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The synthesis of 2-nitroaryl-1,2,3,4-tetrahydroisoquinolines, nitro-substituted 5,6-dihydrobenzimidazo[2,1-*a*]isoquinoline *N*-oxides and related heterocycles as potential bioreducible substrates for the enzymes NAD(P)H: quinone oxidoreductase 1 and *E. coli* nitroreductase

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ABSTRACT

A series of 2-nitroaryl-1,2,3,4-tetrahydroisoquinolines **10** and nitro-substituted 5,6-dihydrobenzimidazo[2,1-*a*]isoquinoline *N*-oxides **11** have been synthesised and evaluated as potential bioreducible substrates for the enzymes NAD(P)H: quinone oxidoreductase 1 (NQO1) and *Escherichia coli* nitroreductase (NR). Also prepared and evaluated were 2-(3,5-dinitropyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline **12** and 5,6-dihydro-10-nitropyrido[3″,2″:4′,5′]imidazo[2′,1′-*a*]isoquinoline 12-oxide **13**. Both compounds **10b** and **13** were reduced faster by human NQO1 than by CB-1954 [5-(aziridin-1-yl)-2,4-dinitrobenzamide].

Many nitroaromatic pro-drugs[1] have been assessed as potential substrates for the enzyme NAD(P)H: quinone oxidoreductase 1 (NQO1), also known as DT-diaphorase, because elevated levels of NQO1 are often associated with tumour tissues.[2] *Escherichia coli* nitroreductase (NR) has also generated interest as an activator of nitroaromatic pro-drugs through use in either anti-body directed enzyme pro-drug therapy (ADEPT) or virus/gene-directed enzyme pro-drug therapy (VDEPT/GDEPT).[3] An important landmark in the development of bioreducible nitroaromatic pro-drugs was the observation that CB-1954 (Fig. 1) exhibited cytotoxicity against rat Walker 256 carcinomas.[4] Unfortunately, human cell lines, including those expressing high levels of NQO1, were unresponsive. However, CB-1954 was reduced more efficiently by *E. coli* NR and this property has stimulated interest in using ADEPT or VDEPT/GDEPT as potential methods for activating CB-1954 in tumours.

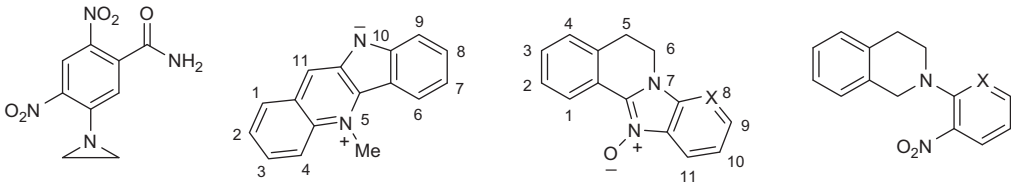

Figure 1. CB-1954, Cryptolepine and potential anti-cancer targets.

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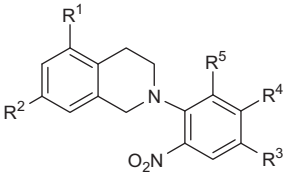
The alkaloid, Cryptolepine **1** (Fig. 1), has been shown to possess a range of biological activities including anti-cancer activity.[5] A series of nitro-analogues of Cryptolepine **1** have recently been prepared as potential bioreducible anti-cancer agents and these compounds have been shown to be human NQO1 substrates and also have displayed cytotoxic activity.[6] However, the mechanism of action of these Cryptolepine nitro-derivatives is as yet unclear because it has been demonstrated that these compounds are localised within the cell cytoplasm and are unable to penetrate into the nucleus.

In view of our previous work describing the synthesis of 5,6-dihydrobenzimidazo[2,1-*a*]isoquinoline *N*-oxides **2** and the 5,6-dihydropyrido[2′,3′:5,4]imidazo[2,1-*a*]isoquinoline 12-oxide **3**,[7] as well as our work on other bioreducible nitroaromatics,[8] we envisaged that nitro-containing derivatives of these compounds might also have the potential to act as bioreducible anti-cancer agents (Fig. 1). These heterocycles could be prepared by cyclodehydration of the corresponding 2-nitroaryl-1,2,3,4-tetrahydroisoquinoline derivatives **4** and **5**, respectively.[9] Thus, derivatives of heterocycles **4** and **5** might provide useful scaffolds for the future development of bioreducible pro-drugs. A series of nitro-derivatives of heterocycles **2–5** have therefore been prepared and evaluated as substrates for human NQO1 and *E. coli* NR in order to assess their bioreducibility profiles.

