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ARTICLE

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An effective green and ecofriendly catalyst for synthesis of bis(indolyl)methanes as promising antimicrobial agents

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Abstract

An effective and suitable meglumine-catalyzed high-yielding process was considered and engaged for the synthesis of new bis(indolyl)methanes at ambient temperature under aqueous conditions. The catalytic reaction proceeds very smoothly. Clean reaction, ease of product isolation/purification, easily available reactant and ecofriendly reaction conditions are the notable advantages of the present methodology. All the title compounds were characterized by IR, 1H, ^{13}C NMR, and mass spectra. All the synthesized compounds were tested for antimicrobial activity, and the results indicated that most of the synthesized compounds exhibited excellent activity against the tested microorganisms. In this new series, compound **3l**, having nitro substituent on the aromatic ring, showed exceptional potent inhibitory activity against *Pseudomonas aeruginosa* and *Penicillium chrysogenum*.

1 | INTRODUCTION

Organic N-heterocycles such as BIM's (bisindole methanes) and their derivatives are advanced molecules and have become indispensable as potential bioactive molecules in modern medicinal chemistry. In the recent years, because of their biological versatility, the BIM's have emerged as potential target of synthesis for medicinal chemist. Bis(indolyl)methanes (BIMs) and their derivatives are promising nitrogen-containing compounds that are present in a variety of natural products and synthetic compounds that find applications in pharmaceutical drug research as HT6 receptor antagonist.[1] Derivatives of indoles find their current applications ranging from design and development, evaluation of novel drug delivery systems, catalysis to green chemistry, cosmetics, and agrochemicals.[2] Researchers are employing BIM's (bisindole methanes), Figure 1,[3] to distinguish promising drugs and avoid failures in potent drugs and have found to possess wide range of applications in the pharmaceutical industry.[4] In fact, the continued expansion of developments of BIM's are now driven mainly by the curiosity in exhibiting broad spectrum bioactivities such as antifungal,[5] antiviral,[6] antimicrobial,[7] anti-inflammatory[8] and antioxidant.[9] In addition, BIM's are evidently reported to exhibit anticancer activity, inhibit the proliferation of cancerous cells, including those of lung, pancreatic, colon, and cervical prostrate.[10] The most significant metabolite of indole-3-carbinol dimeric 3,3′-bis(indolyl methane) plays a significant role in inhibiting breast cancer.[11] Indole carbazole derivatives are known to function as triplet energy materials, while oxidized BIM's are used as colorimetric sensors and dyes.[12] Inferable from their explicit organic and pharmacological properties, there is growing interest for the development of substantial routes for synthesizing BIM's.[13]

Green chemistry is a transforming technology that provides access to novel techniques and robust catalysts, which enables to enhance conventional methodologies. In past decades, electrophilic substitution of indoles with carbonyl compounds is usually mediated by Lewis acids or bronsted acids, hetero polyacids, ionic liquids, use of

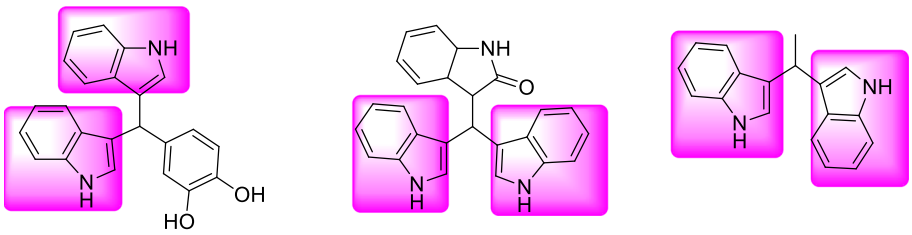


FIGURE 1 Bio-active BIMs derivatives [Color figure can be viewed at wileyonlinelibrary.com]

toxic reagents, volatile organic solvents, excess catalyst loading, and use of expensive catalysts are drawbacks of these methods.[14,15] To overcome these problems, in recent years, researchers are striving for developing advanced green chemical methodologies to reduce costs, hazards, waste and energy consumption using ecofriendly reagents, and economical reaction conditions for achieving successful synthetic procedures.[16] There are a wide range of interpretations about the organic practices that are consistent with the objectives of sustainable and green chemistry. In this context, the usage of environmentally benign solvents (ethylene glycol, glycerol, and water), solvent free conditions, and non-conventional methods such as microwaves, ultrasound and grinding constitute influential and green chemical protocols for cost effective synthesis.[17] In this study, we examine meglumine, which is an amino sugar sequestered from sorbitol-possessing chemical notation $C_7H_{17}NO_5$, as an efficient and reusable catalyst for the synthesis of novel BIM's derivatives[18] (Scheme 1).

