Published in final edited form as:

Bioorg Med Chem. 2015 October 1; 23(19): 6305–6316. doi:10.1016/j.bmc.2015.08.027.

Synthesis, antiproliferative activity and possible mechanism of action of novel 2-acetamidobenzamides bearing the 2-phenoxy functionality

Demetrio Raffa^{a,*}, Benedetta Maggio^{a,*}, Fabiana Plescia^a, Stella Cascioferro^a, Maria Valeria Raimondi^a, Gabriella Cancemi^a, Antonella D'Anne^b, Marianna Lauricella^c, Maria Grazia Cusimano^a, Ruoli Bai^d, Ernest Hamel^d, and Giuseppe Daidone^a

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

^b Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, Laboratory of Biochemistry, University of Palermo, 90123 Palermo, Italy

^c Department of Experimental Biomedicine and Clinical Neurosciences, Laboratory of Biochemistry, University of Palermo, 90123 Palermo, Italy

^d Screening 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 2-(2-phenoxyacetamido)benzamides **17a–v**, **21** and **22** were synthesized by stirring in pyridine the acid chlorides **16a–e** and the appropriate 5-*R*-4-*R*₁-2-aminobenzamide **15a–e** and initially evaluated in vitro for antiproliferative activity against the K562 (human chronic myelogenous leukemia) cell line. Some of synthesized compounds were evaluated for their in vitro antiproliferative activity against the full NCI tumor cell line panel derived from nine clinically isolated cancer types (leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast). The most active compounds caused an arrest of K562 cells in the G0–G1 phase of cell cycle and induction of apoptosis, which was mediated by caspase activation.

Keywords

2-(2-Phenoxyacetamido)benzamides; Antiproliferative activity; G0/G1 arrest; Apoptosis; Pro-caspase 3

* Corresponding authors. Tel.: +39 91 23891917 (D.R.), +39 91 23891949 (B.M.). demetrio.raffa@unipa.it (D. Raffa), benedetta.maggio@unipa.it (B. Maggio)..

Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.bmc.2015.08.027>.

1. Introduction

Acetamidobenzamides represent a class of biologically active substances of great importance in medicinal chemistry. Despite their wide range of biological activities,^{1–6} a review of the literature revealed that no anticancer activity had been described for these compounds. This omission led us to explore the potential of this class of compounds as anticancer agents by synthesizing and screening for antileukemic activity a series of novel 2-cinnamamido, 2-(3-phenylpropiolamido) and 2-(3-phenylpropanamido)benzamides.^{7,8} Among the synthesized compounds, **1–5** showed the best antiproliferative activity (Fig. 1).

Several examples of active compounds containing the phenoxyacetamido scaffold in their structure include the kinase inhibitor **6**,⁹ the carboxypeptidase R39 inhibitor **7**,¹⁰ the HIF- α prolyl hydroxylase inhibitor **8**,¹¹ the FLT3 inhibitor **9**¹² and the HIV-protease inhibitor **10**¹³ (Fig. 2).

Thus, following our studies in the new anticancer agents,^{14–17} and on the basis of our previous work on acetamidobenzamides,^{7,8} as well as of data reported for some active compounds containing the phenoxyacetamido scaffold,^{9–13} we decided to investigate the influence of the cinnamamido and 3-phenylpropiolamido scaffolds when substituted for the phenoxyacetamido scaffold. Moreover, the displacement of the 2-phenoxyacetamido moiety from the *ortho* to the *para* and *meta* positions was investigated.

A series of novel 2-(2-phenoxyacetamido)benzamide derivatives, including the 3-(2-phenoxyacetamido) and the 4-(2-phenoxyacetamido)benzamide derivatives, were therefore synthesized and screened for their antileukemic activity.

2. Results and discussion

2.1. Chemistry

The synthesis of 2-(2-phenoxyacetamido)benzamides **17a**,¹⁸ **17b**, **17c**,¹⁹ **17e–f**, **17g**,¹⁹ and **17h–v** was achieved as described in Scheme 1. A mixture of the appropriate acid chlorides **16a–e** and the appropriate 5-*R*-4-*R*₁-2-aminobenzamides **15a–f** in pyridine was stirred magnetically in an ice bath. Crude benzoylchlorides **16a–e**, not commercially available, were obtained starting from the corresponding acid by treatment with thionyl chloride.²⁰

The anthranilamide **15a** is commercially available, while the 5-*R*-4-*R*₁-2-aminobenzamides **15b–f** were obtained according to Scheme 1.

In particular, the 5-iodo-2-aminobenzamide (**15c**) was obtained, as reported in the literature,²¹ by treatment of anthranilamide (**15a**) with iodine in an aqueous solution of sodium bicarbonate.

The 5-methylantranilamide (**15d**), the 5-methoxyantranilamide (**15e**) and the 4,5-dimethoxyantranilamide (**15f**) were obtained by reduction of 2-nitro-5-methyl benzamides **13a–c** as shown in Scheme 1. Compounds **13a–c** were obtained by reaction of the acids

11a–c with thionyl chloride to afford **12a–c**, followed by treatment of **12a–c** with aqueous ammonia.

The anthranilamide derivative **15b** was obtained by stirring the 6-chloro-1*H*-benzo[*d*][1,3]oxazine-2,4-dione **14a** in a 25% aqueous ammonia solution (Scheme 1).²²

The 4-(2-phenoxyacetamido)benzamide (**21**) and the 3-(2-phenoxyacetamido)benzamide (**22**) were obtained, following the same synthetic route reported for compounds **17a–v**, first by transformation of the commercially available nitrobenzoyl chlorides **18a,b** to nitrobenzamides **19a,b**,^{7,8} then by reduction with palladium on activated charcoal in a Parr apparatus (Scheme 2).

The structures of the new compounds were determined by analytical and spectroscopic measurements. In particular, **17a–v**, **21** and **22** showed ¹H NMR signals attributable to aromatic protons in the range 6.24–8.67 δ and a singlet in the range 11.99–12.92 δ , exchangeable with D₂O, for the amidic NH. The signals of the NH₂ benzamido protons, exchangeable with D₂O, showed a different behavior depending on the solvent. As shown in Figure 3a for **17b**, taken as an example, the spectrum recorded in chloroform showed a broad signal attributable to the NH₂ moiety at 6.07 ppm. In DMSO-*d*₆ as solvent, the presence of an intramolecular hydrogen bond rendered the benzamido NH₂ protons diastereotopic. H_a appeared as a singlet at 7.39 δ whereas H_b was found at a lower field as a singlet at 8.25 δ (Fig. 3b). The presence of an intramolecular hydrogen bond was confirmed by performing the ¹H NMR spectrum of **17b** at 90 °C. Figure 3c showed that, at 90 °C, the two singlets collapsed into a single broad signal at 7.64 δ . Furthermore, the ¹H NMR spectrum of **17b** in a diluted solution of DMSO-*d*₆ was similar to that in a concentrated solution, proving that no intermolecular interaction occurred.

Moreover, the ¹H NMR spectra of methyl and methoxy-substituted derivatives showed signals in the range 2.24–2.39 δ and 2.24–3.90 δ , respectively, arising from the methyl and methoxyl groups. Finally, a signal in the range 4.61–4.91 δ was found, and this was due to the methylene group of the phenoxy moiety.

2.2. Biological activity

All synthesized 2-(2-phenoxyacetamido)benzamido derivatives **17a–v**, **21** and **22** were initially tested in vitro for their antileukemic activity against the K562 (human chronic myelogenous leukemia) cell line; colchicine, whose antileukemic activity is well known, 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 Table 1.

These data showed that the best antiproliferative agent was compound **17j**, with an IC₅₀ value of 0.16 μ M.

As far as structure–activity relationships are concerned, it seems that on average the substitution of the cinnamamido moiety with the phenoxyacetamido moiety had little effect

on inhibition of K562 cell growth (Table 1). In particular, the average inhibitory activity at 10 μ M on K562 cell growth, showed by all the previously tested compounds,^{7,8} was about 46%⁷ and 44%,⁸ respectively, while with the phenoxyacetamido moiety, average inhibition was 49%. Moreover, shifting the phenoxyacetamido moiety from the *ortho* (compound **17a**) to the *para* or *meta* position (compounds **21** and **22**, Table 1) resulted in reduced activity.

As for the previously reported benzamide analogs,^{7,8} our data (Table 1) showed positive effects following substitution at the 5 position of the benzamido moiety (**17f**, **17j**, **17m**, **17r** and **17u**, Table 1), especially when the substituent(s) was iodine or 4,5-dimethoxy (compounds **17j** and **17u**, Table 1). Moreover, it seems that the simultaneous introduction of a substituent into both the benzamido and phenoxyacetamido moieties is unfavourable for inhibition of K562 cell growth relative to the unsubstituted 2-cinnamamidobenzamide **4** (Fig. 1), considering that antiproliferative activity was only maintained in the *ortho*-phenoxyacetamido derivatives (compounds **17g**, **17l**, **17n**, **17s** and **17v**, Table 1).

