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Novel Water Soluble, Substituted Pyrrolo[3,2-*d*]pyrimidines: Design, Synthesis and Biological Evaluation as Antitubulin Antitumor Agents

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Abstract

Purpose—To study the effects of a regioisomeric change on the biological activities of previously reported water soluble, colchicine site binding, microtubule depolymerizing agents.

Methods—Nine pyrrolo[3,2-*d*]pyrimidines were designed and synthesized. The importance of various substituents was evaluated. Their abilities to cause cellular microtubule depolymerization, inhibit proliferation of MDA-MB-435 tumor cells and displace colchicine binding to tubulin were studied. One of the compounds was also evaluated in the National Cancer Institute preclinical 60 cell line panel.

Results—Pyrrolo[3,2-*d*]pyrimidine analogs were more potent than their pyrrolo[2,3-*d*]pyrimidine regioisomers. We identified compounds with submicromolar potency against cellular proliferation. The structure-activity relationship study gave insight into substituents that were crucial for activity and those that improved activity. The compound that was tested in the NCI 60 cell line is a 2-digit nanomolar (GI₅₀) inhibitor of 8 tumor cell lines.

Conclusion—We have identified substituted pyrrolo[3,2-*d*]pyrimidines that are water-soluble colchicine site microtubule depolymerizing agents. These compounds serve as leads for further optimization.

INTRODUCTION

Our efforts to elucidate the plausible binding modes of RTK-inhibitors led to the discovery of highly potent water-soluble antimitotic antiproliferative agents: *N*-(4-methoxyphenyl)-*N*,2-dimethyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine **1** and *N*-(4-methoxyphenyl)-*N*,2,6-trimethyl-6,7-dihydro-5*H*-cyclopenta[*d*]pyrimidin-4-amine (±)-**2**•HCl (figure 1) (1, 2). These compounds bind in the colchicine site of tubulin, inhibit microtubule assembly and cause cellular microtubule disassembly. Compounds **1** and (±)-**2**•HCl also inhibit the growth of cancer cells with GI₅₀'s in the nanomolar range. They overcome the two most clinically relevant tumor resistance mechanisms that limit the utility of microtubule targeting agents (1, 2): overexpression of P-glycoprotein (3, 4) and βIII-tubulin (5–9).

Pyrrolo[3,2-*d*]pyrimidine analogs of the lead compounds were designed to evaluate the effect of intramolecular hydrogen bonding (if any) mediated conformational restriction of the lead analogs **1** and ($\pm$)-**2**•HCl.

Compound **3** was designed as a regioisomer of the pyrrolo[2,3-*d*]pyrimidine **1** and as an isostere of the cyclopenta[*d*]pyrimidine ($\pm$)-**2**•HCl scaffold. Compounds **4**, **5** and **6** were designed to evaluate the importance of the 2-CH₃, 4'-OCH₃ and 4-NCH₃ moieties, respectively. Compound **7** was designed by incorporating a 2'-OCH₃ group to explore the effect of substituents in this position. Compounds **8** and **9** were synthesized to study the effect of a 6-CH₃ group and a 2-NH₂ group respectively. Compound **10** was designed to evaluate the importance of the 4-NCH₃ moiety. Compound **11** was designed as a conformationally restricted analog of **3**. Figure 2 summarizes the structures of compounds **3–11**.

Compounds **12** (**10**) and **13** (**11**) (Scheme 1) were chlorinated with phosphorus oxychloride to generate **14** and **15**, respectively. Compounds **14**, **16** (**12**) and **17** (**13**) were subjected to nucleophilic displacement reactions using appropriately substituted anilines to afford **3–8** and **11**, which were in turn converted to their hydrochloride salts using hydrogen chloride gas. Compound **15** was subjected to nucleophilic displacement reactions using appropriately substituted anilines, and the resulting compounds were subsequently subjected to amide hydrolysis under basic conditions to produce **9** and **10**.

