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Anti-inflammatory evaluation of novel mannich bases of 2, 5-disubstiututed indoles

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ABSTRACT

A series of mannich bases of 2,5-disubstituted indoles have been synthesized by reaction of 2,5-disubstituted indoles with secondary amine and formaldehyde in acetic acid. The title 2-substituted indole were synthesized using a Fischer-indole synthesis gave substituted acetophenone phenylhydrazone and Cyclization of the hydrazone by polyphosphoric acid at 120°C afforded the 2-substituted indole. Nitration of 2-substituted indole was carried out in presence of equal amount of concentrated sulphuric acid and concentrated nitric acid. Most of the test compounds showed appreciable inhibition of the oedema size in comparison with diclofenac sodium. The compound ***MB11*** *had shown pronounced activity after 4th hour interval, almost equipotent with the standard drug diclofenac sodium. The compound* ***MB4, MB5, MB10, MB15*** *and* ***MB16*** *showed excellent protection against inflammation with 68%, 70%, 76%, 74% and 72% inhibition, respectively. The structures were confirmed by FTIR, ^{1}H NMR.*

Key words: Synthesis, mannich bases of 2,5-disubstituted indoles, anti-inflammatory activity.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) remain among the most widely prescribed drugs worldwide for the treatment of inflammation including pain releasing, anti-pyretic and rheumatoild arthritis. The mechanism of action was through their inhibition of prostaglandin biosynthesis via the enzyme cyclooxygenase- 2 (COX-2) [1]. Thus selective inhibition of COX-2 over COX-1 is useful for the treatment of inflammation-associated disorders with reduced gastrointestinal toxicities when compared with the traditional NSAIDs. Current research has focused on developing safer NSAIDs selective COX-2 inhibitors. Several selective COX-2 inhibitors such as Celecoxib [4], Rofecoxib [5] and Valdecoxib [6] have been marketed as a new generation of NSAIDs structurally featuring with vicinal diarylheterocycles inhibitors [7]. Diarylheterocycle class of compounds has been investigated extensively as COX-2 inhibitors. The other two categories of selective COX-2 inhibitors [8] are sulfonanilide inhibitors [9] and modifications of classical NSAIDs [10].

Other reports disclosed that NSAIDs may enhance the progression of bacterial infection. Hence, a dual anti-inflammatory- antibacterial agent with an improved safety profile is required for improved therapeutic benefits and better patient compliance [11]. Indole derivatives have been reported to possess promising biological activities including analgesic [11], antipyretic [12], antifungal [13], anti-inflammatory [14], cardiovascular [11], anticonvulsant [15], antimicrobial [16] and selective COX-2 inhibitory activities [17]. Thus the efficient synthesis of novel substituted indole derivative compounds still represent highly pursued target [18]. The substitution of heterocyclic moiety at 3-position of the indole ring obviously influenced the anti-inflammatory activity [19].

Mannich reaction provides a suitable method to introduce aminoalkyl substituent in a molecule and mannich side chain increases the bioavailability of the drug molecule. Mannich derivatives exhibit better activity than the corresponding parent analogues *vide infra* [20]. They are also potential lead molecules for anti-inflammatory drugs.

These findings prompted us to construct a new molecular framework of mannich bases of indole ring with a hope of developing a compound that possesses good anti-inflammatory activity.

MATERIALS AND METHODS

All starting materials were either purchased from commercial. Solvents and reagents were used without further purification. To monitor the reaction and establish the purity of reactants and products, single spot thin layer chromatography (TLC) was performed using silica gel G plates. Melting points were taken by using ELICO Melting point apparatus in open glass capillary tubes and are uncorrected. IR spectra were recorded on a Shimadzu FTIR spectrometer using potassium bromide. ^{1}H NMR spectra were determined by Bruker Avance II 400 spectrometer (1H, 400 MHz). Chemical shifts are reported as ppm (δ) relative to TMS as an internal standard, abbreviations: singlet (s), doublet (d), triplet (t), multiplet (m).

Procedure followed for synthesis

Preparation of Substituted 2-Phenylindole (4a-c) [25]

Synthesis of 2-substituted indole (4a-c) was carried out by procedure of Fisher indole synthesis. P-substituted acetophenone phenylhydrazone (3a-c) was synthesized by refluxing the mixture for 2-3 hours of 10g (0.08mol) of substituted acetophenone and 9 g (0.08mol) phenylhydrazine with 30 ml of ethanol and few drops of glacial acetic was added to it. After completion reaction mixture was filtered, the solid was washed with 0.1N (10ml) hydrochloric acid followed by 12ml cold rectified spirit.

The crude phenylhydrazone (16g) in a 250-ml beaker containing of 90 g polyphosphoric acid. The mixture was heated over a boiling water bath with continous stirring and maintaing 100-120 ℃ for 10 minutes (the reaction is exothermic). Further added 265 ml of cold water and stirred well until the solid separated out. Neutralized the reaction mixture with 10% sodium hydroxide. Filtered and washed the solid well with cold water. Further, 175 ml of rectified spirit was added to the crude solid and heated till the solid get dissolved. Added small quantity of decolourising charcoal, filtered and washed with hot rectified spirit. The filterate was cooled to room temperature and white crystals were precipitated out.

