Synthesis and Biological Evaluation of some novel coumarin derivatives used as a potent agent

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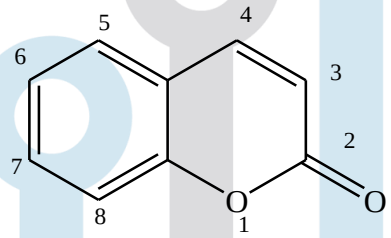
Abstract: Coumarins have been isolated from plants. In the present study, we report synthesis of new coumarin derivatives as prospective therapeutic agents and investigate their potent properties.Coumarin (2H-chromen-2-one) is natural organic chemical compound which is first isolated in 1822 (Isolated by Voleg) and in laboratory it is synthesized from 1868(Synthesized by Perkin). It is obtained from the lactone containing substance with benzopyrone family which consist of the fused benzene ring and the alpha pyrone ring . It comes from the word "Coumarou" and their common name is 'Tonka beans' (Dipteryx odorata) from which coumarin is extracted. The coumarin and its derivatives possess most importance due to their wide range of biological activity like anti-inflammatory, anticancer, anticoagulant, anti tubercular, antioxidant, anti allergic, analgesic, antimicrobial, antiviral, antimalarials etc

Keywords: Coumarin, Anti-Tubercular Agents, Anti-Cancer Agents, Anti-Oxidant Agents.

1. Introduction

1.1 Chemistry of Coumarin: [1-7]



2*H*-chromen-2-one

Coumarin (2H-chromen-2-one) is natural organic chemical compound which is first isolated in 1822 (Isolated by Voleg) and in laboratory it is synthesized from 1868(Synthesized by Perkin). It is obtained from the lactone containing substance with benzopyrone family which consist of the fused benzene ring and the alpha pyrone ring . It comes from the word "Coumarou" and their common name is 'Tonka beans' (Dipteryx odorata) from which coumarin is extracted. The coumarin and its derivatives possess most importance due to their wide range of biological activity like anti-inflammatory, anticancer, anticoagulant, anti tubercular, antioxidant, anti allergic, analgesic, antimicrobial, antiviral, antimalarials etc. 2H-chromen-2-one also can be synthesized in laboratory or extracted from original plant.

Table No.1.1.-Physicochemical Properties of Coumarin:-

Sr.No.	Parameters	Properties
1	Molecular Formula	$C_9H_6O_2$
2	Molecular Weight	146.14
3	Colour	Colourless
4	Odour	Pleasant and Characteristic
5	Melting Point	68-70^{0}C
6	Boiling Point	303^{0}C
7	Solubility	Freely soluble in Ethanol, Chloroform Sparingly soluble in boiling water Slightly soluble in cold water.

1.2:- Introduction of Anti-Tubercular Agents:-[8-9]

Tubercle bacillus identified by the Robert Koch (1882) and its known as Mycobacterium tuberculosis. The first antitubercular chemotherapy discovered in 1938 and later sulfonamide derivative dapsone was found clinically. TB mainly affects the lungs and also affects other parts of body. As on year 2019 near about 10 million active cases are found in worldwide and 1.4 million died from it. Tuberculosis making number one cause of mortality from an infectious disease at that time. The recommended treatment for TB as of 2010 is six months of a combined dose therapy.

