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Short communication

Cytotoxic Mannich bases of 6-(3-aryl-2-propenoyl)-2(3*H*)-benzoxazolones

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Abstract

A series of 12 new Mannich bases with chalcone core structure were synthesized as potential antineoplastic agents, via N-aminomethylation of two parent 6-(3-aryl-2-propenoyl)-2(3*H*)-benzoxazolones. The newly synthesized compounds as well as the chalcone prototypes were evaluated for cytotoxicity in the human pre-B-cell leukemia cell line BV-173 using the MTT-dye reduction assay. The tested compounds exhibited concentration-dependent cytotoxic effects at low micromolar concentrations. Ten of the Mannich bases characterized by significant activity in BV-173 were further evaluated against the chronic myeloid leukemia cell line K-562 and were found to suppress the growth of these cells at relatively higher concentrations as compared to the former tumor model. Selected Mannich bases induced programmed cell death in BV-173 at a concentration of 2.5 μM as evidenced by the encountered DNA-laddering. Taken together our data suggest that the presented heterocyclic chalcone derived Mannich bases necessitate detailed pharmacological evaluation in order to define further the structure activity relationships, in a larger spectrum of tumor models and to elucidate the mechanisms implicated in the observed cytotoxic effects.
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Keywords: Chalcone; 2(3*H*)-Benzoxazolone; Mannich base; Cytotoxicity; MTT assay; Apoptosis

1. Introduction

Chalcone (1,3-diaryl-2-propen-1-one) derivatives constitute an important group of secondary metabolites found ubiquitously in the plant kingdom. These compounds exhibit a constellation of biological activities e.g. analgesic, anti-inflammatory, antibacterial, antimycotic, antiviral, antiprotozoal, chemopreventive, anticancerous, etc [1–5]. The complex pharmacological activities together with the easy synthetic reproduction and derivatization of the core structure **1** (depicted in Fig. 1) have solicited considerable interest towards the exploitation of the unique chalcone template for the discovery of prospective lead compounds [1,3].

The tumor inhibiting properties of chalcones pose special interest and have been found to arise from their effects on malignant cell proliferation, tumor angiogenesis and/or on the established neoplastic vasculature. In contrast to the majority of conventional cytotoxic agents the chalcones are characterized by a low propensity to interact with DNA, which to a great extent eliminates the risk of mutagenicity and carcinogenicity common in various chemotherapeutics [1]. This favorable toxicological profile has solicited intensive research with natural and synthetic chalcones in view of developing novel, patient-friendly antineoplastic agents [1,3,6]. The mechanisms underlying the antiproliferative effects of chalcones appear to be complex and frequently multimodal and include binding to tubulin with consequent disruption of the microtubule assembly [7–9], interference with the p-53-MDM2 interactions [10], tyrosine kinase inhibition [11], depletion of cellular thiol depots [1], and/or triggering of

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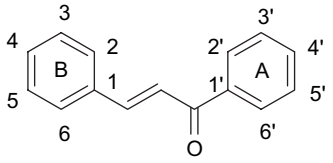


Fig. 1. Structure of the chalcone (1,3-diphenyl-2-propen-1-one), indicating the ring A and ring B atom numbering.

apoptotic cell-signaling pathways [3,12]. Owing to the complex, multimodal pharmacodynamics and the structural diversity of synthetic and natural chalcones, the structural features, affording optimal cytotoxicity vary with the predominant mode of action and there are no generally applicable structure activity rules, valid for the various classes of chalcone analogues [3]. Nevertheless the abundance of the enone system and the substitution of the aryl moieties appear to be crucial for the cytotoxic activity of chalcones [1,3,8,9].

In addition to the plethora of chalcone derivatives bearing hydroxy, methoxy and/or methyl functions in the aryl rings that have been designed as potential cytotoxic agents [7,13], attention has been brought to the development of tumor inhibiting chalcones via introduction of nitrogen-containing substituents as well. Thus, a series of Mannich bases with 1,3-diaryl-2-propen-1-one core structure [1] and more recently 4′-aminochalcones and related Schiff bases and maleamic acids have been reported to exert considerable cytotoxic activity at low micromolar concentrations against P388 and L1210 murine leukemia cell lines [14]. In line with these advances we prepared a series of 12 Mannich bases, derived from a chalcone template with a condensed oxazole ring and evaluated their cytotoxic effects in vitro.

