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Original article

Design, synthesis and pharmacological evaluation of novel substituted quinoline-2-carboxamide derivatives as *human* dihydroorotate dehydrogenase (*h*DHODH) inhibitors and anticancer agents

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ABSTRACT

In continuation of our research for novel *human* dihydroorotate dehydrogenase (*h*DHODH) inhibitors, herein we reported design, synthesis and pharmacological evaluation of novel substituted quinoline-2-carboxamide derivatives. *Human* DHODH enzyme inhibition assay was used to screen the synthesized compounds as *h*DHODH inhibitors. The synthesized compounds were also evaluated for their anti-proliferative effects on the cancer cell lines (HEP-3B and A-375) to establish a proof as anticancer agents. The chemical structures of compounds were confirmed by ^{1}H, ^{13}C NMR, IR, MS and elemental analysis. The purity of compounds was also checked by HPLC analysis. Compounds with bulky groups ($-OCH_3$, $-OCF_3$ and $-CF_3$) at C6-position of quinoline ring showed good activity.

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1. Introduction

Dihydroorotate dehydrogenase (DHODH) [EC 1.3.99.11] is a flavin mononucleotide (FMN) dependent enzyme, which catalyzes oxidation of dihydroorotate (DHO) to orotate (ORO) and reduction of flavin mononucleotide (FMN) to dihydroflavin mononucleotide ($FMNH_2$). The fourth step in *de novo* pyrimidine biosynthesis pathway is a rate limiting step [1]. Pyrimidine bases are required for the biosynthesis of DNA, RNA, glycoproteins and phospholipids [2]. Rapidly proliferating cells, such as cancer cells, in order to meet their increased demand for nucleic acid precursors and other cellular components depend heavily on *de novo* pyrimidine synthesis. The significance of pyrimidine bases for cell proliferation, metabolism and multiplication determines *h*DHODH as a target for the development of new drug candidate [3,4]. Inhibitors of *h*DHODH have proven efficacy for the treatment of cancer [5] and immunological disorders, such as, rheumatoid arthritis and multiple sclerosis. Leflunomide (arava) is an isoxazole-based prodrug, which after administration rapidly converts to the active metabolite A77 1726 (teriflunomide/aubagio) [6]. Teriflunomide is a potent inhibitor of mammalian DHODH [7] (Fig. 1). Brequinar is an inhibitor of *h*DHODH, which is a quinoline carboxylic acid derivative. Brequinar was evaluated in clinical trials as a potential anticancer agent, however it was not approved for clinical use [8] (Fig. 1). Leflunomide and teriflunomide were also reported for the treatment of central nervous system trauma such as spinal cord injury in a recent patent [9]. Recently the effect of leflunomide was reported on melanoma cells proliferation, but not of teriflunomide [10]. In one study, leflunomide was reported for a marked decrease in melanoma growth both *in vitro* and in mouse xenograft studies, which suggested that leflunomide suppressed transcriptional elongation [11].

Concerning current research on *h*DHODH inhibitors, in the year 2011 [12], we had listed many DHODH inhibitors as anticancer and antimalarial agents, and reviewed many chemical compounds for the treatment of autoimmune diseases, such as, rheumatoid arthritis and multiple sclerosis. In continuation of search for novel *h*DHODH inhibitors, earlier we performed 2D [13,14] and 3D QSAR [15] studies using CoMFA and CoMSIA models on a series of aryl carboxylic acid amide derivatives, a 2D and 3D QSAR study on amino nicotinic acid and isonicotinic acid derivatives [16], and docking-based 3D QSAR study on substituted quinoline derivatives as *h*DHODH inhibitors [17]. These studies helped us in the design of novel quinoline-based *h*DHODH inhibitors. Generated CoMFA and

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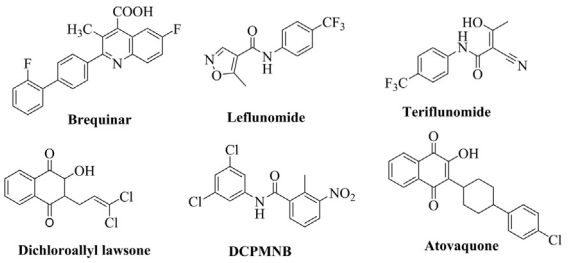


Fig. 1. Chemical structure of the DHODH inhibitors.

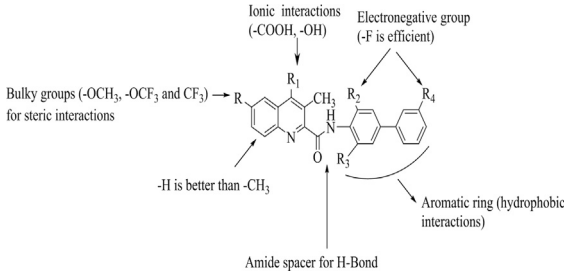


Fig. 2. Design strategy for key structural requirements of quinolone-2-carboxamide derivatives for *h*DHODH inhibition (hypothetical interaction model).

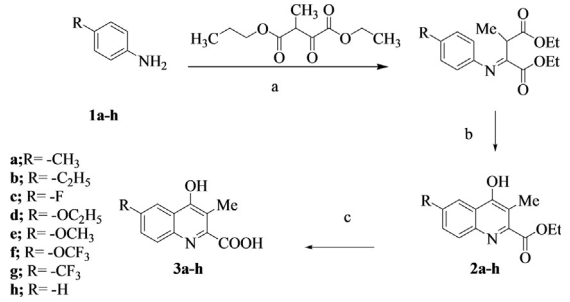
CoMSIA models in QSAR modeling helped in the prediction of the activity of designed compounds prior to their synthesis, and structure-based virtual screening and *in silico* ADMET prediction further helped in the identification lead compounds for the synthesis and pharmacological evaluation as *h*DHODH inhibitors [17]. The strategies used in the design of novel quinoline derivatives were the introduction of functional groups, which were found significant with the QSAR modeling and contour maps analysis. Molecular hybridization, bio-isosterism and molecular simplification methods were also taken into the consideration for design of the compounds, which have structural profile similar to brequinar analogs. Quinoline ring was maintained as a main scaffold in the designed compounds. An amide spacer was introduced between quinoline and biphenyl ring system. At R_1 position of quinoline ring −COOH and −OH groups were substituted. Carboxylic acid containing DHODH inhibitors are currently in discovery, and it was reported in the past that some of hydroxy derivatives (Fig. 1) were also found to inhibit DHODH enzyme activity. In the designed molecules, we retained −OH group at C4-position in some compounds and −COOH group in other compounds. Methyl group at C3 position of quinoline ring, and substitution variations with alkyl ($-CH_3$, $-C_2H_5$) and steric bulky ($-OCH_3$, $-OC_2H_5$, $-CF_3$, $-OCF_3$) groups at R position of quinoline ring were maintained. A biphenyl ring system was maintained for aromatic hydrophobic interactions, and in biphenyl ring system electronegative −F group at R_2, R_3 and R_4 position was substituted in most of the compounds. Some of the compounds were designed without any substitutions at R position of quinoline ring and R_2, R_3 and R_4 positions in biphenyl ring system. These strategies (Fig. 2) enabled the formation of new quinoline-2-carboxamide derivatives with all structural requirements necessary for *h*DHODH inhibition.

In the present study, we have synthesized and evaluated the designed compounds as *h*DHODH inhibitors and anticancer agents. The synthesized compounds were characterized by ^1H, ^{13}C NMR, mass spectroscopy (MS) and elemental analysis, and evaluated for *h*DHODH inhibition in a colorimetric assay with recombinant *h*DHODH enzyme. Finally, antiproliferative effects of synthesized compounds were evaluated against *human* hepatoma (HEP-3B) and melanoma (A-375) cell lines and their structure activity relationship (SAR) studies were discussed.

