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Microwave assisted synthesis of novel 3-mercapto-4,5-disubstituted 1,2,4-triazole derivatives and evaluation of antimicrobial, antitubercular activity

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Abstract
A series of 3-Mercapto-4,5-disubstituted 1,2,4-triazole derivatives (T1-T18) has been synthesized under conventional and microwave irradiation and compared the results. The reaction time decreases hours to minutes along with yield enhancement. The structures of these compounds were characterized by means of FT-IR, ^{1}H-NMR, ^{13}C NMR. Representative compounds have been screened for their antimicrobial activity by tube dilution method and antitubercular activity by Micro plate Alamar Blue assay method. Compound T9, T11, T13 and T15 showed excellent antimicrobial activity. Compound T-3, T-9 showed excellent antitubercular activity against MTB H37Rv (MIC 6.25 µg/ml). In the present study classification models have been developed for the prediction of antimicrobial activity of 1,2,4-triazole analogs.

Keywords: 1,2,4-triazole, conventional, microwave, antimicrobial, antitubercular

1. Introduction
Over the past few years scientists shows interest in the synthesis of organic compounds under microwave irradiation. The feasibility of microwave-assisted synthesis has been demonstrated in various transformations such as condensation [1], cycloaddition [2], alkylation [3], oxidation [4]. synthesis of various heterocyclic compounds [5-7] and many other chemical reactions. Microwave irradiation prevents wall heat transfer as in case of an water/oil bath but produces heat transfer throughout the sample resulting in even heating throughout the sample [8]. The microwave assisted reactions takes less time, safe and product yield is also high. A large number of 1,2,4-triazole ring , have been incorporated into large variety of therapeutic drugs having anti-inflammatory, CNS-stimulant, sedative, antianxiety and antimicrobial activities [9-11], antimycotic activity such as fluconazole, intraconazole, and voriconazole [12-13]. Drugs containing 1,2,4-triazole moiety are triazolam , alprazolam , etizolam and furacylin [14]. Antibiotics are much important in medicine, but resistance developed by bacteria. Antibiotic-resistance bacteria are the germs that are not killed by commonly used antibiotics. When bacteria are exposed to the same antibiotics again and again, the bacteria able to change its shape and are not affected by the drug. There are various mechanism by which bacteria become antibiotic-résistance. They may changes their structure so the antibiotic not work on them for long time. By different ways they inactivate or neutralize the antibiotic. By transfer gene coding bacteria never exposed to an antibiotic to acquire resistance from those which have. Therefore, there is an urgent requirement to develop new classes of drugs to treat microbial infections [15]. Tuberculosis (TB), is the worldwide challenging problem of health caused by *Mycobacterium tuberculosis* (MTB), still remains the leading cause of the worldwide death among the infectious disease [16-17]. The synergy between tuberculosis and the AIDS epidemic as well as the surge of multidrug-resistant isolates of MTB has become a therapeutic challenge to a pharmacist, scientist for selecting novel antitubercular agents [18]. The alarming in TB treatment is the emergence of resistant by strains to two of the best antitubercular drugs, Isoniazid (INH) and Rifampicin (RIF). The current TB treatment comprises of 3–4 drugs for a period of 6–9 months. Novel drugs are urgently required which can shorten this long-treatment period and target multidrug-resistant strains of TB [19–21].

