Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2017, 9(13):61-64
(http://www.derpharmachemica.com/archive.html)

Synthesis, Characterization and Pharmacological Evaluation of New Mannich Bases with Coumarin Derivatives

Kalyani G[1*], Bethi Srinivas[2], Sastry KV[3], Vijaya Kuchana[1]

[1]Department of Pharmaceutical Chemistry, TKR College of Pharmacy, Hyderabad, India
[2]Department of Pharmaceutical Chemistry, Talla Padmavathi College of Pharmacy, Warangal,
[3]Department of Pharmaceutics, Alliance Institute of Advanced Pharmaceutical & Health Sciences, Hyderabad, India

ABSTRACT

In the current investigation, we have synthesized a series of new 4-({1-[(diethylamino)methyl]-1H-benzimidazol-2-yl}methyl)-2H-chromen-2-onederivatives have by the Mannich condensation reaction of 4-(1H-benzimadazole-2-ylmethyl)-2H-chromen-2-one(II) with different secondary amines and formaldehyde and the structures of compounds were characterized by Proton Nuclear Magnetic Resonance (^{1}H-NMR), (Infra-red) IR and mass spectral analysis. The newly synthesized compounds are evaluated for their antibacterial activity against Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative) bacteria and also for anti-inflammatory potentials. Compounds 8 and 7 resulted in relatively higher antibacterial against S. aureus and E. coli respectively when compared to streptomycin. Dose level of 50 mg/kg of test compounds reported significantly higher anti-inflammatory activity when compared to dos level of 20 mg/kg. Moreover, compound 8 (50 mg/kg) resulted in similar anti-inflammatory activity when compared with celecoxib (20 mg/kg).

Keywords: Coumarin, Philips condensation, Mannich condensation, Anti-inflammatory, Antibacterial

INTRODUCTION

Coumarin is a natural plant derivative belongs to Apiaceae family, with a structural imprint of fused benzene and benzopyronone. Coumarin derivatives are naturally isolated as well as synthesized [1]. Earlier research reported various pharmacological actions of coumarin containing compounds which mainly includes antibacterial [2,3], anticancer [4], antioxidant [5], antifungal [6], Recent research on coumarin and its derivatives reported to epitomize anti-inflammatory activity as one of its potent physiological activities [7]. Inflammation is a biological defense reception of damaged vascular tissues to harmful stimuli, either by injury, irritants, or by pathogens. Complex inflammation system response thru multiple pathophysiological pathways like cytokines, interleukin, NF-kB, protein kinases, tyrosine kinases and by various other immunological responses [8]. Upon literature search it was found that coumarin derivative with a 7-azomethine linkage has exhibited *in vivo/in vitro* anti-inflammatory activity. Likewise, our research is postulated on Mannich base linked to coumarin derivatives possess anti-inflammatory activity [9]. Mannich bases are beta-amino-ketones, formed by Mannich reaction. A nucleophilic addition reaction of a non-enolizable aldehyde with primary/secondary amine, forming resonance stabilized imine. Reactivity of these imines with carbanion produces the Mannich bases. In the present study, we have designed and synthesized a novel series of coumarin derivatives with Mannich bases and evaluated for their antibacterial and anti-inflammatory activity.

MATERIALS AND METHODS

Chemistry

All the chemicals for synthesis were obtained from S.D fine chem. Limited (Mumbai). Purity is analyzed and affirmed by using TLC on silica gel-G plate; R_f values produced for each compound were equating with the literature and found to be pure. The solvent used was acetone and methanol (2:2) iodine chamber was used for the visualization of the spots. Characterizations of synthesized compounds were interpreted by FT-IR, ^{1}H-NMR, ^{13}C-NMR, Mass spectroscopy.

General procedure for synthesis

Step A: Synthesis of 2H-1-benzopyran-2-one-4-acetic acid (I)

Equimolar quantities of 9.6 g of citric acid and 16 ml of concentrated H_2SO_4 was taken in a beaker and stirred well upon heating until excess foaming was avoided and it was maintained at 60-65°C then it was cooled at room temperature. And 7.7 g of napthol was added to the above solution and it was stirred well upon cooling over a period of 2 h using mechanical stirrer and it was allowed to stand for 24 h.

