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2-Cinnamamido, 2-(3-phenylpropiolamido), and 2-(3-phenylpropanamido)benzamides: Synthesis, antiproliferative activity, and mechanism of action

Demetrio Raffa^a, Benedetta Maggio^{a,*}, Maria Valeria Raimondi^a, Maria Grazia Cusimano^a, Amico Giandomenico^{b,c}, Anna Carollo^d, Pier Giulio Conaldi^{b,c}, Ruoli Bai^e, Ernest Hamel^e, and Giuseppe Daidone^a

^aDipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche, Via Archirafi, 32, 90123 Palermo, Italy

^bDepartment of Laboratory Medicine and Advanced Biotechnologies, ISMETT Mediterranean Institute for Transplantation and Advanced Specialized Therapies, Palermo, Italy

^cUnit of Regenerative Medicine and Biomedical Technologies, Ri.MED. Foundation, Palermo, Italy

^dServizio Farmacia Clinica, ISMETT Mediterranean Institute for Transplantation and Advanced Specialized Therapies, Palermo, Italy

^eScreening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick National Laboratory for Cancer Research, Frederick, MD 21702, USA

Abstract

Several new benzamides **4a–q** were synthesized by stirring in pyridine the acid chlorides **3a–q** with the appropriate anthranilamide derivatives **2a–g**. Some of the synthesized compounds were evaluated for their *in vitro* antiproliferative activity against a panel of 5 human cell lines (K562 human chronic myelogenous leukemia cells, MCF-7 breast cancer cells, HTC-116 and HT26 colon cancer cells and NCI H460 non-small cell lung cancer cells).

Keywords

2-Cinnamamidobenzamides; 2-(3-Phenylpropiolamido)benzamides; 2-(3-Phenylpropanamido)benzamides; Antiproliferative activity; Apoptosis

1. Introduction

Cinnamoyl anthranilates represent a class of biologically active substances of great importance in medicinal chemistry. Some examples of biological activities of this class of compounds include activity against human coronary artery smooth muscle and endothelial cells [1], antifibrotic and antiinflammatory properties [2,3], inhibition of cornea pterygium

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* Corresponding author. Tel.: +39 91 23891949. demetrio.raffa@unipa.it (D. Raffa), benedetta.maggio@unipa.it (B. Maggio).

Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at <http://dx.doi.org/10.1016/j.ejmech.2013.04.068>. These data include MOL files and InChIKeys of the most important compounds described in this article.

progression and blood vessel development [4,5] and prevention and treatment of renal glomerular diseases [6]. In a previous paper from our laboratories [7], we reported the synthesis, antiproliferative activity and mechanism of action of a series of 2-([2*E*]-3-phenylpropenamido)benzamides **1a–v** (Fig. 1).

Synthesized benzamides **1a–v** were initially tested *in vitro* for their antileukemic activity against the K562 human chronic myelogenous leukemia cell line. In addition, compounds **1a–c,t,u** were evaluated by the NCI against a panel of approximately 60 human cell lines derived from seven cancer types (lung, colon, melanoma, renal, ovarian, brain, and leukemia). The data reported [7] show that the 2-([2*E*]-3-phenylpropenamido)benzamides **1a–c,t,u** caused growth inhibition against many tumor cell lines. These five compounds inhibited proliferation at low micromolar (**1a**, **1b**, **1c**) or sub-micromolar concentrations (**1t**, **1u**) against every tumor cell line investigated. The best activity was obtained when the 5 position of the benzamido moiety was substituted with an iodine moiety. COMPARE analysis, effects on tubulin polymerization in cells and with purified tubulin, and effects on cell cycle distribution, including induction of apoptosis, indicate that these new antiproliferative compounds act as antitubulin agents.

On the basis of these results, we synthesized new derivatives that, starting from the most active previously synthesized compounds, allowed us to determine the best substituents to insert in the molecules to improve their activity and expand our understanding of the SAR in this class of compounds.

In particular, we carried out the following structural modifications on the cinnamamidobenzamide skeleton: introduction of new substituents on the benzamido and cinnamamido groups and substitution of the vinylene group with ethylene and ethynylene moieties, with the goal of identifying new antitubulin agents (Fig. 2).

2. Results and discussion

2.1. Chemistry

The synthesis of benzamido derivatives **4a–e**, **4f** [4,5], **4g–k**, **4l** [8], **4m–q**, **4r** [9], **4s–w** was achieved as described in Scheme 1. A mixture of the appropriate acid chloride **3a–h** and the appropriate 5-R-4-R₁-2-aminobenzamide **2a–g** was stirred magnetically in an ice bath. The attempt to obtain (*Z*)-cinnamamidobenzamides **4f–k**, starting from the appropriate *cis*-2-methoxycinnamic acid failed due to stereochemical conversions during the synthetic procedures, so that only (*E*)-derivatives were isolated.

Crude benzoyl chlorides **3a–q**, not commercially available, were obtained starting from the corresponding acid by treatment with thionyl chloride [10].

The anthranilamide **2a** is commercially available, while the 5-R-4-R₁-2-aminobenzamides **2b–g** were obtained according to Scheme 2.

In particular, the 5-iodo-2-aminobenzamide **2e** was obtained, as reported in the literature [11], by treatment of anthranilamide **2a** with iodine in an aqueous solution of sodium bicarbonate.

The 5-methylantranilamide **2d**, the 5-methoxyantranilamide **2f** and the 4,5-dimethoxyantranilamide **2g** were obtained by reduction of 2-nitro-5-methyl benzamides **7a–c** as shown in Scheme 2. Compounds **7a–c** were obtained by reaction of the acids **5a–c** with thionyl chloride to afford **6a–c**, followed by treatment of **6a–c** with aqueous ammonia.

The anthranilamide derivatives **2b,c** were obtained by stirring the appropriate 2*H*-3,1-benzoxazine-2,4(1*H*)-dione **8a,b** in a 25% aqueous ammonia solution (Scheme 2) [12].

The structures of the new compounds were determined by analytical and spectroscopic measurements. In particular, all derivatives **4a–w** showed signals attributable to aromatic protons in the range 6.18–8.77 δ and a singlet in the range 11.00–13.43 δ , exchangeable with D₂O, for the amidic NH. Different chemical shift values were detected for the signals of the NH₂ benzamido protons according to the different registration solvent. In fact, for compounds **4a–c,f–k,m–q**, whose ¹H NMR spectra were recorded in DMSO, this signal falls together with the aromatic signals, while, in the case of compounds **4d,e,s–w**, recorded in CDCl₃, a splitting of the signals for the two protons of the NH₂ group at 5.84 and 6.29 δ was observed. Moreover, the ¹H NMR spectra of 5-methyl, 5-methoxy and 4,5-dimethoxy-substituted derivatives showed signals at about 2.30 δ and 3.84–3.99 δ , respectively, arising from the methyl and methoxyl groups. ¹H NMR spectra of compounds **4a–k** were consistent with an *E*-olefinic structure. They showed signals attributable to the β -olefinic protons at 6.86–6.96 δ , with coupling constants of 15.6–15.9 Hz, as required for *E*-structures [13], while the α -olefinic hydrogens were found along with aromatic multiplets. As regards the 2-(3-phenylpropiolamido)benzamido derivatives **4l–q**, the IR spectra showed the characteristic band around 2210 cm⁻¹ attributable to acetylenic bond stretching. Finally, ¹H NMR spectra of 2-(3-phenylpropanamido)benzamides **4s–w** showed two triplets at 2.70 and 3.00 δ , respectively, due to the ethylene chain.

