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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4822–4825

Synthesis and antiproliferative activity of substituted benzopyranoisoindoles: A new class of cytotoxic compounds

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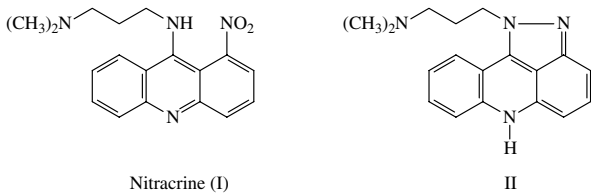
Received 25 May 2006; revised 21 June 2006; accepted 21 June 2006
Available online 7 July 2006

Abstract—A series of novel aminosubstituted benzopyranoisoindoles possessing structural analogy to an active nitracrine metabolite are reported. The compounds exhibited interesting cytotoxic activity against a panel of cell lines, which was maximized by the presence of both 1-dialkylaminoethyl and 3-nitro substituents.

1-Nitro-9-[3′-(dimethylamino)propylamino]acridine (I, nitracrine, Fig. 1) has been used clinically for several years in Poland for the treatment of mammary, ovarian, lung, and colon tumors.[1] A number of in vitro studies have indicated that this drug undergoes metabolic reduction of the 1-nitro group to form a reactive species, while inhibition of this reaction by oxygen transforms the drug into an extremely potent, hypoxia selective cytotoxic agent.[2] Intercalative DNA binding does not appear to be directly responsible for cytotoxicity, but it may contribute to the high potency of the drug, by serving to target reactive, reduced cellular metabolites to the DNA.[3] Unfortunately, the in vivo effectiveness of nitracrine on solid tumors is limited, probably because reductive metabolism is too rapid to allow efficient distribution through hypoxic tumor areas.[4] In an effort to elucidate the bioreduction pathway, extensive structural studies of the cellular metabolites performed in various biological systems revealed the presence of highly reactive intermediates. The initially generated 1-aminoderivative of nitracrine is susceptible to intramolecular cyclization, giving rise to the dihydropyrazoloacridine II (Fig. 1). This key metabolite was found to be a reactive species, as it easily undergoes transformations in the presence of electrophilic carbon atoms, resulting in the formation of a six-membered ring attached to positions 1- and 9- of the acridine core.[5]

During our work directed toward the synthesis of various xanthenone derivatives and the evaluation of their antiproliferative activity, we have prepared aminosubstituted pyranoxanthenones, their pyrazole-fused counterparts, and some related benzopyranoindazoles, which have shown interesting cytotoxicity against a panel of tumor cell lines.[6–8] As part of our ongoing efforts in this field, we have recently reported on the synthesis of a novel benzopyranoisoindole ring system.[9] This scaffold could potentially provide bioisosters of the active nitracrine metabolite (II) and with this prospect in mind, we report the preparation of a number of related aminosubstituted derivatives and the evaluation of their in vitro cytotoxic activity.

For the synthesis of the target compounds commercial 2-iodobenzoic acid (**1**, Scheme 1) was first con-



Nitracrine (I) II

Figure 1. Structures of Nitracrine and its active metabolite.

Keywords: Aminosubstituted benzopyrano[4,3,2-*c*,*d*]isoindoles; Antiproliferative activity; DNA-content analysis.
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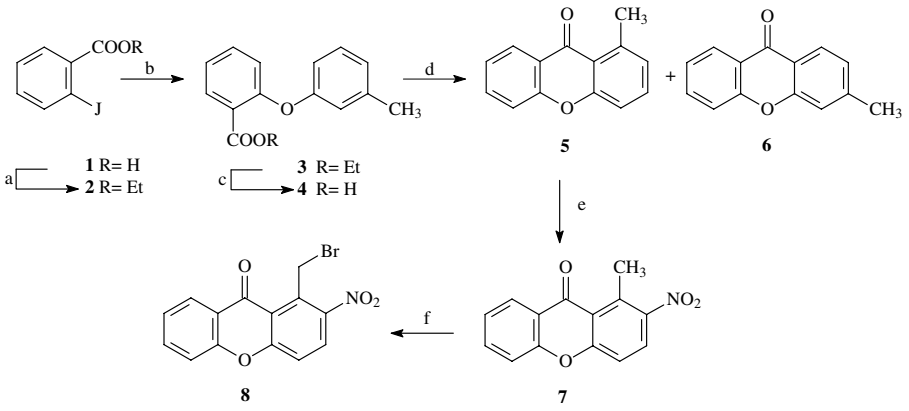
doi:10.1016/j.bmcl.2006.06.074