To obtain more insights into the cytotoxic potential of the test compounds for normal human cells, compounds **17a**, **17f**, **17g**, **17j**, **17k**, **17l**, **17m**, **17r** and **17u** were also tested in vitro for their cytotoxicity against HuDe cells, a primary cell culture from human epidermis (Table 2).

The data in Table 2 show that the K562-active compounds were significantly less cytotoxic against the HuDe cell line (HuDe/K562 index ranging from 21 to 1987), indicating the possibility that these compounds may have limited toxicity in vivo at doses that would limit tumor growth. This contrasts with a very low HuDe/K562 index (0.85) obtained for colchicine.

Compounds **17f**, **17j**, **17l**, **17r** and **17u** were selected by the NCI to evaluate in their in vitro antiproliferative assay at a single high dose (10^{-5} M) against 60 human cell lines derived from nine clinically isolated human cancer types (leukemia, lung, colon, melanoma, renal, ovarian, brain, breast and prostate) according to the NCI standard protocol.²³

The antitumor activity is given by three parameters for each cell line; GI₅₀ (compound's molar concentration inhibiting 50% net cell growth), TGI (compound's molar concentration for total inhibition of net cell growth), and LC₅₀ (compound's molar concentration inducing 50% net cell death).

Among these, **17f**, **17j**, **17r** and **17u**, showing the best mean graph midpoints (MG_MID) (Table 3), and therefore they were progressed to the 5-dose assay against the NCI full panel of 60 cell lines.²³

The GI₅₀ values for the test compounds were reported in Table 4. All the compounds **17f**, **17j**, **17r** and **17u** tested by NCI were found to possess low cytotoxicity. On the other hand, they turn out to be inhibitor of cell growth with GI₅₀ values ranging from 0.101 to 42.8 μ M. Among these, compounds **17j**, **17r** and **17u** were particularly active, against the majority of type of tumor cell lines investigated, in the sub micromolar concentration. The best activity was showed by compound **17j** that caused 50% growth inhibition at submicromolar concentrations against 44 of 56 cellular lines. Compounds **17r** and **17u** resulted less active

with respect to **17j** causing 50% growth inhibition at submicromolar concentrations against 30 of 56 and 26 of 58 cellular lines, respectively. On the contrary, compound **17f** resulted less active with respect to **17j**, **17r** and **17u** being a micro molar inhibitor with GI₅₀ values at submicromolar concentrations only against 3 of 57 cellular lines.

Moreover, a mean graph midpoint (MG_MID) is calculated for the GI₅₀, TGI and LC₅₀ parameters, giving an average activity parameter for all the cell lines. For the calculation of the MG_MID, insensitive cell lines are included and assigned as their values the highest concentration tested. Considering the MG_MID values (Table 5), the most active compound of the series was derivative **17j**, at the GI₅₀, TGI and LC₅₀ levels, followed by **17r**.

In our previous studies on the cinnamamido and 3-phenylpropiolamido benzamides (compounds **1–5**, Fig. 1),^{7,8} the major evidence that suggested these agents interacted with tubulin was derived from cellular studies, such as an increased proportion of cells in the G2/M phase of the cell cycle, increased proportion of apoptotic cells, disappearance of cellular microtubules and COMPARE analysis of NCI cytotoxicity data.

Therefore we first wondered whether the same mechanism of action occurred with the phenoxyacetamido scaffold and we examined the most active compounds in the series (**17f**, **17j**, **17r** and **17u**) for potential effects on the assembly of purified tubulin and on the inhibition of colchicine binding to tubulin, but negligible effects were observed (data not presented). Our initial studies with the K562 cells did yield evidence for mitotic arrest following treatment with the four compounds. However further studies using compounds **17j** and **17u**, which were the most effective antiproliferative agents, failed to validate the initial observations.

In order to elucidate the antiproliferative effect of **17j** or **17u** compounds, K562 cells were treated with different doses of the agents for 48 h. Clear morphological changes were observed in cells exposed to both the compounds.

In fact, under light microscopy, control cells appeared with a regular rounded shape (Fig. 4a), whereas after incubation with **17j** or **17u** cells detached themselves from poly-D lysine coated plates and showed a remarkable reduction in cell number and cell size.

The effect, which appeared with a 10 μ M dose of each compound (Fig. 4b and d), was further enhanced with 25 μ M **17u** (Fig. 4e), while a consistent cytotoxic effect was already reached at the lowest concentration examined of **17j** (Fig. 4b).

To gain additional insight into the mechanism of action of the compounds, a 10 μ M dose of **17j** and **17u** was used in subsequent experiments.

After propidium iodide (PI) staining of DNA, flow cytometry analyses were performed to investigate the distribution of cells in the different phases of the cell cycle. These studies showed that both compounds caused an arrest of cells in the G0/G1 phase at 24 h. As shown in Figure 5, the proportion of K562 cells in the G0/G1 phase increased from 54.9% in control cells (Fig. 5a) to 78.5% in 10 μ M **17j**-treated cells (Fig. 5b, upper panel) and to 80% in 10 μ M **17u**-treated cells (Fig. 5c, upper panel).

Prolonging the time of incubation with the compounds to 48 h a decrease in the proportion of cells in the G0/G1 phase occurred, while the percentage of cells in sub-G0/G1 region, characteristic of apoptotic cells with fragmented DNA, increased, reaching values equal to 32.9% and 20.6% with 10 μ M **17j** and **17u**, respectively (Fig. 5b and c, lower panels).

In order to establish whether the reduction in cell number and cell size as well as the observed DNA fragmentation were related to the apoptotic cell death, additional analyses were performed.

Apoptosis is a programmed cell death that occurs in physiological and pathological conditions.²⁴ Defects in apoptotic pathways are now to contribute to malignant transformation of cells, cancer progression and resistance to anticancer drugs.²⁴ On the other hand, many compounds exerts their cytotoxic activity in tumor cells by inducing apoptosis. Apoptosis is characterized by a series of typical morphological features, such as cell shrinkage and detachment from the surrounding tissue, nuclear condensation, fragmentation into membrane-bound apoptotic bodies which are rapidly engulfed via phagocytosis by neighboring cells. Biochemical hallmarks of apoptosis include activation of proteases termed caspases, cleavage of proteins and DNA and exposure of phosphatidylserine on the cell surface.²⁴

Therefore K562 cells were treated with 10 μ M of **17j** or **17u** for 48 h and examined for the characteristic morphological features of apoptotic cell death under a fluorescence microscope. Using Hoechst 33342 to stain the nuclei, it was observed (Fig. 6) that, in comparison with normal cells, treatment with 10 μ M **17j** or **17u** induced in a high percentage of cells chromatin condensation and nuclear fragmentation, which are morphological changes typical of apoptosis.²⁵

One of the earlier events of apoptosis includes translocation of phosphatidylserine (PS), a phospholipid preferentially present in the inner side of membrane, to the surface of membrane. This allows early recognition of dead cells by macrophages, resulting in phagocytosis without the release of pro-inflammatory cellular components.²⁴ In our study the effects of **17j** and **17u** treatment on early and late apoptosis were examined using combined annexin V/PI staining.²⁶ Annexin V, a Ca^{2+} -dependent phospholipid-binding protein, has high affinity for PS, and fluorochrome-labeled annexin V can be used to specifically target and identify cells in the early stages of apoptosis.²⁶ Propidium iodide (PI) is a DNA-intercalating dye which enters in the cells only when plasma membrane integrity is lost, a typical event of late apoptotic or necrotic cells. Annexin V-FITC and PI positive populations can easily be distinguished using a flow cytometer. In the scatter plot of a double variant flow cytometry (Fig. 7), the lower left quadrant of the histograms shows the viable cells, which exclude PI and are negative for annexin V-FITC staining. The lower right quadrant represents the early apoptotic cells, which are PI negative and annexin V positive indicating that cell population endowed with cytoplasmic membrane integrity and PS externalization. The upper right quadrant represents the non-viable necrotic and late-stage apoptotic cells, which are positive for annexin V binding and PI uptake. This population has loss of membrane integrity and presents externalization of PS. Finally, the upper left quadrant provides information about necrotic cell population, which has loss of

cell membrane integrity (PI positive) with no PS externalization (Annexin-V negative). In our experiments, to distinguish early from late apoptotic cells, the analysis was performed after 24 h incubation with **17j** or **17u**. As shown in Figure 7, after exposure to 10 μ M **17j**, 24.5% of the K562 cells were found to be in early apoptosis and 12.5% in late apoptosis, while no necrotic cells were detected. Similar results were obtained in cells treated with **17u**.

In light of these results, we focused on the possible activation of caspases, known molecular players in the process of apoptosis. Caspases are cysteine aspartyl proteases involved in the activation of apoptotic cell death, being critical for certain processes associated with the dismantling of the cell and the formation of apoptotic bodies.²⁷ Caspases are normally present as pro-caspases, inactive proenzymes which must be cleaved and transformed in functional caspases.²⁷ There are two classes of apoptotic caspases: initiator caspases, which are associated with the initiation of apoptosis (caspases 2, 8, 9, 10), and effector caspases, which cleave cellular substrates needed for the cell's survival (caspases 3, 6, 7).²⁷ To demonstrate the involvement of caspases in apoptosis induced by **17j** and **17u** in K562 cells, activation of the effector caspases 3 and 6 was evaluated by Western blotting analysis. As shown in Figure 8, with respect to the basal level, the cellular content of pro-caspase 3 protein decreased by 40% and 50% after 48 h treatments with 10 μ M **17j** or **17u**, respectively, thus indicating activation of caspase 3.

