Bioorganic & Medicinal Chemistry Letters 20 (2010) 4611–4613

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Dimeric cyclohexane-1,3-dione oximes inhibit wheat acetyl-CoA carboxylase and show anti-malarial activity

Theola Louie [a,b], C. Dean Goodman [c], Georgina A. Holloway [a], Geoffrey I. McFadden [c], Vanessa Mollard [c], Keith G. Watson [a,*]

[a] The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville 3050, Victoria, Australia
[b] Department of Medical Biology, The University of Melbourne, Parkville 3010, Victoria, Australia
[c] School of Botany, University of Melbourne, Parkville 3010, Victoria, Australia

ARTICLE INFO

Article history:
Received 23 April 2010
Accepted 1 June 2010
Available online 8 June 2010

Keywords:
Acetyl-CoA carboxylase
Antimalarial
1,3-Cyclohexanedione

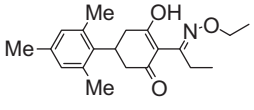
ABSTRACT

A series of dimeric 1,3-cyclohexanedione oxime ethers were synthesized and found to have significant antiplasmodial activity with IC_{50}'s in the range 3–12 μM. The most active dimer was tested in the *Plasmodium berghei* mouse model of malaria and at a dose of 48 mg/kg gave a 45% reduction in parasitaemia. Several commercial herbicides, all known to be inhibitors of maize acetyl-CoA carboxylase, were also tested for antimalarial activity, but were essentially inactive with the exception of butroxydim which gave an IC_{50} of 10 μM.

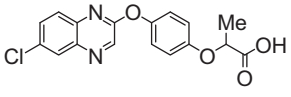
Acetyl-CoA carboxylase (ACC) performs the first and generally rate-limiting step in fatty acid biosynthesis and ACC's are found in most living organisms including bacteria, fungi, plants, animals and humans.[1] ACC catalyses the production of malonyl-CoA which is used in the biosynthesis of long chain fatty acids vital for many cellular functions including the cell membranes. The ACC catalysed formation of malonyl CoA from acetyl-CoA and CO_2 takes place in two stages. Thus, the biotin carboxylase (BC) sub-unit of ACC catalyses the ATP-dependent carboxylation of biotin and then the carboxyltransferase (CT) sub-unit catalyses the transfer of this activated carboxyl group to the acceptor acetyl-CoA. As the supplier of malonyl-CoA and the control point for fatty acid synthesis (FAS) ACC is an excellent target for inhibitor studies and drug discovery.[2] Until recently it was believed that the malaria parasite, *Plasmodium* sp., relies entirely on the host erythrocyte and serum for its fatty acid requirements, but recent studies have shown that *Plasmodium falciparum* and other apicomplexan parasites possess a plastid known as the apicoplast, a similar organelle to plant chloroplasts, which includes all the biochemical machinery to carry out FAS.[3] Thus the *P. falciparum* genome contains FAS genes and a single copy of ACC which is closely related to the ACC from the plastid of many grasses. This indicates that malaria uses the type II FAS pathway common to plant chloroplasts and bacteria, but distinct from the type I FAS pathway of animals including humans.[4] It also suggests that inhibitors of ACC or enzymes in the type II FAS pathway would be good candidates for testing as antimalarials.[5]

Two major classes of herbicide, the cyclohexane-1,3-dione oximes (dims, e.g., tralkoxydim **1**) and the aryloxyphenoxypropionic acids (fops, e.g., quizalofop **2**), which were both discovered in the early 1970s are important to agriculture because of their selective activity against grass weeds in broad-leaf crops (Fig. 1).[6] The mode of action of the dims and fops was a mystery for many years, but in 1987-88 several laboratories independently reported that both types of herbicide are selective inhibitors of ACC's from grass plants.[7] Waller et al. have reported that the dim herbicide tralkoxydim **1**, and also the fops diclofop and fenoxaprop, show weak activity (IC_{50}'s of 181, 210 and 144 μM, respectively) against *P. falciparum*,[4] so we decided to test a wider range of these compound classes for antimalarial activity and herein report our findings.

As a preliminary set of compounds for testing on *P. falciparum* we selected three of the most active commercially available dim herbicides clethodim **3**, tepraloxydim **4** and butroxydim **5**, plus the cyclohexane-1,3-dione oximes **6**, **7** and **8** which are also known



Tralkoxydim, 1



Quizalofop, 2

Figure 1. Structures of a representative dim **1** and fop **2** herbicide.

