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Coumarin based Mannich adducts and their antimycobacterial activities

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Abstract: A series of new (*E*)-7-hydroxy-8-(amino-methyl)-4-styryl-2*H*-chromen-2-one derivatives has been synthesized. The protocol involves the reaction of Knoevenagel condensation product of 7-hydroxy-4-methylcoumarin with secondary aliphatic amine and equimolar formaldehyde under Mannich conditions. *In vitro* antimycobacterial activity of the developed compounds has been assessed against *Mycobacterium smegmatis,* a surrogate of the causal organism of tuberculosis *Mycobacterium tuberculosis*, by broth dilution assay. Among the tested compounds, **7a-f** shows MIC value against the concentration 1000µg/mL.

Keywords: Coumarin nucleus, Mannich reaction, Knoevenagel condensation, Tuberculosis, Antimycobacterial

1. Introduction

The heterocyclic compound, coumarin (2H-1-benzopyran-2-one), often known as "*coumarou*" was first discovered from the *tonka bean* in 1820[1]. These are among the most significant types of often-occurring natural chemicals in nature[2,3]. It has been widely noted that natural compounds can serve as important models for the construction of molecules that are synthetic[4–6]. The Coumarin derivatives shows a diverse range of biological and pharmacological attributes, such as anti-bacterial[7], antifungal[8], antiviral[9], antimalarial[10], anticancer[11], anti-alzheimer[12], anti-inflammatory[13], antioxidant[14], anticoagulants[15], anti-

influenza[16], neuroprotective[17], free radical scavengers, lipoxygenase, cyclooxygenase, and tyrosine kinase[18] inhibitory activities. These activities may be attributed to the fact that diversely substituted coumarin moiety, can exert non-covalent interactions with the active sites of various target proteins and enzymes[19]. Hence, the tremendous collection of these biological effects of compounds based on coumarin is linked to the coumarin main structural component.

A possible method for creating a variety of synthetic and semi-synthetic coumarin derivatives which might serve as useful therapeutic interventions for the treatment of disorders is the hybridization of coumarin moiety by using bioactive pharmacophores[20]. As a result, coumarin-containing hybrids play a crucial role in the creation of new therapies. Over the years, numerous synthetic coumarin derivatives and coumarin-containing hybrids (**Figure 1**) have been synthesized including coumarin-azoles[11,21–23], coumarin-chalcones[24], coumarin dimers[25], coumarin-furoxan[26], coumarin-imine[27], coumarin-indole/isatin[28], coumarin-pyridine/pyrimidine[29], coumarin-sulfonamides[30] and so on. Furthermore, many coumarin analogues are being investigated as promising lead applicants for drug discovery and development to endow pharmacological properties, low toxicological profile, less adverse reactions, ability to overcome resistance to drugs, higher bioavailability, a wider spectrum, and more effective therapeutic effects.

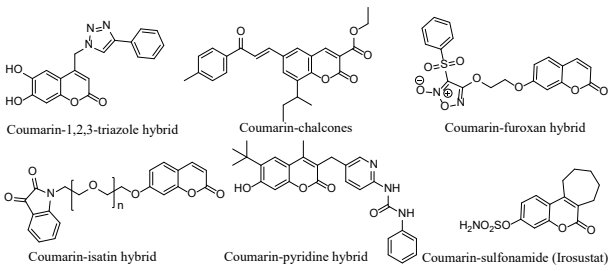


Fig. 1. Synthetic coumarin derivatives and coumarin-containing hybrids.

Tuberculosis (TB) caused by single bacterium *Mycobacterium tuberculosis* (MTB) pose a very serious threat to life for ages, and because of the rise in resistance to drugs. The problem is still complicated and requires novel therapeutic alternatives. *Mycobacterium smegmatis* is surrogate because of the requirement of less containment facility and fast-growing life cycle in comparison to MTB. From the literature, we found that the anti-mycobacterial activity of 7-hydroxy-4-methylcoumarin is certainly related to the substitution at its 8thposition[31]. These findings have made coumarin an attractive lead for further transformations at the 8th-position with non-polar substituents. In light of these results and the continuation of our research[32,33] aimed at discovering coumarin−based antimycobacterial agents, herein, an attempt has been made to synthesize a novel series of 7-hydroxy-4-methylcoumarin based derivatives.

