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Original Research Article

Antibacterial Activity of Bis(Indolyl) Methane Derivatives against *Staphylococcus Aureus*

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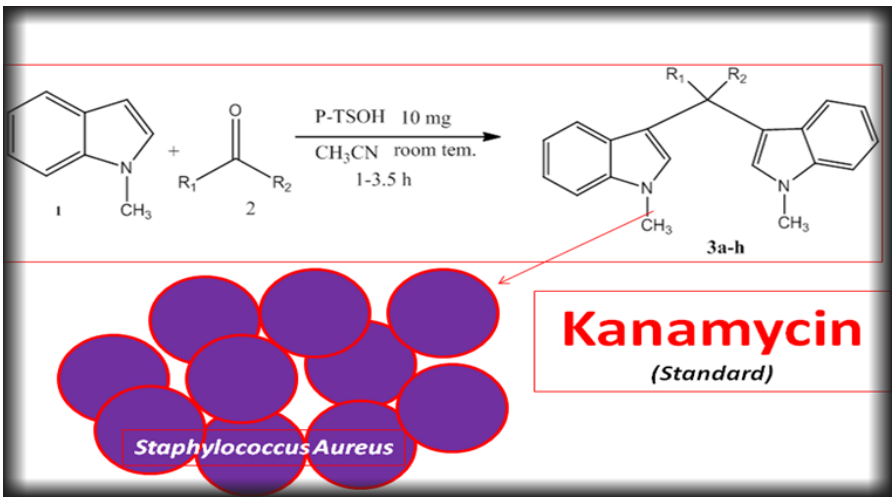
ABSTRACT

In the present study, we successfully synthesized a series of indoles with a variety of aromatic aldehydes *via* one-pot route. The excellent results of the bis(indoly) methane (BIM) derivatives was obtained at the presence of 5.0 mol% *p*-toluene sulfonic acid in acetonitrile at room temperature using conventional process. All the as-synthesized compounds were bioactive. The synthesized BIM derivatives were evaluated for the antibacterial activity (*in vitro*) against the *Staphylococcus aureus*, and the results were compared with the standard Kanamycin. The results confirmed that, majority of the as-synthesized compounds revealed splendid antibacterial activity.

GRAPHICAL ABSTRACT



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Introduction

The pharmaceutical and biological importance of *N*-heterocycles which constitute an important class of indoles have attracted the attention of researchers [1]. Indole and their derivatives were found to have valuable applications in the area of pharmacology, materials, biomedical, and agrochemicals [2–4]. In fact bis(indolyl) alkanes possess all-purpose of biological studies *viz.,* cytotoxic [5,6], antitumor [7], antimicrobial [8], anti-inflammatory [9], antioxidant [10,11]. BIM, a prominent derivative of indoles are very active cruciferous materials that help in evaluating estrogen metabolism and covers apoptosis in human cancer cells [12]. Some of the important drugs involving indole as a functional moiety are Indomethacin [13], Tenidap [14], Sumatriptan and Zolmitriptan [1]. Because of the pharmacological activities and the presence of the indole component in diverse natural drugs, a substantial efforts has been made for the synthetic protocols for the design and synthesis of bis(indolyl) alkanes [15-19]. Moreover, most of the conventional processes for the formation of bis(indolyl) methane involves one equivalent of aromatic aldehydes and two equivalents of indoles in suitable solvent *via* acid catalyzed multi-component condensation reaction. Consequently, it is the acid which is responsible for the formation of indole molecule by attacking the aldehydes carbon and increasing the electrophilicity of aldehydes carbon through coordinating with oxygen atom of –CHO functional group. However, using the toxic reagents, high temperature, and alterable organic solvents are among the most common drawbacks of this procedure [20]. In consequence, there is a requisite for a novel, coherent, and rational synthetic schemes in line with green chemistry for the synthesis of heterocyclic compounds. Herein, we explored the exercise of *p*-toluenesulfonic acid as a efficient catalyst for the synthesis of BIM derivatives and evaluated their antimicrobial activity against the *Staphylococcus aureus*.

