Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Advances in Applied Science Research, 2015, 6(3):64-71

ISSN: 0976-8610
CODEN (USA): AASRFC

Synthesis, spectroscopic and biological activity of 3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole derivatives

Gaurav K. Patel and Hasmukh S. Patel*

Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India

---

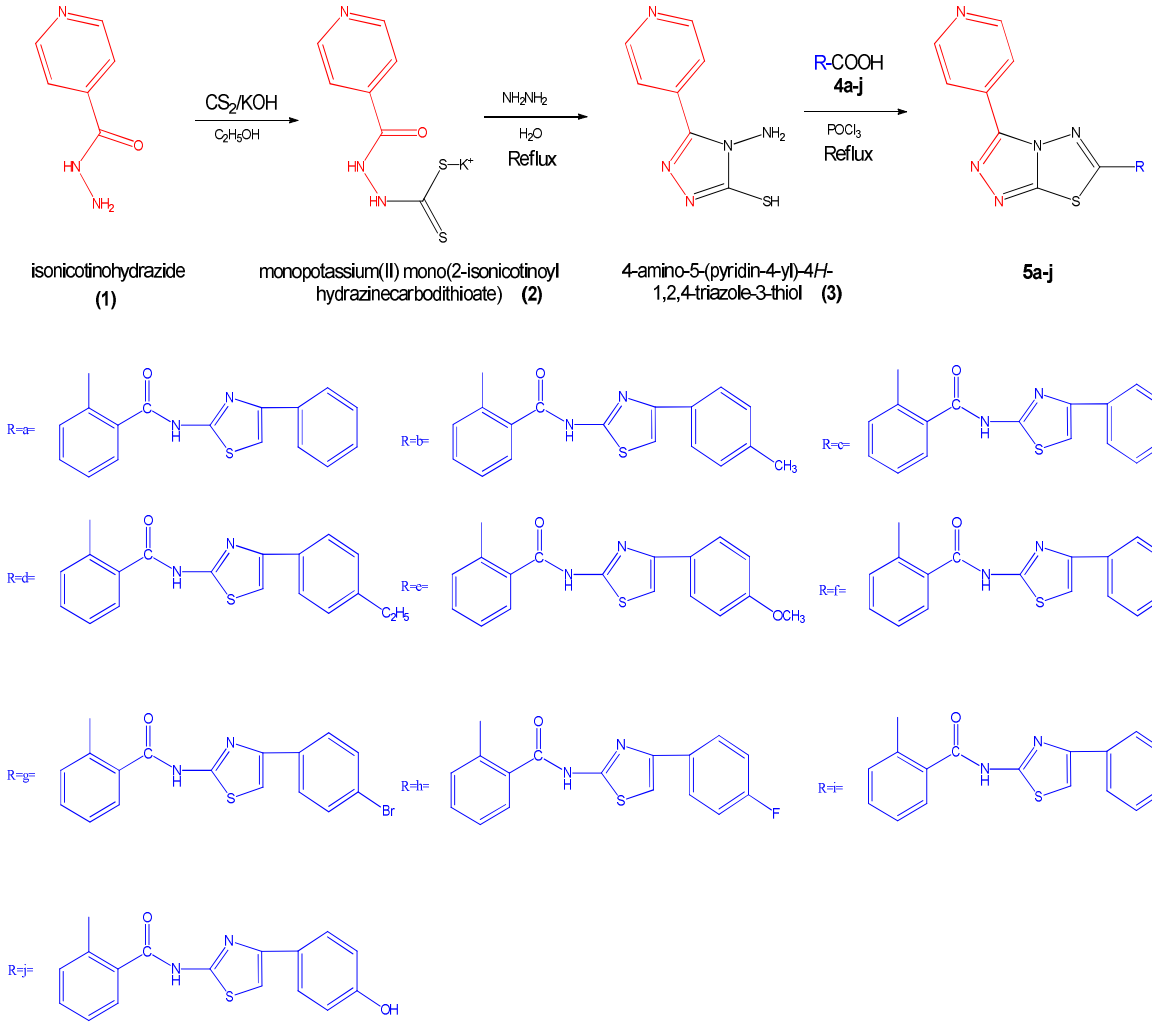
ABSTRACT

*A novel series of heterocyclic compounds N-(4-phenylthiazol-2-yl)-2-(3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzamide derivatives **5a–j** have been synthesized by the reaction of 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol **3** and 2-((4-phenylthiazol-2-yl)carbamoyl)benzoic acid derivatives **4a–j**. Synthesized heterocyclic compounds were characterized by elemental analysis, ^{1}H NMR, ^{13}C NMR, FT–IR and LC-MS spectral studies. Antibacterial activities of all the compounds were studied against gram positive and gram negative bacteria and antifungal activities of all the compounds were studied against various fungi.*

Keywords: heterocyclic compounds, isonicotinohydrazide**,** amino-1,2,4-triazoles, triazolothiadiazoles, 4-phenylthiazol-2-amine.

