Synthesis and cytotoxic activity of new heterocyclic analogues of resveratrol, containing benzoxazolone ring

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Dedicated to Acad. Bogdan Kurtev on the occasion of his 100th birth anniversary

New heterocycle analogues of resveratrol were designed and synthesized as potential anticancer agents. The compounds contain 3,5-dimethoxy- or 3,5-dihydroxystyryl fragment attached to the C5 or C6 position of a benzoxazolone ring. The compounds were tested for their cytotoxic activity against three human cancer cell lines (HL-60, MGF-7 and MDA-MB-321) and some of them were found to exhibit significant antiproliferative effect. Generally, the obtained 5-styrylbenzoxazolones were more active in compare to the corresponding 6-styrylbenzoxazolone positional isomers.

Key words: resveratrol; benzoxazolone; stilbene; cytotoxicity

INTRODUCTION

Resveratrol (Fig. 1) belongs to a group of naturally occurring polyphenols possessing the trans-stilbene scaffold. Found in more than 70 plants, the compound has been shown to exhibit a variety of health-beneficial properties such as antioxidant, anti-inflammatory, anti-diabetic, cardio- and neuroprotective activities [1–5]. Additionally, resveratrol has been recognized as a promising chemopreventive and anticancer agent due to its capability to inhibit tumorigenesis by modulation of several cellular process including apoptosis, cell cycle progression as well as angiogenesis [2, 6–9]. A number of methoxy derivatives of resveratrol have been also reported to exert high cytotoxicity against various human cancer cell lines [10–14]. Some of the synthetic analogues showed better activity compared to the natural compound [10, 11, 14].

In search of new anticancer agents, we have planned the synthesis of a small series of heterocyclic derivatives of resveratrol, in which the 4’-hydroxyphenyl moiety in the parent molecule was replaced with a benzoxazolone (Fig. 1).

Considered to be a "privileged scaffold" in medicinal chemistry, the benzoxazolone heterocyclic system has been extensively used in drug discovery as a phenol and pyrocatechol bioisostere [15]. The 3,5-dihydroxyphenyl fragment of the parent resveratrol molecule was left intact or replaced with a 3,5-dimethoxyphenyl moiety, with the aim to systematically evaluate the role of the isolated fragments on the biological activity of the compounds.

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Fig. 1. Chemical structure of resveratrol, 2(3H)-benzoxazolone and target 5- and 6-styryl-2(3H)-benzoxazolones.

Thus, in continuation of our previous studies on the synthesis of heterocyclic stilbenes [16], here we report the preparation of 5- and 6-(3,5-dimethoxy-
or 3,5-dihydroxy(styryl)-2(3H)-benzoxazolones as closely related resveratrol analogues. Their in vitro cytotoxicity was examined against three human cancer cell lines.

EXPERIMENTAL

Chemistry

Melting points (mp) were determined on a Boetius hot-stage microscope and are uncorrected. Infrared spectra (IR) were recorded on a Specord 71 spectrometer. 1H NMR spectra were obtained on a Bruker DRX250 or Bruker DRX400 spectrometers. Chemical shifts were reported in parts per million (ppm, δ) relative to the solvent peak (CDCl3, 7.26 ppm; DMSO-d6, 2.50 ppm; acetone-d6, 2.05 ppm). Elemental analyses (C, H, N) were performed on a Vario III microanalyzer (Elementar, Hanau, Germany). Analytical samples were prepared by sublimation.

H NMR spectroscopy was recorded on a Bruker DRX 250 or Bruker DRX 400 spectrometers. Chemical shift values were recorded relative to the solvent peak (CDCl3, 7.26 ppm, 50 MHz); δ 3.38 (s, 3H, Me). Elemental analyses (C, H, N) were performed on a Vario III microanalyzer (Elementar, Hanau, Germany). Analytical samples were prepared by sublimation.

