Research Article

Synthesis and Antibacterial Activities of Coumarins with An Acyl Amino Side Chain

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ABSTRACT

Coumarin is a heterocyclic moiety containing benzo pyran ring structure. It is widely distributed in the plant kingdom as a glycoside. Many compounds of medicinal and pharmaceutical importance possess coumarin ring structure. Drugs with coumarin structure can be synthesized by many well-known synthetic reactions. Coumarin derivatives were found to be antibacterial, anticoagulant, anti-inflammatory, anti-HIV, anti-cancer and antioxidant. As part of the search for new and effective antibacterial agents, eight novel derivatives of coumarins were synthesized by Mannich reaction. All the compounds were evaluated for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* using ciprofloxacin as standard and DMSO as solvent. Three of the synthesized derivatives exhibited good antibacterial activity against *Staphylococcus aureus* and *Escherchia coli*. The work must be extended so as to develop coumarin derivatives into highly effective antibacterial agents.

Keywords: Coumarin, Mannich reaction, amino acid, *Staphylococcus aureus*, *Escherichia coli*, zone of inhibition.

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INTRODUCTION

Coumarin, a major class of naturally occurring compounds possess an oxygen heterocyclic benzo pyran system belonging to the lactone family.[1] Their synthesis can be achieved by any one of the methods named Pechmann condensation[2],Claisen rearrangement,[3] Knoevenagel reaction,[4] Reformatsky synthesis,[5] Wittig reaction[6] and Mannich reaction.[7]An important property of coumarin moiety is it's ability to form C-glycosides by combining with sugar units.[8]The benzopyran structure enables coumarin derivatives to combine with biological macromolecules like drug receptors, enzymes etc. This combination arises due to the weak bond interactions of coumarin derivatives with various drug receptors.[9] The bonding ability offers immense potential for coumarin derivatives as therapeutic agents. Coumarin derivatives are an essential constituent not only to the pharmaceutical industry but also to the cosmetic and perfumery industries. Coumarin analogs are very well known in pharmaceutical industry as drugs and also as intermediates in drug synthesis. For example, Warfarin, Dicoumarol, Acenocoumarol all coumarin derivatives which are used as anti coagulants,[10] Novobiocin Chlorobiocin and Coumermycin A1 as antibiotics.[11] Umbelliferone, a 7 hydroxy coumarin is present in about 150 different plant species, distributed over 30 different families.[12]

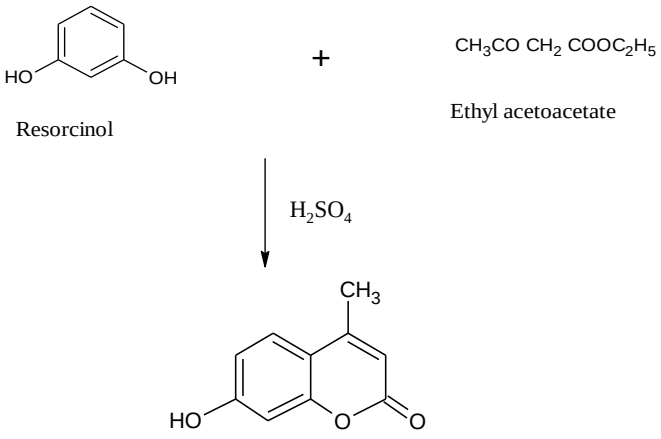
A detailed and comprehensive survey of the published research articles shows that coumarin derivatives possess an array of pharmacological activities viz anti coagulant,[13] anti fungal, [14]anti inflammatory, [15]anti HIV,[16] anticancer[17] and antioxidant.[18] In addition, extensive scanning of the literatures revealed the antibacterial activities of coumarin compounds against a wide spectrum of pathogenic bacterial organisms.[19-22]

As the number of drug resistant strains of bacteria are increasing at a rapid pace, the need for developing newer antibacterial agents is an immediate priority for medicinal chemists. These facts along with the proven antibacterial potency of coumarins prompted us to synthesize newer analogs of coumarin by Maanich reaction and evaluate their activity on gram positive and gram negative organisms.

