Recent Developments in the Medicinal Chemistry and Therapeutic Potential of Dihydroorotate Dehydrogenase (DHODH) Inhibitors

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Abstract: Dihydroorotate dehydrogenase (DHODH) is a flavin-dependent mitochondrial enzyme that catalyzes fourth reaction of pyrimidine *de-novo* synthesis. Pyrimidine bases are essential for cellular metabolism and cell growth, and are considered as important precursors used in DNA (thymine and cytosine), RNA (uracil and cytosine), glycoproteins and phospholipids biosynthesis. The significance of pyrimidines biosynthesis in DNA and RNA makes them ideal targets for pharmacological intervention. Inhibitors of DHODH have proven efficacy for the treatment of malaria, autoimmune diseases, cancer, rheumatoid arthritis and psoriasis. *Plasmodium falciparum* dihydroorotate dehydrogenase (*Pf*DHODH) represents an important target for the treatment of malaria. Many of the clinically relevant anti-tumor and immunosuppressive drugs target human dihydroorotate dehydrogenase (*h*DHODH), and the two most promising drugs of such kinds are brequinar (antitumor and immunosuppressive) and leflunomide (immunosuppressive). X-ray crystal structures of DHODH in complex with inhibitors reveal common binding region shared by each inhibitor. A number of compounds are identified by high-throughput screening (HTS) of chemical libraries and structure-based computational approaches as selective DHODH inhibitors. Based upon the understanding of molecular interaction of DHODH inhibitors with binding site, some of the common structural features are identified like ability of compounds to interact with ubiquinone (CoQ) binding site and substituents linked to a variety of heterocyclic and heteroaromatic rings responsible for H-bonding with binding site. These findings provide new approaches to design DHODH inhibitors and highlights DHODH as a target for chemotherapeutics. This review is mainly focused on the recent developments in the medicinal chemistry and therapeutic potential of DHODH inhibitors as a target for drug discovery.

Keywords: Dihydroorotate dehydrogenase (DHODH), pyrimidines biosynthesis, DHODH inhibitors, cancer, arthritis, malaria.

1. INTRODUCTION

The flavoenzyme dihydroorotate dehydrogenase (DHODH) [EC 1.3.99.11] [1] is a fourth enzyme of pyrimidine *de-novo* synthesis that catalyses oxidation of intermediate dihydroorotate (DHO) to orotate (ORO). Pyrimidines are required for the biosynthesis of DNA, RNA, glycoproteins, and phospholipids, and are linked by phosphodiester bridges to purine nucleotides in double-stranded DNA, both in nucleus and mitochondria [2]. There are two routes for the synthesis of pyrimidines in most of the organisms; salvage pathways and *de novo* synthesis from small metabolites. Requirement of pyrimidine depends on cell type and developmental stage, involvement of *de novo* pathway is small in resting or fully differentiated cells where cell acquire pyrimidine mainly by the salvage pathways [3]. In contrast, activated T cells and other rapidly proliferating cells, in order to meet their increased demand for nucleic acid precursors and other cellular components, depends heavily on *de novo* nucleotide synthesis [4]. DHODH catalyzes conversion of DHO to ORO (Fig. **1**), which represents the rate limiting step in *de novo* pyrimidine biosynthesis [5].

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The significance of pyrimidine for cell proliferation, metabolism and multiplication determines DHODH as targets for the development of new drug candidate [6]. Inhibition of DHODH leads to reduced levels of essential pyrimidine nucleotides. Inhibitors of human DHODH (*h*DHODH) have proven efficacy for treatment of cancer [7,8] and immunological disorders, such as rheumatoid arthritis and multiple sclerosis [9-11]. DHODHs are attractive chemotherapeutic targets in various pathogens, such as *Plasmodium falciparum*, *Helicobacter pylori*, *Enterococcus faecalis* [12-15] and as antifungal agents [16]. DHODH has a number of properties that make it a particularly strong candidate as a new drug target. Brequinar [17] and leflunomide [18, 19] (Fig. **2**) are two examples of such compounds. Brequinar is an antitumor and immunosuppressive agent, while leflunomide, a prodrug which is converted to active metabolite **A77 1726** [20], shows immunosuppressive activity. Many excellent research publications characterize the exponential growth in therapeutic potential of DHODH inhibitors. In 1999 Batt, D.G. [21] compiled the literature of DHODH inhibitors and described the earlier attempts to find DHODH inhibitors based on ORO and CoQ binding site. Leban, J. and Vitt, D. [22] published a review describes DHODH inhibitors as a promising agent for the treatment of autoimmune and inflammatory diseases. The scope of this review is to update the key advances in the medicinal chemistry and biology of DHODH inhibitors as a new target for drug discovery. In the later sec-

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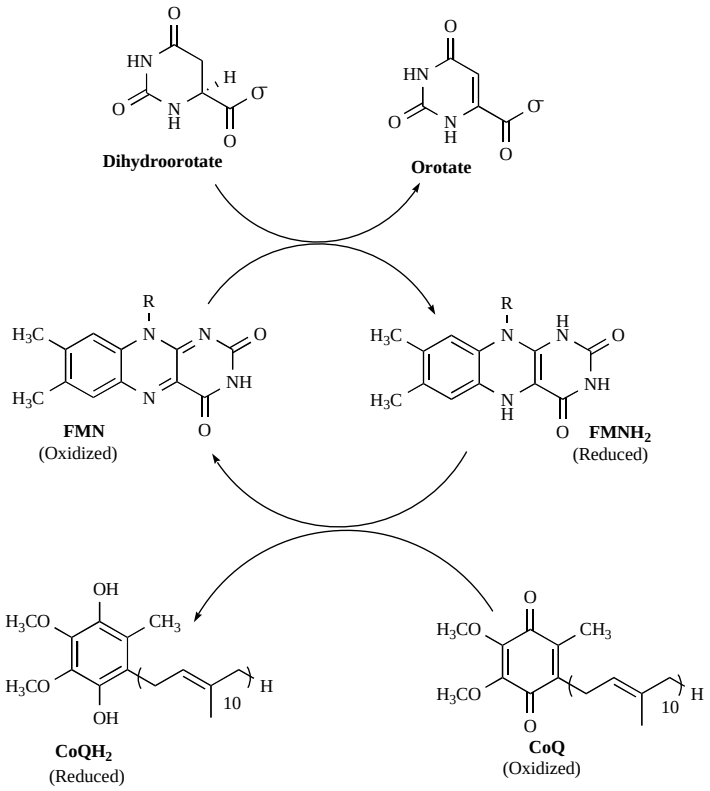


Fig. (1). Reactions catalyzed by DHODH.

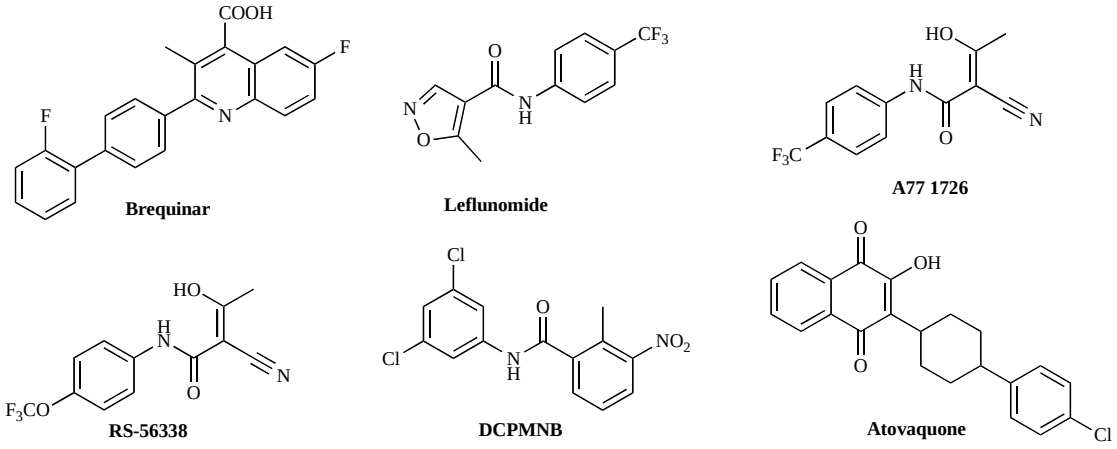


Fig. (2). DHODH inhibitors.

tion, major impetus is given to medicinal chemistry of DHODH inhibitors as therapeutic potential agents.