2. Chemistry

Twelve new 3-(aminomethyl)-6-(3-aryl-2-propenoyl)-2(3*H*)-benzoxazolone derivatives (compounds **4a**–**l**) were synthesized. The synthesis of Mannich bases was carried out by the sequence of reactions summarized in Scheme 1.

2(3*H*)-Benzoxazolone was acylated in $AlCl_3$–DMF with acetyl chloride by a modified method of Friedel–Crafts. In acylation, since both the 3-nitrogen and 1-oxygen atoms are electron-donating, both 5- and 6-positions are activated and therefore, the regioselectivity in the C-acylation of 2(3*H*)-benzoxazolone cannot be predicted. However, it was reported that the substitution was directed by the nitrogen atom of 2(3*H*)-benzoxazolone ring and only the 6-acyl derivative was formed [15].

The chalcone **3a** and its 4-methoxy analogue **3b** was synthesized by a classic Claisen–Schmidt condensation of 6-acetyl-2(3*H*)-benzoxazolone **2** with benzaldehyde and 4-methoxybenzaldehyde [16].

The Mannich reaction was used for the synthesis of compounds **4a**–**l**. In this method the initial compounds **3a** and **3b** are heated in ethanol with 37% formaldehyde solution and secondary amines. Even though the classical literature suggests using acid or base as catalysts in some applications of the Mannich reaction, this was not necessary in our study as reported previously [17]. The physical properties were determined and structures were confirmed by IR, NMR and elemental analyses.

All spectral data are in accordance with the assumed structures. IR spectra of the compounds showed no absorption bands at 3100–3400 cm^{-1}, indicating the absence of an NH-group, which is an evidence for the addition reaction. The lactam and ketone C=O stretching bands were seen at about 1760 cm^{-1} and 1650 cm^{-1}.

In ^1H NMR, methylene protons between the two nitrogen atoms in 2(3*H*)-benzoxazolone were observed as a singlet at 4.65 ppm. The aromatic ring protons were observed at 7.2–8.0 ppm. All of the synthesized Mannich bases were initially *E*-conformers, which was proved by coupling constant value $J = 15.6$ Hz for vinyl protons, observed at 7.4–7.8 ppm. The details of protons belonging to the secondary amines are given in Section 5.

3. Pharmacology

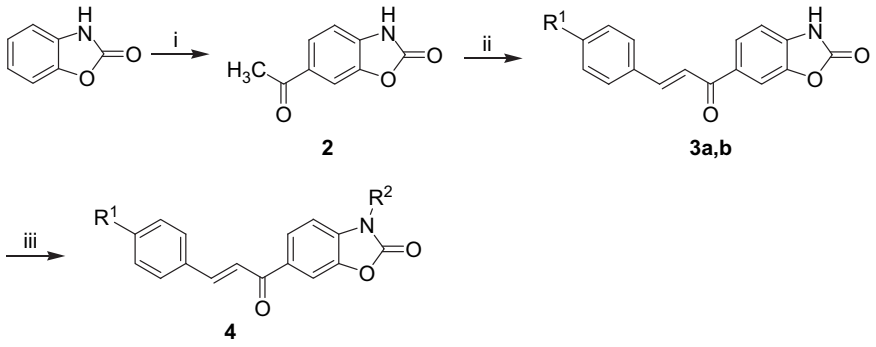
The chalcones **3a**, **b** and the derived Mannich **4a**–**l** bases under investigation were tested on the BV-173 pre-B-cell leukemia-derived cell line (following 72 h exposure) as a pre-screen test system, using the MTT-dye reduction assay. Selected compounds were thereafter evaluated on the chronic myeloid leukemia K-562 cells as well. The clinically utilized anticancer drugs etoposide and cisplatin were used as positive controls throughout the cytotoxicity determination studies.