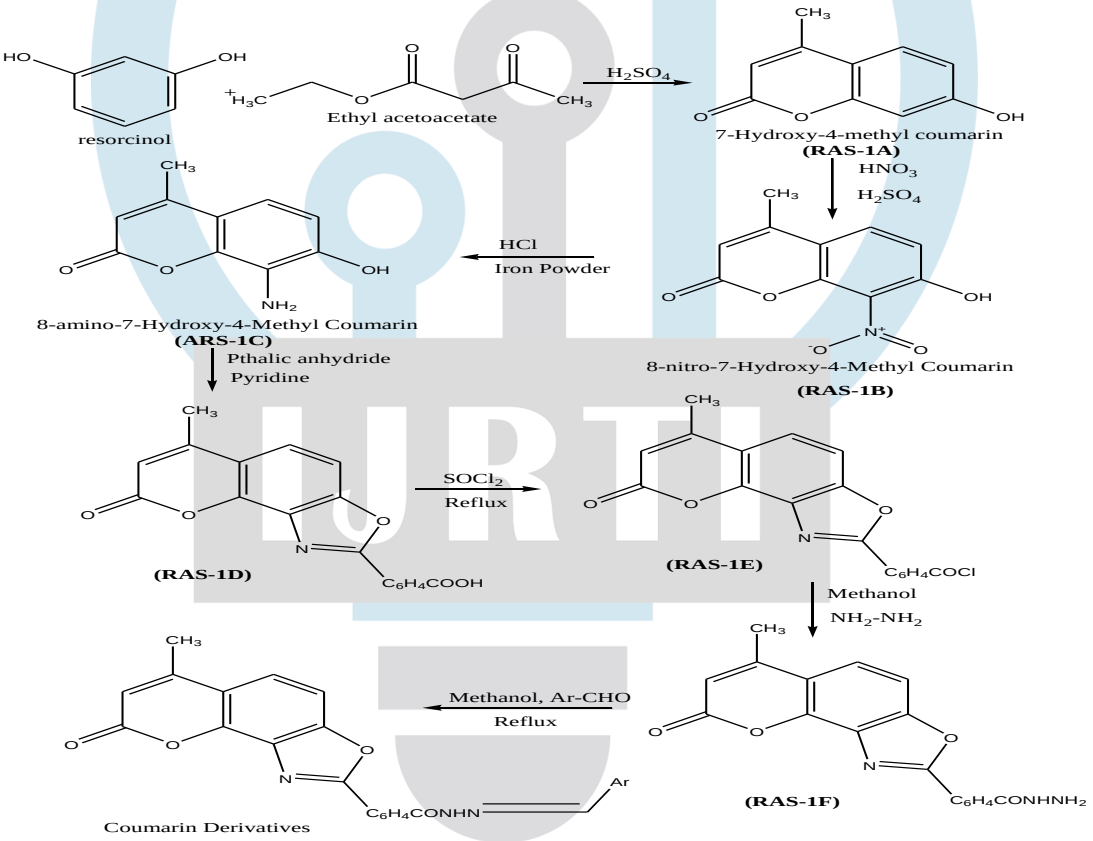
Experimental Work:

As per the study planned in the present research work, with the help of the proposed scheme required chemicals of analytical grade were procured from sigma Aldrich,fisher scientific, as well as SD fine chemicals. TLC confirmed completion of the reaction on silica gel-G plates and with the help of visualizing agents likeUV chamber or Iodine vapours able to visualize the appear spots on TLC plates.

Spectra Data:

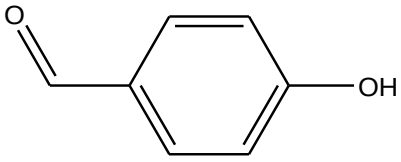
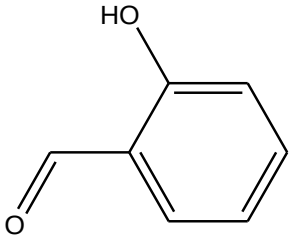
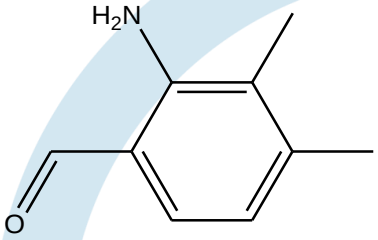
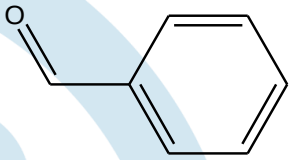
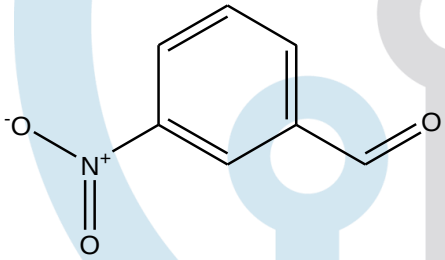
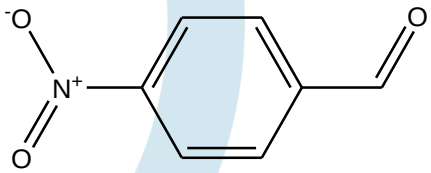
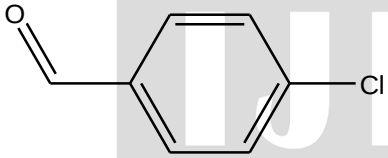
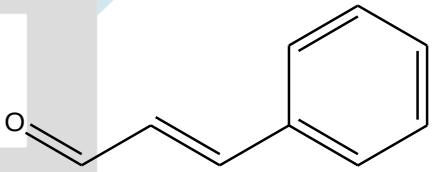
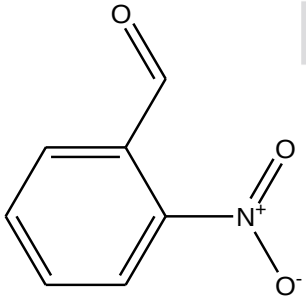
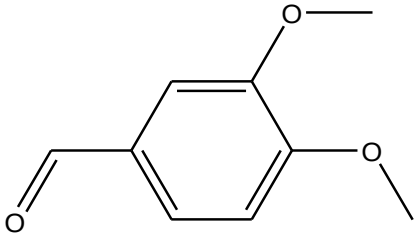
The characterization was done through determined for their melting points with the help of precision melting point apparatus, the study of IR spectra were recorded by using on KBr pellets on a Jasco FTIR-460 plus spectrophotometer, a survey of ^{1}HNMR, as well as ^{13}CNMR spectra, were recorded with the help of spectrometer specifically BRUKER 400MHz and helped of Mass spectroscopy study to confirming their molecular weight of desired molecules.

