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Discovery of a novel series of hDHODH inhibitors with anti-pulmonary fibrotic activities

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ABSTRACT

Human dihydroorotate dehydrogenase (hDHODH) is a flavin-dependent enzyme essential to pyrimidine *de novo* biosynthesis, which serves as an attractive therapeutic target for the treatment of autoimmune disorders. A novel series of hDHODH inhibitors was developed based on a lead which was obtained by a medicinal chemistry exploration. Most compounds showed moderate to significant potency against hDHODH, compounds **5d**, **5e**, and **6a** effectively inhibited the activities of hDHODH with IC_{50} values from 0.9 to 2.8 μM. Further studies showed that compound **5e** also effectively suppressed proliferation of the activated PBMCs (IC_{50} = 20.35 μM). Surprisingly, compound **5e** also showed anti-pulmonary fibrotic activity similar to that of pirfenidone *in vitro* assay. Therefore, compound **5e** might have potential to be developed as a novel hDHODH inhibitors for autoimmune diseases therapy.

1. Introduction

Human dihydroorotate dehydrogenase (hDHODH) is a flavin-dependent enzyme essential to pyrimidine *de novo* biosynthesis. It has been identified as a therapeutic target for the treatment of autoimmune disorders such as rheumatoid arthritis and multiple sclerosis [1–3]. Leflunomide (Fig. 1**a**) and Brequinar (Fig. 1**c**) are two representative hDHODH inhibitors. The former was approved more than 20 years ago for the treatment of rheumatoid arthritis [1]. leflunomide acts as a prodrug and is rapidly converted into its active metabolite teriflunomide (Fig. 1**b**) which was approved for treatment of multiple sclerosis. While, brequinar was initially evaluated in clinical trials as a potential anticancer agent. However, it was discarded due to severe side effects [4]. Recently, Johann Leban [5] and Honglin Li [6] respectively reported several hDHODH inhibitors which bore thiophene carboxylic acid (Fig. 1**d**) and thiazole-scaffold (Fig. 1**e**) with IC_{50} 1 nM and 18 nM. However, despite recent efforts, the quest to explore the therapeutic potential of hDHODH inhibitors remains an urgent area of research.

In our previous studies, compound **f** (Fig. 2) has been identified as a potential hDHODH inhibitor (IC_{50} = 1.5 μM). Docking analysis showed that compound **f** adopted a binding mode similar with brequinar: The amide group forms two hydrogen bonds with residues Gln47 and Arg136 of the enzyme and the terminal phenyl ring extends into the hydrophobic pocket.

In order to improve the potency, we described a novel series of hDHODH inhibitors designed by replacing the phenyl moiety (Fig. 2 blue part) with thiophene which exists in the effective hDHODH inhibitor compound **d**. The most promising compound **5e** (IC_{50} = 0.9 μM) exhibited 1.6-fold more potent than compound **f** and displayed efficient anti-inflammation effect. Unexpectedly, some of the target compounds also showed anti-pulmonary fibrotic activities. It suggested that they might be developed as novel immune-suppressants without drug-induced pulmonary fibrotic.

2. Results and discussion

2.1. Chemistry

The synthetic procedure for the target compounds is described in Scheme 1. 3-amino-2-thiophenecarboxylate methyl ester **1** was treated with $NaNO_2$ to yield the diazonium salt, which were not isolated but directly treated with NaN_3 to obtain azido derivative **2** [7] which was

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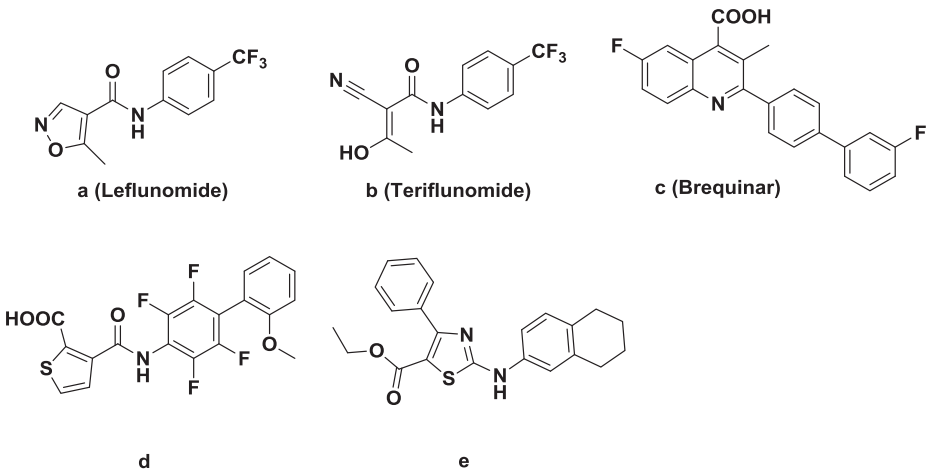


Fig. 1. Selected Structures of the Reported DHODH Inhibitors.

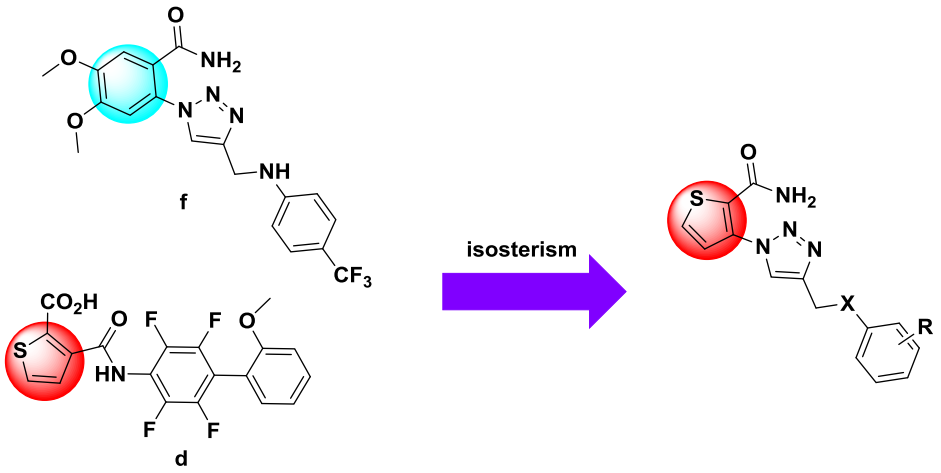
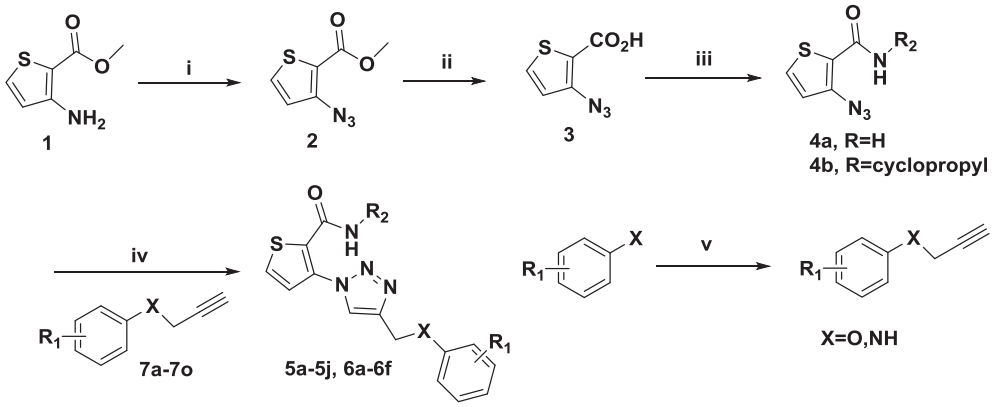


