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SYNTHESIS AND *IN VITRO* PHARMACOLOGICAL PROPERTIES OF SOME NOVEL COUMARIN DERIVATIVES

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ABSTRACT

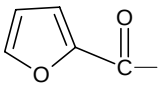
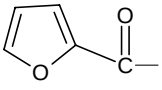
7-hydroxy-4-methyl coumarin (1) was prepared by cyclization of resorcinol with ethylacetoacetate in presence of sulphuric acid. Furan substituted coumarin molecules at C-4 position (1a) and substituted at C-4 and C-7 positions (1c) are prepared by condensation of 7-hydroxy-4-methyl coumarin (2). All the newly synthesized compounds were characterized by IR, ^{1}H NMR and EI-MS. The antioxidant activity, anti-inflammatory and antibacterial activity of newly synthesized compounds 1a and 1c were evaluated. The result of these newly synthesized compounds containing furan substitution exhibit good antioxidant activity, anti-inflammatory and antibacterial activity.

INTRODUCTION

Coumarin derivatives continue to be investigated over the years due to their importance to organic and medicinal chemists because of their huge biological activities[1]. Coumarin and its derivatives are associated with various biological activities viz. anti-inflammatory[2], anti-convulsant[3], anticoagulant[5], antioxidant[6], antibacterial[7], antifungal[8], anti-HIV[9], anti-carcinogenic material[10]. Apart from this, they are attracting considerable attention of chemists as a large number of natural products contain this heterocyclic nucleus and are widely used as additives, in food, perfumes, cosmetics, pharmaceutical[12],optical brighterners[13], in dispersed fluorescent and laser dyes [14].

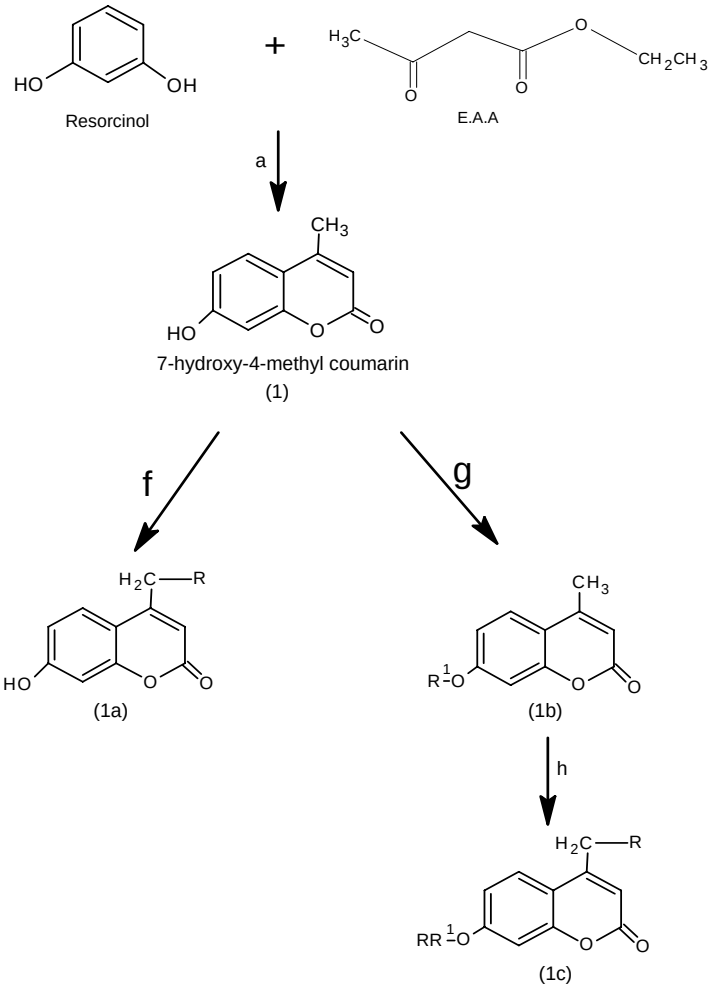
Coumarin derivatives have been reported for antimicrobial[15], antiallergic, anticancer[16] and anti proliferative and antiviral[17] activities. Coumarins and their derivatives have been found to exhibit different biological and pharmacological activities[18]. The 4-methylcoumarins have been found to possess cholesretic[19], antispermatogenic[20] and diuretic[21] properties. Apart from the medicinal applications coumarins are also used as sweetener, fixative of perfumes[22], enhancer of natural oils such as lavender, a food additive in combination with vanillin, a flavour/odour stabilizer in tobacco[22], an odour masker in paints and rubber. Owning to the widespread applications, synthetic and biological activity evaluation of coumarins and their derivatives has been a subject of intense investigations.