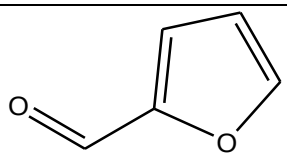
5.1 Methods:-



5.1 Scheme-I

Different aromatic aldehyde attachment with coumarin derivatives. (ARS-1a to ARS-1k)

Compound Code	Ar-CHO	Compound Code	Ar-CHO
ARS-1a	 4-hydroxy benzaldehyde	**ARS-1g**	 salicylaldehyde
ARS-1b	 p-dimethyl amino benzaldehyde	**ARS-1h**	 benzaldehyde
ARS-1c	 3-nitrobenzaldehyde	**ARS-1i**	 4-nitrobenzaldehyde
ARS-1d	 p-chlorobenzaldehyde	**ARS-1j**	 cinnamaldehyde
ARS-1e	 2-nitrobenzaldehyde	**ARS-1k**	 3,4-dimethoxy benzaldehyde

ARS-1f	 furfuraldehyde	

General Procedure:

Synthesis of 7- hydroxy, 4- methyl coumarin (RAS-1A):

Procedure:

In 500 ml beaker containing 150 ml.conc. H_2SO_4 was stirred with surrounding ice water. Cooling the beaker until temperature of acid becomes near about 5-^{0}C -10^{0}C. Another beaker containing 37 gm powdered resorcinol was added to 45 ml. of ethyl acetoacetate until clear solution was obtained. The said solution was slowly added to a beaker containing Conc. H_2SO_4.During addition the temperature should not be rise above 10^{0}C and stirring was continued for 15-20 min. the given mixture is poured in to ice cold water and solid substance was obtained. Filter out the product and dried. The crude product was recrystallized from ethanol. The resultants yields was given 80%, Meltimg Point: 191-193 ^{0}C, Molecular Mass: 176.17 and Mol. Formula $C_{10}H_8O_3$ and Solubility: Pyridine,Methanol,Ethanol.

Synthesis of 8-nitro-7-hydroxy-4-methyl coumarin(RAS-1B):

Procedure:

In a conical flask 12 gm, 7- hydroxy, 4- methyl coumarin was dissolved in 100ml conc. H_2SO_4 and the flask keep in a ice bath. Inside the flask temperature is below 1^{0}C, add 20 ml of nitrating mixture containing Conc. HNO_3 and Conc. H_2SO_4 in 1:3 ratio. Precaution is taken that the temperature is does not rise above 10^{0}C,when complete the addition remove the flask from ice bath and keep it at R.T. for 1 hrs. During this period flask is shaked occasionally and after 1 hrs poured the flask with stirring in a beaker containing crushed ice. Then filter the product which is a mixture of 6 and 8 nitro derivative washed with cold water. Transfer the product in a flask containing ethanol and boiled. The residue is 6- nitro isomer. Concentrated the filtrates and cooled in ice bath, 8-nitro derivative crystallized out. Recrystellized it from ethanol and collect 8-nitro-7-hydroxy-4-methyl coumarin. M.P.257^{0}C -258^{0}C, Molecular Mass: 221.17 and Mol. Formula $C_{10}H_7O_5$ and Solubility: Methanol, Ethanol.

