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Synthesis Characterization and Antimicrobial Evaluation of Indole Based Heterocyclic Compounds

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ABSTRACT

In the present work a few 2-phenylindole derivatives were derivatized to yield indole-thiadiazole conjugates and evaluated for their antibacterial potential. The synthesis was achieved in four steps in which aryl hydrazone is prepared by condensation of phenyl hydrazine and aromatic ketone followed by Fisher indole synthesis of indole via cyclization in the presence of acid catalyst. In the next step the nitrogen of the 2-phenyl indole is reacted with ethyl chloroacetate to yield the acetate derivative which was finally cyclized by reaction with thiosemicarbazide to yield the indole-thiadiazole conjygate. The structure of the compounds was elucidated using IR, Mass and 1H NMR spectral studies while TLC was carried out to monitor the completion of reactions. Antibacterial studies of these compounds indicated that the compounds were having moderate antibacterial activity. The zone of inhibition exhibited by 4e was highest amongst all the conjugates.

Keywords: Indole, antimicrobial, disc diffusion, heterocyclic, thiadiazole

Introduction

Microbial resistance is now frequently confronted to common antibiotics being used in clinical settings, and there is an imperative demand for newer anti-infective agents to overcome emerging multi-drug resistance [1]. Today, the need for novel antimicrobials has been greater than ever in the face of increasing resistance to the older ones and increasingly tough management of bacterial infections. In spite of urge for such agents, the scientific progression in terms of antimicrobial research and discovery of new antibacterial molecules has declined dramatically in the past few years.

Heterocyclic compounds either of synthetic or natural origins have found applicability in several physiological neurotransmitters as well as in the modulators of certain neurotransmission processes [2]. Indole is a benzopyrrole in which the benzene and pyrrole rings are fused at the 2-and 3-positions of the pyrrole nucleus. The Indole nucleus (Figure 1) has ten π-electrons which are circulating over nine atoms. Hence, Indole is an electron rich ring system [3].

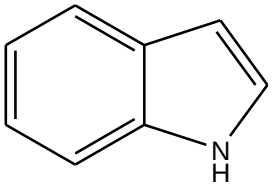


Figure 1. Indole

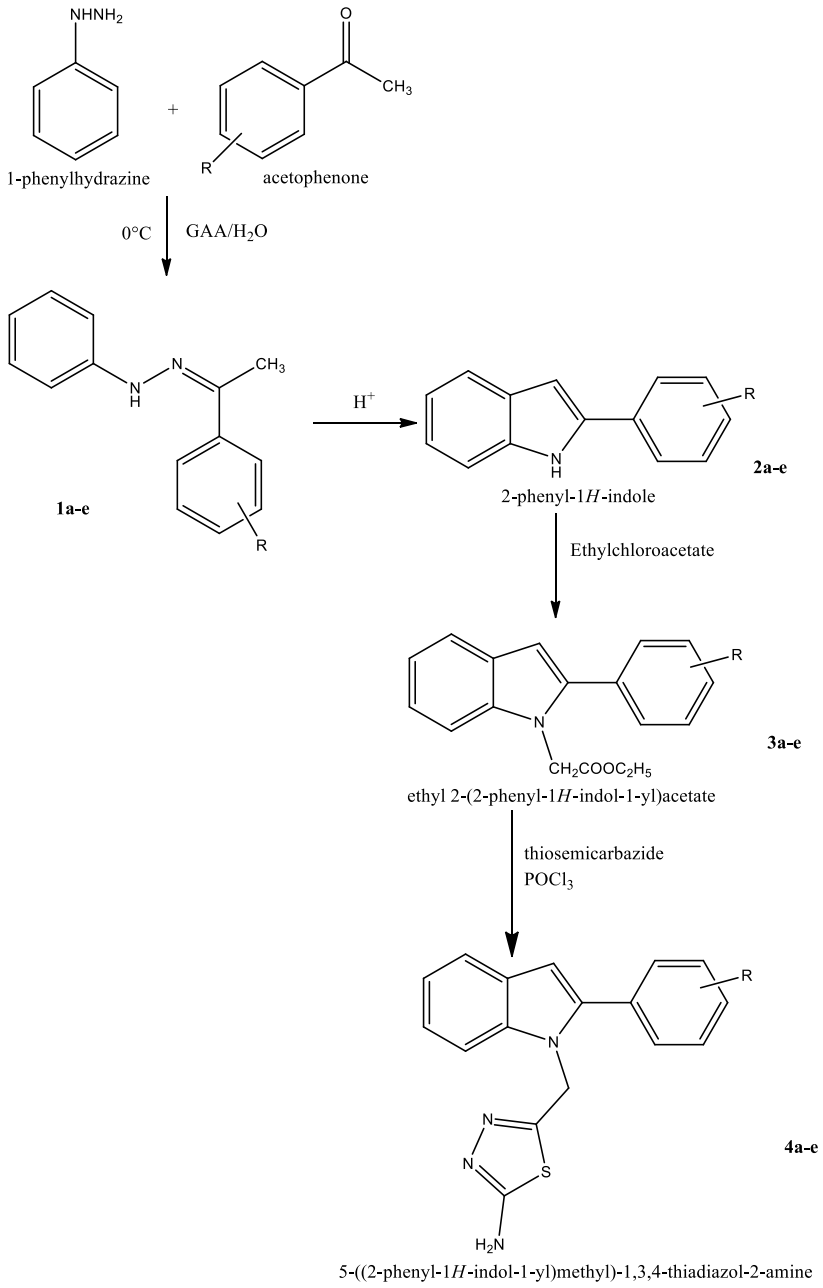
The indole ring due to its presence in several biological systems, has been widely explored for its pharmacological and biological potential [4-15]. The primary objective of the current work was to synthesize some indole derivatives and assess their antimicrobial potential in culture medium.