2.2. Biological activity

Synthesized benzamido derivatives **4a–w** were initially tested *in vitro* for their antiproliferative activity against a panel of 5 human cell lines: K562 (human chronic myelogenous leukemia), MCF-7 (breast cancer), HTC-116 (colon cancer), HT26 (colon cancer) and NCI H460 (non-small cell lung cancer); colchicine was used as a reference compound.

The percent growth inhibition at a screening concentration of 10 μ M and the IC₅₀ values for compounds that exhibited at least 50% growth inhibition in the 10 μ M screening assay are shown in Tables 1 and 2, respectively.

These data showed that the best antiproliferative agent was compound **4l**, which showed antiproliferative activity against all cell lines investigated, and it yielded the lowest IC₅₀ value in the K562 cells, in particular. Overall, however, this series of compounds was most active in the K562 cell line.

To obtain more insights into the cytotoxic potential of test compounds for normal human cells, compounds **4d,g–l,p,q,s,v** were also tested *in vitro* for their cytotoxicity against HuDe (IZSL) cells, a primary cell culture from human epidermis (Table 3).

The data in Table 3 showed that the K562-active compounds were significantly less cytotoxic against the HuDe cell line (HuDe/K562 index ranging from 7.3 to 478), indicating the possibility that these compounds may have limited toxicity *in vivo* at doses that would limit tumor growth; data from Table 3 also showed a very low HuDe/K562 index (0.85) for colchicine.

As far as structure–activity relationships are concerned, it seems that on average the substitution of the double bond with either a triple (compounds **4l–q**) or single (compounds **4r–w**) bond had little effect on inhibition of K562 cell growth (Table 1). With a double bond (**4a–k**), average inhibition by 10 μ M compound of K562 cell growth was 44.4%, while with a triple (**4l–q**) or single (**4r–w**) bond, average inhibition was 47.5 and 38.4%, respectively.

In the cinnamamidobenzamido series (compounds **4a–k**), as previously reported [7], the activity was only maintained in the *ortho*-styryl derivatives (compounds **4g–j**). Moreover, substitutions in the 4 position of the benzamido moiety reduced K562 cell growth irrespective of the substituent (compounds **4e** and **4k**). In the 2-(3-phenylpropiolamido)benzamido series (compounds **4l–q**), antiproliferative activity against the K562 cell line was greatest in the unsubstituted compound **4l**. Finally, with regard to the 2-(3-phenylpropanamido)benzamides (compounds **4r–w**), the unsubstituted **4r** was the least active of the series against the K562 cells, while the chloro (**4s**) and methoxy (**4v**) derivatives were the most active among the compounds with substituents.

The effects (Table 2) of one the most active compounds of each series (**4d** for 2-cinnamamidobenzamides, **4l** for 2-(3-phenylpropiolamido)benzamides and **4s** for 2-(3-phenylpropanamido) benzamides, respectively) on cell cycle distribution were analyzed in K562 cells. Cells were cultured for 24 h in the presence of each compound at 10 μ M. Flow cytometric analyses of cell cycle distribution are summarized in Table 4.

The three compounds increased the proportion of cells in the G2/M phase, although the effect of colchicine was greater. The increase in G2/M cells occurred at the expense of cells in G0–G1, which decreased following treatment with all compounds.

Selected compounds (**4d**, **4l** and **4s**) were also evaluated in human Burkitt lymphoma CA46 cells by flow cytometry, because this cell line generally produces a very high proportion of cells arrested in G2/M when treated with antitubulin agents. The IC₅₀ values obtained in the Burkitt cells were $5.0 \pm 1 \mu\text{M}$ for **4d**, $20 \pm 2 \mu\text{M}$ for **4s**, and $>100 \mu\text{M}$ for **4l**. For flow cytometry, the cells were treated with 100 μM of the three agents. The proportion of cells in G2/M was over 90% following treatment with either **4d** or **4s**, but the cells treated with **4l** showed a control pattern of cell cycle distribution. A morphological analysis was also performed with Burkitt cells treated with **4d** and **4s** at 100 μM . A mitotic index of 32% was obtained following **4d** treatment and of 23% following **4s** treatment (control value, 3%; value following treatment with the potent antitubulin agent combretastatin A-4, 48%).

Since G2/M arrest and, especially, an increase in the mitotic index is associated with antitubulin agents, we evaluated a number of the active compounds for potential effects on the assembly of purified tubulin. None of the examined compounds (**4d**, **4e**, **4l**, **4m**, **4r**, **4s**, **4t**, **4w**) had any effect on tubulin assembly at 20 μM , in contrast to combretastatin A-4, which yielded an IC₅₀ of $1.1 \pm 0.1 \mu\text{M}$ in these experiments.

Nevertheless, since antitubulin agents invariably cause apoptosis in cells treated with these compounds, we evaluated compounds **4a–e**, **i**, **l–w** for apoptotic effects on the cell line HepG2. We used the annexin V assay. In the early stages of apoptosis, changes occur at the cell surface. One of these is the translocation of phosphatidylserine (PS), which is normally located exclusively at the inner side of the plasma membrane, to the external surface of the cell. The Ca²⁺-dependent phospholipid-binding protein annexin V has a high affinity for PS. It can be used as a sensitive probe for PS that is exposed on the outer leaflet of the cell membrane. However, due to the loss of membrane integrity in necrotic cells, annexin V can enter the cells and bind to the PS exposed on the inner leaflet of the cell membrane. Simultaneous staining with propidium iodide (PI), a DNA stain that is not cell membrane permeable, distinguishes apoptotic cells from necrotic cells. Apoptotic cells will stain annexin V positive, but be PI negative because the PI cannot cross the intact cell membrane. Necrotic cells will stain positive for both annexin V and PI because the cell membrane is no longer intact.

A comparison of the percentage of apoptotic cells in the absence of drug (13.9%) with the percentage following a 72 h treatment with the compounds at 10 μ M is shown in Table 5. The greatest apoptotic activity occurred with compounds **4i** (50.9%), **4l** (28%), **4p** (41.7%), **4q** (67.9%), **4s** (26.0%), **4t** (34.9%) and **4v** (40.8%). With the reference compound colchicine there were 40.5% apoptotic cells. Among these, compounds **4i** and **4q** showed a marked apoptotic effect compared with cell death (apoptosis/dead index 1.80 and 2.18, respectively), significantly greater than the ratio obtained with colchicine.

3. Conclusion

To summarize, we have two suggestive pieces of evidence that the most active compounds in this series may act through an antitubulin mechanism of action. The compounds cause G2/M and mitotic arrest in treated cells, and they cause an apoptotic cell death. In contrast, however, we could detect no activity with purified tubulin. There are several potential explanations for such findings. Possibly the target is another intracellular protein. Possibly our derivatives are metabolized intracellularly and converted to more active compound(s). On the other hand, the most potent antitubulin agents have IC₅₀ values in the low nanomolar range and even much lower (the most active spongistatins and cryptophycins arrest cell growth in the low picomolar range). Our reference agents here are good examples, with colchicine having a 20 nM IC₅₀ value in the K562 cells and combretastatin A-4 a 20 nM IC₅₀ in the Burkitt cells. In other words, cells are substantially more sensitive to these agents than is the purified protein (the most active agents in our hands yield assembly IC₅₀ values no lower than 0.5 μ M), possibly because the intracellular concentration of agents is much higher than the nominal concentration in the culture medium.