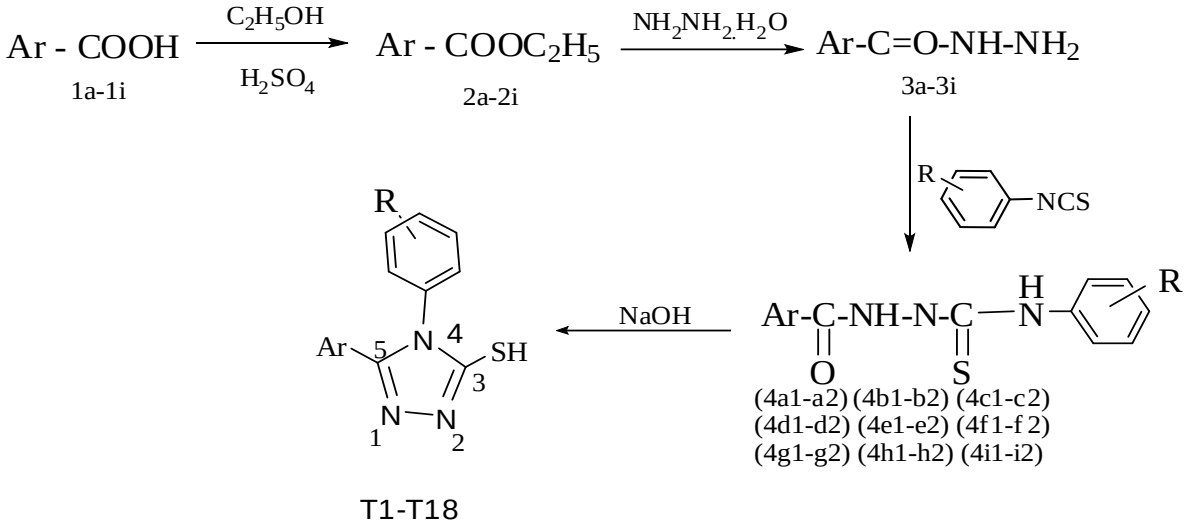
2. Materials and Methods
2.1 General
Melting points were determined by automated melting point apparatus (model 51142501) and are uncorrected. ^{1}H and ^{13}C NMR spectra were determined by Agilent 400 MHz NMR

spectrometer ulventsing DMSO-d6 solvent and are expressed in parts per million (δ, ppm). NMR data are given as multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet) and number of protons. IR spectra were recorded on Brucker 12060280, software OPUS 7.2.139.1294 ATR spectrophotometer in a KBr disc. Reaction progress was observed by thin layer chromatography.

2.2 Chemistry

In the present work eighteen novel derivatives of 1,2,4-triazole were synthesized by using conventional and microwave methods, following the synthetic route given in scheme1.The starting material benzoic acid ester derivatives (2a- 2i), for the synthesis of desired compounds were obtained by Esterification of benzoic acid derivatives (1a-1i) with ethanol in the presence of sulphuric acid as catalyst using both reflux and microwave methods .Then the compounds (2a-2i) on the treatment with aromatic hydrazine hydrate provided aryl carbonyl hydrazide derivatives (3a-3i). In the next step (3a-3i) reacts with aryl thiocyanate gave 1-(aryl carbonyl)-4-arylthiocarbazides ($4a_1$-a_2) ($4b_1$-b_2) ($4c_1$-c_2) ($4d_1$-d_2) ($4e_1$-e_2) ($4f_1$-f_2) ($4g_1$-g_2) ($4h_1$-h_2) ($4i_1$-i_2). Finally cyclization of 1-(aryl carbonyl)-4 arylthiocarbazides derivatives with NaOH provided the desired products 3-Mercapto-4,5-disubstituted 1,2,4-triazole (Table 1). Mechanism involved for synthesis has been summarized. The synthesized compounds were purified and recrystallized using ethanol and acetone. The characterization of these new derivatives were done by spectroscopy (FT-IR, ^{1}H NMR).

Scheme 1. Synthetic scheme for 3-Mercapto-4,5-disubstituted -1,2,4-triazole derivatives (T1-T18).



2.2.1 Synthesis of aryl carboxylic acid ester (2a- 2i)

Conventional Method: A mixture of pure aryl carboxylic acid (0.1 mol) and absolute ethanol (0.4 mol) refluxed in a round bottom flask for 4 h in the presence of 2-3 drops of conc. Sulphuric acid. After refluxing the resulting product was kept at room temperature and then poured into about 250 ml of water in a separating funnel. Lower layer of ester was collected at the bottom. Ester of aryl carboxylic acid shook with a strong solution of potassium hydrogen carbonate until all free acid was neutralized. Washed once with water, and dried by pouring into a dry china dish containing about 2 gm of magnesium sulphate.

Microwave Method: Aryl carboxylic acid (0.01mol), ethanol (0.025mol) and 1-2 drops of sulfuric acid were mixed in a glass vessel with teflon stopper. The reaction was performed in the microwave at an engaged power of 360 W for 5 min. The alcohol was removed in a rota evaporator, the obtained product dissolved in ether and extracted with aqueous solution of sodium hydroxide (pH 9). The ether extract was washed with water to neutralize and dried over anhydrous Na_2SO_4. The m.p., IR and ^{1}HNMR data are in agreement with those obtained for the products synthesized by other reported method [22].