General procedure for the synthesis of stilbene derivatives via Wittig reaction

A mixture of appropriate phosphonium bromide 1a-b (1.51 g, 3 mmol), 3,5-dimethoxybenzaldehyde (0.50 g, 3 mmol), powdered potassium carbonate (1.38 g, 10 mmol) and 18-crown-6 (0.01 g) in THF/DCM (20 mL, 2:1 v/v) was refluxed for 3 h (monitored by TLC). The inorganic salts were filtered off and the filtrate was concentrated under reduced pressure to obtain a mixture of the corresponding E- and Z-stilbenes and triphenylphosphine oxide. Both diastereomers were isolated by column chromatography using petroleum ether:acetone (10:1 v/v) as eluent.

(Z)-5-(3,5-Dimethoxystyril)-3-methyl-2(3H)-benzoxazolone (3a)

Yield: 48% (0.48 g), colourless oil. IR (capillary film, cm⁻¹): 1780 (C=O). 1H NMR (CDCl3, 250 MHz): δ 3.27 (s, 3H, NCH3), 3.67 (s, 6H, OCH3), 6.34 (t, 1H, ArH, J = 2.3 Hz), 6.39 (d, 2H, ArH, J = 2.3 Hz), 6.57 (s, 2H, =CH), 6.84 (br s, 1H, ArH), 7.04–7.05 (m, 2H, ArH). 1H NMR (acetone-d6, 500 MHz): δ 3.29 (s, 3H, NCH3), 3.65 (s, 6H, OCH3), 6.36 (t, 1H, ArH, J = 2.2 Hz), 6.42 (d, 2H, ArH, J = 2.1 Hz), 6.58 (d, 1H, =CH, J = 12.2 Hz), 6.65 (d, 1H, =CH, J = 12.2 Hz), 7.04–7.06 (m, 2H, ArH), 7.12 (d, 1H, ArH, J = 8.0 Hz). Anal. Calcd. for C18H17NO4 (311.34): C 69.44, H 5.50, N 4.50. Found: C 69.62, H 5.62, N 4.30.

(E)-5-(3,5-Dimethoxy(styril))-3-methyl-2(3H)-benzoxazolone (3a)

Yield: 43% (0.40 g), mp 164–165 °C. IR (nujol, cm⁻¹): 1760 (C=O). 1H NMR (CDCl3, 400 MHz): δ 3.44 (s, 3H, NCH3), 3.84 (s, 6H, OCH3), 6.41 (t, 1H, ArH, J = 2.1 Hz), 6.67 (d, 2H, ArH, J = 2.3 Hz), 6.99 (s, 1H, =CH, J = 16.2 Hz), 7.08 (s, 1H, =CH, J = 16.3 Hz), 7.12 (br s, 1H, ArH), 7.17 (d, 1H, ArH, J = 8.3 Hz), 7.23 (dd, 1H, ArH, J = 1.0 Hz, J = 8.3 Hz). Anal. Calcd. for C18H17NO4 (311.34): C 69.44, H 5.50, N 4.50. Found: C 69.21, H 5.37, N 4.53.

(Z)-6-(3,5-Dimethoxystyril)-3-methyl-2(3H)-benzoxazolone (3b)

Yield: 48% (0.48 g), mp 89–91 °C. IR (nujol, cm⁻¹): 1770 (C=O). 1H NMR (CDCl3, 250 MHz): δ 3.37 (s, 3H, NCH3), 3.68 (s, 6H, OCH3), 6.34 (t, 1H, ArH, J = 2.3 Hz), 6.39 (d, 2H, ArH, J = 2.3 Hz), 6.54 (s, 2H, =CH), 6.81 (d, 1H, ArH, J = 8.4 Hz), 7.09–7.13 (m, 2H, ArH). 1H NMR (acetone-d6, 250 MHz): δ 3.38 (s, 3H, NCH3), 3.67 (s, 6H, OCH3), 6.37 (t, 1H, ArH, J = 2.1 Hz), 6.42 (d, 2H, ArH, J = 2.1 Hz), 6.56 (d, 1H, =CH, J = 12.2 Hz), 6.64 (d, 1H, =CH, J = 12.2 Hz), 7.08 (d, 1H, ArH, J = 8.1 Hz), 7.11 (br s, 1H, ArH), 7.16 (dd, 1H, ArH, J = 1.1 Hz, J = 8.4 Hz). Anal. Calcd. for C18H17NO4 (311.34): C 69.44, H 5.50, N 4.50. Found: C 69.52, H 5.83, N 4.23.