2. DIHYDROOROTATE DEHYDROGENASE (DHODH)

Dihydroorotate dehydrogenase (DHODH) catalyses the oxidation/reduction reaction in pyrimidine nucleotide biosynthesis: Oxidation of dihydroorotate (DHO) to orotate (ORO) and reduction of flavin mononucleotide (FMN) to dihydroflavin mononucleotide ($FMNH_2$) (Fig. **1**) comprise two half reactions of redox couple. The co-substrate electron acceptor used by DHODH varies in different organisms. In *h*DHODH, flavin cofactor is FMN and ubiquinone (CoQ) is second substrate. DHODH from higher eukaryotes exhibits a two-site ping-pong mechanism with flavin mononucleotide (FMN) serving as an intermediate in the electron transfer and CoQ acting as the final electron acceptor [23, 24]. Human DHODH enzyme is composed of two domains, a large C-terminal (Met78 to C- terminal) and small N-terminal domain (Met 30 to Leu 68) linked by an extended loop. The large C-terminal domain can be expressed best as an α/β barrel fold with eight parallel β strands forming the barrel and α helices wrapped around the outside. The redox site formed by the substrate binding site of cofactor flavine mononeuclotide (FMN) is located on this large C-terminal domain. The small N-terminal domain, on the other hand consists of two α helices, α1 and α2 connected by a short loop and a binding site for CoQ [25, 26]. DHODH enzymes

are divided into two families, based upon their localization, amino acid sequence, substrate/cofactor dependence, and cellular localization [27, 28]. Family-1 enzymes are located in the cytosol, electron acceptors involved in second half reaction of redox process are either fumarate or NAD^+ whereas family-2 enzymes transfer electrons to ubiquinone (CoQ), to which *h*DHODH belongs, is distinguished from family-1 by the use of ubiquinone (CoQ) for reoxidizing FMN. N-terminus is proposed as binding site for electron acceptor, which is responsible for association of enzyme with inner mitochondrial membrane [29]. Family-1 enzymes are further divided into family-1A, homodimeric proteins which contain a cysteine residue as their active site base and use fumarate as electron receptor [30-32] and family-1B (NAD-dependent) enzymes, hetero tetramers, which contain not only FMN but also an iron–sulfur cluster and FAD, where the enzyme is oxidized by NAD^+ [33] and a new type 1S identified in *Sulfolobus solfataricus* [34]. Family-1S DHODH can use CoQ and molecular oxygen as electron acceptors.

3. STRUCTURE OF DHODHS

Clardy, J. *et al.* [1] solved high-resolution crystal structures of *h*DHODH in complex with two different inhibitors **A77 1726** and brequinar, and refined them to crystallographic R factors of 16.8% and 16.2% at resolutions of 1.6 Å and 1.8 Å respectively. Human DHODH has two domains: α/β-barrel domain containing the active site and an α-helical domain that forms the opening of a tunnel leading to the active site. Both the inhibitors share a common binding site in this tunnel, and differences in binding region govern drug sensitivity or resistance. Fishwick, C.W.G. and colleagues [35] reported X-ray crystal structure of *h*DHODH and *Pf*DHODH with inhibitors and observed different binding modes. In *Pf*DHODH-inhibitor complex, three direct hydrogen bonds can be observed (to His185, Arg265 and Tyr528), whereas in *h*DHODH-inhibitor complex, there are water-mediated hydrogen bonds to Arg265 and Gln47 in addition to direct H-bond to Tyr356. Walse, B. *et al.* [36] determined the crystal structure of N-terminally truncated DHODH in complex with brequinar analogue (6-chloro brequinar, in which 6-fluoro group of brequinar is replaced with a 6-chloro group) and a novel inhibitor (fenamic acid derivative), and presented a crystal structure of the inhibitor-free N-terminally truncated (Met30-Arg396) *h*DHODH. In this study extensive network of interactions provides the basis of high affinity of brequinar analogues for *h*DHODH. Findings of this study suggested a new approach to design DHODH inhibitors. Baumgartner, R. *et al.* [37] solved five high-resolution X-ray structures in complex with low molecular weight inhibitors. The data provides clear and excellent electron densities for the inhibitors and therefore, enables a detailed analysis of prevailing interactions and modes of binding. The structure of *Pf*DHODH with a bound inhibitor was described by Hurt, D.E. *et al.* [24] *Pf*DHODH and *h*DHODH have different sensitivities to inhibitors. The three-dimensional structure (2.4 Å, R = 20.1%) of *Pf*DHODH-leflunomide complex was determined by X-ray crystallography. Comparison of the structures of *h*DHODH and *Pf*DHODH reveals structural differences between the CoQ-binding tunnels and an absolutely different binding mode for the same inhibitor is observed. Gojkovic, Z. *et al.* [38] investigated crystal structure of *Candida albicans* DHODH and proposed that the enzyme is a member of the DHODH family-2. In this study full-length DHODH and N-terminally truncated DHODH, were sub-cloned from *Candida albicans*, recombinantly expressed in *Escherichia coli*, purified and characterized for their kinetics and substrate specificity. The result of this study provides a background for development of DHODH inhibitors for decreasing pyrimidine nucleotide pools in *Candida albicans*. T7 RNA polymerase expression system in *Escherichia coli* was used to produce *h*DHODH as a fusion protein containing an amino-terminal decahistidine tag [39]. Kinetic analysis of this recombinant *h*DHODH indicates that it has a two site ping-pong mechanism where dihydroorotate (DHO) is oxidized at one site and second substrate CoQ is reduced at other. The functional expression of *h*DHODH in *pyr4* mutants of *Ustilago maydis* was studied by Bolker M. *et al.* [40]. Engineered *U. maydis* strains can be used in *in vivo* assays for development of novel *h*DHODH and fungal DHODH inhibitors. Chargas disease is considered as a public health problem in Latin America caused by hemoflagellate protozoan *Trypanosoma cruzi*. Nonato, M.C. *et al.* [41] reported the crystal structure of DHODH from *Trypanosoma cruzi* strain and solved at 2.2Å resolution. Crystal structure of *T. cruzi* DHODH suggested potential sites for the design of highly specific inhibitors.

4. MOLECULAR MECHANISM OF ACTION OF DHODH INHIBITORS

Inhibition of DHODH can cause a lowering of intracellular pyrimidine nucleotides pools in cells, which makes DHODH as an attractive target for drug design in different biological and clinical applications for cancer, arthritis and malaria. The mechanism of action of DHODH inhibitors was studied in several kinetic studies by Bennett, L. and colleagues at Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama [42]. They investigated the mechanism of toxicity of dichloroallyl lawsone (DCL) to tumor cells in culture. DCL specifically inhibits biosynthesis of pyrimidine nucleotides by inhibition of DHO and it was confirmed in studies with isolated mitochondria from mouse liver. Cleaveland, E.S. *et al.* [43] discovered a novel DHODH inhibitor (NSC 665564) and proposed that it has the same mechanism of inhibition as brequinar. Cytotoxicity results were experimentally determined using MOLT-4 lymphoblast (the site of DHODH) and found the IC_{50} of brequinar (0.5 μM) and NSC 665564 (0.3 μM) were comparable. The induced cytotoxicity was reversed using either uridine or cytidine. Davis, J.P. *et al.* [44] investigated the effects of leflunomide and its active metabolites (**A77 1726**) on the activity of purified recombinant *h*DHODH. It was found that **A77 1726** is a potent inhibitor of DHODH (Ki = 179 +/- 19 nM), while the parent compound leflunomide had no inhibitory effect (Concentration >1 μM). These result suggested that **A77 1726** is a competitive inhibitor of the CoQ binding site and is noncompetitive with respect to DHO. Williamson, R.A. *et al.* [45] isolated and characterized target protein from mouse spleen, purified and identified as mitochondrial DHODH by a series of structural and functional criteria. Inhibition of the DHODH activity by **A77 1726** and its analogues clearly showed antiproliferative effect on a wide

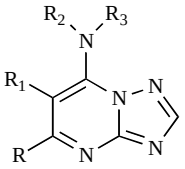
range of cells due to depletion of intracellular pools of uridyl triphospahtate (UTP) and cytidyl triphospahtate (CTP).

5. MEDICINAL CHEMISTRY OF DHODH INHIBITORS

DHODH is a central enzyme of pyrimidine biosynthesis that catalyzes oxidation of dihydroorotate to orotate. DHODH inhibitors are considered as promising targets for the development of antiproliferative, antiparasitic and immunosuppressive drugs since rapid cell proliferation often depends on *de novo* synthesis of pyrimidine nucleotides. The X-ray structures of *Pf*DHODH [24] and *h*DHODH [26] are determined. Orotate and FMN load against each other in the center of the β/α barrel, and the inhibitor binding site is formed nearby to this site by two α-helices, that are positioned between predicted N-terminal transmembrane domain and the canonical β/α barrel domain. Binding mode studies of DHODH with inhibitors have suggested that inhibitor binding-pocket has extensive variation in amino acid sequence between *Pf*DHODH and *h*DHODH. The intention of the next section is to review all known DHODH inhibitors to cover recent advances in the medicinal chemistry and therapeutic potential of this class of agents.

5.1. Triazolopyrimidine-Based DHODH Inhibitors

Phillips, M.A. *et al*. [46] synthesized triazolopyrimidines based inhibitors of *Pf*DHODH with potent antimalarial activity in whole cell assay. Compound **1** (5-methyl-[1,2,4] triazolo[1,5-*a*]pyrimidin-7-yl)naphthalen-2-ylamine (Fig. **3**) was a representative compound of triazolopyrimidines based inhibitors and showed potent activity (IC_{50} *Pf* DHODH = 0.047 μM) against *P. falciparum*. Compound **1** was discovered by HTS of a 220,000 compound library of "druglike" molecules using a colorometric enzyme assay. Structure activity relationship study revealed that substitutions at R and R_1 position of triazolopyrimidine with alkyl group (Fig. **3**) **2** (IC_{50} *Pf* DHODH = 0.21 μM), **3** (IC_{50} *Pf* DHODH = 0.19 μM) **4** (IC_{50} *Pf* DHODH = 0.16 μM) resulted in modest decrease in activity, while the substitutions at R_2 (Fig. **3**) compound **5** (IC_{50} *Pf* DHODH = 3.0 μM) and **6** (IC_{50} *Pf* DHODH = 93 μM) resulted in a loss of potency. Introduction of heteroatoms on, or in, the napthyl ring reduce activity **7** (IC_{50} *Pf* DHODH >200 μM), naphthalene ring attached at 1-position showed reduced activity **8** (IC_{50} *Pf* DHODH = 45 μM) and anthracene moiety at 2-position **9** (IC_{50} *Pf* DHODH = 0.056 μM) showed potent inhibitory activity.



