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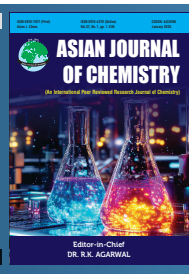

Design, Synthesis, Characterization and *in vitro* Antimicrobial, Anti-inflammatory and Antioxidant Activities of Novel Indole-Based Mannich Base Derivatives

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The object of the present investigation is to synthesize and estimate the antimicrobial, anti-inflammatory and antioxidant activities of indole-based Mannich base derivatives (**M1-M5**). These analogues were synthesized by condensation technique between indole, formaldehyde/furfuraldehyde and substituted aliphatic/aromatic amines. These analogue structures were characterized by FTIR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy. Initially, indole-based Mannich base derivatives therapeutic activities were assessed from AUTODOCK score after the successful binding with *Staphylococcus aureus* DNA gyrase B and cyclooxygenase 2. Each mannich base derivative had revealed an excellent affinity towards the target. Furthermore, the derivatives were also screened for antimicrobial, anti-inflammatory and antioxidant activities by *in vitro* methods. The study findings demostrated that these analogues have considerable antimicrobial activity against *E. coli*, *B. subtilis*, *A. flavus* and *A. niger*. Whereas, the anti-inflammatory activity result suggested that the novel indole-based Mannich derivatives significantly controlled the protein denatured and RBC haemolysis. Furthermore, the antioxidant activity result reveals that compounds exhibited worthy activity due to the presence of the phenyl, piperidine, morpholine ring as a part of the structure. Analogues **M2**, **M3** and **M5** had shown remarkable activity compared to the other compounds. Therefore, these analogues can be considered for the development of new antimicrobial, anti-inflammatory and antioxidant agents.

Keywords: Indole, Mannich base, Antimicrobial, Anti-inflammatory, Antioxidant activity.

INTRODUCTION

The indole nucleus is a trusted fused heterocyclic ring consisting of nitrogen as a hetero atom found in many natural and synthetic molecules [1]. In this nucleus, the pyrrole ring is fused with benzene in 2nd and 3rd positions [2]. Indole and their derivatives play a vital role in the synthesis of novel drugs and are responsible for biological actions such as anticancer [3], anti-inflammatory [4], antipyretic [5], antibacterial [6], anti-fungal [7], anticonvulsant [8], anthelmintic [9], antitubercular [10], analgesic [11], antiHIV [12], antimalarial [13], antipsychotic [14], antiviral agent [15], *etc*. Therefore, the synthesis of novel indole nucleus containing drugs is needed for a better effect on chronic diseases. Similarly, Mannich base derivatives also perform vital pharmacophores that have been prepared by condensation reaction of active hydrogen donating agents, aldehyde and aliphatic/aromatic amines [16]. They are responsible for different biological activities such as antipyretic [17], anti-inflammatory [18], antihypotensive [19], anticonvulsant [20], antiviral [21], antitumour [22], fungicidal [23], herbicidal [24], antimicrobial [25], plant-growth regulator [26], *etc*.

Moreover, Mannich bases may incorporate several heterocyclic systems throughout their structure. Thus, they are a promising subject for the chemical modifications that may produce biologically active molecules and due to the high therapeutic profiles of indole and Mannich bases, the aforesaid observations

promoted us to synthesize the novel indole-based Mannich base derivatives and screened *in vitro* antimicrobial, anti-inflammatory and antioxidant activities.

EXPERIMENTAL

The chemicals, reagents and solvents were obtained from Sigma-Aldrich, Ranbaxy and Dr Reddies companies. The melting points were determined in open capillaries and are uncorrected. The functional groups present in the synthesized analogues were identified from Perkin-Elmer FT-IR spectrophotometer in the 4000-400 cm^{-1} range using KBr method. Bruker ^1H NMR spectra (400 MHz) was used to estimate the various protons present in synthesized analogues from the chemical shifts using TMS as internal standard. Shimadzu mass spectrometer was employed to find the weight of the analogue. Perkin Elmer analyzer (2400 CHN) was utilized to confirm the atoms in the analogues.

