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Some Pyridyl- and Thiophenyl- Substituted 1,2,4-Triazolo[3,4-*b*]1,3,4-thiadiazole Derivatives as Potent Antibacterial

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The target compounds **6-11a-e** were synthesized by condensing 4-amino-5-aryl-3*H*-1,2,4-triazole-3-thiones **5a-f** with various aromatic carboxylic acids in the presence of phosphorous oxychloride. The structures of newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, elemental analysis and mass spectrometric studies. All the synthesized compounds were screened for their antibacterial activity. Almost all the tested compounds were potent against four different strains of bacteria when compared with that of reference drug ciprofloxacin. Compounds **6c**, **6e**, **8d**, **9b**, **9e**, **11a** and **11b** showed nearly equal or lower MIC values than standard drug, against all four tested bacterial strains but rest of the compounds showed excellent antibacterial activities.

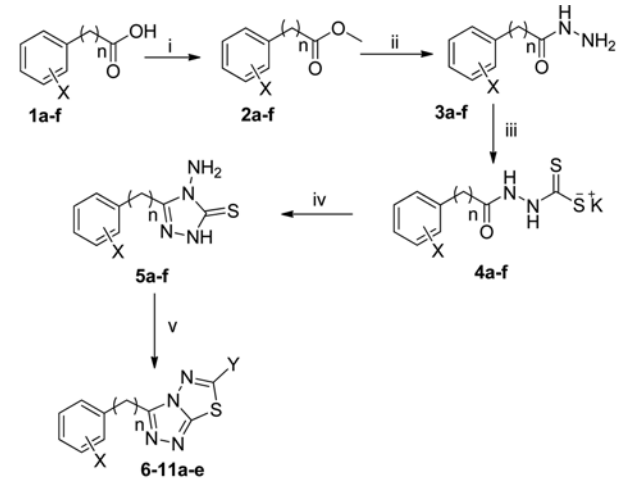
Key Words : Triazolothiadiazoles, Antibacterial activities

Introduction

Recently triazolothiadiazole derivatives have received considerable attentions owing to their synthetic and potent pharmacological importance. Heterocyclic compounds bearing 1,2,4-triazole nuclei and their 3,6-disubstituted 1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazole derivatives have been reported to show a broad spectrum of pharmacological properties such as antimicrobial,[1] anti-inflammatory,[2] anticonvulsant,[3] anticancer,[4] antitubercular[5] and antitumor activities[6] depending upon the nature of heterocyclic rings, hydrophobic, hydrophilic groups and other substituted aromatic systems attached at position 3 and 6 of the fused triazolothiadiazole moiety. Literature survey reveals that 3,6-disubstituted-1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazoles bearing substituted pyridyl and thiophenyl rings at position 3 and 6 have not been paid much attention for their antibacterial studies.

Hence, currently the research has been focused towards development of new antibacterial agents which may result in the discovery of novel effective compounds.[7] However, to get such compounds novel strategies are required to be adapted. Therefore, compounds having antibacterial properties can serve as multi-prong strategies to tackle highly challenging antibiotics resistant strains. Synthetic method, adopted is very simple and we get pure product by killing the reagent through just putting the reaction mixture in ice cold water. There is no side product after work up and it has advantage over other methods where complex chromatographic techniques are required for purification of the target compounds.

The target compounds **6-11a-e** were synthesized by condensing 4-amino-5-aryl-3*H*-1,2,4-triazole-3-thiones **5a-f** with various aromatic carboxylic acids in the presence of phosphorous oxychloride shown in Scheme 1.



1-5a, X = 3-Br	n = o	**6,** X = 3-Br	**a,** Y = Pyridin-2-yl	n = 0
b, X = 4-Br	n = 0	**7,** X = 4-Br	**b,** Y = Pyridin-4-yl	n = 0
c, X = 2-Cl	n = 0	**8,** X = 2-Cl	**c,** Y = 3,5-Difluoropyridin-2-yl	n = 0
d, X = 4-Cl	n = 0	**9,** X = 4-Cl	**d,** Y = Thiophen-2-yl	n = 0
e, X = 2-F	n = 1	**10,** X = 2-F	**e,** Y = Thiophen-2-ylmethyl	n = 1
f, X = 4-OCH$_3$	n = 0	**11,** X = 4-OCH$_3$		

Scheme 1. Reagent and conditions (i) Methanol, sulfuric acid (2-3 drops), reflux, 4-6 h; (ii) Hydrazine hydrate, reflux, 10-12 h; (iii) Potassium hydroxide, carbon disulfide, 0-5 °C stirring, 2-3 h; (iv) Hydrazine hydrate (80%), water and reflux, 8-10 h; (v) Phosphorous oxychloride, substituted acids, reflux, 5-6 h.