MATERIALS AND METHODS

All the chemicals and reagents were of analytical grade and was procured from Sara fine chemicals, spectrum reagents and NICE chemicals. The melting points of the starting materials were uncorrected. UV spectra was recorded using JASCO V-630 spectrophotometer, IR spectra was recorded using AVATAR 370 and ^{1}HNMR spectra was recorded using BRUKER AVANCELL. Mass spectra was recorded using Q-Tof mass spectrophotometer. The progress of the reactions was monitored using TLC. The bacterial strains were procured from National Collection of Industrial Micro organisms (NCIM), Pune.

Scheme of Synthesis

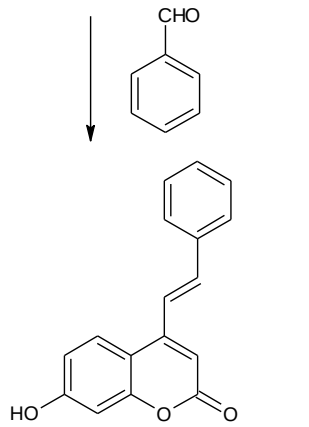


Resorcinol

Ethyl acetoacetate

7-hydroxy-4-methyl-2*H*-chromen-2-one

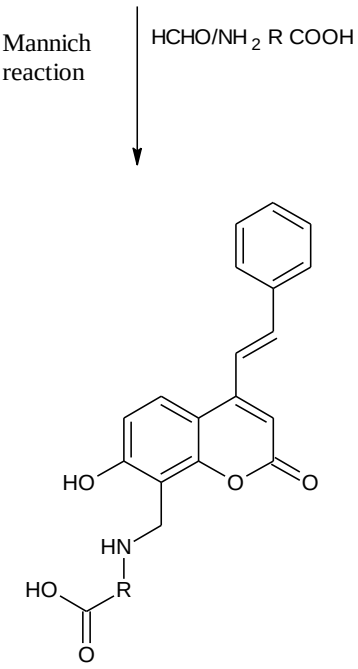
(1)



7-hydroxy-4-[(*E*)-2-phenylethenyl]-2*H*-chromen-2-one

(2)

Mannich reaction



3-5{7-hydroxy-2-oxo-4-[(*E*)-2-phenylethynyl]-2-*H*-chromone-8-yl} methyl)]amino acid

Preparation of 7-hydroxy-4-methyl coumarin

A measured volume of 100ml concentrated sulphuric acid was taken in a conical flask and immersed in an ice bath. Added 12.5gm of resorcinol dissolved in 17.5ml of ethylaceto acetate drop wise with stirring and the temperature was maintained below 10ºC. The reaction mixture was kept at room temperature for 1 hour and poured it with vigorous stirring to a mixture of crushed ice and water in a beaker. Collected the precipitate by filtration and washed with cold water. The crude product was dried at 100ºC and recrystalised from ethanol and dried. The purity of the product was established by a single spot on the TLC. The mobile phase was n-hexane:ethyl acetate in the ratio 8:2. The M.P of the compound was found to be 168º C. Yield 82%. Spectral details of 7-hydroxy -4-methyl coumarin is as follows:

IR: 3478 (OH str), 1724 (C=O,lactone str), 1527(C=C,Ar str),

^{1}H NMR (400 MHz, DMSO-d6): δ 2.36 (s, 3H,-CH3), 6.12(s, 1H), 6.78-6.81 (m, 1H, Ar-H), 10.52(s, 1H, -OH).

MS: 176 (M+)

Elemental analysis: Calcd C (68.18%), H(4.58%) O(27.25%)

Found C (68.14%), H(4.56%) O(27.27%)

Preparation of 7-hydroxy-4-[(*E*)-2-phenylethenyl]-2*H*-chromone-2-one.

Weighed out equimolar quantities of 7-hydroxy-4-methyl coumarin and benzaldehyde and are fused together with 2 to 3 drops of concentrated sulphuric acid at a temperature of 120-130ºC for 1 hour. The obtained product is treated with ethanol and it is then poured into crushed ice. The formed precipitate is filtered and is recrystallised from ethanol and dried.The purity of the product was established by a single spot on the TLC. The mobile phase was n-hexane:ethyl acetate in the ratio 8:2. M.P 245 º C. Yield -70%. Spectral values of **7**-hydroxy-4-[(*e*)-2-phenylethenyl]-2*h*-chromone-2-one are given below.