As evident from the IC_{50} values, summarized in Table 1 all tested compounds exerted cytotoxic effects on BV-173 lymphoid cells in a concentration-dependent manner, causing 50% inhibition of the malignant cell proliferation at low micromolar concentrations, ranging between 4.7 and 18.4 μM.

The comparative evaluation of the N non-substituted compounds **3a** and **3b** (Scheme 1) revealed that the 4-methoxy function in the B-ring of the latter affords a substantial, 2-fold increase in the cytotoxic efficacy against BV-173. Such a trend was not unambiguously encountered with the Mannich bases and even more, in case of **4g** and **4h** the presence of the methoxy substituent caused significant decrease in potency than their corresponding analogues **4a** and **4b**.

The juxtaposition of the cytotoxic effects within non-methoxy substituted analogues **4a**–**f** and their prototype **3a** revealed that all of the Mannich bases bearing bulky basic substituents at the nitrogen of the oxazole ring were more potent than the prototype. Within the series of methoxy chalcones, namely **3b** and its N-substituted analogues **4g**–**l** the similar or even equivalent activity encountered thereof does not allow clear-cut structure activity relationships to be defined.

Compounds **4a**–**f** and **4i**–**l**, were tested on the chronic myeloid leukemia-derived K-562 cell line as well (Table 1). They induced 50% inhibition of the malignant cell growth at higher concentrations relative to their effects on BV-173. Most probably this is due to the higher expression of the non-receptor tyrosine kinase bcr-abl in K-562 as compared to BV-173,



Reagents and conditions: (i) CH_3COCl, $AlCl_3$-DMF; (ii) $R^1C_6H_4CHO$, aq. KOH, EtOH; (iii) secondary amine, 37% CH_2O, EtOH

Compound	R^1	R^2	Compound	R^1	R^2
3a	-H	-H	**3b**	$-OCH_3$	-H
4a	-H	$-CH_2-N(CH_3)_2$	**4g**	$-OCH_3$	$-CH_2-N(CH_3)_2$
4b	-H	$-CH_2-N$	**4h**	$-OCH_3$	$-CH_2-N$
4c	-H	$-CH_2-N$O	**4i**	$-OCH_3$	$-CH_2-N$O
4d	-H	$-CH_2-N$S	**4j**	$-OCH_3$	$-CH_2-N$S
4e	-H	$-CH_2-N$N-C_6H_4-OCH_3	**4k**	$-OCH_3$	$-CH_2-N$N-C_6H_4-OCH_3
4f	-H	$-CH_2-N$N-$CH_2-C_6H_5$	**4l**	$-OCH_3$	$-CH_2-N$N-$CH_2-C_6H_5$

Scheme 1. Schematic representation of the synthesis and chemical structures of the tested compounds: prototype chalcones **2** and **3** and the respective series of Mannich bases **4a–l**.

which renders the former cell line less responsive to pro-apoptotic stimuli including chemotherapeutic agents [18].

In order to elucidate the mechanisms underlying the established cytotoxicity the methoxy-compounds **4j** and **4k** were tested for their ability to trigger DNA fragmentation. Both compounds were applied at concentrations lower than their IC_{50} and were found to evoke a typical DNA-laddering phenomenon in BV-173 (Fig. 2). These results indicate that the induction of apoptosis at least partly mediates the cytotoxic activity of the investigated compounds as repeatedly reported for diverse chalcones and related plant polyphenols [3,12,19].

4. Conclusions

The results obtained from the conducted bioassay of the presented compounds give us reason to conclude that this series of heterocyclic chalcones represents a prospective class of cytotoxic agents. Notwithstanding the fact that the intimate molecular targets and the precise mode of action of these novel synthetic chalcones are yet to be determined, it is clearly evident from the encountered DNA-laddering that the induction of apoptosis at least partly mediates their cytotoxic activity. Currently novel large series of analogues have been synthesized and are subjected to pharmacological testing in view of defining prospective lead compounds for further evaluations.

