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Short Communication

Synthesis and antitumor activity of 1,3-benzodioxole derivatives

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Abstract

A series of 1,3-benzodioxoles (**5**–**19**) was synthesized and evaluated for their in vitro antitumor activity against human tumor cell lines. Some derivatives exhibited tumor growth inhibition activity. In particular, 6-(4-aminobenzoyl)-1,3-benzodioxole-5-acetic acid methyl ester **8**, the most active compound of the series, possesses a significant growth inhibitory activity on 52 cell lines at concentrations ranging from 10^{-7} to 10^{-5} M.

Keywords: 1,3-Benzodioxoles; Antitumor activity

1. Introduction

The ongoing efforts of research on treatment of malignancy are focused on the discovery of novel products that, differently from currently-used drugs, might have a higher selective cytotoxicity and act with different mechanisms of action.

Random screening continues to be one of the main routes to discover new leads in the field of antineoplastic agents and the National Cancer Institute (NCI, Bethesda, USA) plays a pivotal role in this area. The NCI screening program [1] is based on the selection of compounds characterized by a peculiar chemical structure.

It has recently been reported that 1,3-benzodioxole derivatives possess cytotoxic activity against several human tumor cell lines including human colon carcinoma cells [2] and multidrug-resistant nasopharyngeal carcinoma cells [3].

On this basis and in pursuing our interest in the study of new anticancer agents [4–6], we have now focused our attention on new 1,3-benzodioxole derivatives. We report here the synthesis, and the physical–chemical properties of a set of new derivatives (**5**–**19**) along with the results of a primary antitumor screening performed by NCI.

The ability of the NCI selected compounds **5**–**17** and **19** to inhibit the growth of three human tumor cell lines in vitro was used as a preliminary indication of their anticancer activity. Compounds **8**, **12**, **13**, **15**, **16** and **19**, which passed criteria for activity in the primary assay, were evaluated against the full panel of 60 human tumor cell lines.

2. Chemistry

The synthesis of novel compounds was accomplished following the reaction sequence reported in Scheme 1. The methyl ester of 1,3-benzodioxole-5-acetic acid (**3**) and 1-(1,3-benzodioxol-5-yl)-2-propanone (**4**) were prepared by reacting commercially available 1,3-benzodioxole-5-acetonitrile (**1**) with a methanol solution of hydrochloric acid and methylmagnesium bromide, respectively. Ketone **4** was also obtained [7] via a pyridinium chlorocromate (PCC) oxidation of alcohol **2**. By condensing alcohol **2** with 4-nitrobenzaldehyde, isochromane derivative **5** was obtained, predominantly as the *trans* diastereoisomer, in analogy to related transformations [8]. The reduction of ketoester **7**, prepared via Friedel–Crafts acylation of **3** with 4-nitrobenzoic acid in the presence of phosphorous pentoxide, with

