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Synthesis of novel bridgehead heterocycles via cyclization of 4-amino-1,2,4-triazole-3(4*H*)-thiones and their antimicrobial screening

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ABSTRACT

*Synthesis of new fused heterocycles (**1a-d** to **5a-d**) by the interaction of (4-amino-5-(5-(substituted/unsubstituted benzofuran-2-yl)-1-phenyl-1H-pyrazol-3-yl)-2H-1,2,4-triazole-3 (4H)-thiones **(a-d)** with dissimilar reagents. Constitutions of the title compounds have been established on the basis of chemical transformations, elemental analysis, IR, ^{1}H NMR, ^{13}C NMR and Mass spectral studies. In addition the invitro antibacterial and antifungal properties were tested for these synthesized compounds compared with Ampicillin and Clotrimazole as reference drugs. Most of the synthesized compounds were found to possess moderate to excellent activity against selected strains.*

Keywords: triazole-3(4*H*)-thiones, thiadiazoles-6-one, antimicrobial activity

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INTRODUCTION

Sulphur containing heterocycles represent an important group of compounds that are promising for use in practical applications. On the other hand the electronic structure of sulphur imbues sulphurous organic compounds, with chemical reactivity's beyond those of the corresponding oxygen or nitrogen containing analogues. 4-Amino-3-mercapto-5-substituted-1,2,4-triazole has been synthesized by different coworkers [1-6] and has proved to be a versatile precursor for the synthesis of various heterocyclic compounds possessing fused or functionalized triazole ring like thiazolotriazoles, triazolothiadiazoles, triazolothiazines, triazolothiazepines and triazolothiadiazines respectively. It is also reported that [1,2,4] triazolo[3,4-b][1,3,4]thiadiazoles and [1,2,4]triazolo[3,4-b][1,3,4]thiadiazines are incorporated into a wide variety of therapeutically important compounds possessing a broad spectrum of biological activities [7,8] including antimicrobial [9], antioxidants and anticancer agents [10]. The biological activities of [1,3,4]thiadiazoles may be due the presence of the (=N-C-S) NCS moiety [11]. Literature survey reveals that, the newly synthesized title compounds in our laboratory are not reported earlier. In view of these findings and in continuation to our previous work [12-14] here, we wish to first time report a facile one pot synthesis of some novel bridgehead heterocycles such as [1,2,4]triazolo[3,4-b][1,3,4] thiadiazoles and [1,3,4]thiadiazines by the reaction of 1,2,4-triazole-3(4*H*)-thiones along with antibacterial and antifungal activities scheme 1.

