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Synthesis and in-vitro antimicrobial activity of new 1,2,4-triazoles

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Abstract

We have described the synthesis of new 1,2,4-triazoles and have evaluated their antimicrobial profile. Antitubercular activity was determined in triplicate using the Lowenstein-Jensen medium. A loopful of *Mycobacterium tuberculosis* suspension was inoculated on the surface of each Lowenstein-Jensen media containing the test compounds (100, 10 or 1 μg mL^{-1}). To evaluate in-vitro antibacterial activity, compounds (50, 5 or 0.5 μg) were evaluated against *B. subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Staphylococcus typhi* by the disc diffusion method. To evaluate antifungal activity Sabourauds Dextrose agar medium was used. Some of the compounds (5, 0.5 or 0.05 μg mL^{-1}) were screened for activity against *Aspergillus niger* 88 and *Aspergillus niger* 90 and others were screened for activity against *T. rubrum* TR1, *T. rubrum* R6, *T. rubrum* R7 and *T. mentagrophyte* M1, using the cup plate method. Our results show that the triazoles with a pyrazine moiety at position 3 were more active as antitubercular and antifungal agents compared with the triazoles which had a pyrazine moiety at position 4 of the molecule.

Introduction

With the introduction of streptomycin, aminosalicylic acid and isoniazid, decline in the tuberculosis mortality rate has been observed in developed countries. However, no such downward trend has been seen in most of the developing countries and tuberculosis has emerged to be one of the leading causes of death of the population. Drugs such as isoniazid, rifampicin, pyrazinamide, streptomycin and ethambutol are the first line of drugs used in the management of tuberculosis. Second-line drugs include ethionamide, aminosalicylic acid, cycloserine, amikacin and capreomycin. Currently tuberculosis is treated with combinations of drugs, mainly to ensure that the mutants resistant to a single drug do not emerge. According to a WHO report (1998), nearly 3 million people die due to tuberculosis and approximately 8–10 million new cases are reported every year. The emergence of AIDS and multi-drug resistant mutants have contributed to the resurgence of tuberculosis. Therefore there is a need for new antitubercular drugs. Encouraged by results of earlier work on triazoles (Udupi & Bhat 1996), we have synthesized 1,2,4-triazoles containing a pyrazine moiety and investigated the in-vitro antitubercular, antibacterial and antifungal activities of these synthesized compounds.

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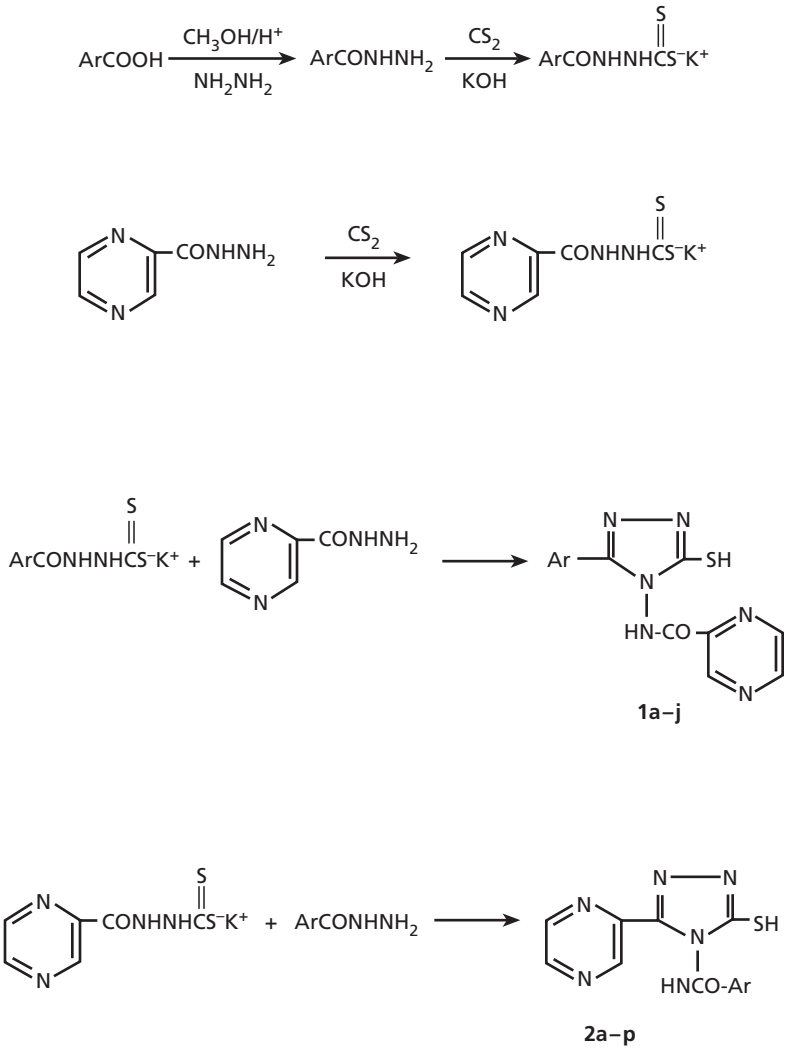

Figure 1 Scheme for the synthesis of **1a**–**j** and **2a**–**p**.

