Journal of Saudi Chemical Society (2017) **21**, S403–S414

King Saud University

Journal of Saudi Chemical Society

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ORIGINAL ARTICLE

A facile synthesis of new 5-aryl-thiophenes bearing sulfonamide moiety via Pd(0)-catalyzed Suzuki–Miyaura cross coupling reactions and 5-bromothiophene-2-acetamide: As potent urease inhibitor, antibacterial agent and hemolytically active compounds

Mnaza Noreen [a], Nasir Rasool [a,*], Yasmeen Gull [a], Faiz-ul-Hassan Nasim [b], Ameer Fawad Zahoor [a], Asma Yaqoob [b], Shazia Kousar [a], Muhammad Zubair [a], Iftikhar Hussain Bukhari [a], Usman Ali Rana [c]

[a] *Department of Chemistry, Government College University, Faisalabad 38000, Pakistan*
[b] *Department of Chemistry, The Islamia University of Bahawalpur, Bahawalpur 63000, Pakistan*
[c] *Deanship of Scientific Research, College of Engineering, King Saud University, PO Box 800, Riyadh 11421, Saudi Arabia*

Received 13 December 2013; revised 17 April 2014; accepted 26 April 2014
Available online 16 May 2014

KEYWORDS

Thiophene sulfonamides;
Palladium;
Suzuki cross coupling;
Catalysis;
Antibacterial activity;
Urease inhibition activity;
Hemolytic activity

Abstract The present study reports a convenient approach for the synthesis of thiophene sulfonamide derivatives (**3a**–**3k**) via Suzuki cross coupling reaction. This method of synthesis involved the reactions of various aryl boronic acids and esters with 5-bromthiophene-2-sulfonamide (**2**) under mild and suitable temperature conditions. The compounds synthesized in the present study were subjected to urease inhibition and hemolytic activities. The substitution pattern and the electronic effects of different functional groups (i.e., Cl, CH_3, OCH_3, F etc.) available on the aromatic ring are found to have significant effect on the overall results. The compound *5-Phenylthiophene-2-sulfonamide* **3a** showed the highest urease inhibition activity with IC_{50} value ~ 30.8 μg/mL compared with the thiourea (used as standard) having IC_{50} value ~ 43 μg/mL. Moreover, almost all of the compounds were examined for the hemolytic activity against triton X-100 with positive results obtained

* Corresponding author. Tel.: +92 332 7491790; fax: +92 41 9201032.
E-mail address: nasirhej@yahoo.co.uk (N. Rasool).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.jscs.2014.04.007

in most of the cases. In addition, the antibacterial activities of the derivatives of 5-arylthiophene-2-sulfonamide and 5-bromothiophene-2-acetamide were also investigated during the course of the study.

1. Introduction

The Suzuki cross coupling reaction has a great deal of importance in the synthesis of substituted aromatic compounds of biological importance. The relatively easy formation of C-C bonds via Suzuki cross coupling reaction may have its origin in the high tolerance of functional group through this reaction pathway [13]. Many heterocycles such as arylated furans and thiophenes have been synthesized in high yield using this reaction scheme [14]. The major interests in the developments of thiophenes are due to their wide range of device applications such as light emitting devices, dye sensitized organic solar cells, and organic TFT's and many others [2]. Some of the important characteristics that make thiophenes and thiophenes based polymers an important class of chemical compounds are their high electrical conductivity [Poly(3,4-ethylenedioxythiophene) or PEDOT is a conducting polymer], liquid crystalline characteristics and interesting light responsive characteristics [19]. In view of these interesting characteristics of thiophene, it is highly desirable to synthesize thiophene with different functional groups, and one such facile way to synthesize thiophene derivatives is via palladium catalyzed Suzuki cross coupling reactions. Being versatile in nature and demonstrating compatibility with functional groups, the Suzuki cross coupling reaction is the method of choice for reactions involving C—C bond formation [17].

Among the wide range of pharmaceutical compounds, the sulfonamide functional groups are found to play a key role in designing the drugs [3]. Sulfonamides represent an important class of biologically productive molecules, which exhibit broad diversity of biological activities [12]. Some of their important applications include the treatment of many microbial infections by inhibiting the growth of gram negative and gram positive bacteria [24]. Significant biological activities have been shown by compounds bearing sulfonamide moieties, which make them important in drug discovery [10]. Sulfonamides usually get attached to the Dihydropteroate (DHPS) enzyme, which then catalyzes the bacterial pathways in folic acid and few eukaryotic cells [6]. In the case of human cells, mechanism does not obey [1]. Thiophene-2-sulfonamides are also known as carbonic anhydrase inhibitors and the literature suggests that many of its simple derivatives also show diuretic activity [5]. The barbituric and thiobarbituric acid based sulfonamides contain coordinating sites to nickel (II) urease sites. Earlier, it was reported that the electronic effects and presence of different functional groups on aromatic rings can affect the urease inhibitory activity. Electronic and Steric factors also showed great influence on the biological activities [27]. Jahan and coworkers reported that the compounds having halogen functional groups exhibit the highest hemolytic activity [16]. In routine, the sulfonamides are prepared by the reaction of a sulfonyl chloride with ammonia or with primary or secondary amines [20], but this methodology has some limitations and drawbacks. As an example, sulfonyl chlorides are not suitable for long term storage and due to their instability, handling of sulfonyl chlorides is rather difficult [8]. A rather convenient route to synthesize sulfonamides is via intermolecular free radical reactions of pentafluorophenylvinyl sulfonate with subsequent aminolysis reaction [7].

In order to explore an yet easy and facile method to synthesize thiophene based derivates, the aim of the present study is to synthesize different derivatives of 5-bromothiophene-2-sulfonamide via Suzuki cross coupling reactions using different aryl boronic acids and aryl boronic esters with 5-bromothiophene-2-sulfonamide. The results from the present study revealed that the 5-bromothiophene-2-sulfonamide has been found active against many diseases. In view of this finding, we investigated the anti-urease, antibacterial and hemolytic or cytotoxicity activities of these derivatives. To the best of our knowledge, this work has not been reported elsewhere so far.

2. Experimental

2.1. General

For synthesis, analytical grade reagents and chemicals were purchased from Sigma Aldrich and Alfa Aesar. The Buchi melting point B-540 apparatus was used to record melting points. The Proton ^{1}H-NMR and Carbon 13, ^{13}C-NMR spectra were obtained in $CDCl_3$ or CD_3OD on the Bruker Aspect AM-400 NMR. The chemical shift values were recorded in Delta (δ) ppm, whereas the coupling constant was obtained and recorded in the units of Hertz (Hz). JMS-HX-110 spectrophotometer with a data system was used to record EI-MS spectra. Column chromatography technique was used to purify compounds. For column chromatography, silica gels of mesh size 230–400 and 70–230 were used. Moreover, the Merck silica gel $60PF_{254}$ TLC cards were used to monitor the reaction and newly synthesized compounds were detected/visualized by UV lamp (254–365).

