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Synthesis, characterization and biological evaluation of thiazole incorporated triazole compounds

Abhinav Kumar Jha[a], Parjanya Kumar Shukla[a]*, Neetu Soni[b] and Amita Verma[b]

[a]*Krishnarpit Institute of Pharmacy, Allahabad, India*
[b]*Department of Pharmaceutical Sciences, Faculty of Health Science, Sam Higginbottom Institute of Agriculture, Technology & Sciences, Allahabad, India*

ABSTRACT

Literature survey reveals that thiazole moieties clubbed with triazoles have attracted considerable attention of medicinal chemists as they are endowed with a wide range of diverse biological activities such as anti-inflammatory, analgesic, antimicrobial, anticonvulsant, anti-oxidant activity etc. Hence, the objective of present project is to synthesize and characterize newer analogues 3-[4-(substituted phenyl)-1,3-thiazol-2-ylamino]-4-(substituted phenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thiones and evaluate their anti-microbial and anti-fungal activity. A series of new thiazole clubbed with triazoles derivatives were prepared by condensation of Ethyl [4-(substituted phenyl)-1,3-thiazol-2-yl]carbamates and N-(substituted phenyl)hydrazinecarbothioamides. The structures of new compounds are supported by their IR, ^{1}H NMR, MASS spectoscopy and Elemental analysis. All these compounds were screened for antimicrobial activity against Gram Positive, Gram Negative bacterial and fungal stains. All the compounds tested showed significant anti-fungal and antibacterial activity against the standard drugs used. Result showed better Antifungal activity in comparison to Antibacterial activity. Amongst the entire compound G1 (2-Nitro-phenyl substitution) showed most potent activity against fungus.

Key words: Triazole, Thiazole, Antibacterial activity, Antifungal activity

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INTRODUCTION

The mutated bacteria have acquired new genes producing novel machinery to overcome the action of many antibiotics. As a result, now with every possible bacterial infection, resistance to antibiotic treatment is a common phenomenon [1]. Hence the research still on antimicrobial agents is continuously going on to develop novel molecules. The search and synthesis of chemotherapeutic drugs with a combination of different mechanisms of action and low side effects constitute an important proceeding with clear objective to overcome the antimicrobial resistance. Due to the dependency of humans on the drugs which are derivatives of heterocyclic rings, heterocycles and medicines are both interrelated to each other [2]. Heterocycles and their derivatives have attracted the attention of chemists, mainly because of broad spectrum biological and pharmacological activities associated with this class of compounds specially having nitrogen, Sulphur and oxygen or three hetero atoms [3].

Thiazoles and their derivatives have attracted continuing interest over the years because of their varied biological activities[4] recently found application in drug development for the treatment of allergies[5], hypertension[6], inflammation[7], schizophrenia[8], bacterial[9], cardiotonic[10], fungicidal[11], HIV infection[12], mental retardation in children[13], age related and neurodegenerative brain damage (Alzheimer's disease, Parkinsonism disease) and as

new inhibitors of bacterial DNA gyrase[14]. It follows from the literature survey that, depending on the type of substituent, the derivatives of [1,2,4]triazole have a high potential for biological activity, possessing a wide range of antifungal and antibacterial[15-18], anticonvulsant and antipsychotic[19-20] and antitumour[21-22] properties. The other ones show also anti-inflammatory[23-24], antitubercular[25-26], urease inhibition [27] and antioxidant [28] activities.

Thus, in continuation to our lasting interest toward chemistry and pharmacological properties of thiazoles and Triazoles, in present study, we synthesized a series of compounds having both these two heterocycles together with different functionality and determined their antimicrobial activity against various bacterial and fungal stains.

