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Research Article

Synthesis of Novel Cinnoline Fused Mannich Bases: Pharmacological Evaluation of Antibacterial, Analgesic and Anti-Inflammatory Activities

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ABSTRACT

In the present study, we have designed and synthesized a series of novel cinnoline fused Mannich bases by the condensation reaction of 4-methyl-3-acetyl cinnoline with different secondary aromatic and aliphatic amines with and the structures of compounds were characterized by H^1-NMR, IR and Mass spectral analysis. The biological potentials of the newly synthesized compounds are evaluated for their antibacterial activity against *Staphylococcus aureus* (Gram positive), and *Eeshricia coli* (Gram negative) bacteria, in vivo analgesic and anti-inflammatory activities. Compounds 4 and 3, which are having larger hydrophobic amino substitutions resulted in relatively higher antibacterial against *S. aureus* and *E. coli* when compared to streptomycin. Similarly, compound 4 reported higher analgesic activity when compared to diclofenac at 120 mins and 180 mins. From anti-inflammatory evaluations, dose level of 50 mg/kg of test compounds reported significantly higher activity when compared to dose level of 20 mg/kg. Moreover, compound 4 (50 mg/kg) resulted in similar anti-inflammatory activity when compared with celecoxib (20 mg/kg).

Keywords: Cinnoline, Mannich condensation, analgesic activity, anti-inflammatory, anti-bacterial.

INTRODUCTION

Cinnoline is a 1,2-diazanaphtalene or benzo[c]-1,2-diazine, that follows Hantsch-Widmann system and is an isosteric relative to either quinoline or isoquinoline[1] Cinnolines exhibit distinct physical properties such as luminescence in pyrrolo[1,2-b]cinnolines[2]. Mannich bases are reported for their high reactivity and plays a vital role in construction of nitrogen containing compounds and also act as vital pharmacophore for potential bioactive leads[3]. Various Mannich bases with aminoalkyl chain are proved for their clinical usefulness includes atropine, cocaine, biperiden, ethacrynic acid, fluoxetine, procyclidine, ranitidine, and trihexyphenidyl[4-6]. Mannich bases are earlier reported for their biological, non-biological, and chemical applications[3,7-9]. Biological activities of Mannich bases includes analgesic and anti-inflammatory[10], anticancer[11], antifilarial[12], antibacterial[13], and other pharmacological activities. Additionally, Mannich bases and their derivatives are key intermediates for the synthesis of various bioactive molecules[14]. Recently a series of cinnoline-pyrazole derivatives are reported with anti-inflammatory potentials[15]. In the current investigations, a novel series of cinnoline fused Mannich bases are designed and synthesized and evaluated for their various pharmacological actions such as antibacterial, analgesic and anti-inflammatory activities.

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. Synthesis of cinnoline fused mannich bases is shown in Scheme 1.

General Procedure for Synthesis

Synthesis of Benzene Diazonium Chloride

Equimolar mixture of Benzene diazoniumchloride was prepared by dissolving Sodium nitrite (7.4 g, 0.1 mole) in 26ml of water and added drop wise to a solution of aniline(10 g in 1N HCl) at 0 ^{0}C under Stirring for about 10mins.Then orange coloured precipitate was collected by filtration and dried. M.P: ; product yield.

Synthesis of Phenyl Hydrazone Acetyl Acetone

The synthesized benzene diazonium was added to stirred solution of ethanol 30ml, water 500ml and acetyl acetone at 0 ^{0}C under continuous stirring. Then sodium acetate was added to keep the reaction alkaline to litmus after 3hrs under stirring at 0-5 ^{0}C A crude product was obtain that is filtered and washed with water and air dried. Later it is recrystallised from ethanol in order to get the pure form of