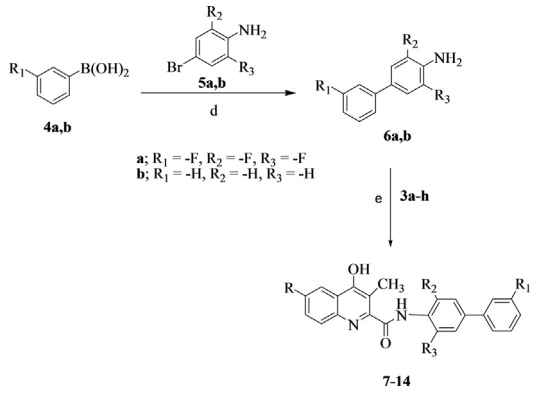
2. Results and discussion

2.1. Chemistry

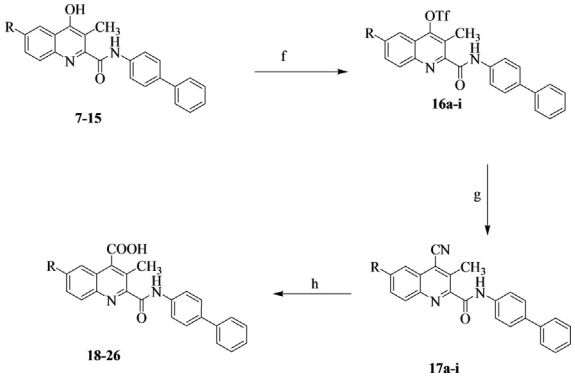
The quinoline scaffold was synthetically derivatized to obtain a series of quinoline-2-carboxamide compounds and characterized as target compounds. For the synthesis of substituted 4-hydroxyquinoline-2-carboxamides, first step in the synthesis of intermediate was based on Conrad–Limpach methodology [18]. The reaction of 4-substituted anilines (**1a**–**h**) and diethyl oxalpropionate in toluene afforded corresponding Schiff base anilino-maleate. Schiff bases on thermal cyclization in diphenyl ether at 250 °C yielded 6-substituted-4-hydroxyquinoline-2-carboxylic ethyl esters (**2a**–**h**), which upon hydrolysis with LiOH afforded 6-substituted-4-hydroxyquinoline-2-carboxylicacids (**3a**–**h**) (Scheme 1). 2,6-Difluoro-4(3-fluorophenyl)amines and unsubstituted biphenylamines (**6a**,**b**) were synthesized via Suzuki coupling reaction of 3-fluorophenyl boronic acid (**4a**) with 2,6-difluoro-4-bromoaniline (**5a**) and coupling of phenyl boronic acid (**4b**) with 4-bromoaniline (**5b**), respectively using catalytic quantity of $Pd(PPh_3)_4$ in refluxing toluene, EtOH and water (4:4:2) for 14–17 h under the inert environment (nitrogen gas) [19,20] (Scheme 2). Substituted 4-hydroxyquinoline-2-carboxamides were synthesized via acid–amine coupling reaction of appropriate 6-substituted-4-hydroxy-3-methyl-quinoline-2-carboxylicacids (**3a**–**h**) with substituted biphenylamines (**6a**,**b**) using dicyclohexyl carbodiimide (DCC), triethylamine (TEA) and 1-hydroxybenzotriazol in dimethylformamide (DMF) for 12–36 h under the inert environment (nitrogen gas) (Scheme 2) [21]. In Scheme 3, aryl triflates (**16a**–**i**) were obtained via treatment of compounds **7**–**15** with triflic anhydride (Tf_2O) in the presence of triethylamine in toluene at 0–5 °C [22,23]. The reaction was followed by direct Pd^0 catalyzed cyanation of the crude triflates to obtained nitriles (**17a**–**i**). Hydrolysis of nitriles in the presence of basic solution of NaOH yielded corresponding carboxylic acid derivatives (**18**–**26**) [24,25]. Chemical structure of all the compounds was confirmed by IR, NMR, mass spectral and elemental analysis data. The significant feature of ^1H NMR spectra, is the appearance of different sets of hydrogen atom resonances corresponding to the protons in the quinoline ring, biphenyl ring system, amide proton and hydorxy and carboxylic acid protons. For synthesized compounds, the set of protons corresponding to the quinoline ring appear in the range of δ 7.5–8.5 ppm, showing the expected multiplicity and integration values. The resonance peaks, in the spectral region from δ 6.0–7.4 ppm, corresponding to the biphenyl protons. These Ar–H of ^1H NMR spectrum are correlated with C atoms (δ



Scheme 1. Synthetic strategy for 6-substituted-4-hydroxyquinoline-2-carboxylic acids (**3a**–**h**) via Conrad–Limpach and base catalyzed hydrolysis of esters reactions. Reagents and conditions (a) AcOH, toluene, Dean–Stark trap, reflux 3–18 h, (b) reflux, 15 min diphenyl ether, (c) LiOH, H_2O, THF, stirring, 4–5 h, RT.



Scheme 2. Synthetic strategy for substituted biphenyl amine derivatives (**6a**,**b**) via Suzuki coupling reaction and for 6-substituted-4-hydroxyquinoline-2-carboxamide derivatives (**7**–**14**) via acid–amine coupling reaction. Reagents and conditions: (d) $Pd(Ph_3)_4$, K_2CO_3, EtOH, H_2O, toluene, reflux 14–17 h, (e) TEA, DCC, HOBt, DMF, stirring 0–5 °C (8–12 h) to RT (12–36 h).



Scheme 3. Synthetic strategy for 6-substituted-4-carboxylicacidquinoline-2-carboxamide derivatives (**18**–**26**). (f) Tf_2O, TEA, toluene, stirring 0–5 °C (2 h) to RT 36–42 h, (g) $Zn(CN)_2$, $Pd(Ph_3)_4$, DMF, reflux 20–24 h (h) NaOH, H_2O.

104–170 ppm) in ^{13}C NMR spectrum. The signals for the amide proton (–NH) appear as singlets. They are observed in the region δ 9–10 ppm, which is typical for this group and correlated with C atom of amide (C=O) form δ 170–175 ppm in ^{13}C NMR spectrum. For –OH and –COOH a singlet appear above chemical shift of δ 10 ppm. The methyl moiety in the quinoline ring at C-3 position, showed a resonance peak in the range δ 2.2–3 ppm.

2.2. Pharmacological evaluation

Pyrimidine biosynthesis pathways are necessary for DNA and RNA synthesis. Fourth step in pyrimidine *de novo* pathway is catalyzed by DHODH enzyme, which represent a rate limiting step in the synthesis. Inhibition of *h*DHODH leads to reduced levels of essential pyrimidine nucleotides, especially in fast proliferating cells, such as cancer cells. The main assay used to assess the activity of synthesized compounds was a colorimetric enzyme inhibition assay with recombinant *h*DHODH enzyme. All the compounds were also screened for antiproliferative activity against two different *human* cancer cell lines (HEP-3B and A-375), to further obtain biologically relevant information as anticancer agents.

2.2.1. Human DHODH enzyme inhibition assay

Human DHODH inhibition assay was performed using chromogen reduction method with 2,6-dichlorophenolindophenol (DCIP) dye. Dihydroorotate (DHO) oxidation as well as ubiquinone (CoQ) reduction was coupled with chromogen reduction. Enzyme inhibition was resulted in loss of chromogen absorbance at 600 nm. The preliminary activity was measured at 10 μM concentration of synthesized compounds (Table 1) along with the standard brequinar sodium. The IC_{50} value as a practical readout of the enzyme inhibition of synthesized compounds under comparable conditions was obtained from dose–response plots (Fig. 3) using GraphPad Prism 5.00 software (GraphPad Software Inc., SanDiego, CA, U.S.A.) for those compounds which showed significant inhibition in the preliminary study. The IC_{50} values of synthesized compounds against *h*DHODH were summarized in Table 2.

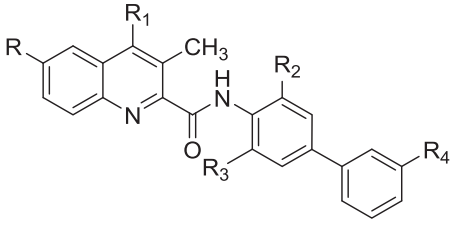
2.2.2. Antiproliferative activity

The synthesized compounds along with two standards brequinar sodium and doxorubicin were evaluated for antiproliferative activity against two different cancer cell lines named *human* hepatoma (HEP-3B) and melanoma (A-375). Dose–response curve (Figs. 4 and 5) against both the cell lines were plotted with 10 analysis point i.e. with 10 different compounds concentrations (Tables S2–S7 under Supplementary materials). The concentration causing 50% cell growth inhibition (IC_{50}) values were determined from dose–response curves (Table 3) using GraphPad Prism 5.00 software (GraphPad Software Inc., SanDiego, CA, U.S.A.).

2.2.3. Structure activity relationship (SAR) studies

In order to explore SAR preliminarily, the compounds with different electron withdrawing and electron releasing groups at R position (C6), –OH and –COOH groups at R_1 position (C4) of quinoline ring, and –F group (R_2, R_3 and R_4) at biphenyl ring were synthesized and evaluated for enzyme inhibition assay and antiproliferative activity. In enzyme inhibition assay compounds with bulky groups at C6-position (R), such as **11**, **14** and **25** with $-OCH_3$

Table 1
Preliminary study with the 10 μM concentration of the compounds for the inhibition of *h*DHODH in chromogen reduction enzyme inhibition assay using DCIP, L-DHO and C_QD, Tris–HCl buffer, pH 8, at 30 °C indicated as % inhibition.



