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Design, synthesis and biological evaluation of indole and N-benzylated indole Mannich bases as potent antitubercular agents

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Though effective medicines have been available for treating drug-susceptible tuberculosis infections, the chances go from bleak to null as we move from multidrug resistant (MDR) to extremely drug resistant (XDR) or totally drug resistant (TDR) disease. Coordinated efforts made by international community have resulted in the identification of a few important anti TB agents like BM212, SQ109, AZD5847 and Sutezolid which are in the late phases of clinical trials. After Rifampicin, only two drugs Bedaquiline (2013) and Delaminid (2014) have been approved for the treatment of tuberculosis. This clearly shows the need for new leads towards fighting tuberculosis. In our present work, we have synthesized a series of indole and substituted indole Mannich bases designed by using structural features of BM212. Further, these synthesized derivatives have been analyzed by IR, NMR and mass spectral studies and are screened for anti-tubercular and antimicrobial activity. Among these, 23 compounds have shown potent anti TB activity with a MIC ≤3.12 µg/mL against *M. tuberculosis* H37Rv. We report here the synthesis, screening data and SAR studies of indole and substituted indole derivatives as antitubercular agents.

Keywords: BM212 analogs, indole Mannich bases, pharmacophore, *M. tuberculosis* H37Rv, antitubercular

Tuberculosis (TB) is one of the oldest debilitating infectious diseases, which affectedmore than 10 million lives in the year 2018-19 [1]. With sustained efforts of the scientific community, effective implementation of DOTS program and public awareness on sanitation has significantly reduced mortality rate due to TB. But the emergence of multi drug resistance (MDR) and extremely drug resistance (XDR) TB in the last 15 years regained global emergency status to this disease. It is estimated that in 2018-19 alone, over half-a million cases of drug resistant TB were reported, in which 78% are resistant to rifampicin. In case of relapsed cases the incidence of drug resistance is close to 82% and rate of fatality is consistently raising in the MDR TB cases[2,3]. An estimated 9.7% of people with MDR-TB have XDR-TB. Treatment of MDR-TB/XDR TB complicates the treatment protocol due to scarcity of effective drugs, drug toxicity and patient non-compliance[4-6]. Bedaquiline (in 2013) and Delamanid (in 2014) are the only two novel drugs approved for treatment of MDR-TB[7]. Hence there remains a pressing requisite to discover a novel, potent and safe anti TB agent.

The 1,5-diarylpyrrole derivative **BM212** was previously shown to be active against multidrug-resistant clinical isolates of *M. tuberculosis*. It was found to be inhibiting the function of the protein MmpL3 involved in the transport of trehalose monomycolate, a critical precursor for mycolic acid synthesis, across cell membrane[8-10]. This molecule is also effective against dormant strains surviving in macrophages. We used the pharmacophoric features of BM212 and designed indole Mannich bases using shape based virtual screening method (Figure 1). Indole Mannich bases are chosen as they are known to possess a wide range of biological activities like antibacterial, antiviral, antifungal, anti-inflammatory analgesic, antitubercular and antidepressant[11,12]. Here we have recently identified potent anti TB activity in indole derivatives using *in silico* screening techniques[13]. Inspired by these results we synthesized a library ofindole Mannich bases in order to optimize anti TB activity and to study comprehensive structure activity relationship. The results of our study are discussed in this manuscript.

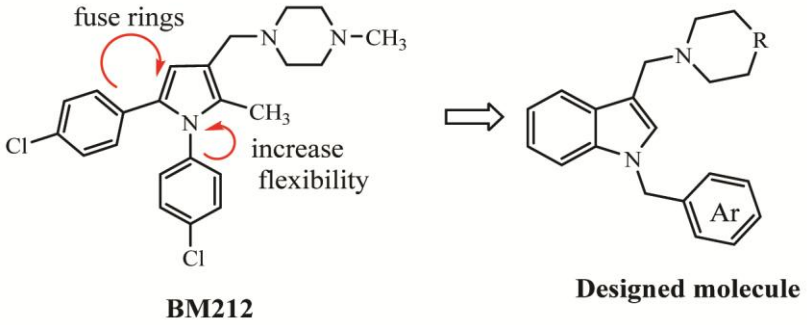
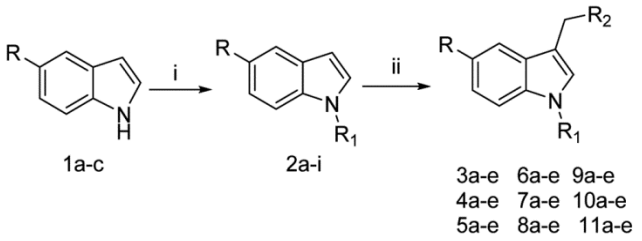


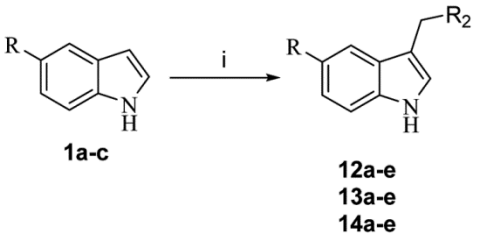
Figure 1 — Design of synthesized compounds from BM212 obtained from vROCS and molecular hybridization technique

Results and Discussion

A total of 60 Mannich bases were synthesized with five secondary amines, three indoles (12a-e to 14a-e series) and nine N-benzylated indoles (3a-e to 11a-e series) using established protocols[14,15]. For the 3a-e to 11a-e series compounds (Scheme I, Table I), an appropriately substituted indole is made to react with respective benzyl chloridesin the presence of KOH as catalyst. The obtained N-benzylindoles were then subjected to Mannich reaction with appropriate secondary amine and glacial acetic acid as catalyst. Here we found that stirring at RT for 3 days gave us relatively pure compounds. To obtain the compounds of the 12a-e to 14a-e series (Scheme II, Table I), the indoles were directly used for Mannich reaction with secondary amines. The compounds were characterized by using spectral analysis including NMR, IR and Mass spectrometry. The secondary amines were selected in order to probe the role of bulk and nucleophilicity of amine group on anti TB activity. Except indole with phenyl piperazine as secondary amine (12e) all the remaining are new to literature. The synthesized compounds were screened for antitubercular and antimicrobial activities.



Scheme I — (i) Benzyl chlorides, DMSO, KOH, (ii) Acetic acid, HCHO, secondary amines



Scheme II — (i) HCl, HCHO, secondary amines

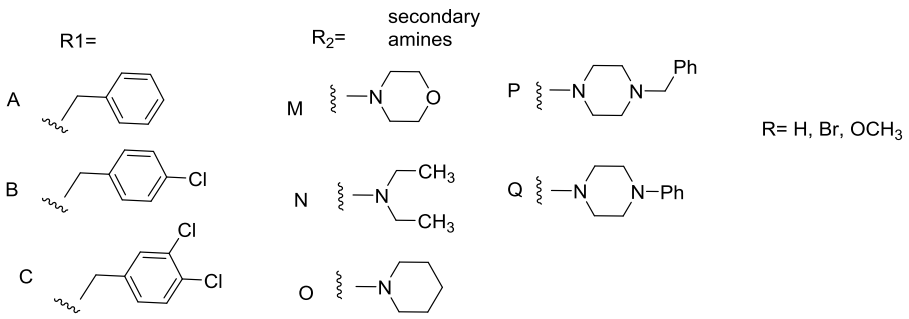
Antimicrobial and tubercular activity

In the antitubercular activity screening of these compounds against *Mycobacterium tuberculosis* H37Rv using MABA method resulted in the identification of potent anti TB activity (MIC 1.6 µg/mL) in 16 compounds (3d, 4b, 4c, 5a, 5b, 5c, 5e, 8a, 8b, 8c, 8e, 11c, 11e, 12e, 13e and 14e). This is highly significant when compared with the standard drugs pyrazinamide (MIC 3.125 µg/mL) and ciprofloxacin (3.125 µg/mL)

Among the indole Mannich bases of the series 12a-12e, 13a-13e and 14a-14e potent bioactivity is observed in the Mannich bases formed from phenylpiperazine. In this series, the substitution at 5th position did not show any significant influence. Order of the secondary amines activity in this case is: Phenylpiperazine > Piperidine > Benzyl piperazine > Diethylamine > Morpholine. This observation specifies that presence or absence of electron withdrawing or electron donating element on indole shows no significant effect over the antitubercular activity.

In the N-benzylindole Mannich bases, except 3d (MIC 1.6 µg/mL) and 3c (MIC 3.125 µg/mL) no other product obtained from unsubstituted benzyl chloride (A) showed activity (MIC) below 25 µg/mL. As observed in the case of 4b and 4c(MIC 1.6µg/mL), introduction of chlorineon to N-benzyl unit significantly improves activity. Further, potent anti TB activity (MIC ≤ 6.25 µg/mL) was observed in the compounds synthesized from 3,4-di Cl benzyl chloride, where 10 off 15 compounds synthesized have shown MIC 1.6 µg/mL.

Table I — Physicochemical constants of the synthesized compounds



Compd	R	R^1	R^2	Yield (%)	m.p. °C	Compd	R	R^1	R^2	Yield (%)	m.p. °C
3a	-H	A	M	60	79-81	**9a**	$-OCH_3$	A	M	75	97-99
3b	-H	A	N	35	88-90	**9b**	$-OCH_3$	A	N	72	108-110
3c	-H	A	O	35	108-110	**9c**	$-OCH_3$	A	O	68	123-125
3d	-H	A	P	30	99-101	**9d**	$-OCH_3$	A	P	63	146-148
3e	-H	A	Q	35	99-101	**9e**	$-OCH_3$	A	Q	70	145-147
4a	-H	B	M	25	124-126	**10a**	$-OCH_3$	B	M	60	92-94
4b	-H	B	N	30	109-111	**10b**	$-OCH_3$	B	N	67	97-99
4c	-H	B	O	20	138-140	**10c**	$-OCH_3$	B	O	58	112-114
4d	-H	B	P	45	157-159	**10d**	$-OCH_3$	B	P	62	122-124
4e	-H	B	Q	65	159-161	**10e**	$-OCH_3$	B	Q	70	126-128
5a	-H	C	M	66	104-106	**11a**	$-OCH_3$	C	M	71	119-120
5b	-H	C	N	69	152-154	**11b**	$-OCH_3$	C	N	74	126-128
5c	-H	C	O	79	138-140	**11c**	$-OCH_3$	C	O	69	119-121
5d	-H	C	P	70	149-150	**11d**	$-OCH_3$	C	P	71	127-129
5e	-H	C	Q	72	144-146	**11e**	$-OCH_3$	C	Q	62	128-130
6a	-Br	A	M	68	86-88	**12a**	-H	-H	M	59	117-119
6b	-Br	A	N	62	99-101	**12b**	-H	-H	N	68	160-162
6c	-Br	A	O	68	111-113	**12c**	-H	-H	O	58	157-159
6d	-Br	A	P	69	146-147	**12d**	-H	-H	P	58	159-161
6e	-Br	A	Q	65	156-158	**12e**	-H	-H	Q	62	167-169
7a	-Br	B	M	75	109-111	**13a**	-Br	-H	M	65	143-145
7b	-Br	B	N	65	115-117	**13b**	-Br	-H	N	33	145-147
7c	-Br	B	O	79	113-115	**13c**	-Br	-H	O	52	Oil
7d	-Br	B	P	70	138-140	**13d**	-Br	-H	P	35	144-146
7e	-Br	B	Q	68	139-141	**13e**	-Br	-H	Q	48	181-183
8a	-Br	C	M	72	109-111	**14a**	$-OCH_3$	-H	M	25	89-91
8b	-Br	C	N	61	129-131	**14b**	$-OCH_3$	-H	N	38	90-92
8c	-Br	C	O	66	136-138	**14c**	$-OCH_3$	-H	O	38	123-125
8d	-Br	C	P	68	145-147	**14d**	$-OCH_3$	-H	P	40	136-138
8e	-Br	C	Q	77	140-142	**14e**	$-OCH_3$	-H	Q	40	138-140