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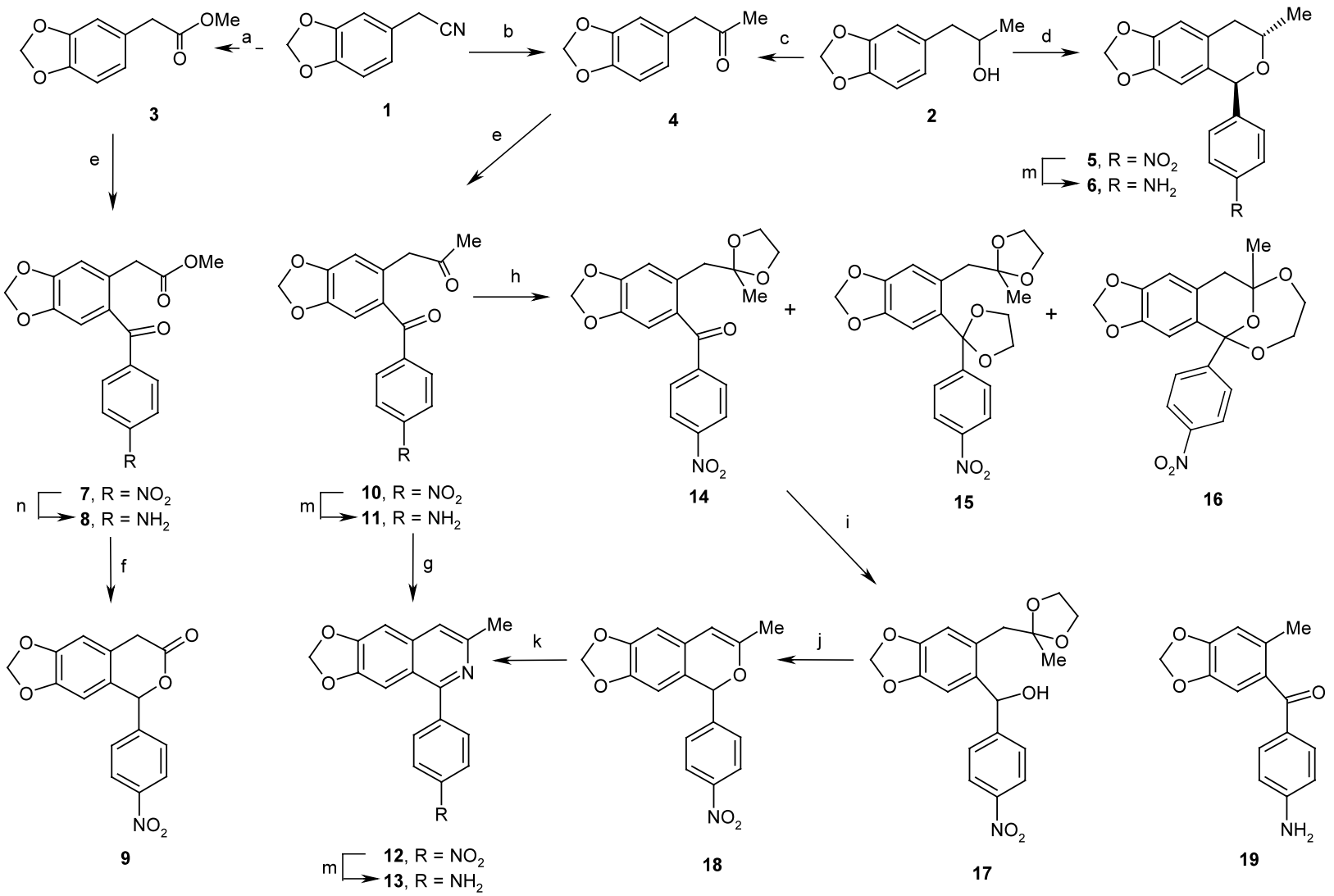

Scheme 1. (a) MeOH/HCl, 0 °C, 8 h; (b) MeMgBr, THF, CuBr, Δ, 30 min; (c) PCC/CH_2Cl_2, rt, 12 h; (d) $O_2NC_6H_4CHO$, HCl, (e) $O_2NC_6H_4COOH$, P_2O_5, $(CH_2)Cl_2$, rt, 12 h; (f) $NaBH_4$, MeOH, 0 °C, 1 h; (g) $NH_2NH_2 \cdot H_2O$, EtOH, rt, 2 h; (h) ethylene glycol, benzene, *p*-TSOH, Δ, 2 h; (i) $NaBH_4$, CH_2Cl_2/MeOH, rt, 2h; (j) $MeSO_2Cl$, CH_2Cl_2/Et_3N, rt, 30 min; (k) $NH_2NH_2 \cdot H_2O$, cat. HCl, MeOH, rt, 24 h; (m) 10% Pd-C/H_2, MeOH, rt, 4 h; (n) $SnCl_2$, MeOH, 70 °C, 1.5 h.

$NaBH_4$ yielded directly isochromanone derivative **9**, whereas diketoderivative **10**, obtained similarly from **4**, gave isoquinoline **12** by treatment with hydrazine hydrate [7]. The reaction of diketoderivative **10** with 1 equiv. of ethylene glycol primarily involved the aliphatic ketone group. Nevertheless, monoketal **14** was contaminated by minor amounts of diketal **15** (9%) and trioxepane derivative **16** (12%), which derives from the involvement of the neighboring carbonyl group as already observed in related transformations [9]. Compound **14** was also converted by treatment with sodium borohydride into alcohol **17**, which was then transformed into benzopyran derivative **18** and isoquinoline **12**. The reduction of the nitro group of derivatives **5**, **7**, **10** and **12** to yield the corresponding amino derivatives **6**, **8**, **11** and **13** was carried out under standard conditions. The elemental analysis (C, H, N) and ^1H NMR spectral data of all the synthesized compounds are in full agreement with the proposed structures and are reported in Section 4.

