2-Arylamino-4-Amino-5-Aroylthiazoles. "One-Pot" Synthesis and Biological Evaluation of a New Class of Potent Inhibitors of Tubulin Polymerization

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SUPPORTING INFORMATION

Characterization for compounds **5a-y,** Figures **1-4** and biological assays.

Chemistry. Materials and Methods. ^{1}H NMR spectra were recorded on a Bruker AC 200 spectrometer. Chemical shifts (δ) are given in ppm upfield from tetramethylsilane as internal standard, and the spectra were recorded in appropriate deuterated solvents, as indicated. Positive-ion electrospray ionization (ESI) mass spectra were recorded on a double-focusing Finnigan MAT 95 instrumental with BE geometry. Melting points (mp) were determined on a Buchi-Tottoli apparatus and are uncorrected. All products reported showed ^{1}H NMR spectra in agreement with the assigned structures. The purity of tested compounds was determined by combustion elemental analyses conducted by the Microanalytical Laboratory of the Chemistry Department of the University of Ferrara with a Yanagimoto MT-5 CHN recorder elemental analyzer. All tested compounds yielded data consistent with a purity of at least 95% as compared with the theoretical values. All reactions were carried out under an inert atmosphere of dry nitrogen, unless otherwise indicated. Standard syringe techniques were applied for transferring dry solvents. Reaction courses and product mixtures were routinely monitored by TLC on silica gel (precoated F_{254} Merck plates), and compounds were visualized with aqueous $KMnO_4$. Flash chromatography was performed using 230-400 mesh silica gel and the indicated solvent system. Organic solutions were dried over anhydrous Na_2SO_4.

Arylisothiocyanate **6a-f** and α-bromoketones **7a-e** and **7g-i** are commercially available and used as received. Compound **7f** was synthesized following the procedure reported in the article: Cushman, M.; Nagarathnam, D.; Gopal, D.; He, H.-M; Lin, C.M.; Hamel, E. Synthesis and evaluation of analogues of (Z)-1-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene as potential cytotoxic and antimitotic agents. *J. Med. Chem.* **1992**, *35*, 2293-2306.

(4-Amino-2-(phenylamino)thiazol-5-yl)(phenyl)methanone (5a). Following the general procedure, compound **5a** was purified by chromatography eluting with petroleum ether-ethyl acetate (3:7). Yellow solid, yield: 42%, mp 182-184 °C. ^{1}H NMR (DMSO-d_6) δ: 7.08 (t, *J*=7.6 Hz, 1H), 7.38 (t, *J*=7.6 Hz, 2H), 7.48 (m, 3H), 7.60 (t, *J*=7.6 Hz, 2H), 7.67 (m, 2H), 8.22 (bs, 2H), 10.8 (s, 1H). Anal. ($C_{16}H_{13}N_3OS$): C, H, N.

[4-Amino-2-(4-fluorophenylamino)-thiazol-5-yl]-phenylmethanone (5b). Following the general procedure, compound **5b** was recrystallized from ethyl ether. Yellow solid, yield: 48%, mp 238-240 °C. ^{1}H NMR (DMSO-d_6) δ: 6.98 (t, *J*=6.8 Hz, 3H), 7.22 (t, *J*=8.8 Hz, 1H), 7.49 (m, 1H), 7.64 (m, 4H), 8.32 (bs, 2H), 10.2 (s, 1H). Anal. ($C_{16}H_{12}FN_3OS$): C, H, N.

[4-Amino-2-(4-chlorophenylamino)-thiazol-5-yl]-phenylmethanone (5c). Following the general procedure, compound **5c** was purified by chromatography eluting with petroleum ether-ethyl acetate (1:1). Yellow solid, yield: 42%, mp 208-210 °C. ^{1}H NMR (DMSO-d_6) δ: 7.33 (m, 5H), 7.44 (d, *J*=6.4 Hz, 2H), 7.73 (d, *J*=6.4 Hz, 2H), 8.03 (bs, 2H), 10.6 (s, 1H). Anal. ($C_{16}H_{12}ClN_3OS$): C, H, N.

(4-Amino-2-p-tolylamino-thiazol-5-yl)-phenyl-methanone (5d). Following the general procedure, compound **5d** was purified by chromatography eluting with petroleum ether-ethyl acetate (1:1). Yellow solid, yield: 43%, mp 146-148 °C. ^{1}H NMR (DMSO-d_6) δ: 2.35 (s, 3H), 7.21 (m, 4H), 7.43 (m, 3H), 7.73 (d, *J*=8.0 Hz, 2H), 8.21 (bs, 2H), 10.7 (s, 1H). Anal. ($C_{17}H_{15}N_3OS$): C, H, N.