The solution was poured into ice cold water. Then the precipitate was filtered the crude product was dissolved in sodium bicarbonate solution. The solution was decolorized with activated charcoal and filtered. The residue was acidified with concentrated hydrochloric acid to give respective Coumarin-4-acetic acid.

Yield: 75%; MP: 102°C; ^{1}H-NMR (DMSO-d6): 1.3-2.1, 7.03 (m, 3H, aromatic-H), 12.64 (s, 1H, OH), 11.64 (s, 1H, NHCO), 3.91; ^{13}C-NMR (DMSO-d6): 151.3, 149.4, 143.1, 138.2, 129.3, 29.2.

Step B: Synthesis of 4-(1h-benzimidazol-2-ylmethyl)-2h chromen-2-one (II)

Equimolar quantities of Coumarin-4-acetic acid (2.04 g) and orthophenylene diamine were taken in round bottom flask then these were dissolved in 25 ml of anhydrous phosphoric acid as condensing medium this reaction mixture was kept in oil bath at 170-180°C upon reflux for 4-5 h. Then the contents were cooled at room temperature, then it was poured in cooled water and stirred well and it is basified with 10% of NaOH and filtered. It was treated with 5% of sodium carbonate to remove unreacted orthophenylene diamine.

^{1}H-NMR (DMSO-d6): 6.13-7.17 (m, 3H, aromatic-H), 3.88; ^{13}C-NMR (DMSO-d6): 172.9, 151.3, 149.4, 143.1, 138.2, 132.4, 131.2, 129.3, 31.4; m/z: 289; FTIR: 2924, 1455, 1514; MP: 130°C; Yield: 65%.

Step C: Synthesis of new Mannich bases (1-8)

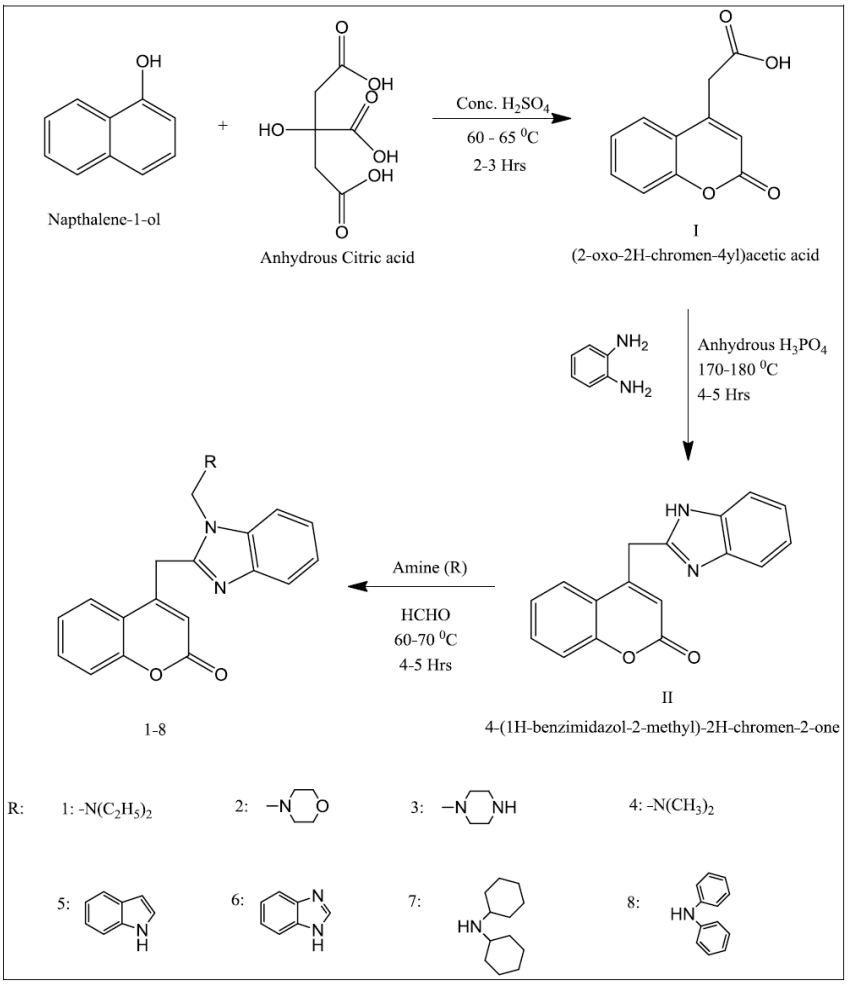
Equimolar quantities of 0.27 g 4-(1h-benzimidazol-2-ylmethyl)-2h chromen-2-one (0.01 M), different secondary amine 0.87 ml (0.01 M) and 0.30 ml (0.01 M) formaldehyde were taken in Round Bottom Flask (RBF) then these were dissolved in 25 ml ethanol as condensing medium this reaction mixture was kept in water bath at 60°C to 65°C upon reflux for 4-5 h. Then the contents were cooled at room temperature and then it was poured into petridish collect the crystals.

