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Synthesis and cytotoxic activity of 1,3-benzodioxole derivatives. Note II

Nicola Micale *, Maria Zappalà, Silvana Grasso

Dipartimento Farmaco-Chimico, Università di Messina, Viale Annunziata, Messina 98168, Italy

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Abstract

A series of 1,3-benzodioxoles (**2**–**12**) were synthesized and evaluated for their in vitro ability to inhibit the growth of three human tumor cell lines. No cytotoxic effects were noticed with any of the test compounds at a concentration of 10^{-4} M.

Keywords: 1,3-Benzodioxoles; Cytotoxic activity

1. Introduction

1,3-Benzodioxole moiety occurs commonly in many antimitotic agents such as podophyllotoxin, steganacin, and combretastatin A-2 [1].

In mid 80s, a number of 1,3-benzodioxoles were reported to be active in vivo against P388 lymphocytic leukaemia and other tumors and, like podophyllotoxin, to be potent tubulin binders and antimitotic agents [2,3].

Furthermore, it has been recently reported that a series of 1,3-benzodioxole derivatives possess a cytotoxic activity against several human tumor cell lines including human colon carcinoma cells [4] and multidrug-resistant nasopharyngeal carcinoma cells [5].

On this basis and in pursuing our interest in the discovery of new anticancer agents [6,7], we have recently focused our attention on new 1,3-benzodioxole derivatives [8]. In particular, we found that 6-(4-aminobenzoyl)-1,3-benzodioxole-5-acetic acid methyl ester (**1**) (Fig. 1) is provided with high growth inhibitory properties against a panel of human tumor cell lines [8].

As an extension of our investigation on the structure–activity relationship of 1,3-benzodioxoles as antiproliferative agents, we prepared a number of analogues of the prototypic molecule **1** and assayed their cytotoxic activity.

Herein, we report the synthesis, the physical–chemical properties of derivatives **2**–**12** (Fig. 1), along with the results of a primary antitumor screening performed in vitro on a panel of three human tumor cell lines. The biological investigation was carried out at the National Cancer Institute (NCI), Bethesda, MD.

2. Chemistry

Compounds **2** and **3** were obtained as previously reported [9]. The synthesis of novel compounds **4**–**8** was accomplished following the reaction sequence reported in Scheme 1. 1,3-Benzodioxole-5-acetic acid methyl or ethyl esters (**13**, **14**), prepared from commercially available 1,3-benzodioxole-5-acetic acid under standard conditions, were reacted with the appropriate acid in the presence of phosphorous pentoxide to give ketoesters **4** and **15**–**17**. The nitro group of **15** and **17** was reduced with ammonium formate in the presence of Raney-Ni to yield amino derivatives **5** and **6**, respectively. Intermediate **16** was sequentially reacted with acetylhydrazide and ammonium formate in the presence of Raney-Ni to give amino derivative **7**.

Derivative **18** was transformed into compound **8** through the straightforward sequence of three steps reported in Scheme 1.

Scheme 2 describes the synthesis of compounds **9**–**12**. 5,8-Dihydro-5-(4-nitrophenyl)-7*H*-[1,3]dioxolo[4,5-

* Corresponding author.
E-mail address: nmicale@pharma.unime.it (N. Micale).

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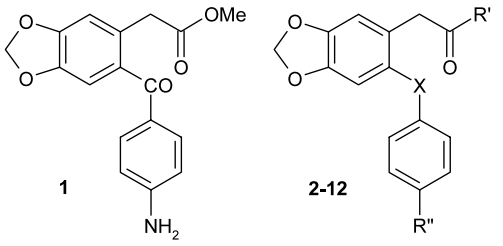


Comp.	R'	X	R''
2	OMe	CO	H
3	OMe	CO	F
4	OMe	CO	Cl
5	OEt	CO	NH_2
6	OMe	$COCH_2$	NH_2
7	OMe	C=NNHAc	NH_2
8	NMe_2	CH_2	NH_2
9	NHMe	CHOH	NO_2
10	$NHNH_2$	CHOH	NO_2
11	NHMe	CO	NO_2
12	$NHNH_2$	CO	NO_2

Fig. 1. Structure of 1,3-benzodioxoles **1**–**12**.

g][2]benzopyran-7-one (**19**), obtained as previously reported [10], was reacted with methylamine or hydrazine to yield **9** and **10**, respectively. Derivatives **9** and **10** were oxidized with MnO_2 to give the corresponding keto derivatives **11** and **12**.