None of the synthesized compounds have very poor antibacterial activity (MIC > 50 µg/mL)against tested bacteria (*Staphylococcus aureus*, and *Escherichiacoli*) and fungi (*Candida albicans* and *Aspergillus niger*) (MIC > 100 µg/mL). Selected compounds (3c, 5e, 6a, 8e, 10d, 11e, 12c, 12e, 13e and 14e) were screened for cytotoxicity activity by MTT assay method against A549 cell line (lung carcinoma). In this,at a concentration of 10 µg/mL5e and 8e had shown percentage inhibition of 86.08 and 81.48 respectively over A549 cell line. 3c and 11e compounds had shown percentage inhibition of 79.08 and 77.16 respectively over the same cell line. From this evidence, it is assumed that the presence of benzylation at the N-1 position increase the Antitubercular activity. The selected potent anti Tb molecules had shown more than 75% of inhibition over the A549 cell line.

In conclusion, our study resulted in identification of a very potent and highly selective anti TB agent and studies its structure activity relations. We also found potent anti-cancer activity in some of the compounds we had synthesized.

Experimental Section

All the chemicals were purchased from Aldrich Chemical Company (USA) and were used without further purification. The reactions were monitored by pre-coated aluminum silica gel TLC plates. Iodine vapors are used as visualizing agents. Melting points (m.p.) were determined using an SRS-EZ Melt automated melting point instrument, and are uncorrected. The IR spectra were recorded on Bruker FT-IR (software - OPUS 6.4) spectrometer using KBr disc method and the values were expressed in cm^{-1}. The ^1H NMR spectra of the compounds were recorded in DMSO-d_6 or $CDCl_3$ with Bruker AVANCE 400 MHz NMR spectrometer (software – Topspin 3.2) and chemical shifts were expressed in δ (ppm). Shifts reported are relative to the signal of the solvent used in each case and coupling constants are reported in Hz (s: singlet, bs: broad singlet, d: doublet, t: triplet, dd: double doublet, dt: double triplet, m: multiplet). The mass spectra were recorded on Agilent QQQ LC-MS (ESI-MS) spectrometer (software - Mass Hunter B.03.01).

General procedure for the synthesis of N-benzylated series of indole and substituted indoles 2a-i [16]

The indole compounds (**1a-c**) (0.01mol) were treated with aryl chloride (0.012mol) and a strong base, KOH (0.04mol) in 15 mL of DMSO. The solution was kept for stirring for 3hrs. The completion of the reaction mixture was optimized by using thin layer chromatography. After that, the reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was collected, dried over sodium sulphate and concentrated. Pure product was obtained by column chromatography and recrystallized by using hexane.

General procedure for the synthesis of N-benzylated series of indole and substituted indoles Mannich bases 3a-e to 11a-e [15]

The formed N-benzylated indoles (**2a-i**) (0.01 mol) were treated with *p*-formaldehyde (0.02 mol) and various secondary amines (0.01 mol) in presence of ethanol. The total reaction was kept for stirring. The completion of the reaction mixture was optimized by using thin layer chromatography. After completion of the reaction, the mixture was cooled, concentrated and then made alkaline by using sodium hydroxide solution. Then the mixture was extracted by using ethyl acetate. The organic layer was collected and washed with water and dried over anhydrous sodium sulphate and concentrated under reduced pressure to obtain the crude product. Pure products were obtained either by recrystallization from methanol or column chromatography.

4-((1-Benzyl-1*H*-indol-3-yl)methyl)morpholine, 3a: Anal. Calcd for $C_{20}H_{22}N_2O$: C, 78.44; H, 7.22; N, 9.16; O, 5.26. Found: C, 78.40; H, 7.24; N, 9.14; O, 5.22%. R_f = 0.57 (35% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3058 (C-H aromatic), 1713 (C-H), 1610 (C=C, aromatic), 1291 cm^{-1} (C-O-C); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.83-7.812(d, Ar-H 1H), 7.33-7.11(m, 9H, Ar-H), 5.34(s, 2H, CH_2), 3.71(s, 2H, CH_2), 3.74(s, 4H, CH_2), 2.55(s, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 137.23, 136.44, 128.65, 127.71, 127.62, 126.57, 124.32, 121.74, 119.81, 117.92, 111.68, 109.62, 66.74, 54.82, 53.65, 51.35; ESI-MS: *m/z* 307[M+1].

N-((1-Benzyl-1*H*-indol-3-yl)methyl-N-ethylamine, 3b: Anal. Calcd for $C_{20}H_{24}N_2$: C, 82.19; H, 8.24; N, 9.61. Found: C, 82.15; H, 8.27; N, 9.58%. R_f = 0.52 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 1586 (C-H aromatic), 3114 (C-H aromatic), 2935 cm^{-1} (C-H, aliphatic);^1H NMR ($CDCl_3$, 400 MHz): δ 7.78-7.76(d, Ar-H 1H), 7.40-7.12(m, 9H, Ar-H), 5.31(s, 2H, CH_2), 3.84(s, 2H, CH_2), 2.59-5.54(qt, 4H, CH_2), 1.13-1.11(t, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 137.82, 137.55, 128.54, 127.63, 127.55, 125.94, 124.76, 119.63, 118.14, 112.55, 108.75; 53.78, 51.56, 46.73, 13.63; ESI-MS: *m/z* 291[M-1].

1-Benzyl-3-(piperidin-1-ylmethyl)-1*H*-indole, 3c: Anal. Calcd for $C_{21}H_{24}N_2$: C, 82.89; H, 7.97; N, 9.22. Found: C, 82.85; H, 7.95; N, 9.20%. R_f = 0.69 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 1526 (C=C aromatic), 3005 (C-H aromatic), 2931 cm^{-1} (C-H, aliphatic); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.77-7.58(d, J=7.6, 1H), 7.12-7.41(m, 9H, Ar-H), 5.31(s, 2H, CH_2), 3.75(s, 2H, CH_2), 2.55(br m, 4H, CH_2), 1.45-1.64(m, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 137.62, 128.43, 127.72, 127.62, 125.48, 124.42, 121.72, 119.67, 119.41, 112.73, 109.47, 54.82, 51.78, 24.82, 24.16; ESI-MS: *m/z* 303[M-1].

1-Benzyl-3-((4-benzylpiperazin-1-ylmethyl)-1*H*-indole, 3d: Anal. Calcd for $C_{27}H_{29}N_3$: C, 82.02; H, 7.42; N, 10.64. Found: C, 81.99; H, 7.39; N, 10.62%. R_f = 0.51 (25% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 1613 (C=C aromatic), 3059 (C-H aromatic), 2963 cm^{-1} (C-H, aliphatic); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.75-7.73(d, J=8, 1H), 7.09-7.32(m, 14H, Ar-H), 5.92(s, 2H, CH_2), 3.76(s, 2H,

CH_2), 3.52(s, 2H, CH_2), 2.51-2.58(m, 8H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 139.43, 138.96, 129.84, 128.67, 127.96, 127.54, 127.28, 125.91, 124.82, 121.85, 119.64, 119.43, 112.26, 109.68, 61.8, 53.85, 52.78, 51.82, 51.74, 24.41; ESI-MS: *m/z* 396[M+1].

1-Benzyl-3-((4-phenylpiperazin-1-ylmethyl)-1*H*-indole, 3e: Anal. Calcd for $C_{26}H_{27}N_3$: C, 81.88; H, 7.08; N, 11.05. Found: C, 81.85; H, 7.13; N, 11.01%. R_f = 0.51 (40% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 1794 (C=C aromatic), 3105 (C-H aromatic), 2853 cm^{-1} (C-H, aliphatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.80-7.78(d, J=8Hz, 1H), 7.37-7.12(m, 13H, Ar-H), 6.93-6.91(d, 1H, Ar-H), 6.86-6.84(d, 1H, Ar-H), 5.31(s, 2H,CH_2), 3.80(s, 2H, CH_2), 3.21(s, 2H, CH_2), 3.05(s, 2H, CH_2), 2.69(s, 2H, CH_2), 1.63(s, 2H,CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 151.57, 137.64, 136.83, 130.22, 129.18, 128.88, 128.84, 128.53, 127.72, 126.95, 121.96, 119.93, 119.59, 119.44, 116.11, 115.79, 111.69, 109.79, 53.68, 53.18, 50.28, 50.07, 49.30; ESI-MS: *m/z* 382[M+1].

4-((1-Chlorobenzyl-1*H*-indol-3-yl)methyl) morpholine, 4a: Anal. Calcd for $C_{20}H_{21}N_2OCl$: C, 70.52; H, 6.18; N, 8.24; O, 4.70; Cl, 10.44. Found: C, 70.48; H, 6.21; N, 8.22; O, 4.69; Cl, 10.40%. R_f = 0.57 (35% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3058 (C-H aromatic), 1713 (C-H), 1610 (C=C, aromatic), 1291 cm^{-1} (C-O-C); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.83-7.81(d, J=8Hz, Ar-H 1H), 7.33-7.11(m, 8H, Ar-H), 5.34(s, 2H, CH_2), 3.71(s, 2H, CH_2), 3.74(s, 4H, CH_2), 2.55(s, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 134.43, 134.22, 131.84, 126.72, 126.14, 121.92, 119.83, 119.14, 112.45, 109.83, 66.82, 54.25, 53.21, 50.19; ESI-MS: *m/z* 341[M+1].

