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Synthesis of S-Phenacylated Trisubstituted 1,2,4-Triazole Incorporated with 5-(Benzofuran-2-yl)-1-Phenyl-*1H*-Pyrazol-3-yl Moiety and their Antibacterial Screening

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ABSTRACT

Starting with a concise and practical synthesis of acyl thiosemicarbazides (3a-h), a series of new 1,2,4-triazole derivatives (4a-h), have been synthesized by intramolecular cyclization in alkaline medium. The acyl thiosemicarbazides required for this purpose were obtained by reaction of carbohydrazides (1a-b) with appropriate aromatic isothiocyanates (2a-h). Subsequently, 4a-h which upon treatment with phenacyl bromide underwent S-phenacylation in presence of triethylamine afforded 5a-h. The structural identities of 3a, 4a and 5a compound were established on the basis of elemental analysis and spectral studies such as ^{1}H NMR, IR, ^{13}C NMR and mass while rest of the compounds were characterized by elemental analysis and IR spectroscopy. The synthesized compound acyl thiosemicarbazide (3a) and 1,2,4-triazole (5a) were evaluated for their in vitro antibacterial activity against pathogenic bacteria and the results were comparable with Chloramphenicol antibiotic.

Keywords: 1,2,4-triazole, Acylthiosemicarbazide, Arylisothiocyate, Carbohydrazides

INTRODUCTION

The rapid development of bacterial resistance to conventional drugs is one of the major difficulties in the treatment of bacterial infection, thus it is still necessary to search for new antibacterial agent. Triazole derivatives have occupied a unique position in heterocyclic chemistry due to their antimicrobial activities. 1,2,4-triazoles as antibacterial agents can be grouped according to the mode of action, i.e., the ability to inhibit the synthesis of the cell wall, cell membrane, proteins and nucleic acids of bacteria. 1,2,4-triazoles exhibit a wide range of therapeutical properties like antibacterial [1-5], antifungal [6], anti-inflammatory [7], antituberculosis [8], anticancer [9], antioxidant [10], InhA inhibitory activity [11], antidepressant [12], etc. Some of the modern day drugs with triazole nucleus are as fluconazole, itraconazole, terconazole, posaconazole, voriconazole. Moreover, now a day researchers prefer to synthesize hybrids of heterocycles to enhance the therapeutically activities. 1,2,4-triazoles with other heterocyclic derivatives possess a wide spectrum of biological activities. The huge number of 1,2,4-triazoles containing hybrid systems exhibits anticonvulsant and CNS depressant [13], anticancer [14], antioxidant [15] activity, etc.

1,2,4-triazole have been prepared by different methods. One of the most common routes to these compounds involves cyclodehydration of acylthiosemicarbazides with a variety of basic reagents, such as sodium hydroxide [16-18], potassium hydroxide [19], sodium carbonate [20], triethylamine [21], etc. In addition, it is known that acylthiosemicarbazides, the versatile key intermediates itself has various pharmacological activities like analgesic [22], antibacterial [23], antifungal [24,25], antitubercular [26], etc.

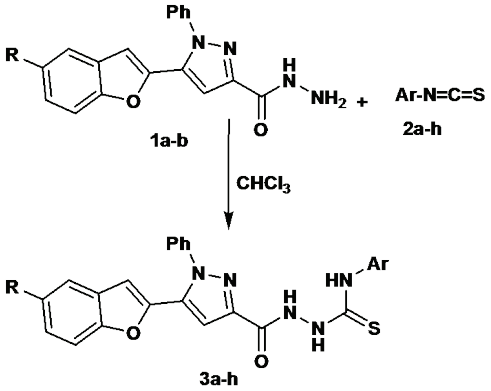
In view of these above findings the present paper reports on the synthesis of a some new 1,2,4-triazoles derivatives bearing 5-(benzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl obtained through cyclodehydration of acyl thiosemicarbazides in basic medium followed by S-phenacylation with phenacyl bromide in weak base and investigates the potential antibacterial activity.