2.2.2 Synthesis of aryl carbonyl hydrazide (3a-3i) hydrazinolysis

Conventional method: In this step the compounds (2a-2i) (0.1mol) and hydrazine hydrate (0.116mol) were refluxed in presence of 25.5 ml of ethanol for 6 h. Excess of ethanol was distilled off, the reaction mixture was cooled. The solid thus separated out was dried and recrystallized from ethanol.

Microwave method: Compounds (2a-2i) (0.01 mol) and hydrazine hydrate (0.012 mol) in a glass vessel with teflon stopper at 900 W for 1-2 min. When reaction completed excess of ethanol was distilled off under reduced pressure and residue poured into ice cold water. The solid obtained was filtered and recrystallized from ethanol. The m.p., IR and ^{1}HNMR data are in agreement with those obtained for the products synthesized by other reported method.

2.2.3 Synthesis of aryl carbonyl hydrazinecarbothioamide ($4a_1$-a_2) ($4b_1$-b_2) ($4c_1$-c_2) ($4d_1$-d_2) ($4e_1$-e_2) ($4f_1$-f_2) ($4g_1$-g_2) ($4h_1$-h_2) ($4i_1$-i_2)

Conventional method: Mixture of compounds (3a-3i) (0.1mol) and aryl thiocyanate (0.12 mol) were taken in a of round bottom flask, 20 ml of 10% HCl solution was added drop wise to this mixture and refluxed for 3-4 h. The solid crude product was separated by filtration, washed twice with cold water. Then the product was recrystallized from acetone.

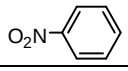
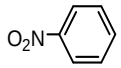
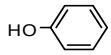
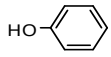
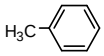
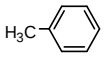
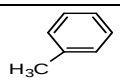
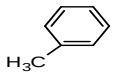
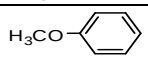
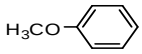
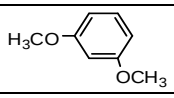
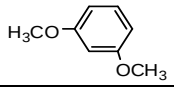
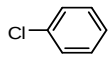
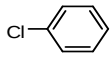
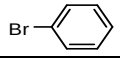
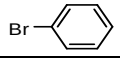
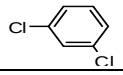
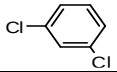
Microwave method: Mixture of compounds (3a-3i) (0.1mol) and arylthiocyanate (0.12mol) were mixed thoroughly in a mortar. Then two drops of ethanol was added and the reaction mixture was irradiated with microwave at 900 W for 2-5 minutes. The solid crude product was purified by recrystallization from ethanol. The m.p., IR and ^{1}HNMR data are in agreement with literature [23].

2.2.4 Synthesis of 3-Mercapto- 4, 5-aryl substituted -1,2,4-triazole (T1-T18)

Conventional method: 5 ml solution of 2M NaOH (8%solution) was added portion wise to solid aryl carbonyl thiosemicarbazide derivatives (0.01mol) and refluxed for 4 h. After the completion of reaction, the reaction mixture was treated with activated charcoal and filtered. Then filtrate was acidified with hydrochloric acid for pH 9. The resulting precipitate was filtered and recrystallized from ethanol to obtain the final derivatives.

Microwave method: 2M NaOH added to solid aryl carbonyl thiosemicarbazide (0.004mol) and kept in microwave for 2-5 minutes. After completion of reaction, cool the mixture and acidified with 2M HCl. The precipitate obtained was recrystallized from acetonitrile.

Table 1: A comparative study of compounds synthesized by conventional and microwave methods

