Original Article

Design, Synthesis, Physicochemical Characterization And Biological (Antioxidant And Antiurease) Evaluation Of Newer Coumarin Derivatives

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Abstract

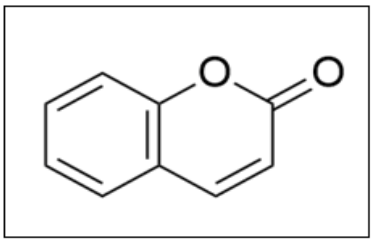
Recently wider research community has become more interested in the antioxidant activity of organic compounds, particularly to prevent the harmful consequences induced by free radicals in the human body. Coumarin continues to be the scaffold of choice despite repeated attempts to find antioxidant agents that are more potent. It has proven useful in squenching free radicals, and as a result, it offers enormous promise for investigation as a candidate molecule for drug discovery to treat illnesses brought on by oxidative damage. Also urease inhibition helps to fight against a number diseases that are mentioned above so there is a need to develop more compounds with anti-urease potential. So, in our present work we have synthesised novel coumarin derivatives (**D_1-D_{10}**) and tested them for various biological functions (antioxidant and antiurease). The antioxidant and urease inhibitory activities of the compounds **D_9** and **D_5** revealed that they were the most active ones as compared to the reference.

Keywords: Synthesis, Coumarin, antioxidant, urease inhibitors

1. INTRODUCTION

Coumarin, also known as 2*H*-chromen-2one, is a member of the benzopyrone family and is also known as benzopyrane-2-one[1]. The primary chemical structure of a class of phytochemicals that are naturally present in many plant species is an oxygen heterocycle known as coumarin. But they have also been discovered in bacteria and animal sources. Additionally, coumarin serves as the basis for a number of compounds with significant therapeutic applications, including novobiocin, coumaromycin, and chartesium. This family of chemicals was divided into various categories as a result of the structural diversity that was discovered there, ranging from simple coumarins to numerous types of coumarins. Simple coumarin is well known for having a scent that is similar to vanilla. In order to make scents and essences, coumarins have primarily been manufactured or created artificially[2].

The Basic structure of coumarin moiety is shown below:



Basic structure of coumarin moiety

Different properties of coumarin are shown in **Table 1.**

Table 1: Properties of Coumarin

S. No.	Property	Description
1.	IUPAC Name	2*H*-1-Benzopyran-2-one
2.	Molecular Formula	$C_9H_6O_2$
3.	Boiling point	301.7°C
4.	Melting point	71°C
5.	Molar mass	146.147g/mol
6.	Pubchem CID	323

7.	Appearance	Colour less to white powder or crytals
8.	Solubility	Water(0.17g/mol) and easily soluble in ether, diethyl ether, chloroform etc.
9.	Log P	1.39
10.	Vapour pressure	1.3hPa
11.	Magnetic susceptibility	$-82.5X10^{-6}cm^3/mol$.
12.	Flash point	150°C
13.	LD_{50}	293mg/kg[Rat,oral]

Coumarins are a broad class of heterocyclic compounds having a benzo-pyrone component that are found in both natural and synthetic sources. Sweet clover and Tonka bean are two examples of plants that naturally contain the chemical coumarin. It was utilized as a flavoring additive because of its aromatic odour, which is often compared to the sweet scent of newly mown hay. Plants contain coumarins in large quantities; for instance, coumarin, the most typical molecule, was first discovered in tonka bean (*Dipteryx odorata Wild*), and it has received substantial research in both the biochemical and medicinal disciplines. Dicoumarol, a naturally occurring anticoagulant, was found in mouldy, moist, sweet-clover hay. While scoparone, with potential pharmacological qualities including immunosuppression and vasorelaxation, was found in *Artemisia scoparia*, osthole, which has recently attracted significant attention due to its wide range of pharmacologic activity, was discovered in *Cnidium monnieri*[3].
Numerous medications containing coumarin moeity are available in the market as follows:

- Simple Coumarin: Esculetin has Antioxidant activity[4]
- Furano coumarins: Bergapten has Anti-TB activity[5]
- Dihydrofurano coumarins: Felamidin has Antibacterial activity[6]
- Pyrano coumarins: Grandivittin has Antibacterial activity[7]
- Phenyl coumarins: Disparinol has Analgesic activity[8]
- Bicoumarins: Dicoumarol has Anticoagulant activity[9]

Urease, an enzyme, helps urea break down into ammonia and carbon dioxide. Then, urea hydrolyzes spontaneously at a rate that is around 1014 times quicker than the uncatalyzed process to produce carbonic acid and a second molecule of ammonia. In recent years, urease has been implicated in the pathogenesis of a number of clinical illnesses[10]. Hence there is a need to search for more compounds which can be utilised as effective urease inhibitors.
A class of naturally occurring phenolic compounds known as coumarins, which have a number of biological activities, are made up of fused benzene and pyrone rings. Coumarins may be employed in specific types of human illnesses such auto-immune disease, cardiovascular, brucellosis, burns, rheumatic, cancer, neurological, and hepatic[11]. The said moeity has been more useful as intermediates in the synthesis of novel active chemicals in medicinal chemistry in recent years. Coumarins are an important heterocyclic nucleus with a wide range of biological activities, attracting researchers from all over the world to synthesise and test numerous coumarin derivatives for their biological activities[12].
Because of their biological characteristics, coumarins are of tremendous interest[13]. According to the literature, Coumarin analogues have found favour in a number of therapeutic fields, including antimicrobial[14], antiviral[15], anti-inflammatory[16], antioxidant[17], cytotoxic[18], anti-cancer[19], antimycobacterial[20], antihistaminic[21], anthelmintic[22], analgesic[23], antidepressant[24], antiparkinson[25] and anticoagulant[26].
The literature reveals that the coumarin is a unique template which is related with a wide spectrum of biological activities like antimicrobial, anticancer, antioxidant, antiurease etc. The therapeutic potential of coumarin derivatives attract the researchers for new inventions in pharmaceutical field.
Design of coumarin analogues based on biological profile is depicted in **fig 1**.

