Synthesis characterization and antimicrobial evaluation of indole based heterocyclic compounds

[1]Kalyani Shashikant Bokde, [2]Mehta Parulben D

Lakshmi Narain College of Pharmacy
Bhopal, Madhya Pradesh, India.

***Abstract*- In the present work a few 2-phenylindole derivatives were synthesized and conjugated with thiadiazole nucleus via a linker and the compounds were assessed for their antimicrobial potential. 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 N-O (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. The antimicrobial action of the synthesized compounds was testing using two bacteria, Escherichia coli and staphylococcus aureus. The standard drug norfloxacin and the test compounds 4e and 4a were found to significantly inhibit the growth of the microbes in culture medium presenting IC_{50} value of 66.94 µg/mL for *E. coli* and 62.21 µg/mL for *S. aureus* (4e) *and* 78.03 µg/mL for *E. coli* and 107.33 µg/mL for *S. aureus* (4a)**

***Keywords*: Indole, antimicrobial, thiadiazole, cup and plate, minimum inhibitory concentration.**

Introduction

Design of novel and newer potent molecules has been the thirst of researchers worldwide. An important approach towards this incorporates the modification of the existing molecules with established biological actions utilizing the various approaches for drug designing. Indole (Figure 1a) is an electron rich benzopyrrole ring system in which the benzene and pyrrole rings are fused at the 2-and 3-positions of the pyrrole nucleus [1]. The indole ring system is probably the most ubiquitous heterocycle in nature. Owing to the great structural diversity of biologically active indoles, it is not surprising that the indole ring system has become an important structural component in many pharmaceutical agents. Thiadiazole (Figure 1b) is a five membered heterocyclic ring system containing two nitrogen atom and one sulfur atom [2].

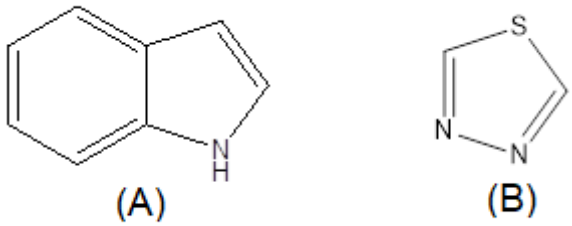


Figure 1. (A) Indole; (B) Thiadiazole

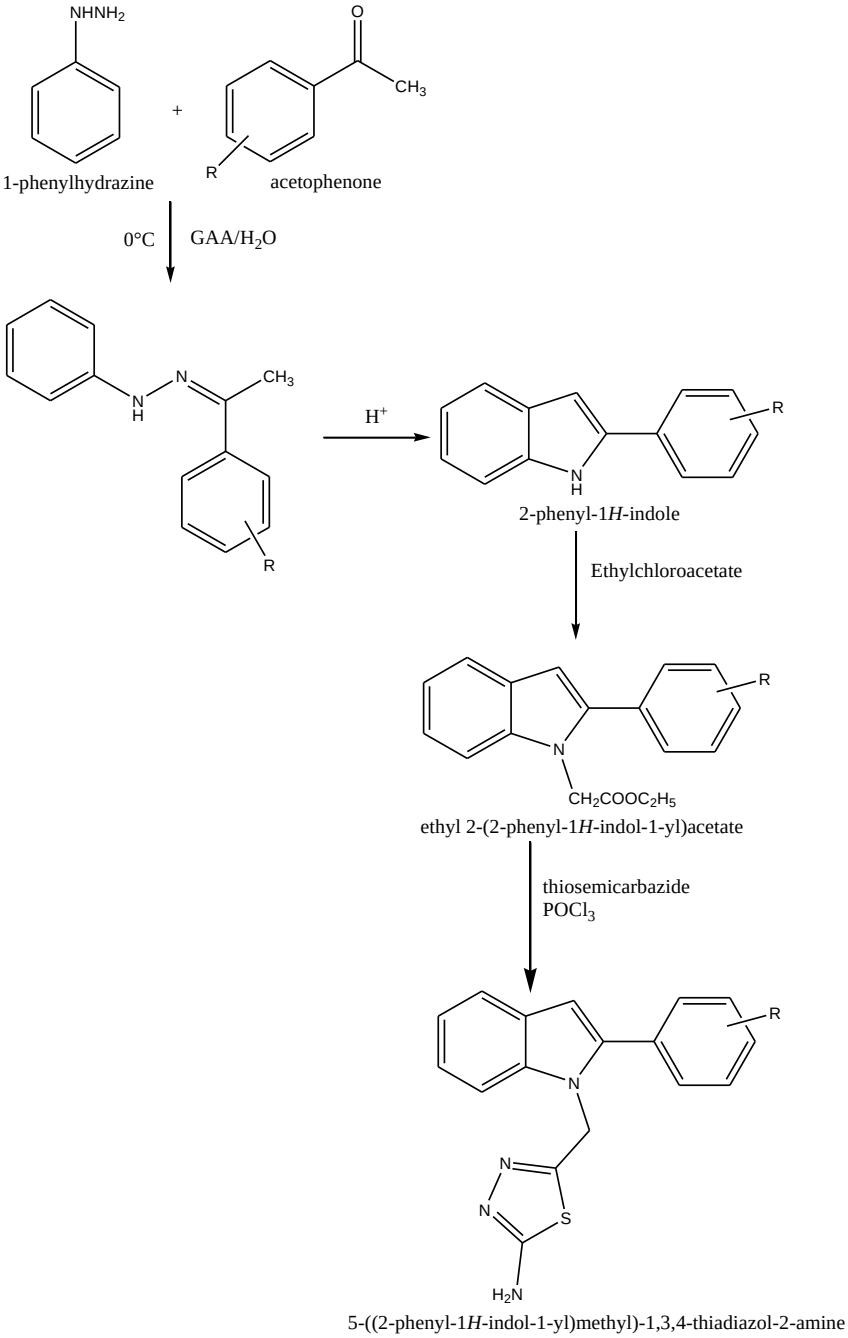
Various congeners of indole, thiadiazole or fused as well as co-linked indole and thiadiazole heterocycles have been widely investigated for various pharmacological actions [2-7]. This prompted us to investigate the antimicrobial potential of some indole derivatives linked with thiadiazole.

