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Synthesis of 1,2,4-Triazoles, 1,3,4-Thiadiazoles and their antimicrobial Activities

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Abstract:

The synthesis of some new 3,5-disubstituted-1,2,4-triazole substituted 1,3,4 -thiadiazole and their derivatives through the intramolecular cyclization of 1,4- substituted thiosemicarbazides. Elemental analysis, IR, 'H-NMR and mass spectral data elucidated the structures of all newly synthesized compounds. Synthesized compounds were studied for their antibacterial and antifungal activities. Some of the tested compounds should exhibit significant pharmacological activities.

Index Terms: Synthesis of 1,2,4-Triazoles, 1,3,4-Thiadiazoles, spectral data and antimicrobial activities of synthesis compounds.

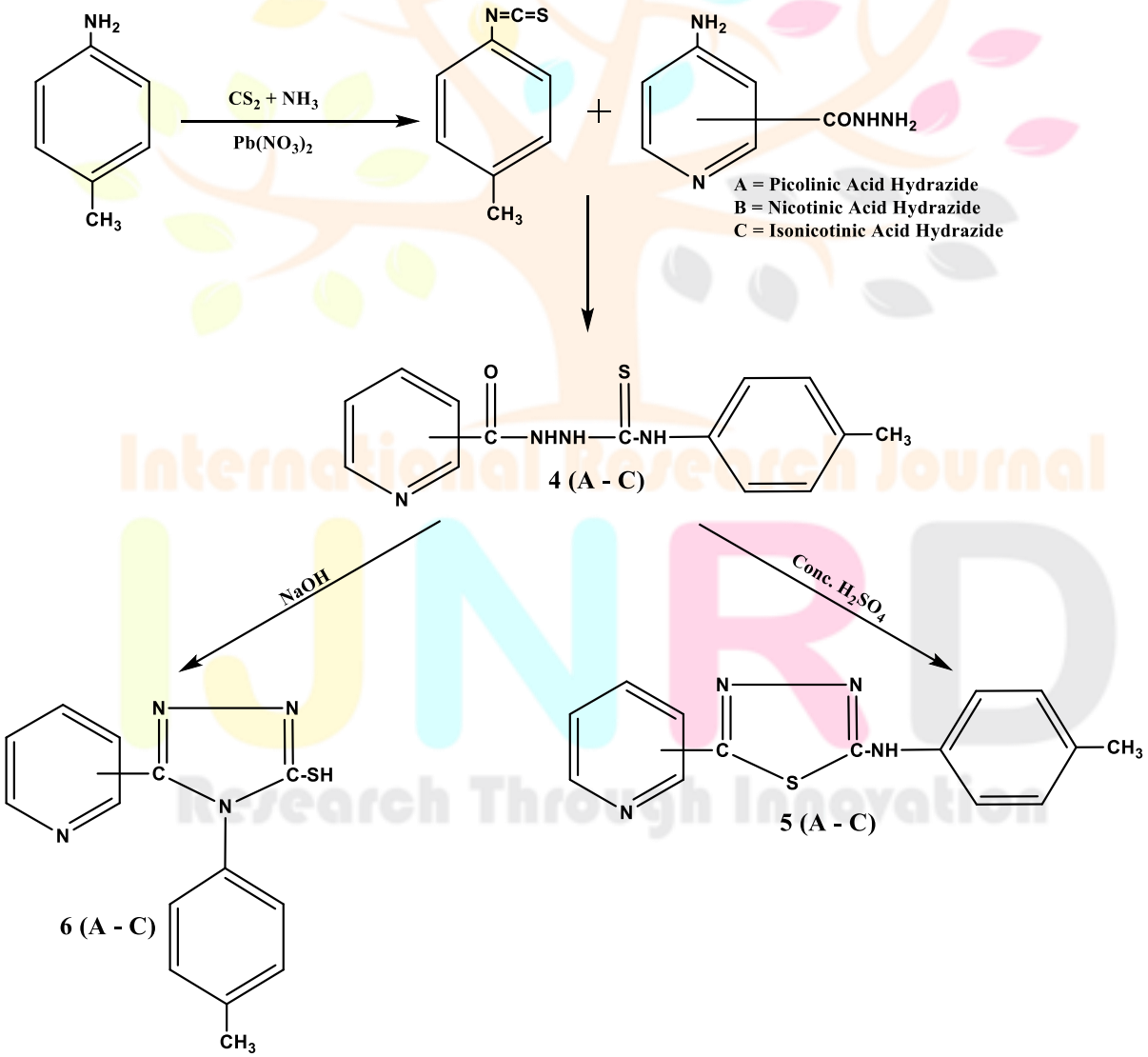
Introduction

The bio-significance, the synthesis and study of biological properties of heterocyclic compounds have gained enough momentum in recent years. Extensive exploratory work has been done on biologically active heterocyclic compounds & compounds of heterocyclic systems and their derivatives incorporating nitrogen, sulphur and oxygen have been synthesized and found to be of commercial importance as biocides. The 1,2,4-triazole and their derivatives constitute an important class of organic compounds with diverse agricultural, industrial and biological activity including anti-microbial[3,4,5], anti-inflamatory[6] as well as anti-cancer[7] activities. Moreover 1,3,4-thiadiazole nucleus constitutes the active part of several biologically active compounds including anti-bacterial[,8,9,10], anti-mycotic[11] and anti-inflammatory[12] agents. We reported the synthesis of some new 3,5- disubstituted, 1,2,4-triazole, substituted 1,3,4-thiadiazole and their derivatives

through intramolecular cyclization of 1,1-disubstituted thiosemicarbazides and potent anti-microbial and anti-inflammatory activities as shown in the scheme.

Experimental