MATERIALS AND METHODS

The melting points were recorded in open capillary in paraffin bath and are uncorrected. IR spectra were recorded on a Shimadzu IR Spectrophotometer (KBr, v max in cm^{-1}). ^1H NMR spectra are recorded on a Bruker AM 400 instrument (400 MHz) using tetramethylsilane (TMS) as an internal reference and DMSO-d_6 as solvent and ^{13}C NMR spectra are recorded on a Bruker AM 400 instrument (100 MHz) and DMSO-d_6 as solvent. Chemical shifts are given in parts per million (ppm). Positive-ion Electro Spray Ionization (ESI) mass spectra were obtained with a Waters Micromass Q-TOF Micro, Mass Spectrophotometer. Elemental (CHN) analysis was done using Thermo Scientific (Flash-2000). The compounds were analyzed for carbon, hydrogen, nitrogen and sulphur and the results obtained are in good agreement with the calculated values. Chemicals used for the synthesis were of AR grade of Merck, S.D. Fine and Aldrich. The reactions were monitored by E. Merck TLC aluminum sheet silica $gel_{60}F_{254}$ and visualizing the spot in UV Cabinet and iodine chamber.

Experimental

General procedure for the synthesis of 1-(5-(5-H/Br benzofuran-2-yl)-1-phenyl-*1H*-pyrazole-3-carbonyl)-4-substituted/unsubstituted phenyl thiosemicarbazides (3a-h): A mixture of 1a-b (10 mmol) and appropriate aromatic isothiocyanates 2a-h (11 mmol) in chloroform (30 mL) was refluxed for 1.5 h. The reaction mixture was cooled, excess of solvent was removed under reduced pressure, solid obtained was washed with water, filtered and further purified by recrystallization using 1,4-dioxane to give 3a-h (**Scheme 1**).



Entry	R	Ar
a	H	4-CH_3-C_6H_4
b	Br	4-Br-C_6H_4
c	Br	4-Cl- C_6H_4
d	Br	3-Cl- C_6H_4
e	Br	2-CH_3-C_6H_4
f	Br	3-CH_3-C_6H_4
g	Br	4-CH_3-C_6H_4
h	Br	-C_6H_5

Scheme 1: Synthesis of 1-(5-(5-H/Br benzofuran-2-yl)-1-phenyl-*1H*-pyrazole-3-carbonyl)-4-substituted/unsubstituted phenyl thiosemicarbazides (3a-h).

1-(5-(benzofuran-2-yl)-1-phenyl-*1H*-pyrazole-3-carbonyl)-4-p-tolylthiosemicarbazide (3a): White crystalline solid; solvent for recrystallization, 1,4-dioxane; mp, 224-226°C; yield, 94%; IR (KBr v max in cm^{-1}): 3114, 3146, 3227, 3312 (NH), 3035, 3066 (ArH), 1650 (C=O), 1234 (C=S), 1441, 1481 (C=C), 1261, 1063 (C-O-C), 1513 (C=N); ^1H NMR(DMSO-d_6) δ (ppm): 2.28 (s, 3H, Ar-CH_3), 6.60 (s, 1H, pyrazole CH), 7.11-7.61 (m, 14H, ArH), 9.73 (s, 1H, NH-CS-NHC_6H_5), 9.78 (s, 1H, NH-CS-NHC_6H_5), 10.44 (s, 1H, -CONH NH-CS-NHC_6H_5); MS: *m/z* 468 $[M+H]^+$, 469 $[M+2]^+$, 470 $[M+3]^+$, 490 $[M+Na]^+$, 491 $[(M+H)+Na]^+$. Elemental Anal. Calcd: for $C_{26}H_{21}O_2N_5S$; C, 66.79; H, 4.53; N, 14.98; S, 6.86; Found: C, 66.12; H, 4.24; N, 14.33; S, 6.23.

1-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazole-3-carbonyl)-4-(4-bromophenyl) thiosemicarbazide (3b): White crystalline solid; recrystallization solvent, 1,4-dioxane; mp, 255-257°C; yield, 89%; IR (KBr v max in cm^{-1}): 3146, 3226, 3315 (NH), 3030, 3056 (ArH), 1618 (C=O), 1227 (C=S), 1435, 1486 (C=C), 1267 (C-O-C), 1502 (C=N). Elemental Anal. Calcd: for $C_{25}H_{17}Br_2N_5O_2S$; N, 11.46; S, 5.25; Found: N, 11.03; S, 5.11.

1-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazole-3-carbonyl)-4-(4-chlorophenyl) thiosemicarbazide (3c): White crystalline solid; recrystallization solvent, 1,4-dioxane; mp, 246-250°C; yield, 82%; IR (KBr v max in cm^{-1}): 3148, 3223, 3315 (NH), 3027, 3038 (ArH), 1618 (C=O), 1228 (C=S), 1436, 1488 (C=C), 1267 (C-O-C), 1510 (C=N). Elemental Anal. Calcd: for $C_{25}H_{17}BrClN_5O_2S$; N, 12.35; S, 5.66; Found: N, 12.17; S, 5.02.