Table 1. Physicochemical properties of coumarin derivatives

Compd.	Substitution		% yield	Solvent [a]	M. point [b]	R_f [c]	Molecular formula
	R	R^1					
1a		-	43.80	Chloroform	164	0.84	$C_{15}H_{10}O_5$
1c		CH_3CO-	29.00	Ethanol	90	0.69	$C_{22}H_{14}O_8$

[a] Recrystallization solvent, [b] Melting point ^{o} C, [c]R_f values (Chloroform: Ethyl acetate, 4:1) as a mobile phase and U.V Chamber as visualizing agent.

Scheme



a = conc.H_2SO_4 f,h, = Dry acetone, K_2CO_3

g = Distilled acetic anhydride

***IN VITRO* ANTIOXIDANT ACTIVITY**

Reactive oxygen species (ROS) are well recognized to be the pathogenesis of various diseases such as atherosclerosis, diabetes, cancer, arthritis etc. thus a scavenger of ROS is expected to prevent these free radical mediated diseases. The use of antioxidants, both synthetic and natural, in the prevention and cure of various diseases is gaining wide importance in the medicinal field. Currently we have evaluated our synthesized compounds for four different *in vitro* antioxidant methods; DPPH, nitric oxide radical scavenging, hydrogen peroxide scavenging, lipid peroxide assay methods, total antioxidant capacity and reducing power of the compounds.

Scavenging of DPPH radical activity

DPPH has widely used to evaluate the free radical scavenging activity of various antioxidant substances. The method is based on the reduction of DPPH radical in the presence of oxidizing agent or hydrogen donating antioxidants due to the formation of non radical form stable DPPH molecule. By the reaction with DPPH our study determines the antiradical power of coumarin derivatives by measuring of decreased absorbance of DPPH at 517 nm.[23] All the compounds tested for antioxidant activity against scavenging of DPPH radical activity. Compound 1, 2, and 1a at 50, 25, 12.5 and 6.25 µg/ml showed comparable percentage of inhibition when compared to the standard rutin. Compounds 1c were found to be moderate antioxidant. The results were given in table 2.

Table 2. Antioxidant activity of coumarin derivatives by scavenging of DPPH radical activity

Compound Code	% Inhibition ± S.D.*			
	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml
1	60.33 ± 0.04	56.69 ± 0.40	52.17 ± 0.24	48.88 ± 0.19
2	60.83 ± 0.52	52.74 ± 0.23	51.84 ± 0.21	49.63 ± 0.34
1a	61.15 ± 0.17	53.03 ± 0.15	47.29 ± 0.34	41.72 ± 0.23
1c	56.90 ± 0.17	52.02 ± 0.52	46.5 ± 0.35	42.87 ± 0.11
Standard (Rutin)	70.60 ± 0.03	55.81 ± 0.10	37.69 ± 0.25	21.79 ± 0.09

Average of three determination ± S.D.

Nitric oxide radical inhibitory assay

Nitric oxide radical inhibition assay was determined by using Geiss Ilsway method. Nitrite ions react with oxygen and get converts to citric oxide radical and the reaction produces purple colour. The antioxidants by donating the electrons to nitric oxide radical, decreases the intense purple colour.[24] All the compounds tested for antioxidant activity against nitric oxide radical. Compound 1, 1a and 1c at 31.25 µg/ml showed highest percentage of inhibition when compared to the standard ascorbic acid. (Table 3).