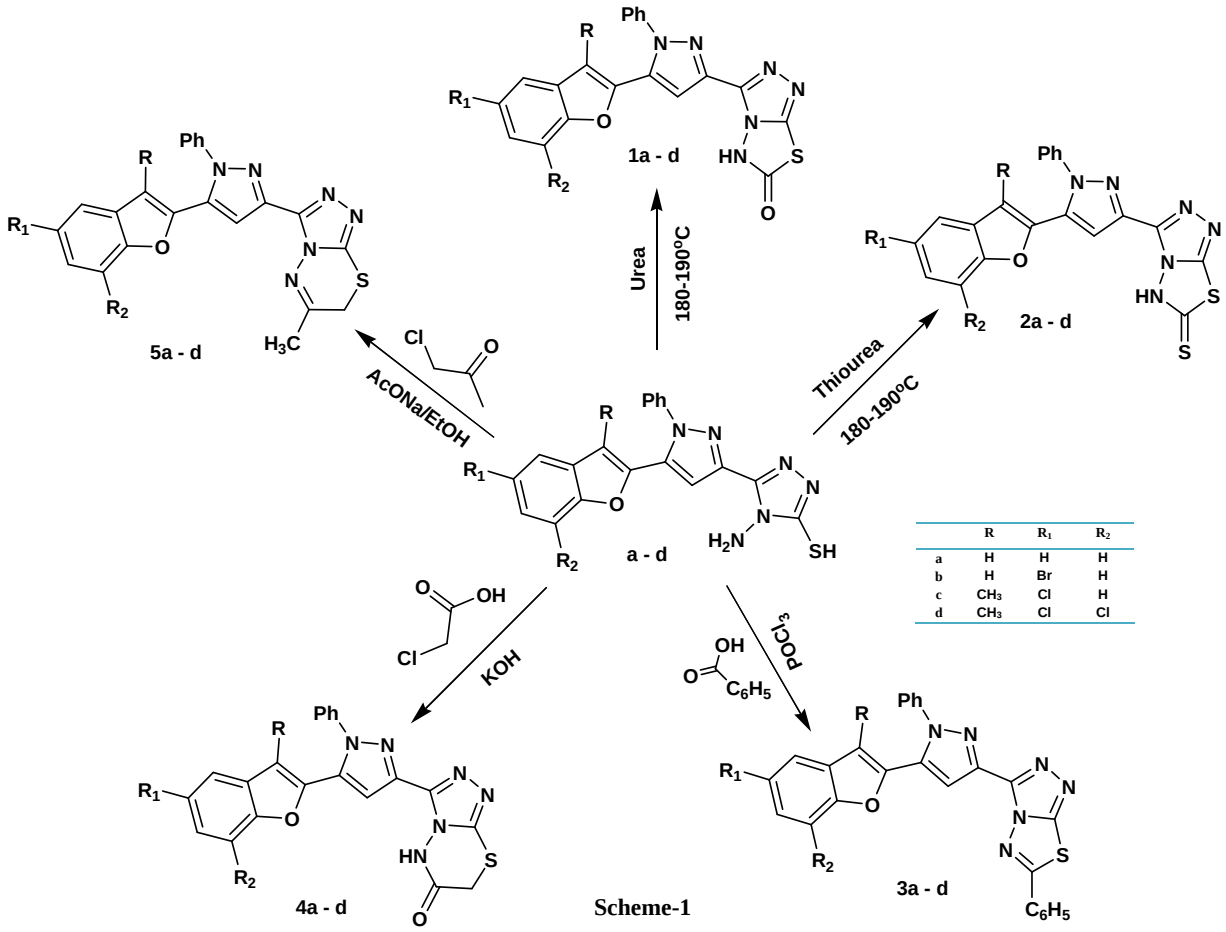
MATERIALS AND METHODS

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}). ^{1}HNMR and ^{13}C 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. Chemical Shifts are given in parts per million (ppm). Positive-ion electrospray ionisation (ESI) mass spectra were obtained with a Waters Micromass Q–TOF Micro, Mass Spectrophotometer. Elemental (CHN) analysis was done using - Vario EL III Elemental Analyzer. All the chemicals used for the synthesis were of AR grade of Merck, S. D.

Fine and Aldrich. The reaction are monitored by E. Merck TLC Aluminum sheet silica $gel_{60}F_{254}$and visualizing the spot in UV light and iodine chamber.

General procedure of the synthesis of 3-(5-(substituted/unsubstitutedbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazol-6(5*H*)-ones (1a-d): A mixture of **a** (10 mmol) and urea (12 mmol) were fused at 180-190°C for 3h. Then it was cooled and diluted with water. The white crystalline solid **1a** was filtered off and then further purified by recrystallization in ethanol. Same procedure was followed to synthesize **1b-d** utilizing **b-d.**

General procedure for the synthesis of 3-(5-(substituted/unsubstitutedbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole-6(5*H*)-thiones (2a-d): A mixture of **a** (10 mmol) and thiourea (12 mmol) were fused at 180-190°C for 3h. Then it was cooled and diluted with water. The white crystalline solid **2a** was filtered off and then further purified by recrystallization using ethanol. Similarly, **2b-d** was synthesized from **b-d** by adopting the same procedure followed for **2a.**



Scheme-1

General procedure for the synthesis of 3-(5-(substituted/unsubstitutedbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles (3a-d): A mixture of **a** (1 mmol) and benzoic acid (1 mmol) in phosphorous oxychloride ($POCl_3$, 10mL) was refluxed for 2h. The reaction mixture was cooled, added to water-ice mixture and neutralized by ammonium hydroxide. The solid obtained was filtered, washed with water, dried and further purified by recrystallization from ethanol to get white crystalline solid **3a**. **3b-d** was then synthesized from **b-d** by extension of the same procedure followed for **3a.**

General procedure for the synthesis of 3-(5-(substituted/unsubstitutedbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-6-methyl-7*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazines (4a-d): A mixture of **a** (1 mmol), sodium acetate (2.5 mmol) and chloroacetone (1 mmol) in absolute ethanol (10 mL) were refluxed for 2h. After cooling, the solvent was removed under pressure; the precipitate formed was washed with water, filtered and further purified by recrystallization from ethanol to give **4a**. **4b-d** was also synthesized from **b-d** by following the same procedure as **4a.**