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

Primarily, we immersed on improving the reaction conditions for the development of meglumine catalyzed procedure for the synthesis of new bis(indolyl)methanes. We designated indole **1a** (1.0 mmol) benzaldehyde **2a** (0.5 mmol) for the typical reaction (Scheme 1). The above reaction was carried out at room temperature in water in presence of $KHSO_4$ catalyst to inaugurate the actual ability of the catalyst. As presented in Table 1, it was detected that only a low yield of product was fashioned even after the reaction time was extended to 13 hours (Table 1, entry 1). Then, we tried to enhance the reaction conditions with altered catalysts, which might help to decrease the reaction time and develop the yield of the objective product. After observing numerous catalysts, it can be noticed that Cu $(OTf)_2$ displayed a slight catalytic activity to provide the product in a low yield (78%, Table 1, entry 5). An improvement was detected when numerous catalysts such as $KHSO_4$, $FeCl_3$, $LiClO_4$, and sulfamic acid were used. Finally, the preferred product was obtained in 35% to 67% yield (Table 1, entries 1-4). Additional investigation designated that meglumine was the greatest catalyst for this conversion and afforded the preferred product in 96% in 0.25 hours (Table 1, entry 6). Some solvents such as H_2O, MeOH, THF, PEG 400, and ethanol-water mixture were verified for the model reaction. It was detected that the mixture of water and ethanol was the most effective solvent, and the present reaction continued strongly, giving the highest yield. It is notable that when the reaction was done in solvent-free conditions, low yield of target product was acquired (Table 1, entry 7). Then, the influence of catalyst loading was calculated in the model reaction at room temperature in H_2O.

Apart from these, to find out the influence of the catalyst concentration, the reaction of indole (**1**) and benzaldehyde (**2**) was carried out with different concentrations of catalyst in H_2O at room temperature. The results presented in Table 2 showed that 10 mol% of meglumine is best to attain high yield in smaller reaction time. There is no effect by enhancing the quantity of catalyst on the product yield. On the other hand, employing a lower fraction of meglumine caused a reduced yield of the preferred product (Table 2).

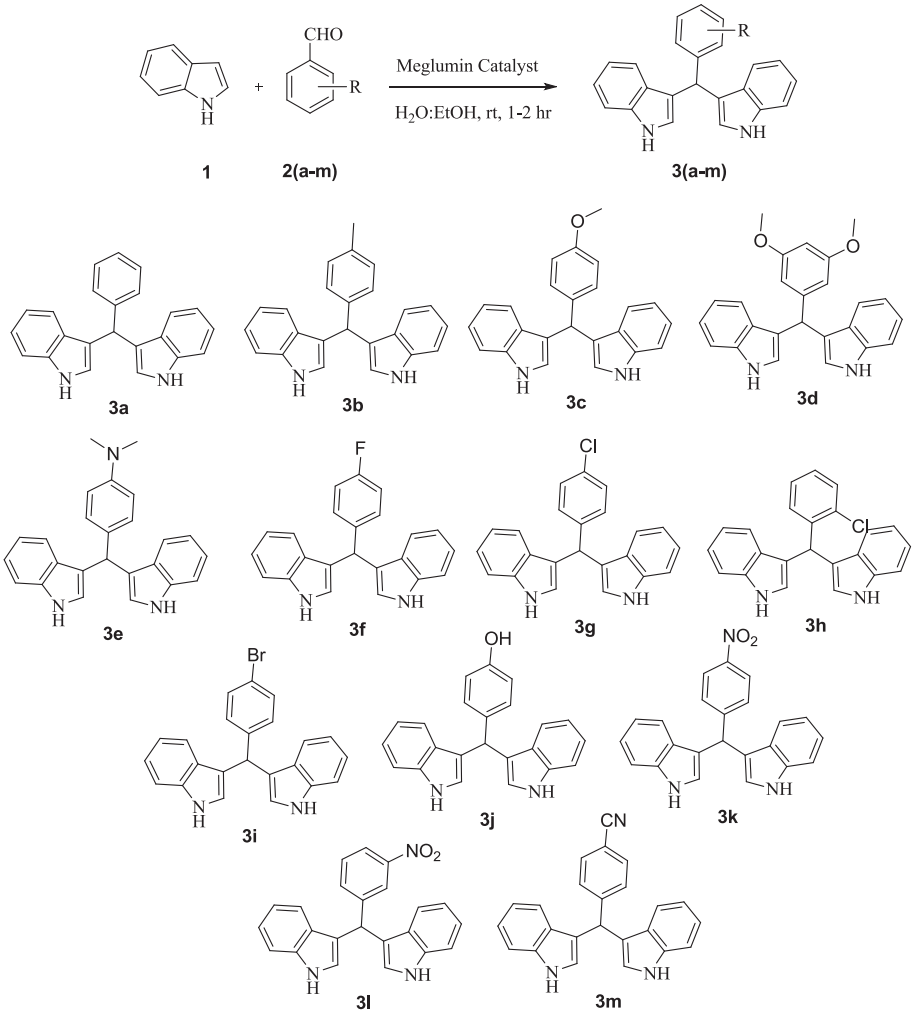
All compounds were accomplished in open atmosphere and are not sensitive to air and moisture. All the synthesized compounds have been characterized by analytical data.