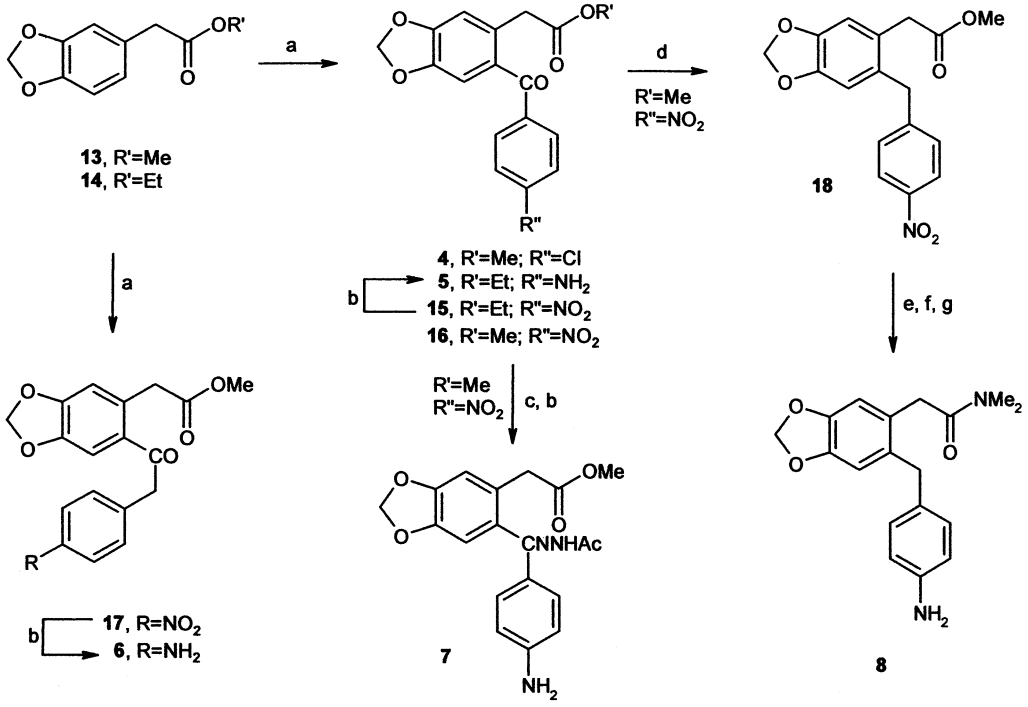
Analytical (C, H, and N) and ^{1}H NMR data of all the synthesized compounds are in full agreement with the proposed structures (see Section 4). The *Z* configuration was assigned to the C=N bond of compound **7**, in analogy to the outcome of a previous investigation carried out on analogous semicarbazono derivatives [10].

3. Pharmacological results and conclusions

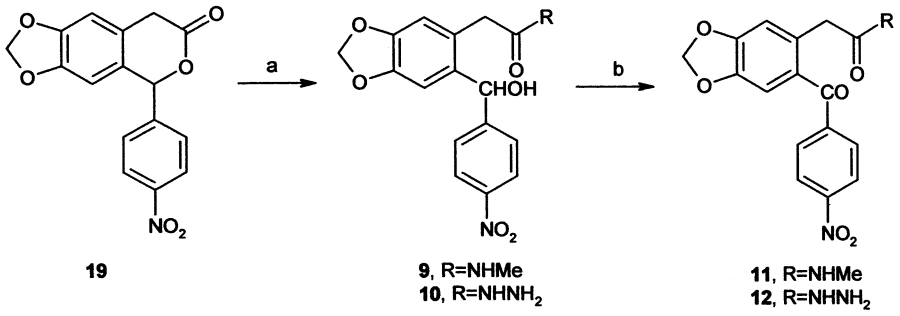
Evaluation of the cytotoxicity of compounds **2**–**12** was performed at NCI. The compounds were preliminarily evaluated in vitro at a concentration of 10^{-4} M against three human tumor cell lines derived from the following neoplastic diseases: lung (NCI-H460), CNS (SF-268), and breast (MCF7) cancers (Table 1).

None of the tested compounds passed the NCI criteria for activity in such a primary assay, i.e. they did not reduce the growth of any one of the cell lines to 32% or less.

The very poor activity of the test compounds does not allow a sound SAR evaluation. All the structural modifications accomplished on the prototype **1** abolished its cytotoxic activity. Such a result suggests that the amino group at C-4′, e.g. **2**–**4**, the carbonyl, e.g. **6** and **7**, and the methyl ester, e.g. **5**, functionalities of **1** are structural requirements essential for cell growth inhibition.