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 was purchased from Sigma 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.

All the synthesized compounds were characterized for their melting point (open capillary method), solubility (qualitative) and retention factor (R_f value) (TLC). The FT-IR was done using Bruker spectrophotometer and 1H-NMR using Jeol NMR spectrophotometer.

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 hydrazine, 1a-e

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, 2a-e

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, 3a-e

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 the synthesis of thiazole-linked compounds, 4a-e

A mixture of compound **3a-e** (0.01 mol), thiosemicarbazide (0.91 g ; 0.01 mol) and (5 mL) phosphorus oxychloride was refluxed for 8 hrs. The cold reaction mixture was poured on crushed ice and neutralized by adding sodium hydroxide solution. The resulting solid was filtered and recrystallized from chloroform to give a white crystals of amino thiadiazole derivatives, **4a-e**.

Antimicrobial 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. The lyophilized cultures obtained from IMT, Chandigarh were revived by adding 0.3 mL of nutrient broth to the culture ampoules to obtain a suspension of the bacteria.

of a slight heat and sterilized using autoclave at 121°C and 15 lbs pressure for 15 min.

Agar plates were prepared by pouring the sterilized medium into sterilized petridishes suitably marked and labeled. The plates were allowed to solidify in the laminar flow bench and stored packed for culturing with microbes and antimicrobial screening.

The synthesized Schiff's bases were dissolved in DMSO to obtain the solutions of 25, 50, 75 & 100 µg/mL. These solutions were used as the test samples.

Screening Procedure (zone of inhibition)

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 [8].

Wells were bored into the agar plate at equal distances using cork borer (10mm) and 200µL of the Schiff's bases (25, 50, 75 & 100 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.

Screening Procedure (Minimum inhibitory concentration)

The broth dilution technique was used to determine the minimum inhibitory concentration of the synthesized compounds. The final inoculum size (of bacterial culture) was maintained to 10^5 CFU/mL. A set of tubes containing only the inoculated broth was used as the growth control, and one containing only the broth was used to ensure the sterility of the medium. A set of six tubes was labelled as 1 to 6 and to each tube was added 3 mL of nutrient broth. To the first tube was added 300µL of 1mg/mL of the test sample. The contents were mixed by swirling between hands and 300µL of content from the 1st tube was transferred to 2nd tube. The process was repeated from 2nd to 3rd tube up to the 6th tube. From the 6th tube, 300µL of content was withdrawn and discarded. To each of these tubes was added 200µL of the bacterial inoculum. All the tubes were incubated at 37°C for 24-48 h to allow for growth of micro-organism. After incubation, the optical density of the content from each tube was observed at 600 nm using UV-Visible spectrophotometer. The concentration that led to half of the optical density (50%) of the growth control tube was observed for each sample and standard (norfloxacin) [9].