Compound	AR	R	Molecular Formula	Mol. Wt.	Reaction Time		Yield (%)		Melting Point (^{0}C)
					Conv. (h)	Micro. (Sec)	Conv.	Micro.	
T1		H	$C_{14}H_{10}N_4O_2S$	298.05	5.0	130	75	88	232-234
T2		CH3	$C_{15}H_{12}N_4O_2S$	312.35	4.5	140	78	90	231-233
T3		H	$C_{14}H_{11}N_3OS$	296.32	5.0	130	80	87	188-189
T4		CH3	$C_{15}H_{13}N_3OS$	283.35	5.0	130	72	84	189-190
T5		H	$C_{15}H_{13}N_3S$	267.35	4.5	130	85	89	210-212
T6		CH3	$C_{16}H_{15}N_3S$	281.38	5.0	200	83	89	211-213
T7		H	$C_{15}H_{13}N_3S$	267.38	5.0	130	70	88	234-236
T8		CH3	$C_{16}H_{15}N_3S$	281.38	4.5	120	70	80	235-237
T9		H	$C_{15}H_{13}N_3S$	263.35	5.5	120	77	86	230-232
T10		CH3	$C_{16}H_{15}N_3S$	281.38	5.0	130	80	87	232-234
T11		H	$C_{16}H_{15}N_3O_2S$	313.37	4.5	120	86	97	246-248
T12		CH3	$C_{17}H_{17}N_3O_2S$	327.40	4.5	130	85	89	247-249
T13		H	$C_{14}H_{16}ClN_3S$	287.77	5.0	210	75	85	249-251
T14		CH3	$C_{15}H_{12}ClN_3S$	301.79	5.0	130	75	88	250-252
T15		H	$C_{14}H_{16}BrN_3S$	287.77	5.5	110	75	86	184-186
T16		CH3	$C_{15}H_{12}BrN_3S$	301.79	5.0	130	80	87	187-189
T17		H	$C_{14}H_9Cl_2N_3S$	332.21	4.5	120	86	97	190-192
T18		CH3	$C_{15}H_{11}Cl_2N_3S$	336.24	4.5	140	70	85	192-194

(4-nitrophenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (T1): IR v_{max} cm^{-1} (KBr): 3067.12 (C-H str., aromatic), 1255.34 (C-N str., aromatic), 1625.44 (C=N str., aromatic), 1507.48 (C=C str., aromatic), 1345.31 (N-O sym. str., aromatic). ^{1}H NMR (DMSO) δ; 6.06-7.87 (9H, m, ArH), 3.35 (1H, s, SH).

4-(4-methylphenyl)-5-(4-nitrophenyl)-4H-1,2,4-triazole-3-thiol(T2): IR v_{max} cm^{-1} (KBr): 3098.77 (C-H str., aromatic), 1345.77 (C-N str., aromatic), 1407.22 (C=C str., aromatic), 1531.94 (N-O sym. str., aromatic), 2904.11 (C-CH3 sym.). ^{1}H NMR (DMSO) δ; 6.55-7.95 (8H, m, ArH), 4.15 (1H, s, SH), 2.31 (3H, s, $ArCH_3$).

5-(4-hydroxyphenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (T3): IR v_{max} cm^{-1}(KBr): 3067.12 (C-H str., aromatic), 1255.34 (C-N str., aromatic), 1625.44 (C=N str., aromatic), 1507.48 (C=C str., aromatic), 1311.84 (N-O sym.str., aromatic). ^{1}H NMR (DMSO) δ; 6.63-8.05 (9H, m, ArH), 3.35 (1H, s, SH), 5.0 (IH, s, OH).

4-(4-methylphenyl)-5-(4-hydroxyphenyl)-4H-1,2,4-triazole-3-thiol (T4): IR v_{max} cm^{-1}(KBr): 3045.86 (C-H str., aromatic), 1250.88 (C-N str., aromatic), 1688.98 (C=N str., aromatic), 1588.96 (C=C str., aromatic), 3538.45 (O-H str.). ^{1}H NMR (DMSO) δ; 6.64-8.26 (8H, m, ArH), 3.35 (1H, s, SH), 2.37 (3H,s, $ArCH_3$), 4.90 (IH, s, OH).

5-(2-methylphenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (T5): IR v_{max} cm^{-1}(KBr) 3015.00(C-H str., aromatic), 1250.69 (C-N str., aromatic), 1664.96 (C=N str., aromatic), 1507.66 (C=C str., aromatic), 2963.966 (C-CH3 str.). ^{1}H NMR(DMSO) δ; 6.11-8.66 (9H, m, ArH), 3.35 (1H, s, SH), 2.35 (3H, s, $ArCH_3$).