Experimental

Chemicals and apparatus

All the chemicals were purchased from the Sigma-Aldrich and Merck India and were used as such. Melting points were measured using the open capillary tube. TLC was used for evaluating the purity of the compounds (silica gel 60 F254) using the hexane/ethyl acetate (7:3) on F254 silica-gel pre-coated sheets (Merck). FTIR Bruker Alpha II ECO-ATR spectrometer was used to record the FTIR spectra's. The ^{1}H-NMR spectra were studied on a JEOL 500 MHz spectrometer in DMSO-d_6 using TMS as the internal standard and ^{13}C-NMR spectra were recorded on JEOL 125MHz using DMSO-d_6 as solvent.

*General procedure for synthesis of bim derivatives under conventional method (**3a-e**)*

A stirred solution of substituted indole (1) (2.0 mmol), a variety of aromatic aldehydes (2) (1.1 mmol) in acetonitrile (5 mL) and 5.0 mol% of *p*-toluene sulfonic acid (as a catalyst) were mixed. The reaction mixture was vigorously stirred at room temperature for 1-3 H to obtain the excellent yields of BIM. The organic layer was washed with NaCl + H2O, dried over Na2SO4 and secluded by filtration. The solvent was separated under the vacuum, and the ultimate crude product was decontaminated by column chromatography (silica gel, 100-200 mesh) using the n-hexane-ethyl acetate (7:3) as an eluent.

In vitro biological evaluation assay

Antibacterial activity

Well diffusion method was used to evaluate the antibacterial activity of BIM's on bacterial species [21]. Antibacterial activity of the

prepared compound or desired compounds were assessed against the microbial strain *Staphylococcus aureus* (MTCC 1144), purchased from the microbial type culture collection institute in Chandigarh, (India). Broth medium of Luria bertani was inoculated with strain MTCC 1144 and cultivate till logarithmic phase (A600 nm=1) & spread 100 µL of 24 h old culture on the solid surface of Mueller Hinton medium with the help of *L*-shaped spreader. Subsequently wells of 8mm diameter were punched into the agar medium and filled with 100 µL (50,100, 150, 200 µg/mL) of desired compounds and allowed to diffuse at room temperature for an hour. The plates were then incubated in the upright position at 37 °C for 24 h. Same amount (100 µL) of DMSO was used as a negative control while as standard antibiotic discs of Kanamycin (30 mcg) were used as positive control. Following this bacterial zone-inhibition diameters were observed and measured carefully.

Results and discussion

p-Toluene sulfonic acid was used as a potential catalyst by most of the synthetic chemists due to its cost-effective nature and availability. One-pot method was employed for the synthesis of BIM derivatives. The reaction conditions were optimized for the preparation of BIM derivatives **3a-h** (Scheme 1). The reaction of indole (1) (2.0 mmol) and benzaldehyde (2) (1.1 mmol) was carried out for the synthesis of **3a** by employing different catalysts (Table 1) at the presence of solvent acetonitrile. The *p*-toluene sulfonic acid showed highest catalytic activity with a yield of 90%. The optimized concentration of the catalyst *p*-toluene sulfonic acid was also determined (Table 2), and the results procured stipulated that 5.0 mol% of *p*-toluene sulfonic acid was optimum to accomplish high yield in stunted reaction time. Moreover the reaction conditions were optimized by diverse solvents (Table 3) as well; in which acetonitrile was found to be most effective, as the yield was high and reaction time was 2.5 h at room temperature. In accordance to the optimized reaction conditions, various BIM derivatives (Table 4) were synthesized through conventional method and were obtained in good percentage yield. All the synthesized compounds **3a-h** was examined for their antibacterial activity using the Kanamycin as standard. Out of all compounds examined, compound **3d** exhibited the highest antibacterial activity against the *Staphylococcus aureus.*

Scheme 1. Synthesis of bis(indolyl) methane derivatives (**3a-h**)

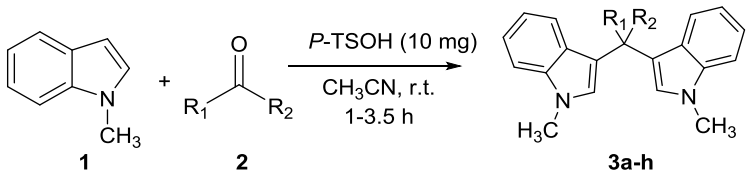


Table 1. Reaction of indole (1) with benzaldehyde (2) in the presence of various catalysts using acetonitrile as a solvent

