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Synthesis and antimicrobial evaluation of new conjugates containing indole and triazine scaffold

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ABSTRACT

In the present work, 2-indolyl-4,6-trisubstituted 1,3,5-triazine analogs were synthesized using the nucleophilic substitution on cyanuric chloride in presence of dioxane or acetone. The FT-IR spectrum of compounds exhibited sharp peaks at 3300-2700 cm^{-1} (aliphatic C-H), 1400-1700 cm^{-1} (aromatic ring), in all compounds while vibrations of N-H (3300-3400), NO_2 (1300-1400 cm^{-1}), C-Cl (850-550 cm^{-1}) & C-Br (520-690 cm^{-1}) were also found in the corresponding compounds. The ^1H NMR spectra exhibit chemical shifts at δ 6.5-7.9 (aromatic protons), 4.0 (N-H), 2.5 (CH_2) and 1.5 (CH_3) in corresponding compounds. The antimicrobial potential of the synthesized compounds was also evaluated and the combined data reveals that all the synthesized compounds show MIC values between 62.5 and 15.625 µg/mL against all the screened microorganisms. The compounds **C3** and **C5** exhibited the best results (IC_{50} – 15.625 µg/mL) against both the bacterial strains, other compounds exhibited MIC value of more than or equal to 32.5 µg/mL.

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Introduction

The triazine structure is a heterocyclic ring, analogous to the six-membered benzene ring but with three carbons replaced by nitrogens. Its analogues, melamine, cyanuric acid and cyanuric chloride are important starting compounds for various materials with wide range of applications in textile, plastic, pharmaceuticals and rubber industries. 1,3,5-Triazines represent a broadly used lead structure with remarkable applications in various fields[1]. s-Triazine derivatives are an important class of compounds showing many pharmacological activities (like antimicrobial activities)[2]. Indole is also known as benzopyrrole which contains benzenoid nucleus and has 10 π-electrons (two from lone pair on nitrogen and double bonds provide eight electrons) which makes them aromatic in nature[3]. Indole is reported to undergo electrophilic substitution mainly at position 3 of the nucleus. When position 3 of the indole nucleus is occupied by substituents other than hydrogen, position 2 is the most reactive one and when positions 2 and 3 are occupied, the electrophile occupies a position in the benzene ring[4].

Several reports have stated the antimicrobial actions for both indole and triazine molecules. Hence in the present investigation it was attempted to combine both the molecules in single entity and evaluate its antimicrobial activity.

Material and Methods

The scheme involved nucleophilic subsitution of cyanuric chloride in successive steps to yield the desired compounds[5] (Scheme 1).

General procedure for synthesis of 6-chloro-N^2,N^4-substituted-1,3,5-triazine-2,4-diamine (3)

The synthesis of (3) was carried out in two step manner each involving nucleophilic substitution of one chloro group of triazine with the amine. Same procedure was followed for both the steps. Briefly, appropriate amine (0.02 mol in 10mL acetone) was added slowly to cyanuric chloride (0.01 mol, 1.845g in 30mL acetone) with constant stirring for 5 hours at 0 to 5°C. Intermittently, sodium carbonate solution (0.005 mol, 0.53g in 10 mL water) was added drop wise to neutralize the HCl evolved over the course of the reaction. The progress of the reaction was monitored by TLC. The content of the flask was poured into crushed ice and the solid that separated out was filtered, washed with water, dried and recrystallised from alcohol to give the product.

Procedure for synthesis of indole conjugated compounds, C1-C5

Indole (0.01 mol) and compound **3a-e** (0.01 mol) were dissolved in acetone (40mL). The reaction mixture was refluxed for 8 hours. Intermittently, sodium carbonate solution (0.005 mol, 0.53g in 10 mL water) was added drop wise to neutralize the HCl evolved over the course of the reaction. The progress of the reaction was monitored by TLC. The content of the flask was poured into crushed ice and the solid that separated out was filtered, washed with water, dried and recrystallised from alcohol to give the indole conjugated triazine derivative.

N^2,N^2,N^4,N^4-tetraethyl-6-(2H-isoindol-2-yl)-1,3,5-triazine-2,4-diamine, ***C1***

^{1}H NMR Spectra (d, 300 MHz, $CDCl_3$): 6.823-7.916 (CH-Benzene), 2.995 (CH_2), 1.592 (CH_3); IR (KBr): 3149-2917 cm^{-1} (C-H aliphatic),

1644-1458 cm^{-1} (C-C Ar), 2346 cm^{-1} (C=N); Mass – 338.22 (Calculated)

6-(2H-isoindol-2-yl)-N2,N2,N4,N4-tetraphenyl-1,3,5-triazine-2,4-diamine, ***C2***