Fig. 2. Design strategy of the target compounds.



Scheme 1. Reagents and conditions: (i) $NaNO_2$, HCl; NaN_3, 1 h; (ii) NaOH, EtOH/H_2O reflux; (iii) a: $SOCl_2$, reflux, 5 h; b: $NH_3{\cdot}H_2O$/ cyclopropylamine, rt, 2 h; (iv) alkyne, Sodium ascorbate, $CuSO_4$, *t*-BuOH/H_2O (1:1), rt, 3–4 h. (v) a: X = NH, 3-bromopropyne, K_2CO_3, DMF, rt, overnight; b: X = O, 3-bromopropyne, K_2CO_3, MeCN, reflux.

converted to intermediate **3** by hydrolysis. Intermediate **3** was treated with $SOCl_2$ under reflux to obtain 2-theonyl chloride derivative which reacted with ammonia or cyclopropylamine to give the intermediates **4a-4b** [8]. Intermediates **7a-n** were synthesized by reacting suitable aniline or phenol with propargyl bromide [9–11]. The target compounds were yielded by click chemistry with copper-catalyzed alkyne-azide 1,3-dipolar cycloaddition (CuAAC) [12] between intermediates **2**, **3**, **4a-4b** and intermediates**7a-o** at room temperature. The target compounds were purified by column chromatography on silica gel. The chemical structures of the target compounds were confirmed by ^{1}H NMR, ^{13}C NMR and ESI-MS.

In order to determine the stereochemistry of the target compounds, the NOESY experiment of compound **5e** was carried out, and an evident NOE signal was observed between the proton (d, 7.42 ppm) on the 4-postion of the thiophene amide moiety and the proton (s, 8.70 ppm) on the 1,2,3-triazole, which existed only in the **A-5e** form due to the appropriate intramolecular H—H distance (Fig. 3 and see Supporting Information).

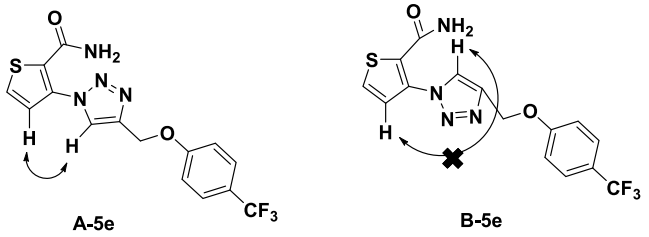


Fig. 3. NOESY effect of the representative compound **5e**.

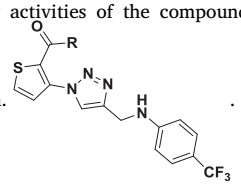
2.2. Biological evaluation

2.2.1. Inhibition of hDHODH and structure-activity relationships

To get a preliminary insight into SARs, the thiophene derivatives with different R group were synthesized and evaluated for enzyme inhibition assay. Human DHODH inhibition assay was conducted using chromogen reduction method with 2,6-dichlorophenolindophenol (DCIP). Dihydroorotate (DHO) oxidation and coenzyme Q (CoQ) reduction was coupled with chromogen reduction. Enzyme inhibition was resulted in loss of chromogen absorbance at 600 nm. The preliminary activities were measured at 10 μM concentration of synthesized compounds. The results (Table 1). showed that compound **4c** (R = NH_2) exhibited higher potency than **2a** and **3a**.

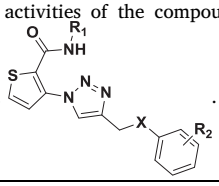
Due to the relatively weak activity of **4c**, further modification of the target compounds with different groups was undertaken. The results (Table 2) showed that the position and property of R_2 groups had a dramatic impact on activities against hDHODH. When **X** is oxygen atom, the incorporation of a polar group such as CN or CF_3 group to 4-position of the terminal phenyl group led to a significant improvement in potency (**5d**, **5e** vs **4c**). However, compounds **5a** and **5c** which possessed 4-position halogen were inactive. Shifting fluorine from 4-position (**5a**) to 3-position (5**b**) increased the activity, while introduction CF_3 group on the 3-position showed a significant decreased potency (**5 g** vs **5e**). Besides, conversion of the R_1 substituted phenyl ring to naphthalene (**5j**) also obtained low potency. When X is nitrogen atom, compound **6a** and **6c** showed high potency (**6a**, **6c** vs **4c**), compounds **6e** and **6f** with 3,4-disubstituted halogen were inactive. Moreover, the introduction of cyclopropyl at R_1 result in the decline of activities against hDHODH (**5f** vs **5e**, **6d** vs **4c)** The results indicated that amide without substitution and a suitable substitution on the terminal phenyl moiety were crucial for high potency.

Based on the preliminary assays, four compounds which showed significant potency were selected for further test IC_{50} values against hDHODH *in vitro*. The results were summarized in Table 3. As predicted, all of the selected compounds displayed good to significant potency against hDHODH with IC_{50} values ranging from 0.9 to 4.6 μM and compound **5e** (IC_{50} = 0.9 μM) exhibited relatively higher potency than teriflunomide.

Table 1
Inhibitory activities of the compounds against hDHODH under 10 μM concentration.  .

Compound	R	% Inhibition ± SD[a] at 10 μM.
2a	OMe	21 ± 0
3a	OH	12 ± 0
4c	NH_2	52 ± 0

[a] SD: standard deviation.

Table 2
Inhibitory activities of the compounds against hDHODH under 10 μM concentration.  .