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.

Five derivatives of indole were synthesized using five aromatic acetophenones. These compounds were substituted on the nitrogen of indole and modified to obtain thiadiazole linked compouns. 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.

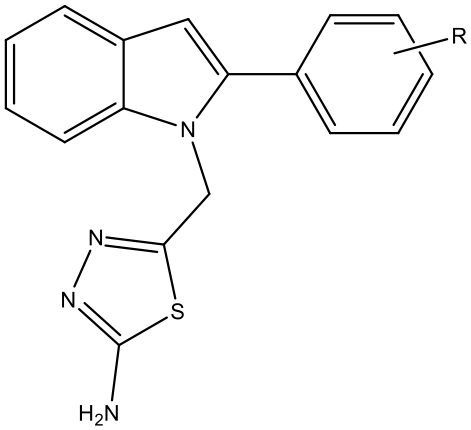


Table 1. Characters of Synthesized Compounds

Code	Color	M.P (˚C)	% Yield	R_f value
4a	Brown	233-235	63	0.67
4b	Yellow	206-208	69	0.48
4c	Yellow	249-251	70	0.63
4d	Brown	263-265	72	0.54
4e	Brown	259-261	75	0.43

All the compounds were found to be soluble in chloroform and methanol.

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

^{1}H NMR Spectra (δ, 300 MHz, $CDCl_3$): 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: 306.38

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

^{1}H NMR Spectra (δ, 300 MHz, $CDCl_3$): 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: 322.38

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

^{1}H NMR Spectra (δ, 300 MHz, $CDCl_3$): 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: 322.38

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

^{1}H NMR Spectra (δ, 300 MHz, $CDCl_3$): 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: 320.41

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

^{1}H NMR Spectra (δ, 300 MHz, $CDCl_3$): 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: 351.38

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 N-O (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.

5.3 Antimicrobial activity

5.1.2.1 Zone of Inhibition

The zone of inhibition was measured to assess the preliminary antibacterial activity of the indol-thiadiazole compunds. 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 inhibition exhibited by compounds

Compound Code	**Zone of Inhibition (mm)***							
	E. coli				***S. aureus***			
	25µg	50µg	75µg	100µg	25µg	50µg	750µg	100µg
4a	-	-	12	13	-	-	-	12
4b	-	-	13	14	-	-	11	12
4c	-	-	16	17	-	-	13	14
4d	-	-	14	16	-	-	14	16
4e	-	-	**19**	**25**	-	-	**17**	**19**
Norfloxacin	**23**	-	-	-	**24**	-	-	-

Minimum Inhibitory Concentration (MIC)

The MIC value of the test compounds was determined using broth dilution method by measuring the optical density of the broth solution incubated with diluted drug samples. The concentration that resulted in 50% optical density in comparison to the growth tube was taken as MIC of the test sample (Figure 2 & 3). The IC50 value was calculated from the plot (Table 3).