4-(4-methylphenyl)-5-(2-methylphenyl)-4H-1,2,4-triazole-3-thiol (T6) : IR v_{max} cm^{-1}(KBr): 3015.00 (C-H str., aromatic), 1284.72 (C-N str., aromatic), 1664.98 (C=N str., aromatic), 1507.66 (C=C str., aromatic), 2963.96 (C-CH3 str.). ^{1}H NMR (DMSO) δ; 7.49-8.02 (8H, m, ArH), 3.35 (1H, s, SH), 1.45 (6H, s, $ArCH_3$).

5-(3-methylphenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (T7): IR v_{max} cm^{-1}(KBr): 3015.00 (C-H str., aromatic), 1284.72 (C-N str., aromatic), 1664.98 (C=N str., aromatic), 1507.66(C=C str., aromatic), 2963.96 (C-CH3 str.). ^{1}H NMR (DMSO) δ; 7.49-8.02 (8H, m, ArH), 3.35 (1H, s, SH), 1.40 (3H,s, $ArCH_3$).

4-(4-methylphenyl)-5-(3-methylphenyl)-4H-1,2,4-triazole-3-thiol (T8): IR v_{max} cm^{-1}(KBr): 3038.36 (C-H str., aromatic), 1284.12 (C-N str., aromatic), 1624.80 (C=N str., aromatic), 1589.40 (C=C str., aromatic), 2941.93 (C-CH3 str.). ^{1}H NMR (DMSO) δ; 6.71-8.74 (8H, m, ArH), 3.35 (1H, s, SH), 2.34 (6H,s ,$ArCH_3$).

5-(4-methoxyphenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (T9): IR v_{max} cm^{-1}(KBr): 3197.33 (C-H str., aromatic), 1324.18 (C-N str., aromatic), 1601.28 (C=N str., aromatic), 1530.51(C=C str., aromatic), 2887.32 (C-CH3 str.). ^{1}H NMR (DMSO) δ; 7.49-8.02 (9H,m,ArH), 3.35 (1H,s,SH), 2.33 (3H,s,$COCH_3$).

4-(4-methylphenyl)-5-(4-methoxyphenyl)-4H-1,2,4-triazole-3-thiol (T10): IR v_{max} cm^{-1}(KBr): 3000.67 (C-H str., aromatic), 1216.77 (C-N str., aromatic), 1678.80 (C=N str., aromatic), 1456.09 (C=C str., aromatic), 2938.10 (C-CH3 str.). ^{1}H NMR (DMSO) δ; 6.71-8.73 (8H, m, ArH), 3.35 (1H, s, SH), 1.43 (3H,s,$COCH_3$) , 2.35 (3H,$ArCH_3$).

5-(2,4-dimethoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol *(T11)* : IR v_{max} cm^{-1}(KBr): 3035.87 (C-H str., aromatic) , 1311.84 (C-N str., aromatic), 1455.08 (C=C str. aromatic ring), 821.88 (C-Cl alkyl halide). ^{1}H NMR (DMSO) δ; 6.65-7.85 (8H, m, ArH), 3.29 (1H, s, SH), 3.33 (6H, s, $ArOCH_3$) .

4-(4-methylphenyl)-5-(2,4-dimethoxyphenyl)-4*H*-1,2,4-triazole-3-thiol*(T12)*: IR v_{max} cm^{-1}(KBr): 3110.35 (C-H str., aromatic), 1204.00 (C-N str., aromatic), 1628.25 (C=N str., aromatic), 1444.08 (C=C str., aromatic), 2879.99 (C-CH3 str.). 6.65-7.83 (8H, m, ArH), 3.33 (1H, s, SH), 3.94 (6H, s, $COCH_3$), 2.35 (3H,$ArCH_3$).

5-(4-chlorophenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (T13): IR v_{max} cm^{-1}(KBr): 3044.22 (C-H str., aromatic), 1252.00 (C-N str., aromatic), 1624.74 (C=N str., aromatic), 1648.72 (C=C str., aromatic), 852.62 (C-Cl alkyl halide). ^{1}H NMR (DMSO) δ; 6.64-8.26 (9H, m, ArH), 3.35 (1H, s, SH).

4-(4-methylphenyl)-5-(4-chlorophenyl)-4H-1,2,4-triazole-3-thiol (T14): IR v_{max} cm^{-1}(KBr): 3044.22 (C-H str., aromatic), 1252.00 (C-N str., aromatic), 1624.74 (C=N str., aromatic), 1430.97 (C=C str., aromatic), 852.62 (C-Cl alkyl halide). ^{1}H NMR (DMSO) δ; 6.64-8.02 (8H, m, ArH), 3.35 (1H, s, SH), 2.37 (3H, s, $ArCH_3$).