Synthesis of indole-based Mannich base derivatives (M1-M5): Indole (0.1 mol), furfuraldehyde (0.1 mol) and substituted amine (0.1 mol) were mixed in 30 mL of ethanol. The mixture was refluxed for 5 h and left overnight at room temperature [17,27]. The product obtained was filtered, washed with distilled water and recrystallized from ethanol (**Scheme-I**).

1-(Furan-2-yl)-(1*H*-indol-3yl)-*N*,*N*-dimethylmethanamine (M1): Yield: 87%; m.p.: 365-368 ºC; colour: white crystalline powder; FT-IR (KBr, ν_{max}, cm^{-1}): 3042 (*str.*, Ar-H), 1627 (*str.*, Ar-C=C), 1057 (Ar-C-O), 2865 (Ar-CH), 3326 (NH), 1138 (C-N); ^1H NMR (100 MHz, $CDCl_3$, δ ppm): 2.34 (s, 6H, CH_3-proton), 5.62 (s, 1H, CH-proton), 6.45 (d, 1H, *J* = 6.8 Hz, Ar-proton), 6.81 (t, 2H, *J* = 9.4 Hz, Ar-H), 7.12-7.30 (m, 1H, Ar-H), 7.24 (s, 2H, Ar-H), 7.32-7.43 (m, 1H, Ar-H), 7.62-7.73 (m, 2H, Ar-H), 10.28 (s, 1H, NH-H); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 43.6 (C-14,15), 77.5 (C-13), 106.5 (C-12), 110.5 (C-11), 111.2 (C-6), 112.7 (C-2), 118.1 (C-3), 119.5, (C-4), 122.5 (C-5), 122.8 (C-1), 127.2 (C-7), 136.9 (C-8), 142.6, (C-9), 152.3 (C-10); MS (*m/z*, %): 240 (35) [M^+], 172 (42), 129 (100), 91 (28), 66 (33), 44 (37), 38 (25); Anal. calcd. (found) % for $C_{15}H_{16}N_2O$ (m.w.: 240): C, 74.97 (74.26); H, 6.71 (6.10); N, 11.66 (11.29), O, 6.66 (6.73).

***N*-(Furan-2-yl(1*H*-indol-3-yl)methyl)-*N*-phenylaniline (M2):** Yield: 92%; m.p.: 423-425 ºC; colour: white powder; FT-IR (KBr, ν_{max}, cm^{-1}): 3056 (*str.*, Ar-H), 1635 (*str.*, Ar-C=C), 1042 (Ar-C-O), 2873 (Ar-CH), 3305 (NH), 1142 (C-N); ^1H NMR (100 MHz, $CDCl_3$, δ ppm): 5.32 (s, 1H, CH-proton), 6.26-6.37 (m, 1H, Ar-proton), 6.41 (t, 1H, *J* = 7.4 Hz, Ar-proton), 6.76 (t, 2H, *J* = 10.8 Hz, Ar-H), 7.18-7.35 (m, 4H, Ar-H), 7.40-7.49 (m, 3H, Ar-H), 7.55-7.62 (m, 4H, Ar-H), 7.71-7.79 (m, 3H, Ar-H), 10.35 (s, 1H, NH-proton); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 77.4 (C-9), 106.2 (C-10), 110.1 (C-11), 112.4 (C-12), 118.6 (C-4), 119.7 (C-15,16,18,20), 121.3 (C-6), 123.3 (C-1) 127.4 (C-3), 129.3 (C-17,19,21,22)), 136.2 (C-8), 139.6 (C-5,7), 136.2 (C-13,14), 142.8 (C-2), 149.5 (C-23,24), 152.6, (C-25); MS (*m/z*, %): 364 (54) [M^+], 196 (100), 129 (37), 91 (28), 66 (22), 40 (39), 38 (26); Anal. calcd. (found) % for $C_{25}H_{20}N_2O$ (m.w. 364): C, 82.39 (81.75); H, 5.53 (5.71); N, 7.69 (7.98), O, 4.39 (4.18).