IR: 3504 (OH str), 3056,(C-H str Ar) 1654 (C=O,lactone str), 1705 (CH=CH bending) 1618

(C=C,Ar str)

^{1}H NMR (400 MHz, DMSO-d6): δ 2.56 (s, 3H,-CH3), 7.25 - 7.48 (d, 1H, Ar-H), 11.32(s, 1H, -OH).

MS:264(M+)

Elemental analysis: Calcd C (77.26%), H(4.58%) O(18.16%)

Found C (777.32%), H(4.53%) O(27.22%)

Preparation of {7-hydroxy-2-oxo-4-[(*E)*-2-phenylethinyl]-2-*H*-chromone-8-YL }methyl)] Amino Acid (CR1-CR8)

Weighed 1.32gm of 7-hydroxy-4-[(*E*)-2-phenylethenyl]-2*H*-chromone-2-one and was dissolved in ethanol by warming. To this 0.83g of amino acid and 0.45ml of formaldehyde was added. The precipitate obtained was filtered, recrystallised from ethanol and dried. The reaction was monitored using TLC using n-hexane: ethyl

acetoacetate as mobile phase. The physical datas of the compounds are presented in table number 1.

Synthesis of [({7-hydroxy-4-[(*Z*)-2-phenylethenyl]-3,4-dihydro-2*H*-1-benzopyran-8-yl}methyl)amino] acetic acid.(CR1)

IR : 3432 (NH str), 2922(Aromatic CH str) 2679(Alkyl CH Str) 1776 (C=O str) 1550(Aromatic C-C str) 1214 (C-N Str)

^{1}H NMR: δ 2.03 (2H,), 3.52 (s 2H,),), 4.15 (2H,ddd), 6.58 (1H, s), 7.17-7.35 (m,10H, Ar),10.5(s,1H,NH),11.32 (s,1H,OH)

^{13} C NMR:163.15(C=O), 155.25,151.34,144.78,30.15

MS:351(M+)

Elemental analysis : Calcd C(68.37%) H(4.88%) N(3.99%) O(22.77%)

Found C(68.48%) H(4.81%) N(3.75%) O(22.72 %)

Synthesis of 2-[({7-hydroxy-2-oxo-4-[(*E*)-2-phenylethenyl]-3,4-dihydro-2*H*-1-benzopyran-8-yl}methyl)amino] propanoic acid. (CR2)

IR : 3314 (NH str), 3071(Aromatic CH str) 2679(Alkyl CH Str) 1764 (C=O str)

1550(Aromatic C-C str) 1200(C-N Str)1021(C-O Str)

^{1}H NMR: δ 1.35 (d, 3H,) 6.58 (s, 1H), 7.18-7.37 (m,10H, Ar) , 10.20(s,1H,NH),11.36(s,1H,OH)

^{13} C NMR: 166 27 (C=O), 152.25,148.34, 143.78, 28.75.

MS:365 (M+)

Elemental analysis: Calcd C(69.03%) H(5.24%) N(3.83%) O(21.89%)

C(69.223%) H(5.18 %) N(3.74 %) O(22.08%)

Synthesis of 3-phenyl 2-[({7-hydroxy-2-oxo-4-[(*E*)-2-phenylethenyl]-3,4-dihydro-2*H*-1-benzopyran-8-yl} methyl)amino] propanoic acid. (CR3)

IR : 3314 (NH str), 3071(Aromatic CH str) 2679(Alkyl CH Str) 1767 (C=O str) 1538 (Aromatic C-C str) 1210(C-N Str)

^{1}H NMR: δ 1.89 (ddddd ,1H,) 7.17-7.35 (m,10H Ar)10.45(s,1H,NH), 11.24(s,1H,OH)

^{13} C NMR: 165.16 (C=O), 164.25, 153.15,149.15,31.45.