Materials and Methods

Chemical procedures

Melting points were determined on a Toshniwal apparatus in an open capillary and were uncorrected. IR spectra (KBr) were recorded on a Perkin Elmer 599-B spectrophotometer and NMR spectra (DMSO-d_6) on a Varian EM 390 spectrophotometer. Purity of the compounds was checked by thin layer chromatography.

Preparation of acid hydrazides

A solution of aromatic acid (0.01 mol) in methanol (50 mL) containing a few drops of conc. sulphuric acid was heated under reflux for 15 h. Hydrazine hydrate (99 %, 1 mL, 0.02 mol) was added and the reaction mixture was refluxed for a further 10 h. Excess methanol was removed under reduced pressure and the reaction mixture was poured into 200 mL ice-cold water. The acid hydrazide obtained was purified by crystallization from ethanol (50 %). The melting point and percentage yield were in agreement with reported values (Udupi 1995).

*Synthesis of 3-aryl 4-*N*-[pyrazin-2-carboxamido]-1,2,4-triazole-5-thiol (**1a**–**j**; Figure 1)*

To a solution of aromatic acid hydrazide (0.1 mol) and potassium hydroxide (7.0 g, 0.125 mol) in 10 mL ethanol, carbon disulphide (7.6 g, 0.1 mol) was added dropwise and the reaction mixture was stirred for 2 h. The

Table 1 Physical properties of compounds **1a**–**j**.

Compound	Ar	mp (°C)	Yield (%)	Elemental analysis					
				Calculated (%)			Found (%)		
				C	H	N	C	H	N
1a	Phenyl	154–156	59	52.35	3.36	28.18	52.14	3.40	28.06
1b	4-Pyridyl	204–206	65	48.16	3.01	32.77	48.02	2.98	32.56
1c	2-Chlorophenyl	140–142	69	46.98	2.71	25.30	46.74	2.78	25.08
1d	4-Nitrophenyl	100–102	62	45.48	2.62	28.57	45.20	2.50	28.38
1e	4-Nitrophenoxymethyl	174–176	66	45.04	2.95	26.27	44.90	2.84	26.04
1f	4-Methylphenoxymethyl	102–104	60	52.63	4.09	24.56	52.46	3.94	24.50
1g	3-Methylphenoxymethyl	260–262	68						
1h	2-Methylphenoxymethyl	220–222	65						
1i	4-Chlorophenoxymethyl	106–108	72						
1j	2-Chloro 5-methylphenoxymethyl	104–106	65	47.80	3.45	22.31	47.62	3.20	22.02

precipitated potassium β-acyl dithiocarbazinate was removed by filtration, washed with cold methanol and used for the next step directly. A mixture of potassium-β-acyl dithiocarbazinate (0.01 mol) and pyrazin-2-carboxylic acid hydrazide (0.01 mol) was heated on an oil bath at 160–180°C for 6 h. The product was dissolved in 25 mL water and the solution acidified with conc. hydrochloric acid. The precipitated triazole was filtered and purified by crystallization from ethanol. The physical properties of compounds **1a**–**j** are detailed in Table 1.

*3-phenyl-4-*N*-[pyrazin-2-carboxamido]-1,2,4-triazole-5-thiol (**1a**).* Yield 59%; mp 154–156°C (ethanol). Anal: C, H, N ($C_{13}H_{10}N_6OS$). ^{1}H NMR-DMSO-d_6 δ: 3.0–4.5(s, 1H, NH), 7.6–9.3 (m, 8H, ArH and pyrazinyl). Mass: 298 (molecular ion). IR (KBr, cm^{-1}) 1600 (C=N), 1700 (C=O), 720 (Ar), 3140 (NH), 1390 (C=S).

*3-(2-chloro 5-methyl-phenoxymethyl)-4-*N*(pyrazin-2-carboxamido)-1,2,4-triazole-5-thiol (**1j**).* Yield 65%; mp 104–106°C (ethanol). Anal: C, H, N ($C_{15}H_{13}N_6O_2SCl$). ^{1}H NMR-DMSO-d_6 δ: 2.32 (s, 3H, CH_3), 4.66 (s, 2H -OCH_2) 6.8–9.2 (m, 6H, ArH and pyrazinyl), 10.35 (s, 1H, NH), 10.78 (s, 1H, SH). IR (KBr, cm^{-1}) 1660 (C=N), 1700 (C=O), 820 (Ar), 3200 (NH), 1380 (C=S).

