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Hybrid α -bromoacryloylamido chalcones. Design, synthesis and biological evaluation

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Abstract

Research into the anti-tumor properties of chalcones has received significant attention over the last few years. Two novel large series of α -bromoacryloylamido chalcones **1a–m** and **2a–k** containing a pair of Michael acceptors in their structures, corresponding to the α -bromoacryloyl moiety and the α,β -unsaturated ketone system of the chalcone framework, were synthesized and evaluated for antiproliferative activity against five cancer cell lines. Such hybrid derivatives demonstrated significantly increased anti-tumor activity compared with the corresponding amino chalcones. The most promising lead molecules were **1k**, **1m** and **2j**, which had the highest activity toward the five cell lines. Flow cytometry with K562 cells showed that the most active compounds resulted in a large proportion of the cells entering the apoptotic sub-G0–G1 peak. Moreover, compound **1k** induced apoptosis through the mitochondrial pathway and activated caspase-3.

Among currently identified anti-tumor agents, chalcones represent an important class of natural small molecules useful in cancer chemotherapy.¹ Chalcones (1,3-diaryl-2-propen-1-ones, Chart 1) are known to exhibit antimitotic properties caused by inhibition of tubulin polymerization by binding to the colchicine-binding site.² Chemically, they are open-chained molecules consisting of two aromatic rings linked by a three-carbon enone fragment. Several research groups have shown that the *s-cis* conformation of chalcones is important for their biological activity.³ The double bond of the enone system is the essential moiety for chalcones as anti-tumor agents.⁴ It has been reported that hydrogenation or bromination across the

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Supplementary data

Detailed biological protocols, synthesis and spectroscopic data for compounds α -bromoacryloylamido chalcones **1a–m** and **2a–k**. General procedures for the synthesis of nitrochalcones **5a–m/6a–k** and aminochalcones **7a–m/8a–k** are available. Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.bmcl.2009.02.038.

carbon–carbon double bond or its transformation into the corresponding epoxide dramatically reduces chalcone activity.⁵ Their simple structure and the ease of preparation make chalcones an attractive scaffold for structure–activity relationship (SAR) studies, and a wide number of substituted chalcones have been synthesized to evaluate effects of various functional groups on biological activity.¹

The pyrroloiminoquinone cytotoxic alkaloids Discorhabdin A⁶ and Discorhabdin G⁷ are characterized by the presence of an α -bromoacryloyl alkylating moiety of low chemical reactivity, an unusual feature for cytotoxic compounds. In fact, α -bromoacrylic acid is not *per se* cytotoxic (IC₅₀ for L1210 cells being greater than 120 μ M).⁸ The same moiety is present in a series of potent anticancer distamycin-like minor groove binders, for example, PNU-166196 (brotallicin), which is currently undergoing Phase II clinical trials.⁹ PNU-166196 is an α -bromoacrylamido derivative of a four-pyrrole distamycin homologue ending with a guanidino moiety (Chart 1).⁹

The reactivity of the α -bromoacryloyl moiety has been hypothesized to be based on a first-step Michael-type nucleophilic attack, followed by a further reaction of the former vinylic bromo substituent alpha to the carbonyl, leading successively either to a second nucleophilic substitution or to beta elimination.¹⁰

Furthermore, several studies confirmed that the α,β -unsaturated ketone system of chalcones acts as a Michael acceptor, suggesting that alkylation of the β -position of the reactive enone system by biological nucleophiles may be one mechanism by which antiproliferative activity is exerted *in vitro*.¹¹

The observations that both the chalcone and the α -bromoacryloyl group can act as trapping agents of cellular nucleophiles led us to prepare and evaluate two novel and unusual classes of synthetic conjugates with general formulae **1a–m** and **2a–k** (Chart 1), incorporating these two moieties within their structures. If such processes occur, the α -bromoacryloylamido chalcone derivatives **1a–m** and **2a–k**, characterized by the presence of two potential sites for electrophilic attack on cellular constituents, should be more potent than the corresponding compounds containing only one nucleophilic center.