3-(Furan-2-yl(piperidin-1-yl)methyl)-1*H*-indole (M3): Yield: 76%; m.p.: 278-280 ºC; colour: pale yellow; FT-IR (KBr, ν_{max}, cm^{-1}): 3217 (*str.*, piperidine N), 3024 (*str.*, Ar-H), 1652 (*str.*, Ar-C=C), 1047 (Ar-C-O), 2856 (Ar-CH), 3374 (NH), 1153 (C-N); ^1H NMR (100 MHz, $CDCl_3$, δ ppm): 1.32-1.45 (m, 6H, CH_2-proton), 2.35-2.56 (m, 2H, CH_2-proton), 5.41 (s, 1H, CH-proton), 6.23-6.38 (m, 1H, Ar-proton), 6.43-6.51 (m, 2H, Ar-proton), 7.13-7.29 (m, 3H, Ar-H), 7.35-7.45 (m, 2H, Ar-H), 7.61-7.76 (m, 2H, Ar-H), 10.15 (s, 1H, NH-proton); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 24.5 (C-16,17), 25.1 (C-18), 57.3 (C-14,15), 72.8 (C-8), 106.9 (C-10), 110.6 (C-11), 111.5 (C-12), 112.7 (C-13) 118.2 (C-4), 119.4 (C-5), 121.7 (C-2), 123.8 (C-9), 126.9 (C-3), 136.5 (C-6), 142.3 (C-7), 152.8 (C-1); MS (*m/z*, %): 280 (27) [M^+], 238 (35), 196 (100), 116 (24), 91 (32), 78 (35), 42 (42), 26 (18); Anal. calcd. (found) % for $C_{18}H_{20}N_2O$ (m.w. 280): C, 77.11 (76.83); H, 7.19 (7.25); N, 9.99 (10.25), O, 5.71 (5.28).

3-(Furan-2-yl(piperazin-1-yl)methyl)-1*H*-indole (M4): Yield: 82%; m.p.: 294-296 ºC; colour: light brown; FT-IR (KBr, ν_{max}, cm^{-1}): 3257 (*str.*, piprazine N), 3172 (*str.*, Ar-H), 1681 (*str.*, Ar-C=C), 1098 (Ar-C-O), 2904 (Ar-CH), 3301 (NH), 1163 (C-N); ^1H NMR (100 MHz, $CDCl_3$, δ ppm): 2.02 (d, 1H, NH-proton), 2.81-3.06 (m, 8H, CH_2-H): 5.62 (s, 1H, CH-H), 6.31 (d, *J* = 9.8 Hz, 1H, Ar-proton), 6.48 (t, 2H, *J* = 5.6 Hz, Ar-proton), 7.20-7.34 (m, 3H, Ar-H), 7.41-7.49 (m, 1H, Ar-H), 7.63-7.70 (m, 2H, Ar-H), 10.22 (s, 1H, NH-proton); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 45.8 (C-12,13), 52.6 (C-14,15), 72.8 (C-9), 106.4 (C-10), 110.2 (C-11), 111.3 (C-6), 112.6 (C-2), 118.5 (C-3), 119.7 (C-4), 121.5 (C-5), 123.6 (C-1), 127.9 (C-7), 136.5 (C-8), 142.8 (C-16), 152.1 (C-17); MS (*m/z*, %): 281 (36) [M^+], 238 (100), 196 (18), 129 (42), 116 (38), 91 (24), 53 (42), 49 (36); Anal. calcd. (found) % for $C_{17}H_{19}N_3O$ (m.w. 281): C, 72.52 (71.89); H, 6.79 (6.35); N, 14.89 (14.05), O, 5.69 (5.61).

