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Studies relating to the synthesis, enzymatic reduction and cytotoxicity of a series of nitroaromatic prodrugs

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ABSTRACT

A series of *N*-nitroarylated-3-chloromethyl-1,2,3,4-tetrahydroisoquinoline derivatives, several of which also possessed a trifluoromethyl substituent, were prepared and assessed as potential nitroaromatic prodrugs. The enzymatic reduction of these compounds and their cytotoxicities were studied. The compounds were cytotoxic, but this is probably not related to their enzymatic reduction.

The reductive activation of the nitroaromatic prodrug CB 1954 **1** (Fig. 1) produces a bifunctional DNA-alkylating agent that is capable of producing DNA-DNA interstrand crosslinks.[1–3] In rats, reductive metabolism of the 4-nitro-group by the enzyme NAD(P)H: quinone oxidoreductase 1 (NQO1, also known as DT-diaphorase) resulted in the formation of the corresponding 4-hydroxylamine derivative **2** that subsequently underwent acylation generating the cytotoxic species **3**.[2,3] DNA alkylation then occurred through the acylated hydroxylamine-group (*via* a putative nitrenium species) and presumably the aziridine moiety, thus creating the DNA crosslinks.[4] Since the highest levels of NQO1 are often found in tumour tissues (breast, colon, lung, and liver), with lower levels detected in bone marrow, this enzyme became an attractive target for nitroaromatic-prodrug therapies in humans.[5] CB 1954 **1** has previously been shown to exhibit substantial and selective cytotoxicity against rat Walker 256 carcinomas but, disappointingly, human cell lines, even those cells expressing high levels of NQO1, were unresponsive towards this agent. A change in the amino acid residue 104 (tyrosine in the rat enzyme and glutamine in the human enzyme) was attributed to the poor catalytic response of human NQO1 towards CB-1954 **1**.[6,7] CB 1954 **1** was, however, reduced more efficiently by *E. coli* nitroreductase (NR)[8] and this property has stimulated interest in using anti-body directed enzyme prodrug therapy (ADEPT) or virus/gene-directed enzyme prodrug therapy (VDEPT/GDEPT) as activation protocols for CB 1954 **1** and related structures in tumours.[9–17] The reduction of the 2-nitro-group in CB 1954 **1** also occurred in the presence of *E. coli* NR resulting in the ultimate formation of amine derivative **4**, a monofunctional alkylating agent which exhibited a significant bystander effect.[18] Analogues of CB 1954 **1** have also been prepared and studied as potential cytotoxic agents[19] as have the structurally related nitrogen-mustard derivatives SN 23862 **5** and its analogues.[20–26] The 2-nitro-group in SN 23862 **5** is reduced by *E. coli* NR producing the amine derivative **6** thus facilitating the formation of an aziridinium species **7** from the mustard moiety.

In this Letter, we report the synthesis and evaluation (enzymatic and cytotoxicity) of a series of *N*-nitroarylated 1,2,3,4-tetrahydroisoquinoline derivatives with a core structure represented by formula **8** as potential nitroaromatic prodrugs. In view of the current interest in fluorinated compounds in medicinal chemistry,[27–30] structures **8b**–**8d** which possess the strongly electron-withdrawing trifluoromethyl group[31] have been prepared and compared with the non-trifluoromethylated mono- and di-nitro compounds **8a** and **8e** respectively. It was anticipated that if metabolic reduction of the nitro-group occurred in these molecules **8**, the resulting hydroxylamine (or amine) derivative would facilitate the formation of an aziridinium ion **9** (*i.e.* a similar activation process of transforming SN 23862 **5** into the aziridinium ion **7**). With compounds **8d** and **8e** (which are both associated with