3 | PHARMOCOLOGY

3.1 | Antimicrobial activity

3.1.1 | Antibacterial activity

The compounds **3(a-m)** were evaluated for antibacterial activity at four different concentrations 12.5, 25, 50, and 100 μg/well in contradiction of *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive bacteria), and *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* (Gram-negative



SCHEME 1 Synthesis of bis(indolyl)methane derivatives (**3a-m**)

TABLE 1 Optimization of the reaction conditions for the synthesis of **3(a-m)**

Entry	Catalyst	Solvent	Time (hrs)	Yield (%)
1	$KHSO_4$	H_2O	13	55
2	$FeCl_3$	H_2O	12	59
3	$LiClO_4$	H_2O	12	35
4	Sulfamic acid	H_2O	10	67
5	Cu $(OTf)_2$	H_2O	6	78
6	Meglumine	H_2O:EtOH (1:1)	0.25	96
7	Meglumine	No	5	16
8	Meglumine	MeOH	4	60
9	Meglumine	EtOH:H_2O (1: 9)	4	65
10	Meglumine	THF	4	41
11	Meglumine	PEG 400	4	52

TABLE 2 Effect of concentration of meglumine on the reaction of indole (**1**) with benzaldehyde (**2**)

Entry	Mole % of catalyst	Time (min)	Yield (%)
1	1	50	50
2	2.5	45	65
3	5	40	70
4	7.5	20	75
5	10	15	96
6	12.5	15	96
7	15	15	96

bacteria) by means of chloramphenicol as reference drug. The results of antibacterial activity revealed in Table 3 & Figure S1 designated that Gram-negative bacteria were more vulnerable in the direction of the verified compounds than Gram-positive ones. It was detected that the compounds (**3l** and **3m**) exhibited slightly higher activity than the respective. This may be because of the presence of electron-withdrawing groups, as well as electronegative atoms such as NO_2, CN, F, Cl, and Br. In fact, the compound **3l** displayed outstanding activity against

TABLE 3 The in vitro antibacterial activity of compounds **3(a-m)**

Compound	Zone of Inhibition, mm															
	Gram-positive bacteria								Gram-negative bacteria							
	Staphylococcus aureus				*Bacillus subtilis*				*Pseudomonas aeruginosa*				*Klebsiella pneumoniae*			
	12.5 μg/well	25 μg/well	50 μg/well	100 μg/well	12.5 μg/well	25 μg/well	50 μg/well	100 μg/well	12.5 μg/well	25 μg/well	50 μg/well	100 μg/well	12.5 μg/well	25 μg/well	50 μg/well	100 μg/well
3a	-	-	07 ± 3	08 ± 2	09 ± 3	10 ± 1	12 ± 1	14 ± 2	08 ± 3	10 ± 3	12 ± 2	14 ± 2	11 ± 2	13 ± 3	14 ± 2	15 ± 3
3b	-	-	10 ± 1	14 ± 3	07 ± 1	09 ± 2	11 ± 3	15 ± 2	11 ± 3	13 ± 3	15 ± 1	17 ± 2	10 ± 3	12 ± 3	14 ± 3	16 ± 2
3c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3e	-	-	12 ± 1	14 ± 3	8 ± 2	9 ± 3	11 ± 2	14 ± 1	12 ± 2	14 ± 1	16 ± 3	18 ± 3	11 ± 3	13 ± 2	15 ± 3	17 ± 2
3f	20 ± 3	21 ± 1	22 ± 3	24 ± 3	25 ± 1	26 ± 2	27 ± 3	29 ± 1	22 ± 1	23 ± 2	25 ± 2	27 ± 1	26 ± 2	28 ± 2	30 ± 3	32 ± 1
3g	15 ± 2	17 ± 2	19 ± 2	22 ± 3	18 ± 2	19 ± 1	20 ± 2	22 ± 2	17 ± 2	19 ± 1	21 ± 3	23 ± 3	20 ± 3	22 ± 3	24 ± 3	26 ± 3
3h	16 ± 1	17 ± 3	18 ± 1	20 ± 2	21 ± 2	22 ± 1	23 ± 1	25 ± 2	20 ± 3	22 ± 3	24 ± 1	26 ± 2	22 ± 2	24 ± 1	26 ± 3	28 ± 3
3i	12 ± 3	14 ± 1	16 ± 3	20 ± 1	13 ± 2	14 ± 2	15 ± 3	17 ± 3	15 ± 1	17 ± 2	19 ± 1	21 ± 2	16 ± 1	18 ± 1	20 ± 2	22 ± 1
3j	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3k	17 ± 1	19 ± 1	21 ± 3	23 ± 1	21 ± 3	22 ± 3	23 ± 3	25 ± 3	20 ± 3	22 ± 3	24 ± 1	26 ± 1	28 ± 2	30 ± 2	32 ± 2	34 ± 2
3l	22 ± 2	23 ± 1	24 ± 2	26 ± 3	28 ± 2	29 ± 2	30 ± 1	32 ± 3	24 ± 3	26 ± 3	28 ± 2	34 ± 3	32 ± 2	34 ± 3	36 ± 2	38 ± 2
3m	19 ± 3	20 ± 2	21 ± 3	23 ± 1	25 ± 3	26 ± 1	27 ± 2	29 ± 3	21 ± 2	23 ± 2	25 ± 3	27 ± 2	23 ± 1	25 ± 2	27 ± 2	29 ± 1
Chloramphenicol	30 ± 1	32 ± 2	35 ± 2	37 ± 1	32 ± 3	34 ± 2	36 ± 1	40 ± 1	25 ± 2	27 ± 2	29 ± 2	32 ± 1	38 ± 2	40 ± 2	42 ± 1	44 ± 2
Control (DMSO)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note. −, no activity; ±, standard deviation.

