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Synthesis of some Antifungal and Anti-tubercular 1, 2, 4-Triazole Analogues

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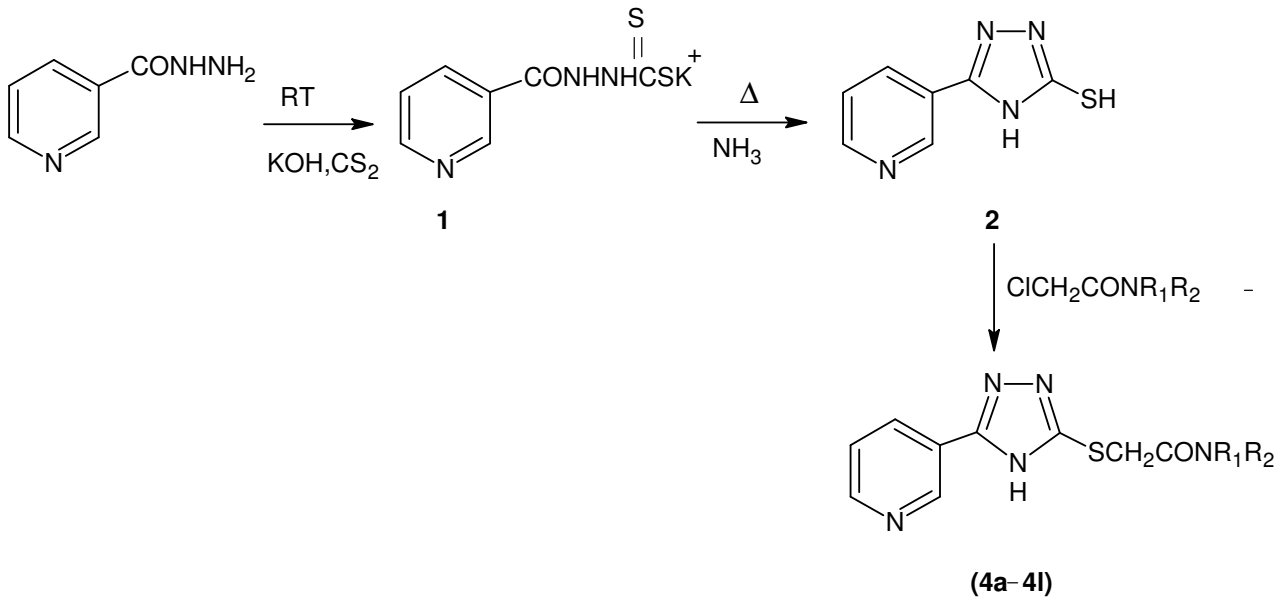
Abstract: Reaction of 3-(3'-pyridyl)-1, 2, 4-triazole-5-thiol **(2)** with appropriately N-substituted-α–chloroacetanilides **(3a-3l)** in aq. potassium hydroxide yielded corresponding 5-(N-substituted carboxamidomethylthio)–3-(3'-pyridyl) - 1, 2, 4-triazoles **(4a-4l).** Structure of these compounds was established by means of elemental analysis and spectral data. These compounds were subjected to antifungal and anti-TB activities. Anti-fungal activity was carried out against *C. albicans* and *A. niger* at the concentrations of 50 and 100 µg/mL using Fluconazole as the standard and in-vitro anti-tubercular activity was done at 50 µg/mL against *Mycobacterium tuberculosis* H_{37} Rv.
Keywords: Disubstituted-1, 2, 4-triazole, Chloroacetanilides, Antifungal, Anti-TB.

Introduction

Tuberculosis (TB) is believed to be present in about one third of the world's population. [1] Active disease following new infection, as well as reactivation of latent tuberculosis, is particularly prevalent in individuals with compromised immune systems, such as those that are HIV positive. In addition, the emergence of drug-resistant strains of *M. tuberculosis* has led to increased pressure on current chemotherapy regimes. [2]Hence there exist urgent needs for the newer molecules which may have potential to curb this disease. 1, 2, 4-triazoles and *N*-bridged heterocycles derived from them are also found to be associated with diverse pharmacological activities. [3,4] Particularly substituted-1, 2, 4-triazoles are among the various heterocycles that have received the most attention during the last two decades as potential antimicrobial agents. [5-9]