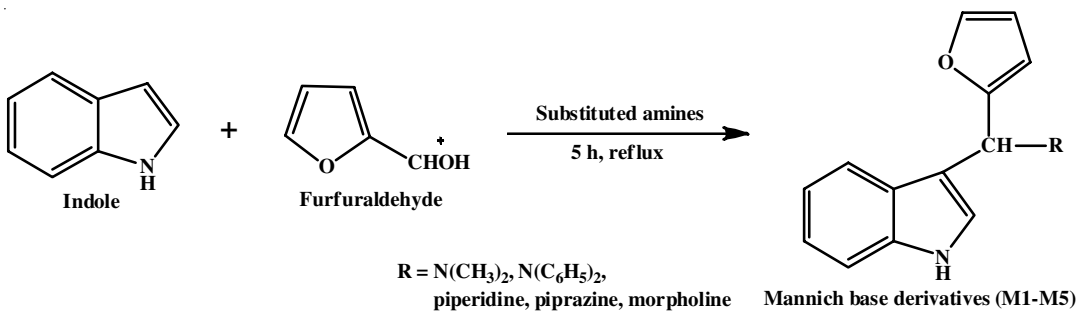

Fig. 1. Synthetic protocol for various potent indole-based Mannich base derivatives (**M1-M5**)

4-(Furan-2-yl(1*H*-indol-3-yl)methyl)morpholine (M5): Yield: 83%; m.p.: 317-319 ºC; colour: creamy white powder; FT-IR (KBr, ν_{max}, cm^{-1}): 3348 (*str.*, morpholine N), 3193 (*str.*, Ar-H), 1627 (*str.*, Ar-C=C), 1023 (Ar-C-O), 2958 (Ar-CH), 3364 (NH), 1147 (C-N); ^{1}H NMR (100 MHz, $CDCl_3$, δ ppm): 2.43-2.52 (m, 4H, CH_2-proton), 3.83-3.91 (m, 4H, CH_2-proton), 5.52 (s, 1H, CH-proton), 6.29 (d, *J* = 8.4 Hz, 1H, Ar-H), 6.51 (t, 2H, *J* = 10.8 Hz, Ar-H), 7.15-7.26 (m, 3H, Ar-H), 7.44-7.50 (m, 1H, Ar-H), 7.87-7.95 (m, 2H, Ar-H), 10.09 (s, 1H, NH-proton); ^{13}C NMR (100 MHz, DMSO-d_6, δ ppm): 50.4 (C-12,13), 66.1 (C-14,15), 73.4 (C-9), 106.6 (C-10), 109.8 (C-11), 111.7 (C-6), 113.5 (C-2), 117.7 (C-3), 119.2 (C-4), 122.6 (C-5), 125.4 (C-1), 128.3 (C-7), 136.4 (C-8), 143.7 (C-16), 152.6 (C-17); MS (*m/z*, %): 282 (100) [M^+], 223 (28), 197 (38), 105 (45), 91 (31), 66 (48), 58 (37), 26 (24); Anal. calcd. (found) % for $C_{17}H_{18}N_2O$ (m.w. 282): C, 72.32 (72.18); H, 6.43 (6.17); N, 9.92 (9.08), O, 11.33 (10.92).

***In silico* docking study:** *In silico* screening has a substantial role in drug discovery and development. It rapidly searches for appropriate drug molecules to bind to the targeted receptor/protein. Herein, we conducted the docking study using Autodock Vena software [28]. The crystal structures of *Staphylococcus aureus* DNA gyrase B, cyclooxygenase 2 and cytochrome c peroxidase were taken from the protein data bank. A set of 5 different ligands 2D structures was drawn in ChemDraw and later transformed to the 3D structure. The perfect conformation of the ligand analogue was selected and used to work out the force of the bond between the ligand and *S. aureus* DNA gyrase B, cyclooxygenase 2 and cytochrome C peroxidase. All the novel indole-based Mannich base derivatives are indispensable components for binding with the mentioned drug target.