While compounds **1a–m** were designed to evaluate the SAR arising from different substitutions (both electron-releasing and electron-withdrawing groups), as well as different positions on the phenyl ring, for hybrids **2a–k** we focused on the synthesis of methoxylated derivatives with one or more methoxy groups at different positions on the aryl moiety.

Synthesis of derivatives **1a–m** and **2a–k** was carried out by the general methodology shown in Scheme 1. Nitrochalcones **5a–m** and **6a–k** were synthesized in high yields (81–95%) by the Claisen–Schmidt aldol condensation of 4-nitrobenzaldehyde or 4-nitroacetophenone with the corresponding appropriately functionalized acetophenones **3a–m** or benzaldehydes **4a–k**, respectively, in the presence of 50% w/v aqueous solution of sodium hydroxide.¹² Coupling constants (*J*) from the proton nuclear magnetic resonance (¹H NMR) spectra clearly indicated that derivatives **5a–m** and **6a–k** were both geometrically pure and were exclusively *trans* (*E*) isomers (*J*_{transC=C} = 15–16 Hz). The cluster of compounds **6a–k** may be referred to as ‘reversed chalcones’, whereby the carbonyl and ethylene groups present in the series **5a–m** are interchanged. Aminochalcones **7a–m** and **8a–k** were generated from the corresponding nitro derivatives **5a–m** and **6a–k** by reduction with iron in a refluxing solution mixture of 37% HCl in water and ethanol (1:2.5 v/v).

Finally, the hybrid compounds **1a–m** and **2a–k** were prepared, in acceptable yields (54–68%), by the condensation of α -bromoacrylic acid with aminochalcones **7a–m** and **8a–k**, respectively. This condensation was performed using an excess (2 equiv) both of 1-ethyl-3-[3-

(dimethylamino)propyl]carbodiimide hydrochloride (EDCI) and 1-hydroxy-1,2,3-benzotriazole (1-HOBt), in dry DMF as solvent at room temperature and with identical reaction times (18 h).

Tables 1 and 2 summarize the antiproliferative effects of α -bromoacryloylamido chalcones **1a–m** and **2a–k**, respectively, against the growth of murine leukemia (L1210), murine mammary carcinoma (FM3A), human T-lymphoblastoid (Molt/4 and CEM) and human cervix carcinoma (HeLa) cells, using aminochalcones **7a–m** and **8a–k** as reference compounds.

In general, the α -bromoacryloylamido chalcones **1a–m** and **2a–k**, were 10–100-fold more active than the corresponding amino chalcones **7a–m** and **8a–k**, respectively, demonstrating that the presence of an α -bromoacryloyl moiety significantly enhanced antiproliferative activity. The compounds displaying the greatest potency were **1k** (*p*-EtO), **1m** (2-thienyl) and **2j** (*o*, *m*, *p*-3OMe), with IC₅₀ values of 0.24–0.63, 0.52–0.68, 0.55–0.68, 0.73–0.84 and 0.34–0.75 μ M against the L1210, FM3A, Molt4, CEM and HeLa cell lines, respectively. The bioisosteric replacement of phenyl (**1a**) with a thiophene ring (**1m**) greatly increased activity, with IC₅₀ values 0.25–0.73 μ M versus 3.1–5.0 μ M against the five tumor cell lines. A positive effect was also observed for the 1-naphthyl derivative **1l**, which had IC₅₀ values of 1.1–3.1 μ M, as compared with **1a**.

The data presented in Tables 1 and 2 demonstrate effects of different substituents on the phenyl rings linked to the carbonyl and at the β -position of the enone system, respectively. With compounds **1a–k** (Table 1), the substitution pattern significantly affected potency. The introduction of either electron-releasing (ERG) or electron-withdrawing (EWG) substituents (derivatives **1b–k**) enhanced antiproliferative activity as compared with the unsubstituted analogue **1a**, and there was no clear difference between them. Ignoring the derivative with a *p*-I (**1f**), the greatest enhancement of activity occurred with the bulkier substituents, *p*-Br (**1e**) and *p*-EtO (**1k**).