Preparation of 2, 5-disubstituted indoles (5a-c) [26]

Concentrated nitric acid (12ml) was taken in 250 ml beaker and dropwise added an equal amount of concentrated sulphuric acid. The mixture was kept in ice cold water and 2-substitued indole (6.32g) was added in portion over a period of 30 minutes with continuous stirring at room temperature. The reaction mixture was poured over crushed ice. The precipitated product was filtered out, washed with cold water and dried.

Preparation of Mannich base of 2, 5-disubstituted indoles (6a-c) [27]

A solution of 2,5-disubstituted indole (0.004 mol) in 12 ml of glacial acetic acid was treated with slight excess of different secondary amine (0.01 mol) and then with 37.7% solution of formalin (0.01 mol). The reaction mixture was stirred at 50– 55 °C for 8 h. 40 ml of water was added in one portion. The resultant mixture was adjusted to pH 10 with sodium hydroxide. The solid was filtered and recrystalized with ethanol. The physiochemical parameters of all the synthesiszed compounds are summarized in Table 1.

3-((4-methylpiperazin-1-yl)methyl)-5-nitro-2-p-tolyl-1H-indole (MB1)

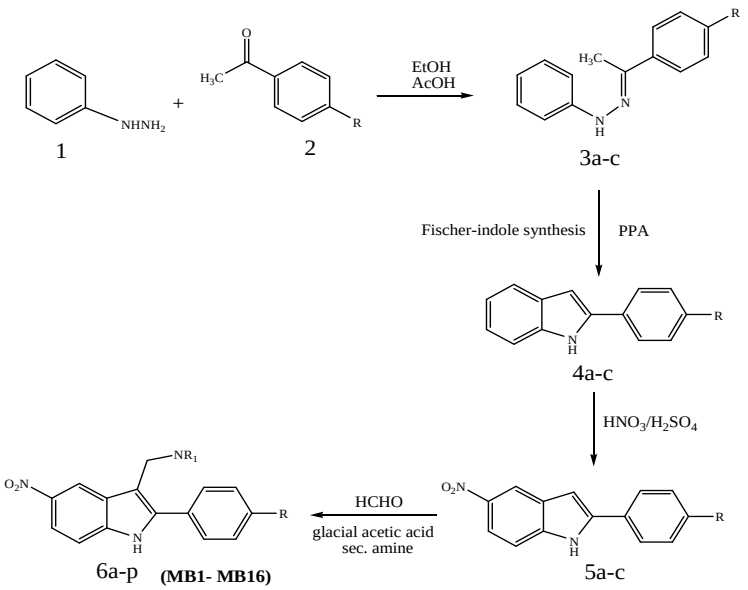
Yield-72.3%, mp-168-170°C, IR (KBr) cm-^{1}: 3313 (-NH str., aromatic), 3101 (-CH str., aromatic), 2983 (-CH str. aliphatic), 1525 (C-NO_2 str., assym.), 1342 (C-NO_2 str., sym.), 1610 (C=C str., aromatic), 1450 (-CH_2 bending), 1219 (-C-N str.,). ^{1}H NMR (400 MHz, DMSO; δ, ppm): 8.58 (1H, s, NH), 8.32-8.38 (3H, m, aromatic), 7.71-7.73 (2H, d, aromatic), 7.41-7.43 (2H, d, aromatic), 3.32 (2H, s, CH_2), 2.61-2.68 (11H, br s, N-methyl piperazine), 2.45 (3H, s, CH_3).

N-((5-nitro-2-p-tolyl-1H-indol-3-yl)methyl)diethanolamine (MB2)

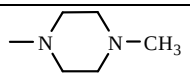
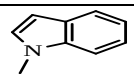
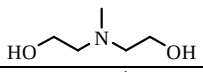
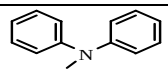
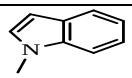
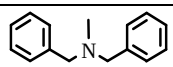
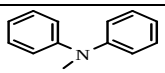
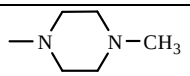
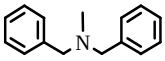
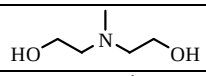
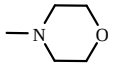
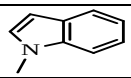
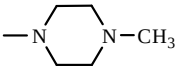
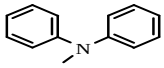
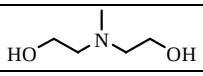
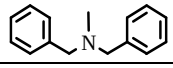
Yield-63.4%, mp-188-190°C, IR (KBr) cm-^{1}: 3234(-NH str., aromatic), 3093 (-CH str., aromatic), 1525 (C-NO_2 str., assym.), 1342 (C-NO_2 str., sym.), 1612 (C=C str., aromatic), 1456 (-CH_2 bending), 1220 (-C-N str.,). ^{1}H NMR (400 MHz, DMSO; δ, ppm): 8.59 (1H, s, NH), 8.32-8.38 (3H, m, aromatic), 7.71-7.73 (2H, d, aromatic), 7.42-7.43 (2H, d, aromatic), 3.33 (2H, s, CH_2), 2.63-2.65 (8H, m), 2.51 (3H, s, CH_3).

