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Investigation of pyrazole and tetrazole derivatives containing 3,5 disubstituted-4*H*-1,2,4-triazole as a potential antitubercular and antifungal agent

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ABSTRACT

A number of 5-methyl-2-[(5-substituted aryl-4*H*-1,2,4-triazol-3-yl)methyl]-2,4-dihydro-3*H*-pyrazol-3-one (4a-g) and 5-phenyl-1-[(5-substituted aryl-4*H*-1,2,4-triazol-3-yl)methyl]-1*H*-tetrazole (8a-g) derivatives were evaluated for *in vitro* antitubercular and antifungal activity. Compounds 4a-g and 8a-g were evaluated against *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC27294) in BACTEC 12B medium using a broth micro dilution assay. The minimum inhibitory concentration (MIC) was determined for compounds that demonstrated≥ 90% growth inhibition in the primary screening. Results of the biological assay showed that 4-chloro, 3-nitro, 4-methoxy and 2-chloro substituted compounds were found to be antimycobacterial agents at MIC value of 6.25 µg/ml. MIC values for *in vitro* antifungal activity were determined by liquid broth method. From the newly synthesized series compounds substituted with 4-chloro, 2-chloro and 4-methoxy group exhibited significant antifungal activity against *Candida albicans* NCIM 3471 and *Aspergillus niger* NCIM 1196.

Keywords: *1,2,4-Triazole, antitubercular, antifungal activity, MIC*

1. INTRODUCTION

Tuberculosis is a disease known to man from the earliest recorded history. It is a disease which is characterized as a chronic bacterial infection caused by *Mycobacterium tuberculosis*, an acid-fast aerobic bacillus. It is estimated that today one-third to one-half of the world population is infected with *Mycobacterium tuberculosis* leading to approximately 6% of all death worldwide. A triazole antifungal agent now plays a leading role in the treatment of a variety of fungal infections. Triazoles act as pharmacophore for important antifungal and anticancer therapeutic agents like Terconazole, Fluconazole, Voriconazole, Itraconazole, Anastrazole and Letrozole [1,2]. 1,2,4- triazoles are pharmacological scaffolds displaying a wide range of biological activities such as anticancer [3], antibacterial [4,5], anticonvulsant [6], anti-inflammatory, analgesic [7], antifungal [8,9], antidepressant [10], antituberculine [11], antimalarial [12] and hypoglycemic [13] activities. The above mentioned facts inspires several chemists and medicinal chemists to prepare newer triazoles

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by different synthetic routes while incorporating a variety of a known pharmacophore into their molecular systems and evaluating them for their possible pharmacological properties. In the recent years there has been an increasing interest in the chemistry of pyrazole because of their biological significance. Various pyrazole derivatives have been widely investigated for therapeutic uses especially as antiinflammatory, analgesic, antipyretic [14], antibacterial, antifungal1[5,16], anticancer [17,18], antituberculine [19], antimalarial [20] activities. Tetrazoles are also known to posses diverse pharmacological activities like antifungal [21], antibacterial, anticonvulsant [22], analgesic [23], antiinflammatory [24, 25], anticancer [26], antituberculine [27] activities. We have reported that 5-methyl-2-[(5-substituted aryl-4*H*-1,2,4-triazol-3-yl)methyl]-2,4-dihydro-3*H*-pyrazol-3-one (4a-g) and 5-phenyl-1-[(5-substituted aryl-4*H*-1,2,4-triazol-3-yl)methyl]-1*H*-tetrazole (8a-g) had significant anticancer activity specially on renal cancer cell lines (UO-31) and also *in vitro* antibacterial activity reported against gram positive bacterial *S. aureus* NCIM 2079, *B. subtillis* NCIM 2063 and gram negative bacterial *E. coli* NCIM 2065, *P. aeruginosa* NCIM 2863 strains [28]. More recently, we have also found that pyrazole and tetrazole derivatives containing 1,2,4-triazole have superior analgesic activity [29]. So the present exertion is to evaluate the *in vitro* antitubercular and antifungal activity of newly synthesized series of 5-methyl-2-[(5-substituted aryl-4*H*-1,2,4-triazol-3-yl)methyl]-2,4-dihydro-3*H*-pyrazol-3-one and 5-phenyl-1-[(5-substituted aryl-4*H*-1,2,4-triazol-3-yl)methyl]-1*H*-tetrazole (Figure 1).