Biological activities

***In vitro* antimicrobial activity:** To carry out the antibacterial activity, the disc diffusion assay was used. After the addition of 25 mL of PDA/NA media to the Petri dishes, the microbe was transferred to a plate of solidified agar, spread out and allowed to dry for 10 min. The medium surfaces were injected with bacteria from a NA culture. A sterile cotton swab was used to evenly inoculate the whole surface of the NA/PDA plates with a standardized microbiological test suspension. Microbes were injected onto the NA/PDA plates with the help of sterile forceps and the sterile filter sheets contained different concentrations of 50, 100 and 150 µL of indole-based Mannich base derivatives, a standard solution comprising 30 µL of chloramphenicol, fluconazole and a solvent mixture [29].

In vitro anti-inflammatory activity

Albumin denaturation assay: A 5 mL of mixture containing 2 mL of different concentrations of indole-Mannich base derivatives (100, 200, 300, 400 and 500 µg/mL), 2.8 mL of phosphate-buffered (pH 6.4) and clean egg albumin (0.2 mL) was heated to 70 ºC for 5 min after being incubated for 15 min at 37 ± 2 ºC. The absorbance was measured at 660 nm, with a vehicle serving as the blank. To determine the absorbance, 500 µg/mL diclofenac was utilized as a reference [30,31]. The percentage of albumin denatured was determined by using the following equation:

$$\text{Inhibition } (\%) = \frac{A_t - A_c}{A_c} \times 100$$

where A_t = absorbance of sample, A_c = absorbance of control.

Assay of membrane stabilizing activity

Preparation of RBC suspension: Prior to the experiment, a healthy volunteer who had not used any drugs for 14 days had their blood drawn. An equivalent quantity of sterilized Alsevers solution was then added to the blood. The crowded cells in this blood solution were isolated after centrifuging it at 3000 rpm. After making a 10% v/v suspension with isosaline, the packed cells were cleaned using an isosaline solution. The anti-inflammatory property was predicted using this HRBC suspension [32,33].

***In vitro* antioxidant activity:** To evaluate the antioxidant activity, the novel indole-based Mannich base derivatives were dissolved into methanol (100 µg/mL). At the same time, a solution of DPPH was also prepared in another beaker with methanol (100 µg/mL). Each indole-based Mannich base derivative (4 mL) was added to 4 mL of DPPH solution and kept aside for 30 min at room temperature and then absorbance was screened at 517 nm using a Shimadzu ultraviolet spectrometer [34]. Standard and blank absorbances were also calibrated. The antioxidant activity of indole-based Mannich base analogues was estimated using the following equation:

$$\text{Antioxidant activity} = \frac{A_{blank} - A_{test}}{A_{blank}} \times 100$$

RESULTS AND DISCUSSION

Indole-based Mannich base derivatives (**M1-M5**) were synthesized by condensation reaction between phenol/malonic acid/acetophenone, guanidine and formaldehyde/furfuraldehyde. In FT-IR spectral analysis, the peaks exhibited the characteristic peaks of both indole as well as Mannich base in the form of the morphonline proton, aromatic C-C, C=C, C-O, CH bond, NH bond and C-N bond present in the titled compounds. The C-H aromatic stretching peak was observed at 3193-3045, 2958-2856 and 1681-1627 cm^{-1}. The peak at 3374-3301 cm^{-1} is due to the presence of amine proton (NH) group present in the compounds. An intensive absorption peak at 1163-1138 cm^{-1} indicated the existence of C-N stretching absorptions. A peak at 3348 cm^{-1} appears due to the presence of the heterocyclic morpholine NH group. The number of protons that exists in the synthetic compounds was calculated by ^{1}H NMR spectroscopy. A singlet at δ 5.32-5.62 ppm corresponds to CH-H, whereas a singlet and multiplet at δ 2.81-3.91 ppm are related to CH_2-H. Similarly, a singlet at δ 2.34 ppm is related to CH_3-H and a doublet at δ 2.20 ppm related to N-H, In aromaticity, a singlet at δ 7.23 ppm related to aromatic proton (Ar-H); a doublet at δ 6.29-6.45 ppm related to Ar-proton; a triplet at δ 6.41-6.81 ppm relating to Ar-proton; a multiplet at δ 6.23-7.95 ppm relating to Ar-proton; a singlet at δ 10.09-10.35 ppm relating to -NH proton.