2.2. Synthesis of 5-bromothiophene-2-sulfonamide (2)

For the synthesis of **2** from **1**, to a solution of 40–60 mmol of freshly distilled chlorosulfonic acid in 6.00 mL of CCl_4, 12 mmol of bromothiophene was added drop wise with vigorous stirring and subsequent cooling up to −30 °C. Stirring was done at −25 °C for another 30 min and then at room temperature for 30 min. After this, the solution was poured onto the crushed ice. In this way, the organic layer got separated, and the solvent was removed under reduced pressure. Later on, about 50 ml of 25% ammonia solution was mixed with the residue and was kept for 3 h and neutralized with 10% HCl. The final product (5-bromothiophene-2-sulfonamide precipitates)

was filtered, dried and later compared with the previously reported compound [31].

2.3. Preparation of 5-bromothiophene-2-sulfonyl acetamide (*4*)

A mixture of 5-bromothiophene-2-sulfonamide (2 mol) and acetic anhydride (3.1 mol) in acetonitrile (5.00 mL) was treated with few drops of concentrated sulfuric acid under nitrogen atmosphere. The reaction mixture was stirred for 40 min at 60 °C and then 15–20 mL distilled water was added to this mixture with continuous stirring to form precipitates. Further stirring was carried out for 1 h at room temperature. The solution containing precipitates was then filtered and the obtained precipitates were subsequently washed with water and dried later. Further purification and identification of the final product were done by flash chromatography and spectroscopic techniques, respectively [21].

2.4. General procedure for the synthesis of 5-arylthiophene-2-sulfonamide (*3a–k*)

In a 0.704 mmol solution of 5-bromothiophene-2-sulfonamide about 5 mol% $Pd(PPh_3)_4$ was added in dioxane (4.00 mL) under nitrogen atmosphere. The reaction mixture was then stirred for 30 min at room temperature. After facile mixing, the aryl boronic acids, aryl boronic esters (0.774 mmol) and potassium phosphate (1.409 mmol) were added with the addition of 1.00 mL water (solvent/H_2O 4:1) under nitrogen atmosphere. The solution was stirred at 95 °C for 30 h and then subsequently cooled to room temperature. Extraction was carried out with ethyl-acetate to obtain the organic layer that was later filtered and dried by the addition of $MgSO_4$. The solvent was then removed under reduced pressure. The residue obtained was purified by column chromatography using ethyl-acetate and *n*-hexane with the 50% ratio to obtain the desired product. The final product was characterized by spectroscopic techniques.

2.5. Hemolytic activity

The cytotoxicity studies of newly synthesized derivatives of 5-bromothiophene-2-sulfonamide were carried out by the use of hemolytic activity according to the method reported by [29]. The solutions of the compounds under investigation were prepared at a concentration of 1 mg/mL in 10% ethyl acetate solvent. Heparinized three ml human fresh blood was homogeneously mixed and spilled into 15.00 mL sterile falcon tube and centrifuged for 5 min. Sterile isotonic phosphate buffer saline chilled (4 °C) solution (5.00 mL) with pH range 7.4 was used to wash it for three times after removing supernatant. Washed red blood cells were suspended in 20.00 mL chilled PBS. Hemocytometer was used to count the erythrocytes. 7.068×10^8 red blood cells per mL count were maintained for each essay. In 20 μL of compound 180 μL diluted blood cell was added 180 μL and suspended in eppendrofs, and incubated at 37 °C for 35 min. After incubation, place the tubes in ice bath for 5 min and again centrifuge for another 5 min. Once, the process of centrifugation completed, the supernatant was collected carefully, diluted with the addition of chilled 900 μL of PBS. Later on, all eppendrofs were kept in ice bath and from each eppendrof about 200 μL solution was added into 96 well plates. Triton X-100 with the concentration of 0.1% was taken for each essay as positive control, while phosphate buffer was taken as negative control. Absorbance of the solutions was determined at 576 nm with a micro plate reader [26].

2.6. Urease inhibition activity

25 μL of the enzyme Jack bean urease was added to the 55 μL of 100 mM urea containing buffer solution in a conical flask. This mixture of solution was then incubated with (0.5 mM conc.) 15 μL of newly synthesized compounds for 15 min at 30 °C in 96-well plates. Anti-urease activity was examined by using the Indophenol method (13) of determining the production of ammonia. In each well, 45 μL of phenol reagent (0.005 % W/V sodium nitroprusside and 1% W/V phenol) and 70 μL of alkali reagent (NaOH 0.5% W/V and 0.1% NaOCl) were added. After 50 min, the increase in absorbance values of each sample was measured at 630 nm by using a micro plate reader. All reactions were repeated three times to obtain ~200 μL of the final volume. Softmax pro software was used to record the change in absorbance values. All assays were carried out at the specific pH value ~6.8. The % inhibition value was calculated using the relation [100-(OD test well/OD control) × 100]. Thiourea was used as the standard urease inhibitor [11]. EZ-fit kinetic database was used to determine IC_{50} values [25]. While, in the cases of colored compounds, blank samples were also prepared. The absorbance value of the blank sample was subtracted from the absorbance value of the sample in order to obtain the corrected absorbance of sample. These corrected values of absorbance for each sample were used to calculate % age inhibition.

2.7. Antibacterial assay

The antibacterial activities of newly synthesized compounds (5-arylthiophene-2-sulfonamide and 5-bromothiophene-2-acetamide) were determined by using the method described by Patel et al. According to this method, an increase in the growth of microbial cells results in an increase in the microbial number [28]. In the present study, antibacterial activity of as-synthesized compounds were determined against the bacterial strains [such as *Escherichia coli* (Gram negative), *Staphylococcus aureus* (Gram positive), *Pseudomonas aeroginosa* (Gram negative), *Bacillus subtilis* (Gram positive), *Shigellasonnei* (Gram negative) and *Salmonella typhi* (Gram negative)], which were acquired from the Agha khan University in Karachi, Pakistan. Streptomycin was used as positive control to compare the sensitivity of the strains. The antibacterial activity of all compounds under investigation was measured by the 96 well plate method optimized in our laboratory. In each well, about 175 μL of sterilized broth was added and inoculated with 5 μL glycerol stock of a specific bacterial strain. The initial absorbance reading was maintained strictly between 0.12–0.19, and the bacteria were allowed to grow overnight in an incubator. After a waiting time (~12 h), about 20 μL of the test sample was added to the pre-determined wells, (test concentration of sample was 20 μg/well) and the total volume of each well was kept 200 μL. The plates were further incubated for 16–24 h at 37 °C. Elisa plate reader was used to measure the absorbance at 630 nm. The difference in absorbance values was recorded and was later used as an index of bacterial

growth. The results of antibacterial activity of newly synthesized compounds are outlined in the Tables 5–7.