MATERIALS AND METHODS

Melting point of synthesized compounds were determined using open capillary tubes Hicon melting point apparatus and were found uncorrected. Synthesis of compound checked by TLC using silica gel G as stationary phase and various combinations of Benzene: Toluene, as mobile phase. The completion of reaction of ascertained as indicated by visualizing the spot on TLC with the help of iodine vapors and Calculated R_f value of each compounds.The IR spectra of synthesized compounds were recorded using KBr pellets in range of 4000-400 cm^{-1} on a Fourier Transform IR Spectrometer and the frequency were recorded in wave number. ^1H NMR Spectra of synthesized compounds were recorded on Bruker Avance II 400 MHz NMR spectrometer and DMSO using tetramethylsilane (TMS) as internal standard. Mass Spectra of synthesized compounds were recorded in range of 10-1000 Da on a DARTMS and provided in [M+H] +ion.

Synthesis of 2-Bromo-1-(substituted phenyl)ethanones (A): Solution of bromine in chloroform (0.11 mol in 50 ml) was added dropwise into the solution of substituted acetophenones (0.1 mol) in chloroform (50 ml), for a period of 15 min. The reaction mixture was stirred for an additional 2 h at room temperature. When the reaction was complete, the reaction mixture was concentrated and cooled to get the crystals of brominated acetophenones.

Synthesis of 4-(Substituted phenyl)-1,3-thiazol-2-amines (B): A solution of compound(A) (0.1 mol) in 150 mL of acetic acid, was refluxed with thiourea (0.1 mol) for 2 h and cooled to get thiazoles as crystals, which were filtered, washed with water and recrystallized with ethanol.

Synthesis of Ethyl [4-(substituted phenyl)-1,3-thiazol-2-yl]carbamates (C): Ethylchloroformate (0.11 mol) and triethylamine(25 ml) were added to the Solution of substituted thiazoles(B) (0.1 mol) in benzene, and the reaction mixture was refluxed for 3 h. After cooling the reaction mixture was poured into cold dil. HCl (50%) and the carbamate thus formed was recrystallized from benzene.

Synthesis of 1-(Substituted phenyl)thioureas (D): Substituted anilines(0.1 mol) were taken in water and warmed with dilute hydrochloric acid (5 ml) until a clear solution was obtained. To this solution, ammonium thiocyanate (0.11 mol) dissolved in water(25 ml), was added gradually. The reaction mixture was boiled and evaporated to less than half of the volume. It was then cooled to get the precipitate of phenyl thiourea which were filtered, washed with water and recrystallized from ethanol to get the target compounds.

Synthesis of N-(substituted phenyl)hydrazinecarbothioamides (E): In the solution of substituted phenylthioureas(D) (0.1 mol) in ethanol, hydrazine hydrate (0.11 mol) was added and the reaction mixture was refluxed for 16 h. It was then concentrated, cooled and poured over crushed ice to get the precipitate which was filtered, washed with water and recrystallized from ethanol to get hydrazinecarbothioamides(E).

Synthesis of 2-[(Substituted phenyl)carbamothioyl]-N-[4-(substituted phenyl)-1,3-thiazol-2-yl]hydrazine carboxamides (F): The solution of carbamates (C) (0.01 mol) and hydrazinecarbothioamides (E) (0.01 mol) in ethanol (25 ml)was refluxed for 4 h. The residue was concentrated, cooled and poured over crushed ice to precipitate, which was filtered, washed with water, dried and recrystallized from ethanol to get the targeted compounds (F).

Synthesis of 4-(Substituted phenyl)-5-[{4-(substituted phenyl)-1,3-thiazol-2-yl}amino]-2,4-dihydro-3H-1,2,4-triazole-3-thiones (G1-G8): A mixture of substituted hydrazinecarboxamides(F) (0.01 mol) and 30 ml of 2% aq. NaOH solution was refluxed for 6 h. After completion of reaction, the reaction mixture was filtered and the filtrate was neutralized with conc. HCl till pH was adjusted to 7. The mixture was kept aside for few minutes. A distinctive

precipitate thus obtained was filtered, washed with water, and recrystallized from ethanol to get the titled compounds (G).