(E)-6-(3,5-Dimethoxystyril)-3-methyl-2(3H)-benzoxazolone (3b)

Yield: 35% (0.33 g), mp 164–165 °C. IR (nujol, cm⁻¹): 1770 (C=O). 1H NMR (CDCl3, 400 MHz): δ 3.42 (s, 3H, NCH3), 3.84 (s, 6H, OCH3), 6.41 (t, 1H, ArH, J = 2.2 Hz), 6.66 (d, 2H, ArH, J = 2.2 Hz), 6.92–6.99 (m, 2H, =CH, ArH), 7.07 (s, 1H, =CH, J = 16.2 Hz), 7.31 (dd, 1H, ArH, J = 1.2 Hz, J = 8.1 Hz), 7.40 (br s, 1H, ArH). Anal. Calcd. for C18H17NO4 (311.34): C 69.44, H 5.50, N 4.50. Found: C 69.38, H 5.37, N 4.53.

General procedure for the demethylation of the methoxy groups with BBr3

Boron tribromide (1.7 M in DCM, 0.53 mL, 0.9 mmol) was added to a stirred solution of corresponding 3,5-dimethoxystyril (E)-stilbene 3a-b (0.16 g, 0.5 mmol) in anhydrous DCM (10 mL) at −10 °C. The resulting mixture was stirred for 1 h at −10 °C, allowed to warm to room temperature, and stirred for another 48 h. Then,
water (15 mL) was added and the obtained precipitate was filtered off and air-dried. The product was purified by recrystallization.

\[(E)-5(3,5-Dihydroxystyrlyl)-3-methyl-2(3H)-benzoxazole\ (4a)\]

Yield: 70% (0.10 g), mp 252–254 °C (ethanol). IR (nujol, cm\(^{-1}\)): 3200-3400 (OH), 1720 (C=O). \(^1\)H NMR (DMSO-\(d_6\), 400 MHz): \(\delta\) 3.38 (s, 3H, NCH), 5.16 (t, 1H, ArH, \(J = 2.1\) Hz), 4.63 (d, 2H, ArH, \(J = 2.1\) Hz), 7.08 (s, 2H, =CH), 7.29 (s, 2H, ArH), 7.58 (s, 1H, ArH), 9.26 (s, 2H, OH). \(^1\)H NMR (acetone-\(d_6\), 500 MHz): \(\delta\) 3.43 (s, 3H, NCH), 6.30 (br s, 1H, ArH), 6.57 (d, 2H, ArH, \(J = 1.9\) Hz), 7.09 (d, 1H, =CH, \(J = 16.3\) Hz), 7.13 (d, 1H, =CH, \(J = 16.3\) Hz), 7.21 (d, 1H, ArH, \(J = 8.2\) Hz), 7.29 (dd, 1H, ArH, \(J = 1.3\) Hz, \(J = 8.2\) Hz), 7.47 (br s, 1H, ArH), 8.46 (br s, 2H, OH). Anal. Calcd. for C\(_{17}\)H\(_{15}\)NO\(_4\): C 67.84, H 4.63, N 4.94. Found: C 67.54, H 4.81, N 4.71.

\[(E)-6(3,5-Dihydroxystyrlyl)-3-methyl-2(3H)-benzoxazole\ (4b)\]