N-((1-Chlorobenzyl-1*H*-indol-3-yl)methyl-N-ethylamine, 4b: Anal. Calcd for $C_{20}H_{23}N_2Cl$: C, 73.52; H, 7.11; N, 8.54; Cl, 10.89. Found: C, 73.49; H, 7.09; N, 8.57; Cl, 10.85%. R_f = 0.57 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 1579 (C-H aromatic), 3125 (C-H aromatic), 2948 cm^{-1} (C-H, aliphatic);^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.76-7.74(d, J=8Hz, Ar-H 1H), 7.39-7.13(m, 8H, Ar-H), 5.32(s, 2H, CH_2), 3.82(s, 2H, CH_2), 2.56-2.3(qt, 4H, CH_2), 1.25-1.23(t, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.43, 135.24, 132.85, 127.93, 126.553, 126.27, 121.54, 119.83, 118.45, 112.93, 109.65,, 53.95, 50.83, 47.13, 13.34; ESI-MS: *m/z* 327 [M+1].

1-(4-Chlorobenzyl)-3-(piperidin-1-ylmethyl)-1*H*-indole, 4c: Anal. Calcd for $C_{21}H_{23}N_2Cl$: C, 74.47; H, 6.81; N, 8.29; Cl, 10.50. Found: C, 74.43; H, 6.84; N, 8.27; Cl, 10.46%. R_f = 0.69 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 1526 (C=C aromatic), 3005 (C-H aromatic), 2931 cm^{-1} (C-H, aliphatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.74-7.72(d, J=8Hz, 1H), 7.45-7.15(m, 8H, Ar-H), 5.37(s, 2H, CH_2), 3.73(s, 2H, CH_2), 2.54 -2.50(br m, 4H, CH_2), 1.62-1.48(m, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 134.44, 134.12, 131.93, 127.91, 127.73, 126.15, 121.76, 119.83, 118.64, 112.83, 109.13, 54.82, 54.21, 50.18, 25.96, 24.64; ESI-MS: *m/z* 339 [M+1].

1-(4-Chlorobenzyl)-3-((4-benzylpiperazin-1-ylmethyl)-1*H*-indole, 4d: Anal. Calcd for $C_{27}H_{28}N_3Cl$: C, 75.46; H, 6.53; N, 9.74; Cl, 8.29. Found: C, 75.43; H, 6.56; N, 9.77; Cl, 8.25%. R_f = 0.51 (25% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 1533 (C=C aromatic), 3010 (C-H aromatic), 2899 cm^{-1} (C-H, aliphatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.34-6.87(m, 14H, Ar-H), 5.21(s, 2H, CH_2), 3.73(s, 2H, CH_2), 3.49(s, 2H, CH_2), 2.54-2.48(m, 8H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 138.41, 136.43, 135.25, 130.85, 129.83, 128.62, 127.76, 127.30, 126.13, 121.92, 119.81, 118.96, 112.47, 109.88, 61.83, 53.84, 52.83, 50.48; ESI-MS: *m/z* 430[M+1].

1-(4-Chlorobenzyl-3-((4-phenylpiperazin-1-ylmethyl)-1*H*-indole, 4e: Anal. Calcd for $C_{26}H_{26}N_3Cl$: C, 75.11; H, 6.27; N, 10.12; Cl, 8.56. Found: C, 75.07; H, 6.30; N, 10.10; Cl, 8.52%. R_f = 0.51 (25% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 1503 (C=C aromatic), 3030 (C-H aromatic), 2905 cm^{-1} (C-H, aliphatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.56-6.85(m, 13H, Ar-H), 6.53(s, 1H, Ar-CH), 5.58(s, 2H, CH_2), 3.22(s, 2H,CH_2), 2.69 (s, 4H,CH_2), 2.64(s, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 148.43, 129.45, 136.66, 131.82, 135.24, 128.42, 127.86, 126.42, 122.16, 121.66, 119.73, 118.65, 114.33, 112.44, 109.64, 53.93, 51.89; ESI-MS: *m/z* 416[M+1].

4-((1-(3,4-Dichlorobenzyl)-1*H*-indol-3-yl) methyl)morpholine, 5a: Anal. Calcd for $C_{20}H_{20}ON_2Cl_2$: C, 64.17; H, 5.34; N, 7.48. Found: C, 64.12; H, 5.29; N, 7.42%. R_f = 0.66 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 1569 (C=C aromatic), 3016 (C-H aromatic), 2946 cm^{-1} (C-H, aliphatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.74-7.72(d, J=7.6, 1H), 7.38-6.92(m, 7H, Ar-H), 5.26(s, 2H, CH_2), 3.89(s, 2H, CH_2), 2.64(s, 4H, CH_2), 1.70-1.67(t, 2H, CH_2), 1.49-1.48(d, 2H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.4, 134.7, 131.9, 130.8, 130.4, 130.1, 127.7, 126.8, 126.1, 121.9, 119.4, 118.9, 112.4, 109.4, 66.7, 54.6, 53.4, 51.3; ESI-MS: *m/z* 375 [M+1].

4-((1-(3,4-Dichlorobenzyl)-1*H*-indol-3-yl)methyl) N-ethylethanamine, 5b: Yield 69%; m.p. 152-154°C; Anal. Calcd for $C_{20}H_{22}N_2Cl_2$: C, 64.17; H, 5.34; N, 7.48. Found: C, 64.12; H, 5.29; N, 7.42%. R_f = 0.69 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 1569 (C=C aromatic), 3016 (C-H aromatic), 2946 cm^{-1} (C-H, aliphatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.76-7.57(d, J=7.6, Ar-H 1H), 7.39-7.13(m, 8H, Ar-H), 5.32(s, 2H, CH_2), 3.82(s, 2H, CH_2), 2.58-2.56(d, 4H, CH_2), 1.25-1.21(t, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.6, 134.7, 131.4, 130.4, 127.5, 126.4, 126.2, 121.3, 119.7, 118.3, 111.9, 108.6, 53.9, 51.21, 46.5, 13.6; ESI-MS: *m/z* 361 [M+1].

1-(3,4-Dichlorobenzyl)-3-(piperidin-1-ylmethyl)-1*H*-indole, 5c: Anal. Calcd for $C_{21}H_{22}N_2Cl_2$: C, 67.74; H, 5.91; N, 7.52. Found: C, 67.72; H, 5.89; N, 7.49%. R_f = 0.69 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 1569 (C=C aromatic), 3016 (C-H aromatic), 2946 cm^{-1} (C-H, aliphatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.74-7.72(d, J=8Hz, 1H), 7.38-7.17(m, 6H, Ar-H), 6.94-6.92(d, 1H, Ar-H), 5.26(s, 2H, CH_2), 3.89(s, 2H, CH_2), 2.64(s, 4H, CH_2), 1.70-1.67(t, 4H, CH_2), 1.49-1.48(d, 2H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 140.01, 136.46, 131.61, 130.41, 129.39, 129.03, 128.85, 126.74, 121.95, 120.00, 119.53, 111.12, 110.43, 53.98, 53.74, 48.16, 25.79, 24.31, 21.78; ESI-MS: *m/z* 373 [M+1].

3-((4-Benzyliperazin-1yl)methyl)-1-(3,4-dichlorobenzyl)-1*H*-indole, 5d: Anal. Calcd for $C_{27}H_{27}N_3Cl_2$: C, 69.97; H, 5.83; N, 9.07. Found: C, 69.94; H, 5.81; N, 9.04%. R_f = 0.65 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 1543 (C=C str), 3036 (C-H str), 2987 cm^{-1} (C-H str); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.34-6.87(m, 13H, Ar-H), 5.21(s, 2H, CH_2), 3.73(s, 2H, CH_2), 3.49(s, 2H, CH_2), 2.54-2.48(m, 8H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 140.0, 138.7, 136.5, 131.6, 131.2, 130.4, 129.3, 129.2, 128.7, 128.5, 127.7, 127.2, 121.9, 120.0, 119.4, 111.5, 110.3, 62.5, 53.3, 53.1, 52.9, 48.1; ESI-MS: *m/z* 464 [M+1].

1-(3,4-Dichlorobenzyl)- 3-((4-phenylpiperazin-1-yl)methyl)-1*H*-indole, 5e: Anal. Calcd for $C_{26}H_{25}N_3Cl_2$: C, 69.48; H, 5.56; N, 9.35. Found: C, 69.44; H, 5.53; N, 9.32%. R_f = 0.67 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 1519 (C=C str), 3039 (C-H str), 2917 cm^{-1} (C-H str); ^{1}H NMR ($CDCl_3$, 400 MHz)δ: 7.56-6.85(m, 12H, Ar-H), 6.53(s, 1H, Ar-CH), 5.58(s, 2H, CH_2), 3.22(s, 2H, CH_2), 2.66(s, 4H, CH_2), 2.59(s, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.4, 136.6, 134.7, 131.4, 130.5, 130.3, 130.01, 129.4, 127.8, 126.4, 122.1, 121.9, 119.7, 119.6, 112.4, 109.6, 114.3, 53.8, 52.4, 50.4, 49.7; ESI-MS: *m/z* 450 [M+1].

4-((1-Benzyl-5-bromo-1*H*-indol-3-yl)methyl) morpholine, 6a: Anal. Calcd for $C_{20}H_{21}BrN_2O$: C, 62.33; H, 5.45; N, 7.27; Br, 20.51; O, 4.15. Found: C, 62.15; H, 5.39; N, 719; Br, 20.79; O, 4.13%. R_f = 0.58 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3096 (C-H aromatic), 1209 (C-O), 1507 (C=C, aromatic); 606 cm^{-1} (C-Br); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.89(s, 1H, Ar-H), 7.29-7.09(m, 8H, Ar-H), 5.26(s, 2H, CH_2), 3.73(s, 2H, CH_2), 3.17-.3.09(m, 4H, CH_2), 2.50-2.44(m, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.83, 134.65, 131.42, 129.46, 128.62, 127.14, 125.95, 122.41, 122.15, 118.43, 113.62, 111.96, 61.86, 61.42, 54.47, 54.44; ESI-MS: *m/z* 387 [M+2].

4-((1-Benzyl-5-bromo-1*H*-indol-3-yl)methyl)-N-ethylethanamine, 6b: Anal. Calcd for $C_{20}H_{23}BrN_2$: C, 64.69; H, 6.19; N, 7.54; Br, 21.29. Found: C, 64.21; H, 6.09; N, 7.51; Br, 21.73%. R_f = 0.64 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3084 (C-H aromatic), 1512 (C=C, aromatic); 598 cm^{-1} (C-Br); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.85(s, 1H, Ar-H), 7.77-6.49(m, 8H, Ar-H), 5.30(s, 2H, CH_2), 3.76(s, 2H, CH_2), 2.62(s, 4H, CH_2), 1.12-1.04(t, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.41, 134.92, 131.15, 129.82, 128.47, 124.93, 122.84, 121.65, 117.82, 114.64, 112.42, 59.64, 54.32, 49.63, 13.64; ESI-MS: *m/z* 371 $[M]^+$.