Thin layer chromatography was used to access the completion of the reaction as well as purity of compounds synthesized. Melting points are taken in open glass capillary tubes using Thiele's tube containing liquid paraffin and are uncorrected. I.R. spectra were carried out on Perkin Elmer FT-IR spectrophotometer (cm^{-1} in KBr) H^1-NMR and C^{13}-NMR spectra were recorded on a Bruker spin spectrometer (400 MHz) in DMSO-d_6 and TMS was used as internal standard. Peak value are shown in ppm, δ and mass spectra were recorded on a Waters L.C.-M.S. Elemental analysis were carried out on a Perkin Elemer analyser, starting material were obtained from S.D. fine and Aldrich chemicals.



Scheme: 1 Preparation of Compounds 4, 5 & 6 (A-C)

Preparation of 4-methylphenyl isothiocyanate (3)

A mixture of 4-methylphenyl amine (0.25 mol. 26.75%) carbon disulfide (0.39 mol. 37.4 ml) and methanol (95% 60 ml) was cooled to about 10°C. Ammonia (33% 0.32 mol) was added drop-wise to the reaction mixture with continuous stirring. The mixture was allowed to stand over-night. Water was added to the mixture (100 ml). An aqueous solution of lead nitrate (0.25 mol. 82.7 g was slowly added to the solution. The mixture was then steam distilled to yield 4-methylphenyl isothiocyanate (3) as a low melting point solid. The infrared spectra (in KBr) of the isolated isothiocyanate indicates a prominent characteristic absorption band at 2070 cm^{-1} attributed to an –N=C=S group (yield 52%).

Preparation of 1-(4-methylphenyl)-4-(isomeric pyridyl) thiosemicarbazides 4 (A - C)

Respective substituted pyridine carboxylic acid hydrazides (1 A - C) (0.004 mol) were dissolved in absolute ethanol (50-80 ml), depending upon the solubility of the compounds. The 4-methylphenyl isothiocyanate (3) (0.004 mol) was separately dissolved in absolute ethanol (30 ml). Then the solution of the isothiocyanate was poured into the solution of hydrazide with continuous stirring. The reaction mixture was then refluxed. Each reaction required different times determined by TLC. After the completion of the reaction, the mixture cooled to room temperature. As a result a white solid crystal appeared. The crude solid was then filtered and recrystallized from appropriate solvent to yield the compounds 4 (A - C).

