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Synthesis and evaluation of some novel 1,2,4-triazole derivatives for antmicrobial, antitubercular and anti-inflammatory activities

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A series of 1,2,4-triazole derivatives have been synthesized and evaluated for antimicrobial, antitubercular and anti-inflammatory activities. The newly synthesized compounds have been characterized by IR, ^{1}H NMR and CHN analysis. All the compounds have shown promising antimicrobial, antitubercular and anti-inflammatory activities when compared with standard drug Norfloxacin, Griseofulvin, Streptomycin and Diclofenac sodium respectively.

Keywords: Antimicrobial, anti-inflammatory, antitubercular activity, CHN analysis, 1,2,4-triazole

Tuberculosis (abbreviated as TB for Tubercle Bacillus) is a common and deadly infectious disease that is caused by mycobacteria, primarily TB. Tuberculosis most commonly affects the lungs (as pulmonary TB) but can also affect the central nervous system, the lymphatic system, the circulatory system, the urinogenital, bones, joints and even the skin. Other mycobacteria such as *Mycobacterium bovis*, *Mycobacterium africanum*, *Mycobacterium canetti* and *Mycobacterium microti* can also cause tuberculosis, but these species do not usually infect healthy adults[1].

There are two fundamental types of inflammation[2]
(i) Acute inflammation and
(ii) Chronic inflammation.

Various derivatives of 1,2,4-triazoles possess a wide spectrum of activity ranging from antibacterial, antifungal, antiinflammatory, anticonvulsant, antineoplastic, antimalarial, antiviral, anticancer, anti-TB, and antiproliferative[3].

It is well known that the mercapto group attached to heterocyclic nucleus enhances the fungicidal activity[4].

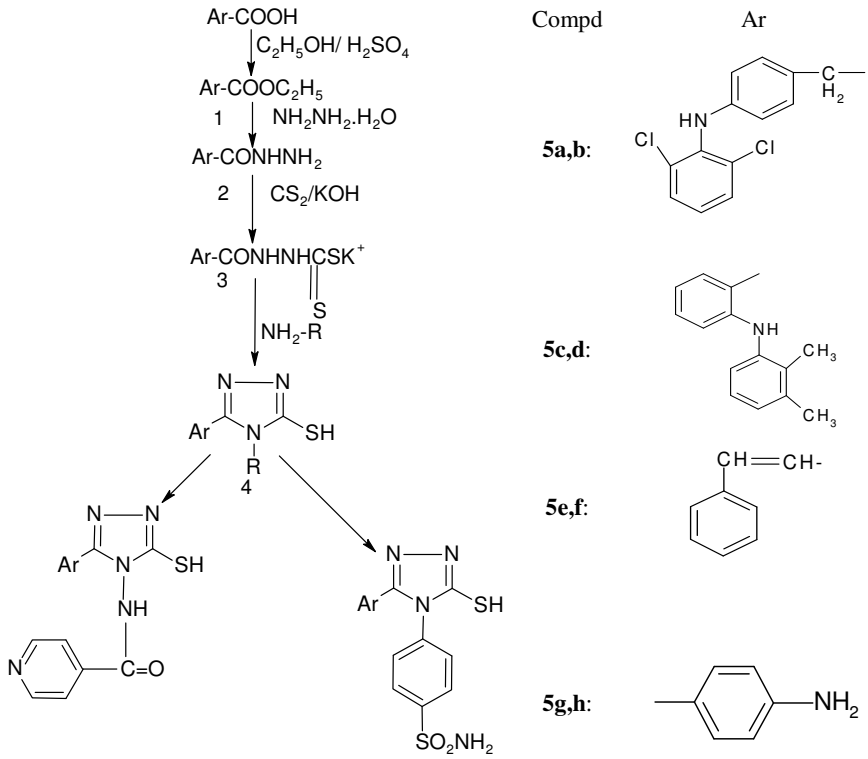
Results and Discussion

Eight new derivatives of triazole were synthesized. The synthesized compounds were subjected to antimicrobial, antitubercular and anti-inflammatory activity studies. Compound **5b, 5d, 5f, 5g** and **5h** have shown promising antibacterial and antifungal activity. Compounds **5e** and **5g** have shown promising antitubercular activity at 25 µg/mL, 50 µg/mL and 100 µg/mL concentration. Compounds **5b** and **5d** have shown promising anti-inflammatory activity.

The compounds were incorporated with SO_2NH_2 in **5b**, **5d**, **5f**, **5g** and **5h**. The compounds were incorporated with isoniazide, hence enhanced antitubercular activity was observed in compound **5e** and **5g**. Compounds **5b** and **5d** were incorporated with Diclofenac and mefanamic acid hence enhanced anti-inflammatory activity was observed in these compounds (**Scheme I**, **Table I**). With still further molecular modification and manipulation these compounds can be expected to be promising therapeutic agents in the future.

