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Synthesis, antibacterial and anti-inflammatory activity of bis(indolyl)methanes

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ABSTRACT

A series of bioactive bis(indolyl)methanes are synthesized by one-pot green reaction of indole with various substituted aldehydes by microwave irradiation under solvent free conditions. The antibacterial activity against *Staphylococcus aureus* and anti-inflammatory activity of the synthesized bis(indolyl)-methanes are evaluated *in vitro* and compared to standard drugs tetracycline and diclofenac, respectively. The majority of the compounds showed good antibacterial and anti-inflammatory activity. Interestingly, compounds **3j**, **3i**, **3k** and **3g** exhibited much higher anti-inflammatory activity than the standard diclofenac drug and thus qualify for clinical trials to be used as an anti-inflammatory compound.

1. Introduction

Staphylococcus aureus, a gram-positive coccal bacterium, infects tissues when the skin or mucosal barriers are breached. Its infections spread through contact with pus from an infected wound, skin-to-skin contact with an infected person by producing hyaluronidase that destroys tissues and contact through the objects used by an infected person. It is estimated that 20% of human population are long term carriers of *S. aureus* [1]. It remains still as one of the five most common causes of nosocomial infections and is often the cause of post-surgical wound infections. *S. aureus*, the chief culprit, is also a common source of community acquired infections, and causes illnesses that range from minor skin infections and abscesses to life-threatening diseases such as severe pneumonia, meningitis, joint infections, and heart and blood stream infections [2]. Most current bactericidal compounds inhibit DNA, RNA, cell walls, and protein synthesis [3]. In the case of *S. aureus*, infection occurs by inhibition of cell wall synthesis by non-lytic cell death. Drug resistant bacterial infections are becoming more prevalent and are a major challenging health issue faced today. This rise of drug-resistance has limited our repertoire of effective antimicrobials that could combat/overcome the problem of drug-resistance.

Inflammation is a complex biological response of vascular tissues to harmful stimuli like pathogens, cells, and irritants [4]. The therapeutic anti-inflammatory effect of nonsteroidal anti-inflammatory drugs (NSAIDs) occurs through inhibition of prostaglandin biosynthesis and the selective inhibition of cyclo-oxygenase. NSAIDs are able to overcome the side-effects of steroid therapy through this mechanism [5,6]. Further, NSAIDs have many drawbacks such as gastrointestinal toxicity, etc. [6]. In view of this, a new generation of bis(indolyl)methanes as therapeutic agents which nullify these drawbacks are developed. The indole is the most ubiquitous heterocyclic moiety in many biological systems that show pharmacological activity [7,8]. During the past few years, a large number of natural products containing bis(indolyl)methanes (BIM's) and bis(indolyl)ethane's (BIE's) have been isolated from marine sources. The BIM's are highly beneficial in promoting estrogen metabolism in women and men [9]. They also exhibit antibacterial [10], cytotoxic [11], insecticidal [12], analgesic [13] and anti-inflammatory activities [13]. Due to this, special interest has been focused on their synthesis [14].

Bis(indolyl)methanes are obtained by reactions of indoles with various aldehydes *via* azafulvenium salt intermediate in the presence of several Bronsted and Lewis acid catalysts such as $LiClO_4$ [15], CAN [16], $ZrOCl_2$ [17], $InCl_3$ [18], $AlPW_{12}O_{40}$ [19], ionic liquids [20], trichloro-1,3,5 triazine [21], PFPAT [22], HFIP [23],

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$KHSO_4$ [24], and molecular iodine [25]. But many of these methods have several drawbacks such as use of expensive reagents, longer reaction times, cumbersome workup, and low product yields. All these procedures involve the use of environmentally toxic solvents and hazardous chemical substrates. Solvent-free organic reactions have become the choice in green chemical organic synthesis [26]. Reports on condensation of indoles with carbonyl compounds under neat conditions are scarce in the literature [27]. Many synthetic chemists have made alternative sustainable and efficient heating procedures to replace the classical thermal heating in synthetic methods. Application of microwave irradiation (MWI) is one such technique employed in effecting organic reactions [28]. MWI assisted organic synthesis requires mild conditions, short reaction times and high product yields. In MWI, the chemical reactions are accelerated because of selective absorption of microwave energy by polar molecules [29,30].