MATERIALS AND METHODS

Chemistry

Analytical samples were dried in vacuo (0.2 mm Hg) in a CHEM-DRY drying apparatus over P₂O₅ at 80°C. Melting points were determined on a MEL-TEMP II melting point apparatus with a FLUKE 51 K/J electronic thermometer and are uncorrected. Proton nuclear magnetic resonance spectra (¹H NMR) were recorded on a Bruker WH-400 (400 MHz) spectrometer. The chemical shift values are expressed in parts per million (ppm) relative to tetramethylsilane as an internal standard: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad singlet. Thin-layer chromatography (TLC) was performed using Whatman Sil G/UV254 silica gel plates with a fluorescent indicator, and the spots were visualized under 254 and 366 nm illumination. Proportions of solvents used for TLC are by volume. Column chromatography was performed with a 230–400 mesh silica gel (Fisher, Somerville, NJ) column. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Element compositions are within 0.4% of the calculated values. Fractional moles of water or organic solvents found in some analytical samples of the compounds could not be prevented in spite of 24–48 h of drying in vacuo and were confirmed where possible by their presence in the ¹H NMR spectra. All solvents and chemicals were purchased from Aldrich Chemical Co. or Fisher Scientific and were used as received.

4-Chloro-2-methyl-5H-pyrrolo[3,2-*d*]pyrimidine (14)—2-Methyl-3*H*-pyrrolo[3,2-*d*]pyrimidin-4(5*H*)-one (**12**, 1 g, 6.7 mmol) was added to phosphorus oxychloride (20 mL) and heated at reflux for 4 h. The solvent was evaporated in vacuo, and the pH of the residue was adjusted to 8 with ammonia solution. The resulting precipitate was filtered and purified by column chromatography (CHCl₃: MeOH; 100:1 to 50:3; v/v) to give a light yellow solid (726 mg, 75%). TLC *R*_f 0.32 (MeOH: CHCl₃; 1:10); Mp, 138–139°C; ¹H NMR, DMSO-*d*₆: δ 2.60 (s, 3H), 6.6 (s, 1H), 7.9 (s, 1H), 12.26 (s, 1H).

N-(4-Chloro-5H-pyrrolo[3,2-*d*]pyrimidin-2-yl)pivalamide (15)—*N*-(4-Oxo-4,5-dihydro-3*H*-pyrrolo[3,2-*d*]pyrimidin-2-yl)pivalamide (**11**) (**13**, 1.16 g, 4.67 mmol) was

added to phosphorus oxychloride (30 mL) and heated at reflux for 3 h. The solvent was evaporated in vacuo, and the pH of the residue was adjusted to 8 with ammonia solution. The resulting precipitate was filtered and purified by column chromatography (CHCl₃: MeOH; 50:1; v/v) to give a white solid (1.1 g, 86%). TLC *R_f* 0.35 (MeOH: CHCl₃; 1:5); Mp, 162–163°C; ¹H NMR, DMSO-*d*₆: δ 1.19 (s, 9 H), 2.47 (s, 3 H), 6.33 (s, 1 H), 9.85 (s, 1 H), 12.07 (s, 1 H). Anal. Calcd. for C₁₂H₁₅ClN₄O: C, 54.04; H, 5.67; N, 21.01; Cl, 13.29. Found C, 54.06; H, 5.78; N, 20.93; Cl, 13.49.