Antimicrobial activity[5,6]

Antimicrobial activity was carried out by cup-plate agar diffusion method using nutrient agar. The



5a, **5c**, **5e**, **5g 5b**, **5d**, **5f**, **5h**

Scheme I

Table I — Analytical data of the synthesized compounds

Compd	Mol. Formula	Mol.Wt	m.p. (°C)	Yield (%)	R_f Value	Calcd % (Found) C	H	N
5a	$C_{18}H_{20}N_4S$	471	108	78	0.56	69.57 (69.40	6.7 6.06	16.23 16.15)
5b	$C_{11}H_{13}N_3S$	522	110	92	0.65	56.84	5.04	11.05
5c	$C_{21}H_{18}N_6OS$	402	96	68	0.56	65.85 (65.70	6.01 65.08	16.69 16.50)
5d	$C_{21}H_{21}N_6OS$	453	102	69	0.52	64.84	5.44	18.90
5e	$C_{16}H_{13}N_5OS$	323	96	62	0.66	60.28 (59.60	4.89 4.67	23.55 23.45)
5f	$C_{16}H_{15}N_5S_2O_2$	373	103	92	0.54	58.51	5.40	20.47
5g	$C_{14}H_{21}N_6OS$	312	112	68	0.63	61.32 (61.12	6.11 6.06	22.342 2.30)
5h	$C_{14}H_{14}N_6O_2S_2$	362	122	80	0.66	52.41 (52.30	4.89 4.76	27.16 27.10)

The combustion analysis of compounds synthesized is within the limits of permissible errors. (±0.4)

compounds were tested *in-vitro* for their antibacterial activity against two microorganisms *viz. Escherichia coli* (NCTC 10418), and *Staphylococcus aureus (*NCTC 6571) which are pathogenic to human beings. The anti-fungal screening was carried out by cup-plate agar diffusion method using nutrient agar. Inoculation of the test organisms *Aspergillus niger* (NCIM 596) and *Candida albicans* (NCIM 3102) fungal cultures were made in the Sabouraud-Dextrose agar and then incubated at 37°C for 18-24 hr.

Table II — Antibacterial activities of the synthesized compounds

Compd	Zone of inhibition at 200 µg/mL (in mm)			
	E. coli	*S. aureus*	*A. niger*	*C. albicans*
5a	18	17	19	18
5b	22	21	23	22
5c	19	19	19	20
5d	21	22	23	23
5e	18	17	16	18
5f	23	23	22	23
5g	20	20	21	23
5h	24	25	24	23
Norfloxacin	25	25	--	--
Griseofulvin	--	--	24	24

Compounds **5b**, **5d**, **5f**, **5g**, **5h** have shown promising antibacterial and antifungal activities. Norfloxacin and Griseofulvin were used as standard drugs

Table III — Anti-tubercular activity of the synthesized compounds

Compd	Concentration (µg/mL)		
	25	50	100
5a	R	S	S
5b	R	R	R
5c	R	S	S
5d	R	R	R
5e	S	S	S
5f	R	R	R
5g	S	S	S
5h	R	R	R
Streptomycin	S	S	S

R- Resistant S- Sensitive

Compounds **5e** and **5g** have shown significant antitubercular activity at all concentrations. H_{37} Rv strain was used as standard tubercular organism. Streptomycin was used as standard drug.

Standard drugs Norfloxacin and Griseofulvin were used. The concentration was 200 µg/mL (**Table II**).

Anti-tubercular activity[7]

The antitubercular screening was carried out by Middlebrook 7H9 agar medium against H_{37}Rv strain. Middlebrook 7H9 agar medium contains different derivatives, standard drug as well as control. Middlebrook 7H9 agar medium was inoculated with *Mycobacterium tuberculosis* H37Rv strain. The inoculated bottles were incubated at 37°C for 4 weeks. At the end of 4 weeks they were checked for growth (**Table III**).

Anti-inflammatory activity[8]

Acute anti-inflammatory method: Carrageenan induced rat hind paw edema was produced using type IV Lambada Carrageenan from Sigma Laboratories. Foot volumes were measured in a plethysmograph by water displacement. The instrument was calibrated before performing the experiment using standard calibrated probe number and standard drug Diclofenac Sodium (**Table IV**).

Experimental Section

Melting points were determined by open capillary method and are uncorrected. Homogenecity of the compounds was checked on silica gel TLC plates. IR spectra were recorded on Thermo Nicole IR 200 spectrophotometer using KBr disc method. ^{1}H NMR spectra were recorded on Bruker AMX-400 instrument with $CDCl_3$ as solvent and TMS as internal standard. Combustion analyses were found to be within the limits of permissible errors (**Table I**).