Results and Discussions

Synthetic pathway for target compounds, 3,6-disubstituted 1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazoles **6-11a-e** is illustrated in Scheme 1. Substituted aromatic esters **2a-f** were synthesized by the reaction of corresponding substituted aromatic acids **1a-f** in the presence of catalytic amount of sulfuric acid, the esters **2a-f** were converted into corresponding aromatic acid hydrazides **3a-f** by refluxing with hydrazine hydrate (80%) in methanol. Reaction of acid hydrazides **3a-f** with carbon disulfide in alcoholic potassium hydroxide solution yielded potassium dithiocarbazate salts **4a-f** and were used for next step without purification. 5-Substituted-4-amino-1,2,4-triazoles **5a-f** were synthesized by refluxing potassium dithiocarbazate salts **4a-f** in dilute solution of hydrazine hydrate. Structures of amino 1,2,4-triazoles **5a-f** were characterized by IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. In IR spectra, a peak in the range of 3209-3044 cm^{-1} for NH stretching and a relatively strong peak with shoulder in the range of 3350-3273 cm^{-1} for NH_2 stretching were observed. In the ^{1}H NMR, NH proton in the range of 14.13-12.66 ppm was observed. This proton exists as thione-thiol tautomeric form. The dominant form is thione, as there was no peak for SH group in IR spectrum. Synthesis of 3,6-disubstituted 1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazoles **6-11a-e** were confirmed by IR as well as NMR spectra by the disappearance of signals for NH and NH_2. In IR spectra, sp^2 CH stretching vibrations occur in the range of 3111 to 3012 cm^{-1} and two to three peaks of bending aromatic vibrations occur in the range of 1663 to 1440 cm^{-1}. In proton NMR spectra, aromatic protons resonate in the range of 8.93 to 6.94 ppm. ^{13}C NMR spectra of **6-11a-e** showed verifiable carbon signals for corresponding compounds. The structure of the target molecules **6-11a-e** were also confirmed by mass spectrometry using ESI (positive) probe and ethyl acetate was used as solvent. Samples were diluted with LC-MS grade methanol and filtered through PTFE membrane filter (0.45 μm pore size) before injecting them using direct syringe pump. Compounds showed clear molecular ion $(M + H)^+$ peak.

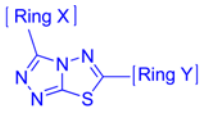
Pharmacology.

Antibacterial Activity: Antibacterial activity of the synthesized 3,6-disubstituted-1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazoles derivatives were tested against two strains of each category, Gram-positive bacteria namely *Staphylococcus aureus*, *Bacillus subtilis*, and Gram-negative bacteria namely, *Escherichia coli*, *Shigella flexneri*. Ciprofloxacin was used as a reference drug for the assay. The results of activities are reported in Table 1. The minimum inhibitory concentrations (MIC) were determined using the broth micro-dilution method, as recommended by the National Committee for Clinical Laboratory Standards. All of the tested compounds **6-11a-e** showed comparable antibacterial activity against the tested strains. In particular, compounds **6e**, **8d**, **9b** and **11b** were found to have the most potent activity (MIC: 0.156 μg/mL) against all the tested strains and were equi-potent *in vitro* as compared to Ciprofloxacin (MIC: 0.156 μg/mL) against *E. coli* and *S. aureus*. However, these compounds were four times more potent against *Bacillus subtilus* and two times more potent against *Shigella flexneri* than reference drug.

Table 1. The MIC (μg/mL) values of 3,6-disubstituted-1,2,4-triazolo[3,4-*b*]1,3,4-thiadiazoles derivatives **6-11a-e** against different bacterial strains and reference Ciprofloxacin

Compounds	Bacterial strains and their MIC μg/mL			
	E. coli	*B. subtilis*	*S. aureus*	*S. flexneri*
6a	0.313	0.156	0.625	0.313
6b	0.313	0.156	0.313	0.156
6c	0.156	0.313	0.156	0.156
6d	0.156	0.625	0.156	0.313
6e	0.156	0.156	0.156	0.156
7a	0.313	0.156	0.313	0.625
7b	0.313	0.313	0.313	0.156
7c	0.313	0.313	0.156	0.156
7e	0.625	0.156	0.156	0.313
8a	0.313	0.156	0.156	0.313
8b	0.156	0.313	0.313	0.625
8c	0.313	0.625	0.313	0.156
8d	0.156	0.156	0.156	0.156
9b	0.156	0.156	0.156	0.156
9e	0.156	0.313	0.156	0.156
10b	0.313	0.156	0.313	0.625
11a	0.313	0.156	0.313	0.156
11b	0.156	0.156	0.156	0.156
11d	0.156	0.625	0.313	0.156
11e	1.250	0.313	0.156	0.156
Ciprofloxacin	0.156	0.625	0.156	0.313

MIC: minimum inhibitory concentration.