Compound	R	R_1	R_2	R_3	R_4	% Inhibition at 10 μM Conc.
7	CH_3	OH	F	F	F	18.92
8	C_2H_5	OH	F	F	F	64.86
9	F	OH	F	F	F	43.24
10	OC_2H_5	OH	F	F	F	27.03
11	OCH_3	OH	F	F	F	54.05
12	OCF_3	OH	F	F	F	8.11
13	CF_3	OH	F	F	F	51.35
14	OCH_3	OH	H	H	H	78.38
15	H	OH	H	H	H	32.43
18	CH_3	COOH	F	F	F	35.14
19	C_2H_5	COOH	F	F	F	18.92
20	F	COOH	F	F	F	56.76
21	OC_2H_5	COOH	F	F	F	51.35
22	OCH_3	COOH	F	F	F	10.81
23	OCF_3	COOH	F	F	F	67.57
24	CF_3	COOH	F	F	F	54.05
25	OCH_3	COOH	H	H	H	54.05
26	H	COOH	H	H	H	35.14
Brequinar sodium						97.29

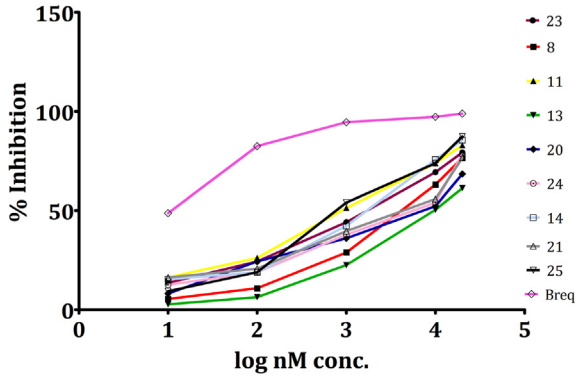


Fig. 3. Dose–response curves for *h*DHODH inhibition. Five different concentrations 10 nM, 100 nM, 1 μM, 10 μM and 20 μM of the compounds were tasted and IC_{50} values were determined.

Table 2
IC_{50} values describing the effect of synthesized derivatives on *human* DHODH enzyme.

Compound	IC_{50} ± SD (μM)	Compound	IC_{50} ± SD (μM)
8	3.21 ± 0.79	**21**	2.83 ± 0.47
11	0.94 ± 0.06	**23**	1.50 ± 0.22
13	6.09 ± 1.96	**24**	3.44 ± 0.94
14	2.28 ± 0.78	**25**	1.09 ± 0.36
20	4.58 ± 1.03	**Brequinar sodium**	0.015 ± 0.002

Results are means of three determinations and are given ± SD.

group, **23** with $-OCF_3$ and **21** with $-OC_2H_5$ group showed good activity against *h*DHODH enzyme. The activity data revealed a clear preference for the potency when substitutions at R position were $-OCH_3$ and $-OCF_3$ groups, which indicated that a more steric group at the C6-position (R) of quinoline ring contributed to the potency of synthesized compounds in following rank order: $-CF_3 < -OCF_3 < -OCH_3$. For comparison of −OH and −COOH groups at R_1 position, compound **11** (R = $-OCH_3$, R_1 = −OH, IC_{50} = 0.94 μM) displayed slightly improved activity over **23** (R = $-OCF_3$, R_1 = −COOH, IC_{50} = 1.5 μM). Further investigations were performed to study the effect of −F group on the biphenyl ring. The results showed that biphenyl ring substituted with −F groups have a marked impact on activity against *h*DHODH enzyme. Compounds with −F groups at R_2, R_3 and R_4 positions and −COOH groups at R_1 position showed better activity with exception of compound **25** (R = $-OCH_3$, R_1 = −COOH, R_2 = R_3 = R_4 = H, IC_{50} = 1.09 μM). In case of antiproliferative activity, SAR study demonstrated a substantial increase in the potency of quinoline-2-carboxamide derivatives that were substituted at C6-position (R) with a bulky groups. Compounds **11** (IC_{50} = 5.03 μM against A-375, and IC_{50} = 6.98 μM against HEP-3B), **25** (IC_{50} = 9.43 μM against A-375) with $-OCH_3$ group and compound **23** (IC_{50} = 4.80 μM against A-375, and IC_{50} = 22.03 μM against HEP-3B) with $-OCF_3$ group were found the most potent compounds. Compound **11** and **23** exhibited potent antiproliferative activities against A-357 and HEP-3B cell lines, which were equally active or more active than brequinar sodium and doxorubicin. Further investigations were performed to study the effects of R_1–OH and R_1–COOH groups on C4-position (R_1) of quinoline ring for antiproliferative activity. Among R–OCH_3 (C6) substituted compounds, **22** (IC_{50} = 14.34 μM against A-375) with R_1–COOH showed less activity as compared to **11** with R_1–OH against A-357 cell lines. The R-position (C6) of quinoline ring is sensitive to the substituent's steric size. Compounds **9** with −F at R (C6) (R = R_2 = R_3 = R_4 = F, IC_{50} > 100 μM against A-375) and compound **10** with larger bulky $-OC_2H_5$ group at R (C6) (IC_{50} > 100 μM against A-375) were found inactive. Introduction of electron withdrawing −F group on biphenyl ring showed different influences on the activity. 3,3′,5-Trifluoro-substituted biphenyl analogs were active than −F unsubstituted biphenyl ring. SAR studies based on IC_{50} values (Tables 2 and 3) showed that compounds **11** and **23** were the most potent compound in both the screening methods with $-OCH_3$ and $-OCF_3$ group at C6-position (R) and electron withdrawing −F groups in biphenyl ring system, respectively. Compounds **21** and **24** exhibited comparable anticancer activity. Biological activity data suggested that a proper degree of electron density on quinoline ring was necessary to retain the activity of synthesized compounds.

3. Conclusion

In this study, novel quinoline-2-carboxamide derivatives were synthesized and screened as *h*DHODH inhibitors as well as anticancer agents against different cancer cell lines. The compounds were synthesized in good yield. The synthesized compound were characterized by IR, ^1H and ^{13}C NMR, mass spectra (ESI-MS) and

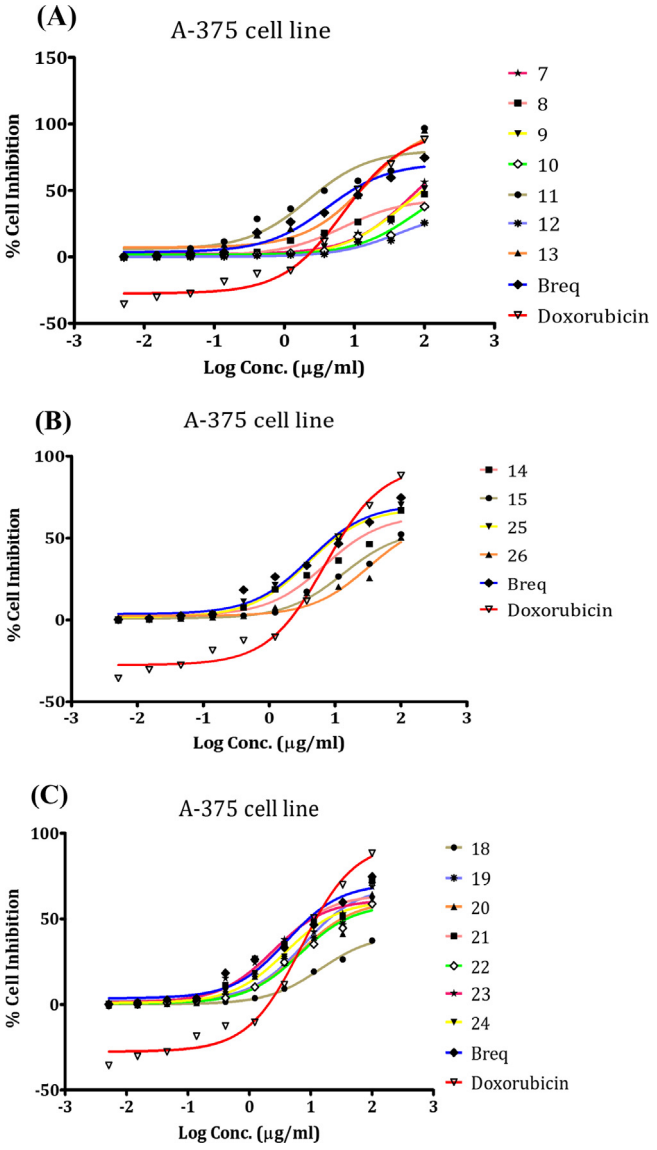


Fig. 4. Dose–response effect of compounds **7**–**13** (A), compounds **14**, **15**, **25**, **26** (B) and compounds **18**–**24** (C) against A-375 cell lines using MTT assay with brequinar sodium (Breq) and doxorubicin as standards.

