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Original article

Expeditious synthesis, antileishmanial and antioxidant activities of novel 3-substituted-4-hydroxycoumarin derivatives

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ABSTRACT

A series of novel 3-substituted-4-hydroxycoumarin derivatives **6(a–l)** were synthesized in high yield using one-pot three component coupling reaction catalyzed by ceric ammonium nitrate. These compounds were evaluated for antileishmanial activity against *Leishmania donovani* promastigotes and antioxidant activity (DPPH-radical scavenging activity). Two compounds, **6h** (IC_{50} = 9.90 μmol/L) and **6i** (IC_{50} = 6.90 μmol/L) displayed potent antileishmanial activity when compared with standard antileishmanial agents pentamidine (IC_{50} = 16.15 μmol/L) and miltefosine (IC_{50} = 12.50 μmol/L). Three compounds, **6c** (IC_{50} = 10.79 μmol/L), **6h** (IC_{50} = 10.60 μmol/L), and **6i** (IC_{50} = 10.73 μmol/L) showed significant antioxidant activity favorably with the antioxidant standards butylated hydroxy toluene (IC_{50} = 16.47 μmol/L) and ascorbic acid (IC_{50} = 12.69 μmol/L). A molecular docking study of compounds **6(a–l)** suggested a possible mode of binding with the *Adenine phosphoribosyltransferase* enzyme of *L. donovani*. ADME properties were predicted *in silico* and support the potential of **6(a–l)** to show favorable drug-like properties.

1. Introduction

Leishmaniasis is a family of parasitic diseases caused by infection of macrophages by obligate intracellular parasites of genus *Leishmania*. It is a major health problem worldwide and over 20 million people in 88 countries have one of the various forms of the disease [1]. Parasites transmissions occur by way of female sandflies *via* anthroponotic or zoonotic cycles [2]. Leishmaniasis is classified as cutaneous, visceral (Kala Azar), mucosal or mucocutaneous and diffused cutaneous on the basis of the evaluation of the patients and parasite [3]. Visceral leishmaniasis (VL or Kala-azar) is the most devastating form and arises from invasion of the reticuloendothelial system (spleen, liver and bone marrow) by the haemo flagellate protozoan parasite *Leishmania donovani* (*L. donovani*) [4]. While several drug treatment strategies are known, these drugs have the drawbacks of high expense, requirement of prolonged treatment, high toxicity to liver and heart, and the development of clinical resistance after a few weeks of treatment. These issues contribute to poor treatment outcomes and to an increase in leishmaniasis-AIDS co-infections in some countries [5]. Therefore, there is an urgent need for development of new, inexpensive, effective and safe drugs having potential antileishmanial activity. Oxidative and free-radical-mediated processes reactions seem to play an important role in the progression of various diseases, including cancer and coronary heart disease [6]. Radical damage has also been implicated in neurodegeneration occurring both through normal aging processes and in diseases such as Alzheimer's disease [7]. Thus, there is also a need for development of safe and effective antioxidants to prevent radical-mediated damage.

The coumarins are an important heterocyclic core structure that is present in numerous natural products with wide range of biological activities [8]. Flavokavain B, for example, is a natural coumarin derivative that is effective against the promastigote form of *L. donovani* parasites [9]. Also, many coumarins known to have antioxidant effect [10]. A number of other heterocyclic core structures, such as, indoles, quinolines, and furans, are also present in various biologically active natural products and drugs, including those with potent antileishmanial activity [11–13]. Pleiocarpine (an indole alkaloid) [14], cryptolepine (a quinoline alkaloid) [15], and brasanquinone (a furan derivative) [16] are specific examples of natural antileishmanial agents with activity against *L. donovani* parasites (Fig. 1).

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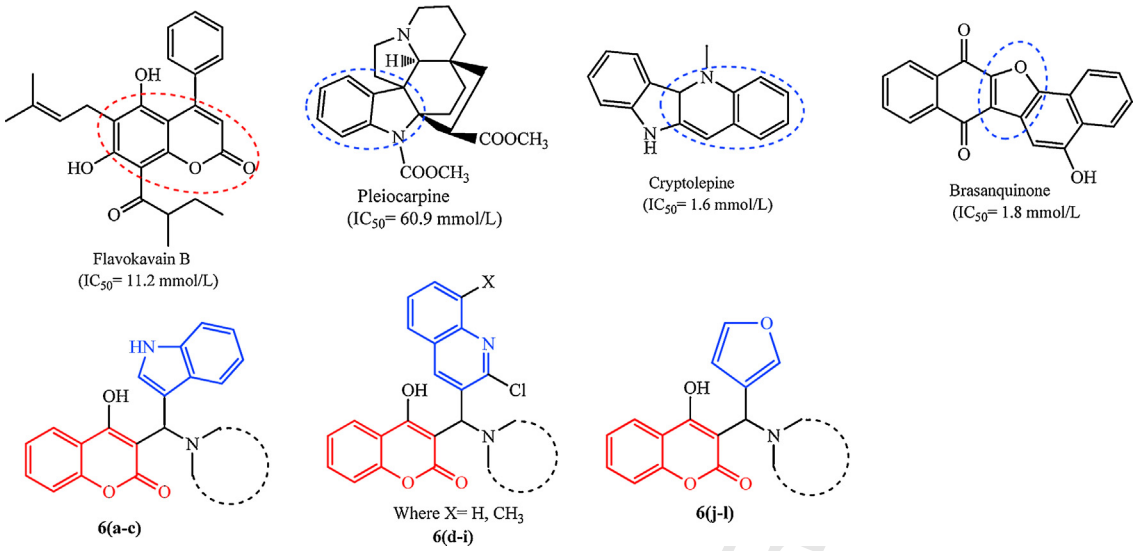