4. Experimental protocols

4.1. Chemistry

4.1.1. General—Reaction progress was monitored by TLC on silica gel plates (Merck 60, F₂₅₄, 0.2 mm). Organic solutions were dried over Na₂SO₄. Evaporation refers to the removal of solvent on a rotary evaporator under reduced pressure. All melting points were determined on a Büchi 530 capillary melting point apparatus and are uncorrected. IR spectra were recorded with a Perkin–Elmer Spectrum RXI FT-IR System spectrophotometer, with compound as a solid in a KBr disc. ¹H NMR spectra were obtained using a Bruker AC-E 300 MHz spectrometer (tetramethylsilane as internal standard); chemical shifts are expressed in δ values (ppm). Merck silica gel (Kieselgel 60/230–400 mesh) was used for flash chromatography columns. Microanalyses data (C, H, N) were obtained by an Elemental Vario EL III apparatus and were within $\pm 0.4\%$ of the theoretical values. Yields refer to purified products and are not optimized. The names of the products were obtained using the ACD/I-Lab Web service (ACD/IUPAC Name Free 8.05).

4.1.2. General procedure for preparation of cinnamoyl chlorides 3a–f, 3-phenylpropioloyl chloride 3g 3-phenylpropanoyl chloride 3h and 5-R-4-R₁-2-nitrobenzoyl chloride 6a–c—Substituted benzoyl and acryloyl chlorides **3a–h**, and **6a–c** were obtained by refluxing for 5 h the appropriate acid derivatives (0.01 mol) with thionyl chloride (7.25 ml) [10]. After evaporation under reduced pressure, the crude liquid residue was used for subsequent reactions without purification.

4.1.3. Preparation of 5-R-4-R₁-2-nitrobenzamides 7a–c—To 0.01 mol of 5-R-4-R₁-2-nitrobenzoyl chlorides **6a–c** 10 ml of an aqueous ammonia solution (25%) and 33 ml of acetonitrile were added. The solution was first refluxed for 8 h, then evaporated under reduced pressure to give pure **7a–c**.

4.1.4. Preparation of 5-R-4-R₁-2-aminobenzamides 2d,f,g—To a magnetically stirred suspension of stannous chloride (0.038 mol) in concentrated HCl (37%), 0.013 mol of **7a–c** was added (15 ml) at a rate so that the temperature of the slurry was maintained below 5 °C (about 1 h). After addition was complete, the mixture was stirred for 24 h. The white slurry thus obtained was diluted with cold water (150 ml), and sodium hydroxide (40%) was added until the tin salt dissolved. The solution was extracted with ethyl acetate (3 × 150 ml), and the extracts dried and evaporated *in vacuo* to obtain pure **2d,f,g**.

4.1.5. General procedure for preparation of 2-amino-5-chlorobenzamide 2b and 2-amino-5-bromobenzamide 2c [12]—A mixture of 0.01 mol of 6-R-7-R₁-1H-benzo[d][1,3] oxazine-2,4-diones **8b,c** and 25 ml of aqueous ammonia solution (25%) was stirred for 1 h. The solid precipitate was removed by filtration, washed with an aqueous ammonia solution (5%) and crystallized from ethanol.

4.1.6. General procedure for preparation of 2-amino-5-iodobenzamide 2e [11]—Powdered iodine (11.7 g, 46.2 mmol) was added portion-wise over 1 h to a stirred solution of 2-aminobenzamide **2a** (5.72, 42.0 mmol) and NaHCO₃ (3.52 g, 42.0 mmol) in water (1.3 l). The solution was stirred overnight at room temperature. Afterward NaHSO₃ (0.87 g, 8.40 mmol) was added. The solution was extracted with ethyl acetate (3 × 800 ml). After being dried with Na₂SO₄, the organic phase was removed under reduced pressure. The crude product was recrystallized with water/methanol (10:1 v/v, 600 ml) to yield pure **2e**; yield 95%, mp 197–198 °C.

4.1.7. General procedure for preparation of 5-R-4-R₁-2-cinnamamidobenzamides 4a–e, 4f[4,5], 4g–k, 5-R-4-R₁-2-(3-phenylpropiolamido)benzamide 4l [8], 4m–q and 5-R-4-R₁-2-(3-phenylpropanamido)benzamide 4r [9] 4s–w—To a cold (0–5 °C) stirred suspension of aminobenzamides **2a–g** (0.016 mol) in pyridine (13 ml), 0.016 mol of the appropriate cinnamoyl chloride **3a–e**, 3-phenylpropioloyl chloride **3f–k** and 3-phenylpropanoyl chloride **3l–o** was added over 30 min. After addition was complete, the solution was stirred for 24 h and then poured onto crushed ice. The precipitate was removed by filtration, washed with water, and crystallized from the appropriate solvent.

4.1.7.1. (E)-2-(3-(3-Bromophenyl)acrylamido)-5-iodobenzamide (4a): Yield 38.0%; mp 252–254 °C (dioxane); I.R. (KBr) cm^{−1} 3347, 3145 (NH, NH₂), 1659, 1620 (2× CO); ¹H NMR (DMSO) δ 6.96 (d, 1H, *J* = 15.9 Hz, olefinic CH); 7.36–8.41 (a set of signals, 9H, aromatic protons, olefinic CH and exchangeable NH₂); 11.76 (s, 1H, NH, exchangeable). Anal. (C₁₆H₁₂BrIN₂O₂) C, H, N.

4.1.7.2. (E)-2-(3-(3-Chlorophenyl)acrylamido)-5-iodobenzamide (4b): Yield 26.4%; mp 254–256 °C (dioxane); I.R. (KBr) cm^{−1} 3354, 3150 (NH, NH₂), 1657, 1618 (2× CO); ¹H NMR (DMSO) δ 6.96 (d, 1H, *J* = 15.6 Hz, olefinic CH); 7.46–8.44 (a set of signals, 10H, aromatic protons, olefinic CH and exchangeable NH₂); 11.82 (s, 1H, NH, exchangeable). Anal. (C₁₆H₁₂ClIN₂O₂) C, H, N.

4.1.7.3. (E)-2-(3-(4-Chlorophenyl)acrylamido)-5-iodobenzamide (4c): Yield 31.6%; mp 250–252 °C (dioxane); I.R. (KBr) cm^{−1} 3377, 3218 (NH, NH₂), 1660, 1616 (2× CO); ¹H NMR (DMSO) δ 6.86 (d, 1H, *J* = 15.9 Hz, olefinic CH); 7.42–8.40 (a set of signals, 10H, aromatic protons, olefinic CH and exchangeable NH₂); 11.78 (s, 1H, NH, exchangeable). Anal. (C₁₆H₁₂ClIN₂O₂) C, H, N.

4.1.7.4. 2-Cinnamamido-5-methoxybenzamide (4d): Yield 38.3%; mp 196–198 °C (ethanol); I.R. (KBr) cm^{-1} 3371, 3188 (NH, NH₂), 1660, 1616 (2× CO); ¹H NMR (CDCl₃) δ 3.85 (s, 3H, OCH₃); 5.68 (broad s, 1H, NH–H, exchangeable); 6.27 (broad s, 1H, NH–H, exchangeable); 6.58 (d, 1H, *J* = 15.6 Hz, olefinic CH); 7.42–8.40 (a set of signals, 9H, aromatic protons, olefinic CH); 11.03 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (CDCl₃) 55.74 (OCH₃), 113.17 (CH_{Ar}), 117.92 (CH_{Ar}), 119.90 (C_{Ar}), 122.06 (C_{Ar}), 123.43 (CH_{Ar}), 128.01 (2× CH_{Ar}), 128.83 (2× CH_{Ar}), 129.82 (CH_{Ar}), 133.42 (C_{Ar}), 134.80 (CH_{Ar}), 141.63 (C_{Ar}), 154.78 (CH_{Ar}), 164.07 (CO), 170.98 (CO). Anal. (C₁₇H₁₆N₂O₃) C, H, N.