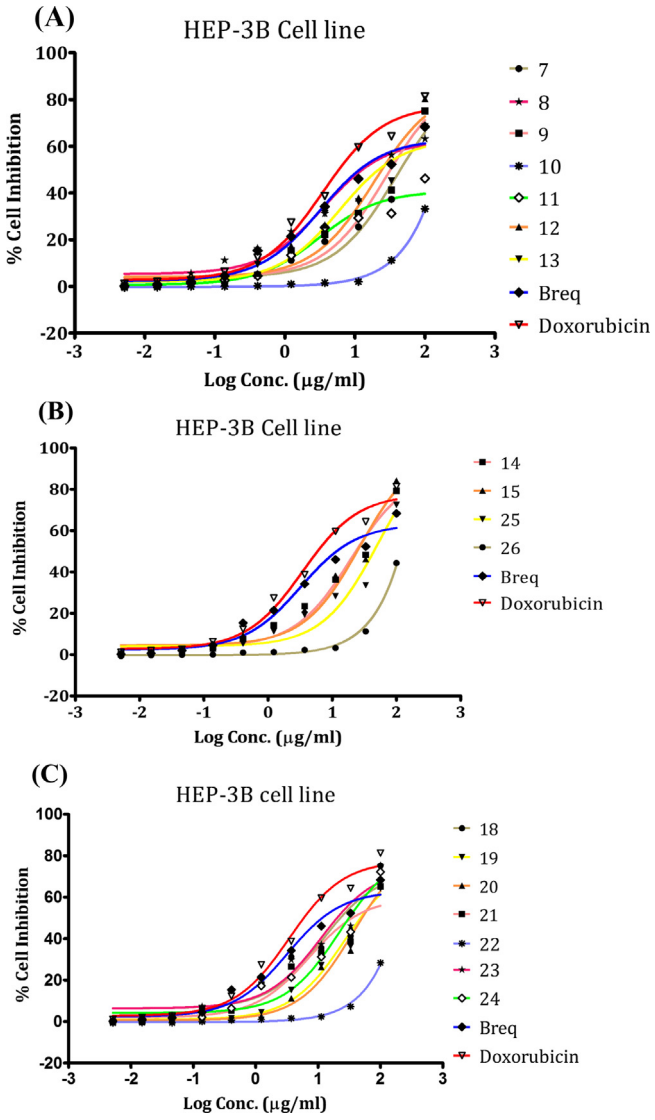


Fig. 5. Dose–response effect of compounds **7**–**13** (A), compounds **14**, **15**, **25**, **26** (B) and compounds **18**–**24** (C) against HEP-3B cell lines by MTT assay with brequinar sodium (Breq) and doxorubicin standards.

elemental analysis. The purity of the compounds were determined using HPLC analysis. *In vitro h*DHODH enzyme inhibition and antiproliferative assays were used to screen the synthesized compounds. Compounds with bulky groups ($-OCH_3$ and $-OCF_3$) at C6-position were shown good activity. When these results are taken together, compound **11** and **23** were found as the most potent compounds in both the screening methods. Ligand and structural-based design and extensive synthesis provided quinoline-2-carboxamide derivatives that target *h*DHODH enzyme involved in pyrimidine *de novo* biosynthesis pathway with micromolar activities.

4. Experimental

4.1. Chemistry

Melting points (mp) were determined using a digital melting point apparatus and by open capillary method, which were found uncorrected. Precoated silica plates were used for TLC to monitor progress of the reaction. UV and iodine chambers were used for detection of spots. IR spectra were recorded on SHIMADZU FTIR 6100 by KBr dispersion method. Mass spectra were recorded using ESI as ion source (Agilent-6310 ion trap). ^{1}H NMR spectra were recorded at room temperature on a Bruker AM-400 and on Bruker Avance II 600 spectrometer, and ^{13}C NMR spectra at 100 MHz in DMSO-*d6*. Chemical shifts were measured in parts per million (ppm, δ) downfield from an internal tetramethylsilane (TMS) standard. Column chromatography was used for the purification of compounds. HPLC equipment consisted of a Waters 510 pump, a Waters 486 UV–vis detector, a Waters 746 data module, and an RP C-18 column. The elemental analysis was performed on a Perkine Elmer elemental analyzer. All the reactions were performed in fume hood. Chemicals were procured from Sigma Aldrich, SD-Fine Himedia, Merck and Spectrochem. All commercial reagents and solvents were used as received from their perspective supplier.

4.1.1. General procedure for the synthesis of 6-substituted-4-hydroxyquinoline-2-carboxylic acid derivatives (**3a**–**h**)

In a 250 ml round-bottom flask (RBF) attached with a Dean–Stark trap and a reflux condenser was charged with diethyl oxalpropionate (β-ketoester) (0.045 mol), toluene (20 ml), glacial acidic acid (1 ml) and corresponding *p*-substituted anilines (**1a**–**h**) (0.045 mol). The reaction mixture was refluxed at 110 °C until no more water was separated (3–18 h) to afford corresponding Schiff bases. Toluene was distilled under reduced pressure, and resulting crude intermediates were than used in the next step (thermal cyclisation). Biphenyl ether (25 ml) was stirred and heated at reflux, while crude intermediates were added rapidly through the dropping funnel. Stirring and refluxing continued for 10–15 min until no more ethanol separated within Dean–Stark trap. The mixture was then allowed to cool at room temperature while precipitation arose. Solids were filtered off and washed. 6-Substituted-4-hydroxyquinoline-2-carboxylicethylesters (**2a**–**h**) were dissolved in required amount of tetrahydrofuran. Lithium hydroxide (0.0031 mol) and water (0.012) mol were added, reaction mixture was allowed to stir for 4–6 h. Reaction mixture was neutralized to pH 6.5 by addition of 1% HCl. Obtained precipitates were filtered, washed, and recrystallized from ethanol.

4.1.2. General procedure for the synthesis of substituted biphenylamines (**6a**,**b**)

In a mixture of EtOH, toluene and water (4:4:2), K_2CO_3 (0.046 mol) was added under nitrogen gas. The reaction mixture was heated to 85–90 °C for 30–35 min, cooled to room temperature, substituted 4-bromoaniline (0.038 mol) and catalytic quantity of $Pd(PPh_3)_4$ were added to reaction mixture. The reaction mixture was heated to reflux for 45 min, than substituted phenyl boronic acid (0.038 mol) was added and stirred for 12–18 h. Reaction mixture was diluted with ethylacetate and filtered. Organic layer was separated, washed with water followed by brine and finally with water. Organic layer was dried over sodium sulphate. Thus, the residues obtained were dissolved in 50 ml ethylacetate. The crude products were purified using column chromatography over silica column.

4.1.3. General procedure for the synthesis of 6-substituted-4-hydroxyquinoline-2-carboxamide derivatives (**7**–**15**)

6-Subsituted-4-hydroxy-3-methyl-quinoline-2-carboxylicacids (0.011 mol) (**3a**–**h**) were dissolved in required amount of DMF, than triethylamine (0.002 mol), 1-hydroxy benzotriazole (0.0012 mol) and dicyclohexyl carbodiimide (0.002 mol) were added at 0–5 °C under the nitrogen gas. Reaction mixture was allowed to stir for 1 h, than biphenylamines (0.011 mol) were added to reaction mixture and allowed to stir for 8–12 h at 0–5 °C. Reaction mixture was taken to room temperature and allowed to stir for 12–36 h.

Table 3
Antiproliferative effects of quinoline-2-carboxamides on A-375 and HEP-3B cell lines with standards brequinar sodium (Breq) and doxorubicin (Dox).

A-375 (melanoma cell lines)				HEP-3B (hepatoma cell lines)			
Compound	IC_{50} (μM)	Compound	IC_{50} (μM)	Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
7	>100	**19**	16.87	**7**	90.53	**19**	57.82
8	15.84	**20**	14.73	**8**	8.13	**20**	81.85
9	>100	**21**	5.80	**9**	65.25	**21**	14.88
10	>100	**22**	14.34	**10**	>100	**22**	>100
11	5.03	**23**	4.80	**11**	6.98	**23**	22.03
12	85.49	**24**	7.57	**12**	38.33	**24**	42.82
13	29.91	**25**	9.43	**13**	12.61	**25**	>100
14	18.10	**26**	83.47	**14**	50.61	**26**	>100
15	37.22	**Breq**	10.22	**15**	75.45	**Breq**	8.10
18	31.61	**Dox**	13.25	**18**	26.04	**Dox**	6.35

IC_{50} values are determined as the mean ± SD of three independent experiments performed.