3-((1H-indol-1-yl)methyl)-5-nitro-2-p-tolyl-1H-indole (MB3)

Yield-82.9%, mp-274-278°C, IR (KBr) cm-^{1}: 3398 (-NH str., aromatic), 3050 (-CH str., aromatic), 2918 (-CH str. aliphatic), 1531 (C-NO_2 str., assym.) 1340 (C-NO_2 str., sym.), 1612 (C=C str., aromatic), 1465 (-CH_2 bending), 1220 (-C-N str.,). ^{1}H NMR (400 MHz, DMSO; δ, ppm): 8.59 (1H, s, NH), 8.23-8.37 (7H, m, aromatic), 7.71-7.73 (2H, d, aromatic), 7.41-7.43 (2H, d, aromatic), 7.27-7.33 (2H, m, aromatic), 3.32 (2H, s, CH_2), 2.51 (3H, s, CH_3).



Scheme1. General synthetic scheme for the synthesis of the derivatives

Comp.	R	R_1	Comp.	R	R_1
MB1	$-CH_3$		MB9	-Br	
MB2	$-CH_3$		MB10	-Br	
MB3	$-CH_3$		MB11	-Br	
MB4	$-CH_3$		MB12	$-OCH_3$	
MB5	$-CH_3$		MB13	$-OCH_3$	
MB6	-Br		MB14	$-OCH_3$	
MB7	-Br		MB15	$-OCH_3$	
MB8	-Br		MB16	$-OCH_3$	

2-(4-bromophenyl)-3-(morpholinomethyl)-5-nitro-1H-indole (MB6)
Yield-63.9%, mp-202-204°C, IR (KBr) cm-^{1}: 3244(-NH str., aromatic), 3107 (-CH str., aromatic), 2827 (-CH str. aliphatic), 1521 (C-NO_2 str., assym.) 1338 (C-NO_2 str., sym.), 1597 (C=C str., aromatic), 1446 (-CH_2 bending), 1217 (-C-N str.,), 1074 (-C-Br str.,). ^{1}H NMR (400 MHz, DMSO, δ, ppm): 8.61 (1H, s, NH), 8.26-8.28 (3H, m, aromatic), 7.83-7.85 (2H, d, aromatic), 7.77-7.79 (2H, d, aromatic), 3.32 (2H, s, CH_2), 2.51 (11H, br s, morpholine).

2-(4-bromophenyl)-3-((4-methylpiperazin-1-yl)methyl)-5-nitro-1H-indole (MB7)
Yield-73.5%, mp-192-194°C, IR (KBr) cm-^{1}: 3502(-NH str., aromatic), 3101 (-CH str., aromatic), 2818 (-CH str. aliphatic), 1521 (C-NO_2 str., assym.) 1340 (C-NO_2 str., sym.), 1649 (C=C str., aromatic), 1446 (-CH_2 bending), 1226 (-C-N str.,), 1076 (-C-Br str.,). ^{1}H NMR (400 MHz, DMSO, δ, ppm): 8.55 (1H, s, NH), 8.37-8.39 (3H, m, aromatic), 7.77-7.84 (2H, d, aromatic), 7.65-7.71 (2H, d, aromatic), 3.33 (2H, s, CH_2), 2.51 (11H, br s, N-methyl piperazine).

N-((2-(4-bromophenyl)-5-nitro-1H-indol-3-yl)methyl)diethanolamine (MB8)
Yield-66.4%, mp-196-198°C, IR (KBr) cm-^{1}: 3493(-NH str., aromatic), 3093 (-CH str., aromatic), 2872 (-CH str. aliphatic), 1529 (C-NO_2 str., assym.) 1342 (C-NO_2 str., sym.), 1597 (C=C str., aromatic), 1444 (-CH_2 bending),

1242 (-C-N str.,), 1074 (-C-Br str.,). ^{1}H NMR (400 MHz, DMSO, δ, ppm): 9.4 (1H, s, NH), 8.27-8.39 (3H, m, aromatic), 7.72-7.83 (4H, d, aromatic), 3.32 (2H, s, CH_2), 2.51 (8H, m), 1.94 (2H, s, OH)

N-((2-(4-bromophenyl)-5-nitro-1H-indol-3-yl)methyl)-N-phenylbenzenamine (MB10)
Yield-80.25%, mp-291-293°C, IR (KBr) cm-^{1}: 3390 (-NH str., aromatic), 3024 (-CH str., aromatic), 2914 (-CH str. aliphatic), 1514 (C-NO_2 str., assym.) 1311 (C-NO_2 str., sym.), 1598 (C=C str., aromatic), 1452 (-CH_2 bending), 1217 (-C-N str.,), 1074 (-C-Br str.,). ^{1}H NMR (400 MHz, DMSO, δ, ppm): 8.37 (1H, s, NH), 8.25-8.27 (3H, m, aromatic), 7.77-7.82 (4H, q, aromatic), 6.97-7.08 (6H, m, aromatic), 6.76-6.95 (4m, m, aromatic) 3.78 (2H, s, CH_2).

