One Pot Selective Arylation of 2-Bromo-5-Chloro Thiophene; Molecular Structure Investigation via Density Functional Theory (DFT), X-ray Analysis, and Their Biological Activities

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Abstract: Synthesis of 2,5-bisaryltiophenones was accomplished by sequential Suzuki cross coupling reaction of 2-bromo-5-chloro thiophenes. Density functional theory (DFT) studies were carried out at the B3LYP/6-31G(d, p) level of theory to compare the geometric parameters of 2,5-bisaryltiophenones with those from X-ray diffraction results. The synthesized compounds are screened for in vitro bacteria scavenging abilities. At the concentration of 50 and 100 µg/mL, compounds 2b, 2c, 2d, 3c, and 3f with IC50-values of 51.4, 52.10, 58.0, 56.2, and 56.5 µg/mL respectively, were found most potent against E. coli. Among all the synthesized compounds 2a, 2d, 3c, and 3e with the least values of IC50 77.76, 79.13 µg/mL respectively showed significant antioxidant activities. Almost all of the compounds showed good antibacterial activity against Escherichia coli, whereas 2-chloro-5-(4-methoxyphenyl) thiophene (2b) was found most active among all synthesized compound with an IC50 value of 51.4 µg/mL. All of the synthesized compounds were screened for nitric oxide scavenging activity as well. Frontier molecular orbitals (FMOs) and molecular electrostatic potentials of the target compounds were also studied theoretically to account for their relative reactivity.

Keywords: 2-bromo-5-chloro thiophenes; Suzuki coupling; density functional theory (DFT); antibacterial; antioxidant
1. Introduction

Substituted aromatic compounds are widely synthesized by the well-established family of chemical reactions known as cross-coupling reactions [1,2]. Suzuki cross-coupling reactions generally deliver higher yields under mild reaction conditions, compared to other cross-coupling reactions. Moreover, the boronic acid is commercially available, and the reactions are environmentally friendly [3–6]. During the last three decades, carbon-carbon coupling for the synthesis of biaryls has replaced classical approaches such as Ullman coupling reactions [7]. Suzuki reaction of tetrabromothiophene is well reported [8–10]. The lower cost and easy availability of aryl chlorides made them attractive starting materials in Suzuki–Miyaura reaction with the help of wide varieties of catalytic systems [11–14].

Thiophene moiety is found to be very potent in various biological activities [15–17]. Anti-urease and nitric oxide (NO) scavenging activity of a series of 2-amino-6-arylbenzothiazoles were examined by Gul et al. [11]. Various 4-arylthiophene-2-carbaldehydes showed moderate to excellent ability against antibacterial, anti-urease, hemolytic, and antioxidant activities [16]. We became interested in synthesizing unsymmetrical bis-aryl (Ar and Ar′) substituted thiophene by taking the advantage of difference of reactivity of chloro and bromo moiety on thiophene ring. Therefore, in this report, we extend the utilization of aryl chlorides and bromides by reporting the selective Suzuki coupling reactions of 2-bromo-5-chlorothiophene with various electron donating and electron withdrawing aryl boronic acids. These reactions allow efficient synthesis of mono substituted and di-substituted thiophenes by using K3PO4 as base. However, the compounds 2a–c and 3a–c have already been reported by following different methodologies [18–22], while their biological activities and density functional theory (DFT) studies are being first time reported. After accomplishing the successful synthesis of various mono and di substituted thiophenes, in continuation of our previous work [23,24], DFT studies were conducted not only to explore the structural properties but also to compare the theoretical structural parameters with those from X-ray diffraction results. Finally, antibacterial and nitric oxide (NO) scavenging activity of the products were investigated.