Preparation of 2-(4-methylphenylamino)-5-(isomeric pyridyl)-1,3,4-thiadiazole (5 A-C)

Each thiosemicarbazide 4 (A-C) ($7x10^{-4}$ mol. 0.2 g) was added portionwise to 25 ml of concentrated sulfuric acid at 0°C with continuous stirring. The reaction mixture was stirred further for 3 Hr at room temperature and then allowed to stand overnight. Neutralization with diluted sodium hydroxide precipitated a crude solid, which was filtered, and washed with water. The crude product was then recrystallized from a mixture of acetic acid and water (1/1 or 1/2) to furnish disubstituted 1,3,4 thiadiazole 5 (A-C).

Preparation of 2,4-Dihydro-4-(4-methylphenyl)-5-(isomeric pyridyl)-3H-1,2,4-triazole 6 (A-C)

Solid thiosemicarbhazides 4 (A-C) ($4x10^{-4}$mole) were added portionwise to 15 ml and 2M sodium hydroxide solution. The reaction mixture was refluxed and completion of the reaction checked by using TLC. After the completion of the reaction, the mixture was allowed to cool and then filtered. The filtrate was acidified with 2M hydrochloric acid and the resulting solid was filtered, washed thoroughly with water, dried and recrystallized from ethanol/water.

Results and Discussion

In the present work 1-(4-methylphenyl)-4-(isomeric pyridoyl) (thiosemicarbazides 4 (A-C) were used as the key intermediates for the synthesis of heterocyelic compounds. Various thiosemicarbazides were synthesized by condensing 4-methylphenyl isothiocyante (3) with isomeric pyridine carboxylic acid hydrazides 1 (A-C).The required isothiocyanate was prepared from the treatment of 4-methyl aniline with carbon disulfide and ammonia in methanol and then reacted with lead nitrate. The acid or base catalyzed intramolecular dehydrative cyclization of the thiosemicarbazides 4 (A-C) furnished the corresponding substituted 1,3.4-thiadiazole 5 (A-C) and 1,2,4-triazole 6 (A-C) respectively.

The infrared spectra of compounds 4 (A-C) (Table 2) exhibited a characteristic strong absorption at 1240-1258 cm^{-1} attributable to the C=S of the thiourca reside. The carbonyl absorption in these compounds was observed at 1655 – 1652 cm^{-1}. The dehydrative cyclization of 4 (A-C) in sodium hydroxide or concentrated sulphuric acid afforded corresponding substituted 1,2,4-triazole 6 (A-C) and substituted 1,3,4-thiadiazole 5 (A-C) respectively. In the IR spectra of compounds 5 & 6 (A-C), the absence of signals in the region 1655-1682 cm^{-1} revealed the lack of C=O orders.

Table 1: Yields, Melting points, Formulas and Elemental Analysis for Compounds.

Compd.	R	R'	Yield (%)	M.P. °C	Formula	Found (required) (%)		
						C	H	N
4A	2-Pyridyl	4-CH_2Ph	85	192	$C_{14}H_{14}N_4SO$			
4B	3-Pyridyl	4-CH_2Ph	75	188	$C_{14}H_{14}N_4SO$			
4C	4-Pyridyl	4-CH_3Ph	82	202	$C_{14}H_{14}N_4SO$	58.27(58.72)	5.02(4.93)	19.49(10.67)
5A	2-Pyridyl	4-CH_3Ph	71	251	$C_{14}H_{14}N_4SO$			
5B	3-Pyridyl	4-CH_3Ph	56	223	$C_{14}H_{14}N_4SO$			
5C	4-Pyridyl	4-CH_3Ph	64	280	$C_{14}H_{12}N_4SO$	62.38(62.66)	4.47(4.51)	11.70(11.93)
6A	2-Pyridyl	4-CH_3Ph	89	232	$C_{14}H_{14}N_4SO$			
6B	3-Pyridyl	4-CH_3Ph	72	263	$C_{14}H_{12}N_4SO$			
6C	4-Pyridyl	4-CH_3Ph	87	270	$C_{13}H_{12}N_4SO$	62.48(62.66)	4.62(4.61)	11.78(11.93)