1: R = CH_3 R_1 = H R_2 = H R_3 = 2-naphthyl

2: R = CF_3 R_1 = H R_2 = H, R_3 = 2-naphthyl

3: R = C_2H_5 R_1 = H R_2 = H, R_3 = 2-naphthyl

4: R = CH_3 R_1 = CH_3 R_2 = H, R_3 = 2-naphthyl

5: R = CH_3 R_1 = H R_2 = CH_3 R_3 = 2-naphthyl

6: R = CH_3 R_1 = H R_2 = H R_3 = 2-naphthyl

7: R = CH_3 R_1 = H R_2 = H R_3 = 2-benzamide

8: R = CH_3 R_1 = H R_2 = H R_3 = 1-naphthyl

9: R = CH_3 R_1 = H R_2 = H R_3 = 2-anthracenyl

10: R = CH_3 R_1 = H R_2 = H R_3 = 3-F- 4-CF_3-Ph

Fig. (3). Triazolopyrimidine-based derivatives.

In another work the authors [47] studied species differences in inhibitor potency between *Pf*DHODH and *P. berghei* dihydroorotate dehydrogenase (*Pb*DHODH). Compound **1** (IC_{50} *Pb*DHODH = 0.23μM) and **9** (IC_{50} *Pb*DHODH = 3.7 μM) do not suppress parasite levels in *P. berghei* mouse model. Analogs of compound **1** were synthesized by replacing the naphthyl moiety with substituted phenyl groups and tested for activity against recombinant enzyme *Pf*DHODH, *Pb*DHODH, *h*DHODH and in whole cell *P. falciparum* parasite assays. Compound **10** contained para and meta substitutions (Fig. **3**) with the best activity against *Pf*DHODH (IC_{50} = 0.077 μM), which has similar potency to compound **1** and showed equal potency against *Pf*DHODH and *Pb*DHODH. Binding mode and specific selectivity of compound **1** was studied in detail to gain insight into the structural requirement for the selective and potent binding of **1** to *Pf*DHODH. Steady-state kinetic analysis showed that the *Pf*DHODH inhibitory activity increased linearly with increasing CoQD concentration**.** The authors found that compound **1** inhibited the oxidative half-reaction and prevented transfer of electrons from $FMNH_2$ to CoQ, without affecting DHO dependent reductive half-reaction. It was suggested that all the inhibitors utilized the same mechanism to inhibit DHODH. Binding mode of **1** and its derivatives in receptor cavity explained the species-selective binding due to variation in amino acid sequence between the human and malarial DHODH.

Phillips, M. A. and Rathod P.K. published a review [48] on identification of several different classes (triazolopyrimidine, phenylbenzamides, thiofuran) of compounds by high throughput screening (HTS) as potent inhibitors of *Pf*DHODH. The most common chemical classes identified by the authors were phenylbenzamides **11**, ureas **12** and naphthamides **13** (Fig. **4**). These compounds showed strong selectivity for the *Pf*DHODH against *h*DHODH. The authors identified a potent and selective triazolopyrimidine-based compound **14**, showed potent activity against *P. falciparum* 3D7 in whole cell assays. The X-ray structures of *Pf*DHODH bound to triazolopyrimdine-based inhibitors (**1**, **9** and **10**) were solved recently, provided the first structures of *Pf*DHODH bound to a parasite selective potent inhibitor [49]. Binding-site is composed of two sites for triazolopyrimidine based inhibitor: 1) a completely hydrophobic site, which binds naphthyl (**1**), anthracenyl (**9**) and phenyltrifluoromethyl (**10**), and 2) triazolopyrimidine pocket capable of H-bond interactions. Both the sites provides a signifi-

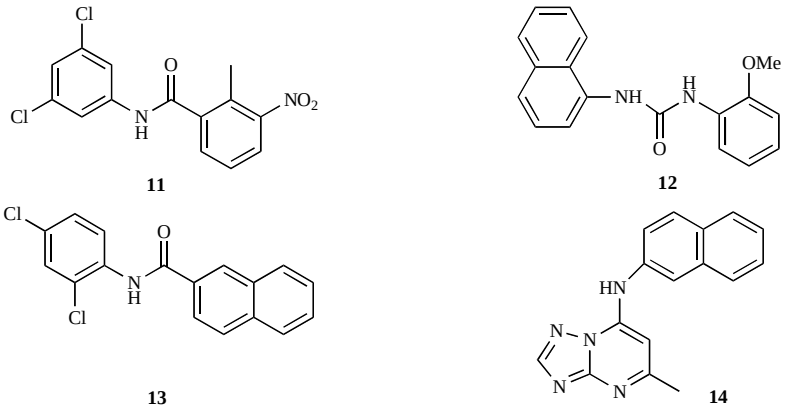
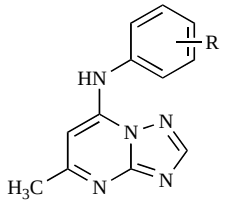


Fig. (4). Analogs of triazolopyrimidine identified by HTS.

cant amount of binding energy to the inhibitor series; π-stacking interactions are observed between the naphthyl and two Phe residues (227 and 188), and H-bonds to H185 and R265.

The triazolopyrimidine based inhibitors overlap the binding site of the **A77 1726** observed in the previously reported *Pf*DHODH-**A77 1726** crystal structure [25]. The naphthyl binding-site access an earlier unidentified pocket formed by rotation of Phe188, which moves this residue into a position that overlaps the phenyl portion of **A77 1726**. The authors suggested that these data provides information on structural features as to how *Pf*DHODH is able to bind different classes of compounds with high affinity.

Recently QSAR and molecular docking studies [50] were performed on triazolopyrimidine based DHODH inhibitors [46, 47] as antimalarial agents. The QSAR studies revealed that spatial, electronic, thermodynamic and structural descriptors play an important role in determining DHODH inhibitory activity of triazolopyrimidine derivatives. Docking studies inferred that hydrophobicity is necessary for better DHODH inhibitory activity. The authors designed some new compounds (**15-17**) (Fig. **5**), performed their docking studies and observed similar binding interaction pattern. Docking study suggested different binding mode of substituted triazolopyrimidine derivatives. 2-Methyltriazolopyrimidine ring interacts with some polar (Arg265 and His185) and some nonpolar amino acids (Ileu263, Gly181, Leu176, Cys175, Cys184, Val532 and Leu172), whereas the substituted phenyl rings interact with a hydrophobic pocket of the DHODH, created by some nonpolar amino acid residues. Ligand-receptor interaction studies inferred that volume and hydrophobicity of the para substituents of phenyl ring should be high but limited, while meta substituents with reasonable volume and moderate hydrophobicity were essential for better DHODH inhibitory activity. Designed compounds showed good *in silico* predicted activity as per the developed QSAR models.



15: R = 3-I, 4-$CH(CH_3)_2$ Dock score = 34.384

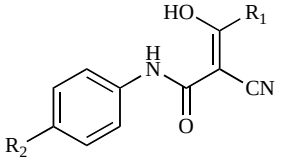
16: 3-Br, 4-I Dock score = 32.916

17: 3-CH_3, 4-I Dock score = 32.449

Fig. (5). Designed triazolopyrimidine-based DHODH Inhibitors with dock scores.

5.2. Trifluoromethy Phenyl Butenamide Derivatives

Fishwick, C.W.G. *et al.* [35] applied SPROUT-LeadOpt, a software package for computational structure based lead optimization exercise aiming for improving binding of **A77 1726** to CoQ binding cavity in DHODH. The authors synthesized and tested analogs of **A77 1726** against human and *P. falciparum* DHODH in inhibition assays. The SAR study of analogs of **A77 1726** revealed that substitution of methyl group for a cyclopropyl group as in **18** led to increase in binding affinity for both *Plasmodium* and human DHODH (IC_{50} *Pf*DHODH = 92.5 μM and IC_{50} *h*DHODH = 0.117 μM). Large hydrophobic substituents at this position (as in compounds **19, 20**) (Fig. **6**) resulted in an adverse effect upon DHODH inhibition. Replacement of the trifluoromethyl group in **A77 1726** with phenyl **21 (**IC_{50} *Pf*DHODH = 25.7



18: R_1 = Cyclopropanyl R_2 = CF_3

19: R_1 = Cyclobutanyl R_2 = CF_3

20: R_1 = Cyclopentanyl R_2 = CF_3

21: R_1 = CH_3 R_2 = Phenyl

22: R_1 = Cyclopropanyl R_2 = Phenyl

Fig. (6). Trifluoromethy phenyl butenamide derivatives.