Compound	X	R_1	R_2	% Inhibition ± SD[a] at 10 μM.
5a	O	H	4-F	NA
5b	O	H	3-F	49 ± 0
5c	O	H	4-Br	NA
5d	O	H	4-CN	86 ± 4
5e	O	H	4-CF_3	89 ± 0
5f	O	cyclopropyl	4-CF_3	49 ± 4
5 g	O	H	3-CF_3	20 ± 11
5 h	O	H	3,4-2F	NA
5i	O	H	2-Br-4-F	49 ± 0
5j	O	H		25 ± 11
6a	N	H	4-F	83 ± 4
6b	N	H	4-Br	57 ± 4
6c	N	H	4-OMe	72 ± 0
6d	N	cyclopropyl	4-CF_3	12 ± 7
6e	N	H	3,4-2F	NA
6f	N	H	3-Cl-4-F	NA

[a] SD: standard deviation.

Table 3
IC_{50} values of synthesized derivatives against hDHODH.

Compound	IC_{50}[a] (μM)	Compound	IC_{50}[a] (μM)
5d	2.8	**6a**	4.6
5e	0.9	**6c**	2.7
Teriflunomide[b]	1.1		

[a] Values are the means of two independent experiments.
[b] Used as positive control.

2.2.2. Immunosuppression assay and metabolic stability

Based on the enzymatic assays, compound **5e** was selected for further study the immunosuppressive effect on the proliferation of stimulated peripheral blood mononuclear cells (PBMCs) compared with teriflunomide. As shown in Table 4, compound **5e** was found to act similarly to teriflunomide, inhibiting activated PBMCs proliferation (IC_{50} = 20.35 μM). Moreover, we performed the study of **5e** in incubation with human liver microsomes *in vitro*. The studies revealed that **5e** possessed high $T_{1/2}$ < 120 min and exhibited moderate CL. It demonstrated that **5e** was stable in human liver microsomes.

2.2.3. Anti-pulmonary fibrotic assay

Drug-induced interstitial lung disease (ILD), particularly pulmonary fibrosis, is a serious clinical concern. For example, hDHODH inhibitor leflunomide, imatinib and gefitinib, are known to induce ILD (pulmonary fibrosis). it can lead to patient death, thereby restricting the therapeutic use of these drugs [13–15]. Recently, a rapid method for anti-fibrotic drugs screening based on cellular stiffness was developed

Table 4
Inhibition of proliferation of stimulated PBMCs and metabolic stability.

Compound	IC_{50}[a] (μM)	$T_{1/2}$ [c](min)	CL(μL/min/mg)
5e	20.35	198.2	7.0
teriflunomide[b]	14.17		

[a] Values are the means of two independent experiments.
[b] Used as positive control.
[c] Conducted in human liver microsomes with final liver microsomal protein (0.5 mg/mL).

Table 5
Alteration of cellular stiffness of TGF-β1 stimulated A549 cells.

Compound	Stiffness[a] (Kpa)	Compound	Stiffness[a] (Kpa)
5d	6.40 ± 1.79	**6c**	7.29 ± 1.51
5e	6.23 ± 1.58	**Control**[b]	4.48 ± 1.96
Model[c]	9.25 ± 1.02	**pirfenidone**[d]	6.09 ± 0.30

[a] Values are the means of at least two independent experiments
[b] A549 cells without any stimulation
[c] TGF-β1 stimulated A549 cells without any compound
[d] Used as positive control.

[16]. A549 cells *in vitro* were stimulated with transforming growth factor-β1 (TGF-β1).It led the increase of the cellular stiffness which may be reversed by the compounds with anti-pulmonary fibrotic activities. The alteration of cellular stiffness was measured utilizing atomic force microscopy (AFM). The anti-pulmonary fibrotic activities of compound **5d**, **5e** and **6c** were evaluated by the method above and anti-pulmonary fibrotic drug pirfenidone was used as positive control. The results are summarized in Table 5. Compared with pirfenidone, compound **5e** exhibited best potency which could reduce the cellular stiffness of TGF-β1 stimulated A549 cells from 9.25 Kpa to 6.23 Kpa.

2.2.4. Binding mode analysis

To further elucidate the binding mode, molecular docking of the potent compound **5e** was performed in the binding site cavity of hDHODH. Crystal structure of hDHODH (PDB ID: 1D3G) [17] and the target compound was pretreated by Discovery Studio 3.0. All docking runs were utilizing CDOCKER protocol of Discovery Studio 3.0 systems. The image files were generated by the PyMOL (The PyMOL Molecular Graphics System, Version 1.4.1. Schrödinger, LLC).

Fig. 4 shows the binding model overlay of the binding model of compound **5e** with brequinar. It can be observed that compound **5e** adopted a brequinar-like binding mode with matching occupancy of the hydrophobic pocket. The carboxyl group on the thiophene amide moiety formed two hydrogen bonds with residues Gln47 and Arg136. However, unlike brequinar, sulfur atom of thiophene moiety was founded to reach the small hydrophobic pocket and the hydrogen on the amide nitrogen atom formed additional hydrogen bond with residues Thr360 which would be beneficial to increase the binding affinity.

3. Conclusions

A previously discovered hDHODH inhibitor was further improved by replacing the phenyl moiety by thiophene, a series of thiophene derivatives were designed, synthesized and evaluated as hDHODH inhibitors. It led us to discover compound **5e** which displayed higher potency than teriflunomide. Compound **5e** also showed high anti-proliferative activity against stimulated PBMCs and possessed acceptable metabolic stability. Besides, compound **5e** reduced the stiffness of TGF-β1 stimulated A549 cells similar with anti-pulmonary fibrotic drug pirfenidone. Taken together, compound **5e** may serve as a lead compound for the development of novel immunosuppressant without inducing pulmonary fibrotic.

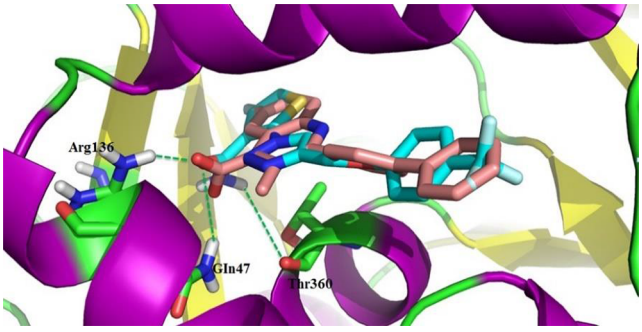


Fig. 4. Docking mode of compound **5e** with hDHODH (PDB ID:1D3G) a representation of the overlap of docking model of **5e** (blue) with brequinar (brown) in the binding site cavity of hDHODH. For clarity, only interacting residues are displayed. The H-bond (Green arrows) are displayed as dotted arrows.