Specifically with the *para*-halogen substituted **1bcef** (Table 1), activity increased in the following order: Br (**1e**) > Cl (**1c**), F (**1b**) > I (**1f**). Insertion of a second chlorine atom, to yield the *m,p*-dichloro derivative **1d**, resulted in about a twofold reduction in activity. Turning to the effects of an ERG on the phenyl moiety, we found that replacement of *p*-methyl (**1g**) with a *p*-methoxy (**1h**) group caused only a minor improvement in anti-proliferative activity. Moreover, a twofold reduction in activity was observed by shifting the methoxy group from the *para* to the *meta* position (**1i**). Replacement of the *p*-MeO group (**1h**) with a *p*-EtO moiety (**1k**) caused a twofold increase in potency, while the *o,m,p*-trimethoxy derivative **1j** was only slightly more active than **1h**.

In the case of the second series (Table 2), compounds **2b–j**, with methoxy substituents on the phenyl ring, had antiproliferative activity that, in general, was comparable with that of the unsubstituted **2a**. Similarly, the antiproliferative activity of the *N,N'*-dimethylamino derivative **2k** was not greatly different from that of the unsubstituted **2a**.

A comparison of the antiproliferative activity of compounds with the same substituent on the phenyl ring (**1a** vs **2a**, **1h** vs **2b**, **1i** vs **2c** and **1j** vs **2i**), the 'reversed' α -bromoacryloylamido derivatives **2a**, **2b**, **2c** and **2i** generally had higher IC₅₀ values than did the corresponding analogues **1a**, **1h**, **1i** and **1j**.

Chalcones are known to block cells in the G2-M phase of the cell cycle, which is consistent with their ability to inhibit tubulin assembly.² We therefore determined whether these hybrid molecules behaved in a similar manner.

The most active compounds (**1ekm** and **2abdej**) were evaluated for their inhibitory effects on tubulin polymerization and on the binding of [³H]colchicine to tubulin.¹³ With the exception of **2d**, all tested compounds were ineffective as inhibitors of tubulin assembly (IC₅₀ >40 μM). However, all compounds had poor solubility in the assay medium (all precipitated in the 40 μM assays). **2d** showed relatively weak activity as a tubulin polymerization inhibitor, with an IC₅₀ value of 8.3 ± 0.9 μM, and, at 50 μM, **2d** weakly inhibited the binding of 5 μM [³H] colchicines to tubulin (37 ± 10% inhibition). These data make it unlikely that the antiproliferative activity of these hybrid compounds results from a direct interaction with tubulin and that it is unlikely that they act as microtubule depolymerizing agents.

To confirm this idea, we next examined the effects of the most active compounds on the cell cycle by performing a flow cytometric analysis of K562 human chronic myelogenous leukemia cells, which are usually employed by our research group to determine the alteration of cell cycle parameters following exposure to anti-tumor compounds.¹⁴ Cells were cultured for 72 h in the absence or presence of each compound at the concentrations summarized in Table 3, and the cells were then stained with propidium iodide (Fig. 1, panels A and B). Unlike chalcones, which induced a substantial recruitment of cells into the G2-M phase of the cell cycle, derivatives **1ekm** and **2abdej** uniformly caused a decrease in the proportion of cells in all phases of the cell cycle (G0–G1, S and G2-M), with a proportionate increase in the number of apoptotic cells in the sub-G1 phase region of the histogram. The absence of the typical increase in G2-M cell number, together with the minimal effect on tubulin assembly, makes it unlikely that microtubule disruption underlies the potent apoptotic effect caused by these hybrid compounds.

The induction of apoptosis was confirmed by the annexin V test¹⁵ with compounds **2j** and **2k** (Fig. 1, panels C and D). The increase in annexin V-positive cells demonstrated activation of the apoptotic pathway by these compounds. Figure 2C shows the extensive binding of annexin V to **2j**-treated cells, and Figure 2D shows how time and concentration of **2k** affect the proportion of cells that become positive for the marker. Similar observations were made with the other most active compounds.