2-(4-methoxyphenyl)-3-((4-methylpiperazin-1-yl)methyl)-5-nitro-1H-indole (MB12)
Yield-74.6%, mp-182-184°C, IR (KBr) cm-^{1}: 3309 (-NH str., aromatic), 3095 (-CH str., aromatic), 2920 (-CH str. aliphatic), 1531 (C-NO_2 str., assym.) 1346 (C-NO_2 str., sym.), 1616 (C=C str., aromatic), 1473 (-CH_2 bending), 1282 (-C-O-C str.,). ^{1}H NMR (400 MHz, DMSO, δ, ppm): 9.57 (1H, s, NH), 8.34-8.45 (3H, m, aromatic), 7.60-7.62 (2H, d, aromatic), 7.50-7.52 (2H, d, aromatic), 4.05 (3H, s, OCH_3), 3.33 (2H, s, CH_2), 2.51 (11H, br s, N-methyl piperazine).

3-((1H-indol-1-yl)methyl)-2-(4-methoxyphenyl)-5-nitro-1H-indole (MB14)
Yield-80.59%, mp-256-260°C, IR (KBr) cm-^{1}: 3390 (-NH str., aromatic), 3152 (-CH str., aromatic), 2919 (-CH str. aliphatic), 1531 (C-NO_2 str., assym.) 1342 (C-NO_2 str., sym.), 1612 (C=C str., aromatic), 1465 (-CH_2 bending), 1220 (-C-N str.,). ^{1}H NMR (400 MHz, DMSO, δ, ppm): 9.50 (1H, s, NH), 8.19-8.26 (3H, m, aromatic), 7.74-7.63 (10H, m, aromatic), 4.03 (3H, s, OCH_3), 3.32 (2H, s, CH_2).

Table 1 The physicochemical characterization of mannich bases of 2,5-disubstituted Indoles

S.NO	Comp.	Molecular Formula	Mol. Wt	R_f Value	M.P.(°C)	% yield
1.	MB1	$C_{21}H_{24}N_4O_2$	364.44	0.71	168-170	72.3
2.	MB2	$C_{20}H_{23}N_3O_4$	369.17	0.67	188-190	63.4
3.	MB3	$C_{24}H_{19}N_3O_2$	381.43	0.76	274-278	82.9
4.	MB4	$C_{28}H_{23}N_3O_2$	433.5	0.63	290-292	78.6
5.	MB5	$C_{30}H_{27}N_3O_2$	461.55	0.86	282-284	57.2
6.	MB6	$C_{19}H_{18}BrN_3O_3$	416.27	0.73	202-204	63.9
7.	MB7	$C_{20}H_{21}BrN_4O_2$	429.31	0.75	192-194	73.5
8.	MB8	$C_{19}H_{20}BrN_3O_4$	434.28	0.69	196-198	66.4
9.	MB9	$C_{23}H_{16}BrN_3O_2$	446.3	0.74	286-288	83.5
10.	MB10	$C_{27}H_{20}BrN_3O_2$	498.37	0.62	291-293	80.2
11.	MB11	$C_{29}H_{24}BrN_3O_2$	526.42	0.83	273-275	56.3
12.	MB12	$C_{21}H_{24}N_4O_3$	380.44	0.82	182-184	74.6
13.	MB13	$C_{20}H_{23}N_3O_5$	385.41	0.72	194-196	65.9
14.	MB14	$C_{24}H_{19}N_3O_3$	397.43	0.78	256-260	80.5
15.	MB15	$C_{28}H_{23}N_3O_3$	449.5	0.65	284-288	79.8
16.	MB16	$C_{30}H_{27}N_3O_3$	477.55	0.81	260-262	58.7

TLC solvent system; Toluene: Ethyl acetate: Formic acid = 7:2:1

N-((2-(4-methoxyphenyl)-5-nitro-1H-indol-3-yl)methyl)-N-phenylbenzenamine (MB15) Yield-79.8%, mp-284-288°C, IR (KBr) cm-^{1}: 3315 (-NH str., aromatic), 3026 (-CH str., aromatic), 2900 (-CH str. aliphatic), 1504 (C-NO_2 str., assym.) 1346 (C-NO_2 str., sym.), 1604 (C=C str., aromatic), 1442 (-CH_2 bending), 1286 (-C-O-C str.,). ^{1}H NMR (400 MHz, DMSO, δ, ppm): 8.79 (1H, s, NH), 8.35-8.39 (3H, m, aromatic), 8.14-8.30 (10H, m, aromatic), 7.53-7.55 (2H, d, aromatic), 7.45-7.49 (2H, d, aromatic), 4.19 (3H, s, OCH_3), 3.32 (2H, s, CH_2)

N-benzyl-N-((2-(4-methoxyphenyl)-5-nitro-1H-indol-3-yl)methyl)(phenyl)methanamine (MB16)
Yield-58.7%, mp-260-262°C, IR (KBr) cm-^{1}: 3477 (-NH str., aromatic), 3030 (-CH str., aromatic), 1529 (C-NO_2 str., assym.), 1342 (C-NO_2 str., sym.), 1612 (C=C str., aromatic), 1456 (-CH_2 bending), 1257 (-C-O-C str.). ^{1}H NMR (400 MHz, DMSO, δ, ppm): 9.58 (1H, s, NH), 7.91-8.19 (3H, m, aromatic), 7.25-7.58 (14H, m, aromatic), 4.03-4.19 (7H, m, OCH_3), 3.32 (2H, s, -CH_2-).