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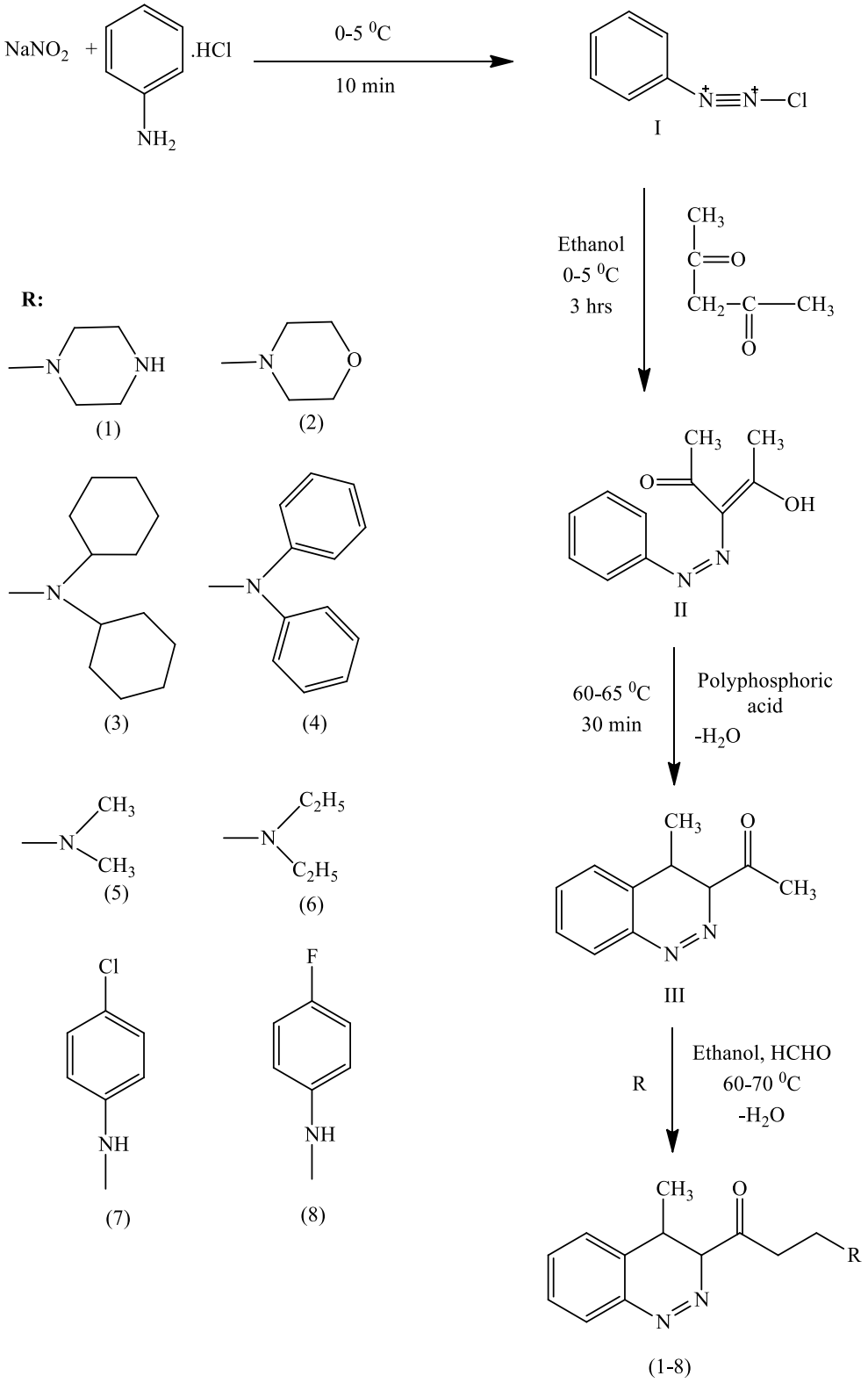

Scheme 1: Synthesis of Novel Cinnoline fused Mannich Bases.

yellow needles i.e., Phenyl hydrazone acetylcaetone crystals (M.P: 80 ^{0}C; yield- 15.2 g).

Synthesis of 4-Methyl-3-Acetyl Cinnoline

Phenyl hydrazone acetylacetone (10g, 0.05 mole) and polyphosphoric acid (16 g 7.216 ml) in small lots over 30 mins and the temperature was maintained at 60-65 ^{0}C ,the reaction was kept for additional 2 hrs. Reaction was monitored by TLC. After completion of reaction chilled water (200 ml) was added carefully to decompose the black residue at 0-5 ^{0}c. Later the product was then extracted with ethyl acetate. Ethyl acetate layer was treated with charcoal and concentrated to get the crude product as

Table 1: Antibacterial Activity of Newly Synthesized Cinnoline Derivatives in Comparison with Streptomycin as a Standard.

Compound	Zone of Inhibition	
	S. aureus	*E. coli*
1	12 mm	10 mm
2	16 mm	14 mm
3	21 mm	20 mm
4	25 mm	21 mm
5	17 mm	18 mm
6	19 mm	17 mm
7	13 mm	17 mm
8	22 mm	15 mm
Control	0 mm	0 mm
Streptomycin	22 mm	20 mm

Table 2: % Analgesic Activity of Novel Cinnoline Derivatives.