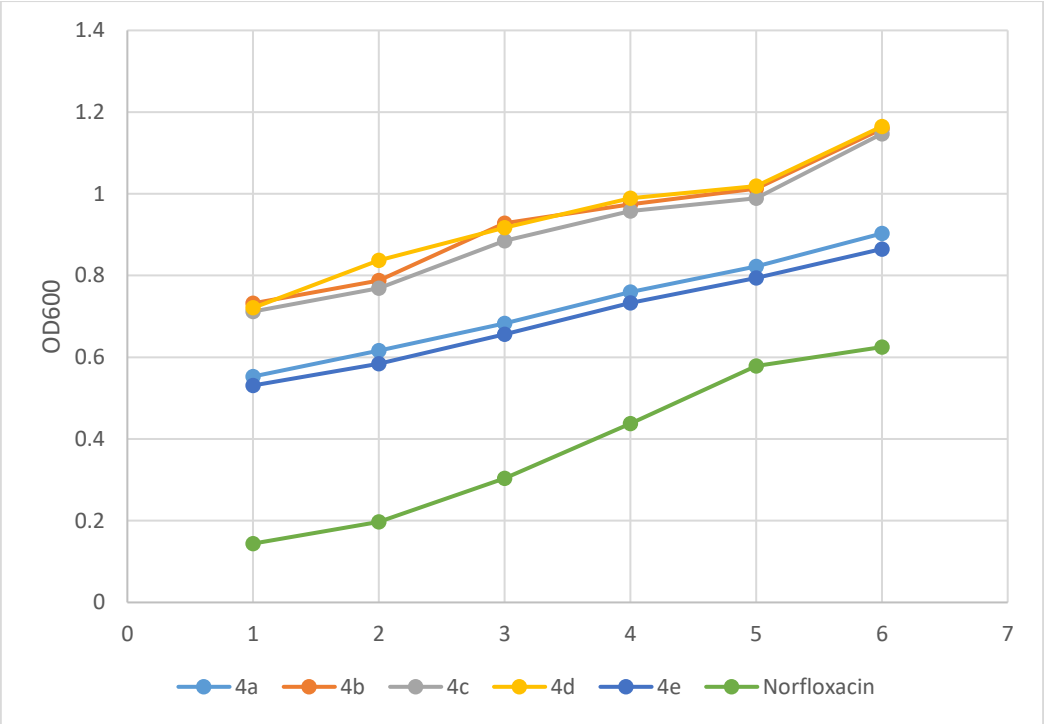


Figure 2. Plot of optical density vs. concentration for *E. coli*

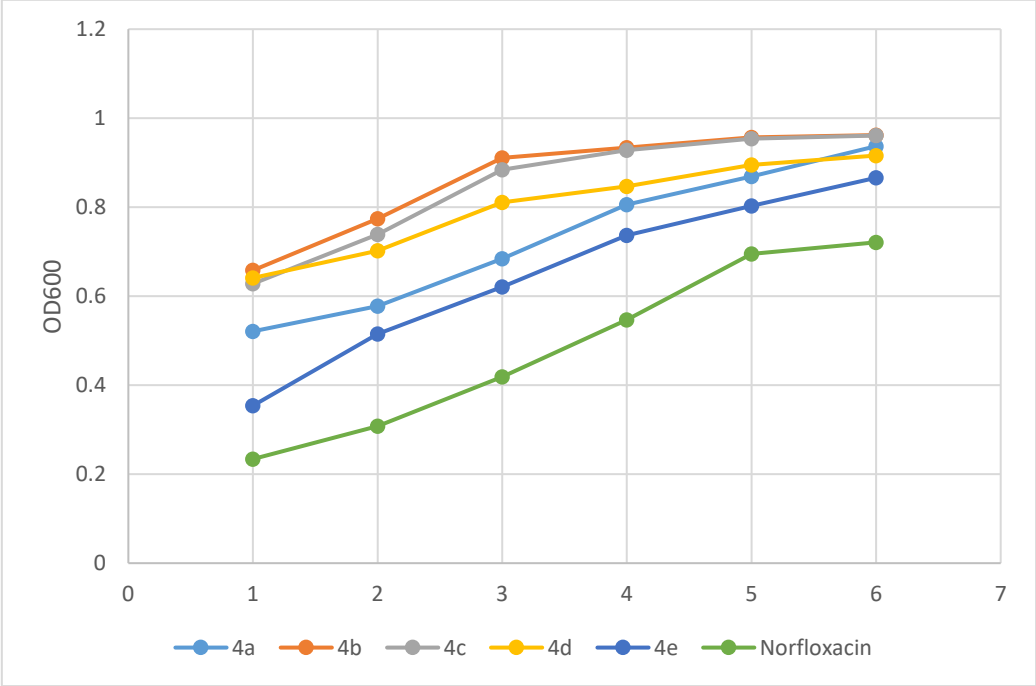


Figure 3. Plot of optical density vs. concentration for *S. aureus*

Table 3. Calculated IC_{50} values of the test samples

Test sample	IC50 (µg/mL)	
	E. coli	***S. aureus***
4_a	78.03	107.33
4_b	147.08	161.27
4_c	140.11	146.20
4_d	141.25	169.38
4_e	66.94	62.21
Norfloxacin	0.89	0.01

As visible from the results, the IC_{50} value for 4e was the lowest against both the pathogen (66.94 µg/mL for *E. coli* and 62.21 µg/mL for *S. aureus*). Also form the results it was very evident that attachment of electron donating groups in the molecule increased the IC50 (reduced antimicrobial potency) as seen in compounds 4b, 4c and 4d. In contrast non substituted compound, 4a was having a higher antibacterial activity in comparison to the electron donor substitution.

Conclusions

The present work focused on synthesizing 2-phenyl indole derivatives conjugated with thiadiazole possessing antimicrobial potential. The synthesized compounds with diverse substitution pattern were able to exhibit antimicrobial action. 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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