Table: 2 IR (KBr CM-1) spectral data for compounds

NO	C=O	C=N, C=C	C=S	NH
4A	1655	1584	1240	3250
4B	1658	1595	1258	1300
4C	1682	1570	1255	3200
5A	-	1550	-	9800
5B	-	1540	-	2764
5C	-	1541	-	2886
6A	-	1541	1248	2885
6B	-	1543	1275	2730
6C	-	1606	1205	3430

Table: 3 ^{1}H NMR spectral data for compounds

No.	^{1}H NMR (DMSO 2D 66ppm)
4A	2.23 (s, 3H, CH_3) 7.16 (d, 2H, Ar-H), 7.33 (d, 2H, Ar-H), 7.64 (m, HI, P5-H), 8.00.s. 08 (m, 2H, Py-H), 8.68 (m, 1H, Py-H), 9.71 (bs, 1H, NH), 10.69 (bs, 1H, NH)
4B	2.25 (s, 3H, CH_3) 7.15 (d, 2H, Ar-H), 7.41 (d, 2H, Ar-H), 7.55 (m, 1H, P2-H), 8.27 (m, 1H, Py-H), 8.75 (m, 1H, Py-H), 9.10 (m, 1H, Py-H), 9.71, 9.82, 10.72 (3 bs, 3H, 3NH)
4C	2.28 (s, 3H, CH_3) 7.13 (d, 2H, Ar-H), 7.20 (d, 2H, Ar-H), 7.84 (m, 2H, Py-H), 8.76 (m, 2H, Py-H), 8.77 (m, 2H, Py-H), 9.74 (bs, 1H, NH), 9.79 (bs, 1H, NH), 10.83 (bs, 1H, NH)
5A	2.35 (s, 3H, CH_3) 7.25 (m, 2H, Ar-H), 7.29 (m, 2H, Ar-H), 7.39 (m, 1H, Py-H), 7.68 (m, 1H, Py-H), 8.49 (m, 1H, Py-H), 8.58 (m, 1H, Py-H), 14.23 (bs, 1H, NH)
5B	2.33 (s, 3H, CH_3) 7.21 (m, 2H, Ar-H), 7.32 (m, 2H, Ar-H), 7.39 (m, 1H, Py-H), 7.77 (m, 1H, Py-H), 7.89 (m, 1H, Py-H), 8.37 (m, 1H, Py-H), 14.81 (bs, 1H- NH)
5C	2.37 (s, 3H, CH_3) 7.24 (m, 2H, Ar-H), 7.26 (m, 2H, Ar-H), 7.32 (m, 2H, Py-H), 8.56 (m, 2H, Py-H), 14.35 (bs, 1H- NH)
6A	2.33 (s, 3H, CH_3) 3.37 (bs, 1H- SH), 7.11 (m, 2H, Ar-H), 7.22 (m, 2H, Ar-H), 7.38 (m, 1H, Py-H), 7.77 (m, 1H, Py-H), 7.88 (m, 1H, Py-H), 8.36 (m, 1H, Py-H)
6B	2.35 (s, 3H, CH_3) 3.25 (bs, 1H- SH), 7.21 (d, 2H, Ar-H), 7.28 (d, 2H, Ar-H), 7.37 (m, 1H, Py-H), 7.63 (m, 1H, Py-H), 8.46 (dd, 1H, Py-H), 8.54 (dd, 1H, Py-H)
6C	2.29 (s, 3H, CH_3) 6.43 (bs, 1H- SH), 7.08 (d, 2H, Ar-H), 7.32 (d, 2H, Ar-H), 7.66 (m, 2H, Py-H), 8.81 (m, 2H, Py-H)

The mass spectra of compound 4-6 (A-C) were studied. The moleculer ion peak indicated its presence in all the compounds. The ^{13}C-NMR spectral data of most of the synthesized compounds are presented in Table: 4.