MS:441(M+)

Elemental analysis: Calcd C(73.46%) H(5.25%) N(3.17%) O(18.12%)

C(73.49%) H(5.1 4%) N(3.11%) O(18.02 %)

Synthesis of 3-hydroxy-2-[({7-hydroxy-2-oxo-4-[(*E*)-2-phenylethenyl]-3,4-dihydro-2*H*-1-benzopyran-8-yl}methyl)amino] propanoic acid. (CR4)

IR : 3314 (NH str), 3071(Aromatic CH str) 2679(Alkyl CH Str) 1750(C=O str) 1550(Aromatic C-C str) 1200(C-N Str)1021(C-O Str)

^{1}H NMR: δ 1.89 (ddddd ,1H,) 7.45- 8.15(m,10H,Ar), 10.35(s,1H,NH),11.25(s,1H,OH)

^{13} C NMR: 163.26 (C=O), 160.18, 151.15,151.15,28.76.

MS:381(M+)

Elemental analysis: Calcd: C(66.13%) H(5.02%) N(3.67%) O(25.17%)

C(66.22 %) H(4.96 %) N(3.58 %) O(25.35 %)

Synthesis of 2-[({7-hydroxy-2-oxo-4-[(*E*)-2-phenylethenyl]-3,4-dihydro-2*H*-1-benzopyran-8-yl}methyl)amino]-3-sulfanyl propanoic acid. (CR5)

IR : 3314 (N-H str), 2985 (Aromatic C-H str) 2679(Alkyl C-H Str) 1763 (C=O str) 1525 (Aromatic C-C str) 1264 (C-N Str)

^{1}H NMR: δ 1.89 (dddd,1H, CH_2) 7.24-8.35(m,10H,Ar), 10.43 (s,1H,NH),11.28 (s,1H,OH)

^{13} C NMR: 164.26 (C=O), 162.25, 150.15,148.15,29.78.

MS: 411 (M+)

Elemental analysis: Calcd: C(63.46%) H(4.82%) N(3.52%) O(20.13%) S(8.07%).

C(63.29 %) H(4.84%) N(3.55%) O(20.18%) S(8.12%).

Synthesis of 3-(4-hydroxy) phenyl 2-[({7-hydroxy-2-oxo-4-[(*E*)-2-phenylethenyl]-3,4-dihydro-2*H*-1-benzopyran-8-yl} methyl)amino] propanoic acid. (CR6)

IR : 3358 (NH str), 2881(Aromatic CH str) 2679(Alkyl CH Str) 1745 (C=O str) 1550(Aromatic C-C str) 1205 (C-N Str)

^{1}H NMR: δ 1.89 (dddd, 1H, CH_2) 2 .53-2.69 (dd,2H,CH) 7.17-7.35 (m,10H,Ar), 10.35 (s,1H,NH),11.43 (s,1H,OH)

^{13} C NMR: 162.38 (C=O), 159.25, 153.15,147.18, 28.65.

MS: 457(M+)

Elemental analysis: Calcd:C(70.89%) H(5.07%) N(3.06%) O(20.98%)

C(71.03 %) H(5.22%) N(3.12 %) O(21.05 %)

Synthesis of 2-methyl- 2-[({7-hydroxy-2-oxo-4-[(*E*)-2-phenylethenyl]-3,4-dihydro-2*H*-1-benzopyran-8-yl} methyl)amino] butanoic acid. (CR7)

IR : 3314 (NH str), 3112 (Aromatic CH str) 2679(Alkyl CH Str) 1757 (C=O str) 1532 (Aromatic C-C str) 1225 (C-N Str)

^{1}H NMR: δ 1.89 (1H, CH_2) 2.12 (1H, dddd, CH) 7.17-7.35 (m. 10H Ar ,) 10.38 (s,1H,NH) 11.15(dd,1H,OH) .

^{13} C NMR: 161.16 (C=O), 159.25, 151.15,149.15,30.73.

MS:393(M+)

Elemental analysis: Calcd : C(70.21%) H(5.89%) N(3.56%) O(20.33%)

C(70.24 %) H(5.94%) N(3.58%) O(20.37 %)

Synthesis of 3-hydroxy - 2-[({7-hydroxy-2-oxo-4-[(*E*)-2-phenylethenyl]-3,4-dihydro-2*H*-1-benzopyran-8-yl} methyl)amino] butanoic acid. (CR8)

IR : 3314 (NH str), 29941(Aromatic CH str) 2679(Alkyl CH Str) 1760(C=O str) 1550(Aromatic C-C str) 1223 (C-N Str)

^{1}H NMR: δ 1.89 (1H, ddddd, CH_2) 7.20 -7.45 (m, 10H, Ar,) 10.42 (s,1H,NH) 11.25(dd,1H,OH)

^{13} C NMR: 164.24 (C=O), 159.33, 152.23,149.15,29.45.