* Corresponding author. Tel.: +61 3 93452113; fax: +61 3 93452211.
E-mail address: kwatson@wehi.edu.au (K.G. Watson).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.06.007

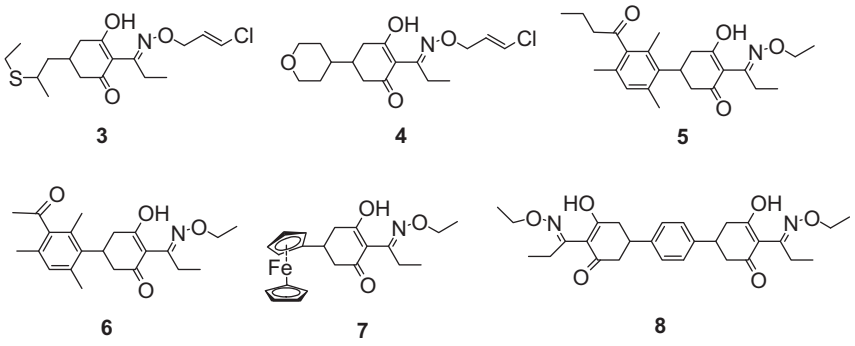


Figure 2. Structures of cyclohexanedione herbicides tested on *P. falciparum*.

to have high herbicidal activity (Fig. 2).[8] In addition we selected for testing quizalofop **2** which is one of the most active and widely used fop herbicides. Since it is well known that fop herbicides show better activity when applied to plants as alkyl esters, which are much more cell-permeable and the esters are readily hydrolysed in vivo to the active acid form,[7] we therefore also tested the quizalofop ethyl ester **2a**.

The compounds were tested for antiplasmodial activity using an in vitro SyberGreen stain method for determining *P. falciparum* cell viability after exposure to the compound for 48 h.[9] Compounds were tested against the laboratory strain D10 using chloroquine as a control and the results are summarized in Table 1. Included in Table 1 are representative reported IC_{50} values for the inhibition of maize ACC for most of the compounds. The results reveal that compounds **1–4** are essentially inactive (IC_{50} >100 μM) and compounds **6–7** are only weak inhibitors of *P. falciparum*. The two compounds showing the best activity were butroxydim **5** (IC_{50} = 10 μM) and the dimeric cyclohexane-1,3-dione **8** (IC_{50} = 6 μM). These two cyclohexanediones **5** and **8** were also tested for activity against the resistant strain W2mef and an alternative strain 3D7 with both compounds showing very similar IC50's to that found with the D10 strain. Compounds **5** and **8** were also tested for inhibition of wheat ACC and, as expected, both compounds showed complete inhibition of ACC activity at 20 μM.

To try to improve the activity of the promising dimeric compound **8** a series of dimers **8–15** (Table 2) was prepared in which the acyl grouping R_1, the oxime ether substituent R_2 and the orientation of the cyclohexane-dione ring substitution on the central phenyl group were varied. The synthesis of the dimers was carried out using the simple general synthetic method shown in Figure 3 for the 1,4-di-substituted compounds **8–10**.

All the dimers **8–15** were tested using the in vitro antiplasmodium assay and the results are summarized in Table 2. The data shows that a smaller acyl group, as in compound **9** (R_1 = Me), reduces the activity, but a larger R_1 (*n*-Pr) is tolerated, with compound **10** showing similar activity to compound **8**. Altering the central phenyl ring substitution from the 1,4 arrangement in compound **8** to the isomeric 1,3 orientation in compound **11** significantly improved the activity with **11** having an IC_{50} of 3 μM. The results for variation of the oxime ether group clearly show that larger R_2 groups are not tolerated with the benzyl oxime ether **13** being less active and the diphenyl-methyl oxime ether **14** only weakly active. On the other hand compound **12** with an allyl oxime ether group shows similar activity to **11**. In general terms the results from variation of the R_1 and R_2 groups in this set of dimeric compounds are consistent with what would be predicted from the known herbicidal structure–activity studies with cyclohexane-1,3-dione oximes, with optimal R_1 being ethyl or propyl and optimal R_2 being ethyl or allyl.[15]

To test the importance of the length of the central spacing group in the dimeric cyclohexane-1,3-diones, two extra dimers **16** and **17** (Fig. 4) were prepared starting from the appropriate di-aldehydes and following a similar reaction sequence to that shown in Figure 3. In the in vitro antiplasmodium assay dimers **16** and **17** showed IC_{50}'s of 12 and 5 μM, respectively, suggesting that the length of the spacer group is not critical to the activity.