^1H NMR Spectra (d, 300 MHz, $CDCl_3$): 6.828-7.913 (C-H, Aromatic); IR (KBr): 3153-2835 cm^{-1} (C-H aliphatic), 1644-1455 cm^{-1} (C-C Ar), 2347 cm^{-1} (C=N); Mass – 530.22 (calculated)

N^2,N^4-bis(2-nitrophenyl)-6-(2H-isoindol-2-yl)-1,3,5-triazine-2,4-diamine, ***C3***

^1H NMR Spectra (d, 300 MHz, $CDCl_3$): 8.025-6.833 (CH-Benzene), 3.974 (N-H); IR (KBr): 3300 (N-H), 3125-2983 cm^{-1} (C-H aliphatic), 1716-1522 cm^{-1} (C-C Ar), 2341 cm^{-1} (C=N), 1358 (N-O); Mass – 468.13 (Calculated)

N^2,N^4-bis(2-bromophenyl)-6-(2H-isoindol-2-yl)-1,3,5-triazine-2,4-diamine, ***C4***

^1H NMR Spectra (d, 300 MHz, $CDCl_3$): 7.913-6.828 (C-H aromatic), 4.010 (N-H); IR (KBr): 3300 (N-H), 2934-2855 cm^{-1} (C-H aliphatic), 1631-1457 cm^{-1} (C-C Ar), 2460 cm^{-1} (C=N), 699 cm^{-1} (C-Br); Mass – 533.98 (Calculated)

N^2,N^4-bis(2-chlorophenyl)-6-(2H-isoindol-2-yl)-1,3,5-triazine-2,4-diamine, ***C5***

^1H NMR Spectra (d, 300 MHz, $CDCl_3$): 8.037-6.849 (CH-Benzene), 3.985 (N-H); IR (KBr): 3300 (N-H), 2934-2855 cm^{-1} (C-H aliphatic), 1631-1457 cm^{-1} (C-C Ar), 2460 cm^{-1} (C=N), 699 cm^{-1} (C-Cl); Mass – 446.08 (Calculated)

Physicochemical characterization

The synthesized compounds were observed for color, practical yield, solubility, melting point and determination of retention factor (R_f) value by thin layer chromatographic technique (TLC).

Evaluation of antibacterial action[6]

The antibacterial action of the synthesized compounds was evaluated against one gram positive (*Bacillus subtilis*) and one gram negative bacteria (*Escherichia coli*).

Preparation of test solutions

The synthesized triazine-indole conjugates were dissolved in dimethyl sulfoxide (DMSO) and the further dilutions of the test compounds were prepared at the required quantities of 1000 µg/mL concentrations with nutrient broth medium.

Preparation of Inoculum

Overnight culture of both the bacteria were prepared separately in nutrient broth, and used as a microbial source for the determination of minimum inhibitory concentration (MIC).

Determination of MIC

The sterile capped test tubes were numbered from 1 to 8 and all of the steps were carried out using aseptic technique. 10 ml of drug sample solution (1000 µg/mL) was added to the first tube while 2.0 ml of nutrient broth to all other tubes. 2.0 ml of the drug sample was transferred from the first tube to the second tube (500 µg/mL). Using a separate pipette, the contents of this tube were mixed and 2.0 mL was transferred to the third tube (250 µg/mL). Using a separate pipette, the contents of this tube were mixed and 2.0 mL was transferred to the fourth tube (125 µg/mL). Using a separate pipette, the contents of this tube were mixed and 2.0 mL was transferred to the fifth tube (62.5 µg/mL). Using a separate pipette, the contents of this tube were mixed and 2.0 mL was transferred to the sixth tube (31.25 µg/mL). Using a separate pipette, the contents of this tube were mixed and 2.0 mL was transferred to the seventh tube (15.625 µg/mL). The dilutions were continued in this manner up to tube number 8 (7.81 µg/mL), making sure that the pipettes were changed between each of the tubes in order to prevent carryover of the drug sample. From the tube number 8, 2.0 ml was removed and discarded. The 9^{th} tube, which serves as a control, received no drug sample. Norfloxacin (1.0 µg/ml) was used as standard drug. An accurately measured quantity of 0.2 µl of the di-

luted bacterial culture suspension (broth) was added to each of the tubes using a micropipette. All the tubes were incubated overnight at 37°C in a bacteriological incubator. The tubes were examined for visible signs of bacterial growth. The highest dilution without growth was recorded as the minimal inhibitory concentration (MIC).

Results and Discussion

Five triazine derivatives were synthesized by both conventional heating and microwave heating and characterized by using TLC, IR, and NMR analysis.