Table 1
Growth percentages at 10^{-4} M in three-cell line for compounds **5**–**17** and **19**

Comp.	Lung (NCI-H460)	Breast (MCF7)	CNS (SF-268)
5	72	68	62
6	67	53	68
7	68	42	44
8	4	16	20
9	76	39	75
10	54	50	51
11	76	58	79
12	16	26	22
13	31	46	52
14	56	43	54
15	38	26	55
16	86	67	20
17	74	45	77
19	21	12	34

Table 2
Mean response parameters of in vitro antitumor activity test for compounds **8**, **12**, **13**, **15**, **16** and **19**

Comp.	GI_{50}	TGI	LC_{50}
8	5.71 ± 1.37	4.51 ± 1.70	4.01 ± 0.72
12	4.48 ± 0.95	4.05 ± 0.35	4.00 ± 0.11
13	4.43 ± 0.68	4.05 ± 0.35	4.00 ± 0.00
15	4.15 ± 0.46	4.00 ± 0.10	4.00 ± 0.00
16	4.23 ± 0.60	4.04 ± 0.33	4.00 ± 0.00
19	4.52 ± 1.94	4.10 ± 1.92	4.01 ± 0.38

The response parameters GI_{50}, TGI and LC_{50} (±SE) are averaged values referred to all cell lines and represent the molar concentration (expressed as $-\log_{10}$) at which the percentage growth is +50, 0 and −50, respectively.

3. Pharmacological results and conclusions

The growth inhibitory effects of compounds **5**–**17** and **19** were evaluated in vitro at a concentration of 10^{-4} M against three human tumor cell lines derived from three neoplastic diseases, i.e. lung (NCI-H460), CNS (SF-268), and breast (MCF7) cancers (Table 1).

Compounds **8**, **12**, **13**, **15**, **16** and **19** passed the NCI criteria for activity in the primary assay, i.e. they reduce the growth of any one of the cell lines to 32% or less, and, consequently, their cytotoxic and/or growth inhibitory effects were evaluated in vitro against approximately 60 human tumor cell lines derived from nine neoplastic diseases namely leukemia, melanoma, non-small cell lung, colon, CNS, ovarian, renal, prostate and breast cancers.

The data reported in Table 2 are average values referred to all cell lines, obtained by interpolation on dose–response curves and which represent concentrations that produced 50% cell growth inhibition (GI_{50}), total cell growth inhibition (TGI, 0% growth) and 50% cell death (LC_{50}, −50% growth. The activity against specific cell lines is shown in Table 3. Data are reported only when the value of GI_{50}, TGI or LC_{50} is higher than 5.

The inhibitory activity of the tested compounds falls into the range of 10^{-5}–10^{-4} M, except **8** which proved to be the most active derivative within this set of derivatives; it displays a GI_{50} mean value of 5.71.

As can be seen in Table 3, compound **8** exhibited high inhibitory properties on 52 of the tested cell lines at GI_{50} level and on 15 cell lines at TGI level at 10^{-5}–10^{-7} M, however, no significant differential cellular or subpanel sensitivity has been observed. For compounds **12** and **19**, only sporadic significant growth inhibition values are recorded.

The lack of a homogeneous series of derivatives, together with the very poor activity of the tested compounds except **8**, does not allow a sound SAR evaluation. In fact, the results obtained with derivatives **7**, **8** and **11** should suggest that both the amino and the ester groups are structural requirements needed for cell growth inhibition. However, such considerations are not always true since other nitro and amino derivatives, e.g. **10** versus **11** and **12** versus **13**, are equipotent and, furthermore, a comparison of the activity of derivatives **8** and **19** makes doubts on the role played by the ester functionality.

In conclusion, this study lead to 6-(4-aminobenzoyl)-1,3-benzodioxole-5-acetic acid methyl ester (**8**), the most active compound of the series, which exhibits good cytotoxicity; thus the optimization of the substitution is a future development goal.