Fig. 1. Structure of some natural antileishmanial compounds active against *L. donovani* promastigotes and model structure of titled compounds **6(a–l)**.

Natural products constitute a large portion of marketed drugs, particularly in the area of infectious diseases, and often show remarkable potency, selectivity and drug-like properties [17]. However, the numbers of natural products are limited; hence incorporating either different natural products or drug fragments may provide millions of combinations which will be enriched in biological activity and less toxicity. Taking into account all of the aforementioned, and as a part of our ongoing effort towards identifying novel bioactive compounds [18,19], we decided to explore ways of combining the coumarin nucleus with fragments present in other antileishmanial agents, heterocycles such as indoles, quinolines, and furans. We have synthesized a novel series of 3-substituted-4-hydroxycoumarin hybrids **6(a–l)** and evaluated them as antileishmanial agents against *L. donovani* (Fig. 1). Coumarin such as isopimpinellin is reported to act as antileishmanial agents through the inhibition of enzyme *Adenine phosphoribosyltransferase* enzyme [20]. Accordingly, we opted to dock our synthesized compounds against the *Adenine phosphoribosyltransferase* of *L. donovani* to understand features of the molecules that may be responsible for their antileishmanial activity. Because coumarins have also found use as antioxidants, the synthesized compounds were also evaluated for antioxidant activity using DPPH-radical scavenging method. We have also used *in silico* method to predict ADME properties to suggest the suitability of any of the new compounds for further drug development, particularly with respect to activity.

2. Experimental

2.1. Chemistry

All reagents and solvents used were purchased from the Sigma and Avra synthesis. The completion of reaction was checked by ascending thin layer chromatography (TLC) on silica gel-G (Merck) coated aluminum plates, visualized by iodine vapor. Infrared (IR) spectra were recorded for the compounds on JASCO FTIR (PS 4000) using KBr pallet. ^{1}H NMR and ^{13}C NMR spectra were recorded using Brucker Avance II. Multiplicities are recorded as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass spectra were taken with Micromass-QUATTRO-II of WATER mass spectrometer. Elemental analyses (C, H, and N) were undertaken with a Shimadzu's FLASHEA112 analyzer and all analyses were consistent with theoretical values (within ±0.4%), unless indicated.

2.1.1. General procedure for synthesis of 3-substituted-4-hydroxycoumarin derivatives ***6(a–l)***

A mixture of 4-hydroxycoumarin 3 (1.0 mmol), heterocyclic aldehydes 4 (1.0 mmol) and secondary amines 5 (1.0 mmol) was stirred at room temperature (25–30 °C) in ethanol (15 mL) in the presence of catalytic amount of ceric ammonium nitrate (10 mol%) for 15–20 min. After completion of the reaction as monitored by TLC analysis, the reaction mixture was poured into water. The solid product formed was filtered, dried and recrystallized using ethanol.

2.2. Biological evaluations

2.2.1. In vitro *antileishmanial activity*

The assay for *in vitro* antileishmanial activity on culture of *L. donovani* promastigotes (NHOM/IN/80/DD8) was carried out in 96-well tissue culture plates using reported procedure [21]. The promastigotes culture was maintained at 22 °C in modified RPMI 1640 pH 7.4 (without phenol red) with 10% FCS medium. Drug dilutions were prepared in DMSO and appropriate concentration of each drug was used in triplicate. Plates were incubated at 22 °C for 72 h and evaluated using modified MTT assay, where the conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan by mitochondrial enzymes served as an indicator of cell viability and the amount of formazan produced was directly proportional to the number of metabolically active cells. Accordingly, absorbance at 492 nm represented the number of live cells. The concentration that decreased cell growth by 50% (IC_{50}) was computed from growth inhibition curve. Pentamidine and miltefosine were used as standard drugs.