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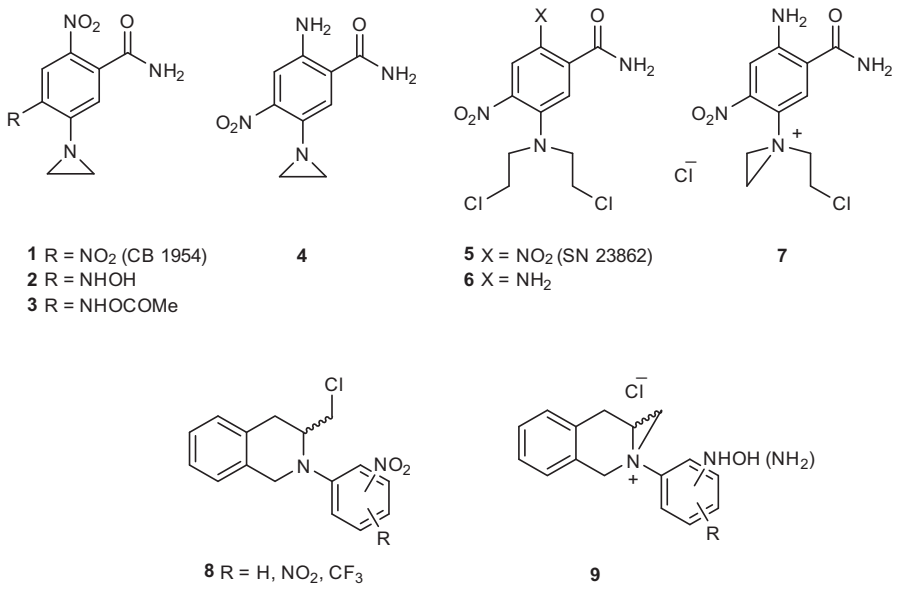
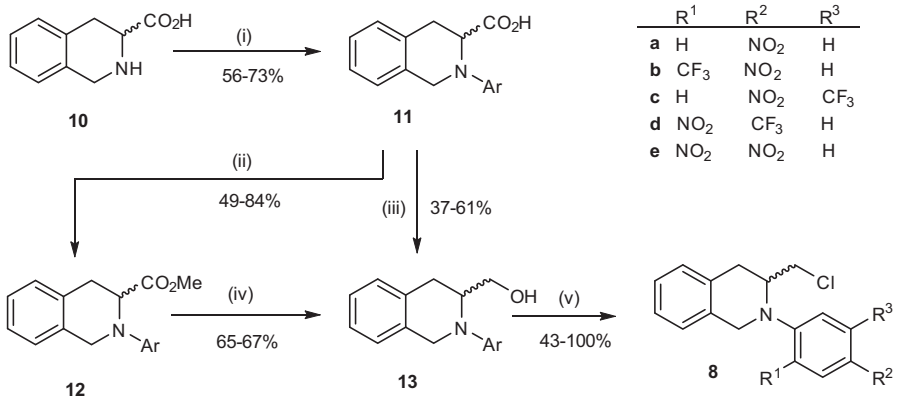


Fig. 1. Nitroaromatic prodrugs and their active metabolites.



Scheme 1. Synthesis of the prodrugs **8a–e**. Reagents and conditions: (i), ArF, DMSO, K_2CO_3, 80 °C then dil. HCl; (ii) Me_2SO_4, acetone, reflux; (iii) EtOCOCl, Et_3N, THF, −15 °C then $NaBH_4$, MeOH, 10 °C; (iv) $LiBH_4$, $B(OMe)_3$ (cat.), Et_2O, reflux; (v) $SOCl_2$, CH_2Cl_2, reflux.

$R^1 = NO_2$), subsequent acylation of the hydroxylamine-group (if formed) might then afford a potential bifunctional alkylating agent, structurally similar to the CB 1954 metabolite **2**. Compounds **8a–8e** (in which $R^2 = NO_2$) would not be expected to produce bifunctional alkylating species, but their corresponding amines (if formed), may exhibit a bystander effect similar to amine **4**.[18]

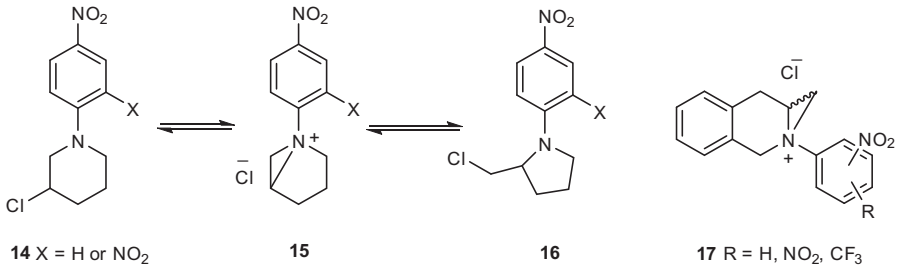
Compounds **8a–e** were therefore prepared from racemic 1,2,3,4-tetrahydroisoquinoline **10** as outlined in Scheme 1 (see Supplementary information for experimental details). Thus, compound **10** was reacted with an appropriate arylfluoride in warm DMSO solution in the presence of K_2CO_3 yielding, after acidification, the arylated carboxylic acid derivatives **11a–d**. Compound **11e** was prepared using a similar procedure except that boiling aqueous EtOH was used as the solvent. These products (with the exception of compounds **11d** and **11e**) were converted into their corresponding methyl esters **12** by treatment with dimethyl sulphate under basic conditions. Reduction of these esters **12** with $LiBH_4$ in the presence of a catalytic quantity of $B(OMe)_3$ afforded the alcohols **13**.[32] The alcohols **13** could also be prepared directly from the carboxylic acids **11** by formation of a mixed anhydride with ethyl chloroformate under basic conditions followed by $NaBH_4$ reduction.[33,34] The required chloromethyl derivatives **8**

Table 1
Specific activities of CB 1954 **1** and prodrugs **8a–e**.

Compound	Human NQO1		*E. coli* NR	
	(μmol/min/mg)	Relative to CB 1954 **1**	(μmol/min/mg)	Relative to CB 1954 **1**
CB 1954 **1**	0.0062	1.000	1.860	1.000
8a	<0.0001	<0.01	<0.01	<0.001
8b	0.0270	4.355	0.166	0.089
8c	0.0120	1.936	0.106	0.057
8d	0.0177	2.855	<0.01	<0.001
8e	0.0033	0.532	0.254	0.137

Table 2
IC_{50} values (μmol) of prodrugs **8a–e** and CB 1954 **1**.