In the nitric oxide scavenging activity method, compounds 1 exhibited highest antioxidant activity with the IC_{50} value of 76.33 ± 1.52 µg/ml.

Table 3. Antioxidant activity of coumarin derivatives by Nitric oxide radical inhibitory assay

Compound Code	% Inhibition ± S.D.*			
	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml
1	65.51 ± 0.06	53.28 ± 0.0	52.15 ± 0.04	47.89 ± 0.05
2	60.23 ± 0.02	50.15 ± 0.03	36.64 ± 0.06	20.77 ± 0.03
1a	57.63 ± 0.16	38.66 ± 0.23	35.42 ± 0.03	34.33 ± 0.11
1c	62.40 ± 0.11	47.41 ± 0.10	39.36 ± 0.15	31.00 ± 0.01
Standard (Ascorbic acid)	54.52 ± 0.55 (62.5 µg/ml)	24.47 ± 1.42 (31.25 µg/ml)	2.35 ± 0.55 (15.64 µg/ml)	0.18 ± 0.01 (7.81 µg/ml)

*Average of three determination ± S.D.

Scavenging of hydrogen peroxide

The newly synthesized coumarin derivatives were screened for their antioxidant activity against H_2O_2 radical. The scavenging of all synthesized compounds of the H_2O_2 radical was evaluated. H_2O_2 is strong oxidizing nature with many oxidizing capacity of enzymes such as superoxide dismutase and catalase. H_2O_2 crosses the membrane and slowly oxidize number of compounds present in the body.[25] The ability of coumarin derivatives to scavenge H_2O_2 was determined. All the compounds tested for antioxidant activity against scavenging of hydrogen peroxide. Compound 1a at 100, 50 and 25 µg/ml showed highest percentage of inhibition when compared to the standard BHA. Compound 1 and 1c has shown comparable percentage of inhibition with the standard. (Table 4).

Table 4. Antioxidant activity of coumarin derivatives by scavenging of hydrogen peroxide

Compound Code	% Inhibition ± S.D.*		
	100 µg/ml	50 µg/ml	25 µg/ml
1	89.92 ± 0.29	70.62 ± 0.14	26.16 ± 0.29
2	178.48 ± 0.07	158.72 ± 0.13	66.21 ± 0.04
1a	121.15 ± 0.29	89.24 ± 0.25	20.09 ± 0.19
1c	101.95 ± 0.23	53.78 ± 0.67	24.27 ± 0.12
Standard (BHA)	82.42 ± 1.04	73.36 ± 0.59	56.63 ± 0.71

Average of three determination ± S.D.

Lipid peroxidation inhibitory activity

Results of antioxidant activity was given in the Table 5. All the twelve compounds tested for antioxidant activity against lipid peroxidation inhibitory activity. In lipid peroxidation formation of lipid hydroperoxides called inhibition step.[26] We have investigated the effect of coumarin derivatives on the inhibition of lipid peroxidation. The result given in Table 5. The coumarin derivatives shown very good antioxidant property but when 7-hydroxy group is acetylated with the acetic anhydride shown much less inhibition against lipid peroxide.

Compound 2 at 250, 125, 62.5 and 31.25 µg/ml showed highest percentage of inhibition when compared to the standard BHA. Compound 1a has shown comparable percentage of inhibition with the standard. Compounds 1 and 1c were found to be moderate antioxidant activity. The compounds 1 and 2 have shown highest percentage inhibition and compared with the well known antioxidant BHA and it was observed that 7-hydroxy coumarin derivatives were much better antioxidant than BHA. Blocking of the 7-hydroxy group and substitution of furans with side chain at the 4th position on coumarin suppresses the inhibition activity of coumarin derivatives against lipid peroxidation.

