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Synthesis, structure-activity relationship and binding mode analysis of 4-thiazolidinone derivatives as novel inhibitors of human dihydroorotate dehydrogenase†

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† The authors declare no competing interests.

A series of 4-thiazolidinone derivatives were synthesized and evaluated as novel human dihydroorotate dehydrogenase (*h*DHODH) inhibitors. Compounds **26** and **31** displayed IC_{50} values of 1.75 and 1.12 μM, respectively. The structure-activity relationship was summarized. Further binding mode analysis revealed that compound **31** could form a hydrogen bond with Tyr38 and a water-mediated hydrogen bond with Ala55, which may be necessary for maintaining the bioactivities of the compounds in this series. Further structural optimization of the *para*- or *meta*-position of the phenyl group at R will lead to the identification of more potent *h*DHODH inhibitors.

Introduction

Human dihydroorotate dehydrogenase (*h*DHODH), a critical rate-limiting enzyme in pyrimidine *de novo* biosynthesis pathway, plays a key role in the biosynthesis of nucleotide and cell proliferation.[1-2] The significance of DHODH in rapidly proliferating cells such as tumor cells and active T and B lymphocytes makes it become an ideal target for pharmacological intervention.[3] Some inhibitors of DHODH have proven efficacy for the treatment of malaria,[4-5] autoimmune diseases,[6-8] cancer,[9]

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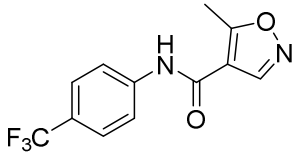
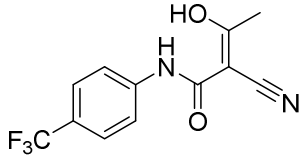
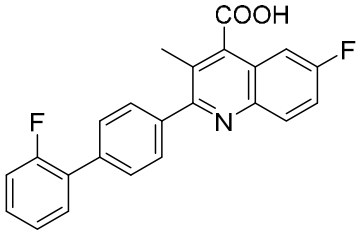
psoriasis,[10] virus proliferation [11-13] and acute myeloid leukemia.[14] Leflunomide (**1**) and brequinar (**3**) are two representative examples of such DHODH inhibitors (Fig. 1) Leflunomide and its active metabolite teriflunomide, have been approved for the treatment of rheumatoid arthritis and multiple sclerosis.[3, 15-16] However, administration of leflunomide for an extended period would cause numerous adverse effects including gastrointestinal symptoms, liver toxicity, hypertension, interstitial lung disease and birth defect, preventing it from being utilized widely.[17-19] Brequinar was used as antitumor and immunosuppressive agent in phase II clinical trials but failed due to its narrow therapeutic window.[20-22] Compound **4** has already exhibited promising results for inflammatory bowel disease in the phase IIa clinical trial, and it is now focused on Crohn's disease in a phase IIb trial.[23] Consequently, there remains intensively needs to discover novel and potent *h*DHODH inhibitors for further development.



1. Leflunomide



2. Teriflunomide



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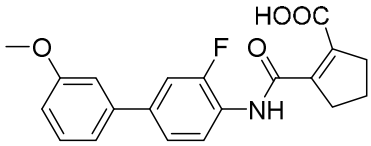

4. Vidofludimus

Figure 1. Structures of representative inhibitors of *h*DHODH.

4-Thiazolidinones, a class of heterocycle including nitrogen and sulfur atoms, have been reported as a biologically important scaffold and possess broad biological activities, such as anticonvulsant activity, cardiovascular effects, antibacterial activity, anticancer activity, antihistaninic activity (H1-antagonist), and anti-inflammatory activity *et al*.[24-27] As part of our continuing studies on 4-thiazolidinones,[28] compound **5** was found to have the inhibitory activity against *h*DHODH by random screening. Then, a serious of 4-thiazolidinones were synthesized and evaluated for their inhibitory activities against *h*DHODH.

Herein we described the discovery and the SAR study of 4-thiazolidinone derivatives as novel *h*DHODH inhibitors. The binding modes of compounds **21** and **31** were also studied by molecular docking to help further elucidate the SAR.