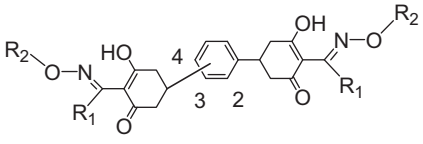
Butroxydim **5** plus three of the most active dimeric compounds **8**, **10** and **11** were tested for in vivo antimalarial activity using the *Plasmodium berghei*-mouse model. Mice were inoculated with 1.5×10^7 parasites followed by four daily subcutaneous injections of placebo, chloroquine or the test compounds. The daily dosages received were 10 mg/kg of chloroquine; 160 mg/kg of butroxydim **5**; 20 mg/kg of dimer **8**; 20 mg/kg of dimer **10** and 48 mg/kg of **11**.

Table 1
Antiplasmodial and ACC activity of **1–8**

Compound no.	IC_{50} (μM) *P. falciparum*	IC_{50} (μM) maize ACC
1	>100	0.52[10]
2	>100	0.016[11]
2a	>100	>1[11]
3	>250	0.02[12]
4	>100	0.7*,[13]
5	10	0.3[14]
6	>10 < 100	ND
7	>10 < 100	ND
8	6	ND
Chloroquine	0.015	ND

* IC_{50} for Giant foxtail ACC; ND not determined.

Table 2
Antiplasmodial activity of the dimeric cyclohexane-1,3-diones **8–15**



Compound no.	Acyl group R_1	Oxime subst. R_2	Substitution of the phenyl ring	IC_{50} (μM) *P. falciparum**
8	Et	Et	1,4	6
9	Me	Et	1,4	12
10	*n*-Pr	Et	1,4	5
11	Et	Et	1,3	3
12	*n*-Pr	Allyl	1,3	2.5–5.0
13	*n*-Pr	Benzyl	1,3	12.5
14	*n*-Pr	$(Phenyl)_2CH$	1,3	~25
15	*n*-Pr	Et	1,3	4
Chloroquine	—	—	—	0.015

* All assays were run in triplicate and the results shown are the average value.

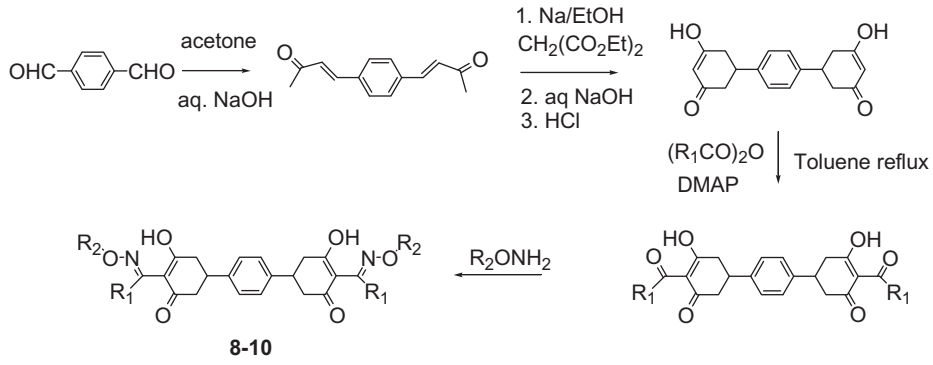


Figure 3. Synthetic method for the dimeric cyclohexane-1,3-diones.

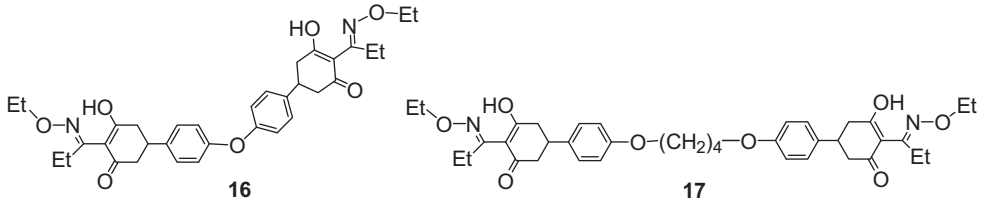


Figure 4. Dimeric cyclohexane-1,3-diones with longer central spacer groups.