***In silico* docking method:** Indole-based Mannich base derivatives (**M1-M5**) therapeutic activities have been assessed from Autodock score after the successful binding with *S. aureus* DNA gyrase B and cyclooxygenase 2. The effective indole-Mannich base derivatives were docked to a hydrophilic and hydrophobic centre of the *S. aureus* DNA gyrase B and cyclooxygenase 2 and exhibited excellent scores. All the indole-Mannich base derivative (**M1-M5**) had revealed an excellent affinity towards the target due to the development of strong hydrophobic and hydrophobic connections with the heterocyclic ring structures such as piperidine and morpholine. The synthesized compounds **M3** and **M5** showed a better binding nature like a standard drugs. The overall declining order of synthesized indole-Mannich base derivatives against *S. aureus* DNA gyrase B was found to be **M2 > M5 > M3 > M4 > M1**, whereas the overall decreasing order against cyclooxygenase 2 was found to be **M3 > M2 > M5 > M1 > M4** (Table-1). The interaction of each Mannich base derivative with the targets is shown in Fig. 1.

Antimicrobial activity: The antimicrobial activity results reveal that the synthesized indole-Mannich bases at different concentrations of 50, 100 and 150 µg/mL have shown significant activity against *B. subtilis*, *E. coli*, *A. flavus* and *A. niger* with the greater zone of inhibition. Particularly at high concentration of 150 µg/mL of **M2** and **M5**, the synthesized indole-Mannich base derivatives exhibited highest zone of inhibition against *B. subtilis* (9.78 ± 0.41, 8.59 ± 0.18), *E. coli* (9.46 ± 0.46, 8.39 ± 0.75), *A. niger* (7.13 ± 0.68, 7.02 ± 0.29) and *A. flavus* (8.82 ± 0.36, 8.16 ± 0.18). These values were compared to standard drugs like chloramphenicol (12.65 ± 0.54, 11.85 ± 0.31) and fluconazole (9.50 ± 0.78, 10.55 ± 0.26). *In vitro* antimicrobial results of different concentrations of indole-Mannich base derivatives are summarized in Table-2.

TABLE-1
BINDING AFFINITY OF INDOLE-BASED MANNICH BASE AGAINST *Staphylococcus aureus* DNA gyrase B AND CYCLOOXYGENASE 2 PROTEIN

Compound	*Staphylococcus aureus* DNA gyrase B protein	Cyclooxygenase 2 protein
M1	-5.6	-7.1
M2	-8.1	-7.8
M3	-6.8	-9.4
M4	-6.3	-6.1
M5	-6.9	-7.4
Chloramphenicol	-9.7	–
Diclofenac sodium	–	-9.8

Egg albumin assay: The effect of synthesized indole-Mannich base derivatives (**M1-M5**) was analyzed against the level of denaturation of egg albumin. Diclofenac sodium drug was employed as a standard drug. Two of the indole-Mannich base analogues (**M3**, **M2**) exhibited different inhibition levels of egg albumin such as 18.37 ± 1.32, 15.28 ± 1.17 against 20.50 ± 1.21 for diclofenac sodium at 100 µL/mL. The highest percentage of inhibition 86.29 ± 1.34, 82.94 ± 1.06 were exhibited in compounds **M5** and **M4** as against 92.03 ± 1.06 for diclofenac sodium at 500 µL/mL. The half inhibition concentration of **M3**