Synthesis of diethyl amine derivative (1)

Yield: 60%; MP: 134°C; ^{1}H-NMR (DMSO-d6): 6.11-7.18 (m, 3H, aromatic-H), 3.74, 2.33-2.47 (t, 4H), 2.53-2.59 (q, 4H, N-$(CH_2)_2$), 2.10-2.19 (t, 6H, $(CH_3)_2$). ^{13}C-NMR (DMSO-d6): 172.4, 151.9, 145.2, 144.5, 138.9, 132.4, 131.2, 129.8, 56.3, 54.6, 50.3, 47.4, 28.5; m/z: 360; FT-IR: 3743, 1447, 1581.

Synthesis of morpholine derivative (2)

Yield: 139%; MP: 150°C; ^{1}H-NMR (DMSO-d6): 6.08-7.14 (m, 3H, aromatic-H), 3.71, 2.19 (-NH), 3.99, 2.35, 2.10 (s, 6H, N-$(CH_3)_2$). ^{13}C-NMR (DMSO-d6): 171.2, 152.1, 147.6, 146.4, 139.1, 132.3, 130.1, 129.5, 56.7, 54.3, 51.3, 48.9; m/z: 376; FTIR: 2979, 3742, 1453, 1504 (Scheme 1).



Scheme 1: Synthesis of new Mannich bases with coumarin derivatives

Pharmacological evaluation

Antibacterial activity

The antibiotic potency can be determined using the cup plate method and *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) as test organisms. Initially, the prepared nutrient agar medium was sterilized by autoclaving method at 15 lbs pressure and 121°C for 25 min. Agar media was cooled to room temperature and the organism was inoculated to the media.

15 ml of media was transferred to a petri plates aseptically. Synthesized Compounds were dissolved in water and diluted to get 10 mg/ml of concentration, whereas, streptomycin is used as standard drug at a concentration of 10 µg/ml. The cultured plates were incubated at 37^{o}C for 24 h. The zone of inhibition produced by test compounds and coumarone were recorded in mm [10].

***In vivo* anti-inflammatory activity**

Anti-inflammatory activity of the newly synthesized coumarin derivatives was evaluated by carrageenan induced paw edema assay in rats [11]. Synthesized test compounds with dose level 20 and 50 mg/kg were administered and compared with that of standard drug celecoxib (20 mg/kg). The paw volumes were measured using the mercury displacement technique with the help of plethysmograph immediately before and 1 h after carrageenan injection. The percent inhibition of paw edema was calculated from percent inhibition formula:

$$\%\text{inhibition } (I)=100[1-(a-x)/(b-y)]$$

Where, x=mean paw volume of rats before the administration of carrageenan and test compounds or reference compound (test group), a= mean paw volume of rats after the administration of carrageenan in the test group (drug treated), b=is the mean paw volume of rats after the administration of carrageenan in the control group, y=mean paw volume of rats before the administration of carrageenan in the control group.

RESULTS AND DISCUSSION

Chemistry

All the newly synthesized compounds were purified and separated using column chromatography or recrystallization method. The compounds were dried for about 12 h under high vacuum. Synthesized compounds were characterized by using ^{1}H-NMR, ^{13}C-NMR and Mass spectrometric studies. The orientation of protons in the analysed compounds fully supported by the integration curves. Furthermore, all the coumarin derivatives demonstrated the characteristic chemical shifts for the coumarin nucleus. Additionally, synthesized coumarin derivatives were analyzed by mass spectra under ESI conditions and indicate no difference in the fragmentation pattern among the set of synthesized series.