Moreover, a modest reduction in the level of pro-caspase 6 was observed in **17j**-treated cells (–20%) and a greater decrease in **17u**-treated cells (–50%). These results demonstrate that the cytotoxic effect exerted in K562 by both **17j** and **17u** was due to the induction of apoptosis mediated by caspase activation.

3. Conclusion

The data reported here show that the 2-(2-phenoxyacetamido) benzamides **17** caused growth inhibition against many tumor cell lines. The best agents inhibited proliferation at low micromolar and submicromolar concentrations in every investigated tumor cell lines. It seems that, on average, the substitution of the cinnamamido moiety with the phenoxyacetamido moiety had little positive effect on inhibition of K562 cell growth, but moving the phenoxyacetamido moiety from the *ortho* to the *meta* or *para* position had negative effects on activity. As in our earlier studies with this class of compounds,^{7,8} we initially suspected tubulin as a target, but the most cytotoxic compounds had little antitubulin activity. In the experiments presented here, we found different behavior in compounds **17a–v**, bearing a phenoxyacetamido scaffold (G0/G1 arrest of cell cycle), in comparison with previously studied cinnamamido and 3-phenylpropiolamido benzamides (G2/M arrest of cell cycle).^{7,8}

Compounds **17j** and **17u** caused a G0/G1 arrest of the cell cycle. Prolonging the treatment to 48 h demonstrated that they caused an apoptotic cell death through the activation of pro-caspase 3 and 6.

4. Experimental section

4.1. Chemistry: general procedures

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 disk. ¹H and ¹³C NMR spectra (250 and 62.90 MHz, respectively) 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).

4.1.1. General procedure for preparation of 5-*R*-4-*R*₁-2-nitrobenzoyl chlorides 12a–c and 2-phenoxyacetyl chlorides 16a–e²⁰—Substituted benzoyl and phenoxyacetyl chlorides **12a–c** and **16a–e** were obtained by refluxing for 5 h the appropriate acid derivatives (0.01 mol) with thionyl chloride (7.25 mL). After evaporation under reduced pressure, the crude liquid residue was used for subsequent reactions without purification.

4.1.2. Preparation of 5-*R*-4-*R*₁-2-nitrobenzamides 13a–c—To 0.01 mol of 5-*R*-4-*R*₁-2-nitrobenzoyl chlorides **12a–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 to give pure **13a–c**.

4.1.3. Preparation of 5-*R*-4-*R*₁-2-aminobenzamides 15d,e,f—To a magnetically stirred suspension of stannous chloride (0.038 mol) in concentrated HCl (37%), 0.013 mol of **13a–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 NaOH (40%) was added until the tin salt dissolved. The solution was extracted with ethyl acetate (3 $\times$ 150 mL), and the extracts dried and evaporated to obtain pure **15d,e,f**.

4.1.4. Preparation of 2-amino-5-chlorobenzamide 15b¹⁸—A mixture of 0.01 mol of 6-*R*-7-*R*₁-1H-benzo[d][1,3]oxazine-2,4-diones **14a** 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.

4.1.5. Preparation of 2-amino-5-iodobenzamide (15c)²¹—Powdered iodine (11.7 g, 46.2 mmol) was added portion-wise over 1 h to a stirred solution of 2-aminobenzamide (**15a**) (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. Afterwards NaHSO₃ (0.87 g, 8.40 mmol) was

added. The solution was extracted with ethyl acetate (3×800 mL). After being dried with Na_2SO_4 , the organic phase was evaporated. The crude product was recrystallized with water/methanol 10:1 v/v (600 mL) to yield pure **15c**; yield 95%, mp 197–198 °C.

4.1.6. General procedure for the preparation of compounds 19a, b—To 0.01 mol of 5-*R*-4-*R*₁-benzoyl chlorides **18a,b** 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 to give pure **19a,b**.²⁸

4.1.7. General procedure for the preparation of compounds 20a,b—To²⁸ a solution of 0.8 mmol of **19a,b** in 20 mL of hot ethanol, 15 mg of 10% palladium on activated charcoal as a catalyst was added. The mixture was left under hydrogenation in a Parr apparatus at 40 psi for 24 h to obtain **20a,b** after crystallization.^{29,30}

4.1.8. General procedure for preparation of phenyl 2-(2-phenoxyacetamido)benzamides 17a,¹⁸ 17b–c,¹⁹ 17d 17e–f, 17g,¹⁹ 17h–v, 4-(2-phenoxyacetamido)benzamide (21) and 3-(2-phenoxyacetamido)benzamide (22)—To a cold (0–5 °C) stirred suspension of aminobenzamides **15a–f** and **20a,b** (0.016 mol) in pyridine (13 mL), 0.016 mol of the appropriate phenoxyacetyl chloride **16a–e** 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 indicated solvent.

4.1.8.1. 2-(2-(*o*-Tolyloxy)acetamido)benzamide (17b).: White solid (1.27 g, 28% yield); mp 170–173 °C (ethanol); I.R. (KBr) cm^{-1} 3347, 3145 (NH, NH₂), 1659, 1620 ($2 \times \text{CO}$); ¹H NMR (DMSO) δ 2.39 (s, 3H, CH₃); 4.70 (s, 2H, OCH₂); 6.87–8.62 (a set of signals, 10H, aromatic protons and exchangeable NH₂); 12.28 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 16.68 (CH₃), 67.75 (OCH₂), 111.73 (CH_{Ar}), 120.85 (C_{Ar}), 120.88 (CH_{Ar}), 121.76 (CH_{Ar}), 123.61 (CH_{Ar}), 126.90 (C_{Ar}), 127.41 (CH_{Ar}), 129.01 (CH_{Ar}), 131.21 (CH_{Ar}), 132.67 (CH_{Ar}), 138.85 (C_{Ar}), 155.65 (C_{Ar}), 167.71 (CO), 170.91 (CO). Anal. Calcd for C₁₆H₁₆N₂O₃: C, 67.59; H, 5.67; N, 9.85. Found: C, 67.52; H, 5.60; N, 9.50.

4.1.8.2. 2-(2-(*p*-Tolyloxy)acetamido)benzamide (17c).: White solid (1.54 g, 34% yield); mp 205–208 °C (ethanol); I.R. (KBr) cm^{-1} 3378, 3318, 3195 (NH, NH₂), 1694, 1651 ($2 \times \text{CO}$); ¹H NMR (DMSO) δ 2.24 (s, 3H, CH₃); 4.63 (s, 2H, OCH₂); 6.96–8.63 (a set of signals, 10H, aromatic protons and exchangeable NH₂); 12.53 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 20.52 (CH₃), 67.88 (OCH₂), 115.18 ($2 \times \text{CH}_{\text{Ar}}$), 120.38 (C_{Ar}), 120.43 (CH_{Ar}), 123.50 (CH_{Ar}), 129.07 (CH_{Ar}), 130.40 ($2 \times \text{CH}_{\text{Ar}}$), 130.94 (C_{Ar}), 132.81 (CH_{Ar}), 139.05 (C_{Ar}), 155.56 (C_{Ar}), 167.73 (CO), 171.06 (CO). Anal. Calcd for C₁₆H₁₆N₂O₃: C, 67.59; H, 5.67; N, 9.85. Found: C, 67.46; H, 5.54; N, 10.10.

4.1.8.3. 2-(2-(2,4-Dichlorophenoxy)acetamido)benzamide (17d).: Sand-colored solid (1.08 g, 20% yield); mp 185–187 °C (ethanol); I.R. (KBr) cm^{-1} 3362, 3314, 3222 (NH, NH₂), 1695, 1628 ($2 \times \text{CO}$); ¹H NMR (DMSO) δ 4.85 (s, 2H, OCH₂); 7.15–8.56 (a set of signals, 9H, aromatic protons and exchangeable NH₂); 12.23 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 68.85 (OCH₂), 116.00 (CH_{Ar}), 120.99 (C_{Ar}), 121.05

(CH_{Ar}), 123.41 (C_{Ar}), 123.74 (CH_{Ar}), 126.09 (C_{Ar}), 128.52 (CH_{Ar}), 128.97 (CH_{Ar}), 129.95 (CH_{Ar}), 132.63 (CH_{Ar}), 138.72 (C_{Ar}), 152.45 (C_{Ar}), 166.76 (CO), 170.82 (CO). Anal. Calcd for C₁₅H₁₂Cl₂N₂O₃: C, 53.12; H, 3.57; N, 8.26. Found: C, 52.95; H, 3.50; N, 7.93.