Reaction mixture was neutralized with 1% HCl solution, organic layer was collected, dried with anhydrous sodium sulfate and evaporated. The compounds were purified using column chromatography over silica column.

*4.1.3.1. 4-Hydroxy-3,6-dimethyl-N-(3,3′,5-trifluorobiphenyl-4-yl) quinoline-2-carboxamide (**7**).* Compound **7** was synthesized according to the synthetic procedure given above as yellowish-white solid in yield of 69.21%, mp 246–248 °C; FT-IR (cm^{-1}) 3492.21 (NH), 1810.91 (C═O); ESI-MS *m/z*: 423 $(M+H)^+$, 424 $(M+2H)^+$; ^1H NMR (400 MHz, DMSO-*d*6) δ 13.88 (s, 1H, −OH), 10.84 (s, 1H, −NH), 7.89 (m, 3H, Ar−H), 7.53 (m, 2H, Ar−H), 7.43 (m, 2H, Ar−H), 7.05 (d, J = 10.4 Hz, 2H, Ar−H), 3.77 (d, J = 6.9 Hz, 3H, $-CH_3$), 3.32 (s, 3H, $-CH_3$); ^{13}C NMR (100 MHz, DMSO-*d*6) δ ^{13}C NMR (100 MHz, DMSO-*d*6) δ 188.15, 182.98, 181.46, 176.64, 173.04, 167.31, 156.10, 154.99 (2C), 149.52, 145.01 (2C), 140.14, 134.80, 133.69, 124.29, 123.42, 120.68, 117.11, 104.24, 14.55, 13.98, 11.4; Anal. Calcd for $C_{24}H_{17}F_3N_2O_2$: C, 68.24; H, 4.06; F, 13.49; N, 6.63; O, 7.58 Found: C, 68.21; H, 4.02; F, 13.52; N, 6.58; O, 7.53.

*4.1.3.2. 6-Ethyl-4-hydroxy-3-methyl-N-(3,3′,5-trifluorobiphenyl-4-yl)quinoline-2-carboxamide (**8**).* Compound **8** was synthesized according to the synthetic procedure given above as white solid in yield of 68.68%, mp 245–247 °C; FT-IR (cm^{-1}) 3420.31 (NH), 1805.63 (C═O); ESI-MS *m/z* 437.1 $(M+H)^+$,438.1 $(M+2H)^+$; ^1H NMR (400 MHz, DMSO-*d*6) δ 11.86 (s, 1H, −OH), 9.91 (s, 1H, −NH), 7.88 (m, 3H, Ar−H), 7.70 (m, 3H, Ar−H), 7.58 (m, 3H, Ar−H), 4.43 (q, J = 7.2 Hz, 3H, $-C_2H_5$), 2.21 (s, 3H, $-CH_3$), 1.37 (t, 2H, J = 6.8 Hz, $-C_2H_5$); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 177.15, 171.92, 167.23, 163.12, 159.92 (2C), 154.92, 150.04, 145.50, 141.06, 136.40 (2C), 127.97, 125.51, 123.64, 123.31, 122.79, 122.74, 122.08, 120.51, 119.95 (2C), 27.23, 18.89, 11.23; Anal. Calcd for $C_{25}H_{19}F_3N_2O_2$: C, 68.80; H, 4.39; F, 13.06; N, 6.42; O, 7.33 Found C, 68.78; H, 4.32; F, 13.03; N, 6.38; O, 7.31.

*4.1.3.3. 6-Fluoro-4-hydroxy-3-methyl-N-(3,3′,5-trifluorobiphenyl-4-yl)quinoline-2-carboxamide (**9**).* Compound **9** was synthesized according to the synthetic procedure given above as yellowish-white solid in yield of 63.21%, mp 232–233 °C; FT-IR (cm^{-1}) 3423.30 (NH), 1837.41 (C═O), ESI-MS *m/z* 426.9 $(M+H)^+$, 427.9 $(M+2H)^+$; ^1H NMR (400 MHz, DMSO-*d*6) δ 12.08 (s, 1H, −OH), 10.53 (s, 1H, −NH), 9.07 (d, J = 7.2 Hz, 1H, Ar−H), 8.47 (m, 2H, Ar−H), 7.50 (m, 2H, Ar−H), 7.21 (m, 2H, Ar−H), 7.01 (m, 2H, Ar−H); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 192.03, 187.15, 182.58 (2C), 181.06, 176.80, 173.15, 168.51, 163.25, 157.87, 155.18, 152.51, 149.86, 144.07, 137.68, 136.17 (2C), 125.97, 123.47, 121.72, 118.58, 115.78 (2C), 13.92; Anal. Calcd for $C_{23}H_{14}F_4N_2O_2$: C, 64.79; H, 3.31; F, 17.82; N, 6.57; O, 7.51 Found: C, 64.74; H, 3.33; F, 17.86; N, 6.52; O, 7.55.

*4.1.3.4. 6-Ethoxy-4-hydroxy-3-methyl-N-(3,3′,5-trifluorobiphenyl-4-yl)quinoline-2-carboxamide (**10**).* Compound **10** was synthesized according to the synthetic procedure given above as yellowish-white solid in yield of 60.46%, mp 261–263 °C; FT-IR (cm^{-1}) 3417.16 (NH), 1796.34 (C═O); ESI-MS *m/z* 454.17 $(M+H)^+$, 454.15 $(M+2H)^+$; ^1H NMR (400 MHz, DMSO-*d*6) δ 12.03 (s, 1H, −OH), 9.93 (s, 1H, −NH), 7.88 (m, 3H, Ar−H), 7.70 (m, 3H, Ar−H), 7.57 (m, 3H, Ar−H), 4.43 (q, J = 7.2 Hz, 3H, $-C_2H_5$) 2.21 (s, 3H, $-CH_3$), 1.41 (t, 2H, J = 6.8 Hz, $-C_2H_5$); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 186.1, 185.3, 173.2, 171.6, 169.7, 168.4, 168.2, 163.2, 140.5, 138.9, 138.3, 138.0, 124.5, 124.3, 124.2, 121.1 (2C), 120.3, 116.3, 116.1, 111.2, 102.1, 22.51, 13.98, 11.4; Anal. Calcd for $C_{25}H_{19}F_3N_2O_3$: C, 66.37; H, 4.23; F, 12.60; N, 6.19; O, 10.61 Found: C, 66.35; H, 4.19; F, 12.64; N, 6.15; O, 10.65.

*4.1.3.5. 6-Methoxy-4-hydroxy-3-methyl-N-(3,3′,5-trifluorobiphenyl-4-yl)quinoline-2-carboxamide (**11**).* Compound **11** was synthesized according to the synthetic procedure given above as yellowish-white solid in yield of 66.56%, mp 242–243 °C; FT-IR (cm^{-1}) 3443.12 (NH), 1756.40 (C═O); ESI-MS *m/z* 439.6 $(M+H)^+$, 440.3 $(M+2H)^+$; ^1H NMR (400 MHz, DMSO-*d*6) δ 13.88 (s, 1H, −OH), 11.34 (s, 1H, −NH), 87.95 (m, 5H, Ar−H), 7.52 (d, J = 6.9 Hz, 1H, Ar−H), 7.32 (m, 1H, Ar−H), 6.99 (m, 1H, Ar−H), 6.88 (d, J = 7.8 Hz, 1H, Ar−H), 3.95 (d, J = 6.9 Hz, 3H, $-OCH_3$), 3.4 (s, 1H, CH_3); ^{13}C NMR (100 MHz, DMSO-*d*6): δ 175.1, 171.1, 167.4, 166.7, 159.7, 159.3, 158.9, 143.2, 138.9, 138.3, 137.4, 130.4, 124.5, 125.8, 112.5, 112.3, 112.1, 111.4, 111.1, 109.1, 108.2, 104.2, 102.3, 14.5, 11.2; Anal. Calcd for $C_{24}H_{17}F_3N_2O_3$: C, 65.75; H, 3.91; F, 13.00; N, 6.39; O, 10.95 found: C, 65.72; H, 3.88; F, 13.05; N, 6.33; O, 10.91.