5. Experimental protocols

5.1. Chemistry

Melting points were determined on a Boetius hot-stage microscope and were uncorrected. IR spectra (nujol) were

Table 1
Cytotoxic activity of tested heterocyclic chalcone compounds and the reference cytotoxic agents on BV-173 and K-562 cells after 72 h incubation (MTT assay)

Compound	IC_{50} value (μM) ± SD	
	BV-173	K-562
3a	18.4 ± 2.2	N.d.[a]
3b	9.2 ± 1.4	N.d.
4a	6.6 ± 0.9	27.2 ± 1.7
4b	8.2 ± 1.3	38.3 ± 3.3
4c	7.4 ± 1.7	31.7 ± 2.4
4d	7.9 ± 2.1	42.2 ± 3.1
4e	9.2 ± 2.7	30.1 ± 1.7
4f	9.4 ± 2.3	29.5 ± 2.8
4g	17.7 ± 3.1	N.d.
4h	15.3 ± 1.3	N.d.
4i	4.7 ± 1.1	22.7 ± 3.1
4j	6.1 ± 1.6	21.2 ± 3.9
4k	5.4 ± 1.2	12.4 ± 1.6
4l	6.2 ± 2.1	24.2 ± 2.7
Etoposide	0.9 ± 0.5	3.1 ± 1.3
Cisplatin	7.9 ± 1.8	29.2 ± 2.9

[a] Not determined.

recorded on a Specord 71 spectrometer. ^{1}H NMR spectra were recorded on a Bruker AM 250 operating in a field strength of 250 MHz. Chemical shifts are given in parts per million (δ) values downfield from TMS as internal standard and coupling constants (J) in Hz. The results of elemental analyses for C, H and N were within ±0.4% of the theoretical values.

5.1.1. 6-Acetyl-2(3H)-benzoxazolone ***2***

Dimethylformamide (13 ml, 172 mmol) was slowly added to aluminum chloride (80 g, 600 mmol). The mixture was stirred and maintained at 45 °C, while 2(3*H*)-benzoxazolone

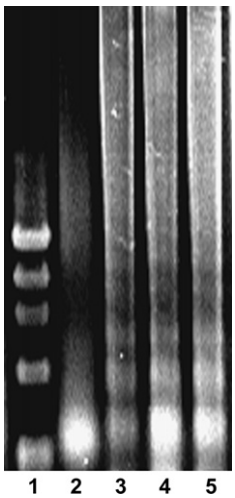


Fig. 2. Imaging of DNA-laddering, evidencing for induction of apoptosis, following treatment of BV-173 with **4j** and **4k** at a concentration lower than their IC_{50} values (2.5 μM). DNA was extracted from the cytosolic fraction of 1×10^6 exponentially growing BV-173 cells exposed for 24 h to either 2.5 μM **4j** (lane 3), 2.5 μM **4k** (lane 4) or to the reference agent etoposide (at 0.25 μM, lane 5); solvent treated cells served as controls (lane 2); lane 1 represents a DNA-laddering marker. Thereafter the isolated DNA was analyzed by 0.8% agarose gel electrophoresis, ethidium bromide staining and UV-transillumination.

(8.1 g, 60 mmol) and acetyl chloride (6.4 ml, 90 mmol) were added. The reaction mixture was heated at 80 °C under stirring for 2 h. Then mixture was poured on ice and conc. HCl (30 ml). The crude product was collected by filtration, air-dried and recrystallized from ethanol. Yield 6.7 g (63%); m.p. 227–228 °C [Ref. [20], m.p. 228 °C].

5.1.2. General procedure for preparation of 6-(3-aryl-2-propenoyl)-2(3H)-benzoxazolones ***3a, b***

Compounds **3a**, **b** were prepared following reported procedures [16]. To a solution of 6-acetyl-2(3*H*)-benzoxazolone (2.65 g, 10 mmol) in mixture of 10% aq. KOH (10 ml) and ethanol (5 ml), the appropriate aldehyde (11 mmol) was added. After stirring for a few hours at room temperature, the mixture precipitated. The mixture was poured on 200 ml water, warmed and acidified with 10% HCl. The crystalline product was filtered, washed to neutrality, dried and recrystallized from ethanol.