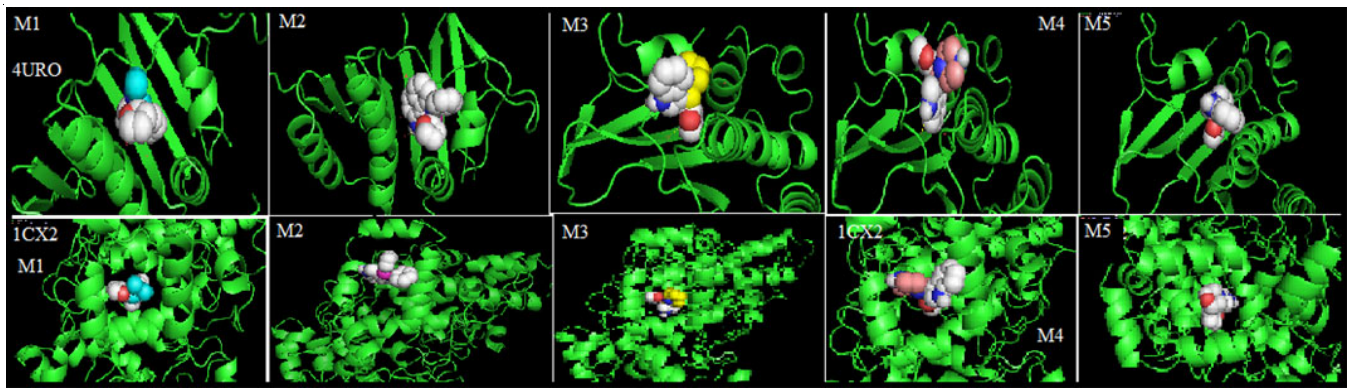

Fig. 1. Docking images of indole-based Mannich base with *Staphylococcus aureus* DNA gyrase B and cyclooxygenase 2 protein

TABLE-2
ANTIMICROBIAL ACTIVITY OF SYNTHESIZED MANNICH BASE DERIVATIVES (**M1-M5**)

Compound	*Bacillus subtilis* (mm)			*Escherichia coli* (mm)			*Aspergillus niger* (mm)			*Aspergillus flavus* (mm)		
	50 µg/mL	100 µg/mL	150 µg/mL	50 µg/mL	100 µg/mL	150 µg/mL	50 µg/mL	100 µg/mL	150 µg/mL	50 µg/mL	100 µg/mL	150 µg/mL
M1	1.98	5.92	6.18	2.34	5.98	6.65	1.02	4.17	6.28	1.09	3.27	6.64
M2	3.58	7.62	9.78	3.42	7.31	9.46	1.89	5.04	7.13	1.63	5.74	8.82
M3	2.83	6.58	7.83	2.81	6.37	7.43	1.14	4.59	6.72	1.21	3.84	7.76
M4	2.05	6.12	6.38	2.56	6.04	6.93	1.07	4.23	6.35	1.16	3.35	6.83
M5	3.41	7.54	8.59	3.27	7.14	8.39	1.74	4.93	7.02	1.45	4.92	8.16
Chloramphenicol (30 µL)		12.65			11.85			–			–	
Fluconazole (30 µL)		–			–			10.55			9.50	
Control (30 µL)		3.45			3.10			1.50			1.30	

Standard: Chloramphenicol for bacteria, fluconazole for fungi; control as DMSO.

and **M2** compounds was found to be 275.34 µg/mL, 286.74 µg/mL and standard was 252.29 µg/mL (Table-3).

