Bioorganic & Medicinal Chemistry Letters 22 (2012) 6583–6586

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design, synthesis, and evaluation of potential prodrugs of DFMO for reductive activation

Yanhui Yang [a], Andrew Voak [c], Shane R. Wilkinson [c], Longqin Hu [a,b,*]

[a] Department of Medicinal Chemistry, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, USA
[b] The Cancer Institute of New Jersey, New Brunswick, NJ 08901, USA
[c] Queen Mary Pre-Clinical Drug Discovery Group, School of Biological & Chemical Sciences, Queen Mary University of London, Mile End Road, London E1 4NS, UK

ARTICLE INFO

Article history:
Received 19 June 2012
Revised 31 August 2012
Accepted 4 September 2012
Available online 13 September 2012

Keywords:
α-Difluoromethylornithine
Nitroreductase
Prodrug design
Reductive activation
Trypanocidal

ABSTRACT

A series of potential DFMO prodrugs was designed through the incorporation of 4-nitrobenzyl ester or carbamate groups for potential activation by trypanosomal nitroreductase. It was found that only modification of N^{ε}-amino group of DFMO by 4-nitro-2-fluorobenzyloxycarbonyl resulted in significant trypanocidal activity and could serve as a lead for further investigation.

α-Difluoromethylornithine (DFMO or Eflornithine) is an irreversible inhibitor of ornithine decarboxylase (EC 4.1.1.17), an enzyme that catalyzes the first step in polyamine biosynthesis. Inhibition of this enzyme activity results in depletion of putrescine and spermidine, and consequently slows down cell proliferation. Although originally developed in the 1970's as a treatment for cancer, DFMO was shown to be ineffective in treating malignancies. The lack of anti-tumor activity stemmed from the fast turnover rate of ornithine decarboxylase displayed in mammalian cells ($t_{1/2}$ = 10–30 min) such that DFMO-inhibited enzyme is rapidly cleared from the cell and replaced with newly synthesized active protein.[1] Subsequently, it was found that DFMO is highly effective against human African trypanosomiasis (African sleeping sickness), especially the West African form (*Trypanosoma brucei gambiense*), due to the slow turn over ($t_{1/2}$ = 18–19 h) of its ornithine decarboxylase.[2,3]

Intravenous infusions of DFMO were approved in 1990 by US Food and Drug Administration as a treatment for the meningoencephalic stage of the human African trypanosomiasis. The current recommended regimen for all age groups is 400 mg/kg body weight/day administered over 4 intravenous infusions daily for 14 days. Intravenous DFMO given either alone or in combination with oral doses of nifurtimox has proven to be effective and safe with 2-year cure rates of 97–100% against West African trypanosomiasis.[3] The major drawback of intravenous DFMO is, however, related to its high dose and cost of treatment, and the inconvenience of intravenous administration especially in understaffed rural hospitals of disease endemic countries. Oral DFMO would be logistically cheaper and much easier, especially for use in rural settings. Even though the oral bioavailability of DFMO is not a problem, its elimination from the blood is rapid so that high doses are necessary to obtain the desired therapeutic effect.[4] Therefore, the development of DFMO prodrugs could be one approach to overcome these drawbacks of DFMO.

Prodrug design is an important strategy that has proven to work for many drugs by improving their undesirable physico-chemical and biological properties.[5–9] Nitroaromatic prodrug conjugates have been developed that rely on specific activation by a designated *E. coli* type I nitroreductase, which can be delivered site-specifically to tumor cells and tissues using antibody-directed enzyme prodrug therapy (ADEPT) or gene-directed enzyme prodrug therapy (GDEPT).[5,8,10,11] We have shown that trypanosomes also express a type I nitroreductase that can activate nitrobenzyl phosphoramide mustard and azirinindyl nitrobenzamidine prodrugs.[12–15] Herein, we report the synthesis and evaluation of four types of DFMO prodrugs that incorporate a nitroaromatic functionality designed to exploit the trypanosomal type I nitroreductase as the activating enzyme to help potentially increase target specificity and improve bioavailability.

