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Synthesis and antiproliferative activity of 2,4-disubstituted 6-aryl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides

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ABSTRACT

A series of 2,4-disubstituted 6-aryl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides were synthesized and in vitro antiproliferative activities were examined in the human solid tumor cell lines A2780, HBL-100, HeLa, SW1573, T-47D, and WiDr. The most potent analog induced considerably growth inhibition in the range 0.35–2.0 μM. Cell cycle studies in the breast and lung cancer cells revealed arrest in the G_2/M compartment. The results showed that the title compounds bearing alkylamino or dialkylamino moieties in position 2 of the pyrimidine ring are more active than those bearing hydrogen or methylthio groups.

1. Introduction

The pyrrolo[3,2-*d*]pyrimidine heterocyclic framework constitutes the basis of an important class of compounds possessing remarkable biological activities. These compounds are 9-deazaanalogs of biogenic purines and have been reported to be inhibitors of purine nucleoside phosphorilase[1] and thymidylate synthase;[2] in addition to antagonists of the neuropeptide Y5,[3] and the A_1 and A_2 adenosine receptors.[4]

Recently, we have developed a novel, concise, and high-yielding formation of pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides via smooth cycloisomerization of 4-amino-6-arylethynyl-5-nitropyrimidines in the presence of pyridine.[5] Moreover, it was showed that the substituent at the C-2 position of the pyrrolo[3,2-*d*]pyrimidine heterocyclic system could be easily modified via one-pot oxidation/substitution of a methylthio group.[6]

We report herein on the synthesis of a series of 2,4-disubstituted 6-aryl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides and the evaluation of the cytotoxicity of the title compounds. The antiproliferative profile of the obtained derivatives was evaluated in vitro against a panel of six human solid tumor cell lines: A2780 (ovarian), HBL-100 (breast), HeLa (cervix), SW1573 (non-small cell lung), T-47D (breast), and WiDr (colon). The most active derivative was further studied to determine disturbances on the cell cycle.

2. Results and discussion

2.1. Chemistry

Our strategy to synthesize 2,4-disubstituted 6-aryl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides was based on our previous reported methods.[5,6] Scheme 1 outlines the general synthetic pathway. The 2,4-disubstituted 6-arylpyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides (**2a–i**) were prepared via pyridine initiated smooth cycloisomerization of 2,4-disubstituted 5-nitro-6-arylethynylpyrimidines (**1a–i**). Reactions were performed in boiling 2-propanol for 10–30 min and the resulting compounds **2a–i** were obtained in high yields (Table 1). The modification of the C-2 position of the pyrrolo[3,2-*d*]pyrimidine heterocyclic system was achieved by oxidation of methylthio moiety of compound **2d** and subsequent nucleophilic displacement of the resulting methylsulfonyl group by the appropriate alkylamine or dialkylamine. With this straightforward strategy we obtained the corresponding derivatives **4a–k** in yields ranging 52–92% (Scheme 1, Table 1).

2.2. Antiproliferative activity

Our synthesized 2,4-disubstituted 6-aryl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides **2**–**4** were tested for antiproliferative activity against the representative panel of human solid tumor cell lines A2780 (ovarian cancer), HBL-100 (breast), HeLa (cervix), SW1573 (non-small cell lung), T-47D (breast), and WiDr (colon). The in vitro activity was evaluated with the National Cancer

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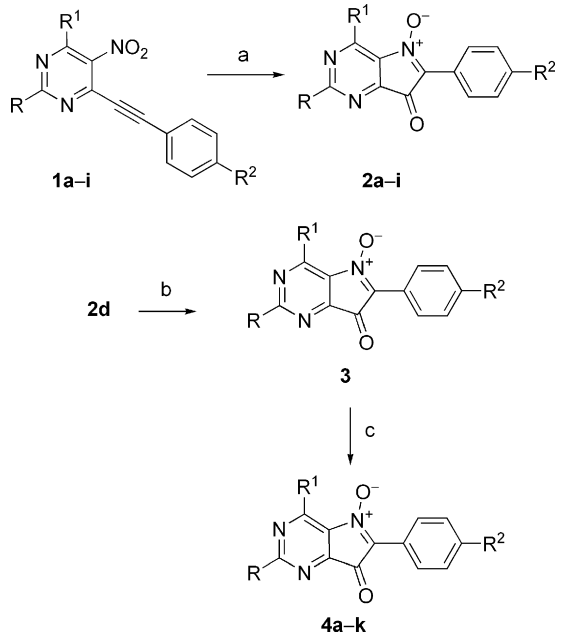


Scheme 1. Reagents and conditions: (a) pyridine (cat), 2-PrOH, reflux, 10–30 min, 79–95%; (b) mCPBA, DCM, rt, 1 h, 85%; (c) amine, rt, 3–8 h, 52–92%.