P. aeruginosa when associated with the standard drug chloramphenicol. This may be because of the presence of more electronegative nitro group on the aromatic ring. Furthermore, it was perceived that the compounds **3f**, **3g**, **3h**, **3i**, and **3k** presented considerable activity; **3a**, **3b**, and **3e** demonstrated reasonable activity; and the compounds **3c**, **3d**, and **3j** were inactive.

3.1.2 | Antifungal activity

All the tested compounds reserved the spore germination. In general, most of the compounds revealed slightly higher antifungal activity towards *Penicillium chrysogenum* than *Aspergillus niger*. Among all the compounds, **3l** displayed greater inhibitory activity, particularly against *P. chrysogenum* when compared with the standard drug ketoconazole (Table 4 & Figure S2). Besides, the compounds **3f, 3g, 3h, 3i,** and **3 k** showed good activity.

3.1.3 | Minimum inhibitory concentration, minimum bactericidal concentration, and minimum fungicidal concentration of the compounds 3f, 3l, and 3m

The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungi-

TABLE 4 The in vitro antifungal activity of compounds **3(a-m)**

	Zone of Inhibition (mm)							
	Aspergillus niger				*Penicillium chrysogenum*			
Compound	**12.5 μg/well**	**25 μg/well**	**50 μg/well**	**100 μg/well**	**12.5 μg/well**	**25 μg/well**	**50 μg/well**	**100 μg/ well**
3a	-	08 ± 1	10 ± 2	10 ± 3	-	-	11 ± 1	14 ± 3
3b	-	09 ± 2	11 ± 1	13 ± 1	-	12 ± 2	14 ± 3	16 ± 2
3c	-	-	-	-	-	-	-	-
3d	-	-	-	-	-	-	-	-
3e	-	09 ± 2	11 ± 2	13 ± 3	12 ± 1	13 ± 3	15 ± 2	17 ± 2
3f	25 ± 2	27 ± 1	29 ± 3	31 ± 3	29 ± 3	34 ± 2	36 ± 3	35 ± 2
3g	21 ± 2	23 ± 3	25 ± 3	27 ± 3	28 ± 3	30 ± 1	32 ± 2	34 ± 3
3h	22 ± 1	24 ± 3	26 ± 2	28 ± 2	25 ± 2	27 ± 3	29 ± 2	31 ± 3
3i	15 ± 2	17 ± 3	19 ± 1	21 ± 3	29 ± 2	32 ± 3	34 ± 2	37 ± 3
3j	-	-	-	-	-	-	-	-
3k	23 ± 3	25 ± 2	27 ± 1	29 ± 2	30 ± 2	32 ± 2	34 ± 1	36 ± 1
3l	27 ± 3	29 ± 2	31 ± 1	32 ± 3	34 ± 1	35 ± 2	37 ± 1	41 ± 2
3m	24 ± 1	26 ± 1	28 ± 1	30 ± 2	31 ± 2	33 ± 3	35 ± 3	38 ± 2
Ketoconazole	29 ± 3	31 ± 2	34 ± 1	37 ± 1	34 ± 1	36 ± 1	37 ± 2	39 ± 2
Control (DMSO)	-	-	-	-	-	-	-	-

Note. −, no activity; ±, standard deviation.