INTRODUCTION

Fused Heterocyclic compounds bearing triazolothiadiazole nucleus have shown a wide range of pharmacological properties such as antimicrobial [1], anti-inflammatory [2], anticonvulsant [3], anticancer [4], antitubercular [5] and antitumor activities [6]. Other heterocyclic derivatives say 4-phenylthiazol-2-amine also finds the pharmaceutical properties [7]. Looking to the pharmacological importance, it was thought to prepare heterocyclic compounds which possess comparable biological activity up to some extent by introducing triazolothiadiazoles and aminothiazole segments together. Literature survey reveals that, not a single report was found in regarding the triazolothiadiazoles arylthiazole-amide combined moieties. Hence the initial work pertinent to this in this direction has been carried out by us [8]. In continuation of this the present work comprises the novel N-(4-phenylthiazol-2-yl)-2-(3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzamide containing 4-aryl-2-aminothiazole segment as shown in Scheme-1.



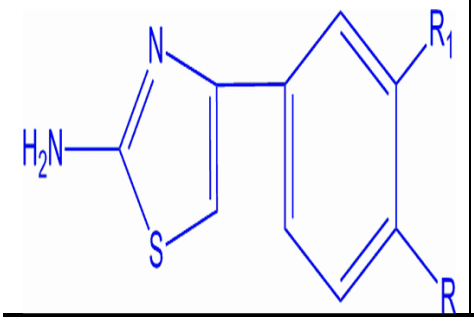
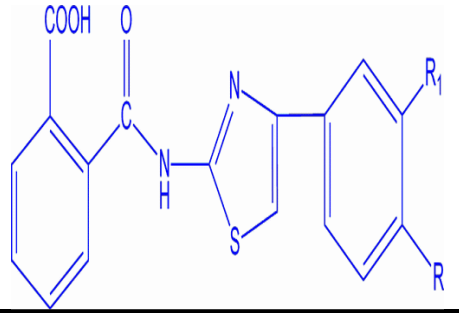
Scheme 1: Synthesis of compounds 5a–j

MATERIALS AND METHODS

Materials and measurements

All common reagents and solvents including isoniazid used were as analytical grade. The 4-amino-5-(pyridin-4-yl)-4H-1,2,4-triazole-3-thiol (3) was prepared by method reported [10]. 4-arylthiazol-2-amines and their derivatization into amide by treatment with phthalic anhydride was carried out by reported method [11,12]**. (listed in Table-1)**

Table-1

4-phenylthiazol-2-amine [11]	*Derivatized product with Phthalic anhydride [12]*
	

Where: R = -H, -CH_3, -CH_3, -C_2H_5, -OCH_3, -Cl, -Br, -F, -NO_2, -OH
R_1 = -H, -H, -CH_3, -H, -H, -H, -H, -H, -H, -H

Alumina supported pre-coated silica gel 60 F254 thin layer chromatography (TLC) plates were purchased from the E. Merck (India) Limited, Mumbai and were used to check purity of compounds and, to study the progress of the

reaction whereby TLC plates were illuminated under Ultraviolet light (254 nm), evaluated in I_2 vapors and visualized by spraying with Draggendorff's reagent. Column chromatography was performed on silica gel (60-120 mesh). LC-MS of all novel samples taken on LCMS 8030 with Nexera UHPLC instrument. Infrared spectra (FT–IR) were obtained from KBr pellets in the range of 4000–400 cm^{-1} with a Perkin Elmer spectrum GX spectrophotometer (FT–IR) instrument. ^1H NMR and ^{13}C NMR spectra were acquired at 400 MHz on a Bruker NMR spectrometer using DMSO–d_6 (residual peak at δ ~2.5 or ~ 39.5 ppm, 300 $^{\circ}K$) as a solvent as well as TMS an internal reference standard. Micro analytical (C, N, H) data was obtained by using a Perkin–Elmer 2400 CHN elemental analyzer. The melting points were checked by the standard open capillary method and were uncorrected.

Synthesis of 5a-j

Compounds 5a-j were synthesized by the general method given below.

An equimolar mixture (0.10 mol) of 4-amino-5-substituted-3-merapto-(4H)-1,2,4-triazoles (2) and 2-((4-phenylthiazol-2-yl)carbamoyl)benzoic acid (Table-1) in phosphorus oxychloride (10 mL) was refluxed for 7 h. The reaction mixture was cooled to room temperature and then gradually poured onto crushed ice with stirring. The mixture was allowed to stand for 5 h. The solid precipitates separated out was filtered, treated with dilute sodium hydroxide solution and washed thoroughly with cold water. The compound obtained was purified by column chromatography, air-dried and recrystallized from ethanol. Products were designated as **5a–j** and characterized by elemental, IR, NMR, CMR and LC-MS analyses.

N-(4-phenylthiazol-2-yl)-2-(3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzamide ***(5a)***

Compound 5a (M. Wt. 481.55g) was obtained in 63% yield as a dark brown color solid; mp > 250 °C (dec); ^1H NMR: δ 9.17 (s, 1H, -NH-), 7.91, 8.76 (m, 4H, pyridine), 7.53-8.08 (m, 4H, Ar-H), 7.43, 7.54, 7.75 (m, 5H, Ar-H), 7.67 (s, 1H, thiazol); ^{13}C NMR: δ 174.5 (-N=C-S-), 167.8 (-N=C-S-), 164.1 (-N=C-S-), 164.6 (-C=O), 151.3 (-N=C-N-), 149.6 (-N=C-C-,Py), 121.7, 134.5, 149.6 (Pyridine), 127.4, 127.9, 128.7, 131.7, 132.8, 135.6 (Ar-H), 105.4, 150.1 (Thiazole), 127.3, 128.9, 129.4, 133.2 (Ar-H); FT-IR: υ 3072 (-C-H=Aromatic stretching), 1683 (-C=O stretching), 1536 (-C=C- stretching), 1235 (-N-N=C- stretching), 694 (-C-S-C- = triazolo-thiadiazole) cm^{-1}; LC-MS *m/z* 481.08 [M–H]+, (M=481.55); Anal. Calcd for $C_{24}H_{15}N_7OS_2$: C 59.86, H 3.14, N 20.36, S 13.32% Found: C 59.83, H 3.11, N 20.32, S 13.30%

