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Original Article

Invitro anti-inflammatory activity of newly synthesized mannich base 2-[(1,3-benzothiazol-2-ylamino)methyl]-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one and its derivatives

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Abstract

The present study was aimed to synthesize a series 2-[(1,3-benzothiazol-2-ylamino)methyl]-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (3 a-l) and to evaluate their *in-vitro* anti-inflammatory activity. The starting material (1a-l) was prepared by the application of cyclization reaction. *In-vitro* anti-inflammatory activity of synthesized compounds [3a-l] was evaluated using inhibition of bovine serum albumin denaturation method. 3c and 3l have shown significant *in-vitro* anti-inflammatory activity. The findings of present study clearly demonstrate that chloro functional group possess inhibition of bovine serum albumin denaturation capacity and has *in-vitro* anti-inflammatory activity. However methyl, methoxy and dimethyl derivatives show mild to moderate *in-vitro* anti-inflammatory activity.

Keywords: *Mannich base, Invitro anti-inflammatory activity.*

1. Introduction

Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation damaged by microbes, physical agents or chemical agents, the injury is in the form stress. Inflammation of tissue is due to response to stress. It is a defensive response that is characterized by redness, pain, heat and swelling and loss of function in the injured area. Loss of function occurs depends on the site and extent of injury. Since inflammation is one of the body's nonspecific internal systems of defense, the response of a tissue to an accidental cut is similar to the response that results from other types of tissue damage, caused by burns due to heat, radiation, bacterial or viral invasion. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. Several experimental protocols of inflammation are used for evaluating the potency of drugs [1]. Pyrazolone has been used as analgesic, antimicrobial agent, fungicides and hypoglycemic agent.

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They inhibit the production of TNF-α and decrease the level of pro-inflammatory cytokines and thereby reduce inflammation and prevent further tissue destruction in disease such as rheumatoid arithritis, osteoarthritis and cronhs disease [2]. Benzthiazole containing organic compounds forms a significant group of drugs which exhibit an array of biological activities ranging from anti-inflammatory [3], antibacterial, antifungal [4], anti-neoplastic [5,6] anti-convulsant [7] anti-viral [8] anthelmintic [9]. Major mechanism of action of nonsteroidal anti-inflammatory drugs (NSAIDs) is the lowering of prostaglandin (PG) production through inhibition of the cyclooxygenase (COX) enzyme, which catalyzes the conversion of arachidonic acid into PG. Since PG has a dual function, mediation of inflammation and cytoprotection in the stomach and intestine, long-term usage of NSAIDs to relieve the symptoms of inflammation and pain always results in gastrointestinal (GI) disorders and renal toxicity. It is known that bacterial infections often produce pain and inflammation. In normal practice, chemotherapeutic, analgesic, and anti-inflammatory drugs are prescribed simultaneously, which increases the risk of developing NSAIDs-related complications, especially in the elderly, patients with a prior history of peptic ulcer disease and patients with impaired kidney functions. Hence, there is a need for drugs having both antimicrobial and analgesic/anti-inflammatory activities with minimum adverse effects [10]. Mannich bases are generally formed by reaction between formaldehyde, a secondary amine and a compound containing are active hydrogen atoms, process known as mannich reaction. Mannich bases display varied biological activities such as anti-cancer[11], anti-microbial[12], cytotoxic [13],anti-inflammatory[14] and anti-convulsant[15] properties. Deamination process is

important to evoke biological responses. Thus, an aminoketone possessing at least one activated hydrogen atom β to amino group can undergo deamination in vivo under simulated in vitro conditions to generate the corresponding unsaturated ketone. The biological activites of mannich base have been attributed to these liberated αβ- unsaturated ketones, which alkylate nucleophiles [11-15]. The starting 1a-g (2-amiobenzothiazole) was prepared by the application of the cyclization [10, 16-18]. Treatment of starting material with formaldehyde and Pyrazolone [23] gave the title compounds 3 (a-l). All the synthesized compounds were characterized by their physical and spectral data. The IR spectra of compound 3(a-l) absence of carbonyl group C=O. The formation of Mannich bases 3(a-l) was confirmed by the presence of a carbonyl group C=O peak at 1709-1689 cm-1. The 1H NMR spectra of compound 3(a-l) exhibited all the expected protons. Mass spectra of compound 3a exhibited M+ ion peak at 293(60%) indicating that this molecule is rather unstable at TOF MS ES-231 and undergo fragmentation to form daughter ions. Appearance of M+ ion and their characteristic daughter ions confirm the structure proposed for the compounds.