General procedure for the synthesis of 3-(5-(substituted/unsubstitutedbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-5*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6(7*H*)-ones (5a-d): A mixture of **a** (1 mmol), chloroacetic acid (1 mmol) and KOH (1 mmol) in water (25 mL) was refluxed for 6h. The reaction mixture was cooled, filtered and acidified with diluted HCl. The precipitate formed was filtered off, washed with water and further purified by recrystallization from ethanol to obtain **5a**. Similarly, **5b-d** was synthesized from **b-d** by adopting the same procedure followed for **5a.**

Antimicrobial Screening:

The novel synthesized compounds were screened for their *in vitro* antimicrobial activity against two Gram positive strains, *Bacillus subtilis* and *Staphylococcus aureus* and two Gram negative strains, *Escherichia coli* and *Pseudomonas aeruginosa* in addition to fungi *Aspergillus niger*. Antibacterial activity was assessed by serial (broth) dilution technique, using Mueller Hinton Agar broth for bacteria and Sabouraud dextrose agar for fungus. Antimicrobial activity of dimethylsulphoxide against the organisms selected was investigated, it was found to be nil. The stock solutions of synthesized compounds were prepared in DMSO as a solvent, starting with maximum concentration of 1000 µg/mL or 800µg/mL and then reducing it successively by two fold dilution methods using a calibrated micropipette to get concentrations of 500-15 µg/mL or 400-12.5 µg/mL in nutrient broth. Similarly, serial dilution tubes for standard drug with its stock solution 100 µg/mL were also prepared so that the concentrations of standard drug in five tubes were 50, 25, 12.5, 6, 3µg/mL. MIC of the sample was carried out by inoculation of these serial dilutions with test organisms. The inoculum size was approximately 10^6 colony forming units (CFC/mL). The inoculated tubes were incubated for 24h at 37(±1) $^{\circ}$C (bacteria) and for 72h at 28°C (fungus). After 24h and 72h the inoculated culture tubes were macroscopically examined for turbidity. MIC was considered to be the lowest concentration of the tested compound which inhibits the visible growth of bacteria or fungus after a period of incubation.

RESULTS AND DISCUSSION

The synthesis of the title compounds **1a-d** to **5a-d** from **a-d** [14] is described in the given reaction scheme**.** The amino and mercapto groups in **a-d** are ready made nucleophilic centre for the synthesis of condensed heterocyclic compounds such as traizolothiadiazoles and traizolothiadiazines which exist as thiol-thione tautomers as indicated by their IR and ^{1}H NMR spectra. Fusion of **a-d** with urea at 180-190°C to form **1a-d,** the structural identities of **1b** shown by IR absorption bands at 1619 and 3271 cm^{-1} due to C=O and NH stretch respectively and in ^{1}H NMR singlet at δ 13.9222 ppm due to NH proton, therefore confirmed the formation of thiadiazole-6-one ring. Similarly, fusing **a-d** and thiourea gave good yields of **2a-d**, and structural uniqueness of **2b** proved by IR absorption band at 1187 cm^{-1} due to C-S stretch whereas bands at 3271, 3138 cm^{-1} due to –NH stretch, while singlet at δ 14.0271 ppm due to NH proton in ^{1}H NMR spectrum confirmed the identities of **2b** by molecular ion peak at 495, and it is consistent with molecular formula $C_{20}H_{11}ON_6S_2Br$.

Reaction of **a-d** with benzoic acid in $POCl_3$ afforded **3a-d**, the disappearance of the signal of NH_2 protons and appearance of the multiplet at δ 7.4470-8.0660 ppm due to fourteen aromatic protons in ^{1}H NMR spectra of **3b.** A molecular ion peak at 562 $[M+Na]^+$ in mass spectra, has further proved that the cyclodehydration has occurred followed by ring closure, as it is in good agreement with the molecular formula $C_{26}H_{15}ON_6SBr$.

Further, reaction of **a-d** with chloroacetone in ethanol afford **4a-d,** the disappearance of signal due to NH_2, NH in IR of **4c** and simultaneously appearance of singlet at δ 2.3572 and δ 4.4419 ppm in ^{1}H NMR and at δ 23 and 28 ppm in ^{13}C NMR due to CH_3 and CH_2 of **4c** was recorded. This was further confirmed by molecular ion peak at 461 $[M+H]^+$, and 483 $[M+Na]^+$ in mass spectrum; it is consistent with the molecular formula $C_{23}H_{17}ON_6SCl$.

