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Synthesis, characterization, antibacterial, antifungal evaluation of novel mannich bases of 2,5-disubstiututed indoles

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ABSTRACT

In an effort to develop potent antimicrobial a series of mannich bases of 2,5-disubstututed indoles were prepared for investigating their antimicrobial activities. All compounds were found to be significantly active against gram positive bacteria: S. aureus, B. subtilus Gram negative bacteria: E.coli, P.aeruginosa and fungal strains: C. albicans, A. niger by tube dilution method. Compound N-((2-(4-bromophenyl)-5-nitro-1H-indol-3-yl)methyl)-N-phenyl benzenamine ***(MB10)*** *and N-benzyl-N-((2-(4-bromophenyl)-5-nitro-1H-indol-3-yl) methyl) (phenyl) methanamine* ***(MB11)*** *emerged as the best antimicrobial agent in the present study. The structure of the synthesized compounds was confirmed by physico-chemical characteristics and spectroscopic investigations. Docking results of these two compounds with COX-2 (PDB ID: 4COX) also exhibited a strong binding profile.*

Key words: mannich bases of 2,5-disubstituted indoles, antimicrobial activity, antifungal activity, minimum inhibitory concentration(MIC)

INTRODUCTION

Antimicrobial chemotherapy has conferred huge benefits on human health. Thereafter, antimicrobial chemotherapy made remarkable advances during the 20th century, resulting in the overly optimistic view that infectious diseases would be conquered in the near future. The first antimicrobial agent in the world was salvarsan was synthesized by Ehrlich in 1910. In 1935, sulphonamides were developed by Domagk. In 1928, Fleming discovered penicillin. During the subsequent two decades, new classes of antimicrobial agents were developed one after another, leading to a golden age of antimicrobial chemotherapy [1]. The development of new antimicrobial agents is needed to counter the increasing number of multi-drug resistant (MDR) strains [2].

Indole derivatives have been reported to possess promising biological activities such as antimicrobial and antifungal agents in several studies [3].

The Mannich reaction has been useful in the preparation of various antimicrobial molecules. Moreover, the antibacterial mannich products displayed much less cytotoxicity, which is a vital requirement for a molecule to be developed as a drug. The presence of mannich side chain increases the water solubility of drug molecule. Hence, the mannich derivatives are easily transported to the site of action and they were found to be more potent than the parent molecule [2]. Based on the fact discussed above we try to design some new mannich bases of indole and evaluate them as potential antimicrobial agents.

MATERIALS AND METHODS

Reactions progress as well as completion was confirmed by single spot of TLC by 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. ^{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).

General procedure for the synthesis of Substituted 2-Phenylindole (4)

In 250 ml round bottom flask 9 g (0.08mol) phenylhydrazine was taken in 30 ml of ethanol. 10g (0.08mol) of substituted acetophenone was added to it, followed by the addition of few drops of glacial acetic acid. Refluxed the mixture for 2-3 hours, after completion reaction mixture was cooled, the solid was washed with 0.1N (10ml) hydrochloric acid followed by 12ml cold rectified spirit.

Placed 16g of the crude phenylhydrazone in a 250-ml beaker containing of 90 g polyphosphoric acid. Heated on a boiling water bath with continuous stirring and maintaining 100-120°C for 10 minutes (the reaction is exothermic). Further added 260 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 water. Further, 175 ml of rectified spirit was added to the crude solid and heated till the solid get dissolved. Added a little decolourising charcoal, filtered and washed with hot rectified spirit. The filterate was cooled to room temperature and white crystals were precipitated out. Dried and stirred in a vacuum desiccator over anhydrous calcium chloride.

General procedure for the synthesis 2, 5-disubstituted indoles (5)

12 ml of concentrated nitric acid 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 product got precipitated out filtered and washed with ice cold water. The yellowish brown product was obtained. The synthesis was confirmed by single spot TLC.

General procedure for Mannich base of 2, 5-disubstituted indoles (6)

To a 12 ml of acetic acid were successively added different secondary amine (0.01 mol), 37.7% solution of formalin (0.01 mol), and 2,5-disubstituted indole (0.004 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.

Spectral data

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).

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-((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).

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).

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-).

Antibacterial activity

Tube dilution method was used for the evaluation of antimicrobial activity. This method depends upon the inhibition of growth of a microbial culture in an uniform solution of antibiotic in a fluid medium that is favorable for its rapid growth in the absence of the antibiotic [4, 5, 6]. Minimum inhibitory concentration (MIC) – The MIC is the lowest concentration of the antibiotic that completely inhibits growth of the specific organism being tested [7]. Ciprofloxacin and Fluconazole are the standard drugs for antibacterial and antifungal activity evaluation respectively.