1-Benzyl-5-bromo-3-(piperidin-1-ylmethyl)-1*H*-indole, 6c: Anal. Calcd for $C_{21}H_{23}BrN_2$: C, 65.79; H, 6.20; N, 7.31; Br, 20.62. Found: C, 65.43; H, 5.94; N, 7.25; Br, 20.94%. R_f = 0.58 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3095 (C-H aromatic), 1510 (C=C, aromatic); 605 cm^{-1} (C-Br); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.85(s, 1H, Ar-H), 7.30-7.07(m, 8H, Ar-H), 5.26(s, 2H, CH_2), 3.65(s, 2H, CH_2), 2.45-2.93(m, 4H, CH_2), 1.61-1.55(m, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.43, 134.74, 130.92, 130.45, 129.43, 126.72, 125.46, 121.82, 121.14, 116.42, 112.85, 110.28, 57.45, 54.82, 54.32, 24.95; ESI-MS: *m/z* 385 $[M+2]^+$.

1-Benzyl--3-((4-benzylpiperazin-1-ylmethyl)-5-bromo-1*H*-indole, 6d: Anal. Calcd for $C_{27}H_{28}BrN_3$: C, 68.35; H, 5.90; N, 8.86; Br, 16.66. Found: C, 65.43; H, 5.94; N, 7.25; Br, 16.62%. R_f = 0.58 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3089 (C-H aromatic), 1506 (C=C, aromatic); 598 cm^{-1} (C-Br); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.86(s, 1H, Ar-H), 7.32-7.06(m, 13H, Ar-H), 5.25(s, 2H, CH_2), 3.66(s, 2H, CH_2), 3.51(s, 2H, CH_2), 2.92(s, 8H, CH_2);

^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.47, 136.21, 135.19, 131.45, 129.14, 128.73, 128.92, 127.44, 125.94, 122.51, 121.42, 118.46, 113.63, 112.94, 59.87, 58.37, 54.32, 54.14, 50.36, 50.14; ESI-MS: *m/z* 476 [M+2].

1-Benzyl--3-((4-phenylpiperazin-1-ylmethyl)-5-bromo-1*H*-indole, 6e: Anal. Calcd for $C_{26}H_{26}BrN_3$: C, 67.82; H, 5.65; N, 9.13; Br, 17.17. Found: C, 67.22; H, 5.59; N, 10.37; Br, 17.14%. R_f = 0.57 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3096 (C-H aromatic), 1516 (C=C, aromatic); 601 cm^{-1} (C-Br); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.91(s, 1H, Ar-H), 7.34-6.85(m, 13H, Ar-H), 5.28(s, 2H, CH_2), 3.80(s, 2H, CH_2), 3.26-3.24(t, 4H, CH_2), 2.74-2.72(t, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 151.28, 136.97, 135.38, 130.40, 129.42, 129.15, 128.92, 127.89, 127.02, 126.80, 124.83, 122.32, 119.77, 117.17, 116.17, 115.86, 112.98, 111.36, 110.43, 101.34, 53.12, 52.81, 50.28, 49.09, 48.96, 45.61; ESI-MS: *m/z* 462 [M+2].

4-((5-Bromo-1-(4-chlorobenzyl)-1*H*-indol-3-yl)methyl)morpholine, 7a: Anal. Calcd for $C_{20}H_{20}BrN_2OCl$: C, 59.25; H, 4.93; N, 6.91; Br, 19.53; O, 3.95; Cl, 8.76. Found: C, 59.05; H, 4.89; N, 6.89; Br, 19.45; O, 3.92; Cl, 8.78%. R_f = 0.58 (15% Ethyl acetate in Hexane); 3085 (C-H aromatic), 1215 (C-O), 1516 (C=C, aromatic); 596 cm^{-1} (C-Br); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.90(s, 1H, Ar-H), 7.34(s, 1H, Ar-H), 7.26-6.99(m, 6H, Ar-H), 5.23(s, 2H, CH_2), 3.71(br s, 4H, CH_2), 3.64(s, 2H, CH_2), 2.50(s, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.72, 134.64, 131.76, 130.83, 129.12, 124.41, 121.43, 118.42, 113.86, 112.64, 64.22, 58.48, 54.32, 52.93; ESI-MS: *m/z* 407 [M+2]$^+$.

4-((1-Benzyl-5-bromo-1*H*-indol-3-yl)methyl)-N-ethylethanamine, 7b: Anal. Calcd for $C_{20}H_{22}BrN_2Cl$: C, 61.38; H, 5.62; N, 7.16; Br, 20.17; Cl, 9.06. Found: C, 61.34; H, 5.58; N, 7.11; Br, 20.14; Cl, 9.02%. R_f = 0.59 (15% Ethyl acetate in Hexane); 3085 (C-H aromatic), 1258 (C-N), 1516 (C=C, aromatic); 596 cm^{-1} (C-Br); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.90(s, 1H, Ar-H), 7.34(s, 1H, Ar-H), 7.26-6.99(m, 6H, Ar-H), 5.23(s, 2H, CH_2), 3.64(s, 2H, CH_2),3.51(s, 4H, CH_2), 2.69-2.62(m, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.83, 134.94, 131.72, 130.93, 129.25, 127.12, 126.45, 122.15, 121.92, 117.65, 114.16, 112.82, 58.45, 54.28, 49.74, 13.83; ESI-MS: *m/z* 391 [M-2]$^+$.

5-Bromo-1-(4-chlorobenzyl)-3-(piperidin-1-ylmethyl)-1*H*-indole, 7c: Anal. Calcd for $C_{21}H_{22}BrN_2Cl$: C, 62.53; H, 5.45; N, 6.94; Br, 19.57; Cl, 8.80. Found: C, 62.49; H, 5.41; N, 6.91; Br, 19.54; Cl, 8.78%. R_f = 0.63 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3095 (C-H aromatic), 1265 (C-N), 1509 (C=C, aromatic); 605 cm^{-1} (C-Br); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.87(s, 1H, Ar-H), 7.77(s, 1H, Ar-H), 7.27-6.49(m, 6H, Ar-H), 5.27(s, 2H, CH_2), 3.63(s, 2H, CH_2), 2.43-2.41(t, 4H, CH_2), 1.73-1.56(m, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δESI-MS: *m/z* 404 [M+1].

5-Bromo-1-(4-chlorobenzyl)-3-((4-benzylpiperazin-1-yl)methyl)-1*H*-indole, 7d: Anal. Calcd for $C_{27}H_{27}BrN_3Cl$: C, 65.38; H, 5.44; N, 8.47; Br, 15.94; Cl, 7.16. Found: C, 65.29; H, 5.42; N, 8.39; Br, 15.91; Cl, 7.12%. R_f = 0.58 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3095(C-H aromatic), 1258 (C-N), 1516 cm^{-1} (C=C, aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.859(s, 1H, Ar-H), 7.31-7.05(m, 12H, Ar-H), 5.25(s, 2H, CH_2), 3.67(s, 2H,CH_2), 3.52(s, 2H, CH_2), 2.87-2.85(t, 4H, CH_2), 2.39-2.37(t, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.73, 135.22, 134.84, 131.62, 130.84, 130.43, 128.93, 128.44, 127.92, 122.14, 121.75, 117.45, 113.42, 112.85, 59.43, 58.32, 54.76, 51.95, 51.83, 49.92; ESI-MS: *m/z* 496 [M+1].

5-Bromo-1-(4-chlorobenzyl)-3-((4-phenylpiperazin-1-yl)methyl)-1*H*-indole, 7e: Anal. Calcd for $C_{26}H_{25}BrN_3Cl$: C, 64.79; H, 5.19; N, 8.73; Br, 16.42; Cl, 7.37. Found: C, 64.73; H, 5.14; N, 8.69; Br, 16.35; Cl, 7.32%. R_f = 0.58 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3098 (C-H aromatic), 1260 (C-N), 1512 cm^{-1} (C=C, aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.91(s, 1H, Ar-H), 7.23-6.85(br m, 12H, Ar-H), 5.24(s, 2H, CH_2), 3.74(s, 2H, CH_2), 3.23(brs, 4H, CH_2), 2.68(brs, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 151.33, 135.51, 133.71, 130.39, 129.33, 129.13, 129.08, 128.91, 128.08, 124.95, 122.55, 120.94, 119.68, 117.16, 116.11, 113.03, 111.37, 111.15, 53.39, 53.04, 49.12, 45.51; ESI-MS: *m/z* 480 [M-1].

4-((5-Bromo-1-(3,4-dichlorobenzyl)-1*H*-indol-3-yl) methyl)morpholine, 8a: Anal. Calcd for $C_{20}H_{19}BrON_2Cl_2$: C, 52.86; H, 4.18; N, 6.16. Found: C, 52.84; H, 4.15; N, 6.14%. R_f = 0.64 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3064 (C-H str), 1219 (C-O-Cstr), 1526 cm^{-1} (C=C, str); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.90(s, 1H, Ar-H), 7.34(s, 1H, Ar-H), 7.26-6.99(m, 5H, Ar-H), 5.23(s, 2H, CH_2), 3.64(s, 2H, CH_2), 2.50(s, 4H, CH_2), 1.69(s, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.74, 134.89, 131.92, 130.28, 128.94, 126.42, 121.31, 117.84,

113.43, 112.48, 61.92, 59.21, 54.28, 53.24; ESI-MS: *m/z* 455 [M+1].

N-((5-Bromo-1-(3,4-dichlorobenzyl)-1*H*-indol-3-yl)methyl)-N-ethylethanamine, 8b: Anal. Calcd for $C_{20}H_{21}BrN_2Cl_2$: C, 54.54; H, 4.77; N, 6.36. Found: C, 54.52; H, 4.74; N, 6.34%. R_f = 0.65 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3099 (C-H str), 1296 (C-N str), 1528 (C=C str), 603 cm^{-1} (C-Br str); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.90(s, 1H, Ar-H), 7.34(s, 1H, Ar-H), 7.26-6.99(m, 6H, Ar-H), 5.23(s, 2H, CH_2), 3.71(br s, 4H, CH_2), 3.64(s, 2H, CH_2); 2.50(s, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 175.44, 136.73, 134.87, 133.17, 132.23, 131.48, 130.99, 130.32, 128.64, 126.01, 125.56, 121.48, 113.98, 111.55, 52.43, 52.19, 49.03, 20.83; ESI-MS: *m/z* 441 [M+1].