4. Experimental section

4.1. Chemistry

Reagents and solvents were obtained from commercial sources and used without further purification. Flash chromatography was performed using silica gel (300–400 mesh) from Qingdao Ocean Chemicals (Qingdao, Shandong, China). All reactions were monitored by TLC on silica gel plates. Melting points were determined on a Büchi Melting Point B-540 apparatus (Büchi Labortechnik, Flawil, Switzerland). The ^{1}H and ^{13}C NMR were recorded on Bruker ARX-400 or ARX-600 spectrometer (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. Mass spectra (MS) were measured in ESI mode on an Agilent 1100 LC-MS (Agilent, Palo Alto, CA, USA). Elemental analysis was determined on a Carlo-Erba 1106 Elemental analysis instrument (Carlo Erba, Milan, Italy). Propargyl intermediate **(7a-7o)** were prepared following already described procedures [9–11].

4.1.1. Preparation of 3-azidothiophene-2-carboxylate methyl ester (2)

3-amino-2-thiophenecarboxylate methyl ester **1** (0.06 mol) was dissolved in concentrated hydrochloric acid (15 mL), and ice (45 g) was added. When the mixture was cooled to 0 °C, saturated sodium nitrite (5.2 g, 0.075 mol) aqueous solution was added keeping the temperature below 5 °C. After 10 min, resinous sediment (if it was formed) should be filtered. To the filtrated solution of the diazonium salt, sodium azide (3.9 g; 0.06 mol) in 5 mL of water was added dropwise. The solution was left for 30 min at room temperature and the azide was filtered as a white solid which was used without subsequent cleaning. Yield: 80% (8.7 g) m.p.:170.6-172.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 7.95 (d, J = 5.4 Hz, 1H), 7.17 (d, J = 5.4 Hz, 1H), 3.79 (s, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ_C 161.15, 142.21, 133.45, 123.74, 116.78, 52.51. ESI-MS m/z: 206.3 $[M+Na]^+$.

4.1.2. Preparation of 3-azidothiophene-2-carboxylic acid (3)

Intermediate **2** (8.0 g, 0.043 mol) and NaOH (5.1 g, 0.13 mol) were added to a solution of EtOH and H_2O (80 mL, EtOH:H_2O = 5:1). The mixture was stirred at 90 °C for 2 h. Upon cooling to room temperature, ethanol was removed under vacuum, and then H_2O (40 mL) was added to the mixture. The solution was adjusted to pH 2–3 with 3 N HCl, and then the resulting precipitate was filtered and dried under reduced pressure to afford a brown solid. Yield: 82% (5.9 g) m.p.:137.2–139.1 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 13.16 (s, 1H), 7.88 (d, J = 5.4 Hz, 1H), 7.10 (d, J = 5.4 Hz, 1H).^{13}C NMR (100 MHz, DMSO-d_6) δ_C 162.25, 141.34, 132.50, 123.87, 112.64. ESI-MS m/z: 168.2 $[M-H]^-$.

4.1.3. General procedure for preparation of 3-azidothiophene-2-carboxamide (4a-4b)

At room temperature, $SOCl_2$ (20 mL) and 2 drops of DMF were added to intermediate **3** (4 g, 30.6 mmol) and the mixture was refluxed for 4 h. The excess $SOCl_2$ was evaporated. Then, 25% $NH_3{\cdot}H_2O$ (20 mL) was added to the residue at 0 °C and the mixture was stirred for 2–3 h. A white precipitate was isolated by filtration. The precipitate was washed with water and dried in vacuo to afford 2-azidobenzamide.

*4.1.3.1. 3-azidothiophene-2-carboxamide **(4a)***. Yield: 72% (2.8 g), pale yellow solid, m.p.:151.3–152.4 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 7.83 (d, J = 5.4 Hz, 1H), 7.70 (s, 1H), 7.24 (d, J = 5.4 Hz, 1H), 7.15 (s,

1H); ^{13}C NMR (100 MHz, DMSO-d_6) δc 162.01, 136.56, 131.29, 123.80, 122.13; ESI-MS *m/z*: 169.2 $[M+H]^+$.

4.1.3.2. 3-azido-N-cyclopropylthiophene-2-carboxamide ***(4b)***. Yield: 76% (3.7 g), yellow solid, m.p.:137.5–139.3 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 7.82 (d, J = 5.4 Hz, 1H), 7.66 (s, 1H), 7.21 (d, J = 5.4 Hz, 1H), 2.78 (m, 1H), 0.73 – 0.69 (m, 2H), 0.61 – 0.58 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δc 161.62, 136.06, 130.82, 123.61, 122.20, 23.34, 6.58; ESI-MS *m/z*: 209.3 $[M+H]^+$.

4.1.4. General procedure for preparation of the target compounds. ***(2a, 3a,4c, 5a-5i, 6a-6f)***

A mixture of the appropriate azido derivative (0.2 g, 1.2 mmol), the appropriate alkyne derivative (1.4 mmol), copper sulphate (0.1 mmol) and sodium ascorbate (0.5 mmol) in 4 mL tBuOH/H_2O (1:1) were stirred at room temperature for 3–4 h. The reaction mixture was diluted with water and the precipitate was collected by filtration. After being washed with cold water (2 × 5 mL), the precipitate was dried under vacuum. when necessary, they were purified by chromatograpy.

4.1.4.1. 3-(4-(((4-(trifluoromethyl)phenyl)amino)methyl)-1H-1,2,3-triazol-1-yl) thiophene-2-carboxylate methyl ester ***(2a)***. Yield: 77% (0.32 g), White solid, M.p.: 140.2–141.8 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 8.51 (s, 1H), 8.10 (d, J = 5.3 Hz, 1H), 7.48 (d, J = 5.3 Hz, 1H), 7.38 (d, J = 8.7 Hz, 2H), 6.96 (t, J = 5.9 Hz, 1H), 6.79 (d, J = 8.7 Hz, 2H), 4.46 (d, J = 5.9 Hz, 2H), 3.68 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δc: 156.74, 147.91, 141.19, 134.39, 129.46, 123.43, 122.85, 122.81, 122.12, 119.59, 119.57, 108.39, 49.02, 34.41; ESI-MS *m/z*: 383.3 $[M+H]^+$. Anal. calcd. For $C_{16}H_{13}F_3N_4O_2S$ (%):C, 50.26; H, 3.43; N, 14.65. Found (%): C, 50.23; H, 3.47; N, 14.59.