Material and Methods

Acetophenone, 3-hydroxy acetophenone, 4-hydroxy acetophenone, 4-methyl acetophenone, 4-nitro acetophenone, phenyl hydrazine, and polyphosphoric acid were obtained from Avra Chemicals. Ethyl chloroacetate and thiosemicarbazide was purchased from Loba. Ethanol, methanol, glacial acetic acid, hydrochloric acid, acetone, chloroform and dimethyl sulfoxide were obtained from Rankem. Any other reagent used was of synthetic grade and used as procured.

Vacuum desiccator (polylab), electrical heating mantle (Biotechnics India), magnetic stirrer with hot plate ((Biotechnics India), water bath (Biotechnics India), melting point apparatus (Biotechnics India) and vacuum pump (Value) were used in the present study. All glassware used were of Borosilicate grade, washed using chromic acid cleaning mixture, rinsed with distilled water and dried in hot air oven (Biotechnics India) before using.

The synthetic scheme (Scheme 1) for the synthesis of indoles was designed using the schemes reported earlier.



Scheme 1. Synthetic route for substituted indoles

General method for synthesis of substituted phenyl hydrazone

A mixture of 0.167 mol of appropriate acetophenone and 0.167 mol of phenyl hydrazine was prepared in 60 mL ethanol and a few drops of glacial acetic acid were added to it. The mixture as cooled to 0°C using an ice bath to obtain a solid. The solid was filtered and washed with dilute HCl and then by rectified spirit. The product was recrystallized using ethanol and white product obtained was filtered and stored in air tight container.

General method for synthesis of substituted phenyl indole

0.15 mol of the substituted phenyl hydrazone was placed in a beaker containing excess of polyphosphoric acid (180 g). The mixture was heated on a boiling water bath, stirring the mixture and maintaining the temperature at 100-120°C for 10 min. To the reaction mixture was added 450 mL of cold water and it was well stirred in order to dissolve the polyphosphoric acid completely. The solid was filtered at pump and washed with cold water several times to remove any trace of acid. The solid was refluxed with 300 mL of rectified spirit and a little amount of decolorizing charcoal was added to it and filtered. The filtrate was cooled to room temperature to obtain the white crystals of phenyl indole which were dried in desiccator over anhydrous calcium chloride and stored in air tight container.

General method for synthesis of N-substituted phenyl indole

Anhydrous K_2CO_3 (0.006 mol) was added to a solution of the appropriate phenyl indole (0.003 mol) and ethylchloroacetate (0.003 mol) dissolved in anhydrous DMF (10 mL) in a round bottom flask and refluxed for 1-2 h. The mixture was poured onto crushed ice to precipitate the solid product. The product was filtered at pump using Buchner funnel.

General method for synthesis of thiadiazole conjugated phenylindole

Compounds **3a-e** (0.001 mol) and thiosemicarbazide (0.001 mol) were dissolved in chloroform (20 mL) and 8-10 drops of phosphorus oxychloride and refluxed for 8 h. To the reaction mixture, 15 mL water was added and sodium bicarbonate solution was added to neutralize the phosphorus oxychloride and the precipitate obtained was filtered, washed with chloroform and hexane to obtain **4a-e.**

All the synthesized compounds were characterized for their melting point, solubility and retention factor (R_f value) by TLC.

Antibacterial study

The microorganisms used for the antimicrobial study were procured from Institute of Microbial Technology, Chandigarh (MTCC). *Escherichia coli* (MTCC 40), and *Staphylococcus aureus* (MTCC 3160) were used for the present investigation.