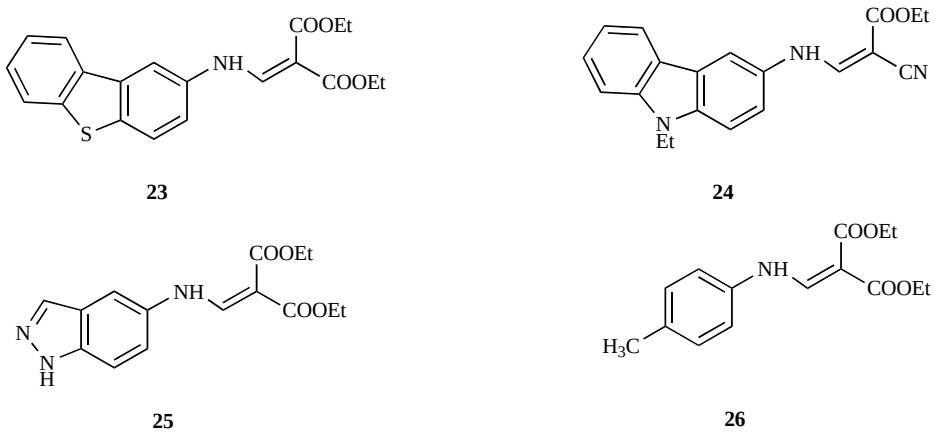


Fig. (7). Structures of ethoxy aromatic amide-based DHODH inhibitors.

μM and IC_{50} *h*DHODH = 0.09 μM**)** resulted in a significant improvement in binding affinities for both the enzymes. Arrangement of these features in compound **22** resulted in good inhibition of *h*DHODH but an 8-fold reduction in activity against *Pf*DHODH.

The authors investigated interaction of inhibitors with *h*DHODH using X-ray crystallography. Co-crystallized structure of *h*DHODH-inhibitors complex suggested that head moiety of inhibitors occupied same part of the binding cavity as **A77 1726.** In case of *Pf*DHODH- **A77 1726** complex, three direct hydrogen bonds were observed (to His185, Arg265, and Tyr528), whereas in case of *h*DHODH- **A77 1726** complex, there were water-mediated hydrogen bonds to Arg265 and Gln47 as well as the direct hydrogen bond to Tyr356. The biphenyl ring (tail) of each inhibitor was predicted to extend toward the surface of the protein and bind in large hydrophobic region of binding cavity. All the inhibitors made direct H-bonding interaction with binding cavity. The cocrystal structures of **21** involved two water mediated H-bonds to Arg136 and Gln47, and a direct hydrogen bond involving the hydroxyl of Tyr356. The authors predicted binding mode of inhibitors in *Pf*DHODH and found that inhibitors binds in a similar fashion to that observed in **21**, with head group making direct H-bonds to residues Arg265, His185 and Tyr528. The biphenyl tail was predicted to bind in the large hydrophobic region of the CoQ binding cavities in a manner similar to that found for these inhibitors in *h*DHODH. All the designed inhibitors showed enhanced levels of inhibition for both *Plasmodium* and human DHODH enzymes compared to that observed for the existing inhibitor **A77 1726**.

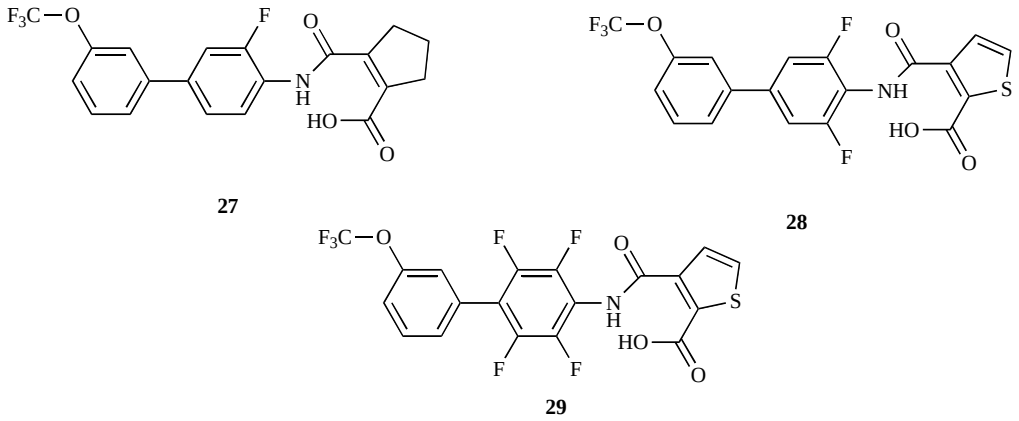
5.3. Ethoxy Aromatic Amide-Based DHODH Inhibitors

A set of compounds were designed and synthesized [51] as *Pf*DHODH inhibitors with high binding affinities for analyzing the mode of binding through modeling and mutagenesis. Two different docking systems Autodock 3.0 and eHITS were used for docking of **23** (IC_{50} *Pf*DHODH = 0.16 μM) and **24** (IC_{50} *Pf*DHODH = 0.44μM) (Fig. **7**) into DHODH structures with DHO. Docking studies suggested that inhibitors (**23, 24**) form H-bonds to H185, R265, and Y528 in a similar manner to that observed for **A77 1726** binding in the *Pf*DHODH crystal structure. Mutagenesis studies further emphasized that H185 and R265 residue were involved in binding of these inhibitors. H-bonding and hydrophobic interactions were favorable interaction of inhibitors with CoQ binding channel.

Synthesized compounds were tested against a recombinant *Pf*DHODH expressed from a synthetic gene in which codon usage was optimized for expression in *E. coli*. The tricyclic compounds (**23** and **24**) were more active than monocyclic or bicyclic compounds **25** (IC_{50} *Pf*DHODH = 27.78 μM) and **26** (IC_{50} *Pf*DHODH = 40.00μM) (Fig. **6**). The compounds showed considerably lower affinity for *h*DHODH, a 1200-fold higher relative affinity for parasite enzyme **23** (IC_{50} *h*DHODH =29.57μM) and **24** (IC_{50} *h*DHODH = 491.4 μM).

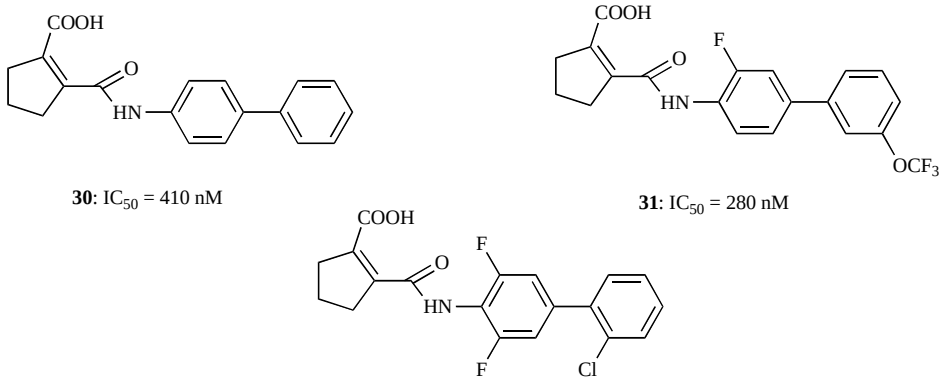
5.4. Cyclic Aliphatic and Aromatic Carboxylic Acid Amide Derivatives

Baumgartner, R. *et al.* [37] disclosed a novel series of DHODH inhibitors, based on virtual screening methods and presented high-resolution X-ray structures of *h*DHODH in complex with a novel class of low molecular weight compounds. Molecules that displayed structure activity relationship in an *in vitro* assay with IC_{50} values in nanomolar range were selected and co-crystallized with *h*DHODH. Selected compounds showed interesting binding modes termed as "nonbrequinar-like" **27** (IC_{50} = 280 nM) and "brequinar-like" **28** (IC_{50} = 2 nM) (Fig. **8**) and some compounds showed interestingly a dual binding mode **29** (IC_{50} = 7 nM) strongly depending on the nature of chemical substitution. In "brequinar-like" binding mode, 6-fluoro-3-methyl-4-quinoline carboxylic acid moiety formed salt bridge to the side chain of Arg136. Additionally H-bonds were formed between carboxylic acid and the side chain of Gln47. The biphenyl ring system showed several hydrophobic contacts with binding cavity, which mainly, consist of hydrophobic amino acids. In case of compound **27**, different binding mode was observed and the carboxylic acid moiety was located in the opposite direction protruding toward the protein's interior. This binding mode was termed as "nonbrequinar-like". "Brequinar-like" binding mode was more favorable with smaller measured IC_{50} values. The authors performed superposition of binding sites, revealed conformational differences for amino acids (Gln47 and Arg136), whereas conformational differ-



27 **28** **29**

Fig. (8). Cyclic aliphatic and aromatic carboxylic acid amide derivatives.



30: IC_{50} = 410 nM **31**: IC_{50} = 280 nM **32**: IC_{50} = 18 nM

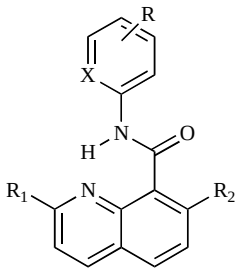
Fig. (9). Structures and activity of cyclopentene carboxylic acid amide derivatives.

ences were very minimum for the rest of the binding cavity. The binding site displayed enough volume to hold a variety of ring substitutions and compound conformations, especially for the hydrophobic part of the binding site.