4-(2-Nitro-phenyl)-5-(4-phenyl-thiazol-2-ylamino)-2,4-dihydro-[1,2,4]triazole-3-thione (G1). Percentage yield 52 %, Melting Point 60^0 C. FTIR Spectra (KBr) ln cm^{-1} 3588.37 N-H stretch(triazole), 2925.15 C-H stretch(benzene), 1692.11 C=N stretch(thaizole), 1597.94 N-H Bending(NH), 1511.83 NO_2 Asymetrical stretch(2-nitrophenyl), 1430.43 C-H Bend in plane(2-nitrophenyl), 1345.75 NO_2 Symetrical stretch(2-nitrophenyl), 1171.23C-C stretch(benzene), 872.61 N-H Rocking(triazole), 695.72 C-S Strech(triazole), 777.14 C-H Rocking(benzene), ^{1}H-NMR (400Hz, DMSO, δ in ppm): 2.29 (s, 3H, CH3), 5.43 (s, 1H, ArH thiazole), 6.74–7.79 (m, 9H, ArH), Mass Spectra 398.44 $(M+H)^+$, 302.35 (23), 230.24 (42).

4-(3-Nitro-phenyl)-5-(4-phenyl-thiazol-2-ylamino)-2,4-dihydro-[1,2,4]triazole-3-thione (G2). Percentage yield 71 %. Melting Point 105^0 C. FTIR Spectra (KBr) ln cm^{-1} 3333.31 N-H Stretch(triazole), 2926.61 C-H Stretch(benzene), 1693.93 C=N Stretch(thiazole), 1624.90 N-H Bending(NH), 1521.48 NO_2 Asymmetrical stretch(2-nitrophenyl), 1483.64 C-H Bend in plane(benzene), 1348.46 NO_2 Symmetrical stretch(2-nitrophenyl), 1090.31 C-H Stretch(benzene), 868.62, N-H Rocking(triazole), 714.91 C-H Rocking(benzene), 669.30 C-S Strech(triazole). ^{1}H-NMR (400Hz, DMSO, δ in ppm): 5.576(s, 1H, Ar-OH), 3.433 (s, 2H, CH_2), d 3.53 (s, 3H, OCH3), 5.51 (s, 1H, ArH thiazole), 6.86–7.83 (m, 9H, ArH),6.888(s, 5H, Ar-H, Benzene), 7.934-7.794(m, 4H, Ar-H, Benzene). Mass Spectra 398.67 (100) $(M+H)^+$, 305.45 (55), 240.67 (28).

4-(4-Nitro-phenyl)-5-(4-phenyl-thiazol-2-ylamino)-2,4-dihydro-[1,2,4]triazole-3-thione (G3). Percentage yield 67 %, melting Point 120^0 C. FTIR Spectra (KBr) ln cm^{-1} : 3534.54 N-H stretch(triazole), 2825.15 C-H stretch(benzene), 1686.34 C=N stretch(thaizole), 1577.64 N-H Bending(NH), 1521.53 NO_2 Asymetrical stretch(2-nitrophenyl), 1434.43 C-H Bend in plane(2-nitrophenyl), 1325.35 NO_2 Symetrical stretch(2-nitrophenyl), 1157.13 C-C stretch(benzene), 852.61 N-H Rocking(triazole), 694.12 C-S Strech(triazole), 773.17 C-H Rocking(benzene), ^{1}H-NMR (400Hz, DMSO, δ in ppm): 3.436 (s, 2H, CH_2), d 2.27 (s, 3H, CH3), 5.41 (s, 1H, ArH thiazole), 6.69–7.62 (m, 9H, ArH),7.582-7.414(m, 4H, Ar-H, Benzene), 7.930(s, 5H, Ar-H, Benzene). Mass Spectra 416.89 (100) $(M+H)^+$, 380.34 (72), 295.67 (25).