Biological activity
Carrageenan induced paw oedema method
Anti-inflammatory activity of the synthesized compounds determined by the carrageenan induced rat paw oedema method [11, 21, 22]. The present method depends on the inhibition of oedema caused by carrageenan. The thickness of rat paw is measured by a vernier caliper.

Animals: Adult Wistar albino rats (150-200g) were used to measure anti-inflammatory activity. The animals were organized randomly in groups of six and each animal was distinctly marked within its group. Rats were purchased

from the Disease free Small Animal House Lala Lajpat Rai University of Veterinary and Animal Science, Hisar (Haryana). The animals were housed properly in the animal facility of Pharmaceutical Sciences, GJU & T, Hisar, under standard conditions of temperature (25±2°C) and 12hr/hr light/dark cycles. The study protocol was approved by the Institutional Animal Ethics Committee of the Lala Lajpat Rai University of Veterinary and Animal Science, Hisar (Haryana) under reg. No 3495 (23-5-2013). The animals were subjected to fasting before experimentation.

Route of administration: Standard drug (Diclofenac sodium) and test compounds were administered with a dose of 50 &100 mg/kg body weight orally as a suspension in a 0.5% w/v solution of carboxymethyl cellulose (CMC). Carrageenan suspension in saline was given to the hind paw by subcutaneous (s.c.) route.

Procedure: The animals were divided into different groups each consisting of 6 rats. Group I, which served as the control, received only 0.5% w/v carboxymethyl cellulose (CMC). Group II and the other groups received diclofenac sodium and the test compounds, respectively, at a dose level of 50 & 100 mg/kg respectively. The hind paw oedema was induced in each rat by the sub-planter injection of 0.1 ml of 1% carrageenan solution in saline 1 h after the administration of the test compounds and standard drug. The volume of the paw oedema (ml) was determined by a vernier caliper before and after 1, 2, 3 and 4 h the carrageenan injection. The percent oedema inhibition was calculated according to the following formula:

Percent oedema inhibition = 100 $(1-V_t/V_c)$

Where Vc = The mean increase in paw thickness of control group
Vt = The mean increase of paw volume after treatment with the test compound and standard drug.

3.4.2 Statistical analysis

Results are presented as mean ± S.E.M of six rats. Difference in mean value between groups were analysed by using one-way ANOVA followed by Dunnett's test for n = 6. A probability value less than 0.01 was consider as statistical significant.

RESULTS AND DISCUSSION

Anti-inflammatory evaluation

Anti-inflammatory activity of mannich bases of 2, 5-disubstituted indoles was carried out using carrageenan induced rat paw oedema method[11, 21, 22]. Diclofenac sodium was used as standard drug at dose level of 50 mg/kg. Results are presented as Mean±SEM and % inhibition. Comparison was done with control by using one-way ANOVA followed by Dunnett's test for n = 6. The results of anti-inflammatory activity by carrageenan induced rat paw oedema method are presented in Table 2.

Most of the test compounds showed appreciable inhibition of the oedema size in comparison with diclofenac sodium. The compound **MB4, MB5, MB10, MB11, MB15** and **MB16** showed excellent protection against inflammation (68%, 70%, 76%, 78%, 74% and 72% inhibition) respectively, whereas compounds **MB2, MB8,** and **MB13** were found to have the least inhibitory effect. Compounds **MB3, MB9** and **MB14** also showed good anti-inflammatory activity with a percent inhibition of 65%, 62% and 63%, respectively, in comparison to diclofenac sodium,which (83.39%). The rest of the compounds exhibited weak to moderate anti-inflammatory. SAR was deduced from the results of the anti-inflammatory activity which is as follows:

1. Compounds which are substituted at 3-position of indole ring with aromatic secondary amine dibenzylamine, diphenylamine and indole ring **(MB5, MB16, MB11, MB4, MB10, MB16, MB3, MB9** and **MB14**) have shown significant contribution towards the anti-inflammatory activity.
2. It has been observed that compounds which are substituted at 3-position of indole ring with N-methyl piperazine and morpholine ring (**MB1, MB7, MB12, MB6)** have shown moderate to good anti-inflammatory activity.
3. The presence of electronegative group at para position of 2-phenyl ring of indole in compounds **(MB9, MB10, MB11)** have shown significant contribution towards anti-inflammatory activity which are similar to result obtained by Chavan *et al.*[5]
4. Compounds which are substituted with diethanolamine at 3-position of indole nucleus have shown least activity.

From the discussion above made it can be postulated that by keeping $-NO_2$ at 5-position of indole ring, substituting the 2-position with para-Br or para-OCH_3, substituted aromatic ring and 3-position preferably with aromatic and alicyclic ring lead to synthesis of potential anti-inflammatory compounds. The presence of methylene bridge between 3-position of indole ring and secondary amine may be responsible for effective binding.