1-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazole-3-carbonyl)-4-(3-chlorophenyl) thiosemicarbazide (3d): White crystalline solid; recrystallization solvent, 1,4-dioxane; mp, 260-262°C; yield, 80%; IR (KBr v max in cm^{-1}): 3198, 3227, 3312 (NH), 3027, 3053 (ArH), 1647 (C=O), 1222 (C=S), 1432, 1478 (C=C), 1261 (C-O-C), 1508 (C=N). Elemental Anal. Calcd: for $C_{25}H_{17}Br_2N_5O_2S$; N, 12.35; S, 5.66; Found: N, 12.40; S, 5.36.

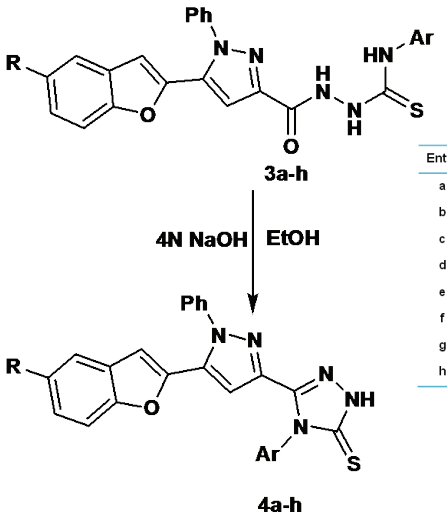
1-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazole-3-carbonyl)-4-o-tolyl thiosemicarbazide (3e): White crystalline solid; recrystallization solvent, 1,4-dioxane; mp, 243-245°C; yield, 86%; IR (KBr v max in cm^{-1}): 3195, 3226, 3317 (NH), 3024, 3048 (ArH), 1652 (C=O), 1234 (C=S), 1432, 1488 (C=C), 1253, 1060 (C-O-C), 1517 (C=N). Elemental Anal. Calcd: for $C_{26}H_{20}BrN_5O_2S$; N, 12.82; S, 5.87; Found: N, 12.26; S, 5.54.

1-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazole-3-carbonyl)-4-m-tolyl thiosemicarbazide (3f): White crystalline solid; recrystallization solvent, 1,4-dioxane; mp, 236-240°C; yield, 84%; IR (KBr v max in cm^{-1}): 3251, 3307 (NH), 3022, 3033 (ArH), 1645 (C=O), 1242 (C=S), 1433, 1485 (C=C), 1262 (C-O-C), 1567 (C=N). Elemental Anal. Calcd: for $C_{26}H_{20}BrN_5O_2S$; N, 12.82; S, 5.87; Found: N, 12.31; S, 5.38.

1-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazole-3-carbonyl)-4-p-tolyl thiosemicarbazide (3g): White crystalline solid; recrystallization solvent, 1,4-dioxane; mp, 238-240°C; yield, 78%; IR (KBr v max in cm^{-1}): 3241, 3314 (NH), 3066 (ArH), 1642 (C=O), 1228 (C=S), 1434, 1494 (C=C), 1265 (C-O-C), 1516 (C=N). Elemental Anal. Calcd: for $C_{26}H_{20}BrN_5O_2S$; N, 12.82; S, 5.87; Found: N, 12.13; S, 5.46.

1-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazole-3-carbonyl)-4-phenyl thiosemicarbazide (3h): White crystalline solid; recrystallization solvent, 1,4-dioxane; mp, 243-245°C; yield, 86%; IR (KBr v max in cm^{-1}): 3193, 3245, 3326 (NH), 3045, 3053 (ArH), 1646 (C=O), 1231 (C=S), 1454, 1489 (C=C), 1261 (C-O-C), 1513 (C=N). Elemental Anal. Calcd: for $C_{25}H_{18}BrN_5O_2S$; N, 13.15; S, 6.02; Found: N, 12.94; S, 5.86.

General procedure for the synthesis 5-(5-(H/Br benzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-Substituted/unsubstituted phenyl-*2H*-1,2,4-triazole-3(*4H*)-thione (4a-h): Thiosemicarbazide 3a-h (0.002 M) in sodium hydroxide (4N, 6 mL) solution and ethanol (20 mL) was heated under reflux for 3 h. The reaction mixture was cooled to room temperature and acidified with diluted acetic acid. The product thus separated was filtered, washed with excess of water and recrystallized from ethanol to afford compound 4a-h (**Scheme 2**).



