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

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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 appropriate5-*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.

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

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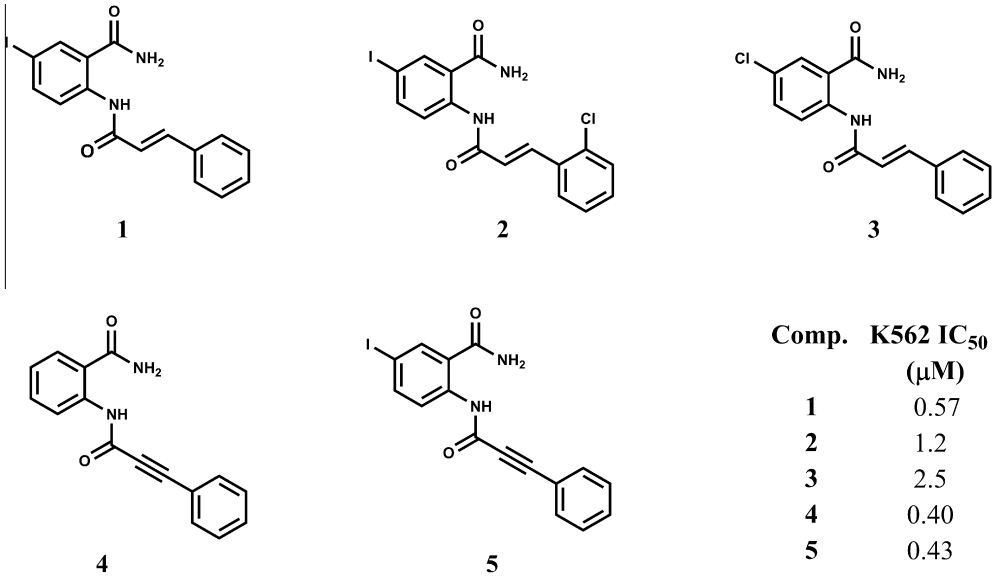

Comp.	K562 IC_{50} (μM)
1	0.57
2	1.2
3	2.5
4	0.40
5	0.43

Figure 1. The most active compounds among the previously synthesized benzamides.[7,8]

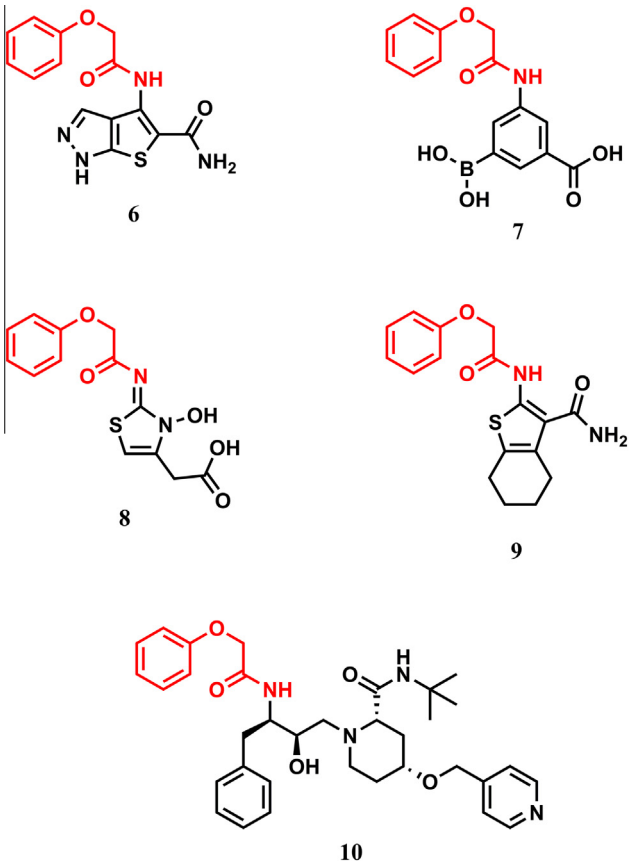

Figure 2. Some examples of active compounds from the literature bearing the phenoxyacetamido scaffold.

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-methylanthranilamide (**15d**), the 5-methoxyanthranilamide (**15e**) and the 4,5-dimethoxyanthranilamide (**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 ^{1}H 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 D_2O, for the amidic NH. The signals of the NH_2 benzamido protons, exchangeable with D_2O, 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 NH_2 moiety at 6.07 ppm. In DMSO-d_6 as solvent, the presence of an intramolecular hydrogen bond rendered the benzamido NH_2 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 ^{1}H 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 ^{1}H 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 ^{1}H 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