Compound	% Analgesic Activity			
	30 min	60 min	120 min	180 min
1	20.2	28.6	34.2	30.7
2	21.9	31.0	37.8	32.1
3	32.9	42.7	48.6	40.8
4	35.1	43.9	52.5	41.6
5	27.8	36.1	42.9	35.2
6	30.7	39.7	41.3	30.1
7	24.3	30.4	40.1	33.8
8	25.1	33.9	39.4	36.0
Diclofenac	36.4	44.2	49.1	39.2
Control	1.6	2.1	1.3	2.5

Table 3: Anti-inflammatory Activity of Synthesized compounds (% inhibition of Paw Edema).

Compound	% Inhibition of Paw Edema	
	20mg/kg	50 mg/kg
1	20.1	58.1
2	21.4	62.3
3	38.1	82.4
4	42.3	85.9
5	35.5	78.5
6	33.9	76.1
7	26.2	69.2
8	30.7	71.9
Celecoxib (20 mg/kg)	86.1	

brownish black residue .Then recrystallize from methanol will give light yellow crystals. (M.P: 65 ^{0}C, yield: 1.2 g). 3 -CH3 1.4(s), Ar-H 7.5(m), H1NMR, FT IR 3 3444.37 cm-1, 1628.99 cm-1,2925.19cm-1,1369.87cm-1, mass 187m\z.

4-Methyl-3-Acetylcinnoline Reacts with Secondary Amine - General Procedure

4-methyl-3-acetyl cinnoline was dissolved in a suitable solvent such as methanol and taken in a RBF along with a secondary amine such as diphenylamine, dicyclohexamine, morpholine, piperidine individually and formaldehyde solution in the same proportion and then reflux for 2hrs by maintaining 60-65 ^{0}C.After refluxing the contents are taken in china dish and dried there a brownish yellow colour crystal.

MP: 68 ^{0}C; mass: 285 m\z.

^{1}HNMR: 1.4(s) -CH3, O=C-CH2 2.85(t), Ar-H 7.6(m), CH2-NH 3.3(t) 3a; mass 285m/z; FTIR: 3418.56 cm-1, 1666.81 cm-1, 2933.84 cm-1, 1371.51cm-1.

^{1}HNMR: CH3,1.4(s), O=C-CH2 2.5(t), Ar-H 7.8(m), CH2-NH 3.8(t) mass 286m/z,FTIR 3422.3 cm-1, 1622.22 cm-1,2925.19cm-1,1367.75cm-1; mass 187m\z3b.

^{1}HNMR: CH_3 1.4 (s), O=C-CH_2 2.4 (t), Ar-H 7.2 (m), CH_2-NH 3.2 (t); mass: 380m/z; FTIR: 3005.59 cm-1, 591.78 cm-1, 411.75 cm-1;

^{1}HNMR: -CH_3 1.6 (s), O=C-CH_2 2.5 (t), Ar-H 7.6 (m), CH_2-NH 3.6 (t) 3d; mass: 283m/z, FTIR: 3067.0 cm-1, 1591.03 cm-1, 2932.56 cm-1, 416.13 cm-1.

Antibacterial Activity

The antibacterial activity of the newly developed cinnoline fused mannich bases was assessed using the Cup plate method and *Staphylococcus aureus* (Gram positive), and *Eeshricia coli* (Gram negative) as test organisms[16]. The prepared nutrient agar medium was sterilized by autoclaving method at 15 lbs pressure and 121 ^{0}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. Test compounds were dissolved in water and diluted to get 10mg/ml of concentration, whereas, streptomycin is used as standard drug at a concentration of 10µg/ml. The cultured plates were incubated at 37oC for 24 hrs. The zone of inhibition produced by test compounds and coumarone were recorded in mm.

Analgesic Activity

The analgesic activity of the newly synthesized compounds was performed by the tail-flick technique[17] using either sex of albino mice, weighing between 25-35 g and were selected by the random sampling technique. Diclofenac sodium (10 mg/kg) was administered orally as a reference drug. The test compounds at a dose level of 10 mg/kg were administered orally. The reaction time was recorded at 30 minutes, 1, 2, and 3 hours after the treatment, and cut-off time was 10 seconds. The percent analgesic activity (PAA) was calculated by the following formula:

$PAA = [T_2-T_1/10-T_1] \times 100$

where, T_1 is the reaction time (s) before treatment and T_2 is the reaction time (s) after treatment.