N-(4-Methoxyphenyl)-N,2-dimethyl-5H-pyrrolo[3,2-d]pyrimidin-4-amine (3) as hydrochloride salt—Compound **14** (0.2 g, 1.05 mmol) and 4-methoxy-phenylamine (0.12 g, 1.05 mmol) were dissolved in isopropanol (20 mL) and heated at reflux for 4 h. The solvent was evaporated in vacuo, and the residue was purified by column chromatography (CHCl₃: MeOH; 50:1; v/v) to give a brown solid (1.1 g, 86%). TLC *R_f* 0.42 (MeOH: CHCl₃; 1:10). The product obtained was dissolved in a minimum amount of ethyl acetate and diethyl ether (10 mL) was added to the solution. Hydrogen chloride gas was bubbled through for 2–3 mins. The precipitate obtained was collected by filtration. Mp, 184–185°C; ¹H NMR, DMSO-*d*₆: δ 2.63 (s, 3H), 3.62 (s, 3H), 3.84 (s, 3H), 6.46 (s, H), 7.1 (d, 2H, J = 8.9 Hz), 7.42 (d, 2H, J = 8.86 Hz), 9.661 (s, 1H), 14.996 (s, 1H). Anal. Calcd. for C₁₅H₁₆N₄O·HCl·H₂O: C, 55.81; H, 5.93; N, 17.36; Cl, 10.98. Found C, 55.94; H, 5.87; N, 17.17; Cl, 10.97.

N-(4-Methoxyphenyl)-N-methyl-5H-pyrrolo[3,2-d]pyrimidin-4-amine (4) as hydrochloride salt—Compound **7** (synthesized from **16** (12) as described for **3**): yield 72%; TLC *R_f* 0.33 (MeOH: CHCl₃; 1:5); Mp, 150–151°C; ¹H NMR, DMSO-*d*₆: δ 3.65 (s, 3H), 3.84 (s, 3H), 6.55 (s, H), 7.1 (d, 2H, J = 8.8 Hz), 7.4 (d, 2H, J = 8.7 Hz), 7.56 (s, 1H), 8.71 (s, 1H), 9.96 (s, 1H), 14.39 (s, 1H). Anal. Calcd. for C₁₄H₁₄N₄O·HCl·H₂O: C, 54.46; H, 5.55; N, 18.15; Cl, 11.48. Found C, 54.21; H, 5.46; N, 17.9; Cl, 11.26.

N,2-Dimethyl-N-phenyl-5H-pyrrolo[3,2-d]pyrimidin-4-amine (5) as hydrochloride salt—Compound **5** (synthesized from **14** as described for **3**): TLC *R_f* 0.42 (MeOH: CHCl₃; 1:5); Mp, 253–254°C; ¹H NMR, DMSO-*d*₆: δ 2.62 (s, 3H), 3.7 (s, 3H), 6.49 (s, H), 7.47–7.59 (m, 6H), 10.07 (s, 1H), 15.02 (s, 1H). Anal. Calcd. for C₁₅H₁₆N₄O·0.95 HCl·0.25H₂O: C, 60.61; H, 5.61; N, 20.1. Found C, 60.59; H, 5.6; N, 20.00.

N-(4-Methoxyphenyl)-2-methyl-5H-pyrrolo[3,2-d]pyrimidin-4-amine (6) as hydrochloride salt—Compound **6** (synthesized from **14** as described for **3**): yield 81%; TLC *R_f* 0.4 (MeOH: CHCl₃; 1:5); Mp, 260°C (decomp); ¹H NMR, DMSO-*d*₆: δ 2.61 (s, 3H), 3.77 (s, 3H), 6.51 (s, H), 7.0 (d, 2H, J = 9 Hz), 7.82 (d, 2H, J = 8.8 Hz), 7.89 (s, 1H), 11.43 (s, 1H), 12.94 (s, 1H), 14.44 (s, 1H). Anal. Calcd. For C₁₄H₁₄N₄O·HCl: C, 57.83; H, 5.20; N, 19.27; Cl, 12.19. Found C, 57.73; H, 5.38; N, 19.05; Cl, 12.07.