General representations of ring X and ring Y

These compounds contain halogenated phenyl group as X except **11b**, which has methoxy phenyl group. Compounds **6e** and **8d** have thiophen-2-yl group as Y whereas compounds **9b** and **11b** contains pyridine-4-yl group as Y. Compounds **6c**, **6d**, **8b**, **9e** and **11d** showed equal activity against *E. coli* as compared to that of standard drug. All compounds **6-11a-e** showed 2 to 4 fold more potent antibacterial activity against *B. Subtilis* except compounds **6d** and **11d** showing equal activity as that of standard drug. Compound **6c** and **9e** have moderate antibacterial activity, while **6d**, **7e** and **11d** have same value of IC_{50} as that of reference drug. Compound **11e** is least active in this series and it contains methoxy group as X and thiophen-2-yl methyl group as Y. Among all the tested compounds, **6a**, **7a**, **8b**, **8c** and **10b** showed fourfold less activity than standard against some Gram negative and Gram positive bacterial strains depending on structure activity relationship.

In conclusion, compounds **6c**, **6e**, **8d**, **9b**, **9e**, **11a** and **11b** were found to possess excellent potential against tested bacterial strains.

Experimental

All the common solvents and chemicals were of analytical grade or dry distilled. The qualitative analysis of the synthesized compounds were ascertained by thin layer chromatography and the R_f values were determined by employing precoated silica gel aluminium plates, Kieselgel 60 F_{254} from Merck (Germany), using *n*-hexane:ethyl acetate (8:2) as an eluent and TLC was visualized under UV lamp. Melting points were determined on a Stuart melting point apparatus (SMP3) and are uncorrected. The IR spectra were recorded on Bruker Optics Alpha FT-IR spectrophotometer. NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer with TMS as an internal standard. The multiplicities were expressed as s = singlet, d = doublet, t = triplet, q = quartet, dt = doublet of triplets. Mass spectra were recorded on Mass Spec LTQXL, by ESI (positive) probe using ethyl acetate as solvent. Samples were diluted with LC-MS grade methanol and filtered through PTFE membrane filter (0.45 μm pore size) before injecting them using direct syringe pump. The elemental analysis was performed on Leco CHNS-932 Elemental Analyzer, Leco Corporation (USA).

Synthesis of Substituted Aromatic Esters 2a-f and Aromatic Acid Hydrazides 3a-f. Substituted aromatic acid **1a-f** was esterified **2a-f** by refluxing in methanol and in the presence of catalytic amount of sulfuric acid for 4-6 h. Substituted aromatic esters **2a-f** were converted into their corresponding acid hydrazides **3a-f** by refluxing in hydrazine hydrate in methanol through literature procedure.[8,9]

Synthesis of 3-Substituted 4-Amino-5-aryl-3*H*-1,2,4-triazole-3-thiones 5a-f. Potassium hydroxide (0.125 mol) was dissolved in dry methanol (50 mL). To the solution, aryl acid hydrazide **3** (0.125 mol) was added and cooled the solution in ice. To this, carbon disulfide (0.125 mol) was added in small portions with constant stirring for 2-3 h. The solid product of potassium dithiocarbazate salts **4** formed, was filtered, washed with chilled diethyl ether and dried. It was directly used for next step without purification. Potassium dithiocarbazate salts **4** was taken in water (20 mL) and hydrazine hydrate (0.250 mol) was added, and followed by refluxed for 8-10 h. The reaction mixture turned to green with evolution of hydrogen sulfide and finally it became homogeneous. It was then poured in crushed ice and acidified with 37% hydrochloric acid. The white precipitates was filtered, washed with cold water and recrystallized from aqueous methanol.[10,11]

3,6-Disubstituted-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazoles 6-11a-e: A mixture of 4-amino-5-aryl[1,2,4]triazole-3-thione (1 mM) and substituted aromatic acids (1.1 mM) in phosphorous oxychloride (8-10 mL) was refluxed for 5-6 h. The reaction mixture was slowly poured in crushed ice with stirring and neutralizes it with sodium hydrogen carbonate. Solid material was filtered, washed with cold water and dried to furnish triazolothiadiazole as white solid.[12]