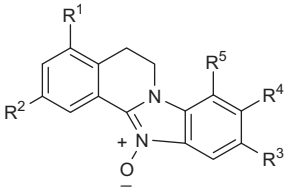
The reaction of 1,2,3,4-tetrahydroisoquinoline **6**, 5-nitro-1,2,3,4-tetrahydroisoquinoline **7**[10] or 7-nitro-1,2,3,4-tetrahydroisoquinoline **8**[11] with the appropriate dinitrochloro- or dinitrofluoro-aromatic compounds **9** under basic conditions afforded the corresponding 2-dinitroaryl-1,2,3,4-tetrahydroisoquinoline derivatives **10a–10o** (14–95%) (Scheme 1, Table 1). A range of compounds **10** were selected for conversion into their corresponding *N*-oxide derivatives **11** (25–99%) by heating in propionic acid at reflux.[7,9] Heterocycles **12** (97% yield) and **13** (quantitative yield) were prepared similarly. Included in the potential bioreducible substrates were some trifluoromethylated derivatives because of the general interest of this functionality in medicinal chemistry.[12] Experimental details are given in the Supplementary data.

The compounds prepared in Table 1 were evaluated as human NQO1 and *E. coli* NR substrates and both their specific activities (i.e. rate of reduction of the substrate by the appropriate enzyme) and their relative activities compared to CB-1954 are presented in Table 2. The compounds' specific activities were determined by monitoring their rate of disappearance as a function of time by HPLC from which their initial rate of reaction was derived. In some cases overlapping substrate and product peaks were observed by HPLC, and in these cases, an estimation of the specific activity was obtained by measuring the decrease in NADH concentration with the assumption that 2 equiv of NADH are required for reduction of the nitro-group to the corresponding hydroxylamine group. Thus, the rate of decrease of NADH