Impairment of mitochondrial function, is an early event in the executory phase of programmed cell death in different cell types, and it occurs as the consequence of a preliminary reduction of the mitochondrial transmembrane potential ($\Delta\psi_{mt}$).^{16,17} Early $\Delta\psi_{mt}$ disruption results from an opening of mitochondrial permeability transition pores, and this permeability transition triggers the release of apoptogenic factors, such as apoptosis inducing factor and cytochrome c, which in turn lead to later apoptotic events.^{16,17}

We used the lipophilic cation 5,5',6,6'-tetrachloro-1,1'.3,3'-tetra-ethylbenzimidazol-carbocyanine (JC-1) to monitor changes in $\Delta\psi_{mt}$ induced by **1k**. The method is based on the ability of this fluorescent probe to enter selectively into the mitochondria, and its color changes reversibly from green to orange as membrane potential increases.¹⁸ This property is due to the reversible formation of JC-1 aggregates upon membrane polarization. Aggregation causes a shift in the emitted light from 530 nm (i.e., emission by JC-1 monomers) to 590 nm (emission by JC-1 aggregates) following excitation at 490 nm.

K562 cells were treated with compound **1k** for 24 and 48 h at different concentrations. As shown in Figure 2 (panels A and B), compound **1k** induces substantial mitochondrial depolarization in a time- and concentration-dependent manner. The disruption of the $\Delta\psi_{mt}$ is associated with the appearance of sub-G1 cells and with the marked increase in the percentage of annexin V-positive cells.

Mitochondrial membrane depolarization is associated with mitochondrial production of reactive oxygen species (ROS).^{19,20} To investigate the effects of **1k** on the production of

oxygen species during apoptosis, we utilized the fluorescence indicator hydroethidine (HE), which fluoresces if reactive oxygen species are generated.²¹ As shown in Figure 2 (panel C), there was an increase in cells producing ROS that closely paralleled the increase in cells with low $\Delta\psi_{mt}$, a function of both the concentration of **1k** and treatment time.

We also evaluated the damage caused by ROS in mitochondria by assessing the oxidation state of cardiolipin, a phospholipid restricted to the inner mitochondrial membrane. We used 10 N-nonyl acridine orange (NAO) a fluorescent probe which is independent of mitochondrial permeability transition.²² The dye interacts stoichiometrically with intact, non-oxidized cardiolipin. Somewhat unexpectedly, cells did not show reduction in NAO fluorescence, but rather, a marked increase, especially after 48 h of treatment with **1k** (Fig. 3 panels A and B). This effect suggests an increase in cardiolipin content as a consequence of increased mitochondrial mass. This has been observed in some tumor cell lines following treatment with herbimycin A,²³ genistein,²⁴ and the acronicyne derivative S23906-1²⁵ and following oxidative stress.²⁶

Several caspases have been shown to be key executioners of apoptosis mediated by various inducers.²⁷ Caspase-3, in particular, is essential to the propagation of the apoptotic signal after exposure of cells to many DNA-damaging agents and other anticancer drugs.^{27,28} We therefore determined whether caspase-3 is involved in the apoptosis induced by compound **1k**. We used a monoclonal antibody specifically for the active fragment of caspase-3. As shown in Figure 4, with 0.6 and especially 1.25 μ M **1k** there was increased activated caspase-3 after 24 h of treatment, and a further increase after 48 h.

In summary, our plan to optimize the potency of a single Michael acceptor by providing multiple sites of reactivity towards cellular nucleophiles led us to synthesize two series of α -bromoacryloylamido chalcones **1a–m** and **2a–k**. These compounds were derived from the hybridization of two kinds of Michael acceptors, corresponding to the α,β -unsaturated ketone system of chalcone and the α -bromoacryloyl moiety. While the amino chalcones **7a–m** and **8a–k** showed weak or no antiproliferative activity against five different cancer cell lines, their conversion into the corresponding α -bromoacryloylamido derivatives **1a–m** and **2a–k** was accompanied by a 10–100-fold increase in potency. More noteworthy was that compounds **1k** (52%), **2b** (68%), **2d** (83%) and **2j** (57%) induced over half the cells to enter a sub-G1 population as compared with untreated control cells (11%). As demonstrated with **1k**, their mechanism of action appears to induce apoptosis mediated by the involvement of mitochondria and by the activation of caspase-3. Thus, the mitochondrial apoptotic pathway plays a major role, in generation of the sub-G1 cell population. Further studies to clarify additional details of the molecular mechanism of action of these compounds and the selectivity to inhibit the growth of cancer cells are underway. On the other hand, the most active compounds should be analyzed for effects on normal primary normal human cells of different histotype, in order to determine potential therapeutic windows supporting further studies on experimental tumor-bearing animals finalized to verify possible *in vivo* anticancer activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Representative histograms of flow cytometry data of untreated control K562 cells (panel A) or K562 cells treated for 3 days with $0.7 \mu\text{M}$ **2j** (IC_{75} , see Table 3) (panel B). After 3 days of incubation the cells were labelled with propidium iodide and analyzed by flow cytometry as described in Materials and Methods. Panel C. Representative histograms of untreated K562 cells (black line) and K562 cells treated for 3 days with $0.2 \mu\text{M}$ **2j** (IC_{50}) (green line). After 48 h cells were stained with annexin V-FITC and analyzed by flow cytometry. Panel D. Effect of **2j** concentration on the proportion of annexin V-positive cells after treatment for 24 and 48 h.