Institute (NCI) protocol[7] after 48 h of drug exposure using the sulforhodamine B (SRB) assay.[8] The sensitivities expressed as GI50 are listed in Table 2. In addition to the antitumor activity, the lipophilicity (Clog *P*) of the compounds was evaluated by in silico calculation based on their chemical structure. Clog *P* values were calculated to correlate lipophilicity with antitumor activity.[9] The Clog *P* values for the compounds reported in this study are in the range −1.1 to 3.2. Taken as a whole, lipophilicity is not sufficient to explain the observed differences in growth inhibition.

The GI_{50} values allow classifying the compounds in two groups. The first group is comprised of 2,4-disubstituted 6-aryl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides **2a–i** and **3**. These compounds showed overall a modest antiproliferative activity with GI_{50} values higher than 24 μM. From this series, only three compounds were active against all cell lines; the derivatives **2c**, **2e**, and **2g**, with GI_{50} values in the range 26–82 μM.

The second group is formed by all derivatives bearing *N*-alkylamino (**4a–g**) or *N,N*-dialkylamino (**4h–k**) substituents at C-2

Table 1
2,4-Disubstituted 6-aryl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides (**2–4**)

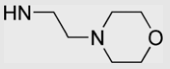
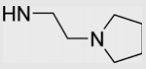
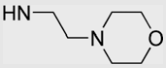
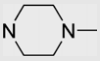
Compd	R	R^1	R^2	Yield (%)
2a	H	NH_2	H	95
2b	H	NH_2	CH_3	90
2c	H	NH_2	C_2H_5	97
2d	SCH_3	NH_2	H	95
2e	SCH_3	NH_2	CH_3	89
2f	SCH_3	NH_2	C_2H_5	92
2g	SCH_3	$NHCH_2C_6H_5$	H	91
2h	SCH_3		H	79
2i	SCH_3		H	95
3	$SOCH_3$	NH_2	H	85
4a	$NHCH_2CH{=}CH_2$	NH_2	H	79
4b	$NHCH_2CO_2CH_3$	NH_2	H	52
4c	$NHCH_2C_6H_5$	NH_2	H	78
4d	$NH(CH_2)_2C_6H_5$	NH_2	H	80
4e		NH_2	H	79
4f		NH_2	H	75
4g	$NH(CH_2)_3N(CH_3)_2$	NH_2	H	69
4h		NH_2	H	79
4i		NH_2	H	83
4j		NH_2	H	85
4k		NH_2	H	92

Table 2
Lipophilicity and GI_{50} values of 2,4-disubstituted 6-aryl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides (**2–4**) against human solid tumor cells[a]

Compd	CLog *P*	A2780 (ovarian)	HBL-100 (breast)	HeLa (cervix)	SW1573 (lung)	T-47D (breast)	WiDr (colon)
2a	−0.2	30 (±11)	72 (±30)	>100	85 (±25)	>100	>100
2b	0.3	>100	>100	>100	>100	>100	>100
2c	0.8	39 (±9.6)	54 (±7.6)	75 (±29)	40 (±6.7)	82 (±17)	70 (±23)
2d	1.0	nt	nt	nt	nt	nt	nt
2e	1.5	93 (±6.8)	28 (±7.9)	>100	31 (±7.3)	>100	>100
2f	2.0	54 (±7.1)	37 (±3.3)	80 (±20)	37 (±5.3)	43 (±13)	53 (±4.6)
2g	3.2	59 (±5.4)	27 (±7.8)	36 (±4.7)	26 (±4.3)	81 (±27)	79 (±30)
2h	2.0	>100	24 (±0.9)	>100	>100	>100	>100
2i	2.5	>100	>100	>100	>100	>100	>100
3	−1.1	79 (±19)	32 (±6.1)	>100	>100	>100	>100
4a	1.3	1.9 (±0.06)	2.7 (±0.9)	7.6 (±1.3)	6.8 (±0.7)	2.8 (±0.9)	4.7 (±0.03)
4b	0.3	1.9 (±0.4)	2.8 (±0.02)	19 (±4.1)	12 (±6.4)	14 (±1.6)	18 (±2.1)
4c	2.0	3.7 (±0.7)	5.1 (±1.0)	29 (±4.0)	30 (±7.9)	4.0 (±1.1)	24 (±10)
4d	2.7	37 (±7.3)	17 (±4.3)	>100	22 (±4.6)	41 (±30)	71 (±41)
4e	1.4	2.6 (±0.3)	1.8 (±0.3)	16 (±1.9)	6.2 (±2.3)	13 (±0.7)	14 (±0.6)
4f	0.8	2.9 (±1.0)	3.4 (±0.8)	15 (±5.9)	14 (±1.5)	17 (±1.3)	16 (±0.4)
4g	1.1	2.3 (±0.2)	2.0 (±0.6)	20 (±4.3)	2.1 (±0.5)	16 (±3.1)	19 (±1.0)
4h	1.9	2.9 (±0.2)	1.7 (±0.3)	3.5 (±0.6)	3.3 (±0.7)	2.7 (±0.8)	3.7 (±1.2)
4i	0.3	37 (±0.4)	22 (±4.9)	38 (±12)	27 (±2.3)	7.5 (±0.6)	20 (±7.3)
4j	−0.1	24 (±7.1)	18 (±1.1)	>100	23 (±2.1)	>100	46 (±3.3)
4k	2.4	1.8 (±0.2)	1.4 (±0.6)	2.0 (±0.3)	1.3 (±0.1)	0.35 (±0.19)	1.8 (±0.8)

