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Characterization, evaluation of products synthesized in the interaction of 4-(N-substituted)-3-pyridyl- 5- mercapto-*S*-triazole with secondary amines

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ABSTRACT

In the present study, the interaction of 4-(N-substituted)-3-pyridyl-5-mercapto-s-triazole with secondary amines was explored. The Isonicotinic acid hydrazide was converted into the corresponding potassium dithiocarbazinate, by reacting with carbon disulphide in alkaline medium which undergoes ring closure reaction after further treatment with aqueous potassium hydroxide to give 5-pyridyl-2-mercapto-1,3,4-oxadiazole. 4-(N-pyridylcarboxamido)-3- pyridyl-5-mercapto-s-triazole and 3-pyridyl-4-amino-5-mercapto-s-triazole were obtained in one pot reaction by heating equimolar quantities of oxadiazole with isonicotinic acid hydrazide and hydrazine hydrate respectively. Condensation of triazole with secondary amines in anhydrous dimethyl sulfoxide results in the formation of corresponding quaternary salts. The synthesized compounds were confirmed by IR, ^{1}HNMR spectra and elemental analysis. All the compounds were screened for their preliminary in-vitro antibacterial and antifungal activity.

Keywords: 1,3,4-oxadiazole, quaternary compound, morpholine, pyrrolidine..

INTRODUCTION

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including emerging infectious diseases and the increasing number of multi-drug resistant microbial pathogens. In spite of a large number of antibiotics and chemotherapeutics available for medical use, at the same time the emergence of old and new antibiotic resistance created in the last decades revealed a substantial medical need for new classes of antimicrobial agents. There is perceived need for the discovery of new compounds endowed with antimicrobial activity, possibly acting through mechanisms of action, which are distinct from those of well-known classes of antibacterial agents to which many clinically relevant pathogens are now resistant. Though the various molecules were designed and synthesized for this aim, it was demonstrated that the mercapto- and thione- substituted 1,2,4-triazoles and their derivatives could be considered as possible antimicrobial agents. The substituted triazoles are heterocyclic compounds, which serve both as biomimetic and reactive pharmacophores and many are key elements with potential biological activities such as analgesic[1], anti-inflammatory [2-6], antimicrobial [7-11], tuberculostatic [12-13],and can be used as fungicides [14] and antitumour agents [15-16] .

In this study, the interaction of 4-(N-substituted)-3- pyridyl-5-mercapto-*s*-triazole with secondary amines, pyrrolidine **(a)** and morpholine **(b)**) was explored. The Isonicotinic acid hydrazide (INH) **(1)** was converted in to the corresponding potassium dithiocarbazinate, by reacting with carbon disulphide in alkaline medium which undergoes ring closure reaction after further treatment with aqueous potassium hydroxide to give 5-pyridyl-2-mercapto-1,3,4-oxadiazole **(2)**. 4-(N-pyridyl carboxamido)-3-pyridyl-5-mercapto-s-triazole **(3)** and 3-pyridyl-4-amino-5-mercapto-s-triazoles **(4)** were obtained in one pot reaction by heating equimolar quantities of oxadiazole with INH and hydrazine hydrate respectively, according to Reid and Heindel procedure with slight modification [17]. Condensation of both triazole **(3& 4)** with secondary amines **(a&b)** when heated at 80^0C in anhydrous dimethyl sulfoxide (DMSO) results in the formation of corresponding 4-(N-pyridylcarboxamido)-3-pyridyl-*s*-triazole-5-thiolate **(3a & 3b)** and 3-pyridyl-4-amino-s-triazole-5-thiolate **(4a & 4b)** as a quaternary salts as shown in **Scheme-I**. This technique of conversion of oxadiazole to triazole is similar to well-known transformation of furans to pyrrols with nucleophilic amines. In this oxadiazole was intermediate in the hydrazinolysis of dithiocarbazinate to triazole. The identity of those was confirmed by elemental analysis and spectral data. All the novel compounds were evaluated for their preliminary *in-vitro* antibacterial and antifungal activity.

Biological evaluation

Antibacterial activity

Studies on the antibacterial activity of the synthesized compounds **(3a-3b & 4a-4b)** have been screened using cup-plate agar diffusion method [18] against four pathogenic organisms, viz., *Staphylococcus aureus* (G^+), *Klebsiella pnemoniae* (G^-), *Escherichia coli* (G^-), and *Pseudomonas aeruginosa* (G^-) by measuring the inhibition zone in mm at two concentrations (100 and 150 μg/ml). Streptomycine (100 and 150 μg/ml) was used as a standard and was also screened under similar conditions for comparison. The results of the antibacterial studies are shown in **Table-1**.