Scheme 1. (a) ArCOOH or $ArCH_2COOH$, P_2O_5, 1,2-dichloroethane, r.t., 16 h; (b) $HCOONH_4$/Raney-Ni, EtOH, Δ, 2 h; (c) $AcNHNH_2$, MeOH, HCl cat., Δ, 48 h; (d) $NaBH_3CN$, BF_3OEt_2, anhydrous THF, Δ, 6 h; (e) H_2O/HCl cat., Δ, 9 h; (f) $SOCl_2$, dry benzene, Δ, 4 h; and (g) Me_2NH, dry benzene, Δ, 2 h.



Scheme 2. (a) $MeNH_2$ or NH_2NH_2, MeOH, Δ, 1 h and (b) MnO_2, dry benzene, Δ, 2 h.

Table 1
Cytotoxic activity of compounds **1**–**12** at 10^{-4} M expressed in growth percentages

Comp.	Cell lines		
	NCI-H460	MCF7	SF-268
1	4	16	20
2	79	53	92
3	92	74	100
4	93	82	104
5	96	61	105
6	57	75	68
7	111	84	101
8	89	103	105
9	85	89	107
10	97	77	91
11	81	96	92
12	119	104	90

4. Experimental

4.1. Chemistry

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Elemental analyses were carried out on a C. Erba Model 1106 Elemental Analyzer for C, H, and N, and the results are within ±0.4% of the theoretical values. Merck silica gel 60 F_{254} plates were used for analytical TLC; column chromatography was performed on Merck silica gel 60 (70–230 meshes). ^{1}H NMR spectra were recorded in $CDCl_3$ by means of a Varian Gemini 300 spectrometer. Chemical shifts are expressed in δ (ppm) relative to TMS as internal standard and coupling constants (J) in Hz. The synthesis and physical and analytical properties of compounds **2** and **3** have been previously described [9].

4.1.1. Synthesis of 6-(4-chlorobenzoyl)-1,3-benzodioxole-5-acetic acid methyl ester (4)

To a stirred solution of **13** (200 mg, 1.03 mmol) in 1,2-dichloroethane (25 ml) was added 4-chlorobenzoic acid (210 mg, 1.34 mmol) followed by phosphorous pentoxide (1.6 g, 11.26 mmol). The mixture was stirred at room temperature overnight, and then water (20 ml) was cautiously added and extracted with chloroform (2 × 20 ml). The organic layer was separated and sequentially treated with 10% NaOH (20 ml), brine (20 ml), and water (2 × 20 ml). The organic phase was dried (Na_2SO_4) and the solvent removed under reduced pressure to yield crude **4** which was purified by a silica gel column chromatography (eluant:ethyl ether/light petroleum, 6:4) to afford pure **4**.

m.p. 67–70 °C; $R_f = 0.38$ ($CHCl_3$/light petroleum, 8:2); yield 63% (216 mg); ^{1}H NMR: 3.57 (s, 3H, CH_3), 3.79 (s, 2H, CH_2-5), 5.97 (s, 2H, OCH_2O), 6.80 (s, 1H, H-7), 6.82 (s, 1H, H-4), 7.39 (d, 2H, $J = 8.5$, H-2′-6′), 7.71 (d, 2H, $J = 8.5$, H-3′-5′). Anal. $C_{17}H_{13}ClO_5$ (C, H, N).

4.1.2. Synthesis of 6-(4-aminobenzoyl)-1,3-benzodioxole-5-acetic acid ethyl ester (5)

Compound **5** was prepared from **14** with the same procedure employed for **4**, using 4-nitrobenzoic acid. The successive reduction of the nitro group was accomplished with ammonium formate and Raney-Ni according to a procedure previously reported [10].

m.p. 150–153 °C; $R_f = 0.55$ (EtOAc/light petroleum, 5:5); yield 61% (192 mg); ^{1}H NMR: 1.41 (t, 3H, $J = 7.1$, CH_3), 3.70 (s, 2H, CH_2-5), 4.04 (q, 2H, $J = 7.1$, CH_2CH_3), 4.15 (bs, 2H, NH_2), 6.02 (s, 2H, OCH_2O), 6.63 (d, 2H, $J = 8.8$, H-3′-5′), 6.83 (s, 1H, H-7), 6.86 (s, 1H, H-4), 7.66 (d, 2H, $J = 8.8$, H-2′-6′). Anal. $C_{18}H_{17}NO_5$ (C, H, N).