(2-(4-Methoxyphenylamino)-4-aminothiazol-5-yl)(phenyl)methanone (5e). Following the general procedure, compound **5e** was recrystallized from ethyl ether. Yellow solid, yield: 50%, mp 202-203 °C. ^{1}H NMR (DMSO-d_6) δ: 3.74 (s, 3H), 6.94 (d, *J*=7.2 Hz, 2H), 7.48 (m, 5H), 7.64 (d, *J*=7.2 Hz, 2H), 8.12 (bs, 2H), 10.6 (s, 1H). Anal. ($C_{17}H_{15}N_3O_2S$): C, H, N.

[4-Amino-2-(3-methoxyphenylamino)-thiazol-5-yl]-phenylmethanone (5f). Following the general procedure, compound **5f** was recrystallized from ethyl ether. Yellow solid, yield: 58%, mp 152-153 °C. ^{1}H NMR (DMSO-d_6) δ: 3.76 (s, 3H), 6.65 (dd, *J*=8.0 and 2.6 Hz, 1H), 7.11 (dd, *J*=8.0 and 2.6 Hz, 1H), 7.24 (d, *J*=8.2 Hz, 1H), 7.34 (s, 1H), 7.49 (m, 3H), 7.66 (m, 2H), 8.20 (bs, 2H), 10.8 (s, 1H). Anal. ($C_{17}H_{15}N_3O_2S$): C, H, N.

(4-Amino-2-(phenylamino)thiazol-5-yl)(4-methoxyphenyl)methanone (5g). Following the general procedure, compound **5g** was recrystallized from ethyl ether. Yellow solid, yield: 62%, mp 230-231 °C. ^{1}H NMR (DMSO-d_6) δ: 3.82 (s, 3H), 7.00 (d, *J*=7.6 Hz, 2H), 7.08 (t, *J*=7.6 Hz, 1H), 7.36 (t, *J*=7.2 Hz, 2H), 7.62 (t, *J*=7.6 Hz, 2H), 7.68 (d, *J*=6.8 Hz, 2H), 8.22 (bs, 2H), 10.7 (s, 1H). Anal. ($C_{17}H_{15}N_3O_2S$): C, H, N.

[4-Amino-2-(4-fluorophenylamino)-thiazol-5-yl]-(4-methoxyphenyl)-methanone (5h). Following the general procedure, compound **5h** was recrystallized from ethyl ether. Yellow solid, yield: 81%, mp 223-225 °C. ^{1}H NMR (DMSO-d_6) δ: 3.81 (s, 3H), 6.99 (t, *J*=9.2 Hz, 2H), 7.21 (t, *J*=9.2 Hz, 2H), 7.67 (m, 4H), 8.12 (bs, 2H), 10.7 (s, 1H). Anal. ($C_{17}H_{14}FN_3O_2S$): C, H, N.

[4-Amino-2-(4-chlorophenylamino)-thiazol-5-yl]-(4-methoxyphenyl)-methanone (5i). Following the general procedure, compound **5i** was purified by recrystallization from ethyl ether. Yellow solid, yield: 50%, mp 228-230 °C. ^{1}H NMR (DMSO-d_6) δ: 3.82 (s, 3H), 7.03 (d, *J*=6.8 Hz, 2H), 7.40 (d, *J*=8.8 Hz, 2H), 7.64 (d, *J*=8.8 Hz, 2H), 7.70 (d, *J*=6.8 Hz, 2H), 8.17 (bs, 2H), 10.8 (s, 1H). Anal. ($C_{17}H_{14}ClN_3O_2S$): C, H, N.

(2-(4-Methoxyphenylamino)-4-aminothiazol-5-yl)(4-methoxyphenyl)methanone (5j). Following the general procedure, compound **5j** was recrystallized from ethyl ether. Yellow solid, yield: 55%, mp 196-197 °C. ^{1}H NMR (DMSO-d_6) δ: 3.74 (s, 3H), 3.80 (s, 3H), 6.94 (d, *J*=8.8 Hz, 2H), 7.00 (d, *J*=8.8 Hz, 2H), 7.47 (d, *J*=8.8 Hz, 2H), 7.64 (d, *J*=8.8 Hz, 2H), 8.10 (bs, 2H), 10.6 (s, 1H). Anal. ($C_{18}H_{17}N_3O_3S$): C, H, N.

(4-Amino-2-phenylamino-thiazol-5-yl)-(3-methoxy-phenyl)-methanone (5k). Following the general procedure, compound **5k** was recrystallized from ethyl ether. Yellow solid, yield: 59%, mp 179-180 °C. ^{1}H NMR (DMSO-d_6) δ: 3.80 (s, 3H), 7.06 (m, 2H), 7.18 (s, 1H), 7.24 (d, *J*=8.0 Hz, 1H), 7.38 (m, 3H), 7.62 (d, *J*=8.0 Hz, 2H), 8.12 (bs, 2H), 10.9 (s, 1H). Anal. ($C_{17}H_{15}N_3O_2S$): C, H, N.