2.2.2. Antioxidant activity (DPPH radical scavenging method)

The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple colored methanol solution of 1,1-diphenyl-1-picrylhydrazyl (DPPH) [22]. The spectrophotometric assay uses the stable radical DPPH as a reagent. 1 mL of various concentrations of the test compounds (5, 10, 25, 50 and 100 μg/mL) in methanol was added to 4 mL of 0.004% (w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. The natural antioxidant ascorbic acid and synthetic antioxidant BHT (butylated hydroxy toluene) were used

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as standards. The percent of inhibition (*I*%) of free radical production from DPPH was calculated by the following equation.

$$\%\ \text{of scavenging} = \left[\frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{blank}}}\right] \times 100$$

where A_{control} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound.

2.2.3. In vitro *cytotoxicity study*

Cytotoxic study of the synthesized compounds on HeLa cell line was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [23]. In brief, exponentially growing Hela cells (1×10^4 cells/well) were seeded in a 96-well culture plate and incubated at 37 °C in a 5% CO_2 incubator for 24 h. The drugs were dissolved in 0.1% DMSO and then diluted with the medium. The cells were then exposed to different concentrations of drug (0–10 μg/mL) and incubated for 72 h. The cells in the control wells received medium containing the same volume of DMSO (0.1%). After incubation, 5 mg/mL of MTT reagent was added in each well and further incubated for additional 4 h. The formazan produced by the viable cells was stabilized by addition of 0.1 mL DMSO. The absorbance of each well at 550 nm was determined by a microplate spectrophotometer. The cells were also seen under the microscope (Ziess, Germany) at 10× magnification.

2.3. Computational studies

2.3.1. Docking study

Docking study of synthesized compounds **6(a–l)** was performed using VLife MDS 4.3 package [24]. With this purpose, crystal structure of *Adenine phosphoribosyltransferase* of *L. donovani* (PDB ID: 1QB8) [25] was obtained from the Protein Data Bank in order to prepare protein for docking study. The cavities in the receptor were mapped to assign an appropriate active site. The basic features used to map the cavities were the surface mapping of the protein and identifying the geometric voids as well as scaling the void for its hydrophobic characteristics using V Life MDS analyze tool. Hence all the cavities that are present in *Adenine phosphoribosyltransferase* protein were identified and ranked based on their size and hydrophobic surface area. Considering the dimensions and hydrophobic surface area, ranked-1 cavity was chosen for docking study.

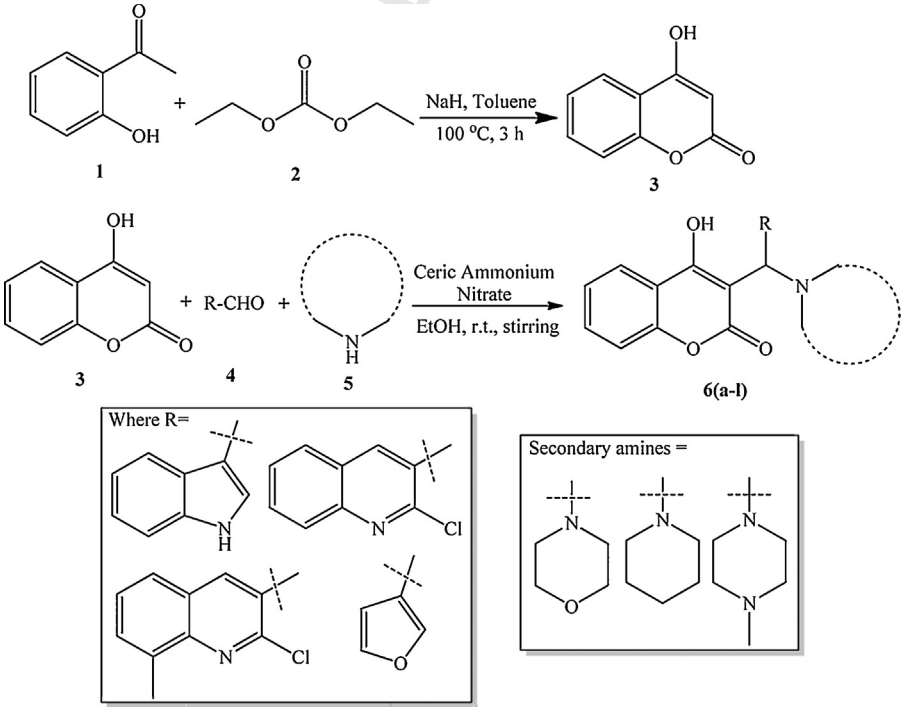
2.3.2. ADME prediction

A computational study of synthesized compounds **6(a–l)** was performed for prediction of ADME properties. In this study, we calculated molecular volume (MV), molecular weight (MW), logarithm of partition coefficient (miLog *P*), number of hydrogen bond acceptors (*n*-ON), number of hydrogen bonds donors (*n*-OHNH), topological polar surface area (TPSA), number of rotatable bonds (*n*-ROTB) and Lipinski's rule of five [26] using Molinspiration online property calculation toolkit [27]. Absorption (% ABS) was calculated by: %ABS = 109 − (0.345 × TPSA) [28].