3-(3-Bromophenyl)-6-(pyridin-2-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (6a): Yellow solid; Yield: 91%; mp 232-234 °C. TLC R_f (7:3 = chloroform:methanol): 0.74; IR (ν/cm^{-1}) 3070, 1591, 1463, 1447, 1068; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.70 (d, 1H, ^3J = 4.8 Hz), 8.55 (s, 1H), 8.31-8.27 (m, 2H), 7.96-7.92 (m, 1H), 7.63-7.59 (m, 1H, ^3J = 8.1 Hz), 7.54-7.50 (m, 1H), 7.42-7.38 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.71, 155.54, 150.11, 147.25, 145.26, 137.45, 133.26, 130.51, 129.11, 127.41, 127.23, 124.76, 123.07, 120.92; ESI/MS (*m/z*, + ion mode): 359.97 $(M + H)^+$; Anal. Calcd. for $C_{14}H_8BrN_5S$: C, 46.94; H, 2.25; N, 19.55; S, 8.95. Found: C, 46.78; H, 2.18; N, 19.44; S, 8.61.

3-(3-Bromophenyl)-6-(pyridin-4-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (6b): Yellow solid; Yield: 75%; mp 250-252 °C. TLC R_f (7:3 = chloroform:methanol): 0.73; IR (ν/cm^{-1}) 3075, 3043, 1593, 1470, 1411, 1068; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.93-8.90 (d, 2H, ^3J = 5.4 Hz), 8.57-8.54 (s, 1H), 8.35-8.32 (d, 1H, ^3J = 7.8 Hz), 7.85 (d, 2H, ^3J = 5.7 Hz), 7.68-7.64 (m, 1H), 7.47-7.44 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.12, 163.45, 153.16, 151.34, 136.67, 133.35, 130.54, 129.16, 127.41, 124.77, 124.73, 123.07, 120.19, 120.03; ESI/MS (*m/z*, + ion mode): 359.94 $(M + H)^+$; Anal. Calcd. for $C_{14}H_8BrN_5S$: C, 46.94; H, 2.25; N, 19.55; S, 8.95. Found: C, 46.90; H, 2.21; N, 19.45; S, 8.87.

3-(3-Bromophenyl)-6-(3,5-difluoropyridin-2-yl)-[1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazole (6c): Brown solid; Yield: 56%; mp 229-231 °C. TLC R_f (7:3 = chloroform:methanol): 0.76; IR (ν/cm^{-1}) 3111, 1589, 1442, 1393, 1069; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.59-8.56 (t, 1H, ^3J = 7.8 Hz), 8.49-8.46 (m, 1H), 8.35-8.31 (m, 1H), 7.63-7.59 (m, 1H), 7.52-7.48 (m, 1H), 7.43-7.40 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.28, 162.55, 158.90, 155.21, 145.36, 135.16, 133.35, 132.16, 130.54, 129.16, 127.29, 124.77, 123.07, 113.70; ESI/MS (*m/z*, + ion mode): 395.95 $(M + H)^+$; Anal. Calcd. for $C_{14}H_6BrF_2N_5S$: C, 42.66; H, 1.53; N, 17.77; S, 8.13. Found: C, 41.96; H, 1.52; N, 17.73; S, 8.10.

3-(3-Bromophenyl)-6-(thiophen-2-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (6d): Brown solid; Yield: 78%; mp 270-271 °C. TLC R_f (7:3 = chloroform:methanol): 0.74; IR (ν/cm^{-1}) 3087, 1552, 1455, 1421, 980; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55-8.51 (t, 1H, ^4J = 1.8 Hz), 8.34-8.30 (m, 1H), 7.66-7.62 (m, 3H), 7.43 (t, 1H, ^3J = 7.8 Hz), 7.22-7.19 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.82, 154.17, 145.30, 133.31, 131.70, 130.68, 130.51, 129.20, 128.47, 127.41, 124.79, 123.03; ESI/MS (*m/z*, + ion mode): 364.94 $(M + H)^+$; Anal. Calcd. for $C_{13}H_7BrN_4S_2$: C, 42.98; H, 1.94; N, 15.42; S, 17.65. Found: C, 41.98; H, 1.85; N, 15.10; S, 9.12.

3-(3-Bromophenyl)-6-(thiophen-2-ylmethyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (6e): White solid; Yield: 75%; mp 250-251 °C. TLC R_f (7:3 = chloroform:methanol): 0.69; IR (ν/cm^{-1}) 3071, 3012, 2922, 2850, 1606, 1570, 1460, 1038; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.52 (t, 1H, ^3J = 7.8 Hz), 8.33-8.30 (m, 1H), 7.63-7.60 (m, 1H), 7.41 (t, 1H, ^3J = 7.8 Hz), 7.35-7.31 (m, 1H), 7.11-7.09 (m, 1H), 7.06-7.01 (m, 1H), 4.62 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.50, 155.21, 144.94, 135.11, 133.35, 130.54, 129.16, 128.15, 127.68, 127.41, 126.69, 124.77, 123.07, 32.66; ESI/MS (*m/z*, + ion mode): 378.91 $(M + H)^+$; Anal. Calcd. for $C_{14}H_9BrN_4S_2$: C, 44.57; H, 2.40; N, 14.85; S, 17.00. Found: C, 44.42; H, 2.36; N, 14.65; S, 16.99.

