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Physicochemical properties, antioxidant and anti-inflammatory activities of coumarin-carbonodithioate hybrids

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ABSTRACT

Objective: To study physicochemical properties, antioxidant and anti-inflammatory activities of coumarin-carbonodithioate hybrids. **Methods:** The substituted 4-bromomethyl coumarins were synthesized in first step by the cyclization. Then the reaction of substituted coumarins (a-e) with potassium O-ethyl/methyl carbonodithioate (1) by using absolute ethanol as solvent, afforded coumarin-carbonodithioate (1a-1j) derivatives under microwave irradiation and the conventional method. The spectroscopic analysis was used for the characterization of coumarin derivatives. The title (1a-1j) compounds were confirmed by spectroscopic methods. Antioxidant property was evaluated by using DPPH free radical-scavenging ability assay method and anti-inflammatory activity was evaluated by protein denaturation procedure using diclofenac sodium as a standard. Drug-likeness. *In-silico* toxicity was predicted with LD_{50} value and bioactivity score was also calculated for all the compounds. **Results:** All coumarin (1a-1j) compounds exhibited promising *in-vitro* antioxidant and anti-inflammatory properties in comparison to standard drugs. All tested compounds were used for evaluating their physicochemical properties as set by Lipinski rule. It was observed that the synthesized compounds followed rule of five, indicating more 'drug-like' nature. **Conclusions:** All the screened coumarin-carbonodithioates display promising *in vitro* antioxidant and anti-inflammatory activities. From the physicochemical properties of coumarin derivatives, it is found that none of the compounds violate the Lipinski rule and they fall well in the range of rule of five. It is concluded that the coumarin-carbonodithioate hybrids act with more 'drug-like' nature.

1. Introduction

Many organic reactions are carried out in microwave (MW) irradiators for chemical transformations, which has a great importance[1]. This technology has played a significant role in the development, such as enhanced reaction rates and good yields with high purity[2,3]. In these conscious days of deteriorating environment, eco-friendly reactions are successfully carried out by MW irradiators to obtain most important bioorganic molecules such as coumarin containing heterocycles[4].

Design of drug development work aims for small active molecules,

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which leads to ingestible capsules or tablets. If the tablet dissolves in the digestive tract, the active contents of the drug are absorbed as small molecules, and chemical composition of the drug often helps to easily enter into the cell membranes and transport to almost any region in the body[5,6]. From these above information, in 1997, Christopher Lipinski, a medicinal chemist and co-workers[7], examined the physicochemical parameters of over 2 000 drugs and concluded that a compound, if it matches the following criterion, is more liable to be a drug candidate and easily retained in the body:

•A molecular weight of the chemical compound should fall inside 500 Daltons.

•Lipophilicity of the compound, expressed as a quantity logP is below 5 (given by logP < 5).

•The number of functionalities in the compound that can give hydrogen atoms to hydrogen bonds is less than 5.

•The number of functionalities that can approve hydrogen atoms to form hydrogen bonds should be below 10.

•Molar refractivity should be between 40-130.

Today, the rule of five (RO5) is widely used by medicinal chemists worldwide to assess not only the absorption of compounds but also specific drug-similarity[8]. Hence, with these highlights in mind, we designed coumarin compounds and analyzed them for their physicochemical properties set by RO5, drug-likeness, toxicity prediction with LD_{50} value, and bioactivity scores. It was found that none of the derived conjugates go against the rule and they are accomplished within the frame of RO5.

Naturally derived products are greatly employed as medicines; amongst them coumarins possess vital and remarkable biological activities. Along with the natural sources of coumarins, there are various synthetic routes that have been developed with enhanced bio-medicinal properties[9]. In particular, coumarin compounds play an important role in drug discovery due to its unique structure. They have a special attribution, which permits their derivatives to easily bind by weak bond interactions with different receptors in enzymes and in organisms, and display more pharmacological properties as medicinal drugs. The various properties of natural and synthetic coumarins depend on their chemical structures. Coumarins are the class of oxygen-containing heterocycles. Across the globe, a lot of researchers have designed and synthesized coumarin containing heterocycles for the treatment of various diseases[10]. Thus, a particular potentiality with binding capacity and their superior physiological feature that coumarin containing drugs possess in the eradication of diseases such as cancer, HIV, Tuberculosis, *etc*., have become an extreme highlight[11,12].