Table 5. Antioxidant activity of coumarin derivatives by lipid peroxidation inhibitory activity

Compound Code	% inhibition ± S.D.*			
	250 µg/ml	125 µg/ml	62.5 µg/ml	31.25 µg/ml
1	70.40 ± 0.53	64.17 ± 0.06	58.99 ± 0.02	31.68 ± 0.18
2	185.86 ± 0.15	143.55 ± 0.37	64.45 ± 0.49	44.81 ± 0.22
1a	82.32 ± 0.25	69.62 ± 0.38	49.53 ± 0.40	26.65 ± 0.26
1c	72.84 ± 0.18	60.66 ± 0.30	47.55 ± 0.10	33.43 ± 0.39
Standard(BHA)	86.40 ± 0.11	61.69 ± 0.37	48.75 ± 0.12	22.43 ± 0.20

Average of three determination ± S.D.

Evaluation of total antioxidant capacity

In total antioxidant capacity method, phosphomolybdenum (VI) reduces to phosphomolybdenum (V) by complexing with antioxidants and produces green colour with maximal absorbance at 695 nm.[27] The synthesized furan derivatives were performed for total antioxidant capacity. Total antioxidant activity determination was performed in mM equivalent to BHA and the highest total antioxidant capacities were shown by the compounds 3a, 3b, 4a and 4d (Table 6).

IC_{50} values of newly synthesized coumarin derivatives in different *in vitro* antioxidant methods:

Among all derivatives tested for i*n vitro* antioxidant activity compounds 1, 2, 1a and 1c in scavenging of DPPH radical method and the IC_{50} values were found to be (8.10 ± 0.10, 8.54 ± 0.05, 17.61 ± 0.12, 20.16 ± 1.04 µg/ml) respectively, In lipid peroxidation inhibitory method compounds 1, 2, 1a and the IC_{50} values were found to be (50.03 ± 0.45, 40.0 ± 0.5, 74.46 ± 0.45 µg/ml) respectively, exhibited potent antioxidant activity and shown in Table No 6. The values were found to be potent antioxidant than standard used. All other compounds found to be moderate to low antioxidant activity in scavenging of all radicals, which were tested (Table 6).

Table 6. IC_{50} values of synthesized compounds and standard in different antioxidant methods.

Compound Code	IC_{50} ± S.D*µg/ml				
	DPPH assay	NO radical inhibition	Hydrogen peroxide	Lipid peroxidation	Total antioxidant capacity (mM)[a]
1	8.10 ± 0.10	50.0 ± 0.5	45.36 ± 0.32	50.03 ± 0.45	1.61 ± 0.04
2	8.54 ± 0.05	125.1 ± 0.28	22.7 ± 0.26	40.0 ± 0.5	0.50 ± 0.01
1a	17.61 ± 0.12	204.23 ± 0.25	75.48 ± 0.45	68.3 ± 0.51	5.22 ± 0.10
1c	20.16 ± 1.04	149.5 ± 0.5	49.05 ± 0.48	80.26 ± 0.46	0.21 ± 0.03
Standard					
Rutin	26.26 ± 0.25	-	-	-	-
Ascorbic acid	-	60.5 ± 0.5		-	-
BHA	-	-	25.5 ± 0.45	-	-
BHA	-	-	-	76.36 ± 0.32	-

*Average of three determinations [a] mM equivalent to BHA

Reducing power assay

Results for antioxidant activity were given in the Table 10. The reducing capacity of the synthesized derivatives was determined by the method of Oyaizu.[28] The compounds 1, 2, 1a and 1b have shown good reducing capacity of reduction (Table 7).

Table 7. Reducing power of coumarin derivatives

Compound Code	Absorbance (Mean ± S.D.)*			
	500 µg/ml	**250 µg/ml**	**125 µg/ml**	**62.5 µg/ml**
1	0.104 ± 0.0004	0.079 ± 0.0006	0.065 ± 0.001	0.015 ± 0.0006
2	0.086 ± 0.0011	0.040 ± 0.001	0.025 ± 0.0008	0.019 ± 0.0005
1a	0.015 ± 0.0010	0.009 ± 0.0006	0.007 ± 0.0004	0.004 ± 0.0002
1c	0.097 ± 0.0013	0.071 ± 0.0007	0.069 ± 0.0015	0.065 ± 0.0015

Average of three determination ± S.D.

