Bioorganic & Medicinal Chemistry Letters 21 (2011) 3110–3112

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Design, synthesis and antiproliferative activity of novel aminosubstituted benzothiopyranoisoindoles

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ARTICLE INFO

Article history:
Received 19 January 2011
Revised 4 March 2011
Accepted 5 March 2011
Available online 11 March 2011

Keywords:
Thioxanthone analogues
Fuzed isoindoles
Nitracrine
Cytotoxic activity
Multi-drug resistance

ABSTRACT

The synthesis of a number of new benzothiopyrano[4,3,2-*cd*]isoindole aminoderivatives designed as structural analogues of the key metabolite of the anticancer agent Ledacrine (nitracrine) and their in vitro cytotoxic activity evaluation against HCT-116, MES-SA, and MES-SA/Dx cancer cell lines is reported. The majority of the derivatives possessed noticeable cytotoxicity in a low μM range indicating an interesting structure–activity relationship.

The supply of blood to large solid tumors is disorganized and hypoxic cells exist in the non-vasculated internal regions of such tumors. These cells are resistant to ionizing radiation, relatively inaccessible to chemotherapeutic drugs and furthermore, are refractory to treatment by many of these agents. Therefore, the need for effective therapeutic strategies designed to eliminate hypoxic cancer cells is widely recognized.[1] Among the studied compounds, are certain nitroaromatics or heterocyclic nitroderivatives which can be reduced in hypoxic tissues to produce active intermediate species. These are relatively stable in the absence of oxygen and selectively cytotoxic to the hypoxic cancer cells. The 1-nitroacridine derivatives are potent DNA binding agents presenting significant antiproliferative activity against hypoxic solid tumors[2,3] and were developed as anticancer agents that may not affect the bone marrow.[1] 1-Nitro-9-[3′-(dimethylamino)propylamino]acridine (nitracrine, I, Fig. 1) has been used clinically since the early 1980s in Poland for the treatment of ovarian, lung, colon and breast cancers.[4] Although further development of this drug was discontinued due to severe side effects, a second generation of 1-nitroacridine derivatives has been synthesized. These modified compounds retain the anticancer activity of the parent drug and enhance its therapeutic efficacy.[5–7] Since the metabolism of nitracrine is too rapid to allow efficient distribution of the active metabolites through hypoxic tumor areas and in order to decrease the ability of the 1-nitro group to undergo metabolic reduction, the introduction of an electron-donating methyl group at C4 of the acridine core was effected.[8] Indeed, C1748 (Fig. 1), is a new clinical candidate, as it induces apoptosis, necrosis or senescence in human cancer cell lines, and possesses high antitumor activity in nude mice while demonstrating low mutagenicity[9] and low systemic toxicity.[10] Like nitracrine, C1748 binds covalently to DNA upon activation by cellular enzymes and induces DNA crosslinking leading to cell cycle perturbation.[11]

While the bioreduction pathway of both drugs is generally unknown, the study of nitracrine metabolites revealed a number of

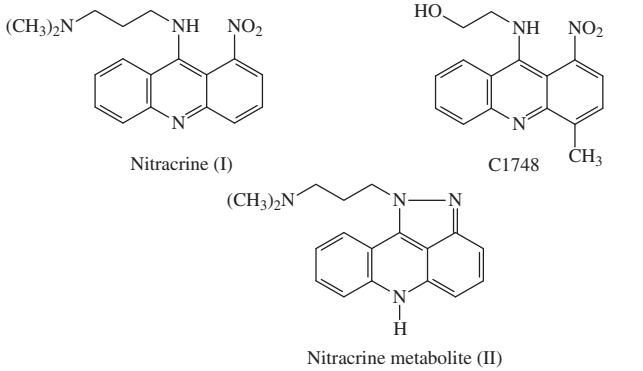

Figure 1. Structure of nitracrine and bioactive analogues.

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doi:10.1016/j.bmcl.2011.03.021

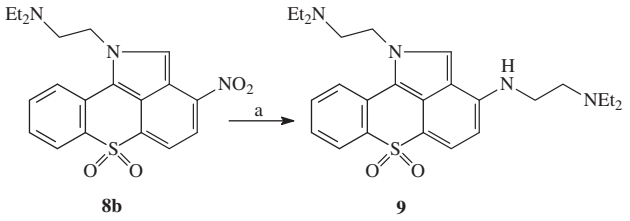
active species, including dihydropyrazoloacridine II (Fig. 1), which results from the intramolecular cyclization of the initially generated 1-aminoderivative of nitracrine. This key metabolite undergoes further transformations in the presence of electrophilic carbon atoms, resulting in the formation of a six-membered ring attached to positions 1- and 9- of the acridine core.[12]

Pursuant to our study of new aminosubstituted xanthenones[13,14] and related fused xanthones[15,16] as cytotoxic agents, we have reported on the synthesis of some benzopyrano[4,3,2-*cd*]isoindoles with a remarkable profile of cytotoxicity against cancer cells.[17] With this in mind we decided to investigate activity within the closely related benzothiopyranoisoindoles, in order to better understand structure–activity relationships around this scaffold.