Lastly, heating **a-d** with chloroacetic acid in presence of KOH as basic catalyst afforded **5a-d**. The structural identities of the product from carboxymethylation of 4-amino-5-mercapto[1,2,4]triazoles was found in literature [15,16] to be either un-cyclized to form amino acid or cyclized into triazolothiadiazinone. Appearance of absorption bands of **5c** at 1717 and 3337 cm^{-1} due to C=O and NH stretch respectively and disappearance of singlet at δ 5.8907 due to $–NH_2$ protons in ^{1}H NMR spectra of **a**, but appearance of a broad singlet at δ 6.1702 ppm due to NH, similarly molecular ion peak at 463 $[M+H]^+$ in mass spectra proved that cyclization has occurred and hence it is consistent with the molecular formula $C_{22}H_{15}O_2N_6SCl$.

The spectral data such as IR, ^{1}H NMR, ^{13}C NMR, and Mass spectrum of the newly synthesized compounds in the reaction schemes are in accordance with the proposed structures. All the carbons in the ^{13}C NMR spectra were seen at their expected chemical shifts and the mass spectrum [17] of these novel compounds revealed a molecular ion peak at *m/z* $[M+H]^+$ and $[M+Na]^+$, besides two molecular ion peaks were observed due to the isotopic chlorine or bromine atom in the molecule which are in agreement with molecular formulas of all the synthesized compounds.

Characterization and analytical data:

4-Amino-5-(5-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-yl)-2*H*-1,2,4-triazole-3(4*H*)-thione (a): White crystalline solid; mp: 222-224^{0}C; yield 68%; M. F. $C_{19}H_{14}ON_6S$; ^{1}H NMR:5.8907 (s, 2H, NH_2), 6.3924 (s, 1H, pyrazole CH), 7.2063-7.6373 (m, 10H, ArH), 13.9184 (s, 1H, NH); ^{13}C NMR:105, 107, 110, 121, 123, 125, 127, 129, 134, 139, 143, 144(Ar-C_1-C_{12}), 153(C-O), 165(C=S); MS:*m/z* 375 $[M+H]^+$, 397$[(M+Na)^+$, ^{35}Cl]; Calculated: C, 60.96; H, 3.74; N, 22.45: S, 8.56 Found: C, 60.84; H, 3.68; N, 22.42; S, 8.40.

4-Amino-5-(5-(bromobenzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-yl)-2*H*-1,2,4-triazole-3 (4*H*)-thione (b): White crystalline solid; mp: 246-248^{0}C; yield 72%; M. F. $C_{19}H_{13}ON_6SBr$, IR: 3268, 3154 (NH), 3062 (ArH), 1628, 1596 (C=N), 1512, 1499, 1475, 1455 (C=C), 1258 (C-O-C),1189 (C=S); ^{1}H NMR: 5.7872 (s, 2H, NH_2), 6.3613 (s, 1H, pyrazole CH), 7.2630-7.6977 (m, 9H, ArH), 13.9121 (s, 1H, NH); Calculated: C, 50.33; H, 2.87; N, 15.54; S, 7.06 Found: C, 50.26; H, 2.66; N, 15.74; S, 7.00.

4-Amino-5-(5-(5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-yl)-2*H*-1,2,4-triazole-3(4*H*)-thione (c): White crystalline solid; mp: 225-226^{0}C; yield 70%; M. F. $C_{20}H_{15}ON_6SCl$; ^{1}H NMR:2.1501 (s, 3H, CH_3), 5.9125 (s, 2H, NH_2), 7.2784-7.6027 (m, 9H, pyrazole CH +ArH), 13.9424 (s, 1H, NH); Calculated: C, 56.74; H, 3.55; N, 19.86; S, 7.56 Found:C, 56.92; H, 3.39; N, 19.96; S, 7.43.

4-Amino-5-(5-(5,7-dichloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-yl)-2*H*-1,2,4-triazole-3(4*H*)-thione(d): White crystalline solid; mp: 210-212^{0}C; yield 65%; M. F. $C_{20}H_{14}ON_6SCl_2$, Calculated: C, 52.52; H, 3.06; N, 18.38: S, 7.00 Found: C, 52.52; H, 3.00; N, 18.29; S, 6.99.