3, 5-disubstituted-1, 2, 4-triazole and its derivatives have been reported to possess wide spectrum of activities ranging from anti-bacterial[10] , anti-inflammatory[11] , anti-convulsant[12], anti-neoplastic[13], antimalarial[14] , anti-viral[15], anticancer [16] , anti-TB[17], and anti-proliferative[18] . Literature has also suggested that 5-aryl/heteroaryl-5-(N-substituted carboxamidomethylthio)-1, 2, 4-triazoles have potential anti-fungal, anti-bacterial[19] and anti-tubercular activities[20] . Pyridine, a heterocyclic nucleus, played a vital role in the development of different medicinal agents and in the field of agrochemicals. This nucleus is present in many products such as drugs, vitamins, food, flavorings, plants dyes, adhesives and herbicides[21] .Nicotinic acid (pyridine-3-carboxylic acid), also known as niacin or vitamin B_3, is found in various plants and animals and has vital role in biological processes as production of energy, signal transduction, regulation of genes expression and synthesis of fatty acids, cholesterol and steroids[22] .The substituted nicotinic acid is among the various heterocycles that have received most attention during last three decades as potential biomolecules. Nicotinic acid derivatives exhibit anti-bacterial, anti-oxidant, anti-inflammatory and anti-carcinogenic activities. It is seen from the current literature that pyridine congeners are associated with different biological properties like pesticidal[23,24] , insecticidal[25] and fungicidal[26] activity. These reports encouraged us to modify 1, 2, 4-triazole scaffold into various bioactive structures (Scheme of synthesis) and their subsequent evaluation for antifungal and anti-tubercular activities, as reported in the present communication.



Scheme of Synthesis

Pyridyl-3-carbohydrazide was synthesized as per literature method. [27] Hydrazide was then agitated with potassium hydroxide and carbon disulphide to yield potassium 3-aroyldithiocarbazate salt **(1)**. This salt was refluxed with ammonia for 4-6 h to yield 3-(3'-pyridyl)-1, 2, 4-triazole-5-thiol **(2)** in moderate to higher yields. The structures were confirmed by IR (KBr) spectral data as characteristic absorption bands were observed around 2800-2851 cm^{-1} (C-H from Ar-H stretch), 1640-1560 cm^{-1} (C=N), 1255-1220 (C=S) and 929, 850 (C-N-C of 1, 2, 4- triazole ring). The compound **2** may exist in thione-thiol tautomeric forms [28,29], but our investigation showed that in this particular case the thiol structure dominated in the solid state, as indicated by the IR and NMR data of the compound **2**. Various substituted α-chloroacetanilides (**3a-3l**) were synthesized from the reaction of chloroacetyl chloride and corresponding aromatic/ aliphatic amines in glacial acetic acid- sodium acetate medium as reported. [28] Finally the titled compounds (**3a-3l**) were achieved by coupling chloroacetanilide and 1, 2, 4-triazole-5-thiol under heating for aromatic chloroacetanilides and at room temperature for aliphatic compounds. Subsequent purification yielded condensed compounds in quantitative yields. Elemental analysis and spectral (IR, NMR) data supported the structures assigned. The physical and analytical data of compounds (**3a-3l**) is summarized in Table 1.

Biological evaluation

Antifungal acticity[30]

All the newly synthesized compounds were screened for antifungal activity against *C. albicans* and *A. niger* at 50 µg/mL and 100 µg/mL concentration using fluconazole as reference standard. Among all the tested compounds **4a - 4d, 4f** and **4h** displayed better activity against *C. albican* and *A. niger* at 100 µg/mL concentration, while **4a** and **4d** exhibited excellent antifungal activity against *C. albican* and *A. niger* even at 50 µg/mL concentration.

The filter paper disc method was employed using sabouraud dextrose agar. The agar media were inoculated by using glass sprecidar technique with 0.5 mL and of the 24 h. liquid cultures containing 10^7 microorganism/ml. Filter paper disc (5 mm diameter) saturated with solution of each compound (concentration 50mg/mL, 100mg/mL) were placed on the indicated agar medium. The inoculation time was 24 h at 27^0C for *Candida* species. Discs with only DMF were used as control. Inhibitory activity was measured (in mm) as the diameter of the observed inhibition zones. The tests were repeated to confirm the findings and the average of the reading was taken into consideration. The figures obtained are reported as the mean of three readings. The data of antifungal screening is given in Table 2.

Anti-tb avtivity [31]

Anti-TB activity was carried out against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA) using rifampicin as standard. Compounds exhibiting fluorescence are tested in the BACTEC 460-radiometric system.

Experimental

General

Unless otherwise specified, starting materials were obtained from commercial suppliers and used without further purification. All the melting points were determined on 'Veego' VMP-D apparatus and are uncorrected. Silica gel G plates of 3x8 cm (Sigma-Aldrich) were used for TLC and spots were located by UV or in iodine chamber. The IR spectra were recorded in the 4000-400 cm^{-1} range using KBr discs on FT-IR 8400 SHIMADZU spectrometer. ^1H NMR spectra were recorded on Varian Mercury (300MHz) spectrometer in DMSO-d6 with TMS as an internal standard and values are expressed in ppm. Elemental analyses were performed for C, H, N (Indian Institute of Technology, Mumbai) and were within ±0.4% of theoretical values.