For the synthesis of the target derivatives we have used commercial thiosalicylic acid (**1**, Scheme 1) which was converted to the corresponding benzoic acid **2** upon treatment of potassium thiosalicylate with 5-chloro-2-nitrotoluene. Compound **2** was then ring-closed with the use of Eaton's reagent, providing the isomeric thioxanthenones **3** and **4** (in a 3:1 ratio), which were separated by column chromatography and spectroscopically identified. Bromide **5** was prepared by the reaction of **3** with NBS in the presence of benzoyl peroxide and was then used for the reaction with suitable ethanediamines to provide in one step and in high yield the isoindole derivatives **6a–d**. As reported,[18] the presence of the 2-nitro group in compound **5** is necessary for the effective ring closure of the intermediate diamines, which result from the nucleophilic substitution of the bromine atom by the dialkylaminoethylamines, giving rise, in turn, to spontaneous cyclization, also evident from the observed characteristic violet color of the reaction solution.

In order to synthesize of the target *S*,*S*-dioxides, we initially oxidized the nitro derivative **3**, but our attempts to achieve the subsequent bromination of the resulting dioxide were not successful. Consequently, we oxidized the bromide **5** using chromium trioxide to obtain the rather unstable compound **7**, which was then converted into compounds **8a–d** upon reaction with the suitable diamines.

In order to verify our previous results, concerning the significance of the nitro group for biologic activity, we also considered preparing an analogue where this group would have been replaced by a second aminosubstituted side chain. The corresponding analogues of the benzopyranoisoindole series showed considerably reduced cytotoxicity when compared to the nitroderivatives.[17] A possible synthetic approach for the preparation of this kind of analogues could proceed through reduction of the nitro group, followed by suitable treatment of the resulting aminoderivative. However, such a reduction is troublesome, due to the ease of reduction of the isoindole nucleus.[19] Thus we decided to insert the side-chain through the nucleophilic displacement of the nitro group. This reaction was effective only in the case of dioxides **8**, probably due to the electron withdrawing character of the sulfonyl group of these derivatives. On the basis of this fact, we treated **8b** with *N*,*N*-diethylethylenediamine and prepared amine **9** in high yield (Scheme 2).



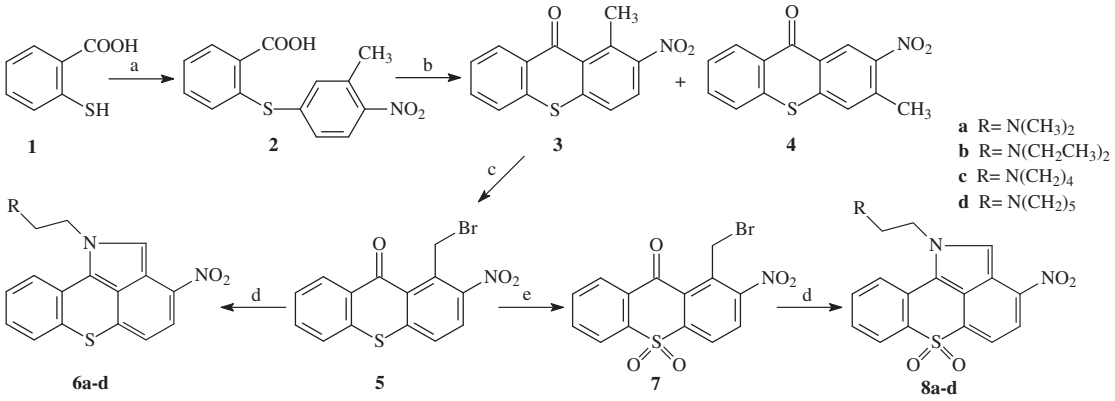
Scheme 2. Reagents and conditions: (a) *N*,*N*-diethylethylenediamine, EtOH, reflux, 24 h, 67%.

As previously reported,[18] the corresponding benzopyrano[4,3,2-*cd*]isoindoles possess a highly acidic 2-aromatic proton and in the corresponding ^{1}H NMR spectra (recorded in 1/9 solution of CD_3OD/$CDCl_3$), a H–D exchange occurred readily under mild conditions. This phenomenon was also observed in the series of compounds **6** and **8**, although the exchange was very slow in the case of **6**; on the contrary, it was extremely rapid for compounds **8**, presumably facilitated by the strong inductive effect of the sulfonyl group.

The in vitro cytotoxic activity of the new compounds was evaluated using the MTT dye reduction assay[20] in three human solid tumor cell lines, namely the colorectal adenocarcinoma HCT-116, the uterine sarcoma MES-SA as well as its variant MES-SA/Dx5, reported to be 100 fold resistant to doxorubicin.[21] The 50% inhibitory concentrations (IC_{50}) of the compounds on each cell line, including as reference compounds doxorubicin (Dx), and mitoxantrone (Mx), are presented in Table 1.