5-(4-bromophenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (T15) : IR v_{max} cm^{-1}(KBr): 3044.22 (C-H str., aromatic), 1252.00 (C-N str., aromatic), 1624.74 (C=N str., aromatic), 1678.88 (C=C str., aromatic), 852.62 (C-Br alkyl halide). ^{1}H NMR (DMSO) δ; 6.66-7.85 (9H, m, ArH), 3.35 (1H, s, SH).

4-(4-methylphenyl)-5-(4-bromophenyl)-4H-1,2,4-triazole-3-thio (T16): IR v_{max} cm^{-1}(KBr): 3000 (C-H str., aromatic), 1097 (C-N str., aromatic), 1541 (C=C str., aromatic ring), 1369 (C-CH3 str.), 751 (C-Br alkyl halide). ^{1}H NMR (DMSO) δ; 6.06-7.87 (8H, m, ArH), 3.35 (1H, s, SH), 2.34 (3H, s, CH_3).

5-(2,4-dichlorophenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (T17) : IR v_{max} cm^{-1}(KBr): IR v_{max} cm^{-1}(KBr): 3163.26 (C-H str., aromatic), 1286.19 (C-N str., aromatic), 1728.66 (C=N str., aromatic), 1445.08 (C=C str., aromatic), 845.42(C-Cl alkyl halide). ^{1}H NMR (DMSO) δ; 6.71-8.74 (8H, m, ArH), 3.35 (1H, s, SH).

4-(4-methylphenyl)-5-(2,4-dichlorophenyl)-4H-1,2,4-triazole-3-thiol (T18) : IR v_{max} cm^{-1}(KBr): 3063.29 (Ar C-H), 1284.07 (C-N str), 1674.24 (C=N str., aromatic), 1601.87 (C=C str., aromatic), 825.16 (C-Cl alkyl halide), 2843.74 (C-CH_3 str.). ^{1}H NMR (DMSO) δ; 7.44-8.02 (7H, m, ArH), 3.35 (1H, s, SH). 2.33 (3H, s, $ArCH_3$).

2.3 *In-vitro* antimicrobial assays/studies

2.3.1 Minimal inhibitory concentrations

The antimicrobial activity was performed against Gram-positive bacteria: *Staphylococcus aureus (*MTCC 2901), *Bacillus subtilis* (MTCC 2063), Gram-negative bacterium *Escherichia coli* (MTCC 1652), and several fungal strains: *Candida albicans* (MTCC 227), and *Aspergillus niger* (MTCC 8189) using tube dilution method [24]. Dilutions of test and standard compounds were prepared in double strength nutrient broth - I.P. (bacteria) or Sabouraud dextrose broth - I.P. [25]. The samples were incubated at 37°C for 24 h (bacteria), at 25°C for 7 d (*A. Niger*) and at 37°C for 48 h (*C. albicans*) and the results were recorded in terms of minimum inhibitory concentration (MIC). MIC was defined as the lowest conc. of compound that inhibited visible growth of microbes after incubation at 35^0C for 24 h. for bacteria and 48 h for fungi.

Table 2: Minimum inhibitory concentration (MIC) in µg/ml of 3-Mercapto-4,5-disubstituted -1,2,4-triazole derivatives against bacterial and fungal strains.

Compound	Bacterial Stain			Fungal Strain	
	Gram Positive		Gram Negative		
	MIC_{ec}	MIC_{bs}	MIC_{sa}	MIC_{ca}	MIC_{an}
T1	4.68	6.25	6.25	4.68	6.25
T2	6.25	4.28	6.25	6.25	6.25
T3	9.37	6.25	6.25	6.25	s6.25
T4	6.25	12.5	6.25	6.25	9.37
T5	12.5	9.37	12.5	9.35	12.5
T6	9.37	12.5	6.25	12.5	12.5
T7	12.5	12.5	9.37	12.5	9.37
T8	25	9.37	18.75	12.5	18.75
T9	3.12	4.68	3.12	3.12	4.68
T10	4.68	4.68	6.25	6.25	6.25
T11	2	3.12	3.12	2	3.12
T12	3.12	4.68	3.12	3.12	4.68
T13	3.12	2	2	2	3.12
T14	4.68	3.12	4.68	3.12	4.68
T15	3.12	3.12	2	2	2
T16	6.25	6.25	3.12	4.68	3.12
T17	12.5	25	12.5	9.37	12.5
T18	9.37	6.25	12.5	12.5	25
Std.	01.56_A	01.56_A	01.56_A	01.56_B	01.56_B