Catalyst[a]	Time (h)	Yield (%)[b]
p-Toluene sulfonic acid	2.5	90
$LiClO_4$	10	40
$FeCl_3$	9	65
H_3BO_3	12	62
H_3NSO_3	10	70
Fe_3O_4	2.5	78

[a] 5.0 mol% of the catalyst was used, [b] Yields referred to the isolated yield

Table 2. Optimization of *p*-toluene sulfonic acid on the reaction of indole (1) with benzaldehyde (2)

Catalyst (Mol %)	Time (h)	Yield (%)[a]
1.0	3.5	55
2.5	3.0	78
5.0	2.5	90
7.5	2.5	90
10	2.5	90

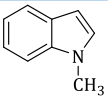
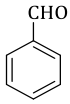
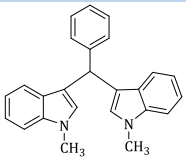
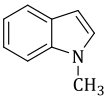
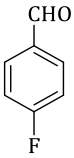
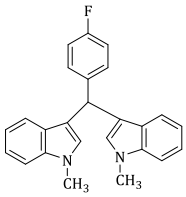
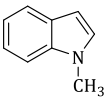
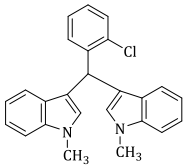
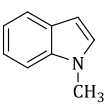
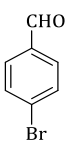
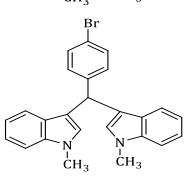
[a] Yields referred to the isolated yield

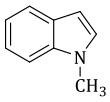
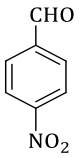
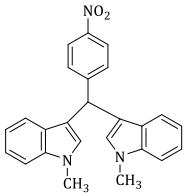
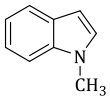
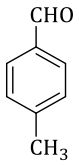
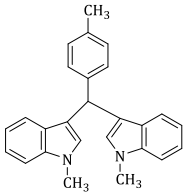
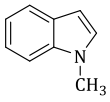
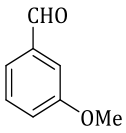
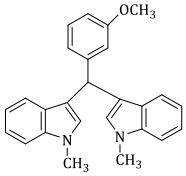
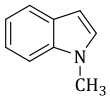
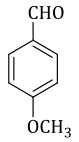
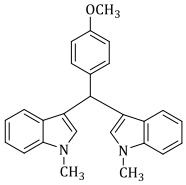
Table 3. Optimization of solvents in the reaction of indole (1) with benzaldehyde (2) catalyzed by *p*-toluene sulfonic acid

Entry	Solvent	Time (h)	Yield (%)[a]
1	Water	9	72
2	Methanol	18	60
3	Ethanol	9.5	62
4	Ethanol-water (1:1)	8	65
5	THF	7	80
6	Acetonitrile	2.5	90
7	Acetonitrile-water (3:1)	3	86
8	DCM	16	50
9	Toluene	22	40
10	1,4-Dioxane-water (3:1)	13	55

[5] THF: Tetrahydrofuran, [8] DCM: Dichloromethane, [a] Yields referred to the isolated yield

Table 4. Reaction time and yield of BIM derivatives **3a-h**

Entry	Substituted Indole	carbonyl compound	products	Time (h)	Yield (%)
3a				2.5	88
3b				2.5	82
3c				2.5	85
3d				3	86

3e				2	90
3f				3	84
3g				3.5	70
3h				3.5	72

In vitro antibacterial activity

As seen in Table 5, compounds **3a**, **3b**, **3c**, **3e**, **3g**, and 3h demonstrated moderate growth inhibitory activities compared to Kanamycin as a standard against the *Staphylococcus aureus* while as **3d** has exhibited a highest antibacterial activity with MIC value of 40 µg/mL. The BIM moiety in these derivatives is replaced with electron withdrawing groups. However, methyl-substituted bis(indolyl) methanes **3f** displayed weak antibacterial activity as revealed by the diameter of its inhibition zone (MIC value of 120 µg/mL). The, electron withdrawing groups particularly Bromo group strengthen the antibacterial activity of as prepared -(Phenylmethylene) bis(1-methyl-1H-indole) which might be attributed to the binding of molecules at the target active sites.