TABLE 5 MIC, MBC, and MFC of compounds **3f**, **3l,** and **3m**

	Minimum Inhibitory Concentration					
	MIC (MBC/MFC) μg/mL					
Compound	***Staphylococcus aureus***	***Bacillus subtilis***	***Pseudomonas aeruginosa***	***Klebsiella pneumoniae***	***Aspergillus niger***	***Penicillium Chrysogenum***
3f	50(200)	25(100)	12.5(50)	25(>100)	25(100)	12.5(50)
3l	25(>100)	25(100)	6.25(12.5)	12.5(50)	12.5(50)	12.5(25)
3m	50(>200)	50(>200)	25(100)	25(>100)	25(100)	50(>200)
Chloramphenicol	6.25	6.25	6.25	12.5	-	-
Ketoconazole	-	-	-	-	6.25	12.5

Abbreviations: MBC, minimum bactericidal concentration; MFC, minimum fungicidal concentration; MIC, minimum inhibitory concentration.

cidal concentration (MFC) values of the compounds tested are listed in Table 5. MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism. The MBC/MFC is the lowest concentration of antibiotic required to kill a particular bacterium/fungus. The MBC/MFC involves an additional set of steps performed once the MIC is determined. The antimicrobials are usually regarded as bactericidal/fungicidal if the MBC/MFC is not greater than four times the MIC.[19] The compound **3l** exhibited low MIC values when compared with **3f** and **3m**. In addition, MBC value in **3l** is 2 × MIC in case of *P. aeruginosa* and MFC value is 2 × MIC in case of *P. chrysogenum*. However, the other compounds showed bactericidal and fungicidal effects greater than 2 × MIC. The nitro substituted bis(indolyl)methane, **3l** exhibited excellent antibacterial activity against *P. aeruginosa* with an inhibition zone of 34 mm at 100 μg/well and MIC and MBC of 6.25 and 12.5 μg/mL, respectively. The compound **3l** also displayed strong antifungal activity against *P. chrysogenum* with an inhibition zone of 41 mm at 100 μg/well and MIC and MFC of 12.5 and 25 μg/mL, respectively. Moreover, it was observed that the compounds having nitro substituent on aromatic ring enhanced the activity when compared with all other compounds.

4 | CONCLUSION

In conclusion, we have successfully developed competent methodology for the synthesis of bis(indolyl)methanes at room temperature under aqueous conditions in high yields by reacting aldehydes with indoles in the presence of meglumine catalyst, which could be beneficial meriting further investigations. Our findings suggest that the present methodology will open a new route for the synthesis of bis(indolyl)methane derivatives. All the entitled compounds were characterized by IR, ^{1}H, ^{13}C NMR, mass spectra. All the lead compounds were tested for antimicrobial activity. The compound **3l** having nitro substituent on the aromatic ring is a potential and promising bioactive compound against *P. aeruginosa* and *P. chrysogenum*. We anticipate that simple synthetic accessibility and intriguing biological properties of new bis(indolyl)methane derivatives could address unmet challenges in the pharmaceutical industry.

5 | EXPERIMENTAL

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The homogeneity of the compounds was checked by TLC (silica gel H, BDH, hexane/ethyl acetate, 3:1). The ^{1}H NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Jeol JNM λ-400 MHz spectrometer. The ^{13}C NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Jeol JNM spectrometer operating at λ-100 MHz. High-resolution mass spectra were recorded on Micromass Q-TOF micro mass spectrometer using electro spray ionization. All chemical shifts were reported in δ (ppm) using TMS as an internal standard. The microanalyses were performed on a Perkin-Elmer 240C elemental analyzer. The temperature was measured by flexible probe throughout the reaction. The microanalyses were performed on a Perkin-Elmer 240C elemental analyzer (Massachusetts). The progress of the reaction was monitored by TLC using silica gel plates (silica gel 60 F254 0.25 mm), and components were visualized by observation under ultraviolet (UV) light (254 and 365 nm).

General procedure for the synthesis of bis(indolyl) methane derivatives (**3a-3m**).

To a stimulated solution of substituted indole (**1**) (2.0 mmol) and carbonyl compound (**2**) (1 mmol) in H_2O (10 mL), meglumine (0.10 mmol) was added and continued the stirring at room temperature for 15 minutes and monitored by TLC. After completion of the reaction, precipitate was formed, filtered, and washed with water. The subsequent product was found to be pure enough for characterization.