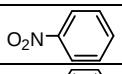
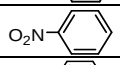
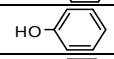
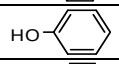
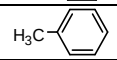
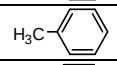
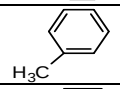
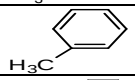
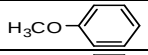
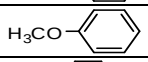
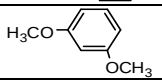
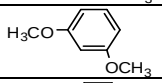
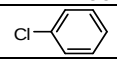
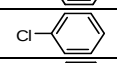
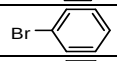
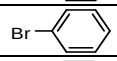
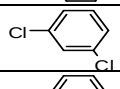
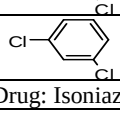
Bacterial Stain: Gram-positive bacteria: *Staphylococcus aureus* (MTCC 2901), *Bacillus subtilis* (MTCC 2063), Gram-negative bacterium *Escherichia coli* (MTCC 1652),) and several fungal strains: *Candida albicans* (MTCC 227),, and *Aspergillus niger* (MTCC 8189) Std. (Standard drugs) A-Norfloxacin, B-Fluconazole.

2.3.2 *Determination of Antitubercular activity*

The antitubercular activity of compounds synthesized by microwave method (T1-T18) were carried out against *M.tuberculosis* using MABA [26]. This method is non-toxic and uses reagents which are stable and shows good association with radiometric BACTEC [27]. 200 micro liters of sterile deionized water was added to all the wells of 96 sterile wells plate to minimize the medium evaporation in case of incubation.

100 micro liters of the Middle brook broth (7H9) is added in 96 wells and serial dilution of derivatives were made on the plate directly.100-0.2 µg /micro liter was concentration of final drug tested. Fully covered plates were sealed and covered with Para film incubation was done at 37^0 C for 5 days. After this, 25micro liter of mixture of 10% of tween 80 and almar blue in the ratio of 1:1 was added to the plate and incubation was done for 24 hours. No growth of bacteria was interprertended by blue colour in the well, and colour of pink was indicated or scored as bacterial growth. The minimum inhibitory concentration i.e. MIC was expressed here as the lowest concentration of drug which prevented the changes from blue colour to pink colour in antitubercular activity.

Table 3: Antitubercular activity of the synthesized triazole derivatives (T1-T18)

Compound	AR	R	Minimum Inhibitory concentration (MIC) (µg/ml) of MTB H37 Rv
T1		H	12.5
T2		CH3	25.0
T3		H	6.25
T4		CH3	12.5
T5		H	12.5
T6		CH3	25
T7		H	18.75
T8		CH3	12.5
T9		H	6.25
T10		CH3	12.5
T11		H	6.25
T12		CH3	18.75
T13		H	12.5
T14		CH3	18.75
T15		H	12.5
T16		CH3	25
T17		H	25
T18		CH3	25
Standard Drug: Isoniazid			2.5