4.1.8.4. 2-(2-(2,3-Dichlorophenoxy)acetamido)benzamide (17e).: White solid (1.52 g, 28% yield); mp 202–205 °C (ethanol); I.R. (KBr) cm⁻¹ 3225–2915 (NH, NH₂), 1673 (broad, 2 × CO); ¹H NMR (DMSO) δ 4.88 (s, 2H, OCH₂); 7.16–8.58 (a set of signals, 9H, aromatic protons and exchangeable NH₂); 12.24 (s, 1H, NH, exchangeable). Anal. Calcd for C₁₅H₁₂Cl₂N₂O₃: C, 53.12; H, 3.57; N, 8.26. Found: C, 52.75; H, 3.86; N, 8.63.

4.1.8.5. 5-Chloro-2-(2-phenoxyacetamido)benzamide (17f).: White solid (0.97 g, 20% yield); mp 197–200 °C (ethanol); I.R. (KBr) cm⁻¹ 3377, 3296, 3178 (NH, NH₂), 1698, 1655 (2 × CO); ¹H NMR (DMSO) δ 4.70 (s, 2H, OCH₂); 6.70–8.67 (a set of signals, 10H, aromatic protons and exchangeable NH₂); 12.51 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 67.67 (OCH₂), 115.33 (2 × CH_{Ar}), 122.01 (C_{Ar}), 122.19 (2 × CH_{Ar}), 127.27 (C_{Ar}), 128.72 (CH_{Ar}), 130.10 (2 × CH_{Ar}), 132.45 (CH_{Ar}), 137.94 (C_{Ar}), 157.55 (C_{Ar}), 167.70 (CO), 169.73 (CO). Anal. Calcd for C₁₅H₁₃ClN₂O₃: C, 59.12; H, 4.30; N, 9.19. Found: C, 58.90; H, 4.11; N, 9.29.

4.1.8.6. 5-Chloro-2-(2-(*o*-tolylxy)acetamido)benzamide (17g).: White solid (0.51 g, 10% yield); mp 218–220 °C (ethanol); I.R. (KBr) cm⁻¹ 3411, 3182 (NH, NH₂), 1665 (broad, 2 × CO); ¹H NMR (DMSO) δ 2.38 (s, 3H, CH₃); 4.71 (s, 2H, OCH₂); 6.90–8.64 (a set of signals, 9H, aromatic protons and exchangeable NH₂); 12.21 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 16.72 (CH₃), 67.73 (OCH₂), 111.80 (CH_{Ar}), 121.65 (CH_{Ar}), 122.26 (CH_{Ar}), 126.85 (C_{Ar}), 127.17 (C_{Ar}), 127.36 (CH_{Ar}), 128.71 (CH_{Ar}), 130.61 (C_{Ar}), 131.20 (CH_{Ar}), 132.34 (CH_{Ar}), 137.98 (C_{Ar}), 157.63 (C_{Ar}), 167.22 (CO), 169.48 (CO). Anal. Calcd for C₁₆H₁₅ClN₂O₃: C, 60.29; H, 4.74; N, 8.79. Found: C, 60.34; H, 5.03; N, 8.95.

4.1.8.7. 5-Chloro-2-(2-(*p*-tolylxy)acetamido)benzamide (17h).: White solid (1.12 g, 22% yield); mp 225–227 °C (ethanol); I.R. (KBr) cm⁻¹ 3390, 3253, 3248 (NH, NH₂), 1660 (broad, 2 × CO); ¹H NMR (DMSO) δ 2.24 (s, 3H, CH₃); 4.65 (s, 2H, OCH₂); 6.95–8.66 (a set of signals, 9H, aromatic protons and exchangeable NH₂); 12.48 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 20.51 (CH₃), 67.86 (OCH₂), 115.19 (2 × CH_{Ar}), 122.01 (C_{Ar}), 122.17 (CH_{Ar}), 127.25 (C_{Ar}), 128.71 (CH_{Ar}), 130.40 (2 × CH_{Ar}), 131.00 (C_{Ar}), 132.45 (CH_{Ar}), 137.94 (C_{Ar}), 155.52 (C_{Ar}), 167.85 (CO), 169.70 (CO). Anal. Calcd for C₁₆H₁₅ClN₂O₃: C, 60.29; H, 4.74; N, 8.79. Found: C, 60.39; H, 4.50; N, 8.53.

4.1.8.8. 5-Chloro-2-(2-(2,4-dichlorophenoxy)acetamido)benzamide (17i).: White solid (0.65 g, 11% yield); mp 215–218 °C (ethanol); I.R. (KBr) cm⁻¹ 3378, 3254, 3246 (NH, NH₂), 1684, 1644 (2 × CO); ¹H NMR (DMSO) δ 4.91 (s, 2H, OCH₂); 6.24–8.64 (a set of signals, 8H, aromatic protons and exchangeable NH₂); 12.19 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 63.83 (OCH₂), 116.10 (CH_{Ar}), 122.72 (C_{Ar}), 122.82 (CH_{Ar}), 123.43 (C_{Ar}), 126.17 (C_{Ar}), 127.54 (C_{Ar}), 128.53 (CH_{Ar}), 128.60 (CH_{Ar}), 129.95 (CH_{Ar}), 132.96 (CH_{Ar}), 137.57 (C_{Ar}), 152.40 (C_{Ar}), 166.88 (CO), 169.45 (CO). Anal. Calcd for C₁₅H₁₁Cl₃N₂O₃: C, 48.22; H, 2.97; N, 7.50. Found: C, 48.38; H, 2.65; N, 7.66.

4.1.8.9. 5-Iodo-2-(2-phenoxyacetamido)benzamide (17j): Sand-colored solid (2.41 g, 38% yield); mp 206–209 °C (ethanol); I.R. (KBr) cm^{-1} 3377, 3295, 3178 (NH, NH₂), 1694, 1652 (2 × CO); ¹H NMR (DMSO) δ 4.69 (s, 2H, OCH₂); 6.99–8.46 (a set of signals, 10H, aromatic protons and exchangeable NH₂); 12.50 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 67.73 (OCH₂), 87.03 (C_{Ar}), 115.13 (2 × CH_{Ar}), 122.20 (CH_{Ar}), 122.50 (C_{Ar}), 122.57 (CH_{Ar}), 130.11 (2 × CH_{Ar}), 137.17 (CH_{Ar}), 138.74 (C_{Ar}), 141.19 (CH_{Ar}), 157.55 (C_{Ar}), 167.70 (CO), 169.62 (CO). Anal. Calcd for C₁₅H₁₃IN₂O₃: C, 45.47; H, 3.31; N, 7.07. Found: C, 45.40; H, 3.59; N, 7.25.

4.1.8.10. 5-Iodo-2-(2-(*p*-tolylloxy)acetamido)benzamide (17k): White solid (1.57 g, 24% yield); mp 220–222 °C (ethanol); I.R. (KBr) cm^{-1} 3386, 3297, 3195 (NH, NH₂), 1693, 1651 (2 × CO); ¹H NMR (DMSO) δ 2.24 (s, 3H, CH₃); 4.63 (s, 2H, OCH₂); 6.94–8.44 (a set of signals, 9H, aromatic protons and exchangeable NH₂); 12.44 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 20.53 (CH₃), 67.85 (OCH₂), 87.05 (C_{Ar}), 115.16 (2 × CH_{Ar}), 122.42 (C_{Ar}), 122.53 (CH_{Ar}), 130.41 (2 × CH_{Ar}), 130.98 (C_{Ar}), 137.16 (CH_{Ar}), 138.74 (C_{Ar}), 141.18 (CH_{Ar}), 155.47 (C_{Ar}), 167.96 (CO), 169.86 (CO). Anal. Calcd for C₁₆H₁₅IN₂O₃: C, 46.85; H, 3.69; N, 6.83. Found: C, 47.00; H, 3.98; N, 6.79.

4.1.8.11. 2-(2-(2,4-Dichlorophenoxy)acetamido)-5-iodobenzamide (17l): White solid (1.04 g, 14% yield); mp 213–215 °C (ethanol); I.R. (KBr) cm^{-1} 3376, 3261, 3144 (NH, NH₂), 1670, 1665 (2 × CO); ¹H NMR (DMSO) δ 4.84 (s, 2H, OCH₂); 7.17–8.38 (a set of signals, 8H, aromatic protons and exchangeable NH₂); 12.11 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 68.93 (OCH₂), 87.38 (C_{Ar}), 116.10 (CH_{Ar}), 122.92 (C_{Ar}), 123.02 (CH_{Ar}), 123.41 (C_{Ar}), 126.07 (C_{Ar}), 128.50 (CH_{Ar}), 129.96 (CH_{Ar}), 137.10 (C_{Ar}), 138.66 (CH_{Ar}), 140.99 (CH_{Ar}), 152.47 (C_{Ar}), 166.84 (CO), 169.35 (CO). Anal. Calcd for C₁₅H₁₁Cl₂IN₂O₃: C, 38.74; H, 2.38; N, 6.02. Found: C, 38.37; H, 2.59; N, 5.88.