4.1.4.2. 3-(4-(((4-(trifluoromethyl)phenyl)amino)methyl)-1H-1,2,3-triazol-1-yl) thiophene-2-carboxylic acid ***(3a)***. Yield: 68% (0.29 g), White solid, M.p.: 189.1–191.0 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 13.54 (s, 1H), 8.53 (s, 1H), 8.02 (d, J = 5.3 Hz, 1H), 7.44 (d, J = 5.3 Hz, 1H), 7.38 (d, J = 8.5 Hz, 2H), 6.94 (t, J = 5.4 Hz, 1H), 6.78 (d, J = 8.5 Hz, 2H), 4.44 (d, J = 5.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δc: 161.54, 151.75, 144.74, 137.65, 132.39, 127.22, 126.65, 126.61, 126.05, 124.87, 116.10, 112.15, 38.25; ESI-MS *m/z*: 367.0 $[M-H]^-$. Anal. calcd. For $C_{15}H_{11}F_3N_4O_2S$ (%):C, 48.91; H, 3.01; N, 15.21. Found (%): C, 48.95; H, 2.97; N, 15.23.

4.1.4.3. 4.1.4.3.3-(4-(((4-(trifluoromethyl)phenyl)amino)methyl)-1H-1,2,3-triazol-1-yl) thiophene-2-carboxamide ***(4c)***. Yield: 75% (0.33 g), White solid, M.p.: 182.7–184.1 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 8.49 (s, 1H), 7.88 (d, J = 5.3 Hz, 1H), 7.84 (s, 1H), 7.69 (s, 1H), 7.41 (d, J = 5.3 Hz, 1H), 7.39 (d, J = 8.6 Hz, 2H), 6.93 (t, J = 5.7 Hz, 1H), 6.79 (d, J = 8.6 Hz, 2H), 4.44 (d, J = 5.7 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δc: 161.97, 151.79, 145.33, 133.99, 129.12, 129.05, 126.67, 126.64, 125.71, 125.16, 112.16, 99.99, 38.26; ESI-MS *m/z*: 390.3 $[M+Na]^+$. Anal. calcd. For $C_{15}H_{12}F_3N_5OS$ (%):C, 49.04; H, 3.29; N, 19.06. Found (%): C, 49.01; H,3.27; N, 19.09.

4.1.4.4. 3-(4-((4-fluorophenoxy)methyl)-1H-1,2,3-triazol-1-yl)thiophene-2-carboxamide ***(5a)***. Yield: 81% (0.30 g), White solid, M.p.: 136.5–137.9 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 8.68 (s, 1H), 7.90 (d, J = 5.3 Hz, 1H), 7.86 (s, 1H), 7.68 (s, 1H), 7.44 (d, J = 5.3 Hz, 1H), 7.18 – 7.14 (m, 2H), 7.13 – 7.09 (m, 2H), 5.21 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δc: 161.92, 158.37, 154.85, 142.93, 134.05, 129.46, 128.96, 126.83, 125.87, 116.58, 116.35, 61.91; ESI-MS *m/z*: 341.5 $[M+Na]^+$. Anal. calcd. For $C_{14}H_{11}FN_4O_2S$ (%):C, 52.82; H, 3.48; N, 17.60. Found (%): C, 52.85; H, 3.46; N, 17.63.

4.1.4.5. 3-(4-((3-fluorophenoxy)methyl)-1H-1,2,3-triazol-1-yl)thiophene-2-carboxamide ***(5b)***. Yield: 84% (0.32 g), Brown solid, M.p.: 168.1–169.2 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 8.69 (s, 1H), 7.89 (d, J = 5.3 Hz, 1H), 7.86 (s, 1H), 7.67 (s, 1H), 7.44 (d, J = 5.3 Hz, 1H), 7.34 (dd, J = 15.5, 8.1 Hz, 1H), 6.99 (m, 1H), 6.92 (m, 1H), 6.80 (m, 1H), 5.24 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δc: 161.90, 159.97, 142.59, 134.07, 131.25, 131.15, 129.44, 128.93, 126.98, 125.88, 111.62, 108.04, 102.78, 61.69; ESI-MS *m/z*: 341.3 $[M+Na]^+$. Anal. calcd. For $C_{14}H_{11}FN_4O_2S$ (%):C, 52.82; H, 3.48; N, 17.60. Found (%): C,52.83; H, 3.50; N, 17.58.

4.1.4.6. 3-(4-((4-bromophenoxy)methyl)-1H-1,2,3-triazol-1-yl)thiophene-2-carboxamide ***(5c)***. Yield: 85% (0.38 g), White solid, M.p.: 134.2–134.9 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 8.68 (s, 1H), 7.89 (d, J = 5.3 Hz, 1H), 7.85 (s, 1H), 7.66 (s, 1H), 7.48 (d, J = 9.0 Hz, 2H), 7.43 (d, J = 5.3 Hz, 1H), 7.06 (d, J = 9.0 Hz, 2H), 5.22 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δc: 161.90, 157.81, 142.69, 134.06, 132.65, 129.46, 128.95, 126.94, 125.87, 117.57, 112.86, 61.59; ESI-MS *m/z*: 401.4 $[M+Na]^+$. Anal. calcd. For $C_{14}H_{11}BrN_4O_2S$ (%):C, 44.34; H, 2.92; N, 14.77. Found (%): C, 44.31; H, 2.93; N, 14.79.

4.1.4.7. 3-(4-((4-cyanophenoxy)methyl)-1H-1,2,3-triazol-1-yl)thiophene-2-carboxamide ***(5d)***. Yield: 91% (0.35 g), Brown solid, M.p.: 190.3–192.1 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 8.71 (s, 1H), 7.89 (d, J = 5.1 Hz, 1H), 7.85 (s, 1H), 7.81 (d, J = 8.5 Hz, 2H), 7.66 (s, 1H), 7.43 (d, J = 5.1 Hz, 1H), 7.27 (d, J = 8.5 Hz, 2H), 5.34 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δc: 161.93, 161.89, 142.20, 134.70, 134.08, 129.47, 128.95, 127.20, 125.89, 119.55, 116.34, 103.73, 61.71; ESI-MS *m/z*: 326.4 $[M+H]^+$. Anal. calcd. For $C_{15}H_{11}N_5O_2S$ (%):C, 55.38; H, 3.41; N, 21.53. Found (%): C, 55.41; H, 3.38; N, 21.55.