[a] Values are given in μM and are means of two to six experiments, standard deviation is given in parentheses (nt = not tested).

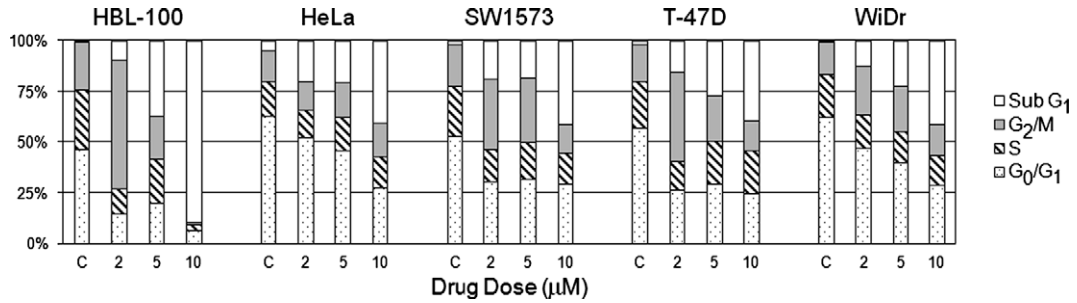


Figure 1. Cell cycle phase distribution in untreated cells (*C*) and cells treated with compound **4k** for 24 h at 2, 5, and 10 μM.

position of the pyrrolo[3,2-*d*]pyrimidine heterocyclic framework. This set of compounds exhibited the best results. The vast majority of the compounds were able to induce antiproliferative effects in all cell lines. Only derivatives **4d** and **4j** showed inactive against one or two of the cell lines. The most potent derivatives were compounds **4a**, **4h**, and **4k**, bearing allylamino, piperidino, and azepanyl substituents, respectively. Their antiproliferative activity was similar against the six cell lines, showing GI50 values within the range 1.9–7.6 μM for **4a**, 1.7–3.7 μM for **4h**, and 0.35–2.0 μM for **4k**. This is a remarkable effect, since the general observation for conventional antitumor drugs is that WiDr, T-47D, and HeLa cancer cells are more drug resistant than A2780 and HBL-100 cancer cells.[10]

From the biological activity data, some structure–activity relationships can be inferred. The presence at C-2 position of the pyrimidine ring of hydrogen (**2a**–**c**), methylthio (**2d**–**i**) or methylsulfonyl (**3**) moieties resulted in modest or inactive compounds. Neither the alkyl substituent at the phenyl group (R^2) nor the amines at C-4 position of the pyrimidine ring (R^1) appear to be crucial for the modulation of the antiproliferative activity. In contrast, the *N*-alkylamino (**4a**–**g**) or *N,N*-dialkylamino (**4h**–**k**) substituents at C-2 position induced an enhancement of the biological activity. Overall, the derivatization at C-2 of the pyrimidine ring with phenylethylamino (**4d**), morpholino (**4i**) or *N*-methylpiperazino (**4j**) moieties produced a decrease of the biological activity. The amines that led to the most potent derivatives were allylamine (**4a**), piperidine (**4h**), and azepane (**4k**).

2.3. Cell cycle disturbances

When exposed to cytotoxic agents, damaged cells may suffer a stop in their cell cycle. If the cell cannot recover from the damage, cell death occurs through apoptosis. This stop in cell division is known as cell cycle arrest and can occur at any of the cell cycle phases, namely G_0/G_1, S, or G_2/M phase.

We studied cell cycle phase distribution by flow cytometry to determine if cell growth inhibition involved cell cycle changes. For these studies we selected **4k**, the most active compound from the series. The effect on the cell cycle was investigated after 24 h exposure. Cells were exposed to compound **4k** at three different drug concentrations: 2, 5, and 10 μM. The drug doses were chosen based on two premises.[11] On the one hand, the GI_{50} values against each cell line. On the other hand, the sensitivity of the cell line to drug treatment, since at higher drug doses large cell death prevents examination of the cell cycle phase distribution. Control cells were incubated in the absence of test drug. The results are shown in Figure 1.