Table 3
GI_{50} and TGI values against different tumor cell lines for compounds **8**, **12** and **19**

Panel/cell line	**8**		**12**	**19**	
	GI_{50}	TGI	GI_{50}	GI_{50}	TGI
Leukemia					
CCRF-CEM	5.79	5.22			
HL-60 (TB)	5.88	5.16			
K-562	6.13				
MOLT-4	6.02				
RPMI-8226	5.72				
SR	7.08	5.92		5.29	
Non-small cell lung cancer					
A549/ATCC	5.46				
EKVX	5.53				
HOP-62	5.55				
HOP-92			5.42		
NCI-H23	5.21				
NCI-H322M	5.01				
NCI-H522	6.58	5.71			
Colon cancer					
COLO 205	5.68				
HCC-2998	6.62	6.01			
HCT-116	5.97				
HCT-15	5.59				
HT29	6.01	5.46			
KM12	6.50				
SW-620	5.83				
CNS cancer					
SF-268	5.99	5.03			
SF-295	6.13	5.46			
SF-539	5.44				
SNB-19	5.40				
SNB-75	5.92	5.23			
U251	5.57				
Melanoma					
LOX IMVI	5.41				
M14	6.13				
SK-MEL-2	6.11				
SK-MEL-28	5.29				
SK-MEL-5	6.63	5.78			
UACC-257	5.89				
UACC-62	5.89				
Ovarian cancer					
IGROV1	6.23				
OVCAR3	6.42			6.46	6.02
OVCAR-5	5.21				
OVCAR-8	5.19				
SK-OV-3	5.36				
Renal cancer					
786-0	5.24				
A498	5.90	5.12			
CAKI-1	5.74				
RXF 393	5.81	5.24			
SN12C	5.14				
TK-10	5.41				
Prostate cancer					
PC-3	5.84	5.13			
DU-145	5.59				
Breast cancer					
MCF7	6.20				
NCI/ADR-RES	6.11				
MDA-MB231/ATCC	5.30				
HS 578T	5.60				
MDA-MB-435	6.93	6.21		5.11	
MDA-N	6.88	5.78			
T-47D	5.62				

Growth inhibition (GI_{50}) and total growth inhibition (TGI) dose represent the concentration (expressed as $-\log_{10}$) at which the percentage growth is +50 and 0 as compared with control untreated cells (+100), respectively.

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 mesh). ^{1}H NMR spectra were recorded in $CDCl_3$ by means 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, physical and analytical properties of compound **10** have been previously described [7].

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

1,3-Benzodioxole-5-acetonitrile (**1**) (2 g, 12.4 mmol) was dissolved in a freshly prepared solution of MeOH/MeCOCl (1/1, v/v, 60 ml) and the reaction mixture was stirred at 0 °C for 8 h. The solvent was removed under vacuum and the residue was treated with water (60 ml) and extracted with ethyl ether (2 × 80 ml). The pooled organic extracts were dried (Na_2SO_4) and the solvent was evaporated at reduced pressure to yield **3** (2.3 g, 95%) as an oil [7].

4.1.2. Synthesis of 1-(1,3-benzodioxol-5-yl)-2-propanone (**4**)

To a stirred solution of **1** (4 g, 24.8 mmol) and methyl magnesium bromide (3.0 M solution in THF, 9.5 ml, 28.5 mmol), in THF (80 ml) was added CuBr (64 mg, 0.43 mmol), and the mixture was refluxed under nitrogen for 30 min. After cooling to 0–5 °C, 5 ml of H_2O was cautiously added, followed by 30 ml of 15% H_2SO_4. After stirring for 14 h, 60 ml of ether was added, the phases were separated, and the aqueous layer was extracted twice more with 50 ml portions of ether. The combined organic phase was dried (Na_2SO_4) and the

solvent removed under reduced pressure to afford **4** (yield 30%, 1.32 g) as a light brown oil.

R_f (cyclohexane/EtOAc, 60/40) 0.56; ^{1}H NMR: 2.14 (s, 3H, CH_3), 3.59 (s, 2H, CH_2), 5.93 (s, 2H, OCH_2O), 6.61–6.77 (m, 3H, Ar). *Anal*. ($C_{10}H_{10}O_3$) C, H, N.