Figure 2.

Induction of loss of $\Delta\psi_{mt}$ and production of ROS in K562 cells after treatment with **1k** at different concentrations. Panel A shows representative histograms of K562 cells incubated with and without 1.25 μ M **1k** and stained with the fluorescent probe JC-1 after 24 and 48 h of treatment. The horizontal axis shows fluorescence intensity of JC-1 monomers, and the vertical axis shows fluorescence of JC-1 aggregates. Panel B shows the percentage of cells with low $\Delta\psi_{mt}$ following treatment with different concentrations of **1k** for the indicated times. Panel C. K562 cells were treated with the indicated concentrations of **1k** and at 24 and 48 h cells were harvested and incubated with HE. Analysis of intracellular fluorescence was conducted by flow

cytometry. The data are represented as percentage of ethidium positive cells and expressed as mean $\pm$ SEM of three independent experiments.

Figure 3.

Increase of mitochondrial mass in K562 cells treated with compound **1k**. Panel A. Representative histograms of cells incubated for 24 and 48 h in the presence of **1k** (2.5 μM) and stained with the fluorescent probe NAO. Black line = controls, red line = **1k**. Panel B. K562 cells were treated as above and the relative NAO intensity was analysed by flow cytometry. The results are expressed as percentage of the fluorescence intensity with respect to the untreated control. Data expressed as mean ± SEM of three independent experiments.

Figure 4.

Caspase-3 activity induced by **1k**. K562 cells were treated with the indicated concentrations of **1k**. After 24 h and 48 h cells were harvested and stained with an anti-human active Caspase-3 fragment monoclonal antibody conjugated with FITC. Data obtained by flow cytometric analysis is expressed as percentage of caspase-3 active fragment positive cells.

Scheme 1.

Reagents and conditions: (a) 50% aqueous NaOH solution, *p*-NO₂C₆H₄CHO for **3** or *p*-NO₂C₆H₄COCH₃ for **4**, EtOH, rt, 18 h; (b) Fe, HCl 37% in water, EtOH, reflux, 3 h; (c) α -bromoacrylic acid, EDCI, HOBt, DMF, 18 h, rt.

General structure of chalcones
 R_1 or $R_2 = \text{H, OH, OMe, halogen, NO}_2, \text{NH}_2$

Discorhabdin A

Discorhabdin G

PNU-166196 or Brostallicin

- 1a**, Ar=C₆H₅
1b, Ar=4-F-C₆H₄
1c, Ar=4-Cl-C₆H₄
1d, Ar=3,4-Cl₂-C₆H₄
1e, Ar=4-Br-C₆H₄
1f, Ar=4-I-C₆H₄
1g, Ar=4-Me-C₆H₄
1h, Ar=4-OMe-C₆H₄
1i, Ar=3-OMe-C₆H₄
1j, Ar=3,4,5-(OMe)₃-C₆H₃
1k, Ar=4-OEt-C₆H₄
1l, Ar=1-naphtyl
1m, Ar=2-thienyl

- 2a**, Ar=C₆H₅
2b, Ar=4-OMe-C₆H₄
2c, Ar=3-OMe-C₆H₄
2d, Ar=2-OMe-C₆H₄
2e, Ar=2,3-(OMe)₂-C₆H₄
2f, Ar=3,4-(OMe)₂-C₆H₄
2g, Ar=2,4-(OMe)₂-C₆H₄
2h, Ar=3,5-(OMe)₂-C₆H₄
2i, Ar=3,4,5-(OMe)₃-C₆H₃
2j, Ar=2,3,4-(OMe)₃-C₆H₃
2k, Ar=4-N(CH₃)₂-C₆H₄

Chart 1.