In another work Leban, J. *et al.* [52] designed *via* docking, synthesized and improve *via* QSAR a novel series of DHODH inhibitors. Docking of **30** (Fig. **9**) into crystal structure of DHODH resulted in a positioning similar to that of brequinar. Docking study suggested that carboxylic acid group formed an ionic interaction to amino acid residue Arg-136, and the biphenyl ring showed hydrophobic interaction in receptor pocket. Introduction of small side chains into the biphenyl ring of lead **30** resulted in compound **31, 32** with additional interactions in hydrophobic pocket of CoQ binding cavity. QSAR analysis resulted in identification of important features of a good DHODH inhibitor. In an *in vitro* enzyme assay, IC_{50} values for DHODH Inhibition were determined using N-terminally truncated recombinant *h*DHODH.

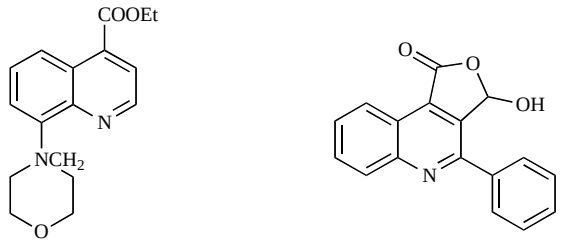
5.5. Aromatic Quinoline Carboxamide Derivatives

The authors [53] designed and synthesized quinoline-8-carboxamide derivatives as immunosuppressant to prevent xenograft rejection, dependent on inhibition of antibodies (Ab) produced by B-cells, independently of T-cell signals.



Comp.	R	R_1	R_2	X
33	4-CF_3	H	H	C
34	4-CF3	H	H	N
35	H	H	H	C
36	2-CF_3	H	H	C

Fig. (10). Aromatic quinolinecarboxamides derivatives.



37: IC_{50} *Pf*DHODH = 38 μM
IC_{50} *h*DHODH = 38 μM

38: IC_{50} *Pf*DHODH = 162 μM
IC_{50} *h*DHODH = 80 μM

Fig. (11). Structures and activity of 2-phenylquinoline-4-carboxylic acid derivatives.

Compounds **33**-**36** (Fig. **10**) were synthesized, *via* coupling of acid chloride of quinoline-8-carboxylic acid with corresponding anilines. Pharmacological screening of compounds based on modulation of Ab production by trinitrophenyl-lipopolysaccharide (TNP-LPS) stimulated murine B-cells. T-cell immunosuppressive activity and cytotoxicity of compounds were assessed in mouse mixed lymphocyte reaction (MLR) and Jurkat proliferation assays.

Compound **33** discriminates between B- and T-cells and showed 10-fold B-cell selectivity over T-cell selectivity. Structure activity relationship (SAR) study revealed the presence and influence of trifluoromethyl substituent. In comparison to **33**, the corresponding phenyl derivative **35** was a weak B-cell inhibitor, whereas the ortho trifluoromethyl substituted derivative **36**, although equipotent to leflunomide has a cytostatic character because it was devoid of selectivity toward T-cells and Jurkat cells.

5.6. 2-Phenylquinoline-4-Carboxylic Acid Derivatives

Boa, A.N. *et al.* [54] synthesized a series of 2-phenylquinoline-4-carboxylic acid derivatives related to brequinar and evaluated as inhibitors of *Pf*DHODH (antimalarial agents). Selected compounds (**37, 38**) (Fig. **11**) were evaluated for inhibition of *Pf*DHODH and they were found more active than brequinar. The active compounds were also screened for inhibition of *h*DHODH. Synthesized 2-phenylquinoline-4-carboxylic acid derivatives were considerably less active against *h*DHODH than brequinar.

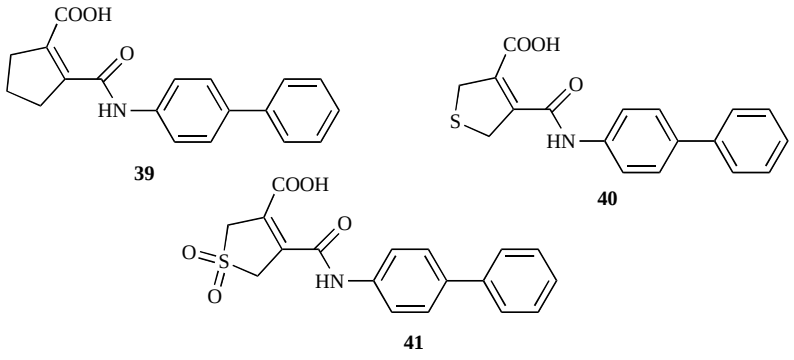
5.7. Cyclopentene Dicarboxylic Acid Amides Derivatives

Leban, J. *et al.* [55] synthesized novel DHODH inhibitors by the introduction of heteroatoms into the cyclopentene ring and attached a hydroxyl group to it**.** Compounds were synthesized by reacting dicarboxylic acid anhydrides with anilines in an inert solvent. DHODH inhibition was measured in an *in vitro* enzyme assay using N-terminally truncated recombinant *h*DHODH. SAR study revealed that introduction of sulfur atom into the petacyclic ring of lead compound **39** (IC_{50} = 0.41 μM) resulted in equipotent compound **40**(IC_{50} = 0.667 μM) (Fig. **12**).

Oxidation of sulfide to sulfone led to less active compound **41** (IC_{50} = 3.8μM). Introduction of oxygen atom in pentacyclic ring system resulted in compounds with lower DHODH inhibitory activity. X-ray crystallography showed a brequinar-like binding mode of these compounds with increasing number of fluoro substituents in the first aromatic ring.

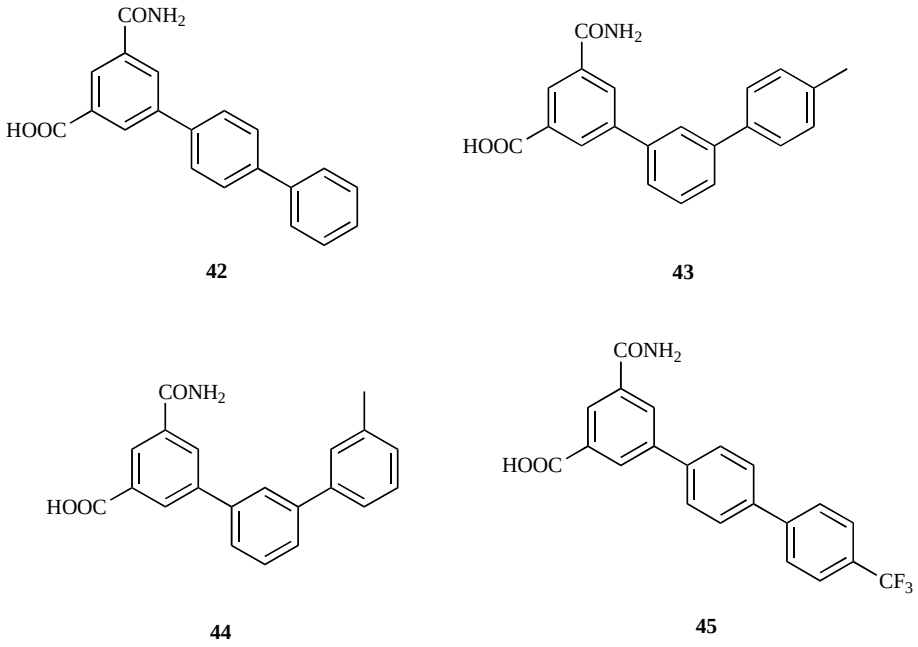
5.8. Terphenyl Carboxylic Acid Amide Derivatives

Clardy, J. *et al*. [56] synthesized a series of potentially selective inhibitors of DHODH (**42**-**45**) (Fig. **13**) *via* iterative, chemoselective Suzuki cross-couplings reaction using biaryl chlorides. The authors [57] disclosed brequinar-derived asymmetric terphenyl compounds**.** X-ray crystallographic structure of an analog of brequinar bound to *h*DHODH was determined. Terphenyl compound **42** bound to CoQ binding site in a similar fashion to brequinar, phenyl tail bound in pocket by hydrophobic interactions, and carboxylic acid moiety formed H-bonds with R136 and amide group forms H-bonds with Y356 near FMN group. Docking study with eHiTS suggested that most of the compounds dock well to human and rat DHODH, confirmed by their docking scores and matched with experimentally determined DHODH inhibition. Synthesized compounds were screened



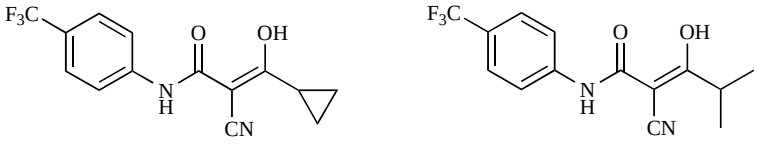
39 **40** **41**

Fig. (12). Structures of cyclopentene dicarboxylic acid amides derivatives.



42 **43** **44** **45**

Fig. (13). Structures of terphenyl carboxylic acid amide derivatives.



46 **47**

Fig. (14). Structures of cyclopropane carbonyl derivatives.

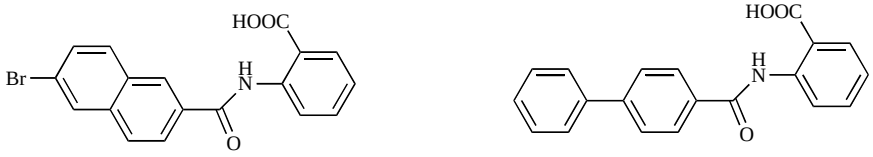
via a crude visual enzyme assay based on the color change in the dye 2,6-dichloroindophenol (DCIP) (ε595 = 18.8 mM^{-1} cm^{-1}), required to established a threshold concentration for DHODH inhibition.