Overall, cell cycle distributions of samples collected from control and treated cells show in all cell lines an increase of the sub G_1 compartment in a dose dependent manner. The results indicate that compound **4k** produces net cell killing. However, the data revealed a diverse pattern of sensitivity between HBL-100 cells and the remaining cell lines HeLa, SW1573, T-47D, and WiDr. In this particular context, HBL-100 cells are more sensitive to compound **4k** as observed in the increased sub G_1 compartment at 5 and 10 μM. Additionally, a clear cell cycle arrest in the G_2/M phase was observed for the breast cancer cell lines HBL-100 and T-47D, and for the lung cancer cells SW1573 when exposed at 2 μM of **4k** (Fig. 2). This increase was concomitant with a decrease in both the G_1 and S compartments. On the contrary, cell cycle arrest was not apparent in HeLa and WiDr cells.

Although the mechanism of action of the title compounds remains unclear, the first results suggested a significant role of the lipophilic 2-alkylamino and 2-dialkylamino fragment in the enhancement of the antiproliferative activity of 6-aryl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides. The observation that the major part of the *N*-alkylamino (**4a**–**g**) or *N,N*-dialkylamino (**4h**–**k**) derivatives evaluated in this study present antiproliferative activity lead us to consider the 6-aryl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxide as a privileged structure with the substituents on the pyrimidine ring (R and R^1) modulating the biological activity.

Privileged structures, with their inherent drug-likeness, represent an ideal source of core scaffolds to obtain combinatorial libraries targeted at various receptors. A number of these libraries have proved to be an extremely powerful tool to aid the rapid discovery and optimization of potent and selective drugs for a wide variety of cellular targets.[12] The straightforward synthesis of the 6-aryl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides reported in this study, together with the high chemical yields, make these compounds valuable scaffolds for obtaining combinatorial libraries in which the cytotoxic activity of the molecules is anticipated.

In conclusion, a series of 2,4-disubstituted 6-aryl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxides were prepared and in vitro antiproliferative activities were examined in the human solid tumor cell lines A2780, HBL-100, HeLa, SW1573, T-47D, and WiDr. The in vitro experiments showed that the analogs containing *N*-alkylamino or *N,N*-dialkylamino substituents in position 2 of the pyrrolo[3,2-*d*]pyrimidine heterosystem induced considerable growth inhibition in the panel of six diverse human solid tumor cells. Cell cycle studies demonstrated arrest in the G_2/M phase when the breast and lung cancer cells were exposed to compound **4k**. The title products appear as good lead molecules for the development of novel antitumor agents.

3. Experimental

3.1. General

Melting points were determined in open capillaries and are uncorrected. IR spectra were run in Nujol mulls or in KBr discs on a Perkin–Elmer FT spectrophotometer Spectrum BX II. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Unity INOVA spectrometer (300 MHz) using tetramethylsilane as internal standard. Elemental analysis (C, H, N) results were found to be in good agreement (±0.4%) with the calculated values. All reactions and