2-(3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)-N-(4-(p-tolyl)thiazol-2-yl)benzamide ***(5b)***

Compound **5b** (M. Wt. 495.09 g) was obtained in 68% yield as a dark brown color solid; mp > 250 °C (dec); ^1H NMR: δ 9.14 (s, 1H, -NH-), 7.96, 8.79 (m, 4H, pyridine), 7.51-8.06 (m, 4H, Ar-H), 7.25, 7.65 (m, 4H, Ar-H), 2.32 (s, 3H, $-CH_3$), 7.64 (s, 1H, thiazol)**;** 13C NMR: δ 174.9 (-N=C-S-), 164.5 (-N=C-S-), 164.3 (-N=C-S-), 164.3 (-C=O), 151.7 (-N=C-N-), 149.3 (-N=C-C-,Py), 121.4, 134.8, 149.4 (Pyridine), 127.4, 128.3, 128.8, 131.4, 132.6, 135.4, (Ar-H), 125.3, 129.3, 130.4, 131.5 (Ar-H), 105.7, 150.3 (Thiazole), 21.5 (-CH3); FT-IR: υ 3076 (-C-H=Aromatic stretching), 1688 (-C=O stretching), 1534 (-C=C- stretching), 1233 (-N-N=C- stretching), 698 (-C-S-C- = triazolo-thiadiazole) cm^{-1}; LC-MS *m/z* 496.1[M–H]+, (M=495.5); Anal. Calcd for $C_{25}H_{17}N_7OS_2$: C 60.59, H 3.46, N 19.78, S 12.94%. Found: C 60.54, H 3.42, N 19.75, S 12.91%.

N-(4-(3,4-dimethylphenyl)thiazol-2-yl)-2-(3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzamide ***(5c)***

Compound **5c** (M. Wt. 509.6g) was obtained in 65% yield as a dark brown color solid; mp > 250 °C (dec); ^1H NMR: δ 9.12 (s, 1H, -NH-), 7.92, 8.79 (m, 4H, pyridine), 7.54-8.06 (m, 4H, Ar-H), 7.14, 7.44, 7.62 (m, 3H, Ar-H), 2.36 (s, 3H, $-CH_3$), 2.39 (s, 3H, $-CH_3$), 7.69 (s, 1H, thiazol); 13C NMR: δ 174.7 (-N=C-S-), 167.4 (-N=C-S-), 164.5 (-N=C-S-), 164.3 (-C=O), 151.6 (-N=C-N-), 149.7 (-N=C-C-,Py), 121.4, 134.7, 149.7 (Pyridine), 127.5, 128.1, 128.9, 131.7, 132.2, 135.4 (Ar-H), 122.4, 129.2, 129.7, 130.4, 136.6, 137.8 (Ar-H), 105.1, 150.5 (Thiazole), 18.6 (-OCH3), 19.4 (-OCH3); FT-IR: υ 3071 (-C-H=Aromatic stretching), 1684 (-C=O stretching), 1533 (-C=C- stretching), 1236 (-N-N=C- stretching), 693 (-C-S-C- = triazolo-thiadiazole) cm^{-1}; LC-MS *m/z* 509.1[M–H]+, (M=509.6); Anal. Calcd for $C_{26}H_{19}N_7OS_2$: C 61.28, H 3.76, N 19.24, S 12.58%. Found: C 61.25, H 3.73, N 19.22, S 12.56%.

N-(4-(4-ethylphenyl)thiazol-2-yl)-2-(3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzamide ***(5d)***

Compound **5d** (M. Wt. 509.6g) was obtained in 66% yield as a light brown color solid; mp > 250 °C (dec); ^1H NMR: δ 9.16 (s, 1H, -NH-), 7.98, 8.73 (m, 4H, pyridine), 7.57-8.07 (m, 4H, Ar-H), 7.38, 7.79 (m, 3H, Ar-H), 1.25, 2.56 (m, 5H, $-C_2H_5$), 7.62 (s, 1H, thiazol); 13C NMR: δ 174.5 (-N=C-S-), 164.3 (-N=C-S-), 167.8 (-N=C-S-), 164.2 (-C=O), 151.5 (-N=C-N-), 149.6 (-N=C-C-,Py), 121.7, 134.4, 149.6 (Pyridine), 127.3, 128.1, 128.7, 131.3, 132.8, 135.8 (Ar-H), 125.5, 129.4, 130.6, 144.7 (Ar-H), 105.5, 150.3 (Thiazole), 14.4, 28.6, ($-C_2H_5$); FT-IR: υ 3074 (-C-H=Aromatic stretching), 1687 (-C=O stretching), 1535 (-C=C- stretching), 1238 (-N-N=C- stretching), 691 (-C-S-C- = triazolo-thiadiazole) cm^{-1}; LC-MS *m/z* 509.1[M–H]+, (M=509.6); Anal. Calcd for $C_{26}H_{19}N_7OS_2$: C 61.28, H 3.76, N 19.24, S 12.58%. Found: C 61.25, H 3.72, N 19.21, S 12.55%.

