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REGULAR ARTICLE

Synthesis and biological evaluation of novel benzothiophene derivatives

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Abstract. Benzothiophene derivatives were synthesized regioselectively using coupling reactions and electrophilic cyclization reactions. Antimicrobial properties of isolated compounds were tested against indicator microorganisms such as *C. albicans* ATCC 10231, *B. subtilis* ATCC 6633, *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. 3-(4-aminobenzoethynyl)-2-(thiophen-2-yl) benzo[*b*]thiophene (**12E**), 3-ethynyl-2-(thiophen-2-yl) benzo[*b*]thiophene (**12L**) and 3-(2-aminobenzoethynyl)-2-(thiophen-2-yl) benzo[*b*]thiophene (**12J**) displayed high antibacterial activity against *S. aureus*. Further, 3-iodo-2-(thiophen-2-yl) benzo[*b*]thiophene (**10**) and 3-(trimethylsilylethynyl)-2-(thiophen-2-yl) benzo[*b*] thiophene (**12K**) were found to have potentials to be used as antifungal agents against current fungal diseases. Novel 3-(1H-indole-2-yl)-2-(thiophen-2-yl) benzo[*b*] thiophene (**16**) and 3-(4-aminobenzoethynyl)-2-(thiophen-2-yl) benzo[*b*] thiophene (**12E**) also showed quite high antioxidant capacities with TEAC values of 2.5 and 1.1, respectively; which surpassed the antioxidant capacity of an universally accepted reference of trolox.

Keywords. Benzothiophenes; heteroaromatic compounds; biological properties; antioxidant capacity; antimicrobial agents.

1. Introduction

Heteroaromatic compounds have very important roles in the discovery and development of new drug candidates due to their biological and pharmacological properties.[1,2] They have been used as anti-parasitic,[3] anti-bacterial,[4] anti-cancer,[5] anti-fungal,[6] anti-inflammatory[7] and antioxidant[8] drugs for years. In addition, they are known as strong inhibitors of lipid peroxidation,[9] potassium channel openers,[10] topoisomerase inhibitors,[11] and L1210 cell selectors.[12]

Benzothiophenes and thiophenes are well-known candidates of heteroaromatic compounds and used as pharmaceuticals like Raloxifene **1**,[13] Zileuton **2**,[14] and Sertaconazole **3** (Scheme 1).[15] Raloxifene, whose commercial brand name is Evista, is used for the treatment of breast cancer. Moreover, Raloxifene has less side-effects than another popular anti-cancer drug, Tamoxifen, with similar biological properties.[16] Recently, a number of 3-(4-pyridinyl) amino benzothiophenes, which were selective serotonin re-uptake inhibitors, have been tested for their contribution on the treatment of central nervous system disorders such as Alzheimer's disease.[17] Therefore, synthetic and medicinal chemists have tried to find novel organic compounds containing sulfur atoms for decades.

Over the past years, benzothiophenes have been generally obtained from intramolecular cyclization and Claisen rearrangement reactions. For example, benzothiophenes were regioselectively synthesized via intramolecular cyclization reaction of *o*-alkinylthioanisoles.[18] Recently, Mohanakrishnan *et al.,* published a new method for the synthesis of benzothiophene and dibenzothiophene starting from thiophenes and 2,5-dimethoxy-THF using Lewis Acid catalyst.[19]

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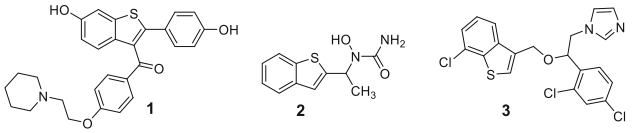


Scheme 1. Structures of Raloxifene **1**, Zileton **2** and Sertaconazole **3**.

Antimicrobial resistance to discovered antibiotics and other drugs used in the treatment of bacterial, fungal and viral infections is a serious problem threatening all the world population. Antimicrobial agents with unique chemical structures and strong antimicrobial potentials are urgently needed. However, the discovery rate of biologically active compounds has decreased. In this respect, chemically designed and synthesized sulfur containing benzothiophene compounds are among the promising members for the treatment of infections.[20] Further, those benzothiophene derivatives have been tested and used as antioxidant agents for years. Antioxidants have essential roles in overall metabolism because of their potential in repairing damages generated by reactive oxygen species (ROS) and simple deactivation of those active compounds. Therefore, they are participants in hindering and reprieving various diseases such as cancer,[21] diabetes,[22] and cardiovascular problems.[23,24] Besides natural antioxidants, nowadays, synthetic antioxidants, also, have an important place in food and pharmaceutical industries.[25]

In the present study, novel benzothiophene derivatives were synthesized using intramolecular electrophilic cyclization reactions and Palladium catalyzed Sonogashira coupling reactions. Additionally, antimicrobial and antioxidant capacities of new derivatives were also tested to find their potentials as novel drug candidates.