Design and synthesis

Examination of the structure of DFMO suggested that there are three possible sites for the attachment of a promoiety. As shown in

* Corresponding author. Tel.: +1 848 445 5291; fax: +1 732 445 6312.
E-mail address: LongHu@rutgers.edu (L. Hu).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.09.005

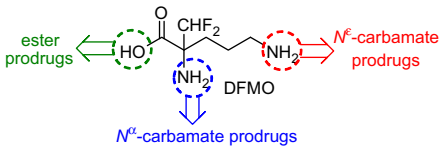


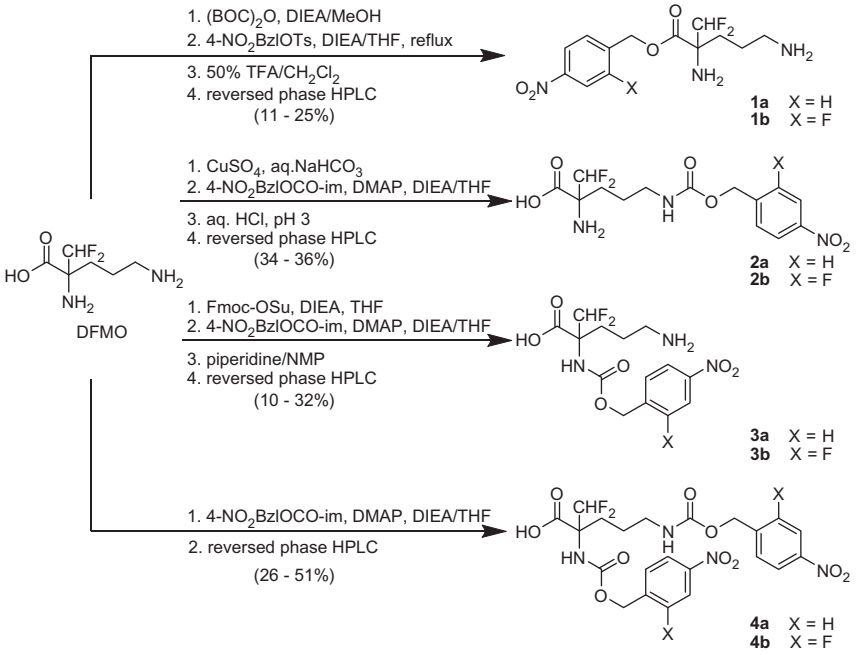
Figure 1. Structure of DFMO and sites for the attachment of a promoiety in the design of potential prodrugs.

Figure 1, DFMO contains two amino groups and one carboxylic acid group; each of these functional groups could be attached to a nitrobenzyl promoiety to form either a carbamate or ester prodrug that can potentially be activated by nitroreduction. We selected 4-nitrobenzyl and 4-nitro-2-fluorobenzyl as the promoieties since both have been shown in our earlier efforts to be substrates of trypanosomal nitroreducases.[12,13]

As shown in Scheme 1, the prodrugs were readily synthesized through varying protection and deprotection strategies using DFMO as the starting material. Boc protection of the primary amines in DFMO followed by S_N2 reaction of 4-nitrobenzyl tosylate with the protected DFMO carboxylate anion gave the diBoc protected DFMO esters, which upon TFA deprotection afforded the desired DFMO 4-nitrobenzyl ester prodrugs **1a** and **1b**. For the synthesis of N^{ε}-(4-nitrobenzyloxycarbonyl)-DFMO prodrugs **2a** and **2b**, the N^{α}-primary amine was selectively protected through copper(II) complexation during carbamoylation. For the synthesis of N^{α}-(4-nitrobenzyloxycarbonyl)-DFMO prodrugs **3a** and **3b**, the more basic N^{ε}-amino group was first selectively protected with Fmoc before carbamoylation. After reaction with 4-nitrobenzyloxycarbonyl imidazole, the Fmoc was removed using 20% piperidine to the desired N^{α}-carbamate DFMO prodrugs **3a** and **3b**. The dicarbamate DFMO prodrugs **4a** and **4b** were prepared from DFMO in the presence of excess 4-nitrobenzyloxycarbonyl imidazole. The prodrugs were all purified to homogeneity by preparative HPLC and structures confirmed by NMR and MS. The overall yields for the synthesis of these prodrugs starting from DFMO were between 10% and 51% and were not optimized.

