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Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 1523–1525

Synthesis of novel 2-nitroimidazole-tethered tricyclic quinolines, bearing a second heteroatom, and their in vitro evaluation as hypoxia-selective cytotoxins and radiosensitizers

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Received 18 September 2003; accepted 29 December 2003

Abstract—Two novel nitroimidazole-based bioreductive compounds, 10-[3-(2-nitroimidazolyl)-propylamino]-3,4-dihydro-1*H*-thiopyrano[4,3-*b*]quinoline hydrochloride (**8a**) and 10-[3-(2-nitroimidazolyl)propylamino]-2-methyl-1,2,3,4-tetrahydro-benzo[*b*]-1,6-naphthyridine hydrochloride (**8b**) have been synthesized and evaluated in V79 cells as hypoxia-selective cytotoxins and radiosensitizers that target DNA through weak intercalation. Both compounds were relatively good radiosensitizers ($C_{1.6}$ values of 40.0 ± 0.8 and 59.0 ± 0.4 μM for **8a** and **8b**, respectively) but neither of the compounds was superior to **2** which does not carry a second heteroatom in the DNA-intercalating chromophore.

DNA-intercalating bioreductive drugs demonstrate increased potency as selective cytotoxins and radiosensitizers of hypoxic cells in vitro.[1,2] However, chromophores capable of tight DNA binding, such as acridinic and phenanthridinic derivatives, also demonstrate slow dissociation kinetics which lead to restricted extravascular diffusion to hypoxic regions of tumors and thus, ineffectiveness in vivo.[1d,2] In addition, they tend to be potent cytotoxins by mechanisms independent of a bioreductive activation (i.e., by hindering the movement of polymerases or interfering with the action of topoisomerases I or II along DNA), which decreases hypoxic selectivity.[3] Another limitation associated with strong DNA-intercalators is their aerobic radioprotection which has been observed both in vitro and in vivo.[1b–d]

The development of weak DNA-intercalating bioreductive compounds was therefore undertaken as the next logical step to ensure DNA affinity high enough to produce toxicity yet low enough to permit efficient extravascular diffusion and penetration to hypoxic tumor tissue. We define as weak DNA-intercalators, compounds whose range of concentrations (C_{50}) for 50% ethidium bromide (EB) displacement (from an EB–DNA complex) lies between 40–100 μM. Low DNA-binding through intercalation can be obtained either by eliminating a ring in the chromophore, for example, replacing an acridinic chromophore with a quinolinic system, as in 4-[3-(2-nitro-1-imidazolyl)-propylamino]-7-chloroquinoline hydrochloride **1** (NSC 709257),[4] or by disturbing the planarity of the chromophore, as in the fused alicyclic quinolinic derivatives 9-[3-(2-nitro-1-imidazolyl)-propylamino]-1,2,3,4-tetrahydroacridine hydrochloride[3d] **2** and 9-[3-(2-nitro-1-imidazolyl)-propylamino]-cyclopenteno[*b*]quinoline hydrochloride[5] **3** (Scheme 1). Thus, compounds **1**, **2**, and **3**, which are weak DNA-intercalators, do not inhibit topoisomerases I/II neither demonstrate aerobic radioprotection and show better activity in vivo in combination with radio/chemotherapy than the corresponding acridinic analogues (e.g., **4**).[3d,5,6]

However, several fused tricyclic quinolines with a second heteroatom are known to demonstrate DNA intercalative properties, ability to induce topoisomerase II dependent DNA cleavage and antitumor activity.[7] In the present paper we report the synthesis of 10-[3-(2-nitroimidazolyl)-propylamino]-3,4-dihydro-1*H*-thiopyrano[4,3-*b*]quinoline hydrochloride (**8a**) and 10-[3-(2-nitroimidazolyl)-propylamino]-2-methyl-1,2,3,4-tetrahydro-benzo[*b*]-1,6-naphthyridine hydrochloride (**8b**) and their evaluation as weakly DNA-intercalating hypoxia-selective cytotoxins and radiosensitizers in vitro.