Table 8. *In vitro* anti-inflammatory activity of the coumarin derivatives

Compound Code	Absorbance (Mean ± S.D.)*				IC_{50} ± S.D. µg/ml
	500 µg/ml	**250 µg/ml**	**125 µg/ml**	**62.5 µg/ml**	
1	62.5 ± 0.10	58.33 ± 0.29	55.87 ± 0.09	46.6 ± 0.20	85.23 ± 0.25
2	65.37 ± 0.08	63.56 ± 0.15	59.75 ± 0.14	43.10 ± 0.49	92.16 ± 0.28
1a	31.18 ± 0.21	14.25 ± 0.02	-61.36 ± 0.31	-147.1 ± 0.27	<500
1c	55.83 ± 0.05	45.48 ± 0.06	35.4 ± 0.05	16.62 ± 0.05	380.4 ± 0.36
Standard Diclofenac sodium	87.78 ± 1.19	84.08 ± 1.28	78.09 ± 1.41	48.24 ± 1.30	70.43 ± 0.40

*Average of three determination

Table 9. Antibacterial activity of coumarin derivatives at different concentration by well diffusion method (values in mm)

Compound code	Mean zone of inhibition (in mm)															
	S .Aureus				***K. Pneumonia***				***E. coli***				***P. Auruginosa***			
	62.5 µg/ml	**125 µg/ml**	**250 µg/ml**	**500 µg/ml**	**62.5 µg/ml**	**125 µg/ml**	**250 µg/ml**	**500 µg/ml**	**62.5 µg/ml**	**125 µg/ml**	**250 µg/ml**	**500 µg/ml**	**62.5 µg/ml**	**125 µg/ml**	**250 µg/ml**	**500 µg/ml**
2	-	-	-	-	-	-	-	-	-	-	-	-	3	5	8	11
1a	-	-	-	-	3	7	10	16	-	-	-	-	-	-	-	-
1c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Standard Ciprofloxacin[a]	11	13	15	17	12	13	15	17	13	15	16	18	10	12	14	16

*Average of three determination

[a] Ciprofloxacin for bacteria was used at the concentration of 100, 50, 25. 12.5 µg/ml

The results for anti-inflammatory activity are given in the (Table 8). The coumarin derivatives synthesized were tested to evaluate their *in vitro* anti-inflammatory and antibacterial activities at 500, 250, 125, 62.5 µg/ml respectively. The studies showed (Table 8 and 9) Synthesized compounds were tested for anti-inflammatory activity and compared to standard diclofenac sodium. Among all the derivatives tested for i*n vitro* anti-inflammatory activity, compound 1**c** showed comparable activity with the standard diclofenac sodium and the IC_{50} value was found to be 380.4 ± 0.36 µg/ml. Other compounds showed less anti-inflammatory activity when compared to standard diclofenac sodium and compounds were active against ***K. pneumonia***. **1a** was found to be the most active derivative against ***K. pneumonia***.

Experimental:

Melting points of the synthesized compounds were determined using the microcontroller based melting point apparatus (Uniline) and were found uncorrected. The IR spectra of the synthesized compounds were recorded using KBr pellets in range of 4000 - 400 cm^{-1} on a Fourier transform IR spectrometer (Therma Nicolet 380, SAC College of Pharmacy, B.G.Nagara, India) and the frequencies were recorded in wave numbers. ^{1}H NMR (400 MHz) spectra were recorded in Brucker, liquid state NMR spectrometer (NMR research Center, Indian institute of science Bangalore, India). Chemical shifts (δ) were reported in parts per million downfield from internal reference Tetra Methyl Saline (TMS). Mass spectra were recorded on GCMS in dimethyl sulphoxide **(**University Science Instrument Center, Dharwad, India). The homogeneity of the compounds was described by TLC on aluminum silica gel plates detected by UV light (254 nm) and iodine vapours. The reagents used were all analytical grade reagents.