Antifungal Activity

The antifungal activity studies of the novel triazole derivatives**(3a-3b & 4a-4b)** have been screened using cup-plate agar diffusion method [18] against the four fungi *Aspergillus flavus, A.fumigatus, penicillium* and *Trichophyton* by expressing the zone of inhibition in mm at two concentrations (100 and 150 μg/ml). Griseofulvin (100 and 150 μg/ml) was used as a standard and was also screened under similar conditions for comparison. The results of the antifungal studies are shown in **Table-2.**

MATERIALS AND METHODS

Experimental

General

Melting points were taken using microprocessor based melting point apparatus (Veego make) containing liquid paraffin and are uncorrected. Thin layer chromatography was used to monitor the progress of the reaction on silica gel-G precoated plates by using chloroform and ethyl acetate (1:1) as the eluent and observed in UV light. IR spectra in KBr were recorded on Shimadzu-8400 FTIR spectrophotometer, ^{1}H NMR Spectra were recorded on Bruker spectrophotometer(400MHz) in DMSO-d_6/$CDCl_3$ using trimethylsilane as an internal standard (chemical shifts are expressed in δ, ppm). Isonicotinic acid hydrazide and all reagents were used of analytical grade purchased from commercial suppliers without further purification.

Preparation of 4-(N-pyridyl carboxamido)-3-pyridyl-5-mercapto-s-triazole (3)

To a solution of Potassium hydroxide (0.015mol) in absolute ethanol, 5-pyridyl-2-mercapto-1, 3, 4-oxadiazole (0.01mol) and carbon disulphide (0.01mol) were added and mixture was stirred for 24 hrs. The solid separated out, to this Isonicotinic acid hydrazide (0.01mol) was added and refluxed at 140^0 for 8 hrs. The reaction mixture was cooled and diluted with water. On acidification with hydrochloric acid the solid obtained was filtered, washed with water and recrystallized from aqueous ethanol[17] yield 84%, m.p.290^0.

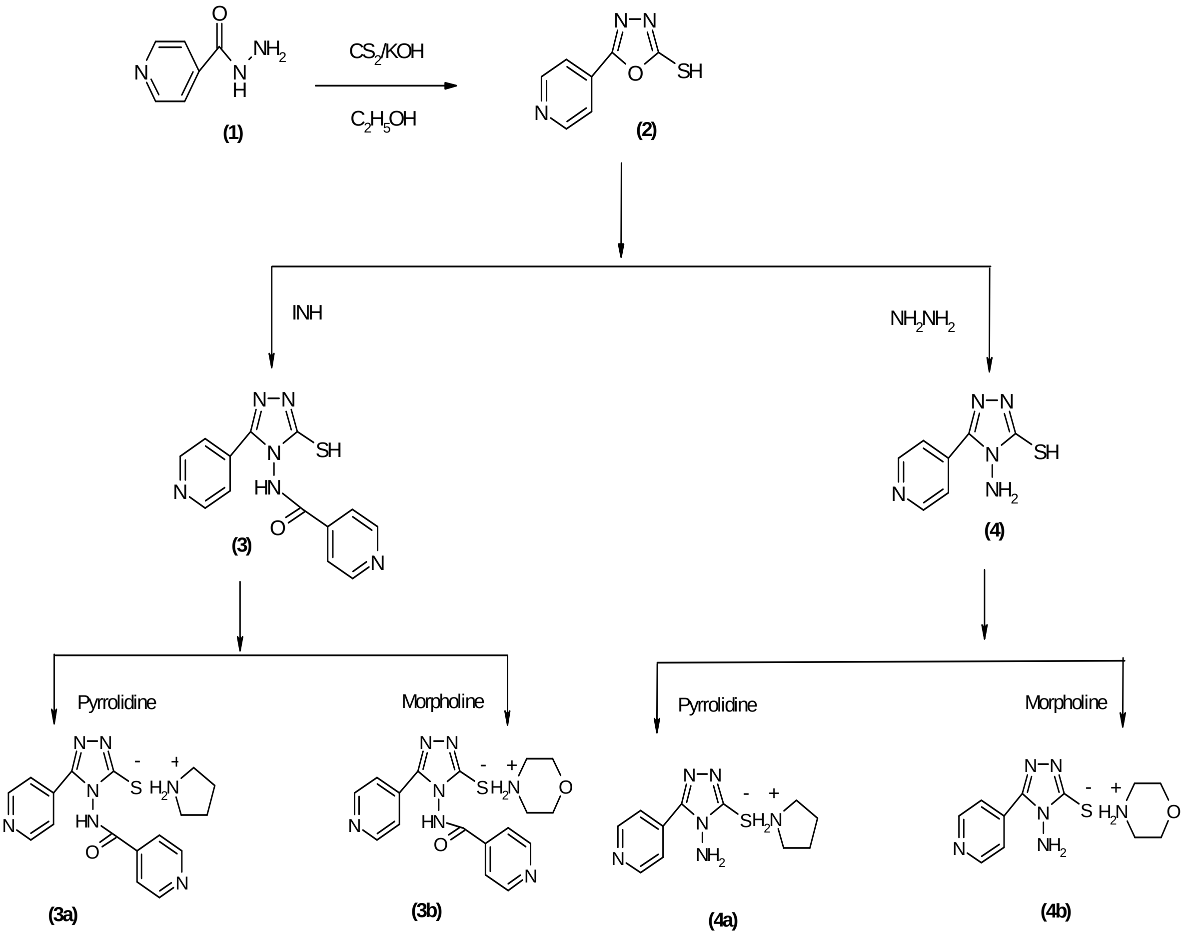
Preparation of 3-pyridyl-4-amino-5-mercapto-s-triazole (4)