Table 1
Synthesis of compounds **10** and **11**



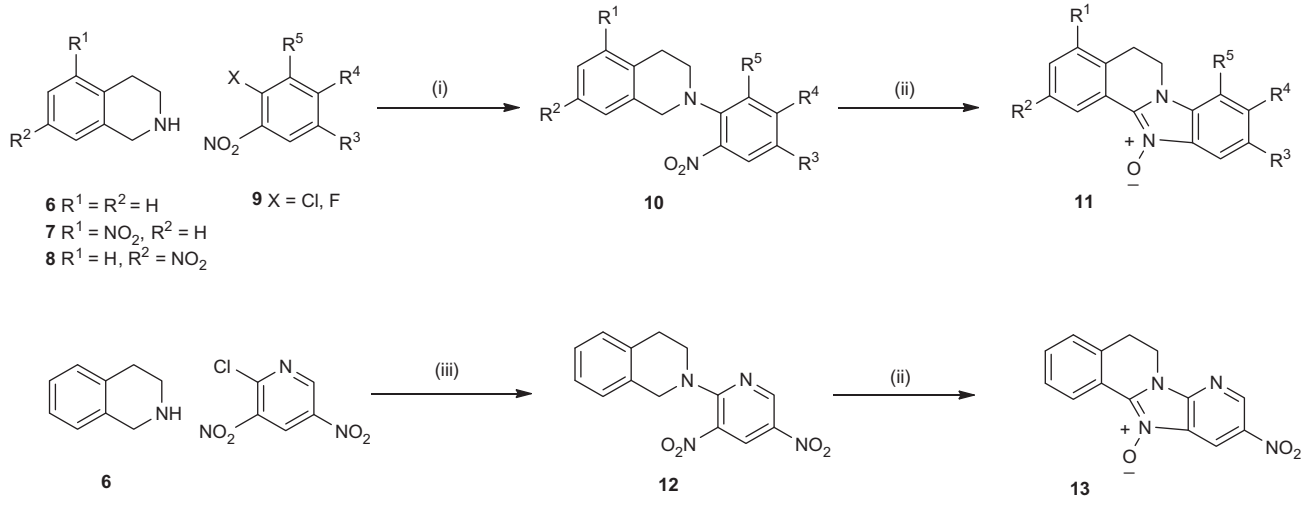
10



11

	R^1	R^2	R^3	R^4	R^5	**10** Method[a]	**10** Yield (%)	**11** Yield (%)
a	H	H	NO_2	H	H	A	95	99
b	H	H	NO_2	H	CF_3	A	60	93
c	H	H	NO_2	H	CO_2H	B	70	[b]
d	H	H	NO_2	H	$CONH_2$	A	75	89
e	H	H	NO_2	$CONH_2$	H	A	68	74
f	H	H	CO_2H	H	NO_2	B	75	[b]
g	H	H	$CONH_2$	H	NO_2	C	27	[b]
h	NO_2	H	NO_2	H	H	A	90	82
i	NO_2	H	NO_2	H	CF_3	A	49	61
j	NO_2	H	NO_2	H	CO_2H	D	60	[b]
k	NO_2	H	NO_2	$CONH_2$	H	A	75	71
l	H	NO_2	NO_2	H	H	A	45	52
m	H	NO_2	NO_2	H	CF_3	A	14	[b]
n	H	NO_2	NO_2	H	CO_2H	D	51	[b]
o	H	NO_2	NO_2	$CONH_2$	H	A	68	[c]

[a] Method A: $EtOH/H_2O$, $NaHCO_3$, reflux; Method B: (i) DMSO, K_2CO_3, ca 60 °C, (ii) concd HCl; Method C: DMSO, K_2CO_3, ca 60 °C; Method D: (i) $EtOH/H_2O$, $NaHCO_3$, reflux, (ii) concd HCl.
[b] Not prepared.
[c] Compound **11o** could not be characterised satisfactorily due to its insolubility.



Scheme 1. Synthesis of nitro-substituted 5,6-dihydrobenzimidazole *N*-oxides and related derivatives. Reagents and conditions: (i) see Table 1; (ii) propionic acid, reflux; (iii) $EtOH/H_2O$, $NaHCO_3$, 50–60 °C.

Table 2
Activity data of potential human NQO1 and *E. coli* NR substrates as determined by HPLC

Compound	Human NQO1		*E. coli* NR	
	Specific activity[a] (μmol/min/mg)	Relative to CB-1954	Specific activity[a] (μmol/min/mg)	Relative to CB-1954
CB-1954	6.2×10^{-3}	1.0	1.86	1.0
10a	<0.001	<0.16	0.44	0.2
10b	0.110	17.7	41.49	22.3
10c	0.011	1.8	4.22	2.3
10d	0.053	8.6	(2.82)	(1.5)
10e	0.042	6.8	4.40	2.4
10f	<0.001	<0.16	0.02	0.01
10g	<0.001	<0.16	<0.001	<0.001
10h	<0.001	<0.16	0.44	0.2
10i[b]	—	—	—	—
10j	(0.038)	(6.1)	(9.85)	(5.3)
10k	0.0014	0.23	1.11	0.6
10l[c]	—	—	—	—
10m[d]	0.076	12.3	2.39	1.3
10n	(0.020)	(3.2)	(14.8)	(7.4)
10o	<0.001	<0.16	1.67	0.9
11a	0.016	2.6	0.50	0.3
11b[e]	—	—	—	—
11d	<0.001	<0.16	(1.62)	(0.9)
11e	<0.001	<0.16	<0.001	<0.001
11h	<0.001	<0.16	(1.51)	(0.8)
11i	<0.001	<0.16	<0.001	<0.001
11k	<0.001	<0.16	<0.001	<0.001
11l	<0.001	<0.16	(1.3)	(0.6)
12	0.013	2.1	1.49	0.8
13	0.170	27.4	54.0	29.0

[a] Numbers in parenthesis determined by monitoring the decrease in NADH concentration. Other values determined by monitoring the decrease in substrate concentration. Specific activities determined at 37 °C.
[b] Compound was insoluble in 10 mM phosphate buffer, pH 7 + 10% DMSO.
[c] The compound precipitated out of solution in buffer with 10% DMSO + 500 μM NADH.
[d] Compound shows poor solubility. Enzyme work carried out using 50 μM compound in the buffer with 10% DMSO. Rates assume a starting concentration of 50 μM compound and no effect of the DMSO on the enzyme activity.
[e] Multiple reduction products observed.

concentration has been divided by 2 in order to produce an estimation of specific activity and these values are given in parenthesis in Table 2.