4.1.7.5. 2-Cinnamamido-4,5-dimethoxybenzamide (4e): Yield 41.6%; mp 214–216 °C (ethanol); I.R. (KBr) cm^{-1} 3327–3221 (NH, NH₂), 1657, 1610 (2× CO); ¹H NMR (CDCl₃) δ 3.89 (s, 3H, OCH₃); 3.99 (s, 3H, OCH₃); 5.92 (broad s, 2H, NH₂, exchangeable); 6.59 (d, 1H, *J* = 15.6 Hz, olefinic CH); 6.97–8.61 (a set of signals, 8H, aromatic protons, olefinic CH); 11.73 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (CDCl₃) 56.15 (OCH₃), 56.52 (OCH₃), 104.79 (CH_{Ar}), 109.52 (C_{Ar}), 110.00 (CH_{Ar}), 122.12 (CH_{Ar}), 128.07 (2× CH_{Ar}), 128.86 (2× CH_{Ar}), 129.92 (CH_{Ar}), 134.71 (C_{Ar}), 136.77 (C_{Ar}), 141.88 (CH_{Ar}), 144.09 (C_{Ar}), 153.16 (C_{Ar}), 164.54 (CO), 171.15 (CO). Anal. (C₁₈H₁₈N₂O₄) C, H, N.

4.1.7.6. (E)-5-Chloro-2-(3-(2-methoxyphenyl)acrylamido)benzamide (4g): Yield 36%; mp 235–240 °C (ethanol); I.R. (KBr) cm^{-1} 3365, 3160 (NH, NH₂), 1689, 1654 (2× CO); ¹H NMR (DMSO) δ 3.89 (s, 3H, OCH₃); 6.80 (d, 1H, *J* = 15.6 Hz, olefinic CH); 6.98–8.64 (a set of signals, 10H, aromatic protons, olefinic CH and exchangeable NH₂); 11.85 (s, 1H, NH, exchangeable). Anal. (C₁₇H₁₅ClN₂O₃) C, H, N.

4.1.7.7. (E)-2-(3-(2-Methoxyphenyl)acrylamido)-5-methylbenzamide (4h): Yield 36%; mp 216–220 °C (ethanol); I.R. (KBr) cm^{-1} 3384, 3166 (NH, NH₂), 1652, 1621 (2× CO); ¹H NMR (DMSO) δ 2.32 (s, 3H, CH₃); 3.89 (s, 3H, OCH₃); 6.74 (d, 1H, *J* = 16.0 Hz, olefinic CH); 6.79–8.50 (a set of signals, 10H, aromatic protons, olefinic CH and exchangeable NH₂); 11.81 (s, 1H, NH, exchangeable). Anal. (C₁₈H₁₈N₂O₃) C, H, N.

4.1.7.8. (E)-5-Iodo-2-(3-(2-methoxyphenyl)acrylamido)benzamide (4i): Yield 32%; mp 240–242 °C (ethanol); I.R. (KBr) cm^{-1} 3390, 3214 (NH, NH₂), 1662, 1613 (2× CO); ¹H NMR (DMSO) δ 3.88 (s, 3H, OCH₃); 6.77 (d, 1H, *J* = 15.6 Hz, olefinic CH); 6.97–8.41 (a set of signals, 10H, aromatic protons, olefinic CH and exchangeable NH₂); 11.79 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 55.64 (OCH₃), 86.14 (C_{Ar}), 111.71 (CH_{Ar}), 120.70 (CH_{Ar}), 110.00 (CH_{Ar}), 122.10 (C_{Ar}), 122.40 (CH_{Ar}), 122.58 (CH_{Ar}), 122.65 (C_{Ar}), 128.40 (CH_{Ar}), 131.53 (CH_{Ar}), 136.04 (CH_{Ar}), 136.63 (CH_{Ar}), 139.28 (C_{Ar}), 140.49 (CH_{Ar}), 157.72 (C_{Ar}), 163.91 (CO), 169.28 (CO). Anal. (C₁₇H₁₅IN₂O₃) C, H, N.

4.1.7.9. (E)-5-Methoxy-2-(3-(2-methoxyphenyl)acrylamido)benzamide (4j): Yield 36%; mp 158–161 °C (ethanol); I.R. (KBr) cm^{-1} 3382, 3234 (NH, NH₂), 1662, 1619 (2× CO); ¹H NMR (DMSO) δ 3.81 (s, 3H, OCH₃); 3.89 (s, 3H, OCH₃); 6.82 (d, 1H, *J* = 15.9 Hz, olefinic CH); 6.98–8.50 (a set of signals, 10H, aromatic protons, olefinic CH and exchangeable NH₂); 11.57 (s, 1H, NH, exchangeable). Anal. (C₁₈H₁₈N₂O₄) C, H, N.

4.1.7.10. (E)-4,5-Dimethoxy-2-(3-(2-methoxyphenyl)acrylamido) benzamide (4k): Yield 36%; mp 240–243 °C (ethanol); I.R. (KBr) cm^{-1} 3333, 3202 (NH, NH₂), 1654, 1607 (2× CO); ¹H NMR (DMSO) δ 3.83 (s, 3H, OCH₃); 3.85 (s, 3H, OCH₃); 3.90 (s, 3H, OCH₃); 6.75 (d, 1H, *J* = 15.6 Hz, olefinic CH); 6.98–8.47 (a set of signals, 9H, aromatic protons, olefinic CH and exchangeable NH₂); 12.40 (s, 1H, NH, exchangeable). Anal. (C₁₉H₂₀N₂O₅) C, H, N.

4.1.7.11. 5-Chloro-2-(3-phenylpropiolamido)benzamide (4m): Yield 95%; mp 239–240 °C (ethanol–water); I.R. (KBr) cm^{-1} 3390, 3215 (NH, NH₂), 2208 (C≡C), 1666, 1649 (2 × CO); ¹H NMR (DMSO) δ 7.47–8.52 (a set of signals, 8H, aromatic protons); 8.55 (s, 2H, NH₂, exchangeable); 12.29 (s, 1H, NH, exchangeable). Anal. (C₁₆H₁₁ClN₂O₂) C, H, N.

4.1.7.12. 5-Bromo-2-(3-phenylpropiolamido)benzamide (4n): Yield 95%; mp 239–240 °C (ethanol–dioxane); I.R. (KBr) cm^{-1} 3392, 3217 (NH, NH₂), 2209 (C≡C), 1664, 1651 (2 × CO); ¹H NMR (DMSO) δ 7.45–8.43 (a set of signals, 8H, aromatic protons); 8.43 (s, 2H, NH₂, exchangeable); 12.26 (s, 1H, NH, exchangeable). Anal. (C₁₆H₁₁BrN₂O₂) C, H, N.

4.1.7.13. 5-Methyl-2-(3-phenylpropiolamido)benzamide (4o): Yield 95%; mp 239–240 °C (ethanol–dioxane); I.R. (KBr) cm^{-1} 3393, 3213 (NH, NH₂), 2207 (C≡C), 1666, 1647 (2 × CO); ¹H NMR (DMSO) δ 2.32 (s, 3H, CH₃); 7.35–8.27 (a set of signals, 8H, aromatic protons); 8.30 (s, 2H, NH₂, exchangeable); 12.26 (s, 1H, NH, exchangeable). Anal. (C₁₇H₁₄N₂O₂) C, H, N.