*Synthesis of 4-*N*(aryl carboxamido)-3-(pyrazin-2-yl)-1,2,4-triazole-5-thiol (**2a**–**p**; Figure 1)*

Carbon disulphide (7.6 g, 0.1 mol) was added dropwise to a solution of pyrazin-2-carboxylic acid hydrazide (0.1 mol) and potassium hydroxide (7.0 g, 0.125 mol) in 10 mL ethanol, and the reaction mixture was stirred for 2 h. The precipitated potassium β-acyl dithiocarbazinate was removed by filtration, washed with cold methanol and used for the next step directly. A mixture of potassium β-acyl dithiocarbazinate (0.01 mol) and aromatic acid hydrazide (0.01 mol) was heated on an oil bath at 160–180°C for 8 h. The product was dissolved in 25 mL water and the solution acidified with conc. hydrochloric acid. The precipitated triazole was filtered and purified by crystallization from ethanol. The physical properties of compounds **2a**–**p** are detailed in Table 2.

*4-*N*(phenyl carboxamido)-3-(pyrazin-2-yl)-1, 2, 4-triazole-5-thiol (**2a**).* Yield 72%; mp 268–270°C (ethanol). Anal: C, H, N ($C_{13}H_{10}N_6OS$). ^{1}H NMR-DMSO-d_6 δ: 3.0–4.5 (s, 1H, NH), 7.6–9.2 (m, 8H, ArH and pyrazinyl). Mass: 298 (molecular ion). IR (KBr, cm^{-1}) 1600 (C=N), 1700 (C=O), 720 (Ar), 3140 (NH), 1390 (C=S).

Evaluation of in-vitro antitubercular activity

Antitubercular activity was determined in triplicate using the Lowenstein-Jensen medium (Cruickshank et al 1975). Stock solutions of the test compounds were prepared in dimethylsulfoxide (DMSO) at 1000, 100 and 10 $\mu g\,mL^{-1}$. Solution of the test compound (0.8 mL) was added to 7.2 mL of the Lowenstein-Jensen media taken in sterile McCartney bottles to achieve a final concentration of 100, 10 or 1 $\mu g\,mL^{-1}$. Controls received

Table 2 Physical properties of compounds **2a**–**p**.

Compound	Ar	mp (°C)	Yield (%)	Elemental analysis					
				Calculated (%)			Found (%)		
				C	H	N	C	H	N
2a	Phenyl	268–270	72	52.35	3.36	28.18	52.24	3.42	28.00
2b	4-Chlorophenyl	108–110	68						
2c	4-Methylphenyl	122–124	64	53.85	3.85	26.92	53.50	3.50	26.70
2d	4-Hydroxyphenyl	274–276	70	49.68	3.18	26.75	49.46	3.02	26.58
2e	4-Aminophenyl	164–166	75	49.84	3.51	31.30	49.63	3.40	31.06
2f	2-Chlorophenyl	130–132	77						
2g	4-Nitrophenyl	256–358	68	45.48	2.62	28.57	45.32	2.40	28.35
2h	2-Hydroxyphenyl	144–146	74						
2i	4-Pyridyl	266–268	76	48.16	3.01	32.77	48.00	2.81	32.49
2j	Phenoxymethyl	124–126	80	51.21	3.65	25.60	50.84	4.00	24.98
2k	4-Chlorophenoxymethyl	212–214	78						
2l	2-Chlorophenoxymethyl	190–192	75	46.34	3.04	23.26	46.50	3.00	23.50
2m	4-Methylphenoxymethyl	124–126	80						
2n	3-Methylphenoxymethyl	248–250	82						
2o	2-Methylphenoxymethyl	228–230	71	52.63	4.09	24.56	52.80	4.50	25.00
2p	4-Methoxyphenoxymethyl	120–122	74	50.27	3.91	23.46	50.30	4.00	23.70

Table 3 Antitubercular activity of compounds **1a**–**i** and **2a**–**p**.

Compound	100 μg mL^{-1}	10 μg mL^{-1}	1 μg mL^{-1}
1a	−	+	+
1b	−	+	+
1c	−	+	+
1d	−	+	+
1e	−	+	+
1f	−	+	+
1g	−	+	+
1h	−	+	+
1i	−	+	+
2a	−	−	−
2b	−	−	−
2c	−	−	−
2d	−	−	−
2e	−	−	−
2f	−	−	−
2g	−	−	−
2h	−	−	−
2i	−	−	−
2j	−	−	−
2k	−	−	−
2l	−	−	−
2m	−	−	−
2n	−	−	−
2o	−	−	−
2p	−	−	−
Streptomycin	−	−	+
Control	+	+	+

Growth present, +; growth absent, −.

