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Research Article

SYNTHESIS AND BIOLOGICAL EVALUATION OF SOME NEW 1,3,4-THIADIAZOLE DERIVATIVES FOR THEIR ANTIMICROBIAL ACTIVITIES

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ABSTRACT

In the present study, novel Thiadiazoles were synthesized by reaction of benzoic and 2-hydroxybenzoic acid with thiosemicarbazide to synthesize 5-phenyl-1,3,4-thiadiazol-2-amine**(A)** and 2-(5-amino-1,3,4-thiadiazole-2-yl) phenol**(B).**From these compounds various derivatives of 1,3,4-Thiadiazole derivatives (A1-A4 and B1-B4) have been synthesized. The chemical structures of the synthesized compounds were confirmed by means of IR, ^{1}H NMR, and nitrogen estimation. These compounds were screened for antibacterial (*Staphylococcus aureus* ATCC 9144*, Becillus Cereus* ATCC *11778, Escherichia coli* ATCC 25922*, Pseudomonas aeruginosa* ATCC 2853 and anti-fungal (*Aspergillus niger* ATCC 9029 and *Aspergillus fumigatus* ATCC 46645) by paper disc diffusion technique.

Keywords: 1,3,4-Thiadiazole, Synthesis, Antibacterial, Antifungal, NMR.

INTRODUCTION

The structures of imidazo[2,1-b] [1,3,4]thiadiazoles are closely related to the biologically vibrant imidazo[1,3,4]thiazole heterocycles, in which the CH group in the thiazole ring is substituted by the isosteric nitrogen atom, but their properties often possess marked differences. The practically planar and rigid heteroaromatic imidazo[2,1-b][1,3,4] thiadiazole ring system may therefore have interesting physicochemical and biological properties, because of the presence of four heteroatoms and two condensed heterocycles with different π-conjugation. Thiadiazole play a prominent role in nature. For example, the thiazolium ring present in vitamin B1 serves as anelectron sink and its coenzyme form is important for thedecarboxylation of α-keto acids[1]. Thiazoles were reported to possess anti-microbial[2], analgesic[3], anti inflammatory[4], anti-cancer[5], anti-tubercular[6], anthelmintic[7] & diuretic[8] activities. Thiazoles are easily metabolized by routine biochemical reactions and are non-carcinogenic in nature[9]. In addition, pyrazoles are reported as anti-microbial[10], analgesic[11], anti inflammatory[12], anti hypertensive[13], anti-depressant[14] and anticancer[15] agents. Above observation prompted us to synthesize the title compounds **(A1-A4 and B1-B4)** with presumption that drug intermediates based on thiosemicarbazide with various amines would produce novel thiadiazole derivatives with potent biological activities. Their chemical structure was confirmed by IR, ^{1}HNMR, and nitrogen estimation. These compounds were screened for their antibacterial activity against two gram + ve bacteria (*Staphylococcus aureus* ATCC 9144, *Becillus Cereus* ATCC *11778* and two gram - ve bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 2853) and anti-fungal (*Aspergillus niger* ATCC 9029 and *Aspergillus fumigatus* ATCC 46645) activities by paper disc diffusion technique.

EXPERIMENTAL

All chemicals used in this study were purchase from Aldrich Chemicals and were used without further purification. All melting points were taken in open capillary tube and are uncorrected. The purity of the compounds was checked by TLC on pre-coated SiO_2 gel (HF_{254}, 200 mesh) aluminum plates (E Merk) using Butanol: Acetic acid: water (4:1:5) visualized in iodine chamber. FTIR spectra were recorded with Perkin Elmer spectrophotometer. The ^{1}HNMR spectra were determined with Brucker 400 MHz FTNMR spectrometer.

Synthesis of 5-phenuyl-1, 3, 4-thiadiazole-2-amine

A mixture of thiosemicarbazide (9.11 g, 0.1 mol),benzoic acid (12.2g,0.1 mol), and conc. Sulphuric acid (5 ml) in 50 ml of ethanol was refluxed for 1.5 hour and poured onto crushed ice. The solid separated out was filtered, washed with cold water and recrystallized from ethanol to separate the first step product.

General method for synthesis of $A_1 – A_4$.