MS:409(M+)

Elemental analysis: Calcd :: C(66.83%) H(5.35%) N(3.54%) O(24.28%)

C(66.45%) H(5.46 %) N(3.46 %) O(24.25%)

Antibacterial Screening

Placed agar plates right side up in an incubator heated to 37ºC for 10-20 minute with the covers adjusted so that the plates are slightly opened. Label the covers of each of the plates with the name of test organisms to be inoculated using sterile technique, inoculate all agar plates with *E.coli* and *S.aureus*. Using the swab, streak the entire agar surface horizontally, vertically and around the outer edge of the plate to ensure a heavy growth over the entire surface. Allow the culture plates to dry for about 5 minute. After solidification of the media, a well with the help of borer (0.85cm) was made and 0.1ml of synthetic compound dissolved in DMSO was inoculated into the well. Controls are performed (for each bacterial strains), where 0.1ml of pure solvent was inoculated into the well. The plates were inoculated for 24 hours at 37ºC. The zone of inhibition of bacterial growth exhibited by the compounds against the particular test bacterial strains determined, the antibacterial activities of the synthetic compound.[23] The zone of inhibition measured for the compounds against *Staph Aureus* and *E Coli* are depicted in table number 2.

Table 1: Physical Properties of {7-hydroxy-2-oxo-4-[(*E*)-2-phenylethinyl]-2-*H*-chromone-8-yl }methyl)] Amino Acid (CR1-CR8)

Compound Code	R	Molecular Formula	Molecular Weight (D)	Melting Point (^{0}C)	Rf Value	Yield (%)
CR1	-CH_2-	$C_{20}H_{17}NO_5$	351	259	0.67	70
CR2	-CH-CH_3	$C_{21}H_{19}NO_5$	365	284	0.69	64
CR3	-CH-CH_2-C_6H_5	$C_{27}H_{23}NO_5$	441	263	0.79	62
CR4	-CH-CH_2-OH	$C_{21}H_{19}NO_6$	381	280	0.64	63
CR5	-CH-CH_2-SH	$C_{21}H_{19}NO_5S$	411	265	0.72	59
CR6	-CH-$CH_2C_6H_4OH$	$C_{27}H_{23}NO_6$	457	246	0.67	68
CR7	-CHCH(CH_2)CH_2	$C_{23}H_{23}NO_5$	393	301	0.65	61
CR8	-CH-CH(CH_2)OH	$C_{22}H_{21}NO_6$	409	256	0.69	68

Table 2: Zone of Inhibition of compounds against *Staphylococcus aureus* and *Escheichia coli*

Sl No	Codes of Compounds	*Staphylococcus Aureus*		*Escherichia Coli*	
		***50* µgm/ml**	***100* µgm/ml**	***50* µgm/ml**	***100* µgm/ml**
1	CR1	04	12	03	10
2	CR2	02	09	05	11
3	CR3	06	11	04	10
4	CR4	06	19	06	17
5	CR5	13	22	09	20
6	CR6	06	20	05	18
7	CR7	05	12	03	09
8	CR8	06	13	04	10
9	Ciprofloxacin (30µgm/ disc)	29		28	
10	Solvent (DMSO)	0		0	

RESULTS AND DISCUSSION

Eight derivatives of coumarin were synthesized and screened for their antibacterial activity.

The reactions were monitored by TLC analysis and the melting points were determined. The structures of the respective prototypes were confirmed by UV, IR NMR and mass spectra. Antibacterial activity was determined by measuring the zone of inhibition of the compounds against *Staphylococcus Aureus* and *Escherichia Coli* at concentrations of 50 µg/ml and 100 µg/ml using ciprofloxacin 30µg/ml as standard and DMSO as solvent.