4.1.3. Synthesis of trans-7,8-dihydro-7-methyl-5-(4-nitrophenyl)-5H-[1,3]dioxolo[4,5-g][2]benzopyrane (5)

To a stirred solution of **2** (3 g, 16.6 mmol) in dioxane (100 ml) was added 4-nitrobenzaldehyde (3 g, 19.8 mmol) and 12N HCl (3 ml). The reaction mixture was refluxed for 1 h and the solvent was removed under vacuum. The resulting residue was dissolved in $CHCl_3$ and washed with water (100 ml). The organic layer was dried (Na_2SO_4), the solvent was removed at reduced pressure, and the residue was purified by crystallization from EtOH to give 4.99 g (94%) of **5**.

R_f (cyclohexane/EtOAc, 70/30) 0.74; m.p. 134–136 °C; ^{1}H NMR: 1.38 (d, 3H, $J = 6.0$, CH_3), 2.65–2.89 (m, 2H, CH_2), 4.02 (m, 1H, H-7), 5.74 (s, 1H, H-5), 5.86 (m, 2H, OCH_2O), 6.04 and 6.61 (s, 2H, H-4 and H-9), 7.52 (d, 2H, $J = 8.8$, H-2′,6′), 8.22 (d, 2H, $J = 8.8$, H-3′,5′). *Anal*. ($C_{17}H_{15}NO_5$) C, H, N.

4.1.4. Synthesis of trans-5-(4-aminophenyl)-7,8-dihydro-7-methyl-5H-[1,3]dioxolo[4,5-g][2]benzopyrane (6)

To a stirred solution of **5** (1.1 g, 3.5 mmol) in MeOH (50 ml) 10% Pd/C (50 mg) was added. The mixture was shaken under hydrogen at atmospheric pressure for 4 h and the Pd/C was filtered out with Celite pad. The solvent was removed in vacuo and the resulting residue was purified by column chromatography eluting with cyclohexane/EtOAc (70/30) as eluant and recrystallized from EtOH to give **6** (677 mg, 68%).

R_f (cyclohexane/EtOAc, 70/30) 0.55; m.p. 86–88 °C; ^{1}H NMR: 1.35 (d, 3H, $J = 6.0$, CH_3), 2.60–2.79 (m, 2H, CH_2), 3.30 (bs, 2H, NH_2), 3.94 (m, 1H, H-7), 5.52 (s, 1H, H-5), 5.84 (m, 2H, OCH_2O), 6.16 and 6.56 (s, 2H, H-4 and H-9), 6.67 (d, 2H, $J = 8.5$, H-3′,5′), 7.10 (d, 2H, $J = 8.5$, H-2′,6′). *Anal*. ($C_{17}H_{17}NO_3$) C, H, N.

4.1.5. Synthesis of 6-(4-nitrobenzoyl)-1,3-benzodioxole-5-acetic acid methyl ester (7)

To a stirred solution of **3** (2.3 g, 11.6 mmol) in 1,2-dichloroethane (150 ml) 4-nitrobenzoic acid (2.53 g, 15.1 mmol) and phosphorous pentoxide (16.5 g, 117 mmol) were added. The mixture was stirred at room temperature overnight, cautiously treated with water (120 ml) and extracted with chloroform (2×80 ml). The organic layer was separated and sequentially treated with 10% NaOH (120 ml), brine (100 ml) and water (3×70 ml). The organic phase was dried (Na_2SO_4) and the solvent removed under reduced pressure to yield crude **7** which was purified by a silica gel column chromatography (eluant: ethyl ether/light petroleum, 60/40) to afford pure **7** as yellow solid (2.6 g, 65%).

R_f (ethyl ether/light petroleum, 50:50) 0.61; m.p. 135–137 °C; ^{1}H NMR: 3.62 (s, 3H, CH_3), 3.87 (s, 2H, CH_2), 6.06 (s, 2H, OCH_2O), 6.80 and 6.84 (2s, 2H, H-4 and H-7), 7.92 (d, 2H, $J = 8.5$, H-2′ and H-6′), 8.32 (d, 2H, $J = 8.5$, H-3′ and H-5′). *Anal*. ($C_{17}H_{13}NO_7$) C, H, N.