3. Results and discussion

3.1. Chemistry

The starting material 4-hydroxycoumarin (**3**) was synthesized using 2-hydroxyacetophenone (**1**) and diethyl carbonate (**2**) as per reported method [29]. Then a one-pot three component reaction [30] was used to synthesize 3-substituted-4-hydroxycoumarin derivatives **6(a–l)** as shown in Scheme 1. These products were obtained *via* the reaction of 4-hydroxycoumarin (**3**) with various heterocyclic aldehydes (**4**) and secondary amines (**5**) in ethanol at room temperature, using ceric ammonium nitrate (CAN, 10 mol%) as catalyst, through a Mannich type reaction mechanism. CAN has received considerable attention as an relatively inexpensive, non-toxic, water soluble and easily handled catalyst for the construction of carbon-carbon and carbon-heteroatom bonds [31]. The formation of the desired compound indicates that CAN play an important role for the rapid formation of the imine intermediate.



Scheme 1. Synthetic protocol for titled compounds **6(a–l)**.

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Table 1
Physical data for 3-substituted-4-hydroxycoumarin derivatives **6(a–l)**.

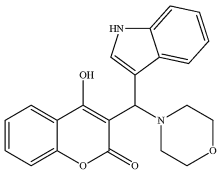
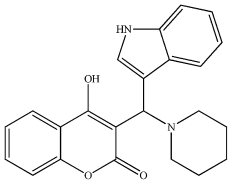
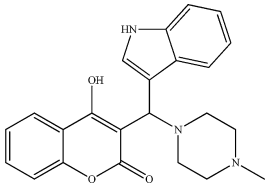
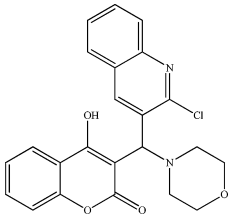
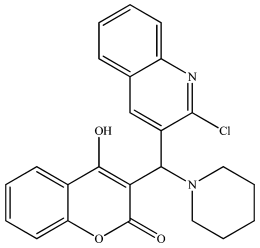
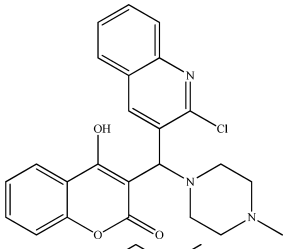
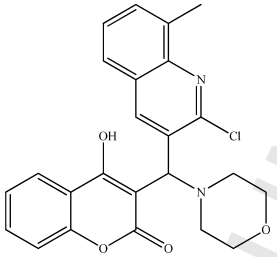
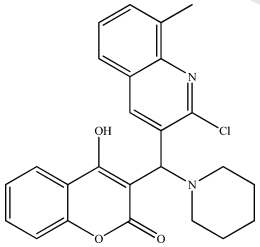
Entry	Structure	Molecular formula	Reaction time (min)	Yield (%)	R_f value	Mp (°C)
6a		$C_{22}H_{20}N_2O_4$	15	98	0.48	180–182
6b		$C_{23}H_{22}N_2O_3$	15	96	0.58	166–168
6c		$C_{23}H_{23}N_3O_3$	20	92	0.54	158–160
6d		$C_{23}H_{19}ClN_2O_4$	15	95	0.64	142–144
6e		$C_{24}H_{21}ClN_2O_3$	20	90	0.81	130–132
6f		$C_{24}H_{22}ClN_3O_3$	15	93	0.67	170–172
6g		$C_{24}H_{21}ClN_2O_4$	20	95	0.69	198–200
6h		$C_{25}H_{23}ClN_2O_3$	20	91	0.86	134–136

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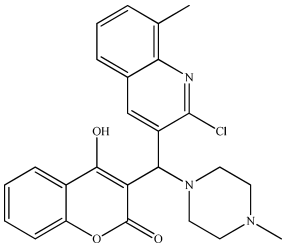
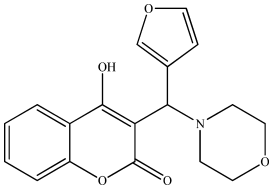
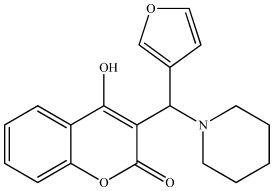
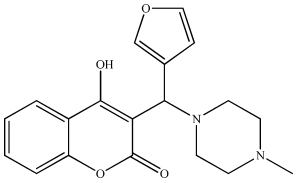
Table 1 (*Continued*)

Entry	Structure	Molecular formula	Reaction time (min)	Yield (%)	R_f value	Mp (°C)
6i		$C_{25}H_{24}ClN_3O_3$	15	98	0.73	156–158
6j		$C_{18}H_{17}NO_5$	20	90	0.37	110–114
6k		$C_{19}H_{19}NO_4$	20	92	0.53	94–96
6l		$C_{19}H_{20}N_2O_4$	15	90	0.45	122–124

Solvent of recrystallization was ethanol; eluants used in TLC were ethyl acetate/*n*-hexane (9:1) for all compounds.