2. Experimental & Methods

Step I-General synthesis of 1,3-benzothiazole-2-amine[16-18]

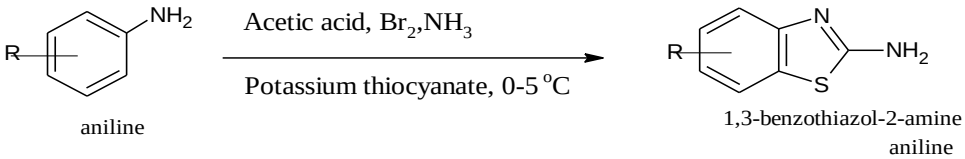
To glacial acetic acid (20ml) precooled to 5oC were added 8gm (0.08mol) of potassium thiocyanate and (0.01 mol) substituted aniline. Mixture was placed in freezing mixture of ice and salt stirred while 1.6ml of bromine in 6ml of glacial acetic acid was added from a dropping funnel at such a rate that the temperature never rises beyond 0-5oC. After all the bromine was added (105min.), the solution was stirred for 2 hours below 0oC and at room temperature for 10 hours, it was then allowed to stand overnight, during which period an orange precipitate settle at the bottom, water (6ml) was added quickly and slurry was heated at 85oC and filtered hot. Orange residue was placed in a reaction flask and treated with 10ml of glacial acetic acid heated again to 85oC and filtered hot. Combined filtrate was cooled and neutralized by ammonia solution to pH 6, precipitate was collected and recrystallized.

Step II – Synthesis of Mannich Base [19-22]

Corresponding equimolar solution of substituted 2-aminobenzothiazole derivatives in DMF and 3-methyl-5-pyrazolone in DMF were refluxed for around 3-5hrs in presence of formaldehyde and concentrated HCl. The resultant mixture was added into crush ice or ice-cold water with vigorous stirring till precipitation occurs. All synthesized compound was recrystallized with DMF: ethanol.

3. Reaction Scheme

Synthesis of Subsituted 1,3-Benzothiazole-2-Amine

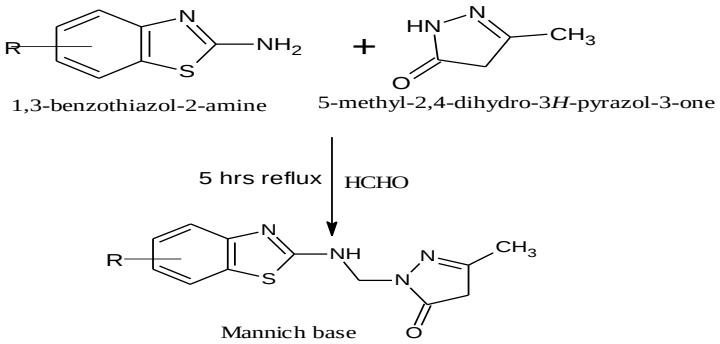


aniline

1,3-benzothiazol-2-amine
aniline

Scheme 1.

Synthesis of Mannich Base



1,3-benzothiazol-2-amine

5-methyl-2,4-dihydro-3*H*-pyrazol-3-one

Mannich base

4. Experimental protocols

Melting points were determined in open capillaries and are uncorrected. Purity of the compounds was checked by TLC on silica gel plates. The solvent system used to carry out the TLC is n-hexane: Toluene: formic acid (5:4:1). Spectral data IR spectra (cm^{-1}) were recorded in KBr on a bruker alpha T FTIR spectrometer. ^{1}H NMR δ (ppm) in DMSO-d_6 using TMS as reference on BrukerVANCE 400 SAIF. Mass spectra by using TOF MS ES.

Table 1

Physicochemical properties of the newly synthesized compounds.

Sr.No	R	MW (g/mol)	m.p (^{0}C)	R_f value	% yield
3a	4-Cl	294.75	206-208	0.45	74.55
3b	5-Cl	294.75	210-212	0.42	79.95
3c	6-Cl	294.75	208-210	0.44	70.44
3d	4-CH_3	274.341	258-260	0.41	75.20
3e	4,7-CH_3	288.368	260-262	0.45	65.20
3 f	5-CH_3	274.341	254-256	0.41	68.20
3g	4-OCH_3	290.340	260-262	0.47	70.55
3h	5-OCH_3	290.340	258-260	0.46	75.66
3 i	6-OCH_3	290.340	264-268	0.48	75.69
3 j	6-Br	339.210	248-250	0.44	60.65
3k	6-NO_2	305.312	258-262	0.44	77.57
3 l	6-Cl,7-F	312.31	250-252	0.46	64.55

