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DESIGN AND SYNTHESIS OF BIOLOGICAL ACTIVE N-(5-P-TOLYL-1,3,4-THIADIAZOL-2-YL) BENZAMIDEANDITS DERIVATIVES WITH EVALUATION OF THEIR ANTI-BACTERIAL ACTIVITY

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Keywords:

Thiadiazole, Benzamide, Antibacterial, Gram-positive bacteria, Gram-negative bacteria, Aromatic acid

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ABSTRACT: A number of N-(5-p-tolyl-1,3,4-thiadiazole-2-yl) benzamide derivatives were synthesized from aromatic acid. The antibacterial activity of these newly synthesized N-(5-p-tolyl-1,3,4-thiadiazole-2-yl) benzamide derivatives against Gram-positive bacteria and Gram-negative bacteria was also studied using the minimum inhibition concentration method. The synthesized compounds were showed better results for antibacterial evaluation against gram-positive (*Staphylococcus aureus, Bacillus subtilis*), gram-negative (*Escherichia coli, Pseudomonas aeruginosa*) and fungal strains (*Candida albicans, A. niger*) and were found to be good antibacterial and antifungal agents. Most of the derivatives showed significant antibacterial and antifungal activity against standard. Compared to other methods, the advantageous features of this methodology are operational simplicity, including excellent yields and short reaction time. The developed synthetic protocol represents a very simple and easy to handle for the synthesis of substituted N-(5-p-tolyl-1,3,4-thiadiazole-2-yl) benzamide derivatives.

INTRODUCTION: The 1,3,4-thiadiazole ring is one of the most important and well-known heterocyclic nuclei, which have been wide scope in pharmaceutical industry because of their interesting biological activities such as antibacterial and antifungal and specially used in the preparation of antibacterial therapy of microbes and also many thiadiazole drugs have been developed and successfully used from many years for the treatment of bacterial and fungal diseases [1]. The thiadiazole rings also have common and basic features in natural products [2].

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Thiadiazole show a broad range of biological activities and are found in many potential biological active molecules such as antimicrobial [3-8] antibacterial [9] and anticonvulsant drugs [10-11] anticancer [12] anti-inflammatory activity [13] anti-tubercular [14] and antifungal activities [15]. Due to the resistance of pathogenic bacteria towards available antibacterial drugs is rapidly becoming a main worldwide problem, the design and synthesis of new class of chemical moieties to handle with resistant bacteria has become one of the significant areas of antibacterial research today.

On the other hand, fungal infections are also increasing rapidly because of the increased number of immune-compromised patients. As known, not only biochemical similarity of the human cell and fungi forms a handicap for selective activity, but also easily gained resistance is the main problem noticed in developing safe and efficient antifungals.

In the pharmaceutical field, new drugs are discovered by molecular modification of the lead compound of established activity.

So an attempt was made to synthesize, new substituted 1,3,4-thiadiazoles compounds as antimicrobial and antifungal agents. Hence, the synthesis of a different derivative of 1,3,4-thiadiazoles was carried out along with other substituted aromatic acid.

MATERIALS AND METHODS: The entire chemicals and solvent used in the present project were purchased from commercial suppliers and used without further purification. The completion of reactions was monitored by thin-layer chromatography (TLC) using silica gel coated aluminum sheets. Mass spectra were recorded on Shimadzu mass-spectrometer. NMR spectra were recorded on a Bruker advance II 400 NMR Spectrometer.

Scheme:

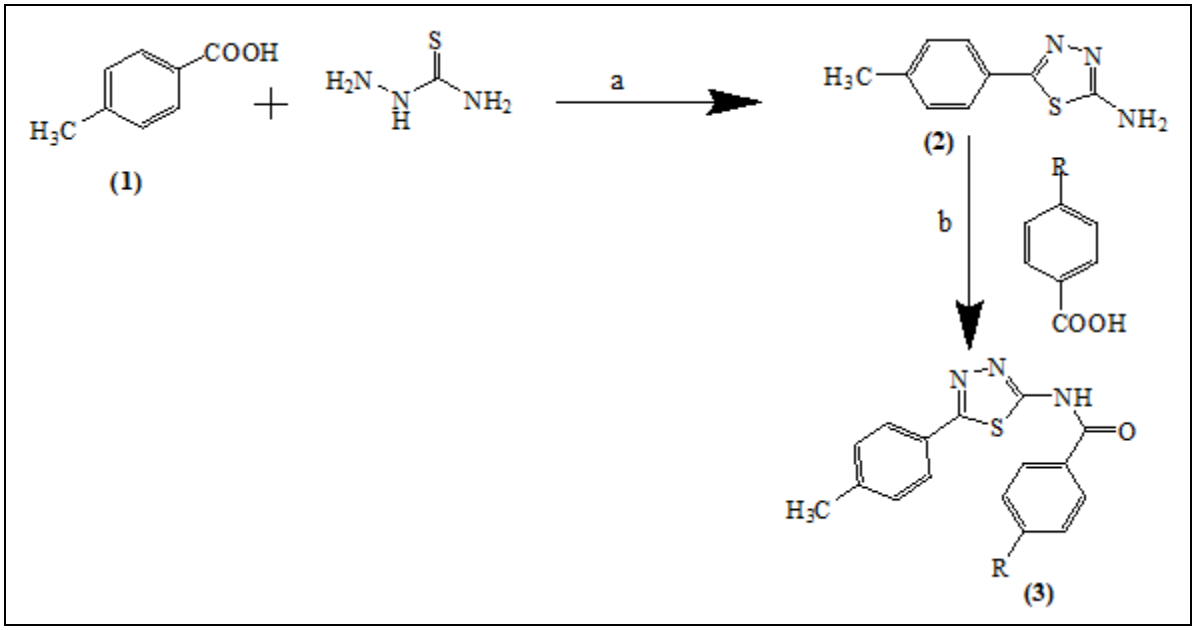

FIG. 1: SYNTHETIC ROUTE OF TARGET COMPOUNDS

Where R=H, CH_3, OCH_3, Cl, Br; Synthesis of compounds (a) $POCl_3$, 60–65 °C; (b) DCM, DCC, DMAP, 0-5 °C

RESULTS AND DISCUSSION: Resultant compounds (3) were synthesized using an identical efficient and conventional synthetic route outlined in Scheme. Compound (3) was synthesized by reacting with 4-methyl benzoic acid, and hydrazine carboxamide in the presence of phosphorus oxy-chloride at 60-65 °C form a compound (2) and (2) reacted with substituted aromatic acid in the presence of Dicyclohexylcarbodiimide and Dimethylamino-pyridine in DCM as a solvent at 0-5 °C to form substituted compound (3) as a product in good yield. The structures of these compounds were verified by means of, MS, 1H NMR.

General Procedure for the Synthesis of the Derivative 5-p-tolyl-1,2,4-thiadiazol-2-yl) benzamide: 5-p-tolyl-1,3,4-thiadiazol-2-amine (2): In a clean 4 Neck RBF, take a solution of 4-methyl benzoic acid (0.05 mol) was refluxed with thio-semicarbazide (0.05 mol) in the presence of phosphorus oxychloride (15 ml) for 1 h. The reaction mixture was cooled and diluted with water and again refluxed for 4 h. The reaction was monitored by thin-layer chromatography and filtered after completion. The filtrate was basified with potassium hydroxide, and the precipitate was filtered off and then recrystallized from ethanol to give the desired compound (2). 1H-NMR *d* (ppm): 2.34 (H s, CH_3); 7.29-7.68 (4H, d, Ar-H); 7.22 (2H, s, -NH_2) MS: m/z 192 (M +1).

5-p-tolyl-1,2,4-thiadiazol-2-yl) Benzamide (3): In a clean 4 Neck RBF, takea solution of substituted aromatic carboxylic acid and compound (3) in 15 mL CH_2Cl_2 was added catalytic DMAP 0.1 mole was added dropwise solution of DCC 1.3 mole dissolved in 5 ml DCM after stirring 1.0 hr completion of reaction was confirmed by TLC,

Filter the reaction mass and discarded the DCU obtained as bi product, washed the filtrate with 5% $NaHCO_3$ solution and the layer was separated, the product was obtained after vacuum distillation of solvent, product (3). The target compounds were achieved in good yield.

1. N- (5-tolyl-1,3,4-thiadiazol-2-yl) Benzamide: $C_{16}H_{13}N_3OS$: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.34 (H, s, -CH_3), 7.29-7.68 (4H, m, Ar-H), 7.63-8.10 (5H, m, Ar-H), 12.83 (1H, s, -NH_2). M/Z: m/z 296 [M+1H].

2. 4- methyl- N- (5-p-tolyl-1,3,4-thiadiazol-2-yl) Benzamide: $C_{17}H_{15}N_3OS$: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.36 (H, s, -CH_3), 2.34 (H, s, -CH_3), 7.27-7.69 (4H, m, Ar-H),7.40-7.93 (4H, m, Ar-H), 2.34 (H, s, -CH_3), 12.85 (1H, s, -NH_2). M/Z: m/z 310 [M+1H].

3. 4- methoxy- N- (5-tolyl-1,3,4-thiadiazol-2-yl) Benzamide: $C_{17}H_{15}N_3O_2S$ M/Z 326: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.33 (H, s, -CH_3), 3.84 (H, s, -CH_3), 7.28-7.67 (4H, m, Ar-H), 7.16-7.93 (4H, m, Ar-H), 3.84 (H, s, -CH_3), 12.82 (1H, s, -NH-). M/Z: m/z 326 [M+1H].