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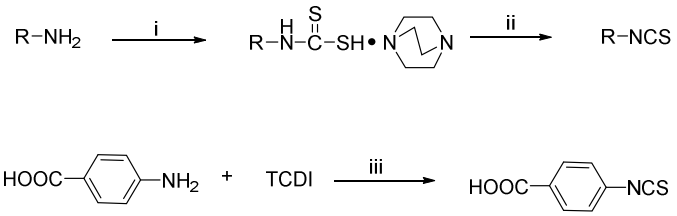
Results and discussion

Chemistry

The synthesis of starting materials aryl isothiocyanates was shown in Scheme 1. Dithiocarbamate salts were prepared by reaction of triethylenediamine and carbamodithioic acids which were formed by treatment of aromatic amines with carbon bisulfide. Isothiocyanates were then obtained by treated dithiocarbamate salts with BTC. 4-isothiocyanatobenzoic acid was prepared by reaction of 4-aminobenzoic acid with TCDI in the presence of TEA.

4-Thiazolidinones and their analogs were prepared according to the routes depicted in Scheme 2. Aryl isothiocyanates were treated with active methylene compounds and potassium hydroxide in DMF to provide ketene-N,S-acetal salts. These ketene-N,S-acetal salts were further reacted with 2-chloroacetyl chloride, 3-bromopropanoyl chloride or 1,2-dibromoethane to give compounds **5-11** and **13-31**. Compound **12** was prepared by de-protection of compound **11** with TFA in DCM. Reaction of isothiocyanate with 2-mercaptoacetic acid offered an intermediate 2-(carbamothioylthio)acetic acid, which was subsequently cyclized to produce the additional analogue **32**. All the final compounds were fully characterized by spectroscopic techniques.



Scheme 1. Synthesis of intermediates. (i) DABCO, CS_2, acetone, r.t., 12 h; (ii) BTC, $CHCl_3$, r.t., 4 h, 70%-95%; (iii) TEA, DCM, r.t. 90%.

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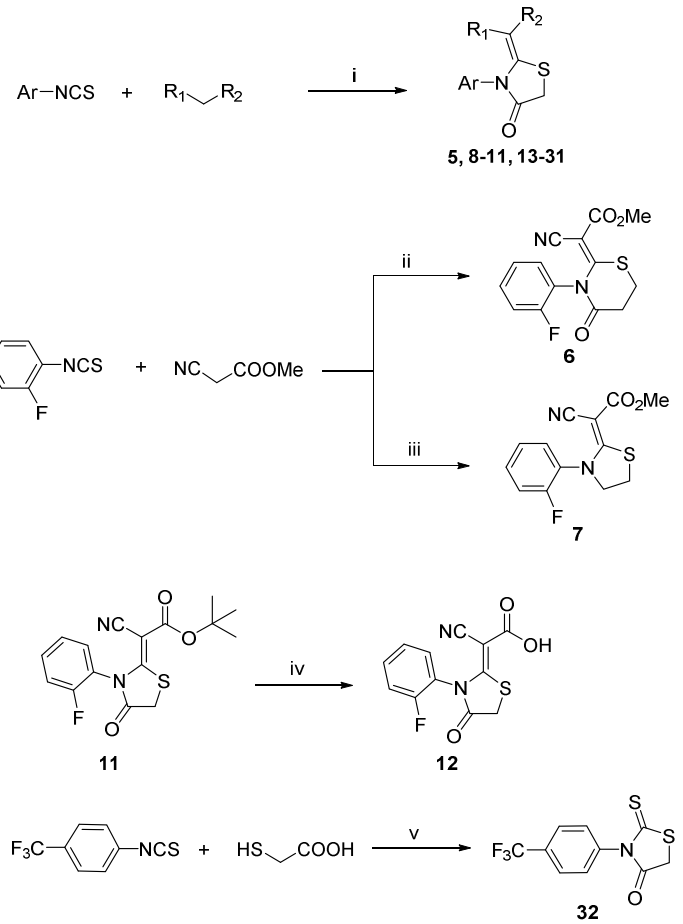

Scheme 2. Synthesis of compounds **5-32**. (i) KOH, DMF, r.t. 15-30 min; $ClCH_2COCl$, 0 ^oC; r.t., 12 h, 68%-80%. (ii) KOH, DMF, r.t. 15 min; $BrCH_2CH_2COCl$, 0 ^oC; r.t., 12 h, 60%. (iii) KOH, DMF, r.t. 15 min; $BrCH_2CH_2Br$, 0 ^oC; r.t., 12 h, 48%. (iv) TFA, DCM, r.t. 85%. (v) TEA, dioxane, reflux, 65%.