Parasitaemia was monitored by blood smears on day 4. Four replicate mice were used for each treatment and the results averaged and standard deviations and *t*-tests performed. In summary it was found that treatment with butroxydim **5** gave a 35% reduction in parasitaemia at the 160 mg/kg dose and dimer **11** gave a 45% reduction of parasitaemia with the 48 mg/kg dose (both results were statistically significant with $p < 0.01$). The chloroquine treated mice were completely cleared of all parasites whereas dimers **8** and **10** were essentially inactive albeit at lower doses than used for **5** and **11**.

The high resolution crystal structure of tepraloxydim **4** in complex with the CT domain of yeast ACC was recently solved and reveals that the small molecule inhibitor is bound in the active site at the interface of a dimer of the enzyme.[16] We were intrigued that the most active compounds from our exploration are dimeric cyclohexanedione oximes with each end of the molecules potentially able to bind into an ACC-CT active site. However, a cursory look at the ACC-CT X-ray structure in complex with **4** shows that the oxime ether moiety is close to the interface of the protein dimer and thus the inhibitor is the wrong way around to allow simultaneous binding of both ends of dimers such as **8**–**17**.

The fact that the aryloxyphenoxy propionic acid **2** and many of the cyclohexanedione herbicides do not show significant activity on *P. falciparum* in the in vitro assay suggests that neither class of molecule are good inhibitors of malaria ACC. However, recent reports indicate that cultured *P. falciparum* parasites can survive in the absence of type II FAS and that fatty acid synthesis is only essential during the liver stage of the life cycle in the mouse malaria model *P. berghei*.[17] Thus, it appears that ACC inhibitors will only show full activity in liver stage testing. Alternatively, it is possible that the antiplasmodial activity of butroxydim **5** and dimers such as **11** may be due to activity on some other as yet unidentified target.

References and notes

1. Wakil, S. J.; Stoops, J. K.; Joshi, V. C. *Annu. Rev. Biochem.* **1983**, *52*, 537.
2. Tong, L. *Cell. Mol. Life Sci.* **2005**, *62*, 1784.
3. Ralph, S. A.; van Dooren, G. G.; Waller, R. F.; Crawford, M. J.; Fraunholz, M. J.; Foth, B. J.; Tonkin, C. J.; Roos, D. S.; McFadden, G. I. *Nat. Rev. Microbiol.* **2004**, *2*, 203.
4. Waller, R. F.; Ralph, S. A.; Reed, M. B.; Su, V.; Douglas, J. D.; Minnikin, D. E.; Cowman, A. F.; Besra, G. S.; McFadden, G. I. *Antimicrob. Agents Chemother.* **2003**, *47*, 297.
5. Goodman, C. D.; McFadden, G. I. *Curr. Pharm. Des.* **2008**, *14*, 901.
6. *The Pesticide Manual*; Tomlin, C., Ed., 10th ed.; Crop Protection Publications: UK, 1995; pp 1336–1337.
7. Gronwald, J. W. *Biochem. Soc. Trans.* **1994**, *22*, 616.
8. The synthesis and herbicidal activity of compounds **6** and **8** is described in the patent literature, for example, U.S. patents 4,666,510 and 4,746,350, respectively.
9. Smilkstein, M.; Sriwilaijaroen, N.; Kelly, J. X.; Wilairat, P.; Riscoe, M. *Antimicrob. Agents Chemother.* **2004**, *48*, 1803.
10. Secor, J.; Cseke, C. *Plant Physiol.* **1988**, *86*, 10.
11. Nakahira, K.; Haga, M.; Uchiyama, M.; Suzuki, K. *Nippon Noyaku Gakkaishi* **1990**, *15*, 189 (*Chem. Abstr.* **1991**, *115*, 66750).
12. Rendina, A. R.; Felts, J. M. *Plant Physiol.* **1988**, *86*, 983.
13. Takahashi, A.; Yamada, S.; Tanaka, K. *Weed Biol. Manag.* **2002**, *2*, 84.
14. Jenkins, C. L. D. (CSIRO Division of Plant Industry) Personal communication, 1989.
15. Iwataki, I. In *Rational Approaches Struct., Act., Ecotoxicol. Agrochem.*; Draber, W., Fujita, T., Eds.; CRC: Boca Raton, FL, 1992; p 397. Chapter 16.
16. Xiang, S.; Callaghan, M. M.; Watson, K. G.; Tong, L. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 20723.
17. Vaughan, A. M.; O'Neill, M. T.; Tarun, A. S.; Camargo, N.; Phuong, T. M.; Aly, A. S. I.; Cowman, A. F.; Kappe, S. H. I. *Cell. Microbiol.* **2009**, *11*, 606.