The reactive oxygen species (ROS) plays a vital part in the normal physiological process. In eukaryotic cells, these ROS are generated along with a result of aerobic metabolism. In cell physiology, the concentrations of ROS at low-to-high make an impact over activity, *viz*, directive of cellular signal transduction pathways, resistance against pathogens and cell development[13,14]. Simultaneously an extra production of unstable, highly ROS is regarded as the main donor to cellular and metabolic variations. In many degenerative diseases, the oxidative stress has to play the main role in the pathogenesis *i.e.* inflammatory, cancer, diabetes; tumor growth and Alzheimer's disease are contributed by increased cell oxidation[15]. In modern drug development system, maintaining the balance between antioxidant defense system and ROS formation is believed to be a crucial concept for healthy biological systems[16]. Consequently, this is of urgency to invent new derivations with less priced, chemically derived antioxidants for formulations in food and pharmaceutical areas. Most of the coumarin compounds are of plant origin, and chemically derived molecules, which have essential components and can be used to regulate oxidation and in stress-related chronic diseases such as cardiovascular diseases and diabetes. The potential properties of coumarins are directly related to their chemical nature to eliminate free radicals[17] by undergoing oxidation, producing toxic compounds, which generate effects on infective micro-organisms[18]. As a normal prophylactic response of damaged tissue, inflammation is a common symptom and has impact on some chronic diseases[19]. Inflammation is the denaturation of proteins at tissue level, which is known fact that leads to inflammatory and arthritic diseases[20]. Under inflammatory effects, some chemical mediators are released[21]. Various heterocyclic compounds containing coumarins have been used to treat inflammatory disorders since last two decades. From the literature, it is evident that most of the drugs containing coumarins are helpful for the treatment of the inflammatory disorder[22,23].

In continuation of our earlier research work on coumarin-carbonodithioates[24] synthesis, we extended work for *in-vitro* biological evaluation of their immune-modulatory potential. Here we described a novel multi-purpose tool for performing nucleophilic condensation reactions, leading to coumarin-carbonodithioates through thioether linkage, with a short reaction time, acutely non-toxic, easy to handle and easy work up without using any purification techniques.

2. Materials and methods

The substituted 4-bromomethyl coumarin (a-e) was synthesized using cyclization reaction of phenols and 4-Bromo ethyl acetoacetate[11]. Further condensation of substituted coumarins (a-e) with potassium O-ethyl/methyl carbonodithioate[25] (1) in ethanol solvent afforded coumarin (1a-1j) conjugates under both MW and conventional method (Figure 1). It was determined that MW synthesis is evidenced as exceedingly fast method which comes out

as better percentage of yields than the conventional method. The improvement was the speed of reaction, being 35-40 times quicker compared to other method.

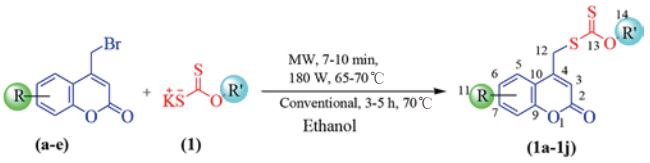

Figure 1. Syntheses of coumarin-carbonodithioate derivatives.

2.1. Representative procedure for synthesis of (1a-1j)

MW method: A mixture of substituted coumarins (0.01 mol) and potassium-O-ethyl dithiocarbonate (0.01 mol) with 5 mL of dry ethanol in the 10mL vial was put into MW irradiator at 60-70 ℃ for 6-10 min and cooled. The reaction progress was monitored by thin layer chromatography. The reaction mass was quenched onto the ice; after filtered, the solid obtained was washed with water.

Conventional method: A mixture of substituted coumarins (0.01 mol) and potassium-O-ethyl dithiocarbonate (0.01 mol) with 5 mL of dry ethanol in 10 mL RB flask was refluxed at 70 ℃ for 3-6 h and cooled. The product formation was confirmed by thin layer chromatography and followed the procedure as prescribed in MW method.