Mobile phase n-hexane: Toluene: formic acid (5:4:1)

4.1. Spectral data

4.1.1.2-{[(4-chloro-1,3-benzothiazol-2 yl) amino] methyl}-5-methyl-2,4-dihydro-3H-pyrazol-3-one; ***(1)*** (3a); 3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67-1659.74 (HC=N); 827.19-811.08 (C-N); 721.85 (C-Cl); ^{1}H NMR DMSO- δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 5.1 (s 2H –CH_2), 2.58-2.59 (d 2H –CH_2), 2.0(s 3H –CH_3); Mass (TOF MS ES) m/z: 293 (M^+) RA (60%) 295 (M^{+2}) RA (25%) Elemental Analysis Calculated (found) C 48.90 % (48.72 %) H 3.76 % (3.78 %) N 19.01 % (19.07 %) O 5.43 % (5.49 %).

4.1.2.2-{[(5-chloro-1,3-benzothiazol-2-yl) amino] methyl}-5-methyl-2,4-dihydro-3H-pyrazol-3-one; ***(2)*** (3b); 3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67-1659.74 (HC=N); 827.19-811.08 (C-N); 721.85 (C-Cl); ^{1}H NMR DMSO- δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 5.1 (s 2H –CH_2), 2.58-2.59 (d 2H –CH_2), 2.0(s 3H –CH_3); Mass (TOF MS ES) m/z: 293 (M^+) RA (60%) 295 (M^{+2}) RA (30%) Elemental Analysis Calculated (found) C 48.90 % (48.72 %) H 3.76 % (3.78 %) N 19.01 % (19.07%) O 5.43 % (5.49 %).

4.1.3.2-{[(6-chloro-1,3-benzothiazol-2-yl) amino] methyl}-5-methyl-2,4-dihydro-3H-pyrazol-3-one; ***(3)*** (3c); 3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67-1659.74 (HC=N); 827.19-811.08 (C-N); 721.85 (C-Cl); ^{1}H NMR DMSO- δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 5.1 (s 2H –CH_2), 2.58-2.59 (d 2H –CH_2), 2.0(s 3H –CH_3); Mass (TOF MS ES) m/z: 293 (M^+) RA (60%) 295 (M^{+2}) RA (25%) Elemental Analysis Calculated (found) C 48.90 % (48.72%) H 3.76 % (3.78 %) N 19.01 %(19.07 %) O 5.43 % (5.49 %).

4.1.4.5-methyl-2-{[(4-methyl-1,3-benzothiazol-2-yl) amino] methyl}-2,4-dihydro-3H-pyrazol-3-one; ***(4)***

(3d); 3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67-1659.74 (HC=N); 827.19-811.08 (C-N); 721.85; ^{1}H NMR DMSO- δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 2.2(s 3H –CH_3), 5.1 (s 2H –CH_2), 2.5-2.6 (d 2H –CH_2), 2.0(s 3H –CH_3), Mass (TOF MS ES) m/z: 273 (M^+) RA (30%) Elemental Analysis Calculated (found) C 56.91 % (56.93 %) H 5.14 % (5.15 %) N 20.42 % (20.42 %) O 5.83 %(5.90) %).

4.1.5.5-methyl-2-{[(4,7-dimethyl-1,3-benzothiazol-2-yl) amino] methyl}-2,4-dihydro-3H-pyrazol-3-one; ***(5)***

(3e); 3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67-1659.74 (HC=N); 827.19-811.08 (C-N); ^{1}H NMR DMSO- δ

(ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 2.2(s 3H $–CH_3$), 2.3(s 3H $–CH_3$), 5.1 (s 2H $–CH_2$), 2.58-2.59 (d 2H $–CH_2$), 2.0(s 3H $–CH_3$); Mass (TOF MS ES) m/z: 287 (M^+) RA(20%) Elemental Analysis Calculated (found) C 58.31 % (58.29 %) H 5.59 % (5.61 %) N 19.43 % (19.43 %) O 5.55 % (5.57 %).