Table: 4 ^{13}C-NMR (DMSO 4) 66ppm)

No.	^{13}C-NMR (DMSO 4) 66ppm)
4A	0.49, 122.30, 122.41, 126.86, 128.27, 128.42, 133.03, 136.62, 137.63, 148.44, 149.25, 181.32
4B	20.52, 122.33, 123.37, 128.44, 129.86, 135.54, 136.52, 139.20, 148.62, 152.31, 168.86, 181.10
4C	20.54, 121.67, 126.01, 128.52, 134.31, 136.52, 139.60, 150.158, 164.45, 181.14
5A	20.73, 124.00, 124.83, 128.04, 129.16, 132.41, 135.25, 138.56, 148.22, 148.26, 150.26, 169.08
5B	20.72, 123.00, 123.40, 126.41, 129.67, 132.41, 135.25, 138.56, 148.22, 148.26, 150.26, 169.08
5C	20.83, 123.59, 127.32, 128.27, 130.14, 131.25, 139.63, 145.42, 147.19, 169.70
6A	20.73, 122.36, 123.46, 128.48, 129.85, 131.58, 135.78, 139.17, 148.49, 148.60, 150.91, 168.86
6B	20.73, 124.00, 121.92, 128.04, 129.19, 132.46, 137.24, 138.16, 145.20, 149.20, 149.61, 169.10
6C	20.73, 121.83, 128.31, 129.96, 131.57, 133.23, 139.34, 148.40, 150.06, 169.31

The antimicrobial results show that some of the compounds are active against the tested microbial strain, but was interesting to note that compounds 4A, 5A, 5C, 6B and 6C were very sensitive to all tested organisms comparable to the standards used at the concentration of 30 µ/ml.

Antimicrobial effect of some synthesized compounds were reported in **Table: 5** and **Table: 6** respectively.

Table 5: Antifungal activities of compounds

Compounds		***C. albucans*[1]**				***A. niger*[2]**	
		10-µg. /ml ±SD*	**20 -µg. /ml ±SD***	**30-µg. /ml ±SD***	**40-µg. /ml ±SD***	**20-µg. /ml ±SD***	**30-µg. /ml ±SD***
4	**A**	5.64 ±1.16	12.84 ±1.73	18.78 ±1.53	6.04 ± 0.58	11.63 ±0.58	17.58 ±1.16
	B	5.82±0.58	11.37±0.58	17.95±1.73	6.14±1.16	11.54±1.53	16.72±0.58
	C	6.27±0.58	14.84±1.16	19.36±1.53	5.92±0.58	11.00±0.00	17.67±0.58
5	**A**	6.18±1.16	13.82±0.58	18.75±0.61	5.62±0.47	12.62±0.58	18.54±0.27
	B	5.84±0.53	12.62±0.58	19.00±2.00	6.00±1.00	13.46±0.58	17.42±1.16
	C	5.48±0.58	12.00±1.00	17.48±1.53	6.25±0.58	13.00±1.00	16.72±0.27
6	**A**	6.48±0.58	13.92±1.73	18.00±1.00	6.40±0.61	12.85±0.58	19.10±1.53
	B	6.14±0.58	13.66±0.47	17.82±0.27	6.32±0.61	13.00±1.00	19.00±0.00
	C	5.78±0.58	11.00±1.00	19.24±1.53	6.14±0.58	12.00±1.00	19.10±0.58
Griseofulvin				24.57±1.45		23.13±2.00	