3-(5-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6(5*H*)-one (1a): White crystalline solid; mp 253-254^{0}C; yield 78%; M. F. $C_{20}H_{12}O_2N_6S$, **Calculated:**C, 60.00; H, 3.00; N, 21.00; S, 8.00 **Found:** C, 60.24; H, 2.99; N, 21.22; S, 7.99.

3-(5-(5-bromobenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazol-6(5*H*)-one (1b): White crystalline solid; mp: 283-285^{0}C; yield 85%; M. F. $C_{20}H_{11}O_2N_6SBr$, IR: 3271 (NH), 1619 (C=N), 1508, 1477 (C=C), 1284 (C-O-C), 694 (C-S), ^{1}H NMR:6.3671 (s, 1H, pyrazole CH), 7.4025-8.0302 (m, 9H, ArH),13.9222 (s, 1H, NH). MS:*m/z* 478 $[M]^+$ Calculated: C, 50.10; H, 2.29; N, 17.54; S, 6.68 Found: C, 49.93; H, 2.09; N, 17.14; S, 6.28.

3-(5-(5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazol-6(5*H*)-one (1c): White crystalline solid; mp: 220-222^{0}C; yield 73%; M. F. $C_{21}H_{13}O_2N_6SCl$, Calculated: C, 56.25; H, 2.90; N, 18.75; S, 7.14 Found: C, 56.12; H, 3.00; N, 18.95; S, 7.36.

3-(5-(5,7-dichloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazol-6(5*H*)-one (1d): White crystalline solid; mp: 268-270^{0}C; yield 65%; M. F. $C_{21}H_{12}O_2N_6SCl_2$, Calculated: C, 52.28; H, 2.49; N, 17.43; S, 6.64 Found: C, 52.10; H, 2.52; N, 17.01; S, 6.58.

3-(5-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole-6(5*H*)-thione (2a): White crystalline solid; mp: 218-220^{0}C; yield 83%; M. F. $C_{20}H_{12}ON_6S_2$ Calculated: C, 57.69; H, 2.88; N, 20.19; S, 15.38 Found: C, 57.78; H, 3.01; N, 9.99; S, 15.01.

3-(5-(5-bromobenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole-6(5*H*)-thione (2b): White crystalline solid; mp: 238-240^{0}C; yield 85%; M. F. $C_{20}H_{11}ON_6S_2Br$, IR: 3138 (NH), 3013 (ArH), 1622, 1595 (C=N-N=C), 1508, 1478, 1446, 1417 (C=C), 1260 (C-O-C),1187 (C=S), 694 (C-S). ^{1}H NMR: 6.4924 (s, 1H, pyrazole CH), 7.4797-7.8696 (m, 9H, ArH),14.0271 (s, 1H,-NH). ^{13}C NMR:104, 107, 112, 115, 123, 125, 127, 129, 134, 138, 139, 143, 145(Ar-C_1-C_{13}), 152(C-O), 165(C=S), MS: *m/z* 495 M^+, 497 $[M+2]^+$, Calculated: C, 48.48; H, 2.22; N, 16.97; S, 12.43 Found: C, 48.20; H, 2.11; N, 17.03; S, 11.98.

3-(5-(5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazole-6(5*H*)-thione (2c): White crystalline solid; mp: 216-218^{0}C; yield 80%; M. F. $C_{21}H_{13}ON_6S_2Cl$, Calculated: C, 54.31; H, 2.80; N, 18.10; S, 13.79 Found: C, 54.34; H, 2.78; N, 18.00; S, 13.64.

3-(5-(5,7-dichloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole-6(5*H*)-thione (2d): White crystalline solid; mp: 212-214^{0}C; yield 75%; M. F. $C_{21}H_{12}ON_6S_2Cl_2$, Calculated: C, 50.60; H, 2.41; N, 16.86; S, 12.85 Found: C, 51.04; H, 2.45; N, 17.18; S, 13.02.

3-(5-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4]-thiadiazole (3a): White crystalline solid; mp: 258-260^{0}C; yield 67%; M. F. $C_{26}H_{16}ON_6S$, Calculated: C, 67.83; H, 3.48; N, 18.26; S, 6.96 Found: C, 67.48; H, 3.27; N, 18.11; S, 6.55.