4.1.6.5-methyl-2-{[(6-methyl-1,3-benzothiazol-2-yl)amino]methyl}-2,4-dihydro-3H-pyrazol-3-one; ***(6)*** (3f); 3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67-1659.74 (HC=N); 827.19-811.08 (C-N); ^{1}H NMR DMSO- δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 2.2(s 3H $–CH_3$), 5.1 (s 2H $–CH_2$), 2.58-2.59 (d 2H $–CH_2$), 2.0(s 3H $–CH_3$); Mass (TOF MS ES) m/z: 273 (M^+)RA(40%) Elemental Analysis Calculated (found) C 56.91 % (56.93 %) H 5.14 % (5.15 %) N 20.42 % (20.42 %) O 5.83 %(5.90) %).

4.1.7.2-{[(4-methoxy-1,3-benzothiazol-2-yl)amino] methyl}-5-methyl-2,4-dihydro-3H-pyrazol-3-one ; ***(7)*** (3g); 3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67-1659.74 (HC=N); 827.19-811.08 (C-N); ^{1}H NMR DMSO- δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 3.3(s 3H $–OCH_3$), 5.1 (s 2H $–CH_2$), 2.58-2.59 (d 2H $–CH_2$), 2.0(s 3H $–CH_3$); Mass (TOF MS ES) m/z: 289 (M^+) RA (30%) Elemental Analysis Calculated (found) C 53.78% (53.75%) H 4.86 % (4.88 %) N19.30 % (19.30 %) O 11.02 % (11.05 %).

4.1.8.2-{[(5-methoxy-1,3-benzothiazol-2-yl) amino] methyl}-5-methyl-2,4-dihydro-3H-pyrazol-3-one; ***(8)*** (3h); 3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67-1659.74 (HC=N); 827.19-811.08 (C-N); ^{1}H NMR DMSO- δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 3.4(s 3H $–OCH_3$), 5.1 (s 2H $–CH_2$), 2.58-2.59 (d 2H $–CH_2$), 2.0(s 3H $–CH_3$); Mass (TOF MS ES) m/z: 289 (M^+) RA (60%) Elemental Analysis Calculated C 53.78% (53.75%)H 4.86 % (4.88 %) N19.30 % (19.30 %) O 11.02 % (11.05 %)

4.1.9.2-{[(6-methoxy-1,3-benzothiazol-2-yl) amino] methyl}-5-methyl-2,4-dihydro-3H-pyrazol-3-one; ***(9)*** (3i); 3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67-1659.74 (HC=N); 827.19-811.08 (C-N); ^{1}H NMR DMSO- δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 3.40(s 3H $–OCH_3$), 5.1 (s 2H $–CH_2$), 2.58-2.59 (d 2H $–CH_2$), 2.0(s 3H $–CH_3$); Mass (TOF MS ES) m/z: 289 (M^+) RA (20%) Elemental Analysis Calculated (found) C 53.78% (53.75%) H 4.86 % (4.88 %) N19.30 % (19.30 %) O 11.02 % (11.05 %).

4.1.10.2-{[(6-bromo-1,3-benzothiazol-2-yl) amino] methyl}-5-methyl-2,4-dihydro-3H-pyrazol-3-one; ***(10)*** (3j); 3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67-1659.74 (HC=N); 827.19-811.08 (C-N); 802-810 (C-Br); ^{1}H NMR DMSO- δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 5.1 (s 2H $–CH_2$), 2.58-2.59 (d 2H $–CH_2$), 2.0(s 3H $–CH_3$); Mass (TOF MS ES) m/z: 338 (M^+) RA (40%) Elemental Analysis Calculated (found) C 53.78% (53.75%) H 4.86 % (4.88 %) N19.30 % (19.30 %) O 11.02 % (11.05 %).

4.1.11.2-{[(6-nitro-1,3-benzothiazol-2-yl) amino] methyl}-5-methyl-2,4-dihydro-3H-pyrazol-3-one; ***(11)*** (3k); 3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67-1659.74 (HC=N); 827.19-811.08 (C-N); 1355-1320(-NO_2); ^{1}H NMR DMSO- δ (ppm) 8.2 (s 1 H -NH), 7.5- 8.5 (m Ar-H), 5.1 (s 2H $–CH_2$), 2.58-2.59 (d 2H $–CH_2$), 2.0(s 3H $–CH_3$); Mass (TOF MS ES) m/z: 304 (M^+) RA (30%) Elemental Analysis Calculated (found) C 47.21 % 47.21%) H 3.36 % (3.65 %) N 22.94 % (22.94 %) O 15.72 % (15.70 %).