4.1.6. Synthesis of 6-(4-aminobenzoyl)-1,3-benzodioxole-5-acetic acid methyl ester (8)

To a stirred solution of **7** (252 mg, 0.73 mmol) in anhydrous MeOH (40 ml) tin(II) chloride (825 mg, 3.67 mmol) was added. The reaction mixture was heated at 70 °C for 1.5 h, the solvent was removed under vacuum and the resulting residue was dissolved in ethyl acetate and neutralized with 2N NaOH (2×100 ml). The organic layer was dried (Na_2SO_4) and the solvent was removed at reduced pressure. The residue was purified (202 mg, 88%) by silica gel column chromatography using EtOAc/light petroleum (50/50) as eluant and recrystallized from light petroleum to give **8**.

R_f (EtOAc/light petroleum, 50/50) 0.38; m.p. 124–126 °C; ^{1}H NMR: 3.58 (s, 3H, CH_3), 3.70 (s, 2H, CH_2), 4.19 (bs, 2H, NH_2), 6.01 (s, 2H, OCH_2O), 6.62 (d, 2H, $J = 8.8$, H-3′ and H-5′), 6.81 and 6.85 (2s, 2H, H-4 and H-7), 7.64 (d, 2H, $J = 8.8$, H-2′ and H-6′). *Anal*. ($C_{17}H_{15}NO_5$) C, H, N.

4.1.7. Synthesis of 5,8-dihydro-5-(4-nitrophenyl)-7H-[1,3]dioxolo[4,5-g][2]benzopyran-7-one (9)

To a cooled (0–5 °C) solution of **7** (2.1 g, 6.12 mmol) in MeOH (60 ml) was slowly added $NaBH_4$ (341 mg, 9.18 mmol) and the mixture was stirred for 1 h. The solvent was removed under reduced pressure and the resulting residue was poured into water and extracted with chloroform (2×80 ml). The combined extracts were dried over Na_2SO_4. The solvent was removed under reduced pressure and the resulting residue was purified by column chromatography using cyclohexane/EtOAc (50/50) as eluant to give **9** (1.1 g, 58%).

R_f (cyclohexane/EtOAc, 50/50) 0.61; m.p. 204–206 °C; ^{1}H NMR: 3.53 and 3.69 (dd, 2H, $J = 18.4$, CH_2), 6.00 (s, 2H, OCH_2O), 6.36 (s, 1H, H-5), 6.37 (s, 1H, H-9), 6.74 (s, 1H, H-4), 7.53 (d, 2H, $J = 8.5$, H-2′,6′), 8.29 (d, 2H, $J = 8.5$, H-3′,5′). *Anal*. ($C_{16}H_{11}NO_6$) C, H, N.

4.1.8. Synthesis of 1-[6-(4-aminobenzoyl)-1,3-benzodioxol-5-yl]-2-propanone (11)

Compound **11** was obtained with a procedure similar to that reported for compound **6** starting from **10** (219 mg, 0.58 mmol). The product was purified by column chromatography with EtOAc/cyclohexane/*i*-PrOH (60/30/10) as eluant and recrystallized from EtOH to give **11** (159 mg, 80%).

R_f (EtOAc/cyclohexane/*i*-PrOH, 60/30/10) 0.83; m.p. 83–86 °C; ^{1}H NMR: 2.15 (s, 3H, CH_3), 3.77 (s, 2H, CH_2), 4.18 (bs, 2H, NH_2), 6.01 (s, 2H, OCH_2O), 6.62 (d, 2H, $J = 8.5$, H-3′,5′), 6.72 (s, 1H, H-4), 6.88 (s, 1H, H-7), 7.63 (d, 2H, $J = 8.5$, H-2′,6′). *Anal*. ($C_{17}H_{15}NO_4$) C, H, N.

4.1.9. Synthesis of 7-methyl-5-(4-nitrophenyl)-1,3-dioxolo[4,5-g]isoquinoline (12)

18 (280 mg, 0.88 mmol) was dissolved in MeOH/cat. HCl and to the solution was added an excess of hydrazine hydrate (0.15 ml, 3.1 mmol). The mixture reaction was stirred at room temperature for 24 h. The solvent was removed under reduced pressure and the resulting residue was dissolved in chloroform, washed with water, saturated $NaHCO_3$ and then with NaCl. The organic phase was dried (Na_2SO_4), the solvent evaporated and the residue purified by silica gel column chromatography (eluant: cyclohexane/EtOAc, 60/40) of the residue gave 198 mg (72%) of **12**; m.p. 178–180 °C [7].