5.1 | 3,3′-(Phenylmethylene)bis(1H-indole) (3a)

Red solid; Yield: 94%; mp: 140°C-142°C; IR (KBr, cm^{-1}): 3315 (NH); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.89 (s, 2H, NH), 7.28-6.62 (m, 15H, Ar—H), 5.91 (s, 1H, CH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 144.4, 136.9, 129.7, 128.5, 127.4, 126.8, 123.4, 121.7, 120.3, 119.9, 118.6, 111.7 (aromatic carbons), 41.2 (CH) ppm. MS (EI) *m/z*: 322.4033 [M$^+$]; Anal. Calcd. for $C_{23}H_{18}N_2$: C, 85.68; H, 5.63; N, 8.69; Found: C, 85.78; H, 5.61; N, 8.90%.

5.2 | 3,3′-(p-Tolylmethylene)bis(1H-indole) (3b)

White solid; Yield: 96%; mp: 93°C-95°C; IR (KBr, cm^{-1}): 3362 (NH); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.42 (s, 2H, NH), 7.41-6.97 (m, 14H, Ar—H), 5.91 (s, 1H, CH), 2.73 (s, 3H, CH_3) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 141.6, 136.6, 135.3, 130.8, 128.1, 127.6, 124.7, 121.3, 119.8, 119.4, 118.1, 111.9 (aromatic carbons), 41.5 (CH), 21.9 (CH_3) ppm. MS (EI) *m/z*: 336.4298 [M$^+$]; Anal. Calcd. for $C_{24}H_{20}N_2$: C, 85.68; H, 5.99; N, 8.33; Found: C, 85.81; H, 5.97; N, 8.56%.

5.3 | 3,3′-((4-Methoxyphenyl)methylene) bis(1H-indole) (3c)

Brown solid; Yield: 92%; IR (KBr, cm^{-1}): 3359 (NH); ^1H NMR (DMSO-d_6, 400 MHz): δ 8.64 (s, 2H, NH), 7.73-7.14 (m, 14H, Ar—H), 5.71 (s, 1H, CH), 3.05 (s, 3H, OCH_3) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 158.3, 136.8, 135.4, 130.4, 127.5, 124.1, 122.5, 121.0, 120.6, 119.7, 113.9, 111.6 (aromatic carbons), 55.8 (OCH_3), 42.7 (CH) ppm. MS (EI) *m/z*: 352.1578 [M^+]; Anal. Calcd. for $C_{24}H_{20}N_2O$: C, 81.79; H, 5.72; N, 7.95; Found: C, 81.91; H, 5.74; N, 8.14%.

5.4 | 3,3′-((3,5-dimethoxyphenyl) methylene)bis(1H-indole) (3d)

Red solid; Yield: 89%; mp: 196°C-198°C; IR (KBr, cm^{-1}): 3323 (NH); ^1H-NMR (DMSO-d_6, 400 MHz) δ 7.89 (s, 2H, NH), 7.19-6.68 (s, 16H, Ar—H), 5.85 (s, 1H, CH), 3.76 (s, 6H, 2-OCH_3) ppm; ^{13}C-NMR (DMSO-d_6, 100 MHz) δ 161.4, 140.8, 136.3, 127.7, 123.9, 121.0, 119.7, 118.9, 116.5, 113.2, 111.8, 105.9, 97.6 (aromatic carbons), 55.8 (OCH_3), 42.4 (CH); MS (EI) *m/z*: 352.1578 [M^+]; Anal. Calcd. for $C_{25}H_{22}N_2O_2$: C, 78.51; H, 5.80; N, 7.32; Found: C, 78.62; H, 5.83; N, 7.57%.

5.5 | 3,3′-((4-N,N-dimethylaniline) methylene)bis(1H-indole) (3e)

Pink solid; Yield: 86%; mp 170°C-171°C; IR (KBr, cm^{-1}): 3346 (NH); ^1H NMR (DMSO-d_6, 400 MHz): δ 8.01 (s, 2H, NH), 7.61-6.68 (m, 14H, Ar—H), 5.88 (s, 1H, CH), 3.16 (s, 6H, CH_3) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 151.7, 137.5, 133.0, 130.2, 126.9, 123.7, 121.9, 118.5, 118.1, 117.3, 116.2, 111.1, 108.2 (aromatic carbons), 39.7 (CH), ppm. MS (EI) *m/z*: 365.4714 [M^+]; Anal. Calcd. for $C_{25}H_{23}N_3$: C, 82.16; H, 6.34 N, 11.50; Found: C, 82.28; H, 6.36; N, 11.75%.