Scheme 1. Synthesis of compound **8**. Reagents and conditions: (a) HCl/EtOH, reflux, 8 h, 93%; (b) *m*-cresol, Cs_2CO_3, CuCl, pyridine, reflux, 3 h, 89%; (c) 1—NaOH (40%), CH_3OH, rt, 2 h; 2—HCl (36%), 99% (d) $(CF_3CO)_2O$, CH_2Cl_2, rt, 3 h, 57%; (e) HNO_3 65%, H_2SO_4 98%, 0 °C, 2 h, 56%; (f) NBS, benzoyl peroxide, CCl_4, reflux, 18 h, 82%.

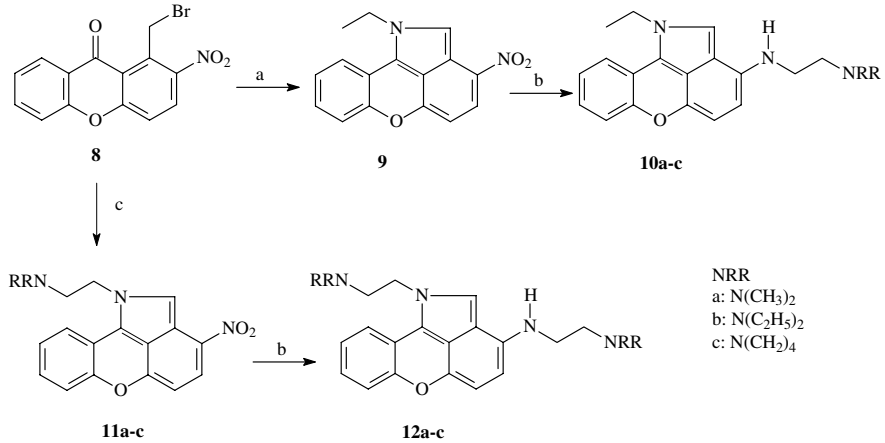
verted to the corresponding ethyl benzoate **2** and then treated with *m*-cresol in the presence of cesium carbonate and cuprous chloride to provide the diarylether **3** in 89% yield. This diarylether has been previously prepared,[10] but the yield of the reaction was clearly improved, through the alterations reported herein. Compound **3** was first saponified and then ring-closed, upon treatment with trifluoroacetic anhydride, to result in a mixture of the isomeric xanthenones **5** and **6**, which were separated by column chromatography (silica gel, cyclohexane/EtOAc 15:1) and identified. The use of trifluoroacetic anhydride, as an alternative to the reported acetic anhydride/sulfuric acid,[10a] or PPA,[10b] improved the yield of both isomers **5** and **6**, and provided a larger amount of the required compound **5** (57%, instead of the reported 42%). 1-Methylxanthenone (**5**) was then nitrated to provide only the 2-nitroderivative **7**,[9] which was treated with NBS in the presence of a catalytic amount of benzoyl peroxide to give the 1-bromomethyl analogue **8**.

Reaction of the bromide **8** with ethylamine provided quantitatively, in one step, the isoindole derivative **9** (Scheme 2).[9] The presence of the nitro group in compound **8** is necessary for effective ring closure. Indeed, when 1-bromomethylxanthen-9(9*H*)-one[11] was heated at reflux with an ethanolic solution of 2-diethylaminoethylamine, only *N*,*N*-diethyl-*N*′-[(9-oxo-9*H*-xanthen-1-yl)methyl]ethane-1,2-diamine was obtained.

The nitro group of **9** could be displaced easily and upon treatment with 2-dialkylaminoethylamines resulted in the target derivatives **10a**–**c**.[12] Similarly, when the bromide **8** was treated with suitable ethanediamines, it provided in one step the amines **11a**–**c**,[13] which upon nucleophilic substitution of the nitro group yielded the target derivatives **12a**–**c**.[14]

The in vitro cytotoxic activity of the new compounds was evaluated by using the MTT assay[6–8] in the colorectal adenocarcinoma cell line HT-29, the uterine sarcoma MES-SA as well as its variant MES-SA/Dx5, reported to be 100-fold resistant to doxorubicin.[15] The results, including reference compounds mitoxantrone and doxorubicin, are presented in Table 1.