2. Experimental

2.1 *General information*

Solvents, reagents, and chemicals used for reactions were purchased from commercial suppliers. Synthesized molecules were analyzed by ^{1}H and ^{13}C NMR. ^{1}H and ^{13}C NMR spectra were recorded on an Agilent NMR (400 MHz) spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from an internal TMS (trimethylsilane) reference. Coupling constants (J) were reported in Hertz (Hz). In addition, spin multiplicities were presented by the following symbols: s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). DEPT ^{13}C NMR information was given in parentheses as C, CH, CH_2 and CH_3. Flash chromatography was done using thick-walled glass columns and 'flash grade' silica 60 (Merck 230–400 mesh). Thin layer chromatography (TLC) was performed using commercially prepared 0.25 mm silica gel plates (Silica gel 60, F254) and visualization was effected with short wavelength UV lamp. The relative proportions of a mixture of solvents in chromatography were indicated by volume to volume ratio. All commercially available reagents were used directly without purification unless otherwise stated. All the solvents used in reaction experiments were distilled for purity. The inert atmosphere was created by slight positive pressure (ca. 0.1 psi) of argon. All reactions were carried out in flame-dried glassware.

2.2 *Synthesis of 2-trimethylsilanylethynylthioanisole (**5**)*

To a stirred mixture of the 2-iodothioanisole (2.5 mmol, 620 mg), THF (8 mL), ethynyltrimethylsilane (3 mmol, 303 mg), triethylamine (12.4 mmol, 1.72 mL), $PdCl_2$ $(PPh_3)_2$ (0.06 mmol, 42.1 mg) was added CuI (0.06 mmol, 11.4 mg) under argon atmosphere. The resulting mixture was stirred at room temperature for 12 h. Then, the mixture was quenched with water (30 mL), and extracted with DCM (3 × 30 mL). The organic phase was dried over anhydrous $MgSO_4$ and filtered. The solvent was removed under vacuum, and the residue was purified by column chromatography over silica gel with hexane to afford 2-trimethylsilanylethynylthioanisole (**5**) as light yellow oil in **96%** yield. ^{1}H NMR (400 MHz, $CDCl_3$) 7.43 (dd, J = 7, 7, 1.5 Hz; 1H), 7.27 (td, J = 7.5, 1.5 Hz; 1H), 7.11 (d, J = 7.4 Hz; 1H), 7.05 (td, J = 7.5, 1.2 Hz; 1H), 2.46 (s, 3H), 0.32 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.2, 132.7, 129.1, 124.1, 123.9, 121.1, 102.3, 101.4, 15.0, 0.13; IR (ATR) υ_{max} (cm^{-1}): 3059.5, 2980.5, 2920.7, 2890.6, 2154.7 (C ≡ C), 1435.5, 1248.3 (Si–CH_3), 838.1, 747.6, 685.2 (S-C). The spectral data were in agreement with those reported previously for this compound.[26]

2.3 *Synthesis of 2-ethynyl thioanisole (**6**)*

To a stirred mixture of **5** (500 mg, 2.27 mmol), methanol (60 mL), and THF (20 mL) was added K_2CO_3 (939 mg, 6.81 mmol). The mixture was stirred at room temperature for 60 min. The reaction mixture extracted with EtOAc (3 × 15 mL). The organic extracts were dried over anhydrous $MgSO_4$ and filtered. The solvent was removed under vacuum, and the residue was purified by column chromatography over silica gel with hexane to afford 2-ethynylthioanisole (**6**) as yellow oil in **91%** yield: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.46 (dd, J = 7.6, 1.5 Hz; 1H), 7.30 (td, J = 8.2, 1.4 Hz; 1H), 7.15 (d, J = 8.0 Hz; 1H), 7.08 (td, J = 8.5, 1.0 Hz; 1H), 3.5 (s, 1H, alkyne), 2.48 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.0, 133.1, 129.3, 124.2, 124.1, 120.1, 83.7, 81.0, 60.3; IR (ATR) υ_{max} (cm^{-1}): 3058.32, 2982.06, 3281.39 (≡ C−H), 2920.25, 2101.87 (C ≡ C), 1432.71, 747.94, 614.6 (S-C); HRMS calcd for C_9H_9S, 149.0425 $[M+H]^+$ found 149.0248 $[M+H]^+$. The spectral data were in agreement with those reported previously for this compound.[26]