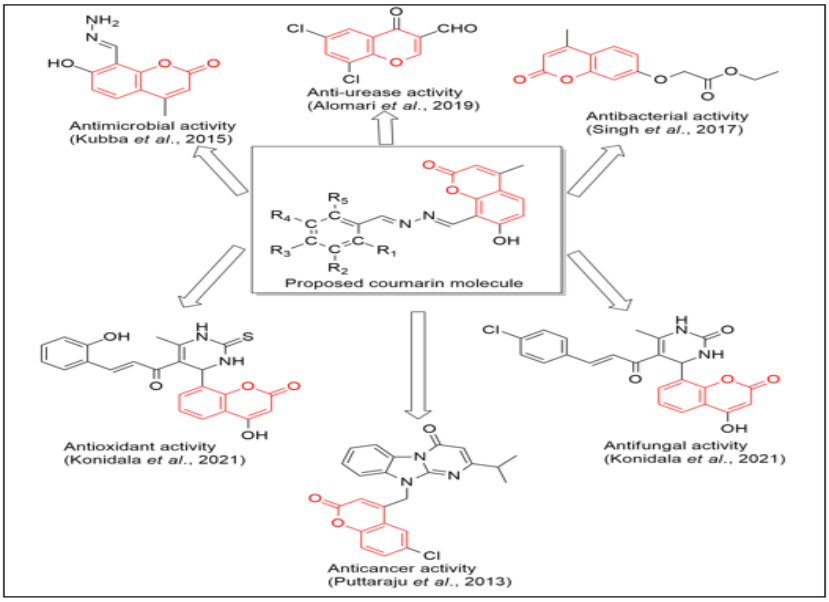


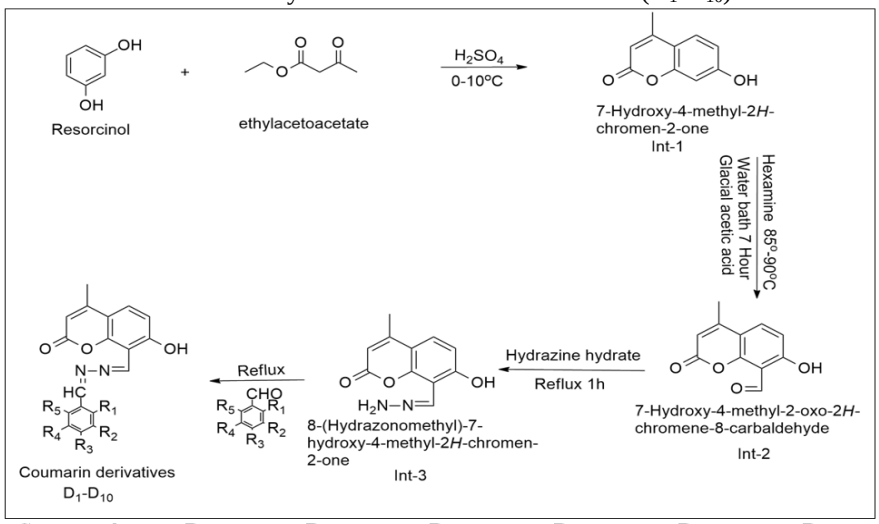
Fig 1: Biological Profile of Coumarin derivatives

2. EXPERIMENTAL WORK

2.1. Material and instruments

The starting material, solvents and reagents were purchased from Loba chemie. Glassware was purchased from Borosil. On a calibrated weighing balance, the raw material was weighed. Using ChemDraw Ultra 12.0 the synthetic scheme was drawn. TLC was performed for reaction confirmation at each stage. Bruker 12060280, Software: OPUS 7.2.139.1294 spectrometer using ATR for IR spectra (cm^{-1}) and Bruker Avance III at 600 NMR and 150 MHz for 1H (DMSO-d6, δ ppm) were used for spectral characterizations of synthesised derivatives.

Scheme 1: Synthesis of coumarin derivatives (D_1-D_{10})



Compound	R	R_1	R_2	R_3	R_4	R_5
D_1	-	H	H	Br	H	H
D_2	-	H	H	H	Cl	H
D_3	-	OH	H	H	Br	H
D_4	-	H	H	OH	H	H
D_5	-	H	H	OCH_3	OCH_3	H
D_6	-	H	H	H	NO_2	H
D_7	-	H	H	H	OH	H
D_8	-	Cl	H	H	H	H
D_9	-	H	H	CHO	H	H
D_{10}	-	OCH_3	H	H	H	H

2.2. Synthetic Procedure for synthesis of coumarin derivatives (D_1-D_{10})

Step 1:- Synthesis of 7-hydroxy-4-methyl coumarin (3)

Compound 3 was prepared by careful addition of 100 ml of conc. sulfuric acid in the ice bath, resorcinol (0.01 mole, 11 g) and ethyloacetoacetate (0.01 mole, 13 ml). All chemicals were added dropwise over the course of 30 minutes with constant stirring. After being held at room temperature for three hours, the reaction mixture was put into a container with ice and water and was vigorously stirred. To obtain the pure product, ppt was filtered out, washed by water, then dried, and then recrystallized from ethanol.

Step 2:- Synthesis of 8-formyl-7-hydroxy-4-methyl coumarin (4)

Hexamethylenetetramine (0.07 moles, 9.8 g) was added in **3** and warmed at 85–90ºC for seven hours on a water bath. The hexamine adduct thus produced was hydrolyzed using 75 ml of 20% HCl, and the mixture was then heated for an additional 30 minutes. Diethyl ether (50 ml) was twice extracted from the reaction mixture after cooling, and the ether layer was then evaporated. The yellow crystals that were produced after recrystallizing with ethanol.