Following formula was used to determine Percentage inhibition.

$$\text{Percentage Inhibition} = \frac{\text{O.D. of positive control} - \text{O.D. of sample} \times 100}{\text{O.D. of positive control}}$$

*2.7.1. 5-Phenylthiophene-2-sulfonamide (**3a**)*

M.P. 183–186 °C; ^{1}H-NMR (400 MHz, $CDCl_3$ + CD_3OD): δ = 7.50–7.10 (m, 5H-Ar, 2H-Thiophene), 7.49 (s, 2H-NH_2). ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD): δ = 127.1 128.0, 128.9, 129.5, 130.0, 132.0, 135.1, 139.5; EIMS (*m*/*z*, +ion mode): 239.00 $[M]^+$; $[M–NH_2–C_6H_6]^+$ = 147. Anal calcd for $C_{10}H_9NO_2S_2$: C, 50.19; H, 3.79; N, 5.85; O, 13.37; S, 26.80. found: C, 50.25; H, 3.92; N, 5.98; O, 13.80; S, 26.98.

*2.7.2. 5-(3-cyno-5-(trifluoromethyl)phenyl)thiophene-2-sulfonamide (**3b**)*

M.P. 155–158 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.06–7.0 (m, 3H-Ar, 2H-Thiophene), 7.51 (s, 2H, NH_2)[a]. ^{13}C-NMR (100 MHz, $CDCl_3$ + CD_3OD): δ = 114.1, 119.1, 124.0, 127.1, 128.1, 128.7, 129.2, 130.9, 132.8, 134.9, 135.1, 139.2; EIMS (*m*/*z*, + ion mode): 331.99 $[M]^+$; $[M–CF^3–CN]^+$ = 240.1 ; $[M–O_2]^+$ = 301.08; [M - NH_2]$^+$ = 317.08. Anal calcd for $C_{12}H_7N_2F_3O_2S_2$: C, 43.37; H, 2.12; N, 8.43; O, 9.63; S, 19.30. found: C, 43.50; H, 2.92; N, 8.98; O, 9.80; S, 19.62.

*2.7.3. 5-(3,5-bis(trifluoromethyl)phenyl)thiophene-2-sulfonamide (**3c**)*

M.P. 147–150 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.9–7.10 (m, 3H-Ar, 2H-Thiophene), 7.9 (s, 2H-NH_2). ^{13}C-NMR (100 MHz, $CDCl_3$ + CD_3OD): δ = 123.3, 125.1, 127.3, 129.2, 130.4, 133.2, 134.6, 136.4, 139.8; EIMS (*m*/*z*, +ion mode): 375.00 $[M]^+$. Anal calcd for $C_{12}H_7NF_6O_2S_2$: C, 38.40; H, 1.88; N, 3.73; O, 8.53; S, 17.09. found: C, 39.00; H, 1.92; N, 3.79; O, 8.61; S, 17.12.

*2.7.4. 5′-chloro-2,2′-bithiophene-5-sulfonamide (**3d**)*

M.P. 152–153 °C;^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.67–7.11 (m 3H-Thiophene), 7.52 (s, 2H-NH_2), 7.1 (d, *J* = 4,1H-thiophene). ^{13}C NMR(100 MHz, $CDCl_3$ + CD_3OD): δ = 112.5,127.9,128.6,129.4,130.6,132.9,137.1,138.5; EIMS (*m*/*z*, −ion mode): 278.01 $[M]^-$; $[M–NH_2]^-$ = 264; $[M–O_2]^-$ = 242.08. Anal calcd for $C_8H_6NClO_2S_3$: C, 34.34; H, 2.16; N, 5.01; O, 11.44; S, 34.38. found: C, 34.42; H, 2.20; N, 5.34; O, 11.48; S, 34.42.

*2.7.5. 5′-methyl-2,2′-bithiophene-5-sulfonamide (**3e**)*

M.P. 154–156 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.73–7.09 (m, 3H-Thiophene), 7.51 (s, 2H-NH_2), 7.0 (d, *J* = 4.2, 1H-Thiophene), 2.40 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$ + CD_3OD): δ = 16.1,125.5,127.9,128.4,129.2,133.2,137.6,137.1, 139.3; EIMS (*m*/*z*, +ion mode): 260.0 $[M]^+$; $[M–NH_2–SO_2]^+$ = 180.01; $[M–O_2]^+$ = 226.0. Anal calcd for $C_9H_9NO_2S_3$: C, 41.68; H, 3.50; N, 5.40; O, 12.34; S, 37.09. found: C, 41.72; H, 3.58; N, 5.48; O, 12.40; S, 37.10.

*2.7.6. 5-p-tolylthiophene-2-sulfonamide (**3f**)*

M.P. 144–147 °C;^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.68–7.11 (m, 4H-Ar, 2H-Thiophene), 7.49 (s, 2H,NH_2), 2.36 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$ + CD_3OD): δ = 29.6, 127.4, 127.5, 128.1, 130.3, 132.4, 134.2, 134.1, 139.0; EIMS (*m*/*z*, +ion mode): 254.25 $[M]^-$; $[M–CH_3–SO_2]^-$ = 175.05. Anal calcd for $C_{11}H_{11}NO_2S_2$: C, 52.15; H, 4.38; N, 5.53; O, 12.63; S, 25.31. found: C, 52.24; H, 4.42; N, 5.58; O, 12.72; S, 25.38.

*2.7.7. 5-(4-methoxyphenyl)thiophene-2-sulfonamide (**3g**)*

M.P. 145.2–146 °C;^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.6–6.9 (m, 4H-Ar, 2H-Thiophene), 7.49 (s, 2H,NH_2), 3.82(s, 3H,OCH_3). ^{13}C-NMR (100 MHz, $CDCl_3$ + CD_3OD): δ = 55.4, 114.9, 127.9, 128.1, 129.0, 130.1, 135.0, 139.1, 158.0; EIMS (*m*/*z*, +ion mode): 270.00 $[M]^+$; $[M–NH_2–SO_2]^+$ = 190.05; $[M–OCH3]^+$ = 240.17; $[M–Benzene]^+$ = 190.00. Anal calcd for $C_{11}H_{11}NO_3S_2$: C, 49.05; H, 4.12; N, 5.20; O, 17.82; S, 23.81. found: C, 50.02; H, 4.18; N, 5.26; O, 17.88; S, 23.88.

*2.7.8. 5-(4-chlorophenyl)thiophene-2-sulfonamide (**3h**)*

M.P. 131.8–133.4 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.63–7.0 (m, 4H-Ar, 2H-Thiophene), 7.51 (s, 2H, NH_2). ^{13}C-NMR (100 MHz, $CDCl_3$ + CD_3OD): δ = 128.1, 129.2, 130.1, 131.4, 132.0, 133.4, 133.2, 139.0; EIMS (*m*/*z*, −ion mode): 272.08 $[M]^-$; $[M–SO_2–NH_2]^-$ = 192.92; $[M–Cl–Benzene]^-$ = 161.17. Anal calcd for $C_{10}H_8NClO_2S_2$: C, 43.87; H, 2.95; N, 5.12; O, 11.69; S, 23.43. found: C, 43.92; H, 2.98; N, 5.20; O, 11.80; S, 23.48.