2. Experimental

Solvents and reagents were procured from Sigma–Aldrich & Merck and are used as such without further purification. Melting points were determined using a calibrated thermometer by Guna Digital Melting Point apparatus. IR spectra of samples were recorded as potassium bromide pellet on a Bruker Vector 21 FT-IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded as solutions in $CDCl_3$ on a Bruker AMX 500 MHz NMR spectrometer operating at 400 MHz for ^{1}H spectra, and 100 MHz for ^{13}C spectra using tetra methyl silane (TMS) as an internal standard. LCMS mass spectra were recorded on a Jeol SX 102 DA/600 Mass spectrometer. Elemental analysis was performed on a Thermo Finnegan Instrument.

2.1. General procedure

A mixture of indole (2 mmol), aldehyde (1 mmol) is placed in 25 mL conical flask and was irradiated by microwave (CATA.4R, Catalyst 300 W, 5 min). After completion of the reaction, the reaction mixture is extracted with ethyl acetate and concentrated under reduced pressure. The obtained crude product is purified by column chromatography on silica gel adsorbent using petroleum ether–ethyl acetate mixtures as eluent to obtain known pure **3a**, **3b**, **3d**, **3f**, **3i**, **3j**, **3k**, **3l**, **3m**, **3n** and **3a**, **3c**, **3e**, **3g**, **3h** and **3o** new compounds.

Spectral data for 3,3′-((4-(methylthio)phenyl)methylene) bis(1 H-indole) (3e): Orange solid; isolated yield: (90%); mp 187–189 °C; IR: ν = 3401 (NH), 2928, 1684, 1497, 1217, 1085, 1014 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79 (brs, 2H, NH) 7.37, 6.58 (m, 14H, Ar-H), 5.82 (s, 1H, Ar-CH), 2.43 (s, 3H, S-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 141.2, 136.7, 130.1, 129.3, 127.1, 126.79, 123.7, 119.9, 53.5, 16.1; MS (LCMS): m/z 391 $[M + Na]^+$; Anal. Calcd. for $C_{24}H_{20}N_2S$: C, 78.23, H, 5.47, N, 7.60, S, 8.70. Found: C, 78.20, H, 5.44, N, 7.40, O, 8.66.

The compound **3e** was also characterized from its powder XRD data (Fig. 1).

2.2. Biological assay

2.2.1. Antibacterial assay

Well diffusion method was used to evaluate the antibacterial activity of BIM's on bacterial species [31]. Bacterial inoculums are prepared by growing a single colony overnight in nutrient broth and adjusting the turbidity to 0.5 McFarland standards. Mueller–Hinton agar (MHA) plates were inoculated with this bacterial suspension and the compounds (1–15) 100 μg/mL were added to a center well with a diameter of 8 mm. These plates were incubated at 37 °C for 24 h. The zone of inhibition (ZOI) was measured by subtracting the well diameter from the total inhibition zone diameter. Tetracycline is used as a positive control for bacterial species. All experiments were done in triplicates, and the results are consistent.

2.2.2. In vitro *anti-inflammatory assay*

Alsever's solution was prepared by dissolving 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% of sodium chloride in distilled water followed by sterilization [32]. Blood was collected from healthy volunteers. The collected blood was mixed in equal volumes with Alsever's solution. The mixture was centrifuged at 3000 rpm for 10 min and the packed cells were washed three times with isosaline (0.85%, pH 7.2) and 10% (v/v) suspension was made. Test samples with concentrations of 50 μg/mL and 100 μg/mL