The spectroscopic data of title compounds were confirmed. For compound (1a), in IR spectrum the stretching band at 1 712 cm^{-1} assigned for -C=O, the band at 1 044 cm^{-1} assigned to the thiocarbonyl group, stretching frequency of S-OEt group observed at 942 cm^{-1}. Further proton-NMR spectra, one triplet corresponding to –C7-H and –C8-H coumarin resonated at 7.33-7.37 ppm (J = 6.2 Hz). Adjacent to this, –C5-H appeared at δ 7.22 ppm as a singlet, one more singlet corresponding to –C3-H appeared at δ 6.54 ppm. Remaining all protons resonated at their expected values. ^{13}C NMR furnished extra support for the compound 1a. The thiocarbonyl and the lactone carbonyl carbons resonated at δ 211.86 and 160.66 ppm respectively. The remaining carbons have shown signals in their expected values. Finally, mass spectrum provided an extra supporting data to the architecture of compound 1a {m/z at 294 [M^+]}. The remaining coumarin derivatives gave satisfactory results, which were correlated with their structures.

2.2. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability assay

The radical scavenging property of coumarin (1a-1j) molecules was examined by DPPH free radical-scavenging ability assay, and DPPH radical was used as a reagent[26]. One hundred μL of a stock solution in ethanol (60 μM) was thoroughly stirred with 100 μL of the sample solution in 0.5% DMSO (at different w/v). The resultant solution was placed for incubation at RT for half an hour in the dark and then measured absorbance at 517 nm using UV-VIS spectrophotometer. The reference standard used was ascorbic acid. The DPPH scavenging activity of all coumarin compounds was examined using the following equation:

% inhibition = $(A_c-A_t)/A_c\times100$

A_c is the absorbance of control solution, and A_t is the absorbance of all derivative samples. Experiment was carried out in triplicate. The indication of higher free radical properties was based on lower absorbance of the compound mixtures.

2.3. Evaluation of in vitro anti-inflammatory activity

The anti-inflammatory property of coumarin (1a-1j) compounds was investigated by protein denaturation method[27]. Diclofenac sodium, a powerful non-steroidal drug, was used as a standard compound. A mixture of 2 mL of varied concentrations of coumarin conjugates (500 μg/mL) with diclofenac sodium and 2.8 mL of pH 6.4 phosphate buffer solution was stirred thoroughly with 2 mL of egg albumin and placed in incubation at (27±1) ℃ for 15 min. Denaturation was brought about by maintaining the final solution at 70 ℃ for 10 min, followed by cooling and measuring its absorbance at 660 nm using double distilled-water as a blank and was calculated in triplicate. The % inhibition is examined by using the following expression:

% inhibition = $(A_t-A_c)/A_c\times100$

Where A_t is the absorbance of sample solution and A_c is the absorbance of control.

2.4. Physicochemical properties

The theoretical calculation of adsorption, distribution, metablism, and excretion - toxicity properties for synthesized compounds was done and compared with RO5. This was expressed as octanol/water partition coefficient, also called as logP. Besides, other theoretical calculations were carried out such as topological polar surface area, number of hydrogen bond acceptors (n-ON) and number of hydrogen bond donors (n-OHNH).

2.5. Statistical analysis

The result of all compounds was expressed as mean ± SD. To examine the variation and level of statistical significances, one-way ANOVA was used between groups. $P < 0.05$ was considered statistically significant difference.

3. Results

3.1. In vitro antioxidant assay

Here the different concentrations of coumarin compounds were subjected to DPPH free radical scavenging technique. The antioxidant capacity of all the molecules was compared with standard antioxidant. The results revealed that 1b, 1c, 1e, 1f, and 1j among the compounds were highly active. Similarly, the compounds 1a, 1d, 1h and 1i showed good activity, whereas the only compound 1g found least active compared with standard ascorbic acid. The results were demonstrated in Table 1.

3.2. In vitro anti-inflammatory assay

Obtained results revealed a concentration-dependent inhibition by determining anti-inflammatory activity of coumarin derivatives concentration and standard drug diclofenac sodium at 100 μg/mL. The results of the coumarin compounds were comparable to that of diclofenac sodium. A significant difference in the thermally induced inhibition of denaturation of protein was found in 1b, 1f, 1h, and 1i bearing methyl and methoxy derivatives and these were highly active. Similarly, 1a, 1c, 1d, 1e, 1g, and 1j derivatives showed moderate inhibitory activity. The results were given in Table 2. From Table 2, it could be concluded that synthetic compounds showed effective inhibition of the protein denaturation. With this, evidence-based studies were required to regulate the components behind its anti-inflammatory actions and its mechanisms.