Entry	R	Ar
a	H	4-CH_3-C_6H_4
b	Br	4-Br-C_6H_4
c	Br	4-Cl- C_6H_4
d	Br	3-Cl- C_6H_4
e	Br	2-CH_3-C_6H_4
f	Br	3-CH_3-C_6H_4
g	Br	4-CH_3-C_6H_4
h	Br	-C_6H_5

Scheme 2: Synthesis 5-(5-(H/Br benzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-Substituted/unsubstituted phenyl-*2H*-1,2,4-triazole-3(*4H*)-thione (4a-h).

5-(5-(benzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-p-tolyl-*2H*-1,2,4-triazole-3(*4H*)-thione (4a): White crystalline solid; recrystallization solvent, Ethanol; mp, 230-232°C; yield, 88%; IR (KBr v max in cm^{-1}): 3316 (N-H), 3064 (ArH), 1610 (C=N), 1435 (C=C), 1248 (C-O-C), 1154 (C=S); ^{1}H NMR(DMSO-d_6) δ (ppm): 2.47 (s, 3H, Ar-CH3), 6.61 (s, 1H, C_3-H of Benzofuran), 6.30 (s, 1H, pyrazole CH), 7.18-7.48 (m, 14H, ArH); ^{13}C NMR δ (ppm): 21, 106, 107, 111, 121, 123, 125, 127, 128, 129, 130, 131, 135, 139, 140, 141, 146, 154, 169; Elemental Anal. Calcd: for $C_{26}H_{19}N_5OS$; N, 15.58; S, 7.13; Found: N, 15.21; S, 6.98.

5-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-(4-bromophenyl)-*2H*-1,2,4-triazole-3(*4H*)-thione (4b): White crystalline solid; recrystallization solvent, Ethanol; mp, 222-224°C; yield, 83%; IR (KBr v max in cm^{-1}): 3314 (N-H), 3066 (ArH), 1609 (C=N), 1434, 1494 (C=C), 1256, 1228 (C-O-C), 1152 (C=S). Elemental Anal. Calcd: for $C_{25}H_{15}Br_2N_5OS$; N, 11.80; S, 5.40; Found: N, 11.24; S, 5.22.

5-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-(4-chlorophenyl)-*2H*-1,2,4-triazole-3(*4H*)-thione (4c): White crystalline solid; recrystallization solvent, Ethanol; mp, 246-248°C; yield, 80%; %; IR (KBr v max in cm^{-1}): 3312 (N-H), 3055 (ArH), 1597 (C=N), 1432, 1491 (C=C), 1245 (C-O-C), 1153 (C=S). Elemental Anal. Calcd: for

$C_{25}H_{15}BrClN_5OS$; N, 12.76; S, 5.84; Found: N, 12.54; S, 5.68.

5-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-(3-chlorophenyl)-*2H*-1,2,4-triazole-3(*4H*)-thione (4d): White crystalline solid; recrystallization solvent, Ethanol; mp, 226-228°C; yield, 78%; IR (KBr v max in cm^{-1}): 3316 (N-H), 3062 (ArH), 1597 (C=N), 1432, 1489 (C=C), 1245 (C-O-C), 1152 (C=S). Elemental Anal. Calcd: for $C_{25}H_{15}BrClN_5OS$; N, 12.76; S, 5.84; Found: N, 12.48; S, 5.57.

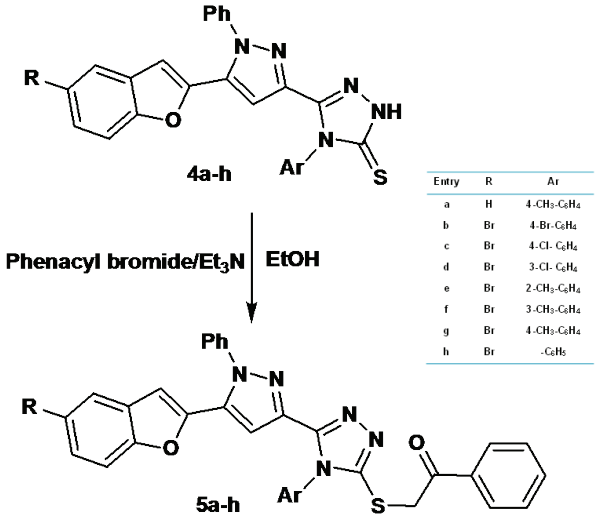
5-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-o-tolyl-*2H*-1,2,4-triazole-3(*4H*)-thione (4e): White crystalline solid; recrystallization solvent, Ethanol; mp, 243-245°C; yield, 83%; IR (KBr v max in cm^{-1}): 3313 (N-H), 3061 (ArH), 1607 (C=N), 1432 (C=C), 1253 (C-O-C), 1154 (C=S). Elemental Anal. Calcd: for $C_{26}H_{18}BrN_5OS$; N, 13.25; S, 6.07; Found: N, 12.97; S, 6.02.