*2.7.9. 5-(3,4-dichlorophenyl)thiophene-2-sulfonamide (**3i**)*

M.P. 123–124.7 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.70–7.0 (m, 3H-Ar, 2H-Thiophene), 7.51 (s, 2H, NH_2). ^{13}C-NMR (100 MHz, $CDCl_3$ + CD_3OD): δ = 127.0, 128.2, 129.5, 129.7, 133.1, 133.6, 134.0, 135.1, 139.2; EIMS (*m*/*z*, +ion mode): 308.17 $[M]^+$; $[M–NH_2]^+$ = 293.0; $[M–NH_2–SO_2]^+$ = 230.00; $[M–SO_2]^+$ = 244.9; $[M–NH_2–2Cl]^+$ = 224.00. Anal calcd for $C_{10}H_7NCl_2O_2S_2$: C, 38.97; H, 2.29; N, 4.54; O, 10.38; S, 20.18. found: C, 39.00; H, 2.32; N, 4.60; O, 10.44; S, 20.22.

*2.7.10. 5-(3,5-dimethylphenyl)thiophene-2-sulfonamide (**3j**)*

M.P. 138–140 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.67–6.90 (m, 3H-Ar, 2H-Thiophene), 7.51 (s, 2H,NH_2), 2.35 (s, 3H,2CH_3). ^{13}C-NMR (100 MHz, $CDCl_3$ + CD_3OD): δ = 22.1, 127.0, 127.9, 129.9, 131.0, 131.6, 134.2, 139.0, 139.3; EIMS (*m*/*z*, +ion mode): 268.08 $[M]^+$; $[M–NH_2–SO_2]^+$ = 188.08. Anal calcd for $C_{12}H_{13}NO_2S_2$: C, 53.91; H, 4.90; N, 5.24; O, 11.97; S, 23.99. found: C, 54.02; H, 4.96; N, 5.32; O, 12.00; S, 24.12.

*2.7.11. 5-(4-chloro-3-fluorophenyl)thiophene-2-sulfonamide (**3k**)*

M.P. 144–146 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.68–7.0 (m, 3H-Ar, 2H-Thiophene), 7.52 (s, 2H,NH_2). ^{13}C-NMR (100 MHz, $CDCl_3$ + CD_3OD): δ = 118.1, 121.0, 125.2, 127.1, 130.1, 131.2, 132.1, 134.2, 139.1, 164.0; EIMS (*m*/*z*, +ion mode): 292.08 $[M]^+$; $[M–NH_2–SO_2]^+$ = 212.08; $[M–F]^+$ = 274.92; $[M–Cl]^+$ = 259.1. Anal calcd for $C_{10}H_7$-

$NFClO_2S_2$: C, 41.17; H, 2.42; N, 4.80; O, 10.97; S, 21.98. found: C, 41.22; H, 2.52; N, 4.90; O, 11.00; S, 22.08.

2.7.12. N-(5-Bromothiophne-2-ylsulfonyl)acetamide (4)

M.P. 118–120 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.44 (s, 1H, NH), 7.62 (d, J = 4.4, 1H-Thiophene), 7.09 (d, J = 4, 1H-Thiophene), 2.12 (s, 3H, CH_3), ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD): δ = 23.6, 122.2, 130.2, 135.4, 174.2; EIMS (m/z, +ion mode): 284.00 $[M]^+$; 282.00; [M–NH and Acetyl fragment]$^+$ = 226; [M–SO_2 and Acetyl fragment]$^+$ = 178. Anal calcd for $C_6H_6NBrO_3S_2$: C, 25.36; H, 2.13; N, 4.93; O, 16.89; S, 22.57. found: C, 25.46; H, 2.52; N, 5.12; O, 16.92; S, 22.66.

3. Results and discussion

3.1. Synthesis

Herein, we describe the application of Suzuki cross coupling reactions [22] to synthesize thiophene sulfonamide derivatives. To the best of our knowledge, Suzuki cross coupling reactions of 5-bromothiophene-2-sulfonamide have not been explored so far, except for the coupling of phenyl boronic acid with 5-bromothiophene-2-sulfonamide [4]. In the current experiments, $Pd(PPh_3)_4$ was used as a catalyst, while $K_3PO_4$was used as a base. The reactions were carried out at 95 °C, and moderate to excellent yields of the desired products were obtained under these conditions. 5-Bromothiophene-2-sulfonamide (**2**) was prepared by the reaction of 2-bromothiophene with chlorosulfonic acid, followed by the addition of aqueous ammonia according to the previously reported method [31]. The Suzuki reaction of **2** (0.704 mmol) with different aryl boronic acids and boronic esters (0.774 mmol) produced 5-arylthiophene-2-sulfonamides (**3a**–**k**) in moderate to good yields (Scheme 1, Table 1) [33]. The solvent showed a significant effect on the yield of the reaction. It was also observed that the relatively high solubility of oxygen and halogen containing aryl boronic acids in 1,4-dioxane (compared to toluene) results in greater yields of the products in 1,4-dioxane. On the other hand, the major benefit of using toluene is its high boiling point that makes it useful for several high temperature reactions. A number of different 5-Arylthiophene-2-sulfonamides (**3a**–**e**) (Table 1) were synthesized by using various aryl boronic esters, while, the other types of thiophene derivatives [**3f**–**k** (Table 1)] were synthesized by using arylboronic acids. It is important to note that the aryl boronic esters and acids did not influence the yield of **3a**–**k** compounds.

Different derivatives of 5-bromothiophene-2-sulfonamide were synthesized by Scheme 1 and the desired compounds (**3a**–**3k**) were obtained in moderate to good yields. It was observed that the products were synthesized in better yields when 1,4-dioxane was used as the solvent (entries 1–7). Using toluene as solvent, only moderate yields were obtained. The other experimental conditions such as temperature, nature of solvent and the water content also showed a great influence on the final yield of the product. The optimum solvent/water ratio is found to be 4:1 (solvent/water), as previously reported by [15].

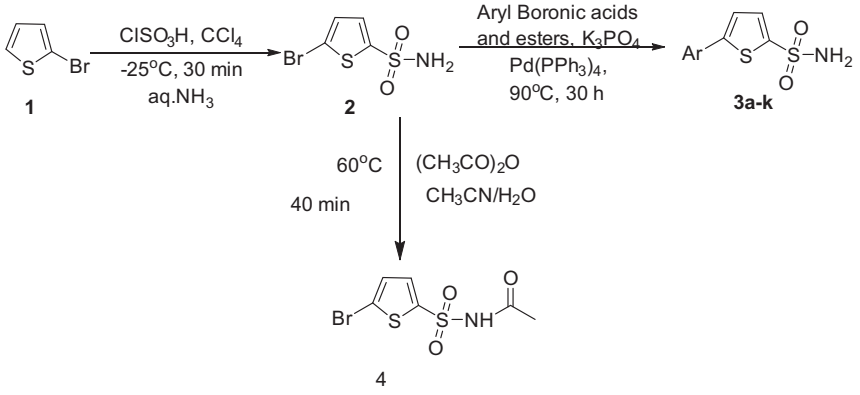
3.2. Pharmacology

3.2.1. Urease inhibition activity

The chemical nature of 5-bromothiophene-2-sulfonamide encouraged us to study the antiurease activities of our newly synthesized compounds. An insight understanding about using these compounds as urease inhibitors via enzyme catalyzed mechanism can be established from the report of [34]. An initial screen of these compounds for their potential application as urease inhibitor suggests that these compounds can be categorized into the following two groups;

- Group 1: Relatively less reactive group (compounds **3a**, **3c**–**f** and **4**).
- Group 2: Relatively more reactive group (compounds **3b** and **3g**–**k**).

