Bioorganic & Medicinal Chemistry Letters 26 (2016) 5851–5854

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

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

Philip J. Burke [a], Lai Chun Wong [b], Terence C. Jenkins [a], Richard J. Knox [a], Ian T. Meikle [b], Stephen P. Stanforth [b,*]

[a] Morvus Technology Ltd, Llanvetherine Court, Llanvetherine, Abergavenny NP7 8NL, UK
[b] Department of Applied Sciences, University of Northumbria, Newcastle-upon-Tyne NE1 8ST, UK

ARTICLE INFO

Article history:
Received 17 October 2016
Revised 10 November 2016
Accepted 10 November 2016
Available online 11 November 2016

Keywords:
Nitrogen mustards
NQO1
Nitroreductase
CB 1954
Nitroaromatic prodrugs

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

* Corresponding author.
E-mail address: steven.stanforth@northumbria.ac.uk (S.P. Stanforth).

http://dx.doi.org/10.1016/j.bmcl.2016.11.024
0960-894X/

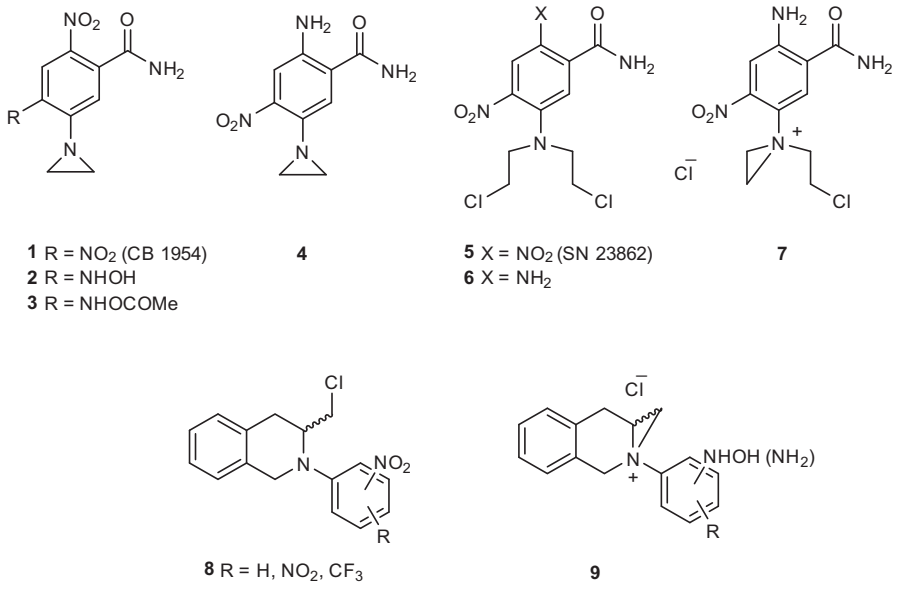
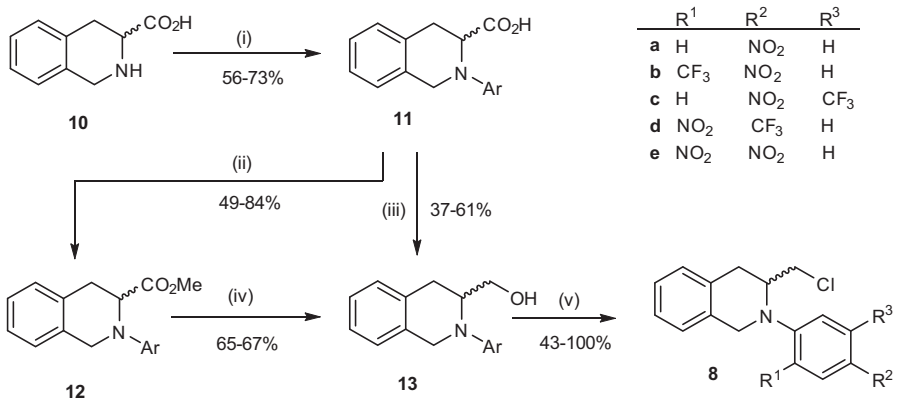


Fig. 1. Nitroaromatic prodrugs and their active metabolites.



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