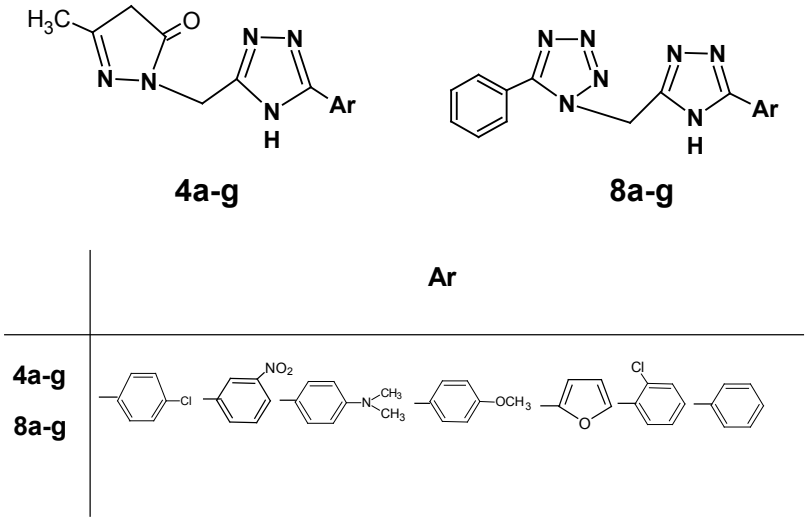

Figure 1: Various Substitutions of compound 4a-g and 8a-g

2. EXPERIMENTAL SECTION

2.1. Bacterial strains and growth conditions. *Mycobacteruim tuberculosis* ATCC 27294 ($H_{37}Rv$) was obtained from the American Type Culture Collection (Rockville, Md.). The microbial cultures of *Candida albicans* (NCIM 3471) and *Aspergillus niger* (NCIM 1196) were procured from National Centre for Industrial Microorganisms (NCIM), Pune, India. $H_{37}Rv$ inocula was first passaged in radiometric 7H12 broth (BACTEC 12B; Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) until the growth index (GI) reached 800 to 999. $H_{37}Rv$ was cultured identically except that Casitone was omitted. Cultures were incubated in 500 ml nephelometer flasks on a rotary shaker (New Brunswick Scientific, Edison, N.J.) at 150 rpm and 37°C until they reached an optical density of 0.4 to 0.5 at 550 nm. Bacteria were washed and suspended in 20 ml of phosphate-buffered saline and passed through an 8 mm pore size filter to eliminate clumps. The filtrates were aliquoted, stored at -80°C and used within 30 days. Isoniazid and Rifampicin were obtained from Sigma. Sabouraud

Dextrose broth (SDB), Sabouraud Dextrose Agar (SDA), Peptone water and standard antifungal drug Fluconazole were procured from Hi-media laboratories, Mumbai, India. DMSO was procured from E. Merck Ltd., Mumbai, India.

2.2. Antitubercular Activity. The compounds were screened for their antituberculosis activity under the direction of the US National Institute of Health, NIAID division. All compounds were initially screened against *Mycobacterium tuberculosis* strain $H_{37}Rv$ at a single concentration of 6.25 µg/ml in BACTEC 12B medium using a broth microdilution assay. Compounds demonstrating growth inhibition≥90% in the primary screening were considered active. The active compounds were re-tested by serial dilution beginning at the concentration of 6.25µg/ml against *Mycobacterium tuberculosis* $H_{37}Rv$ to determine the actual minimum inhibitory concentration (MIC) in the BACTEC 460 radiometric system and BACTEC 12B medium. The MIC is defined as the lowest concentration reducing fluorescence to 90% of controls. The significance of this value depends on several factors such as compound structure, novelty, toxicity, and potential mechanism of action. Isoniazide (MIC= 0.025- 0.05 µg/ml) and Rifampicin (0.025- 0.125 µg/ml) were used as positive control drugs [30]. Antitubercular activity data indicated in Table 1.