2.4 *Synthesis of 2-[(2-(methylthio) phenyl]ethynyl) thiophene 9 via Sonogashira palladium-catalyzed cross-coupling reaction between 2-ethynylthioanisole (**6**) and 2-bromothiophene (**8**)*

To a solution of 2-bromothiophene (456.4 mg, 2.8 mmol) in 1, 4-dioxane (6 mL), CuI (32.4 mg, 0.17 mmol), $PdCl_2(PhCN)_2$ (65.2 mg, 0.17 mmol) were added, followed by **6** (3.0 mmol, 444 mg), diisopropylamine (1.38 g, 13.7 mmol), and P(*t*-Bu)$_3$ (52.9 mg, 0.33 mmol) at room temperature under argon. The mixture was stirred at room temperature for 12 h, and extracted with DCM (3 × 20 mL). The organic layer was dried over anhydrous $MgSO_4$ and filtered. The solvent was removed under vacuum, and the residue was purified by column chromatography over silica gel with Hexane/EtOAc (10/1) to afford 2-[(2-(methylthio) phenyl)ethynyl]thiophene (**9**) as an orange oil in **77%** yield. ^{1}H NMR (400 MHz, $CDCl_3$) 7.49 (d, J = 7.7 Hz; 1H), 7.36–7.30 (m, 3H), 7.19 (d, J= 8,0 Hz; 1H), 7.13 (td, J = 8, 0, 1.1 Hz; 1H), 7.04 (m, 1H), 2.53 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 141.8, 132.3, 132.2, 129.1, 127.8, 127.3, 124.4, 124.3, 123.2, 121.1, 90.7, 89.2, 15.3; IR (ATR) υ_{max} (cm^{-1}): 3086.06, 2911.91, 2980.6, 2199.72(C ≡ C),1431.12, 753.46, 699.33 (S-C); HRMS calcd for $C_{13}H_{11}S_2$, 231.0302 $[M+H]^+$ found 231.0313 $[M+H]^+$. The spectral data were in agreement with those reported previously for this compound.[27]

2.5 *Synthesis of 2-[(2-(methylthio) phenyl) ethynyl] thiophene 9 via Sonogashira palladium-catalyzed cross-coupling reaction between 2-ethynylthioanisole (**6**) and 2-iodothiophene (**7**)*

To a mixture of 2-iodothiophene (707 mg, 3.37 mmol), **6** (500 mg, 3.37 mmol), DMF (6 mL), $PdCl_2(PPh_3)_2$ (118.3 mg, 0.17 mmol), and triethylamine (1.9 mL, 13.7 mmol) at 0 °C under argon gas was added CuI (32.1 mg, 0.17 mmol). The mixture was stirred at room temperature for 4 h and extracted with ether (3 × 20 mL). The organic layer was dried over anhydrous $MgSO_4$ and filtered. The solvent was removed under vacuum, and the residue was purified by column chromatography over silica gel with Hexane/EtOAc (10/1) to afford compound **9** as orange oil in **71%** yield.

2.6 *Synthesis of 3-iodo-2-(thiophen-2-yl) benzo[b] thiophene (10) via electrophilic cyclization reaction*