5-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-m-tolyl-*2H*-1,2,4-triazole-3(*4H*)-thione (4f): White crystalline solid; recrystallization solvent, Ethanol; mp, 252-254°C; yield, 84%; IR (KBr v max in cm^{-1}): 3317 (N-H), 3068 (ArH), 1579 (C=N), 1444, 1493 (C=C), 1251 (C-O-C), 1152 (C=S). Elemental Anal. Calcd: for $C_{26}H_{18}BrN_5OS$; N, 13.25; S, 6.07; Found: N, 13.08; S, 5.99.

5-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-p-tolyl-*2H*-1,2,4-triazole-3(*4H*)-thione (4g): White crystalline solid; recrystallization solvent, Ethanol; mp, 242-244°C; yield, 84%; IR (KBr v max in cm^{-1}): 3315 (N-H), 3066 (ArH), 1595 (C=N), 1437, 1499 (C=C), 1249 (C-O-C), 1152 (C=S). Elemental Anal. Calcd: for $C_{26}H_{18}BrN_5OS$; N, 13.25; S, 6.07; Found: N, 13.03; S, 6.03.

5-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-phenyl-*2H*-1,2,4-triazole-3(*4H*)-thione (4h): White crystalline solid; recrystallization solvent, Ethanol; mp, 258-260°C; yield, 84%; IR (KBr v max in cm^{-1}): 3314 (N-H), 3049 (ArH), 1583 (C=N), 1445, 1479 (C=C), 1252 (C-O-C), 1153 (C=S). Elemental Anal. Calcd: for $C_{25}H_{16}BrN_5OS$; N, 13.61; S, 6.23; Found: N, 13.23; S, 6.09.

General procedure for the synthesis 2-(5-(5-(H/Bromo benzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-substituted/unsubstituted phenyl-*4H*-1,2,4-triazol-3-ylthio)-1-phenylethanone (5a-h): A mixture of 4a-h (0.001 mmol) and phenacyl bromide (0.001 mmol) were dissolved in ethanol (15 mL) and 2-3 drops of triethylamine was added as a catalyst. The reaction mixture was heated for 3h and cooled to room temperature then content was poured into cold water and product separated was filtered, washed with ethanol and recrystallized from 1,4-dioxane to give 5a-h (**Scheme 3**).



Entry	R	Ar
a	H	4-CH_3-C_6H_4
b	Br	4-Br-C_6H_4
c	Br	4-Cl- C_6H_4
d	Br	3-Cl- C_6H_4
e	Br	2-CH_3-C_6H_4
f	Br	3-CH_3-C_6H_4
g	Br	4-CH_3-C_6H_4
h	Br	-C_6H_5

Scheme 3: Synthesis 2-(5-(5-(H/Bromo benzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-substituted/unsubstituted phenyl-*4H*-1,2,4-triazol-3-ylthio)-1-phenylethanone (5a-h).

2-(5-(5-(benzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-p-tolyl-*4H*-1,2,4-triazol-3-ylthio)-1-phenylethanone (5a): White crystalline solid; solvent for recrystallization, 1,4-dioxane; mp, 218-220°C; yield, 86%; IR (KBr v max in cm^{-1}): 3056, 3037 (ArH), 2954, 2917, 2849 (CH_3 and CH_2), 1685 (C=O), 1466, 1579, 1595 (C=C), 1513 (C=N), 1256, 1072 (C-O-C), 1045 (N-N), 691 (C-S-C); ^{1}H NMR(DMSO-d_6) δ (ppm): 2.41 (s, 3H, Ar-CH_3), 4.99 (s, 2H, CH_2), 6.65 (s, 1H, pyrazole CH), 7.03-8.06 (m, 19H, ArH); MS: *m/z* 568 $[M+H]^+$, 569 $[M+2]^+$, 590 $[M+Na]^+$. Elemental Anal. Calcd: for $C_{34}H_{25}N_5O_2S$; C, 71.94; H, 4.44; N, 12.34; S, 5.65; Found: C, 71.12; H, 4.21; N, 12.13; S, 5.58.