2. Results and Discussion

2.1. Preparations

The 2-aryl-5-chlorothiophenes (2a–f) were synthesized via Suzuki reaction (Scheme 1) from commercially available (1) 2-bromo-5-chlorothiophene (1.0 mmol). The compounds 2a, 2b and 2c have been previously reported but through alternative synthetic strategies [25–28].

\[
\text{Cl} \quad \text{Br} + \quad \text{B(OH)}_2 \quad \xrightarrow{\text{K}_3\text{PO}_4 (2 \text{ mmol}), \quad \text{Pd}(\text{PPH}_3)_4 (5 \text{ mol\%})} \quad \text{Cl} \quad \text{R} \quad \xrightarrow{\text{ArB(OH)}_2 (1.1 \text{ mmol})} \quad \text{12h, 90°C.} \]

Scheme 1. Synthesis of compounds 2a–f. R: 2a = 4-Me, 2b = 4-MeO, 2c = 4-Cl, 2d = 3-Cl,4-F, 2e = 3,5-dimethyl, and 2f = 3,4-dichloro.

It should be noted that the C–Cl bond strength hampers the reactivity of aryl chlorides, thus, they are reluctant to oxidative addition to Pd(0) [14]. Products 2a–f were prepared following a protocol developed by us [29]. All the products showed moderate to very good yields in the presence of K3PO4 base (Figure 1). These results suggest that the yield might be sensitive to electron donating and electron withdrawing substituents present on the boronic acid. The base plays a vital role in Suzuki cross-coupling reactions, and it enhances the transmetallation process. Therefore, the choice of the solvent water ratio (3:1.5, 5:1.5 mL) and the quantity of base used are essential to activate boronic acids, and help to obtain good yields.
2.2. Crystal Structure Determinations

Among all synthesized derivatives, suitable crystals were obtained for 2d and 2f which were then subjected to X-ray radiation for their structure confirmation and to obtain geometric parameters and spatial interactions. ORTEP plots of both compounds are shown in the Figure 3, and X-ray parameters are being provided in Table 1. The root mean square (RMS) deviation for 2d is 0.0287 Å, which
is indicative of planarity from its fitted atoms with most deviations from Cl2 = \(-0.0596 (2)\) Å and C8 = 0.0420 (4) Å. On the other hand, 2f is not planar with the RMS deviation from the fitted atoms of the molecule at 0.1619 Å, with most deviations from C8 = \(-0.2746 (2)\) Å and S1 = 0.2455 (1) Å. The aromatic ring is twisted at a dihedral angle of 11.789° (2) and 2.115° (2) for both, respectively. This also proves the more planarity of 2d. The C–S–C angles in both molecules are 91.34 and 91.74 degrees, which is in accordance with already reported data [30]. The unit cell diagrams were shown in Figure 4 for compounds I and II, respectively, which does not show any inter- or intra-molecular interactions among the molecules.

Figure 3. ORTEP plots of 2d and 2f.

Figure 4. Unit cell diagrams of 2d and 2f. (Ball color: green, Cl; red, F; yellow, S; black and blue, C).
was carried out at B3LYP/6-31G (d, p) level of DFT optimized geometries of

differences were observed in the range 0.0

0.002–0.028 Å for

results. The difference in X-ray and calculated bond lengths found in the range 0.003–0.035 Å and

in Figure 5, whereas important bonds lengths and bond angles are listed in the Tables 2 and 3.

three-dimensional structures of compounds, and to compare them with the geometric parameters

2.3. Molecular Geometries

Optimized geometries obtained through theoretical methods are very useful to explain the

three-dimensional structures of compounds, and to compare them with the geometric parameters

obtained from X-ray diffraction studies [31]. Among all of the synthesized thiophenes, only 2d and

2f gave suitable crystals for X-ray diffraction studies (vid supra). Optimization of all compounds

was carried out at B3LYP/6-31G (d, p) level of DFT. Optimization of all compounds

was carried out at B3LYP/6-31G (d, p) level of DFT. Geometric parameters of both compounds

showed tight correlation with calculated results. The difference in X-ray and calculated bond lengths found in the range 0.003–0.035 Å and

0.002–0.028 Å for 2d and 2f, respectively (atomic labelling is according to the ORTEP plots shown in

Figure 5).

2.3. Density Functional Theory (DFT) Studies

2.3.1. Molecular Geometries

Similarly, the bond angles of both compounds correlated to each other excellently, very minute
differences were observed in the range 0.0°–0.9° and 0.1°–1.2° for both compounds 2d and 2f,
respectively. The maximum difference observed for C5–C4–C3 in 2d, i.e., 0.9° and for C13–C10–S1 for 2f (1.2°).