Synthesis of 8-amino-7-hydroxy-4-methyl coumarin(RAS-1C):

Procedure:

4 gm iron powder was added slowly with stirring to a warm mixture of 8-nitro-7-hydroxy-4-methyl coumarin 2.2.gm in ethyl alcohol 10 ml and conc. HCl 30 ml at a reflux temperature. After addition completed in RBF refluxing was continued for 7 Hrs. after cooling a white precepited was formed, which was filtered off, then washed with water, dried and recrystallized. M.P.281^{0}C -282^{0}C, Molecular Mass: 191.2 and Mol. Formula $C_{10}H_9NO_3$ and Solubility: DMSO,Pyridine.

General procedure for synthesis of intermediate compound (RAS-1D):

In round bottom flask add 0.42g 8-amino-7-hydroxy-4-methyl coumarin (RAS-1C) and mixture of pyridine (10 ml) in phthalic anhydrides (0.3 g). The above reaction mixture was refluxed on water bath for 10 hours. The completion of reaction was ascertained by TLC (Ethyl acetate: n-hexane/4:6 or Benzene: Methanol/4:2). After that, the reaction mixture was cooled and filters the residue and washed with water. Add 5 % solution of sodium hydroxide and solution of dil. HCl.precipitate formed. The obtained solid crystals were separated through Buchner funnel and dried product was recrystallized from the ethanol.

General procedure for synthesis of intermediate compound (RAS-1E):

In round bottom flask add intermediate compound (RAS-1D) and thionyl chloride (10 ml). The above reaction mixture was refluxed on water bath for 10 hours. The completion of reaction was ascertained by TLC (Ethyl acetate: n-hexane/4:6 or Benzene: Methanol/4:2). After that, the reaction mixture was cooled and filters the residue and washed with water. Add 5 % solution of sodium hydroxide and solution of dil. HCl.precipitate formed. The obtained solid crystals were separated through Buchner funnel and dried product was recrystallized from the ethanol.

General procedure for synthesis of intermediate compound (RAS-1F):

In round bottom flask add add intermediate compound (RAS-1E) and hydrazine hydrate (3 ml) in 10 ml methanol used as a solvent. The above reaction mixture was refluxed on water bath for 10 hours. The completion of reaction was

ascertained by TLC (Ethyl acetate: n-hexane/4:6 or Benzene: Methanol/4:2). After that, the reaction mixture was cooled and filters the residue and washed with water. Add 5 % solution of sodium hydroxide and solution of dil. HCl.precipitate formed. The obtained solid crystals were separated through Buchner funnel and dried product was recrystallized from the ethanol.

General procedure for synthesis of coumarin derivatives (ARS-1a to ARS-1k):
To a solution of RAS-1F synthesized compound (1.0g) in ethanol (10ml), an appropriate aromatic aldehyde was added. The above reaction mixture was refluxed on water bath for 6 hours. The completion of reaction was ascertained by TLC (Ethyl acetate: n-hexane/4:6 or Benzene: Methanol/4:2). After that, the reaction mixture was cooled to room temperature and neutralized with 2N aqueous solution of sodium hydroxide and filtered. The obtained solid crystals were separated through Buchner funnel and dried product was recrystallized from the ethanol.

Result and Discussion:

The given research study, the synthesized substituted novel coumarin derivatives and the completion of reaction was confirmed by TLC with the help of visualizing agents like Iodine vapours or UV chambers able to visualize the appear spot on TLC plates.

The characterization was done through the melting points with the help of precision melting point apparatus, IR spectra was recorded by using Jasco FTIR-460 plus spectrophotometer.

Recording ^{1}HNMR and ^{13}CNMR spectra with the help of BRUKER 400 MHz spectrophotometer along with confirming molecular weight of desired molecules by liquid chromatography-mass spectrometric (LC-MS) techniques.

The synthesized substituted novel coumarin derivatives were screened for their in vitro antitubercular activity, anticancer activity and antioxidant activity.2D-QSAR analysis studies performed against in vitro antitubercular activity (MLR and PLS two models would be selected), in vitro antimicrobial activity (MLR model would be chosen) and in vitro anticancer activity (MLR model would be chosen).