(2-(4-Methoxyphenylamino)-4-aminothiazol-5-yl)(3-methoxyphenyl)methanone (5l). Following the general procedure, compound **5l** was recrystallized from ethyl ether. Yellow solid, yield: 55%, mp 122-123 °C. ^{1}H NMR (DMSO-d_6) δ: 3.74 (s, 3H), 3.79 (s, 3H), 6.93 (d, *J*=7.2 Hz, 2H), 7.00 (d, *J*=7.2 Hz, 1H), 7.15 (s, 1H), 7.20 (d, *J*=8.0 Hz, 1H), 7.37 (t, *J*=8.0 Hz, 1H), 7.47 (d, *J*=8.8 Hz, 2H), 8.10 (bs, 2H), 10.6 (s, 1H). Anal. ($C_{18}H_{17}N_3O_3S$): C, H, N.

(4-Amino-2-phenylamino-thiazol-5-yl)-(2-methoxyphenyl)-methanone (5m). Following the general procedure, compound **5m** was recrystallized from petroleum ether. Yellow solid, yield: 53%, mp 207-209 °C. ^{1}H NMR (DMSO-d_6) δ: 3.76 (s, 3H), 6.96 (d, *J*=7.2 Hz, 1H), 7.02 (m, 3H), 7.15 (s, 1H), 7.23 (d, *J*=7.6 Hz, 1H), 7.37 (m, 3H), 7.54 (d, *J*=7.6 Hz, 2H), 7.94 (bs, 2H), 10.6 (s, 1H). Anal. ($C_{17}H_{15}N_3O_2S$): C, H, N.

(2-(4-Methoxyphenylamino)-4-aminothiazol-5-yl)(2-methoxyphenyl)methanone (5n). Following the general procedure, compound **5n** was purified by chromatography eluting with petroleum ether-ethyl acetate (1:1). Yellow solid, yield: 40%, mp 116-118 °C. ^{1}H NMR (DMSO-d_6) δ: 3.72 (s, 3H), 3.75 (s, 3H), 6.96 (t, *J*=8.4 Hz, 1H), 7.05 (t, *J*=8.4 Hz, 1H), 7.08 (d, *J*=6.8 Hz, 2H),

7.21 (d, *J*=7.6 Hz, 1H), 7.37 (t, *J*=8.4 Hz, 1H), 7.41 (d, *J*=8.8 Hz, 2H), 8.00 (bs, 2H), 10.5 (s, 1H). Anal. ($C_{18}H_{17}N_3O_3S$): C, H, N.

(4-Amino-2-phenylamino-thiazol-5-yl)-(3,4-dimethoxyphenyl)-methanone (5o). Following the general procedure, compound **5o** was recrystallized from petroleum ether. Yellow solid, yield: 58%, mp 215-217 °C. ^{1}H NMR (DMSO-d_6) δ: 3.80 (s, 3H), 3.81 (s, 3H), 7.03 (d, *J*=8.4 Hz, 1H), 7.07 (t, *J*=7.6 Hz, 1H), 7.32 (m, 4H), 7.65 (d, *J*=8.8 Hz, 2H), 8.12 (bs, 2H), 10.8 (s, 1H). Anal. ($C_{18}H_{17}N_3O_3S$): C, H, N.

(2-(4-Methoxyphenylamino)-4-aminothiazol-5-yl)(3,4-dimethoxyphenyl)methanone (5p). Following the general procedure, compound **5p** was recrystallized from ethyl ether. Orange solid, yield: 46%, mp 148-150 °C. ^{1}H NMR (DMSO-d_6) δ: 3.74 (s, 3H), 3.79 (s, 3H), 3.80 (s, 3H), 6.93 (d, *J*=7.2 Hz, 2H), 7.00 (d, *J*=8.0 Hz, 1H), 7.26 (m, 2H), 7.49 (d, *J*=8.8 Hz, 2H), 8.05 (bs, 2H), 10.5 (s, 1H). Anal. ($C_{19}H_{19}N_3O_4S$): C, H, N.

(4-Amino-2-(phenylamino)thiazol-5-yl)(3,4,5-trimethoxyphenyl)methanone (5q). Following the general procedure, compound **5q** was recrystallized from ethyl ether. Orange solid, yield: 49%, mp 113-114 °C. ^{1}H NMR (DMSO-d_6) δ: 3.73 (s, 3H), 3.83 (s, 6H), 7.00 (s, 2H), 7.07 (m, 1H), 7.37 (t, *J*=7.6 Hz, 2H), 7.63 (d, *J*=8.0 Hz, 2H), 8.12 (bs, 2H), 10.7 (s, 1H). Anal. ($C_{19}H_{19}N_3O_4S$): C, H, N.