N-(2,4-Dimethoxyphenyl)-2-methyl-5H-pyrrolo[3,2-d]pyrimidin-4-amine (7) as hydrochloride salt—Compound **7** (synthesized from **14** as described for **3**): yield 76%; TLC *R_f* 0.6 (MeOH: CHCl₃; 1:10); Mp, 262–264°C; ¹H NMR, DMSO-*d*₆: δ 2.5 (s, 3H), 3.8 (s, 6H), 6.5 (m, 1H), 6.59 (dd, 1H, J = 8.7, 2.6), 6.7 (d, 1H, J = 2.6 Hz), 7.48 (d, 1H, J = 8.7 Hz), 7.85 (s, 1H), 10.4 (s, 1H), 12.65 (s, 1H), 14.86 (s, 1H). Anal. Calcd. for C₁₅H₁₆N₄O₂·HCl·0.2H₂O: C, 55.54; H, 5.41; N, 17.27; Cl, 10.93. Found C, 55.74; H, 5.28; N, 17.21; Cl, 10.85.

N-(4-Methoxyphenyl)-N,2,6-trimethyl-5H-pyrrolo[3,2-d]pyrimidin-4-amine (8) as hydrochloride salt—Compound **7** (synthesized from **17** (13) as described for **3**): yield

69%; TLC R_f 0.3 (MeOH: CHCl_3 ; 1:10); Mp, 224–226°C; ^1H NMR, DMSO- d_6 : δ 2.38 (s, 3H), 2.55 (s, 3H), 3.68 (s, 3H), 3.83 (s, 3H), 6.32 (s, H), 7.07 (d, 2H, $J = 8.9$ Hz), 7.36 (d, 2H, $J = 8.9$ Hz), 10.31 (s, 1H), 14.55 (s, 1H). Anal. Calcd. for $\text{C}_{17}\text{H}_{18}\text{N}_4\text{O} \cdot \text{HCl} \cdot 0.25 \text{H}_2\text{O}$: C, 60.82; H, 5.86; N, 16.71; Cl, 10.57. Found C, 60.61; H, 5.91; N, 16.56; Cl, 10.80.

N^4 -(4-Methoxyphenyl)- N^4 ,6-dimethyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (9)—Compound **15** (0.2 g, 1.05 mmol) and 4-methoxy-phenylamine (0.12 g, 1.05 mmol) were dissolved in isopropanol (20 mL), followed by the addition of 2–3 drops of conc. HCl. The mixture was heated at reflux for 45 min. The solvent was evaporated in vacuo, 1,4-dioxane (10 mL) and 15% aqueous KOH solution (10 mL) were added. The resulting mixture was heated at reflux overnight. After cooling, the reaction solution was neutralized with 1N HCl, and evaporated in vacuo to dryness and the residue was purified by column chromatography (CHCl_3 : MeOH; 50:1; v/v) to give a brown solid (1.1g, 86%). TLC R_f 0.42 (MeOH: CHCl_3 ; 1:5); Mp, 201–202°C; ^1H NMR, DMSO- d_6 : δ 2.34 (s, 3H), 3.78 (s, 3H), 5.33 (s, 2H), 5.75 (s, 1H), 6.86 (d, 2H, $J = 6.3$ Hz), 7.72 (d, 2H, $J = 6.3$ Hz), 8.45 (s, 1H), 8.52 (s, 1H). Anal. Calcd. for $\text{C}_{14}\text{H}_{15}\text{N}_5\text{O} \cdot 0.71\text{CHCl}_3 \cdot 0.81\text{HCl}$: C, 45.93; H, 4.33; N, 18.19. Found C, 45.97; H, 4.53; N, 18.08.

N^4 -(4-Methoxyphenyl)-6-methyl-5H-pyrrolo[3,2-d]pyrimidine-2,4-diamine (10)—Compound **10** (synthesized from **15** as described for **9**): yield 47%; TLC R_f 0.48 (MeOH: CHCl_3 ; 1:5); Mp, 161–163°C; ^1H NMR, DMSO- d_6 : δ 2.12 (s, 3H), 3.37 (s, 3H), 3.78 (s, 3H), 5.31 (s, 2H), 5.70 (s, 1H), 6.96 (d, 2H, $J = 5.4$ Hz), 7.15 (d, 2H, $J = 5.4$ Hz), 8.16 (s, 1H). Anal. Calcd. for $\text{C}_{15}\text{H}_{17}\text{N}_5\text{O} \cdot 0.78\text{H}_2\text{O}$: C, 60.55; H, 6.20; N, 23.54. Found C, 60.54; H, 5.94; N, 23.51.