A methanolic solution of first step product (2 gm, 0.001mole) was charged into a three nack flask equipped with a stirred and dropping funnel. The solution was stirred to dissolve it completely. To this methanolic solution, formaldehyde (7 ml, 37%) was added dropwise during 15-20 minutes. The resulting mixture was stirred during half an hour to complete reaction of formaldehyde and to yield methylol derivative. To this reaction mixture, the methanolic solution of amine (1.5 gm, 0.001 mol) was added dropwise with stirring in about half an hour at 30 ^{o} C temperature and refluxed for two hour at 65-70 ^{o}C. it was allowed to cool and poured in ice water. The solid obtained was filtered off washed thoroughly with hot water and air dried. The reaction scheme for these compounds are shown in figure-2.

Synthesis of 5-(substituted)-2-amino-thiadiazole

A mixture of thiosemicarbazide (9.11 g, 0.1 mol), Salicylic acid (13.8 g,0.1 mol), and conc. Sulphuric acid (5 ml) in 50 ml of ethanol was refluxed for 1.5 hour and poured onto crushed ice. The solid separated out was filtered, washed with cold water and recrystallized from ethanol to separate the first step product (figure-3).

General procedure for synthesis of B1 – B_4

A methanolic solution of first step product (2 gm, 0.001mole) was charged into a three nack flask equipped with a stirred and dropping funnel. The solution was stirred to dissolve it completely. To this methanolic solution, formaldehyde (7 ml, 37%) was added dropwise during 15-20 minutes. The resulting mixture was stirred during half an hour to complete reaction of formaldehyde and to yield methylol derivative. To this reaction mixture, the methanolic solution of amine (1.5 gm, 0.001 mol) was added drop wise with stirring in about half an hour at 30 ^{o} C temperature and refluxed for two hour at 65-70 ^{o}C. It was allowed to cool and poured in ice water. The solid obtained was filtered off washed thoroughly with hot water and air dried. The reaction scheme for these compounds are shown in figure-4.

The data of physical characteristics of synthesized compounds are shown in table-1. Percentage of nitrogen was estimated by Kjeldahl method. All the synthesized compounds were characterized by IR and ^{1}H NMR. The spectral data of synthesized compounds are shown in table-2.

Antibacterial activity

All the newly synthesized 1, 3, 4-thiadiazole derivatives were screened for their antibacterial and antifungal activity. For antibacterial studies microorganisms employed were *Staphylococcus aureus* ATCC 9144, *Becillus Cereus* ATCC *11778*, *Escherichia coli ATCC 25922*, *Pseudomonas aeruginosa ATCC 2853*. For antifungal, *Aspergillus niger ATCC 9029 and Aspergillus fumigatus ATCC 46645* were used as organism. Both microbial studies were assessed by Minimum Inhibitory Concentration (MIC) by serial dilution method. For this, the compound whose MIC has to be determined is dissolved in serially diluted DMF Then a standard drop of the culture prepared for the assay is added to each of the dilutions, and incubated for 16–18 hrs at 37^{0}c. MIC is the highest dilution of the compound, which shows clear fluid with no development of turbidity. The results are shown in the table: 3.

RESULT AND DISCUSSION

The structures of the synthesized compound were determined on the basis of their FTIR and ^{1}H NMR data. The spectral data for FTIR and 1 H NMR are elaborated in table-2, which confirms the structure of synthesized compounds. *In vitro* antibacterial activity data of 1,3,4-Thiadiazole derivatives against tested organisms displayed significant activity with a wide degree of variation. It is found that compound **A1, A2 and B2** have shown significant antibacterial activity against gram positive bacteria. Rest of the compounds has exhibited significant to substantial activity against the same strain. Compound **A2, A4** and **B2** have shown highest

activity against gram negative bacteria. Substantial activity is achieved in case of compounds **A2** against *S. aureus, B.cereus, E.coli, P. aeruginosa* and the remaining compounds are significantly active against the same species. All the 1,3,4-Thiadiazole derivatives have exhibited significant to moderate activity against gram positive and gram negative bacteria. Derivatives **A3** and **B2** has exhibited substantial activity against *A.niger*. and *C.* While derivatives **A1, A4, B1**and **B2** have shown higher activity against *A. fumigates.* Comparatively weak to moderate activity has been reported by remaining compounds. *E. coli* was found to be more susceptible than rest of the other strains of bacteria. From *in vitro* antifungal activity (Table 3), data reveals that all the newly synthesized compounds displayed higher to week activity in comparison to standards. Thus, it is obvious from the structure-activity profile of substituted 1,3,4-Thiadiazole derivatives; a small structural variation may induce an effect on antibacterial activity.