[4-Amino-2-(4-fluorophenylamino)-thiazol-5-yl]-(3,4,5-trimethoxyphenyl)-methanone (5r). Following the general procedure, compound **5r** was recrystallized from ethyl ether. Orange solid, yield: 48%, mp 125-127 °C. ^{1}H NMR (DMSO-d_6) δ: 3.72 (s, 3H), 3.82 (s, 6H), 6.99 (s, 2H), 7.22 (t, *J*=9.2 Hz, 2H), 7.66 (m, 2H), 8.10 (bs, 2H), 10.6 (s, 1H). Anal. ($C_{19}H_{18}FN_3O_4S$): C, H, N.

[4-Amino-2-(4-chlorophenylamino)-thiazol-5-yl]-(3,4,5-trimethoxyphenyl)-methanone (5s). Following the general procedure, compound **5s** was recrystallized from ethyl ether. Yellow solid, yield: 44%, mp 136-138 °C. ^{1}H NMR (DMSO-d_6) δ: 3.72 (s, 3H), 3.83 (s, 6H), 7.00 (s, 2H), 7.40 (d, *J*=9.0 Hz, 2H), 7.69 (d, *J*=9.0 Hz, 2H), 8.24 (bs, 2H), 10.9 (s, 1H). Anal. ($C_{19}H_{18}ClN_3O_4S$): C, H, N.

(4-Amino-2-p-tolylaminothiazol-5-yl)-(3,4,5-trimethoxyphenyl)-methanone (5t). Following the general procedure, compound **5t** was recrystallized from ethyl ether. Yellow solid, yield: 47%, mp 178-179 °C. ^{1}H NMR (DMSO-d_6) δ: 2.27 (s, 3H), 3.72 (s, 3H), 3.82 (s, 6H), 6.99 (s, 2H), 7.16 (d, *J*=8.2 Hz, 2H), 7.50 (d, *J*=8.2 Hz, 2H), 8.22 (bs, 2H), 10.8 (s, 1H). Anal. ($C_{20}H_{21}N_3O_4S$): C, H, N.

(2-(4-Methoxyphenylamino)-4-aminothiazol-5-yl)(3,4,5-trimethoxyphenyl)methanone (5u). Following the general procedure, compound **5u** was recrystallized from ethyl ether. Orange solid, yield: 47%, mp 131-132 °C. ^{1}H NMR (DMSO-d_6) δ: 3.72(s, 3H), 3.74 (s, 3H), 3.77 (s, 3H), 3.80 (s, 3H), 6.92 (d, *J*=7.2 Hz, 2H), 6.97 (s, 2H), 7.62 (d, *J*=7.2 Hz, 2H), 8.10 (bs, 2H), 10.6 (s, 1H). Anal. ($C_{20}H_{21}N_3O_5S$): C, H, N.

[4-Amino-2-(3-methoxyphenylamino)-thiazol-5-yl]-(3,4,5-trimethoxyphenyl)-methanone (5v). Following the general procedure, compound **5v** was recrystallized from ethyl ether. Yellow solid, yield: 60%, mp 154-156 °C. ^{1}H NMR (DMSO-d_6) δ: 3.72 (s, 3H), 3.76 (s, 3H), 3.83 (s, 6H), 6.64 (dd, *J*=8.0 and 2.4 Hz, 1H), 7.00 (s, 2H), 7.14 (dd, *J*=8.0 and 2.4 Hz, 1H), 7.27 (t, *J*=8.0 Hz, 1H), 7.38 (s, 1H), 8.22 (bs, 2H), 10.8 (s, 1H). Anal. ($C_{20}H_{21}N_3O_5S$): C, H, N.

[4-Amino-2-(4-methoxyphenylamino)-thiazol-5-yl]-(4-chlorophenyl)-methanone (5w). Following the general procedure, compound **5w** was purified by recrystallization from ethyl ether. Yellow solid, yield: 52%, mp 183-185 °C. ^{1}H NMR (DMSO-d_6) δ: 3.75 (s, 3H), 6.93 (d, *J*=6.8 Hz,

2H), 7.44 (d, *J*=8.8 Hz, 2H), 7.55 (d, *J*=8.8 Hz, 2H), 7.67 (d, *J*=6.8 Hz, 2H), 8.22 (bs, 2H), 10.6 (s, 1H). Anal. ($C_{17}H_{14}ClN_3O_2S$): C, H, N.

[4-Amino-2-(4-methoxyphenylamino)-thiazol-5-yl]-(4-bromophenyl)-methanone (5x). Following the general procedure, compound **5x** was purified by recrystallization from ethyl ether. Yellow solid, yield: 53%, mp 181-183 °C. ^{1}H NMR (DMSO-d_6) δ: 3.74 (s, 3H), 6.93 (d, *J*=9.0 Hz, 2H), 7.48 (d, *J*=9.0 Hz, 2H), 7.58 (d, *J*=8.4 Hz, 2H), 7.66 (d, *J*=8.4 Hz, 2H), 8.21 (bs, 2H), 10.7 (s, 1H). Anal. ($C_{17}H_{14}BrN_3O_2S$): C, H, N.