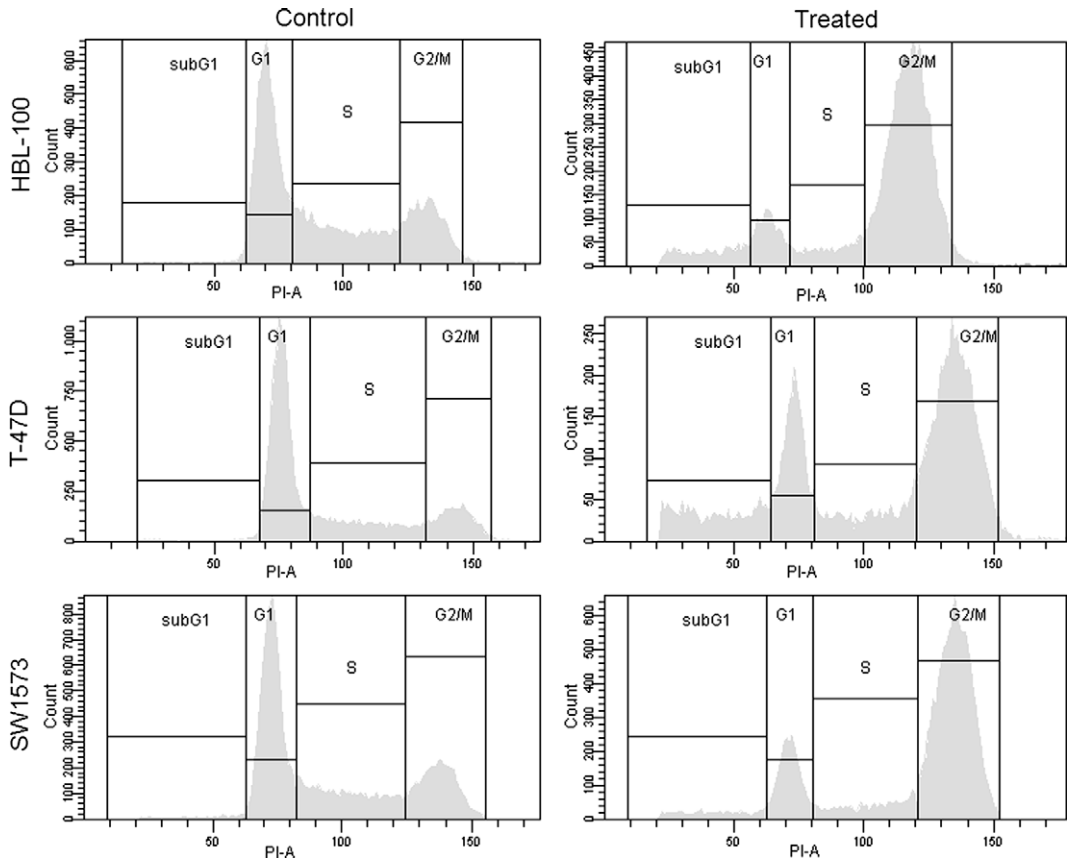


Figure 2. Cell cycle phase distribution in control and treated HBL-100, T-47D, and SW1573 cells, after 24 h exposure to compound **4k** at 2 μM.

purity of the synthesized compounds were monitored by TLC using Silica Gel 60 F_{254} aluminium plates (Merck). Visualization was accomplished by UV light.

Compounds **2a–i**, **3**, and **4a–k** were prepared according the methods we have described earlier.[5,6] Data for compounds **2a**–**b**,[5a] **2d**,[5b] **2g**,[6] **2i**,[6] **3**,[6] **4c**,[6] **4f**,[6] **4h**–**i**[6] have been described previously.

3.1.1. 4-Amino-6-(4-ethylphenyl)-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxide (2c)

Mp 235–236 °C (toluene); IR (KBr) ν cm^{-1} 3341, 3291 (NH_2), 1724 (CO); ^{1}H NMR (DMSO, 300 MHz): δ ppm: 1.22 (3H, t, J = 7.5 Hz, CH_3), 2.67 (2H, q, J = 7.5 Hz, CH_2), 7.42 (2H, d, J = 8.0 Hz, ArH), 7.47 (1H, br s, NH), 8.23 (1H, br s, NH), 8.33 (2H, d, J = 8.0 Hz, ArH), 8.72 (1H, s, CH); ^{13}C NMR (DMSO, 75 MHz): δ 15.0, 28.3, 119.1, 123.5, 126.7, 127.8, 146.2, 150.8, 160.6, 185.3. Anal. Calcd for $C_{14}H_{12}N_4O_2$ (268.27): C, 62.68; H, 4.51; N, 20.88. Found: C, 62.49; H, 4.40; N, 20.71.

3.1.2. 4-Amino-6-(4-methylphenyl)-2-methylthio-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxide (2e)

Mp 230–232 °C (2-PrOH); IR (KBr) ν cm^{-1} 3427, 3246 (NH_2), 1721 (CO); ^{1}H NMR (DMSO, 300 MHz): δ ppm: 2.33 (3H, s, CH_3), 2.53 (3H, s, SCH_3), 7.29 (2H, d, J = 7.8 Hz, ArH), 7.36 (1H, br s, NH), 8.18 (1H, br s, NH), 8.25 (2H, d, J = 7.8 Hz, ArH); ^{13}C NMR (DMSO, 75 MHz): δ 13.9, 25.2, 119.7, 123.0, 126.9, 127.9, 146.0, 151.8, 174.6, 187.3. Anal. Calcd for $C_{14}H_{12}N_4O_2S$ (300.34): C, 55.99; H, 4.03; N, 18.65. Found: C, 56.17; H, 4.16; N, 18.79.

3.1.3. 4-Amino-6-(4-ethylphenyl)-2-methylthio-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxide (2f)

Mp 211–213 °C (2-PrOH); IR (KBr) ν cm^{-1} 3405, 3398 (NH_2), 1713 (CO); ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm: 1.29 (3H, t, J = 6.3 Hz, CH_3), 2.62 (3H, s, SCH_3), 2.72 (2H, q, J = 6.3 Hz, CH_2), 5.56 (1H, br s, NH), 7.00 (1H, br s, NH), 7.31 (2H, d, J = 7.8 Hz, ArH), 8.43 (2H, d, J = 7.8 Hz, ArH); ^{13}C NMR (DMSO, 75 MHz): δ 13.9, 15.2, 28.2, 119.5, 123.2, 126.7, 127.9, 146.1, 150.8, 173.6, 186.3. Anal. Calcd for $C_{15}H_{14}N_4O_2S$ (314.36): C, 57.31; H, 4.49; N, 17.82. Found: C, 57.19; H, 4.46; N, 18.01.