*4.1.3.6. 6-Trifluoromethoxy-4-hydroxy-3-methyl-N-(3,3′,5-trifluorobiphenyl-4-yl)quinoline-2-carboxamide (**12**).* Compound **12** was synthesized according to the synthetic procedure given above as white solid in yield of 56.78%, mp 239–241 °C; FT-IR (cm^{-1}) 3414.10 (NH), 1731.14 (C═O), ESI-MS *m/z* 493.1 $(M+H)^+$, 494.1 $(M+2H)^+$; ^1H NMR (600 MHz, DMSO-*d*6) δ 11.35 (s, 1H, −OH), 10.54 (s, 1H, −NH), 7.75 (d, 1H, J = 7.2 Hz, Ar−H), 7.42 (m, 1H, Ar−H), 7.22 (d, 1H, J = 7.2 Hz, Ar−H), 7.13 (t, 1H, J = 7.8 Hz, Ar−H), 6.95 (d, 2H, J = 2.4 Hz, Ar−H), 6.60 (m, 3H, Ar−H), 3.64 (s, 3H, $-CH_3$); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 187.15, 182.58 (2C), 181.06, 176.80, 173.71, 168.29, 165.96, 163.24, 158.06, 155.71, 153.57, 149.27, 144.06, 137.68, 136.17, 125.97, 123.46, 121.71, 118.57, 115.77, 99.51, 13.91, 11.25; Anal. Calcd for $C_{24}H_{14}F_6N_2O_3$: C, 58.54; H, 2.87; F, 23.15; N, 5.69; O, 9.75 found C, 58.50; H, 2.85; F, 23.19; N, 5.62; O, 9.72.

*4.1.3.7. 6-Trifluoromethyl-4-hydroxy-3-methyl-N-(3,3′,5-trifluorobiphenyl-4-yl)quinoline-2-carboxamide (**13**).* Compound **13** was synthesized according to the synthetic procedure given above as white solid in yield of 58.67%, mp 249–251 °C; FT-IR (cm^{-1}) 3435.17 (NH), 1725.61(C═O); ESI-MS *m/z* 477.3 $(M+H)^+$, 478.3

$(M+2H)^+$; ^{1}H NMR (600 MHz, DMSO-*d*6) δ 11.29 (s, 1H, −OH), 10.41 (s, 1H, −NH), 7.79 (d, 1H, *J* = 7.8 Hz, Ar−H), 7.42 (m, 1H, Ar−H), 7.22 (d, 1H, *J* = 7.8 Hz, Ar−H), 7.15 (t, 2H, *J* = 7.2 Hz, Ar−H), 7.09 (d, 1H, *J* = 7.8 Hz, Ar−H), 6.80 (m, 2H, Ar−H), 6.55 (d, *J* = 7.8 Hz, 2H, Ar−H), 2.98 (s, 3H, CH_3); 176.71 (2C), 171.29, 166.87, 163.35, 159.58, 157.17, 151.17, 146.92, 143.13, 139.71, 135.78 (2C), 124.13 (2C), 124.06, 121.79, 121.71, 121.42, 121.17, 117.63, 108.75 (2C) 13.91, 11.24; Anal. Calcd for $C_{24}H_{14}F_6N_2O_2$: C, 60.51; H, 2.96; F, 23.93; N, 5.88; O, 6.72 Found C, 60.43; H, 2.92; F, 23.91; N, 5.82; O, 6.68.

*4.1.3.8. N-biphenyl-4-yl-4-hydroxy-6-methoxy-3-methylquinoline-2-carboxamide (**14**).* Compound **14** was synthesized according to the synthetic procedure given above as white solid in yield of 54.42%, mp 256–258 °C; FT-IR (cm^{-1}) 3322.75 (NH), 1699.94 (C═O); ESI-MS *m*/*z* 385.6 $(M+H)^+$, 386.5 $(M+2H)^+$; ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.23 (s, 1H, −OH), 9.66 (s, 1H, −NH), 8.15 (d, 2H, *J* = 8.8 Hz, Ar−H), 8.07 (m, 2H, Ar−H), 7.61 (m, 2H, Ar−H), 7.43 (d, 2H, *J* = 8.8 Hz, Ar−H), 7.29 (d, 1H, *J* = 8.8 Hz, Ar−H), 6.89 (d, 3H, *J* = 8.8 Hz, Ar−H), 6.75 (t, *J* = 8.8 Hz, 1H, Ar−H), 4.74 (d, 1H, *J* = 8.8 Hz, $-OCH_3$), 2.50 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 178.8, 174.0, 170.0, 165.0, 161.6, 159.6, 152.7, 150.5, 142.5, 136.5, 135.6, 133.7, 130.1, 129.6, 128.4, 122.5, 122.3, 118.8, 116.01, 111.04, 109.39, 21.13; Anal. Calcd for $C_{24}H_{20}N_2O_3$ C, 74.98; H, 5.24; N, 7.29; O, 12.49 Found: C, 74.94; H, 5.22; N, 7.22; O, 12.44.

*4.1.3.9. N-biphenyl-4-yl-4-hydroxy-3-methylquinoline-2-carboxamide (**15**).* Compound **15** was synthesized according to the synthetic procedure given above as white solid in yield of 58.18%, mp 246–248 °C; FT-IR (cm^{-1}) 3325.64 (OH), 1647.88 (C═O); ESI-MS *m*/*z* 355.1 $(M+H)^+$, 356.1 $(M+2H)^+$; ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.107 (s, 1H, −OH), 8.37 (s, 1H, −NH), 8.25 (d, 1H, *J* = 7.5 Hz, Ar−H), 7.94 (m, 3H, Ar−H), 7.53 (t, 2H, *J* = 8 Hz, Ar−H), 7.46 (t, 3H, *J* = 7.5 Hz, Ar−H), 7.32 (m, Ar−H), 3.37 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-*d*6): δ 163.6, 160.2, 151.5, 142.8, 137.1 (2C), 136.8, 132.9, 129.8, 128.6, 128.5, 128.3, 127.3, 125.7, 125.3, 125.1, 122.9, 119.7, 115.0, 114.7 (2C), 110.3, 14.4; Anal. Calcd for $C_{23}H_{18}N_2O_2$: C, 77.95; H, 5.12; N, 7.90; O, 9.03 Found C, 77.91; H, 5.13; N, 7.85; O, 9.01.

*4.1.4. General procedure for the synthesis of 6-substituted-4-carboxylicacidquinoline-2-carboxamide derivatives (**18**–**26**)*

In a cooled (0–5 °C) solution of **7**–**15** (0.0047 mol) and triethylamine (TEA) (0.014 mol) in toluene under inert environment triflic anhydride (Tf_2O) (0.009 mol) was added drop wise, stirred at room temperature for 36–42 h. Reaction mixture was treated with $NaHCO_3$ solution and extracted with chloroform, which afforded quinoline triflates (**16a**–**i**). A mixture of quinoline triflate (0.002 mol), $Zn(CN)_2$ (0.004 mol), $Pd-(Ph_3P)_4$ (0.00016 mol), in DMF was stirred at 150 °C for 20–24 h. After cooling, saturated sodium carbonate and water were added, and mixture was extracted with ethylacetate, washed with brine and dried over anhydrous sodium sulfate, which afforded **17a**–**i**. In 4-cyano quinoline derivatives (**17a**–**i**) (0.0011 mol), 10% solution of NaOH was added and reflux gently for 1–2 h. After cooling, 6 M HCl with stirring was added until the solution become acidic and precipitation of residue was complete. The residues were collected by vacuum filtration and washed with cold water. Finally, the compounds (**18**–**26**) were subjected to silica column chromatography.

*4.1.4.1. 3,6-Dimethyl-2-[(3,3′,5-trifluorobiphenyl-4-yl)carbamoyl] quinoline-4-carboxylic acid (**18**).* Compound **18** was synthesized according to the synthetic procedure given above as white solid, in yield of 42.61%, mp 213–215 °C; FT-IR (cm^{-1}) 3340.13 (COOH); ESI-MS *m*/*z* 450.1 $(M+H)^+$, 451.9 $(M+2H)^+$; ^{1}H NMR (600 MHz, DMSO-*d*6) δ 11.31 (s, 1H, −COOH), 10.13 (s, 1H, −NH), 7.75 (d, 1H, *J* = 7.8 Hz, Ar−H), 7.42 (m, 1H, Ar−H), 7.22 (d, 1H, *J* = 8.4 Hz, Ar−H), 7.13 (t, 2H, *J* = 7.2 Hz, Ar−H), 6.83 (d, 1H, *J* = 8.4 Hz, Ar−H), 6.58 (d, 1H, *J* = 7.8 Hz, Ar−H), 5.90 (s, 1H, Ar−H), 3.35 (d, 1H, *J* = 6.9 Hz, $-CH_3$), 3.11 (s, 3H, $-CH_3$); ^{13}C NMR (100 MHz, DMSO) δ 211.38, 184.98, 162.2, 160.1, 159.7, 157.57, 151.38, 149.03, 136.36, 136.24 (2C), 131.1 (2C), 124.9 (2C), 121.56, 116.02, 115.97, 114.99, 111.91 (2C), 104.33, 13.93, 11.26; Anal. Calcd for $C_{25}H_{17}F_3N_2O_3$: C, 66.67; H, 3.80; F, 12.65; N, 6.22; O, 10.66 Found C, 66.62; H, 3.83; F, 12.63; N, 6.18; O, 10.62.