Table IV — Anti-inflammatory activity of synthesized compound

Compd	Mean paw oedema volume ± SE					% inhibition
	0 hr	1 hr	2 hr	3 hr	4 hr	at 4th hr
Ct	0.975±0.025	1.475±0.025	1.650±0.028	1.775±0.025	1.842±0.012	
Std	0.975±0.025	1.225±0.025**	1.325±0.025**	1.350±0.028**	1.275±0.025**	44.47
5a	0.975±0.025	1.400±0.041	1.490±0.025*	1.420±0.025**	1.364±0.028**	35.04
5b	1.000±0.021	1.350±0.028	1.425±0.045**	1.500±0.040**	1.425±0.047**	40.26
5c	0.975±0.025	1.350±0.028	1.500±0.040*	1.600±0.0**	1.370±0.025*	34.45
5d	1.060±0.0	1.375±0.025	1.435±0.028**	1.440±0.025**	1.320±0.040**	39.54

One way ANOVA followed by Dunnett's 't' test **P<0.01
Compounds **5b** and **5d** have shown significant anti-inflammatory activity. Diclofenac sodium was used as a standard drug.

General method for synthesis of ester, 1

A mixture of 0.01 mole of acid, 10 mL of absolute ethanol and 2 mL of concentrated sulphuric acid was refluxed for 4 hr. Work-up was carried out as per reported procedures[9].

General method for synthesis of acid hydrazide, 2

A mixture of 0.01 mole of ester and 0.2 moles (10 mL) of hydrazine hydrate were refluxed in 50 mL of 95% ethanol for 2 hr. The resultant mixture was concentrated, cooled and poured into crushed ice. The solid mass thus separated out was filtered, dried and purified by recrystallization from ethanol[9].

General method for synthesis of potassium salt of dithiocarbazinate, 3

Potassium hydroxide (0.01 mole) was dissolved in absolute ethanol (75 mL). To the above solution, acid hydrazide **2** (0.01 mole) was added with stirring and cooling in ice. To this, carbon disulphide (10 mL) was added in small portions. The reaction mixture was refluxed for 4-6 hr. The potassium dithiocarbazinate separated was filtered, washed several times with ether and dried in vacuum. The dithiocarbazinates were obtained in quantitative yield. As most of the potassium salts of dithiocarbazinates were moisture sensitive, they were employed directly for the preparation of aminomercapto triazoles without further purification[10,11].

General method for synthesis of 5-mercepto-1,2,4-triazole derivatives, 4

Aqueous solution of 10% NaOH was added in 0.01 mole of primary amines (Isoniazid, sulphanilamide) and the reaction mixture was refluxed for 2 hr. The resulting solution was treated with charcoal, cooled and filtered. The filtrate was acidified with 10% HCl to adjust the *p*H between 5-6. The solid mass was precipitated, filtered, washed with ice cold water and purified by recrystallization from ethanol[12]. The melting point and percentage yield are reported in **Table I**.

Spectral data

5a: IR (KBr): 3110 (Ar-CH str), 2835 (CH_2 str), 1693 (C=O str), 2500 (SH str), 3540 (NH str), 1550 (C=N str), 367 (C-N str), 678 cm^{-1} (C-Cl str); ^{1}H NMR: δ 3.0 (s, 1H, CH_2), 9.2 (s, 1H, NH), 4.0 (s, 2H, NH_2), 2.8 (s, 1H, SH), 7.0-8.0 (d, 4H, pyridyl), 6.2-6.8 (m, 7H, phenyl).

5b: IR (KBr): 3010 (Ar-CH str), 2845 (CH_2 str), 2500 (SH str), 3520 (NH str), 1550 (C=N str), 1300 (C-N str), 678 cm^{-1} (C-Cl str); ^{1}H NMR: δ 2.0 (s, 2H, CH_2), 3.0 (s, 1H, SH), 6.2-7.8 (m, 11H, ArH), 3.4 (s, 1H, NH), 3.6-4.0 (s, 2H, NH_2).

5c: IR (KBr): 3120 (ArCH str), 2835 (CH_2 str), 1698 (C=O str), 2500 (SH str), 3545 (NH str), 1550 cm^{-1} (C=N str).

5d: IR (KBr): 3108 (Ar-CH str), 2840 (CH_2 str), 1695 (C=O str), 2500 (SH str), 3450 (NH str), 1550 cm^{-1} (NH str).

5e: IR (KBr): 3085 (CH=CH str), 1690 (C=O str), 2500 (SH str), 3539 (NH str), 1550 (C=N str), 1352 cm^{-1} (C-N str).

5f: IR (KBr): 3030 (CH=CH str), 1698 (C=O str), 2500 (SH str), 3530 (NH str), 1550 (C=N str), 1354 cm^{-1} (C-N str).

5g: IR (KBr): 3109 (ArCH str), 1689 (C=Ostr), 2500 (SH str), 3542 (NH str), 1550 (C=N str), 1359 cm^{-1} (C-N str); ^{1}H NMR: δ 3.0 (s, 1H, SH), 8.8 (d, 4H, pyridinyl), 7.2-7.4 (m, 4H, phenyl), 4.0 (s, 2H, NH_2), 9.2 (s, 1H, NH).

5h: IR (KBr): 3106 (Ar-CH str), 1694 (C=O str), 2500 (SH str), 3540 (NH str), 1550 (C=N str), 1345 cm^{-1} (C-N str).

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