4-(4-Fluoro-phenyl)-5-(4-phenyl-thiazol-2-ylamino)-2,4-dihydro-[1,2,4]triazole-3-thione (G4). Percentage yield 48 %, melting Point 125^0 C. FTIR Spectra (KBr) ln cm^{-1} 3432.11 N-H Stretch(triazole), 2856.21 C-H Stretch(benzene), 1690.33 C=N Stretch(thiazole), 1664.50 N-H Bending(NH), 1527.48 NO_2 Asymmetrical stretch(2-nitrophenyl), 1488.34 C-H Bend in plane(benzene), 1388.16 NO_2 Symmetrical stretch(2-nitrophenyl), 10967.31 C-H Stretch(benzene), 863.22, N-H Rocking(triazole), 717.09 C-H Rocking(benzene), 6697.33 C-S Strech(triazole). ^{1}H-NMR (400Hz, DMSO, δ in ppm) 3.453 (s, 2H, CH_2), d 5.47 (s, 1H, ArH-thiazole), 6.81–7.78 (m, 10H, ArH), 7.700 (s, 5H, Ar-H, Benzene), 8.086-8.051(d, 2H, J=14, Ar-H, Benzene) 7.830-7.792(d, 2H, J=15.2, Ar-H, Benzene). Mass Spectra 427.50 (100) $(M+H)^+$, 278.90 (8).

5-[4-(4-Bromo-phenyl)-thiazol-2-ylamino]-4-(2-nitro-phenyl)-2,4-dihydro-[12,,4]triazole-3-thione (G5).
Percentage yield 59 %, melting Point 65^0 C. FTIR Spectra (KBr) ln cm^{-1} 3478.29 N-H Stretch(triazole), 3018.16 C-H Stretch(benzene), 1629.87 C=N Stretch(thiazole), 1597.64 N-H Bending(NH), 1512.25 NO_2 Asymetrical Stretch(2-nitrophenyl), 1498.42 C-H Bend in plane(benzene), 1345.32 NO_2 Symmetrical Stretch(2-nitrophenyl), 1171.87 C-C Stretch (benzene), 872.37,846.78 N-H Rocking(triazole), 778.06 C-H Rocking (benzene), 696.26 C-S Stretch(triazole), 557.49 C-Br Stretch(Aromatic). ^{1}H-NMR (400Hz, DMSO, δ in ppm) 2.509(s, 6H, CH_3, N-$(CH_3)_2$), 3.397 (s, 2H, CH_2), d 2.27 (s, 3H, CH3), 5.40 (s, 1H, ArH-thiazole), 6.98–7.95 (m, 8H, ArH) 6.763(s, 5H, Ar-H, Benzene), 7.551-7.467(d, 2H, J=13.6, Ar-H, Benzene), 7.711-7.690(d, 2H, J=8.4, Ar-H, Benzene). Mass Spectra 425.55 (100) $(M+H)^+$, 310.67 (12), 250.78 (10).

5-[4-(4-Bromo-phenyl)-thiazol-2-ylamino]-4-(3-nitro-phenyl)-2,4-dihydro-[12,,4]triazole-3-thione (G6).
Percentage yield 63 %, melting Point 105^0 C. FTIR Spectra (KBr) ln cm^{-1} 3433.82 N-H Stretch(triazole), 3076.03 C-H Stretch(benzene), 1625.16 C=N Stretch(thiazole), 1581.55 N-H Stretch(NH), 1522.47 NO_2 Asymetrical Stretch(2-nitrophenyl), 1485.09 C-H Bend in plane(benzene), 1348.71 NO_2 Symetrical Stretch(2-nitrophenyl), 1090.44 C-C Stretch(benzene), 869.54 N-H Rocking(triazole), 817.22 C-H Out of plane(benzene), 736.43 C-H Rocking(benzene), 669.60 C-S Stretch(triazole), 555.31 C-Br Stretch(Aromatic). ^{1}H-NMR (400Hz, DMSO, δ in ppm): 3.429 (s, 2H, CH_2), d 3.51 (s, 3H, OCH3), 5.65 (s, 1H, ArH-thiazole), 7.15–8.03 (m, 8H, ArH),, 6.065(s, 5H,

Ar-H, Benzene), 8.099-8.082(d, 2H, Ar-H, Benzene), 7.791-7.743(d, 2H, Ar-H, Benzene). Mass Spectra 461.34 (100) $(M+H)^+$, 350.45 (45), 290.78 (65).