5.9. Cyclopropane Carbonyl Derivatives

Yang, D.Y. *et al.* [58] synthesized cyclopropane carbonyl analogues as inhibitors of 4-hydroxyphenylpyruvate dioxygenase (HPPD) and DHODH. The authors reported X-ray crystal structures, and suggested that cyclopropanecarbonyl derivative **46** (IC_{50} = 21 nM) was 14 times more potent than the corresponding isopropylcarbonyl derivative **47** (IC_{50} = 295 nM) in rat DHODH inhibition due to H-bonding of cyclopropanecarbonyl oxygen of **46** to hydroxyl group of Tyr356. This specific H-bonding induces cyclopropyl group in **46** to adopt the fixed bisected conformation, causing **46** to function as a conformation-restricted DHODH inhibitor.

5.10. Biaryl Carboxyamide Derivatives

Fishwick, C.W.G. *et al.* [59] applied *de novo* molecular design program SPROUT to design novel inhibitors of *Pf*DHODH. The authors made a detailed **c**omparison of the crystal structure of *Pf*DHODH and *h*DHODH in the dimensions and topography of the hydrophobic CoQ binding channel. The study suggested that the topography *of h*DHODH is flattened by the projection of a methyl group in the region which was occupied by the aromatic ring of the bound inhibitor and required inhibitors of this type to be essentially planar. In case of *Pf*DHODH, same region was much less congested unlike that in *h*DHODH and could accommodate



48: IC_{50} *Pf*DHODH = 142.6 μM

IC_{50} *h*DHODH = 8.4 μM

49: IC_{50} *Pf*DHODH = 153.3 μM

IC_{50} *h*DHODH = 5.0 μM

Fig. (15). Structures and activity of biaryl carboxyamide derivatives**.**

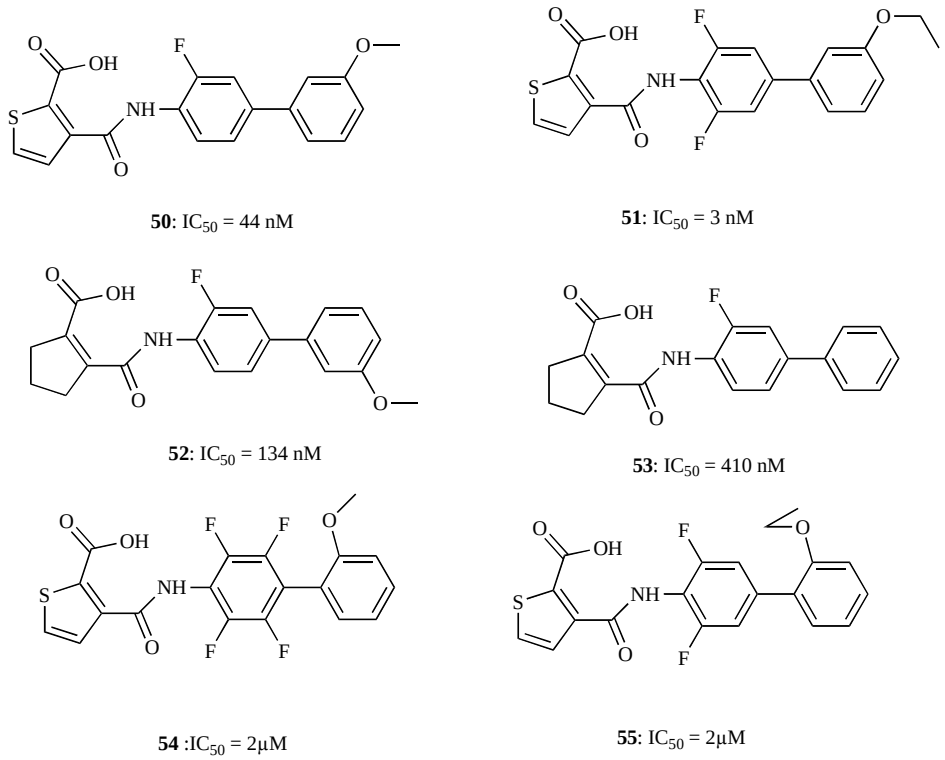


Fig. (16). Structures and activity of biphenyl-4-ylcarbamoyl thiophene/cyclopentene carboxylic acids derivatives.

inhibitors that were cylindrical in shape. Two amino acids His185 and Arg265, made direct H-bonding contacts to the designed inhibitors. *Pf*DHODH and *h*DHODH inhibition was measured by crude visual assay based on the color change in the dye DCIP, using saturated concentrations of DHO and decylubiquinone (CoQd). Structure based *de novo* design produced 20 different small molecule templates, binding affinities of some of them were predicted in micromolar range (**48, 49**) (Fig. **15**).

5.11. Biphenyl-4-ylcarbamoyl Thiophene/Cyclopentene Carboxylic Acids Derivatives

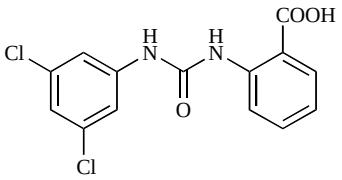
Replacement of cyclopentene ring by aromatic heterocycles resulted in a series of biphenyl-4-ylcarbamoyl thiophene carboxylic acids derivatives (**50, 51**) [60] (Fig. **16**). SAR study revealed that compounds with thiophene ring (**50, 51**) were more potent as compared to cyclopentene ring **(52, 53)**. The authors compared X-ray crystal structures of **52** and **55**, and found that cyclopentene derivatives **(52**) showed a single binding mode (non-brequinar like) and thiophene analog (**55)** showed a dual binding mode which was "brequinar like" and "non-brequinar like". DHODH inhibition was measured in an *in vitro* enzyme assay using N-terminally truncated recombinant *h*DHODH. Compounds **54** (IC_{50} = 2μM) and **55** (IC_{50} = 2μM) displayed potent antiproliferatory effects on peripheral blood mononuclear cells (PBMCs), and suggested potential of such compounds for treatment of autoimmune diseases.

5.12. Amino-Benzoic Acid Derivatives

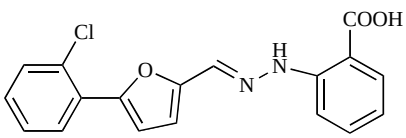
Recently, novel amino-benzoic acid derivatives [61] were disclosed as DHODH inhibitors by virtual screening and X-ray crystallographic studies. The authors determined binding mode of selected hits by X-ray crystallography, suggested the H-bonding interaction to the side-chain of either Arg136 or Tyr365 and a hydrophobic interaction to the side-chain of Val134 and Tyr356. H-bond network was created around the acidic group that made bifurcated H-bonds to the guanidinyl group of Arg136. One carboxylate oxygen atom formed H-bond to a crystallographic water molecule (H-bonds to Gln-47), other carboxylate oxygen formed H- bonds to a second crystallographic water (H-bonds to the NHs of the urea linker of the inhibitor and the backbone carbonyl oxygen of Thr-360). The benzoic acid phenyl ring occupied hydrophobic pocket formed by the FMN and the side-chains of Val134 and Tyr356 amino acid residue. The software program GLIDE was used for virtual screening which resulted in 200 hits with IC_{50} <10 μM. The binding modes of the compounds **56, 57** were revealed by co-crystal structures with *h*DHODH. Selected compounds were screened *via* enzymatic assay using recombinant *h*DHODH.

5.13. N-Arylaminomethylene Malonate

Johnson, A.P. *et al.* [62] studied the effects of substituents on the selectivity of binding of N-arylaminomethylene malonate derivatives to DHODH. Molecular modeling study suggested that substitution at aryl portion with H-bond acceptors (**58-60**) (Fig. **18**) increased selectivity of these inhibitors for *Pf*DHODH. The authors described the synthesis of novel series of DHODH inhibitors containing either carboxylic acid or ester functionality. Synthesized compound were screened for binding affinity against *Pf*DHODH and *h*DHODH at 50 μM using the DCIP dye. X-ray crystal structures of selective inhibitors bound to *Pf*DHODH showed

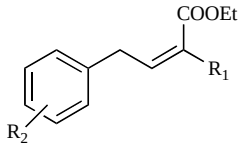


56: IC_{50} = 0.7 μM



57: IC_{50} = 0.5 μM

Fig. (17). Structures and activity of amino-benzoic acid derivatives.



Compound	R1	R2	*Pf*DHODH(50 μM)	*h*DHODH(50 μM)
58	CO_2Et	*m*-CO_2Et	0 ± 4	5 ± 2
59	CO_2Et	*p*-CO_2Et	15 ± 4	4 ± 2
60	CN	*p*-CO_2Et	2 ± 4	2 ± 2

Fig. (18). Structures and activity of N-arylaminomethylene malonate derivatives.

mutation of His185 to Ala causing a large reduction in binding affinity. N-acyl-2-aminomethylene malonate inhibitors bound to DHODH in a similar fashion to that of **A77 1726**. Examination of binding cavities for both *Pf*DHODH and *h*DHODH revealed that, in addition to a large hydrophobic cavity, a smaller hydrophobic cavity was also located near to Arg265 in *Pf*DHODH. The docking studies indicated that, volume of the small hydrophobic cavity accommodated one of the two ester-derived ethyl groups.