2-(5-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-(4-bromophenyl)-*4H*-1,2,4-triazol-3-ylthio)-1-phenylethanone (5b): White crystalline solid; solvent for recrystallization, 1,4-dioxane; mp, 254-256°C; yield, 82%; IR (KBr v max in cm^{-1}): 3052, 3033 (ArH), 2949, 2915 (CH_3 and CH_2), 1673 (C=O), 1434, 1493 (C=C), 1585 (C=N), 1248 (C-O-C), 685 (C-S-C). Elemental Anal. Calcd: for $C_{33}H_{21}Br_2N_5O_2S$; N, 9.84; S, 4.51; Found: N, 9.13; S, 4.26.

2-(5-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-(4-chlorophenyl)-*4H*-1,2,4-triazol-3-ylthio)-1-phenylethanone (5c): White crystalline solid; solvent for recrystallization, 1,4-dioxane; mp, 256-258°C; yield, 80%; IR (KBr v max in cm^{-1}): 3044, 3037 (ArH), 2948, 2917 (CH_3 and CH_2), 1671 (C=O), 1430, 1494 (C=C), 1583 (C=N), 1248 (C-O-C), 683 (C-S-C). Elemental Anal. Calcd: for $C_{33}H_{21}BrClN_5O_2S$; N, 10.50; S, 4.81; Found: N, 9.98; S, 4.37.

2-(5-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-(3-chlorophenyl)-*4H*-1,2,4-triazol-3-ylthio)-1-phenylethanone (5d): White crystalline solid; solvent for recrystallization, 1,4-dioxane; mp, 214-218°C; yield, 79%; IR (KBr v max in cm^{-1}): 3060, 3036 (ArH), 2946, 2911 (CH_3 and CH_2), 1676 (C=O), 1429, 1484 (C=C), 1582 (C=N), 1252 (C-O-C), 685 (C-S-C). Elemental Anal. Calcd: for $C_{33}H_{21}BrClN_5O_2S$; N, 10.50; S, 4.81; Found: N, 10.12; S, 4.32.

2-(5-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-o-tolyl-*4H*-1,2,4-triazol-3-ylthio)-1-phenylethanone (5e): White crystalline solid; solvent for recrystallization, 1,4-dioxane; mp, 290-294°C; yield, 77%; IR (KBr v max in cm^{-1}): 3056, 3040 (ArH), 2942, 2916 (CH_3 and CH_2), 1670 (C=O), 1430, 1484 (C=C), 1583 (C=N), 1256, 1062 (C-O-C), 685 (C-S-C). Elemental Anal. Calcd: for $C_{34}H_{24}BrN_5O_2S$; N, 10.83; S, 4.96; Found: N, 10.52; S, 4.77.

2-(5-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-m-tolyl-*4H*-1,2,4-triazol-3-ylthio)-1-phenylethanone (5f): White crystalline solid; solvent for recrystallization, 1,4-dioxane; mp, 206-208°C; yield, 77%; 3058, 3038 (ArH), 2952, 2917 (CH_3 and CH_2), 1672 (C=O), 1431, 1488 (C=C), 1585 (C=N), 1256 (C-O-C), 687 (C-S-C). Elemental Anal. Calcd: for $C_{34}H_{24}BrN_5O_2S$; N, 10.83; S, 4.96; Found: N, 10.52; S, 4.67.

2-(5-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-p-tolyl-*4H*-1,2,4-triazol-3-ylthio)-1-phenylethanone (5g): White crystalline solid; solvent for recrystallization, 1,4-dioxane; mp, 218-220°C; yield, 74%; IR (KBr v max in cm^{-1}): 3053, 3042 (ArH), 2929, 2917 (CH_3 and CH_2), 1671 (C=O), 1433, 1490 (C=C), 1585 (C=N), 1253, 1028 (C-O-C), 687 (C-S-C). Elemental Anal. Calcd: for $C_{34}H_{24}BrN_5O_2S$; N, 10.83; S, 4.96; Found: N, 10.18; S, 4.72.