2.3. Antifungal Activity

2.3.1. Preparation and Standardization of Stock cultures. Cultures on receipt were sub cultured in SDA plates and further stored in slants as stock cultures. For the experiments, stock culture was prepared by inoculating each culture from slants to flask in sterile SDB and incubated at 28°C for 48 h. The stock culture was serially diluted by ten fold with sterile peptone water and 0.1ml from each dilution was spread over SDA plates and incubated at 28°C for 48 h. The number of colony forming units (CFU) was counted on plates of each dilution and there by the total CFU was calculated in the stock culture. For the antimicrobial screening the stock cultures of $1x10^5$ CFU per ml were used.

2.3.2. Determination of Minimum Inhibitory Concentration (MIC). The Minimum Inhibitory Concentration (MIC) of the tested substances against *Candida albicans* and *Aspergillus niger* was determined by liquid broth method using two fold serial dilution technique [31]. In this assay, the minimum concentration of each test substance required to inhibit the growth of microorganism was determined. For this assay, a series of assay tubes were prepared containing uniform volume (1ml) of sterile SD broth and equal volume of known concentration of test substance was added. The test substance in the first tube was serially diluted in twofold decreasing concentrations. The tubes with the test substances were inoculated with 1 ml of inoculum ($1x10^6$ CFU per ml). The final concentration of test substance ranged from 1000 to 15.62 µg/ml. Solvent control and sterility controls were maintained in the experiment. The tubes were incubated at 28°C for 48 h. Standard antifungal drug Fluconazole was tested at concentrations ranging from 100 to 3.12 µg/ml. The tubes were inspected visually to determine the growth of the organism as indicated by turbidity. In experimental terms the MIC is the concentration of the drug present in the last clear tube, i.e. in the tube having the lowest concentration in which growth is not observed. Antifungal testing statistics compounds 4a-g and 8a-g tabulated in Table 2.

3. RESULTS SECTION

The *in vitro* antitubercular activity of pyrazole and tetrazole derivatives carrying 1,2,4-triazole were found appreciable, while the phenyl substituted 4-methoxy, 3-nitro, 2-chloro, 4-chloro group of 1,2,4-triazole nucleus exhibited antitubercular activity at MIC 6.25 µg/ml (compound 4a, 4b, 4d, 8b,

and 8f). 5-position of 1,2,4-triazole nucleus may influence the antitubercular activity when phenyl ring is substituted by electron withdrawing group. Furan and phenyl substitution on 5-position confers weak antitubercular activity (compound **4e**, **4g, 8e** and **8g**) in both series. Electron withdrawing substituents like chloro, nitro, methoxy exhibited good assortment of antitubercular activity. 4-dimethyl aminophenyl substituted compound **4c** and **8c** found to be weekly active.

Table 1: Antitubercular screening of compounds 4a-g and 8a-g

Sr No.	Compounds	MIC (µg/ml)
1	4a	6.25
2	4b	6.25
3	4c	> 6.25
4	4d	6.25
5	4e	> 6.25
6	4f	> 6.25
7	4g	> 6.25
8	8a	> 6.25
9	8b	6.25
10	8c	> 6.25
11	8d	6.25
12	8e	> 6.25
13	8f	6.25
14	8g	> 6.25
15	Isoniazid	0.05
16	Rifampicin	0.125

Legend: " > " more than- MIC value observed

Table 2: Minimum Inhibitory Concentrations of compounds 4a-g and 8a-g against *Candida albicans* and *Aspergillus niger*

Sr No.	Compounds	Minimum Inhibitory Concentration (MIC)	
		Candida albicans	***Aspergillus niger***
1	4a	62.5	62.5
2	4b	1000	1000
3	4c	500	1000
4	4d	250	250
5	4e	1000	1000
6	4f	1000	1000
7	4g	250	250
8	8a	31.25	31.25
9	8b	125	125
10	8c	250	250
11	8d	62.5	125
12	8e	1000	500
13	8f	62.5	31.25
14	8g	125	125
15	Fluconazole	6.25	6.25