The urease inhibition activity (IC_{50}) values were measured for all compounds. Thiourea was used as control having the



Scheme 1 Synthesis of 5-Bromothiophene-2-sulfonamide (2) and 5-arylthiophene sulfonamide (**3a**–**k**). Reagents and conditions: (i) **1** Bromothiophene (12 mmol), Chlorosulfonic acid (40–60 mmol), solvent (CCl_4,6 ml); (ii) **2** (0.704 mmol), Aryl boronic acids and Aryl boronic acid pinacol esters (0.774 mmol), K_3PO_4 (1.409 mmol), $Pd(PPh_3)_4$ (5 mol %), solvent/H_2O (4:1), (see Table 1), 95 °C, 30 h. Synthesis of 5-Bromothiophene-2-sulfonyl acetamide 4. Reagents and conditions: (i): 2 (0.002 mmol), Acetic anhydride (0.0031 mmol), Acetonitrile (5 mL).

Table 1 Synthesis of 5-arylthiophene-2-sulfonamide (**3a**–**k**).

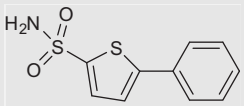
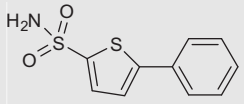
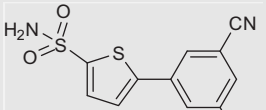
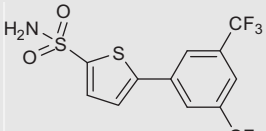
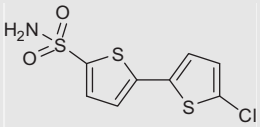
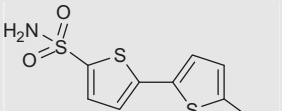
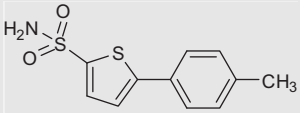
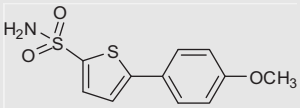
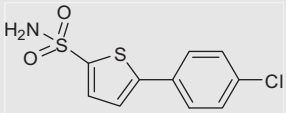
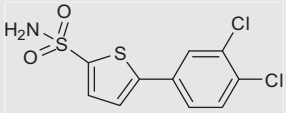
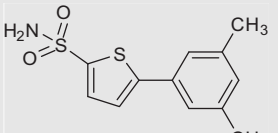
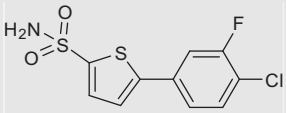
Entry	Aryl boronic acids and esters	Product	Solvent/H2O(4:1)	Yield%
1	C6H4B(OR)2	 3a	Toluene	67
2	C6H4B(OR)2	 3a	Dioxane	78
3	3-CF3,5-CN,C6H3B(OR)2	 3b	Dioxane	68
4	3,5- CF3-C6H3 B(OR)2	 3c	Dioxane	55
5	5-Cl,2-Thienyl B(OR)2	 3d	Dioxane	40
6	5-Me,2-Thienyl B(OR)2	 3e	Dioxane	67
7	4-Me-C6H4 B(OH)2	 3f	Toluene	44
8	4-MeO-C6H4 B(OH)2	 3g	Dioxane	62

Table 1 (*continued*)

Entry	Aryl boronic acids and esters	Product	Solvent/H2O(4:1)	Yield%
9	4-Cl-C6H4 B(OH)2	 3h	Dioxane	63
10	3,4-Cl2-C6H3 B(OH)2	 3i	Dioxane	61
11	3,5-Me_C6H3 B(OH)2	 3j	Dioxane	62
12	3-F,4-Cl-C6H3 B(OH)2	 3k	Dioxane	55

[a] isolated yield conditions: (95 °C, 30 h).

Table 2 Urease inhibition data of 5-arylthiophene-2-sulfonamide based compounds [(**3a**), (**3c**–**3f**) and (**4**)].

Compounds	Percentage activity at 25 μg/mL	Percentage activity at 50 μg/mL	Percentage activity at 250 μg/mL	IC_{50} μg/mL
3a	37.11 ± 0.001	93.5 ± 0.0007	98 ± 0.007	30.8 ± 0.3
3c	13 ± 0.007	37.11 ± 0.001	76 ± 0.01	116 ± 1.12
3d	33 ± 0.05	64.9 ± 0.007	92 ± 0.005	38.4 ± 0.4
3e	19 ± 0.01	29.6 ± 0.006	54 ± 0.004	218 ± 2.1
3f	11 ± 0.01	22.3 ± 0.0007	56 ± 0.007	214 ± 1.98
4	18 ± 0.0007	36.3 ± 0.01	70 ± 0.01	132 ± 2.21
Standard (thiourea)	24 ± 0.002	60 ± 0.032	95 ± 0.09	43 ± 0.4

percentage activities ~24 ± 0.002, 60 ± 0.032, 95 ± 0.09 for 25, 50 and 250 μg/mL concentrations respectively. The IC_{50} value of thiourea was found to be 43 μg/L (Table 2). Similarly, Table 3 shows that the thiourea percentage urease inhibition activity for concentrations 15, 25 and 50 μg/mL was 34 ± 0.004, 51 ± 0.005 and 72 ± 0.005 respectively, whereas the IC_{50} value was found to be ~24.4 μg/mL. The compounds having such functional groups can bind with active sites of enzyme, which therefore impede the hydrolysis of enzyme [32]. On the basis of these findings, we examined the antiurease activity of these synthesized compounds, where almost all compounds showed moderate to high urease enzyme inhibition activity. Two sets of compounds were investigated for the urease inhibition assay with different concentrations. Compound *5-Phenylthiophene-2-sulfonamide* (**3a**) showed excellent urease inhibition activity at 50 μg/mL concentration with percentage inhibition activity ~93.5 ± 0.0007. Similarly, compound *5-(3-cyno-5-(triflouromethyl)phenyl)thiophene-2-sulfonamide* (**3d**) also showed the highest percentage inhibition 33 ± 0.05 at 25 μg/mL concentration along with IC_{50} value ~38.4 μg/mL. The highest antiurease activity of this compound might be due to the presence of one more thiophene ring attachments. At concentration ~50 μg/mL, compound *5-(3,5-bis(triflouromethyl)phenyl)thiophene-2-sulfonamide*(**3c**), was found a better inhibitor displaying the percentage inhibition value ~37.11 ± 0.04. Antiurease activities of *5′-methyl-2,2′-bithiophene-5-sulfonamide*(**3e**) and *5-p-tolylthiophene-2-sulfonamide* (**3f**) were also investigated at 250 μg/mL concentration, and their

Table 3 Urease inhibition data of 5-arylthiophene-2-sulfonamide based compounds [(**3b**), (**3g**–**3k**)].