Out of the synthesized compounds, three compounds had good activity against *S.aureus* and *E.coli* at a concentration of 100 µg/ml, whereas remaining five compounds exhibited moderate activity against the microorganisms. Among the three compounds which exhibited good antibacterial activity, CR5(2-[({7-hydroxy-2-oxo-4-[(*E*)-2-phenylethenyl]-3,4-dihydro-2*H*-1-benzopyran-8-yl}methyl)amino]- 3-sulfanyl propanoic acid) exhibited the highest activity at a concentration of 100 µg/ml followed by CR6 3-(4-hydroxy) phenyl 2-[({7-hydroxy-2-oxo-4-[(*E*)-2-phenylethenyl]-3,4-dihydro-2*H*-1-benzopyran-8-yl} methyl)amino] propanoic acid) and CR4 **3**-hydroxy-2-[({7-hydroxy-2-oxo-4-[(*E*)-2-phenylethenyl]-3,4-dihydro-2*H*-1-benzopyran-8-yl}methyl) amino] propanoic acid. At a concentration of 50 µg/ml all the compounds showed negligible activity. The comparatively higher activity of CR5 can be attributed to the presence of sulfhydryl group in the aliphatic chain of the molecule. The activities of CR5 and CR4 can be explained on the basis of the presence of aromatic ring in the side chain of the molecule.

CONCLUSION

From the present study, it can be concluded that compounds with 7- hydroxy coumarin ring with an amino acyl side chain attached to the eighth position of the coumarin ring can be developed as effective antibacterial agents against gram-positive and gram-negative bacteria. As infections due to various bacterial organisms are on the rise, as well as resistance of currently available drugs also on the upswing, studies which focus on the development of newer antibacterial agents should be carried out. Hence development of more and more analogues of coumarin should be undertaken, so that the search for effective antibacterial agents get fulfilled.

REFERENCES

1. Stefanachi A, Leonetti, F, Pisani, L, Catto, M, & Carotti, A. Coumarin: A Natural, Privileged and Versatile Scaffold for Bioactive Compounds. *Molecules*, 2018; *23*(2): 250-257. https://doi.org/10.3390/molecules23020250.

2. Majid M, Heravi S, Khaghaninejad M.M, Chapter One - Pechmann Reaction in the Synthesis of Coumarin Derivatives, Advances in Hetrocyclic Chemistry, 2014;112: 41-50.

3. Lončarić M, Gašo-Sokač D, Jokić S, Molnar M. Recent Advances in the Synthesis of Coumarin Derivatives from Different Starting Materials. Biomolecules. 2020 ;10(1):151-160. doi: 10.3390/biom10010151. PMID: 31963362.

4. Abraham GG**,** David M. Aparicio S , Hidemí AM **,** Abraham GR **,** Luis F. R **,** Cuauhtémoc AS **,** Cárlos EL**,** Nancy RC Synthesis of 3-carboxylated Coumarins by Knoevenagel Condensation and Exploratory Anti-inflammatory Activity Evaluation by *in vivo* model. American Journal of Organic Chemistry, 2016; 6(1): 17-28. doi:10.5923/j.ajoc.20160601.03.

5. Donald CD, Qun L, and Dimitry VA Synthesis of Coumarins, 4-Hydroxycoumarins, and 4-Hydroxyquinolinones by Tellurium-Triggered Cyclizations,Journal of Organic Chemistry, 2005;70: 4682-4686.

6. Liu YY, Thom E, Liebman A A, ChemInform Abstract: Coumarins via the Wittig reaction.Synthesis of Methoxsalen -514c, Chemischer Informationsdienst, 2016; 10.1002/chin .1979; 49219 10: 49.

7. Madhura V, Manohar VK, Amol S, Alagawadi KV**,** Mannich Reaction as a New Route for the Synthesis of Tetrahydro Pyrimido Benzimidazolyl Coumarins, Journal of Heterocyclic Chemistry, 2014; 51(6): 1705-1711.

8. Sun, Y, Gao, M., Chen, H., Han, R., Chen, H., Du, K., Zhang, Y., Li, M., Si, Y., & Feng, W.Six New Coumarin Glycosides from the Aerial Parts of *Gendarussa vulgaris*. *Molecules,* 2019; *24*(8): 1456. https://doi.org/10.3390/molecules24081456.