5-[4-(4-Bromo-phenyl)-thiazol-2-ylamino]-4-(4-nitro-phenyl)-2,4-dihydro-[12,,4]triazole-3-thione (G7).
Percentage yield 72 %, melting Point 135^0 C. FTIR Spectra (KBr) ln cm^{-1} 34768.49 N-H Stretch(triazole), 3118.16 C-H Stretch(benzene), 1649.77 C=N Stretch(thiazole), 1593.61 N-H Bending(NH), 1518.27 NO_2 Asymetrical Stretch(2-nitrophenyl), 14986.42 C-H Bend in plane(benzene), 1345.32 NO_2 Symmetrical Stretch(2-nitrophenyl), 1171.87 C-C Stretch (benzene), 846.78 N-H Rocking(triazole), 778.06 C-H Rocking (benzene), 694.26 C-S Stretch(triazole), 554.49 C-Br Stretch(Aromatic). ^{1}H-NMR (400Hz, DMSO, δ in ppm): 5.569(s, 1H, Ar-OH), 3.413 (s, 2H, CH_2), d 3.57 (s, 3H, OCH3), 5.66 (s, 1H, ArH-thiazole), 7.09–7.89 (m, 8H, ArH),8.071-8.052(d, 2H, J=7.6, Ar-H, Benzene), 7.854-7.834(d, 2H, J=8, Ar-H, Benzene), 7.611-7.591(d, 2H, J=8, Ar-H, Benzene), 7.562-7.547(d, 2H, J=6, Ar-H, Benzene). Mass Spectra 477.34 (100) $(M+H)^+$, 295.54 (35).

5-[4-(4-Bromo-phenyl)-thiazol-2-ylamino]-4-(4-fluoro-phenyl)-2,4-dihydro-[12,,4]triazole-3-thione (G8).
Percentage yield 52 %, melting Point 155^0 C. FTIR Spectra (KBr) ln cm^{-1} 3435.67 N-H Stretch(triazole), 2926.70 C-H Stretch(benzene), 1626.18 C=N Stretch(thiazole), 1581.53 N-H Stretch(NH), 1522.47 NO_2 Asymetrical Stretch(2-nitrophenyl), 1485.09 C-H Bend in plane(benzene), 1348.71 NO_2 Symetrical Stretch(2-nitrophenyl), 1090.44 C-C Stretch(benzene), 869.54 N-H Rocking(triazole), 817.22 C-H Out of plane(benzene), 736.43 C-H Rocking(benzene), 649.62 C-S Stretch(triazole), 545.31 C-Br Stretch(Aromatic). ^{1}H-NMR (400Hz, DMSO, δ in ppm): 3.413 (s, 2H, CH_2), 5.42 (s, 1H, ArH-thiazole), 6.95–7.83 (m, 9H, ArH),), 8.108-8.087(d, 2H, J=8.4, Ar-H, Benzene), 7.799-7.779(d, 2H, Ar-H, Benzene), 7.942-7.922(d, J=8, Ar-H, Benzene), 7.726-7.679(d, 2H, J=18.8, Ar-H, Benzene). Mass Spectra 495.80 (100) $(M+H)^+$, 385.80 (55), 275.67 (20).

Biological activity
The bacterial strains (Gram positive and Gram negative strains of bacteria) & fungal strains were selected for *in vitro* antimicrobial screening. These strains were procured from Deptt. of Microbiology, Sam Higginbottom Institute of Agriculture, Technology and Sciences (Deemed To-Be University), Allahabad. All the synthesized compounds were tested for their in-vitro growth inhibitory activity against a panel of standard strains of pathogenic microorganism including Gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cereus)*, Gram negative bacteria (*Escherichia coli*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*) and fungal strains (*Aspergillus*, *A. fumigates* and *C. albicans*).

Antibacterial activity
The petridishes were thoroughly washed and sterilized in hot air oven and 160°C for 1hr. 2/3 part of sterile Mueller Hinton Agar media was poured into sterile petridishes for solidifying. Cultured organism (inoculums) poured in each petridishes. Bores were made on the medium using sterile borer. 0.1mL of test solution (solution of synthesized drug) was added to the respective borers and Cefixime drug used as a standard drug. A control having only DMSO in the cup was maintained in each plate. The petridishes were kept in the refrigerator at 4°C for 15 minutes for diffusion to take place. After diffusion, the petridishes were incubated at 37°C for 24 h and zones of inhibition were observed and measured using a scale.