N-(4-(4-methoxyphenyl)thiazol-2-yl)-2-(3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzamide ***(5e)***
Compound **5e** (M. Wt. 511.6g) was obtained in 65% yield as a light brown color solid; mp > 250 °C (dec); ^{1}H NMR: δ 9.18 (s, 1H, -NH-), 7.93, 8.77 (m, 4H, pyridine), 7.52-8.02 (m, 4H, Ar-H), 7.08, 7.57 (m, 4H, Ar-H), 3.86 (m, 3H, -OCH_3), 7.66 (s, 1H, thiazol); 13C NMR: δ 174.9 (-N=C-S-), 164.7 (-N=C-S-), 167.5 (-N=C-S-), 164.6 (-C=O), 151.8 (-N=C-N-), 149.3 (-N=C-C-,Py), 121.3, 134.2, 149.3 (Pyridine), 127.9, 128.4, 128.6, 131.9, 132.9, 135.4 (Ar-H), 114.2, 125.1, 128.5, 160.8 (Ar-H), 105.4, 150.5 (Thiazole), 55.6 (-OCH); FT-IR: υ 3076 (-C-H=Aromatic stretching), 1688 (-C=O stretching), 1532 (-C=C- stretching), 1224 (-N-N=C- stretching), 696 (-C-S-C- = triazolo-thiadiazole) cm^{-1}; LC-MS *m/z* 511.1[M–H]+, (M=511.6); Anal. Calcd for $C_{25}H_{17}N_7O_2S_2$: C 58.69, H 3.35, N 19.17, S 12.54%. Found: C 58.66, H 3.32, N 19.15, S 12.51%.

N-(4-(4-chlorophenyl)thiazol-2-yl)-2-(3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzamide ***(5f)***
Compound **5f** (M. Wt. 516.0g) was obtained in 68% yield as a light brown color solid; mp > 250 °C (dec); ^{1}H NMR: δ 9.11 (s, 1H, -NH-), 7.95, 8.71 (m, 4H, pyridine), 7.61-8.07 (m, 4H, Ar-H), 7.59, 7.93 (m, 4H, Ar-H), 7.63 (s, 1H, thiazol); 13C NMR: δ 174.7 (-N=C-S-), 164.5 (-N=C-S-), 167.2 (-N=C-S-), 164.6 (-C=O), 151.7 (-N=C-N-), 149.9 (-N=C-C-,Py), 121.4, 134.6, 149.9 (Pyridine), 127.5, 128.3, 128.9, 131.7, 132.4, 135.5 (Ar-H), 128.4, 129.8, 131.5, 134.9 (Ar-H), 105.2, 150.7 (Thiazole); FT-IR: υ 3076 (-C-H=Aromatic stretching), 1686 (-C=O stretching), 1539 (-C=C- stretching), 1238 (-N-N=C- stretching), 682 (-C-S-C- = triazolo-thiadiazole) cm^{-1}; LC-MS *m/z* 515.0[M–H]+, (M=516.0); Anal. Calcd for $C_{24}H_{14}ClN_7OS_2$: C 55.86, H 2.73, N 19.00, S 12.43%. Found: C 55.82, H 2.71, N 18.98, S 12.41%.

N-(4-(4-bromophenyl)thiazol-2-yl)-2-(3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzamide ***(5g)***
Compound **5g** (M. Wt. 560.4g) was obtained in 67% yield as a dark brown color solid; mp > 250 °C (dec); ^{1}H NMR: δ 9.14 (s, 1H, -NH-), 7.98, 8.73 (m, 4H, pyridine), 7.55-8.02 (m, 4H, Ar-H), 7.62, 7.77 (m, 4H, Ar-H), 7.68 (s, 1H, thiazol); 13C NMR: δ 174.3 (-N=C-S-), 164.6 (-N=C-S-), 167.4 (-N=C-S-), 164.8 (-C=O), 151.2 (-N=C-N-), 149.3 (-N=C-C-,Py), 121.6, 134.7, 149.3 (Pyridine), 127.7, 128.1, 128.7, 131.2, 132.8, 135.7 (Ar-H), 123.4, 128.5, 132.2, 132.7 (Ar-H), 105.6, 150.8 (Thiazole); FT-IR: υ 3066 (-C-H=Aromatic stretching), 1688 (-C=O stretching), 1529 (-C=C- stretching), 1233 (-N-N=C- stretching), 698 (-C-S-C- = triazolo-thiadiazole) cm^{-1}; LC-MS *m/z* 560.1[M–H]+, (M=560.4); Anal. Calcd for $C_{24}H_{14}BrN_7OS_2$: C 51.43, H 2.52, N 17.49, S 11.44%. Found: C 51.41, H 2.51, N 17.47, S 11.42%.