5.6 | 3,3′-((4-Fluorophenyl)methylene) bis(1H-indole) (3f)

Red solid; Yield: 95%; IR (KBr, cm^{-1}): 3350 (NH); ^1H NMR (DMSO-d_6, 400 MHz): δ 7.59 (s, 2H, NH), 7.20-6.83 (m, 14H, Ar—H), 5.75 (s, 1H, CH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 162.5, 139.1, 136.2, 131.2, 128.1, 124.3, 123.1, 119.4, 119.1, 118.7, 116.0, 111.9 (aromatic carbons), 39.6 (CH) ppm. MS (EI) *m/z*: 340.3932 [M^+]; Anal. Calcd. for $C_{23}H_{17}FN_2$: C, 81.16; H, 5.03; N, 8.23; Found: C, 81.28; H, 5.05; N, 8.46%.

5.7 | 3,3′-((4-Chlorophenyl)methylene) bis(1H-indole) (3g)

Red solid; Yield: mp: 74°C-76°C, 88%; IR (KBr, cm^{-1}): 3328 (NH); ^1H NMR (DMSO-d_6, 400 MHz): δ 7.94 (s, 2H, NH), 7.06-6.64 (m, 14H, Ar—H), 5.88 (s, 1H, CH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 143.7, 137.4, 132.5, 131.6, 129.1, 128.9, 124.7, 123.3, 119.7, 119.1, 118.6, 111.2 (aromatic carbons), 39.6 (CH) ppm. MS (EI) *m/z*: 356.8479 [M^+]; Anal. Calcd. for $C_{23}H_{17}ClN_2$: C, 77.41; H, 4.80; N, 7.85; Found: C, 77.55; H, 4.81; N, 8.06%.

5.8 | 3,3′-((2-Chlorophenyl)methylene) bis(1H-indole) (3h)

Pink solid; Yield: 91%; mp: 71°C-73°C, IR (KBr, cm^{-1}): 3332 (NH); ^1H NMR (DMSO-d_6, 400 MHz): δ 7.71 (s, 2H, NH), 7.19-6.54 (m, 14H, Ar—H), 6.52 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 141.3, 136.2, 134.1, 131.2, 129.5, 126.6, 126.1, 125.7, 124.6, 122.7, 119.7, 119.3, 118.4, 111.3 (aromatic carbons), 36.2 (CH) ppm. MS (EI) *m/z*: 356.8479 [M^+]; Anal. Calcd. for $C_{23}H_{17}ClN_2$: C, 77.41; H, 4.80; N, 7.85; Found: C, 77.54; H, 4.82; N, 8.09%.

5.9 | 3,3′-((4-Bromophenyl)methylene) bis(1H-indole) (3i)

Pink solid; Yield: 87%; mp: 111°C-113°C, IR (KBr, cm^{-1}): 3341 (NH); ^1H NMR (DMSO-d_6, 400 MHz): δ 7.88 (s, 2H, NH), 7.07-6.62 (m, 14H, Ar—H), 5.87 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 144.2, 137.8, 132.4, 131.6, 128.4, 124.8, 123.2, 120.7, 119.9, 119.5, 119.1, 111.2 (aromatic carbons), 39.9 (CH) ppm. MS (EI) *m/z*: 401.2979 [M^+]; Anal. Calcd. for $C_{23}H_{17}BrN_2$: C, 68.84; H, 4.27; N, 6.98; Found: C, 68.97; H, 4.29; N, 7.19%.

5.10 | 3,3′-((4-Hydroxyphenyl)methylene) bis(1H-indole) (3j)

Red solid; Yield: 84%; IR (KBr, cm^{-1}): 3318 (NH), 3554 (OH); mp: 121°C-123°C, ^1H NMR (DMSO-d_6, 400 MHz): δ 10.8 (s, 2H, NH), 9.04 (s, 1H, OH), 7.06-6.71 (m, 14H, Ar—H), 5.92 (s, 1H, CH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 156.1, 136.6, 135.4, 129.7, 126.3, 123.9, 120.8, 119.5, 118.2, 117.8, 114.3, 111.2 (aromatic carbons), 38.9 (CH) ppm; MS (EI) *m/z*: 338.4020 [M^+]; Anal. Calcd. for $C_{23}H_{18}N_2O$: C, 81.63; H, 5.36 N, 8.28; Found: C, 81.76; H, 5.38; N, 8.50%.

5.11 | 3,3′-((4-Nitrophenyl)methylene) bis(1H-indole) (3k)

Yellow solid; Yield: 90%; mp: 218°-220°C, IR (KBr, cm^{-1}): 3366 (NH); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.57 (s, 2H, NH), 7.09-6.87 (m, 14H, Ar—H), 5.91 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 153.7, 146.7, 135.5, 128.4, 127.2, 122.2, 121.8, 120.9, 118.8, 118.2, 116.3, 114.2 (aromatic carbons), 40.1 (CH) ppm. MS (EI) *m/z*: 367.4006 [M^+]; Anal. Calcd. for $C_{23}H_{17}N_3O_2$: C, 75.19; H, 4.66 N, 11.44; Found: C, 75.33; H, 4.68; N, 11.65%.