4.1.10. Synthesis of 7-methyl-5-(4-aminophenyl)-1,3-dioxolo[4,5-g]isoquinoline (13)

Compound **13** (343 mg, 85%) was obtained with a procedure similar to that reported for compound **6** starting from **12** (447 mg, 1.45 mmol).

R_f (EtOAc/cyclohexane/*i*-PrOH, 60:30:10) 0.53; m.p. 107–109 °C; ^{1}H NMR: 2.65 (s, 3H, CH_3), 3.70 (bs, 2H, NH_2), 6.04 (s, 2H, OCH_2O), 7.01 (s, 1H, H-9), 7.27 (s, 1H, H-4), 7.35 (s, 1H, H-8), 6.80 (d, 2H, $J = 8.2$ Hz, H-3′,5′), 7.45 (d, 2H, $J = 8.2$ Hz, H-2′,6′). *Anal*. ($C_{17}H_{14}N_2O_2$) C, H, N.

4.1.11. Synthesis of [6-(2-methyl-1,3-dioxolan-2yl-methyl)-1,3-benzodioxol-5-yl]-(4-nitrophenyl)-methanone (14), 5-(2-methyl-1,3-dioxolan-2yl-methyl)-6-[2-(4-nitrophenyl)-1,3-dioxolan-2-yl]-1,3-benzodioxole (15) and 4,5-methylenedioxy-9-methyl-1-(4-nitrophenyl)-10,13,14-trioxa-tricyclo[7.4.1.0*2,7*]-tetradeca-2,4,6-triene (16)

To a solution of **10** (1.35 g, 4.11 mmol) in anhydrous benzene (150 ml), ethylene glicol (0.36 ml, 5.34 mmol) and *p*-toluensulfonic acid (0.135 g, 0.71 mmol) were added. The reaction mixture was refluxed for 2 h, was washed with water (2×80 ml) and then dried over anhydrous Na_2SO_4. The organic phase was concentrated in vacuo, and the resulting residue was purified by column chromatography using CCl_4/EtOAc (85/15) as eluant.

14: R_f (CCl_4/EtOAc, 85/15) 0.28; oil (1.07 g, 71%);. ^{1}H NMR: 1.21 (s, 3H, CH_3), 3.13 (s, 2H, CH_2), 3.48–3.68 (m, 4H, OCH_2CH_2O), 6.02 (s, 2H, OCH_2O), 6.72 (s, 1H, H-7), 6.87 (s, 1H, H-4), 7.92 (d, 2H, $J = 8.8$, H-2′,6′), 8.28 (d, 2H, $J = 8.8$, H-3′,5′). *Anal*. ($C_{19}H_{17}NO_7$) C, H, N.

15: R_f (CCl_4/EtOAc, 85:15) 0.30; oil (0.135 g, 9%); ^{1}H NMR: 0.98 (s, 3H, CH_3), 2.81 (s, 2H, CH_2), 3.79–4.15 (m, 8H, 2-OCH_2CH_2O), 5.99 (s, 2H, OCH_2O), 7.17 (s, 1H, H-4), 7.34 (s, 1H, H-7), 7.57 (d, 2H, $J = 9.1$, H-2′,6′), 8.05 (d, 2H, $J = 9.1$, H-3′,5′). *Anal*. ($C_{21}H_{21}NO_8$) C, H, N.

16: R_f (CCl_4/EtOAc, 85:15) 0.37; m.p. 243–247 °C (0.183 g, 12%); ^{1}H NMR: 1.56 (s, 3H, CH_3), 2.92 and 3.09 (dd, 2H, $J = 16.0$, CH_2), 3.58 and 4.27 (m, 4H, OCH_2CH_2O), 5.90 (m, 2H, OCH_2O), 6.47 (s, 1H, H-10), 6.64 (s, 1H, H-4), 7.64 (d, 2H, $J = 9.1$, H-2′,6′), 8.14 (d, 2H, $J = 9.1$, H-3′,5′). *Anal*. ($C_{19}H_{17}NO_6$) C, H, N.