4.1.4.8. 3-(4-((4-(trifluoromethyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl) thiophene-2-carboxamide ***(5e)***. Yield: 86% (0.38 g), Yellow solid, M.p.: 147.9–149.2 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 8.71 (s, 1H), 7.90 (d, J = 5.2 Hz, 1H), 7.86 (s, 1H), 7.69 (d, J = 8.4 Hz, 3H), 7.44 (d, J = 5.2 Hz, 1H), 7.28 (d, J = 8.4 Hz, 2H), 5.33 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δc: 161.34, 160.77, 141.89, 133.51, 128.89, 128.38, 126.91, 126.87, 126.52, 125.32, 121.56, 115.19, 61.08; ESI-MS *m/z*: 369.5 $[M+H]^+$. Anal. calcd. For $C_{15}H_{11}F_3N_4O_2S$ (%):C, 48.91; H, 3.01; N, 15.21. Found (%): C, 48.87; H, 3.04; N, 15.19.

4.1.4.9. N-cyclopropyl-3-(4-((4-(trifluoromethyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)thiophene-2-carboxamide ***(5f)***. Yield: 87% (0.42 g), Yellow solid, M.p.: 146.3–148.5 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 8.65 (s, 1H), 8.50 (d, J = 3.1 Hz, 1H), 7.88 (d, J = 5.3 Hz, 1H), 7.69 (d, J = 8.7 Hz, 2H), 7.45 (d, J = 5.3 Hz, 1H), 7.28 (d, J = 8.7 Hz, 2H), 5.33 (s, 2H), 2.71 (m, 1H), 0.65 – 0.61 (m, 2H), 0.46 – 0.42 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δc: 161.60, 161.31, 142.45, 133.82, 129.05, 128.70, 127.47, 127.44, 126.70, 125.25, 121.96, 115.75, 61.61, 23.37, 6.10; ESI-MS *m/z*: 409.5$[M+H]^+$. Anal. calcd. For $C_{18}H_{15}F_3N_4O_2S$ (%):C, 52.94; H, 3.70; N, 13.72. Found (%): C, 52.95; H, 3.72; N, 13.69.

4.1.4.10. 3-(4-((3-(trifluoromethyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)thiophene-2-carboxamide ***(5 g)***. Yield: 78% (0.34 g), White solid, M.p.: 142.7–144.3 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 8.71 (s, 1H), 7.91 (d, J = 5.3 Hz, 1H), 7.87 (s, 1H), 7.68 (s, 1H), 7.57 (t, J = 7.9 Hz, 1H), 7.45 (d, J = 5.3 Hz, 1H), 7.42 (m, 2H), 7.34 (d, J = 7.9 Hz, 1H), 5.34 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δc: 161.91, 158.81, 142.56, 134.07, 131.24, 130.67, 129.43, 128.95, 127.02, 125.87, 123.11, 119.53, 118.00, 111.92, 61.76; ESI-MS *m/z*: 369.5$[M+H]^+$. Anal. calcd. For $C_{15}H_{11}F_3N_4O_2S$ (%):C, 48.91; H,3.01; N, 15.21. Found (%): C, 48.93; H, 3.02; N,15.24.

4.1.4.11. 3-(4-((3,4-difluorophenoxy)methyl)-1H-1,2,3-triazol-1-yl) thiophene-2-carboxamide ***(5 h)***. Yield: 82% (0.33 g), White solid, M.p.: 145.8–147.6 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 8.68 (s, 1H), 7.89 (d, J = 5.3 Hz, 1H), 7.85 (s, 1H), 7.66 (s, 1H), 7.43 (d, J = 5.3 Hz, 1H), 7.38 (dd, J = 19.8, 9.4 Hz, 1H), 7.25 (m, 1H), 6.91 (m, 1H), 5.22 (s, 2H).; ^{13}C NMR (100 MHz, DMSO-d_6) δc: 161.33, 154.59, 154.51,

141.89, 133.51, 128.88, 128.37, 126.45, 125.32, 117.51, 111.07, 111.00, 104.40, 61.60; ESI-MS *m/z*: 337.4 $[M+H]^+$. Anal. calcd. For $C_{14}H_{10}F_2N_4O_2S$ (%):C, 50.00; H, 3.00; N, 16.66. Found (%): C,50.03; H, 3.01; N, 16.64.

4.1.4.12. 3-(4-((2-bromo-4-fluorophenoxy)methyl)-1H-1,2,3-triazol-1-yl) thiophene-2-carboxamide ***(5i)***. Yield: 73% (0.34 g), White solid, M.p.: 147.9–149.5 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 8.69 (s, 1H), 7.89 (d, J = 5.3 Hz, 1H), 7.86 (s, 1H), 7.68 (s, 1H), 7.57 (dd, J = 8.1, 3.1 Hz, 1H), 7.45 (d, J = 5.3 Hz, 1H), 7.40 (dd, J = 9.1, 4.9 Hz, 1H), 7.26 (m, 1H), 5.30 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δc: 161.37, 156.11, 151.07, 141.98, 133.46, 128.74, 128.37, 126.41, 125.26, 119.88, 115.29, 115.09, 111.36, 62.51; ESI-MS *m/z*: 397.4 $[M+H]^+$. Anal. calcd. For $C_{14}H_{10}BrFN_4O_2S$ (%):C, 42.33; H, 2.54; N, 14.10. Found (%): C,42.31; H, 2.55; N, 14.12

4.1.4.13. 3-(4-((naphthalen-2-yloxy)methyl)-1H-1,2,3-triazol-1-yl) thiophene-2-carboxamide ***(5j)***. Yield: 76% (0.32 g), White solid, M.p.: 161.1–162.8 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 8.74 (s, 1H), 7.90 (d, J = 5.2 Hz, 1H), 7.85 (m, 4H), 7.68 (s, 1H), 7.56 (d, J = 1.5 Hz, 1H), 7.50 – 7.46 (m, 1H), 7.45 (d, J = 5.2 Hz, 1H), 7.37 (t, J = 7.1 Hz, 1H), 7.23 (m, 1H), 5.34 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δc: 161.93, 156.42, 142.93, 134.68, 134.08, 129.87, 129.47, 129.12, 128.96, 128.00, 127.26, 126.92, 126.92,125.89, 124.23, 119.12, 107.75, 61.45; ESI-MS *m/z*: 373.5 $[M+Na]^+$. Anal. calcd. For $C_{18}H_{14}N_4O_2S$ (%):C, 61.70; H, 4.03; N, 15.99. Found (%): C,61.73; H,4.02; N, 15.97