Antibacterial activity

The antibacterial activities of the synthesized compounds against 2 bacterial strains are shown in the Table 1 and the comparison of the activity against control and streptomycin is shown in Figure 1. Zone of inhibition in *S. aureus* screening is ranged from 14 mm (compound 1) to 26 mm (compound 8), whereas, in *E. coli* screening is from 11 mm (compound 1) to 22 mm (compound 7). Zone of inhibition of with streptomycin is 24 mm and 21 mm against *S. aureus* and *E. coli*, respectively. However, no inhibition was observed in control screening. Compounds 8 and 7 exhibited higher zone of inhibition when compared to streptomycin in *S. aureus* and *E. coli* groups, respectively, and also reported the highest activity in the groups. This could be due to the presence of larger hydrophobic substitutions such as di phenyl and di cyclohexane groups at amino group, creating bulkier region.

Table 1: Antibacterial activity of synthesized coumarin derivatives in comparison with streptomycin as a standard

Compound	*Staphylococcus aureus*	*Escherichia coli*
1	14 mm	11 mm
2	18 mm	13 mm
3	14 mm	18 mm
4	21 mm	14 mm
5	18 mm	16 mm
6	21 mm	19 mm
7	20 mm	22 mm
8	26 mm	20 mm
Control	-	-
Streptomycin	24 mm	21 mm

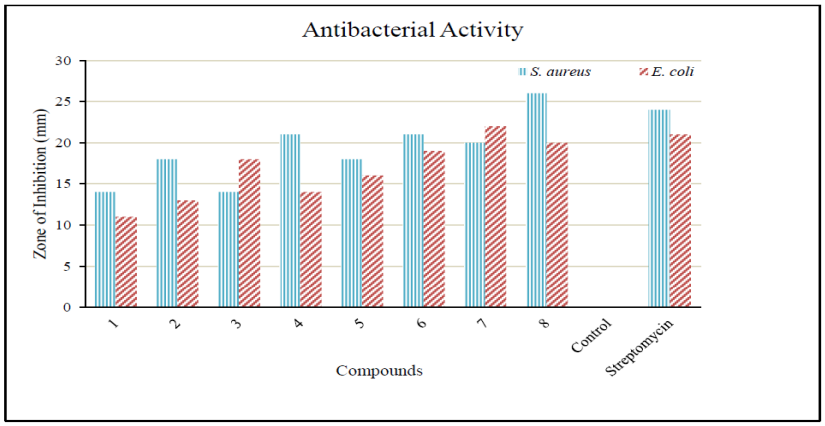


Figure 1: Comparison of antibacterial activity of novel coumarin derivatives with streptomycin

Anti-inflammatory activity

Anti-inflammatory activity of synthesized coumarin derivatives were evaluated by carrageenan induced paw edema bioassay in rats with celecoxib (20 mg/kg) as reference standard. Percentage inhibitions of the molecules are tabulated in Table 2. Percentage inhibition of paw edema with test compounds dose of 20 mg/kg is ranged between 17 and 37.5, whereas, with 50 mg/kg dose, it is between 59.5% and 82.6%.

The results indicated that all the compounds reported significantly higher (two-tailed, paired *t* test; P<0.0001) anti-inflammatory at dose of 50 mg/kg when compared to that of 20 mg/kg dose (Figure 2). However, the anti-inflammatory effect of compound 8 (50 mg/kg) and celecoxib (20 mg/kg) was found to be similar (82.6% vs. 84.6%; P=0.105). The higher anti-inflammatory activity of compound 8 could be due to its higher hydrophobic planar substitutions.