4.1.3. Synthesis of 6-(4-aminophenylacetyl)-1,3-benzodioxole-5-acetic acid methyl ester (6)

Compound **6** was prepared from **13**, using 4-nitrophenylacetic acid as reagent, according to the procedure described for the synthesis of **5**.

m.p. 131–133 °C; $R_f = 0.76$ (EtOAc/light petroleum, 8:2); yield 60% (202 mg); ^{1}H NMR: 3.66 (s, 3H, CH_3), 3.80 (s, 2H, CH_2-5), 4.03 (s, 2H, CH_2-6), 4.25 (bs, 2H, NH_2), 6.01 (s, 2H, OCH_2O), 6.62 (d, 2H, $J = 8.5$, H-3′-5′), 6.69 (s, 1H, H-7), 6.99 (d, 2H, $J = 8.5$, H-2′-6′), 7.33 (s, 1H, H-4). Anal. $C_{18}H_{17}NO_5$ (C, H, N).

4.1.4. Synthesis of (Z)-6-[acetylhydrazono-(4-aminophenyl)-methyl]-1,3-benzodioxole-5-acetic acid methyl ester (7)

To a solution of 6-(4-nitrobenzoyl)-1,3-benzodioxole-5-acetic acid methyl ester (**16**) (200 mg, 0.58 mmol) [8] in MeOH (25 ml), $AcNHNH_2$ (86 mg, 1.16 mmol) and a catalytic amount of 6 N HCl were added. The reaction mixture was heated to reflux for 48 h. After cooling, the solvent was removed under reduced pressure and the residue was subjected to chromatography eluting with EtOAc/cyclohexane (1:1) to afford the acetylhydrazono derivative which was successively reduced with a procedure similar to that reported for compound **5**, to give compound **7**.

m.p. 106–109 °C; $R_f = 0.58$ (EtOAc); yield 55% (118 mg); ^{1}H NMR: 2.40 (s, 3H, $COCH_3$), 3.30 (m, 2H, CH_2), 3.53 (s, 3H, OCH_3), 3.96 (bs, 2H, NH_2), 6.05 (m, 2H, OCH_2O), 6.56 (s, 1H, H-3), 6.60 (d, 2H, $J = 8.0$, H-3′-5′), 6.91 (s, 1H, H-6), 7.34 (d, 2H, $J = 8.5$, H-2′-6′), 8.19 (bs, 1H, NH). Anal. $C_{19}H_{19}N_3O_5$ (C, H, N).

4.1.5. Synthesis of 6-(4-aminobenzyl)-1,3-benzodioxole-5-dimethylacetamide (8)

Compound **18** (200 mg, 0.61 mmol) was subjected to hydrolysis and converted into the corresponding acyl chloride with a procedure previously reported [10], then dissolved in dry benzene (25 ml) and treated with an excess of dimethylamine at reflux (2 h). The solvent was removed in vacuo and the residue was purified by column chromatography with CCl_4/EtOAc/*i*-PrOH (70:20:10) as eluant. The reduction of the nitro group was accomplished with ammonium formate and Raney-Ni to give a crude which was purified by treatment with acetone.

m.p. 120–121 °C; $R_f = 0.37$ (EtOAc); yield 53% (106 mg); ^{1}H NMR: 2.80 and 2.94 (2s, 6H, $N(CH_3)_2$), 3.52 (s, 2H, CH_2-5), 3.58 (bs, 2H, NH_2), 3.76 (s, 2H, CH_2-6), 5.91 (s, 2H, OCH_2O), 6.60 (d, 2H, $J = 7.7$, H-3′-5′), 6.62 (2s, 2H, H-4 and H-7), 6.68 (d, 2H, $J = 7.7$, H-2′-6′). Anal. $C_{18}H_{20}N_2O_3$ (C, H, N).