Table 2 Effect of synthesized derivatives on paw thickness in carrageenan induced paw oedema method in Wistar albino rats

Comp. code	Mean difference in paw thickness in mm (Mean ±SEM) (% inhibition)			
	1h	2h	3h	4h
Control	0.65±0.011	0.86±0.027	0.98±0.017	1.14±0.010
Std.	0.24±0.020** (63%)	0.26±0.015** (69%)	0.22±0.017** (77%)	0.18±0.010** (84%)
MB1	0.44±0.013** (32%)	0.47±0.017** (45%)	0.49±0.008** (51%)	0.54±0.018** (52%)
MB2	0.53±0.010** (18%)	0.67±0.018** (22%)	0.72±0.015** (26%)	0.89±0.074** (21%)
MB3	0.31±0.008** (52%)	0.33±0.011** (61%)	0.34±0.011** (65%)	0.39±0.016** (65%)
MB4	0.29±0.011** (55%)	0.32±0.006** (62%)	0.33±0.009** (66%)	0.36±0.013** (68%)
MB5	0.28±0.016** (56%)	0.30±0.024** (65%)	0.31±0.005** (68%)	0.34±0.012** **(70%)**
MB6	0.38±0.006** (41%)	0.44±0.013** (48%)	0.46±0.012** (53%)	0.51±0.013** (55%)
MB7	0.36±0.009** (44%)	0.37±0.004** (56%)	0.44±0.006** (55%)	0.45±0.033** (60%)
MB8	0.52±0.016** (20%)	0.63±0.013** (26%)	0.68±0.008** (30%)	0.78±0.012** (31%)
MB9	0.33±0.014** (49%)	0.35±0.012** (59%)	0.39±0.009** (60%)	0.43±0.015** (62%)
MB10	0.25±0.018** (61%)	0.26±0.011** (69%)	0.26±0.008** (73%)	0.27±0.014** **(76%)**
MB11	0.26±0.019** (60%)	0.29±0.014** (66%)	0.25±0.012** (74%)	0.25±0.006** **(78%)**
MB12	0.45±0.009** (30%)	0.46±0.011** (46%)	0.50±0.009** (48%)	0.56±0.009** (50%)
MB13	0.46±0.012** (29%)	0.55±0.014** (36%)	0.55±0.008** (43%)	0.65±0.010** (42%)
MB14	0.31±0.023** (52%)	0.44±0.012** (48%)	0.45±0.013** (54%)	0.42±0.016** (63%)
MB15	0.27±0.010** (58%)	0.31±0.022** (63%)	0.27±0.011** (72%)	0.29±0.009** **(74%)**
MB16	0.27±0.005** (59%)	0.30±0.007** (65%)	0.29±0.010** (70%)	0.32±0.010** **(72%)**

*Statistical analysis were performed by one-way ANOVA followed by Dunnett's multiple comparison test for n = 6. *p < 0.05, **p < 0.01 compared to standard drug (Diclofenac sodium)*

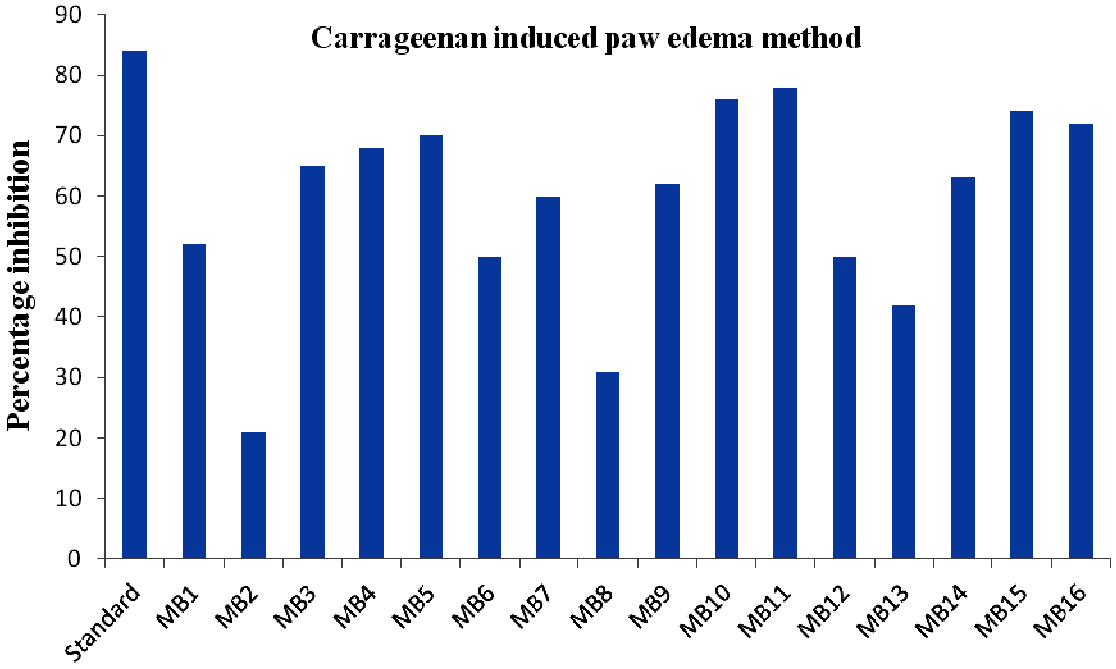


Fig 3.2 % Inhibition at 4th hour after carrageenan injection of all compounds compared to standard drug