The catalyst may induce 4-hydroxy coumarin to act as the Mannich donor for the very fast formation of 3-substituted-4-hydroxycoumarin derivatives. The rapid imine generation and subsequent 'C–C' bond formation within a very short time catalyzed by CAN are the attractive features of this protocol.

To optimize the reaction conditions, we studied a model reaction of 4-hydroxycoumarin (1.0 mmol), indole-3-carbaldehyde (1.0 mmol) and morpholine (1.0 mmol) in ethanol to synthesize compound **6a**. A wide variety of catalysts were surveyed, including oxalic acid, boric acid, sulphamic acid, *p*-toluenesulphonic acid, ZnO, $ZnCl_2$, and ceric ammonium nitrate (CAN), all at 30 mol%. CAN as the catalyst gave the best results in terms of yield (98%) and reaction time (15 min) (Table S1 in Supporting information). Solvent effects were then studied, using water, toluene, dimethylsulfoxide, ethanol, and dichloromethane. Ethanol was found to be best among the studied solvents (Table S2 in Supporting information). For the optimization of catalyst loading, we used CAN as catalyst at 0, 5, 10, 15, 20, and 30 mol%. The results (Table S3 in Supporting information) revealed that 10 mol% CAN is fully sufficient for obtaining a high yield. This low catalyst loading contributes to the efficiency and simplicity of the process.

After optimization of the model reaction, the scope of the transformation was then studied. We further expanded the series and synthesized 12 novel derivatives of 3-substituted-4-hydroxycoumarins **6(a–l)** by reacting 4-hydroxycoumarin **3**, heterocyclic aldehydes **4**, and secondary amines **5** in ethanol using CAN (10 mol%) as catalyst at room temperature (25–30 °C). The reaction preceded smoothly under mild reaction conditions. The isolated yield of synthesized compounds was in the range of 90–98% and reactions were completed in 15–20 min (monitored by TLC). The work-up of these reactions was simple, required only a filtration. The compounds were thus obtained in pure form without aids of any system like chromatography. Melting points were determined in open capillary tubes and are uncorrected. The physical data for the compounds **6(a–l)** are presented in Table 1, with characterization by IR, ^{1}H NMR, ^{13}C NMR, Mass and elemental spectroscopic data, with results in agreement with the proposed structures (Supporting information). This type of Mannish adducts are prone to retro-Mannich decomposition to lose the amine and form a Michael acceptor [32]. In order to check the thermal stability of the synthesized compounds **6(a–l)**, we subjected compound **6i** for stress stability testing as per ICHQ1A 2.1.2 guidelines [33]. Compound **6i** was subjected to forced degradation study at temperatures 105 °C and 157 °C for 1 month. After 1 month of study, compound **6i** was again characterized for physical and for spectroscopic data. The data suggested that compound **6i** was stable even after 1 month of force degradation study (stress study).

3.2. Biological evaluations

3.2.1. In vitro *antileishmanial activity*

The title compounds **6(a–l)** were tested *in vitro* for their antileishmanial activity against a culture of *L. donovani* promastigotes and data obtained is depicted in Table 2. Most of the synthesized compounds showed promising antileishmanial activity. Compounds **6a**, **6c**, **6h**, **6i**, and **6j** (IC_{50} values = 6.90–16.48 μmol/L) were most promising, with activity comparable to that of standard drugs. Compounds **6b**, **6g**, **6k**, and **6l** were less potent in this assay (IC_{50} values = 22.01–38.50 μmol/L). The compounds **6d**, **6e**, and **6f** (*R* = 2-chloroquinolinyl) were found to inactive against culture of *L. donovani* promastigotes. The compounds **6h** (IC_{50} = 9.90 μmol/L) and **6i** (IC_{50} = 6.90 μmol/L) were more potent than standard drugs pentamidine ((IC_{50} = 16.15 μmol/L) and miltefosine (IC_{50} = 12.5 μmol/L).

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Table 2
Biological evaluation and molecular docking statistics of synthesized compounds **6(a–l)**.