4.1.8.12. 5-Methyl-2-(2-phenoxyacetamido)benzamide (17m): White solid (1.91 g, 42% yield); mp 199–202 °C (ethanol); I.R. (KBr) cm^{-1} 3381, 3302, 3182 (NH, NH₂), 1693, 1652 (2 × CO); ¹H NMR (DMSO) δ 2.31 (s, 3H, CH₃); 4.67 (s, 2H, OCH₂); 6.98–8.52 (a set of signals, 10H, aromatic protons and exchangeable NH₂); 12.43 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 20.84 (CH₃), 67.73 (OCH₂), 115.38 (CH_{Ar}), 120.23 (C_{Ar}), 120.31 (CH_{Ar}), 122.02 (CH_{Ar}), 129.44 (CH_{Ar}), 130.05 (CH_{Ar}), 132.33 (C_{Ar}), 133.16 (CH_{Ar}), 136.92 (C_{Ar}), 157.00 (C_{Ar}), 167.14 (CO), 171.08 (CO). Anal. Calcd for C₁₆H₁₆N₂O₃: C, 67.59; H, 5.67; N, 9.85. Found: C, 67.45; H, 5.76; N, 9.90.

4.1.8.13. 5-Methyl-2-(2-(*o*-tolylloxy)acetamido)benzamide (17n): White solid (0.72 g, 15% yield); mp 160–162 °C (ethanol); I.R. (KBr) cm^{-1} 3385, 3344, 3186 (NH, NH₂), 1693, 1652 (2 × CO); ¹H NMR (DMSO) δ 2.03 (s, 3H, CH₃); 2.38 (s, 3H, CH₃); 4.67 (s, 2H, OCH₂); 6.87–8.50 (a set of signals, 9H, aromatic protons and exchangeable NH₂); 12.14 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 16.78 (CH₃), 20.82 (CH₃), 67.78 (OCH₂), 111.79 (CH_{Ar}), 120.70 (CH_{Ar}), 120.78 (C_{Ar}), 121.63 (CH_{Ar}), 126.91 (CH_{Ar}), 127.36 (C_{Ar}), 129.37 (CH_{Ar}), 131.15 (CH_{Ar}), 132.44 (C_{Ar}), 133.00 (CH_{Ar}), 136.72 (C_{Ar}), 155.74 (C_{Ar}), 167.28 (CO), 170.92 (CO). Anal. Calcd for C₁₇H₁₈N₂O₃: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.65; H, 6.34; N, 9.78.

4.1.8.14. 5-Methyl-2-(2-(*p*-tolylloxy)acetamido)benzamide (17o): White solid (1.72 g, 36% yield); mp 216–218 °C (ethanol); I.R. (KBr) cm^{-1} 3390, 3262, 3222 (NH, NH₂), 1665, 1660 (2 × CO); ¹H NMR (DMSO) δ 2.24 (s, 3H, CH₃); 2.30 (s, 3H, CH₃); 4.61 (s, 2H, OCH₂); 6.95–8.51 (a set of signals, 9H, aromatic protons and exchangeable NH₂); 12.39 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 20.65 (CH₃), 20.81 (CH₃), 67.90 (OCH₂), 115.22 (2 × CH_{Ar}), 120.88 (C_{Ar}), 120.23 (CH_{Ar}), 129.42 (CH_{Ar}), 130.35 (2 × CH_{Ar}), 130.76 (C_{Ar}), 132.30 (C_{Ar}), 133.14 (CH_{Ar}), 136.91 (C_{Ar}), 155.66 (C_{Ar}), 167.28 (CO), 171.05 (CO). Anal. Calcd for C₁₇H₁₈N₂O₃: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.46; H, 6.19; N, 9.76.

4.1.8.15. 2-(2-(2,4-Dichlorophenoxy)acetamido)-5-methylbenzamide (17p): White solid (1.02 g, 18% yield); mp 214–216 °C (ethanol); I.R. (KBr) cm^{-1} 3391, 3320, 3201 (NH, NH₂), 1665, 1660 (2 × CO); ¹H NMR (DMSO) δ 2.30 (s, 3H, CH₃); 4.82 (s, 2H, OCH₂); 7.16–8.44 (a set of signals, 8H, aromatic protons and exchangeable NH₂); 12.07 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 20.83 (CH₃), 68.92 (OCH₂), 116.08 (CH_{Ar}), 120.84 (C_{Ar}), 120.90 (CH_{Ar}), 123.41 (C_{Ar}), 125.99 (C_{Ar}), 128.51 (CH_{Ar}), 129.32 (CH_{Ar}), 129.95 (CH_{Ar}), 132.59 (C_{Ar}), 132.96 (CH_{Ar}), 136.57 (C_{Ar}), 152.57 (C_{Ar}), 166.42 (CO), 170.81 (CO). Anal. Calcd for C₁₆H₁₄Cl₂N₂O₃: C, 54.41; H, 4.00; N, 7.93. Found: C, 54.09; H, 4.20; N, 7.58.

4.1.8.16. 2-(2-(2,3-Dichlorophenoxy)acetamido)-5-methylbenzamide (17q): White solid (0.56 g, 10% yield); mp 223–225 °C (ethanol); I.R. (KBr) cm^{-1} 3310–2910 (NH, NH₂), 1670 (broad, 2 × CO); ¹H NMR (DMSO) δ 2.30 (s, 3H, CH₃); 4.85 (s, 2H, OCH₂); 7.14–8.44 (a set of signals, 8H, aromatic protons and exchangeable NH₂); 12.07 (s, 1H, NH, exchangeable). Anal. Calcd for C₁₆H₁₄Cl₂N₂O₃: C, 54.41; H, 4.00; N, 7.93. Found: C, 54.68; H, 3.74; N, 7.47.

4.1.8.17. 5-Methoxy-2-(2-phenoxyacetamido)benzamide (17r): White solid (0.96 g, 20% yield); mp 178–181 °C (ethanol); I.R. (KBr) cm^{-1} 3391, 3313, 3164 (NH, NH₂), 1698, 1654 (2 × CO); ¹H NMR (DMSO) δ 3.80 (s, 3H, OCH₃); 4.66 (s, 2H, OCH₂); 7.00–8.56 (a set of signals, 10H, aromatic protons and exchangeable NH₂); 12.27 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 55.93 (OCH₃), 67.69 (OCH₂), 113.99 (CH_{Ar}), 115.38 (2 × CH_{Ar}), 118.18 (CH_{Ar}), 121.64 (C_{Ar}), 121.86 (CH_{Ar}), 122.01 (CH_{Ar}), 130.05 (2 × CH_{Ar}), 132.58 (C_{Ar}), 154.82 (C_{Ar}), 157.71 (C_{Ar}), 166.79 (CO), 170.69 (CO). Anal. Calcd for C₁₆H₁₆N₂O₄: C, 63.99; H, 5.37; N, 9.33. Found: C, 64.05; H, 5.72; N, 9.64.

4.1.8.18. 5-Methoxy-2-(2-(*o*-tolylloxy)acetamido)benzamide (17s): Brown solid (0.65 g, 13% yield); mp 165–168 °C (ethanol); I.R. (KBr) cm^{-1} 3347, 3289, 3186 (NH, NH₂), 1672 (broad, 2 × CO); ¹H NMR (DMSO) δ 2.39 (s, 3H, OCH₃); 3.90 (s, 3H, OCH₃); 4.67 (s, 2H, OCH₂); 6.87–8.53 (a set of signals, 9H, aromatic protons and exchangeable NH₂); 11.99 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 16.42 (CH₃), 55.38 (OCH₃), 67.39 (OCH₂), 111.62 (CH_{Ar}), 113.52 (CH_{Ar}), 118.07 (CH_{Ar}), 121.98 (CH_{Ar}), 122.96 (C_{Ar}), 123.11 (CH_{Ar}), 126.85 (C_{Ar}), 127.46 (CH_{Ar}), 131.03 (C_{Ar}), 131.30 (CH_{Ar}), 155.26 (C_{Ar}), 155.40 (C_{Ar}), 167.71 (CO), 170.81 (CO). Anal. Calcd for C₁₇H₁₈N₂O₄: C, 64.96; H, 5.77; N, 8.91. Found: C, 65.16; H, 6.16; N, 9.19.

4.1.8.19. 5-Methoxy-2-(2-(*p*-tolylloxy)acetamido)benzamide (17t): Yellow solid (0.85 g, 17% yield); mp 238–240 °C (ethanol); I.R. (KBr) cm^{-1} 3353, 3245, 3191 (NH, NH₂), 1669, 1667 (2 × CO); ¹H NMR (DMSO) δ 2.24 (s, 3H, CH₃); 3.79 (s, 3H, OCH₃); 4.61 (s, 2H, OCH₂); 6.95–8.55 (a set of signals, 9H, aromatic protons and exchangeable NH₂); 12.24 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 20.55 (CH₃), 55.91 (OCH₃), 67.85 (OCH₂), 113.93 (CH_{Ar}), 115.20 (CH_{Ar}), 118.18 (CH_{Ar}), 121.70 (C_{Ar}), 121.92 (CH_{Ar}), 130.38 (CH_{Ar}), 130.82 (C_{Ar}), 132.47 (C_{Ar}), 154.84 (C_{Ar}), 155.64 (C_{Ar}), 167.98 (CO), 170.69 (CO). Anal. Calcd for C₁₇H₁₈N₂O₄: C, 64.96; H, 5.77; N, 8.91. Found: C, 65.21; H, 5.69; N, 9.17.