3-(4-Bromophenyl)-6-(pyridin-2-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (7a): Yellow solid; Yield: 75%; mp 250-252 °C. TLC R_f (7:3 = chloroform:methanol): 0.73; IR (ν/cm^{-1}) 3073, 1591, 1476, 1447, 1068; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.74-8.71 (m, 1H), 8.31-8.28 (m, 3H), 7.97-7.94 (m, 1H), 7.71-7.68 (m, 2H), 7.50-7.46 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.01, 155.14, 150.45, 147.52, 145.26, 137.56, 132.25, 127.73, 127.57, 127.23, 124.80, 124.47, 120.98; ESI/MS (*m/z*, + ion mode): 359.93 $(M + H)^+$; Anal. Calcd. for $C_{14}H_8BrN_5S$: C, 46.94; H, 2.25; N, 19.55; S, 8.95. Found: C, 46.80; H, 2.20; N, 19.45; S, 8.79.

3-(4-Bromophenyl)-6-(pyridin-4-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (7b): White solid; Yield: 78%; mp 218-220 °C. TLC R_f (7:3 = chloroform:methanol): 0.71; IR (ν/cm^{-1}) 3047, 1598, 1473, 1440, 1058; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.93 (d, 2H, ^3J = 5.4 Hz), 8.27-8.25 (m, 2H), 7.85 (d, 2H, ^3J = 5.7 Hz), 7.70-7.66 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.24, 163.56, 153.93, 151.56, 137.75, 136.78, 132.28, 127.75, 124.90, 124.87, 124.34, 120.92, 120.56; ESI/MS (*m/z*, + ion mode): 359.94 $(M + H)^+$; Anal. Calcd. for $C_{14}H_8BrN_5S$: C, 46.94; H, 2.25; N, 19.55; S, 8.95. Found: C, 46.80; H, 2.11; N, 19.35; S, 8.74.

3-(4-Bromophenyl)-6-(3,5-difluoropyridin-2-yl)-[1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazole (7c): Yellow solid; Yield: 78%; mp 219-221 °C. TLC R_f (7:3 = chloroform:methanol): 0.75. IR (ν/cm^{-1}) 3042, 1602, 1582, 1440, 1059. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.50-8.47 (m, 1H), 8.30-7.26 (m, 2H), 7.70-7.65 (m, 2H), 7.50-7.46 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.28, 162.56, 158.25, 155.36, 145.37, 135.69, 132.46, 132.20, 127.75, 124.79, 124.56, 124.11, 113.58. ESI/MS (*m/z*, + ion mode): 394.40 $(M + H)^+$. Anal. Calcd. for $C_{14}H_6BrF_2N_5S$: C, 42.66; H, 1.53; N, 17.77; S, 8.13. Found: C, 41.96; H, 1.58; N, 17.63; S, 8.11.

3-(4-Bromophenyl)-6-(thiophen-2-ylmethyl)-[1,2,4]tri-azolo[3,4-*b*][1,3,4]thiadiazole (7e): White solid; Yield: 80%; mp 210-211 °C. TLC R_f (7:3 = chloroform:methanol): 0.78; IR (ν/cm^{-1}) 3073, 2920, 2852, 1593, 1552, 1463, 1066; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26-8.22 (m, 2H), 7.70-7.67 (m, 2H), 7.35-7.31 (m, 1H), 7.09-7.01 (m, 1H), 6.98-6.95 (m, 1H), 4.60 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.50, 155.21, 144.94, 135.14, 132.20, 128.11, 127.75, 127.68, 126.69, 124.79, 124.48, 124.37, 32.66; ESI/MS (*m/z*, + ion mode): 378.99 $(M + H)^+$; Anal. Calcd. for $C_{14}H_9BrN_4S_2$: C, 44.57; H, 2.40; N, 14.85; S, 17.01. Found: C, 44.41; H, 2.26; N, 14.55; S, 16.95.