Compound	Cytotoxicity (3 days exposure): IC_{50} values (μmol)			
	Control F179	Human NQO1 hDT7	*E. coli* NR T116	Rat NQO1 186/6
CB 1954 **1**	195.9	1.5	0.03	0.05
8a	3.3	2.8	3.1	2.9
8b	37.8	27.5	3.1	22.6
8c	7.3	5.9	2.9	5.8
8d	36.6	31.2	39.1	34.4
8e	49.0	43.1	1.2	36.6



14 X = H or NO_2 **15** **16** **17** R = H, NO_2, CF_3

Scheme 2. *N*-Nitroaryl aziridinium intermediates as potential mono-alkylating agents.

were prepared from the alcohols **13** by their reaction with $SOCl_2$ in CH_2Cl_2 solution at reflux.

The series of prodrugs **8** were assessed against the enzymes human NQO1 and *E. coli* NR and their specific activities (calculated by dividing the initial rate of reaction by the concentration of the enzyme used and quoted as μmoles of compound reduced per minute per mg of protein (μmol/min/mg)) have been compared to CB 1954 **1** (Table 1). Interestingly, all the trifluoromethylated derivatives **8b–d** exhibited higher specific activities with human NQO1 than CB 1954 **1**. The mono-nitro derivative **8a** was a poor substrate for human NQO1, and in the absence of a second electron-withdrawing group located on the *N*-aryl-substituent, this observation was not unexpected. The specific activity of the dinitro-derivative **8e** was lower than the nitro/trifluoromethylated derivatives **8b–d**. All of the series of prodrugs **8a–8e** showed poor specific activities with *E. coli* NR compared to CB-1954 **1**. Noteworthy is the observation that the prodrug **8d**, which lacks a *para*-nitro substituent in the *N*-aryl ring, exhibits very little specific activity with *E. coli* NR compared to prodrugs **8b**, **8d** and **8e**. This correlates with the *E. coli* NR-induced reduction of the nitro-group in both CB 1954 **1** and SN 23862 **5** (*i.e.* the nitro-group *para* to the aziridine/mustard moieties is reduced).

In order to assess the cytotoxicities of the potential prodrugs **8a–e**, their IC_{50} values were determined against constructed cell-lines that expressed the relevant enzyme against a null background using a conventional sulforhodamine-B (SRB) assay.[35] Examination of the cytotoxicity data (Table 2) revealed that prodrugs **8a** and **8c** exhibited broadly similar IC_{50} values across the four cell-lines and that there was no clear differentiation between the control line and the three nitroreductase-expressing cell-lines. Additionally, both compounds displayed a greater cytotoxicity in the control cell-line than CB 1954 **1**. These observations suggested that compounds **8a** and **8c** are associated with a cytotoxic effect that is not related to their nitroreductase activity despite prodrug **8c** showing a higher specific activity to human NQO1 than CB 1954 **1** (Table 1). A possible explanation for this observation is that these prodrugs are behaving as mono-functional alkylating agents, either as alkyl chlorides or *via* aziridinium intermediates. In support of this hypothesis, we have recently shown that the *N*-nitroaryl-3-chloropiperidine derivatives **14** are converted *via* aziridinium intermediates **15** into the *N*-nitroaryl-2-chloropyrrolidines **16** (Scheme 2).[36] Hence compounds **8a** and **8c** may be forming aziridinium intermediates **17** (rather than aziridiniums **9**, Fig. 1) that might be capable of functioning as mono-alkylating agents. Prodrug **8d** also showed broadly similar, but significantly higher IC_{50} values across the four cell-lines compared to compounds **8a** and **8c**. Prodrugs **8b** and **8e** displayed broadly similar IC_{50} values across three of the cell-lines (control, human NQO1 and rat NQO1), but both of these compounds are associated with significantly lower IC_{50} values in the *E. coli* NR cell-line for reasons that are as yet unclear. The relatively high cytotoxicity observed for CB 1954 **1** compared to the poor cytotoxicities seen for the mono-nitro analogues (*i.e.* structure **1** with one nitro-group replaced by hydrogen) in nitroreductase-transfected cell lines has been attributed to the reduction potential of these pro-drugs.[19] The cytotoxicities of the mono and dinitro prodrugs **8a** and **8e** respectively are not correlated with their perceived reduction potentials because compound **8a** is significantly more cytotoxic than compound **8e** in the control, human NQO1 and rat NQO1 cell lines and broadly similar to compound **8e** in the *E. coli* NR cell line. This evidence would also support the hypothesis that the series of prodrugs **8a–8e** may be acting as mono-alkylating agents.

In conclusion, it is clear that all of the prodrugs **8a–8e** examined in this study are significantly less cytotoxic than CB 1954 **1** in the *E. coli* NR and rat NQO1 cell-lines. Compounds **8a** and **8c** displayed IC_{50} values that are reasonably aligned to that of CB 1954 **1**, but only in the human NQO1 cell-line. The cytotoxicity studies suggest that these prodrugs may be functioning as mono-functional alkylating agents.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.11.024.

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