4.1.12.2-[(6-chloro,7-fluro-1,3-benzothiazol-2-ylamino) methyl]-5-methyl-2,4-dihydro-3H-pyrazol-3-one; ***(12)***
(3l); 3346 (–NH str); 3001.98 (Ar-C-H); 1709.97 (C=O); 1590 (–NH bend); 1643.67-1659.74 (HC=N); 827.19-811.08 (C-N); 721.85 (C-Cl); 1150-1100 (C-F). ^{1}H NMR DMSO- δ (ppm) 8.2 (s 1 H -NH), 7.5-8.5 (m Ar-H), 5.1 (s 2H $–CH_2$), 2.58-2.59 (d 2H $–CH_2$), 2.0(s 3H $–CH_3$); Mass (TOF MS ES) m/z: 311 (M^+) RA (60%) 313 (M^{+1}) RA (30%) Elemental Analysis Calculated (found) C 46.08 % (46.08%) H17.91 % (17.91%) N 5.12 % (5.12 %) O 10.25 % (10.25 %).

5. Pharmacological screening

In-vitro Anti-inflammatory activity [24-25]
The synthesized compounds were screened for in-vitro anti-inflammatory activity by inhibition of bovine serum albumin denaturation method according to M.N.A. Rao et al. Experimental design the test compounds were dissolved in minimum amount of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solution was less than 2.5%. Test solution (1 ml) containing different concentrations of drug was mixed with 1 ml of 1mM albumin solution in phosphate buffer and incubated at 27o ±1oC for 15 min. Denaturation was induced by keeping the reaction mixture at 60o ± 1oC in a water bath for 10 min. After cooling the turbidity was measured at 660 nm (Shimadzu 1800 Spectrometer). Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and the average was taken. The percentage of inhibition was calculated from the following formula. The standard solution was also prepared as similar to that of the test solution Diclofenac was used as a standard.

$$\% \text{ Inhibition} = (Vt\text{-}Vc/Vc) \times 100$$

Where, Vt = Drug absorbance of triplicate Average,
Vc = Control absorbance of triplicate average

Table 2
Effect of BMP on heat induced Protein Denaturation.

Sr. No	Treatment	R	Absorbance at 660nm (Mean + SD)	% inhibition of protein denaturation
1	Control	-	0.098 ± 0.009	----
2	3a	4-Cl	0.158±0.002	61.22
3	3b	5-Cl	0.154±0.002	57.14
4	3c	6-Cl	0.168 ± 0.003	70.80
5	3d	4-CH_3	0.122±0.001	24.48
6	3e	4,7-CH_3	0.135±0.003	37.75
7	3f	5-CH_3	0.118 ±0.004	20.40
8	3g	4-OCH_3	0.132 ± 0.003	34.69
9	3h	5-OCH_3	0.128 ± 0.002	30.06
10	3i	6-OCH_3	0.148±0.03	51.2
11	3j	6-Br	0.147 ± 0.002	50.00
12	3k	6-NO_2	0.145 ± 0.004	47.95
13	3l	6-Cl,7-F	0.170 ± 0.002	73.80
14	Diclofenac sodium	-	0.190 ±0.002	93.87

From table 2 In-vitro Anti-inflammatory activity data among all the compounds tested 3c, 3i, 3j, 3k, 3l substitution at (6 position) showed good in-vitro anti-inflammatory activity (more than 50%). The remaining compounds exhibited mild to moderate activities (20% to 40%) compared to the standard Diclofenac (93.87%).

Results and Discussion

The purpose of the present work was to synthesize a series of desired title compounds 3(a-l) 2-[(1,3-benzothiazol-2-ylamino) methyl]-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one by reacting compounds 1 (a-l) with formaldehyde and pyrazolone. All the compounds (3a-l) were screened for in-vitro anti-inflammatory activity. The results are shown in the From table 2 In-vitro Anti-inflammatory activity data among all the compounds tested, 3c, 3i, 3j, 3k, 3l substitution at (6 position) showed good in-vitro anti-inflammatory activity (more than 50%). The remaining compounds exhibited mild to moderate activities (20% to 40%) compared to the standard Diclofenac (93.87%).

Conclusion

In summary, various benzothiazole derivatives were prepared with the objective of developing anti inflammatory agents, from the in-vitro anti-inflammatory activity results; it was observed that both electron donating and electron withdrawing groups on the benzothiazole ring of the compounds influenced the activity. But benzothiazole ring containing electron withdrawing groups had shown more promising result. Among all the compounds tested, 3c with 6'-chloro substitution and 3l with 6'-chloro, 7'-fluro substitution showed good in-vitro anti-inflammatory activity. The remaining compounds exhibited mild to moderate activities compared to the standard Diclofenac.

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