Inhibitory activities against *h*DHODH and SAR study

Table 1 highlights SAR for the 4-thiazolidinone moiety. If 4-thiazolidinone was replaced by 1,3-thiazin-4-one, the inhibitory activity of compound **6** decreased dramatically. Replacement of the 4-thiazolidinone of **5** with thiazolidin provided **7**, with apparently decreased activity again. We next investigated the effect of the R_2 group. Compounds **8-11** showed similar activity with **5**, suggesting that carbon chain length of the ester group probably had no apparent effect on the activity. When the ester structures were transformed into their acid, the activity of compound **12** became very poor with an inhibitory rate of only 14.867 % at 10 μM. Introducing a -$CONH_2$ group to the R_2 position also showed weak activity (compound **13**), which implied that polar groups were not tolerated at the R_2 position. Compounds **14** and **15** with ester groups at R_1 also showed dramatically lower enzyme inhibitory activities than that of the cyano group substituted counterparts (compounds **5** and **8**). The above discussions indicated that the 4-thiazolidinone scaffold, the cyano substitution for R_1

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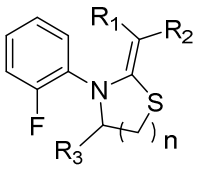
and the ester structure for R_2 are key factors for potent *h*DHODH inhibitors.

Table 1

Structures and activities for 4-thiazolidinone analogs **5-15**



compd	R_1	R_2	R_3	n	Inhibitory Rate at 10 μM (%)	*h*DHODH IC_{50} (μM)[a]
5	CN	CO_2Me	=O	1	34.866	>10
6	CN	CO_2Me	=O	2	15.690	>10
7	CN	CO_2Me	H	1	11.063	>10
8	CN	CO_2Et	=O	1	38.194	>10
9	CN	CO_2Pr	=O	1	33.481	>10
10	CN	CO_2Bu	=O	1	28.770	>10
11	CN	CO_2tBu	=O	1	33.933	>10
12	CN	COOH	=O	1	14.867	>10
13	CN	$CONH_2$	=O	1	11.757	>10
14	CO_2Me	CO_2Me	=O	1	4.271	>10
15	CO_2Et	CO_2Et	=O	1	8.713	>10
Brequinar						0.0084

[a] Attempts to determine IC_{50} values were made if the inhibition rate at 10 μM was larger than 50%.

In order to optimize the activity of lead compound **5**, N-substituted derivatives were then explored (Table 2). Compound **16** with *o*-chlorophenyl or compound **17** with *o*-iodophenyl group showed equal or lower enzyme inhibitory activities compared with that of lead compound **5**. When *m*-Cl, *p*-F and *p*-Br group were introduced to the phenyl of the scaffold (compounds **19-21**), the inhibitory activities were higher than that of compound **5**, which were shown in Table 2. Among them, given the dramatically improved potency observed with 4-bromophenyl analog **21**, further investigations were performed to study the effect of various *para*-substituted phenyls. Many *para*-substituted analogs displayed submicromolar potencies (compounds **20-24** and **26**). Compounds with hydrophobic groups (-F, -Br, $-NO_2$, $-CF_3$, $-OCH_3$ and -*t*-Bu) at the para-position of phenyl showed better potencies. Compound **27** with hydrophilic group (-COOH) displayed decreased potencies. Pyridinyl derivatives (**28** and **29**) were not beneficial for the increasing of inhibitory activities. These results indicated that introduction of bulky hydrophobic groups to R_1 might improve the activity. Based on this idea, naphthalenyl was introduced at the N-position to give analogs **30** and **31**. Gratifying, compound **31** (2-naphthyl) was slightly more potent than **26** with IC_{50} value of 1.12 μM. The poor inhibitory activity of compound **32** further verified the key effect of 2-cyanoacetate substitution for potency.

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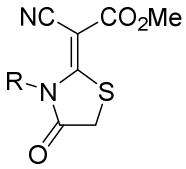
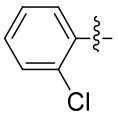
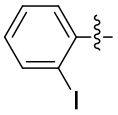
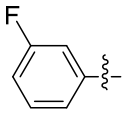
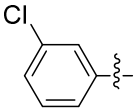
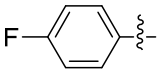
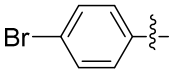
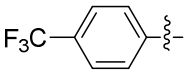
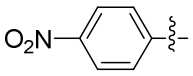
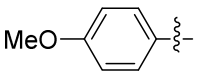
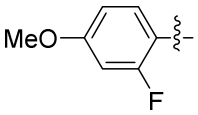
Table 2
Structures and activities for 4-thiazolidinone analogs **16-32**



compd	R	Inhibitory Rate at 10 μM (%)	*h*DHODH IC_{50} (μM)[a]
16		40.010	>10
17		18.377	>10
18		34.109	>10
19		56.856	8.51
20		49.438	10.64
21		68.979	2.68
22		74.431	3.03
23		62.932	4.41
24		52.475	9.45
25		40.378	>10