*4.1.4.2. 6-Ethyl-3-methyl-2-[(3,3′,5-trifluorobiphenyl-4-yl)carbamoyl]quinoline-4-carboxylic acid (**19**).* Compound **19** was synthesized according to the synthetic procedure given above as white solid in yield of 40.32%, mp 230–232 °C; FT-IR (cm^{-1}) 3348.23 (COOH); ESI-MS *m*/*z* 465.1 $(M+H)^+$, 466.9 $(M+2H)^+$; ^{1}H NMR (400 MHz, DMSO-*d*6) δ 10.97 (s, 1H, −COOH), 9.55 (s, 1H, −NH), 7.21 (m, 3H, Ar−H), 7.13 (m, 3H, Ar−H), 6.95 (m, 3H, Ar−H), 4.44 (q, *J* = 7.2 Hz, 3H, $-C_2H_5$) 2.22 (s, 3H, $-CH_3$), 1.38 (t, 2H, *J* = 6.8 Hz, $-C_2H_5$); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 211.4, 185.1, 179.1, 173.4, 172.7, 169.9(2C), 168.6, 153.6, 150.1, 138.1, 135.3, 132.0, 130.8, 123.5(2C), 119.5, 117.9, 117.3(2C), 112.5, 112.3, 111.4, 104.4, 32.12, 13.56; Anal. Calcd for $C_{26}H_{19}F_3N_2O_3$: C, 67.24; H, 4.12; F, 12.27; N, 6.03; O, 10.33 Found C, 67.20; H, 4.09; F, 12.30; N, 6.01; O, 10.28.

*4.1.4.3. 6-Fluoro-3-methyl-2-[(3,3′,5-trifluorobiphenyl-4-yl)carbamoyl]quinoline-4-carboxylic acid (**20**).* Compound **20** was synthesized according to the synthetic procedure given above as yellowish-white solid in yield of 43.81%, mp 241–243 °C; FT-IR (cm^{-1}) 3384.23 (COOH); ESI-MS *m*/*z* 456.1 $(M+H)^+$, 457.2 $(M+2H)^+$; ^{1}H NMR (600 MHz, DMSO-*d*6) δ 11.52 (s, 1H, −COOH), 9.11 (s, 1H, −NH), 8.31 (d, 1H, *J* = 7.2 Hz, Ar−H), 7.75 (d, 1H, *J* = 7.8 Hz, Ar−H), 7.41 (d, 2H, *J* = 4.2 Hz, Ar−H), 7.22 (d, 1H, *J* = 16.8 Hz, CH), 7.13 (t, 2H, *J* = 5.4 Hz, Ar−H), 6.95 (d, 1H, *J* = 3.0 Hz, CH), 6.65 (d, 1H, *J* = 3.0 Hz, CH), 3.64 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-*d*6): δ 211.56, 176.83 (2C) 171.09, 167.87, 163.24, 157.30, 151.89, 148.89, 144.07, 137.68, 136.15, 131.07, 125.97, 123.47 (2C), 121.71, 118.58, 115.77, 108.77(2C), 13.92; Anal. Calcd for $C_{24}H_{14}F_4N_2O$: C, 63.44; H, 3.11; F, 16.72; N, 6.17; O, 10.56 Found C, 63.41; H, 3.12; F, 16.98; N, 6.19; O, 10.55.

*4.1.4.4. 6-Ethoxy-3-methyl-2-[(3,3′,5-trifluorobiphenyl-4-yl)carbamoyl]quinoline-4-carboxylic acid (**21**).* Compound **21** was synthesized according to the synthetic procedure given above as yellowish-white solid in yield of 47.56%, mp 215–217 °C; FT-IR (cm^{-1}) 3362.12 (COOH); ESI-MS *m*/*z* 480.0 $(M+H)^+$, 481.0 $(M+2H)^+$; ^{1}H NMR (400 MHz, DMSO-*d*6) δ 12.03 (s, 1H, −COOH), 10.40 (s, 1H, −NH) 7.40 (m, 3H, Ar−H), 7.39 (t, *J* = 7.2 Hz, 2H, Ar−H), 7.37 (m, 2H, Ar−H), 7.28 (m, 2H, Ar−H), 4.45 (q, *J* = 7.2 Hz, 3H, $-C_2H_5$) 2.23 (s, 3H, $-CH_3$), 1.38 (t, 2H, *J* = 8 Hz, $-C_2H_5$); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 211.4, 185.1, 180.4, 172.7, 169.1, 165.4, 163.2, 158.1, 155.3, 153.2, 140.0, 138.6, 132.1, 130.0 (2C), 121.7 (2C), 113.4, 113.1, 108.3, 105.2, 103.5, 102.9, 102.5, 26.12, 32.12, 13.41; Anal. Calcd for $C_{26}H_{19}F_3N_2O_4$ C, 65.00; H, 3.99; F, 11.86; N, 5.83; O, 13.32 Found C, 65.04; H, 3.96; F, 11.84; N, 5.80; O, 13.28.

*4.1.4.5. 6-Methoxy-3-methyl-2-[(3,3′,5-trifluorobiphenyl-4-yl)carbamoyl]quinoline-4-carboxylic acid (**22**).* Compound **22** was synthesized according to the synthetic procedure given above as yellowish-white solid in yield of 45.86%, mp 210–212 °C; FT-IR (cm^{-1}) 3321.80 (COOH); ESI-MS *m*/*z* 466.0 $(M+H)^+$, 466.9 $(M+2H)^+$; ^{1}H NMR (600 MHz, DMSO-*d*6) δ 11.01 (s, 1H, −COOH), 9.39 (s, 1H, −NH), 8.30 (d, 1H, *J* = 7.8 Hz, Ar−H), 7.79 (d, 1H, *J* = 7.8 Hz, Ar−H), 7.42 (m, 1H, Ar−H), 7.22 (d, 1H, *J* = 10.8 Hz, Ar−H), 7.12 (t, 1H, *J* = 7.2 Hz, Ar−H), 7.01 (d, 1H, *J* = 3.6 Hz, Ar−H), 6.82 (d, 1H, *J* = 7.8 Hz, CH), 3.54 (d, 3H, *J* = 7.2 Hz, $-OCH_3$), 2.91 (s, 3H, $-CH_3$); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 211.56, 176.75(2C), 163.36,

161.34, 157.87, 155.18, 152.51(2C), 149.86, 142.93, 139.51, 135.81, 124.15(2C), 121.81(2C), 121.45, 121.20, 117.63, 108.77(2C), 108.55, 26.09, 11.26; Anal. Calcd for $C_{25}H_{17}F_3N_2O_4$ C, 64.38; H, 3.67; F, 12.22; N, 6.01; O, 13.72 Found C, 64.33; H, 3.62; F, 12.24; N, 6.05; O, 13.73.

*4.1.4.6. 6-Trifluoromethoxy-3-methyl-2-[(3,3′,5-trifluorobiphenyl-4-yl)carbamoyl]quinoline-4-carboxylic acid (**23**).* Compound **23** was synthesized according to the synthetic procedure given above as yellowish-white solid in yield of 38.13%, mp 246–248 °C; FT-IR (cm^{-1}) 3321.80 (COOH), ESI-MS *m/z* 521.09 $(M+H)^+$, 522.09 $(M+2H)^+$, ^1H NMR (600 MHz, DMSO-*d*6): δ 11.21 (s, 1H, −COOH), 9.15 (s, 1H, −NH), 8.32 (d, 1H, $J = 7.8$ Hz, Ar−H), 7.75 (d, 1H, $J = 7.8$ Hz, Ar−H), 7.23 (d, 1H, $J = 9.0$ Hz, Ar−H), 7.14 (m, 2H, Ar−H), 6.82 (d, 2H, $J = 2.4$ Hz, Ar−H), 6.58 (d, 2H, $J = 3.0$ Hz, Ar−H), 3.15 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 211.22, 182.02, 153.50 (2C), 151.03 (2C), 149.35 (2C), 148.74 (2C), 136.49, 128.39, 128.22, 123.76, 123.91, 122.39, 122.30, 117.65, 115.50, 115.32, 109.73, 109.39, 34.78, 27.03; Anal. Calcd for $C_{25}H_{14}F_6N_2O_4$: C, 57.70; H, 2.71; F, 21.91; N, 5.38; O, 12.30 Found C, 57.66; H, 2.68; F, 21.88; N, 5.33; O, 12.28.