2-(5-(5-(5-bromobenzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-phenyl-*4H*-1,2,4-triazol-3-ylthio)-1-phenylethanone (5h): White crystalline solid; solvent for recrystallization, 1,4-dioxane; mp, 218-220°C; yield, 74%; IR (KBr v max in cm^{-1}): 3062, 3046 (ArH), 2927, 2914 (CH_3 and CH_2), 1676 (C=O), 1449, 1493 (C=C), 1584 (C=N), 1258, 1022 (C-O-C), 687 (C-S-C). Elemental Anal. Calcd: for $C_{33}H_{22}BrN_5O_2S$; N, 11.07; S, 5.07; Found: N, 10.88; S, 4.87.

Antibacterial activity

The novel synthesized acyl thiosemicarbazide 3a and 1,2,4-triazole 5a were screened for their *in vitro* antimicrobial activity using cup plate agar disc-diffusion method against two Gram positive bacterial strains, *B. thurengienesis* and *S. aureus* and two Gram negative strains, *E. coli* and *E. aerogenes*. Chloramphenicol was used as standard drug for bacteria.

General procedure

Determination of zone of inhibition by agar disc-diffusion method: Test solutions were prepared with known weight of compound in DMSO and half diluted suitably to give the resultant concentration of 31 µg/mL to 1000 µg/mL. Whatmann no. 1 sterile filter paper discs (6 mm) were impregnated with solution and allowed to dry at room temperature. *In vitro* antibacterial activity was determined by using Mueller Hinton Agar obtained from Himedia Ltd., Mumbai. Petri plates were prepared by pouring 10 mL of Mueller Hinton Agar for bacteria containing microbial culture was allowed to solidify. The discs were then applied and the plates were incubated at 37°C for 24 h (bacteria) and the inhibition zone was measured as diameter in four directions and expressed as mean. The results were compared using chloramphenicol as a standard antibacterial agent. The results of antibacterial activity (i.e. zone of inhibition in mm) of some of the synthesized compounds are given in the **Table 1**.

RESULT AND DISCUSSION

The schemes of the targeted synthesized compounds 3a-h, 4a-h and 5a-h are described in **Schemes 1-3** respectively.

The purity of synthesized compound at every synthetic stage was monitored by TLC technique. The structures of the newly synthesized products were substantiated based on elemental and spectral analysis such as IR, ^{1}H NMR and mass. The synthesis of the starting compound, 5-(5-H/Br benzofuran-2-yl)-1-phenyl-*1H*-pyrazole-3-carbohydrazides (1a-b) were achieved in quantitative yields by adopting published literature method [27].

Table 1: Antibacterial Activity of 3a and 5a.

S. No.	Conc. (µg/mL)	Zone of Inhibition in mm			
		Gram +ve		Gram -ve	
		B. thurengienesis	*S. aureus*	*E. coli*	*E. aerogenes*
3a					
1	**1000**	16	8	15	10
2	**500**	12	15	**22**	12
3	**250**	10	14	15	15
4	**125**	10	8	24	8
5	**63**	8	10	22	8
6	**31**	-	8	8	-
5a					
1	**1000**	15	13	18	10
2	**500**	13	8	**20**	14
3	**250**	18	14	**24**	12
4	**125**	14	18	16	8
5	**63**	16	12	16	10
6	**31**	16	**20**	20	14
Standard Chloramphenicol					
1	**1000**	22	26	24	16
2	**500**	20	30	20	16
3	**250**	21	27	18	17
4	**125**	16	21	17	16
5	**63**	15	18	17	15
6	**31**	16	20	21	15

The reaction of 1a-b with aryl isothiocyanates 2a-h in chloroform as a solvent led to the formation of 3a-h. The IR spectrum of 3a showed -NH stretch of amine at 3312 cm^{-1} and C=O stretching in amide group at 1650 cm^{-1}. The ^{1}H NMR spectrum showed singlet at δ 2.3 ppm for -CH_3 protons, hence it confirms that aryl isothiocyanates has condensed with 5-(benzofuran-2-yl)-1-phenyl-*1H*-pyrazole-3-carbohydrazide to afford 1-(5-(H/Br benzofuran-2-yl)-1-phenyl-*1H*-pyrazole-3-carbonyl)-4-substituted/unsubstituted phenyl thiosemicarbazide derivatives 3a-h. The elemental analysis of this product gave C, 66.12; H, 4.24; N, 14.33 and S, 6.23. The mass spectra of the products revealed a molecular ion peak at *m/z* 468 $[M+H]^+$ which is in agreement with the molecular formula $C_{26}H_{21}O_2N_5S$.