Entry	*L. donovani* (IC_{50} μmol/L)	Antioxidant (IC_{50} μmol/L)	Docking result against *L. donovani* (*Adenine phosphoribosyltransferase*)	
	Mean ± SEM	Mean ± SEM	Binding energy (kcal/mol)	Hydrogen bonding
6a	16.48 ± 0.68	28.22 ± 0.60	−52.88	1 (ARG37–OH)
6b	38.50 ± 1.25	15.23 ± 0.64	−50.88	1(ARG37–OH)
6c	13.11 ± 0.74	10.79 ± 0.69	−55.01	1 (ARG37–O=C)
6d	124.40 ± 2.55	14.52 ± 0.14	−44.01	1 (ARG37–O of coumarin)
6e	136.90 ± 3.48	15.46 ± 0.26	−48.12	1 (GLU120–O of coumarin)
6f	417.81 ± 5.14	22.00 ± 0.35	−43.25	1 (LYS186–O of coumarin)
6g	22.01 ± 0.43	15.63 ± 0.57	−57.64	*
6h	9.90 ± 0.33	10.60 ± 0.48	−61.81	*
6i	6.90 ± 0.12	10.73 ± 0.61	−65.40	2 (ARG37–OH; ARG37–O of coumarin)
6j	14.67 ± 0.98	15.58 ± 0.83	−56.71	*
6k	33.45 ± 1.33	15.47 ± 0.49	−50.87	1 (ARG37–O of coumarin)
6l	28.23 ± 0.21	25.14 ± 0.53	−52.92	1 (ARG37–O of coumarin)
STD 1	16.15 ± 0.85	ND	ND	ND
STD 2	12.50 ± 0.90	ND	ND	ND
STD 3	ND	16.47 ± 0.18	ND	ND
STD 4	ND	12.47 ± 0.85	ND	ND

IC_{50} represents the mean values of three replicates; standard errors were all within 10% of the mean; STD 1: pentamidine; STD 2: miltefosine; STD 3: butylated hydroxy toluene; STD 4: ascorbic acid.
* Donates no hydrogen bond interaction of ligands with protein; ND: Not done.

To establish structure-activity relationship (SAR) in the series, we divided the synthesized compounds into three classes, the indole class **6(a–c)**, quinoline class **6(d–i)**, and furan class **6(j–l)**. The activity mainly depends upon the presence of the different heterocycles and secondary amines at the 3rd position of the 4-hydroxycoumarin scaffold. The indole class showed promising activity, particularly when a 4-methylpiperazine group was present at 3rd position of 4-hydroxycoumarin (**6c**). From the quinolines **6(d–i)**, a 2-chloroquinolinyl group at 3rd position of 4-hydroxycoumarin (compounds **6d**, **6e**, and **6f**) provides much lower activity towards *L. donovani* promastigote cultures. Replacement of the 2-chloroquinolinyl group with 2-chloro-8-methylquinolinyl (compounds **6g**, **6h**, and **6i**) increased the activity by 5–70 folds. Adding a methyl group at the 8th position of quinoline further enhanced activity. Substitution of 2-chloro-8-methylquinolinyl in combination with a 4-methylpiperazinyl group at 3rd position of the 4-hydroxycoumarin core led to the most potent compound of the series (**6i**). From the furan class **6(j–l)**, the furanyl nucleus in combination with morpholine group (compound **6j**) at 3rd position of the 4-hydroxycoumarin gave the good antileishmanial activity.

3.2.2. Antioxidant activity (DPPH radical scavenging method)

Antioxidant activity was assessed *in vitro* by using the DPPH radical scavenging assay method. The DPPH has an odd electron so it can accept an electron or hydrogen free radical. In the presence of antioxidant, this odd electron becomes paired due to H transfer from antioxidant and hence DPPH absorbance decreases. All the synthesized compounds **6(a–l)** showed interesting antioxidant activity (IC_{50} = 10.60–28.22 μmol/L) when compared to the standards (Table 2). The common antioxidant activity in the series is likely due to the presence of electron-releasing hydroxy at the 4th position of coumarin nucleus. All the compounds except **6a** (IC_{50} = 28.22 μmol/L), **6f** (IC_{50} = 22.00 μmol/L), and **6l** (IC_{50} = 25.14 μmol/L) were more active than standard butylated hydroxy toluene (IC_{50} = 16.47 μmol/L). The most potent antioxidants in the series were **6c** (IC_{50} = 10.79 μmol/L), **6h** (IC_{50} = 10.60 μmol/L), and **6i** (IC_{50} = 10.73 μmol/L), comparing favorably to the standards BHT (IC_{50} = 16.47 μmol/L) and ascorbic acid (IC_{50} = 12.69 μmol/L). Among the synthesized compounds, quinoline series **6(d–i)** had shown better activity than indole **6(a–c)** and furan **6(j–l)** series. The higher activity for quinolines may arise due to the presence of an imine unit in the quinoline moiety, which can stabilize an adjacent radical [34]. The higher radical scavenging activity for compounds **6g**, **6h**, and **6i** (*R* = 2-chloro-8-methylquinol-3-yl) can also be explained by noting that the methyl group also helps stabilize an adjacent unpaired electron [35].