4.1.7.14. 5-Iodo-2-(3-phenylpropiolamido)benzamide (4p): Yield 84.0%; mp 235–237 °C (ethanol); I.R. (KBr) cm^{-1} 3402, 3211 (NH, NH₂), 2207 (C≡C), 1666, 1647 (2 × CO); ¹H NMR(DMSO) δ 7.50–8.16 (a set of signals, 8H, aromatic protons); 8.43 (s, 2H, NH₂, exchangeable); 12.26 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 83.99 (CH_{Ar}), 84.98 (C_{Ar}), 87.79 (CH_{Ar}), 119.15 (C_{Ar}), 122.12 (C_{Ar}), 123.00 (CH_{Ar}), 129.23 (2 × CH_{Ar}), 131.01 (CH_{Ar}), 132.58 (2 × CH_{Ar}), 136.97 (CH_{Ar}), 138.45 (C_{Ar}), 140.99 (CH_{Ar}), 150.12 (CO), 169.27 (CO). Anal. (C₁₆H₁₁IN₂O₂) C, H, N.

4.1.7.15. 4,5-Dimethoxy-2-(3-phenylpropiolamido)benzamide (4q): Yield 87.4%; mp 190–193 °C (ethanol); I.R. (KBr) cm^{-1} 3359, 3164 (NH, NH₂), 2208 (C≡C), 1677, 1618 (2 × CO); ¹H NMR (DMSO) δ 3.81 (s, 6H, 2 × OCH₃); 7.40–8.36 (a set of signals, 9H, aromatic protons, exchangeable NH₂); 12.86 (s, 1H, NH, exchangeable). Anal. (C₁₈H₁₆N₂O₄) C, H, N.

4.1.7.16. 5-Chloro-2-(3-phenylpropanamido)benzamide (4s): Yield 76.6%; mp 165–166 °C (ethanol); I.R. (KBr) cm^{-1} 3347, 3173 (NH, NH₂), 1683, 1618 (2 × CO); ¹H NMR (CDCl₃) δ 2.72 (t, 2H, CH₂); 3.05 (t, 2H, CH₂); 5.69 (broad s, 1H, NH–H, exchangeable); 6.15 (broad s, 1H, NH–H, exchangeable); 6.19–8.64 (a set of signals, 8H, aromatic protons); 11.00 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (CDCl₃) 31.29 (CH₂), 39.92 (CH₂), 119.81 (C_{Ar}), 123.04 (CH_{Ar}), 126.25 (CH_{Ar}), 126.99 (CH_{Ar}), 127.56 (C_{Ar}), 128.37 (2 × CH_{Ar}), 128.53 (2 × CH_{Ar}), 133.15 (CH_{Ar}), 138.67 (C_{Ar}), 140.52 (C_{Ar}), 169.81 (CO), 171.09 (CO). Anal. (C₁₆H₁₅ClN₂O₂) C, H, N.

4.1.7.17. 5-Methyl-2-(3-phenylpropanamido)benzamide (4t): Yield 58.8%; mp 155–158 °C (ethanol); I.R. (KBr) cm^{-1} 3294, 3162 (NH, NH₂), 1667, 1630 (2 × CO); ¹H NMR (CDCl₃) δ 2.33 (s, 3H, CH₃); 2.71 (t, 2H, CH₂); 3.06 (t, 2H, CH₂); 5.59 (broad s, 1H, NH–H, exchangeable); 6.13 (broad s, 1H, NH–H, exchangeable); 7.20–8.54 (a set of signals, 8H, aromatic protons); 11.01 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (CDCl₃) 20.78 (CH₃), 31.41 (CH₂), 39.95 (CH₂), 118.48 (C_{Ar}), 121.61 (CH_{Ar}), 126.15 (CH_{Ar}), 127.45 (CH_{Ar}), 128.38 (2 × CH_{Ar}), 128.48 (2 × CH_{Ar}), 132.17 (C_{Ar}), 134.03 (CH_{Ar}), 137.66 (C_{Ar}), 140.73 (C_{Ar}), 170.91 (CO), 171.21 (CO). Anal. (C₁₇H₁₈N₂O₂) C, H, N.

4.1.7.18. 5-Iodo-2-(3-phenylpropanamido)benzamide (4u): Yield 67.4%; mp 183–185 °C (ethanol); I.R. (KBr) cm^{-1} 3388, 3267, 3188 (NH, NH₂), 1654, 1610 (2 × CO); ¹H NMR(CDCl₃) δ 2.71 (t, 2H, CH₂); 3.04 (t, 2H, CH₂); 5.83 (broad s, 1H, NH–H, exchangeable); 6.31 (broad s, 1H, NH–H, exchangeable); 7.19–8.43 (a set of signals, 8H,

aromatic protons); 11.03 (s, 1H, NH, exchangeable). ^{13}C NMR (δ) (CDCl_3) 31.24 (CH_2), 39.97 (CH_2), 85.00 (C_{Ar}), 120.54 (C_{Ar}), 123.43 (CH_{Ar}), 126.24 (CH_{Ar}), 128.36 ($2\times \text{CH}_{\text{Ar}}$), 128.53 ($2\times \text{CH}_{\text{Ar}}$), 135.76 (CH_{Ar}), 139.70 (C_{Ar}), 140.48 (C_{Ar}), 141.89 (CH_{Ar}), 169.62 (CO), 171.13 (CO). Anal. ($\text{C}_{16}\text{H}_{15}\text{IN}_2\text{O}_2$) C, H, N.

4.1.7.19. 5-Methoxy-2-(3-phenylpropanamido)benzamide (4v): Yield 72.1%; mp 156–158 °C (ethanol); I.R. (KBr) cm^{-1} 3382, 3280, 3187 (NH, NH_2), 1652, 1608 ($2\times \text{CO}$); ^1H NMR(CDCl_3) δ 2.75 (t, 2H, CH_2); 3.11 (t, 2H, CH_2); 3.85 (s, 3H, OCH_3); 5.98 (broad s, 1H, NH–H, exchangeable); 6.54 (broad s, 1H, NH–H, exchangeable); 7.06–8.52 (a set of signals, 8H, aromatic protons); 10.76 (s, 1H, NH, exchangeable). ^{13}C NMR (δ) (CDCl_3) 31.43 (CH_2), 39.72 (CH_2), 55.64 (OCH_3), 113.15 (CH_{Ar}), 117.58 (CH_{Ar}), 120.72 (C_{Ar}), 123.31 (CH_{Ar}), 126.19 (CH_{Ar}), 128.35 ($2\times \text{CH}_{\text{Ar}}$), 128.50 ($2\times \text{CH}_{\text{Ar}}$), 132.75 (C_{Ar}), 140.66 (C_{Ar}), 154.69 (C_{Ar}), 170.86 (CO), 171.02 (CO). Anal. ($\text{C}_{17}\text{H}_{18}\text{N}_2\text{O}_3$) C, H, N.