The nitroderivative **9** is inactive against the HT-29 cell line and exhibits moderate activity against MES-SA uterine sarcoma cells, as well as the corresponding



Scheme 2. Synthesis of compounds **10a**–**c**, **11a**–**c**, and **12a**–**c**. Reagents and conditions: (a) ethylamine/CH_3OH, rt, 2 h, 99%; (b) 2-dialkylaminoethylamine, DMSO, 90 °C, 2 h, 78–85%; (c) 2-dialkylaminoethylamine, EtOH, rt, 1 h, 92–95%.

Table 1. Inhibition of proliferation induced after incubation for 72 h with the xanthenone derivatives (IC_{50}[a] values in μM)

Compound	NRR	HT-29	MES-SA	MES-SA/Dx5	RF[b]
9	—	>100	19.8 (±9.12)	11.5 (±5.74)	0.6
10a	$N(CH_3)_2$	44.6 (±6.12)	45.8 (±4.18)	31.9 (±8.83)	0.7
10b	$N(CH_2CH_3)_2$	19.9 (±5.31)	31.0 (±1.20)	15.7 (±7.12)	0.5
10c	$N(CH_2)_4$	46.6 (±4.02)	24.7 (±6.05)	15.5 (±6.94)	0.6
11a	$N(CH_3)_2$	4.2 (±0.78)	0.9 (±0.03)	0.8 (±0.12)	0.9
11b	$N(CH_2CH_3)_2$	6.8 (±4.39)	1.1 (±0.35)	1.4 (±0.26)	1.3
11c	$N(CH_2)_4$	3.3 (±0.38)	0.6 (±0.09)	0.6 (±0.11)	1.0
12a	$N(CH_3)_2$	65.1 (±9.71)	50.4 (±7.30)	40.3 (±8.56)	0.8
12b	$N(CH_2CH_3)_2$	29.8 (±8.57)	>100	20.8 (±7.80)	—
12c	$N(CH_2)_4$	50.5 (±3.67)	60.5 (±8.57)	17.7 (±4.59)	0.3
Mx		0.025 (±0.008)	0.003 (±0.001)	0.028 (±0.002)	9.3
Dx		0.153 (±0.076)	0.0097 (±0.0012)	0.704 (±0.337)	72.6

[a] The results represent mean (±standard deviation) of three independent experiments and are expressed as IC_{50}, the concentration that reduced by 50% the optical density of treated cells with respect to untreated controls.
[b] IC_{50} resistant cells/IC_{50} sensitive cells.

Table 2. DNA-content analysis[a]

Compound[b]	G0/G1	S	G2/M	Apoptosis
9	62.14	37.86	0.00	2.51
10a	74.70	21.55	3.75	1.17
10b	77.69	21.34	0.97	0.79
10c	74.33	23.53	2.14	0.52
11a	57.44	20.65	21.91	4.15
11b	55.57	15.33	29.10	7.36
11c	64.04	33.73	2.23	14.10
12a	68.91	25.87	5.22	0.55
12b	68.09	28.13	3.78	0.54
12c	73.58	26.42	0.00	1.44
Control	63.68	33.19	3.14	0.29

[a] Values represent cell-cycle phase distribution (%), while apoptosis has been calculated as percentage of the total number of events. One out of two similar experiments is depicted.
[b] All compounds were administered at concentration equal to their IC_{50}, except for **12b** and **12c** which were used at 50 μM.

MES-SA/Dx5 variant. Derivatives **10a–c** and **12a–c**, that possess two side chains in positions 1- and 3-, show a low profile of cytotoxicity, in all cell lines. In contrast, the 3-nitro aminoderivatives, **11a–c**, appear to be highly active against both HT-29 and MES-SA cell lines and their IC_{50} values vary typically within the range of 0.6–6.8 μM. This could be considered an important finding, since it is evident that the insertion of an aminosubstituted side chain in position 1- of the molecule in conjunction with the existence of a 3-nitro group substantially increases cytotoxic activity. This improvement is not so pronounced when the nitro group is replaced by an additional aminosubstituted side chain. Among the compounds **11**, the pyrrolidine analogue **11c** is the most cytotoxic derivative, followed by the dimethylamino analogue **11a**. From a direct comparison of activity toward sensitive and resistant cell lines, it is evident that the compounds appear to be active against MES-SA and also possess generally comparable cytotoxicity against the doxorubicin resistant MES-SA/Dx5 cell line. The ability of compounds **11a–c** to overcome multidrug resistance to the MES-SA/Dx5 cell line is clearly indicated by the resistant factor (RF) values, which are practically all equal to 1. These results suggest that the novel compounds are hardly recognized by the protein machinery governing multidrug resistance.