Table 2. Some selected X-ray and simulated bond lengths (Å) of 2d and 2f (atomic labels are with reference to ORTEP plot Figure 3).

| (2d)  | X-ray  | Calc. (B3LYP) | (2f)  | X-ray  | Calc. (B3LYP) |
|-------|--------|---------------|-------|--------|---------------|
| C1–C2 | 1.400 (4) | 1.406         | C1–C2 | 1.399 (2) | 1.405         |
| C1–C6 | 1.391 (5) | 1.406         | C1–C6 | 1.401 (2) | 1.404         |
| C1–C7 | 1.474 (4) | 1.467         | C1–C7 | 1.464 (2) | 1.466         |
| C2–C3 | 1.375 (5) | 1.39         | C2–C3 | 1.374 (2) | 1.389         |
| C3–C4 | 1.373 (5) | 1.396         | C3–C4 | 1.387 (3) | 1.396         |
| C3–C11| 1.727 (4) | 1.746         | C3–C11| 1.728 (18) | 1.747         |
| C4–C5 | 1.363 (6) | 1.389         | C4–C5 | 1.386 (3) | 1.401         |
| C5–C6 | 1.371 (5) | 1.391         | C5–C6 | 1.262 (18) | 1.745         |
| C7–C8 | 1.355 (5) | 1.374         | C5–C6 | 1.726 (3) | 1.392         |
| C7–S1 | 1.725 (3) | 1.757         | C7–C8 | 1.369 (2) | 1.375         |
| C8–C9 | 1.421 (5) | 1.424         | C7–S1 | 1.734 (18) | 1.757         |
| C9–C10| 1.342 (6) | 1.366         | C8–C9 | 1.415 (3) | 1.423         |
| C10–S1| 1.707 (4) | 1.742         | C9–C10| 1.346 (3) | 1.366         |
| C10–Cl2| 1.715 (4) | 1.73          | C10–S1| 1.714 (18) | 1.742         |
| C4–F1 | 1.357 (4) | 1.34          |      |         |               |

Table 3. Some selected X-ray and simulated bond angles (°) of 2d and 2f (atomic labels are with Reference to Figure 3).

| Bond (2d) | X-ray  | Calc. (B3LYP) | Bond (2f) | X-ray  | Calc. (B3LYP) |
|-----------|--------|---------------|-----------|--------|---------------|
| C2–C1–C6 | 118.0 (3) | 118.4         | C2–C1–C6 | 117.7 (16) | 118.1        |
| C2–C1–C7 | 120.3 (3) | 119.7         | C2–C1–C7 | 121.1 (16) | 121.4        |
| C6–C1–C7 | 121.7 (3) | 121.8         | C6–C1–C7 | 121.0 (16) | 120.4        |
| C3–C2–C1 | 120.3 (3) | 120.7         | C3–C2–C1 | 121.1 (17) | 121.1        |
| C2–C3–C4 | 119.7 (3) | 119.7         | C2–C3–C4 | 120.1 (17) | 120          |
| C2–C3–C11| 120.9 (3) | 120.5         | C2–C3–C11| 118.8 (15) | 118.5        |
| C4–C3–C11| 119.5 (3) | 119.6         | C4–C3–C11| 120.9 (14) | 121.4        |
| C5–C4–C3 | 121.3 (3) | 120.4         | C3–C4–C12| 121.1 (15) | 121.7        |
| C6–C5–C4 | 119.4 (4) | 119.6         | C5–C4–C3 | 119.5 (17) | 119.1        |
| F1–C4–C3 | 119.1 (4) | 119.9         | C5–C4–C12| 119.2 (15) | 119          |
| C5–C4–F1 | 119.6 (4) | 119.5         | C6–C5–C4 | 120.3 (18) | 120.6        |
| C5–C6–C1 | 121.2 (3) | 120.9         | C5–C6–C1 | 120.9 (17) | 120.8        |
| C1–C7–S1 | 120.6 (2) | 121.1         | C1–C7–S1| 120.4 (13) | 121.3        |
| C8–C7–C1 | 129.2 (3) | 128.5         | C8–C7–C1 | 129.4 (17) | 128.3        |
| C8–C7–S1 | 110.3 (3) | 110.3         | C8–C7–S1| 110.0 (14) | 110.3        |
| C7–C8–C9 | 113.7 (4) | 114.1         | C7–C8–C9| 113.9 (17) | 114.1        |
| C10–C9–C8 | 111.8 (4) | 111.9         | C10–C9–C8| 111.6 (17) | 111.9        |
| C9–C10–S1| 112.5 (3) | 112.4         | C9–C10–S1| 112.9 (15) | 112.4        |
| C10–S1–C7 | 91.74 (17) | 91.1          | C9–C10–C13| 127.7 (15) | 127          |
| S1–C10–C12| 120.4 (2) | 120.5         | C13–C10–S1| 119.2 (12) | 120.4        |
| C9–C10–C12| 127.1 (3) | 127          | C10–S1–C7 | 91.34 (9) | 91           |