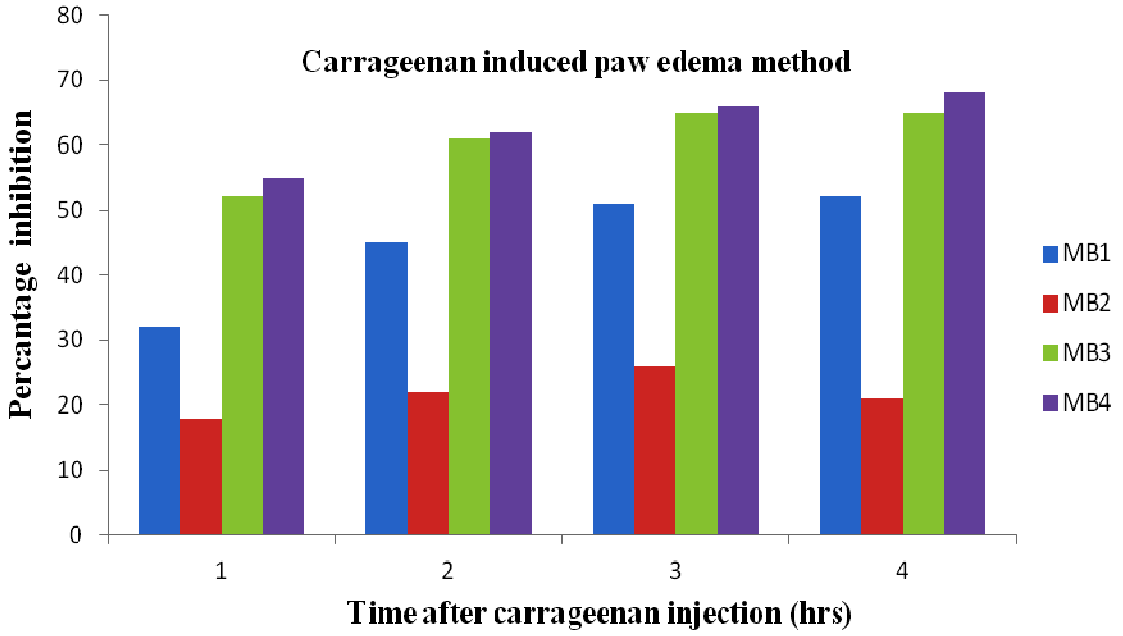


Fig 3.3 % Inhibition of carrageenan induced paw oedema by test compounds (MB1, MB2, MB3, MB4)

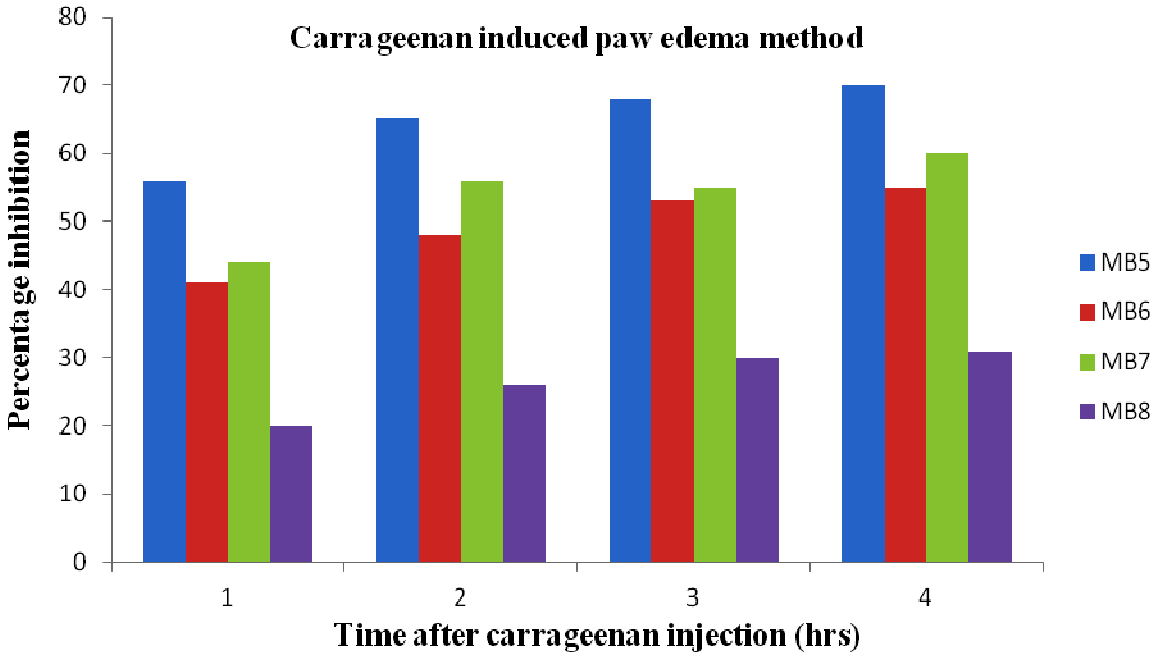


Fig 3.4 % Inhibition of carrageenan induced paw oedema by test compounds (MB5, MB6, MB7, MB8)

