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TARGETING RADIOSENSITIZERS TO DNA BY MINOR GROOVE BINDING: NITROARENES BASED ON NETROPSIN AND DISTAMYCIN

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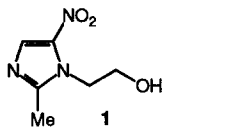
Abstract: Four analogues of the antitumour antibiotics, netropsin and distamycin, containing 2-nitroimidazole or 5-nitrofuran moieties were synthesised. All showed high affinity for DNA, including compounds with only one pyrrole/amidine moiety, but the radiosensitizing efficiencies were poor. The results have implications both for minor groove targeting and the design of radiosensitizers.

The presence of hypoxic cells in a tumour is a result of the rapid growth outstripping the tumour's vascular system, hence reducing the supply of essential nutrients and particularly oxygen. Studies on many types of mammalian cells have shown that cells irradiated under oxic conditions are killed about three times more readily than similar cells under hypoxic conditions.[1,2] A number of ways has been investigated to overcome the resistance of hypoxic tumour cells to radiation without increasing the sensitivity of oxic cells. An approach which has received much attention is the use of electron-affinic nitroaromatics, eg. metronidazole (**1**),[3,4] misonidazole (**2**)[5,6] and etanidazole (**3**).[7,8] These compounds do selectively sensitize hypoxic cells to killing by X-rays and are called radiosensitizers.[9] Unfortunately, the neurotoxicity of **1** and **2**, which appears to be related partly to the lipophilic properties of these compounds and their ability to cross the blood-nerve barriers, substantially limit their clinical usefulness.[6,10,11] Radiosensitizers almost certainly act by oxidizing, or adding to radical centers in DNA, which is the major radiobiological target.[12,13] Targeting of radiosensitizers to DNA



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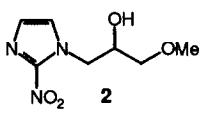

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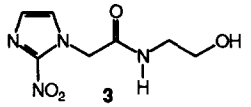

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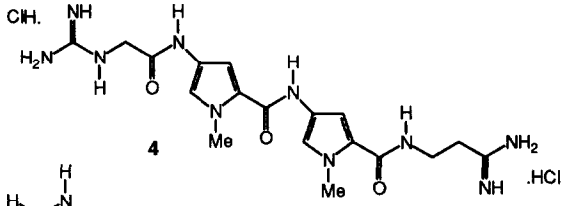

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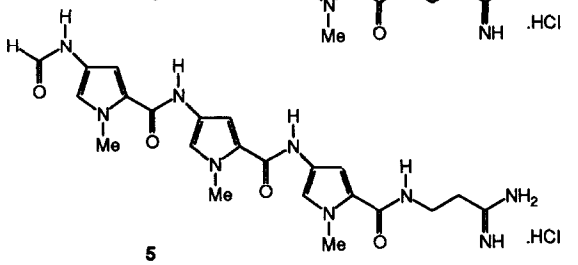