Table 5. Antibacterial activity of the as-synthesized compounds **3a-h**
Diameter of zone of inhibition (mm) for 3,3'-(Phenylmethylene) bis(1-methyl-1H-indole)

Compound code	Molecular weight	Zone of inhibition (mm)*	Minimum inhibitory concentration (µg/ml)
3a	350.46	17.10±0.89	80
3b	368.45	16.18±0.23	>80
3c	384.90	10.32±0.90	160
3d	429.35	22.82±1.35	40
3e	867.45	15.8±0.18	140
3f	364.48	10.02±0.14	>160
3g	380.48	11.04±017	150
3h	380.48	11.01±012	160
Kanamycin	484.5	25.70±0.64	40

*Values are given as mean standard deviation (n=3)

Spectral Data for Selected Compounds

*3,3'-(Phenylmethylene)bis(1-methyl-1H-indole) (**3a**)*: Barn Reddish solid, mp 182–184 °C, FTIR (cm^{-1}): 1625 (C = C), 2940 (C-H), ^1H NMR (500 MHz, DMSO-d_6): δ 5.60 (s, 1H, Ar-CH), 3.70 (s, 6H, 2 × CH_3), 6.98–7.78 (m, 13H, Ar-H), 6.92 (s, 2H, Ar–H), ppm, ^{13}C NMR (125 MHz, DMSO-d_6): δ 39.2 (Ar-CH), 32.7 (N-CH_3), 144.1, 136.1, 128.6, 128.4, 128.1, 127.1, 125.2, 124.1, 120.2, 119.4, 114.1, 110.4, ppm (aromatic carbons).

*3,3'-((4-fluorophenyl)methylene)bis(1-methyl-1H-indole) (*3b*)*: Sepia solid, mp 132–134 °C, FTIR (cm^{-1}): 1620, 1508 (C = C) 2980 (C-H), ^1H NMR (500 MHz, DMSO-d_6): δ 5.65 (s, 1H, Ar-CH), 3.72 (s, 6H, 2× CH_3), 6.99–7.81 (m, 12H, Ar-H), 7.1 (s, 2H, Ar–H), ppm, ^{13}C NMR (125 MHz, DMSO-d_6): δ 39.2 (Ar-CH), 33.9 (N-CH_3), 146.1, 138.1, 129.6, 128.8, 129.1, 127.1, 126.2, 124.1, 122.2, 119.4, 116.1, 111.4, ppm (aromatic carbons).

*3,3'-((2-chlorophenyl)methylene)bis(1-methyl-1H-indole) (**3c**)*: solid, mp 128–130 °C, FTIR (cm^{-1}): 1624 (C = C), 2967(C-H) ,^1H NMR (500 MHz, DMSO-d_6): δ 5.62(s, 1H, Ar-CH), 3.98 (s, 6H, 2× CH_3), 6.98–7.79 (m, 12H, Ar-H), 6.98 (s, 2H, Ar–H), ppm, ^{13}C NMR (125 MHz, DMSO-d_6): δ 39.2 (Ar-CH), 32.9 (N-CH_3), 144.7, 137.1, 128.9, 128.6, 128.4, 127.2, 126.2, 124.1, 121.2, 118.4, 11.1, 110.1, ppm (aromatic carbons).

*3,3'-((4-bromophenyl)methylene)bis(1-methyl-1H-indole) (**3d**)* Sangria solid, mp 182–184 °C, FTIR (cm^{-1}): 1619, 1508, 1450 (C = C), ^1H NMR (500 MHz, DMSO-d_6): δ 5.62 (s, 1H, Ar-CH), 3.84 (s, 6H, 2× CH_3), 6.98–7.78 (m, 12H, Ar-H), 6.84 (s, 2H, Ar–H), ppm, ^{13}C NMR (125 MHz, DMSO-d_6): δ 35.2 (Ar-CH), 32.6 (N-CH_3), 144.1, 136.1, 128.6, 128.4, 128.1, 127.1, 125.2, 124.1, 120.2, 119.4, 114.1, 110.4, ppm (aromatic carbons).