Cell-cycle perturbations induced after incubation of exponentially growing MES-SA uterine sarcoma cells with the new compounds for 24 h were studied by flow-cytometric analysis of DNA content.[8] The sub-diploid peak observed during this analysis was used for the assessment of cell death due to apoptosis. As shown in Table 2, compounds **11a** and **11b** provoke a significant G2/M arrest, as well as cell death by apoptosis. Furthermore, compound **11c** does not seem to block the cell-cycle in the G2 phase at the specific time-point selected for the FACS analysis, however it possesses a strong apoptotic effect, which is in accordance with its high cytotoxic activity. Finally, compounds **10a–c** and **12a–c** induce to a greater or a lesser extent, a G1 block.

In conclusion, we have prepared a series of novel bioactive xanthenone aminoderivatives. This class of compounds constitute new potential anticancer leads and the observed variation of in vitro activity against colon HT-29 and MES-SA uterine sarcoma cell lines offered the opportunity to determine the substitution pattern favorable for cytotoxicity.

Acknowledgment

The present study was supported in part from the Special Account for Research Grants Committee of the National and Kapodistrian University of Athens (Program Kapodistrias).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.06.074.

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12. *N*,*N*-Dimethyl-*N*′-(1-ethyl-1*H*-benzopyrano[4,3,2-*c*,*d*]isoindol-3-yl)-1,2-ethanediamine (**10a**). A solution of **9** (40 mg, 0.14 mmol) and 2-dimethylaminoethylamine (200 μL, 1.4 mmol) in dry dimethylsulfoxide (6 mL) was heated at 90 °C for 2 h. Upon cooling, the reaction mixture was poured into ice/water and extracted with CH_2Cl_2 (3× 40 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated to dryness. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH 95:5) to furnish the target compound (40 mg, 85%); mp 167–170 °C (diethyl ether/*n*-pentane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.31 (t, J = 7.32 Hz, 3H, CH_2CH_3), 2.00 (s, 6H, $N(CH_3)_2$), 2.30 (m, 1H, $HCHCH_2NMe_2$), 2.36 (m, 1H, $HCHCH_2NMe_2$), 3.18 (m, 2H, $HNCH_2CH_2NMe_2$), 3.94 (m, 2H, CH_2CH_3), 5.72 (br s, 1H, D_2O exchangeable, NH), 5.81 (d, J = 8.7 Hz, 1H, H-5), 6.96 (t, J = 7.5 Hz, 1H, H-9), 7.04 (d, J = 7.9 Hz, 1H, H-7), 7.16 (dd, J = 1.7, 7.5 Hz, 1H, H-10), 7.36 (ddd, J = 1.7, 7.2, 8.1 Hz, 1H, H-8), 7.76 (s, 1H, H-2), 8.05 (d, J = 8.7 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 16.66 (CH_2CH_3), 39.56 ($CH_2CH_2NMe_2$), 42.91 (CH_2CH_3), 44.47 (2× NCH_3), 56.18 ($CH_2CH_2NMe_2$), 96.86 (C-5), 113.79 (C-10c), 114.02 (C-2), 117.11 (C-2a), 117.37 (C-7), 117.62 (C-10b), 120.55 (C-9), 120.58 (C-10a), 128.19 (C-3), 128.80 (C-4), 131.42 (C-8), 132.36 (C-10), 150.56 (C-5a), 155.92 (C-6a). Anal. Calcd for $C_{20}H_{23}N_3O$; calcd: C, 74.74; H, 7.21; N, 13.07. Found: C, 74.97; H, 7.12; N, 12.84.