Table 2

In-vitro anti-inflammatory activity results by protein denaturation method.

Samples	Concentration (μg)	% Inhibition
Diclofenac sodium	100	88.883±2.622
1a	100	49.647±3.943
1b	100	81.247±2.085**
1c	100	42.530±5.517
1d	100	46.003±1.672
1e	100	46.177±3.842
1f	100	81.940±1.594
1g	100	46.523±2.165
1h	100	77.773±1.589**
1i	100	85.063±1.589**
1j	100	47.217±1.826

**$P<0.01$.

Table 1

In-vitro antioxidant screening results by DPPH free radical scavenging assay.

Samples	Concentration (μg)				
	10	20	30	40	50
1a	31.760±0.837**	40.420±1.052**	49.820±0.437**	55.870±0.669**	65.170±1.420**
1b	53.160±0.320**	59.910±0.625**	67.600±1.175**	70.590±0.910**	80.650±0.642**
1c	41.470±0.936*	52.530±0.869*	55.490±0.493*	61.230±0.432*	71.180±0.625*
1d	41.510±0.813*	45.860±0.336*	53.160±0.529*	62.420±0.185*	66.280±0.651*
1e	51.390±0.628*	59.180±0.730*	69.310±0.479*	74.070±0.493*	77.620±0.794*
1f	52.290±0.279**	59.150±0.869**	66.840±1.082**	70.410±1.225**	80.580±0.210**
1g	31.450±0.837**	39.830±1.052**	46.890±0.336**	60.780±0.732**	53.680±0.869**
1h	42.550±0.813**	49.540±0.493**	54.240±0.471**	70.870±0.478**	56.640±0.493**
1i	52.920±0.582**	55.560±0.669**	63.460±0.179**	75.640±0.495**	62.380±0.437**
1j	42.600±0.942**	65.480±1.418**	67.320±0.651**	79.190±0.799**	72.330±0.601**
Ascorbic acid	71.040±0.336**	76.270±0.315**	80.060±0.447**	82.740±0.433**	85.550±0.366**

**$P<0.01$; *$P<0.05$.

Table 3

Drug-likeness property (RO5) of compounds (1a-1j).

Compd	Lipinski's parameters				TPSA	Molar volume (A^3)	Drug likeliness	Bioactivity score					
	HBA	HBD	logP	Violations				GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1a	3	0	3.63	0	39.45	250.29	0.41	-0.99	-1.08	-1.05	-0.63	-0.56	-0.38
1b	3	0	3.63	0	39.45	250.29	0.38	-1.00	-1.07	-1.08	-0.67	-0.59	-0.40
1c	4	0	3.24	0	48.68	259.27	0.28	-0.90	-1.02	-0.91	-0.51	-0.51	-0.32
1d	3	0	3.86	0	3.86	247.26	0.23	-0.95	-0.99	-1.01	-0.62	-0.53	-0.35
1e	4	1	2.70	0	59.67	241.74	-0.09	-0.91	-0.96	-0.94	-0.40	-0.52	-0.23
1f	3	0	3.25	0	39.45	233.49	0.26	-1.03	-1.11	-1.06	-0.72	-0.67	-0.35
1g	3	0	3.25	0	39.45	233.49	0.23	-1.03	-1.09	-1.09	-0.76	-0.70	-0.37
1h	4	0	2.86	0	48.68	242.47	0.08	-0.91	-1.02	-0.90	-0.57	-0.58	-0.27
1i	3	0	3.48	0	39.45	230.46	0.07	-0.98	-1.01	-1.02	-0.72	-0.64	-0.32
1j	4	1	2.32	0	59.67	224.94	-0.13	-0.94	-0.98	-0.94	-0.48	-0.62	-0.20

HBA: Number of hydrogen bond acceptors (n-ON); HBD: Number of hydrogen bond donors (n-OHNH); logP: Logarithm of partition coefficient between n-octanol and water (miLogP); TPSA: Topological polar surface area; GPCR: G-protein-coupled receptors.

3.3. Drug-likeness, bioactivity score and toxicity prediction

It was observed that these compounds were in agreement with RO5, indicating more 'drug-like' nature. These calculations for all compounds were summarized in Table 3. It was found that the all coumarin-carbonodithioates indicating more drug-like nature and bioactive computability.