To a solution of 2-[(2-(methylthio phenyl) ethynyl] thiophene (**9**) (230 mg, 1 mmol) in CH_2Cl_2 (10 mL) was added I_2 (762 mg, 3 mmol) at room temperature. After stirring for 30 min, the saturated aqueous solution of $Na_2S_2O_3$ was added into the reaction mixture and extracted with EtOAc (3 × 20 mL). The combined organic phase was dried over anhydrous $MgSO_4$ and filtered. The solvent was removed under vacuum, and the residue was purified by column chromatography over silica gel with hexane/EtOac (19/1) to afford 3-iodo-2-(thiophen-2-yl) benzo[b]thiophene (**10**) as light yellow solid in **99%** yield. ^{1}H NMR (400 MHz, $CDCl_3$) 7.82 (d, J = 8.1 Hz; 1H), 7.75 (d, J = 9.1 Hz; 1H), 7.62 (d.d, J = 4, 0, 1.1 Hz; 1H), 7.48–7.44 (m, 2H), 7.39 (td, J = 8, 0, 1.2 Hz; 1H), 7.17 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 142.4, 138.1, 136.0, 135.9, 128.8, 127.5, 127.4, 126.4, 125.9, 125.8, 122.0, 79.5; IR (ATR) υ_{max} (cm^{-1}): 698.75, 815.0, 1419.47, 2912.23, 2980.61, 3085.91; HRMS calcd for $C_{12}H_7S_2$, 341.9034 found; 341.9059.

2.7 *General procedure of Sonogashira cross-coupling reaction between 3-iodo-2-(thiophen-2-yl) benzo[b]thiophene (10) and alkynes*

To a stirred mixture of 3-iodo-2-(thiophen-2-yl) benzo[*b*] thiophene **10** (0.7 mmol, 239.5 mg), dimethylformamide (DMF) (7.5 mL), alkyne (0.75 mmol), triethylamine (3.0 mL), $PdCl_2$ $(PPh_3)_2$ (0.035 mmol, 24.5 mg) under argon atmosphere was added CuI (0.035 mmol, 6.6 mg). The resulting mixture was stirred at room temperature for 12 h. It was quenched with water (30 mL) and extracted with DCM (3 × 30 mL). The organic layer was dried over anhydrous $MgSO_4$ and filtered. The solvent was removed under vacuum, and the residue was purified by column chromatography over silica gel with Hexane/EtOAc (19/1) to afford compounds (**12A–L**).

3-(Phenylethynyl)-2-(thiophen-2-yl)benzo[*b*] thiophene (12A) was isolated in **94%** yield as a green solid. M.p: 85.1–86.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.99 (d, J = 7.4 Hz; 1H), 7.77 (d, J = 8.0 Hz; 1H), 7.74–7.70 (m, 2H), 7.66 (d, J = 3.72 Hz; 1H), 7.49–7.37 (m, 6H), 7.13–7.15 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.8; 140.2, 137.0, 136.5, 131.8, 128.8, 128.7, 127.5, 127.2, 127.16, 125.7, 125.3, 123.5, 123.3, 122.2, 113.0, 97.9, 84.2; IR (ATR) υ_{max} (cm^{-1}): 3062.96, 2980.48, 2922.72, 2199.68, 1438.03, 1237.22, 746.05, 683.58; HRMS: calcd for $C_{20}H_{13}S_2$, 317.0458 $[M+H]^+$ found, 317.0457 $[M+H]^+$.

3-(p-Tolylethynyl)-2-(thiophen-2-yl)benzo[b]thiophene (12B) was isolated in **65%** yield as an yellow solid. M.p.: 120.0–121.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 8.0 Hz; 1H), 7.76 (d, J = 7.9 Hz; 1H), 7.66 (d, J = 3.8 Hz; 1H), 7.60 (d, J = 8.0 Hz; 2H), 7.48–7.36 (m, 3H), 7.24 (d, J = 7.9 Hz; 2H), 7.15–7.12 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.8, 139.8, 139.0, 137.0, 136.6, 131.7, 129.5, 127.4, 127.04, 127.01, 125.6, 125.2, 123.3, 122.1, 120.4, 113.1, 98.2, 83.6, 21.8; IR (ATR) υ_{max} (cm^{-1}): 3068.87, 2980.59, 2918.12, 1437.45, 806.73, 752.44; HRMS:calcd for $C_{21}H_{15}S_2$, 331.0615 $[M+H]^+$ found 331.0699 $[M+H]^+$.