3.2.3. In vitro cytotoxicity study

To test for cytotoxicity of titled compounds, we exposed HeLa cells to the test compounds **6(a–l)** and observed the cell morphology. None of the synthesized compounds showed cytotoxicity towards the HeLa cell line up to highest tested concentrations, suggesting the potential for low intrinsic toxicity toward human cells. The lack of cytotoxicity effects of compound **6i** on the HeLa cell line is shown in Fig. 2.

3.3. Computational studies

3.3.1. Docking study

A molecular docking study of the synthesized compounds **6(a–l)** was performed against the *Adenine phosphoribosyltransferase* enzyme of *L. donovani* to understand the possible binding mode for the test compounds. *Adenine phosphoribosyltransferase*, a Leishmania enzyme, is responsible for pyrophosphorolysis in sequencing and amplification of nucleic acids in protein synthesis [36] and is a potential target for chemotherapeutic intervention. The docking calculations and hydrogen bonds interactions noted are shown in Table 2. The modeling results for compounds **6(a–l)** correlate in general with the observed antileishmanial activity. The docking results indicated that the 3-substituted-4-hydroxycoumarin core of the compounds **6(a–l)** is held in the active site by a combination of various hydrophobic and van der Waals interactions with the enzyme. Major hydrophobic interactions occur between the 3-substituted-4-hydroxycoumarin core and with the active side chain of VAL39, PRO40, GLU120, TYR121, LYS122, GLU123, ALA124, ALA150, THR151, and LEU181. The various methylene groups (–CH_2–) form strong hydrophobic interactions with active site hydrophobic residues. H-bonding groups in the coumarin core (OH, C=O, and –O–) formed hydrogen bonds with ARG37, GLU120, and LYS186, thus suggesting that the central core is important for inhibiting of *Adenine phosphoribosyltransferase* enzyme of *L. donovani.*

The most active antileishmanial compound **6i** showed the lowest binding energy in the docking study, -65.40 kcal/mol. The

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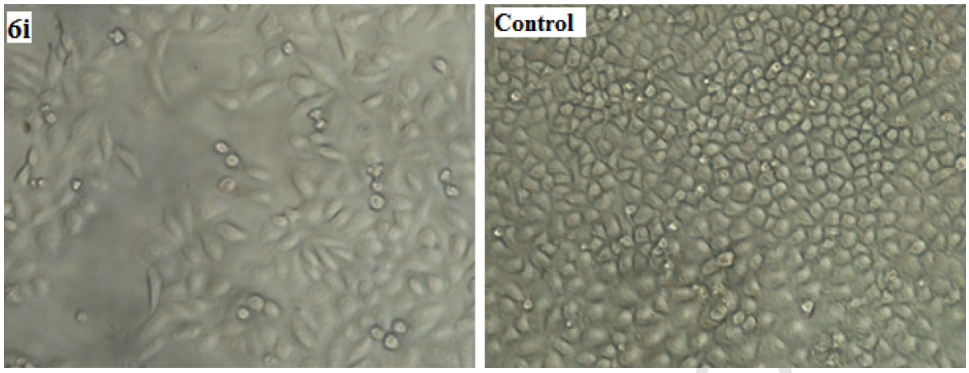

Fig. 2. Cytotoxic study of compound **6i**.

Table 3
Pharmacokinetic parameters important for good oral bioavailability of synthesized compounds **6(a–l)**.

Entry	% ABS	TPSA (A^2)	*n*-ROTB	MV	MW	miLog *P*	*n*-ON acceptors	*n*-OHNH donors	Lipinski's violations
Rule	–	–	–	–	≤500	≤5	≤10	≤5	≤1
6a	81.84	78.70	3	331.95	376.41	3.22	6	2	0
6b	85.03	69.46	3	339.76	374.44	4.29	5	2	0
6c	83.91	72.70	3	352.31	389.45	3.27	6	2	0
6d	82.95	75.80	3	356.34	422.86	4.03	6	1	0
6e	86.03	66.56	3	364.16	420.89	4.89	5	1	0
6f	84.91	69.80	3	376.70	435.91	4.07	6	1	0
6g	82.84	75.80	3	372.90	436.89	4.43	6	1	0
6h	86.02	66.59	3	380.72	434.92	4.79	5	1	0
6i	84.91	69.80	3	393.26	449.93	4.47	6	1	0
6j	82.76	76.05	3	284.54	327.33	2.02	6	1	0
6k	85.95	66.81	3	292.36	325.36	3.08	5	1	0
6l	84.83	70.05	3	304.90	340.37	2.07	6	1	0