[4-Amino-2-(4-methoxyphenylamino)-thiazol-5-yl]-(4-nitrophenyl)-methanone (5y). Following the general procedure, compound **5y** was purified by chromatography eluting with petroleum ether-ethyl acetate (1:1). Brown solid, yield: 47%, mp 198-200 °C. ^{1}H NMR (DMSO-d_6) δ: 3.74 (s, 3H), 6.92 (d, *J*=8.8 Hz, 2H), 7.46 (d, *J*=8.8 Hz, 2H), 7.85 (d, *J*=8.6 Hz, 2H), 8.29 (d, *J*=8.6 Hz, 2H), 8.31 (bs, 2H), 10.7 (s, 1H). Anal. ($C_{17}H_{14}N_4O_4S$): C, H, N.

Growth inhibitory activity. Murine leukemia L1210, murine mammary carcinoma FM3A, human T-lymphocyte Molt 4 and CEM cells and human cervix carcinoma (HeLa) cells were suspended at 300,000-500,000 cells/mL of culture medium, and 100 μL of a cell suspension was added to 100 μL of an appropriate dilution of the test compounds in wells of 96-well microtiter plates. After incubation at 37 °C for two days, the cell number was determined using a Coulter counter. The IC_{50} was defined as the compound concentration required to inhibit cell proliferation by 50%. Data are expressed as the mean ± SE from the dose-response curves of at least three independent experiments.

Effects on tubulin polymerization and on colchicine binding to tubulin. Bovine brain tubulin was purified as described previously.[1] To evaluate the effect of the compounds on tubulin assembly *in vitro,*[2] varying concentrations were preincubated with 10 μM tubulin in glutamate buffer at 30 ˚C and then cooled to 0 ˚C. After addition of GTP, the mixtures were transferred to 0 °C cuvettes in a recording spectrophotometer and warmed-up to 30 °C, and the assembly of tubulin was observed turbidimetrically. The IC_{50} was defined as the compound concentration that inhibited the extent of assembly by 50% after a 20 min incubation. The capacity of the test compounds to inhibit colchicine binding to tubulin was measured as described, [3] except that the reaction mixtures contained 1 μM tubulin, 5 μM [^{3}H]colchicine and 1 μM test compound.

Flow cytometric analysis of cell cycle distribution and apoptosis. For flow cytometric analysis of the DNA content, $5x10^5$ HeLa cells in exponential growth were treated with different concentrations of the test compounds for 24 hours. After an incubation period, the cells were trypsinized and, together with floating cells, centrifuged and fixed with ice-cold ethanol (70%). The cells were then treated with lysis buffer containing RNAseA, and then stained with propidium iodide (PI). Samples were analyzed on a Cytomic FC500 flow cytometer (Beckman Coulter). For cell cycle analysis, DNA histograms were analyzed using MultiCycle for Windows (Phoenix Flow Systems, CA, USA). The percentage of each phase of the cell cycle (G1, S, G2/M) were calculated on living cells. The percentage of apoptotic cells is refered to the cell populations characterized by the appearance of a sub-G1-peak.

Molecular modelling. All molecular modeling studies were performed on a MacPro dual 2.66 GHz Xeon running Ubuntu 8. The tubulin structure was downloaded from the PDB data bank (http://www.rcsb.org/ - PDB code: 1SA0). Hydrogen atoms were added to the protein, using Molecular Operating Environment (MOE) 2007.09,[4] and minimized keeping all the heavy atoms fixed until a RMSD gradient of 0.05 kcal mol^{-1} Å^{-1} was reached. Ligand structures were built with MOE and minimized using the MMFF94x force field until a RMSD gradient of 0.05 kcal mol^{-1} Å^{-1} was reached. The docking simulations were performed using FlexX[5]. The RMSD of the

trimethoxyphenyl moiety for each of the results obtained was calculated in comparison with ring A of the DAMA-colchicine and the results from the docking were scored using this value.[6]

References

(1) Hamel, E.; Lin, C.M. Separation of active tubulin and microtubule-associated proteins by ultracentrifugation, and isolation of a component causing the formation of microtubule bundles. *Biochemistr*y **1984**, *23*, 4173-4184.

(2) Hamel, E. Evaluation of antimitotic agents by quantitative comparisons of their effects on the polymerization of purified tubulin. *Cell Biochem. Biophys.* **2003**, *38*, 1-21.

(3) Verdier-Pinard, P.; Lai J.-Y.; Yoo, H.-D.; Yu, J.; Marquez, B.; Nagle D.G.; Nambu, M.; White, J.D.; Falck, J.R.; Gerwick, W.H.; Day, B.W.; Hamel, E. Structure-activity analysis of the interaction of curacin A, the potent colchicine site antimitotic agent, with tubulin and effects of analogs on the growth of MCF-7 breast cancer cells. *Mol. Pharmacol.* **1998**, *53*, 62-67.

(4) Molecular Operating Environment (MOE 2007.09). Chemical Computing Group, Inc. Montreal, Quebec, Canada. http://www.chemcomp.com.