Given the above facts, in our present study, we have synthesized different coumarin based Mannich bases by reacting (*E*)-7-hydroxy-4-styryl-coumarins with various substituted secondary amines in the presence of formaldehyde. The synthesized 7-hydroxy-8-(amino-methyl)-4-styryl-coumarin derivatives have been evaluated

for their antimycobacterial activities.

2. **Experimental**

2.1 *General*

The top-grade commercially available precursors and extra reagents were all employed without further purification. Thin-layer chromatography (TLC), carried out using pre-coated silica gel 60-F_{254} aluminium plates (Merck plates), was used to track the reactions' progress. Iodine staining and UV light were used to see spots. The melting points were uncorrected and recorded using the melting-point instrument. On 500 MHz spectrometers (JEOL JNM-ECZR 500 RS1), ^{1}H and ^{13}C NMR spectra were captured and referred to leftover solvents. The units of chemical shifts are parts per million (ppm). Spin multiplicities can be categorised as singlets (s), wide singlets (brs), doublets (d), triplets (t), quadruplets (q), or multiplets (m).

2.2 *Synthesis*

*2.2.1. General procedure for the synthesis of 7-hydroxy-4-methylcoumarin (**3**):* A mixture of resorcinol (10.0 g), ethyl acetoacetate (11.8 mL) and conc. H_2SO_4 (90.9 mL) was heated in an oil bath at 100°C for 30 minutes. The resulting solution was cooled with crushed ice and stirred for 15 minutes. The crude was filtered off and washed with water and dried in an oven at 100°C. Yellow solid, yield 89%; Mp: 180−182 °C;^{1}H NMR (500 MHz, DMSO−d_6): δ 2.47(s, 3H, CH_3), 6.08 (s, 1H, H-3), 6.66, (s, 1H, H-8), 6.76 (d, 1H, H-6), 7.55 (d, 1H, H-5), 10.38 (brs, 1H, OH); ^{13}C NMR (125 MHz, DMSO−d_6): δ 161.7 (C-2), 160.8 (C-7), 155.4 (C-9), 154.1 (C-4), 127.1 (C-5), 113.3 (C-6), 112.5 (C-3), 110.7 (C-10), 102.7 (C-8), 18.6 (CH_3).

*2.2.2.General procedure for the synthesis of (E)-7-hydroxy-4-styryl-2H-chromen-2-ones (**5a−c**)*: To a solution of 7-hydroxy-4-methylcoumarin **3** (10 mmol) in methanol, appropriately substituted aromatic aldehyde **4** (10 mmol) was added. To this, piperidine in the catalytic quantity was added. Three hours were spent refluxing the resultant mixture. TLC kept track of the reaction's development and completion. The solid reaction product precipitated out at the completion of the reaction. It underwent filtration, complete methanol washing, and recrystallization from chloroform to afford (*E*)-7-hydroxy-4-styryl-2*H*-chromen-2-ones (**5a−c**) as pure product. (*E*)-7-Hydroxy-4-styryl-2*H*-chromen-2-one (**5a**): Pale brown solid, yield 85%; IR (KBr) 3332 cm^{-1}, 2928 cm^{-1}, 1751 cm^{-1}; ^{1}H NMR (500 MHz, DMSO−d_6): δ 6.70 (d, 1H, J = 7.3 Hz), 6.73 (d, 1H, J = 6.7 Hz), 6.87-7.52 (m, 9H, Ar proton), 10.29 (brs, 1H, OH).