4.1.8.20. 4,5-Dimethoxy-2-(2-phenoxyacetamido)benzamide (17u): White solid (2.38 g, 45% yield); mp 219–222 °C (ethanol); I.R. (KBr) cm^{-1} 3367, 3289, 3173 (NH, NH₂), 1693, 1652 (2 × CO); ¹H NMR (DMSO) δ 3.81 (s, 6H, 2 × OCH₃); 4.67 (s, 2H, OCH₂); 6.98–8.44 (a set of signals, 9H, aromatic protons and exchangeable NH₂); 12.92 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 55.58 (OCH₃), 56.37 (OCH₃), 67.76 (OCH₂), 103.33 (CH_{Ar}), 111.21 (C_{Ar}), 112.13 (CH_{Ar}), 115.38 (2 × CH_{Ar}), 121.98 (CH_{Ar}), 130.03 (2 × CH_{Ar}), 135.01 (C_{Ar}), 144.08 (C_{Ar}), 151.88 (C_{Ar}), 157.75 (C_{Ar}), 167.15 (CO), 170.84 (CO). Anal. Calcd for C₁₇H₁₈N₂O₅: C, 61.81; H, 5.49; N, 8.48. Found: C, 62.06; H, 5.18; N, 8.83.

4.1.8.21. 2-(2-(2,3-Dichlorophenoxy)acetamido)-4,5-dimethoxybenzamide (17v): Yellow solid (0.96 g, 15% yield); mp 248–250 °C (ethanol); I.R. (KBr) cm^{-1} 3363, 3259, 3178 (NH, NH₂), 1664, 1616 (2 × CO); ¹H NMR (DMSO) δ 3.80 (s, 6H, 2 × OCH₃); 4.85 (s, 2H, OCH₂); 7.13–8.35 (a set of signals, 7H, aromatic protons and exchangeable NH₂); 12.60 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 55.87 (OCH₃), 56.28 (OCH₃), 68.96 (OCH₂), 104.33 (CH_{Ar}), 111.70 (C_{Ar}), 111.79 (CH_{Ar}), 113.11 (CH_{Ar}), 121.66 (C_{Ar}), 123.48 (CH_{Ar}), 128.92 (CH_{Ar}), 132.90 (C_{Ar}), 134.43 (C_{Ar}), 144.25 (C_{Ar}), 151.67 (C_{Ar}), 154.87 (C_{Ar}), 166.44 (CO), 170.63 (CO). Anal. Calcd for C₁₇H₁₆Cl₂N₂O₅: C, 51.14; H, 4.04; N, 7.02. Found: C, 51.21; H, 4.05; N, 6.77.

4.1.8.22. 4-(2-Phenoxyacetamido)benzamide (21): Sandcolored solid (2.81 g, 65% yield); mp 216–218 °C (ethanol); I.R. (KBr) cm^{-1} 3379, 3323–3175 (NH, NH₂), 1681, 1645 (2 × CO); ¹H NMR (DMSO) δ 4.74 (s, 2H, OCH₂); 6.96–7.88 (a set of signals, 11H, aromatic protons and exchangeable NH₂); 10.24 (s, 1H, NH, exchangeable). ¹³C NMR (δ) (DMSO) 67.48 (OCH₂), 115.08 (2 × CH_{Ar}), 119.22 (2 × CH_{Ar}), 121.68 (CH_{Ar}), 128.84 (2 × CH_{Ar}), 129.64 (C_{Ar}), 129.99 (2 × CH_{Ar}), 141.48 (C_{Ar}), 158.19 (C_{Ar}), 167.46 (CO), 167.89 (CO). Anal. Calcd for C₁₅H₁₄N₂O₃: C, 66.66; H, 5.22; N, 10.36. Found: C, 66.34; H, 4.93; N, 10.32.

4.1.8.23. 3-(2-Phenoxyacetamido)benzamide (22): Sandcolored solid (1.12 g, 26% yield); mp 168–170 °C (ethanol); I.R. (KBr) cm^{-1} 3400–3212 (NH, NH₂), 1678, 1652 (2 × CO); ¹H NMR (DMSO) δ 4.72 (s, 2H, OCH₂); 6.96–8.13 (a set of signals, 11H, aromatic protons and exchangeable NH₂); 10.24 (s, 1H, NH, exchangeable). Anal. Calcd for C₁₅H₁₄N₂O₃: C, 66.66; H, 5.22; N, 10.36. Found: C, 66.91; H, 5.26; N, 10.24.

4.2. Biology

4.2.1. Percent growth inhibition assay—Human chronic myelogenous leukemia cell line K562 was maintained in suspension culture in RPMI1640 medium (SIGMA–Aldrich, cat. #R8758) supplemented with 5% fetal bovine serum (FBS: SIGMA–Aldrich, cat. #F6178), 100 U/mL penicillin, 100 µg/mL streptomycin and 0.25 Ig/mL amphotericin B (complete medium) at 37 °C in a 5% CO₂ humidified atmosphere.

The trypan blue exclusion assay was performed to assess the effect of all test compounds on the viability of K562 cells, as described previously.³¹ 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 IC₅₀). 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.

4.2.2. MTT assay—The MTT (3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed as described earlier³² and was used to determine the viability of 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 µL 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 and 150 µL of 37% HCl. Cells grown in culture media alone or with appropriate concentrations of DMSO as controls.

4.2.3. Hoechst staining and cell cycle distribution—For these experiments, after plating on poly-(D-lysine) (Sigma–Aldrich, Milan, Italy) coated 96 or 6 well plates, K562 cells were allowed to adhere overnight and then treated with chemicals or vehicle only.

In order to determine either changes in nuclear morphology or plasma membrane damage, the cells were stained with Hoechst 33342 (Sigma–Aldrich, Milan, Italy), a fluorescent cell permeant dye. Specifically, cells (8×10^3 /well) were stained with Hoechst 33342 (2.5 Ig/mL medium) for 30 min, washed with PBS, resuspended in culture medium and treated for various times with compounds. Cell morphology was visualized using a Leica DC 300F microscope (Leica Microsystems, Wetzlar, Germany), and all images were acquired by Leica Q Fluoro Software using appropriate filters to examine Hoechst 33342 (DAPI filter with excitation wavelength of 372 nm and emission wavelength of 456 nm).

Cell cycle distribution was analyzed by flow cytometry using PI staining. Cells were seeded in 6 well plates (1.5×10^5 /well), treated with compounds and harvested for flow cytometric

analysis. Fluorescence of the cells was analyzed by a FACScan (Beckman Coulter Inc., Brea, CA). The proportion of cells with fragmented DNA was estimated by evaluating the percentage of events accumulated in the hypodiploid sub G0/G1 area of each scan. Data were analyzed using Expo32 software (Beckman Coulter Inc., Brea, CA).

4.2.4. Annexin V and PI staining—Cell apoptosis was detected using an Annexin V-FITC Detection Kit (Biotool, Munich, Germany). Briefly, after treatment for 24 h with compounds, K562 cells were harvested, centrifuged at 800 rpm for 10 min and washed in PBS. Then 10^5 cells/sample were incubated in the dark with annexin V-FITC (5 μ l) and PI (5 μ l) for 15 min according to the manufacturer's instructions. After that, stained cells were immediately analyzed by flow cytometry (Beckman Coulter Inc., Brea, CA) measuring the fluorescence emission at 530 nm (FL1) and >575 nm (FL3).

4.2.5. Western blotting analysis—Cell lysates were prepared as previously reported.³³ Protein concentration was determined by Lowry assay, 50 μ g protein/lane were subjected to SDS polyacrylamide gel electrophoresis and then transferred to a nitrocellulose membrane for detection with specific antibodies conjugated with alkaline phosphatase. Analyses of pro-caspase 3 and pro-caspase 6 were performed by using specific antibodies produced by Santa Cruz Biotechnology (Santa Cruz, CA) while antibody for b actin was purchased from Sigma Aldrich (Milan, Italy). The correct protein loading was ascertained by means of both red Ponceau staining and immunoblotting for b-actin. The densitometric analysis of western blot bands was performed by Image J software. All the blots shown are representative of at least three separate experiments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Financial support from 'Fondo di Finanziamento della Ricerca di Ateneo (ex 60%)' is gratefully acknowledged. The authors wish to thank the Developmental Therapeutics Program of the National Cancer Institute of the United States of America for performing the antiproliferative screening of compounds.