N-(4-(4-fluorophenyl)thiazol-2-yl)-2-(3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzamide ***(5h)***
Compound **5h** (M. Wt. 499.5g) was obtained in 65% yield as a dark brown color solid; mp > 250 °C (dec); ^{1}H NMR: δ 9.19 (s, 1H, -NH-), 7.94, 8.76 (m, 4H, pyridine), 7.53-8.03 (m, 4H, Ar-H), 7.36, 8.13 (m, 4H, Ar-H), 7.68 (s, 1H, thiazol)**;** 13C NMR: δ 174.1 (-N=C-S-), 164.4 (-N=C-S-), 167.7 (-N=C-S-), 164.3 (-C=O), 151.2 (-N=C-N-), 149.3 (-N=C-C-,Py), 121.5, 134.2, 149.3 (Pyridine), 127.4, 128.1, 128.7, 131.4, 132.8, 135.6 (Ar-H), 116.7, 128.5, 130.4, 162.7 (Ar-H), 105.4, 150.7 (Thiazole); FT-IR: υ 3068 (-C-H=Aromatic stretching), 1684 (-C=O stretching), 1520 (-C=C- stretching), 1237 (-N-N=C- stretching), 691 (-C-S-C- = triazolo-thiadiazole) cm^{-1}; LC-MS *m/z* 499.1[M–H]+, (M=499.5); Anal. Calcd for $C_{24}H_{14}FN_7OS_2$: C, 57.70, H 2.82, N 19.63, S 12.84%. Found: C, 57.69, H 2.80, N 19.61, S 12.82%.

N-(4-(4-nitrophenyl)thiazol-2-yl)-2-(3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzamide ***(5i)***
Compound **5i** (M. Wt. 526.5g) was obtained in 64% yield as a dark brown color solid; mp > 250 °C (dec); ^{1}H NMR: δ 9.13 (s, 1H, -NH-), 7.98, 8.77 (m, 4H, pyridine), 7.51-8.06 (m, 4H, Ar-H), 8.06, 8.33 (m, 4H, Ar-H), 7.66 (s, 1H, thiazol)**;** 13C NMR: δ 174.7 (-N=C-S-), 164.5 (-N=C-S-), 167.9 (-N=C-S-), 164.2 (-C=O), 151.6 (-N=C-N-), 149.8 (-N=C-C-,Py), 121.7, 134.4, 149.8 (Pyridine), 127.3, 128.4, 128.8, 131.6, 132.7, 135.9 (Ar-H), 124.9, 126.8, 139.5, 147.3 (Ar-H), 105.2, 150.6 (Thiazole); FT-IR: υ 3067 (-C-H=Aromatic stretching), 1689 (-C=O stretching), 1522 (-C=C- stretching), 1238 (-N-N=C- stretching), 696 (-C-S-C- = triazolo-thiadiazole) cm^{-1}; LC-MS *m/z* 526.1[M–H]+, (M=526.5); Anal. Calcd for $C_{24}H_{14}N_8O_3S_2$: C 54.74, H 2.68, N 21.28, S 12.18%. Found: C 54.72, H 2.67, N 21.26, S 12.15%.

N-(4-(4-hydroxyphenyl)thiazol-2-yl)-2-(3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzamide ***(5j)***
Compound **5j** (M. Wt. 497.6g) was obtained in 67% yield as a light brown color solid; mp > 250 °C (dec); ^{1}H NMR: δ 9.16 (s, 1H, -NH-), 7.97, 8.78 (m, 4H, pyridine), 7.56-8.06 (m, 4H, Ar-H), 6.83, 7.47 (m, 4H, Ar-H), 5.39 (s, 1H, -OH), 7.64 (s, 1H, thiazol)**;** 13C NMR: δ 174.5 (-N=C-S-), 164.2 (-N=C-S-), 167.9 (-N=C-S-), 164.1 (-C=O), 151.3 (-N=C-N-), 149.7 (-N=C-C-,Py), 121.8, 134.7, 149.7 (Pyridine), 127.1, 128.4, 128.7, 131.4, 132.9, 135.7 (Ar-H), 116.8, 125.8, 128.3, 158.9 (Ar-H), 105.8, 150.7 (Thiazole); FT-IR: υ 3062 (-C-H=Aromatic stretching), 1686 (-C=O stretching), 1524 (-C=C- stretching), 1234 (-N-N=C- stretching), 693 (-C-S-C- = triazolo-thiadiazole) cm^{-1}; LC-MS *m/z* 497.1[M–H]+, (M=497.6); Anal. Calcd for $C_{24}H_{15}N_7O_2S_2$: C 57.94, H 3.04, N 19.71, S 12.89%. Found: C 57.92, H 3.02, N 19.70, S 12.87%.

RESULTS AND DISCUSSION

Synthesis of compounds 5a–j

To the best of our knowledge, compounds **5a–j** has not been reported previously. The characterization of the reaction product provided the first unambiguous proof of the successful synthesis of N-(4-phenylthiazol-2-yl)-2-(3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzamide derivatives. Elemental analysis of all compounds was in good agreement with proposed structures as mentioned in scheme 1. The structures of all compounds were consistent with the FT–IR, ^{1}H NMR, ^{13}C NMR and LC-MS.

IR spectral features provide valuable information regarding the nature of functional group attached [9]. In order to study the bonding mode of compound 3 to the compound 5a-j, the IR spectrum of compound 3 was compared with the spectra of compound 5a-j. Considerable differences to be expected were observed. The FT–IR spectrum of **5a–j** showed the most relevant peaks of triazolo-thiadiazole ring. The band around 1680 cm^{-1} and 1533 cm^{-1} corresponding respectively to –C=N stretching and –C=C– stretching. The band around 1230 cm^{-1} and 688 cm^{-1} corresponding respectively to –N–N=C– banding and –C–S–C– banding indicating the formation of triazolo-thiadiazole derivatives.