Table 1: Characterization data of synthesized compounds

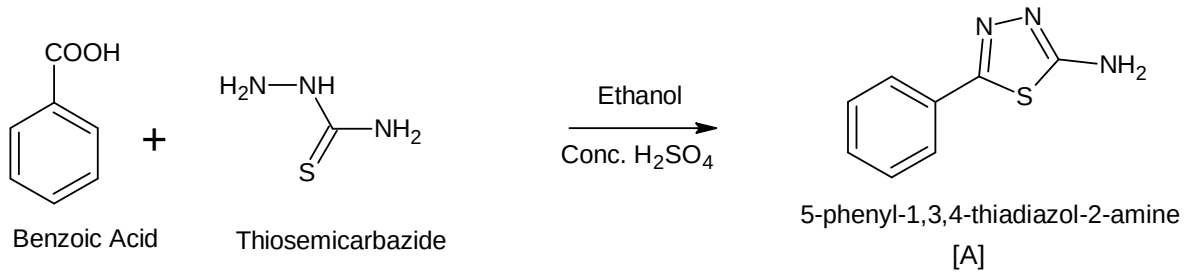
Compd.	Molecular Formula	Melting Point °C	Yield (%)	Mol. Wt gm/mole	Nitrogen (%)	
A	$C_8H_7N_3S$	160	70%	177	23.73	20.12
A1	$C_{17}H_{12}N_4O_2S$	142	68	336	16.67	13.45
A2	$C_{14}H_{15}N_5O_2S$	115	65	315	22.22	20.67
A3	$C_{14}H_{15}N_5O_2S$	145	62	315	22.22	20.67
A4	$C_{14}H_{13}N_4O_2S$	140	68	297	18.85	15.87
B	$C_8H_7N_3OS$	135	69	193	21.76	15.87
B1	$C_{17}H_{12}N_4O_3S$	112	65	342	16.37	15.68
B2	$C_{14}H_{15}N_5O_2S$	116	75	331	21.14	19.08
B3	$C_{14}H_{15}N_5O_2S$	120	72	331	21.14	19.08
B4	$C_{14}H_{14}N_4O_2S$	137	65	302	18.54	15.87