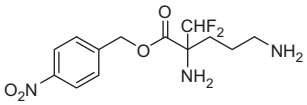
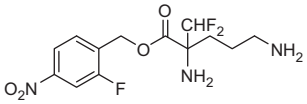
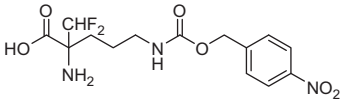
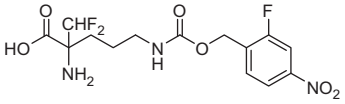
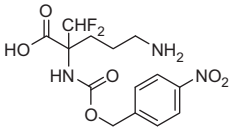
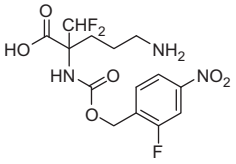
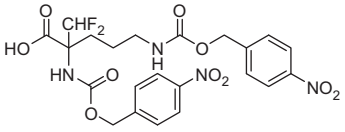
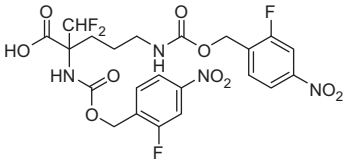
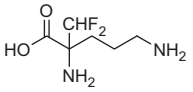
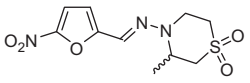
Biological evaluation

All potential prodrugs were evaluated as substrates of *T. brucei* nitroreductase (TbNTR) and screened for trypanocidal activity against bloodstream form *T. brucei* using DFMO and nifurtimox as the controls.[12,13] As shown in Table 1, mono(4-nitrobenzyl)-DFMO ester or carbamate conjugates (**1a**, **2a**, **3a**) without halogen substitutions on the benzyl ring were not effective substrates of TbNTR, giving substrate activity values similar to the background DFMO control. The presence of two 4-nitrobenzylcarbamoyl groups on the DFMO conjugate **4a** did confer TbNTR substrate activity. Addition of a 2-fluoro substitution to the phenyl ring in the mono(4-nitrobenzyl)-DFMO ester or carbamate conjugates as in **1b**, **2b**, and **3b** resulted in an increased TbNTR activity while conjugate **4b** containing two nitrobenzyl carbamates both with 2-fluoro substituents had the highest activity of all. When tested for their antitrypanosomal activity, only N^{ε}-(4-nitro-2-fluorobenzyloxycarbonyl)-DFMO **2b** out of the 8 DFMO conjugates synthesized had significant inhibitory effect on parasite growth at concentrations up to 100 μM with an IC_{50} of 40 μM (Table 1, entry 4). When **2b** was tested against *T. brucei* induced to express elevated levels of nitroreductase, we found that there was no alteration in trypanocidal activity as compared to non-induced controls, suggesting that *T. brucei* nitroreductase does not contribute towards the activation and trypanocidal activity of **2b**; in contrast, cells expressing higher levels of nitroreductase were seven fold more sensitive to the nitroaromatic prodrug nifurtimox than controls. Precisely how **2b** works in producing its trypanocidal activity is still under investigation. As the *T. brucei* nitroreductase is localized to the parasite's single mitochondrion,[15] **2b** may not be able to access this sub-cellular compartment. Alternatively, if **2b** can get into the mitochondrion, the product generated from the nitroreductase-mediated reaction may not be able to gain access into the cytoplasm, the site where the trypanosomal ornithine decarboxylase is found. Why the other DFMO conjugates fail to affect trypanosomal growth is also unclear. However, given that DFMO is actively transported into the parasite by specific amino acid permeases, then the modifications reported here may



Scheme 1. Synthesis of DFMO prodrugs designed.

Table 1
Biological activities of DFMO prodrugs as substrates of trypanosomal NTR and as inhibitors of trypanosomal growth

Entry	Compounds	Structure	TbNTR activity[a] (nmol NADH oxidized/min/mg)	IC_{50} (μM)[b]	
				wild-type *T. brucei*	*T. brucei* with elevated NTR
1	**1a**		60 ± 4	>100	–
2	**1b**		203 ± 12	>100	–
3	**2a**		33 ± 2	>100	–
4	**2b**		260 ± 8	39.5 ± 0.9	39.9 ± 2.0
5	**3a**		42 ± 10	>100	–
6	**3b**		227 ± 6	>100	–
7	**4a**		140 ± 3	>100	–
8	**4b**		312 ± 13	>100	–
9	DFMO		21 ± 4	8.30 ± 1.46	–
10	Nifurtimox		423 ± 45	1.83 ± 0.02	0.26 ± 0.01

