Bioorganic & Medicinal Chemistry Letters 27 (2017) 3878–3882

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Interconvertible geometric isomers of *Plasmodium falciparum* dihydroorotate dehydrogenase inhibitors exhibit multiple binding modes

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ARTICLE INFO

Article history:
Received 3 May 2017
Revised 15 June 2017
Accepted 17 June 2017
Available online 21 June 2017

Keywords:
Dihydroorotate dehydrogenase
DHODH
Plasmodium falciparum
Inhibitor

ABSTRACT

Two new tricyclic β-aminoacrylate derivatives (**2e** and **3e**) have been found to be inhibitors of *Plasmodium falciparum* dihydroorotate dehydrogenase (*Pf*DHODH) with Ki 0.037 and 0.15 μM respectively. ^{1}H and ^{13}C NMR spectroscopic data show that these compounds undergo ready *cis-trans* isomerisation at room temperature in polar solvents. *In silico* docking studies indicate that for both molecules there is neither conformation nor double bond configuration which bind preferentially to *Pf*DHODH. This flexibility is favourable for inhibitors of this channel that require extensive positioning to reach their binding site.

Malaria is one of the main infectious diseases in the world, with an estimated 212 million cases in 2015, centred to a great extent on the countries in central Africa and south east Asia.[1] The numbers are even starker when one considers these cases led to an estimated 429,000 deaths and that approximately 70% of these deaths were of children under five years old. Malaria is considered a 'preventable' disease, largely because of the use of chemotherapeutic agents,[2] however widespread drug resistance[3] has rendered these agents ineffective in many regions of the world. This problem has led to much effort being expended into validating novel drug targets, as well as discovering small molecule enzyme inhibitors to act as leads for development of new antimalarial drugs.[4] One such target which has attracted recent attention is *P. falciparum* dihydroorotate dehydrogenase (*Pf*DHODH),[5] a key enzyme in the obligate *de novo* pyrimidine pathway for uridine monophosphate (UMP) biosynthesis. Early work aimed towards the discovery of *Pf*DHODH inhibitors focussed on modification of the human DHODH (*h*DHODH) inhibitors known at the time, such as brequinar (Fig. 1).[6] This work was naturally followed by high throughput screening[7] and *de novo* design approaches.[8] During the progress of this work, co-crystallisation[9] and *in silico* docking[8,10] experiments revealed that the inhibitors discovered targeted the ubiquinone binding channel, and also revealed important features for the design of selective and potent inhibitors. Firstly amino acid residues H185 and/or R265, at the head of the ubiquinone-binding channel, were found to be essential for hydrogen bonding interactions with the inhibitors. The ubiquinone channel also contained a hydrophobic region which contributes to the binding of the more potent inhibitors reported. Indeed many of the inhibitors reported to date[6–12] reveal broadly this amphiphilic nature, and possess a polar 'head group' and hydrophobic 'tail'. Triazolopyrimidine DSM265 (Fig. 1) is currently the most advanced *Pfal*DHODH inhibitor, and Phase 1 clinical trials for this candidate have recently been reported.[13]

We have reported previously that β-aminoacrylate derivatives of the general structure **1** (Fig. 1) are inhibitors of *Pf*DHODH.[10] In particular, tricyclic derivatives **2a** and **3b** displayed sub-micromolar IC_{50} values against *Pf*DHODH, and in the case of **2a** there was a 1000-fold greater potency compared to the human enzyme (*h*DHODH). An extensive series of experiments in this previous work with this class of inhibitors[10a] showed that compounds **2a** and **3b**, and related structures, were competitive inhibitors with the enzyme cofactor CoQ and reversible. Compounds such as **2a** may in principle exist as a pair of geometric isomers and we considered that different H-bonding groups on either side of the

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http://dx.doi.org/10.1016/j.bmcl.2017.06.052
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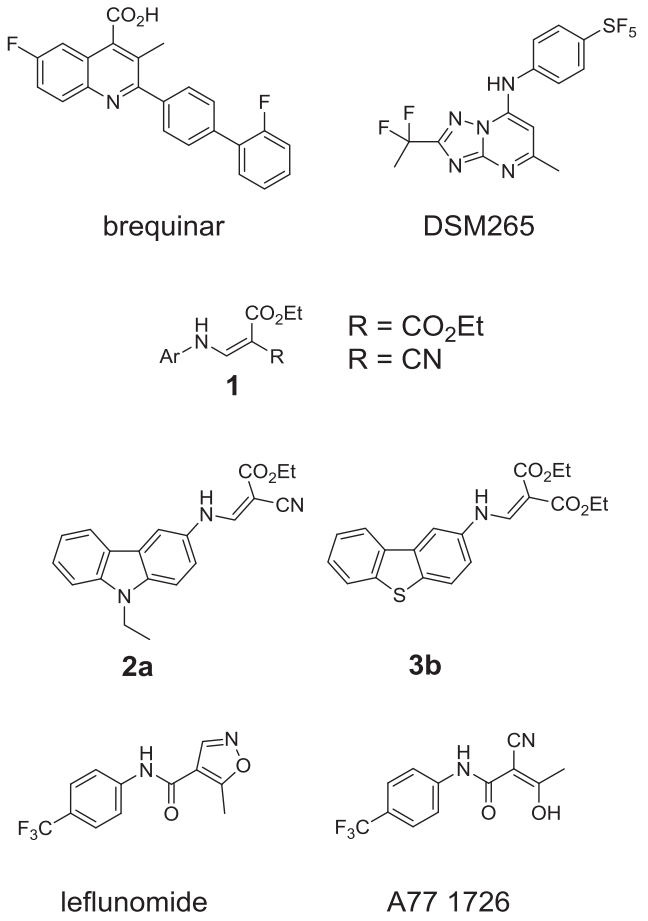
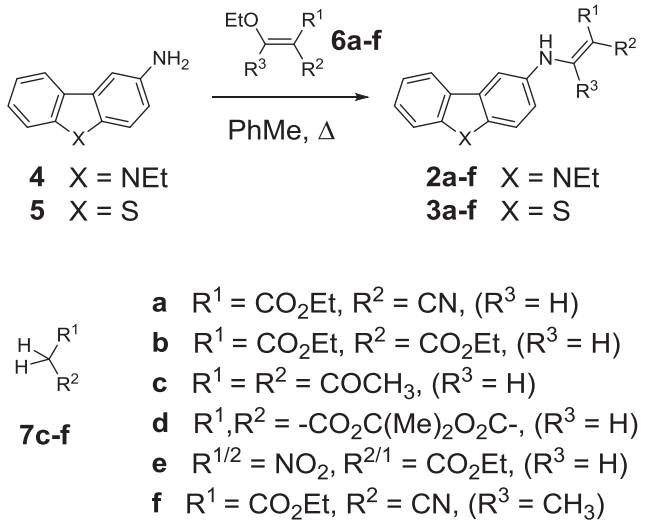