*2.2.3. General procedure for the synthesis of (E)-7-hydroxy-8-(amino-methyl)-4-styryl-2H-chromen-2-ones (**7a−h**)*: An equimolar amount of amine (1.0 mmol) was gradually added into a 10 mL methanolic solution of formaldehyde (1.0 mmol) with cooling. Then, 1.0 mmol of the (E)-7-hydroxy-4-styryl-2H-chromen-2-one (5**a-c**) was added to the mixture. This was followed by a few drops of acetic acid that had been dissolved in methanol. In an oil bath, the reaction mixture was slowly heated to reflux temperature for 5 to 6 hours. The raw material was re-crystallized from hot ethanol once the solution cooled and crystalized. The data of one of the representative reference compounds **7a** is described. The spectroscopic data of all the final Mannich products are in

accordance with the representative (*E*)-7-hydroxy-8-(piperidin-1-ylmethyl)-4-styryl-2*H*-chromen-2-one **7a**. (*E*)-7-hydroxy-8-(piperidin-1-ylmethyl)-4-styryl-2*H*-chromen-2-one (***7a***): Brownish-yellow solid, yield 79%;^{1}H NMR (500 MHz, DMSO−d_6): δ 1.45-160 (m, 6H, piperidine), 2.43-2.51 (m, 4H, piperidine), 4.23 (s, 2H), 6.65 (d, 1H, J = 7.3 Hz), 6.72 (d, 1H, J = 6.8 Hz), 6.79-7.53 (m, 8H, Ar proton), 10.61 (brs, 1H, OH); ^{13}C NMR (125 MHz, DMSO−d_6): δ 24.1(2C), 26.7, 51.4, 56.7 (2C), 103.4, 112.3, 116.1, 120.2, 123.5, 125.7, 128.2 (2C), 129.4 (2C), 132.8,135.4 (2C), 153.4, 155.2, 161.7, 162.3.

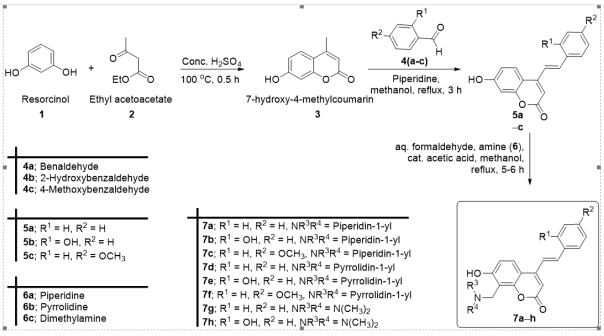
2.3 *Biological testing*

Biological evaluation by broth microdilution assay: The Clinical and Laboratory Standards Institute (CLSI) formerly NCCLS was followed for the determination of MIC. In short, 100 μL of media was placed in each of the wells of the 96-well plate using the broth microdilution technique as stated in method M27-A3 from the NCCLS. The Mannich derivatives were then added together with the residual media and sequentially diluted. In each well of the 96-well plate, 100 μL (in normal saline to an OD6000.1) of *M. smeg.* cell suspension was added, and after 48 hours at 37 °C, OD600 measurements were made. The quantity of the compound that prevented 80% of the growth is defined as MIC_{80} [34,35].

3. **Results and discussion**

3.1 *Chemistry*

The 7-hydroxy-4-methylcoumarin **3** was synthesized from the interaction between resorcinol and ethyl acetoacetate (β-ketoester) when concentrated sulfuric acid is present as a condensing agent under Pechmann reaction conditions. The initial synthesis of β-hydroxy ester, which is followed by cyclization and dehydration to give the resulting 7-hydroxy-4-methylcoumarin, is assumed to be the mechanism of the Pechmann reaction. The resorcinol reacted with great ease and sulphuric acid was used while carefully controlling the temperature to produce a high yield of 7-hydroxy-4-methylcoumarin. The (*E*)-7-hydroxy-4-styryl-2*H*-chromen-2-ones **5a-c** were synthesized by the condensation of 7-hydroxy-4-methylcoumarin **3** with appropriate aromatic aldehydes **4** in the presence of catalytic piperidine in methanol as reaction solvent under refluxing conditions. The (*E*)-7-hydroxy-8-(amino-methyl)-4-styryl-2*H*-chromen-2-one derivatives **7a−h** were synthesized from the corresponding (*E*)-7-hydroxy-4-styryl-2*H*-chromen-2-one derivatives **5a−c**, a variety of secondary amines **6**, and equimolar of 37% aqueous formaldehyde by employing the Mannich reaction conditions in the refluxing methanol (5−6 h) as shown in **Scheme 1**.