Synthesis of Potassium-3-(3′-Pyridyl) Dithiocarbazate[32] (1)

A solution of 8.40 g (0.15 mole) of potassium hydroxide, 200 mL of absolute ethanol, and 13.7g (0.10 mole) of pyridyl-2-carbohydrazide was treated to the addition of 11.4 g (0.15 mole) of carbon disulphide. This mixture was diluted with 150 mL of absolute ethanol and agitated for 12-16 h. It was then diluted with 200 mL of dry ether, and dried at 65 ^oC. The salts, prepared as describe above, were obtained in nearly quantitative yield and were employed without further purification.

Synthesis of 5-Mercapto-3-(3'-PyridyL)-1, 2, 4-Triazole (2)

A suspension of **1** (24 g,0.096 mole), 95% ammonia 20 mL (0.864 mole) and water 45 mL was refluxed with stirring for 3-4 h. The color of the reaction mixture changed to yellow, hydrogen sulfide was evolved and a homogenous solution resulted. A white solid was precipitated by dilution with cold water (100 mL) and acidifying with conc. HCl, filtered and wash with cold water (2 x 30) and recrystallized in ethanol.

Synthesis of 5-(N-substituted Carboxamidomethylthio)-3-(3-Pyridyl)-1, 2, 4-Triazole (4a-4l):

Compound **(1)** 1.79g (0.01mole) was dissolved in aqueous potassium hydroxide solution (0.61g in 10mL water) with stirring till a clear yellow solution was obtained. It was filtered to remove any suspended impurities. Then various aromatic N-substituted-ά-chloracetanilide (0.011mole) were added in small portions with shaking at 50-60^0C for 4-5 h. In case of aliphatic N-substituted-ά-chloracetanilides, the amide was added at room temperature. Then the reaction mixture was left overnight. Next morning, the precipitate that separated was filtered and washed twice with cold water to remove KCl. Table 1 gives the detail physical and analytical data of these compounds.

Result and Discussion

The 3, 5-disubstituted-1, 2, 4-triazole derivatives presented herein showed antifungal and anti-TB activity. Derivatives like **4 b, 4 c, 4 d, 4 e, 4 h, 4 i** exhibited excellent anti-TB activity. Whereas compounds like **4 b, 4 c, 4 f, 4 g, 4 h, 4 i** and **4 l** showed comparable antifungal activity even at lower concentration. The antifungal activity can be attributed to triazole ring as it contains the toxophoric moiety-(N-C-N-). This stresses the fact that the activity is mainly enhanced due to the coupling of the various chloroacetanilides at 3-position with the 3-(5'-pyridyl)-1, 2, 4-triazolyl-2-thiol. Hence it can be concluded that 3, 5-disubstitution on 1, 2, 4-triazole can lead to potential bioactivity.

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Table 1: Physicochemical characteristics of titled compounds **(3a-3l):**