5-Bromo-1-(3,4-dichlorobenzyl)-3-piperidin-1-ylmethyl)-1*H*-indole, 8c: Anal. Calcd for $C_{21}H_{21}BrN_2Cl_2$: C, 55.75; H, 4.64; N, 6.19. Found: C, 55.73; H, 4.62; N, 6.17%. R_f = 0.65 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3120 (C-H str), 1298 (C-N str) 1519 (C=C str), 599 cm^{-1} (C-Br str); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.87(s, 1H, Ar-H), 7.77(s, 1H, Ar-H), 7.27-6.49(m, 6H, Ar-H), 5.27(s, 2H, CH_2), 3.632 (s, 2H, CH_2), 2.439 (m, 4H, CH_2), 1.730-1.562 (m, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.98, 134.84, 133.10, 130.87, 130.81, 128.62, 128.54, 127.67, 121.76, 113.66, 113.34, 110.92, 108.13, 101.96, 52.35, 49.21, 24.51, 23.43, 23.28, 53.37, 49.32; ESI-MS: *m/z* 453 [M+1].

3-((4-Benzylpiperazin-1-yl)methyl-5-bromo-1-(3,4-dichlorobenzyl)-1*H*-indole, 8d: Anal. Calcd for $C_{27}H_{26}BrN_3Cl_2$: C, 59.66; H, 4.78; N, 7.73. Found: C, 59.64; H, 4.74; N, 7.71%. R_f = 0.67 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3089 (C-H str), 1296 (C-N str), 1522 (C=C str), 609 cm^{-1} (C-Br str); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.85(s, 1H, Ar-H), 7.31-7.05 (m, 13H, Ar-H), 5.25(s, 2H, CH_2), 3.67(s, 2H, CH_2), 3.52(s, 2H, CH_2), 2.87(s, 8H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.84, 137.98, 136.87, 131.84, 130.64, 130.28, 128.97, 128.64, 126.84, 121.65, 121.38, 118.92, 114.83, 112.64, 60.12, 59.42, 54.69, 52.38, 49.87; ESI-MS: *m/z* 545 [M+2].

5-Bromo-1-(3,4-dichlorobenzyl)-3-((4-phenylpiperazin-1-yl)methyl)-1*H*-indole, 8e: Anal. Calcd for $C_{26}H_{24}BrN_3Cl_2$: C, 58.97; H, 4.53; N, 7.93. Found: C, 58.95; H, 4.51; N, 7.90%. R_f = 0.68 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3120 (C-H str), 1286 (C-N str), 1522 (C=C str), 606 cm^{-1} (C-Br str); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.91(s, 1H, Ar-H), 7.23-6.85 (br m, 11H, Ar-H), 5.24(s, 2H, CH_2), 3.74(s, 2H, CH_2), 3.23(br s, 4H, CH_2), 2.68(br s, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 150.87, 137.05, 135.05, 133.14, 132.12, 130.95, 130.37, 129.35, 129.31, 129.20, 128.62, 125.97, 125.33, 122.12, 120.20, 116.90, 116.55, 116.41, 111.26, 52.85, 52.59, 49.29, 48.61; ESI-MS: *m/z* 530 [M+1].

4-((1-Benzyl-5-methoxy-1*H*-indol-3-yl)methyl) morpholine, 9a: Anal. Calcd for $C_{21}H_{24}O_2N_2$: C, 75; H, 7.14; N, 8.33; O, 9.52. Found: C, 74.78; H, 7.11; N, 8.29; O, 9.48%. R_f = 0.54 (25% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3084 (C-H aromatic), 2108 (C-O), 1516 cm^{-1} (C=C, aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.31(s, 1 Ar-H), 7.29-6.82(m, 8H, Ar-H), 5.25(s, 2H, CH_2), 3.87(s, 3H, CH_2), 3.73(s, 2H, CH_2), 2.52(s, 4H, CH_2); 1.70(s, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.63, 136.34, 129.92, 129.13, 128.85, 128.74, 126.51, 125.82, 112.55, 112.13, 109.62, 102.13, 66.85, 59.56, 55.49, 54.46, 53.32; ESI-MS: *m/z* 337 $[M+1]^+$.

N-((1-Benzyl-5-methoxy-1*H*-indol-3-yl)methyl)-N-ethylethanamine, 9b: Anal. Calcd for $C_{21}H_{26}ON_2$: C, 78.26; H, 8.07; N, 8.69; O, 4.96. Found: C, 78.18; H, 8.03; N, 8.62; O, 4.91%. R_f = 0.54 (25% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3094 (C-H aromatic), 2119 (C-O), 1507 cm^{-1} (C=C, aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.31(s, 1H, ArH), 7.29-6.81(m, 8H, Ar-H), 5.26(s, 2H, CH_2), 3.87(s, 3H, CH_2), 3.78(s, 2H, CH_2), 2.62-2.56(m, 4H, CH); 1.13-1.09(br t, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.64, 136.35, 129.96, 129.12, 128.83, 128.71, 126.54, 125.83, 112.52, 112.12, 109.65, 102.14, 59.53, 55.93, 54.12, 49.14, 13.43; ESI-MS: *m/z* 323 [M+1].

1-Benzyl-5-methoxy-3-(piperidin-1-ylmethyl)-1*H*-indole, 9c: Anal. Calcd for $C_{22}H_{26}O_2N_2$: C, 79.04; H, 7.78; N, 8.38; O, 4.79. Found: C, 78.98; H, 7.75; N, 8.34; O, 4.73%. R_f = 0.59 (25% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3054 (C-H aromatic), 2097 (C-O), 1511 cm^{-1} (C=C, aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.30-6.85(m, 9H, Ar-H), 5.30(s, 2H, CH_2), 3.86(s, 3H, CH_2), 3.65(s, 2H, CH_2), 2.45(s, 4H, CH_2), 1.64-1.55(m, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.63, 136.34, 128.99, 128.71, 128.18, 126.54, 125.62, 112.52, 112.14, 109.62, 102.14, 59.52, 55.97, 54.46, 54.26, 25.94; ESI-MS: *m/z* 335[M+1].

3-((4-Benzylpiperazin-1-yl)methyl)-1-benzyl-5-methoxy-1*H*-indole, 9d: Anal. Calcd for $C_{28}H_{31}ON_3$: C, 79.05; H, 7.29; N, 9.88; O, 3.76. Found: C, 78.85; H, 7.25; N, 9.83; O, 3.72%. R_f = 0.64 (25% Ethyl

acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3064 (C-H aromatic), 2115 (C-O), 1519 cm^{-1} (C=C, aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.32(s, 1H, ArH), 7.30-6.83(m, 13H, Ar-H), 5.27(s, 2H, CH_2), 3.86(s, 3H, CH_2), 3.75(s, 2H, CH_2), 3.23(s, 2H, CH_2), 2.69-2.65(m, 8H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.64, 136.33, 135.76, 129.95, 128.87, 128.74, 128.53, 127.38, 126.56, 125.69, 112.54, 112.17, 109.68, 102.14, 59.87, 59.64, 55.94, 54.38, 54.13, 52.64; ESI-MS: *m/z* 426 [M+1].

3-((4-Phenylpiperazin-1-yl)methyl)-1-(4-chlorobenzyl)-5-methoxy-1*H*-indole, 9e: Anal. Calcd for $C_{27}H_{29}ON_3Cl$: C, 72.89; H, 6.29; N, 9.44; O, 3.59; Cl, 7.98. Found: C, 72.81; H, 6.25; N, 9.41; O, 3.52; Cl, 7.94%. R_f = 0.57 (25% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3066 (C-H aromatic), 2122 (C-O), 1513 cm^{-1} (C=C, aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.30-6.83(m, 14H, Ar-H), 5.27(s, 2H, CH_2), 3.86(s, 3H, CH_2), 3.76(s, 2H, CH_2), 3.23-3.20(t, 4H, CH_2), 2.70-2.68(t, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.67, 149.64, 136.36, 129.94, 129.74, 128.98, 128.81, 128.74, 126.5, 125.84, 118.46, 114.76, 112.54, 112.13, 109.62, 102.14, 59.64, 55.94, 54.14, 52.27, 51.98; ESI-MS: *m/z* 412[M+1].

4-((1-(4-Chlorobenzyl)-5-methoxy-1*H*-indol-3-yl) methyl)morpholine, 10a: Anal. Calcd for $C_{21}H_{23}O_2N_2Cl$: C, 68.01; H, 6.20; N, 7.55; O, 8.63; Cl, 9.58. Found: C, 67.92; H, 6.15; N, 7.51; O, 8.79; Cl, 9.53%. R_f = 0.67 (25% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3084 (C-H aromatic), 2108 (C-O), 1516 cm^{-1} (C=C, aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.27-6.83(m, 8H, Ar-H), 5.21(s, 2H, CH_2), 3.87(s, 3H, CH_2), 3.58(s, 2H, CH_2), 2.51-2.47(br m, 4H, CH_2), 1.87(s, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 154.13, 134.90, 132.16, 131.92, 131.75, 130.79, 130.50, 129.52, 129.13, 128.41, 127.68, 112.26, 110.67, 101.90, 66.85, 55.94, 54.89, 53.36, 49.17; ESI-MS: *m/z* 371 [M+1].

N-((1-(4-Chlorobenzyl)-5-methoxy-1*H*-indol-3-yl) methyl)-N-ethylethanamine, 10b: Anal. Calcd for $C_{21}H_{25}ON_2Cl$: C, 70.68; H, 7.01; N, 7.85; O, 4.48; Cl, 9.95. Found: C, 70.62; H, 6.97; N, 7.82; O, 4.39; Cl, 9.89%. R_f = 0.53 (25% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3063 (C-H aromatic), 2118 (C-O), 1512 cm^{-1} (C=C, aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.24(s, 1H, Ar-H), 7.22-6.81(m, 7H, Ar-H), 5.22(s, 2H, CH_2), 3.86(s, 3H, CH_2), 3.76(s, 2H, CH_2), 2.61-2.58(qt, 4H, CH_2), 1.12-1.10(t, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 154.25, 132.83, 131.56, 131.73, 130.75, 130.78, 129.48, 129.11, 128.45, 127.86, 112.24, 110.19, 101.72, 55.86, 49.32, 49.15, 47.62, 13.35; ESI-MS: *m/z* 356 [M+1].