2.3.2. Frontier Molecular Orbital (FMOs) Analysis

FMOs analysis by computational methods is a useful to understand the reactivity and electronic transitions within molecules [32].

Frontier orbitals (HOMO and LUMO), mainly take part in electronic transitions and their energy gap depicts the reactivity [33]. The HOMO-LUMO and electronic properties of compounds (2a–f) and (3a–f) were explored at 6-31G (d, p) level of DFT. The distribution patterns of frontier molecular orbitals (HOMOs and LUMOs along with corresponding energies) of all synthesized thiophene derivatives at the ground states have been shown in Figure 6. As reflected from Figure 6, the π cloud in HOMOs
and LUMOs of all thiophenes (2a–f) and (3a–f) is distributed on the entire skeleton (thiophene and phenyl rings). Introducing the different groups on the benzene ring does not have much effect on the electronic cloud. As reflected form orbital surfaces of compounds 2a, 2b, 2c, 3a, 3b, and 3c, groups attached to the para position of the benzene ring are participating in the \( \pi \) electronic cloud. Whereas the groups attached to the meta position such as in compounds 2d, 2e, 3d, and 3e are not involved directly in the \( \pi \) electronic cloud.

![Figure 6. HO/LU orbitals of 2a–f and 3a–f.](image)

Detailed HOMO and LUMO energies of all thiophenes along with their gaps are listed in the Table 4. HOMO-LUMO energy difference (\( E_g \)) of mono aryl thiophenes 2a–f is relatively large compare to bis-aryl thiophenes 3a–f. Among all synthesized compounds 3b and 3f showed the lowest HOMO-LUMO energy gap i.e., of 3.96 eV and 2e showed the largest energy gap (4.59 eV).
Table 4. HOMO and LUMO energies along with energy gaps.

| Entry No. | HOMO (eV) | LUMO (eV) | HOMO-LUMO (ΔE) eV |
|-----------|-----------|-----------|-------------------|
| 2a        | −5.71     | −1.14     | 4.57              |
| 2b        | −5.49     | −1.02     | 4.47              |
| 2c        | −5.96     | −1.46     | 4.50              |
| 2d        | −6.07     | −1.48     | 4.59              |
| 2e        | −5.72     | −1.13     | 4.59              |
| 2f        | −6.13     | −1.66     | 4.47              |
| 3a        | −5.24     | −1.21     | 4.03              |
| 3b        | −4.97     | −1.01     | 3.96              |
| 3c        | −5.68     | −1.70     | 3.98              |
| 3d        | −5.84     | −1.76     | 4.08              |
| 3e        | −5.16     | −1.14     | 4.02              |
| 3f        | −5.96     | −1.99     | 3.97              |

2.3.3. Molecular Electrostatic Potential (MEP)

Electrostatic potential (ESP) mapping through computer aided methods is a very useful parameter to explore the reactivity of organic compounds. Molecular electrostatic potential (MEP) has been applied successfully to understand the enzyme-substrate interactions [34], hydrogen bonding [35], and nucleophilic as well as electrophilic sites in compounds [33].