5.1.2.1. 6-(3-Phenyl-2-propenoyl)-2(3H)-benzoxazolone ***3a***. Yield 84%; m.p. 223–225 °C; IR: 3100–3300 (NH), 1765 (lactam C=O), 1655 (ketone C=O) cm^{-1}; ^{1}H NMR: 7.20 (d, 1H, arom. H, $J = 8.2$ Hz); 7.41 (m, 3H, arom. H); 7.53 (d, 1H, =C*H*CO, $J = 15.6$ Hz); 7.62 (m, 2H, arom. H); 7.84 (d, 1H, ArC*H*=, 15.6 Hz); 7.93 (d, 1H, arom. H, $J = 1.5$ Hz), 7.96 (dd, 1H, arom. H, $J = 1.5$ Hz, $J = 8.2$ Hz), 10.93 (br. 1H, NH).

5.1.2.2. 6-(3-(4-Methoxyphenyl)-2-propenoyl)-2(3H)-benzoxazolone ***3b***. Yield 81%; m.p. 209–211 °C; IR: 3100–3350 (NH), 1760 (lactam C=O), 1645 (ketone C=O) cm^{-1}; ^{1}H NMR: 3.85 (s, 3H, OCH_3); 6.94 (d, 2H, arom. H, $J = 8.7$ Hz); 7.24 (d, 1H, arom. H, $J = 8.2$ Hz); 7.40 (d, 1H, =CHCO, $J = 15.5$ Hz); 7.60 (d, 2H, arom. H, $J = 8.7$ Hz); 7.82 (d, 1H, ArCH=, $J = 15.5$ Hz); 7.98 (d, 1H, arom. H, $J = 1.4$ Hz); 7.95 (dd, 1H, arom. H, $J = 1.5$ Hz, $J = 8.2$ Hz), 11.14 (br. 1H, NH).

5.1.3. General procedure for preparation of Mannich bases ***4a–l***

A mixture of 6-(3-aryl-2-propenoyl)-2(3*H*)-benzoxazolone (0.27 g, 1 mmol), 37% formaldehyde solution (0.1 ml, 1.3 mmol) and ethanol (8 ml) was heated and secondary amine (1 mmol) was added. After standing for 24 h at room temperature the mixture crystallized, filtered off, washed with ethanol, dried and recrystallized from ethanol.

5.1.3.1. 3-(Dimethylaminomethyl)-6-(3-phenyl-2-propenoyl)-2(3H)-benzoxazolone ***4a***. Yield 53%; m.p. 225–227 °C; IR: 1766 (lactam C=O), 1653 (ketone C=O) cm^{-1}; ^{1}H NMR: 2.44 (s, 6H, CH_3); 4.64 (s, 2H, CH_2), 7.22 (d, 1H, arom. H, $J = 8.2$ Hz); 7.43 (m, 3H, arom. H); 7.52 (d, 1H, =C*H*CO, $J = 15.6$ Hz); 7.65 (m, 2H, arom. H); 7.85 (d, 1H, ArC*H*=, $J = 15.6$ Hz); 7.91 (d, 1H, arom. H, $J = 1.5$ Hz), 7.96 (dd, 1H, arom. H, $J = 1.5$ Hz, $J = 8.2$ Hz).

5.1.3.2. 6-(3-Phenyl-2-propenoyl)-3-(1-piperidinylmethyl)-2(3H)-benzoxazolone ***4b***. Yield 78%; m.p. 159–162 °C; IR: 1766

(lactam C=O), 1653 (ketone C=O) cm^{-1}; ^1H NMR: 1.44–1.59 (m, 6H, piperidine H-3, 4, 5); 2.66 (m, 4H, piperidine H-2, 6); 4.69 (s, 2H, CH_2); 7.22 (d, 1H, arom. H, $J = 8.2$ Hz); 7.43 (m, 3H, arom. H); 7.52 (d, 1H, =CHCO, $J = 15.6$ Hz); 7.65 (m, 2H, arom. H); 7.84 (d, 1H, ArCH=, $J = 15.6$ Hz); 7.89 (d, 1H, arom. H, $J = 1.4$ Hz); 7.96 (dd, 1H, arom. H, $J = 8.2$ Hz, $J = 1.5$ Hz).

