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


![Chemical Structure](image)

**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.

**Keywords:** nitrogen mustards, NQO1, nitroreductase, CB 1954, nitroaromatic prodrugs.

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The reductive activation of the nitroaromatic prodrug CB 1954 1 (Figure 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-

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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 agents19 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.

![Chemical structures](image)

**Figure 1.** Nitroaromatic prodrugs and their active metabolites.

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 group31 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 R1 = NO2), 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 R2 = NO2) 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$_2$CO$_3$ 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. 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. The required chloromethyl derivatives 8 were prepared from the alcohols 13 by their reaction with SOCl$_2$ in CH$_2$Cl$_2$ solution at reflux.

Scheme 1. Synthesis of the prodrugs 8a-e. Reagents and conditions: (i), ArF, DMSO, K$_2$CO$_3$, 80 ºC then dil. HCl; (ii) Me$_2$SO$_a$, acetone, reflux; (iii) EtOCOCl, Et$_3$N, THF, -15 ºC then NaBH$_4$, MeOH, 10 ºC; (iv) LiBH$_4$, B(OMe)$_3$ (cat.), Et$_2$O, reflux; (v) SOCl$_2$, CH$_2$Cl$_2$, 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 \textit{E. coli} NR-induced reduction of the nitro-group in both CB 1954 1 and SN 23862 5 (i.e. the nitro-group \textit{para} to the aziridine/mustard moieties is reduced).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Human NQO1 (µmol/min/mg)</th>
<th>Relative to CB 1954 1</th>
<th>\textit{E. coli} NR (µmol/min/mg)</th>
<th>Relative to CB 1954 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB 1954 1</td>
<td>0.0062</td>
<td>1.000</td>
<td>1.860</td>
<td>1.000</td>
</tr>
<tr>
<td>8a</td>
<td>&lt;0.0001</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8b</td>
<td>0.0270</td>
<td>4.355</td>
<td>0.166</td>
<td>0.089</td>
</tr>
<tr>
<td>8c</td>
<td>0.0120</td>
<td>1.936</td>
<td>0.106</td>
<td>0.057</td>
</tr>
<tr>
<td>8d</td>
<td>0.0177</td>
<td>2.855</td>
<td>&lt;0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8e</td>
<td>0.0033</td>
<td>0.532</td>
<td>0.254</td>
<td>0.137</td>
</tr>
</tbody>
</table>

\textbf{Table 1.} Specific activities of CB 1954 1 and prodrugs 8a-e.

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. 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 \textit{via} aziridinium intermediates. In support of this hypothesis, we have recently shown that the N-nitroaryl-3-chloropiperidine derivatives 14 are converted \textit{via} aziridinium intermediates 15 into the N-nitroaryl-2-chloropyrrolidines 16 (Scheme 2). Hence compounds 8a and 8c may be forming aziridinium intermediates 17 (rather than aziridiniums 9, Figure 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 \textit{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. The cytotoxicities of the mono and dinitro prodrugs 8a and
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.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Control F179</th>
<th>Human NQO1 hDT7</th>
<th><em>E. coli</em> NR T116</th>
<th>Rat NQO1 186/6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB 1954 1</td>
<td>195.9</td>
<td>1.5</td>
<td>0.03</td>
<td>0.05</td>
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<tr>
<td>8a</td>
<td>3.3</td>
<td>2.8</td>
<td>3.1</td>
<td>2.9</td>
</tr>
<tr>
<td>8b</td>
<td>37.8</td>
<td>27.5</td>
<td>3.1</td>
<td>22.6</td>
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<tr>
<td>8c</td>
<td>7.3</td>
<td>5.9</td>
<td>2.9</td>
<td>5.8</td>
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<tr>
<td>8d</td>
<td>36.6</td>
<td>31.2</td>
<td>39.1</td>
<td>34.4</td>
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<tr>
<td>8e</td>
<td>49.0</td>
<td>43.1</td>
<td>1.2</td>
<td>36.6</td>
</tr>
</tbody>
</table>

Table 2. IC$_{50}$ values (µmol) of prodrugs 8a-e and CB 1954 1.

Scheme 2. *N*-Nitroaryl aziridinium intermediates as potential 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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References and notes