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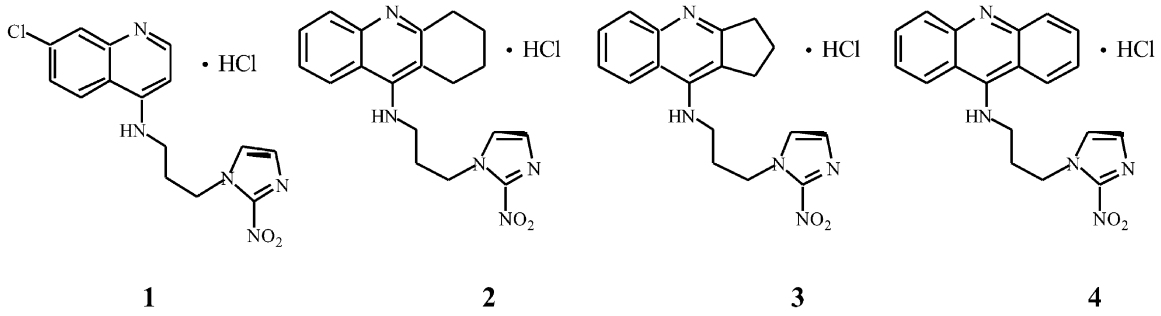
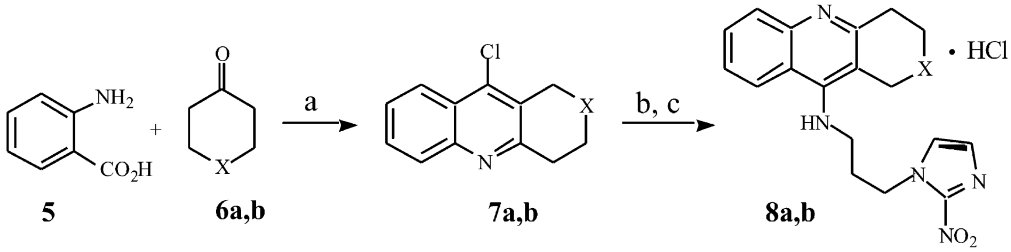
doi:10.1016/j.bmcl.2003.12.102



Scheme 1.



a: X = S
b: X = NMe

Scheme 2. Reagents and conditions: (a) $POCl_3$, reflux; (b) phenol, NaI, 3-(2-nitro-1-imidazolyl)propylamine, 130 °C, 15 min; (c) HCl gas in dioxane.

Refluxing for 2 h of anthranilic acid (**5**) with tetrahydrothiopyran-4-one (**6a**) or 1-methyl-4-piperidone (**6b**) in the presence of phosphorus oxychloride afforded the thiopyranoquinoline **7a** and the tetrahydro-benzo[*b*]-1,6-naphthyridine **7b,** respectively, in 55 and 47% yield, respectively, by the method of Yamato et. al.[7] (Scheme 2). Coupling of the chloro-derivatives **7a**,**b** with 3-(2-nitro-1-imidazolyl)propylamine in the presence of phenol at 130 °C afforded the free amines of compounds **8a** and **8b**, which were isolated by preparative TLC (alumina, ethyl acetate) in 16–27% and 7–22% yields, respectively. The time and temperature of the coupling reaction were crucial factors for the yield of the desired product. Thus, the reaction does not take place at temperatures lower than 130 °C even after 12–24 h of heating. At temperatures $\geq$130 °C tarry side products are formed (presumably due to self-oxidation of the 3-(2-nitro-1-imidazolyl)propylamine) which significantly reduce the yield of the desired products. The optimal time for the coupling reaction was 15 min. Methanol was added to the reaction mixture while it was still hot, to prevent solidification of the tarry materials, followed by chromatographic separation. Finally, the free amines were quantitatively converted to their corresponding (mono)hydrochloride salts by treating with an HCl/dioxane solution.[8]

DNA-binding affinity was measured by determination of a C_{50} value,[4] otherwise known as the concentration that causes 50% decrease in fluorescence in the ethidium bromide displacement assay and the way that compounds **8a**,**b** affect the mobility of supercoiled DNA on an electrophoretic gel.[9] Cytotoxicity and radiosensitizing properties of **8a**,**b** were determined under hypoxic or aerobic conditions in V79 cells by using the clonogenic assay.[4,6]

Table 1. Physicochemical and biological data of compounds **8a**,**b** in V79 cells and comparison with compounds **2** and 9-[3-(2-nitro-1-imidazolyl)propylamino] acridine hydrochloride (**4**)

Parameter	8a	8b	2	4
$PC_{o/w}$[a]	0.40±0.01	0.41±0.01	0.14±0.02[b]	0.07±0.01
C_{50}[c]	58.0	45.0	58.0	9.0
$CT_{50(A)}$[d]	580±10	426±2	360±20	84±3
$CT_{50(H)}$[d]	68±3	196±5	33±3	15±1
HS[e]	9.0	2.2	11.0	5.5
$C_{1.6}$[f]	40.0±0.8	59.0±0.4	19.0±0.5	8.0±0.1
$C_{1.6i}$[g]	932	586	443	885
ER_{max}[h]	2.76 (100)	2.40 (200)	3.10 (100)	2.36 (20)
T.I.[i]	15	7	20	11