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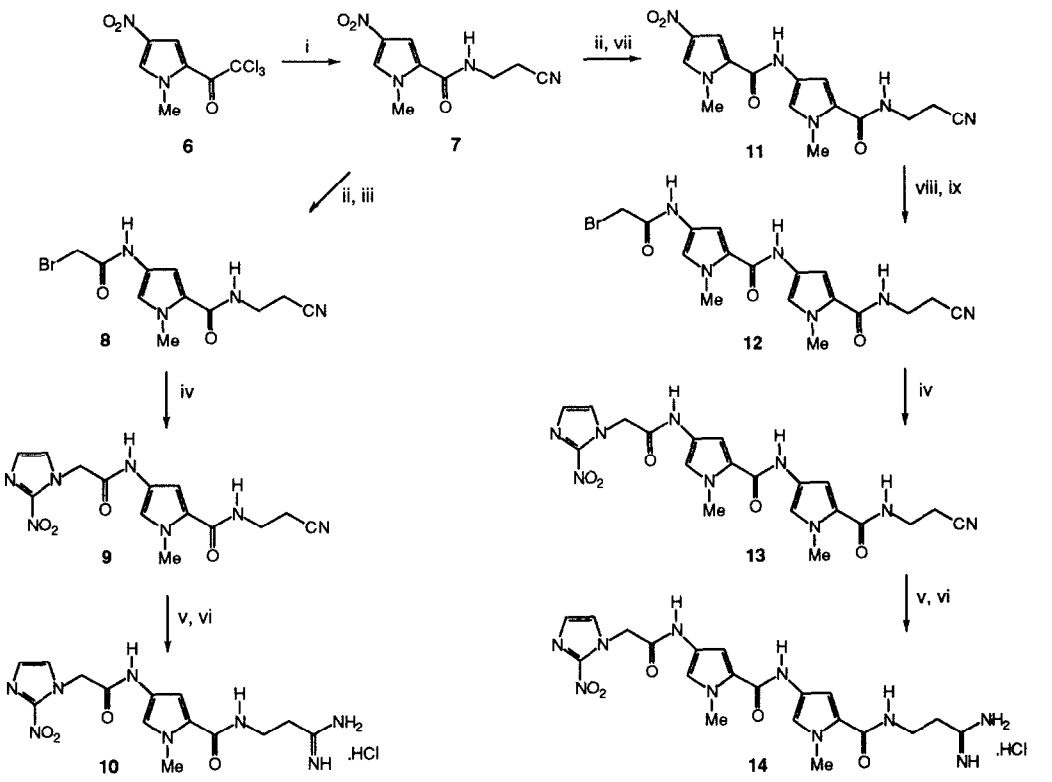
has been explored by linking intercalators to nitroarenes,[14] or by using an intercalator itself as a nitroaromatic moiety.[15] Minor groove binders as a targeting route have not been explored in radiosensitization, although nitro analogues of lexitropsins have been envisaged as potential cytotoxins.[16] The redox properties of nitroarenes control their radiosensitization efficiency[17] and aerobic[18] and hypoxic[19] cytotoxicity, with 2-nitroimidazoles and 5-nitrofurans having electron affinities[20] in the most active range. We have therefore prepared compounds having a nitroarene radiosensitizer moiety linked to a DNA binding ligand, based on netrospin (**4**) or distamycin (**5**), which act by blocking the template function of DNA by binding to the minor groove of duplex DNA.[21,22] We thus hoped to obtain a higher concentration of the radiosensitizer around the supposed active site, i.e. DNA.

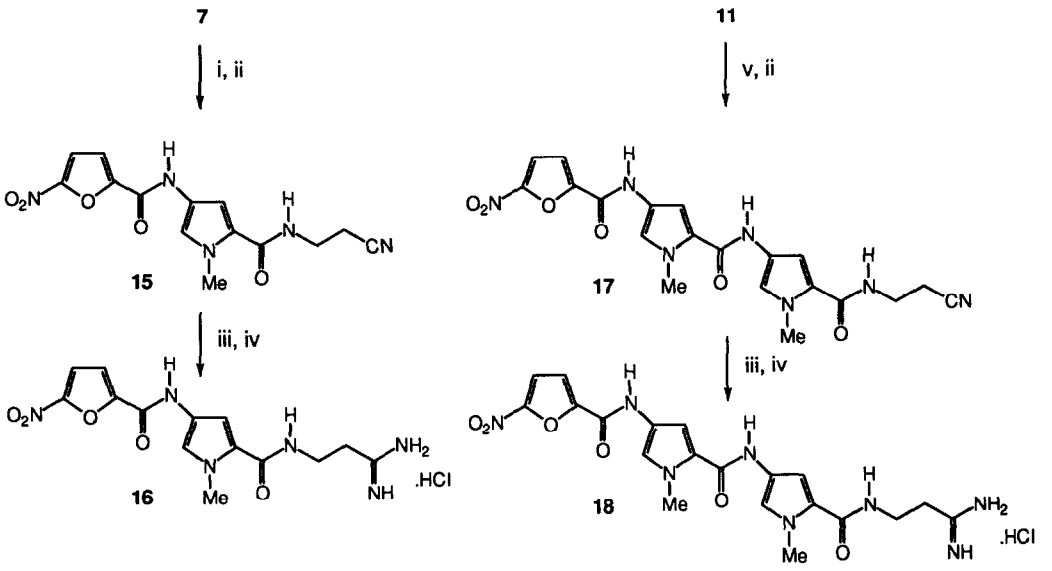
The syntheses of the compounds containing the 2-nitroimidazole ring required as the key starting material, the cyanoamide **7** [23,24] which was prepared (Scheme 1) by condensing 1-methyl-4-nitro-2-trichloroacetylpyrrole (**6**) [24] with 3-aminopropionitrile. Catalytic reduction of **7** in the presence of palladium on carbon yielded the corresponding amine, which, without separation, was treated with bromoacetyl bromide in the presence of diisopropylethylamine to afford the bromo compound **8**. The required compound **9** was then obtained as a bright yellow powder, by alkylation of the dry sodium salt of 2-nitroimidazole with **8** in the presence of 15-crown-5 in acetonitrile.[25]