Inspection of IR spectra of 5a-j, 3 and 4a-g reveals discernible differences. The important band due to –COOH group of 4a-g appeared [9] at 1680 cm^{-1} almost disappeared in IR spectra of 5a-j. The bands due to –NH_2 and –SH groups observed [9] in the spectrum of 3 are almost vanished in the IR spectra of 5a-j.

The ^{1}H NMR spectra of 5a-g are identical in almost all aspects. Only new signal due to substitution group is appeared at its respectable position e.g. 5b, 5c, 5d, 5e and 5j. Other detail data of each compound are presented in experimental section. All the data suggest the predicted structure shown in scheme-1.

The expected structure was thus clearly verified by the spectroscopic analysis which indicated moreover the absence of any detectable impurity, particularly of the two reagents used to prepare 5a–j. which again supported by the LC-MS Spectral features.

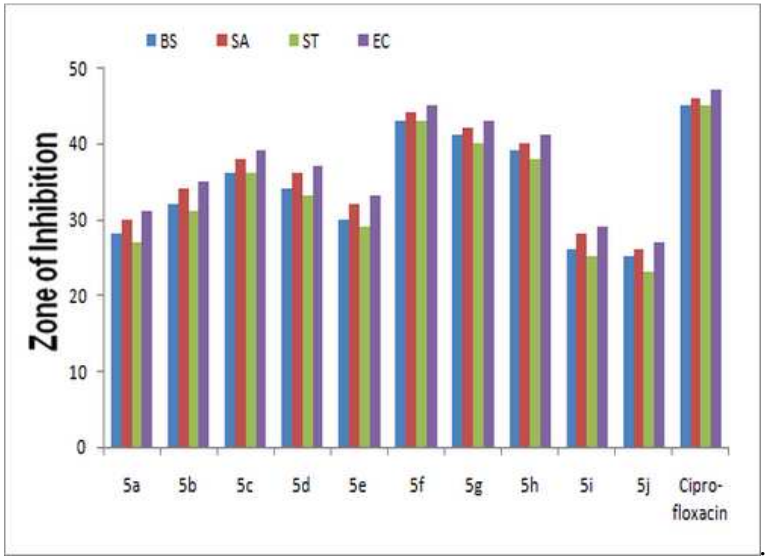


Figure 1: Antibacterial activity of compounds 5a–j

Biological activity

Antibacterial activity

Based on the data from the antibacterial studies against both Gram–positive and Gram–negative bacterial strains (Figure 1), the following observations can be made. All compounds (5a–j) exhibited antibacterial activity against both Gram–positive and Gram–negative bacterial strains with zones of inhibition (ZOI) ranging from 23 mm to 45 mm (Figure 2). Among the analogs 5a–j, compound 5f ($ZOI_{[BS]}$ = 43 mm, $ZOI_{[SA]}$ = 44 mm, $ZOI_{[ST]}$ = 43 mm, $ZOI_{[EC]}$ = 45 mm) and compound 5g ($ZOI_{[BS]}$ = 41 mm, $ZOI_{[SA]}$ = 42 mm, $ZOI_{[ST]}$ = 40 mm, $ZOI_{[EC]}$ = 43 mm) was identified as a potent antibacterial agent against all Gram-positive and Gram-negative bacterial strains. Compound 5h ($ZOI_{[BS]}$ = 39 mm, $ZOI_{[SA]}$ = 40 mm, $ZOI_{[ST]}$ = 38 mm, $ZOI_{[EC]}$ = 41 mm), compound 5c ($ZOI_{[BS]}$ = 36 mm, $ZOI_{[SA]}$ = 38 mm, $ZOI_{[ST]}$ = 36 mm, $ZOI_{[EC]}$ = 39 mm) and compound 5d ($ZOI_{[BS]}$ = 34 mm, $ZOI_{[SA]}$ = 36 mm, $ZOI_{[ST]}$ = 33 mm, $ZOI_{[EC]}$ = 37 mm) had good antibacterial activity against bacterial strains. Compound 5b ($ZOI_{[BS]}$

= 32 mm, $ZOI_{[SA]}$ = 34 mm, $ZOI_{[ST]}$ = 31 mm, $ZOI_{[EC]}$ = 35 mm), compound 5e ($ZOI_{[BS]}$ = 30 mm, $ZOI_{[SA]}$ = 32 mm, $ZOI_{[ST]}$ = 29 mm, $ZOI_{[EC]}$ = 33 mm) and compound 5a ($ZOI_{[BS]}$ = 28 mm, $ZOI_{[SA]}$ = 30 mm, $ZOI_{[ST]}$ = 27 mm, $ZOI_{[EC]}$ = 31 mm) also had comparable antibacterial activity against bacterial strains. Compounds 5i and 5j exhibited less antibacterial activity. Compounds 5a–j exhibited less antibacterial activity as compare to standard antibiotic drug, ciprofloxacin ($ZOI_{[BS]}$ = 45 mm, $ZOI_{[SA]}$ = 46 mm, $ZOI_{[ST]}$ = 45 mm, $ZOI_{[EC]}$ = 47 mm).

