NH). MS: m/z 364 $[M+1]^+$. Anal. ($C_{20}H_{18}N_5Cl$) C, H, N.

Table S1.

Compd.	Elemental analysis (%, calculated, found)			
	C	**H**	**N**	**S**
3a	57.07 56.96	4.56 4.84	15.85 15.91	7.26 7.44
4b	62.74 62.80	5.88 6.03	21.52 21.80	9.85 9.99
5b	57.12 57.24	5.36 5.66	19.59 19.39	8.97 8.93
5c	62.99 63.00	5.05 5.01	16.69 16.72	7.64 7.72
6c	68.98 68.78	6.04 6.29	20.99 20.79	
8	50.29 50.00	4.22 4.20	16.76 16.49	9.59 9.33
9	59.81 59.59	5.61 5.58	20.52 20.49	
10	53.98 54.05	4.53 4.77	18.51 18.35	
11	58.86 58.82	4.72 5.01	17.91 17.83	
13	56.84 56.68	5.77 5.55	17.44 17.48	7.99 8.00
14	52.06 52.00	4.83 4.91	15.98 16.01	7.31 7.52
15a	57.31 57.04	6.34 6.41	18.23 18.12	6.96 7.00
15b	61.34 61.12	5.74 5.94	16.51 16.31	6.30 6.22
15c	59.25 59.50	5.35 5.60	15.95 15.88	6.08 6.06
15d	61.99 62.22	5.97 6.05	16.07 16.00	6.13 6.03
15e	56.71 56.54	4.95 5.24	15.87 15.68	6.06 5.88
15f	60.66 60.57	5.50 5.70	16.98 16.78	6.48 6.25
17a	59.61 59.50	4.21 4.34	21.95 21.75	
17b	59.61 59.50	4.21 4.33	21.95 21.85	
17c	57.15 57.01	4.04 4.18	21.05 21.00	
17d	57.15 57.03	4.04 4.24	21.05 20.95	
19	66.02 65.82	4.99 4.70	19.25 19.20	
23	65.91 65.90	7.01 6.99	15.37 15.25	
24	66.13 65.88	5.55 5.85	22.03 19.94	
25	61.65 61.75	4.80 4.87	20.54 20.72	

Table S2. Structures of the metabolites predicted by Metasite (M1-3) and corresponding ranking.

	**METABOLITES**					
	Metasite Ranking					
COMPOUNDS	**I**	**II**	**III**	**IV**	**V**	**VI**
 **1**		 **M1a**				
 **17c**		 **M1b**	 **M1a**			
 **11**		 **M2**				

 **15b**						
 **6c**		 **M1a**	 **M2**	 **M3a**		 **M3a**
 **19**		 **M2**	 **M3a**			
 **20**		 **M1a**