[a] Partition coefficient (means±SEM) in octanol/water.[10]
[b] Data for compounds **2** and **4** are taken from ref 3d.
[c] Concentration (μM) for 50% reduction in fluorescence in the ethidium bromide displacement assay, a direct measure for DNA-binding.
[d] The drug concentration (μM) required to reduce cell survival to 50% of controls under aerobic (A) or hypoxic (H) conditions, using the clonogenic assay (mean of four independent experiments in quadruplicate±SEM).
[e] The ratio of CT_{50} values in air and N_2 ($CT_{50(A)}/CT_{50(H)}$).
[f] Concentration (μM) for an enhancement ratio (ER: The ratio between two radiation doses in the absence and presence of a compound for the same surviving fraction to be obtained under identical conditions) of 1.6 (mean of three independent experiments in quadruplicate±SEM).
[g] Intracellular concentration (μM) at C1.6, determined as described in ref 4.
[h] Maximum ER values at non toxic concentrations (indicated into parentheses, in μM).
[i] In vitro therapeutic index (ThI): CT50(A)/C1.6.

Compounds **8a**,**b** bind weakly to DNA with C_{50} values of 58 and 45 μM, respectively, similar to that of compound **2** (Table 1). In addition, significantly greater concentrations of either compound are needed (0.8–1.0 mM) compared to the strong DNA-intercalator **4** (50–100 μM) for retardation of DNA mobility on gel

electrophoresis. Although **8a**,**b** are of similar lipophilicity, **8b** demonstrates less uptake in V79 cells (Table 1), presumably due to the possibility of an additional charge. Surprisingly, **8a**,**b** were more lipophilic than **2**.

On a concentration basis, compounds **8a**,**b** were less potent cytotoxins under aerobic or hypoxic conditions, compared to compounds **2** and **4**. Decreased aerobic toxicity can be attributed to the inability of these compounds to inhibit the catalytic activity of topoisomerase I/II (>1000 μM concentrations) in contrast to **4** (12–157 μM),[3c] whereas decreased hypoxic toxicity perhaps implies a decrease in the one electron reduction potential (E_7^1) of the nitro group due to the second heteroatom in the fused ring. However, **8a** was of a similar hypoxic selectivity with **2** and thus superior to **4** (Table 1). Compounds **8a**,**b** were less potent as radiosensitizers of hypoxic cells compared to **2** and **4** on a concentration basis ($C_{1.6}$ values in Table 1), but efficient ER_{max} values were obtained at non toxic concentrations in contrast to **4**.[1c] The in vitro therapeutic index (T.I.) of **8a** was 15, superior to that of compound **4**, suggesting a potential further investigation of this compound in vivo. However, introducing a second heteroatom in a weakly DNA-intercalating chromophore can not guarantee improved hypoxic selectivity or radiosensitizing properties.

Acknowledgements

This work was supported by the Claude Worthington Benedum Foundation and internal funds of the Radiation Medicine Department at Evanston Northwestern Healthcare, Evanston, IL.

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8. (a) Compound **8a**: Recrystallized from an ethanol:ethyl acetate mixture as a white solid; ^{1}H NMR (500 MHz) (D_2O, 4.8 δ) δ 8.06 (d, J = 8.7 Hz, 1H), 7.85 (t, J = 7.6 Hz, 1H), 7.69 (d, J = 8.7 Hz, 1H), 7.54 (t, J = 7.6 Hz, 1H), 7.35 (s, 1H), 6.98 (s, 1H), 4.54 (t, J = 7.5 Hz, 2H), 4.04 (t, J = 6.5 Hz, 2H), 3.63 (s, 2H), 3.28 (t, J = 6.3 Hz, 2H), 3.02 (t, J = 6.3 Hz, 2H), 2.40 (m, 2H). HRMS (VG 70-250SE mass spectrometer): calcd for $C_{18}H_{19}N_5O_2S$ (free amine): m/z 369.125946. Found: 369.1262 Anal. ($C_{18}H_{20}ClN_5O_2S$) C, H, Cl, N, S. (b) Compound **8b**: Recrystallized from an ethanol:ethyl acetate mixture as a white solid; ^{1}H NMR (500 MHz) of the free amine ($CDCl_3$), δ 8.00 (d, J = 8.0 Hz,1H), 7.91 (d, J = 8.0 Hz,1H), 7.66 (t, J = 7.0, 1H), 7.45 (t, J = 7.0 Hz, 1H), 7.20 (s, 1H), 7.04 (s, 1H), 4.57 (t, J = 6.8, 2H), 3.67 (s, 2H), 3.55 (t, J = 6.0 Hz, 2H), 3.28 (t, J = 6.0 Hz, 2H), 2.88 (t, J = 6.0 Hz, 2H), 2.61 (s, 3H), 2.28 (m, 2H). FAB in *m*-nitrobenzylalcohol (VG 70-250SE mass spectrometer): calcd for $C_{19}H_{23}N_6O_2$ (monoprotonated form): m/z 367.1882; Found: 367.1819. Anal. ($C_{19}H_{23}ClN_6O_2$) C, H, N, Cl.
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