In-vivo Anti-Inflammatory activity

Anti-inflammatory activity of the newly synthesized cinnoline derivatives was determined by carrageenan induced paw edema assay in albino rats [18]. Synthesized test compounds with dose level 20 mg/kg and 50 mg/kg were administered and compared with that of standard drug Celecoxib (20mg/kg). The paw volumes were measured using the mercury displacement technique with the help of plethysmograph immediately before and 1h after carrageenan injection. The percent inhibition of paw edema was calculated from percent inhibition formula,

%inhibition (I) = 100[1-(a-x)/(b-y)]

Where,

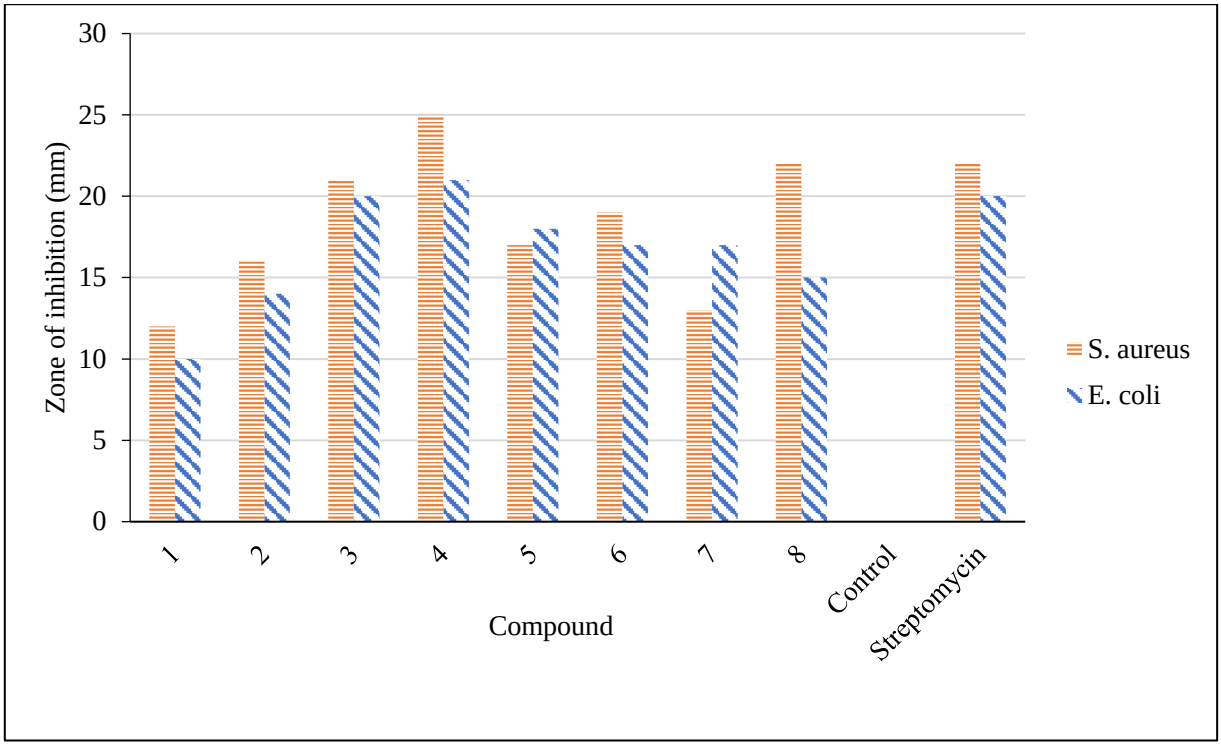

Figure 1: Comparison of Antibacterial Activity of Novel Coumarin Derivatives with Streptomycin.