3.1.4. 2-Methylthio-4-[(2-morpholin-4-ylethyl)amino]-6-phenyl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxide (2h)

Mp 208–210 °C (2-PrOH); IR (KBr) ν cm^{-1} 3401, 3390, 3329 (NH_2, NH), 1712 (CO); ^{1}H NMR (DMSO, 300 MHz): δ ppm: 2.58 (3H, s, SCH_3), 2.70–2.79 (4H, m, $N(CH_2)_2$), 2.83 (2H, t, J = 6.8 Hz, NCH_2), 3.66–3.69 (4H, m, $O(CH_2)_2$), 3.89 (2H, td, J = 6.8, 1.5 Hz, $NHCH_2$), 7.51–7.56 (3H, m, ArH), 8.07 (1H, br s, NH), 8.36 (2H, d, J = 7.5 Hz, ArH); ^{13}C NMR (DMSO, 75 MHz): δ 14.9, 37.4, 53.7, 57.1, 66.8, 120.6, 126.3, 127.5, 128.2, 129.2, 130.7, 149.6, 150.2, 174.9, 186.8. Anal. Calcd for $C_{19}H_{21}N_5O_3S$ (399.47): C, 57.13; H, 5.30; N, 17.53. Found: C, 57.10; H, 5.40; N, 17.33.

3.1.5. 2-Allylamino-4-amino-6-phenyl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxide (4a)

Mp 228–230 °C (2-PrOH); IR (KBr) ν cm^{-1} 3400, 3393, 3322 (NH_2, NH), 1714 (CO); ^{1}H NMR (DMSO, 300 MHz): δ ppm: 3.94–3.99 (2H, m, $NHCH_2$), 5.05 (1H, d, J = 10.2 Hz, CH), 5.16 (1H, d, J = 17.7 Hz, CH), 5.83–5.92 (1H, m, CH), 6.88 (1H, br s, NH), 7.35–7.40 (3H, m, ArH), 7.81 (1H, br s, NH), 7.94 (1H, br s, NH), 8.29 (2H, d, J = 7.5 Hz, ArH); ^{13}C NMR (DMSO, 75 MHz): δ 40.8, 112.5, 120.4, 125.9, 126.4, 126.7, 128.3, 128.9, 147.0, 151.2, 152.7, 162.0, 174.5, 185.9. Anal. Calcd for $C_{15}H_{13}N_5O_2$ (295.30): C, 61.01; H, 4.44; N, 23.72. Found: C, 61.10; H, 4.45; N, 24.01.

3.1.6. 4-Amino-2-methoxycarbonylmethylamino-6-phenyl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxide (4b)

Mp 202–203 °C (2-PrOH); IR (KBr) ν cm^{-1} 3469, 3318, 3251 (NH_2, NH), 1741, 1710 (CO); ^{1}H NMR (DMSO, 300 MHz): δ ppm: 3.82 (3H, s, OCH_3), 4.25 (2H, t, J = 7.8 Hz, NHCH_2), 6.89 (1H, br s, NH), 7.37–7.40 (3H, m, ArH), 7.82 (1H, br s, NH), 7.97 (1H, br s, NH), 8.31 (2H, d, J = 7.5 Hz, ArH); ^{13}C NMR (DMSO, 75 MHz): δ 39.4, 54.8, 118.4, 125.5, 126.6, 126.3, 128.7, 128.9, 151.4, 152.5, 161.5, 164.3, 183.7. Anal. Calcd for $C_{15}H_{13}N_5O_4$ (327.29): C, 55.05; H, 4.00; N, 21.40. Found: C, 54.96; H, 4.18; N, 21.31.

3.1.7. 4-Amino-2-phenylethylamino-6-phenyl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxide (4d)

Mp 239–241 °C (2-PrOH); IR (KBr) ν cm^{-1} 3464, 3386, 3327 (NH_2, NH), 1709 (CO); ^{1}H NMR (DMSO, 300 MHz): δ ppm: 2.87 (2H, t, J = 6.9 Hz, PhCH_2), 3.37–3.53 (2H, m, NHCH_2), 6.99 (1H, br s, NH), 7.31 (H5, br s, ArH), 7.42–7.51 (3H, m, ArH), 7.72 (1H, br s, NH), 8.11 (1H, br s, NH), 8.30 (2H, d, J = 7.5 Hz, ArH); ^{13}C NMR (DMSO, 75 MHz): δ 37.2, 39.0, 117.4, 125.8, 125.9, 126.2, 126.3, 127.1, 127.8, 128.3, 128.5, 135.9, 152.1, 152.5, 162.3, 185.8. Anal. Calcd for $C_{20}H_{17}N_5O_2$ (359.38): C, 66.84; H, 4.77; N, 19.49. Found: C, 67.00; H, 4.89; N, 19.70.