*3,3'-((4-nitrophenyl)methylene)bis(1-methyl-1H-indole) (**3e**)* Sangria solid, mp 262–264 °C, FTIR (cm^{-1}): 1628 (C = C), 2940 (C-H) , ^1H NMR (500 MHz, DMSO-d_6): δ 5.69 (s, 1H, Ar-CH), 3.70 (s, 6H, 2× CH_3), 6.98–7.9 (m, 12H, Ar-H), 6.92 (s, 2H, Ar–H), ppm, ^{13}C NMR (125 MHz, DMSO-d_6): δ 40.2 (Ar-CH), 32.7 (N-CH_3), 148.1, 137.1, 129.6, 130.4, 131.1, 129.1, 127.2, 126.1, 125.2, 120.4, 118.1, 114.4, ppm (aromatic carbons).

*3,3'-(p-tolylmethylene)bis(1-methyl-1H-indole) (**3f**)* Persian solid, mp 144–146 °C, FTIR (cm^{-1}): 1625 (C = C), 2960 (C-H), ^1H NMR (500 MHz, DMSO-d_6): δ 2.28 (s, 3H, Ar-CH_3), 5.76 (s, 1H, Ar-CH), 3.69 (s, 6H, 2× CH_3), 2.29 (s, 3H, CH_3). 6.98–7.78 (m, 12H, Ar-H), 6.5(s, 2H, Ar–H), ppm, ^{13}C NMR (125 MHz, DMSO-d_6): δ 39.0 (Ar-CH), 31.7 (N-CH_3), 20.9 (CH_3) 141.1, 136.1, 133.9, 126.6, 128.1, 126.9, 126.5, 121.9, 119.4, 117.2, 115.4, 109.1, ppm (aromatic carbons).

*3,3'-((3-methoxyphenyl)methylene)bis(1-methyl-1H-indole) (**3g**)* Redwood solid, mp 125–127 °C, FTIR (cm^{-1}): 1626 (C = C), ^1H NMR (500 MHz, DMSO-d_6): δ 3.72 (s,3H, -OCH3), 5.54 (s, 1H, Ar-CH), 2.5 (s, 6H, 2× CH_3), 6.76–7.79 (m, 12H, Ar-H), 6.5 (s, 2H, Ar–H) ppm, ^{13}C NMR (125 MHz, DMSO-d_6): δ 46.4 (Ar-CH), 12.4 (indolyl-CH3), 49.8 (Ar-OCH3), 111.4, 112.4, 114.3, 118.7, 119.6, 121.7, 125.4, 126.2, 130.4, 131.2, 133.6, 136.2, 139.5, 148.7 ppm (aromatic carbons).

*3,3'-((4-methoxyphenyl)methylene)bis(1-methyl-1H-indole) (**3h**)* Red solid, mp 130–132 °C, FTIR (cm^{-1}): 1628 (C = C), ^1H NMR (500 MHz, DMSO-d_6): δ 3.72 (s,3H, -OCH3), 5.54 (s, 1H, Ar-CH), 2.6 (s, 6H, 2× CH_3), 6.78–7.79 (m, 12H, Ar-H), 6.2 (s, 2H, Ar–H) ppm, ^{13}C NMR (125 MHz, DMSO-d_6): δ 42.4 (Ar-CH), 32.4 (N-CH_3), 49.7 (Ar-OCH3), 111.4, 112.4, 114.3, 118.7, 119.6, 121.7, 125.4, 126.2, 130.4, 133.6, 136.2, 146.7 ppm (aromatic carbons).

Conclusion

Synthesis of BIM derivatives **3a-h** was explored successfully by using the one-pot synthetic route. All the as-synthesized compounds were assessed for the antibacterial activity against *Staphylococcus aureus*, interestingly; compound **3a**, **3b**, **3g**, **3e** exhibited acceptable antibacterial activity while as **3d** exhibited the excellent antibacterial activity against *Staphylococcus aureus*. The results obtained can open the plausible way for the pharmaceutical companies for producing highly active antibiotics against the life threatening pathogens.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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