Table 2: Infra Red / ^{1}H NHR spectral study of the synthesized compounds

Compound	IR (cm^{-1})	H – NMR (δ, ppm)
A	3391 (N-H str.), 1400 (Ar.C-H str.), 1628 (Ar.C-C str.), 1450 (C-N str.), 1055 (C-S str.), 1450 (C=C str.) 1250 (C-N str.)	7.8-8.20 (8H, Ar-CH), 6.87 (1H, NH), 4.72 (1H, NH), 4.50 (2H, CH_2)
A1	3420 (N-H str.), 1450 (Ar.C-H str.), 1670 (Ar.C-C str.), 1465 (C-N str.), 1125 (C-S str.), 1550 (C=C str.), 1555 (N-O str.), 1720 (C=O str.)	10.85 (1H, OH), 6.90-8.06 (8H, Ar-CH), 4.85 (2H, CH_2), 6.50 (1H, NH)
A2	3450 (N-H str.), 1475 (Ar.C-H str.), 1605 (Ar.C-C str.), 1420 (C-N str.), 1065 (C-S str.), 1560 (C=C str.) 1565 (N-O asy. str.)	10.05 (1H, OH), 6.80-8.20 (8H, Ar-CH), 4.65 (2H, CH_2), 6.40 (1H, NH)
A3	3310 (N-H str.), 1460 (Ar.C-H str.), 1680 (Ar.C-C str.), 1450 (C-N str.), 1567 (N-O str.), 1020 (C-S str.), 1580 (C=C str.), 1635 (-NH_2 str.)	10.05 (1H, OH), 6.80-8.20 (8H, Ar-CH), 4.65 (2H, CH_2), 6.40 (1H, NH)
A4	3550 (N-H str.), 1550 (Ar.C-H str.), 1690 (Ar.C-C str.), 1450 (C-N str.), 1567 (N-O str.), 1010 (C-S str.), 1500 (C=C str.), 750 (NH str.), 3455 (-OH str.)	10.85 (1H, OH), 6.50-8.25 (8H, Ar-CH), 4.85 (2H, CH_2), 6.70 (1H, NH)
B	3550 (N-H str.), 1550 (Ar.C-H str.), 1690 (Ar.C-C str.), 1010 (C-S str.), 1500 (C=C str.) 1250 (C-N str.), 750 (NH str.), 3455 (-OH str.)	7.0-8.30 (4H, Ar-CH), 4.80(1H, NH), 4.90 (2H, CH_2)
B1	3520 (N-H str.), 1570 (Ar.C-H str.), 1680 (Ar.C-C str.), 1430 (C-N str.), 1547 (N-O str.), 950 (C-S str.), 1500 (C=C str.) 1250 (C-N str.), 750 (NH str.), 3455 (-OH str.), 1740(C=O str.)	10.00 (1H, OH), 6.00-8.00 (8H, Ar-CH), 4.65 (2H, CH_2), 6.20 (1H, NH)
B2	3450 (N-H str.), 1550 (Ar.C-H str.), 1690 (Ar.C-C str.), 1450 (C-N str.), 1470 (N-O str.), 910 (C-S str.), 1500 (C=C str.) 1250 (C-N str.), 750 (NH str.), 3455 (-OH str.)	10.05 (1H, OH), 6.80-8.20 (8H, Ar-CH), 4.65 (2H, CH_2), 6.40 (1H, NH)
B3	3470 (N-H str.), 1520 (Ar.C-H str.), 1650 (Ar.C-C str.), 1410 (C-N str.), 1540 (N-O str.), 980(C-S str.), 1500 (C=C str.) 1220 (C-N str.), 750 (NH str.), 3455 (-OH str.)	10.00 (1H, OH), 6.00-8.00 (8H, Ar-CH), 4.65 (2H, CH_2), 6.20 (1H, NH)
B4	3580 (N-H str.), 1500 (Ar.C-H str.), 1670 (Ar.C-C str.), 1450 (C-N str.), 1567 (N-O str.), 1010 (C-S str.), 1500 (C=C str.) 1240 (C-N str.), 750 (NH str.), 3455 (-OH str.)	10.40 (1H, OH), 6.20-8.00 (8H, Ar-CH), 4.75 (2H, CH_2), 6.30 (1H, NH)

Table 3: Antibacterial and Antifungal data for the newly synthesized Thiadiazoles

Compd.	Antibacterial data in MIC(µg/ml)				Antifungal data in MIC (µg/ml)	
	Gram +ve Bacteria		Gram -ve Bacteria			
	S. aureus	*B. cereus*	*P. aeruginosa*	*E.coli*	*A.niger*	*A.fumigatus*
A1	8	8	5	7	15	17
A2	9	8	10	9	16	15
A3	5	4	7	6	18	14
A4	8	6	9	9	16	18
B1	7	6	6	7	16	17
B2	8	8	9	9	17	17
B3	6	7	4	5	13	12
B4	7	5	8	6	15	14
Streptomycin	10	9	12	10	--	--
Fluconazole	--	--	--	--	20	22



Benzoic Acid Thiosemicarbazide 5-phenyl-1,3,4-thiadiazol-2-amine [A]