Preparation of compounds 7-hydroxy-4-methyl-2*H*-chromen-2-one (1):

A mixture of resorcinol (5.5 g, 0.05 mole), ethyl acetoacetate (6.35 ml, 0.04 mole), sulphuric acid (75%, 50 ml) was refluxed on a water bath for 30 min at 100 °C. The resultant dark green solution was cooled and stirred in 250 g of crushed ice. The crude product was filtered and washed with water. Recrystallized by using methanol to get pale yellow crystals of product. A single spot in TLC indicated the purity of the compound.[29,30]

Preparation of compound 4-[2-(furan-2-yl)-2-oxoethyl]-7-hydroxy-2*H*-chromen-2-one (1a):

A mixture of compound 1(0.69 g, 0.00395 mole) and 2-furoyl chloride (0.52 g, 0.004 mole) and potassium carbonate (0.5 g) was stirred at room temperature in 25 ml of dry acetone for 12-14 h. The reaction mixture was evaporated to remove acetone and added to 100 ml of water. The obtained residue was washed with dilute hydrochloric acid, filtered, dried and recreystallized from dioxane and ethanol mixture (1:1).[31]

Preparation of compound 4-methyl-2-oxo-2*H*-chromen-7-yl acetate (1b):

A mixture of compound 1 (28.2 g, 0.16 mole) and freshly distilled acetic anhydride (52.87 ml, 0.56 mole) was refluxed for 1.5 h under anhydrous conditions. While the solution was hot, it was poured to crushed ice and the separated product was filtered and washed with water. It was crystallized from methanol as fibrous colorless needles.[31]

Preparation of compound 4-[2-(furan-3-yl)-2-oxoethyl]-2-oxo-2*H*-chromen-7-yl 3-(furan-2-yl)-3-oxopropanoate (1c):

To a mixture of 7-acetoxy-4-methyl coumarin 1b (0.86 g, 0.00395 mole) and 2-furoyl chloride (1.04 g, 0.008 mole) and potassium carbonate (0.5 g) was stirred at room temperature in 25 ml of dry acetone for 12-14 h. The reaction mixture was evaporated to remove acetone and added to 100 ml of water. The obtained precipitate was washed with dilute hydrochloric acid,filtered,dried,recreystallized from dioxane-ethanol mixture (1:1)[32]

7-hydroxy-4-methyl-coumarin 1, yield 78.40% (6.9 gm); m.p. 184 ^{o}C; IR (KBr): OH-3494, C=O-1670, C-O-C-1069, C=C-1602 cm^{-1}; ^{1}H NMR (400 MHz, δ ppm): 4.81(s, 3H, -CH_3), 3.30 (s, 1h, -OH), 6.81 (d. 1H, J= 2.0 Hz –CH), 6.79 (d, 1H, J= 2.4 Hz –CH), 6.08 (s, 1H, -CH), 7.59 (s, 1H); GC-MS: *m/z* 176 (M^+).

4-[2-(furan-2-yl)-2-oxoethyl]-7-hydroxy-2*H*-chromen-2-one (1a): yield 43.80% (0.43 gm); m.p. 164 ^{o}C; IR (KBr cm^{-1}): OH-3132, C=O-1727, C-O-C-1089; ^{1}H NMR ($CDCl_3$) δ (ppm): 8.12-7.34 (4H, Ar-H), 7.32-6.82 (3H, furan –H), 3.29 (s, 1H, -OH), 2.499 (s, 2H, -CH_2-). Mass GC-MS : *m/z* 270 (M^+).