Compounds	Percentage activity at 15 μg/mL	Percentage activity at 25 μg/mL	Percentage activity at 50 μg/mL	IC_{50} μg/mL
3b	28 ± 0.005	51 ± 0.001	80.05 ± 0.007	24.5 ± 0.21
3g	34 ± 0.001	55 ± 0.002	88.30 ± 0.004	22.6 ± 0.22
3h	33 ± 0.006	51 ± 0.001	80.43 ± 0.006	24.4 ± 0.23
3i	32 ± 0.03	56 ± 0.004	86.10 ± 0.005	22.5 ± 0.19
3j	22 ± 0.05	53 ± 0.005	83.34 ± 0.002	24 ± 0.18
3k	31 ± 0.006	50 ± 0.001	79.56 ± 0.002	23.6 ± 0.21
Standard (thiourea)	34 ± 0.004	51 ± 0.005	72 ± 0.005	24.4 ± 0.28

IC_{50} values were found to be 218 μg/mL and 214 μg/mL with percentage inhibition activities ~54 ± 0.004 and 56 ± 0.007, respectively (Table 2). Compounds *5-(3,4-dichlorophenyl)thiophene-2-sulfonamide* (**3i**) and *5-(4-methoxyphenyl)thiophene-2-sulfonamide* (**3g**) were found to be the most effective antiurease inhibitors at 50 μg/mL concentration and displayed the percentage activities ~86.1 ± 0.005 and 88.30 ± 0.004 with IC_{50} values of 22.5 and 22.6 μg/mL, respectively. Moreover, compounds *5-(4-chloro-3-flourophenyl)thiophene-2-sulfonamide* (**3k**) and *5-(3,5-dimethylphenyl)thiophene-2-sulfonamide* (**3j**) containing chloro, flouro and methyl moieties present on the thiophene substituted phenyl rings were also found to be efficient urease inhibitors with percentage inhibition values ~79.56 ± 0.002 and 22 ± 0.05. In addition, compound *5-(4-chlorophenyl)thiophene-2-sulfonamide* (**3h**) exhibited antiurease activity with the percentage inhibition value ~80.43 ± 0.006, and IC_{50} value ~24.4 μg/mL at 50 μg/mL concentration. In the same way, compound *5-(3-cyano-5-(triflouromethyl)phenyl)thiophene-2-sulfonamide* (**3b**) also facile inhibitory response against urease enzyme with percentage inhibition value ~80.05 ± 0.007 along with IC_{50} value of 24.5 μg/mL at 50 μg/mL concentration (Table 3). Some of the newly as-synthesized compounds showed relatively higher antiurease activity than others that showed moderate urease inhibition activities. It was also noted that the electron withdrawing functional groups present on the benzene ring have negative effect of the inhibitor activity of these compounds against urease enzyme, while, in contrast, the electron donating functional groups present on the benzene ring led to the occurrence of high inhibitor activity against urease enzyme. Moreover, the electron withdrawing groups also cause a decrease in the metal

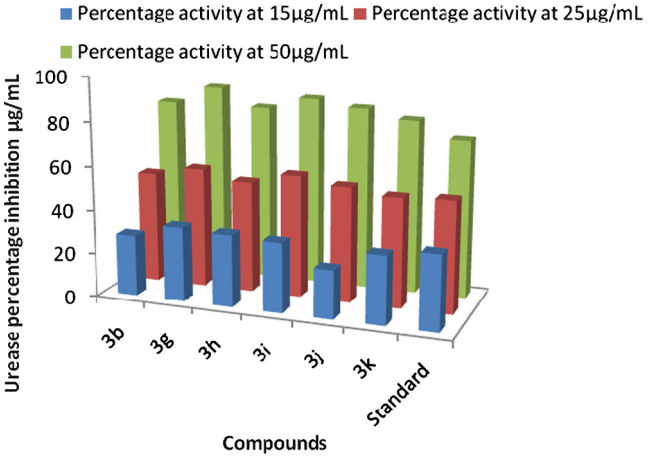


Figure 2 Urease percentage inhibition activity values at μg/mL.

chelating activity and vice versa. Removal/chelation of Ni^{+2} ion will result in inactivation of the enzyme. Therefore, the changes in urease inhibitory activity can be ascribed to the changes in the electronic environments and the position of functional groups in the series of compounds **3a**–**3k** and compound **4** (Figs. 1 and 2).

3.2.2. Hemolytic activity

Compared with the positive control triton X-100 standard; compounds **3a**–**c**, **3f** and **3i** showed moderate hemolytic activity, whereas compounds **3d**, **3g**–**h** and **3j**–**k** exhibited the highest toxicity effects. Compound **3h** exhibited highest % lysis of

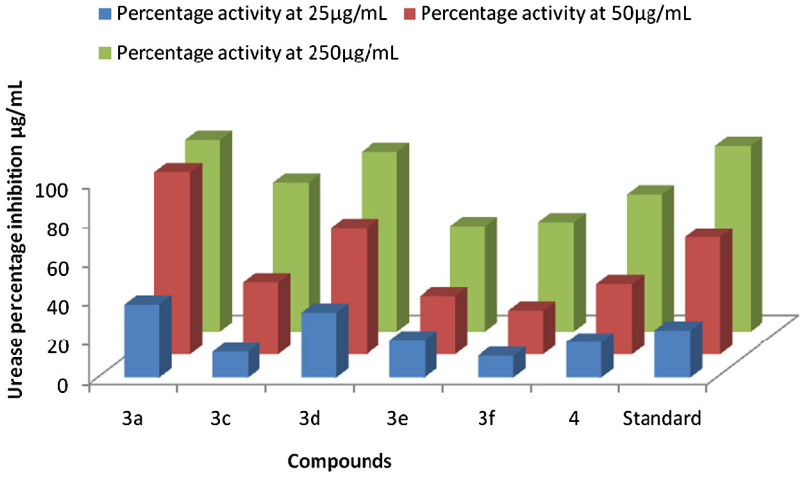


Figure 1 Urease percentage inhibition activity values at μg/mL.

Table 4 Hemolytic activity data of 5-arylthiophene-2-sulfonamide based compounds [(**3a**–**3k**) and (**4**)].

Compounds	% lysis of RBC
3a	4.387 ± 0.115
3b	10.86 ± 0.174
3c	2.602 ± 0.164
3d	20.75 ± 0.164
3e	4.613 ± 0.749
3f	5.907 ± 0.115
3g	27.15 ± 0.339
3h	37.663 ± 0.115
3i	18.303 ± 0.329
3j	29.12 ± 0.223
3k	34.477 ± 0.380
4	14.605 ± 0.242
Triton × 100	99.783 ± 0.912

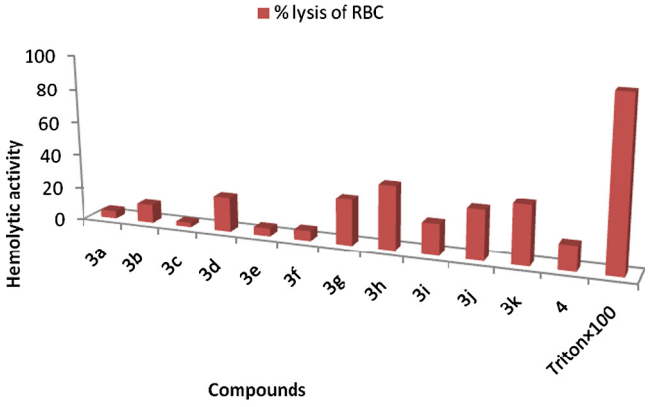


Figure 3 Hemolytic activity graph.