Fig. 1: Synthesis of 5-phenuyl-1,3,4-thiadiazole-2-amine

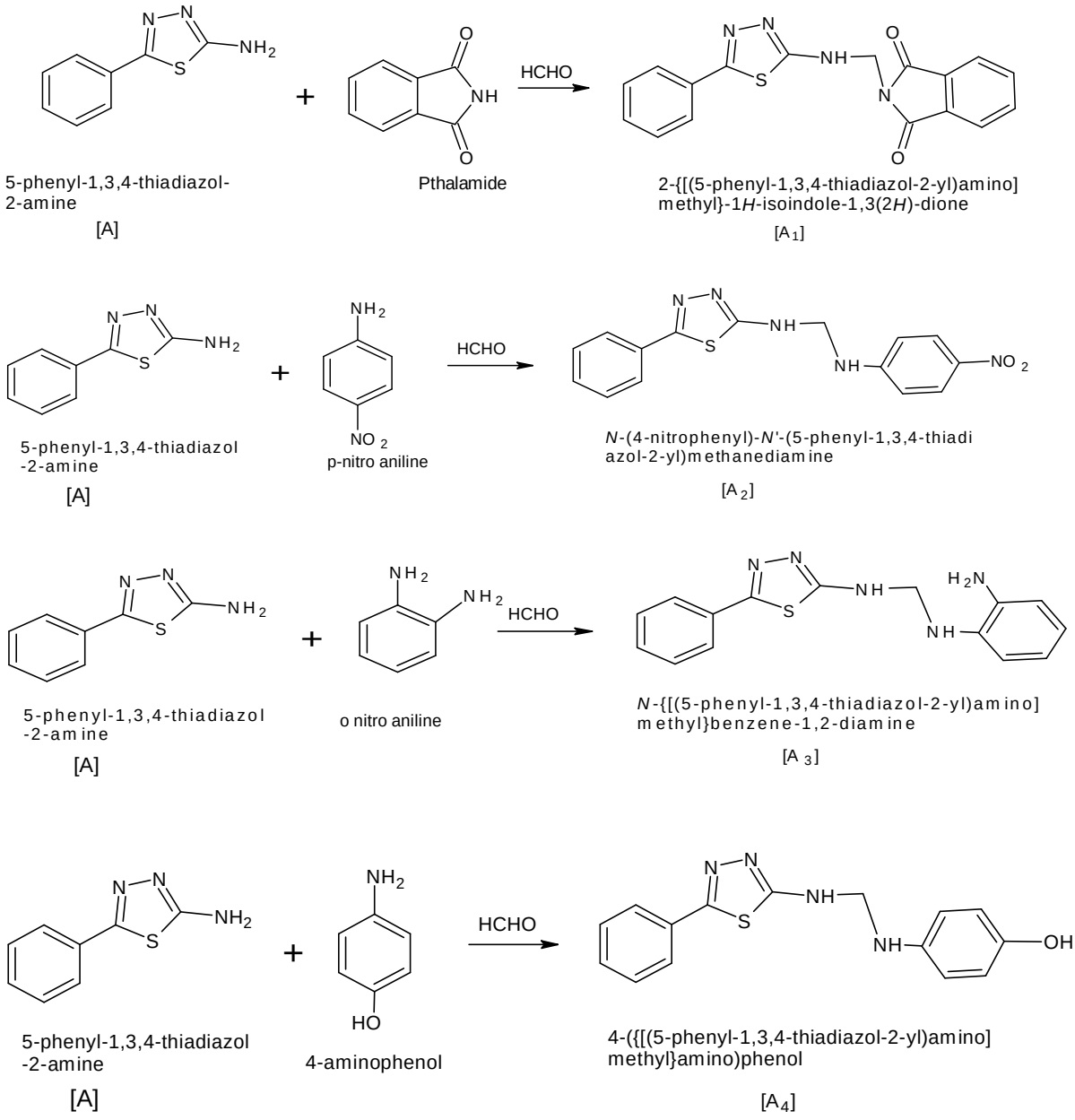


Fig. 2: Synthesis of 5-(substituted)-2-amino-thiadiazoles

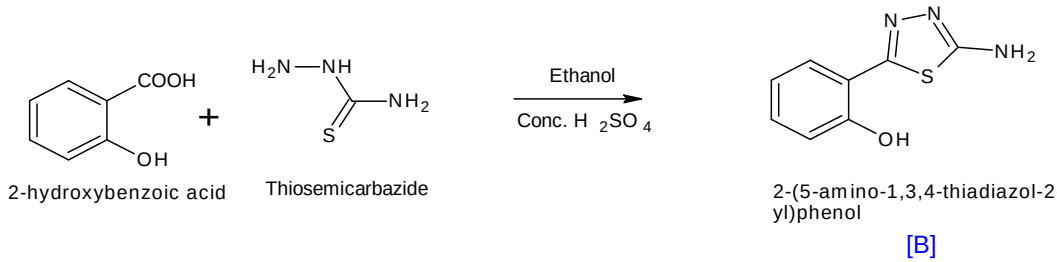


Fig. 3: Synthesis of 2-(5-amino-1,3,4-thiadiazol-2-yl)phenol

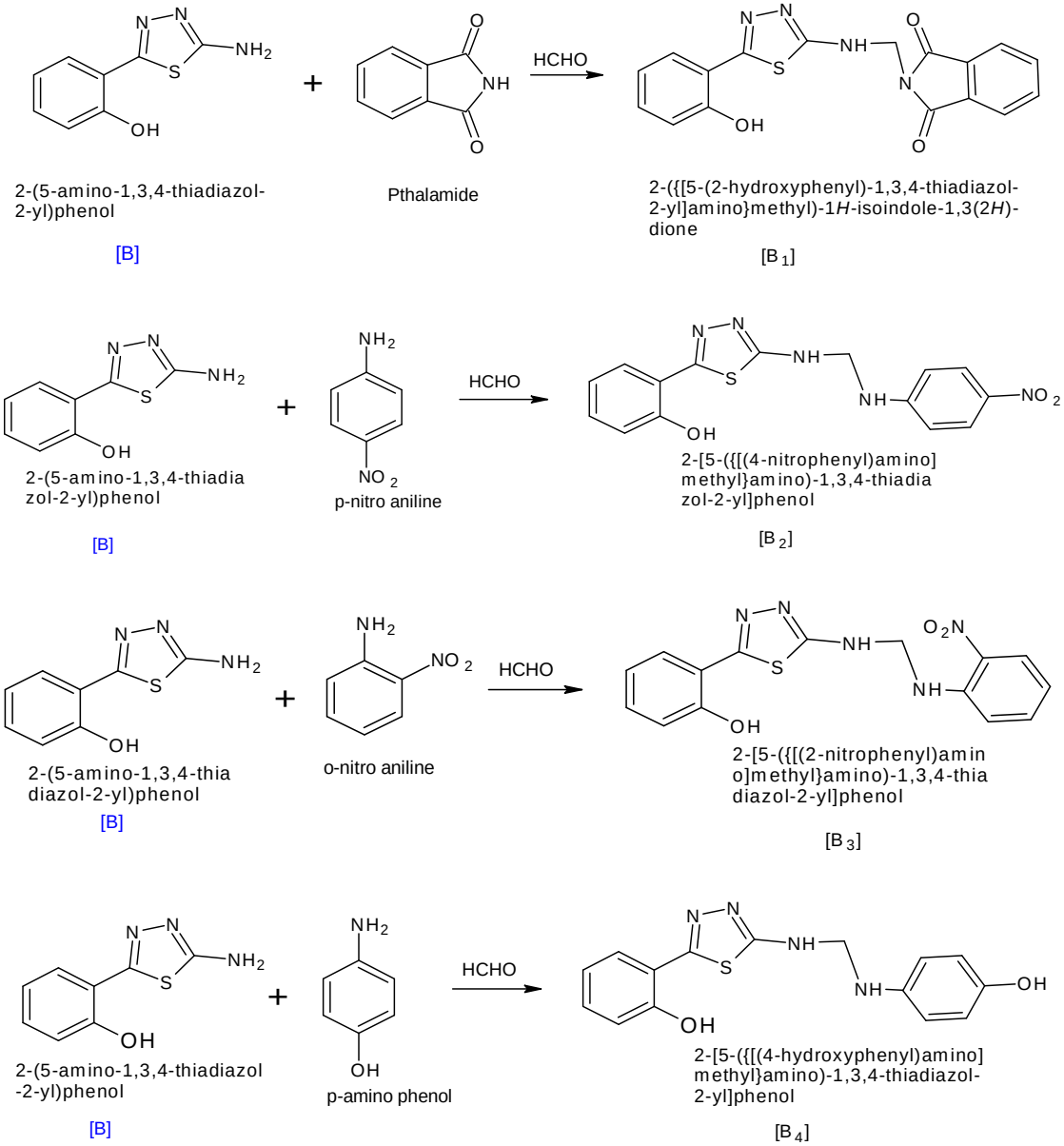


Fig. 4: Synthesis of 5-(substituted)-2-amino-thiadiazole

CONCLUSION

A series of novel 1,3,4-Thiadiazole derivatives were synthesized and the structure of the entire compounds were confirmed by recording by their[1]H NMR, and IR spectra. In conclusion, we feel that the preliminary *in vitro* activity results of this class of compounds may possess potential for design of future molecules with modifications on the aryl substituent's as well as NH2 side chain. All the synthesized compounds

showed moderate activity against bacteria and fungi. The screening studies have demonstrated that the newly synthesized compounds exhibit promising antibacterial and antifungal properties. Therefore, it is concluded that there exists ample scope for further study in this class of compounds.