Step 3:-Synthesis of 8-(hydrazineylidenemethyl)-7-hydroxy-4-methyl-2*H*-chromen-2-one

0.01 mole of hydrazine hydrate 99% was added into **(4)** and refluxed in RBF for 1 hour in the presence of absolute ethanol as a solvent, the obtained ppt was collected by filtration, dried and recrystallized from ethanol and creamy coloured precipitates were collected.

Step 4:- Preparation of coumarin derivatives (D_1-D_{10})

The right amount of compound **(4)** was combined with various substituted aldehydes, some drops of concentrated H_2SO_4 (sulphuric acid), and refluxed for right duration of time, depending upon the completion of reaction which was monitored by TLC, to produce various coumarin derivatives, which were then recrystallized with ethanol.

2.3. Biological studies

2.3.1. *In vitro* antioxidant evaluation

Compounds (**D_1-D_{10}**) were assessed for their DPPH free radical scavenging activity using ascorbic acid as the reference compound and the IC_{50} was computed. The results are summarised in Table 2 and displayed in Figures 2 and 3. With

higher sample compound concentrations, the scavenging effect grew. The relatively stable nitrogen- centered free radical known as DPPH is easily converted. When DPPH radicals interact with suitable reducing agents, the electrons pair up and create the matching hydrazine. As a result, the amount of electrons taken up determines how much colour the solution loses. percent 50 millilitres of various concentrations of the compounds (20, 40, 60, 80, and 100 g/ml) were added. The sample having dark environment & then absorbance had measured at 517 nm in comparison to the control solution. The following equation was used to determine the relative percent of DPPH scavenging activity [31].

$$\mathbf{I\ \% = A_{control} - A_{sample} / A_{control} \times 100}$$

Where $\mathbf{A_{control}}$ is absorbance of control, $\mathbf{A_{sample}}$ is absorbance of test compound

2.3.2. Urease inhibitory evaluation

The Jack Bean Urease by Indophenol technique was used to assess the urease inhibitory ability of each synthesised derivatives (**D_1-D_{10}**). The results are summarised in Table 3 and displayed in Figures 4 and 5. 250 loof jack bean urease (4U) was combined with 250µl of various synthesised derivatives and standard at various concentrations, all of which were dissolved in a 1:1 v/ v combination of DMSO and water. Pre-incubation of the mixture took place in test tubes for an hour at 37 °C. A pH 6.8 phosphate buffer solution containing 5000 mM urea and 0.002 percent phenol red was then added after the sample test tubes had been pre-incubated. After that, the test tubes underwent another incubation at room temperature. The absorbance of the reaction mixture at 570 nm was measured by the UV Spectrophotometer. The urease enzyme converted urea to phosphate buffer, whose peak pH was measured by the colour of the phenol red indicator and elevated from 6.8 to 7.7 by ammonium carbonate[31].

$$\mathbf{I\ \% = A_{control} - A_{sample} / A_{control} \times 100}$$

Where $\mathbf{A_{control}}$ is absorbance of control, $\mathbf{A_{sample}}$ is absorbance of test compound

3. RESULTS & DISCUSSION

3.1. Chemistry

The multistep synthesis process of coumarin derivatives **(D_1-D_{10})** is depicted in **Scheme 1**. Initially, 7-Hydroxy-4-methyl-2*H*-chromen-2-one (**Int-1**) coumarin was prepared by reaction of resorcinol & ethylacetoacetate, after that hexamine was added followed by addition of glacial acetic acid to obtain 7- hydroxy- 4- methyl- 2- oxo- 2*H*- chromene- 8- carbaldehyde (**Int-2**). Hydrazine hydrate was added to obtain 8-(hydrazonomethyl)-7-hydroxy-4-methyl-2*H*-chromen-2-one (**Int-3**). In the final step, title derivatives **(D_1-D_{10})** were obtained by carrying out the reflux with different substituted aromatic aldehydes in ethanol.

The physicochemical characterization of the synthesised derivatives is depicted in the **Table 2.**

Table 2: Physicochemical characterization of synthesized derivatives (D_1-D_{10})

Compound	Mol. Formula	Mol. Wt	Color	R_f value	% Yield
Int-3	$C_{11}H_{10}N_2O_3$	218.21	Pale Yellow	0.75	79%
D_1	$C_{18}H_{13}BrN_2O_3$	385.21	Cream	0.72	69%
D_2	$C_{18}H_{13}ClN_2O_3$	340.76	Orange	0.71	72%
D_3	$C_{18}H_{13}BrN_2O_4$	401.21	Brick Red	0.68	65%
D_4	$C_{18}H_{14}N_2O_4$	322.31	Pale Yellow	0.69	75%
D_5	$C_{20}H_{18}N_2O_5$	366.37	Magenta	0.73	81%
D_6	$C_{18}H_{13}N_3O_5$	351.31	Red	0.63	78
D_7	$C_{18}H_{14}N_2O_4$	322.31	Brown	0.68	69
D_8	$C_{18}H_{13}ClN_2O_3$	340.76	Light Brown	0.62	83
D_9	$C_{19}H_{14}N_2O_4$	334.33	Dark Red	0.65	76
D_{10}	$C_{19}H_{16}N_2O_4$	336.34	Orange	0.69	67

Mobile phase – [Dichloro methane : Methanol : Glacial acetic acid , 9: 0.3: few drops]

Synthesized derivatives of coumarin were confirmed by IR and ^{1}HNMR. The spectro analytical data has been shown in **Table 3**.