Within the series of compounds **10**, the trifluoromethylated derivatives **10b** and **10m** exhibited human NQO1 activities that were higher than CB-1954. The activity of the other trifluoromethylated substrate, compound **10i**, could not be assessed because of the relative insolubility of this compound. Compound **10b** also exhibited the best *E. coli* NR activity of this series of compounds whereas compound **10m** was only comparable in activity to CB-1954. The amide isomers, compounds **10d** and **10e** which bear the closest structural resemblance to CB-1954, were better substrates for human NQO1 by a factor of 8.6 and 6.8, respectively, than CB-1954 but were only slightly better substrates for *E. coli* NR. The two isomeric carboxylic acid derivatives, compounds **10j** and **10n**, in which one nitro-group was present in the 1,2,3,4-tetrahydroisoquinoline moiety of the molecule, both showed higher activities towards human NQO1 and *E. coli* NR than CB-1954 but the rate of disappearance of NADH was used to estimate the activities for these two substrates. Compounds **10f** and **10g**, both bearing a 2,6-dinitro substitution pattern in the *N*-aryl ring were essentially devoid of activity in both the human NQO1 and *E. coli* NR assays.

The relative activities of the nitro-containing 5,6-dihydrobenzimidazo[2,1-*a*]isoquinoline *N*-oxide derivatives **11** compared to CB-1954 were disappointing. Only compound **11a** showed human NQO1 activity greater than CB-1954. All the other derivatives exhibited little, if any, activity with human NQO1 and *E. coli* NR. However, this is in marked contrast to the pyridine analogue, compound **13**, which displayed significantly better relative activities towards both human NQO1 and *E. coli* NR than CB-1954 (27.4 and 29.0, respectively).

In conclusion, the trifluoromethylated derivative **10b** has been established to be a significantly better substrate for human NQO1 and *E. coli* NR than CB-1954. Some other *N*-nitroaryl-1,2,3,4-tetrahydroisoquinoline derivatives were also better human NQO1 and *E. coli* NR substrates than CB-1954 but only marginally so. Most of the *N*-oxide derivatives evaluated showed little, if any, activity towards these two enzymes with the exception of heterocycle **13** which was reduced over twenty times faster than CB-1954. These studies suggest that the synthesis of other trifluoromethylated 1,2,3,4-tetrahydroisoquinoline derivatives would be useful in order to obtain a more detailed structure–activity profile.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.10.044.

References and notes

1. Chen, Y.; Hu, L. *Med. Res. Rev.* **2009**, *29*, 29. and references cited therein.
2. (a) Cresteil, T.; Jaiswal, A. K. *Biochem. Pharmocol.* **1991**, *42*, 1021; (b) Riley, R. J.; Workman, P. *Biochem. Pharmacol.* **1992**, *43*, 1657.
3. (a) Melton, R. G.; Knox, R. J. *Enzyme-Prodrug Strategies for Cancer Therapy*; Kluwer Academic/Plenum: New York, 1999. p. 272; (b) Denny, W. A. *Curr. Pharm. Des.* **1996**, *2*, 281; (c) Denny, W. A. *Eur. J. Med. Chem.* **2001**, *36*, 577; (d) Denny, W. A.; Wilson, W. R. *J. Pharm. Pharmacol.* **1998**, *50*, 387; (e) Denny, W. A.

Curr. Pharm. Des. **2002**, *8*, 1349; (f) Roldán, M. D.; Pérez-Reinado, E.; Castillo, F.; Moreno-Vivián, C. *FEMS Microbiol. Rev.* **2008**, *32*, 474.
4. Cobb, L. M.; Connors, T. A.; Elso, L. A.; Khan, A. H.; Mitchley, B. V. C.; Ross, W. J. C.; Whisson, M. E. *Biochem. Pharmacol.* **1969**, *18*, 1519.
5. Lavardo, J.; Reszka, A. P.; Moreria, R.; Neidle, S.; Paulo, A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7042.
6. Seville, S.; Phillips, R. M.; Shnyder, S. D.; Wright, C. W. *Bioorg. Med. Chem.* **2007**, *15*, 6353.
7. Hedley, K. A.; Stanforth, S. P. *J. Heterocycl. Chem.* **1995**, *32*, 529.
8. Burke, P. J.; Chun Wong, L.; Clegg, W.; Harrington, R. W.; Jenkins, T. C.; Knox, R. J.; Meikle, I. T.; Stanforth, S. P. *Tetrahedron Lett.* **2010**, *51*, 3918.
9. Meth-Cohn, O. *Adv. Heterocycl. Chem.* **1996**, *65*, 1.
10. Zhu, Z.; Furr, J.; Buolamwini, J. K. *J. Med. Chem.* **2003**, *46*, 831.
11. Ajao, J. F.; Bird, C. W. *J. Heterocycl. Chem.* **1985**, *22*, 329.
12. Cherkupally, P.; Beier, P. *Tetrahedron Lett.* **2010**, *51*, 252. and references cited therein.