*4.1.4.7. 6-Trifluoromethyl-3-methyl-2-[(3,3′,5-trifluorobiphenyl-4-yl) carbamoyl]quinoline-4-carboxylic acid (**24**).* Compound **24** was synthesized according to the synthetic procedure given above as white solid in yield of 41.45%, mp 237–239 °C; FT-IR (cm^{-1}) 3404.45 (COOH); ESI-MS *m/z* 505.2 $(M+H)^+$, 506.1 $(M+2H)^+$; ^1H NMR (400 MHz, DMSO-*d*6) δ 11.10 (s, 1H, −COOH), 9.45 (s, 1H, −NH), 8.30 (d, 1H, $J = 7.2$ Hz, Ar−H), 7.79 (d, 1H, $J = 7.8$ Hz, Ar−H), 7.44 (m, 2H, Ar−H), 7.22 (d, 2H, $J = 7.8$ Hz, Ar−H), 7.11 (m, 1H, Ar−H), 6.70 (d, 1H, $J = 3.0$ Hz, Ar−H), 6.55 (m, 2H, Ar−H), 3.66 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 211.06, 176.55, 176.53, 167.87, 163.24, 157.87, 155.18, 149.86, 139.31, 139.00, 131.88, 128.98, 127.31, 126.46, 120.06, 116.86, 113.63, 108.47, 108.25, 29.81, 13.93; Anal. Calcd for $C_{25}H_{14}F_6N_2O_3$ C, 59.53; H, 2.80; F, 22.60; N, 5.55; O, 9.52 Found C, 59.51; H, 2.77; F, 22.54; N, 5.51; O, 9.56.

*4.1.4.8. 2-(Biphenyl-4-ylcarbamoyl)-6-methoxy-3-methylquinoline-4-carboxylic acid (**25**).* Compound **25** was synthesized according to the synthetic procedure given above as yellowish-white solid in yield of 52.61%, mp 248–250 °C; FT-IR (cm^{-1}) 3500.17 (COOH); ESI-MS *m/z* 413.1 $(M+H)^+$, 414.6 $(M+2H)^+$; ^1H NMR (400 MHz, DMSO-*d*6) δ 12.35 (s, 1H, −COOH), 8.73 (s, 1H, −NH), 8.20 (d, 2H, $J = 7.2$ Hz, Ar−H), 8.16 (d, 3H, $J = 6.8$ Hz, Ar−H), 8.06 (d, 3H, $J = 6.8$ Hz, Ar−H), 7.93 (m, 2H, Ar−H), 7.72 (d, 3H, $J = 7.6$ Hz, Ar−H), 7.65 (m, 2H, Ar−H), 7.56 (m, 2H, Ar−H), 7.19 (d, 1H, $J = 10.8$ Hz, Ar−H), 4.15 (d, 3H, $J = 7.2$ Hz, $-OCH_3$); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 180.0, 175.0, 170.0, 164.9, 161.6, 151.4, 142.8, 138.7, 137.2, 134.1, 131.3, 131.2, 130.5, 127.0, 126.2, 126.1, 123.2, 122.9, 119.6, 115.5, 115.2, 110.4, 40.0, 21.1 Anal. Calcd for $C_{25}H_{20}N_2O_4$: C, 72.80; H, 4.89; N, 6.79; O, 15.52 found C, 72.82; H, 4.83; N, 6.75; O, 15.50.

*4.1.4.9. 2-(Biphenyl-4-ylcarbamoyl)-3-methylquinoline-4-carboxylic acid (**26**).* Compound **26** was synthesized according to the synthetic procedure given above as white solid in yield of 43.32%, mp 238–240 °C; FT-IR (cm^{-1}) 3322.75, (COOH); ESI-MS *m/z* 383.1 $(M+H)^+$, 384.5 $(M+2H)^+$; ^1H NMR (400 MHz, DMSO-*d*6) δ 11.82 (s, 1H, −COOH), 8.65 (s, 1H, −NH) 7.90 (s, 2H, Ar−H), 7.67 (m, 2H, Ar−H), 7.40 (m, 2H, Ar−H), 7.36 (m, 3H, Ar−H), 6.92 (m, 3H, Ar−H), 2.50 (s, 3H, CH_3); ^{13}C NMR (100 MHz, DMSO-*d*6) δ 181.0, 176.0, 171.0, 164.7, 161.3, 150.9, 142.5, 136.5, 134.9, 134.2, 131.0, 130.9, 129.8, 128.2, 125.4, 123.2, 122.8, 119.5, 115.4, 115.1, 109.7, 20.0 Anal. Calcd for $C_{24}H_{18}N_2O_3$: C, 75.38; H, 4.74; N, 7.33; O, 12.55 found C, 75.33; H, 4.72; N, 7.31; O, 12.52.

4.2. Pharmacological assays

4.2.1. hDHODH inhibition assay

Enzyme assay was done as described in the literatures [26–28]. Decylubiquinone (C_QD), dichlorophenolindophenol (DCIP) and L-dihydroorotate (L-DHO) were obtained from Sigma Aldrich. Brequinar sodium (6-fluoro-2-(2-fluoro-1,1-biphenyl-4-yl)-3-methyl-4-quinoline carboxylic acid sodium salt, was used as reference compound, which was also obtained from Sigma Aldrich. Stock solution of synthesized compounds was retained in DMSO. Recombinant *human* DHODH was purchased from Prospec Bio (ENZ-642). The assay solution containing 0.1 mM C_QD, 1 mM L-dihydroorotate (L-DHO), 0.06 mM DCIP, 0.1% Triton X-100 in 50 mM Tris–HCl buffer, 150 mM NaCl at pH 8.0 was prepared and 170 μl was transferred to each well. Each compound was prepared as a 200 μM stock in 1% DMSO, and of that, 10 μl was transferred to assay mixture. The assay was started by the addition of 20 μl of 20× stock of enzyme prepared in Tris–HCl buffer at pH 8.0, 300 mM NaCl, and 15% glycerol to give a final enzyme concentration between 10 nM in the assay wells. The assay was allowed to progress for 20 min at room temperature, then the reaction was stopped by addition of 5 μl of 10% sodium dodecyl sulfate. Absorbance of each well was measured at 600 nm and quantified in triplicate using a 96 microplate reader (BioRad-680 instrument) and the data were exported to an Excel (Microsoft) spreadsheet for analysis.

4.2.2. Antiproliferative MTT assay

The compounds were evaluated for antiproliferative activity against HEP-3B (hepatoma) and A-375 (melanoma) cancer cell lines cultures. Stock solution of these cell lines were cultured in DMEM, supplemented with 10% FBS (fetal bovine serum). Cells were also supplemented with 5% HBSS, penicillin, streptomycin and amphotericin B in a humidified atmosphere of 5% CO_2 at 37 °C with 90% humidity. Stock solution of compounds were prepared in 2% DMSO at different concentrations. 100 μM of 1 mM conc. of synthesized compounds were added in to 900 μl of culture media, and as a result 100 μM conc. of synthesized compound was obtained. 100 μl of complete media was added into well number 1–9. Well number 10 contained 150 μl compounds only, from that 50 μl was pipette out and added into well number 9, which already contain 100 μl of complete media lead to 1:3 dilution of compounds. Same procedure was repeated 9 times in order to get final concentration of compounds up to 0.005 μM. Cell lines in exponential growth phase were washed, trypsinized and re-suspended in complete culture media. Cells were seeded at 2×10^4 cells/well in 96 well microtitre plate and incubated for 24 h. The cells were then exposed to various concentrations of synthesized compounds and standards brequinar sodium and doxorubicin. MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium bromide) solution (20 ml of 5 mg/ml) was added to each well, and incubation was continued for additional 3 h. The dark blue formazan crystals formed within the cells were solubilized with DMSO and absorbance was estimated in ELISA plate reader at 550 nm. Absorbance was correlated with the cell number. Experiments were performed in triplicates and values are the average of three ($n = 3$) independent experiments. The inhibitory concentration (IC_{50}) of compounds was assessed using *GraphPad Prism* software (Ver. 5.04) (GraphPad Software, Inc., USA) and Micorsoft Excel 2007 (Microsoft Corporation, USA) application [29,30].

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.05.064.

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