Scheme 1. Synthesis of (E)-7-hydroxy-8-(amino-methyl)-4-styryl-2H-chromen-2-one derivatives 7a−h

With the endeavor to develop procedures for synthesizing new amino-methyl derivatives **7a-h** of 7-hydroxy-4-styryl-

coumarins, we explored the reactions of a variety of Knoevenagel reaction intermediates to obtain the simple Mannich reaction products. Based on the current finding, we observed that 7-hydroxycoumarins seem to be ideal for the Mannich reaction than do the 5-or 6-hydroxyanalogs. The reaction went off without a hitch with moderate to high yields (65−90%) of the final products every time. The 7-hydroxy-8-(amino-methyl)-4-styryl-coumarins **7a−h** were obtained as crude pale-yellow solids which were recrystallized using hot ethanol to obtain pure products.

All the synthesized compounds **7a-h** were based on confirmation of spectroscopic methods. The absence of distinctive signals caused by three protons of the methyl group of 7-hydroxy-4-methylcoumarin in the ^{1}H NMR spectrum of compound **5** establishes the formation of styryl derivatives of 7-hydroxy-4-methylcoumarin. Further, the presence of a broad singlet of phenolic -OH proton resonates between δ 10.0−11.0 ppm. Moreover, in the ^{1}H NMR spectra of **7a–h**, the signal for the H-8 proton of the 7-hydroxycoumarin ring disappears, while the appearance of an additional singlet of two de-shielded aliphatic methylene protons further confirms the product. At the anticipated chemical shift and integral values, the rest of the aromatic protons are seen.

3.2 *Biology*

We have analyzed the anti-mycobacterial activity of these Mannich derivatives of 7-hydroxy-4-styryl-coumarins. The inhibitory concentrations were evaluated by minimum inhibitory concentration (MIC) of the compounds through broth dilution assay (**Table 1** and **Figure 2**) according to Clinical and Laboratory Standards Institute guidelines. The value was considered as MIC_{80} which showed at least 80% inhibition in OD_{600} compared to positive control without any drug, also a negative control was included in the experimental set with no cells. The inhibition was observed at the concentration of 1000 µg/mL. MTB is developing resistance against the available anti-TB drugs and evolved as multi-drug resistant (MDR) TB. Combinatorial therapy is emerging as a new strategy to control MDR-TB. These derivatives with anti-mycobacterial activity could provide valuable insights for further structural modifications of coumarins to yield potential candidates for the treatment of multi-drug-resistant tuberculosis.

[a]The MIC values (in µg/mL) of **7a-h** against *Mycobacterium smegmatis*.

Table 1: MIC VALUES[a] (in µg/mL)

Concentration	7a	7b	7c	7d	7e	7f	7g	7h
3.91µg/mL	0.311	0.347	0.367	0.384	0.393	0.422	0.471	0.473
7.81µg/mL	0.307	0.346	0.365	0.423	0.692	0.397	0.440	0.446
15.62µg/mL	0.325	0.35	0.357	0.364	0.409	0.406	0.425	0.428
31.25µg/mL	0.27	0.341	0.36	0.352	0.437	0.550	0.421	0.425
62.5µg/mL	0.424	0.352	0.314	0.325	0.343	0.253	0.290	0.297
125µg/mL	0.376	0.311	0.337	0.315	0.34	0.274	0.289	0.295
250µg/mL	0.316	0.32	0.374	0.335	0.342	0.299	0.324	0.319

500µg/mL	0.33	0.334	0.36	0.364	0.329	0.308	0.329	0.337
1000µg/mL	0.067	0.057	0.061	0.07	0.06	0.057	0.239	0.234
2000µg/mL	0.043	0.061	0.037	0.0329	0.0222	0.0279	0.0276	0.0286

Column 1 mentions the concentrations in µg/mL and column 2-7 mentions optical density (O.D.) of the cells at different concentrations of **7a-h**.