Preparation of test compounds

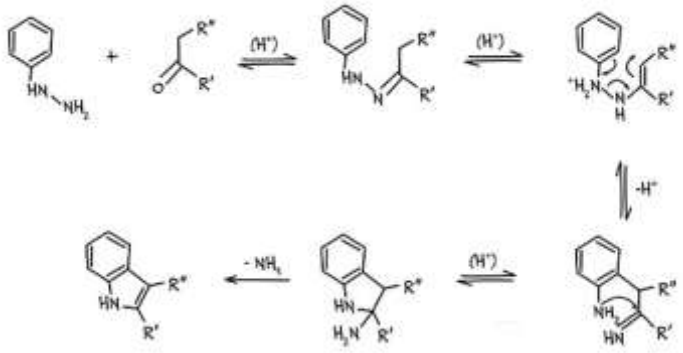
The synthesized indole-thiadiazole conjugates were dissolved in DMSO to obtain the solutions of 50, 75, 100 & 150 µg/mL. These solutions were used as the test samples.

Screening Procedure

About 3 mm thick pre-poured nutrient agar plates were inoculated with a few drops of the bacterial suspension by swabbing on the surface of agar. The antimicrobial action was screened using disc diffusion method [16]. Wells were bored into the agar plate at equal distances using cork borer (10mm) and 200µL of the indole-thiadiazole conjugates (50, 75, 100 & 150 µg/mL) were placed in each hole. The plates were incubated for 24h at 37 ± 0.1°C to allow for microbial growth. The zone of inhibition in each plate was measured in millimeters.

Results and Discussion

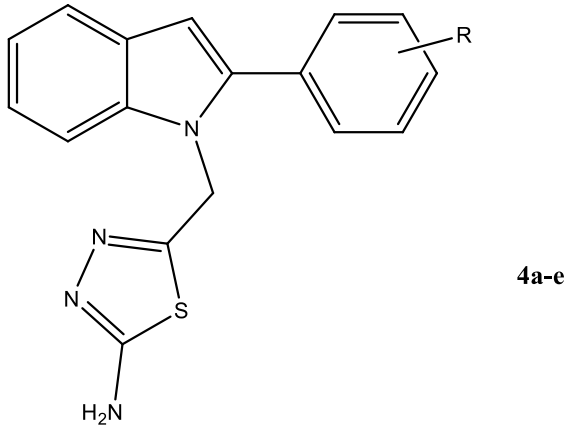
In the first two steps, aryl hydrazone is prepared by condensation of phenyl hydrazine and aromatic ketone followed by Fisher indole synthesis of indole via cyclization in the presence of acid catalyst. In the last step the nitrogen of the phenyl indole is substituted with various alkyl groups. The mechanism involved in formation of phenyl indole is presented in scheme 2.



Scheme 2. Mechanism of Fisher Indole Synthesis

Five derivatives of indole were synthesized using five aromatic acetophenones and four alkyl halides. The compounds were characterized by using TLC and IR. NMR and mass spectral study was carried out on five compounds in order to assure the formation of proper products. The result of the yield, melting point and R_f value of the synthesized compound were depicted in the Table 1

Table 1. Characters of Synthesized Compounds



4a-e

Code	Color	M.P (°C)	% Yield	R_f value	Molecular Formula
4a	Yellow	218-220	61	0.49	$C_{17}H_{14}N_4S$
4b	Yellow	210-213	67	0.47	$C_{17}H_{14}N_4OS$
4c	Yellow	231-234	64	0.61	$C_{17}H_{14}N_4OS$
4d	Brown	226-228	66	0.55	$C_{18}H_{16}N_4OS$
4e	Brown	212-214	69	0.43	$C_{17}H_{13}N_5O_2S$

5-((2-phenyl-1H-indol-1-yl)methyl)-1,3,4-thiadiazol-2-amine, ***4a***

^{1}H NMR Spectra (δ, 300 MHz, DMSO): 7.2-7.5 (H aromatic), 6.5 (H-pyrrole ring), 3.60 (H-methyl); IR (KBr): 3420.33 (C-H), 3161.06 cm^{-1} (CH Ar), 2603.54 (-CH_2-), 1600-1700 cm^{-1} (C-C Ar), 1485.76 cm^{-1} (C=C), 1197.04 cm^{-1} (C-N); m/z: 306.5