4. 3- methyl- N- (5-tolyl-1,3,4-thiadiazol-2-yl) Benzamide: $C_{17}H_{15}N_3OS$: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.36 (H, s, -CH_3), 2.35 (H, s, -CH_3), 7.29-7.67 (4H, m, Ar-H), 7.37-7.85 (4H, m, Ar-H), 2.35 (H, s, -CH_3), 12.84 (1H, s, -NH-). M/Z: m/z 310 [M+1H].

5. 3- methoxy- N- (5-tolyl-1,3,4-thiadiazol-2-yl) Benzamide: $C_{17}H_{15}N_3O_2S$: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.34 (H, s, -CH_3), 3.86 (H, s, -CH_3), 7.26-7.69 (4H, m, Ar-H), 6.90-7.83 (4H, m, Ar-H), 3.83 (H, s, -CH_3), 12.85 (1H, s, -NH-). M/Z: m/z 326 [M+1H].

6. 4- ethyl- N- (5-p-tolyl-1,3,4-thiadiazol-2-yl) Benzamide: $C_{18}H_{17}N_3OS$: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.32 (H, s, -CH_3),1.25 (H, s, -CH_3), 7.23-7.65 (4H, m, Ar-H), 7.37-7.85 (4H, m, Ar-H), 2.36 (H, s, -CH_3), 12.81 (1H, s, -NH-). M/Z: m/z 310 [M+1H].

7. 4- methoxy- 3- methyl- N- (5-p-tolyl-1,3,4-thiadiazol-2yl) Benzamide: $C_{18}H_{17}N_3O_2S$: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.35 (H, s, -CH_3),7.30-7.68 (4H, m, Ar-H), 7.05-7.75 (3H, m, Ar-H), 2.15 (H, s, -CH_3), 3.86 (H, s, -CH_3), 12.84 (1H, s, -NH). M/Z: m/z 340 [M+1H].

8. 4- Chloro- 3, methyl- N (5-p-totyl-1,3,4-thiadiazol-2yl) Benzamide:$C_{17}H_{14}ClN_3OS$: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.32 (H, s, -CH_3), 7.28-7.69 (4H, m, Ar-H), 7.55-7.79 (3H, m, Ar-H), 2.35 (H, s, -CH_3), 12.81 (1H, s, -NH-). M/Z: m/z 344 [M+1H].

9. 4- Chloro- N- (5-p-tolyl-1,3,4-thiadiazol-2yl) Benzamide: $C_{16}H_{12}ClN_3OS$: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.35 (H, s, -CH_3), 7.24-7.65 (4H, m, Ar-H), 7.68-7.98 (4H, m, Ar-H), 12.85 (1H, s, -NH-). M/Z: m/z 330 [M+1H].

10. 4- Bromo- N-(5-p-tolyl-1,3,4-thiadiazol-2-yl): Benzamide$C_{16}H_{12}BrN_3OS$: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.33 (H, s, -CH_3), 7.27-7.66 (4H, m, Ar-H), 7.78-7.93 (4H, m, Ar-H), 12.82 (1H, s, -NH-). M/Z: m/z 374 [M+1H].

11. 4- Chloro- 2- methyl- N- (5-p-tolyl-1,3,4-thiadiazol-2-yl)Benzamide: $C_{17}H_{14}ClN_3OS$: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.36 (H, s, -CH_3), 7.25-7.67 (4H, m, Ar-H), 7.48-7.86 (3H, m, Ar-H), 2.48 (H, s, -CH_3), 12.87 (1H, s, -NH-). M/Z: m/z 346 [M+1H].

12. 4- bromo- 2- methyl- N- (5-p-tolyl-1,3,4-thiadiazol-2-yl) Benzamide: $C_{17}H_{14}BrN_3OS$: ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.35 (H, s, -CH_3), 7.28-7.69 (4H, m, Ar-H), 7.55-7.80 (3H, m, Ar-H), 2.45 (H, s, -CH_3), 12.82 (1H, s, -NH-). M/Z: m/z 388 [M+1H].

TABLE 1: SYNTHESIZED 5-P-TOLYL1,2,4-THIADIAZOL-2-YL) BENZAMIDE DERIVATIVE

S. no.	-R-	Product	Yield (%)
1	-C_6H_5	$C_{16}H_{13}N_3OS$	82
2	4-CH_3-C_6H_4	$C_{17}H_{15}N_3OS$	80
3	4-OCH_3-C_6H_4	$C_{17}H_{15}N_3O_2S$	84
4	3-CH_3-C_6H_4	$C_{17}H_{15}N_3OS$	86
5	3-OCH_3-C_6H_4	$C_{17}H_{15}N_3O_2S$	84
6	4-C_2H_5-C_6H_4	$C_{18}H_{17}N_3OS$	81
7	3-CH_3-4-OCH_3-C_6H_4	$C_{18}H_{17}N_3O_2S$	82
8	4-Cl-3-CH_3-C_6H_4	$C_{17}H_{14}ClN_3OS$	80
9	4-Cl-C_6H_4	$C_{16}H_{12}ClN_3OS$	75
10	4-Br-C_6H_4	$C_{16}H_{12}BrN_3OS$	79
11	4-Cl-2-CH_3-C_6H_4	$C_{17}H_{14}ClN_3OS$	73
12	4-Br-2-CH_3-C_6H_4	$C_{17}H_{14}BrN_3OS$	72