Table 1

In vitro inhibitory effects of compounds **1a–m** and **7a–m** on the proliferation of murine leukemia (L1210), murine mammary carcinoma (FM3A), human T-leukemia (Molt/4 and CEM) and human cervix carcinoma (HeLa) cells

Compound	IC ₅₀ (μM) ^a				
	L1210	FM3A	Molt4	CEM	HeLa
7a	88 ± 12	146 ± 96	10 ± 2	34 ± 1	36 ± 1
7b	123 ± 92	185 ± 3	12 ± 2	39 ± 0	40 ± 4
7c	111 ± 15	178 ± 30	7.8 ± 1.9	21 ± 17	43 ± 1
7d	26 ± 1	33 ± 0	13 ± 0	15 ± 0	31 ± 9
7e	90 ± 12	120 ± 3	10 ± 0	14 ± 1	49 ± 2
7f	50 ± 4	110 ± 9	7.5 ± 0.8	16 ± 1	58 ± 11
7g	49 ± 12	107 ± 25	12 ± 7	31 ± 3	40 ± 1
7h	160 ± 15	282 ± 44	19 ± 13	37 ± 8	98 ± 64
7i	38 ± 15	43 ± 0	20 ± 15	26 ± 24	33 ± 6
7j	14 ± 3	36 ± 6	1.5 ± 0.3	2.5 ± 0.2	1.9 ± 0.2
7k	21 ± 4	55 ± 0	11 ± 1	15 ± 0	41 ± 4
7l	32 ± 9	41 ± 4	12 ± 2	22 ± 14	18 ± 4
7m	87 ± 7	123 ± 20	13 ± 2	27 ± 19	15 ± 3
1a	5.0 ± 1.7	3.9 ± 1.5	3.1 ± 1.4	4.0 ± 2.5	3.9 ± 1.5
1b	1.6 ± 0.9	1.2 ± 0.3	0.66 ± 0.02	1.1 ± 0.9	1.4 ± 0.1
1c	1.1 ± 0.2	1.3 ± 0.0	0.81 ± 0.33	0.95 ± 0.62	1.6 ± 0.2
1d	2.1 ± 0.1	3.2 ± 1.1	1.8 ± 0.2	1.7 ± 0.1	3.2 ± 1.4
1e	0.62 ± 0.20	1.1 ± 0.8	0.68 ± 0.24	0.99 ± 0.31	1.0 ± 0.0
1f	3.4 ± 0.6	8.3 ± 2.6	2.8 ± 0.7	4.0 ± 2.4	7.7 ± 3.4
1g	1.9 ± 0.4	2.1 ± 0.1	1.7 ± 0.1	1.8 ± 0.2	2.1 ± 0.7
1h	0.98 ± 0.96	1.2 ± 0.5	1.3 ± 1.2	1.4 ± 1.2	1.4 ± 0.2
1i	2.1 ± 1.5	2.7 ± 1.2	0.85 ± 0.06	2.3 ± 1.7	0.70 ± 0.10
1j	0.91 ± 0.68	0.95 ± 0.77	0.85 ± 0.30	1.1 ± 0.8	1.1 ± 0.6
1k	0.24 ± 0.05	0.68 ± 0.20	0.61 ± 0.17	0.75 ± 0.20	0.75 ± 0.05
1l	1.1 ± 0.1	2.4 ± 0.8	2.1 ± 0.6	3.1 ± 1.3	2.0 ± 0.5
1m	0.25 ± 0.11	0.52 ± 0.06	0.55 ± 0.18	0.73 ± 0.25	0.34 ± 0.12

IC_{50} = compound concentration required to inhibit tumor cell proliferation by 50%. Data are expressed as the mean $\pm$ SE from the dose-response curves of at least three independent experiments.