Yield: 77% (0.11 g), mp 281–283 °C (acetone/water, 1:1 v/v). IR (nujol, cm\(^{-1}\)): 3150-3400 (OH), 1740 (C=O). \(^1\)H NMR (DMSO-\(d_6\), 400 MHz): \(\delta\) 3.35 (s, 3H, NCH), 6.14 (t, 1H, ArH, \(J = 2.1\) Hz), 6.42 (d, 2H, ArH, \(J = 2.1\) Hz), 7.05 (d, 2H, =CH), 7.23 (d, 1H, ArH, \(J = 8.1\) Hz), 7.42 (dd, 1H, ArH, \(J = 1.1\) Hz, \(J = 8.1\) Hz), 7.65 (d, 1H, ArH, \(J = 1.1\) Hz), 9.23 (s, 2H, OH). \(^1\)H NMR (acetone-\(d_6\), 500 MHz): \(\delta\) 3.41 (s, 3H, NCH), 6.29 (t, 1H, ArH, \(J = 2.0\) Hz), 6.57 (d, 2H, ArH, \(J = 2.0\) Hz), 7.05 (d, 1H, =CH, \(J = 16.3\) Hz), 7.12 (d, 1H, =CH, \(J = 16.3\) Hz), 7.16 (d, 1H, ArH, \(J = 8.1\) Hz), 7.40 (dd, 1H, ArH, \(J = 1.2\) Hz, \(J = 8.1\) Hz), 7.52 (d, 1H, ArH, \(J = 1.1\) Hz), 8.43 (br s, 2H, OH). Anal. Calcd. for C\(_{17}\)H\(_{15}\)NO\(_4\): C 67.84, H 4.63, N 4.94. Found: C 67.68, H 4.57, N 4.73.

**Biology**

Cytotoxicity tests were carried out on three tumor cell lines with different origin, namely HL-60 (human promyelocytic leukemia), MCF-7 (human breast cancer) and MDA-MB-231 (human breast adenocarcinoma). The cells were maintained as suspension type cultures (leukemia and adenocarcinoma) or as adherent culture (breast cancer) in controlled environment: RPMI-1640 medium, supplemented by 10% FBS and 2 mM L-glutamine at 37 °C in a "Heraeus" incubator with humidified atmosphere and 5% CO\(_2\). In order to keep cells in log phase, the cultures were refed with fresh RPMI-1640 medium two or three times a week.

Tested compounds were dissolved in DMSO and the solutions were diluted with RPMI-1640 medium to yield the desired final concentrations. Cytotoxicity of the compounds was assessed using the MTT-dye reduction assay [17], with minor modifications [18]. Exponentially growing cells were seeded in 96-well plates (100 μL/well at a density of 1 × 10^5 cells/mL). After 24 h incubation (37 °C, 5% CO\(_2\) and maximum humidity), they were exposed to various concentrations of the tested compounds (200, 50, 25, 12.5, 6.25 μM) for 72 h. Then, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution (10 mg/mL in PBS) was added (10 μL/well). Plates were further incubated for 3 hours at 37 °C. To dissolve the formazan crystals formed, 5% solution of formic acid in isopropanol (100 μL/well) was used. Absorption was measured on an ELISA reader at 580 nm. A mixture of 100 μL RPMI-1640 medium, 10 μL MTT stock and 100 μL 5% formic acid in isopropanol was used as control. For each concentration tested a set of six separate wells was used. The IC\(_{50}\) value (the concentration that inhibit 50% of cell growth) for each compound was calculated using OriginLab program.

**RESULTS AND DISCUSSION**

As depicted in Scheme 1, the synthesis of target stilbene derivatives 2a-b and 3a-b was achieved by applying the Wittig methodology on 3,5-dimethoxybenzaldehyde and the appropriate heterocyclic ylide, in turn obtained from the phosphonium bromides 1a-b in the presence of potassium carbonate and 18-crown-6. The reactions were carried out in THF/DCM at reflux for 3 h and produced the corresponding 3,5-dimethoxystyrlybenzoxazolones as mixtures of \(\pi\)-diastereomers. The pure \(Z\) and \(E\)-stilbenes (2a-b, respectively 3a-b) were separated by column chromatography. As the natural resveratrol is in the \(E\)-configuration, the obtained methoxy substituted \(E\)-stilbenes 3a-b were subjected to a reaction of demethylation with boron tribromide to afford 4a-b in high yields. The demethylation of the \(Z\)-isomers 2a-b in these conditions led to a mixture of products caused by additionally isomerization of the double bond.

The structures of all newly synthesized benzoxazolone-containing stilbene derivatives 2a-b – 4a-b were confirmed by \(^1\)H NMR spectroscopy. The geometry of the double bond was assigned on the basis of the coupling constants of the olefinic protons signals (\(J = 12.2\) Hz for \(Z\)-stilbene 2a-b.