13. *N*,*N*-Dimethyl-2-(3-nitro-1*H*-benzopyrano[4,3,2-*c*,*d*]isoindol-1-yl)ethylamine (**11a**). A solution of the bromide **8** (400 mg, 1.2 mmol) and 2-dimethylaminoethylamine (850 μL, 6 mmol) in absolute ethanol (7 mL) was stirred at room temperature for 1 h. The reaction mixture was vacuum-evaporated, extracted with CH_2Cl_2 and water, the organic layer was dried (Na_2SO_4) and evaporated to dryness. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH 98:2) to furnish the target compound (380 mg, 92%); mp 189–192 °C (CH_2Cl_2/diethyl ether); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 2.32 (s, 6H, 2× CH_3), 2.79 (t, J = 7.32 Hz, 2H, $Me_2NCH_2CH_2$), 4.49 (t, J = 7.32 Hz, 2H, $Me_2NCH_2CH_2$), 6.11 (d, J = 8.3 Hz, 1H, H-5), 7.16–7.32 (m, 3H, H-7, H-8, H-9), 7.42 (s, 1H, H-2), 7.53 (dd, J = 7.1, 1.7 Hz, 1H, H-10), 8.08 (d, J = 8.3 Hz. 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 46.01 (2× CH_3), 48.80 ($Me_2NCH_2CH_2$), 58.61 ($Me_2NCH_2CH_2$), 98.27 (C-5), 115.25 (C-2a), 116.61 (C-2), 118.19 (C-10a), 118.78 (C-7), 119.63 (C-10b), 120.20 (C-10c), 120.51 (C-10), 125.51 (C-9), 128.19 (C-8), 130.32 (C-4),131.90 (C-3), 153.07 (C-6a), 157.67 (C-5a). Anal. Calcd for $C_{18}H_{17}N_3O_3$; calcd: C, 66.86; H, 5.30; N, 13.00. Found: C, 67.03; H, 5.22; N, 12.79.
14. *N*,*N*-Dimethyl-*N*′-[1-(2-dimethylaminoethyl)-1*H*-benzopyrano[4,3,2-*c*,*d*]isoindol-3-yl]-1,2-ethanediamine (**12a**). This compound was prepared by a procedure analogous to **10a**. Yield: 78%; mp 71–74 °C (diethyl ether/*n*-pentane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) 1.96 (s, 6H, $HNCH_2CH_2N(CH_3)_2$), 2.11 (s, 6H, $(CH_3)_2NCH_2CH_2$), 2.25 (m, 2H, $HNCH_2CH_2N(CH_3)_2$), 2.65 (m, 1H, $(CH_3)_2NCHHCH_2$), 3.04 (m, 1H, $(CH_3)_2NCHHCH_2$), 3.11 (m, 2H, $HNCH_2CH_2N(CH_3)_2$), 3.91 (m, 1H, $(CH_3)_2NCH_2CHH$), 4.07 (m, 1H, $(CH_3)_2NCH_2CHH$), 5.53 (br s, 1H, D_2O exchangeable, NH), 5.82 (d, J = 8.7 Hz, 1H, H-5), 6.99 (t, J = 7.2 Hz, 1H, H-9), 7.08–7.13 (m, 2H, H-7, H-10), 7.42 (ddd, J = 1.7, 7.2, 8.1 Hz, 1H, H-8), 7.86 (s, 1H, H-2), 8.23 (d, J = 8.7 Hz, 1H, H-4); ^{13}C NMR ($CDCl_3$, 50 MHz) δ (ppm) 39.80 ($HNCH_2CH_2N(CH_3)_2$), 44.46 ($HNCH_2CH_2N(CH_3)_2$), 46.18 ($(CH_3)_2NCH_2CH_2$), 46.35 ($(CH_3)_2NCH_2CH_2$), 56.21 ($HNCH_2CH_2N(CH_3)_2$), 60.72 ($(CH_3)_2NCH_2CH_2$), 97.09 (C-5), 113.02 (C-2), 113.04 (C-10c), 118.23 (C-2a), 119.89 (C-10a), 120.36 (C-7), 121.03 (C-9), 123.95 (C-10b), 127.54 (C-3), 129.64 (C-4), 131.95 (C-8), 132.62 (C-10), 151.94 (C-5a), 157.27 (C-6a). Anal. Calcd for $C_{22}H_{28}N_4O$; calcd: C, 72.50; H, 7.74; N, 15.37. Found: C, 72.32; H, 7.69; N, 15.12.
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