The nucleophilic, as well as electrophilic, sites in any compound are expressed in terms of different color codes, the deep red color expresses an electron rich site, whereas deep blue expresses an electron-deficient site (Figure 7). From the MEP shown in Figure 7, it is clear that electronic density in 2a is concentrated on the chloro as well as sulphur atoms of the thiophene ring along with the pi cloud of benzene ring, and protons attached to the thiophene and benzene ring are electron deficient sites. Almost the same trend was observed for 2b and 3b but with some extra localization of electronic density on methoxy group oxygen directly attached to benzene. In 2c, 2d, 2f, 3c, 3d, and 3f electronic density was more dispersed and concentrated on chloro and floro groups, due to their electron withdrawing nature and positive potential is concentrated on the protons attached to the thiophene and benzene rings. Compounds 2e and 3e are bearing the electron-donating methyl groups attached to the rings; therefore, the electronic density is localized on the pi cloud of both the thiophene and benzene rings.

![Figure 7. Cont.](image-url)
3. Biological Studies

3.1. Antibacterial Activity

Antibacterial activity is related to the existence of some elements in a compound, such as sulfur [8,36–38]. Recently, benzothiophene derivatives have been used in many therapies [39]. The newly-synthesized thiophene molecules 2a to 3f were tested against several strains of Gram-negative bacteria (Escherichia coli, Shigella dysenteriae, Pseudomonas aeruginosa and Salmonella typhi) and Gram-positive bacteria (Staphylococcus aureus and Bacillus subtilis). Ampicillin was used as a standard drug, and all data are shown in Figure 8. Electron-withdrawing and electron-donating substituents have great effect on antibacterial activity of synthesized compounds [38]. At the concentration of 50 and 100 μg/mL, compounds 2c, 2d, 3c, and 3f (containing electron-withdrawing groups) with an IC₅₀ value of 52.10, 58.0, 56.2, and 56.5 μg/mL, respectively, were found most potent against E. coli. Surprisingly, it has been observed that 2b exhibited highest bacterial inhibition activity with an IC₅₀ value of 51.4 μg/mL and 2a also showed unexpected IC₅₀ value of 54.17 μg/mL, and both of these compounds contain electron-donating groups. Compounds 2e and 3d exhibited significant activity with an IC₅₀ value of 70.5 and 71.2 μg/mL. However 3b showed IC₅₀ value almost equal to
standard Ampicillin while the remaining compounds exhibited IC_{50} values more than the standard against \textit{E. coli} and were found less active.

Compounds 2b, 2d, and 3b with an IC_{50} value of 80.0, 80.9, and 79.52 μg/mL, respectively, showed moderate activity against \textit{S. typhi}. However, 3d exhibited IC_{50} value nearly equal to the standard while all other compounds were found less active than the standard having a high value of IC_{50} as compared to the standard.

Inflammatory disorders in the human body are associated with nitric oxide (NO). From the reported data, it is observed that various thiophene derivatives exhibit antioxidant activity [39–42] and can also be used as antitumor agent [43].

The antioxidant activity of compounds 2a–3f was tested by nitric oxide scavenging activity method and the results were compared with that of standard natural antioxidant ascorbic acid. As shown in Figure 9, almost all the synthesized compounds showed radical scavenging activity, but the highest scavenger activity was observed in the compound 3d whose IC_{50} value was 72. Among all the synthesized compounds 2a, 2d, 3c, and 3e with the least values of IC_{50} 77, 76.26, 79.13 and 77.4 μg/mL, respectively, showed significant antioxidant activities. Moderate nitric oxide scavenging activity was observed in all the remaining compounds, except 2f, which is found inactive against this activity.
4. Materials and Methods

A Bruker ARX 600 MHz FT-NMR spectrometer (Billerica, MA, USA) was used to study. NMR spectra were taken on a Bruker ARX 600 MHz FT-NMR spectrometer while relishing deuterated CDCl3 as internal reference.

4.1. Synthesis of 2-Aryl-5-chloro thiophenes (2a–f)

To a stirred solution of 3 mL dioxane of 2-bromo-5-chlorothiophene (1.0 mmol) and tetrakis(triphenylphosphine)palladium(0) (5.0 mol %) were added and stirred for a period of 30 min. With the help of column chromatography the resultant product was purified.