3.1.8. 4-Amino-6-phenyl-2-[(2-pyrrolidin-4-ylethyl)amino]-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxide (4e)

Mp 210–213 °C (2-PrOH); IR (KBr) ν cm^{-1} 3400, 3317, 3251 (NH_2, NH), 1714 (CO); ^{1}H NMR (DMSO, 300 MHz): δ ppm: 1.69 (4H, br s, $(CH_2)_2$), 1.74 (4H, br s, $(CH_2)_2$), 2.49 (4H, br s, $(CH_2)_2$), 2.61 (4H, br s, $(CH_2)_2$), 3.43–3.45 (4H, m, N$(CH_2)_2$), 6.99 (1H, br s, NH), 7.35–7.50 (3H, m, ArH), 7.60 (1H, br s, NH), 7.89 (1H, br s, NH), 8.30 (2H, d, J = 7.5 Hz, ArH); ^{13}C NMR (DMSO, 75 MHz): δ 23.1, 40.6, 53.5, 54.3, 113.1, 124.7, 125.8, 126.2, 128.3, 128.9, 151.5, 152.9, 163.2, 186.7. Anal. Calcd for $C_{18}H_{20}N_6O_2$ (352.39): C, 61.35; H, 5.72; N, 23.85. Found: C, 61.21; H, 5.88; N, 24.00.

3.1.9. 4-Amino-2-[(3-dimethylaminopropyl)amino]-6-phenyl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxide (4g)

Mp 201–202 °C (2-PrOH); IR (KBr) ν cm^{-1} 3405, 3319, 3259 (NH_2, NH), 1710 (CO); ^{1}H NMR ($CDCl_3$, 300 MHz): δ ppm: 1.77–1.86 (2H, m, CH_2), 2.31 (6H, s, N$(CH_3)_2$), 2.46 (2H, t, J = 9.2 Hz, NCH_2), 3.56 (2H, br s, NHCH_2), 6.75 (1H, br s, NH), 7.42–7.51 (3H, m, ArH), 7.88 (2H, br s, NH_2), 8.44 (2H, dd, J = 7.5, 4.8 Hz, ArH); ^{13}C NMR (DMSO, 75 MHz): δ 26.2, 29.7, 45.3, 57.8, 114.0, 125.8, 126.9, 127.1, 128.4, 129.6, 140.6, 151.8, 163.7, 187.0. Anal. Calcd for $C_{17}H_{20}N_6O_2$ (340.38): C, 59.99; H, 5.92; N, 24.69. Found: C, 60.28; H, 5.78; N, 24.55.

3.1.10. 4-Amino-2-(4-methylpiperazin-1-yl)-6-phenyl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxide (4j)

Mp 207–209 °C (2-PrOH); IR (KBr) ν cm^{-1} 3472, 3324 (NH_2), 1706 (CO); ^{1}H NMR (DMSO, 300 MHz): δ ppm: 2.23 (3H, s, NCH_3), 2.38 (4H, br s, N$(CH_2)_2$), 3.82 (4H, br s, N$(CH_2)_2$), 7.00 (1H, br s, NH), 7.40–7.53 (3H, m, ArH), 7.70 (1H, br s, NH), 8.30 (2H, d, J = 7.5 Hz, ArH); ^{13}C NMR (DMSO, 75 MHz): δ 44.5, 46.4, 55.1, 14.5, 120.7, 126.8, 126.9, 127.1, 129.1, 129.9, 152.2, 153.1, 162.6, 187.5. Anal. Calcd for $C_{17}H_{18}N_6O_2$ (338.36): C, 60.34; H, 5.36; N, 24.84. Found: C, 60.30; H, 5.24; N, 24.79.

3.1.11. 4-Amino-2-azepan-1-yl-6-phenyl-7*H*-pyrrolo[3,2-*d*]pyrimidin-7-one 5-oxide (4k)

Mp 215–217 °C (2-PrOH); IR (KBr) ν cm^{-1} 3470, 3340 (NH_2), 1704 (CO); ^{1}H NMR (DMSO, 300 MHz): δ ppm: 1.52 (4H, br s, $(CH_2)_2$), 1.74 (4H, br s, $(CH_2)_2$), 3.74–3.80 (4H, m, N$(CH_2)_2$), 6.94 (1H, br s, NH), 7.42 (1H, t, J = 7.5 Hz, ArH), 7.50 (2H, d, J = 7.5 Hz, ArH), 7.62 (1H, br s, NH), 8.29 (2H, d, J = 7.5 Hz, ArH); ^{13}C NMR (DMSO, 75 MHz): δ 26.4, 26.9, 27.7, 46.8, 47.5, 113.4, 125.8, 126.2, 126.3, 128.3, 128.9, 151.4, 152.5, 162.0, 186.9. Anal. Calcd for $C_{18}H_{19}N_5O_2$ (337.38): C, 64.08; H, 5.68; N, 20.76. Found: C, 64.30; H, 5.55; N, 20.78.