4.1.7.20. 4,5-Dimethoxy-2-(3-phenylpropanamido)benzamide (4w): Yield 44.3%; mp 170–172 °C (ethanol); I.R. (KBr) cm^{-1} 3381, 3280, 3230 (NH, NH_2), 1660, 1621 ($2\times \text{CO}$); ^1H NMR (CDCl_3) δ 2.73 (t, 2H, CH_2); 3.06 (t, 2H, CH_2); 3.87 (s, 3H, OCH_3); 3.96 (s, 3H, OCH_3); 5.84 (broad s, 2H, NH_2 , exchangeable); 6.92–8.44 (a set of signals, 7H, aromatic protons); 11.43 (s, 1H, NH, exchangeable). ^{13}C NMR (δ) (CDCl_3) 31.34 (CH_2), 40.02 (CH_2), 56.13 (OCH_3), 56.50 (OCH_3), 104.16 (CH_{Ar}), 109.50 (C_{Ar}), 109.98 (CH_{Ar}), 126.21 (CH_{Ar}), 128.34 ($2\times \text{CH}_{\text{Ar}}$), 128.53 ($2\times \text{CH}_{\text{Ar}}$), 136.38 (C_{Ar}), 140.65 (CH_{Ar}), 143.93 (C_{Ar}), 153.09 (C_{Ar}), 171.06 (CO), 171.17 (CO). Anal. ($\text{C}_{18}\text{H}_{20}\text{N}_2\text{O}_4$) C, H, N.

4.2. Biology

4.2.1. Cell lines and cell cultures—Human chronic myelogenous leukemia cell line K562 was maintained in suspension culture in RPMI 1640 medium (SIGMA–Aldrich, cat.#R8758) supplemented with 10% fetal bovine serum (FBS: SIGMA–Aldrich, cat.#F6178), 100 U/ml penicillin, 100 ($\mu\text{g}/\text{ml}$) streptomycin and 0.25 ($\mu\text{g}/\text{ml}$) amphotericin B (complete medium) at 37 °C in a 5% CO_2 humidified atmosphere.

The hepatocarcinoma HEPG2 cell line was incubated for 72 h with 10^5 cells/well in 24-well flasks with medium VITO 143, with the addition of ITG (insulin, transferrin, and glucagon), phenol red, and penicillin and streptomycin, as above. After 72 h, the cells were harvested with EDTA, washed 2 times with PBS for 5 min with centrifugation at $200\times g$ and resuspended in 100 μl of PBS for flow cytometric analysis.

4.2.2. Trypan blue exclusion assay—The trypan blue exclusion assay was performed to assess the effect of all test compounds on the viability of K562 cells, as described earlier [14]. Briefly, about 2×10^5 cells/ml were cultured for 24 h, and compounds were added at 10 μM (or at different concentrations to determine the IC_{50}). The effects of test compounds on cell viability were assayed on a portion of the cell suspension after 24 h. The cell number was determined with a hemocytometer, and viability was estimated by trypan blue dye exclusion.

4.2.3. MTT assay—MTT (3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed as described earlier [15,16] and was used to determine viability of NCI-H460, HT29, HCT116, MCF-7 and HuDe cells.

MTT is a yellow-colored tetrazolium salt that is taken up and cleaved only by metabolically active cells, which reduce it to a colored, water-insoluble formazan salt. The solubilized formazan product can be quantified via absorbance at 570 nm (690 nm for blank), which is

measured using a 96-well-format spectrophotometer (ELx800-BioTek instruments). The absorbance correlates directly with cell number.

Briefly, about 2×10^5 cells/ml (2×10^4 cells/ml for HuDe) were cultured; different concentrations of test drugs or 0.1% DMSO were added to the wells 24 h after seeding. Cells were collected after 72 h and evaluated by the MTT assay. Cells were incubated with 10 μ l of MTT (5 mg/ml in PBS) at 37 °C for 3 h. The tetrazolium crystals were solubilized by the addition of 4.5 ml isopropyl alcohol 0.5 ml triton X-100 in 150 μ l of 37% HCl. Cells grown in culture media alone or with appropriate concentrations of DMSO were used as controls.

4.2.4. Flow cytometry (FACS)—To analyze the proportion of cells in different phases of the cell cycle and the percentage of apoptotic cells, K562 and HepG2 cells were analyzed, respectively, by flow cytometry. The cells were analyzed after growth for 72 h in the compound being examined. In the apoptotic study, one million cells were treated with fluorescein isothiocyanate (FITC)-conjugated antibody for annexin V and propidium iodide (PI) for staining of necrotic cells. Side scatter and forward scatter were set to include all viable cells. The debris was excluded from the population of cells by applying a gate in the FSC (forward scatter) versus SSC (side scatter). The data were acquired on a Becton–Dickinson FACS Air II instrument and analyzed by FACSDiva software 6.1.2 (BD Biosciences, Franklin Lakes, NJ). We used manual compensation with the instrument to exclude the overlap of the two emission spectra.

4.2.5. Determination of mitotic index—Human Burkitt lymphoma CA46 cells were purchased from the ATCC (Manassas, VA, USA) and grown in RPMI 1640 medium with glutamine (Gibco, Grand Island, NY, USA) supplemented with 20% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA, USA). For mitotic index determinations (that is, percent of cells with condensed chromosomes), 10^6 cells/ml were grown for 16 h at 37 °C in a humidified 5% CO₂ atmosphere. Cells were harvested by centrifugation for 5 min at 1000 rpm in a Beckman Allegra 6KR centrifuge equipped with a GH-3.8A swinging bucket rotor. The supernatant was decanted, and the cells were washed once in 5 ml of phosphate-buffered saline (pH 7.4). After centrifugation again, the cells were resuspended in 0.5 ml of 0.034 M sodium–potassium phosphate (pH 7.2) and left for 10 min at room temperature. After this hypotonic swelling, the cells were recentrifuged and suspended and fixed in 1.5% ethanol–0.5% acetic acid. After 30 min, the cells were again harvested by centrifugation, and the final cell pellet was suspended in 75% ethanol–25% acetic acid. The cell suspension was smeared onto a microscope slide, which was air dried. The cells were stained with Giemsa, and the percentage of cells with condensed chromosomes was determined by visual examination of the slide with a Nikon TMS light microscope.

4.2.6. Evaluation of tubulin assembly—Purified bovine brain tubulin [17] was used in turbidimetric assembly studies. Tubulin and the desired concentration of compound were preincubated at 30 °C for 15 min in a 0.24 ml reaction volume. Reaction mixtures were then chilled on ice, and 10 μ l of 10 mM GTP was added. All concentrations refer to the final reaction volume of 0.25 ml: tubulin at 1.0 mg/ml (10 μ M), 0.8 M monosodium glutamate (pH 6.6 in a 2 M stock solution with HCl), 4% (v/v) dimethyl sulfoxide, and 0.4 mM GTP. The chilled reaction mixtures were transferred to cuvettes held to 0 °C in recording spectrophotometers (Beckman models DU7400 and DU7500) equipped with electronic temperature controllers. After baselines were established at 350 nm, the temperature was jumped to 30 °C in about 30 s, and the IC₅₀ is defined as the concentration of compound that inhibits turbidity development by 50% at 30 min. Detailed method description has been published previously [18]. The method is generally most reliable at compound

concentrations up to 20 μ M. At higher concentrations, compound precipitation and/or absorbance often leads to interference with the simple turbidity readings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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1a	R=CH ₃ , R ₁ =H, R ₂ =H, R ₃ =H	1l	R=Br, R ₁ =Cl, R ₂ =H, R ₃ =H
1b	R=Cl, R ₁ =H, R ₂ =H, R ₃ =H	1m	R=Br, R ₁ =H, R ₂ =Cl, R ₃ =H
1c	R=Br, R ₁ =H, R ₂ =H, R ₃ =H	1n	R=Br, R ₁ =H, R ₂ =H, R ₃ =Cl
1d	R=Cl, R ₁ =Cl, R ₂ =H, R ₃ =H	1o	R=Br, R ₁ =Br, R ₂ =H, R ₃ =H
1e	R=Cl, R ₁ =H, R ₂ =Cl, R ₃ =H	1p	R=Br, R ₁ =H, R ₂ =Br, R ₃ =H
1f	R=Cl, R ₁ =H, R ₂ =H, R ₃ =Cl	1q	R=Br, R ₁ =CH ₃ , R ₂ =H, R ₃ =H
1g	R=Cl, R ₁ =Br, R ₂ =H, R ₃ =H	1r	R=Br, R ₁ =H, R ₂ =CH ₃ , R ₃ =H
1h	R=Cl, R ₁ =H, R ₂ =Br, R ₃ =H	1s	R=Br, R ₁ =H, R ₂ =H, R ₃ =CH ₃
1i	R=Cl, R ₁ =CH ₃ , R ₂ =H, R ₃ =H	1t	R=I, R ₁ =H, R ₂ =H, R ₃ =H
1j	R=Cl, R ₁ =H, R ₂ =CH ₃ , R ₃ =H	1u	R=I, R ₁ =Cl, R ₂ =H, R ₃ =H
1k	R=Cl, R ₁ =H, R ₂ =H, R ₃ =CH ₃	1v	R=I, R ₁ =H, R ₂ =H, R ₃ =CH ₃

Fig. 1.
Structure of cinnamoyl anthranilates.