The predicted LD_{50} with 650-1 500 mg/kg for all the compounds was summarized in Table 4. As claimed by the developer's limits, all the synthesized compounds come under the category of class 4 toxicity except hydroxyl substituted compounds which were of class 5 toxicity category and there were no toxic fragments present. This toxicity prediction study revealed that coumarin compounds could act as the lead compounds for further detailed investigations. Based on the investigation of *in-silico* toxicology, all the compounds have shown median LD_{50} values ranging from 625 to 1 500 mg/kg. Compounds 1b, 1d and 1i showed LD_{50} values of 625 mg/kg while only one compound 1 g showed 1 000mg/kg. Furthermore, compounds 1a, 1c, 1f and 1h have shown LD_{50} values of 1 250 mg/kg and remaining derivatives 1e and 1j LD_{50} values of 1 500 mg/kg. Almost all the targeted compounds belonged to toxicity class of 4 and none of them showed toxicity fragments. The predicted results of all the compounds were tabulated in Table 4.

Table 4

Oral toxicity prediction results of coumarin-carbonodithioate derivatives (1a-1j).

Compounds	Predicted LD_{50} (mg/kg)	Predicted toxicity class	Average similarity (%)	Prediction accuracy (%)	Toxic fragments
1a	1 250	4	60.03	67.38	Nil
1b	625	4	61.52	67.38	Nil
1c	1 250	4	59.70	54.26	Nil
1d	625	4	57.79	54.26	Nil
1e	1 500	5	68.41	67.38	Nil
1f	1 250	4	62.83	67.38	Nil
1g	1 000	4	64.53	67.38	Nil
1h	1 250	4	61.52	67.38	Nil
1i	625	4	60.37	67.38	Nil
1j	1 500	5	71.50	68.07	Nil

Nil: None of toxic fragments were found.

4. Discussion

Natural antioxidants make an impact over the enhancement in the antioxidant capacity of blood plasma and help in the prevention of many diseases. Coumarins are also best-known to have several biological properties such as anti-inflammatory, anti-tumor, and antioxidant activities. Thus, in the present study, the coumarin-based biomolecules are synthesized using MW irradiation technique, which would help in the exploration of novel drugs in synthetic organic chemistry. In summary, a series of new coumarin (1a-1j) compounds were found under MW conditions with a short reaction time, non-toxic, easy to handle and simple work up without any purification techniques.

DPPH free radical scavenging assay is an easy method for screening the *in-vitro* antioxidant properties of coumarin compounds. In the present study, antioxidant activity results revealed that the compounds with substituted coumarins were prime candidates to exhibit excellent activity. Compounds 1b, 1c, and 1e showed higher activity, while the remaining derivatives were moderately active. A significant difference was observed among antioxidant activities of all synthesized compounds and the methyl substituted coumarins came out as a superior total antioxidant capacity, with significantly higher results in all performed.

For many chronic diseases, inflammation is a common symptom. Presently in the market, non-steroidal anti-inflammatory drugs are used for inflammatory diseases but are having side effects of an ulcer, and many others[28]. Drugs which are designed particularly are in demand for management of inflammation due to their fewer side effects and cost-effective. Thus, in the present study simple/ viable protein denaturation method was used to screen the anti-inflammatory activity of the coumarin-carbonodithioates. All the derived biomolecules were subjected to anti-inflammatory assay with diclofenac sodium as a reference drug. In comparison to the reference drug, -chloro substituted coumarins showed higher inhibitory activity followed by methyl substitutions whereas remaining derivatives exhibited moderate activity. However, based on this promising observation, it is immature to arrive at the conclusion on a structure-activity aspect of these molecules and further evaluation is needed for their clinical use.

The toxicity prediction study reveals that coumarin compounds can act as the lead compounds for further investigations and potent applications of pharmacological interest. From overall findings, these studies suggest that all the potentialities are because of oxygenated coumarin heterocycle and later enhanced by condensing carbonodithioates. In conclusion, the coumarin compounds can be utilized as antioxidants to inhibit the occurrence and approach of many diseases. However, the further detailed study of coumarins is needed for the exploration of antioxidant and anti-inflammatory drugs.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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