Antibacterial activity of the synthesized derivatives was tested *in vitro* against Gram positive *Bacillus subtilis, Staphylococcus aureus* and Gram negative *Escerichia coli, Pseudomonas aeruginosa* by serial dilution method using double strength nutrient broth I.P [8].Inoculated tubes were incubated at 37 ± 1^0C for 24hrs for the different bacterial strains used and MIC was observed.

Preparation of standard and test

1000 µg/ml stock solution of standard (Ciprofloxacin) and the test compounds were prepared in DMSO.

Determination of minimum inhibitory concentration

1mL of sterilized media was poured into sterilized test tubes. 1mL of 100µg/ml test solution was transferred in one tube and serially diluted to give a concentration of 50, 25, 12.5, 6.25, 3.12, 1.56µg/ml. To all the test tubes 0.1 ml of suspension of bacteria in saline was added and the tubes were incubated at 37 ± 1^0C for 24hrs (*B. subtilis, S. aureus, P. aeruginos)* and 48hrs (*E. coli*). The growth in the tubes was observed visually for turbidity. MIC was determined by the lowest concentration of the sample that prevented the development of turbidity.

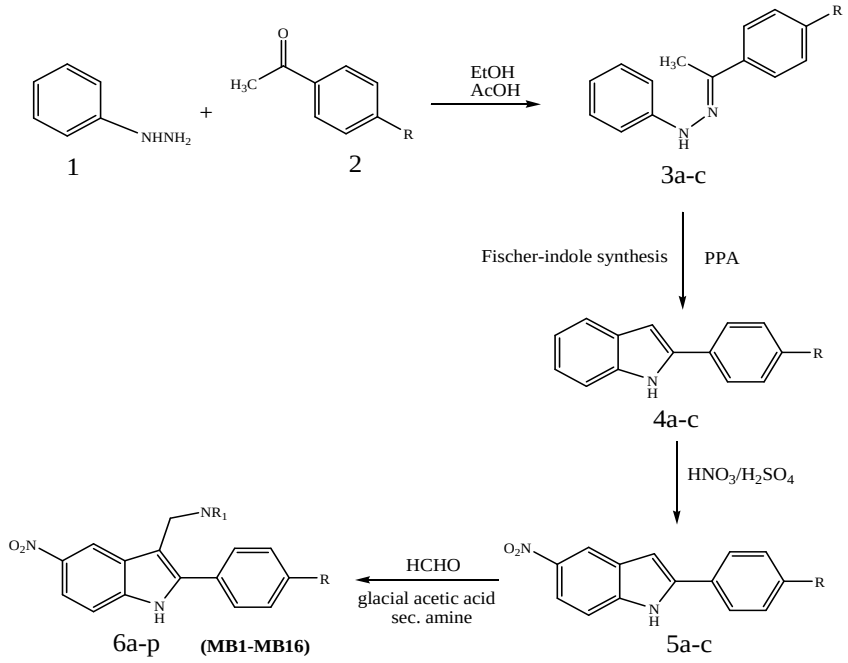
Antifungal activity

Antifungal activity of the synthesized compounds was evaluated against *Candida albicans* & *Aspergillus niger* was performed by serial dilution method similar to antibacterial assay using Sabouraud's dextrose broth I.P [8]. The tubes were incubated at 37 ± 1^0C for 2 days (*Candida albicans*) and 25±1°C for 7 days for (*Aspergillus niger)* respectively.

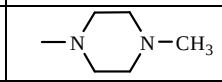
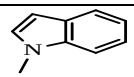
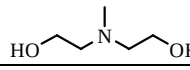
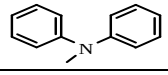
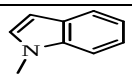
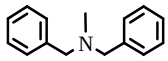
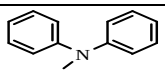
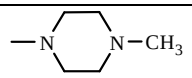
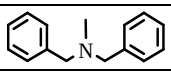
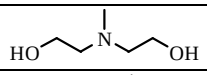
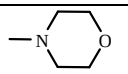
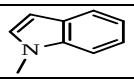
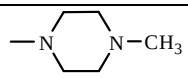
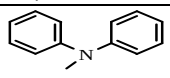
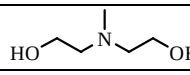
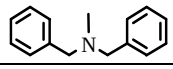
RESULTS AND DISCUSSION