3-(5-(5-bromobenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-6-phenyl-[1,2,4]triazolo[3,4b] [1,3,4]thiadiazole (3b): White crystalline solid; mp: >280^{0}C; yield 75%; M. F. $C_{26}H_{15}ON_6SBr$, IR: 3054 (ArH), 1624, 1596 (C=N-N=C), 1518, 1500, 1464 (C=C), 1262 (C-O-C), 693 (C-S), ^{1}H NMR:6.4994 (s, 1H, pyrazole CH), 7.4470-8.0660 (m, 14H, ArH). MS:*m/z* 541 $[M+2]^+$ Calculated: C, 57.88; H, 2.78; N, 15.58; S, 5.94 Found: C, 57.54; H, 2.63; N, 15.76; S, 5.60.

3-(5-(5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-6-phenyl-[1,2,4] triazolo[3,4-b][1,3,4] thiadiazole (3c):White crystalline solid; mp: 222-224^{0}C; yield 70%; M. F. $C_{27}H_{17}ON_6SCl$, Calculated: C, 63.77; H, 3.35; N, 16.53; S, 6.29 Found: C, 63.58; H, 3.25; N, 16.59; S, 6.08.

3-(5-(5,7-dichloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-6-phenyl-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazole (3d): White crystalline solid; mp: 236-238^{0}C; yield 64%; M. F. $C_{27}H_{16}ON_6SCl_2$, Calculated: C, 59.77; H, 2.95; N, 15.49; S, 5.91 Found: C, 60.01; H, 2.82; N, 15.53; S, 5.87.

3-(5-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-6-methyl-7*H*-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazine (4a): White crystalline solid; mp: 230 (d); yield 72%; M. F. $C_{22}H_{16}ON_6S$, Calculated: C, 64.07; H, 3.88; N, 20.38; S, 7.77 Found: C, 64.18; H, 3.62; N, 20.05; S, 7.82.

3-(5-(5-bromobenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-6-methyl-7*H*-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazine (4b): White crystalline solid; mp: 225-226^{0}C; yield 78%; M. F. $C_{22}H_{15}ON_6SBr$, Calculated: C, 53.76; H, 3.05; N, 17.11; S, 6.52 Found: C, 54.00; H, 3.12; N, 17.00; S, 6.25.

3-(5-(5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-yl)-6-methyl-7*H*-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazine (4c): White crystalline solid; mp: 178-180^{0}C; yield 88%; M. F. $C_{23}H_{17}ON_6SCl$**,** IR: 3058 (ArH), 2954, 2917, 2849 (CH_3, CH_2) 1610, 1596 (C=N-N=C), 1519, 1498, 1459 (C=C), 1260 (C-O-C), 691, 661 (C-S), ^{1}H NMR: 2.1558 (s, 3H, CH_3), 2.3572 (s, 3H, CH_3), 4.4419 (s, 2H, CH_2), 7.2794-7.6132 (m, 9H, ArH), ^{13}C NMR: 8 (benzofuran CH_3), 28(CH_3), 42(SCH_2), 99(CH=C), 109,110, 112, 116, 119, 123, 125, 127, 128, 130, 133, 137, 139, 141(Ar-C_1-C_{14}), 152(C-O), 160(C-S), 163(C=N) MS: *m/z* 461 $[M+H]^+$, 483 $[M+Na]^+$Calculated: C, 60.00; H, 3.69; N, 18.26; S, 6.96 Found: C, 60.15; H, 3.54; N, 18.04; S, 6.76.

3-(5-(5,7-dichloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazole-3-yl)-6-methyl-7H-[1,2,4]triazolo[3,4-b] [1,3,4]thiadiazine (4d): White crystalline solid; mp: 210-212^{0}C; yield 55%; M. F. $C_{23}H_{16}ON_6SCl_2$. Calculated: C, 55.87; H, 3.24; N, 17.00; S, 6.48 Found: C, 55.92; H, 3.44; N, 17.08; S, 6.52.

3-(5-(benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-5*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6(7*H*)-one(5a): White crystalline solid; mp: >280^{0}C; yield 60%; M. F. $C_{21}H_{14}O_2N_6S$, Calculated: C, 60.86; H, 3.38; N, 20.29; S, 7.73 Found: C, 60.54; H, 2.98; N, 20.09; S, 8.00.