References

1. Ogita H, Isobe Y, Takaku H, Sekine R, Goto Y, Misawa S, Hayashi H. *Bioorg. Med. Chem. Lett.* 2001; 11:549. [PubMed: 11229768]
2. Williams SJ, Stapleton D, Zammit S, Kelly DJ, Gilbert RE, Krum H. Patent WO2008003141, 2008; *Chem. Abstr.* 2008; 148:144415.
3. Bo Y, Chakrabarti PP, Chen N, Doherty EM, Fotsch CH, Han N, Kelly MG, Liu Q, Norman MH, Wang X, Zhu J, Ognynanov V. Patent WO03049702, 2003; *Chem. Abstr.* 2003; 139:53025.
4. Harada H, Isaji M, Miyata H, Kusama H, Nonaka Y, Kamata K, Yazaki T, Hotei Y. Patent JP10330254, 1998; *Chem. Abstr.* 1998; 130:105331.
5. Harada H, Isaji M, Kusama H, Taketana Y, Nonaka Y, Kamata T, Futai Y. Patent JP10259129, 1998; *Chem. Abstr.* 1998; 129:321163.
6. Harada H, Kusama H, Nonaka Y, Kamata K, Fotei Y. Patent JP10306024, 1998; *Chem. Abstr.* 1998; 130:47477.

7. Raffa D, Maggio B, Plescia F, Cascioferro S, Plescia S, Raimondi MV, Daidone G, Tolomeo M, Grimaudo S, Di Cristina A, Pipitone RM, Bai R, Hamel E. *Eur. J. Med. Chem.* 2011; 46:2786. [PubMed: 21530013]
8. Raffa D, Maggio B, Raimondi MV, Cusimano MG, Amico G, Carollo A, Conaldi PG, Bai R, Hamel E, Daidone G. *Eur. J. Med. Chem.* 2013; 65:427. [PubMed: 23747810]
9. Akritopoulou-Zanze I, Darczak D, Sarris K, Phelan KM, Huth JR, Song D, Johnson EF, Jia Y, Djuric SW. *Bioorg. Med. Chem. Lett.* 2013; 16:96. [PubMed: 16216502]
10. Inglis SR, Zervosen A, Woon ECY, Gerards T, Teller N, Fischer DS, Luxen A, Schofield CJ. *J. Med. Chem.* 2009; 52:6097. [PubMed: 19731939]
11. Tegley CM, Viswanadhan VN, Biswas K, Frohn MJ, Peterkin TAN, Chang C, Bürlü RW, Dao JH, Veith H, Rogers N, Yoder SC, Biddlecome G, Tagari P, Allen JR, Hungate RW. *Bioorg. Med. Chem. Lett.* 2008; 18:3925. [PubMed: 18579373]
12. Patch RJ, Baumann CA, Liu J, Gibbs AC, Ott H, Lattanze J, Player MR. *Bioorg. Med. Chem. Lett.* 2006; 16:3282. [PubMed: 16580199]
13. Beaulieu PL, Anderson PC, Cameron DR, Croteau G, Gorys V, Grand-Maitre C, Lamarre D, Liard F, Paris W, Plamondon L, Soucy F, Thibeault D, Wernic D, Yoakim C. *J. Med. Chem.* 2000; 43:1094. [PubMed: 10737742]
14. Raffa D, Daidone G, Maggio B, Cascioferro S, Plescia F, Schillaci D. *Il Farmaco.* 2004; 59:215. [PubMed: 14987985]
15. Daidone G, Maggio B, Raffa D, Plescia S, Schillaci D, Raimondi MV. *Il Farmaco.* 2004; 59:413. [PubMed: 15120321]
16. Maggio B, Raffa D, Raimondi MV, Cusimano MG, Plescia F, Cascioferro S, Cancemi G, Lauricella M, Carlisi D, Daidone G. *Eur. J. Med. Chem.* 2014; 72:1. [PubMed: 24333609]
17. Maggio B, Raimondi MV, Raffa D, Plescia F, Cascioferro S, Cancemi G, Tolomeo M, Grimaudo S, Daidone G. *Eur. J. Med. Chem.* 2015; 96:98. [PubMed: 25874335]
18. Purandare AV, Gao A, Wan H, Somerville J, Burke K, Seachord C, Vaccaro W, Witjak J, Poss MA. *Bioorg. Med. Chem. Lett.* 2005; 15:2669. [PubMed: 15863339]
19. Compounds 17b, 17c, 17d and 17g were known (CAS numbers 349474-45-9, 328001-92-9, 349637-46-3 and 708981-97-09, respectively), but no references were found on SciFinder Scholar
20. Palmer MH, McVie GJ. *J. Chem. Soc. B.* 1968:745.
21. Sardon T, Cotting T, Xu J, Giannis A, Vernos I. *ChemBioChem.* 2009; 10:464. [PubMed: 19199284]
22. Dolzhenco-Podchezertseva AV, Korkodinova LM, Vaslyuc MV, Koteyov VP. *Pharm. Chem. J.* 2002; 36:647.
23. Boyd MR, Paull KD. *Drug Dev. Res.* 1995; 34:91.
24. Wong RS. Y. *J. Exp. Clin. Cancer Res.* 2011; 30:87.
25. D'Anneo A, Carlisi D, Lauricella M, Emanuele S, Di Fiore R, Vento R, Tesoriere G. *J. Cell. Physiol.* 2013; 228:952. [PubMed: 22688575]
26. Pietkiewicz S, Schmidt JH, Lavrik IN. *J. Immunol. Methods.* 2015; S0022-1759:00152.
27. Shalini S, Dorstyn L, Dawar S, Kumar S. *Cell Death Differ.* 2015; 22:526. [PubMed: 25526085]
28. Ramon RS, Bosson J, Diez-Gonzales S, Marion N, Nolan SP. *J. Org. Chem.* 2010; 75:1197. [PubMed: 20102222]
29. Shill AK, Sharma D, Guha NR, Das P. *Tetrahedron Lett.* 2012; 53:4858.
30. Rahaim RJ Jr, Maleckza RE Jr. *Synthesis.* 2006; 19:3316.
31. Chiruvella KK, Kari V, Choudhary B, Nambiar M, Ghanta RG, Raghavan SC. *FEBS Lett.* 2008; 582:4066. [PubMed: 19022252]
32. Freimoser FM, Jakob CA, Aebi M, Tuor U. *Appl. Environ. Microbiol.* 1999; 65:3727. [PubMed: 10427074]
33. Carlisi D, Lauricella M, D'Anneo A, Buttitta G, Emanuele S, Di Fiore R, Martinez R, Rolfo C, Vento R, Tesoriere G. *J. Cell. Physiol.* 2015; 230:1276. [PubMed: 25370819]

Figure 1.
The most active compounds among the previously synthesized benzamides.^{7,8}

Figure 2.
Some examples of active compounds from the literature bearing the phenoxyacetamido scaffold.

Figure 3. ¹H NMR spectrum of **17b** in (a) chloroform, (b) DMSO-*d*₆ and (c) DMSO-*d*₆ performed at 90 °C.

Figure 4.

Effects of **17j** and **17u** on cell morphology. Cells (8×10^3 /well) were treated with 10 μ M compound for 48 h, then morphology was observed under light microscopy at 200 $\times$ magnification: (a) control, (b) cells treated with 10 μ M **17j**, (c) cells treated with 25 μ M **17j**, (d) cells treated with 10 μ M **17u**, (e) cells treated with 25 μ M **17u**.

Figure 5.

Cell cycle distribution of K562 cells after exposure to **17j** or **17u**. Cells (1.5×10^5) were incubated with compounds for 24 or 48 h (upper and lower panels, respectively). Then, cells were harvested, stained with PI and subjected to flow cytometry. Percentages of cells in the sub G0/G1 region was considered as an index of apoptosis. At least 10,000 events per sample were measured: (a) control, (b) cells treated with 10 μ M of **17j** for 24 or 48 h, (c) cells treated with 10 μ M of **17u** for 24 or 48 h (upper and lower panels, respectively).

Figure 6.

Fluorescence micrographs showing the effects of **17j** and **17u** on K562 cells after Hoechst 33342 staining. K562 cells (8×10^3 /well) were stained with Hoechst 33342 (2.5 μ g/mL medium; blue fluorescence) and then treated with 10 μ M of **17j** or **17u** for 48 h. Cell morphology was visualized with a Leica DC 300F microscope with a fluorescence filter for DAPI. The images show details of cells with clear signs of apoptosis, such as nuclear shrinkage and condensed and fragmented chromatin: (a) control, (b) cells treated with 10 μ M **17j** for 48 h, (c) cells treated with 10 μ M **17u** for 48 h.

Figure 7.

Flow cytometric analysis of apoptotic effects of **17j** and **17u** in K562 cells. Cells were treated with compounds, then 10^5 cells/sample were analyzed by flow cytometry for PS externalization after double staining with annexin V and PI. To distinguish early apoptotic from late apoptotic cells, the analysis was performed after a 24 h incubation with the compounds. The different cell populations are reported in the C1–C4 areas. The C1 area represents necrotic cells, the C2 area represents late apoptotic cells, the C3 area represents viable cells and the C4 area early apoptotic cells. Analysis of fluorescence intensity of gated cells in FL-1 (annexin V-FITC) and FL-3 (PI) channels was from 10,000 events: (a) control, (b) cells treated with 10 μ M **17j**, (c) cells treated with 10 μ M **17u**. The results shown are representative of three independent experiments.

Figure 8.