4.1.4.14. 3-(4-(((4-fluorophenyl)amino)methyl)-1H-1,2,3-triazol-1-yl) thiophene-2-carboxamide ***(6a)***. Yield: 89% (0.33 g), Yellow solid, M.p.: 154.1–155.3 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 8.46 (s, 1H), 7.88 (d, J = 5.3 Hz, 1H), 7.84 (s, 1H), 7.69 (s, 1H), 7.41 (d, J = 5.3 Hz, 1H), 6.93 (t, J = 8.9 Hz, 2H), 6.67 (dd, J = 8.8, 4.5 Hz, 2H), 6.05 (brs, 1H), 4.35 (d, J = 4.4 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δc: 161.97, 156.17, 153.87, 145.80, 133.96, 129.14, 129.07, 125.66, 125.07, 115.67, 113.68, 39.39; ESI-MS *m/z*: 340.4 $[M+Na]^+$. Anal. calcd. For $C_{14}H_{12}FN_5OS$ (%):C, 52.99; H, 3.81; N, 22.07. Found (%): C,52.31; H,3.78; N, 22.08

4.1.4.15. 3-(4-(((4-bromophenyl)amino)methyl)-1H-1,2,3-triazol-1-yl) thiophene-2-carboxamide ***(6b)***. Yield: 85% (0.38 g), Brown solid, M.p.: 157.7–158.5 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 8.46 (s, 1H), 7.88 (d, J = 5.2 Hz, 1H), 7.84 (s, 1H), 7.69 (s, 1H), 7.41 (d, J = 5.2 Hz, 1H), 7.21 (d, J = 8.5 Hz, 2H), 6.65 (d, J = 8.5 Hz, 2H), 6.38 (brs, 1H), 4.36 (d, J = 5.1 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δc: 161.98, 148.03, 145.74, 133.97, 131.82, 129.14, 129.07, 125.69, 125.10, 114.81, 107.28, 38.70; ESI-MS *m/z*: 400.3$[M+Na]^+$. Anal. calcd. For $C_{14}H_{12}BrN_5OS$ (%):C, 44.46; H, 3.20; N, 18.52. Found (%): C,44.45; H, 3.23; N, 18.54.

4.1.4.16. 3-(4-(((4-methoxyphenyl)amino)methyl)-1H-1,2,3-triazol-1-yl) thiophene-2-carboxamide ***(6c)***. Yield: 78% (0.30 g), Dark yellow solid, M.p.: 185.1–186.7 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 8.44 (s, 1H), 7.88 (d, J = 5.3 Hz, 1H), 7.85 (s, 1H), 7.69 (s, 1H), 7.41 (d, J = 5.3 Hz, 1H), 6.73 (d, J = 8.8 Hz, 2H), 6.64 (d, J = 8.8 Hz, 2H), 5.64 (brs, 1H), 4.33 (s, 2H), 3.64 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δc: 161.98, 151.57, 151.56, 133.98, 129.13, 129.09, 125.65, 124.98, 115.01, 114.10, 114.08, 55.76, 40.65; ESI-MS *m/z*: 330.3 $[M+H]^+$. Anal. calcd. For $C_{15}H_{15}N_5O_2S$ (%):C, 54.70; H, 4.59; N, 21.26. Found (%): C,54.73; H, 4.61; N, 21.23.

4.1.4.17. N-cyclopropyl-3-(4-(((4-(trifluoromethyl)phenyl)amino) methyl)-1H-1,2,3-triazol-1-yl)thiophene-2-carboxamide ***(6d)***. Yield: 83% (0.40 g), Yellow solid, M.p.: 160.1–162.3 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 8.51 (d, J = 2.0 Hz, 1H), 8.40 (s, 1H), 7.85 (d, J = 5.2 Hz, 1H), 7.41 (d, J = 5.2 Hz, 1H), 7.38 (d, J = 8.4 Hz, 2H), 6.93 (t, J = 5.7 Hz, 1H), 6.78 (d, J = 8.4 Hz, 2H), 4.44 (d, J = 5.7 Hz, 2H), 2.69 (m, 1H), 0.61 (m, 2H), 0.41 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δc: 161.12, 151.15, 144.85, 133.19, 128.08, 127.94, 126.09, 126.06, 124.44, 124.07, 115.60, 111.62, 37.66, 22.80, 5.53; ESI-MS *m/z*: 430.5[M +Na]$^+$. Anal. calcd. For $C_{18}H_{16}F_3N_5OS$ (%):C, 53.07; H, 3.96; N, 17.19. Found (%): C,53.06; H, 3.98; N, 17.21.

4.1.4.18. 3-(4-(((3,4-difluorophenyl)amino)methyl)-1H-1,2,3-triazol-1-yl)thiophene-2-carboxamide ***(6e)***. Yield: 81% (0.32 g), Pale yellow solid, M.p.: 174.5–175.6 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 8.53 (s, 1H), 7.94 (d, J = 5.3 Hz, 1H), 7.90 (s, 1H), 7.74 (s, 1H), 7.46 (d, J = 5.3 Hz, 1H), 7.17 (dd, J = 19.9, 9.0 Hz, 1H), 6.72 (m, 1H), 6.51 (d, J = 9.0 Hz, 1H), 6.42 (t, J = 5.8 Hz, 1H), 4.40 (d, J = 5.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δc: 161.97, 150.41, 146.45, 145.52, 141.75, 133.99, 129.13, 129.04, 125.69, 125.11, 117.82, 108.46, 100.87, 39.03; ESI-MS *m/z*: 334.5 $[M-H]^-$ Anal. calcd. For $C_{14}H_{11}F_2N_5OS$ (%):C, 50.15; H, 3.31; N, 20.89. Found (%): C, 50.12; H, 3.34; N, 20.92

4.1.4.19. 3-(4-(((3-chloro-4-fluorophenyl)amino)methyl)-1H-1,2,3-triazol-1-yl)thiophene-2-carboxamide ***(6f)***. Yield: 71% (0.29 g), Yellow solid, M.p.: 151.5–152.5 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ_H: 8.47 (s, 1H), 7.89 (d, J = 5.3 Hz, 1H), 7.85 (s, 1H), 7.68 (s, 1H), 7.41 (d, J = 5.3 Hz, 1H), 7.12 (t, J = 9.1 Hz, 1H), 6.81 (m, 1H), 6.66 – 6.63 (m, 1H), 6.36 (t, J = 5.8 Hz, 1H), 4.36 (d, J = 5.8 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δc: 161.97, 149.73, 146.39, 145.53, 133.98, 129.11, 129.04, 125.68, 125.10, 119.83, 117.33, 113.16, 112.51, 38.99; ESI-MS *m/z*:: 352.4 $[M+H]^+$. Anal. calcd. For $C_{14}H_{11}ClFN_5OS$ (%):C,47.80; H,3.15; N, 19.91 Found (%): C,47.78; H, 3.16 N, 19.93.