3-(1-((5-amino-1,3,4-thiadiazol-2-yl)methyl)-1H-indol-2-yl)phenol, ***4b***

^{1}H NMR Spectra (δ, 300 MHz, DMSO): 7.4 (CH-Benzene), 7.3 (CH-Benzene), 7.2 (CH-Benzene), 2.5 (CH_2), 7.0 (CH-Benzene), 7.1 (CH-Benzene); IR (KBr): 3410.90 (O-H), 3121.21 (C-H Ar), 2602.10 (C-H), 1500-1650 cm^{-1} (C-C Ar), 1481.52 (C=C), 1282.15 (C-N), 1069.29 (C-O); m/z: 322.4

4-(1-((5-amino-1,3,4-thiadiazol-2-yl)methyl)-1H-indol-2-yl)phenol, ***4c***

^{1}H NMR Spectra (δ, 300 MHz, DMSO): 7.4 (CH-Benzene), 7.3 (CH-Benzene), 7.2 (CH-Benzene), 2.5 (CH_2), 7.0 (CH-Benzene), 7.1 (CH-Benzene); IR (KBr): 3240.81 (O-H), 3119.55 (C-H Ar), 2981.44 (C-H), 1500-1650 cm^{-1} (C-C Ar), 1484.01 (C=C), 1396.53 (O-H bending), 1294.08 (C-N), 1092.37 (C-O); m/z: 322.4

5-((2-(p-tolyl)-1H-indol-1-yl)methyl)-1,3,4-thiadiazol-2-amine, ***4d***

^{1}H NMR Spectra (δ, 300 MHz, DMSO): 7.4 (CH-Benzene), 7.3 (CH-Benzene), 7.2 (CH-Benzene), 2.5 (CH_3), 7.0 (CH-Benzene), 7.1 (CH-Benzene); IR (KBr): 3113.84 (C-H Ar), 2943.23 (C-H), 1500-1650 cm^{-1} (C-C Ar), 1461.21 (C=C), 1282.19 (C-N); m/z: 320.4

5-((2-(4-nitrophenyl)-1H-indol-1-yl)methyl)-1,3,4-thiadiazol-2-amine, ***4e***

^{1}H NMR Spectra (δ, 300 MHz, DMSO): 7.1-7.4 (H Aromatic), 8.25 (H adjacent to NO_2), 3.6 (H – methyl); IR (KBr): 3116.02 (C-H Ar), 2978.17, 2809.22 (C-H), 1500-1650 cm^{-1} (C-C Ar), 1525.49 (N-O), 1454.47 (C=C), 1285.93 (C-N); m/z: 351.3

The structure elucidation of the compounds was performed by IR, ^{1}HNMR and mass spectroscopy. The IR spectra of all the compounds exhibited the stretching vibration peaks due to C-N, C=C, C-H Ar, CH aliphatic at 1300-1100 cm^{-1}, 1500-1700 cm^{-1}, 3000-3200 cm^{-1} and 2600-3000 cm^{-1} respectively. The other vibrations that appeared in the spectra included those from C-O (1000-1100 cm^{-1}), O-H (3200-3500 cm^{-1}) and C-S (1400-1500 cm^{-1}).

The ^{1}HNMR spectra obtained displayed the peaks of aliphatic CH and aromatic CH as well as peak of O-H in the corresponding compounds (**4b, 4c**). The mass spectra displayed the molecular ion peak and the isotopic peaks as calculated.

Antibacterial activity

The antibacterial activity of the synthesized benzoxazole-isatin conjugates was determined measuring the zone of inhibition in the agar plate. Four concentrations of the conjugates were tested for antibacterial action. Norfloxacin was used as the standard drug for antibacterial action (Table 2).

Table 2. Zone of inhibitions of indole-thiadiazole conjugates

Compound Code	Zone of Inhibition (mm)*							
	S. auerus				***E.coli***			
	25µg	50µg	100µg	150µg	25µg	50µg	100µg	150µg
4a	-	-	-	12	-	-	-	13
4b	-	-	13	12	-	-	11	14
4c	-	-	12	11	-	-	16	16
4d	-	-	13	13	-	-	14	19
4e	-	-	17	17	-	-	**21**	**25**
Norfloxacin	**22**	-	-	-	**23**	-	-	-

* Below 12 mm – poor activity; 13-18 mm – moderate activity & above 18 mm – good activity

The zone of inhibition exhibited by **4e** was highest amongst all the conjugates. This signifies the importance of the nitro group in antibacterial action of the compound. It was also observed that the synthesized compounds were having poor activity against gram positive bacteria.

Conclusions

The present work focused on synthesizing 2-phenyl indole derivatives conjugated with thiadiazole possessing antibacterial action. The synthesized compounds with variable substitution pattern were able to exhibit moderate antibacterial action against gram negative bacteria. Further studies on new compounds of similar structure would be carried out in order to derive a relation between the structure and activity of the nucleus.

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