3-(2-Chlorophenyl)-6-(pyridin-2-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (8a): White solid; Yield: 76%; mp 204-206 °C. TLC R_f (7:3 = chloroform:methanol): 0.74; IR (ν/cm^{-1}) 3073, 1591, 1476, 1447, 1068; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.72-8.68 (m, 1H), 8.17 (d, 1H, ^3J = 7.8 Hz), 7.87-7.85 (m, 2H), 7.62-7.57 (m, 1H), 7.54-7.50 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.75, 155.63, 150.56, 146.36, 146.25, 137.56, 134.04, 132.10, 131.86, 130.46, 127.36, 127.08, 125.14, 120.96; ESI/MS (*m/z*, + ion mode): 314.01 $(M + H)^+$; Anal. Calcd. for $C_{14}H_8ClN_5S$: C, 53.59; H, 2.57; N, 22.32; S, 10.22. Found: C, 53.41; H, 2.37; N, 22.12; S, 10.06.

3-(2-Chlorophenyl)-6-(pyridin-4-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (8b): White solid; Yield: 76%; mp 209-211 °C. TLC R_f (7:3 = chloroform:methanol): 0.76; IR (ν/cm^{-1}) 3082, 1594, 1512, 1469, 1090; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.83-8.78 (m, 2H), 7.81-7.77 (m, 1H), 7.76-7.73 (m, 2H), 7.61-7.58 (m, 1H), 7.47-7.42 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.57, 162.30, 153.78, 150.98, 136.45, 133.96, 132.10, 131.91, 130.49, 127.18, 127.06, 124.95, 120.45, 120.31; ESI/MS (*m/z*, + ion mode): 314.00 $(M + H)^+$; Anal. Calcd. for $C_{14}H_8ClN_5S$: C, 53.59; H, 2.57; N, 22.32; S, 10.22. Found: C, 53.35; H, 2.37; N, 22.12; S, 10.09.

3-(2-Chlorophenyl)-6-(3,5-difluoropyridin-2-yl)-[1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazole (8c): White solid; Yield: 80%; mp 214-216 °C. TLC R_f (7:3 = chloroform:methanol): 0.78; IR (ν/cm^{-1}) 3070, 1591, 1463, 1447, 1068; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48-8.46 (m, 1H), 7.80-7.76 (m, 1H), 7.60-7.55 (m, 1H), 7.49-7.43 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.28, 162.53, 158.69, 155.64, 145.36, 135.69, 133.87, 132.45, 132.10, 131.91, 130.49, 127.08, 124.80, 113.65; ESI/MS (*m/z*, + ion mode): 410.02 $(M + H)^+$; Anal. Calcd. for $C_{14}H_6ClF_2N_5S$: C, 48.08; H, 1.73; N, 20.02; S, 9.17. Found: C, 47.89; H, 1.72; N, 19.78; S, 9.14.

3-(2-Chlorophenyl)-6-(thiophen-2-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (8d): White solid Yield: 82%; mp 234-236 °C. TLC R_f (7:3 = chloroform:methanol): 0.68; IR (ν/cm^{-1}) 3098, 3055, 1600, 1551, 1461, 1057; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80-7.76 (m, 1H), 7.62-7.58 (m, 2H), 7.48-7.44 (m, 2H), 7.18-7.15 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.82, 154.17, 145.30, 134.04, 132.10, 131.86, 131.56, 130.52, 130.34, 128.36, 127.08, 125.14; ESI/MS (*m/z*, + ion mode): 318.99 $(M + H)^+$; Anal. Calcd. for $C_{13}H_7ClN_4S_2$: C, 48.98; H, 2.21; N, 17.57; S, 20.12. Found: C, 48.45; H, 2.20; N, 17.54; S, 19.94.

3-(4-Chlorophenyl)-6-(pyridin-4-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (9b): White solid Yield: 80%; mp 210-212 °C. TLC R_f (7:3 = chloroform:methanol): 0.78; IR (ν/cm^{-1}) 3034, 1594, 1510, 1469, 1090; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.91-8.87 (m, 2H), 8.48.37 (m, 2H), 7.84-7.79 (m, 2H), 7.55 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.83, 164.15, 154.46, 151.35, 136.82, 136.56, 129.26, 129.11, 127.55, 127.23, 123.93, 120.61, 120.55; ESI/MS (*m/z*, + ion mode): 314.01 $(M + H)^+$; Anal. Calcd. for $C_{14}H_8ClN_5S$: C, 53.59; H, 2.57; N, 22.32; S, 10.22. Found: C, 53.51; H, 2.42; N, 22.11; S, 10.16.