Antifungal activity
The antifungal activity of the synthesized compounds was performed by using poison food technique. All the glass apparatus were thoroughly washed and sterilized in hot air oven at 160°C for 1hr. The molds were grown on Potato dextrose agar (PDA) at 25^oC for 7 days and used as inocula. The 20 ml of molten PDA (45^oC) was poisoned by the addition of 100 µl volume of each compound having concentration of 25 µg/ml. Petri plate was allowed to solidify at room temperature. The solidified poisoned agar plates were inoculated at the centre with fungal plugs, obtained from the actively growing colony and incubated at 25^oC for 7 days. After 7 days the zone of inhibition of compounds against different microorganisms were observed by (mm) scale (Kamboj et al., 2011).

RESULTS AND DISCUSSION

Chemistry
4-(Substituted phenyl)-5-[{4-(substituted phenyl)-1,3-thiazol-2-yl}amino]-2,4-dihydro-3H-1,2,4-triazole-3-thiones (G1-G8) was synthesized in three steps as shown is scheme 1. Appropriate substituted acetophenones were brominated and refluxed with thiourea in acetic acid to get the substituted thiazoles (B). These thiazole derivatives

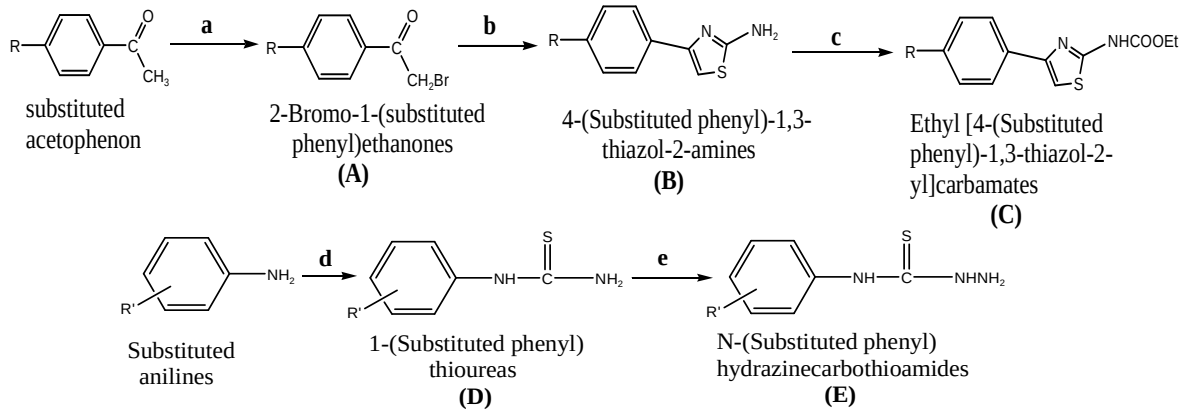
on refluxing with ethylchloroformate and triethylamine afforded the carbamate derivatives (C). On the other hand, the substituted phenylthioureas (D) were obtained by treating the substituted anilines with ammonium thiocyanates in presence of dilute hydrochloric acid. These phenylthioureas were then refluxed with hydrazine hydrate to afford hydrazinecarbothioamides (E). The hydrazinecarbothioamides on condensation with different substituted carbamates (C) in presence of ethanol gave substituted hydrazinecarboxamides (F). Finally the substituted hydrazinecarboxamides were cyclized in presence of aqueous sodium hydroxide to yield the titled compounds (G1-G8). The structures and purity of the final compounds were confirmed in the basis of spectral analysis.