3-(5-(5-bromobenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-5*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6(7*H*)–one (5b): White crystalline solid; mp: 225-226^{0}C; yield 69%; M. F. $C_{21}H_{15}O_2N_6SBr$, Calculated: C, 51.12; H, 2.64; N, 17.03; S, 6.49 Found: C, 51.01; H, 2.88; N, 16.98; S, 6.22.

3-(5-(5-chloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-5*H*-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-6(7*H*)-one (5c):White crystalline solid; mp: 212-214^{0}C; yield 65%; M. F. $C_{22}H_{15}O_2N_6SCl$, IR: 3337, 3275 (NH), 3058 (ArH), 2918 (CH_3), 1717 (C=O), 1613, 1596 (C=N-N=C), 1497, 1482 (C=C), 1263 (C-O-C),688, 664 (C-S), ^{1}H NMR:2.1538 (s, 3H, CH_3), 4.0416 (s, 2H, CH_2), 6.1702 (b, 2H, NH + pyrazole CH), 7.2668-7.5632 (m, 8H, ArH) ^{13}C NMR:8(benzofuran CH_3), 33(SCH_2), 109, 112,115, 119, 123, 125, 127, 128, 130, 132, 139, 140, 141, 148 (Ar-C_1-C_{14}), 152(C-O), 169(C=O) MS: *m/z* 463 $[M+H]^+$, 485 $[M+Na]^+$ Calculated: C, 57.14; H, 3.25; N, 18.18; S, 6.93 Found: C, 57.00; H, 3.41; N, 18.36; S, 6.64.

3-(5-(5,7-dichloro-3-methylbenzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-5*H*-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazin-6(7*H*)-one (5d): White crystalline solid; mp: 210-212^{0}C; yield 59 %; M. F. $C_{22}H_{14}O_2N_6SCl_2$, Calculated: C, 53.23; H, 2.82; N, 16.94; S, 6.45 Found: C, 53.52; H, 2.98; N, 16.55; S, 6.38

Antimicrobial activity:

The data obtained from table no. **1** reported that compounds were able to inhibit the growth of the selected bacteria *in vitro* showing MIC values between 6.25 ≥ 200 µg/mL for bacteria while 12.5-100 µg/mL for fungus. The title compounds were graded as highly active with MIC value 6.25-25 µg/mL, moderately active at 50-100 µg/mL and poorly active at values ≥ 200 µg/mL. The most active compounds are **2b and 5c** (*B. subtilis)*, **4c** (*S. aureus)*, **b, c, 3b, 2b** and **4c** (*E. coli)*, **4c** (*P. aeruginosa)* However, all the tested compounds showed substantial activity in the range of 12.5-100 µg/mL against the fungus *A. niger.* Rests of the compounds with MIC values ≥ 200 µg/mL are found to be poorly active against all the test bacteria and fungus.

Table 1 – Antibacterial and antifungal activities

Sr. No.	Compound Code	Minimum Inhibitory Concentration (MIC[a], µg/mL)				
		B. subtilis **(NCIM 2439)**	***S. aureus*** **(NCIM 2079)**	***E.coli*** **(NCIM 2064)**	***P. aeruginosa*** **(NCIB 8650)**	***A. niger*** **(NCIM 501)**
1.	**b**	100	100	12.5	>200	12.5
2.	**c**	50	>200	12.5	>200	12.5
3.	**1b**	>200	>200	25	>200	>200
4.	**2b**	12.5	>200	>200	>200	100
5.	**3b**	>200	>200	25	>200	50
6.	**4c**	12.5	25	12.5	25	25
7.	**5c**	12.5	50	100	100	50
8.	**Ampicillin**	25	12.5	25	25	-
9.	**Clotrimazole**	-	-	-	-	12.5
10.	**DMSO**	-	-	-	-	-

[a]***MIC:*** *Lowest concentration of an antimicrobial agent that significantly inhibits the visible growth of microorganism after a period of incubation.*

CONCLUSION

In conclusion, we have reported herein a facile one pot synthesis of some new fused nitrogenous heterocycles [1,2,4]triazolo[3,4-b][1,3,4]thiadiazoles and [1,2,4]triazolo[3,4-b][1,3,4] thiadiazines from 4-amino-5-(5-(substituted/unsubstituted benzofuran-2-yl)-1-phenyl-1*H*-pyrazol-3-yl)-2*H*-1,2,4-triazole-3(4*H*)-thione in substantial yields. Synthesized compounds were found to possess moderate to excellent activity against selected strains.

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