A solution of 30 mmoles of 5-pyridyl-2-mercapto-1, 3, 4-oxadiazole, 20ml. of water, and 30 mmoles 95% hydrazine was refluxed for 4 hours, diluted with 200ml. of cold water, acidified by the drop wise addition of concentrated hydrochloric acid, and filtered. The solid was washed with a minimum of cold water, and recrystallized from aqueous ethanol[17], yield 62%, m.p. 206^0.

Synthesis of morpholine-4-ium 4-(N-pyridylcarboxamido)-3-pyridyl-s-triazole-5-thiolate (3b)

30 mmol of 4-(N-pyridylcarboxamido)-3-pyridyl-5-mercapto-s-triazole **(3)**, 30 mmol of Morpholine were added to 20 ml of anhydrous dimethyl sulfoxide and the reaction mixture was stirred vigorously for 12 hr at room temperature under nitrogen atmosphere. Dimethyl sulfoxide was partially recovered under reduced pressure, diluted with 200 ml cold water. Sticky residue obtained which was purified by column chromatography using neutral alumina (25g), using chloroform: methanol (8:2) as an eluent and recrystallized from methanol to obtained desired compound. Yield -32%, m.p. 167^0C. The preparation of the remaining compounds was carried

out as above described procedure. Physicochemical and spectral data of titled compounds **(3a-3b & 4a-4b)** are shown in **Table 3** and **4.**



Scheme-I

Table1. Antibacterial Activity of Synthesized Compounds (3a-b & 4a-b)

Compound	***Staphylococcus aureus***		***Klebsiella pnemoniae***		***Escherichia coli***		***Pseudomonas aeruginosa***	
	100 µg/ml	**150 µg/ml**	**100 µg/ml**	**150 µg/ml**	**100 µg/ml**	**150 µg/ml**	**100 µg/ml**	**150 µg/ml**
3a	09	12	10	16	08	11	12	15
3b	10	13	12	17	10	12	14	18
4a	08	10	11	15	08	11	13	16
4b	09	11	12	16	10	13	14	18
Standard streptomycin	15	18	16	21	13	16	18	22

Zone of inhibition expressed in mm

Table2. Antifungal Activity of Synthesized Compounds (3a-b & 4a-b)

Compound	*Aspergillus flavus*		*Aspergillus fumigates*		*Penicillium*		*Trichophyton*	
	100 µg/ml	150 µg/ml	100 µg/ml	150 µg/ml	100 µg/ml	150 µg/ml	100 µg/ml	150 µg/ml
3a	10	13	10	14	07	10	10	12
3b	11	14	12	16	08	11	11	14
4a	11	13	11	15	08	11	10	12
4b	11	13	13	17	09	10	11	12
Standard griseofulvin	12	15	13	17	11	14	13	16

Zone of inhibition expressed in mm

Table 3. Physicochemical data of Synthesized Compounds (3a-b & 4a-b)