5.1.3.3. 3-(4-Morpholinylmethyl)-6-(3-phenyl-2-propenoyl)-2(3H)-benzoxazolone ***4c***. Yield 67%; m.p. 177–180 °C; IR: 1766 (lactam C=O), 1653 (ketone C=O) cm^{-1}; ^1H NMR: 2.71 (m, 4H, morpholine H-3, 5); 3.71 (m, 4H, morpholine H-2, 6); 4.68 (s, 2H, CH_2); 7.20 (d, 1H, arom. H, $J = 8.2$ Hz); 7.43 (m, 3H, arom. H); 7.52 (d, 1H, =CHCO, $J = 15.6$ Hz); 7.65 (m, 2H, arom. H); 7.84 (d, 1H, ArCH=, $J = 15.6$ Hz); 7.91 (d, 1H, arom. H, $J = 1.4$ Hz); 7.96 (dd, 1H, arom. H, $J = 8.2$ Hz, $J = 1.5$ Hz).

5.1.3.4. 6-(3-Phenyl-2-propenoyl)-3-(4-thiomorpholinylmethyl)-2(3H)-benzoxazolone ***4d***. Yield 87%; m.p. 180–183 °C; IR: 1766 (lactam C=O), 1653 (ketone C=O) cm^{-1}; ^1H NMR: 2.68 (m, 4H, thiomorpholine H-2, 6); 3.00 (m, 4H, thiomorpholine H-3, 5); 4.70 (s, 2H, CH_2); 7.17 (d, 1H, arom. H, $J = 8.2$ Hz); 7.43 (m, 3H, arom. H); 7.52 (d, 1H, =CHCO, $J = 15.6$ Hz); 7.66 (m, 2H, arom. H); 7.85 (d, 1H, ArCH=, $J = 15.6$ Hz); 7.90 (d, 1H, arom. H, $J = 1.4$ Hz); 7.96 (dd, 1H, arom. H, $J = 8.2$ Hz, $J = 1.5$ Hz).

5.1.3.5. 3-((4-(2-Methoxyphenyl)-1-piperazinyl)methyl)-6-(3-phenyl-2-propenoyl)-2(3H)-benzoxazolone ***4e***. Yield 98%; m.p. 149–151 °C; IR: 1766 (lactam C=O), 1653 (ketone C=O) cm^{-1}; ^1H NMR: 2.95–3.09 (m, 8H, piperazine H); 3.82 (s, 3H, CH_3); 4.82 (s, 2H, CH_2); 6.84–6.92 (m, 4H, arom. H); 7.23 (d, 1H, arom. H, $J = 8.2$ Hz); 7.43 (m, 3H, arom. H); 7.52 (d, 1H, =CHCO, $J = 15.6$ Hz); 7.66 (m, 2H, arom. H); 7.85 (d, 1H, ArCH=, $J = 15.6$ Hz); 7.90 (d, 1H, arom. H, $J = 1.4$ Hz); 7.98 (dd, 1H, arom. H, $J = 8.2$ Hz, $J = 1.5$ Hz).

5.1.3.6. 3-((4-Benzyl-1-piperazinyl)methyl)-6-(3-phenyl-2-propenoyl)-2(3H)-benzoxazolone ***4f***. Yield 80%; m.p. 167–170 °C; IR: 1766 (lactam C=O), 1653 (ketone C=O) cm^{-1}; ^1H NMR: 2.50–2.77 (m, 8H, piperazine H); 3.51 (s, 2H, CH_2–Ar); 4.72 (s, 2H, CH_2); 7.17 (d, 1H, arom. H, $J = 8.2$ Hz); 7.28 (m, 5H, arom. H); 7.43 (m, 3H, arom. H); 7.52 (d, 1H, =CHCO, $J = 15.6$ Hz); 7.66 (m, 2H, arom. H); 7.84 (d, 1H, ArCH=, $J = 15.6$ Hz); 7.92 (d, 1H, arom. H, $J = 1.4$ Hz); 7.95 (dd, 1H, arom. H, $J = 8.2$ Hz, $J = 1.5$ Hz).