Anti-inflammatory assay:

Many *in-vitro* assays, each based on a specific biochemical or cellular mechanism have been developed for the initial screening of the anti-inflammatory compounds. Denaturation of proteins as one of the causes of inflammation well documented. A number of anti-inflammatory drugs are known to inhibit the denaturation of proteins as an *in vitro* screening model for anti-inflammatory compounds.[33-35]

The standard drug and test compounds were dissolved in minimum amount of DMF and diluted with phosphate buffer (0.2M, pH 7.4) in such a way that concentration of DMF in all solutions was less than 2.5%. Test solution (1 ml) containing different concentrations of drug was mixed with 1 ml of 1% albumin solution in phosphate buffer and incubated at $27^0 \pm 1^0$C in an incubator for 15 min. Denaturation was induced by keeping the reaction mixture at $60^0 \pm 1^0$ C in a water bath for 10 min. After cooling, the turbidity was measured at 660 nm. Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average is

taken. The Diclofenac was used as standard drug. The percentage of inhibition was calculated using the formula

$$\% \text{ Inhibition} = \left[\frac{V_t - V_c}{V_t} \right] X \ 100$$

Where,

V_t : Absorbance of test compounds

V_c : Absorbance of Control

Antimicrobial assay[36-38]

Compounds 1a and 1c were screened for their antimicrobial activity by using cup–plate agar method at a concentration of 500, 250, 125, 62.5 µg/ml respectively against *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa.*

The antimicrobial activity of the compounds was assayed by antimicrobial susceptibility test.[6] One hundred microliters of 24 h growth of each microorganism was spread on the surface of nutrient agar for bacteria in Petri plates. Twenty microliter compounds at the concentration of 500, 250, 125, 62.5 µg/ml in DMSO saturated in well of 6 mm diameter were poured in agar. The plates were kept in laminar air flow for 2 h to allow prediffusion of the compounds from the well into the agar layer and then incubated at 37 °C for 24 h for bacteria. Zones of inhibition were measured in millimeter and size of the well was subtracted from the zone size to measure final activity. DMSO saturated well served as solvent control or negative control and Ciprofloxacin saturated well (100, 50, 25, 12.5 µg/ml) for bacteria reference or positive control.

CONCLUSION

The present study was aimed at synthesis and characterization of some novel furan derivatives bearing coumarin nucleus. The compounds were screened for anti-oxidant, anti-bacterial and anti-inflammatory activities were found to possess considerable activity.

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REFERENCES

1. Kumar, S.; Kumar, A.; Sandhu, J. S.;ARKIVOC 2007, (xv), 18-23.
2. Lin, C. M.; Huang, S. T.; Lee, F. W.; Sawkuo, H.; Lin,M.H.; Bioorg. Med. Chem. 2006 14, 4402.
3. Bhat, M. A.; Siddiqui, N.; Khan, S. A.; Indian J. Pharm. Sci. 2006, 68,120.
4. Ruszat, R.; Wyler, S.; Forster, T. Reich, O.; Christian, G. S; Thomas, C. G; Sulser, T. Bachmann, A.;. Eur. Assoc. Urol. 2006.
5. Tyagi, A. K; Raj, H. G; Vohra, P.; Gupta, G.; Kumari, R.; Kumar, P.; Gupta, R. K.; Eur J. Med. Chem. 2003, 40, 413.
6. Modrana, J. N.; Nawrot, E.; Graczy, K.; Eur. J.Med. Chem. 2006, 41,1301.
7. Sardari, S.; Mori, Y.; Horita, K.; Micetich, R.G.; Nishibe, S.; Daneshtalab, M.; Bioorg. Med. Chem. 1999, 7, 1933.
8. Huang, L.; Yuon, X.; Yu, D.; Lee, K. H.; Chin,H. C.; Virology 2005, 332, 623.
9. Elinos-Baez, C. M.; Leon,F.; Santos, E.;Cell.Biol. Int. 2005, 29,703.
10. Mohanty, N.; Rath, P. C.; Rout, M. K.; J. IndianChem. Soc. 1967, 44, 1001.
11. Kennedy, R. O.; Tharnes, R. D.; Coumarins Biology, Application and Mode of Action, Wiley & Sons, Chichester, 1997.
12. Zahradnik, M.; the Production and Application of Fluorescent Brightening Agents, Wiley and Sons, 1990.
13. Murray, R. D. H.; Mendez, J.; Brown TheNatural Coumarins: Occurrence, Chemistry and Biochemistry, Wiley and Sons, New York S. A. 1982.
14. Mulwad, V.V.; Shirodkhar, J. M.; Indian J. of Heterocyclic Chem. 2002, 11 192-202.
15. Rajeshwar, R. V.; Srimanth, K;. and VijayaKumar, P.; Indian J. of Heterocyclic chem. 2004, 14, 141-144.