1-(4-Chlorobenzyl)-5-methoxy-3-(piperidin-1-ylmethyl)-1*H*-indole, 10c: Anal. Calcd for $C_{21}H_{25}ON_2Cl$: C, 71.64; H, 6.78; N, 7.59; O, 4.34; Cl, 9.63. Found: C, 71.59; H, 6.72; N, 7.53; O, 4.28; Cl, 9.58%. R_f = 0.63 (25% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3074 (C-H aromatic), 2116 (C-O), 1509 cm^{-1} (C=C, aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.26-6.81(m, 8H, Ar-H), 5.26(s, 2H, CH_2), 3.87(s, 3H, CH_2), 3.66(s, 2H, CH_2), 2.46(s, 4H, CH_2), 1.61-1.56(m, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 154.67, 138.22, 132.45, 131.72, 131.10, 130.98, 129.89, 128.66, 128.39, 125.58, 112.23, 112.14, 110.45, 101.87, 55.84, 54.44, 54.21, 49.03, 26.10, 25.98; ESI-MS: *m/z* 369[M+1].

3-((4-Benzylpiperazin-1-yl)methyl)-1-(4-chlorobenzyl)-5-methoxy-1*H*-indole, 10d: Anal. Calcd for $C_{28}H_{30}ON_3Cl$: C, 73.12; H, 6.52; N, 9.14; O, 3.48; Cl, 7.72. Found: C, 72.98; H, 6.48; N, 9.09; O, 3.41; Cl, 7.65%. R_f = 0.57 (25% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3085 (C-H aromatic), 2109 (C-O), 1514 cm^{-1} (C=C, aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.33-6.80(m, 13H, Ar-H), 5.20(s, 2H, CH_2), 3.85(s, 3H, CH_2), 3.69(s, 2H, CH_2), 3.51(s, 2H, CH_2), 2.50(s, 8H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 139.87, 137.42, 135.34, 133.32, 132.23, 130.43, 130.41, 130.50, 129.88, 129.72, 129.43, 128.55, 128.34, 128.23, 127.53, 125.14, 121.45, 113.56, 111.31, 101.96, 60.56, 55.84, 51.81, 51.45, 49.51, 49.29; ESI-MS: *m/z* 460[M+1].

3-((4-Phenylpiperazin-1-yl)methyl)-1-(4-chlorobenzyl)-5-methoxy-1*H*-indole, 10e: Anal. Calcd for $C_{27}H_{28}ON_3Cl$: C, 72.89; H, 6.29; N, 9.44; O, 3.59; Cl, 7.98. Found: C, 72.81; H, 6.25; N, 9.41%. R_f = 0.57 (25% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3066 (C-H aromatic), 2115 (C-O), 1513 cm^{-1} (C=C, aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.27-6.83(m, 13H, Ar-H), 5.23(s, 2H, CH_2), 3.86(s, 3H, CH_2), 3.75(s, 2H, CH_2), 3.23-3.20(t, 4H, CH_2), 2.70-2.67(t, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 151.45, 136.19, 136.15, 133.45, 131.89, 129.14, 129.11, 128.97, 128.30, 128.26, 128.13, 128.10, 119.57, 117.16, 116.03, 112.09, 111.43, 110.35, 101.90, 55.94, 53.70, 53.12, 49.59, 49.27; ESI-MS: *m/z* 460[M+1].

4-((1-(3,4-Dichlorobenzyl)-5-methoxy-1*H*-indol-3yl)methyl)morpholine, 11a: Anal. Calcd for $C_{21}H_{22}O_2N_2Cl_2$: C, 62.37; H, 5.44; N, 6.93. Found: C, 62.35; H, 5.42; N, 6.91%. R_f = 0.65 (25% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3064 (C-H

str), 2116 (C-O str), 1526 cm^{-1} (C=C str); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.27-6.83(m, 7H, Ar-H), 5.21(s, 2H, CH_2), 3.87(s, 3H, CH_2), 3.68(s, 2H, CH_2), 2.51-2.47(br m, 4H, CH_2), 1.87(s, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 154.23, 137.90, 132.96, 131.79, 131.75, 130.79, 130.50, 129.50, 129.17, 128.41, 127.68, 112.26, 110.27, 101.90, 66.95, 55.94, 53.89, 53.46, 49.13; ESI-MS: *m/z* 405 [M+1].

N-((1-(3,4-Dichlorobenzyl)-5-methoxy-1*H*-indol-3yl)methyl)N-ethylethanamine, 11b: Anal. Calcd for $C_{21}H_{24}ON_2Cl_2$: C, 64.61; H, 6.15; N, 7.17. Found: C, 64.59; H, 6.13; N, 7.15%. R_f = 0.65 (25% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3063 (C-H str), 2123 (C-O str), 1522 cm^{-1} (C=C str); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.22-6.81(m, 7H, Ar-H), 5.22(s, 2H, CH_2), 3.86(s, 3H, CH_2), 3.76(s, 2H, CH_2), 2.61-2.56(qt, 4H, CH_2), 1.12-1.10(t, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 154.25, 132.93, 131.69, 131.63, 130.76, 130.50, 129.50, 129.31, 128.57, 127.68, 112.18, 110.29, 101.62, 55.89, 49.15, 46.32, 47.62, 11.35; ESI-MS: *m/z* 391 [M+1].

1-(3,4-Dichlorobenzyl)-5-methoxy-3-(piperidin-1-ylmethyl)-1*H*-indole, 11c: Anal. Calcd for $C_{22}H_{24}ON_2Cl_2$: C, 65.67; H, 5.97; N, 6.96. Found: C, 65.65; H, 5.94; N, 6.94%. R_f = 0.65 (25% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3074 (C-H str), 2119 (C-O str), 1516 cm^{-1} (C=C str); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.26-6.81(m, 7H, Ar-H), 5.26(s, 2H, CH_2), 3.87(s, 3H, CH_2), 3.66(s, 2H, CH_2), 2.46(s, 4H, CH_2), 1.61-1.56(m, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 154.11, 138.12, 132.88, 131.66, 131.60, 130.71, 129.49, 128.57, 128.20, 125.96, 112.10, 112.04, 110.10, 101.96, 55.86, 54.44, 54.10, 53.11, 49.03, 26.10, 25.98; ESI-MS: *m/z* 403 [M+1].

3-((4-Benzylpiperazin-1-ylmethyl)-1-(3,4-dichlorobenzyl)-5-methoxy-1*H*-indole, 11d: Anal. Calcd for $C_{28}H_{29}ON_3Cl_2$:: C, 68.15; H, 5.88; N, 6.96. Found: C, 68.13; H, 5.85; N, 6.94%. R_f = 0.66 (25% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3079 (C-H str), 2117 (C-O str), 1523 cm^{-1} (C=C str); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.33-6.80(m, 12H, Ar-H), 5.20(s, 2H, CH_2), 3.85(s, 3H, CH_2), 3.69(s, 2H, CH_2), 3.51(s, 2H, CH_2) 2.50-2.48(t, 4H, CH_2), 1.97-1.95(t, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.92, 136.44, 134.93, 133.13, 132.13, 130.95, 130.50, 130.41, 129.52, 129.23, 129.14, 128.64, 128.43, 128.13, 127.61, 125.33, 121.86, 113.68, 111.31, 101.96, 60.42, 51.80, 51.61, 51.32, 49.30, 49.21; ESI-MS: *m/z* 495 [M+2].

1-(3,4-Dichlorobenzyl)-5-methoxy-3-((4-phenylpiperazin-1-yl)methyl)-1*H*-indole, 11e:

Anal. Calcd for $C_{27}H_{27}ON_3Cl_2$: C, 67.64; H, 5.63; N, 8.76. Found: C, 67.62; H, 5.61; N, 8.74%. R_f = 0.65 (25% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3068 (C-H str), 2122 (C-O str), 1519 cm^{-1} (C=C str); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.38-6.86(m, 12H, Ar-H), 5.22(s, 2H, CH_2), 4.52(s, 3H, CH_2), 3.89-3.83(d, 2H, CH_2), 3.29-3.22(m, 4H, CH_2), 2.77-2.07(m, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ154.26, 137.88, 132.98, 131.77, 131.74, 130.81, 130.51, 129.51, 129.22, 129.13, 128.64, 128.58, 126.82, 119.61, 116.14, 112.31, 110.89, 110.31, 101.82, 55.95, 53.43, 52.91, 51.57, 49.18, 49.05; ESI-MS: *m/z* 481 [M+2].

General procedure for the synthesis of indole Mannich bases 12a-e to 14a-e [16]

To a solution of 0.01mol of amine in ethanol (10 mL), an equivalent amount of formaldehyde was added. The entire mixture was kept for reflux for half an hour. To the above solution, 0.01mol of compound (**1a**) in ethanol was added and continue for condensation for about 2hrs with the addition of few drops of conc. HCl for every half an hour. This reaction is not suitable for substituted indoles because of heat, charred products are obtained. So, the substituted indoles (**1b and 1c**), (0.01mol) were treated with *p*-formaldehyde (0.02mol) and various secondary amines (0.01mol) in presence of ethanol. The total reaction was kept for stirring. The completion of the reaction mixture was optimized by using thin layer chromatography. The extraction procedure for all these compounds (**12a-e to 14a-e**) is same as of N-benzylated Mannich base series (3a-e).

4-((1*H*-Indol-3-yl)methyl)morpholine, 12a: Anal. Calcd for $C_{13}H_{16}N_2O$: C, 72.22; H, 7.48; N, 12.98; O, 7.44. Found: C, 72.19; H, 7.46; N, 12.95; O, 7.40%. R_f = 0.65 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3406 (N-H aromatic), 3041 (C-H aromatic), 996 (C-O), 1544 cm^{-1} (C=C, aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.09(s, 1H, NH), 7.77-7.75(d, 1H, Ar-H), 7.36-7.11(m, 4H, CH_2), 3.71(s, 2H, CH_2), 3.69(s, 4H, CH_2), 2.42(s, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.53, 124.24, 121.73, 119.81, 118.82, 112.24, 111.15, 66.74, 53.95, 53.23 ESI-MS: *m/z* 217 [M+1].

N-((1*H*-Indol-3-yl)methyl-N-ethylamine, 12b: Anal. Calcd for $C_{13}H_{18}N_2$: C, 55.05; H, 2.77; N, 12.84. Found: C, 55.08; H, 2.79; N, 12.92%. R_f = 0.54 (15% Ethyl acetate in Hexane); IR (KBr) υ max:

cm^{-1}: 3379 (N-H aromatic), 3105 (C-H aromatic), 2953 cm^{-1} (C-H, aliphatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.05(s, 1H, N-H), 7.61-7.15(m, 5H, Ar-H), 3.54(s, 2H, CH_2), 2.45-2.43(t, 4H, CH_2), 1.19-1.13(m, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.12, 127.53, 123.24, 121.72, 119.32, 118.81, 112.32, 110.67, 53.66, 46.5, 13.3; ESI-MS: *m/z* 203[M+1].