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REFERENCES

1. Sondhi, SM.; Singh, N.; Johar, M.; Kumar, A. Synthesis, anti-inflammatory and analgesic activities evaluation of some mono, bi, tricyclic pyrimidine derivatives. *Bioorg. Med. Chem.*2005;*13*, 6158–6166
2. Mahajan NS, Pattan SR, Jadhav RL,Pimpodkar NV and Manikrao AM, Synthesis of some thiazole compounds of biological interest containing mercapto group, Int. J. Chem. Sci.2008; 6 (2): 800-806.
3. Basavaraja KM, Somasekhar B and Appalaraju S, Synthesis and biological activity of some 2-[3-substituted-2-thione- 1,3,4-thiazole-5-yl] amino benzothiazoles, Indian J. Heterocycl. Chem.2008;18: 69-72.
4. Karabasanagouda T, Adhikari AV, Ramgopal D and Parameshwarappa G, Synthesis of some new 2-(4-alkylthiophenoxy)-4-substituted-1, 3-thiazoles as possible anti-inflammatory andantimicrobial agents, Indian J. Chem.2008; 47B:144-152.
5. Abbs TF, Reji F, Devi SKC, Thomas KK, Sreejalekshmi KG, Manju SL, Francis M, Philip SK, Bharathan A and Rajasekharan KN, ynthesis and cytotoxicity studies of thiazole analogs of the anticancer marine alkaloid dendrodoine, Indian J. Chem.2008; 47B: 1145-1150.
6. Chowki AA, Magdum CS, Ladda PL and Mohite SK, Synthesis and antitubercular activity of 6-nitro-2-[4-formyl-3-(substituted phenyl) pyrazol-1-yl] benzothiazoles, Int. J. Chem. Sci.2008;6 (3): 1600-1605.
7. Bhusari KP, Khedekar PB, Umathe SN, Bahekar RH and Raghu Ram Rao A, Synthesis of 8-bromo-9-substituted-1,3-benzothiazolo-[5,1-b]-1, 3, 4-triazoles and their anthelmintic activity, Indian J. Hetero.Chem.200;9: 275-278.
8. Basawaraj R, Suresh M and Sangapure SS, Synthesis and Pharmacological activities of some 2-arylamino/arylidene hydrazio-4-(5'-chloro-3'-methylbenzofurn-2'- yl) thiazoles, Indian J. Heterocycl. Chem.2005;15: 153-156.
9. Lozach N., Forty years of heterocyclic Sulphur Chemistry in Sulphur reports, 10 (7): (1990).
10. Om Prakash, Rashmi P, Pooja R, Kamaljeet P, Yogita D and Aneja KR, Synthesis and antibacterial activity of 1,3-diaryl-4-cyanopyrazole, Indian J. Chem.2009; 48B: 563-568.
11. Kumar A, Lata S, Saxena KK and Chandra T, Synthesis and evaluation of new substituted indolyl pyrazolines as anti-inflammatory and analgesic activities, Indian Drugs.2009.46 (9): 43-48.
12. Kittur BS, Sastri BS, Pattan SR, Rabara PA and Muchhandi IS, Synthesis and anti-inflammatory activity of some novel 1,3,4- oxadiazole and pyrazole derivatives, Indian drugs.2009; 46 (4): 287-290.
13. El-Hamouly WS, El-Khamry AMA and Abbaa EMH, Synthesis of new 4-arylisoxazolo[5,4-d]pyrimidin-6-one(thione) and 4-aryl-pyrazolo[3,4-d]-pyrimidin-6-one derivatives of potential antihypertensive activity, Indian J. Chem.2006; 45B: 2091-2098.
14. Patil PO, Belsare DP, Kosalge SB and Fursule RA, Microwave assisted synthesis and anti-depressant activity of some 1,3,5- triphenyl-2-pyrazolines, Int. J. Chem. Sci.2008;6 (2): 717-725,
15. Shikha SD and Anjali MR, Synthesis of substituted 2-(5-(2-chloroquinolin-3-yl)-4,5- dihydro-1H-pyrazol-3-yl)phenols as antibacterial and anticancer agents, Indian J. Hetero. Chem.2009;18: 397-398.