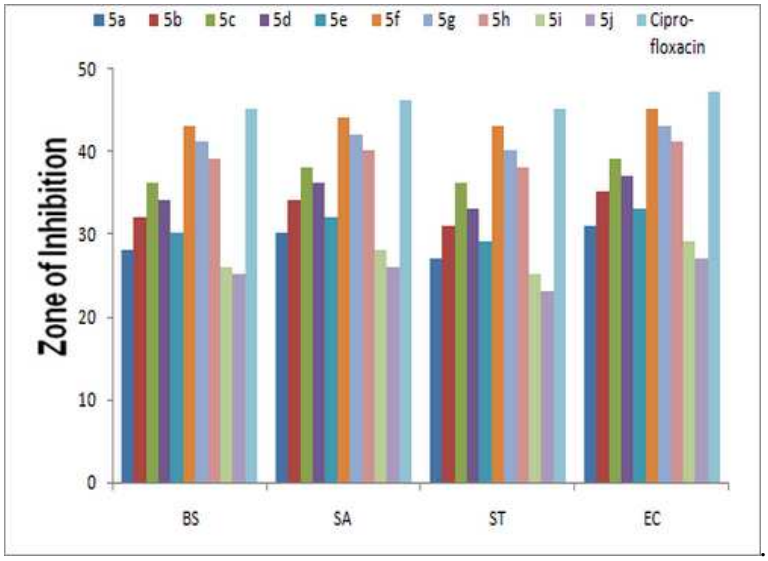


Figure 2: Comparative antibacterial activity of compounds 5a–j

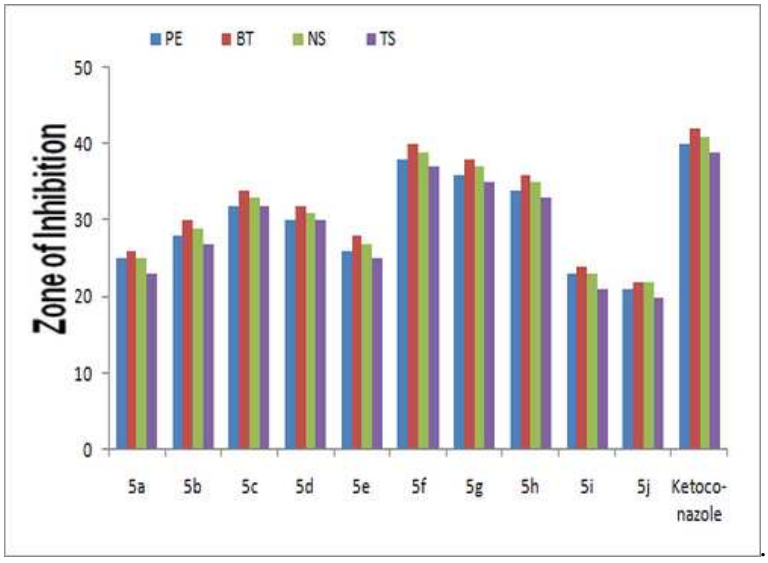


Figure 3: Antifungal activity of compounds 5a–j

Antifungal activity:

Based on the screening data from the antifungal studies (Figure 3), the following observations can be made. All compounds (5a–j) exhibited antifungal activity against different fungal strains (Figure 4). Among the analogs 5a–j, compound 5f ($ZOI_{[PE]}$ = 38 mm, $ZOI_{[BT]}$ = 40 mm, $ZOI_{[NS]}$ = 39 mm, $ZOI_{[TS]}$ = 37 mm) and Compound 5g ($ZOI_{[PE]}$ = 36 mm, $ZOI_{[BT]}$ = 38 mm, $ZOI_{[NS]}$ = 37 mm, $ZOI_{[TS]}$ = 35 mm) was found more active against all fungal strains. Compound 5h ($ZOI_{[PE]}$ = 34 mm, $ZOI_{[BT]}$ = 36 mm, $ZOI_{[NS]}$ = 35 mm, $ZOI_{[TS]}$ = 33 mm), compound 5c ($ZOI_{[PE]}$ = 32 mm, $ZOI_{[BT]}$ = 34 mm, $ZOI_{[NS]}$ = 33 mm, $ZOI_{[TS]}$ = 32 mm) and compound 5d ($ZOI_{[PE]}$ = 30 mm, $ZOI_{[BT]}$ = 32 mm, $ZOI_{[NS]}$ = 31 mm, $ZOI_{[TS]}$ = 30 mm) also had good antifungal activity against fungal strains. Compound 5b ($ZOI_{[PE]}$ = 28 mm, $ZOI_{[BT]}$ = 30 mm, $ZOI_{[NS]}$ = 29 mm, $ZOI_{[TS]}$ = 27 mm), compound 5e ($ZOI_{[PE]}$ = 26 mm, $ZOI_{[BT]}$ = 28 mm, $ZOI_{[NS]}$ = 27 mm, $ZOI_{[TS]}$ = 25 mm) and compound 5a ($ZOI_{[PE]}$ = 25 mm, $ZOI_{[BT]}$ = 26 mm, $ZOI_{[NS]}$ = 25 mm, $ZOI_{[TS]}$ = 23 mm) also had comparable antifungal activity against bacterial strains. Compounds 5i and 5j exhibited less antifungal activity. All compounds (5a–j) exhibited less antifungal activity as compare to standard antibiotic drug, ketoconazole ($ZOI_{[PE]}$ = 40 mm, $ZOI_{[BT]}$ = 42 mm, $ZOI_{[NS]}$ = 41 mm, $ZOI_{[TS]}$ = 39 mm).