6-Methoxy-1-(2-methyl-5H-pyrrolo[3,2-d]pyrimidin-4-yl)-1,2,3,4-tetrahydroquinoline (11) as hydrochloride salt—Compound **7** (synthesized from **14** as described for **3**): yield 79%; TLC R_f 0.36 (MeOH: CHCl_3 ; 1:10); Mp, 230–232°C; ^1H NMR, DMSO- d_6 : δ 2.01 (m, 2H), 2.63 (s, 3H), 2.79 (t, 2H, $J = 6.5$ Hz), 3.8 (s, 3H), 4.1 (t, 2H, $J = 6.3$ Hz), 6.53 (m, 1H, $J = 8.7$, 2.6), 6.8 (dd, 1H, $J = 8.8$, 2.9), 6.94 (d, 1H, $J = 2.8$ Hz), 7.15 (d, 1H, $J = 8.8$ Hz), 7.65 (t, 1H, $J = 2.7$ Hz), 11.05 (s, 1H), 14.85 (s, 1H). $\text{C}_{16}\text{H}_{18}\text{N}_4\text{O} \cdot \text{HCl} \cdot 0.2\text{H}_2\text{O}$: C, 59.61; H, 6.06; N, 17.38; Cl, 11.00. Found C, 59.82; H, 6.05; N, 17.25; Cl, 10.85.

Biological evaluations

The effects of the compounds on interphase and mitotic microtubules in A-10 cells were evaluated using indirect immunofluorescence techniques, and the EC_{50} values (concentration required to cause 50% loss of cellular microtubules) were calculated from a minimum of three experiments as previously described (14).

Antiproliferative effects were evaluated against the drug sensitive MDA-MB-435 melanoma cells using sulforhodamine B assay as previously described and the IC_{50} values (concentration required to cause 50% inhibition of proliferation) were calculated (15–17).

The inhibition of tubulin assembly by these compounds were studied. Tubulin polymerization was measured by turbidimetry at 350 nm in Beckman DU7400 and DU7500 recording spectrophotometers equipped with temperature controllers. The methodology was described in detail previously (18). In brief, 10 μM bovine brain tubulin, purified as described previously (19), was preincubated for 15 min in a 0.24 mL volume at 30°C containing 0.75 M monosodium glutamate (adjusted to pH 6.6 with HCl in a 2 M stock solution), varying compound concentrations, and 4% (v/v) dimethyl sulfoxide (compound solvent). Following the preincubation, which permits detection of activity in slow binding

compounds such as colchicinoids (18), samples were chilled on ice, and 10 μL of 10 mM GTP was added (0.4 mM). The addition of GTP is an absolute requirement for assembly under these reaction conditions. All concentrations refer to the final 0.25 mL reaction volume. Samples were transferred to cuvettes held at 0°C in the recording spectrophotometers, and the temperature was jumped to 30°C, which takes less than a minute. Assembly at 30°C was followed for 30 min, and the compound concentration to inhibit extent of assembly after 30 min was determined by interpolation of data obtained with individual compound concentrations. After determining the likely range for the IC_{50} value, 2–4 individual determinations were made, and the average from these determinations are presented in Table 1. The control compound was combretastatin A-4 (CSA4), a potent colchicine site agent (20) generously supplied by Dr. G. R. Pettit, Arizona State University, Tempe AZ.