All the compounds (**D_1-D_{10}**) showed the peaks of Ar-OH around 3612-3677 cm^{-1} and C=O at around 1700-1743 cm^{-1}. The peak range 1621-1693 cm^{-1} indicated the presence of –C-N str. in all compounds. Peak in the range 2823-2992 cm^{-1} indicated the presence of –C-H str. due to methyl substitution. Peak in the range 1480-1650 cm^{-1} indicated the presence of aromatic C-H str., and C=C str. The peak of Ar-Br in **D_1/ D_3** was in the range 653-699 cm^{-1} whereas the peak of Ar-Cl in **D_2/ D_8** was in the range 676-778 cm^{-1}. The peak in the range 1255-1264 cm^{-1} indicated the presence of -$COCH_3$ str. in **D_5/ D_{10}** The peak of Ar–NO_2 in **D_6** peak was in the range 1461 cm^{-1}.

The obtained compounds spectral signal and suggested molecular structure were visible when the ^{1}HNMR spectra of coumarin derivatives were recorded using DMSO.

We had taken DMSO as solvent for ^{1}H NMR analysis of all derivatives. The triplet signal was present at 7.48- 7.91 δ ppm in **Int-3** and the singlet signal at 1.91 and 8.33 δ ppm showed the availability of -NH_2 and N=CH protons in **Int-3**. The singlet signal 2.34- 2.56 δ ppm showed the availability of -CH_3 protons in synthesized compound **(D_1-D_{10}).** The singlet signal at 8.33-8.98 δ ppm describe the availablity of N=CH protons in synthesized derivatives **D_1-D_{10}** and **Int-3**. The range from 6.23- 8.14 δ ppm shows the availablity of aromatic multiplet protons in **D_1-D_{10}** and **Int-3**. The -OH group proton was identified with singlet signal at 5.31- 5.40 δ ppm in all derivatives and final intermidiate **(Int-3 & D_1-D_{10})**.

Table 3: Spectral characterization of synthesized derivatives

Compound	IR (KBr, cm^{-1}) data	^{1}H NMR (400MHz, DMSO) data
Int-3	3680 (O-H str.), 1732 (C=O str., ketone), 1652 (C=N, N=CH str.), 1258 (-NH_2 str.) 2982 (-CH_3 str.), 1065 (C-O-C str., chromene), 3113 (C-H str., aromatic), 1460 & 1652 (C=C str., aromatic)	1.91 (s, 2H, NH_2), 8.33 (s, 1H, N=CH), 5.31 (s, 1H, OH), 7.48-7.91 (t, 3H, ArH), 2.34 (s, 3H, CH_3)
D_1	3677 (O-H str.), 1704 (C=O str., ketone), 1621 (C=N, N=CH str.), 2992 (-CH_3 str.), 699 (C-Br str.), 1065 (C-O-C str., chromene), 2993 (C-H str., aromatic), 1478 & 1621 (C=C str., aromatic)	6.23-7.91 (m, 7H, ArH), 8.71 (s, 2H, N=CH), 5.38 (s, 1H, OH), 2.56 (s, 3H, CH_3)
D_2	3639 (O-H str.), 1733 (C=O str., ketone), 1653 (C=N, N=CH str.), 2983 (-CH_3 str.), 778 (C-Cl str.), 1066 (C-O-C str., chromene), 2984 (C-H str., aromatic), 1468 & 1653 (C=C str., aromatic)	6.97-7.85 (m, 7H, ArH), 8.50 (s, 2H, N=CH), 5.33 (s, 1H, OH), 2.44 (s, 3H, CH_3)
D_3	3614 (O-H str.), 1743 (C=O str., ketone), 1656 (C=N, N=CH str.), 2836 (-CH_3 str.), 653 (C-Br str.), 1175 (C-O-C str., chromene), 3067 (C-H str., aromatic), 1470 & 1616 (C=C str., aromatic)	6.95-7.90 (m, 6H, ArH), 8.86 (s, 2H, N=CH), 5.37 (s, 1H, OH), 2.40 (s, 3H, CH_3)
D_4	3638 (O-H str.), 1700 (C=O str., ketone), 1641 (C=N, N=CH str.), 2925 (-CH_3 str.), 3705 (C-OH str.), 1162 (C-O-C str., chromene), 2925 (C-H str., aromatic), 1460 & 1607 (C=C str., aromatic)	6.85-7.78 (m, 7H, ArH), 8.59 (s, 2H, N=CH), 5.32 (s, 1H, OH), 2.40 (s, 3H, CH_3)
D_5	3619 (O-H str.), 1706 (C=O str., ketone), 1677 (C=N, N=CH str.), 2923 (-CH_3 str.), 1264 (C-H str., O-CH_3), 1137 (C-O-C str., chromene), 2924 (C-H str., aromatic), 1485 & 1626 (C=C str., aromatic)	7.01-7.58 (m, 6H, ArH), 8.64 (s, 2H, N=CH), 5.39 (s, 1H, OH), 2.40 (s, 3H, CH_3), (s, 6H, OCH_3)
D_6	3615 (O-H str.), 1733 (C=O str., ketone), 1666 (C=N, N=CH str.), 3113 (-CH_3 str.), 1461 (C-NO_2 str.) 1274 (C-O-C str., chromene), 3114 (C-H str., aromatic), 1462 & 1653 (C=C str., aromatic)	6.91-8.12 (m, 7H, ArH), 8.59 (s, 2H, N=CH), 5.34 (s, 1H, OH), 2.37 (s, 3H, CH_3)
D_7	3612 (O-H str.), 1740 (C=O str., ketone), 1693 (C=N, N=CH str.), 3116 (-CH_3 str.), 1162 (C-OH str.), 1271 (C-O-C str., chromene), 3116 (C-H str., aromatic), 1465 & 1646 (C=C str., aromatic)	7-7.93 (m, 7H, ArH), 8.55 (s, 2H, N=CH), 5.35 (s, 2H, OH), 2.41 (s, 3H, CH_3)
D_8	3615 (O-H str.), 1700 (C=O str., ketone), 1666 (C=N, N=CH str.), 3114 (-CH_3 str.) 676 (C-Cl str.), 1167 (C-O-C str., chromene), 3114 (C-H str., aromatic), 1462 & 1652 (C=C str., aromatic)	7.01-8.14 (m, 7H, ArH), 8.98 (s, 2H, N=CH), 5.40 (s, 1H, OH), 2.40 (s, 3H, CH_3)
D_9	3615 (O-H str.), 1700 (C=O str., ketone), 1660 (C=N, N=CH str.), 2987(-CH_3 str.), 1690 (C=O str., aldehyde), 1167 (C-O-C str., chromene), 2987 (C-H str., aromatic), 1435 & 1658 (C=C str., aromatic)	6.99-8.06 (m, 7H, ArH), 8.82 (s, 2H, N=CH), 5.39 (s, 1H, OH), 2.41 (s, 3H, CH_3), 10.09 (s, 1H, CHO)
D_{10}	3615 (O-H str.), 1700 (C=O str., ketone), 1699 (C=N, N=CH str.), 3001 (-CH_3 str.), 1255 (C-H str., O-CH_3), 1167 (C-O-C str., chromene), 3001 (C-H str., aromatic), 1425 & 1587 (C=C str., aromatic)	7.04-8.14 (m, 7H, ArH), 8.51 (s, 2H, N=CH), 5.36 (s, 1H, OH), 2.40 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3)