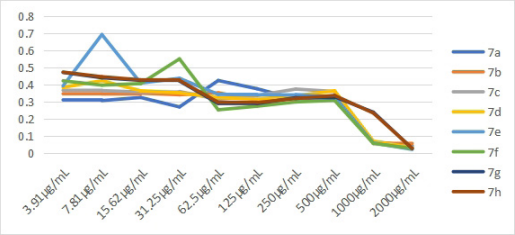


Fig.2. Broth micro dilution assay to determine the MIC_{80} against *Mycobacterium smegmatis* in the presence of Mannich derivatives **7a-h**. On X-axis the concentrations are mentioned in µg/mL and y-axis is the Optical Density (O.D.) of the cells.

As shown in **Fig. 2**, the tested compounds, (*E*)-7-hydroxy-8-(amino-methyl)-4-styryl-2*H*-chromen-2-one derivatives (**7a**, **7b**, **7c**, **7d**, **7e**, **7f**) shows MIC value against the concentration 1000µg/mL while **7g** and **7h** shows MIC above the concentration of 2000µg/mL. The structure-activity relationships also suggest that candidates with better activity may be obtained from a good combination of the substituents at C7 and C8 – positions of the coumarin ring. The compounds with C8–substituent having cyclized amines show better activity than the aliphatic acyclic amines.

4. Conclusion

In conclusion, we have synthesized a series of substituted (*E*)-7-hydroxy-8-(amino-methyl)-4-styryl-coumarins via Mannich reactions in a simple and efficient manner. All of the compounds' antimycobacterial activity was assessed and found that compounds from **7a-f** have MIC 1000µg/mL and compound **7g-h** have 2000µg/mL. Further research is required to evaluate the ways in which synthetic compounds work and to develop a more promising antimycobacterial new chemical entity.

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Conflicts of interest

The authors declared no conflict of interest.

References

1. Sethna, S. M.; Shah, N. M. The chemistry of coumarins. *Chem. Rev.* **1945**, *36*, 1−62.
2. Murray, R. D. H. Coumarins. *Nat. Prod. Rep.***1989**, *6*, 591−624.
3. Stefanachi, A.; Leonetti, F.; Pisani, L.; Catto, M.; Carotti, A. Coumarin: A natural, privileged and versatile scaffold for bioactive compounds. *Molecules,* **2018**, *23*, 250.
4. Newman, D. J.; Cragg, G. M. Natural products as sources of new drugs from 1981 to 2014. *J. Nat. Prod.* **2016**, *79*, 629–661.
5. Chouhan, M.; Senwar, K. R.; Sharma, R.; Grover, V.; Nair, V. A. Regiospecific epoxide opening: a facile approach for the synthesis of 3-hydroxy-3-aminomethylindolin-2-one derivatives. *Green Chem.* **2011**, *13*, 2553–2560.
6. Senwar, K. R.; Sharma, P.; Reddy, T. S.; Jeengar, M. K.; Nayak, V. L.; Naidu, V. G. M.; Kamal, A.; Shankaraiah, N. Spirooxindole-derived morpholine-fused-1,2,3- triazoles: design, synthesis, cytotoxicity and apoptosis inducing studies. *Eur. J. Med. Chem.* **2015**, *102*, 413–424.