[a] Nitroreductase substrate activity expressed as the initial velocity (nmoles of NADH oxidized/min/mg) in the presence of purified His-tagged *T. brucei* nitroreductase (TbNTR) by following the change in absorption at 340 nm.
[b] The blood stream form *T. brucei* parasites were seeded at 1×10^3 mL^{-1} in 200 μL growth medium containing different concentrations of test compounds. After incubation at 37 °C for 3 days, 20 μL alamarBlue® (Biosource UK Ltd) was added to each well and the plates incubated for a further 16 h. Cell viability was determined using fluorescence at 530 nm/ex and 585 nm/em to establish the IC_{50} value for each test compound.

adversely affect prodrug uptake.[16–18] Nevertheless, the weak antitrypanosomal activity of **2b** could serve as a lead for further investigation.

In summary, we attempted to incorporate 4-nitrobenzyl groups into DFMO to form prodrugs for potential activation by trypanosomal nitroreductase and found that only modification of N^{ε}-amino group of DFMO by 4-nitro-2-fluorobenzyloxycarbonyl resulted in significant but weak trypanocidal activity. Further assay against *T. brucei* expressing elevated levels of nitroreductase did not show any increased trypanocidal activity. Additional studies will be necessary to understand the mechanism of the tryapnocidal activity of N^{ε}-(4-nitro-2-fluorobenzyloxycarbonyl)-DFMO **2b**. Because of the increased urgency in finding effective treatments of trypanosomal infections, **2b** could potentially serve as a lead for further investigation.

Acknowledgments

We gratefully acknowledge the financial support of grant SNJ-CCR 700–009 from the State of New Jersey Commission on Cancer Research and grant RSG-03-004-01-CDD from American Cancer Society.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.09.005.

References and notes

1. Russell, D. H.; Snyder, S. H. *Mol. Pharmacol.* **1969**, *5*, 253.
2. Steverding, D. *Parasit. Vectors* **2010**, *3*, 15.
3. Na-Bangchang, K.; Doua, F.; Konsil, J.; Hanpitakpong, W.; Kamanikom, B.; Kuzoe, F. *Eur. J. Clin. Pharmacol.* **2004**, *60*, 269.
4. Loiseau, P. M.; Czok, M.; Chauffert, O.; Bourass, J.; Letourneux, Y. *Parasite* **1998**, *5*, 239.
5. Chen, Y.; Hu, L. *Med. Res. Rev.* **2009**, *29*, 29.
6. Gomes, P.; Vale, N.; Moreira, R. *Molecules* **2007**, *12*, 2484.
7. Hu, L. *IDrugs* **2004**, *7*, 736.
8. Denny, W. A. *Eur. J. Med. Chem.* **2001**, *36*, 577.
9. Shan, D.; Nicolaou, M. G.; Borchardt, R. T.; Wang, B. *J. Pharm. Sci.* **1997**, *86*, 765.
10. Ge, Y.; Wu, X.; Zhang, D.; Hu, L. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 941.
11. Denny, W. A. *Curr. Pharm. Des.* **2002**, *8*, 1349.
12. Hu, L.; Wu, X.; Han, J.; Chen, L.; Vass, S. O.; Browne, P.; Hall, B. S.; Bot, C.; Gobalakrishnapillai, V.; Searle, P. F.; Knox, R. J.; Wilkinson, S. R. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3986.
13. Hall, B. S.; Wu, X.; Hu, L.; Wilkinson, S. R. *Antimicrob. Agents Chemother.* **2010**, *54*, 1193.
14. Bot, C.; Hall, B. S.; Bashir, N.; Taylor, M. C.; Helsby, N. A.; Wilkinson, S. R. *Antimicrob. Agents Chemother.* **2010**, *54*, 4246.
15. Wilkinson, S. R.; Taylor, M. C.; Horn, D.; Kelly, J. M.; Cheeseman, I. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 5022.
16. Baker, N.; Alsford, S.; Horn, D. *Mol. Biochem. Parasitol.* **2011**, *176*, 55.
17. Burkard, G. S.; Jutzi, P.; Roditi, I. *Mol. Biochem. Parasitol.* **2011**, *175*, 91.
18. Vincent, I. M.; Creek, D.; Watson, D. G.; Kamleh, M. A.; Woods, D. J.; Wong, P. E.; Burchmore, R. J. S.; Barrett, M. P. *PLoS Pathog.* **2010**, *6*, e1001204.