TABLE-3
EFFECT OF **M2** AND **M3** ON EGG ALBUMIN DENATURATION

Concentrations (µg/mL)	Inhibition (%)		
	M2	**M3**	Std. (diclofenac)
100	15.28 ± 1.17	18.37 ± 1.32	20.50 ± 1.21
200	30.65 ± 1.51	33.28 ± 1.39	39.82 ± 1.17
300	47.73 ± 1.36	51.06 ± 1.28	60.91 ± 1.09
400	71.21 ± 1.46	74.35 ± 1.32	80.53 ± 1.15
500	82.94 ± 1.06	86.29 ± 1.34	92.03 ± 1.06
IC_{50} (µg/mL)	286.74	275.34	252.29

Values were expressed as mean ± Standard deviation for triplicates; IC_{50}: Half inhibitions concentration

HRBC membrane stabilization activity: The HRBC membrane stabilizing activity of indole-Mannich base derivatives was calculated against RBC haemolysis. The lowest percentage of inhibition 17.36 ± 0.28 and 15.65 ± 1.08 was observed in compounds **M3** and **M2** as against 20.16 ± 1.11 in diclofenac sodium at 100 µL/mL. The highest percentage of inhibitions of 78.29 ± 1.41 and 72.16 ± 1.29 were observed in compounds **M3** and **M2** as against 92.78 ± 1.17 in diclofenac sodium at 500 µL/mL. The half inhibition concentration of **M3** and **M2** was found to be 274.65 µg/mL, 285.42 µg/mL and diclofenac sodium was secured 259.31 µg/mL (Table-4).

TABLE-4
HRBC MEMBRANE STABILIZATION ACTIVITY OF **M2** AND **M3**

Concentrations (µg/mL)	Stabilization (%)		
	M2	**M3**	Std. (diclofenac)
100	15.65 ± 1.08	17.36 ± 0.28	20.16 ± 1.11
200	29.27 ± 0.36	31.44 ± 1.18	36.51 ± 1.07
300	51.27 ± 1.04	53.51 ± 1.02	60.29 ± 1.28
400	69.25 ± 1.45	73.19 ± 1.25	78.34 ± 1.15
500	72.16 ± 1.29	78.29 ± 1.41	92.78 ± 1.17
SC_{50} (µg/mL)	285.42	274.65	259.31

Values were expressed as mean ± Standard deviation for triplicates; SC_{50}: Stabilization concentration.

Antioxidant activity: Compounds **M2**, **M3** and **M5** had shown significant radical scavenging activities such as 47.010%, 43.586% and 36.625% as against 58.072% in ascorbic acid. The overall declining order of synthesized indole-Mannich base derivatives antioxidant activity was found to be **M2 > M3 > M5 > M4 > M1** (Table-5).

TABLE-5
ANTIOXIDANT ACTIVITY OF INDOLE BASED MANNICH BASE DERIVATIVES

Compound	Absorbance (mean ± standard deviation at 517 nm)	Scavenging (%)
M1	0.624 ± 0.014*	22.381
M2	0.405 ± 0.013*	47.010
M3	0.437 ± 0.012*	43.586
M4	0.562 ± 0.010*	30.074
M5	0.492 ± 0.024*	36.625
Blank	0.927 ± 0.008*	NA
Standard (ascorbic acid)	0.321 ± 0.017*	58.072

Conclusion

Among the synthesized novel Mannich base derivatives, compounds **M2**, **M3** and **M5** exhibited excellent *in vitro* antimicrobial, anti-inflammatory and antioxidant activities due to the presence of phenyl, piperidine and morpholine moiety in indole-Mannich base derivatives, which support the compound to fight better against microorganisms, control the inflammation and free radicals. The *in silico* results also indicated that all the analogues demonstrated admirable activity against *Staphylococcus aureus* DNA gyrase B and cyclooxygenase 2. Therefore, all these analogues acted as potent antimicrobial, anti-inflammatory and antioxidant agents. Further studies of antimicrobial, anti-inflammatory and antioxidant activity are needed to support the *in vitro* study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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