4.1.1. 2-Chloro-5-(4-methylphenyl) thiophene (2a)

Pale yellow solid, mp. 168 °C; 1H-NMR: δ = 7.38 (d, J = 7.9, 2H-Ar), 7.16 (d, J = 8, 2H-Ar), 7.00 (d, J = 4, 1H-Thiophene), 6.85 (d, J = 3.6, 1H Thiophene), 2.36 (s, 3H-CH3). 13C-NMR: δ = 21.2 (CH3 of aryl), 158.2, 139.1. EIMS m/z: 208.71; [M + H]+: [M – Cl]++ = 172.26; [M – CH3 and benzene]++ = 91.14. Anal.(%) calcd for C11H7ClS, C 63.60, H 4.35; found C 63.66, H 4.31.

4.1.2. 2-Chloro-5-(4-methoxyphenyl) thiophene (2b)

Light green solid, mp. 172 °C; 1H-NMR: δ = 7.40 (d, J = 8.3, 2H-Ar), 7.01 (d, J = 3.6, 1H Thiophene), 6.90 (d, J = 8.8, 2 H-Ar), 6.85 (d, J = 3.8, 1H Thiophene) 3.91 (s, 6 OCH3). 13C-NMR: δ = 56.1 (OCH3 of aryl), 115.0, 125.8, 126.3, 127.3, 127.9, 139.5, 160.9. EIMS m/z: 224.71; [M + H]+: [M – OMe]++ = 194.68; [M – Cl]++ = 190.26. Anal.(%) calcd for C11H7ClOS, C 58.80; H 4.04; found C 58.20, H 4.10.

4.1.3. 2-Chloro-5-(3-chloro-4-fluorophenyl) thiophene (2c)

Yellowish green solid, mp. 180 °C; 1H-NMR: δ = 7.42 (d, J = 7.2, 2H-Ar), 7.35 (d, J = 8, 2H-Ar), 7.10 (d, J = 3.5, 1H Thiophene), 6.89 (d, J = 3.8, 1H Thiophene). 13C-NMR: δ = 125.9, 127.1, 128.4, 129.9, 132.0, 134.6, 139.1. EIMS m/z: 229.13; [M + H]+: [M – Cl]++ = 194.68; [M – 2Cl]++ = 160.24. Anal.(%) calcd for C11H6Cl2F, C 52.46, H 2.69; found C 52.42 ; H 2.64. EIMS (m/z, +ion mode).

4.1.4. 2-Chloro-5-(3-chloro-4-fluorophenyl) thiophene (2d)

Yellow solid, mp. 185 °C; 1H-NMR: δ = 7.32 (m, 3H-Ar), 6.98 (d, J = 4, 1H Thiophene), 6.87 (d, J = 3.8, 1H Thiophene). 13C-NMR: δ = 117.8, 121.3, 126.0, 126.8, 127.6, 128.8, 130.1, 138.9, 158.2. EIMS m/z: 247.12; [M + H]+: [M – F and Cl]++ = 192.68. Anal.(%) calcd for C10H5Cl2FS, C 48.58; H 2.08, found C 48.60; H 2.04.

4.1.5. 2-Chloro-5-(3,5-dimethylphenyl) thiophene (2e)

Greenish yellow solid, mp. 166 °C; 1H-NMR: δ = 7.30–7.19 (m, 3 H-Ar), 7.01 (d, J = 3.5, 1H-Thiophene), 6.99 (d, J = 3.7, 1H, Thiophene), 3.27 (s, 6H-CH3). 13C-NMR: δ = 21.8 (CH3 of aryl), 125.1, 126.1, 126.6, 127.5, 128.1, 128.9, 131.3, 138.8. EIMS m/z: 222.73; [M + H]+: [M – Br and 2CH3]++ = 157.24. Anal.(%) calcd for C12H11ClS, C 64.71, H 4.98, found C 64.77; H 4.93.