3-(Hept-1-yn-1-yl)-2-(thiophen-2-yl)benzo[b]thiophene (12C) was isolated in **82%** yield as a green solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 8.0 Hz; 1H), 7.74(d, J = 1.08 Hz; 1H), 7.63 (d, J = 4.7 Hz; 1H), 7.45–7.36 (m,3H), 7.14–7.10 (m,1H), 2.65 (t, J = 7.1 Hz; 2H), 1.82–1.74 (m,2H), 1.61–1.53 (m,2H), 1.48–1.40 (m,2H), 0.98 (t, J = 7.3 Hz; 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.3, 138.8, 137.0, 136.7, 127.3, 126.7, 126.5, 125.5, 125.1, 123.3, 122.1, 113.8, 99.8, 75.2, 31.5, 30.0, 28.5, 22.5, 20.3, 14.3; IR (KBr, thin film) υ_{max} (cm^{-1}): 3067.19, 2950.81, 2929.27, 2855.59,

2215.36 (C ≡ C),1455.44, 1420.67, 836.14, 698.05; HRMS calcd. for $C_{19}H_{19}S_2$, 311.0850 $[M+H]^+$; found 311.0843 $[M+H]^+$.

3-[(3-Bromothiophen-2-yl)ethynyl)-2-(thiophen-2-yl]benzo[b]thiophene (12D) was isolated in **71%** yield as an yellow solid. M.p.: 91.7–62.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 (d, J = 7.4 Hz; 1H), 7.78–7.72 (m, 2H), 7.50–7.34 (m, 3H), 7.31 (d, J = 5.3 Hz, 1H), 7.14–7.13 (m, 1H), 7.06(d, J = 5.4, 1H).^{13}C NMR (100 MHz, $CDCl_3$) δ 140.8, 140.6, 137.0, 136.3, 130.6, 127.9, 127.7, 127.4, 127.3, 125.8, 125.5, 123.4, 122.2, 121.2, 116.4, 112.3, 91.8, 89.3; IR (ATR) ν_{max} (cm^{-1}): 3099.53, 3054.27, 2980.48, 2922.74, 2195.37 (C ≡ C),1436.51, 1149.67, 862.56, 688.91; HRMS calcd for $C_{18}H_{10}S_3Br$, 400.9128 $[M+H]^+$ found 400.9131 $[M+H]^+$.

3-(4-Aminobenzoethynyl)-2-(thiophen-2-yl) benzo[b]thiophene (12E) was isolated in **92%** yield as a green solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 10.0 Hz; 1H), 7.76 (d, J = 9.6 Hz; 1H), 7.65 (d.d, J = 3.72 Hz, 1.12 Hz; 1H), 7.51(d, J = 8.7 Hz; 2H), 7.47–7.35 (m, 3H), 7.14–7.11 (m, 1H), 6.70 (d, J = 8.7 Hz; 2H), 3.9 (brs, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.2, 140.9, 138.8, 136.9, 136.8, 133.2, 127.4, 126.8 (including 2C), 125.6, 125.2, 123.4, 122.1, 115.1, 113.6, 112.8, 98.9, 82.2; IR (ATR) υ_{max} (cm^{-1}): 3648.08, 3378.68, 2955.73, 2922.29, 2193.60, 1603.81, 1613.73, 1288.05, 1174.35, 906.16, 825.42, 728.67, 696.05; HRMS:calcd for $C_{20}H_{14}S_2N$, 332.0568 $[M+H]^+$ found 332.0559 $[M+H]^+$.

3-(4-Methoxybenzoethynyl)-2-(thiophen-2-yl) benzo[b] thiophene (12F) was isolated in **70%** yield as a light green solid. M.p.: 99.5–100.8 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98 (d, J = 10.2 Hz; 1H), 7.76(d, J = 7.9 Hz, 1H), 7.66–7.63 (m, 3H), 7.45–7.351(m, 3H), 7.14–7.11 (m,1H), 6.96 (d, J = 14.4 Hz, 2H) 3.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.0, 140.8, 139.4, 136.9, 136.7, 133.3, 127.4, 127.0, 126.9, 125.6, 125.2, 123.4, 122.9, 115.6, 114.4, 113.3, 98.1, 82.9, 55.6; IR (ATR) υ_{max} (cm^{-1}): 3099.03, 3050.45, 2969.85, 2836.00, 2537.06, 2198.92, 1603.21, 1455.42, 1290.88, 1248.09, 1169.13, 1022.60, 834.25, 753.21, 689.80; HRMS: calcd for $C_{21}H_{14}OS_2$, 346.0486, found 346.0488.