RBCs (37.663 ± 0.115) against triton X-100. Similarly, compound **3k** also showed extravagant hemolytic activity value ~34.477 ± 0.380 due to the presence of electron withdrawing F and Cl moieties present on the phenyl ring.

Table 4 shows that compound *5-(3,4-dichlorophenyl)thiophene-2-sulfonamide* (**3i**), showed moderate hemolytic activity value ~18.303 ± 0.329, probably due to the presence of the Cl group at meta and para positions in the aromatic phenyl ring. The data from Table 4 also shows that compounds **3e** and **3f** exhibited low toxicity effect with % lysis values of RBC ~4.613 ± 0.749 and 5.907 ± 0.115, respectively. On the other hand, compound **3j** showed a higher toxicity value ~29.12 ± 0.223. This might be due to the presence of two $-CH_3$ groups. Molongi et al. [23] reported that the anticancer activity is usually enhanced by the presence of electron releasing groups [23]. Therefore, the presence of two $-CH_3$ groups in compound **3j** could be the possible cause of the highest % lysis of RBCs when compared with compound **3f** having one $-CH_3$ group. In view of the observed differences in the % lysis of RBC values, it is suggested that the electron withdrawing and electron donating nature of the attached groups have an influence on the hemolytic activity of the compounds. Moreover, the position of functional groups also has an impact on the hemolytic activity as can be seen from the results. Ding and co-workers [9] reported that the compounds having the Cl functional group at benzene ring exhibited better and higher hemolytic activities than the compounds having H-atom or $O-CH_3$ substitution at the same position [9]. In the present study, we observed that compounds **3b**–**3c** with CF_3 and CN moieties on the benzene ring show less hemolytic activities ~10.86 ± 0.174 and 2.602 ± 0.164, respectively. Overall, it is concluded that the presence of electron withdrawing groups promote hemolytic activity, while, the position of functional groups also contributes toward the hemolytic activity of all newly synthesized sulfonamide series of compounds (**3a**–**k**) in

Table 5 Antibacterial activities (100 μg) of 5-arylthiophene-2-sulfonamide (**3a**, **3c**–**f**) and (**4**).

Compounds	% Activity at 100 μg					
	Bacillus Subtilis	*E coli*	*Staphylococcus*	*Shigella*	*Salmonella typhae*	*Pseudomonas*
3a	22 ± 0.019	12 ± 0.01	20.17 ± 0.02	31.1 ± 0.009	32.51 ± 0.006	32.06 ± 0.004
3c	20 ± 0.002	25 ± 0.04	17.00 ± 0.004	45.3 ± 0.005	37.82 ± 0.007	30.93 ± 0.002
3d	20 ± 0.002	20 ± 0.002	13.8 ± 0.004	32.02 ± 0.000	34.07 ± 0.015	32.5 ± 0.014
3e	19 ± 0.007	12 ± 0.001	19.86 ± 0.001	38.33 ± 0.0007	48.55 ± 0.014	35.05 ± 0.004
3f	14 ± 0.015	15 ± 0.013	13.37 ± 0.0007	26.86 ± 0.004	41.44 ± 0.002	28.18 ± 0.003
4	30 ± 0.014	31 ± 0.002	31.18 ± 0.01	43.62 ± 0.010	31.64 ± 0.0007	35.0 ± 0.014
Streptomycin	60 ± 0.001	60 ± 0.001	60 ± 0.001	60 ± 0.001	60 ± 0.001	60 ± 0.001

Table 6 Antibacterial activities (300 μg) of 5-arylthiophene-2-sulfonamide (**3a**, **3c**–**f**) and (**4**).

Compounds	% Activity at 300 μg					
	Bacillus Subtilis	*E. coli*	*Staphylococcus*	*Shigella*	*Salmonella typhae*	*Pseudomonas*
3a	37 ± 0.114	35 ± 0.144	29.57 ± 0.0007	52.94 ± 0.001	40.2 ± 0.016	38.4 ± 0.003
3c	31 ± 0.07	41 ± 0.07	35.29 ± 0.0005	44.98 ± 0.00	36.2 ± 0.006	40.64 ± 0.03
3d	23.1 ± 0.000	24 ± 0.03	28.11 ± 0.0007	53.80 ± 0.00	28.33 ± 0.009	42.2 ± 0.024
3e	24.5 ± 0.000	25 ± 0.000	28.74 ± 0.0007	49.30 ± 0.023	33.58 ± 0.007	41.86 ± 0.043
3f	26.3 ± 0.000	30 ± 0.013	24.8 ± 0.001	42.67 ± 0.00	29.15 ± 0.001	38.14 ± 0.004
4	41 ± 0.002	41 ± 0.002	45.58 ± 0.0007	48.73 ± 0.04	42.49 ± 0.002	40.93 ± 0.008
Streptomycin	83 ± 0.06	83 ± 0.06	83 ± 0.06	83 ± 0.06	83 ± 0.06	86 ± 0.06

Table 7 Antibacterial activities (1000 μg) of 5-arylthiophene-2-sulfonamide (**3a**, **3c–f**) and (**4**).

Compounds	% Activity at 1000 μg					
	Bacillus Subtilis	*E-Coli*	*Staphylococcus*	*Shigella*	*Salmonella typhae*	*Pseudomonas*
3a	78 ± 0.007	71 ± 0.007	61 ± 0.006	75 ± 0.02	68 ± 0.0004	43 ± 0.5
3c	78 ± 0.00	76 ± 0.00	58 ± 0.001	67 ± 0.005	45 ± 0.006	56 ± 0.09
3d	20 ± 0.06	59 ± 0.06	53 ± 0.00032	64 ± 0.017	61 ± 0.004	55 ± 0.045
3e	34 ± 0.0021	54 ± 0.00	56 ± 0.004	67 ± 0.045	65 ± 0.001	64 ± 0.0012
3f	23 ± 0.002	63 ± 0.002	55 ± 0.005	76 ± 0.032	60 ± 0.00012	53 ± 0.004
4	70 ± 0.010	70 ± 0.010	70 ± 0.01	70 ± 0.005	65 ± 0.01	55 ± 0.004
Streptomycin	95 ± 0.004	95 ± 0.004	95 ± 0.004	95 ± 0.004	95 ± 0.004	95 ± 0.004

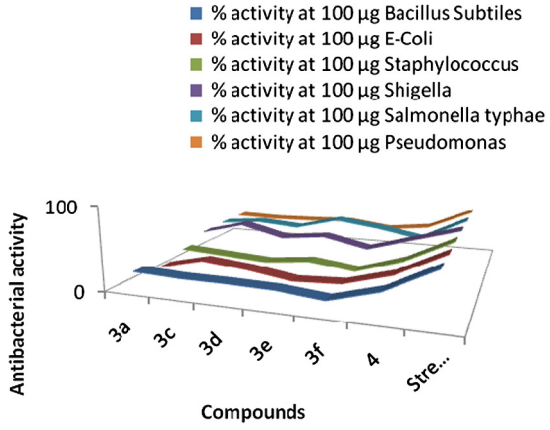


Figure 4 Antibacterial activity graph (100 μg).

the present study. These compounds [(**3a–k**) and (**4**)] showed moderate to high % lysis of RBC and can be used as potential anticancer agents (Fig. 3).