The effect of **17j** and **17u** on pro-caspase 3 and pro-caspase 6 expression. K562 cells (1.5×10^5 /well) were treated for 48 h with 10 μ M **17j** or **17u**. Cell extracts were prepared and, following gel electrophoresis, subjected to Western blotting analysis to determine the levels of pro-caspases 3 and 6. Equal loading of the gel was verified by immunoblotting for β -actin. (a) Cells treated with 10 μ M **17j**. (b) Cells treated with 10 μ M **17u**. The results are representative of three independent experiments.

Scheme 1.

Synthetic pathway for the formation of 2-[(phenoxyacetyl)amino]benzamides **17a–v**.

Reagents and conditions: (i) SOCl₂, (ii) aqueous NH₃, (iii) I₂, aqueous NaHCO₃, (iv) SnCl₂, HCl.

Scheme 2.

Synthetic pathway for the formation of benzamides **20a,b**, 4-(2-phenoxyacetamido)benzamide (**21**) and 3-(2-phenoxyacetamido)benzamide (**22**).

Table 1

Percent growth inhibition of compounds **17a–v** at 10 μ M and IC₅₀ values (μ M) for compounds that exhibited at least 50% growth inhibition in the 10 μ M screening assay against the K562 cell line

Compd	10 μ M	IC ₅₀ (μ M)
17a	57.4 $\pm$ 3.4	8.9 $\pm$ 0.9
17b	19.7 $\pm$ 2.0	
17c	ns	
17d	ns	
17e	ns	
17f	87.0 $\pm$ 4.3	1.3 $\pm$ 0.1
17g	75.9 $\pm$ 1.9	1.61 $\pm$ 0.7
17h	24.1 $\pm$ 0.2	
17i	18.8 $\pm$ 1.0	
17j	73.0 $\pm$ 0.7	0.16 $\pm$ 0.02
17k	49.8 $\pm$ 3.5	
17l	74.7 $\pm$ 1.2	6.9 $\pm$ 0.3
17m	72.0 $\pm$ 1.1	4.7 $\pm$ 0.6
17n	45.6 $\pm$ 0.4	
17o	30.3 $\pm$ 1.8	
17p	35.4 $\pm$ 0.9	
17q	30.8 $\pm$ 1.9	
17r	85.3 $\pm$ 3.2	1.1 $\pm$ 0.17
17s	40.1 $\pm$ 08	
17t	48.1 $\pm$ 0.7	
17u	72.8 $\pm$ 3.1	0.58 $\pm$ 0.03
17v	17.7 $\pm$ 0.1	
21	23.7 $\pm$ 0.8	
22	ns	
Colchicine	63.6 $\pm$ 1.7	0.02 $\pm$ 0.003

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

Table 2Comparison of IC₅₀ values (μM) in HuDe and K562 cells

Compd	HuDe	K562	HuDe/K562
17a	523.0 ± 7.8	8.9 ± .09	58.76
17f	807.8 ± 11.7	1.3 ± 0.1	621.38
17g	27.3 ± 3.9	1.61 ± 0.7	16.96
17j	318.0 ± 15.7	0.16 ± 0.02	1987.5
17k	355.7 ± 12.1	10 ± 1.5	35.57
17l	33.8 ± 2.3	6.9 ± 0.3	4.9
17m	99.3 ± 1.4	4.7 ± 0.6	21.13
17r	941.9 ± 4.7	1.1 ± 0.17	856.28
17u	162.7 ± 1.9	0.58 ± 0.03	280.52
Colchicine	0.017 ± 0.001	0.02 ± 0.003	0.85

Values are the means of at least three independent determinations.

Table 3

Overview of the results of the in vitro single high dose (10^{-5} M) antitumor screening for compounds **17f**, **17j**, **17r** and **17u**^a

Compd	Growth percent ^b MG_MID ^c
17f	46.40
17j	24.31
17l	87.28
17r	25.81
17u	27.38

^aData obtained from the NCI's in vitro disease-oriented human tumor cells screen.

^bGrowth percent is the percent of growth inhibition at the single high dose of 10^{-5} M.

^cMG_MID = mean graph midpoint = arithmetical mean value for all tested cancer cell lines.

Table 4Results of multi-dose growth inhibition assay (GI₅₀, μ M) for compounds **17f**, **17j**, **17r** and **17u**

Cell line		17f	17j	17r	17u
Leukemia	CCRF-CEM	3.07	0.382	0.487	0.562
	HL-60 (TB)	3.29	0.339	0.340	0.293
	K-562	0.854	0.161	0.245	0.310
	MOLT-4	8.14	1.10	2.00	2.91
	RPMI-8226	4.33	0.553	1.17	2.27
	SR	0.777	0.221	0.392	0.515
Non-small cell lung cancer	A549/ATCC	7.06	0.732	0.908	0.917
	HOP-62	5.88	0.824	1.01	1.75
	HOP-92	5.70	1.50	0.414	17.7
	NCI-H226	>100	4.94	21.5	42.8
	NCI-H23	5.90	0.657	1.38	1.32
	NCI-H322M	7.09	0.705	2.10	5.77
	NCI-H460	3.00	0.425	0.484	0.529
	NCI-H522	3.78	0.630	1.19	0.410
Colon cancer	COLO 205	2.51	0.353	0.487	0.453
	HCC-2998	12.1	2.95	2.34	3.33
	HCT-116	2.72	0.517	0.518	0.620
	HCT-15	1.57	0.253	0.432	0.804
	HT29	1.74	0.349	0.388	0.437
	KM12	2.13	0.308	0.388	0.443
	SW-620	2.58	0.454	0.448	0.646
CNS cancer	SF-268	9.89	0.934	2.42	6.77
	SF-295	1.96	0.223	0.650	0.930
	SF539	1.80	0.249	0.441	0.563
	SNB-19	11.7	0.913	3.20	3.31
	SNB75	1.81	0.215	0.245	0.547
	U251	2.34	0.396	0.664	0.777
Melanoma	LOX IMVI	8.33	0.822	0.869	1.81
	M14	2.21	0.389	0.514	4.00
	MDA-MB-435	0.356	0.101	0.121	0.614
	SK-MEL-2	5.08	0.463	0.611	0.203
	SK-MEL-28	6.25	1.79	0.502	24.2
	SK-MEL-5	7.45	0.533	1.68	0.766
	UACC-257	>100	41.7	>100	>100
	UACC-62	5.42	0.701	1.05	2.52
Ovarian cancer	IGROV1	5.60	1.37	4.75	4.07
	OVCAR-3	2.30	0.321	0.375	0.472
	OVCAR-4	>100	nt	>100	>100
	OVCAR-5	3.86	1.68	5.40	15.0

Cell line		17f	17j	17r	17u
Renal cancer	OVCAR-8	7.06	0.904	3.13	3.01
	NCI/ADR-RES	1.43	0.344	0.418	0.487
	SK-OV-3	8.15	3.17	2.99	3.58
	786-0	9.54	1.07	2.10	7.32
	A498	1.41	1.02	0.304	2.77
	ACHN	10.7	1.32	2.25	8.12
	CAKI-1	4.18	0.519	0.721	3.80
	RXF 393	3.93	0.413	1.40	1.69
	SN12C	17.0	0.945	3.09	3.12
Prostate cancer	TK-10	63.8	8.11	23.8	14.4
	UO-31	17.5	2.87	8.72	13.5
	PC-3	3.22	0.428	0.464	2.23
	DU-145	6.01	0.444	1.87	0.914
Breast cancer	MCF7	1.81	0.230	0.497	0.661
	MDA-MB-231	9.01	1.28	2.03	2.39
	HS 578T	4.65	0.630	0.592	0.771
	BT-549	nt	nt	nt	0.770
	T-47D	6.87	0.556	nt	3.46
	MDA-MB-468	1.742	0.351	0.345	0.848

nt = not tested.

Table 5

Overview of the results of the in vitro antitumor screening for compounds **17f,j,r,u**^a

Compd	No. Studied ^e	pGI ₅₀ ^b			pTGI ^c			pLC ₅₀ ^d		
		No. giving positive results ^e	Range	MG_MID ^f	No. giving positive results ^e	Range	MG_MID ^f	No. giving positive results ^e	Range	MG_MID ^f
17f	57	54	6.45–4.00	5.31	10	5.65–4.00	4.14	1	4.13–4.00	4.00
17j	57	57	7.00–4.38	6.16	25	6.46–4.00	4.45	8	4.84–4.00	4.05
17r	56	54	6.92–4.00	6.05	25	6.20–4.00	4.31	2	4.50–4.00	4.01
17u	58	56	6.69–4.00	5.71	23	6.06–4.00	4.31	5	4.70–4.00	4.03

^aData obtained from the NCI's in vitro disease-oriented human tumor cell screen.

^bpGI₅₀ is the –log of the molar concentration that inhibits 50% net cell growth.

^cpTGI is the –log of the molar concentration giving total growth inhibition.

^dpLC₅₀ is the –log of the molar concentration leading to 50% net cell death.

^eRefers to the number of cell lines for which GI₅₀, TGI or LC₅₀, respectively, are 100 μM.

^fMG_MID = mean graph midpoint = arithmetical mean value for all tested cancer cell lines. If the indicated effect was not attainable within the used concentration interval, the highest tested concentration was used for the calculation.