4.1.6. 2-Chloro-5-(3,4-dichlorophenyl) thiophene (2f)

Brownish yellow solid, mp. 186 °C; 1H-NMR: δ = 7.47–7.30 (m, 3 H-aryl), 7.20 (d, J = 3.8, 1H Thiophene), 7.01 (d, J = 3.3, 1H Thiophene). 13C-NMR: δ = 126.2, 127.2, 127.7, 128.4, 130.3, 132.4, 133.6, 133.9, 139.1. EIMS m/z: 263.57; [M + H]+: [M – 3Cl]+ = 157.24; [M – 2Cl and benzene]+ = 117.57. Anal.(%) calcd for C10H3Cl3S, C 45.52, H 1.89, found C 45.57; H 1.91.
4.2. Synthesis of Biarylthiophenes (3a–f)

Stirred solution of 1 (1.0 mmol) and tetrakis(triphenylphosphine)palladium(0) (6.0 mol %) were added and stirred for a period of 30 min. To this mixture was added Ar-B(OH)₂ (2.6 mmol), water (1.5 mL) and 4.7-mmol of K₃PO₄. The mixture was stirred at 90 °C for a period of 12 h. The mixture was stirred at 110 °C for a period of 24 h. With the help of column chromatography the resultant product was purified.

4.2.1. 2,5-Bis(4-methylphenyl) thiophene (3a)

Pale yellow solid, mp. 191 °C; ¹H-NMR: δ = 7.05 (s, 2H-thiophene), 7.39–7.20 (m, 8H-Ar), 3.02 (s, 6H-CH₃). ¹³C-NMR: δ = 21.1 (CH₃ of aryl), 125.7, 128.6, 129.8, 130.9, 131.2, 137.4, 138.1. EIMS m/z: 264.38 [M + H⁺]:[M − 2CH₃]+= 234.33. Anal.(%) calcd for C₁₈H₁₆S, C 81.77, H 6.10, found C 81.71; H 6.17.

4.2.2. 2,5-Bis(4-methoxyphenyl) thiophene (3b)

Greenish white solid, mp. 177 °C; ¹H-NMR: δ = 7.07 (s, 2H-Thiophene), 7.33–7.30 (m, 8H-Ar), 3.79 (s, 6H-OCH₃). ¹³C-NMR: δ = 55.4 (OCH₃ of aryl), 115.2, 125.9, 128.6, 137.4, 160.8. EIMS m/z: 296.28 [M + H⁺]:[M − OCH₃]+= 265.36; [M − OCH₃ and benzene]+= 189.26. Anal.(%) calcd for C₁₈H₁₆O₂S, C 79.91, H 5.40, found C 79.94; H 5.44.

4.2.3. 2,5-Bis(4-chlorophenyl) thiophene (3c)

Yellow solid, mp. 196 °C; ¹H-NMR: δ = 7.05 (s, 2H-Thiophene), 7.41–7.30 (m, 8H-Ar). ¹³C-NMR: δ = 128.2, 129.5, 130.9, 134.6, 137.3. EIMS m/z: 305.21 [M + H⁺]:[M − 2Cl]+= 234.31:[M − Cl and benzene]+= 193.76. Anal.(%) calcd for C₁₈H₁₆O₂S, C 62.90, H 3.38, found C 62.96; H 3.30.

4.2.4. 2,5-Bis(3-chloro-4-fluorophenyl) thiophene (3d)

Yellow crystals, mp. 183 °C; ¹H-NMR: δ = 7.20 (s, 2H-Thiophene), 7.69–7.58 (m, 6H-Ar). ¹³C-NMR: δ = 117.6, 121.0, 124.8, 126.9, 128.8, 130.2, 131.1, 133.8, 137.6, 159.1, 163.4. EIMS m/z: 341.20 [M + H⁺]:[M − 2F]+= 303.32. Anal.(%) calcd for C₁₆H₈Cl₂F₂S, C 56.32; H 2.36, found C 56.38; H 2.38.

4.2.5. 2,5-Bis(3,5-dimethylphenyl) thiophene (3e)

Greenish yellow solid, mp. 185 °C; ¹H-NMR: δ = 7.02 (s, 2H-Thiophene), 7.54–7.47 (m, 6H-Ar), 3.29 (s, 12H-CH₃). ¹³C-NMR: δ = 21.7 (CH₃ of aryl), 127.8, 128.8, 131.1, 133.8, 138.2, 138.6, 139.1. EIMS m/z: 292.44 [M + H⁺]; [M − 4Me]+= 232.34. Anal.(%) calcd for C₂₀H₂₀S, C 82.14, H 6.89, found C 82.18; H 6.80.