16. Nofal, Z. M.;. El-Zahar, M. I,; and. Abd El-Karim, S. S.; Journal of Antimicrobial Chemotherapy. 2005, 5, 483–488.
17. Egan, D.; Kennedy, R. O.; Prosser, E.; Drug Metab. Rev. 1990, 22, 503–529.
18. Takeda, S.; Aburada, M.; J. Pharmacobiodyn. 1981, 4, 724–729.
19. Tyagi, A.; Dixit, V. P.; Joshi, B. C.; Naturwissenschaften. 1980, 67, 104–109.
20. Deana, A. J., Med. Chem. 1983, 26, 580–585.
21. Tyagi, Y. K.; Studies on the synthesis of azides and heterocyclic triazoles and investigation on biotransformation of polyphenols, Thesis, CCS University, Meerut, July 2000 (submitted).
22. HseuYC, Chang WH, Chen C S, Liao JW, Huang C J, Lu F J, et al. Antioxidant activities of toona sinensis leaves extracts using different antioxidant models. Food Chem. Toxicol. 2008; 46:105–114.
23. Ialenti A, Moncada S, Di Rosa M. Modulation of adjuvant arthritis by endogenous nitric oxide. Br. J. Pharmacol. 1993; 110: 701
24. Aoshima H, Tsunoue H, Koda H, KisoY. Aging of whiskey increases 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity. J. Agric. Food Chem. 2004; 52: 5240–44.
25. Coyle JT, Puttfarcken P. Oxidative stress, glutamate and neurodegenerative diseases. Science 1993; 219: 1184.
26. Jaishree V, Badami S, Suresh B. *In vitro* antioxidant activity of *Enicostemmaaxillare*. J. Health Sci. 2008; 54: 524-528.
27. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jpn. J. Nutr. 1986; 44: 307-15.
28. Mann, F. G.; Sounders, B. C.; Practical organic chemistry 4th ed. p. 307.
29. Furniss, B. S.; Hannaford, A. J.; Smith P. W. G.; Tatchell, A. R.; Vogel's textbook of practical organic chemistry 5th ed. p. 1193.
30. Gummudavelly, S.; Sri Ranganath, Y.; Rajkumar, N.; synthesis and biological screening of some novel coumarin derivatives. Asian J. Res. Chem. 2009, 2(1).
31. Flavia, C. M.; Dagoberto, O. S.; Adair, R. S. S.; Gilson, Z.; Joao Batista, T. R.; Cristina, W. N.; Thiophenes and furan derivatives: a new class of potential pharmacological agents.Environ. Toxicol. Phar. 2003, 37, 37-44.

32. Paul, A. I.; Analgesic, Text Book Goodman and Gillman's, The pharmacological basis of therapeutics. International ed, 1995, 9, 617.
33. Rang, H. P.; Dale, M. M.; Ritter, J. M.; Anti-inflammatory and immuno-suppressant drugs. Pharmacology, International Student. ed. 1995, 3.
34. Grover, J. K.; Experiments in pharmacy and pharmacology. 1993, 2(II).
35. World Health Organization, Basic laboratory procedures in clinical bacteriology, Jaypee Brothers, Geneva, 1991, 84, 453.
36. Basile, A.; Antibacterial and antioxidant activities in Saderitalica miller. J. Ethnopharmacol. 2006, 24, 1.
37. Ozsoy, N.; Yilmaz, T.; Kurt, C.; Can, A. C.; Yanardag, R.; *In vitro* antioxidant activity of Amaranthus lividus. J. Agric. Food Chem. 2009, 116, 867.