Table 2: Anti-inflammatory activity of synthesized compounds (% inhibition of Paw Edema)

Compound	% Inhibition of Paw Edema	
	20 mg/kg	50 mg/kg
1	19.2	59.5
2	17	60.4
3	21.8	61.5
4	22.5	57.3
5	28.5	68.7
6	23.2	62.8
7	32.1	77.9
8	37.5	82.6
Celecoxib (20 mg/kg)	84.6	

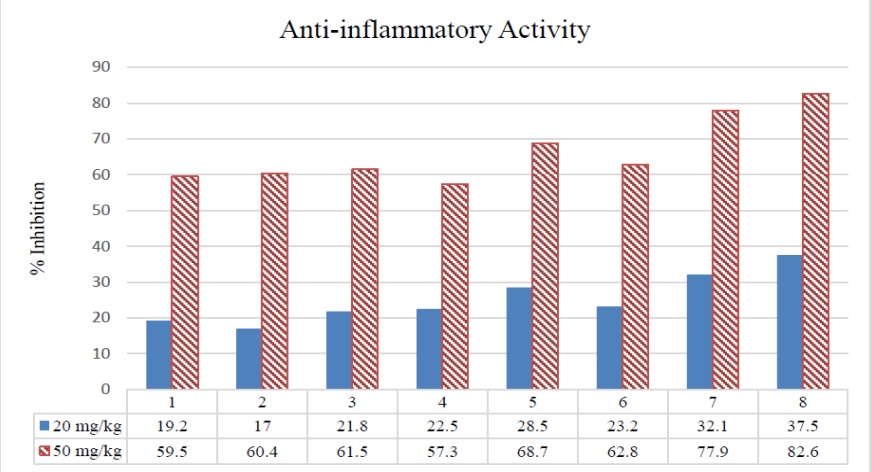


Figure 2: Comparison of anti-inflammatory activity between dose levels of coumarin derivatives

CONCLUSION

A series of new Mannich bases with coumarin derivatives were synthesized and evaluated for their antibacterial and anti-inflammatory activities. Compound 8 and compound 7 have shown relatively higher antibacterial activity and higher when compared to streptomycin against *S. aureus* and *E. coli*, respectively. Similarly, compound 8 also reported highest anti-inflammatory activity among molecules tested at both dose levels and statistically similar activity at 50 mg/kg dose with celecoxib (20 mg/kg). The results demonstrated that anti-inflammatory properties of novel molecules are due to larger hydrophobic groups at amino substitution.

ACKNOWLEDGEMENTS

I thank Teegala Krishna Reddy Education Societies, Hyderabad for providing the facilities for completion of research work.

REFERENCES

[1] P.K. Jain, H. Joshi, *J. App. Pharm. Sci*., **2012**, 2, 236.
[2] D. Iannazzo, A. Piperno, A. Ferlazzo, A. Pistone, C. Milone, M. Lanza, F. Cimino, A. Speciale, D. Trombetta, A. Saija, S. Galvagno, *Org. Biomol. Chem*., **2012**, 10, 1025.
[3] P.Y. Lin, K.S. Yeh, C.L. Su, S.Y. Sheu, T. Chen, K.L. Ou, M.H. Lin, L.W. Lee, *Molecules.*, **2012**, 17, 10846.
[4] S. Rehman, M. Ikram, R.J. Baker, M. Zubair, E. Azad, S. Min, K. Riaz, K.H. Mok, S.U. Rehman, *Chem. Centre J*., **2013**, 7, 68.
[5] K.C. Fylaktakidou, D.J. Hadjipavlou-Litina, K.E. Litinas, D.N. Nicolaides, *Curr. Pharm. Des*., **2004**, 10, 3813.
[6] K.M. Khan, Z.S. Saify, M.Z. Khan, Zia-Ullah., I.M. Choudhary, AttaUr-Rahman, S. Perveen, Z.H. Chohan, C.T. Supuran, *J. Enzyme. Inhib. Med. Chem*., **2004**, 19, 373.
[7] Z. Li, J. Hu, M. Sun, X. Song, G. Li, Y. Liu, G. Li, H. Ji, G. Liu, N. Chen, *Int. Immunopharmacol*., **2013**, 17, 400.
[8] M. Ruslan, *Nature.*, **2008**, 454, 428.
[9] C.A. Kontogiorgis, K. Savvoglou, D.J. Hadjipavlou-Litina, *J. Enzy. Inhib. Med. Chem*., **2006**, 21, 21.
[10] G. Sahoo, N.S.S. Mulla, Z.A. Ansari, C. Mohandass, *Ind. J. Pharm. Sci*., **2012**, 74, 348.
[11] P. Chaitanya, G.D. Reddy, G. Varun, L.M. Srikanth, V.V. Prasad, A. Ravindernath, *Med. Chem*., **2014**, 10, 711.