The reaction of 1-(5-(5-H/Br benzofuran-2-yl)-1-phenyl-*1H*-pyrazole-3-carbonyl)-4-substituted/unsubstituted phenyl thiosemicarbazides 3a-h with NaOH (4N) yielded 5-(5-(H/Br benzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-substituted/unsubstituted phenyl-*2H*-1,2,4-triazole-3(*4H*)-thione derivatives 4a-h. The IR spectrum of 4a reveals that C=O stretching in amide group has disappeared and 1,2,4-triazole endocyclic C=N stretch has appeared at 1610 cm^{-1}. The ^{1}H-NMR spectra revealed singlet signal at δ 2.47 ppm which was attributed to the -CH_3 protons. ^{1}H-NMR spectra showed expected signals for aromatic and aliphatic protons, obtained data assigned to the proposed structure 4a confirms that thiosemicarbazide has been cyclized to form 1,2,4-triazole.

Further, 5-(5-(benzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-p-tolyl-*2H*-1,2,4-triazole-3(*4H*)-thione (4a) treated with phenacyl bromide in presence of triethylamine afforded 2-(5-(5-(benzofuran-2-yl)-1-phenyl-*1H*-pyrazol-3-yl)-4-p-tolyl-*4H*-1,2,4-triazol-3-ylthio)-1-phenyl ethanone (5a).

4a exhibits thione-thiol tautomerization hence undergoes phenacylation when treated with phenacyl bromide in presence of a base. In this reaction, selective S-phenacylation was observed in presence of triethylamine; a weak base to afford 5a, which was confirmed from its spectral data. The IR spectra of 5a showed moderately strong bands around 2954 cm^{-1}, 2917 cm^{-1}, 2849 cm^{-1}, 1685 cm^{-1} and 1045 cm^{-1}, characteristic of the CH_3 and CH_2, C=O and N-N groups respectively. In the ^{1}H NMR spectra, characteristic signal due to the -S-CH_2-CO- protons appeared at δ 4.99 ppm. The signal due to the -CH_3 protons appeared at δ 2.41 ppm. The signals due to the aromatic protons appeared as multiples at δ 7.03-8.06 ppm. Its elemental analysis reveals that % of C, H, N and S are 71.12, 4.21, 12.13 and 5.58

respectively, while its mass spectrum shows a molecular ion peak at *m/z* 568 $[M+H]^+$ matches with the molecular formula $C_{34}H_{25}N_5O_2S$.

Antibacterial activity

Obtained results of *in vitro* antimicrobial activity of 3a and 5a are summarized in the **Table 1** and their comparative study has done. Dark value in the **Table 1** indicated that either zone of inhibition of tested compound at that particular concentration is greater or equal as standard drug. Data revealed that the acylthiosemicarbazide 3a showed good antibacterial activity against Gram positive bacteria, *S. aureus* and *B. thurengienesis*. It is inactive against *S. aureus* only at a concentration of 31 µg/mL. In case of Gram negative bacteria, 3a showed good activity against *E.coli*, and at a concentration of 500 µg/mL it showed high activity compared with standard drug while moderately active against *E. aerogenes* and inactive at 31 µg/mL.

1,2,4-triazole 5a showed good activity against all tested microorganisms at all concentrations. The part of interest is that it possesses high activity against *E. coli* at concentration of 500 µg/mL and 250 µg/mL. At lower concentration that is 31 µg/mL, 5a showed equal zone of inhibition against both the Gram positive bacteria compared with the standard drug chloramphenicol.

CONCLUSION

A series of novel 1,2,4-triazoles (4a-h, 5a-h) were successfully synthesized in good yields. In first step acyl thiosemicarbazides (3a-h) obtained by condensation of carbohydrazides (1a-b) with aromatic isothiocynates (2a-h) then cyclization of acyl thiosemicarbazides to triazole thiones (4a-h) in presence of sodium hydroxide. Finally, 1,2,4-triazole thione was selectively S-phenacylated by phenacyl bromide in presence of triethylamine to give 5a-h. Their purity and confirmation was checked by physical, analytical and spectral data. Antibacterial screening of selected compounds were found to possess high to moderate activity against selected strains of bacteria.

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