Scheme 1. Reagents: i, $H_2NCH_2CH_2CN$, THF; ii, 10% Pd/C, MeOH; iii, $BrCH_2COBr$, *i*-Pr_2EtN, THF; iv, Na salt of 2-nitroimidazole, 15-crown-5, MeCN; v, HCl, EtOH; vi, NH_3, EtOH; vii, (**6**), DMF; viii, 10% Pd/C, MeOH/DMF; ix, $BrCH_2COBr$, *i*-Pr_2EtN, THF/DMF.

The nitro compound **7** was selectively reduced to give an aromatic amine which was not isolated but immediately reacted with **6** to give the nitrodiamide **11**. In a similar way to that described earlier for the conversion of **7** to **8** and to **9**, so **11** was converted to **12** and then to the cyano compound **13**. The amidine hydrochloride derivatives **10** and **14** were prepared from the cyanides **9** and **13** respectively by the Pinner reaction.

The compounds containing the 5-nitrofuran moiety were synthesised (Scheme 2) from the nitro compound **7** by catalytic reduction to the corresponding amine which was then condensed with 5-nitro-2-furoyl chloride to afford **15**. Similarly, regioselective reduction of **11** and subsequent condensation of the resultant amine with 5-nitro-2-furoyl chloride yielded the nitrile **17** as a brown powder. The amidine hydrochloride derivatives **16** and **18** were prepared via the Pinner reaction. All the novel compounds were characterised by ^{1}H n.m.r., i.r., and accurate mass measurement by mass spectroscopy or microanalysis (Table 1).



Scheme 2. Reagents: i, 10% Pd/C, MeOH; ii, 5-nitro-2-furoyl chloride, DMF; iii, HCl, EtOH; iv, NH_3, EtOH; v, 10% Pd/C, MeOH/DMF.

The novel amidine hydrochloride derivatives were evaluated for their binding affinities for DNA, by measuring the concentration required to halve the fluorescence at 600 nm (525 nm excitation) of ethidium bromide (0.5 μg/ml) bound to calf thymus DNA (DNA base:ethidium concentration ratio 1.24:1) at pH 7.4 (10 mM *N*-tris(hydroxymethyl)methyl-2-aminoethane buffer) and 25°C. The compounds were found to show no fluorescence themselves at this wavelength. Clear evidence for the concentration of the compounds at DNA, presumably by binding in the minor groove of the duplex, was found from their efficient displacement of the DNA bound fluorophor, or quenching by other mechanisms obviously reflecting concentrations near DNA.[26] The lower the C_{50} for fluorescence quenching, the greater the interaction with DNA (Table 2). If quenching is by displacement of ethidium, a value of C_{50} of 1.3 μM corresponds to a binding constant of ca. 10^7 M^{-1}.[27]

Table 1. Physical data of the novel compounds

Compound	Yield (%)	M.p. (°C)	Molecular formula
8	57	193-195	$C_{11}H_{13}O_2N_4Br$
9	71	130-132	$C_{14}H_{15}O_4N_7$
10	68	212-214	$C_{14}H_{18}O_4N_8$.HCl
12	51	103-104	$C_{17}H_{19}O_3N_6Br$
13	59	114-115	$C_{20}H_{21}O_5N_9$
14	73	230 (decomp.)	$C_{20}H_{24}O_5N_{10}$.HCl
15	61	207-210	$C_{14}H_{13}O_5N_3$
16	73	248-250	$C_{14}H_{16}O_5N_6$.HCl
17	60	147-149	$C_{20}H_{19}O_6N_7$
18	65	205-210	$C_{20}H_{22}O_6N_8$.HCl

The fluorescence quenching efficiencies were compared with netropsin and distamycin, which quenched the fluorescence at concentrations quite similar to those expected from previous work involving slightly different conditions.[27] Particular noteworthy results are the approximately three orders of magnitude difference in concentration required to achieve a given effect with the novel compounds **10** and **14**, containing the 2-nitroimidazole nucleus, compared with metronidazole (**1**) or misonidazole (**2**); the strong interaction with DNA of the nitrofurans, **16** and **18** compared to a simple neutral 5-nitrofuran, nitrofurazone; and the DNA binding efficiencies of the amides containing only two aromatic rings (**10** and **16**).