3.2. *In vitro* antioxidant screening

DPPH free radical scavenging activity of compound **(D_1-D_{10})** was performed and the absorbance was recorded at 517nm. The reference drug was used as Ascorbic acid. The IC_{50} value of the compounds was calculated from the graph plotted between % Inhibition against Concentration (Fig 2 and Fig 3). Results of antioxidant activity showed that Compound **D_9** was found the most active compound having IC_{50} value 69.76µg/ ml as compared with standard drug (75.47µg/ml) Results are shown in **Table 4**.

Table 4: Antioxidant screening results of the synthesized compounds (D_1-D_{10})

Compounds	% inhibition					IC_{50}(µg/ml)
	20(µg/ml)	40(µg/ml)	60(µg/ml)	80(µg/ml)	100(µg/ml)	
D_1	28	38	46	54	63	70.26
D_2	27	36	45	53	61.5	71.68
D_3	22	31	46	55	62	73.07
D_4	24	34	46	55	65	70.09
D_5	23.5	32	44	50	61	76.98
D_6	26.55	35.84	44.28	52.1	63.42	72.36
D_7	23	33.74	44	55.32	63	72.18
D_8	24.5	33	41.5	52	60	77.33
D_9	28	38	46	54	63	69.76
D_{10}	27	36	45	53	61.5	72.79
Ascorbic Acid	42	46.66	49.51	50	52.45	75.47

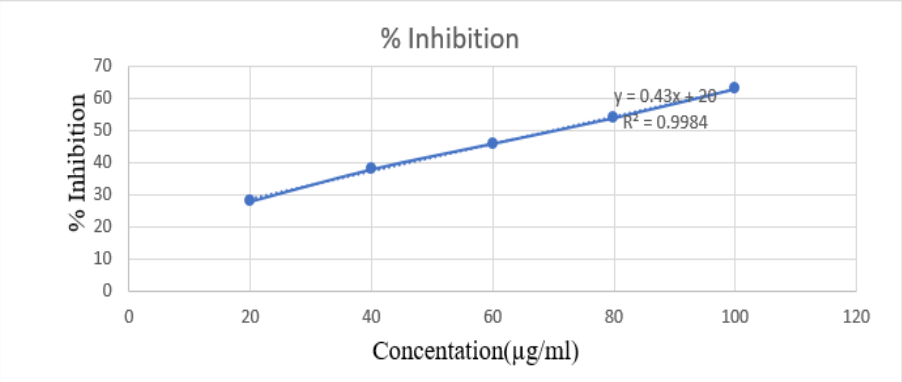


Fig 2:% Inhibition of most active compound D_9

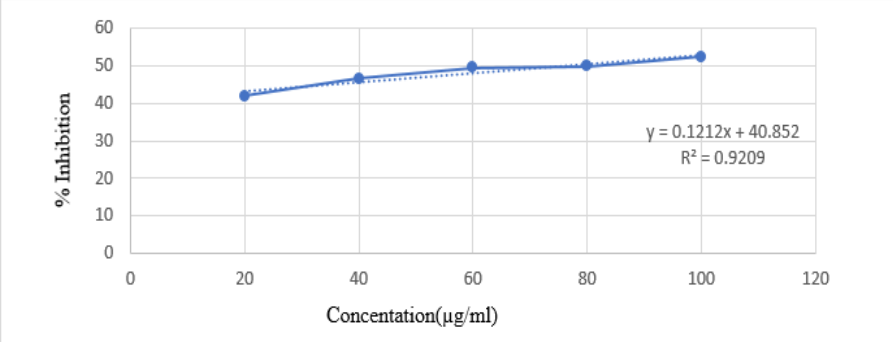


Fig 3: % Inhibiton of Ascorbic Acid

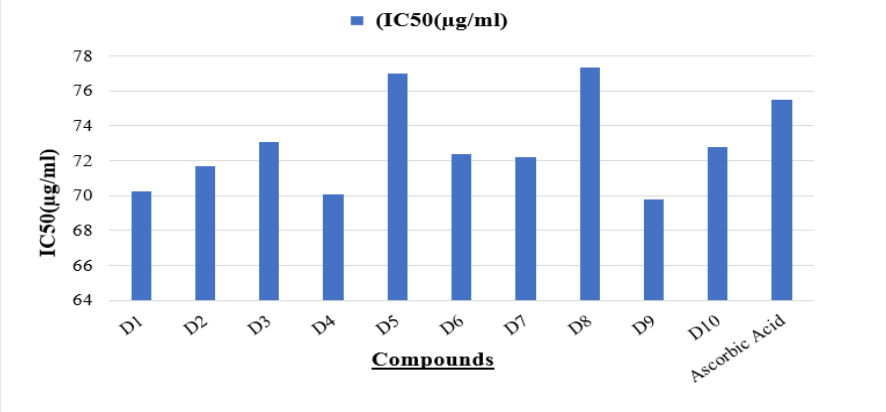


Fig A1: Antioxidant screening results of synthesized derivatives (D_1-D_{10})

3.3. Anti-urease screening

The urease inhibitory activity of the compounds (**D_1-D_{10}**) were performed and the absorbance was recorded at 517nm. Thiourea was used as standard. The IC_{50} value of compounds was calculated from graph plotted between % Inhibition against Concentration (Fig 4 and Fig 5). The results of urease inhibitory activity showed that **D_5** was the most active compound having IC_{50} value (44.69µg/ml) as challenged to standard drug (53.46µg/ml) as shown in **Table 5**.