Fig. 2.
Substitution pattern of compounds 4.

2a	$\text{R}=\text{H}, \text{R}_1=\text{H}$	4f	$\text{R}=\text{H}, \text{R}_1=\text{H}, \text{R}_2=2\text{-OCH}_3, \text{X-Y}=\text{vinylene}$
2b	$\text{R}=\text{Cl}, \text{R}_1=\text{H}$	4g	$\text{R}=\text{Cl}, \text{R}_1=\text{H}, \text{R}_2=2\text{-OCH}_3, \text{X-Y}=\text{vinylene}$
2c	$\text{R}=\text{Br}, \text{R}_1=\text{H}$	4h	$\text{R}=\text{CH}_3, \text{R}_1=\text{H}, \text{R}_2=2\text{-OCH}_3, \text{X-Y}=\text{vinylene}$
2d	$\text{R}=\text{CH}_3, \text{R}_1=\text{H}$	4i	$\text{R}=\text{I}, \text{R}_1=\text{H}, \text{R}_2=2\text{-OCH}_3, \text{X-Y}=\text{vinylene}$
2e	$\text{R}=\text{I}, \text{R}_1=\text{H}, \text{R}_2=\text{H}$	4j	$\text{R}=\text{OCH}_3, \text{R}_1=\text{H}, \text{R}_2=2\text{-OCH}_3, \text{X-Y}=\text{vinylene}$
2f	$\text{R}=\text{OCH}_3, \text{R}_1=\text{H}$	4k	$\text{R}=\text{OCH}_3, \text{R}_1=\text{OCH}_3, \text{R}_2=2\text{-OCH}_3, \text{X-Y}=\text{vinylene}$
2g	$\text{R}=\text{OCH}_3, \text{R}_1=\text{OCH}_3$	4l	$\text{R}=\text{H}, \text{R}_1=\text{H}, \text{R}_2=\text{H}, \text{X-Y}=\text{ethynylene}$
3a	$\text{R}_2=\text{H}, \text{X-Y}=\text{vinylene}$	4m	$\text{R}=\text{Cl}, \text{R}_1=\text{H}, \text{R}_2=\text{H}, \text{X-Y}=\text{ethynylene}$
3b	$\text{R}_2=3\text{-Br}, \text{X-Y}=\text{vinylene}$	4n	$\text{R}=\text{Br}, \text{R}_1=\text{H}, \text{R}_2=\text{H}, \text{X-Y}=\text{ethynylene}$
3c	$\text{R}_2=3\text{-Cl}, \text{X-Y}=\text{vinylene}$	4o	$\text{R}=\text{CH}_3, \text{R}_1=\text{H}, \text{R}_2=\text{H}, \text{X-Y}=\text{ethynylene}$
3d	$\text{R}_2=4\text{-Cl}, \text{X-Y}=\text{vinylene}$	4p	$\text{R}=\text{I}, \text{R}_1=\text{H}, \text{R}_2=\text{H}, \text{X-Y}=\text{ethynylene}$
3f	$\text{R}_2=2\text{-OCH}_3, \text{X-Y}=\text{vinylene}$	4q	$\text{R}=\text{OCH}_3, \text{R}_1=\text{OCH}_3, \text{R}_2=\text{H}, \text{X-Y}=\text{ethynylene}$
3g	$\text{R}_2=\text{H}, \text{X-Y}=\text{ethynylene}$	4r	$\text{R}=\text{H}, \text{R}_1=\text{H}, \text{R}_2=\text{H}, \text{X-Y}=\text{ethylene}$
3h	$\text{R}_2=\text{H}, \text{X-Y}=\text{ethylene}$	4s	$\text{R}=\text{Cl}, \text{R}_1=\text{H}, \text{R}_2=\text{H}, \text{X-Y}=\text{ethylene}$
4a	$\text{R}=\text{I}, \text{R}_1=\text{H}, \text{R}_2=3\text{-Br}, \text{X-Y}=\text{vinylene}$	4t	$\text{R}=\text{CH}_3, \text{R}_1=\text{H}, \text{R}_2=\text{H}, \text{X-Y}=\text{ethylene}$
4b	$\text{R}=\text{I}, \text{R}_1=\text{H}, \text{R}_2=3\text{-Cl}, \text{X-Y}=\text{vinylene}$	4u	$\text{R}=\text{I}, \text{R}_1=\text{H}, \text{R}_2=\text{H}, \text{X-Y}=\text{ethylene}$
4c	$\text{R}=\text{I}, \text{R}_1=\text{H}, \text{R}_2=4\text{-Cl}, \text{X-Y}=\text{vinylene}$	4v	$\text{R}=\text{OCH}_3, \text{R}_1=\text{H}, \text{R}_2=\text{H}, \text{X-Y}=\text{ethylene}$
4d	$\text{R}=\text{OCH}_3, \text{R}_1=\text{H}, \text{R}_2=\text{H}, \text{X-Y}=\text{vinylene}$	4w	$\text{R}=\text{OCH}_3, \text{R}_1=\text{OCH}_3, \text{R}_2=\text{H}, \text{X-Y}=\text{ethylene}$
4e	$\text{R}=\text{OCH}_3, \text{R}_1=\text{OCH}_3, \text{R}_2=\text{H}, \text{X-Y}=\text{vinylene}$		

Scheme 1.

Synthetic pathway for the formation of benzamido derivatives **4**.

Scheme 2. Synthetic pathway for the formation of the 2-aminobenzamides **2**.

Table 1

Percent growth inhibition of compounds **4a–w** at 10 μ M.