Physicochemical data of scheme-I (Intermediates and targeted compounds)

Compound Code	Molecular Formula	Mol.Wt.	% Yield	Melting Point	Mobile Phase	R_f Value
RAS-1A	$C_{10}H_8O_3$	176.17	67.35	182-187^{0}C	Benzene: Methanol(1:1)	0.77
RAS-1B	$C_{10}H_7NO_5$	221.17	70.12	255-270^{0}C	Ethyl acetate: n-hexane (4:6)	0.76
RAS-1C	$C_{10}H_9NO_3$	191.18	65.18	279-284^{0}C	Toluene: Ethyl acetate (1:4)	0.67
RAS-1D	$C_{18}H_{11}NO_5$	321.18	55.42	211-216^{0}C	Benzene: Methanol(1:1)	0.77
RAS-1E	$C_{18}H_{10}ClNO_4$	339.73	60.72	251-256^{0}C	Ethyl acetate: n-hexane (4:6)	0.56
RAS-1F	$C_{18}H_{13}N_3O_4$	335.31	69.80	210-215^{0}C	Toluene: Ethyl acetate (1:4)	0.54
ARS-1a	$C_{25}H_{19}N_3O_5$	441.13	74.20	311-316^{0}C	Benzene: Methanol(1:1)	0.46
ARS-1b	$C_{27}H_{24}N_4O_4$	468.5	71.30	341-346^{0}C	Benzene: Methanol(1:1)	0.62
ARS-1c	$C_{28}H_{18}N_4O_6$	470.43	68.04	366-371^{0}C	Ethyl acetate: n-hexane (4:6)	0.58

ARS-1d	$C_{25}H_{18}ClN_3O_4$	459.88	62.61	349-354^{0}C	Benzene: Methanol(1:1)	0.64
ARS-1e	$C_{25}H_{18}N_4O_6$	470.43	74.50	307-312^{0}C	Toluene: Ethyl acetate (1:4)	0.71
ARS-1f	$C_{23}H_{17}N_3O_5$	415.4	67.84	288-292^{0}C	Benzene: Methanol(1:1)	0.57
ARS-1g	$C_{25}H_{19}N_3O_5$	441.44	80.10	306-311^{0}C	Benzene: Methanol(1:1)	0.49
ARS-1h	$C_{25}H_{19}N_3O_4$	425.44	78.45	365-370^{0}C	Benzene: Methanol(1:1)	0.53
ARS-1i	$C_{25}H_{18}N_4O_6$	470.43	64.43	302-307^{0}C	Toluene: Ethyl acetate (1:4)	0.81
ARS-1j	$C_{28}H_{23}N_3O_4$	465.5	68.10	342-347^{0}C	Ethyl acetate: n-hexane (4:6)	0.67
ARS-1k	$C_{27}H_{23}N_3O_6$	485.49	82.54	285-290^{0}C	Benzene: Methanol(1:1)	0.72

Table: 6.2: Physicochemical data of scheme-II (Intermediates and targeted compounds)

Compound Code	**Molecular Formula**	**Mol.Wt.**	**% Yield**	**Melting Point**	**Mobile Phase**	**R_f Value**
RAS-2 A	$C_{10}H_8O_3$	176.17	67.35	182-187^{0}C	Toluene: Ethyl acetate (1:4)	0.77
RAS-2 B	$C_{10}H_7NO_5$	221.17	70.12	255-270^{0}C	Ethyl acetate: n-hexane (4:6)	0.76
RAS-2 C	$C_{10}H_9NO_3$	191.18	65.18	279-284^{0}C	Toluene: Ethyl acetate (1:4)	0.67
TRS-2 a	$C_{17}H_{12}ClNO_3$	313.05	73.00	305-310^{0}C	Benzene: Methanol(1:1)	0.53
TRS-2 b	$C_{19}H_{18}N_2O_3$	322.13	78.45	347-352^{0}C	Toluene: Ethyl acetate (1:4)	0.68
TRS-2 c	$C_{17}H_{12}N_2O_5$	324.07	75.30	355-360^{0}C	Ethyl acetate: n-hexane (4:6)	0.54
TRS-2 d	$C_{17}H_{12}ClNO_3$	313.05	68.22	292-297^{0}C	Toluene: Ethyl acetate (1:4)	0.58
TRS-2 e	$C_{17}H_{12}N_2O_5$	324.29	72.60	345-350^{0}C	Toluene: Ethyl acetate (1:4)	0.74
TRS-2 f	$C_{15}H_{11}NO_4$	269.25	82.45	368-373^{0}C	Benzene: Methanol(1:1)	0.72
TRS-2 g	$C_{17}H_{13}NO_4$	295.29	75.30	322-327^{0}C	Toluene: Ethyl acetate (1:4)	0.61