3-(Piperidin-1-ylmethyl)-1*H*-indole, 12c: Anal. Calcd for $C_{14}H_{18}N_2$: C, 78.50; H, 8.41; N, 13.10. Found: C, 78.46; H, 8.47; N, 13.07%. R_f = 0.55 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3419 (N-H aromatic), 3100 cm^{-1} (C-H aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.82(s, 1H, NH), 7.77-7.75(d, 1H, Ar-CH), 7.36-7.10(m, 4H, Ar-H), 3.78(s, 2H, CH_2), 1.66-1.61(m, 8H, CH_2), 1.47-1.46(d, 2H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.13, 128.35, 124.24, 121.76, 119.36, 119.34, 112.03, 111.17, 54.32, 53.87, 25.91, 24.35; ESI-MS: *m/z* 215 [M+1].

3-((4-Benzylpiperazin-1-ylmethyl)-1*H*-indole, 12d: Anal. Calcd for $C_{20}H_{23}N_3$: C, 78.68; H, 7.54; N, 13.78. Found: C, 78.65; H, 7.59; N, 13.76%. R_f = 0.67 (35% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3423 (N-H aromatic), 3097 (C-H aromatic), 1347 cm^{-1} (C-N, aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.20(s, 1H, NH), 7.71(d, 1H, Ar-H), 7.80-7.33(m, 9H, Ar-H), 3.73(s, 2H, CH_2), 3.49(s, 2H, CH_2), 2.55-2.44(s, 8H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 138.62, 136.53, 128.82, 128.41, 127.44, 127.24, 118.81, 112.36, 110.86, 61.93, 53.62, 52.52; ESI-MS: *m/z* 306[M+1].

3-((4-Phenylpiperazin-1-ylmethyl)-1*H*-indole, 12e: Anal. Calcd for $C_{19}H_{21}N_3$: C, 78.35; H, 7.21; N, 14.45. Found: C, 78.32; H, 7.26; N, 14.42%. R_f = 0.54 (25% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3420 (N-H aromatic), 3094 (C-H aromatic), 1295 cm^{-1} (C-, N aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.07(s, 1H, NH), 7.78-7.76(d, 1H, Ar-H), 7.76-7.38(m, 7H, Ar-H), 6.92-6.90(d, 2H, Ar-H), 3.78(s, 2H, CH_2), 3.18-3.16(t, 4H, CH_2), 2.66-2.64(t, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 149.62, 136.12, 129.64, 127.53, 123.23, 121.91, 121.75, 119.33, 118.82, 114.33, 112.36, 108.53, 53.69, 52.34, 49.76; ESI-MS: *m/z* 292 [M+1].

4-((5-Bromo-1*H*-indol-3-yl)methyl)morpholine, 13a: Anal. Calcd for $C_{13}H_{15}BrN_2O$: C, 53.06; H, 5.10; N, 9.52; O, 5.44; Br, 26.87. Found: C, 52.90; H, 5.12; N, 9.49; O, 5.42; Br, 27.07%. R_f = 0.58 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3394 (N-H aromatic), 3094 (C-H aromatic), 2109 (C-O), 1507 cm^{-1} (C=C, aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.09(s, 1H, NH), 7.77-7.14(m, 4H, Ar-H), 3.7I(s, 2H, CH_2), 3.70-2.49(m, 8H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 135.22, 129.94, 122.93, 121.22, 121.14, 117.03, 113.32, 112.23, 66.81, 54.13, 53.32; ESI-MS: *m/z* 296 [M+2].

4-((5-Bromo-1*H*-indol-3-yl)methyl)-N-ethylethanamine, 13b: Anal. Calcd for $C_{13}H_{17}BrN_2$: C, 55.51; H, 6.04; N, 9.96; Br, 28.46. Found: C, 52.90; H, 5.12; N, 9.49; O, 5.42; Br, 28.54%. R_f = 0.58 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3394 (N-H aromatic), 3094 (C-H aromatic), 2109 (C-O), 1507 cm^{-1} (C=C, aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.63(br s, 1H), 7.81(s, 1H, Ar-H), 7.31-7.04(m, 3H, Ar-H), 2.55(qt, 4H, CH_2), 1.02(br t, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 135.54, 129.7, 122.93, 121.24, 121.15, 117.01, 113.34, 112.24, 53.83, 49.15, 13.43; ESI-MS: *m/z* 279 [M-2].

5-Bromo-3-(piperidin-1-yl-methyl)-1*H*-indole, 13c: Anal. Calcd for $C_{14}H_{17}BrN_2$: C, 57.53; H, 5.82; N, 9.58; Br, 27.05. Found: C, 57.35; H, 5.84; N, 9.55; Br, 27.25%. R_f = 0.58 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3394 (N-H aromatic), 3094 (C-H aromatic), 2109 (C-O), 1507 cm^{-1} (C=C, aromatic); ^{1}H NMR ($CDCl_3$, 400MHz): δ 8.151 (br s, 1H, NH), 7.626 (s, 1H, Ar-H), 7.30-6.95(m, 3H, Ar-H), 4.22(s, 2H, CH_2), 2.14-2.09(m, 4H, CH_2), 1.27-1.21(m, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100MHz): δ 135.52, 129.63, 124.74, 123.04, 121.01, 116.42, 113.25, 112.23, 54.24, 53.94, 25.96, 24.55; ESI-MS: *m/z* 294 [M+2].

3-((4-Benzylpiperazin-1-yl)methyl)-5-bromo-1*H*-indole, 13d: Anal. Calcd for $C_{20}H_{22}BrN_3$: C, 62.50; H, 5.72; N, 10.93; Br, 20.57. Found: C, 62.24; H, 5.68; N, 10.85; Br, 20.64%. R_f = 0.58 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3394 (N-H aromatic), 3094 (C-H aromatic), 2109 (C-O), 1507 cm^{-1} (C=C, aromatic); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.76(s, 1H, NH), 7.29-7.09(m, 8H, Ar-H), 3.77(s, 2H, CH_2), 3.50(s, 2H, CH_2), 2.64-2.53(m, 8H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 135.96, 135.49, 129.73, 128.92, 128.54, 127.38, 122.93, 121.25, 121.12, 117.01, 113.35, 112.23, 60.13, 53.89, 53.82, 52.63; ESI-MS: *m/z* 386 [M+2].

5-Bromo-3-((4-phenylpiperazin-1-yl)methyl)-1*H*-indole, 13e: Anal. Calcd for $C_{19}H_{20}BrN_3$: C, 61.62; H, 5.40; N, 11.35; Br, 21.35. Found: C, 61.25; H, 5.35; N, 11.29; Br, 21.22%. R_f = 0.58 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3394 (N-H aromatic), 3094 (C-H aromatic), 2109 (C-O), 1507

cm^{-1} (C=C, aromatic); ^1H NMR ($CDCl_3$, 400MHz): δ 7.78-7.76(d, 1H, NH), 7.32-7.08(m, 9H, Ar-H), 3.73-3.70(t, 6H, CH_2), 2.52(s, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100MHz): δ 150.75, 134.86, 129.68, 129.22, 126.70, 125.03, 121.50, 120.30, 116.38, 113.21, 112.97, 108.52, 52.17, 51.96, 50.44, 49.08, 48.27; ESI-MS: *m/z* 368 [M-2].

4-((5-Methoxy-1*H*-indol-3-yl)methyl)morpholine, 14a: Anal. Calcd for $C_{14}H_{17}O_2N_2$: C, 68.29; H, 6.91; N, 11.38; O, 13.00. Found: C, 68.15; H, 6.88; N, 11.35; O, 12.95%. R_f = 0.58 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3398 (N-H aromatic), 3084(C-H aromatic), 1209 (C-O), 1507 cm^{-1} (C=C, aromatic); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.05(br s, 1H, NH), 7.26-6.85(m, 4H, Ar-H), 3.87(s, 3H, CH_2), 3.76(s, 2H, CH_2), 3.21(s, 2H, CH_2), 2.69(s, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100MHz)δ: 155.63, 128.83, 128.54, 122.91, 122.26, 112.17, 109.67, 102.13, 55.97, 66.87, 53.73, 54.13; ESI-MS: *m/z* 247 [M+1].

N-Ethyl-N-((5-methoxy-1*H*-indol-3-yl)methyl) ethylamine, 14b: Anal. Calcd for $C_{14}H_{19}ON_2$: C, 68.29; H, 6.91; N, 11.38; O, 13.00. Found: C, 68.15; H, 6.88; N, 11.35; O, 12.95%. R_f = 0.58 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3386 (N-H aromatic), 3052 (C-H aromatic), 2115 (C-O), 1512 cm^{-1} (C=C, aromatic); ^1H NMR ($CDCl_3$, 400MHz): δ 8.09(s, 1H, NH), 7.24-6.48(m, 4H, Ar-H), 3.86(s, 3H, CH_2), 3.74(s, 2H, CH_2), 2.57-2.55(t, 4H, CH_2), 1.17-1.15(t, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100MHz): δ 155.73, 128.25, 127.95, 122.82, 122.75, 112.56, 109.61, 102.67, 55.83, 54.52, 49.16, 13.42; ESI-MS: *m/z* 233 [M+1].

5-Methoxy-3-(piperidin-1-yl-methyl)-1*H*-indole, 14c: Anal. Calcd for $C_{15}H_{19}N_2O$: C, 73.77; H, 7.78; N, 11.47; O, 6.55. Found: C, 73.64; H, 7.75; N, 11.44; O, 6.52%. R_f = 0.58 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3356 (N-H aromatic), 3056 (C-H aromatic), 2104 (C-O), 1516 cm^{-1} (C=C, aromatic); ^1H NMR ($CDCl_3$, 400MHz): δ 8.15(s, 1H, NH), 7.24-6.80(m, 4H, Ar-H), 3.84(s, 3H, CH_2), 3.63(s, 2H, CH_2), 2.41(m, 4H, CH_2), 1.73-1.59(m, 6H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.36, 127.63, 128.32, 122.14, 122.45, 121.54, 109.76, 102.47, 55.53, 54.64, 54.24, 26.27; ESI-MS: *m/z* 245 [M+1].

3-((4-Benzylpiperazin-1-yl)methyl)-5-methoxy-1*H*-indole, 14d: Anal. Calcd for $C_{21}H_{25}ON_3$: C, 75.22; H, 7.46; N, 12.53; O, 4.77. Found: C, 75.15; H, 7.43; N, 12.49; O, 4.74%. R_f = 0.64 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3394 (N-H aromatic), 3094 (C-H aromatic), 2109 (C-O), 1507 cm^{-1} (C=C, aromatic); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.01(s, 1H, NH), 7.22-6.58(m, 9H, Ar-H), 3.85(s, 3H, CH_2), 3.72(s, 2H, CH_2), 3.56(s, 2H, CH_2), 2.48(s, 8H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 155.43, 135.65, 128.93, 128.54, 128.33, 127.53, 127.35, 122.42, 122.14, 121.53, 109.64, 102.43, 60.14, 55.59, 53.65, 52.66; ESI-MS: *m/z* 336 [M+1].