Comp.	K562	NCI H460	MCF-7	HT29	HCT116
4a	11.1 $\pm$ 1.2	ns	ns	ns	17.1 $\pm$ 1.1
4b	26.6 $\pm$ 3.5	36.6 $\pm$ 0.6	ns	42.9 $\pm$ 3.7	15.3 $\pm$ 1.6
4c	19.4 $\pm$ 1.9	20.6 $\pm$ 0.3	24.0 $\pm$ 1.7	14.6 $\pm$ 1.9	ns
4d	79.0 $\pm$ 2.5	40.6 $\pm$ 1.2	ns	45.7 $\pm$ 2.9	15.0 $\pm$ 0.9
4e	40.7 $\pm$ 4.3	ns	ns	17.7 $\pm$ 2.0	11.0 $\pm$ 1.0
4f	40.0 $\pm$ 3.9	21.3 $\pm$ 1.9	ns	14.9 $\pm$ 2.0	ns
4g	80.0 $\pm$ 0.9	29.8 $\pm$ 0.8	ns	26.3 $\pm$ 1.2	17.2 $\pm$ 0.7
4h	68.0 $\pm$ 1.4	12.6 $\pm$ 1.4	13.7 $\pm$ 1.0	17.5 $\pm$ 1.7	ns
4i	56.0 $\pm$ 0.6	19.3 $\pm$ 2.0	ns	13.5 $\pm$ 1.9	18.1 $\pm$ 2.1
4j	68.0 $\pm$ 2.3	31.4 $\pm$ 3.1	ns	35.1 $\pm$ 0.4	34.5 $\pm$ 2.3
4k	ns	ns	ns	20.2 $\pm$ 1.8	13.9 $\pm$ 1.8
4l	80.0 $\pm$ 0.2	39.7 $\pm$ 4.3	45.2 $\pm$ 4.1	85.0 $\pm$ 0.3	49.9 $\pm$ 0.6
4m	27.5 $\pm$ 1.2	53.6 $\pm$ 3.4	21.1 $\pm$ 1.9	30.7 $\pm$ 1.0	49.9 $\pm$ 2.4
4n	15.0 $\pm$ 1.9	23.6 $\pm$ 3.0	nt	24.2 $\pm$ 1.1	nt
4o	36.2 $\pm$ 2.2	11.9 $\pm$ 0.5	nt	ns	nt
4p	65.0 $\pm$ 0.7	43.0 $\pm$ 0.4	ns	30.3 $\pm$ 0.6	39.0 $\pm$ 1.6
4q	61.1 $\pm$ 2.1	ns	ns	16.5 $\pm$ 0.8	21.6 $\pm$ 1.1
4r	ns	ns	ns	16.0 $\pm$ 1.2	13.2 $\pm$ 0.6
4s	66.7 $\pm$ 2.2	15.6 $\pm$ 1.0	ns	29.5 $\pm$ 3.7	8.6 $\pm$ 0.4
4t	25.7 $\pm$ 1.9	ns	13.2 $\pm$ 0.5	24.5 $\pm$ 1.1	ns
4u	37.8 $\pm$ 1.6	24.1 $\pm$ 1.9	ns	36.2 $\pm$ 1.7	19.0 $\pm$ 0.2
4v	59.7 $\pm$ 5.4	ns	18.9 $\pm$ 2.1	34.4 $\pm$ 0.8	ns
4w	40.6 $\pm$ 5.6	ns	ns	46.8 $\pm$ 1.0	ns
Colchicine	63.6 $\pm$ 0.7	60.1 $\pm$ 2.8	58.7 $\pm$ 1.3	72.3 $\pm$ 2.2	

Values are the mean of at least three independent determinations. ns = not significant (% inhibition < 10%). nt = not tested.

Table 2

IC₅₀ values (μM) for compounds that exhibited at least 50% of growth inhibition in the 10 μM screening assay.

Comp.	K562	HEPG2	NCI H460	HT29
4d	4.4 ± 0.01	>10	>10	>10
4g	3.7 ± 0.003	>10	>10	>10
4h	5.7 ± 0.04	>10	>10	>10
4i	7.9 ± 0.02	>10	>10	>10
4j	7.9 ± 0.009	>10	>10	>10
4l	0.4 ± 0.001	5.9 ± 0.2	>10	1.4 ± 0.03
4m	>10	>10	9.7	>10
4p	0.43 ± 0.009	>10	>10	>10
4q	3.2 ± 0.006	>10	>10	>10
4s	5.6 ± 0.1	>10	>10	>10
4v	8.0 ± 0.07	>10	>10	>10
Colchicine	0.02 ± 0.003	>10	0.71 ± 0.02	0.07 ± 0.001

Values are the mean of at least three independent determination.

Table 3Comparison of IC₅₀ values (μM) in HuDe and K562 cells.

Comp.	HuDe	K562	HuDe/K562
4d	145.1 ± 0.01	4.4 ± 0.01	32.9
4g	191.3 ± 0.9	3.7 ± 0.003	51.7
4h	260.5 ± 2.1	5.7 ± 0.04	45.7
4i	57.5 ± 1.7	7.9 ± 0.02	7.3
4j	51.5 ± 0.4	7.9 ± 0.009	6.52
4l	191.2 ± 0.09	0.4 ± 0.001	478
4p	54.6 ± 0.4	0.43 ± 0.009	127
4q	71.7 ± 2.5	3.2 ± 0.006	22.4
4s	184.4 ± 3.1	5.6 ± 0.1	32.9
4v	52.4 ± 0.01	8.0 ± 0.07	6.55
Colchicine	0.017 ± 0.001	0.02 ± 0.003	0.85

Values are the mean of at least three independent determination.

Table 4

Effects of compounds **4d**, **4l**, **4s** on DNA content/cell following the treatment of K562 cells for 24 h.

	SUB G1 ± SE	G0/G1 ± SE	S ± SE	G2M ± SE
No drug	7.1 ± 1.1	40 ± 1.2	24 ± 0.8	28.9 ± 1.3
4d	3.0 ± 1.7	29.2 ± 2.2	27.2 ± 1.6	40.6 ± 2.5
4l	12.0 ± 4.2	28.8 ± 1.6	23.2 ± 1.7	36.0 ± 3.7
4s	4.0 ± 1.1	27.3 ± 2.1	27.7 ± 0.7	40.9 ± 1.5
Colchicine	5.6 ± 0.4	14.3 ± 0.8	16.8 ± 0.1	63.3 ± 0.5

Table 5Apoptotic activity on HepG2 cell line of compounds **4a–ei, l–w**.

	Annexin V (% apoptotic cells $\pm$ SE)	PI (% dead cells $\pm$ SE)	Apoptosis/dead
No drug	13.9 $\pm$ 1.2	0.7 $\pm$ 0.2	
4a	15.9 $\pm$ 2.8	31.5 $\pm$ 6.3	0.50
4b	11.6 $\pm$ 2.7	25.0 $\pm$ 6.3	0.46
4c	12.2 $\pm$ 3.0	27.9 $\pm$ 7.4	0.44
4d	18.1 $\pm$ 1.7	47.3 $\pm$ 3.3	0.38
4e	16.8 $\pm$ 0.9	38.7 $\pm$ 2	0.43
4i	50.9 $\pm$ 2.2	28.2 $\pm$ 4.1	1.80
4l	28.0 $\pm$ 2.9	62.8 $\pm$ 7.3	0.45
4m	19.1 $\pm$ 0.8	56.6 $\pm$ 2.0	0.34
4n	20.5 $\pm$ 0.2	45.2 $\pm$ 1.8	0.45
4o	11.1 $\pm$ 0.3	34.3 $\pm$ 1.0	0.32
4p	41.7 $\pm$ 0.8	57.0 $\pm$ 2.4	0.73
4q	67.9 $\pm$ 0.1	31.2 $\pm$ 0.4	2.18
4r	14.1 $\pm$ 1.3	39.7 $\pm$ 11.6	0.36
4s	26.0 $\pm$ 1.2	32.2 $\pm$ 3.2	0.81
4t	34.9 $\pm$ 1.1	49.4 $\pm$ 3.0	0.71
4u	15.9 $\pm$ 0.5	27.1 $\pm$ 1.3	0.59
4v	40.8 $\pm$ 3.3	46.2 $\pm$ 2.7	0.88
4w	17.8 $\pm$ 1.7	39.5 $\pm$ 2.5	0.45
Colchicine	40.5 $\pm$ 2.8	41.8 $\pm$ 1.9	0.97