7. Singh, L. R.; Avula, S. R.; Raj, S.; Srivastava, A.; Palnati, G. R.; Tripathi, C. K. M.; Pasupuleti, M.; Sashidhara, K. V. Coumarin–benzimidazole hybrids as a potentantimicrobial agent: synthesis and biological elevation. *J. Antibiot.* **2017**, *70*, 954–961.
8. Hu, X. L.; Xu, Z.; Liu, M. L.; Feng, L. S.; Zhang, G. D. Recent developments of coumarin hybrids as anti-fungal agents. *Curr. Top. Med. Chem.* **2017**, *17*, 3219–3231.
9. Mishra, S.; Pandey, A.; Manvati, S. Coumarin: An emerging antiviral agent. *Heliyon***2020**, *6*, e03217.
10. Hu, X. L.; Gao, C.; Xu, Z.; Liu, M. L.; Feng, L. S.; Zhang, G. D. Recent development of coumarin derivatives as potential antiplasmodial and antimalarial agents. *Curr. Top. Med. Chem.* **2018**, *18*, 114–123.
11. Zhang, L.; Xu, Z. Coumarin-containing hybrids and their anticancer activities. *Eur. J. Med. Chem.* **2019**, *181*, 111587.
12. Yang, J.; Zhang, P.; Hu, Y.; Liu, T.; Sun, J.; Wang, X. Synthesis and biological evaluation of 3-arylcoumarins as potential anti-Alzheimer's disease agents. *J. Enzyme Inhib. Med. Chem.* **2019**, *34*, 651–656.
13. Lv, H. -N.; Wang, S.; Zeng, K. W.; Li, J.; Guo, X. -Y.; Ferreira, D.; Zjawiony, J. K.; Tu, P. -F.; Jiang, Y. Anti-inflammatory Coumarin and Benzocoumarin Derivatives from *Murrayaalata*. *J. Nat. Prod.* **2015**, *78(2)*, 279–285.
14. Wang, G. Liu, Y.; Zhang, L; An, L.; Chen, R.; Liu, Y.; Luo, Q.; Li, Y.; Wang, H.; Xue,Y. Computational study on the antioxidant property of coumarin-fused Coumarins.*Food Chem.* **2020**, *304*, 125446.
15. Lei, L.; Xue, Y. -B.; Liu, Z.; Peng, S. -S.; He, Y.; Zhang, Y.; Fang, R.; Wang, J. -P.; Luo, Z. -W.; Yao, G. -M.; Zhang, J.-W.; Zhang, G.; Song, H. -P.; Zhang, Y. -H. Coumarin derivatives from *Ainsliaea fragrans* and their anticoagulant activity. *Sci. Rep.* **2015**, *5*, 13544.
16. Bizzarri, B. M.; Botta, L.; Capecchi, E.; Celestino, I.; Checconi, P.; Palamara, A. T.; Nencioni, L.; Saladino, R. Regioselective IBX-Mediated Synthesis of Coumarin Derivatives with Antioxidant and Anti-influenza Activities. *J. Nat. Prod*. **2017**, *80*, 3247−3254.
17. Pisani, L.; Farina, R.; Catto, M.; Iacobazzi, R.; Nicolotti, O.; Cellamare, S.; Mangiatordi, G. F.; Denora, N.; Ramón S. –O.; Siragusa, L.; Altomare, C. D.; Carotti,Exploring basic tail modifications of coumarin-based dual acetylcholinesterase- monoamine oxidase b inhibitors: Identification of water-soluble, brain-permeant neuroprotective multitarget agents. *J. Med. Chem.* **2016**, *59*, 6791–6806.
18. Sahoo, C. R.; Paidesetty, S. K.; Dehury, B.; Padhy, R. N. Molecular dynamics and computational study of Mannich-based coumarin derivatives: potent tyrosine kinase inhibitor. *J. Biomol. Struct. Dyn.* **2020**, *38*, 5419–5428.
19. Singh, H.; Singh, J. V.; Bhagat, K.; Gulati, H. K.; Sanduja, M.; Kumar, N.; Kinarivala, N.; Sharma, S. Rational approaches, design strategies, structure activity relationship and mechanistic insights for therapeutic coumarin hybrids. *Bioorg. Med. Chem.* **2019**, *27*, 3477−3510.
20. Fotopoulos, I.; Dimitra H. -L. Hybrids of Coumarin Derivatives as Potent and Multifunctional Bioactive Agents: A Review. *Medicinal Chemistry* **2020**, *16 (3)*,272−306.Xu, Z.; Zhao, S. -J.; Liu,