TRS-2 h	$C_{17}H_{13}NO_3$	279.29	79.31	205-210^{0}C	Benzene: Methanol(1:1)	0.74
TRS-2 i	$C_{17}H_{12}N_2O_5$	324.29	68.20	266-271^{0}C	Ethyl acetate: n-hexane (4:6)	0.57
TRS-2 j	$C_{19}H_{15}NO_3$	305.33	66.87	304-308^{0}C	Toluene: Ethyl acetate (1:4)	0.80
TRS-2 k	$C_{19}H_{18}N_2O_3$	322.36	70.24	221-226^{0}C	Ethyl acetate: n-hexane (4:6)	0.63

Spectral Characterization of Scheme-I:-

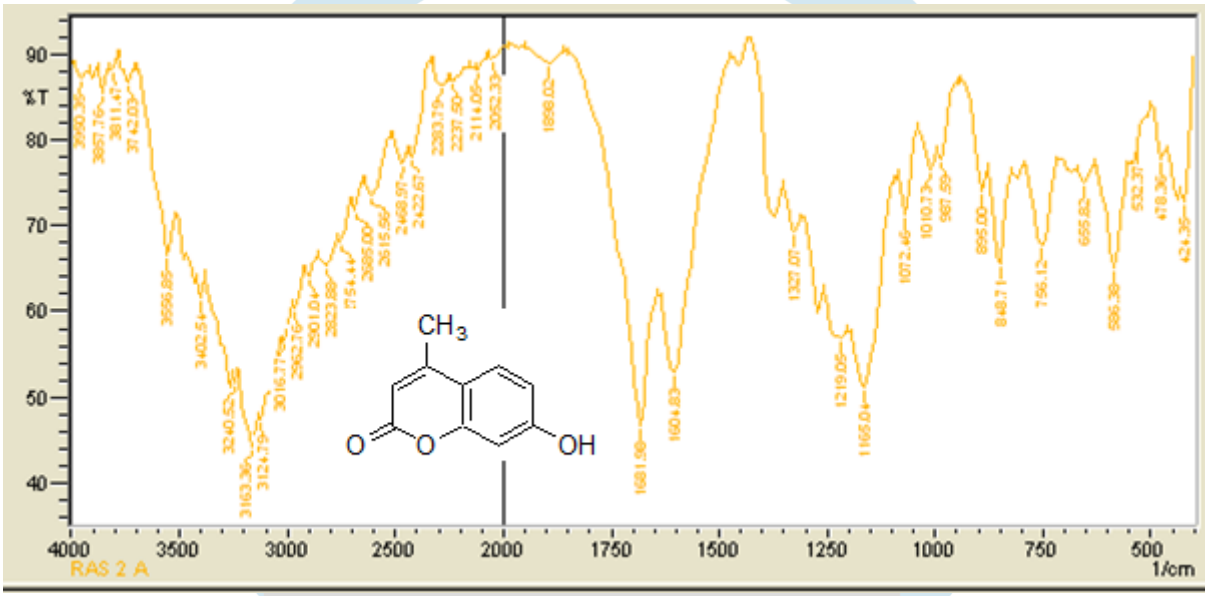


Fig .No:- 6.1 FTIR Spectra of 7- hydroxy, 4- methyl coumarin (RAS-1A)

Table No: 6.3 FTIR Spectrum data of 7- hydroxy, 4- methyl coumarin (RAS-1A)

Sr. No.	Wave Number(cm^{-1})	Functional Group Assigned
1	3163	-OH, Ar
2	2962	C-H, Ar
3	1681	C=O
4	1600	C=C
5	1219	C-O-C

Conclusion:

We have synthesized novel coumarin deriv atives (IIIa–d) and (IVa–d) and evaluated their anti oxidant and reducing power properties. Coumarin derivatives (IIIa), (IIIb), (IVa), and (IVd) were potent antioxidants in most of the tested methods. The cou marin derivatives showed promising reducing capacity. Hydroxyl substituent at position 7 of coumarin moiety enhanced the activity and acetate group at position 7 reduced the activity. Therefore, our findings are a great impact on the coumarin field in search of molecules possessing potent antioxidant activity

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