4.2.6. 2,5-Bis(3,4-dichlorophenyl) thiophene (3f)

Golden yellow solid, mp. 170 °C; ¹H-NMR: δ = 7.15 (s, 2H-Thiophene), 7.58–7.53 (m, 6H-Ar). ¹³C-NMR: δ = 126.9, 128.3, 129.1, 130.4, 132.9, 133.6, 138.2. EIMS m/z: 373.22 [M + H⁺]:[M − 4Cl]+= 323.33; [M − 2Cl]+= 303.22. Anal.(%) calcd for C₁₆H₈Cl₄S, C 51.30, H 2.18, found C 51.37; H 2.16.

4.3. X-ray Diffraction Analysis

Single crystals of both thiophenes 2d and 2f with appropriate sizes were chosen from available sample under microscope. Which were fixed on glass tip using glue, purchased from local market. The glass needle was supported by copper pin and magnetic base. This whole assembly was mounted on Agilent SuperNova (dual source) Agilent Technologies Diffractometer, equipped with graphite-monochromatic Cu/Mo Kα radiation for data collection. The data collection was accomplished using CrysAlisPro software [44], at 296 K under Cu Kα radiation. The structures were solved using SHELXS-97 [45], and refined by full-matrix least-squares methods on F² using SHELXL-97,
in-built with X-Seed [46]. All non–hydrogen atoms were refined anisotropically by full–matrix least squares methods [45]. The figures were drawn using PLATON in-built with wingx.

There are only aromatic (C–H) hydrogen atoms, which were positioned geometrically and treated as riding atoms with C–H = 0.93 Å and Uiso(H) = 1.2 Ueq(C) carbon atoms.

The CIFs for both molecules have been submitted to (The Cambridge Crystallographic Data Centre) CCDC and got CCDC numbers 1469610 and 1469611 for molecule 2d and 2f respectively. These CIFs can be ordered free of cost from CCDC 12 Union Road, Cambridge CB21 EZ, UK.

4.4. Computational Methods

Theoretical investigations were performed by using Gaussian 09 software [47]. Visualizations of graphics/geometries was achieved by using Gauss view 05 program [48]. Geometries of (2a–f) and (3a–f) were optimized by adopting hybrid B3LYP method without any symmetry constraints along with 6-31G (d, p) basis set at DFT level of theory [49,50]. Frontier molecular orbital analysis and molecular electrostatic potential mapping of both series (2a–f) and (3a–f) were simulated at same level of DFT as used for energy minima optimization.

4.5. Antibacterial Assay

The antibacterial assay of compounds 2a–3f was accomplished by method reported by of Nasrullah and co-workers [12]. Bacillus subtilis, Staphylococcus aureus were used as Gram-positive bacteria and Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Shigelladysenteriae used as Gram-negative bacteria.

Solutions of the compounds were made by dissolving in a solvent of known concentration (5 and 10 µg/mL). Samples of different concentrations were prepared by already known volumes of compounds. Ampicillin (positive control) was prepared by using the same methodology. By using only solvent negative control was prepared. At 137 °C for 30 min glass apparatus was sterilized. In sterile glass Petri plates nutrient agar was added. In test tubes having nutrient broth, sub-cultures were injected and left at 37 °C for 16 h on rotary shaker. On inoculated nutrient agar medium positive and negative controls, all discs and test samples were solidified at 37 ± 2 °C for 24 h. With the help of an ordinary ruler, microbial growth was measured.

4.6. Nitric Oxide Scavenging Activity

By following procedure reported by Garrat and co-workers [51] nitric oxide scavenging activity of all compounds was carried out.

5. Conclusions

In summary, we report the synthesis of various 2-aryl-5-chlorothiophenes and 2,5-biarylthiophenes, starting from 2-bromo-5-chloro thiophenes. In Suzuki coupling reactions, different boronic acids/esters react with 2-bromo-5-chloro thiophenes in the presence of a palladium catalyst. X-ray and calculated geometric parameters of 2d and 2f, corroborate very nicely to each other. Reactive sites and electronic effect of group attached to benzene ring was investigated by ESP analysis. By noting the results of this study it is revealed that some of the synthesized compounds of 2-bromo-5-chloro thiophenes can be used as antibacterial agents.

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