5.14. 4-Hydroxycoumarins, Fenamic Acids and N-(alkylcarbonyl) Anthranilic Acids Derivatives

Fritzson, I. *et al.* [63] identified three different classes of *h*DHODH inhibitor based upon virtual screening and structure-guided selection of fragments. The authors created pharmacophores by using structure based virtual screening method. This allowed the identification of some structural features like hydrophobic, donor, acceptor and ionizable groups for interaction. On the basis of structure-guided fragment selection method, it was suggested that substitution at third position of benzoic acid caused polar interaction with Try356 amino acid in inner subside of CoQ biding site. Three different class of compounds (4-hydroxycoumarins, fenamic acids, and N-(alkylcarbonyl)anthranilic acids) were designed, synthesized and evaluated for inhibition of *h*DHODH. Derivatives of N-(alkylcarbonyl) anthranilic acid (**61, 62**) showed potent *h*DHODH inhibitory activity than 4-hydroxycoumarins **(63, 64)** and fenamic acids derivatives **(65, 66)** (Fig. **19**).

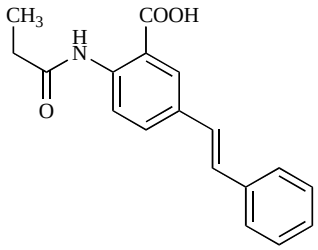
5.15. Inhibitors of *Trypanosoma cruzi* DHODH

Nonato, M.C. *et al.* [64] identified a novel series of *Trypanosoma cruzi* DHODH inhibitors by a combination of virtual screening (structure-based and ligand-based) and isothermal titration calorimetry (ITC) methods. *Trypanosoma cruzi* DHODH (*Tc*DHODH) is a member of family-1A DHODH and *h*DHODH is a member of family-2 DHODHs. Members of family-1A are homodimeric proteins whereas family-2 enzymes are monomeric. The authors have explored active site of DHODHs, to map structural differences between *h*DHODH, *Trypanosoma cruzi* (*Tc*DHODH) and *Leishmania major* (*Lm*DHODH) enzymes. The topographical analysis identified four new regions (S1, S2, S3 and S4) which were able to accommodate inhibitors. The results from ITC assays provided insights into the role of different substituents in determining binding affinity of compounds tested against *Tc*DHODH. The authors proposed SAR study based on the calorimetric assays, together with structural information provided by X-ray diffraction analysis of 3D structures. In compounds **67-72** (Fig. **20**) substitution at C5 was more favorable than C6 (carboxylic oxygen), and rigidification of the carboxyl group resulted in a complete loss of molecular interaction. The authors determined the apparent inhibition constant (Ki^{app}) and elucidated the mechanism of inhibition of compounds.

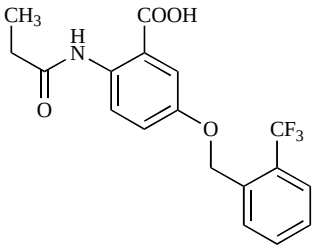
Schematic diagram of important interactions of inhibitor with DHODH is given in Fig. (**21**).

5.16. Alkyl-5-Benzimidazole Thiophene-2-Carboxamide Derivatives

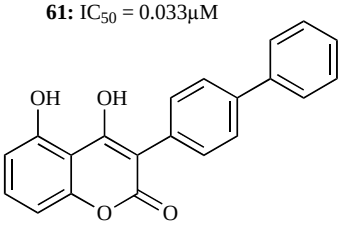
Booker, M.L. *et al.* [65] recently identified novel inhibitors of DHODH. Compounds **73-75** were identified form HTS screening of 215,000 diverse compounds [66]. The authors evaluated i*n vitro* and *in vivo* drug absorption, distribution, metabolism, and excretion properties of inhibitors



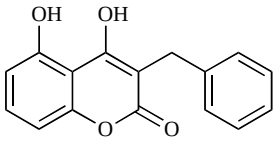
61: IC_{50} = 0.033μM



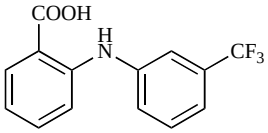
62: IC_{50} = 0.015 μM



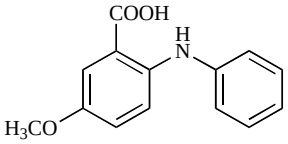
63: IC_{50} = 0.23 μM



64: IC_{50} = 11 μM

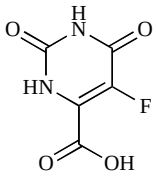


65: IC_{50} = 0.24 μM

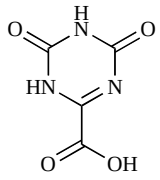


66: IC_{50} = 0.26 μM

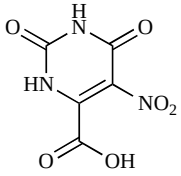
Fig. (19). Structures and activity of 4-hydroxycoumarins, fenamic acids and N-(alkylcarbonyl) anthranilic acids derivatives.



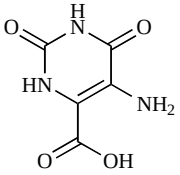
67:*Ki* ^{app} = 19.28 ± 4.01 (μM)



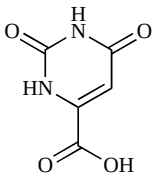
68:*Ki* ^{app} = 21.70 ± 4.36(μM)



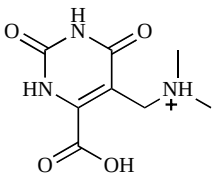
69:*Ki* ^{app} = 72.21 ± 7.41(μM)



70:*Ki* ^{app} = 94.79 ± 14.98 (μM)



71:*Ki* ^{app} = 139.24 ± 26.04(μM)



72:*Ki* ^{app} = 300 ± 20.08(μM)

Fig. (20). Structures of Inhibitors of *Trypanosoma cruzi* DHODH.

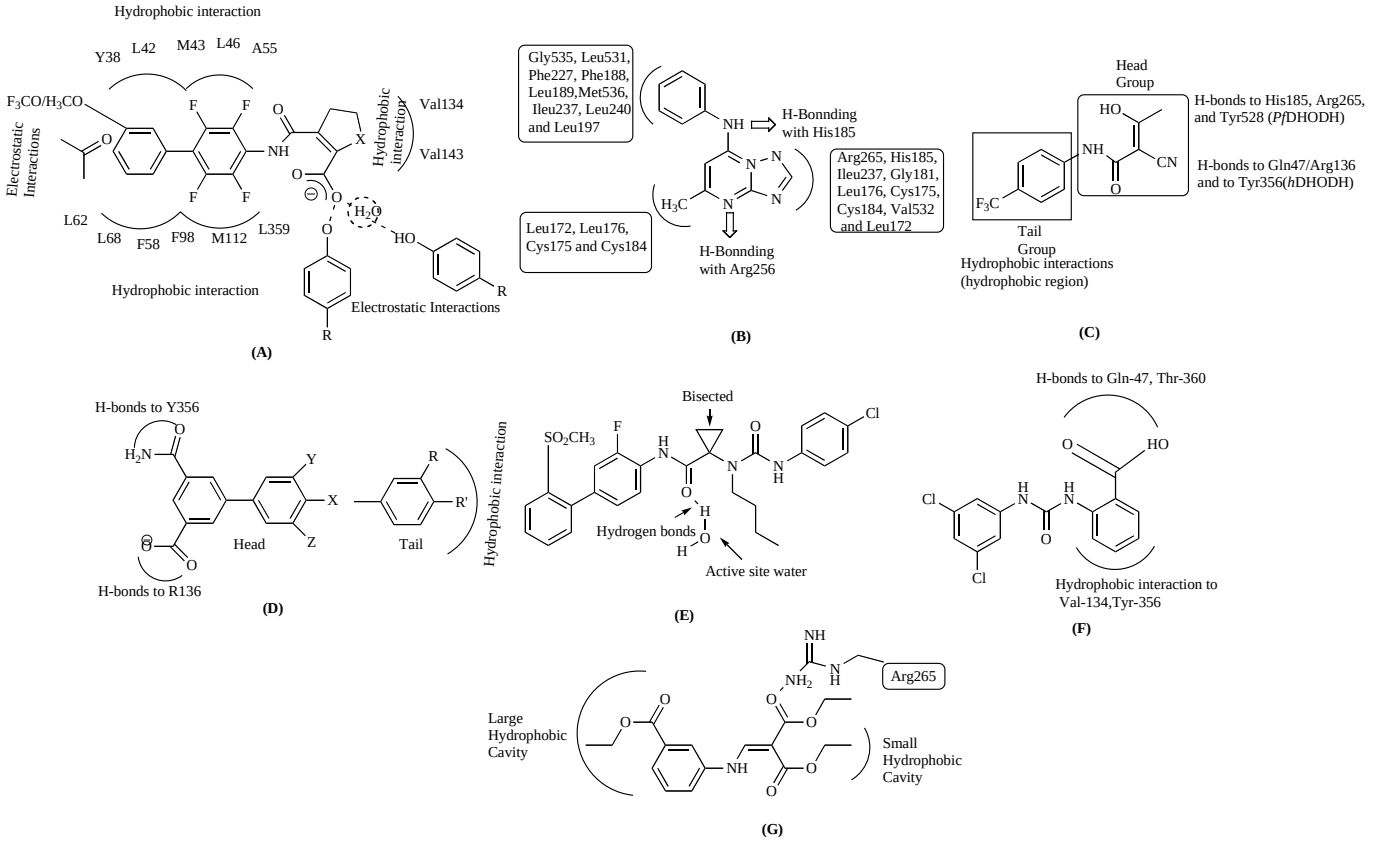
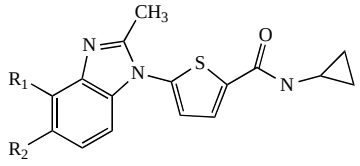