4.2. hDHODH inhibition assay

Coenzyme Q10 (CoQ10), dichlorophenolindophenol (DCIP) and L-dihydro orotate (L-DHO) were obtained from Sigma Aldrich. teriflunomide were used as reference compound, which was obtained from Sigma Aldrich. Stock solution of synthesized compounds was retained in DMSO. Recombinant human DHODH was purchased from Prospec Bio (ENZ-642). Inhibitory activity was assessed by monitoring the reduction of 2,6-dichloroindophenol (DCIP), which is associated with oxidation of dihydroorotate catalyzed by the DHODH enzyme. The enzyme was pre-incubated for five minutes at 37 °C in Tris-buffer solution (pH 8.0) with coenzyme Q10 (100 μM), tested compounds at different concentrations (0.04–10 μM), and DCIP (50 μM). The reaction was initiated by addition of DHO (500 μM) and absorbance of each well was measured at 600 nm. Percent inhibition = (max-conversion)/(max–min) * 100."max" stands for high control; "min" stands for low control. an IC_{50} value was calculated using GraphPad Prism software. Values are means ± SD of two independent experiments.

4.3. Immunosuppression assay

PBMCs were isolated by Ficoll/Isopaque (Lymphoprep) density gradient centrifugation of buffy coat leukopheresis residues from fresh blood samples from healthy donors. Purified cells were grown and maintained in culture medium at 37 °C in 5% CO_2 humidified atmosphere. Cell proliferation was assessed by CellTiter-Glo® assay. Cells were seeded in a white-opaque 96-well plate and exposed to increasing concentrations (0.8–200 μM) of each compound or vehicle (DMSO) for 2 h and then stimulated with 1 μg/mL anti-CD3 and 2 μg/mL anti-CD28 for 72 h. IC_{50} values were determined using nonlinear regression plots with GraphPad Prism software. Values are means ± SD of two independent experiments.

4.4. Microsomal metabolic stability assay

4.4.1. Solutions preparation

The stock solutions of test article and positive control were prepared at a concentration of 10 mM using DMSO as diluents. All stock solutions

were then diluted to working concentrations at 0.25 mM with 70% acetonitrile. The cofactor used in this study was NADPH regenerating system, that was composed of 6.5 mM NADP, 16.5 mM G-6-P, 3 U/mL G-6-PD.The quench reagent was consisted of acetonitrile containing tolbutamide and propanolol (serve as internal standard).The buffer used in this study was 100 mM, phosphate buffer with 3.3 mM $MgCl_2$. Incubation mixtures containing 0.5 mg/mL liver microsomal protein and 1 μM test article/positive control in 100 mM potassium phosphate buffer.

4.4.2. Assay procedure

The 0-minute samples were prepared by addition of an 80 μL aliquot of each incubation mixture to 300 μL quench reagent to precipitate proteins. The samples were vortexed, and then a 20 μL aliquot of the NADPH regenerating system was added in. The reaction was initiated by addition of 80 μL of the NADPH regenerating system to 320 μL of each incubation mixture. The final incubation conditions achieved in 400 μL are: 0.5 mg/mL microsomal protein, 1 μM test article/positive control, 1.3 mM NADP, 3.3 mM glucose 6 phosphate, 0.6 U/mL glucose 6 phosphate dehydrogenase. The mixtures were incubated in a 37 °C water bath with gentle shaking. A 100 μL aliquot of each mixture was removed at 10, 30, 90 min to a clean 96-well plate which contains 300 μL quench reagent to precipitate proteins, and centrifuged (4000 × g, 15 min) 0.80 μL of supernatant are taken into 96-well assay plates pre-added with 160 μL ultrapure water, and then analyzed by LC-MS/MS.

4.5. Anti-pulmonary fibrotic assay

4.5.1. Cell culture and treatment

The human alveolar epithelial cell line A549 was purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in RPMI-1640 (Hyclone, Logan, UT) containing 10% fetal bovine serum (Hyclone), 100 U/ml penicillin and 100 mg/ml streptomycin (Sigma, St. Louis, MO) in an incubator at 37 °C with 5% CO_2. Cells were rendered quiescent by serum free medium for 24 h, and then pretreated with for 3 h before simulation with 5 ng/ml TGF-β1 for another 72 h. A549 cells simulated with PBS only were taken as the control.

4.5.2. AFM imaging

Morphological and ultrastructural assays were performed using a Nanoscope VI Dimension 3100 AFM (Veeco Company, Santa Barbara, CA) and oxide-sharpened Si3N4 tips (MLCT, radius 10–40 nm; Bruker Company, Santa Barbara, CA). Cells were seeded in 70 mm culture disk. For living cell imaging, the triangular cantilever with a normal spring constant 0.01 N/m was employed. The spring constant was calibrated with a Thermal Tune Adapter (Veeco Company). The probe was localized onto the cell surface with the assistance of a CCD camera. The experiments were performed at contact mode. The scan force was 50 pN and the scan rate was 0.3 Hz.

4.5.3. Measurement of cellular stiffness by AFM

The stiffness of cells was also detected at contact mode. Measurements were conducted above the nucleus region of the cell to avoid the influence of the underlying substrate. 100 Force-distance curves were obtained from 10 different cells in each group at the ramp rate of 0.5 Hz. Force curves were obtained at the same loading rate and were analyzed by Matlab 7.6.0.

4.6. Molecular docking

The molecular modelling studies were performed with Accelrys Discovery Studio 3.0. The crystal structure of hDHODH complexed with brequinar (PDB code: 1D3G) was obtained from the RCSB Protein Data Bank (http://www.rcsb.org/pdb/). Compound **5e** was drawn with Chemdraw and fully minimized using the CHARMm force field. Before docking, the crystal structure of the protein underwent an optimization process using the Protein Preparation Wizard tool, implemented in Discovery Studio 3.0. Missing hydrogen atoms were added and bond orders were assigned while missing loops and residues not belonging to the binding site were capped by adding ACE (*N*-acetyl) and NMA (*N*-methylamide) groups. Then, ORO, nonstructural water molecules and impurities (such as solvent molecules) were removed and the co-crystalized ligand brequinar was maintained. In the docking process, the protein protocol was prepared by several operations, including standardization of atom names and insertion of missing atoms in residues. Then, the receptor model was typed with the CHARMm force field and a binding sphere with radius of 9.0 Å was defined through the original ligand (hydroxylated azole) as the binding site. Finally, they were docked into the binding site using the CDOCKER with the default settings. And figures were prepared using PyMOL (The PyMOL Molecular Graphics System, Version 1.4.1. Schrödinger, LLC).

Conflict of interest

The authors have declared no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.01.011.

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