Table 5. Urease inhibitory screening of the synthesized compounds (D_1-D_{10})

Compounds	% inhibition				IC_{50}(µg/ml)
	25(µg/ml)	50(µg/ml)	75(µg/ml)	100(µg/ml)	
D_1	26.0	46.5	71.29	92.35	52.40
D_2	35.50	42.32	56.63	68	61.13
D_3	30.25	46.32	66.23	72	56.13
D_4	23.34	39.25	64.22	85.32	58.90
D_5	39.46	50.25	68.52	91.23	44.69
D_6	22.69	51.23	65.25	93.65	53.45
D_7	32.95	54.85	75.36	92.46	45.03
D_8	27.87	43.21	69.25	80.25	55.47
D_9	23	41.52	65.21	86.32	57.80
D_{10}	35.35	46.12	56.59	89.85	52.47
Thiourea	30.69	45.63	68.65	79.56	53.46

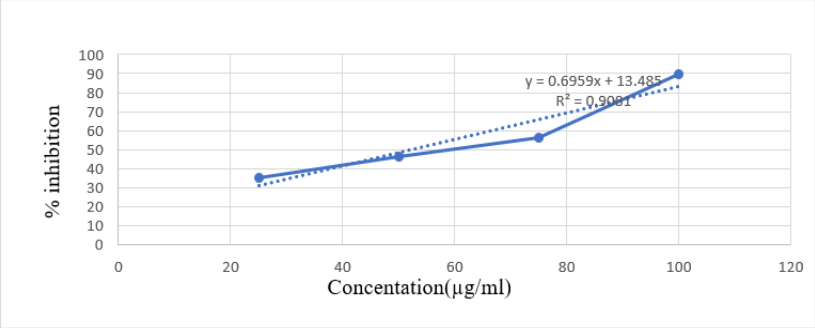


Fig 4:% inhibition of most active compound D_5

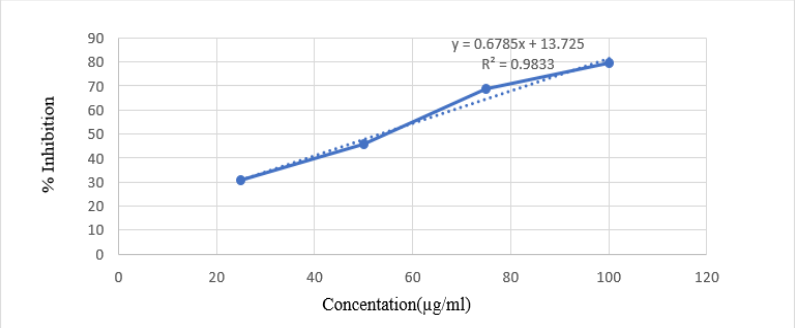


Fig 5: % inhibiton of Thiourea

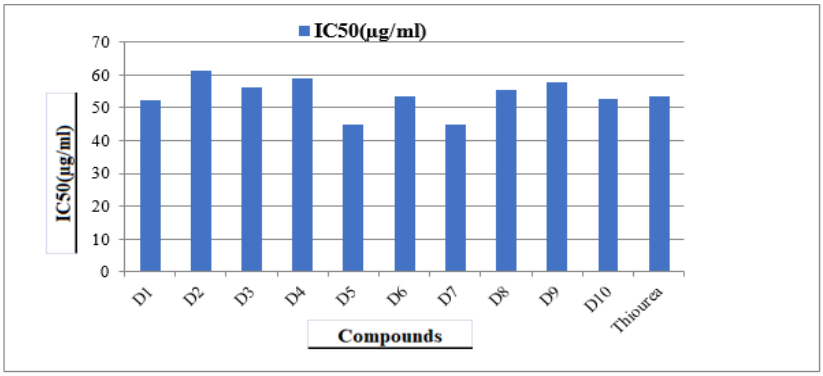


Fig U_1. Urease inhibitory screening results of synthesized derivatives (D_1-D_{10})

3.4. SAR (Structure activity relationship)

- The following structure-activity connection can be obtained from the antioxidant and anti-urease activity results of the synthesised coumarin derivatives (Fig 6).
- Presence of –CHO (electron withdrawing) group at *para* position in **D_9** improved the antioxidant activity.
- Presence of –OCH_3 at *meta* & *para* position which is an electron withdrawing group increased the urease inhibitory activity of the compound **D_5** as compared to standard drug.