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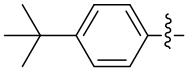
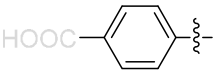
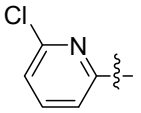
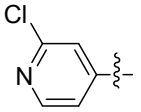
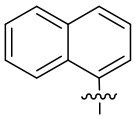
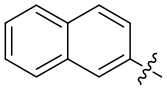
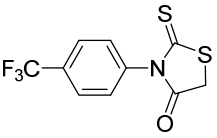
26		79.074	1.75
27		15.064	>10
28		43.738	>10
29		45.360	>10
30		21.886	>10
31		80.327	1.12
32		25.632	>10

[a] IC_{50} values were determined from three independent tests, and attempts to determine IC_{50} values were made if the inhibition rate at 10 μM was larger than 50%.

Binding mode analysis

To further study the action mechanism of this series of derivatives, the binding modes of compounds **21** and **31** were simulated by molecular docking (Fig. 2). Figure 2A tells that the carbonyl group of 4-thiazolidinone forms a hydrogen bond with Tyr38. When 4-thiazolidinone was replaced by 1,3-thiazin-4-one (compound **6**) or thiazolidin (compound **7**), the hydrogen bond mentioned above would be destroyed, leading to a dramatically decreased inhibitory activity. The methyl ester of **21** is located at the entrance of the binding site, interacting with hydrophobic residues like Leu42, Leu46, Leu58 and Phe62. However, it should be noticed that the methyl moiety of the ester in **21** is exposed to the bulk solvent (Fig. 2B), thus prolongation of the carbon chain length of the ester group may contribute little to the bioactivity, that's why compounds **8-11** showed similar activity with **5**. Because of the relatively hydrophobic profile, hydrophilic substituents like -COOH and -$CONH_2$ (compounds **12** and **13**) are not favored at the entrance of the binding site. Figure 2A also shows that the cyano group of **21** orients itself towards the inner side of the binding site, forming a water-mediated hydrogen bond with Ala55. Limited space is left around the

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cyano group for substituting. Larger groups like -CO_2Me and -CO_2Et may clash with residues Leu58 and Ala59, leading to sharply decreased bioactivity (**14** and **15**). Additionally, the 4-bromophenyl group of **21** makes beneficial van der Waals (vdW) interactions with the hydrophobic subsite formed by residues Met43, Ala59, Leu68, Phe98, Met111, Leu359 and Pro364. Obviously, hydrophilic groups like -COOH are disfavored at this hydrophobic subsite, thus compound **27** displayed seriously decreased bioactivity against *h*DHODH compared with **21**. The binding mode of the phenyl group of **21** informs us that little space is remained for substitution at the *ortho*-position of the phenyl, while larger hydrophobic groups are preferred at the *para*- or *meta*-position. This is consistent with the decreased inhibitory activities of **16-17**, **25** and **30**, the increased inhibitory activities of **18-19**, and the improved inhibitory activities of **20-24**, **26** and **31**. As for compound **31**, the large naphthalenyl group accommodates the concave surface well (Fig. 2B) and displayed the most potent bioactivity of this series. Collectively, the 4-thiazolidinone scaffold, the cyano substitution for R_1, the ester structure for R_2 and the hydrophobic substitutions at para- or meta-positions of the phenyl group for R are beneficial for maintaining the bioactivities of the inhibitors in this series.

Compared with the ligand **3X2** in 4LS1 occupying both the hydrophobic and hydrophilic sections of the ubiquinone-binding site of DHODH (Figure S1), compound **31** mainly accommodates the hydrophobic subsite with its naphthalenyl group contacting with residues Met43, Ala59, Leu68, Phe98, Met111, Leu359 and Pro364 through favorable apolar interactions. This obviously distinct binding mode of compound **31** and **3X2** informed us that polar group, such as carboxyl, formyl and hydroxyl, which is connected to a proper linker that substituted to the naphthalenyl group of 31, would generate hydrogen bond or salt bridge interactions with residue Arg136 in the hydrophilic subsite of the ubiquinone-binding site, thus may bring us compounds with improved binding affinity against DHODH. This structural optimization work is undertaken.