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and \( J = 16.2 \) or 16.3 Hz for \( E \)-stilbene 3a-b and 4a-b). Consistent with the coupling constant data, both doublets for the olefinic protons of the Z-isomers appeared at 6.54–6.65 ppm whereas those for \( E \)-stilbenes shifted downfield to 6.99–7.13 ppm.

The synthesized heterocyclic analogues of resveratrol were tested in vitro for their cytotoxicity against three human cancer cell lines (HL-60, MCF-7 and MDA-MB-231), using MTT-dye reduction assay. As presented in Table 1, the obtained results showed that most derivatives exert weak antiproliferative effects on the studied cancer cells lines. Compound 2a bearing (Z)-3,5-dimethoxystyryl fragment on C5 position of benzoxazolone ring exhibited the highest activity with \( IC_{50} \) of 19 \( \mu \)M against HL-60, 42 \( \mu \)M against MCF-7 and 76 \( \mu \)M against MDA-MB-231 cells. The corresponding E-isomer 3a was inactive, but the hydroxy substituted \( E \)-stilbene 4a exerted a similar cytotoxic potential as 2a.

**Table 1.** Cytotoxic effects (expressed as IC\(_{50}\)) of compounds 2a-b – 4a-b on HL-60, MCF-7 and MDA-MB-231 cell lines.

<table>
<thead>
<tr>
<th>Compd</th>
<th>( IC_{50} (\mu M) \pm SD )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HL-60</td>
</tr>
<tr>
<td>2a</td>
<td>19±1.1</td>
</tr>
<tr>
<td>2b</td>
<td>13±1.3</td>
</tr>
<tr>
<td>3a</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>3b</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>4a</td>
<td>38±1.7</td>
</tr>
<tr>
<td>4b</td>
<td>&gt; 200</td>
</tr>
</tbody>
</table>

These results showed that the biological activity of the compounds 2a-b – 4a-b was influenced by the position of the styryl fragment in a benzoxazolone ring as the obtained 5-styrylbenzoxazolones were generally more active in compare to the corresponding 6-styrylbenzoxazolone positional isomers. Disregarding the configuration of the double bond in tested derivatives, the introduction of 3,5-dimethoxystyril or 3,5-dihydroxystyril moiety on C5 position of the heterocyclic system led to compounds closely resembling resveratrol.

**CONCLUSION**

In this study we reported the synthesis of six heterocycle analogues of resveratrol, containing a benzoxazolone ring. Evaluation of the cytotoxicity of the stilbene derivatives on HL-60, MCF-7 and MDA-MB-231 cancer cell lines showed that (Z)-3-methyl-5-(3,5-dimethoxystyril)-2(3H)-benzoxazolone (2a) and (E)-3-methyl-5-(3,5-dihydroxystyril)-2(3H)-benzoxazolone (4a) were the most active in the series.

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M. S. Gerova et al.: Synthesis and cytotoxic activity of new heterocyclic analogues of resveratrol, containing benzoazolone ring

СИНТЕЗ И ЦИТОТОКСИЧНА АКТИВНОСТ НА НОВИ ХЕТЕРОЦИКЛНИ АНАЛОЗИ НА РЕСВЕРАТРОЛ, СЪДЪРЖАЩИ БЕНЗОКСАЗОЛОНОВ ПРЪСТЕН

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(Резюме)

Синтезирани са нови хетероциклени аналоги на ресвератрол като потенциални противоракови средства. Съединенията съдържат 3,5-диметокси- или 3,5-дицидроксистерицифрагмент, въведен в позиция C5 или С6 на бензоксазолов пръстен. Цитотоксичната активност на съединенията е изследвана върху три туморни клетъчни линии (HL-60, MGF-7 и MDA-MB-321) и получените резултати показват, че някои от тях проявяват добър антипролиферативен ефект. В повечето случаи, 5-стирилбензосазоловите са по-активни в сравнение с техните позиционни изомери, съответните 6-стирилбензосазолони.