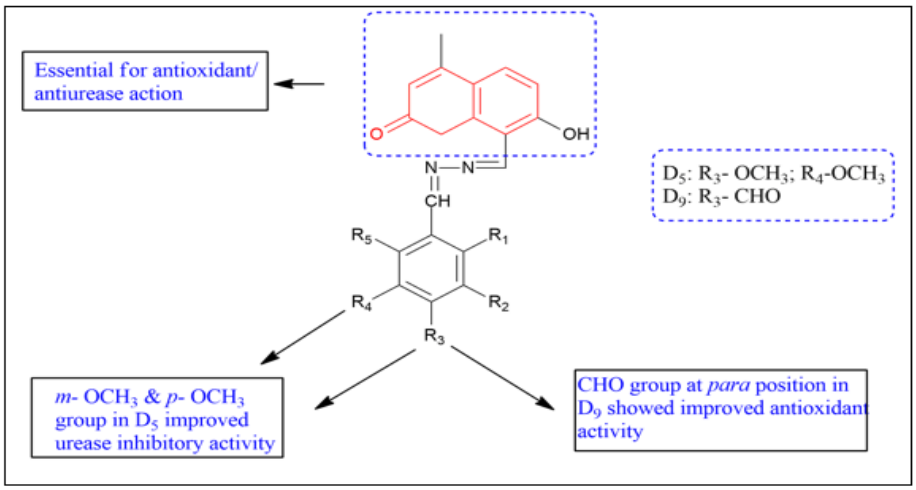
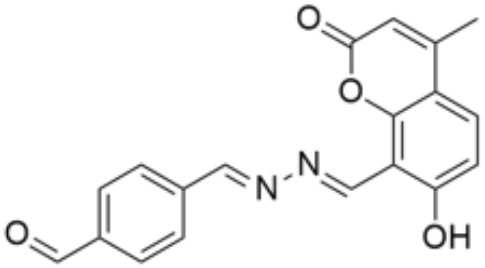


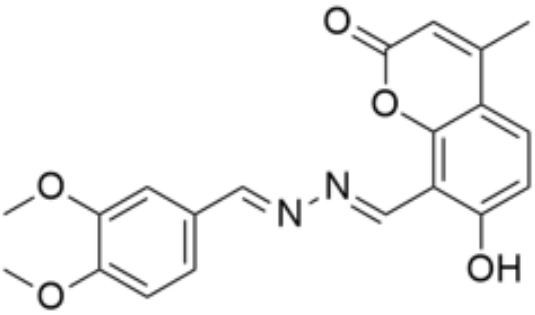
Fig 6: Structure activity relationship of synthesized derivatives (D_1-D_{10})

4. SUMMARY AND CONCLUSION

Coumarins have been more useful as intermediates in the synthesis of novel active chemicals in medicinal chemistry in recent years. Coumarins are an important heterocyclic nucleus with a wide range of biological activities, attracting researchers from all over the world to synthesise and test numerous coumarin derivatives for their biological potentials. We synthesised novel coumarin derivatives (**D_1-D_{10}**) and tested them for antioxidant and antiurease potentials in this study. The activities of the compounds **D_9** and **D_5** revealed that they were the most active antioxidant and urease inhibitor respectively.



(D_9)
(IC_{50} =69.76g/ml)
Fig 7: Most active Antioxidant compound



(D_5)
(IC_{50} =44.69g/ml)
Fig 8: Most active Urease Inhibitor

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

SUPPORTING INFORMATION

The data that supports the findings of this study are available in the supplementary material of this article.

REFERENCES

1. Naz F, Latif M, Salar U, Khan KM, Al-Rashida M, Ali I, Ali B, Taha M, Perveen S. 4-Oxycoumarinyl linked acetohydrazide Schiff bases as potent urease inhibitors. Bioorganic Chemistry. 2020 Dec 1;105:104365.
2. Kulkarni MV, Kulkarni GM, Lin CH, Sun CM. Recent advances in coumarins and 1-Azacoumarins as versatile biodynamic agents. Current medicinal chemistry. 2006 Oct 1;13(23):2795-818.
3. Borges MF, Roleira FM, Milhazes NJ, Villare EU, Penin LS. Simple coumarins: Privileged scaffolds in medicinal chemistry. Frontiers in Medicinal Chemistry. 2010 Dec 10;4:23-85.
4. Witaicenis A, Seito LN, Di Stasi LC. Intestinal anti-inflammatory activity of esculetin and 4-methylesculetin in the trinitrobenzenesulphonic acid model of rat colitis. Chemico-biological interactions. 2010 Jul 30;186(2):211-8.
5. Chiang CC, Cheng MJ, Peng CF, Huang HY, Chen IS. A novel dimeric coumarin analog and antimycobacterial constituents from Fatoua pilosa. Chemistry & biodiversity. 2010 Jul;7(7):1728-36.
6. Rosselli S, Maggio AM, Faraone N, Spadaro V, Morris-Natschke SL, Bastow KF, Lee KH, Bruno M. The cytotoxic properties of natural coumarins isolated from roots of Ferulago campestris (Apiaceae) and of synthetic ester derivatives of aegelinol. Natural product communications. 2009 Dec;4(12):1934578X0900401219.
7. Basile A, Sorbo S, Spadaro V, Bruno M, Maggio A, Faraone N, Rosselli S. Antimicrobial and antioxidant activities of coumarins from the roots of Ferulago campestris (Apiaceae). Molecules. 2009 Feb 27;14(3):939-52.
8. Crombie L, Games DE, McCormick A. Isolation and structure of mammea A/BA, A/AB and A/BB: A group of 4-Aryl-coumarin extractives of mammea americana L. Tetrahedron Letters. 1966 Jan 1;7(2):145-9.
9. Poole SK, Poole CF. Thin-layer chromatographic method for the determination of the principal polar aromatic flavour compounds of the cinnamons of commerce. Analyst. 1994 Jan 1;119(1):113-20.
10. Rehman NU, Khan A, Al-Harrasi A, Khiat M, Hussain H, Wadood A, Riaz M. Natural urease inhibitors from Aloe vera resin and Lycium shawii and their structural-activity relationship and molecular docking study. Bioorganic Chemistry. 2019 Jul 1;88:102955.