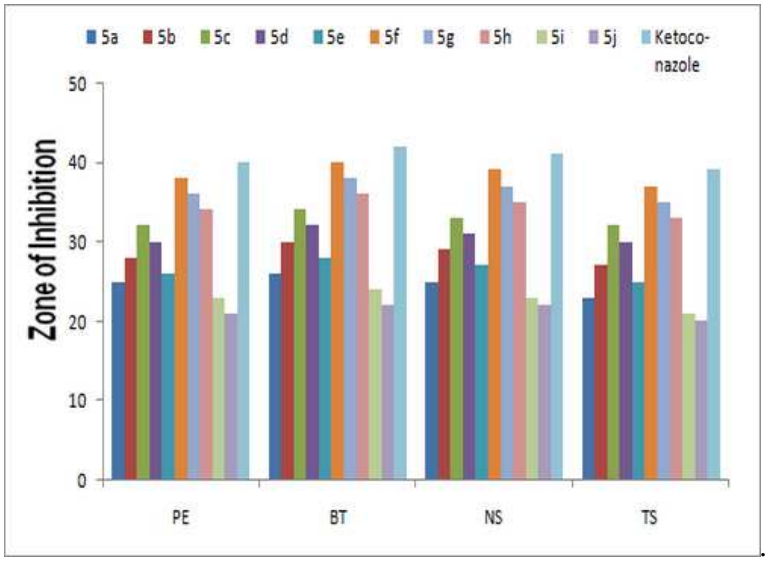


Figure 4: Comparative Antifungal activity of compounds 5a–j

Biological activity

Antibacterial activity (in vitro)

Compounds (5a–j) were screened for in vitro antibacterial activity against Gram-positive bacterial strains (*Bacillus subtilis* [BS] and *Staphylococcus aureus* [SA]) and Gram-negative bacterial strains (*Salmonella typhimurium* [ST] and *Escherichia coli* [EC]) utilizing the agar diffusion assay [13-14]. The wells were dug in the media with the help of a sterile metallic borer. Recommended concentration (100 µl) of the test sample (1 mg/mL in DMSO) was introduced in the respective wells. Other wells supplemented with DMSO and reference antibacterial drug, ciprofloxacin were served as negative and positive controls, respectively. The plates were incubated immediately at 37˚C for 24 hours. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was compared with the standard drug. In order to clarify any participating role of DMSO in the biological screening, separate studies were carried out with the solutions alone of DMSO and they showed no activity against any bacterial strains.

Antifungal activity (in vitro)

Compounds (5a–j) were also examined for antifungal activity against different fungal strains, i.e. *Penicillium expansum* [PE], *Botryodiplodia theobromae* [BT], *Nigrospora sp.* [NS], *Trichothesium sp.* [TS]. The antifungal drug, ketoconazole was used as a positive control. Antifungal screening for compounds (5a–j) and positive control was performed at a recommended concentration. The fungal strains were grown and maintained on potato dextrose agar plates. The cultures of the fungi were purified by single spore isolation technique. Each compound (5a–j) in DMSO solution was prepared for testing against spore germination of each fungus. The fungal culture plates were inoculated and incubated at 25± 2˚C for 48 h. The plates were then observed and the diameters of the zone of inhibition (in mm) were measured. The percentage inhibition for fungi was calculated after five days using the formula given below:

Percentage of inhibition = 100(X-Y) / X

Where, X = Area of colony in control plate
Y = Area of colony in test plate

CONCLUSION

A novel series of heterocyclic compounds N-(4-phenylthiazol-2-yl)-2-(3-(pyridin-4-yl)-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazol-6-yl)benzamide derivatives **5a–j** have been duly synthesized and characterized. Antibacterial activities were studied against gram positive and gram negative bacteria and antifungal activities of all the compounds were studied against various fungi. All the compounds were found biologically active.

Acknowledgements
One of the authors Dr. H. S. Patel is greatly thankful to the authorities of University Grants Commission, New Delhi for awarding him Faculty Fellowship and Gaurav K. Patel is thankful to the authorities of Sardar Patel University for providing necessary research facilities.

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.; Sarafroz, M. *Acta Pol. Pharm. Drug Res*. **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] Shruthy, S. V.; Shakkeela, Y. *Int. J. Pharm. Pharm. Sci*. **2014,** 6**,** 271.
[8] Patel, G. K.; Patel, Y. S.; Patel, H. S. *Int. J. Chem. Tech Appl*. **2014,** 3**,** 1.
[9] Silverstein, R.M.; Webste, F.X. Spectrometric Identification of Organic Compounds, 6th ed.; John Wiley & Sons: New York, **2004**.
[10] Muhammad, H.; Muhammad, S.; Muhammad, T. H.; Nasim, H. R.; Sumera, Z.; Muhammad, A. M. A.; Peter, G. J.; Jamshed, I. *J. Braz. Chem. Soc*. **2012,** 23**,** 854.
[11] Mahajan, D. P.; Bendre, R. S. *Asian J. Biochem. Pharm. Res*. **2014,** 4**,** 103.
[12] Gilani, S. J.; Khan, S. A.; Alam, O.; Siddiqui, N. *Acta Pol. Pharm. Drug Res*. **2011,** 68**,** 205.
[13] Alam, S. *J. Chem. Sci*. **2004,** 166**,** 325.
[14] Pelczar, M. J.; Chan, E.C.S.; Krieg, N.R. Antibiotics and other chemotherapeutic agents in microbiology, 5th ed.; Blackwell Science: New York, **1998**.