5.12 | 3,3′-((3-Nitrophenyl)methylene) bis(1H-indole) (3l)

Yellow solid; Yield: 93%; mp: 220°C-222°C, IR (KBr, cm^{-1}): 3372 (NH); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.83 (s, 2H, NH), 7.12-6.97 (m, 2H, Ar—H), 5.88 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 147.5, 145.4, 136.9, 133.9, 129.6, 126.7, 123.9, 124.6, 123.3, 122.5, 119.1, 119.4, 118.3, 111.3 (aromatic carbons), 40.1 (CH). MS (EI) *m/z*: 367.4035 [M^+]; Anal. Calcd. for $C_{23}H_{17}N_3O_2$: C, 75.19; H, 4.66 N, 11.44; Found: C, 75.29; H, 4.67; N, 11.64%.

5.13 | 3,3′-((4-Phenylnitrile)methylene) bis(1H-indole) (3m)

Slight brown solid; Yield: 85%; mp 205°C-207°C; IR (KBr cm^{-1}): 3357 (NH), 1592 (C═N); ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.96 (s, 2H, NH), 7.64-6.89 (m, 14H, Ar—H), 5.97 (s, 1H, CH) ppm; ^{13}C NMR (DMSO-d_6, 100 MHz): δ 150.9, 136.8, 132.7, 129.5, 126.4, 123.3, 121.5, 118.9, 118.8, 118.3, 116.7, 111.6, 108.9, 38.8 (CH), ppm (aromatic carbons). MS (EI) *m/z*: 347.4124 [M^+]; Anal. Calcd. for $C_{24}H_{17}N_3$: C, 82.97; H, 4.93 N, 12.10; Found: C, 83.06; H, 4.94; N, 12.33%.

6 | ANTIMICROBIAL STUDIES

The compounds **3(a-m)** were dissolved in DMSO at different concentrations of 12.5, 25, 50, and 100 μg/well. Bacterial strains *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *K. pneumoniae* and fungi *A. niger* and *P. chrysogenum*.

The in vitro antimicrobial studies were carried out by agar well diffusion method against test organisms.[20] Nutrient broth (NB) plates were swabbed with 24-hour-old broth culture (100 μL) of test bacteria. Using the sterile cork borer, wells (6 mm) were made into each petriplate. The compounds were dissolved in DMSO of 5 mg/mL and from this, 2.5, 5, 10, and 20 μL (12.5, 25, 50, 100 μg/well) were added into the wells by using sterile pipettes. Simultaneously, the standard antibiotics, chloramphenicol for antibacterial activity and ketoconazole for antifungal activity (as positive control), were tested against the pathogens. The samples were dissolved in DMSO, which showed no zone of inhibition acts as negative control. The plates were incubated at 37°C for 24 hours for bacteria and at 28°C for 48 hours for fungi. After appropriate incubation, the diameter of the zone of inhibition of each well was measured. Duplicates were maintained, and the average values were calculated for eventual antimicrobial activity.

Broth dilution test is used to determine MIC of the previously mentioned samples.[21,22] Freshly prepared nutrient broth was used as diluents. The 24-hour-old culture of the test bacteria *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *K. pneumoniae* and the test fungi *A. niger* and *P. chrysogenum* were diluted 100-fold in nutrient broth (100 μL bacterial cultures in 10 mL NB). The stock solution of the synthesized compounds was prepared in dimethyl sulfoxide (DMSO) by dissolving 5 mg of the compound in 1 mL of DMSO. Increasing concentrations of the test samples (1.25, 2.5, 5, 10, 20, and 40 μL of stock solution contains 6.25, 12.5, 25, 50, 100, and 200 μg of the compounds) were added to the test tubes containing the bacterial and fungal cultures. All the tubes were incubated at 37°C for 24 hours for bacteria and at 28°C for 48 hours for fungi. The tubes were examined for visible turbidity and using NB as control. Control without test samples and with solvent was assayed simultaneously. The lowest concentration that inhibited visible growth of the tested organisms was recorded as MIC.

To determine the MBC[23] and MFC[24] for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes, which did not show any growth and inoculated on sterile nutrient broth (for bacteria) and PDA (for fungi) by streaking. Plates inoculated with bacteria and fungi were incubated at 37°C for 24 hours and at 28°C for 48 hours, respectively. After incubation, the lowest concentration was noted as MBC (for bacteria) or MFC (for fungi), at which no visible growth was observed.

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