SD = Standard deviation

1 = *Candida ablicans*

2 = *Aspergillus niger*

Compounds		10 µg. /ml ±SD*	20 µg. /ml ±SD*	30 µg. /ml ±SD*	10 µg. /ml ±SD*	20 µg. /ml ±SD*	30 µg. /ml ±SD*	10 µg. /ml ±SD*	20 µg. /ml ±SD*	30 µg. /ml ±SD*	10 µg. /ml ±SD*	20 µg. /ml ±SD*	30 µg. /ml ±SD*
4	A	6.86±1.73	11.72±2.52	16.52±2.65	6.72±1.16	11.38±0.58	15.49±0.58	6.82±0.47	11.45±1.16	16.16±1.53	5.82±0.58	10.12±0.58	16.36±0.58
	B	6.08±0.58	11.28±0.47	16.86±1.16	5.92±0.61	10.85±0.58	16.04±1.16	5.76±0.58	10.24±1.53	15.92±0.47	5.12±0.26	10.26±0.61	16.74±0.58
	C	5.16±1.16	9.84±1.53	14.18±2.52	6.10±0.58	10.63±0.58	14.48±0.58	4.80±0.58	9.02±1.16	14.00±1.00	5.04±0.58	10.36±1.16	16.60±0.58
5	A	7.64±0.26	13.18±0.58	17.36±0.45	7.12±0.26	12.04±4.16	17.26±2.09	6.16±0.61	11.84±0.58	16.86±1.51	6.18±0.58	12.69±0.17	17.48±0.58
	B	5.16±1.73	9.12±2.52	16.67±2.89	5.31±1.16	10.26±1.53	16.11±0.58	7.06±1.71	11.10±0.61	16.38±0.45	6.89±0.58	12.06±0.17	16.10±1.16
	C	5.25±1.15	8.80±0.00	16.00±0.00	5.26±0.57	10.12±1.15	16.05±1.15	6.29±0.57	10.18±1.15	16.12±0.57	6.72±0.57	11.68±0.57	16.60±0.57
6	A	5.84±0.58	8.19±0.58	14.20±1.53	4.67±0.45	09.10±0.58	15.53±1.16	5.48±1.16	9.63±0.58	15.80±1.73	0.78±0.58	11.29±0.61	16.00±1.00
	B	6.28±0.45	10.48±0.58	17.00±1.00	6.00±0.00	11.84±0.17	10.82±1.73	7.38±1.16	12.48±0.17	17.10±1.53	7.10±0.58	13.73±0.63	17.92±0.26
	C	8.28±0.26	14.48±0.58	18.78±0.45	7.82±0.26	13.27±1.16	18.71±2.09	7.68±0.61	13.06±0.58	18.80±1.53	7.21±0.58	13.19±0.17	18.12±0.58
Vancomycin		-	-	-	-	-	-	-	-	23.32±1.52	-	-	22.44±0.57
Amikacin		-	-	22.65±0.58	-	-	24.00±1.00	-	-	-	-	-	-

Table: 6

SD= Standard Deviation

= Zone of inhibition in mm

1= *Escherchia coli*

2=*Pseudomonas*

3= *Staphylo coccus aureus*

4= *Bacillus subtilis*

PHARMACOLOGY

Antibacterial and antifungal activities

Applying the agar plate diffusion technique[13] to all these compounds were screened in vitro for antibacterial activity against *Escherichia coli* (E. coli), *Pseudomonas aeruginosa* (*P. aeruginosa*) (gram negative) *staphylococcus aureus* (*S. aureus*) *Bacillus subtilis* (*B. subtilis*) (Gram positive) at 10 µ/ml, 20 µ g/ml, 30 µ g/ml concentration respectively.

Under identical conditions the positive control Amikacin at 30 µ g/ml showed 20 zone inhibition 23-24 mm for gram negative organism and Vancomycin at 100 µg/ml showed zone of inhibition 23 mm for gram positive organism. Similarly the antifungal screening of the compounds were carried out in vitro by Petri dish method against two fungi *Aspergillus niger* (*A. niger*) and *Candida albicans* (*C. albicans*) by using Griseofulvin (30 µg/ml) as the positive control, which showed the 23 mm and 25 mm respectively) as zone inhibition.

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