5.1.3.7. 3-(Dimethylaminomethyl)-6-(3-(4-methoxyphenyl)-2-propenoyl)-2(3H)-benzoxazolone ***4g***. Yield 72%; m.p. 139–142 °C; IR: 1766 (lactam C=O), 1640 (ketone C=O) cm^{-1}; ^1H NMR: 2.44 (s, 6H, NCH_3); 3.87 (s, 3H, OCH_3); 4.64 (s, 2H, CH_2); 6.95 (d, 2H, arom. H, $J = 8.7$ Hz); 7.20 (d, 1H, arom. H, $J = 8.2$ Hz); 7.40 (d, 1H, =CHCO, $J = 15.5$ Hz); 7.63 (d, 2H, arom. H, $J = 8.7$ Hz); 7.82 (d, 1H, ArCH=, $J = 15.5$ Hz); 7.98 (d, 1H, arom. H, $J = 1.4$ Hz); 7.94 (dd, 1H, arom. H, $J = 1.5$ Hz, $J = 8.2$ Hz).

5.1.3.8. 6-(3-(4-Methoxyphenyl)-2-propenoyl)-3-(1-piperidinylmethyl)-2(3H)-benzoxazolone ***4h***. Yield 86%; m.p. 158–160 °C; IR: 1766 (lactam C=O), 1640 (ketone C=O) cm^{-1}; ^1H NMR: 1.39–1.60 (m, 6H, piperidine H-3, 4, 5); 2.66 (m, 4H, piperidine H-2, 6); 3.86 (s, 3H, OCH_3); 4.69 (s, 2H, CH_2); 6.95 (d, 2H, arom. H, $J = 8.8$ Hz); 7.21 (d, 1H, arom. H, $J = 8.2$ Hz); 7.40 (d, 1H, =CHCO, $J = 15.6$ Hz); 7.61 (d, 2H, arom. H, $J = 8.8$ Hz); 7.82 (d, 1H, ArCH=, $J = 15.6$ Hz); 7.88 (d, 1H, arom. H, $J = 1.4$ Hz); 7.95 (dd, 1H, arom. H, $J = 1.5$ Hz, $J = 8.2$ Hz).

5.1.3.9. 6-(3-(4-Methoxyphenyl)-2-propenoyl)-3-(4-morpholinylmethyl)-2(3H)-benzoxazolone ***4i***. Yield 66%; m.p. 168–170 °C; IR: 1766 (lactam C=O), 1640 (ketone C=O) cm^{-1}; ^1H NMR: 2.71 (m, 4H, morpholine H-3, 5); 3.71 (m, 4H, morpholine H-2, 6); 3.86 (s, 3H, OCH_3); 4.68 (s, 2H, CH_2); 6.95 (d, 2H, arom. H, $J = 8.7$ Hz); 7.19 (d, 1H, arom. H, $J = 8.2$ Hz); 7.40 (d, 1H, =CHCO, $J = 15.6$ Hz); 7.62 (d, 2H, arom. H, $J = 8.7$ Hz); 7.82 (d, 1H, ArCH=, $J = 15.6$ Hz); 7.90 (d, 1H, arom. H, $J = 1.4$ Hz); 7.95 (dd, 1H, arom. H, $J = 1.5$ Hz, $J = 8.2$).

5.1.3.10. 6-(3-(4-Methoxyphenyl)-2-propenoyl)-3-(4-thiomorpholinylmethyl)-2(3H)-benzoxazolone ***4j***. Yield 95%; m.p. 181–185 °C; IR: 1766 (lactam C=O), 1640 (ketone C=O) cm^{-1}; ^1H NMR: 2.68 (m, 4H, thiomorpholine H-2, 6); 3.00 (m, 4H, thiomorpholine H-3, 5); 3.86 (s, 3H, OCH_3); 4.70 (s, 2H, CH_2); 6.95 (d, 2H, arom. H, $J = 8.8$ Hz); 7.16 (d, 1H, arom. H, $J = 8.2$ Hz); 7.39 (d, 1H, =CHCO, $J = 15.6$ Hz); 7.62 (d, 2H, arom. H, $J = 8.8$ Hz); 7.82 (d, 1H, ArCH=, $J = 15.6$ Hz); 7.89 (d, 1H, arom. H, $J = 1.4$ Hz); 7.95 (dd, 1H, arom. H, $J = 1.5$ Hz, $J = 8.2$).