5-Methoxy-3-((4-phenylpiperazin-1-yl)methyl)-1*H*-indole, 14e: Anal. Calcd for $C_{20}H_{23}ON_3$: C, 74.76; H, 7.16; N, 13.08; O, 4.98. Found: C, 74.69; H, 7.12; N, 13.02; O, 4.93%. R_f = 0.64 (15% Ethyl acetate in Hexane); IR (KBr) υ max: cm^{-1}: 3360 (N-H aromatic), 3089 (C-H aromatic), 2116 (C-O), 1516 cm^{-1} (C=C, aromatic); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.17(br s, 1H, NH), 7.29-6.86(m, 9H, Ar-H), 3.88(s, 3H, CH_2), 3.77(s, 2H, CH_2), 3.22(s, 4H, CH_2), 2.70(s, 4H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 154.04, 149.62, 129.74, 128.33, 127.66, 122.38, 121.92, 121.53, 118.23, 114.35, 108.72, 101.82, 55.55, 53.87, 52.42, 51.65; ESI-MS: *m/z* 322 [M+1].

Pharmacological activity

In vitro antitubercular activity screening – MABA assay [17]

The compoundssynthesizedwere screened for preliminary anti-TB activity against pathogenic strains of *M. tuberculosis* $H_{37}Rv$ (ATCC 27294), using Microplate Alamar Blue assay (MABA). The $H_{37}Rv$ culture grown on Lowenstein Jensen (LJ) medium was suspended in sterile Middlebrook 7H9 broth supplemented with 0.2% glycerol and 10% OADC (Oleate-Albumin-Dextrose-Catalase) enrichment and a 1:20 dilution used as the inoculum for MABA. 200µl of sterile de-ionized water was added to all outer perimeter wells of sterile 96 well plates to minimize evaporation of medium in the test wells during incubation. To the 96 well plates 100µl of the Middlebrook 7H9 broth was transferred and serial dilutions of compounds were made directly on plate. The final drug concentrations tested were 100 to 0.2 µg/mL. The 96 well Plate was covered and sealed with para film and incubated at 37 ºC for five days. After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs. Appearance of blue color in the well was interpreted as no bacterial growth whereas pink color scored as growth. The lowest drug concentration at which the blue color changes to pink was prevented, that concentration is referred as Minimum Inhibitory Concentration (MIC).

The anti-tubercular activity results were presented in Table II.

In vitro Cytotoxicity screening by MTT Assay [18]

Among all the synthesized series of Compounds from **3a-e to 14a-e**, potent Antitb molecules **3c, 5e, 8e, 11e,12c, 12e, 13e, 14e** and non potent Tb molecules **6a, 10d** were selected randomly and screened for the Cytotoxicity by MTT Assay over A 549 (Lung Carcinoma) cell line. *In vitro* growth inhibition effect of test compounds were assessed by calorimetric or spectrophotometric method by the conversion of MTT into "Formazan blue" by living cells. Remove the supernatant from the plate and add fresh MEM solution and treat with different concentrations of compounds appropriately diluted with DMSO. Control group contains only DMSO. In our study 10, 20, 25, 30 and 50 µL from the stock solution (10mg/mL prepared in DMSO) was prepared and added to respective wells containing 100 uL of the medium. So, the final concentrations were 10, 20, 25, 30 and 50 µg/mL. After 48 hr incubation at 37 ^{0}C in a humidified atmosphere of 5% CO_2, stock solution of MTT was added to each well (20µl, 5mg per mL in sterile PBS) for further 4 hr incubation. The supernatant carefully aspirated the precipitated crystals of "Formazan blue' were solubilized by adding DMSO (100 µL) and Optical Density was measured at a wavelength of 570nm by using LISA plus. The results represent the mean of five readings. The % of the surviving cells in a concentration at which the OD of treated cells was reduced by 50% with respect to the untreated control was measured by the following formula and the results were tabulated in Table III.

$$\text{Surviving cells} = \frac{\text{Mean OD of test compound x 100}}{\text{Mean OD of control}}$$

In vitro antimicrobial screening [19]

The antimicrobial susceptibility testing was performed *in vitro* by Agar well diffusion method. The results were expressed by measuring the zone of inhibition. The zone of inhibition of the synthesized compounds was compared with standard drugs

Table III — MTT assay results of selective potent anti TB synthesized compounds

Compd	% Inhibition A 549 cell line	*M. tuberculosis* H37Rv MIC value (µg/mL)
3c	79.08	1.6
5e	86.08	1.6
6a	NA	25
8e	81.48	1.6
10d	NA	25
11e	77.16	1.6
12c	56.08	3.125
12e	59.42	1.6
13e	61.16	1.6
14e	61.48	1.6

Table II — Anti-tubercular activity of synthesized compounds **3a-e** to **11a-e** series

Compd	MIC (µg/mL)	Compd	MIC (µg/mL)	Compd	MIC (µg/mL)
3a	25	**7a**	25	**11a**	3.125
3b	25	**7b**	50	**11b**	3.125
3c	3.125	**7c**	50	**11c**	1.6
3d	1.6	**7d**	12.5	**11d**	6.25
3e	50	**7e**	25	**11e**	1.6
4a	12.5	**8a**	1.6	**12a**	25
4b	1.6	**8b**	1.6	**12b**	12.5
4c	1.6	**8c**	1.6	**12c**	3.125
4d	12.5	**8d**	3.125	**12d**	6.25
4e	50	**8e**	1.6	**12e**	1.6
5a	1.6	**9a**	25	**13a**	25
5b	1.6	**9b**	25	**13b**	25
5c	1.6	**9c**	25	**13c**	25
5d	6.25	**9d**	25	**13d**	25
5e	1.6	**9e**	25	**13e**	1.6
6a	25	**10a**	25	**14a**	25
6b	25	**10b**	3.125	**14b**	25
6c	25	**10c**	25	**14c**	25
6d	25	**10d**	25	**14d**	25
6e	25	**10e**	25	**14e**	1.6
Pyrazinamide	3.125	**Isoniazid**	6.25	**Ciprofloxacin**	3.125

Table IV — Antimicrobial activity of synthesized compounds at (100 μg) of **3a-e** to **14a-e** series

Compd	Zone of inhibition (in mm)*		Compd	Zone of inhibition (in mm)*		Compd	Zone of inhibition (in mm)*	
	S.aureus	*E.coli*		*S.aureus*	*E.coli*		*S.aureus*	*E.coli*
3a	11±1.83	12±0.11	**6a**	12±1.72	NI	**9a**	12±0.85	13±0.39
3b	11±1.59	13±0.49	**6b**	12±0.91	13±1.36	**9b**	12±0.94	13±0.29
3c	NI	11±0.95	**6c**	NI	NI	**9c**	11±0.78	13±0.58
3d	NI	NI	**6d**	11±0.15	13±0.79	**9d**	14±0.27	NI
3e	13±0.17	11±0.59	**6e**	12±0.72	13±0.49	**9e**	NI	14±1.79
4a	12±1.94	13±0.58	**7a**	12±1.44	13±0.84	**10a**	12±1.09	NI
4b	12±0.38	11±0.27	**7b**	13±0.82	NI	**10b**	11±0.84	12±0.72
4c	NI	NI	**7c**	12±0.49	13±2.42	**10c**	12±0.89	NI
4d	11±0.87	13±0.73	**7d**	NI	NI	**10d**	12±0.42	NI
4e	12±0.93	13±1.82	**7e**	14±1.39	13±1.58	**10e**	11±0.88	13±0.17
5a	NI	NI	**8a**	NI	NI	**11a**	NI	NI
5b	NI	NI	**8b**	NI	NI	**11b**	NI	NI
5c	NI	NI	**8c**	NI	NI	**11c**	NI	NI
5d	NI	NI	**8d**	NI	NI	**11d**	NI	NI
5e	NI	NI	**8e**	NI	NI	**11e**	NI	NI
12a	16±0.47	14±1.20	**13a**	15±1.14	14±0.38	**14a**	15±1.72	14±0.93
12b	15±0.74	16±0.39	**13b**	14±1.03	16±0.08	**14b**	17±0.37	14±0.82
12c	18±1.39	16±0.02	**13c**	16±0.39	14±0.22	**14c**	18±1.02	16±0.57
12d	14±0.27	15±0.44	**13d**	15±2.07	16±0.86	**14d**	15±0.34	14±1.17
12e	16±0.99	14±0.42	**13e**	14±0.48	15±0.92	**14e**	14±0.26	15±0.44
Rifampicin (Standard)	28±0.36	26±0.29		–		Control (DMSO)	–	–

* Mean±S.D. of three replications * NI = No Inhibition

Rifampicin and Ketoconazole. The test organisms used were: Gram-positive bacteria - *Staphylococcus aureus* (NCIM 2122), Gram-negative bacteria- *Escherichia coli* (NCIM 2137)and fungi - *Candida albicans* (NCIM 3102),*Aspergillus niger* (NCIM 652). The test organisms for antibacterial and antifungal screening were subculture using pre-sterilized nutrient agar and potato dextrose agar media respectively. The pre-inoculated medium was transferred aseptically into sterilized Petri plates (4-inch diameter). After solidification of the medium, cups of each of 5mm diameter were made with a sterilized cork borer and labeled accordingly. The standard and test compounds (10 mg) were dissolved in DMSO (10 mL) to give a concentration of 1000 μg/mL. All the compounds were tested at a dose level of 100 μg (0.1 mL) and DMSO was used as a control where as Rifampicin taken as a standard drug. None of the compounds had shown antifungal activity. The results obtained for antibacterial activity were triplicated and tabulated in Table III and Table IV.

Conclusion

In conclusion, the synthesis of a series of novel indole Mannich bases has been achieved from readily accessible starting materials in good yields. The newly synthesized compounds have been evaluated for *in vitro* antitubercular, antimicrobial and cytotoxicity activity. A total of 16 compounds (3d, 4b, 4c, 5a, 5b, 5c, 5e, 8a, 8b, 8c, 8e, 11c, 11e, 12e, 13e and 14e) have shown potent anti TB activity (MIC 1.6 μg/mL).This is highly significant when compared with the standard drugs pyrazinamide (MIC 3.125 μg/mL) and ciprofloxacin (3.125 μg/mL). Except the compounds3c, 5e, 8e and 11e remaining compounds are not very cytotoxic against A549 cell line. Hence, the remaining compounds may be considered for further chemical modification and lead optimizationstudies to obtain desired potency and safety window.

Supplementary Information

Supplementary information is available in the website http://nopr.niscair.res.in/handle/123456789/58776.

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