Fig. 1. Example structures of known *Pfal*DHODH (brequinar, DSM265, **2a** and **3b**) and *h*DHODH (A77 1726) inhibitors.



Scheme 1. Synthesis of β-aminoacrylate derivatives of 9-ethyl-3-aminocarbazole and 3-aminodibenzothiophene in this study.

Table 1
IC_{50} values of selected compounds against *P. falciparum* and human DHODH. The IC_{50} values were determined according to methodology reported previously.[10a] n.d. = not determined; [†]Ki vs *Pfal*DHODH) for **2e** was 0.037 ± 0.012 μM; [‡]Ki (*Pfal*DHODH) for **3e** was 0.150 ± 0.001 μM (cf. Ki = 0.05 and 0.02 μM for **2a** and 3**b** respectively[10a]).

Entry	*Pfal*DHODH IC_{50} (μM)	hDHODH IC_{50} (μM)
2a	0.28 ± 0.05 0.44 ± 0.06[10a]	491 ± 42[10a]
3a	1.2	n.d.
2b	4.4	n.d.
3b	0.16 ± 0.05 0.16 ± 0.05[10a]	30 ± 5.9[10a]
2c	>100	n.d.
3c	>100	n.d.
2d	>100	n.d.
3d	>100	n.d.
2e	0.094 ± 0.031[†]	>100
3e	0.38 ± 0.06[‡]	>100
2f	>100	n.d.
3f	>100	n.d.

double bond, and their position relative to the aromatic portion of the structure through rotation about the C_{Ar}—N bond, would be key in determining their binding to the enzyme. It is perhaps noteworthy to mention that alternative binding modes have been reported with certain *h*DHODH inhibitors,[14] and also differential binding modes for A77 1726, the active metabolite of leflunomide (Fig. 1), when binding to *Pf*DHODH is compared to its principal target *h*DHODH.[9a] We therefore sought to examine whether alterative polar 'head groups' to those investigated would lead to improved activity of members in this class of compound, and the results obtained are reported herein.

Ten new derivatives (**2b–f**, **3a**,**c–f**) based on the 3-aminocarbazole (**4**) and 3-aminodibenzothiophene (**5**) core unit were synthesised using the method reported previously (Scheme 1).[10a] Derivatives **2b** and **3a** were prepared by simply heating the aromatic amine with the appropriate commercially available β-ethoxy-α,β-unsaturated carbonyl compound **6b** and **6a** respectively. For **2c–f** and **3c–f** the relevant active methylene compounds **7c–f** were first heated with an excess of triethylorthoformate (or acetate) in toluene, forming the β-ethoxy-α,β-unsaturated carbonyl compounds **6c–f** *in situ*, followed by addition of either the aromatic amine **4** or **5** and heating for a further short period. After cooling, the products precipitated upon standing, were collected by filtration and then recrystallised.[15]

Table 1 shows the IC_{50} values obtained for the compounds discussed in this work. These values were obtained using the screening methodology reported previously.[10a] The IC_{50} values of derivatives **2c**,**d**,**f** and **3c**,**d**,**f** were above the arbitrary cut-off value of 0.1 mM and thus not further examined. Derivatives **2d** and **3d**, structures based upon Meldrum's acid, are conformationally restricted analogues. We assigned their low activity to the restricted freedom imposed by the six-membered ring to position the polar head group for maximal hydrogen bonding to H-bond donors on either side the co-factor channel.

The activity of the cyanoacetate (**2a**, **3a**) and malonate derivatives (**2b**, **3b**) are not too dissimilar, and the IC_{50} values are probably subtly altered depending on the influence of the second ethyl ester and/or *N*-ethyl group. The lowest activity is seen with **2b**, where both ethyl groups are present, may mean that the molecule is slightly too large to fit well into the ubiquinone binding site. Similarly, derivatives **2f** and **3f**, which only differ from **2a** and **3a** by a single methyl group, showed that substitution at this position cannot be tolerated sterically. The lack of activity in **2c** and **3c** was somewhat surprising, given the similar electron withdrawing effect of the methyl ketone compared to the ethyl ester and cannot so easily be explained.

Nitroacrylate derivatives **2e** and **3e** were as active, if not more so, than cyanoacrylate and malonate derivatives **2a**,**b** and **3a**,**b**. As discussed above, we considered that the conformational prefer-