% ABS: percentage absorption, TPSA: topological polar surface area, *n*-ROTB: number of rotatable bonds, MV: molecular volume, MW: molecular weight, miLog *P*: logarithm of partition coefficient of compound between *n*-octanol and water, *n*-ON acceptors: number of hydrogen bond acceptors, *n*-OHNH donors: number of hydrogen bonds donors.

interactions of compound **6i** with enzyme are shown in Fig. 3. The amino acid ARG37 (2.589 Å) forms hydrogen bonds with the –OH and –O– groups of the 4-hydroxycoumarin nucleus of compound **6i**. Also, both methyl groups of compound **6i** fit well into the active site, forming hydrophobic interactions with the THR151 amino residue. This observation suggests that a 2-chloro-8-methylquinolinyl moiety in combination with 4-methylpiperazinyl group at 3rd position of 4-hydroxycoumarin ring has optimal interactions with the *Adenine phosphoribosyltransferase* enzyme.

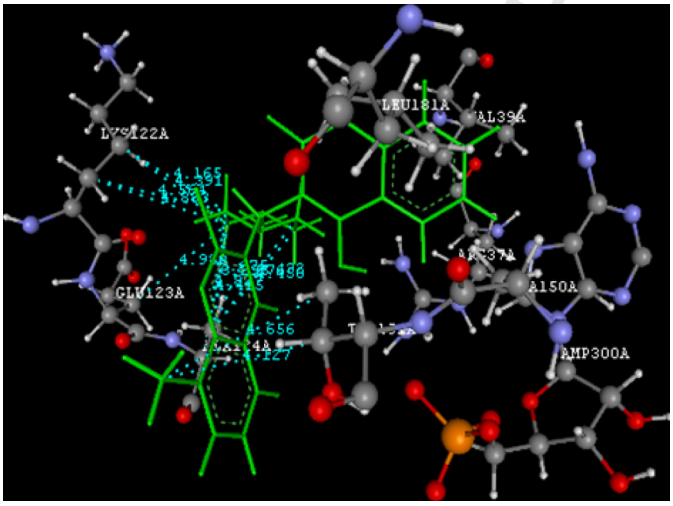

Fig. 3. Docking study of compound **6i** with *Adenine phosphoribosyltransferase* of *L. donovani* (PDB ID: 1QB8). Ligands are shown in green color. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3.2. ADME prediction

Issues of druggability are also important considerations. As shown in Table 3, all of the synthesized compounds exhibited a good % ABS (81.84–86.03%). Furthermore, none of the compounds violated Lipinski's rule of five, suggesting promise for development as good drug candidates. A molecule likely to be developed as an orally active drug candidate should show no more than one violation of the following four criteria: log *P* (octanol–water partition coefficient) ≤ 5, molecular weight ≤ 500, number of hydrogen bond acceptors ≤ 10 and number of hydrogen bond donors ≤ 5 [37]. All the synthesized compounds **6(a–l)** followed the criteria for orally active drug and therefore, these compounds can be further developed as oral drug candidates.

4. Conclusion

In conclusion, we have successfully designed compounds **6(a–l)** based on a natural product-inspired scaffolds. Also, we have developed an expeditious one-pot three component method for the synthesis of novel 3-substituted-4-hydroxycoumarin derivatives **6(a–l)** from 4-hydroxycoumarin, heterocyclic aldehydes and secondary amines in ethanol at room temperature using CAN (10 mol%) as catalyst. The advantages of this method include mild reaction conditions, simplicity, short reaction time, easy work up, and high yields. Several compounds have significant antileishmanial activity against *L. donovani* promastigotes and many also have antioxidant activity. The compounds **6h** (IC_{50} = 9.90 μmol/L) and **6i** (IC_{50} = 6.90 μmol/L) from the quinoline class were the most active antileishmanial agents. The compound **6i** with 2-chloro-8-methylquinolinyl in combination with 4-methylpiperazinyl group at 3rd position of 4-hydroxycoumarin was most potent antileishmanial agent in the series. Three compounds **6c** (IC_{50} = 10.79 μ‴

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mol/L), **6h** (IC_{50} = 10.60 μmol/L), and **6i** (IC_{50} = 10.73 μmol/L) were shown potent antioxidants when compared with standards. None of the synthesized compounds were cytotoxicity to HeLa cell lines upto their highest tested concentrations. A molecular docking study suggested the binding interactions of these compounds with *Adenine phosphoribosyltransferase* of *L. donovani*. Furthermore, analysis of the ADME parameters for synthesized compounds suggested that they have good drug-like properties with potential for oral delivery. The lead compounds will be investigated further in an effort to develop more active, safe and cost-effective antileishmanial and antioxidant agents.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2015.10.028.

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