Chemistry

The aim of this study is to investigate presence of antimicrobial activities of sixteen new mannich bases of 2,5-disubstituted indoles (MB1-16). They were synthesized and the reaction sequence for the synthesis is outlined in Scheme 1. The Mannich bases of 2,5-disubstituted indoles (6a-p) were 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 as shown in Scheme 1. Accordingly, refluxing an ethanolic mixture of phenylhydrazine 1 and an appropriate para-substituted acetophenone 2 on a water bath in presence of few amounts of acetic acid gave substituted acetophenone phenylhydrazone 3a-c. Cyclization of the hydrazone by polyphosphoric acid at 120°C afforded the 2-substituted indole. The required intermediates 2,5-disubstituted indole compounds (5a-c) was obtained from nitration of 2-substituted indole (4a-c) in presence of equal amount of concentrated sulphuric acid and concentrated nitric acid. TLC was performed throughout the reactions to optimize the reaction for purity and completion. The physicochemical parameters of all the synthesized compounds are summarized in Table 1.



Scheme 1. General synthetic scheme for the synthesis of 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	$-OCH_3$	

Spectral analysis

The IR band in range 3502-3107cm^{-1} corresponds to the presence of N-H stretch of indole ring. The appearance of peak between 3107-3024cm^{-1} region indicated the presence of aromatic ring of the synthesized compounds along with C=C aromatic stretch between 1649-1597cm^{-1} which further confirmed the presence of aromatic region of the synthesized derivatives. The presence of IR peak in the region of 1550-1504 and 1346-1311cm^{-1} indicated the presence of nitro group (NO_2). The IR peak value 1076-1022cm^{-1} in **MB6-11** indicated the bromine substitution on aromatic ring. The IR band in range 1286-1257cm^{-1} corresponds to the presence of C-O-C group in **MB12-16** derivatives. The appearance of IR band in the range 1227-1217cm^{-1} demonstrated the C-N stretch of indole ring. The IR band between 1473-1427cm^{-1} region indicated the presence of methyene group which confirmed the formation of mannich bases.

All ^{1}HNMR spectra of the synthesized compounds showed multiplet from δ 6.76-8.45 and two doublet between δ 7.41-7.85 (C_6H_4) for aromatic proton. Formations of compounds **[MB1-MB16]** were confirmed by appearance of

singlet between δ 3.32-3.78 for CH_2 group connecting indole and secondary amine through mannich reaction. The singlet between δ 4.03 & 4.29 appearing in the spectra indicated the proton of OCH_3.

Evaluation of antimicrobial activity

The synthesized compounds were evaluated for their antimicrobial activity against Gram positive *Bacillus subtilis (*MTCC 2063*), Staphylococcus aureus* (MTCC 3160) and Gram negative *Escerichia coli* (MTCC 40) and *Pseudomonas aeruginosa* (MTCC 425) and antifungal activity against fungal strain *Candida albicans* (MTCC 183) & *Aspergillus niger* (MTCC 281) by *in vitro* serial dilution method. The results (pMIC value) obtained by antimicrobial evaluation are shown in the Table 2.

All compounds were found to be significantly active. In case of *Bacillus subtilis*, result showed that six compounds **MB2, MB5, MB6, MB8, MB10** and **MB11** were found to be more active compounds than the standard drug. While two compounds **MB7, MB12** showed similar activity to standard drug and considered to have good antibacterial activity. Five compounds **MB3, MB4, MB9**, **MB13, MB14** exihibited moderate activity In case of antifungal activity eight compounds **MB4, MB5, MB6, MB7, MB8, MB10, MB11** and **MB12** showed excellent activity against *Candida albicans*. Also it was observed that Compound **MB10** and **MB11** were the most active compounds against all tested strains.

Table 2 Antimicrobial activity of synthesized mannich bases of 2,5-disubstituted indoles

Comp. code	pMIC (µM/ml)					
	B.Substilis	***S. aureus***	***E. coli***	***P.aeruginosa***	***A.niger***	***C.albicans***
MB1	1.46	1.46	1.46	1.46	1.46	1.77
MB2	**2.37**	1.47	1.47	1.47	1.47	1.77
MB3	1.79	1.48	1.48	1.48	1.48	1.48
MB4	1.84	1.54	1.54	1.54	1.54	**2.44**
MB5	**2.47**	1.57	1.57	1.57	1.57	**2.47**
MB6	**2.43**	1.52	1.52	1.52	1.52	**2.43**
MB7	**2.14**	1.54	1.53	1.53	1.53	**2.44**
MB8	**2.44**	1.54	1.54	1.54	1.54	**2.14**
MB9	1.85	1.55	1.55	1.55	1.55	1.55
MB10	**2.50**	**1.60**	**1.60**	**1.60**	**1.60**	**2.50**
MB11	**2.53**	**1.62**	**1.62**	**1.62**	**1.62**	**2.53**
MB12	**2.09**	1.48	1.48	1.48	1.48	**2.09**
MB13	1.79	1.49	1.49	1.49	1.49	1.49
MB14	1.80	1.50	1.50	1.50	1.50	1.50
MB15	1.86	1.56	1.56	1.56	1.56	1.86
MB16	1.58	1.58	1.59	1.59	1.59	1.58
Std.	2.33*	2.33*	2.33*	2.33*	1.99**	1.99**

**Ciprofloxacin ** Fluconazole*

From the result of antimicrobial study, following SAR has been derived.