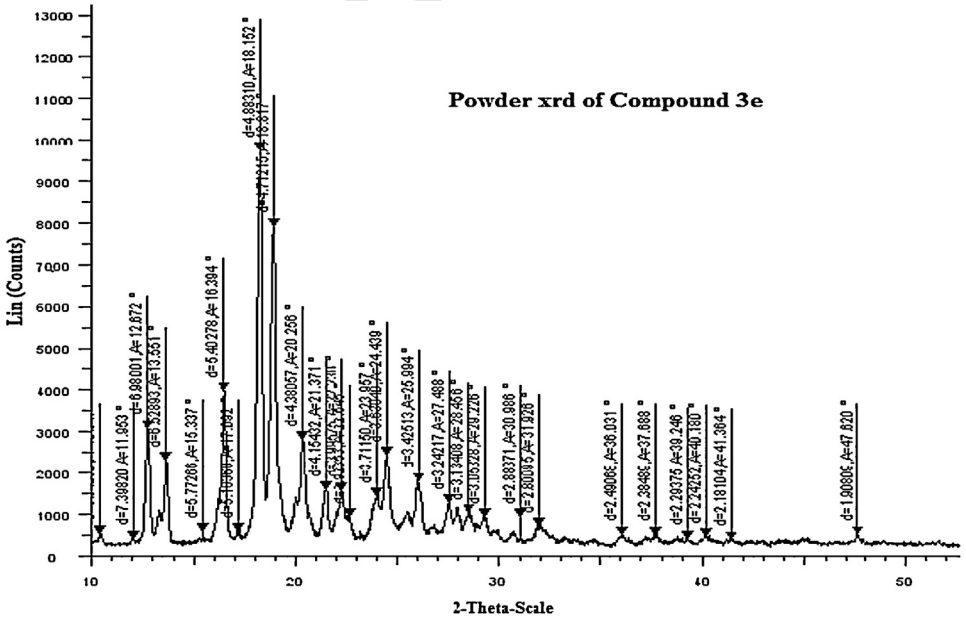

Fig. 1. Powder XRD data of Compound **3e**.

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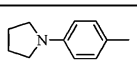
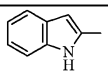
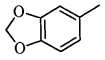
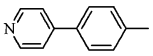
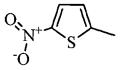
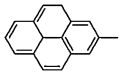
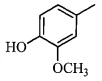
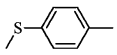
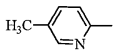
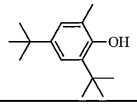
Entry	R	Yield (%)	mp (°C)	Entry	R	Yield (%)	mp (°C)	Entry	R	Yield (%)	mp (°C)
3a		92	152-154	**3f**		88	162-164 [35]	**3k**		82	152-154 [34]
3b		89	146-148 [33]	**3g**		85	204-206	**3l**		80	324-326 [38]
3c		87	112-114	**3h**		86	192-194	**3m**		82	292-294 [39]
3d		89	161-163 [34]	**3i**		81	42-45 [36]	**3n**		85	124-126 [16]
3e		90	187-189	**3j**		83	113-115 [37]	**3o**		87	198-200

Scheme 1. Synthesis of bis(indolyl)methanes.

were prepared by suspending in DMSO. The assay mixture contained the sample, 1 mL phosphate buffer (pH 7.2, 0.1 mol/L), 2 mL hyposaline (0.36%), and 0.5 mL human red blood cells suspension. Hydrocortisone sodium was used as the reference drug and 2 mL of distilled water as control. All the assay mixtures were incubated at 37 °C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using a spectrophotometer at 560 nm. The percentage hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water was 100%.

The percentage of hemolysis and stabilization of HRBC membrane are calculated from the following equations:

$$\%\,\text{Hemolysis} = \frac{OD_T}{OD_C} \times 100, \quad \%\,\text{Protection} = 100 - \left[\frac{OD_T}{OD_C}\right] \times 100$$

where OD_T = optical density of the test sample and OD_C = optical density of the control.