Fig. (21) Schematic diagram of important interactions of inhibitors with DHODH **A**: Aromatic carboxylic acid amide derivatives [37], **B**: Triazolopyrimidine-based derivatives [50], **C**: Trifluoromethy phenyl butenamide derivatives [35], **D**: Terphenyl carboxylic acid amide derivatives [56, 57], **E**: Cyclopropane carbonyl derivatives [58], **F**: Amino-benzoic acid derivatives [61], **G**: N-arylaminomethylene malonate [62].

and found double-digit nanomolar potency against *Pf*DHODH, *Pb*DHODH and *Pv*DHODH, while lacking activity against *h*DHODH (Fig. **22**). Molecular modeling studies suggested that the structure of *Pf*DHODH complexed with **73** was similar to the structures of *Pf*DHODH bound to the triazolopyrimidine based inhibitors and **A77 1726**. X-ray structure determination of **73** in complex with *Pf*DHODH reveled that cyclopropyl ring system binds a largely hydrophobic pocket formed by Val532, Ile272, and Ile263. Ion pair H-bonds between His-185 and the nitrogen of methylformamide, and between Arg265 and oxygen of methylformamide (non-hydrophobic contacts) were observed near to hydrophobic pocket. The benzimidazole ring bound in a hydrophobic pocket formed by Tyr168, Cys175, Phe171, Leu172, Phe188, Leu191, and Leu31 suggested that the selective binding of **73** to *Pf*DHODH was due to amino acid substitutions in the benzimidazole ring.

5.17. Amino Nicotinic Acid and Isonicotinic Acid Derivatives

Castro, P.L.J.C. *et al.* [67] granted a U.S. patent on discloser of a series of amino nicotinic acid and isonicotinic



Comp.	R_1	R_2	*Pf*DHODH	*Pb*DHODH	*Pv*DHODH	*h*DHODH μM
73	H	OCF_3	0.022 μM	0.014 μM	0.042 μM	>30
74	$OCHF_2$	H	0.044 μM	0.012 μM	0.015 μM	>30
75	CN	H	0.050 μM	0.040 μM	0.015 μM	>30

Fig. (22). Structures and activity of Alkyl-5- benzimidazole thiophene-2-carboxamide derivatives.

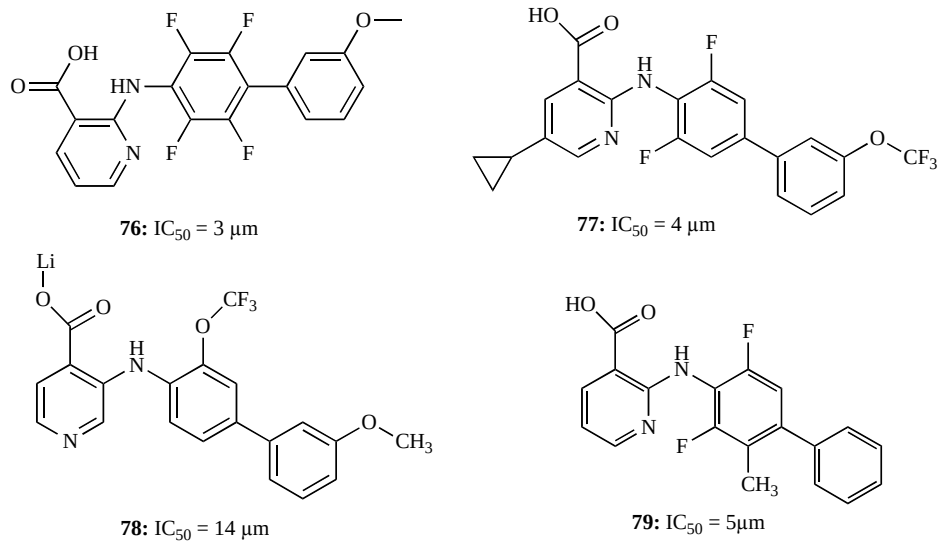


Fig. (23). Structures and activity of amino nicotinic acid and isonicotinic acid derivatives.

acid derivatives as *h*DHODH inhibitors. The authors described the method of preparation of *h*DHODH inhibitors, biological evaluation and use of the compounds in the manufacturing of medicine for treatment of rheumatoid arthritis, psoriatic arthritis, ankylosing spondilytis, multiple sclerosis, Wegener's granulomatosis, systematic lupus erythematosus and psoriasis and method of treatment of pathological conditions or diseases susceptible to amelioration by inhibition of DHODH. Some of the compounds (**76-79**) with *h*DHODH inhibitory activity are shown in Fig. (**23**) [67].

6. CONCLUSION AND PERSPECTIVE

Purine and pyrimidine bases are essential for cellular metabolism and cell growth and are considered as important precursors in DNA and RNA synthesis. DHODH catalyzes the conversion of dihydroorotate to orotate, which is the precursor for uridine and cytidine. This is a rate limiting step in the pyrimidine biosynthesis pathway, necessary for cell growth and proliferation. Rapidly proliferating cells (cancer cells, T-lymphocytes) do not acquire sufficient nucleosides from the salvage pathway for their survival. DHODH inhibitors block the growth of fast proliferating cell (*de novo* synthesis). The cells which grow at normal speed can meet the requirement of pyrimidines base (uracil, cytosine and thymine) from normal metabolic cycle (salvage pathways). Lymphocytes, the primary mediators of the immune response, use pyrimidines exclusively, for their growth and rely mainly on *de novo* synthesis to meet their requirement for activation and proliferation. Compounds that reduce the availability or utilization of pyrimidines are important agents for the treatment of auto immune diseases. A large number of DHODH inhibitors are emerging as potential intervention points for developing therapeutic agents for treatment of malaria, cancer therapy and as an immunosuppressive drug for the treatment of rheumatoid arthritis. The X-ray structure of *h*DHODH in complex with inhibitors is also resolved. All the *h*DHODH inhibitors, reported to date in the literatures, bind to the CoQ binding channel and display favorable activities. The CoQ binding site of all DHODH enzymes contains two highly conserved residues, an arginine and a tyrosine. Binding mode analysis of **A77 1726** with co-crystallized DHODH structures reveals the important of carbonyl group in making H-bond with water, which bounds to Arg136. The variable nature of the CoQ binding site in all the DHODHs is considered to be responsible for species-related inhibition for compounds that bind in the tunnel. The inhibitor-binding pocket can be divided into site A (H-bonding pocket), which forms H-bonding interactions with the bound inhibitors, and site B, which is completely hydrophobic. Knowledge of DHODH structures can help in *de novo* design and discovery of DHODH inhibitors by competing for the CoQ binding site. Binding profile studies confirmed that differences in substituents and their pattern of substitution were largely dictated by different levels of inhibition of *Pf*DHODH and *h*DHODH. Based upon various experimental finding discussed above the important pharmacophoric groups are identified. DHODH is capped by a hydrophobic cavity and inhibitors have to diffuse through a very hydrophobic environment. DHODH inhibitors often have a heterocyclic rings in their structure. Lone pair of electron on N-atom in triazolopyrimidine, qunoline, isoxazol and imidazole ring system act as hydrogen acceptor and responsible for H-bonding with CoQ binding site. Secondly, the biphenyl or aryl ring system showed several hydrophobic interactions in the enzyme binding pocket. The substituted biphenyl (biaryl tail) ring especially with fluoro, trifluoro, methoxy and ethoxy in first aromatic ring led toward better inhibitory activity. Carboxy group attached to five-membered ring (cyclopentene, thiophene) can form a dual binding mode. Compounds containing a carbonyl group at either the meta- or para-positions of the aryl ring, showed H-bonding. Beyond these findings, several other important chemical scaffolds are identified. Compounds containing cyclopropane, cyclopentene, naphthyl, anthracenyl and other pentacyclic aromatic heterocycles showed good binding affinities for both *Plasmodium* and human DHODH. HTS [68] and virtual screening [69] methods have proved a successful strategy to identify potent and highly species selective inhibitors of DHODH. The most common structural features across the different classes of inhibitors are 1) 5 or 6 mem-

bered aromatic or non-aromatic ring system with acidic (carboxylic acid) head group, which interacts (H-bonding) with the Arg136 (CoQ binding cavity), 2) a planar functional group, and 3) a bulky hydrophobic substituent (biphenyl ring system) which fulfills shape requirement in the hydrophobic binding pocket of the CoQ binding cavity. The most significant feature for good DHODH inhibitory activity is the substitution pattern on one of the biphenyl rings with fluoro or trifluoromethyl group. Several structural features necessary for DHODH inhibition are reviewed in present communication. These recent advances in design, synthesis and therapeutic potential of different classes of DHODH inhibitors will aid the structural insights into the development of potent DHODH inhibitors.

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