As observed, the target aminoderivatives **6a–d** and **8a–d** exhibited remarkable cytotoxicity against the tested cell lines HCT-116 and MES-SA and for the majority of them (**6a–c**, **8a–d**), the IC_{50} values varied typically within the range of 1.15–7.04 μM. It is of interest that the benzothiopyranoisoindoles **6** are 2–3 times more active than the corresponding dioxides **8**, with the exception of the piperidine analogue **6d**, which is the less active compound of the series. The pyrrolidine analogue **6c** is the most potent cytotoxic compound in both cell lines; this observation is in accordance with our previously reported data, where synthetic analogues of the benzopyranoisoindole series were tested against the same (MES-SA) or of similar origin (HT-29) cancer cells.[17]

By evaluating the cytotoxic activity of all compounds in the Dx-sensitive (MES-SA) and Dx-resistant (MES-SA/Dx5) cell lines, we found that they could exert a cytotoxic activity in both cell lines



Scheme 1. Reagents and conditions: (a) 5-chloro-2-nitrotoluene, KOH, DMSO, 3 h, 110 °C, 94%; (b) CH_3SO_2OH/P_2O_5, 4 h, 60 °C, 31% for **3**, 10% for **4**; (c) NBS, benzoyl peroxide, CCl_4, 40 min, 70 °C, 51%; (d) EtOH, $RCH_2CH_2NH_2$, rt, 30–60 min for **6a–d**, 12 h for **8a–d**, 69–78%; (e) H_2SO_4/CH_3COOH (1:4 v/v), CrO_3, 110 °C, 90 min, 56%.

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

Compound	HCT-116	MES-SA	MES-SA/Dx5	RF[b]
6a	2.10 ± 0.36	4.44 ± 1.85	1.70 ± 0.05	0.38
6b	2.14 ± 0.28	1.93 ± 0.13	5.08 ± 0.26	2.63
6c	1.15 ± 0.07	1.72 ± 0.10	2.15 ± 0.26	1.25
6d	8.09 ± 0.43	16.74 ± 2.75	9.16 ± 0.90	0.55
8a	5.42 ± 0.55	3.52 ± 0.45	5.18 ± 0.26	1.47
8b	6.02 ± 0.53	3.98 ± 0.94	2.61 ± 0.37	0.66
8c	7.04 ± 0.47	2.30 ± 0.13	1.45 ± 0.31	0.63
8d	5.47 ± 2.12	3.98 ± 0.10	2.55 ± 0.51	0.64
9	60.37 ± 3.57	68.17 ± 2.99	>100	––
Dx	0.16 ± 0.07	0.030 ± 0.008	2.398 ± 0.162	79.93
Mx	0.022 ± 0.003	0.012 ± 0.003	0.080 ± 0.016	6.67

[a] The results represent the mean (±standard deviation) of 3–5 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.

at relatively similar doses. Moreover, estimation of the relative resistant factor (RF) of each compound revealed that, with the exception of **6b**, all other analogues are equally active against both chemo-sensitive and chemo-resistant cells (RF less or close to 1), and therefore, could potentially possess the ability to overcome multidrug resistance. Notably, in some cases (e.g., compounds **6a**, **6d**, **8c**, **8d**) the Dx-resistant (MES-SA/Dx5) cells appeared to be more sensitive, as compared to Dx-sensitive MES-SA cells, to the cytotoxic effects of the compounds under study (Table 1).

Interestingly, compound **9**, in which the 3-nitro group has been replaced by a second basic side-chain, lacks cytotoxicity. As previously noted this compound was prepared on purpose, since we anticipated this lack of activity based on our preceding results.[17] Conclusively, the nitro group at position 3, seems to be a rather important feature of this class of compounds and correlates with enhanced cytotoxic activity against tumor cells.

The observed low IC_{50} values of the new compounds and their ability to overcome multidrug resistance, as they could also efficiently kill the 100 fold Dx-resistant MES-SA/Dx5 cell line, are directly comparable to the effect exhibited by the previously reported benzopyranoisoindole bioisosters.[17] This observation provides significant evidence that these substituted fused ring systems may serve as a good pharmacophore/scaffold and could provide excellent candidates for future anticancer drug development. The in depth study of their exact mechanism of action at the molecular level and the induced effects in DNA damage and repair signaling pathways as well as on the cell cycle and apoptotic cellular machineries is currently under active investigation in our laboratories.

Acknowledgments

The authors would like to thank Dr. H. Pratsinis and Dr. D. Kletsas, who kindly provided MES-SA and MES-SA/Dx5 cell lines as well as Dr. N. Cacoullos for helpful discussions.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.03.021.

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