3.2. Clog *P*

Software-predicted lipophilicity of the compounds (Clog *P*) was calculated with the program CS ChemFinder Ultra, version 9.0, Cambridge-Soft Corporation. Alternatively, Clog *P* values can be calculated with the free program *New and Improved Clog P calculator* accessible via Internet (http://intro.bio.umb.edu/111-112/OLLM/111F98/newclogp.html).

3.3. Biological tests

3.3.1. Cells, culture, and plating

The human solid tumor cell lines A2780, HBL-100, HeLa, SW1573, T-47D, and WiDr were used in this study. These cell lines were a kind gift from Professor Godefridus J. Peters (VU Medical Center, Amsterdam, The Netherlands). Cells were maintained in 25 cm^2 culture flasks in RPMI 1640 supplemented with 5% heat inactivated fetal calf serum and 2 mM L-glutamine in a 37 °C, 5% CO_2, 95% humidified air incubator. Exponentially growing cells were trypsinized and resuspended in antibiotic containing medium (100 units penicillin G and 0.1 mg of streptomycin per mL). Single cell suspensions displaying >97% viability by trypan blue dye exclusion were subsequently counted. After counting, dilutions were made to give the appropriate cell densities for inoculation onto 96-well microtiter plates. Cells were inoculated in a volume of 100 μL per well at densities of 15,000 (WiDr, T-47D, and HeLa) and 10,000 (A2780, SW1573, and HBL-100) cells per well, based on their doubling times.

3.3.2. Antiproliferative tests

Chemosensitivity tests were performed using the SRB assay of the NCI with slight modifications. Briefly, pure compounds were initially dissolved in DMSO at 400 times the desired final maximum test concentration. Control cells were exposed to an equivalent concentration of DMSO (0.25% v/v, negative control). Each agent was tested in triplicates at different dilutions in the range 1–100 μM. The drug treatment was started on day 1 after plating. Drug incubation times were 48 h, after which time cells were precipitated with 25 μL ice-cold 50% (w/v) trichloroacetic acid and fixed for 60 min at 4 °C. Then the SRB assay was performed. The optical density (OD) of each well was measured at 492 nm, using BioTek's PowerWave XS Absorbance Microplate Reader. Values were corrected for background OD from wells only containing medium. The percentage growth (PG) was calculated with respect to untreated control cells (C) at each of the drug concentration levels based on the difference in OD at the start (T_0) and end of drug exposure (T), according to NCI formulas. Therefore, if T is greater than or equal to T_0 the calculation is $100 \times [(T - T_0)/(C - T_0)]$. If T is less than T_0 denoting cell killing the calculation is $100 \times [(T - T_0)/(T_0)]$. The effect is defined as percentage of growth, where 50% growth inhibition (GI_{50}) represents the concentration at which PG is +50. With these calculations a PG value of 0 corresponds to the amount of cells present at the start of drug exposure, while negative PG values denote net cell kill.

3.3.3. Cell-cycle analysis

Cells were seeded in a six well plates at a density of 2.5–5 × 10^5 cells/well. After 24 h the products were added to the respective well and incubated for an additional period of 24 h. Cells were trypsinized, harvested, transferred to test tubes (12 × 75 mm) and centrifuged at 1500 rpm for 10 min at 5 °C. The supernatant was discarded and the cell pellets were resuspended in 200 μL of cold PBS and fixed by the addition of 1 mL ice-cold 70% ethanol. Fixed cells were incubated overnight at −20 °C after which time was

centrifuged at 1500 rpm for 10 min. The cell pellets were resuspended in 500 μL PBS. Then, 5 μL of DNAse-free RNAse (10 mg/mL) was incubated in the dark at 37 °C for 30 min. After incubation 5 μL of propidium iodide (0.5%) was added. Flow cytometric determination of DNA content (25,000 cells/sample) was analyzed by LSR II Flow Cytometer (Becton Dickinson, San José, CA, USA). The fractions of the cells in sub G_1, G_0/G_1, S, and G_2/M phase were analyzed using cell cycle analysis software, FACSDiva 6.0 (Becton Dickinson, San José, CA, USA).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.05.078.

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