(5) FlexX 3.0. BioSolveIT GmbH, Sankt Augustin, Germany. http://www.biosolveit.de.

(6) Code "fragment_rmsd.svl" obtained from SLV Exchange website http://svl.chemcomp.com., Chemical Computing Group, Inc. Montreal, Canada

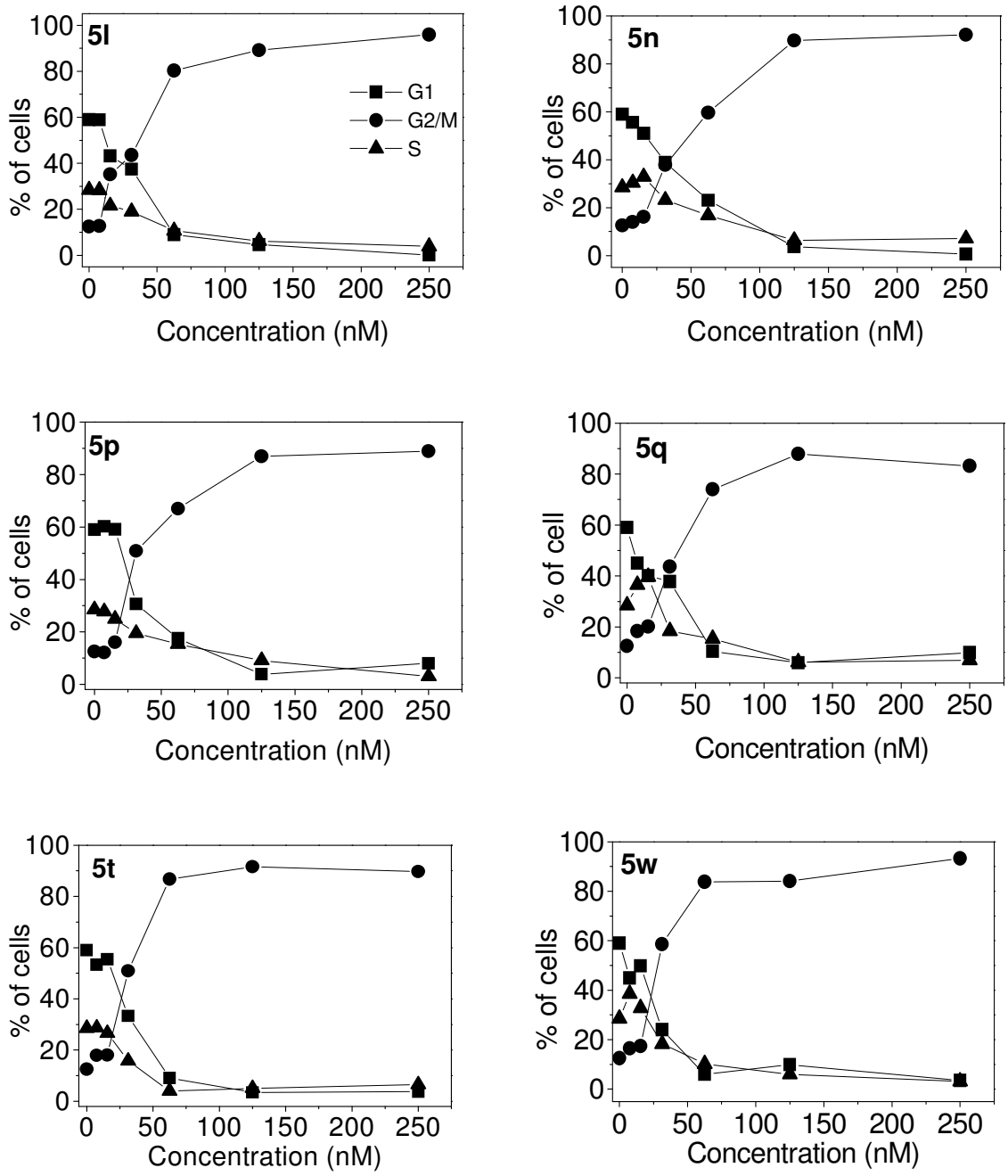


Figure 1. Percentage of each phase of the cell cycle in HeLa cells treated with the indicated compounds at different concentrations for 24 h. Cells were fixed and labeled with propidium iodide (PI) and analyzed by flow cytometry as described in the experimental section.