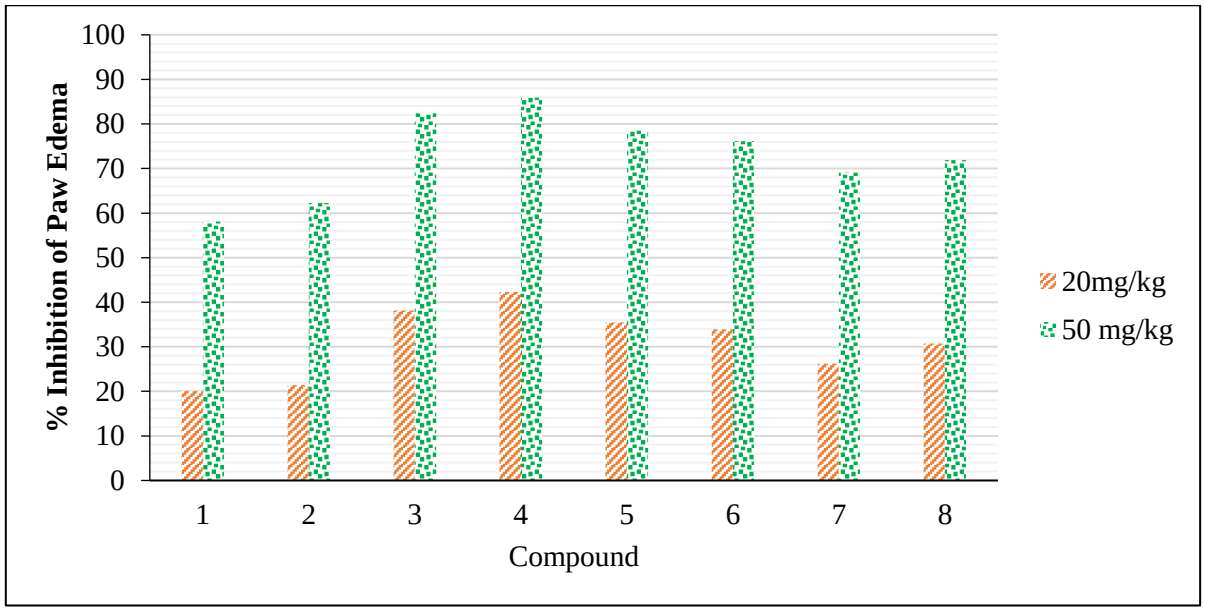

Figure 2: Comparison of Anti-inflammatory Activity between Dose Levels of Cinnoline Fused Mannich Bases.

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 cinnoline derivatives 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, and FTIR techniques. The orientation of protons in the analysed compounds fully supported by the integration curves. Furthermore, all the derivatives

demonstrated the characteristic chemical shifts for the cinnoline nucleus. Additionally, synthesized derivatives were analyzed by mass spectra under ESI conditions and indicates no difference in the fragmentation pattern among the set of synthesized series.

Antibacterial activity

The antibacterial activity of the cinnoline derivatives against *S. aureus* and *E. coli* 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 12 mm (compound 1) to 25 mm (compound 4), whereas, in *E. coli* screening is from 10 mm (compound 1) to 21 mm (compound 4). Zone of inhibition of with streptomycin is 22 mm and 20 mm against *S. aureus* and *E. coli*, respectively, whereas, in control screening no zone of inhibition was observed. Compounds 4 and 3 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, owing to their larger hydrophobic substitutions such as di phenyl and di cyclohexane groups at amino group, creating bulkier region.

In vivo Analgesic Activity

The *in vivo* analgesic activity for the novel cinnoline derivatives was performed by the tail-immersion technique using albino mice. Results for the activity are shown in Table 2. The results from the in vivo analgesic evaluation reported moderate analgesic activity at 30 minutes of reaction time and an increase in activity at 60 mins and was maximum at 120 mins, which declined at 180 mins (Tables 2). Compounds with larger secondary amine substitutions (compounds 3 and 4) reported with higher activity among the tested compounds as well as difclofenac.

In vivo Anti-inflammatory Activity

Anti-inflammatory activity of newly synthesized cinnoline fused mannich bases 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 3 and Figure 2. % inhibition of paw edema with test compounds dose of 20 mg/kg is ranged between 20.1 (compound 1) and 42.3 (compound 4), whereas, with 50 mg/kg dose, it is between 58.1% (compound 1) and 85.9% (compound 4). The results indicated that all the compounds reported significantly higher (two-tailed, paired t test; $P<.0001$) anti-inflammatory activity at dose of 50 mg/kg when compared to that of 20 mg/kg dose. However, the anti-inflammatory effect of compound 4 (50 mg/kg) and celecoxib (20 mg/kg) was found to be similar (85.9% vs. 86.1%; $P = 0.281$). The higher anti-inflammatory activity of compound 4 and 3 could be due to presence of higher hydrophobic planar substitutions.

CONCLUSION

A series of new cinnoline fused mannich bases were designed, synthesized and characterized for chemical confirmation. These newly synthesized derivatives were evaluated for their antibacterial, in vivo analgesic 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 biological potentials of novel molecules (compounds 8 and 7) are due to presence of larger hydrophobic groups at amino substitution.

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