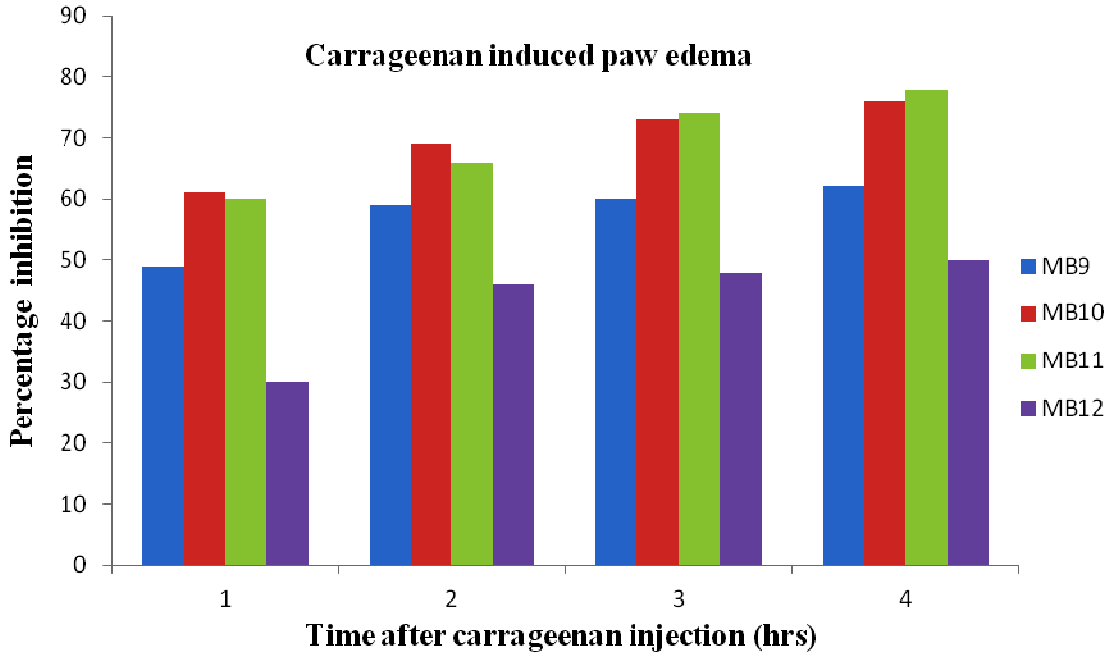


Fig 3.5 % Inhibition of carrageenan induced paw oedema by test compounds (MB9, MB10, MB11, MB12)

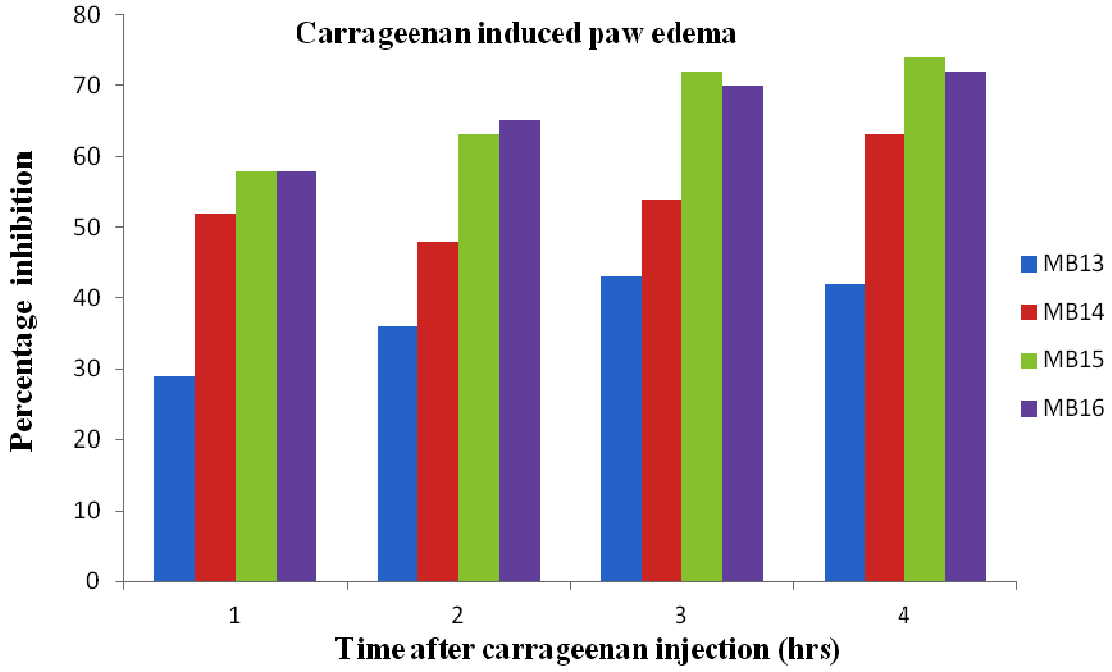


Fig 3.6 % Inhibition of carrageenan induced paw oedema by test compounds (MB13, MB14, MB15, MB16)

CONCLUSION

A series of mannich bases of 2,5-disubstituted indoles have been synthesized in moderate to good yield and examined for their anti-inflammatory activity. The trend of the activity leads to the following conclusions:

❖ Aromatic substituent at 3-position of indole ring enhance the anti-inflammatory activity in comparison to alicyclic and aliphatic substituent.

❖ The presence of electron withdrawing group at para position of 2-phenyl ring of indole is favourable for anti-inflammatory activity of compounds, which is clearly evident from the activity of **MB10, MB11** with 76% and 78% inhibition respectively.

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