3. Results and discussion

3.1. Chemistry

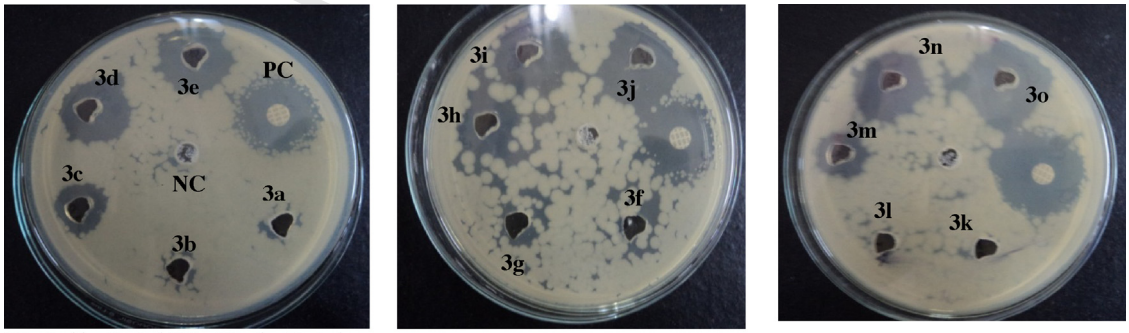
A facile and clean one pot two component reaction of an aldehyde and indole in 1:2 molar ratio is described for the synthesis of bis(indolyl)methanes under microwave irradiation and catalytic free condition (Scheme 1). To optimize the solvent requirements, ethanol, toluene, acetonitrile and THF are used and obtained in 70, 62, 58, 43% yields respectively in 15 min under microwave conditions. Under the same neat condition, 92% product yield is obtained within 5 min. The scope of application of this method is found by reacting different substituted aromatic and heteroaromatic aldehydes. In all cases, corresponding BIM's were obtained in 90% yields for heteroaromatic aldehydes while homoaromatic aldehydes afforded poor yields. Among the heteroaromatic aldehydes, six membered derivatives gave higher yields when compared to that of five membered ones.

3.2. Pharmacology

The antibacterial activity of **3a–o** is investigated by screening them against the gram positive bacterium *S. aureus* by well diffusion technique (Fig. 2) with reference to Tetracycline.

The inhibition zone values are summarized in Table 1. The results showed that **3o** showed good antibacterial activity whereas **3i**, **3j** showed moderate activity compared to that of the standard drug.

All the title compounds are also subjected to *in vitro* anti-inflammatory activity using two different concentrations, 50 μg/mL and 100 μg/mL. Diclofenac is used as the standard. All the tested compounds showed excellent anti-inflammatory activity when compared to their diclofenac except **3m**. Interestingly, **3j**, **3i**, **3k**, **3g** exhibited remarkably higher anti-inflammatory activity than that of the standard drug and thus qualifies for further clinical tests to on them so that they can be used as anti-inflammatory agents. The results are summarized in Table 2.



PC = Positive Control; NC = Negative Control

Fig. 2. The antibacterial activity of **3a–o** by well diffusion analysis.

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Table 1
Anti-bacterial activity of bis(indolyl)methanes.

Compounds	Zone of inhibition (mm)[a]
3a	–
3b	–
3c	2.7
3d	6.6
3e	7.2
3f	–
3g	–
3h	3.4
3i	9.1
3j	9.3
3k	–
3l	–
3m	4.5
3n	7.8
3o	16.2
Tetracycline	18.0

[a] Concentration in 100 μg/mL.

Table 2
In vitro anti-inflammatory activity of bis(indolyl)methanes.

Entry	% Hemolysis		% Protection	
	50[a]	100[a]	50[a]	100[a]
3a	69.44	58.33	30.6	41.67
3b	47.22	30.55	52.78	69.45
3c	52.77	38.8	47.3	61.2
3d	55.83	45.55	44.17	54.45
3e	79.72	67.50	20.28	32.50
3f	63.88	55.55	36.12	44.45
3g	33.33	27.77	66.67	72.23
3h	66.66	52.77	33.34	47.23
3i	41.60	25.00	58.40	75.00
3j	30.55	22.22	69.45	77.78
3k	44.44	27.70	55.56	72.30
3l	72.22	52.77	27.78	47.23
3m	94.40	80.55	5.60	19.45
3n	86.10	66.66	13.90	33.40
3o	77.77	58.33	22.23	41.67
Diclofenac	75.00	66.60	25.00	33.40

[a] Concentration in μg/mL.

4. Conclusion

Green synthesis of bis(indolyl)methanes by microwave irradiation condition of indole with aldehydes under solvent free conditions is reported. This procedure has short reaction time and affords high product yields. Compound **3o** showed good antibacterial activity against *S. aureus*. The anti-inflammatory activity revealed almost all title compounds except **3m** exhibited good anti-inflammatory activity. Compounds **3j, 3i, 3k** and **3g** showed much higher anti-inflammatory activity than the standard diclofenac drug and thus qualifying for further clinical evaluation so that they can be used as effective anti-inflammatory agents.

Q2 Uncited references

[33–39].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2015.08.012.

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