3-(2, 5-Dimethylbenzoethynyl)-2-(thiophen-2-yl) benzo[b] thiophene (12G) was isolated in **69%** yield as an orange solid. M.p.: 124.7–126.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.0 (d, J = 6.0 Hz;1H), 7.8 (d, J = 9.6 Hz; 1H), 7.69 (dd, J= 4.0 Hz, 1.2 Hz;1H), 7.52 (s,1H), 7.50–7.35 (m, 3H), 7.20 (d, J = 7.8 Hz; 1H), 7.16–7.12 (m, 2H), 2.65 (s, 3H), 2.40 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.9, 139.5, 137.2, 137.0, 136.5, 135.4, 132.7, 129.8, 129.7, 127.4, 127.1, 127.0, 125.6, 125.3, 123.3, 123.1, 122.2, 113.4, 97.1, 87.4, 21.1, 20.9; IR (ATR) ν_{max} (cm^{-1}): 3058.44, 2980.45, 2915.37, 2727.39, 2190.46, 1936.28, 1901.14, 1373.30, 1420.57, 1228.57, 813.75, 695.58, 573.38; HRMS calcd for $C_{22}H_{17}S_2$, 345.0772 $[M+H]^+$ found 345.0768 $[M+H]^+$. .

3-(2-Naphthylethynyl)-2-(thiophen-2-yl) benzo[b]thiophene (12H) was isolated in **93%** yield as an yellow solid. M.p.: 140.3–143.3 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.6 (d, J = 8.5 Hz; 1H), 8.1 (d, J= 8.0 Hz; 1H), 7.97–7.91(m, 3H), 7.8 (d, J = 9.0 Hz; 1H), 7.22 (dd, J= 4.0, 1.2 Hz; 1H), 7.68–7.64 (m, 1H), 7.60–7.48 (m, 3H), 7.44–7.40 (m, 2H), 7.16–7.12 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.9, 140.2, 137.1, 136.5, 133.5, 133.3, 131.0, 129.3, 128.6, 127.5, 127.3, 127.25, 127.22, 126.8, 126.6, 125.7, 125.6, 125.5, 123.4, 122.2, 121.2, 113.2, 96.1, 88.8; IR (ATR) ν_{max} (cm^{-1}): 3051.37, 2918.28, 2191.60, 1936.73, 1727.62, 1388.38, 791.93, 768.18, 694.03; HRMS calcd for 367.0615 $C_{24}H_{15}S_2$ $[M+H]^+$ found 367.0602 $[M+H]^+$.

3-(2-Thioanisolethynyl)-2-(thiophen-2-yl) benzo[b]thiophene (12I) was isolated in **81%** yield as a yellow solid. M.p.: 165.3–167.2 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (d, J = 8.0 Hz; 1H), 7.75 (d, J = 7.96 Hz; 1H) 7.72 (dd, J = 3.7 Hz, 1.08 Hz; 1H), 7.66 (dd, J = 7.7 Hz, 1.32 Hz;1H), 7.49–7.33 (m, 4H) 7.27–7.24 (m, 1H), 7-22-711 (m, 2H), 2.56 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.7, 141.0, 136.9, 136.5, 132.64, 130.0, 129.2, 127.6, 127.3, 127.2, 125.7, 125.5, 124.6, 124.5, 123.8, 122.1, 121.7, 113.0, 95.1, 90.5, 15.52; IR (ATR) υ_{max} (cm^{-1}): 3054.12, 2919.23, 2196.83, 1433.38, 1069.32, 745.44, 691.63; HRMS: calcd for $C_{21}H_{15}S_3$, 363.0336 $[M+H]^+$ found 363.0335 $[M+H]^+$.

3-(2-Aminobenzoethynyl)-2-(thiophen-2-yl) benzo[*b*]thiophene (12J) was isolated in **91%** yield as an yellow-green solid. M. p.: 89.9–94.5 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98–7.96 (d, J = 8.0, 1H), 7.78–7.76 (d, J = 8.0, 1H), 7.66–7.64 (m, 1H), 7.56–7.54 (m, 1H), 7.48–7.37 (m, 3H), 7.24–7.20 (m, 1H), 7.13–7.11 (m, 1H), 6.83–6.78 (m, 2H), 4.47 (brs, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148, 140.7, 139.5, 137.0, 136.4, 132.6, 130.4, 127.5, 127.1, 127.0, 125.7, 125.4, 123.2, 122.2, 118.3, 114.7, 113.1, 108.1, 94.68, 89.0; IR (ATR) ν_{max} (cm^{-1}): 3646.46, 3388.01, 3291.45, 2980.39, 2921.93, 2190.03, 1727.63, 1642.99, 1620.79, 1437.80, 1252.95, 1153.55, 753.99, 694.32; HRMS calcd for $C_{20}H_{14}NS_2$ 332.0567 $[M+H]^+$ found 332.0568 $[M+H]^+$.