4.1.6. Synthesis of 6-[(4-nitrophenyl)-hydroxymethyl]-1,3-benzodioxole-5-methylacetamide (9)

To a stirred solution of **19** (200 mg, 0.64 mmol) in MeOH (25 ml), an excess of methyl amine was added and the resulting mixture was refluxed for 1 h. Methanol was removed under vacuum and the oil residue was triturated by treatment with diethyl ether to afford a white solid which was filtered-off to give **9** as pure product.

m.p. 117–119 °C; $R_f = 0.71$ (EtOAc/acetone, 8:2); yield 96% (211 mg); ^{1}H NMR: 2.82 (d, 3H, $J = 4.7$, $NHCH_3$), 3.37 and 3.71 (dd, 2H, $J = -14.8$, CH_2-5), 5.25 (bs, 1H, OH), 5.78 (m, 1H, $NHCH_3$), 5.93 (s, 2H, OCH_2O), 6.03 (s, 1H, CH), 6.50 (s, 1H, H-7), 6.65 (s, 1H, H-4), 7.60 (d, 2H, $J = 8.8$, H-2′-6′), 8.21 (d, 2H, $J = 8.8$, H-3′-5′). Anal. $C_{17}H_{16}N_2O_6$ (C, H, N).

4.1.7. Synthesis of 6-[(4-nitrophenyl)-hydroxymethyl]-1,3-benzodioxole-5-acetic acid hydrazide (10)

With the same procedure described for the synthesis of **9**, **10** was prepared from **19** using hydrazine as reagent.

m.p. 92–94 °C; $R_f = 0.60$ (EtOAc/acetone, 8:2); yield 97% (214 mg); ^{1}H NMR: 1.63 (bs, 2H, NH_2), 3.37 and 3.68 (dd, 2H, $J = -15.1$, CH_2-5), 4.92 (bs, 1H, OH), 5.94 (s, 2H, OCH_2O), 6.04 (s, 1H, CH), 6.49 (s, 1H, H-7), 6.66 (s, 1H, H-4), 7.16 (bs, 1H, NH), 7.58 (d, 2H, $J = 8.5$, H-2′-6′), 8.22 (d, 2H, $J = 8.5$, H-3′-5′). Anal. $C_{16}H_{15}N_3O_6$ (C, H, N).

4.1.8. Synthesis of 6-(4-nitrobenzoyl)-1,3-benzodioxole-5-methylacetamide (11)

To a suspension of **9** (200 mg, 0.58 mmol) in dry benzene (25 ml) was added activated MnO_2 (50 mg, 0.58 mmol) and the resulting mixture was stirred under reflux for 2 h. After cooling, the mixture was filtered-off on a Celite pad and the solvent was removed under reduced pressure. The crude product was purified by column chromatography with EtOAc/cyclohexane (80:20) to give **11**.

m.p. 171–173 °C; $R_f = 0.51$ (EtOAc/cyclohexane, 8:2); yield 68% (135 mg); ^{1}H NMR: 2.75 (d, 3H, $J = 4.7$, $NHCH_3$), 3.58 (s, 2H, CH_2-5), 6.05 (s, 2H, OCH_2O), 6.76 (s, 1H, H-7), 6.92 (m, 1H, $NHCH_3$), 7.03 (s, 1H, H-4), 7.92 (d, 2H, $J = 8.8$, H-2′-6′), 8.31 (d, 2H, $J = 8.8$, H-3′-5′). Anal. $C_{17}H_{14}N_2O_6$ (C, H, N).

4.1.9. Synthesis of 6-(4-nitrobenzoyl)-1,3-benzodioxole-5-acetic acid hydrazide (12)

Compound **12** was prepared from **10**, with the same procedure described for the synthesis of **11**.

m.p. 118–120 °C; $R_f = 0.51$ (EtOAc/cyclohexane, 8:2); yield 56% (111 mg); ^{1}H NMR: 1.66 (bs, 2H, NH_2), 3.69 (s, 2H, CH_2-5), 6.10 (s, 2H, OCH_2O), 6.80 (s, 1H, H-7), 7.26 (s, 1H, H-4), 8.01 (d, 2H, $J = 8.8$, H-2′-6′), 8.39 (d, 2H, $J = 8.8$, H-3′-5′), 10.37 (bs, 1H, NH). Anal. $C_{16}H_{13}N_3O_6$ (C, H, N).

4.2. Primary anticancer assay

Evaluation of cytotoxic activity of compounds **2**–**12** was performed at NCI. As primary anticancer assay, a 3-cell line panel consisting of MCF7 (breast), NCI-H460 (lung), and SF-268 (CNS) was used. Each cell line is inoculated and preincubated on a microtiter plate. Test agents are then added at a single 10^{-4} concentration and the culture incubated for 48 h. End point determinations are made with alamarBlue [11]. Results for each test agent are reported as the percent of growth of the treated cells when compared with the untreated control

cells. Compounds which reduce the growth of any one of the cell lines to approximately 32% or less are passed on for evaluation in the full panel of 60 cell lines over a 5-log dose range.

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