5.1.3.11. 3-((4-(2-Methoxyphenyl)-1-piperazinyl)methyl)-6-(3-(4-methoxyphenyl)-2-propenoyl)-2(3H)-benzoxazolone ***4k***. Yield 86%; m.p. 142–144 °C; IR: 1766 (lactam C=O), 1640 (ketone C=O) cm^{-1}; ^1H NMR: 2.87–3.01 (m, 8H, piperazine H); 3.75 (s, 3H, CH_3); 3.79 (s, 3H, CH_3); 4.74 (s, 2H, CH_2); 6.75–6.94 (m, 4H, arom. H); 6.87 (d, 2H, arom. H, $J = 8.8$ Hz); 7.15 (d, 1H, arom. H, $J = 8.2$ Hz); 7.33 (d, 1H, =CHCO, $J = 15.5$ Hz); 7.54 (d, 2H, arom. H, $J = 8.8$ Hz); 7.74 (d, 1H, ArCH=, $J = 15.5$ Hz); 7.82 (d, 1H, arom. H, $J = 1.5$ Hz), 7.88 (dd, 1H, arom. H, $J = 1.5$ Hz, $J = 8.2$ Hz).

5.1.3.12. 3-((4-Benzyl-1-piperazinyl)methyl)-6-(3-(4-methoxyphenyl)-2-propenoyl)-2(3H)-benzoxazolone ***4l***. Yield 81%; m.p. 168–171 °C; IR: 1766 (lactam C=O), 1640 (ketone C=O) cm^{-1}; ^1H NMR: 2.47–2.74 (m, 8H, piperazine H-2, 6); 3.47 (s, 2H, CH_2–Ar); 3.85 (s, 3H, OCH_3); 4.71 (s, 2H, CH_2); 6.93 (d, 2H, arom. H, $J = 8.8$ Hz); 7.15 (d, 1H, arom. H, $J = 8.2$ Hz); 7.27 (m, 5H, arom. H); 7.37 (d, 1H, =CHCO, $J = 15.6$ Hz); 7.60 (d, 2H, arom. H, $J = 8.8$ Hz); 7.80 (d, 1H, ArCH=, $J = 15.6$ Hz); 7.86 (d, 1H, arom.

H, $J = 1.4$ Hz); 7.91 (dd, 1H, arom. H, $J = 1.5$ Hz, $J = 8.2$).

5.2. Pharmacology

5.2.1. Cell lines, culture conditions and cytotoxicity determination

All of the procedures regarding the cell culture maintenance, drug solution preparation and treatment of cells were carried out in a ''Heraeus'' laminar flow cabinet. The stock solutions of the referent antineoplastic agents – etoposide and cisplatin were prepared using the commercially available sterile dosage forms for clinical application, while the tested chalcones were dissolved in DMSO. The stock solutions were consequently diluted with RPMI-1640 medium to yield the desired final concentrations (in case of DMSO stocks at the final dilutions obtained, the concentration of the solvent never exceeded 0.5%). The human tumor cell lines exploited in this study, namely the pre-B-cell leukemia BV-173 and the chronic myeloid leukemia K-562 cell were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ GmbH, Braunschweig, Germany). The cells were grown as suspension cultures in a controlled environment: RPMI-1640 medium supplemented with 10% FBS and 2 mM L-glutamine at 37 °C in an incubator ''BB 16-Function Line'' Heraeus (Kendro, Hanau, Germany) with humidified atmosphere and 5% CO_2. Cells were maintained in log phase by supplementation with fresh medium after removal of cell suspension aliquots, two or three times a week.

The cytotoxic activity of the tested compounds was assessed by the MTT-dye reduction assay as described by Mosmann [21], with minor modifications [22]. For each concentration tested a set of six separate wells was used. The equieffective concentrations (IC_{50}) were calculated using GraphPad Prism software for PC. All cytotoxicity experiments were carried out in triplicate.

5.2.2. DNA fragmentation analysis

The cytosolic fraction DNA isolation and its electrophoretic analysis was performed as previously described [22].

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