3-(4-Chlorophenyl)-6-(thiophen-2-ylmethyl)-[1,2,4]tri-azolo[3,4-*b*][1,3,4]thiadiazole (9e): White solid; Yield: 64%; mp 269-271 °C. TLC R_f (7:3 = chloroform:methanol): 0.79; IR (ν/cm^{-1}) 3012, 2921, 2852, 1592, 1511, 1455, 1036; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34-8.30 (m, 2H), 7.53-7.49 (m, 2H), 7.37-7.32 (m, 1H), 7.09-7.05 (m, 1H), 7.04-6.99 (m, 1H), 4.59 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.50, 155.21, 144.94, 136.59, 135.15, 129.26, 129.11, 128.58, 127.68, 127.57, 127.54, 126.53, 124.03, 32.66; ESI/MS (*m/z*, + ion mode): 333.03 $(M + H)^+$; Anal Calcd. for $C_{14}H_9ClN_4S_2$: C, 50.52; H, 2.73; N, 16.83; S, 19.27. Found: C, 49.25; H, 2.71; N, 16.32; S, 19.12.

3-(3-Fluorobenzyl)-6-(pyridin-4-yl)-[1,2,4]triazolo[3,4-

***b*][1,3,4]thiadiazole (10b):** White solid; Yield: 78%; mp 247-249 °C. TLC R_f (7:3 = chloroform:methanol): 0.80; IR (ν/cm^{-1}) 3042, 2918, 2837, 1611, 1542, 1459, 1028; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.85-8.79 (m, 2H), 7.72-7.68 (m, 2H), 7.30-7.26 (m, 1H), 7.18-7.15 (m, 2H), 6.96-6.92 (m, 1H), 4.50 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.53, 161.45, 161.28, 153.78, 151.21, 136.46, 127.99, 127.87, 120.49, 120.43, 117.83, 114.43, 114.38; ESI/MS (*m/z*, + ion mode): 312.05 $(M + H)^+$; Anal. Calcd. for $C_{14}H_8FN_5S$: C, 56.56; H, 2.71; N, 23.56; S, 10.79. Found: C, 56.45; H, 2.70; N, 23.45; S, 10.56.

3-(4-Methoxyphenyl)-6-(pyridin-2-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (11a): White solid; Yield: 91%; mp 232-234 °C. TLC R_f (7:3 = chloroform:methanol): 0.74; IR (ν/cm^{-1}) 3043, 2952, 2837, 1593, 1470, 1411, 1068; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.47-8.43 (m, 1H), 7.49-7.46 (m, 1H), 7.26-7.21 (m, 5H), 6.97-6.94 (m, 2H), 4.51 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.14, 160.23, 159.11, 153.35, 151.12, 135.93, 127.63, 127.25, 120.33, 119.99, 118.01, 114.45, 56.21; ESI/MS (*m/z*, + ion mode): 348.04 $(M + H)^+$; Anal. Calcd. for $C_{15}H_{11}N_5OS$: C, 58.24; H, 3.58; N, 22.64; S, 10.37. Found: C, 58.14; H, 3.57; N, 22.54; S, 10.34.

3-(4-Methoxyphenyl)-6-(pyridin-4-yl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (11b): Brown solid; Yield: 82%; mp 234-236 °C. TLC R_f (7:3 = chloroform:methanol): 0.68; IR (ν/cm^{-1}) 3061, 2953, 2842, 1566, 1441, 1300, 994; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.61-8.57 (m, 2H), 8.29-8.25 (m, 2H), 7.84-7.79 (m, 2H), 7.18-7.14 (m, 2H), 3.90 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.16, 162.26, 161.20, 151.80, 136.61, 127.91, 127.83, 127.41, 120.74, 120.63, 118.11, 114.53, 114.33; ESI/MS (*m/z*, + ion mode): 310.07 $(M + H)^+$; Anal Calcd. for $C_{15}H_{11}N_5OS$: C, 58.24; H, 3.58; N, 22.64; S, 10.37. Found: C, 58.14; H, 3.48; N, 22.60; S, 10.30.

3-(4-Methoxyphenyl)-6-(thiophen-2-yl)-[1,2,4]triazolo [3,4-*b*][1,3,4]thiadiazole (11d): Yellow solid; Yield: 71%; mp 238-240 °C. TLC R_f (7:3 = chloroform:methanol): 0.64; IR (ν/cm^{-1}) 3096, 3066, 2958, 2864, 1613, 1478, 1476, 1033; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.17-8.13 (m, 2H), 7.66-7.61 (m, 2H), 7.23-7.18 (m, 1H), 7.09-7.04 (m, 2H), 3.92 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.20, 160.82, 154.17, 145.30, 131.15, 130.56, 130.49, 128.36, 127.91, 118.11, 114.36, 114.27, 55.43; ESI/MS (*m/z*, + ion mode): 410.02 $(M + H)^+$; Anal. Calcd. for $C_{14}H_{10}N_4OS_2$: C, 53.49; H, 3.21; N, 17.82; S, 20.40. Found: C, 52.94; H, 3.12; N, 17.65; S, 19.98.