The radiosensitization efficiencies of the four novel nitroarene minor-groove binders (making the reasonable assumption that the strong interaction with DNA can thus be assigned) were investigated using V79 379A fibroblast-like cells *in vitro*.[17] In spite of the strong interaction with DNA, the radiosensitization efficiencies were uniformly poor (solubility or cytotoxicity restrictions prevented measurements at concentrations higher than those shown). Thus radiosensitization by **10** and **14** was barely detectable at an extracellular concentration of 0.5 mM, and the 5-nitrofuran **16** was less active than nitrofurazone.

It was possible, although unlikely by comparison with the reported intracellular uptake of distamycin,[31] that intracellular access of the novel compounds was less efficient relative to the free passage of misonidazole, etc. across the cytoplasmic membrane.[29] The intracellular uptake of **14** and **18** was therefore measured in V79 cells, using HPLC as previously described.[29] It was found that binding to DNA was so strong that sonication with acidic methanol[29] was not sufficient to free the compounds from cellular material, and digestion with DNase, phosphodiesterase and trypsin was necessary. The *average* intracellular concentrations relative to that in the suspending medium (50 μM) with ~10^6 cells/ml after 30-90 min exposure to **14** or **18** were ca. 5 or 13-fold higher, respectively, than those measured for misonidazole.[29] With such high average concentrations, and the demonstrated strong interaction with DNA, we would expect very high concentrations near the DNA of the novel nitroarene binders. The nitrofurans **16** and **18** were considerably more cytotoxic than nitrofurazone, as might be expected if the novel compounds enter the cell and interact strongly with DNA.

Table 2. DNA binding, radiosensitization, and cytotoxicity of representative compounds

Compound	DNA binding (Ethidium) [a]	Radiosensitization at concentration shown		Cytotoxicity (aerobic) [b]	Cellular uptake [c]
	C_{50} / μM	E.R. [d]	Concn./ mM	C_{50} / mM	C_i / C_e
Netropsin (**4**)	1.1				
Distamycin (**5**)	1.3				
18	4.8	1.16	0.5	<0.01	9.4
10	6.8	1.04	0.5	>0.25	
14	16	1.04	0.5	>0.25	3.6
16	26	1.30	0.05	0.025-0.05	
Nitrofurazone	>1000	1.60	0.05 [e]	0.13 [f]	
Misonidazole (**2**)	2700	1.60	1.0 [g]	1.3 [f]	0.7 [h]
Etanidazole (**3**)	3000	1.55	1.0 [i]		0.4 [i]
Metronidazole (**1**)	3200	1.60	~5 [g]	6.5 [f]	

[a] Concentration required for 50% decrease in fluorescence of ethidium bromide/DNA.
[b] Concentration required for 50% cell kill after *ca.* 7 days' aerobic exposure.[18]
[c] Ratio of intracellular:extracellular concentration in V79 hamster cells *in vitro*.[29]
[d] E.R. (enhancement ratio) = ratio of radiation doses required for similar cell kill in the exponential portion of the survival curve, for cell in anoxia compared to cells with the compound at the extracellular concentration shown. Approximate uncertainty ±0.05.[17]
[e] Ref.17. [f] Ref 18. [g] Ref. 28. [h] Ref. 29. [i] Ref. 30.

We conclude that minor groove binding as a route to targeting nitroarene radiosensitizers appears unlikely to result in therapeutic gain over compounds such as etanidazole. However, the study provides valuable insight into two aspects of DNA targeting.

Firstly, there are two possible explanations why high concentrations near DNA of electron-affinic compounds may not show markedly enhanced radiosensitization efficiciency. The bibenzimidazole minor groove binders, Hoechst 33342 and 33258 lack the nitroarene moiety and show *radioprotection* of both plasmid DNA and clonogenic survival of V79 cells.[32] This could be due to scavenging hydroxyl radicals close to DNA, a property which the novel compounds described here would also share, although other explanations for the protective effect of the bibenzimidazoles have been considered.[32] Another possible explanation is that the critical target radical(s) are immobile and short-lived, requiring good mobility of sensitizer along the DNA to oxidize or add to the target radical. Too strong an interaction, and hence too slow DNA/drug dissociation rates, may be counterproductive. This has been considered in relation to intercalators, where the rationale was originally based on intercepting mobile electrons.[33]

Perhaps the most important result from this work is that strong interaction with DNA results even if only one pyrrole/amidine moiety is present in the binder, making design of potential minor groove binders with other mechanisms of therapeutic action much simpler, although the nature of the interaction needs to be characterized.

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