3-(Trimethylsilylethynyl)-2-(thiophen-2-yl) benzo[b]thiophene (12K) was isolated in **89%** yield as a red oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 10, 0 Hz; 1H), 7.74 (d, J = 8.0 Hz; 1H), 7.68 (d, J = 4, 0 Hz; 1H), 7.47–7.41 (m, 2H), 7.36 (td, J = 8.0, 0.9 Hz; 1H), 7.14–7.11 (m, 1H), 0.40 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.1, 140.9, 136.8, 136.4, 127.4, 127.2, 127.0, 125.6, 125.3, 123.4, 122.0, 112.9, 104.1, 99.2, 0.13; IR (ATR) ν_{max} (cm^{-1}): 2958.21, 2148.01, 1438.15, 1247.46, 908.99, 839.30, 756.33, 696.03; HRMS cald for $C_{17}H_{17}S_2Si$, 313.0541 $[M+H]^+$ found; 313.0537 $[M+H]^+$.

2.8 *General procedure of desilylation reaction for the synthesis of 3-ethynyl-2-(thiophen-2-yl) benzo[b] thiophene (12L)*

To a solution of 3-(trimethylsilylethynyl)-2-(thiophen-2-yl) benzo [b] thiophene (**12l**) (56 mg, 0.17 mmol) in methanol (15 mL) and THF (5 mL) was added K_2CO_3 (74.3 mg, 0.53 mmol) at room temperature for 1 h. Then, organic solvents were removed under reduced pressure and the residue was

extracted with DCM (10 mL × 3). The solvent was removed under vacuum, and the residue was purified by column chromatography over silica gel with Hexane/EtOAc (19/1) to afford 3-ethynyl-2-(thiophen-2-yl) benzo [b] thiophene (**12L**) as a red oil in **83%** yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 8, 0 Hz; 1H), 7.77–7.70 (m, 2H), 7.48–7.34 (m, 3H), 7.16–7.10 (m, 1H), 3.70 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.4, 140.1, 136.9, 136.0, 127.7, 127.4, 127.1, 125.7, 125.4, 123.2, 122.1, 111.8, 85.8, 78.3; IR (ATR) υ_{max} (cm^{-1}): 3286.03, 3101.49, 2922.12, 2851.51, 2095.61, 1940.45, 1791.21, 1437.68, 1188.59, 752.07, 690.36, 647.28, 584.34; HRMS cald for $C_{14}H_9S_2$, 241.0146 $[M+H]^+$ found; 241.0139 $[M+H]^+$.

2.9 *Synthesis of 3-(3-iodobenzo[b]thiophen-2-yl)-2-(thiophen-2-yl) benzo [b] thiophene (**14**)*

To a solution of the 3-(2-thioanisolethynyl)-2-(thiophen-2-yl) benzo [*b*] thiophene**12I** (85 mg, 0.23 mmol) in dichloromethane (5mL) was added I_2 (173.8 mg, 0.69 mmol) under argon atmosphere. The mixture was stirred at room temperature for 1h. It was quenched with sat. sodium thiosulfate (30 mL), and extracted with DCM (3 × 20 mL). The organic layer was dried over anhydrous $MgSO_4$ and filtered. The solvent was removed under vacuum, and the residue was purified by column chromatography over silica gel with Hexane to afforded 3-(3-iodobenzo[b]thiophen-2-yl)-2-(thiophen-2-yl) benzo[b]thiophene (**14**) as a light yellow solid in **81%** yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90–7.85 (m, 3H), 7.57–7.47 (m, 2H), 7.42–7.33 (m, 3H), 7.28–7.27 (m, 1H), 7.24–7.22 (m, 1H), 6.99–6.97 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 141.5, 140.8, 140.5, 137.8, 137.7, 136.3, 135.5, 128.0, 127.5, 127.4, 126.4, 126.1, 125.6, 125.4, 125.3, 124.8, 123.4, 122.8, 122.2, 86.48; IR (ATR) υ_{max} (cm^{-1}): 3104.56, 3057.09, 2930.00, 2851.61, 1728.33, 1430.00, 1244.14, 753.26, 703.55; HRMS calcd for $C_{20}H_{11}IS_3$, 473.9067; found 473.9072.