21. Y. 1,2,3-Triazole-containing hybrids as potential anticancer agents: Current developments, action mechanisms and structure-activity relationships. *Eur. J. Med. Chem.* **2019**, *183*, 111700.Goel, R.; Luxami, V.; Paul, K. Synthesis, in vitro anticancer acti
22. vity and SAR studies of arylated imidazo[1,2-α]pyrazine-coumarin hybrids. *RSC Adv.* **2015**, *5*,37887−37895.
23. Abdellatif, K. R. A.; Abdelgawad, M. A.; Elshemy, H. A. H.; Kahk, N. M.; Amir, D.M. E. Design, synthesis, antioxidant and anticancer activity of new coumarin derivatives linked with thiazole, isoxazole or pyrazole moiety. *Lett. Drug Des. Discov.***2017**, *14*, 773−781.
24. Ashraf, R.; Hamidullah; Hasanain, M.; Pandey, P.; Maheshwari, M.; Singh, L. R.; Siddiqui, M. Q.; Konwar, R.; Sashidhara, K. V.; Sarkar, J. Coumarin-chalcone hybrid instigates DNA damage by minor groove binding and stabilizes p53 through post translational modifications. *Sci. Rep.* **2017**, *7*, e45287.
25. Ren, Q. -C.; Gao, C.; Xu, Z.; Feng, L. -S.; Liu, M. -L.; Wu, X.; Zhao, F.Bis-coumarinDerivatives and Their Biological Activities. *Curr. Top. Med. Chem.* **2018**, *18*, 101−113.
26. Zhang, Z.; Bai, Z. W.; Ling, Y.; He, L. Q.; Huang, P.; Gu, H. X.; Hu, R. F. Design, synthesis and biological evaluation of novel furoxan-based coumarin derivatives as antitumor agents. *Med. Chem. Res.* **2018**, *27*, 1198−1205.
27. Kumar, S.; Saini, V.; Maurya, I. K.; Sindhu, J.; Kumari, M.; Kataria, R.; Kumar, V. Design, synthesis, DFT, docking studies and ADME prediction of some new coumarinyl linked pyrazolylthiazoles: potential standalone or adjuvant antimicrobial agents. *PLoS One,* **2018**, *13*, e0196016.
28. Ding Z.; Zhou M.; Zeng C. Recent advances in isatin hybrids as potential anticancer agents. *Arch Pharm Chem Life Sci.* **2020**, e1900367.
29. Ma, C. C.; Liu, Z. P. Design and synthesis of coumarin derivatives as novel PI3K inhibitors. *Anti Cancer Agents Med. Chem.* **2017**, *17*, 395−403.
30. Ganeshapillai, D.; Woo, L. W. L.; Thomas, M. P.; Purohit, A.; Potter, B. V. L. C-3- and C-4-Substituted bicyclic coumarin sulfamates as potent steroid sulfatase inhibitors. *ACS Omega* **2018**, *3*, 10748−10772.
31. Hu, Y. -Q.; Xu, Z.; Zhang, S.; Wu, X.; Ding, J. -W.; Lv, Z. -S.; Feng, L. -S. Recent developments of coumarin-containing derivatives and their anti-tubercular activity. *Eur. J. Med. Chem.* **2017**, *136*, 122−130.
32. Mehta, S.; Sumit; Goyal, N.; Pathak, S. R. Molecular docking and ADMET studies of 3-phenyl coumarin derivatives for their anti-cancer activity. *Int. J. Pharma. Bio. Sci.* **2016**, *7*, 291−304.

33. Mehta, S.; Pathak, S. R. In silico drug design and molecular docking studies of novel coumarin derivatives as anticancer agents. *Asian J. Pharm. Clin. Res.* **2017**, *10*, 335−340.
34. Hameed, S.; Hans, S.; Nandan, S.; Fatima, Z. Mechanistic insights into the antimycobacterial action of unani formulation, Qurs Sartan Kafoori. *J Tradit Complement Med*. **2021**, *12(2)*, 162−171.
35. Sharma, S.; Hameed, S.; Fatima, Z. Monoterpenoid geraniol improves anti- mycobacterial drug efficiency by interfering with lipidome and virulence of mycobacteria. *Infect Disord Drug Targets*. **2020**, *20(4)*, 467−485.