The spectral characteristics were used to elucidate the structures of the synthesized compounds. Spectral analyses (IR, NMR & Mass) of the compounds satisfactorily supported the structures of the synthesized compounds. The FT-IR spectrum of compounds exhibited sharp peaks at 3300-2700 cm^{-1} (aliphatic C-H), 1400-1700 cm^{-1} (aromatic ring), in all compounds while vibrations of N-H (3300-3400), NO_2 (1300 -1400 cm^{-1}), C-Cl (850-550 cm^{-1}) & C-Br (520-690 cm^{-1}) were also found in the corresponding compounds.

The ^1H NMR spectra exhibit chemical shifts at δ 6.5-7.9 (aromatic protons), 4.0 (N-H), 2.5 (CH_2) and 1.5 (CH_3) in corresponding compounds.

Molecular ion peaks and fragmentation peaks of the synthesized triazines obtained on the mass spectrum adequately corresponds with the structures of the compounds. This spectral data satisfactorily supports the formation of the title compounds.

The antibacterial activity of the synthesized compounds was evaluated at various concentrations to determine the MIC of each compound. The compounds **C3** and **C5** exhibited the best results (IC_{50} – 15.625 µg/mL) against both the bacterial strains, other compounds exhibited MIC value of more than or equal to 32.5 µg/mL. The antimicrobial potential of the synthesized compounds has been evaluated by determining the minimum inhibition concentration values by broth dilution method using nutrient broth for culturing the pathogen. The results obtained indicate that the presence of electron withdrawing substituent on the phenyl substituent of nitrogen was beneficial for the antibacterial potential. In the halogen containing compounds chlorine substituent was more effective compared to bromine substituent.

Conclusion

In the present study, indole conjugated triazines were and the compounds were found to be of good purity and yield as compared to the conventional synthetic procedure. The compounds exhibited good antibacterial potential against both gram negative and gram positive bacterium tested.

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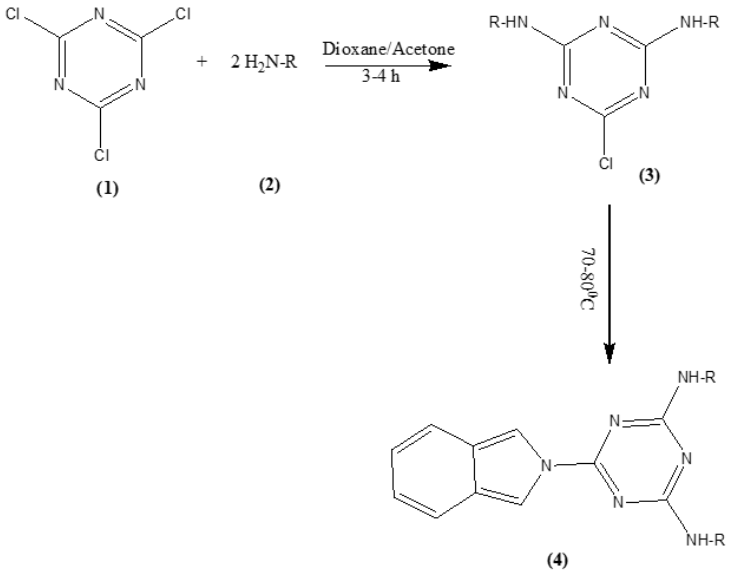
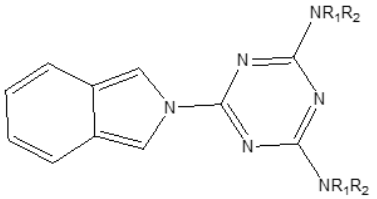


Figure 1 Scheme for synthesis of 1,3,5-triazine-indole conjugates.

Table 1 Substitution present in the synthesized compounds



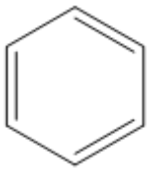
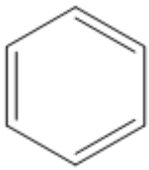
Code	**R_1**	**R_2**
C1	C_2H_5	C_2H_5
C2		
C3	H	2-nitrophenyl
C4	H	2-bromophenyl
C5	H	2-chlorophenyl

Table 2 Physical properties of the synthesized compounds

Code	Yield (%)		Color	Melting point
	Conventional Method	Microwave method	Color	Melting point
C1	32	67	Yellow	259-261
C2	29	62	Brown	173-175
C3	37	79	Yellow	178-183
C4	34	81	Yellow	167-171
C5	39	82	Yellow	154-160

Table 3 MIC of the synthesized compounds against gram positive and gram negative bacteria

Code	MIC (µg/mL)[a,b,c]	
	B. subtilis	*E.coli*
C1	31.25	31.25
C2	62.5	62.5
C3	15.625	15.625
C4	31.25	31.25
C5	15.625	15.625

[a] A set of tubes with only the inoculated broth was used as control to determine MIC

[b]MIC is expressed by measuring the turbidity of test and control dilution tubes. A 50% decrease in turbidity was taken as MIC.

[c]All values are expressed as mean of a set of three experiments

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