The abilities of these compounds to inhibit binding of radiolabeled colchicine to tubulin were measured. The binding of [^3H]colchicine to tubulin was performed by the DEAE-cellulose filter technique (21) with a stack of two filters, as described in detail previously (22). In brief, reaction mixtures contained, in a 0.10 mL volume, 1.0 μM purified tubulin, 5.0 μM [^3H]colchicine, potential inhibitor at 5.0 μM , 5% (v/v) dimethyl sulfoxide (the compound solvent), and other components previously found to stabilize the colchicine binding activity of tubulin for prolonged periods at 37°C (23). Incubation was at 37°C for 10 min, at which time samples were diluted with 2 mL of ice-cold water and poured over the DEAE-filters under mild suction, with several rinses of the reaction vessel and of the filtration chamber. The amount of radiolabel bound to the filters was determined by liquid scintillation counting, and samples containing test compounds were compared to reaction mixtures without compound. The percent inhibition relative to the control was determined for each compound in 2–4 independent experiments.

Compound **3** was evaluated in the National Cancer Institute preclinical 60 cell line panel, as described in detail previously (24).

RESULTS AND DISCUSSION

All target compounds were synthesized in yields varying from 47 to 86%, and effects on cellular microtubule depolymerization in A-10 cells was used as an initial screening assay (Table 1). In addition, selected compounds were evaluated for cytotoxicity in a human breast cancer cell line (MDA-MB-35 cells), and the two most active compounds (**8**•HCl and **11**•HCl) were evaluated for interactions with purified tubulin (inhibition of assembly and inhibition of colchicine binding). Compound **3**•HCl was evaluated in the National Cancer Institute preclinical 60 cancer cell line screen. The GI_{50} against different cell lines is shown in Table 2, and the mean GI_{50} in the cell screen was 44.1×10^{-8} M, with eight cell lines showing less than 10×10^{-8} M GI_{50} values and three cell lines showing micromolar GI_{50} values. These data indicate that **3**•HCl has varied GI_{50} values across the 60 tumor cell line panel as well as within a particular tumor type indicating that the compound does have selectivity for certain tumor cells in culture and is not a general cell-poison.

The biological data show that **3**, the regioisomer of **1**, is about 5-times more potent than **1** as an inducer of cellular microtubule disassembly (Table 1). The 4'-OCH₃ moiety and the methyl group attached to the nitrogen bridge both are crucial for activity (comparing activities of **5** and **6** with that of **3**; and that of **10** with that of **9**). Removal of either of these groups resulted in complete loss of the ability of the compound to induce cellular microtubule disassembly. Removal of the 2-CH₃ group of **3** (as in compound **4**) caused a 2-fold reduction in cytotoxicity in the MDA-MB-435 cell line but only a small loss in ability to cause microtubule disassembly in the A-10 cells, presumably due to slight differences in

compound uptake in the two cell lines. In contrast, addition of a methyl group at C-6 (as in compound **8**) improved the activity of **3** 3-fold as a cytotoxic agent against the MDA-MB-435 cells and improved ability to disassembly microtubules in the A-10 cells 5–6-fold. Replacement of the 2-methyl moiety of **8** with a 2-amino group as in **9** substantially decreased activity in both cellular assays. The conformationally restricted analog **11** was more potent than **3** in the cellular assays, with activities comparable to those of compound **8**. With purified tubulin, compounds **8** and **11** had essentially identical activities, but they were significantly less active than the standard agent CSA4. The reasonably strong inhibition of the binding of colchicine to tubulin by **8** and **11** makes it likely that these compounds are colchicine site agents.

CONCLUSION

In summary, we report the synthesis and biological activities of water-soluble colchicine site microtubule depolymerizing agents as analogs of compounds **1** and ($\pm$)-**2**•HCl that overcome two clinically important tumor resistance mechanisms that limit the activity of microtubule targeting agents, expression of P-glycoprotein and β III tubulin (1, 2). Compounds **3**, **8** and **11** serve as leads for further structural modifications to potentially identify a clinical lead candidate.