3.2.3. Bacteriological study

The sulfonamides display various biological activities including carbonic anhydrase inhibition, insulin releasing, antimicrobial, antitumor and anti-inflammation activities [30]. Keche and co-workers reported that the derivative of 1-Acetyl-3,5-diaryl-4,5-dihydro(1H) pyrazole, having Br at ortho and CF_3 at para position of the benzene ring of terminal sulfonamide is twice more efficient against *salmonella typhimurium* and *staphylococcus aureus*. While, the compound bearing Cl at ortho position and CF_3 at para position is 1.5-fold more potent against *Escherichia coli* and *Bacillus subtilis*. This compound is also found 1.3-fold more potent against *staphylococcus* and *salmonella typhimurium aureus* as well [18]. The study on the antibacterial activities of six newly as-synthesized derivatives of thiophene sulfonamide was examined against two gram positive bacteria and four gram negative bacteria. Table 7 shows that compound **3a** (at 1000 μg concentration) exhibited the highest % inhibition ~78 ± 0.007, 71 ± 0.007 and 75 ± 0.02 against the *Bacillus Subtilis*, *E-coli* and *Shigella*, respectively. The same compound at 100 and 300 μg concentrations showed moderate inhibition activities (Tables 5 and 6) against the same bacterial strains. Interestingly, in the case of compound **3c** (at 1000 μg concentration) bearing the CF_3 group, magnificent % antibacterial activities ~78 ± 0.00, 76 ± 0.00, and 67 ± 0.005 were observed against *Bacillus Subtilis*, *E-coli* and *Shigella* respectively. However, the compounds **3d**, **3e** and **3f** (at 1000 μg concentration) showed moderate to acceptable antibacterial activities (in order of 64.002 ± 0.00, 67 ± 0.045, and 76 ± 0.032, respectively) against *Shigella* (a gram-negative bacterium), while the same compounds exhib-

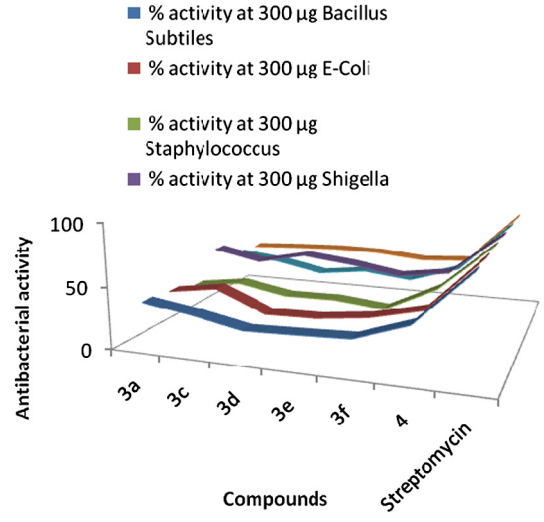


Figure 5 Antibacterial activity graph (300 μg).

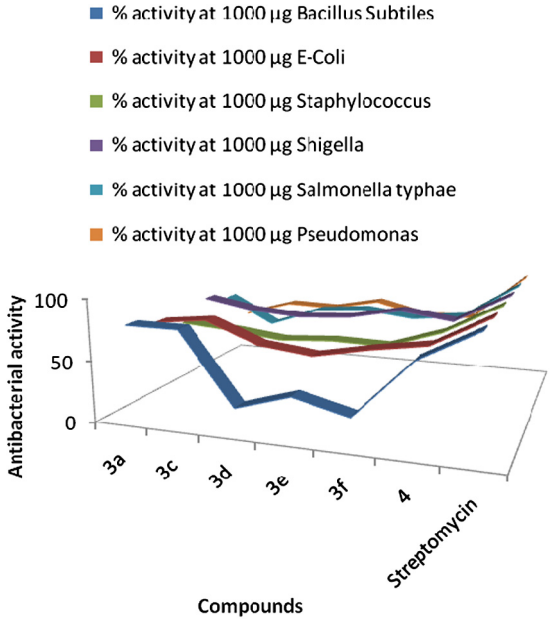


Figure 6 Antibacterial activity graph (300 μg).

ited poor inhibition activities against other gram-negative bacteria, (*E. coli*, *Pseudomonas aeroginosa*, *Salmonella typhi*) and few gram-positive bacteria (*Staphlococcusaureus* and *Bacillus subtilis*). It was interesting to note that the acetylated product *N-(5-Bromothiophne-2-ylsuelfonyl)acetamide* (**4**) exhibited the highest inhibition activities against both types of gram negative and gram positive bacteria at 1000 μg concentration. Moreover, it was also observed that the electron donating groups present at the phenyl ring of substituted thiophene sulfonamide had a positive impact to promote the antibacterial activities of these newly synthesized thiophene based compounds (Figs. 4–6).

4. Conclusions

In the current study, we report a facile route of Suzuki cross coupling reaction to synthesize thiophene sulfonamide derivatives (3a–3k).These newly synthesized compounds (**3a**–**3k**) and compound **4** were identified for their purity and later investigated for the urease enzyme inhibition and antibacterial and hemolytic activities. Most of these thiophene sulfonamide derivatives showed excellent activities with the exception of some compounds that displayed moderate to low biological activities. The detailed investigation in the present study revealed that the electronic factors such as the nature and position of various functional groups might be responsible for the observed low anti-urease activities of some compounds. On the other hand, the elevated anti-urease activity of compound **3d** might be due to the presence of second thiophene ring in the compound. Moreover, the moderate antibacterial activity observed in the case of compound **3e** could be due to the presence of thiophene ring. Our investigation shows that the presence of F and Cl groups has a positive impact to enhance the hemolytic activity of these newly synthesized thiophene sulfonamide derivatives. In such cases, the position of the functional group showed a significant effect on the hemolytic activity of these compounds; an evidence of which can be seen in the case of compound **3h**. Though, few of the compounds showed antibacterial activities, compounds **3e**–**f** and **4** exhibited appreciable % antibacterial activity. Further biological studies need to be done in order to fully explore these 2-bromothiophene derivatives for the treatment of bacterial infections.

Acknowledgments

The data present here is part of Ph.D thesis. The authors gratefully acknowledge the financial support by the Higher Education Commission (HEC), Pakistan through scholarship (PIN NO. 106-2102-Ps6-070) for Mnaza Noreen. We also acknowledge the HEJ Research institute of Chemistry, University of Karachi for NMR analysis. U. A. Rana would like to extend his sincere appreciation to the Deanship of Scientific Research at the King Saud University for their funding through the Research Group Project no RGP-VPP-345.

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