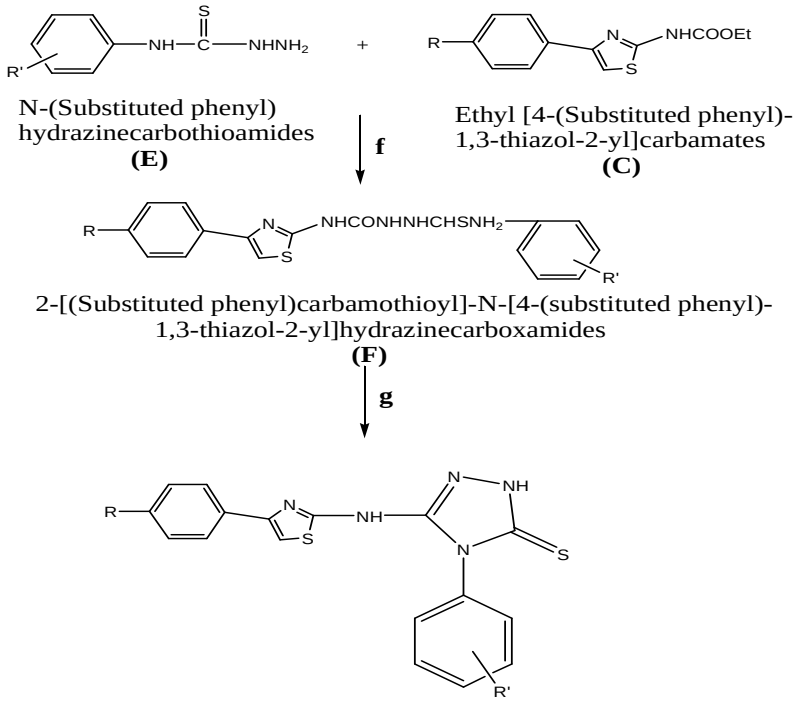
All the reactions were monitored by TLC. The structure elucidations of the newly synthesized compounds were carried out by modern spectroscopic techniques like FTIR, ^{1}H NMR. Further confirmations of the compounds were carried out by mass spectrometry & elemental analysis (Table 1).

Table 1. Physicochemical properties and elemental analysis of synthesized compounds

COMPOUND	MOL. FORM.	MELTING POINT (°C)	MOL. WT.	% YIELD	% of Nitrogen		% of Sulphur	
					Calculated	Found	Calculated	Found
G1	$C_{17}H_{12}N_6O_2S_2$	60	396.45	52	21.20	20.31	16.18	14.31
G2	$C_{17}H_{12}N_6O_2S_2$	105	396.45	71	21.20	23.32	16.18	15.18
G3	$C_{17}H_{12}N_6O_2S_2$	120	396.45	67	21.20	22.34	16.18	11.25
G4	$C_{17}H_{12}FN_5OS_2$	125	369.44	48	18.96	17.83	17.36	16.82
G5	$C_{17}H_{11}BrN_6O_2S_2$	65	475.34	59	17.68	18.62	13.49	12.27
G6	$C_{17}H_{11}BrN_6O_2S_2$	105	475.34	63	17.68	15.01	13.49	14.22
G7	$C_{17}H_{11}BrN_6O_2S_2$	135	475.34	72	17.68	16.92	13.49	10.81
G8	$C_{17}H_{11}BrFN_5S_2$	155	448.34	52	15.62	13.79	14.30	12.97

The IR spectrum of compound G1 showed bands at 3588.37 N-H stretch(triazole), 2925.15 C-H stretch(benzene), 1692.11 C=N stretch(thaizole), 1597.94 N-H Bending(NH), 1511.83 NO_2 Asymetrical stretch(2-nitrophenyl), 1430.43 C-H Bend in plane(2-nitrophenyl), 1345.75 NO_2 Symetrical stretch(2-nitrophenyl), 1171.23C-C stretch(benzene), 872.61 N-H Rocking(triazole), 695.72 C-S Strech(triazole) along with other bands which supports the formation of final products. 1H NMR signals at δ 2.29 (s, 3H, CH3), 5.43 (s, 1H, ArH thiazole), 6.74–7.79 (m, 9H, ArH) further confirmed the formation of product.