Table 2

In vitro inhibitory effects of compounds **2a-k** and **8a-k** on the proliferation of murine leukemia (L1210), murine mammary carcinoma (FM3A), human T-leukemia (Molt/4 and CEM) and human cervix carcinoma (HeLa) cells

Compound	IC ₅₀ (μM) ^a			
	L1210	FM3A	Molt4	HeLa
8a	14 ± 3	34 ± 1	7.9 ± 0.8	8.5 ± 1.5
8b	18 ± 5	35 ± 6	8.6 ± 0.4	14 ± 3
8c	22 ± 15	45 ± 10	21 ± 11	23 ± 15
8d	12 ± 1.0	25 ± 5	8.3 ± 0.9	6.3 ± 1.0
8e	13 ± 8	29 ± 2	8.5 ± 0.3	7.6 ± 1.0
8f	25 ± 17	35 ± 3	8.6 ± 0.2	9.3 ± 0.2
8g	34 ± 12	43 ± 0	20 ± 11	18 ± 7
8h	6.0 ± 4.2	12 ± 1	7.5 ± 0.1	5.7 ± 0.5
8i	7.8 ± 0.1	8.8 ± 0.0	5.6 ± 1.2	2.3 ± 0.3
8j	25 ± 16	29 ± 3	10 ± 1	9.7 ± 0.6
8k	67 ± 33	96 ± 32	9.9 ± 1.0	20 ± 3
2a	0.88 ± 0.59	0.69 ± 0.68	0.50 ± 0.17	1.5 ± 0.0
2b	0.90 ± 0.63	1.0 ± 0.1	0.61 ± 0.55	1.1 ± 0.8
2c	1.1 ± 0.6	1.8 ± 0.5	1.1 ± 0.1	1.8 ± 0.2
2d	1.7 ± 0.3	0.37 ± 0.33	0.24 ± 0.20	0.58 ± 0.53
2e	1.1 ± 0.9	0.55 ± 0.31	0.49 ± 0.07	0.71 ± 0.53
2f	1.3 ± 0.9	1.2 ± 1.1	1.8 ± 0.2	1.6 ± 0.2
2g	0.52 ± 0.05	1.2 ± 0.9	1.5 ± 0.8	1.1 ± 0.3
2h	2.7 ± 0.7	2.0 ± 0.1	1.5 ± 0.0	2.2 ± 0.0
2i	1.5 ± 0.9	1.8 ± 1.9	0.28 ± 0.08	0.33 ± 0.1
2j	0.63 ± 0.12	0.53 ± 0.28	0.68 ± 0.26	0.84 ± 0.2
2k	1.9 ± 1.6	2.8 ± 1.5	1.3 ± 0.3	1.5 ± 0.2

^aIC₅₀ = compound concentration required to inhibit tumor cell proliferation by 50%. Data are expressed as the mean ± SE from the dose-response curves of at least three independent experiments.

Table 3
Cell-cycle distribution of K562 cells after 72 h of treatment with compounds **1e**, **1k**, **1m**, **2a-b**, **2d-e** and **2j**

Compound	IC ₇₅ ^a (μM)	Cell cycle percentage (%)			
		Sub-G1 ^b	G0-G1	S	G2-M
Control	NM	10.8	39.8	30.7	18.7
1e	0.84 ± 0.18	20.4	35.9	27.5	16.2
1k	0.85 ± 0.21	52.3	16.5	19.6	11.6
1m	0.82 ± 0.13	42.8	17.0	23.2	17.0
2a	2.77 ± 0.51	47.6	23.0	16.4	13.0
2b	1.14 ± 0.21	68.2	11.0	11.6	9.2
2d	2.94 ± 1.21	83.4	6.4	6.3	3.9
2e	0.64 ± 0.15	48.4	22.3	15.2	14.1
2j	0.71 ± 0.18	57.8	13.7	14.9	13.6

NM: not meaningful.

^a IC₇₅ = compound concentration required to inhibit tumor cell proliferation by 75%. Data are expressed as the mean ± SE from the dose-response curves of at least three independent experiments.

^b Apoptosis area.