Antibacterial Activity: Standardized disk diffusion method was used in the determination of *in-vitro* antibacterial activity. The total synthesized

compounds (1-12) were tested for their antimicrobial activity. In this project *Staphylococcus aureus, Bacillus subtilis, Escherichia coli,* and *Pseudomonas aeruginosa* bacterial strains were used. The test bacteria were subcultured using nutrient agar medium. Freshly prepared sterilized nutrient agar media were poured 20 ml into each petri plate and allowed to solidify. With the respective strains of bacteria that were transferred aseptically. The plates were kept undisturbed for at least 20 min in the refrigerator to allow diffusion of the solution properly in the nutrient agar medium. The plates were incubated at 37 ± 1 °C for 24 h. Then a well 0.5cm was made in the medium by using sterile cork borer, 50µl of each compound were transferred into separate wells. Then these plates were incubated at 37 °C for 24 h. After the incubation period, the results were observed and measured the diameter of inhibitor zone around each well. The zones of growth inhibition around the well were measure after 24 h at 37 °C. Ciprofloxacin and Streptomycin was used as standard drug for antibacterial activity.

Antifungal Activity: *In-vitro* antifungal activity was determined by agar well diffusion method. All synthesized compounds (1-12) were selected for their antifungal activity. In this work and *Candida albican*s and *A. niger* fungal strains were used. The test fungi were subcultured using potato dextrose agar medium. Freshly prepared sterilized potato dextrose agar media were poured 20 ml into each petri plate and allowed to solidify. The test fungal cultures were evenly spread over using sterile cotton swab. Then a well 0.5cm was made in the medium by using sterile cork borer, 50µl of the each compound were transferred into separate wells. Then these plates were incubated at 27 °C for 48-96 h.

After the incubation period, the results were observed and measured the diameter of the inhibitor zone around each well. The zones of growth inhibition around the well were measure after 48 to 96 h at 28 °C. Nystatin was used as a standard drug for antifungal activity.

TABLE 2: SYNTHESIZED ANTIBACTERIAL DERIVATIVES

S. no.	-R-	Product	Bacteria				Fungi	
			S. aureus ZOI (mm)	*B. subtilis* ZOI (mm)	*E. coli* ZOI (mm)	*P. aeruginosa* ZOI (mm)	*C. albicans* ZOI (mm)	*A. niger* ZOI (mm)
1	-C_6- -H	$C_{16}H_{13}N_3OS$	14	13	15	14	14	15
2	4-CH_3	$C_{17}H_{15}N_3OS$	12	13	12	15	12	11
3	4-OCH_3	$C_{17}H_{15}N_3O_2S$	11	12	11	10	13	12
4	3-CH_3	$C_{17}H_{15}N_3OS$	15	14	16	14	18	20
5	3-OCH_3	$C_{17}H_{15}N_3O_2S$	15	16	15	14	18	17
6	4-C_2H_5	$C_{18}H_{17}N_3OS$	12	11	12	14	12	13
7	3-CH_3-4-OCH_3	$C_{18}H_{17}N_3O_2S$	14	13	14	12	17	19
8	4-Cl-3-CH_3	$C_{17}H_{14}ClN_3OS$	11	11	13	12	15	14
9	4-Cl	$C_{16}H_{12}ClN_3OS$	12	12	11	12	12	14
10	4-Br	$C_{16}H_{12}BrN_3OS$	10	11	12	11	11	13
11	4-Cl-2-CH_3	$C_{17}H_{14}ClN_3OS$	13	14	15	13	13	15
12	4-Br-2-CH_3	$C_{17}H_{14}BrN_3OS$	15	13	14	12	14	13
13	-	Steptomycin (Std)	17	18	20	18	-	-
14	-	Nystatin (Std)	-	-	-	-	24	25

CONCLUSION: In conclusion, we have synthesized thiadiazole based derivatives by suitable methods. Most of the compounds showed antibacterial activities *in-vitro* against gram-positive (*Staphylococcus aureus, Bacillus subtilis),* gram-negative (*Escherichia coli, Pseudomonas aeruginosa)* and also showed antifungal activity against (*Candida albicans, Aspergillus niger*). Maximum compounds have been found to be shown antibacterial and antifungal activity.

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