11. Kiraz AÖ, Koca M, Kurt A, Kara İ, Arıcı C. Synthesis and computational studies on a coumarin derivative: 4-Chloromethyl coumarin-7-yl-methacrylate. Journal of Molecular Structure. 2022 Jul 5;1259:132702.
12. Salehian F, Nadri H, Jalili-Baleh L, Youseftabar-Miri L, Bukhari SN, Foroumadi A, Küçükkilinç TT, Sharifzadeh M, Khoobi M. A review: Biologically active 3, 4-heterocycle-fused coumarins. European Journal of Medicinal Chemistry. 2021 Feb 15;212:113034.
13. Hamid SJ, Kubba AA. Synthesis and characterization of new coumarin derivatives containing various moieties with antibacterial activities. International Journal of Pharmacy and Pharmaceutical Sciences, 2015;7:70-4.
14. Desai NC, Satodiya HM, Rajpara KM, Joshi VV, Vaghani HV. A microwave-assisted facile synthesis of novel coumarin derivatives containing cyanopyridine and furan as antimicrobial agents. Journal of Saudi Chemical Society. 2017 Jan 1;21:S153-62.
15. Liu L, Hu Y, Shen YF, Wang GX, Zhu B. Evaluation on antiviral activity of coumarin derivatives against spring viraemia of carp virus in epithelioma papulosum cyprini cells. Antiviral Research. 2017 Aug 1;144:173-85.
16. Kontogiorgis C, Hadjipavlou-Litina D. Biological evaluation of several coumarin derivatives designed as possible anti-inflammatory/antioxidant agents. Journal of Enzyme Inhibition and Medicinal Chemistry. 2003 Jan 1;18(1):63-9.
17. Gacche RN, Jadhav SG. Antioxidant activities and cytotoxicity of selected coumarin derivatives: preliminary results of a structure–activity relationship study using computational tools. Journal of Experimental & Clinical Medicine. 2012 Jun 1;4(3):165-9.
18. Haque S, Singh V, Katiyar D, Khan MT, Tripathi V, El Enshasy H, Pasupuleti M, Mishra BN. Biotransformation of newly synthesized coumarin derivatives by Candida albicans as potential antibacterial, antioxidant and cytotoxic agents. Process Biochemistry. 2019 Dec 1;87:138-44.
19. Huang XY, Shan ZJ, Zhai HL, Su L, Zhang XY. Study on the anticancer activity of coumarin derivatives by molecular modeling. Chemical Biology & Drug Design. 2011 Oct;78(4):651-8.
20. Angelova VT, Valcheva V, Vassilev NG, Buyukliev R, Momekov G, Dimitrov I, Saso L, Djukic M, Shivachev B. Antimycobacterial activity of novel hydrazide-hydrazone derivatives with 2*H*-chromene and coumarin scaffold. Bioorganic & Medicinal Chemistry Letters. 2017 Jan 15;27(2):223-7.
21. Buckle DR, Outred DJ, Smith H, Spicer BA. N-benzylpiperazino derivatives of 3-nitro-4-hydroxycoumarin with H1 antihistamine and mast cell stabilizing properties. Journal of medicinal chemistry. 1984 Nov;27(11):1452-7.
22. Liu GL, Hu Y, Chen XH, Wang GX, Ling F. Synthesis and anthelmintic activity of coumarin–imidazole hybrid derivatives against Dactylogyrus intermedius in goldfish. Bioorganic & Medicinal Chemistry Letters. 2016 Oct 15;26(20):5039-43.
23. Ghate M, Kusanur RA, Kulkarni MV. Synthesis and in vivo analgesic and anti-inflammatory activity of some bi-heterocyclic coumarin derivatives. European journal of medicinal chemistry. 2005 Sep 1;40(9):882-7.
24. Sashidhara KV, Kumar A, Chatterjee M, Rao KB, Singh S, Verma AK, Palit G. Discovery and synthesis of novel 3-phenylcoumarin derivatives as antidepressant agents. Bioorganic & medicinal chemistry letters. 2011 Apr 1;21(7):1937-41.
25. Olaya P, Viña D, Lopez JL, Guerrero M. Coumarin derivatives as monoamine oxidase B inhibitors with antiparkinsonian like properties. Farma Journal. 2019 Feb 1;4(1):218.
26. Haziri A, Mazreku I, Rudhani I. Anticoagulant Activity of Coumarin Derivatives. Malaysian Applied Biology. 2022 Jun 29;51(2):107-9.
27. Alomari M, Taha M, Imran S, Jamil W, Selvaraj M, Uddin N, Rahim F. Design, synthesis, *in vitro* evaluation, molecular docking and ADME properties studies of hybrid bis-coumarin with thiadiazole as a new inhibitor of Urease. Bioorganic Chemistry. 2019 Nov 1;92:103235.
28. Singh AD, Kumar A, Saini S, Prabha H. Synthesis and biological evaluation some new of 7-Hydroxy-4-methylcoumarin derivative. Asian Pacific Journal of Health Sciences, 2017;4:84.
29. Konidala SK, Kotra V, Danduga RC, Kola PK, Bhandare RR, Shaik AB. Design, multistep synthesis and *in-vitro* antimicrobial and antioxidant screening of coumarin clubbed chalcone hybrids through molecular hybridization approach. Arabian Journal of Chemistry. 2021 Jun 1;14(6):103154.
30. Puttaraju KB, Shivashankar K, Mahendra M, Rasal VP, Vivek PN, Rai K, Chanu MB. Microwave assisted synthesis of dihydrobenzo [4, 5] imidazo [1, 2-a] pyrimidin-4-ones; synthesis, *in vitro* antimicrobial and anticancer activities of novel coumarin substituted dihydrobenzo [4, 5] imidazo [1, 2-a] pyrimidin-4-ones. European Journal of Medicinal Chemistry. 2013 Nov 1;69:316-22.
31. Kumari M, Tahlan S, Narasimhan B, Ramasamy K, Lim SM, Shah SA, Mani V, Kakkar S. Synthesis and biological evaluation of heterocyclic 1, 2, 4-triazole scaffolds as promising pharmacological agents. BMC chemistry. 2021 Dec;15(1):1-6.