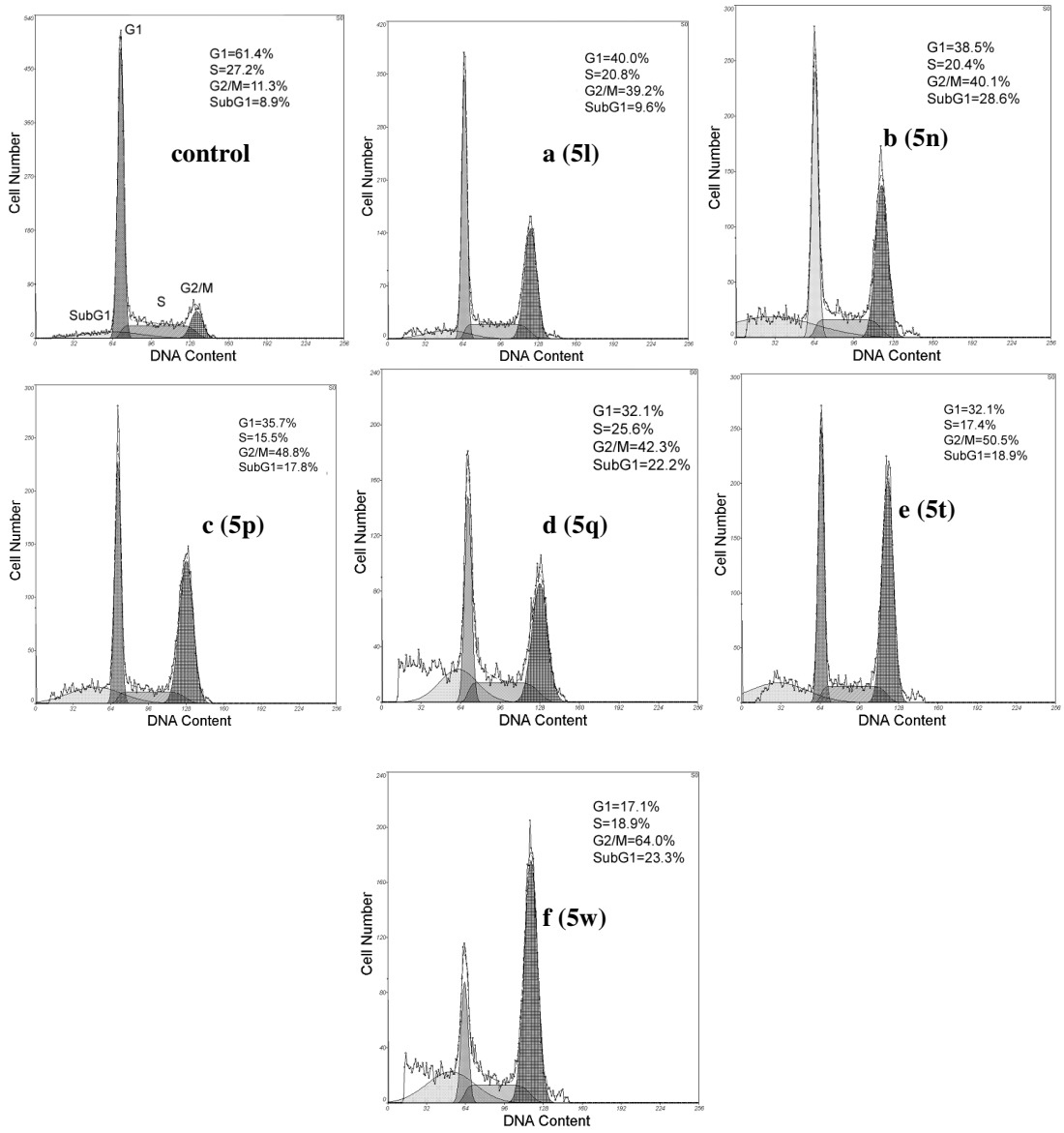


Figure 2. Representative cell cycle distribution patterns by flow cytometry analysis. HeLa cells were treated for 24 h with compounds **5l**, **5n**, **5p**, **5q**, **5t** and **5w** at 30 nM. The cells were analyzed by the standard propidium iodide procedure, as described in the Methods section. The regions of the distribution patterns attributed to the sub-G_1, G_1, S and G_2-M phases of the cell cycle are indicated in the panels showing the pattern obtained with control cells.