0.8 mL DMSO. Streptomycin was used as the reference standard. The bottles were inspissated at 75–80°C for 2 h for three successive days for solidification and sterilization. A sweep from H37 Rv strain culture of *Mycobacterium tuberculosis* was discharged with the help of 22 S. W. Nichrome wire loop of 3-mm external diameter into a sterile bijou bottle containing six 3-mm glass beads and 4 mL sterile distilled water. The bottle was shaken with the aid of a mechanical stirrer. Using a 27 S. W. G. Nichrome wire loop of 3-mm external diameter, a loopful of the suspension was inoculated on the surface of each Lowenstein-Jensen media containing the test compounds. All the bottles were incubated at 37°C for six weeks at the end of which the readings were taken.

Evaluation of in-vitro antibacterial activity

Compounds **1a**–**j** were evaluated for in-vitro antibacterial activity against *B. subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *S. typhi* by disc diffusion method (Stokes & Waterworth 1972). Stock solutions of the compounds were prepared in DMSO and sterile paper discs (6-mm diameter) were impregnated with the stock solution of the test compounds to obtain a concentration of 50, 5 or 0.5 μg of the test compounds per disc. Antibacterial screening was performed in triplicate on Muller-Hinton agar and the antibacterial activity profile was evaluated.

Table 4 Antifungal activity profile of compounds **1a**–**i** and **2a**–**p**.

Compound	*A. niger* 88 (μg mL^{-1})			*A. niger* 90 (μg mL^{-1})			*T. rubrum* TR1 (μg mL^{-1})			*T. rubrum* T6 (μg mL^{-1})			*T. rubrum* T7 (μg mL^{-1})			*T. mentagrophyte* (μg mL^{-1})		
	5.0	0.5	0. 05	5.0	0.5	0.05	5.0	0.5	0.05	5.0	0.5	0.05	5.0	0.5	0.05	5.0	0.5	0.05
1a	+	+	+	+	+	+												
1b	+	+	+	+	+	+												
1c	+	+	+	+	+	+												
1d	+	+	+	+	+	+												
1e	+	+	+	+	+	+												
1f	+	+	+	+	+	+												
1g	+	+	+	+	+	+												
1h	+	+	+	+	+	+												
1i	+	+	+	+	+	+												
2a	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
2b	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
2c	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
2d	+	+	+	+	+	+												
2e	+	+	+	+	+	+												
2f	+	+	+	+	+	+												
2g	+	+	+	+	+	+												
2h	+	+	+	+	+	+												
2i	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
2j	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
2k	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
2l	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2m	+	+	+	+	+	+												
2n	+	+	+	+	+	+												
2o	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
2p	+	+	+	+		+	+											

Growth present, +; growth absent, −.

Evaluation of the in-vitro antifungal activity

Sabourauds Dextrose agar medium was used for the antifungal screening. Compounds **1a**–**i** and **2a**–**i** were screened for activity against *Aspergillus niger* 88 and *Aspergillus niger* 90 by the cup plate method (Clayton 1978) at 5, 0.5 or 0.05 μg mL^{-1}. Compounds **2a**–**c, 2i**–**l** and **2o** were screened for activity against *T. rubrum* TR1, *T. rubrum* R6, *T. rubrum* R7 and *T. mentagrophyte* M1 by the cup plate method in triplicate at 5, 0.5 or 0.05 μg mL^{-1}.

Results and Discussion

Compounds **1a**–**i** and **2a**–**p** were evaluated for in-vitro antitubercular activity against *Mycobacterium tuberculosis* H_{37} Rv strain. A good correlation between the structure and the antitubercular activity was observed (Table 3). Compounds having a pyrazine moiety in the 3 position were found to be more active than the triazoles having a pyrazine moiety at position 4. The nature of the functional group on the benzene ring did not affect the antitubercular activity of the compounds.

Compounds **1a**–**j** did not exhibit antibacterial activity against *B. subtilis*, *E. coli*, *P. aeruginosa*, *S. aureus* or *S. typhi* even at 50 μg/disc. Compounds **2l** and **2o** were found to be active against *Aspergillus niger* 88 and *Aspergillus niger* 90 at 0.05 μg mL^{-1} (Table 4). Compounds **2a**, **2b**, **2i**–**l** and **2o** were found to be active against *T. rubrum* TR1, *T. rubrum* R6, *T. rubrum* R7, and *T. mentagrophyte* M1 at 0.05 μg mL^{-1}.

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