1. Compounds **MB10, MB11** were found to be more active than the standard drug against all the bacterial as well as fungal strains. Further, it was observed, that these compounds have shown excellent activity against *B. substilis* (bacterial strain) and *C. albicans* (fungal strain). This may be attributed towards the presence of lipophilic group i.e dibenzylamine and diphenylamine substitutent at 3-position of indole ring [9].
2. Presence of aliphatic alcohol substituted at position 3 of indole ring **(MB2, Mb8, M13)** were found to be more active than alicyclic substitution against microbial strains. This may be due to the formation of hydrogen bonding by substituent groups which may further be essential for efficient binding at the receptor site.
3. Compounds with electron withdrawing groups i.e *p*-Br and *p*-OCH_3 at 2-phenyl ring of indole were found to possess potent antimicrobial activity, which may be again be responsible for effective binding at receptor site.
4. Substitution with nitro group at position 5 of indole ring is found to be responsible for the antimicrobial activity of almost all the compounds. This fact is supported by the result of Joseph *et al* [10].

From the above discussion made, it can be summarized that all compounds were found to be most effective against *B. substilis* among all the tested strains. In case of fungal strain compounds found to be moe effective against *C. albicans.* Compounds **MB10, MB11** emerged as most potent antimicrobial agent. The aromatic substitution at 3-position, electron withdrawing group at para position of 2-phenyl ring and nitro group at 5-position of indole ring increase the antimicrobial activity. The methylene group which link the indole moiety to secondary amine may be essential for effective binding of compounds to the target. Thus present study shows that different structural requirements are essential for effective antimicrobial activity.

CONCLUSION

Various derivatives of 2,5-disubstituted indole were synthesized and screened for their antimicrobial activities. As per the result of the present study, it could be concluded that, compound N-((2-(4-bromophenyl)-5-nitro-1H-indol-3-yl)methyl)-N-phenyl benzenamine (**MB10)** and N-benzyl-N-((2-(4-bromophenyl)-5-nitro-1H-indol-3-yl) methyl) (phenyl) methanamine (**MB11)** possess a significant *in vitro* antimicrobial activity. Aromatic substitution at 3-position of indole ring increases the antimicrobial activity. Compounds showed excellent activity against *B. subtilis* among all the bacterial strains. In case of fungal strain compounds found to be more effective against *C. albicans*. The observations provide newer insights for designing novel and potential antimicrobial agents by taking 3-indole as an active pharmacophore.

REFERENCES

[1] T Saga ; K Yamaguchi. *JMAJ*, **2009,** 52, 103–10
[2] Subraamaniapillai SG. *J Chem Sci*, **2013,** 125, 467–482
[3] S Olgena ; N Altanlarb ; E Karataylıc ; M Bozday. Z *Naturforsch*, **2008,** 63, 189-195
[4] AA Bakr ; MA Hussein ; SG Abdel-Moty ; AHN Kafafy ; MM Hamdy. *Bull Pharm Sci*, **2006,** 29, 348-370
[5] D Yuksek ; O Algul ; A Dogen ; O Tari ; E Kucuk ; JF Otag ; MS Serin. *African Journal of Microbiology Research*, **2013,** 7, 1708-1715
[6] WJ Brown ; Sautter. RL Five-Hour Minimal Inhibitory Concentration Test of Four, **1977,** 11, 1064-1066
[7] UK Singh ; SN Pandeya ; A Singh ; BK Srivastava ; Pandey M. *International Journal of Pharmaceutical Sciences and Drug Research*, **2010,** 2, 151-154
[8] Indian pharmacopoeia.Published by the The Indian Pharmacopoeia Commission; Government of india, Ministry of Health and Family Welfare, **2007**, 1, 45-55
[9] AC Bajji; KP Channabasavraj; KMK Swamy, *Indian Drugs*, **1994**, 31:269-272
[10] A Dandia; V Sehgal; P Singh, *Indian J Chem*, **1993**, 32:1288-1291.