Compound	Molecular Formula	Molecular Weight	%Yield	Melting point	R_f *
3a	$C_{17}H_{19}N_7OS$	369.44	29	281-84	0.17
3b	$C_{17}H_{19}N_7O_2S$	385.13	32	292-95	0.25
4a	$C_{11}H_{16}N_6S$	264.12	34	248-50	0.24
4b	$C_{11}H_{16}N_6OS$	280.11	37	262-65	0.19

** Mobile Phase - Chloroform: Methanol (8:2)*

Table4. Spectral data of Synthesized Compounds (3a-b & 4a-b)

Compound	IR (KBr)cm^{-1}	^{1}H NMR ($CDCl_3$)	Elemental analysis (%) Calculated (Found)
3a	3090 (Ar C-H),2490(+NH_2) 1670(C=ONH),1629(C=N) 1261(C-N)	2.1(2H,m,$NH^+{}_2$),2.8(4H,m,CH_2NCH_2),1.6(4H,m,2CH_2), 7.5-7.9 (4H, dd,3-H,5-H), 8.5-9.06 (4H,dd, 2-H, 6-H) & 11.6 (1H,s,NH)	C, 55.27; H, 5.18; N, 26.54 (C,55.82; H,5.07; N, 26.19)
3b	3090(ArC-H), 2487(+NH_2) 1670(C=ONH),1629(C=N) 1261(C-N)1114(C-O-C)	2.36(2H,m,$NH^+{}_2$),2.8(4H,m,CH_2NCH_2),3.6(4H,m,CH_2OCH_2),7.6-8.0 (4H,dd,3-H,5-H),8.7-9.0 (4H,dd,2-H,6-H) & 12.6 (1H,s,NH)	C, 52.97; H, 4.97; N, 25.44 (C,53.057;H,4.91;N, 25.18)
4a	3298 (NH_2), 3045 (Ar C-H), 2484(+NH_2),1629(C=N), 1270(C-N)	2.14(2H,m,$NH^+{}_2$),2.7(4H,m,CH_2NCH_2), 1.6(4H,m,2CH_2), 7.7 (2H,d, 3-H,5-H), 8.7 (2H,d,2-H,6-H) &5.9 (2H, s, NH2)	C, 49.98; H, 6.10; N, 31.79 (C,50.12; H,5.98; N, 31.64)
4b	3295(NH_2),2923(ArC-H), 2510(+NH_2),1621(C=N), 1255(C-N),1118(C-O-C)	2.3(2H,m,$NH^+{}_2$),2.9(4H,m,CH_2NCH_2),3.8(4H,m,CH_2OCH_2), 7.7(2H,d,3-H,5-H), 8.6(2H,d,2-H,6-H) & 5.9 (2H,s,NH_2)	C, 47.13; H, 5.75; N, 29.98 (C,47.80; H,5.45; N, 30.14)

RESULTS AND DISCUSSION

Condensation of 4-(N-pyridylcarboxamido)-3-pyridyl-5-mercapto-s-triazole and 3-pyridy-4-amino-5-mercapto-s-triazole with secondary amines when heated at 60^0C in anhydrous dimethyl sulfoxide results in the formation of corresponding 4-(N-pyridylcarboxamido)-3-pyridy-*s*-triazole-5-thiolate and 3-pyridyl-4-amino-s-triazole-5-thiolate as a quaternary salts. The identity of those was confirmed by elemental analysis and spectral data. From the analysis of IR and ^{1}H NMR data, the possibility of the formation of the corresponding thiosemicarbazide is excluded by the absence of the absorption band at 1520 and 1320 cm^{-1}(ascribed to C=S) in the IR spectra and there are no signals of C(S) NH (at 7.8-8.8 ppm) group in the data of ^{1}H NMR spectra of compounds synthesized. From the antifungal screening it was found that the compounds showed significant activity compared to standard Griseofulvin. However no conclusive structural activity relationship has emerged from the antifungal screening. Antibacterial screening revealed that the compounds exhibit weak to moderate activity.

CONCLUSION

Morpholine or pyrrolidine formed quaternary salts when heated with 4-(N-pyridyl carboxamido)-3-pyridy-5-mercapto-s-triazole **(3)** or 3-pyridy-4-amino-5-mercapto-s-triazole **(4)** at 80^{0}C in DMSO. The quaternary salts showed significant antifungal activity in primary screening against *Aspergillus flavus, A.fumigatus* and moderate activity against *penicillium* and *Trichophyton.* Antibacterial screening revealed that the quaternary salts exhibit weak to moderate activity against *Staphylococcus aureus*, *Klebsiella pnemoniae*, *Escherichia coli* and *Pseudomonas aeruginosa*.

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