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Figure 1.
Lead compounds

	R ₁	R ₂	R ₃	R ₄	R ₅
3	CH ₃	CH ₃	OCH ₃	H	H
4	H	CH ₃	OCH ₃	H	H
5	CH ₃	CH ₃	H	H	H
6	CH ₃	H	OCH ₃	H	H
7	CH ₃	H	OCH ₃	OCH ₃	H
8	CH ₃	CH ₃	OCH ₃	H	CH ₃
9	NH ₂	CH ₃	OCH ₃	H	CH ₃
10	NH ₂	H	OCH ₃	H	CH ₃

Figure 2.
Designed compounds

Scheme 1.
Synthesis of target compounds

Table 1

Effects in cellular assays and on purified tubulin

	EC ₅₀ Microtubule Depolymerization (μM)	MDA-MB-435 IC ₅₀ ±SD (nM)	Inhibition of	
			Tubulin Assembly IC ₅₀ ±SD (μM)	% Colchicine Binding inhibited at 5μM compound concentration
1	5.8	183 ± 3.4		
3•HCl	1.2	96.6 ± 5.3	10 ± 0.6	
4•HCl	1.4	193 ± 5.3		
5•HCl	Not Active Up To 10 μM	ND		
6•HCl	Not Active Up To 10 μM	ND		
7•HCl	Not Active Up To 10 μM	ND		
8•HCl	0.22	30.3 ± 2.7	3.1 ± 0.3	65 ± 1
9	8.4	298 ± 19.7		
10	Not Active Up To 40 μM	ND		
11•HCl	0.23	42.7±3.2	3.1 ± 0.08	62 ± 2
CSA4	0.0131	3.47 ± 0.6	1.2 ± 0.01	98 ± 0.3

Table 2

Tumor cell growth inhibitory activity GI₅₀ (10⁻⁸ M) of 3•HCl in NCI 60 cell line panel

Panel/Cell line	GI ₅₀	Panel/Cell line	GI ₅₀	Panel/Cell line	GI ₅₀	Panel/Cell line	GI ₅₀
Leukemia	3•HCl	Colon Cancer	3•HCl	Melanoma	3•HCl	Renal Cancer	3•HCl
CCRF-CEM	33.9	COLO 205	13.4	LOX IMVI	51.2	786-0	44.6
HL-60(TB)	21.7	HCC-2998	12.9	MALME-3M	13.6	A498	4.45
K-562	8.69	HCT-116	29.2	M14	16.7	ACHN	71.2
MOLT-4	40.2	HCT-15	36.3	MDA-MB-435	2.79	CAKI-1	37.2
RPMI-8226	30.1	HT29	18.9	SK-MEL-2	18.5	RXF 393	9.65
SR	4.47	KM12	7.56	SK-MEL-28	405	SN12C	86.3
NSCLC		SW-620	16.5	SK-MEL-5	17.1	TK10	56.6
A549/ATCC	27.1	CNS Cancer		UACC-257	37.0	UO-31	43.5
EKVX	35.2	SF-268	58.6	UACC-62	47.4	Prostate Cancer	
HOP-62	33.7	SF-295	13.0	Ovarian cancer		PC-3	25.9
HOP-92	295	SF-539	25.3	IGROVI	29.5	DU-145	21.2
NCI-H226	55.9	SNB-19	48.9	OVCAR-3	16.0	Breast Cancer	
NCI-H23	32.8	SNB-75	24.1	OVCAR-4	252	MCF7	8.67
NCI-H322M	37.2	U251	26.0	OVCAR-5	45.4	MDA-MB-231/ATCC	46.6
NCI-H460	19.1			OVCAR-8	39.2	HS 578T	17.9
NCI-H522	4.56			NCI/ADR- RES	66.9	BT-549	24.7
				SK-OV-3	23.3	MDA-MB-468	11.6