4.1.12. Synthesis of [6-(2-methyl-1,3-dioxolan-2yl-methyl)-1,3-benzodioxol-5-yl]-(4-nitrophenyl)-methanol (17)

To a solution of **14** (920 mg, 2.46 mmol) in CH_2Cl_2/MeOH (100 ml, 40/60) $NaBH_4$ (466 mg, 12.4 mmol) was added and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured into water, extracted with $CHCl_3$ (60 ml) and dried over Na_2SO_4. The solvent was removed in vacuo and the crude product was purified by silica gel column chromatography (eluant: light petroleum/EtOAc, 70/30).

R_f (eluant: light petroleum/EtOAc, 70/30) 0.35; Oil. (601 mg, 65%); ^{1}H NMR: 1.46 (s, 3H, CH_3), 3.01 and 3.04 (d, 2H, $J = 14.5$, CH_2), 3.95–3.97 (m, 4H, OCH_2-CH_2O), 4.52 (s, 1H, OH), 5.94 (m, 2H, OCH_2O), 6.09 (s, 1H, CH), 6.38 (s, 1H, H-7), 6.84 (s, 1H, H-4), 7.60 (d, 2H, $J = 8.5$, H-2′,6′), 8.23 (d, 2H, $J = 8.5$, H-3′,5′). *Anal*. ($C_{19}H_{19}NO_7$) C, H, N.

4.1.13. Synthesis of 7-methyl-5-(4-nitrophenyl)-5H-1,3-dioxolo[4,5-g][2]benzopyran (18)

To a cooled (0–5 °C) and stirred solution of **17** (475 mg, 1.27 mmol) in CH_2Cl_2 (60 ml) was added triethylamine (264 μl, 1.90 mmol) and methansulfonyl chloride (114 μl, 1.65 mmol). The ice-bath was removed and the mixture was stirred at room temperature for 30 min. The solvent was removed under reduced pressure and the resulting residue was treated with water, extracted with ethyl acetate (2×100 ml) and purified by column chromatography using light petroleum/ethyl acetate (80/20) as eluant.

R_f (light petroleum/ethyl acetate, 70/30) 0.64; m.p. 120–123 °C (301 mg, 75%); ^{1}H NMR: 1.91 (s, 3H, CH_3), 5.58 (s, 1H, H-8), 5.93 (s, 2H, OCH_2O), 6.07 (s, 1H, H-5), 6.27 (s, 1H, H-9), 6.52 (s, 1H, H-4), 7.51 (d, 2H, $J =$ 8.8, H-2′,6′), 8.21 (d, 2H, $J = 8.8$, H-3′,5′). *Anal*. ($C_{17}H_{13}NO_5$) C, H, N.

4.1.14. Synthesis of (6-methyl-1,3-benzodioxol-5-yl)-(4-aminophenyl)-methanone (19)

19 (821 mg, 92%) was obtained with a procedure analogous to that reported for compound **8**, starting from (6-methyl-1,3-benzodioxol-5-yl)-(4-nitrophenyl)-methanone (1.0 g, 3.5 mmol) obtained in turn as reported in literature [10].

R_f (EtOAc/light petroleum, 50/50) 0.41; m.p. 110–112 °C; ^{1}H NMR: 2.23 (s, 3H, CH_3), 4.19 (bs, 2H, NH_2), 5.98 (s, 2H, OCH_2O), 6.63 (d, 2H, $J = 8.8$, H-3′,5′), 6.72 (s, 1H, H-4), 6.78 (s, 1H, H-7), 7.66 (d, 2H, $J = 8.8$, H-2′,6′). *Anal*. ($C_{17}H_{13}NO_3$) C, H, N.

4.2. Pharmacology

Evaluation of anticancer activity of compounds **5**–**17** and **19** was performed at the NCI following the well-known in vitro disease-oriented antitumor screening program [11], which is based upon the use of multiple panels of 60 human tumor cell lines against which our compounds were tested at 10-fold dilutions of five concentrations, ranging from 10^{-4} to 10^{-8} M. The percentage growth was evaluated spectrophotometrically versus controls not treated with test agents. A 48 h continuous drug exposure protocol was followed and a sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth.

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