3-(4-Methoxyphenyl)-6-(thiophen-2-ylmethyl)-[1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazole (11e): Brown solid; Yield: 65%; mp 230-232 °C. TLC R_f (7:3 = chloroform:methanol): 0.75; IR (ν/cm^{-1}) 3096, 3066, 2961, 2922, 1613, 1478, 1476, 1033; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.32-8.28 (m, 2H), 7.35-7.31 (m, 1H), 7.18-7.14 (m, 2H), 7.11-7.08 (m, 1H), 7.06-7.03 (m, 1H), 3.92 (s, 3H), 4.62 (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.50, 161.20, 155.21, 144.94, 135.63, 128.36, 127.91, 127.68, 126.36, 118.11, 114.33, 113.98, 55.32; ESI/MS (*m/z*, + ion mode): 410.02 $(M + H)^+$; Anal. Calcd. for $C_{15}H_{12}N_4OS_2$: C, 54.86; H, 3.68; N, 17.06; S, 19.53. Found: C, 54.56; H, 3.52; N, 17.01; S, 19.25.

Antibacterial Assay: The antibacterial activity was evaluated *in vitro* by minimum inhibitory concentration (MIC) using the serial tube dilution method.[13] For the assay, two gram positive bacteria namely, *Staphylococcus aureus*, *Bacillus subtilis*, and two gram negative bacteria namely, *Escherichia coli*, *Shigella flexneri* were used. Bacterial strains stored in Muller-Hinton broth (Merck), were subcultured for testing in the same medium and were grown at 37 °C. Then the cells were suspended, in saline solution, to produce a suspension of about 10^{-5} CFU mL^{-1} (colony-forming units per mL). Serial dilutions of the test compounds, previously dissolved in *N,N*-dimethylformamide (DMF), were prepared in test tubes to final concentrations of 2.5, 1.25, 0.625, 0.313 and 0.156 μg/mL. 100 μL of 24 h old inoculums was added to each tube. The MIC, defined as the lowest concentration of the test compound, which inhibits the visible growth after 18 h, was determined visually after incubation for 24 h at 37 °C. Tests using DMF as negative control were carried out in parallel. Ciprofloxacin was used as control drug. Because the MIC values are not spectacular, no statistical calculations were made.

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References

1. Karabasanagouda, T.; Adhikari, A. V.; Shetty, N. S. *Eur. J. Med. Chem*. **2007**, *42*, 521.
2. El-Shehry, M. F.; Abu-Hashem, A. A.; El-Telbani, E. M. *Eur. J. Med. Chem*. **2010**, *45*, 1906.
3. Husain, A.; Naseer, M. A.; Sarafaroz, M. *Acta Poloniae Pharmaceutica-Drug Research*. **2009**, *66*, 135.
4. Kamal, A.; Khan, M. N. A.; Reddy, K. S.; Srikanth, Y. V. V.; Sridhar, B. *Chem. Biol. Drug Des*. **2008**, *71*, 78.
5. Joshi, S. D.; Vagdevi, H. M.; Vaidya, V. P.; Gadaginamath, G. S. *Eur. J. Med. Chem*. **2008**, *43*, 1989.
6. Ibrahim, D. A. *Eur. J. Med Chem*. **2009**, *44*, 2776.
7. Nitta, T.; Arai, T.; Takamatsu, H.; Inatomi, Y.; Murata, H.; Iinuma, M.; Tanaka, T.; Ito, T.; Asai, F.; Ibrahim, I.; Nakanishi, T.; Watabe, K. *J. Health Sci*. **2002**, *48*, 273.
8. Cao, S.; Qian, X.; Song, G.; Huang, Q. *J. Flour. Chem*. **2002**, *117*, 63.
9. Hebert, N.; Hannah, A. L.; Sutton, S. C. *Tetrahedron Lett.* **1999**, *40*, 8547.
10. Lam, K. W.; Syahida, A.; Ulhaq, Z.; Rahman, M. B. A.; Lajis, N. H. *Bioorg. Med. Chem. Lett*. **2010**, *20*, 3755.
11. Li, Z.; Gu, Z.; Yin, K.; Zhang, R.; Deng, Q.; Xiang, J. *Eur. J. Med. Chem*. **2009**, *44*, 4716.
12. Li, D.; Fu, H. *Heterocyclic Commun.* **2006**, *12*, 383.
13. Zampini, I. C.; Cuello, S.; Alberto, M. R.; Ordoñez, R. M.; Almeida, R. D.; Solorzano, E.; Isla, M. I. *J. Ethnopharmacol*. **2009**, *124*, 499.