9. Maria JM , Santiago V, Sonja K, André F, Lourdes S, Eugenio U, Fernanda B, Karl NK, Insight Into the Interactions Between Novel Coumarin Derivatives and Human A3 Adenosine Receptors, Chem med Chem, 2014; 9(10): 2245-2253.doi: 10.1002/cmdc. 201402205.

10. Lei, L, Xue, Y, Liu, Z. Coumarin derivatives from *Ainsliaea fragrans* and their anticoagulant activity. *Sci Rep* 2015;5:13544-13553. https://doi.org/10.1038/srep13544.

11. Muratovic S, Duric K, Veljovic E, Osmanovic A, Dzenita Softic ,Davorka Zavrsnik, Synthesis of Biscoumarin Derivatives as Antimicrobial Agents,Asian Journal of Pharmaceutical and Clinical Resaearch, 2013 ;6(3): 132-134.

12. Venugopala KN, Reshmi V, Odhav B Review on Natural Coumarin Lead Compounds for Their Pharmacological Activity, Biomed Research International, 2013;10:01-14. http://dx.doi.org/10.1155/2013/963248.

13. Kasperkiewicz K, Ponczek MB, Owczarek J, Guga P, Budzisz EZ, Antagonists of Vitamin K—Popular Coumarin Drugs and New Synthetic and Natural Coumarin Derivatives, Molecules 2020; 25: 1465-1469. doi:10.3390/molecules25061465.

14. Prusty, J.S. Kumar A, Coumarins antifungal effectiveness and future therapeutic scope. Mol Divers ,2019;24:1367-1383. https://doi.org/10.1007/s11030-019-09992-x.

15. Kayal G, Kayal S, Gokhale N and Jain S: Synthesis and Biological Evaluation of Coumarin Derivatives as Anti-Inflammatory Agents. Int J Pharm Sci Res 2016; 7(6): 2394-24001.doi: 10.13040/IJPSR.0975-8232.7(6).2394-01.

16. Kostova, S, Raleva K Genova P and Argirova R Structure-Activity Relationships of Synthetic Coumarins as HIV-1 Inhibitors, Bioinorganic Chemistry and Applications 2006; Vol 2006: 1-9.

17. Thakur A, Singla R, Jaitak V, Coumarins as anticancer agents: A review on synthetic strategies, mechanism of action and SAR studies, Eur J of Med Chem, 2015; 101: 476-495.

18. Al-Majedy YK, Al-Duhaidahawi DL, Al-Azawi KF, Al-Amiery AA, Kadhum AH, Mohamad AB. Coumarins as Potential Antioxidant Agents Complemented with Suggested Mechanisms and Approved by Molecular Modelling Studies. *Molecules,* 2016; 21(2): 135-142. doi:10.3390/molecules21020135.

19. De Souza SM, Delle MF, Smânia A J. Antibacterial activity of coumarins. *Z* Naturforsch C J Biosci. 2005; 60(9-10):693-700. doi:10.1515/znc-2005-9-1006.

20. Yasameen KA, Abdul AH, Ahmed AK, Amiery AM, Coumarins. The Antimicrobial Agents, Systematic Reviews in Pharmacy, 2017; 8(1): 62-70.

21. Shanawas KM Ubaidulla M, Imtiyaz HM , Tarannum, N , Design, Synthesis and validation of antimicrobial Coumarin derivatives: An efficient green approach. Heliyon, 2019; 5(1): e201615.

22. Maria JM, Saleta VR, Lourdes KS, Eugenio PU, Fuentes EC, Munoz Crego DA,. Looking for New Targets: Simple Coumarins as Antibacterial Agents, Medicinal Chemistry, 2012;8(6):1140-1145.
DOI: 10.2174/1573406411208061140

23. Vinod B, Prasanth V V, Synthesis and Broad Spectrum Antibacterial Activities of 2-Pyrazolines Synthesized from Chalcones, Int J of Pharm Sci, 2019 ;10(12): 5510-5516.

DOI: 10.13040/IJPSR.0975 8232.10(12).5510-16.

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