All synthesized derivatives (4a-g and 8a-g) were also tested for the *in vitro* antifungal activity and MIC values were determined by liquid broth method. Compound **8a, 8f, 4a** and **8d** exhibited fungicidal potential with MIC values 31.25, 62.5, 62.5, and 62.5 µg/ml respectively against *Candida*

albicans and 31.25, 31.25, 62.5 and 125 µg/ml respectively against *Aspergillus niger*. Compound **8b, 8g, 4d, 4g** and **8c** showed moderate inhibitory properties against both the microorganism with MIC values 125, 125, 250, 250 and 250µg/ml respectively (Table 2).The superior antifungal activity is attributed to the presence of pharmacologically active phenyl substituted chloro, nitro, methoxy groups attached to position 5 of 1,2,4- triazole moiety. Remaining tested compounds did not showed any promising activity against the tested fungi (Figure 2). Standard drug Fluconazole showed potent activity against both test organisms with MIC value 6.25µg/ml.

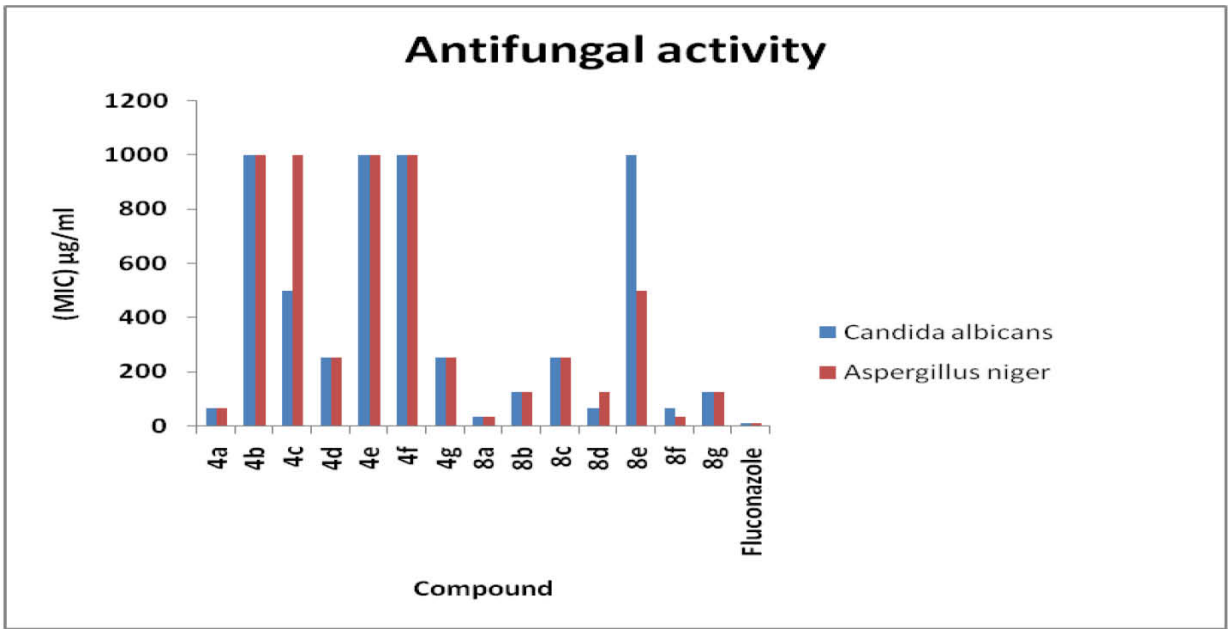

Figure 2: Antifungal activity of compounds 4a-g and 8a-g

4. CONCLUSIONS

In conclusion, a new class of tetrazole and pyrazole containing triazole heterocycle was evaluated for the antitubercular and antifungal activity. The newly synthesized heterocycles exhibited promising antitubercular activity against *Mycobacterium tuberculosis* $H_{37}Rv$. Chloro, nitro and methoxy substituted compounds were found to be effective antimycobacterial agents. All tested compounds containing 1,2,4-triazole moiety exhibited antifungal activity. Compounds 8a, 8f, 4a and 8d exhibited significant antifungal activity against *Candida albicans* and *Aspergillus niger***.** These outcomes make novel tetrazole and pyrazole containing triazole heterocycles are interesting molecules for further synthetic and biological evaluation.

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