3. Result and discussion

3.1 Chemistry

In the present work eighteen derivatives of 3-Mercapto-4,5-disubstituted-1,2,4-triazole were synthesized. Microwave-assisted synthesis gave products with good yield and time reduced from hour to minutes. A comparative data of the synthesized compounds are presented in Table 1. The structures of the synthesized compounds were established by spectral data. According to IR spectroscopic data of compound (T1-T18) showed peaks at ^{1}H NMR, ^{13}C NMR analysis. The IR spectra of all synthesized 1,2,4-triazole derivatives exhibit the absorption bands for Ar-H, C=C, Aromatic ring vibrations, C-N stretching in the regions of 3100-3045 cm-1, 1450- 1600 , 1284-1394 cm-1 respectively. IR stretching band at 1680-1650 cm-1 (C=N str.) confirmed the formation of a Schiff base. Further ^{1}H NMR spectra the signals of the respective protons of the synthesized compounds were confirmed based on their chemical shifts, multiplicities and coupling constants. These spectra showed a singlet at 3.00-3.05 ppm, which corresponds to the SH protons and multipletes at 7.3-8.25 ppm, showed aromatic protons. Specifically, compounds showed a singlet at 2.31-2.26 ppm which corresponds to CH_3 protons on aromatic ring. Similarly spectra shows a singlet at 1.40-1.95 ppm which corresponds to $COCH_3$ protons. Mass spectra of the synthesized compounds also in the favor of the formation of title compounds.

3.2 *In-vitro* antimicrobial assays/studies

All the synthesized derivatives by microwave method (T1-T18) were tested for their *invitro* antimicrobial activity against gram positive bacteria (*S. aureus , Bacillus subtilis)*, gram negative bacteria *E.coli* and fungal strains (*C.albicans, A. niger)* and the results have been presented in Table 2. *C*ompound T11 having *para* 2,4-dimethoxy substituent in the *N-phenyl* ring showed excellent antibacterial activity against *S. aureus* with MIC value 2 μg/ml, less active than the standard drugs Norfloxacin (1.56 μg/ml) but is equivalent to standard drug amoxicillin. This excellent inhibition is attributed to participation of the free electron pairs on the oxygen by resonance and increased electron density in the aromatic system. The introduction of a *para* halogen substituent in *N-phenyl* ring of T13 showed excellent antibacterial activity against *B. subtilis, E.coli* and fungal strains *C.albicans , A. niger* (MIC 2 μg/ml) but less than the standard drug Norfloxacin and Fluconazole (MIC 1.56 µg/ml) Further replacement of halogen groups with methyl, nitro and hydroxyl groups reduced antibacterial potentials and compounds T1, T2, T3, T4 and T5 showed moderate activity. Further introduction of two chloro group on the same phenyl ring (T17) reduced the antimicrobial properties [28].

3.3 Antitubercular activity

The MIC of synthesized compounds compared with Isoniazid, standard antitubercular drugs are summarized in Table 3. MIC assays of the triazole series T1-T18 revealed that T3,T9 and T11 shows good activity (MIC 6.5 µg/ml) but less active as compared to standard drug Isoniazid (MIC 2.5 µg/ml) but comparable or better than some compounds reported in the literature such as ethambutol derivatives SQ109 and pyrazinamide (MIC range in 5-64 µg/ml and 12.5-25 µg/ml respectively [29]. The Schiff bases were readily prepared for evaluation against *M. tuberculosis* good yield. In drug design, halogen atoms are introduced to improve penetration through lipid membranes and tissues. In this research work, the introduction of a *para* halogen substituent in the phenyl ring of T13, T14, T15 and T16 presented a negative effect as they reduced the antitubercular activity. SAR concluded that the size or volume of the halogen is restrictive factor since bromine, the largest halogen, is more deleterious to the antitubercular activity than chlorine. Based on these data, we may infer that substituents (Ar, R) is a steric and restricted position that should be carefully considered in the future design of antitubercular moieties [30].

4. Conclusion

The short reaction time, easy experimental procedure, quantitative yield and expanded reaction range offered by microwave synthesis is demand of today industry and should be encouraged in institutes. It will be very helpful for searching novel compounds against the diseases like tuberculosis which shows resistance and having long duration of treatment. A series of 3-Mercapto- 4, 5-disubstituted 1,2,4-triazole (T1-T18) was synthesized and evaluated for its in vitro antimicrobial and antitubercular activities. Antimicrobial study indicated that compounds T13 found to be the most effective antimicrobial agents but less active than the standard drug Norfloxacin and Fluconazole. Compounds T3, T 9 and T11 showed antitubercular activity (MIC 6.25 µg/ml) less than the standard drug Isoniazid (MIC 2.5. µg/ml). All these results suggest that it will be interesting to prepare analogues of active molecules for finding new compounds that possess better activity and bioavability. Bearing in mind that most biologically active compounds are heterocyclic and the importance in combinatorial chemistry to identify leads and to optimize structures, we believe that the number of applications of microwaves will only increase in the future.

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