R = H, *p*-Br

R' = *o*-NO_2 , *m*-NO_2 , *p*-NO_2 , *p*-F

Reactions and conditions: (a) Br2, Chloroform, (b) Thiourea, glacial acetic acid, reflux (c) Ethylchloroformate, triethylamine, benzene (d) NH_4SCN, HCl (e) $NH_2NH_2H_2O$, ethanol (f) ethanol (g) 2N NaOH

Scheme 1: Synthesis of title Compounds G1-G8

Table 2. Antimicrobial activity of synthesized compounds

S.No.	Code	Zone of Inhibition (mm)								
		Gram positive bacteria			Gram negative bacteria			Antifungal activity		
		S.a.	*B.s.*	*B.c.*	*E.c.*	*P.v.*	*P.a.*	*C. albicans*	*A. fumigatus*	*Aspergillus*
1.	G1	14	14	14	16	15	Nil	19	25	15
2.	G2	14	17	11	18	15	13	18	17	24
3.	G3	16	18	12	18	16	14	16	21	14
4.	G4	16	18	17	17	18	16	24	16	22
5.	G5	15	16	14	Nil	19	17	17	14	17
6.	G6	15	11	11	12	17	14	21	23	17
7.	G7	16	15	15	11	19	16	17	12	16
8.	G8	13	19	12	13	18	16	19	21	16
Control	DMSO	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Standard	Cefixime	21	23	19	22	23	22	-	-	-
Standard	Fluconazole	-	-	-	-	-	-	21	23	20

Antimicrobial Activity

Antibacterial activity

The antibacterial potency (zone of inhibition) of all synthesized compounds were assessed *in-vitro* by Agar diffusion method against standard cultures of Gram positive bacteria viz: *Staphylococcus aureus (NCIM-2079), Bacillus subtilis (NCIM-2063)* and *Bacillus cerus (NCIM-2156)* and Gram negative bacteria viz: *Escherichia coli (NCIM-2065), Proteus vulgaris (NCIM-2027)* and *Pseudomonas aeruginosa (NCIM-2036)* species along with Cefixime as standard drug. The zone of inhibition values of this class of synthesized compounds (Table 2) against tested

organism displayed a potent antibacterial activity. The zones of inhibition of synthesized compound were found to be in the range between 12 to 19 mm.

Compound having 4-Nitro-phenyl (G3) and 4-bromo-phenyl substitution (G8) showed more activity against *B. subtilis* as compared to other organism where as 4-Fluoro-phenyl (G4) showed more activity against *B. subtilis* and *B. cereus*. Compound G3 (4-Nitro-phenyl substitution), G4 (4-Fluoro-phenyl substitution) and G7 (4-bromo-phenyl substitution) showed the maximum antibacterial activity against *S.aureus* bacteria. Compound G5 and G7 showed significant activity against *Proteus vulgaris* as compared to cefixime.

Antifungal Activity

The antifungal potency (zone of inhibition) of all synthesized hybrids of pyrazole-benzotriazole was assessed *in-vitro* by food poisoning technique against three standard cultures of Fungus viz: *Aspergillus fumigatus (NCIM-2081), Candida albicans (NCIM-2087)* and *Aspergillus niger (NCIM-2191)* species along with fluconazole as standard drug. The zone of inhibition values of this class of synthesized compounds (Table 2) against tested organism displayed a potent antifungal activity. The zones of inhibition of synthesized compound were found to be in the range between 12 to 24 mm.

Compounds G4 and G6 showed potent antifungal activity but amongst them G4 (4-Fluoro-phenyl substitution) showed most potent activity against *C.albicans*. Compounds G1, G3, G5 and G6 showed excellent antifungal activity but amongst them G1 (2-Nitro-phenyl substitution) showed most potent activity against *A.fumigatus*. Compounds G2 and G4 showed excellent antifungal activity but amongst them G2 (3-Nitro-phenyl substitution) showed most potent activity against *A.niger*.

CONCLUSION

Ten different derivatives of 1,2,4 triazole was synthesized as per the scheme and their structure was confirmed by FT IR, NMR , Mass analysis. All the synthesized compounds shows good antibacterial and antifungal activity at 100 µg/ml. All synthesized compounds showed better Antifungal activity in comparison to Antibacterial activity. Amongst the entire compound G1 (2-Nitro-phenyl substitution) showed most potent activity against fungus.

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