Compound	R_1	R_2	m.f. (m.w.)	m.p. (°C) (Yield %)	IR (cm^{-1})	^1H NMR
3a/4a	H	4-Methoxyphenyl	$C_9H_{10}ClNO_2$ (199.5)	120-123 (60)	3296(-NH),3136(-CH),1664 (-CONH),831,788 (Ar-CH)	9.20 (s,1H,NH_2),8.28-8.16 (m,4H, Pyridyl),7.50-7.37 (m,4H,Ph), 6.21 (s,1H,NH), 4.36 (s,2H,SCH_2), 3.23 (s, 3H, OCH_3).
3b/4b	H	4-Bromophenyl	$C_8H_7NOClBr$ (248.5)	168-170 (86)	3263(-NH),3076(-CH),1670 (-CONH),821,777 (Ar-CH)	9.23 (s,1H,NH_2),8.30-8.20 (m,4H, Pyridyl),7.60-7.42 (m,4H,Ph), 4.40 (s,2H,SCH_2), 6.23 (s,1H,NH).
3c/4c	H	4-Chlorophenyl	$C_8H_7NOCl_2$ (204)	171-175 (60)	3253(-NH),3080(-CH), 1680 (-CONH),810,780 (Ar-CH)	9.19 (s,1H,NH_2),8.27-8.21 (m,4H, Pyridyl),7.58-7.42 (m,4H,Ph), 4.40 (s,2H,SCH_2),6.13 (s,1H,NH).
3d/4d	H	4-Nitrophenyl	$C_8H_7ClN_2O_3$ (214.5)	175-178 (81)	3258(-NH),3105(-CH),1685, (-CONH),1502(NO_2),810,780 (Ar-CH)	9.27 (s,1H,NH_2),8.35-8.25 (m,4H, Pyridyl),7.55-7.41 (m,4H,Ph), 4.39 (s,2H,SCH_2), 6.20 (s,1H,NH).
3e/4e	H	3-Nitrophenyl	$C_8H_7ClN_2O_3$ (214.5)	96-98 (72)	3268(-NH),3095(-CH),1670, (-CONH), 1510(NO_2) 840,780 (Ar-CH)	--
3f/4f	H	4- Methylphenyl	$C_9H_{10}ClNOCl$ (183.5)	164-166 (62)	3286(-NH), 3126(-CH),1667, (-CONH), 821,770(Ar-CH)	9.19 (s,1H,NH_2), 8.30-8.16 (m,4H, Pyridyl),7.58-7.43 (m,4H,Ph), 4.36 (s,2H,SCH_2), 6.14 (s,1H,NH), 2.11 (s, 3H, CH_3).
3g/4g	H	2-Phenylenediamine	$C_8H_9ClN_2OCl$ (184.5)	197-199 (60)	3252(-NH),3012(-CH),1676, (-CONH), 835,770 (Ar-CH)	9.21 (s,1H,NH_2), 8.23-8.17 (m,4H, Pyridyl),7.50-7.37 (m,3H,Ph), 4.39 (s,2H,SCH_2),6.33-6.11 (s,7H,NH).
3h/4h	H	2, 6-Dichlorophenyl	$C_8H_6NOCl_3$ (238.5)	160-162 (52)	3207(-NH),3037(-CH),1681, (-CONH),873,770 (Ar-CH)	9.18 (s,2H,NH_2),7.65-7.55(m,4H,Pyridyl),7.44-7.35 (m,3H,Ph), 6.21(s,1H,NH),4.3(s,2H,SCH_2).
3i/4i	H	2, 6-Dimethylphenyl	$C_{10}H_{12}NOCl$ (197.5)	147-149 (60)	3213(-NH),3033(-CH),1646, (-CONH),796,709(Ar-CH)	9.18 (s,1H,NH_2),8.30-8.17 (m,4H, Pyridyl),7.39-7.27 (m,3H,Ph), 4.42 (s,2H,SCH_2), 6.23 (s,1H,NH), 2.12-2.24 (s, 6H, $(CH_3)_2$).
3j/4j	H	n-Butylamine	$C_6H_{12}NOCl$ (149.5)	157-159 (68)	3259(-NH),3091(-CH), 1654 (-CONH)	9.18 (s,1H,NH_2),8.27-8.16 (m,4H, Pyridyl), 4.38 (s,2H,SCH_2),6.23 (s,1H,NH), 1.20-1.47 (m, 9H, $(CH_2)_3CH_3$).
3k/4k	Morpholinyl		$C_6H_{10}NO_2Cl$ (163.5)	98-101 (60)	3292(-NH),2923 (-CH),1654, (-CON),1236,1114,1066 (C-O-C).	9.20 (s,1H,NH_2),8.40-7.54 (m,4H,Pyridyl), 4.64 (s, 2H, SCH_2), 3.65-3.50 (m,8H,morpholinyl), 4.41 (s,2H,SCH_2), 6.23 (s,1H,NH).
3l/4l	H	t-Butylamine	$C_6H_{12}NOCl$ (149.5)	160-163 (68)	3270(-NH),2940(-CH), 1660 (-CONH)	9.20 (s,1H,NH_2),8.33-8.30 (m,4H, Pyridyl), 4.33 (s,2H,SCH_2), 6.23 (s,1H,NH), 1.37 (s, 9H, t-butyl).

Table 2: In-vitro anti-fungal activity [#].

Compound	*C. albicans*		*A. niger*	
	50µg/mL	100µg/mL	50µg/mL	100µg/mL
4a	10	16	16	18
4 b	12	20	10	17
4 c	10	20	8	14
4 d	12	16	17	19
4 e	10	15	10	12
4 f	12	19	11	15
4 g	14	14	10	10
4 h	14	18	9	16
4 i	14	14	8	13
4 j	11	14	10	12
4 k	10	13	9	14
4 l	12	12	-	11
DMF	-	-	-	-
Fluconazole	16	18	16	18

#Diameter of zone of inhibition expressed in mm

Table 3: In-vitro anti-TB Activity of **4a-4l**.

Compound	% Inhibition
4 a	55
4 b	81
4 c	98
4 d	100
4 e	98
4 f	72
4 g	51
4 h	99
4 i	100
4 j	22
4k	96
4l	97
Rifampicin	98

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