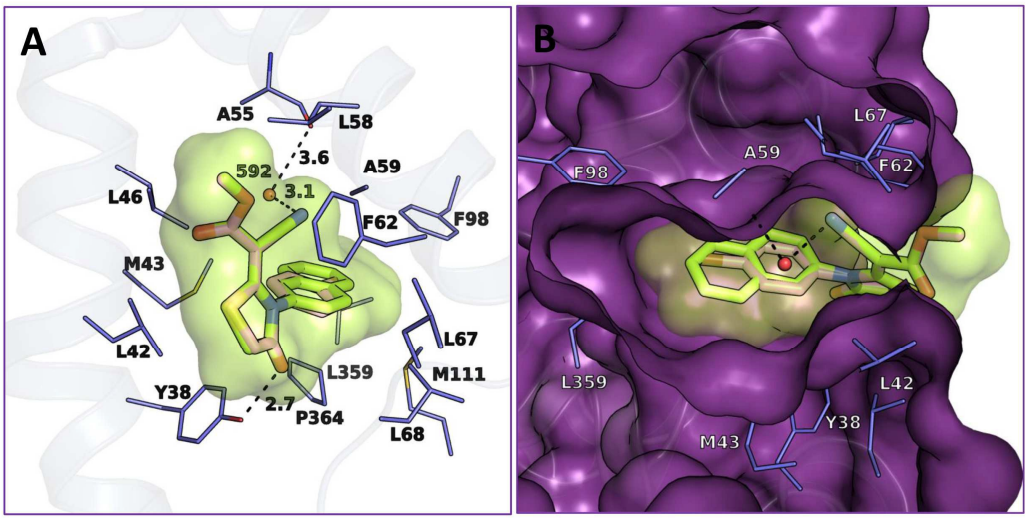

Figure 2. The proposed binding modes for representative compounds **21** and **31**. The

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X-ray crystal structure of *h*DHODH (PDB ID: 4LS1) is shown as transparent purple cartoon (A) or solid surface (B), and the docked inhibitors are represented as sticks with transparent surface. Compound **21** is shown as pink sticks, while compound **31** is displayed as green sticks. For small molecules, oxygen atoms are colored red, nitrogen atoms are colored blue, and the sulfur atoms are colored yellow. Key residues (thin sticks) in the binding site are colored in purple. Potential intermolecular hydrogen bonds are showed in black dashed lines. Water molecule W592 is depicted as red balls.

Conclusions

In conclusion, a series of 4-thiazolidinone derivatives have been identified as *h*DHODH inhibitors. Several compounds exhibited moderate activities against *h*DHODH, especially compounds **26** and **31** with IC_{50} values of 1.75 and 1.12 μM, respectively. The SAR study and binding mode investigation demonstrate that the 4-thiazolidinone scaffold, the cyano substitution for R_1, the ester structure for R_2 and the hydrophobic substitutions at para- or meta-positions of the phenyl group for R are favorable for improving inhibitory activity. For this series of 4-thiazolidinone derivatives, the hydrogen bond with Tyr38 and the water-mediated hydrogen bond with Ala55 were proposed to be indispensable to maintain the inhibitory activity for *h*DHODH. The present SAR indicates that further decoration of the phenyl group at R may bring us more potent *h*DHODH inhibitors.

Acknowledgments

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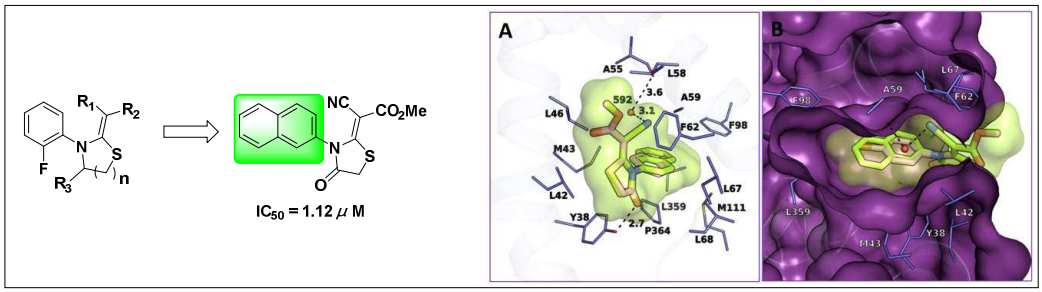
Graphical Abstract



A series of 4-thiazolidinone derivatives were synthesized and evaluated as novel human dihydroorotate dehydrogenase (*h*DHODH) inhibitors.