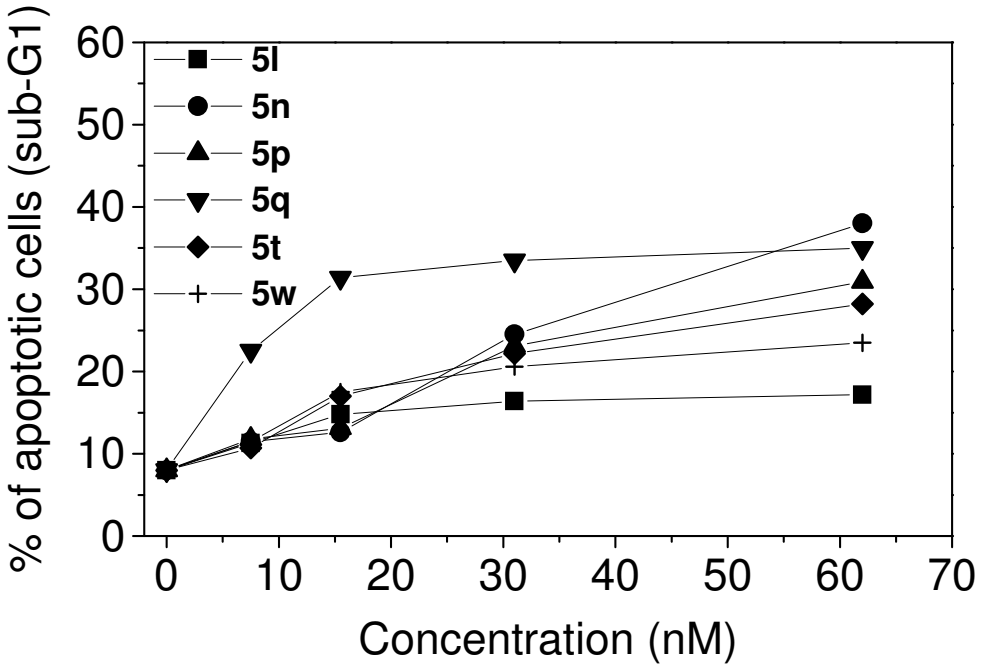
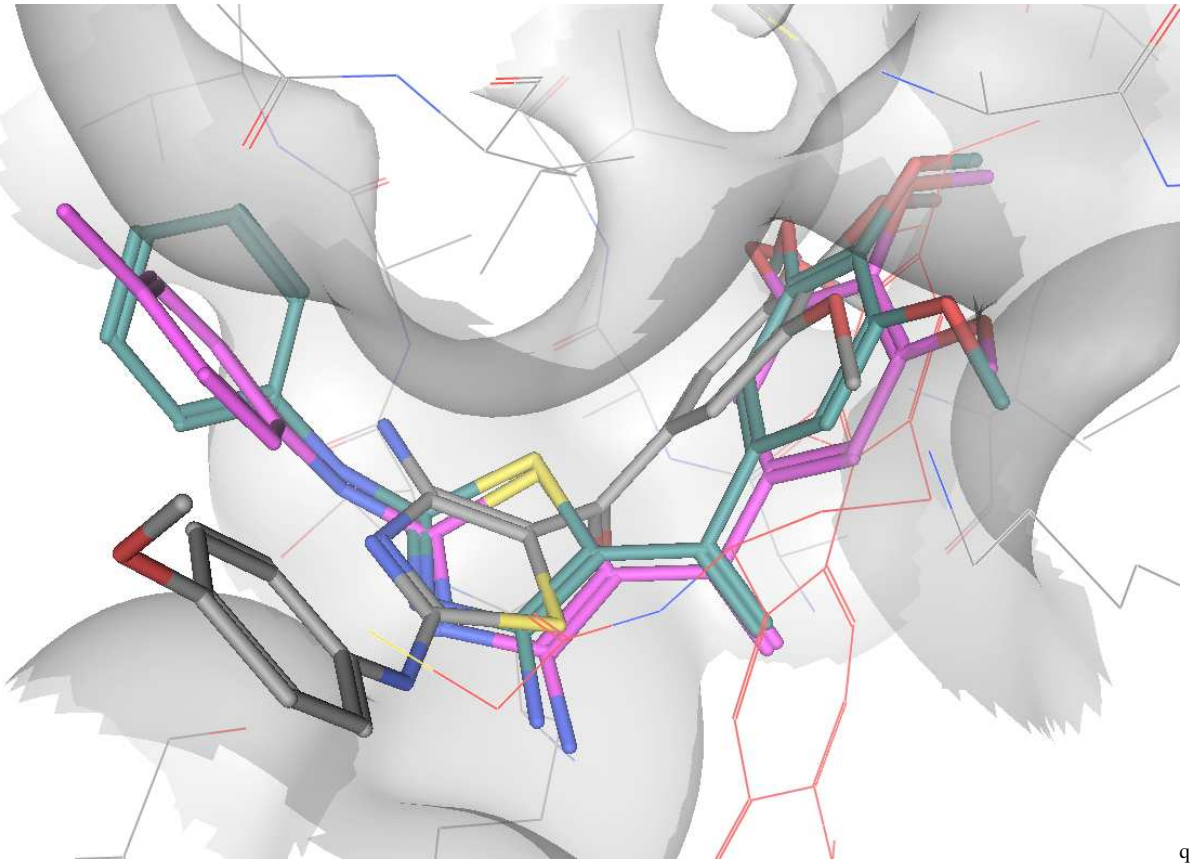


Figure 3. Percentage of apoptotic cells after treatment of HeLa cells with different concentrations of the indicated compounds. Cells were analyzed after 24h of treatment by the standard propidium iodide procedure, as described in the Methods section



q

Figure 4. Compounds **5t** (magenta), **5q** (cyan) and **5u** (grey) docked in the colchicine site. The bound DAMA-colchicine molecule is indicated by the structure shown in red.