2.10 *General procedure for the synthesis of 3-(arylethynyl)-2′-(thiophen-2-yl)-2,3′-bibenzo[b] thiophene (**15**)*

To a stirred mixture of **14** (0.7 mmol), DMF (7.5 mL), alkyne (0.75 mmol), triethylamine (3.0 mL), $PdCl_2$ $(PPh_3)_2$ (0.035 mmol) under argon gas was added CuI (0.035 mmol, 6.7 mg). The resulting mixture was stirred at room temperature for 12 h Water (30 mL) was added to the reaction mixture, and the resulting solution was extracted with DCM (3 × 30 mL). The organic layer was dried over anhydrous $MgSO_4$ and filtered. The solvent was removed under vacuum, and the residue was purified by column chromatography over silica gel with Hexane/EtOAc (19/1) to afford compounds (**15A–D**).

3-(3-(p-Tolylethynyl)benzo[b]thiophen-2-yl)-2-(thiophen-2-yl)benzo[b]thiophene 15A was isolated in **89%** yield as a yellow solid : M.p.: 139.7–141.2 °C; ^{1}H NMR (400 MHz, CDCl3) δ 8.08–8.10 (d, J = 8.0, 1H), 7.88–7.90 (d, J = 8.0, 1H), 7.84–7.86 (m, 1H), 7.66–7.69 (m, 1H), 7.45–7.55 (m, 2H), 7.34–7.40 (m, 2H), 7.23–7.26 (m, 2H), 7.11–7.13 (d, J = 8.0, 2H), 7.03–7.05 (d, J = 8.0, 2H), 6.94–6.97 (m, 1H), 2.31 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.9, 139.9, 139.8, 138.9, 138.6, 138.0, 137.2, 135.8, 131.7, 129.1, 127.68, 127.66, 127.4, 125.6, 125.2, 125.1, 125.0, 124.1, 123.8 (include 2C), 122.7, 122.1, 120.2, 120.1, 95.4, 82.2, 21.7; IR (ATR) v_{max} (cm^{-1}): 698.50, 728.72, 755.38, 814.32, 1435.36, 2205.17, 2853.61, 2920.76; HRMS calcd for $C_{29}H_{19}S_3$, 463.0649 $[M+H]^{+;}$ found 463.0636 $[M+H]^+$.

3-(3-(2,5-Dimethylbenzoethynyl)benzo[b]thiophen-2-yl)-2-(thiophen-2-yl)benzo[b]thiophene 15B was isolated in **90%** yield as a yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12–8.14 (d, J = 8.0, 1H), 7.90–7.92 (d, J = 8.0, 1H), 7.83–7.85 (m, 1H), 7.54–7.59 (m, 2H), 7.47–7.51 (m, 1H), 7.31–7.39 (m, 2H), 7.26–7.28 (m, 1H), 7.21–7.22 (m, 1H), 7.10 (s, 1H), 6.94–6.96 (m, 3H), 2.24 (s, 3H), 1.90 (s, 3H); 13C NMR (100 MHz, $CDCl_3$) δ 141.0, 140.0, 139.8, 138.9, 137.8, 137.4, 137.3, 135.7, 135.0, 132.4, 129.4, 129.38, 127.7, 127.5, 127.3, 125.7, 125.24, 125.16, 125.1, 124.0, 123.8, 123.7, 122.8, 122.7, 122.0, 120.7, 94.3, 86.3, 20.9, 19.8; IR (ATR) vmax (cm^{-1}): 661.07, 729.36, 756.18, 1030.58, 1232.42, 1435.79, 1456.13, 1733.94, 2202.29, 2922.48; HRMS calcd for $C_{30}H_{21}S_3$, 477.0805 $[M+H]^+$; found 477.0770 $[M+H]^+$.

3-(3-(2-Naphthylethynyl)benzo[b]thiophen-2-yl)-2-(thiophen-2-yl)benzo[b]thiophene 15C was isolated in **76%** yield as an yellow solid: M.p.: 153.2–154.7 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21–8.23 (d, J = 8.0, 1H), 7.90–7.95 (m, 2H), 7.73–7.76 (m, 2H), 7.66–7.68 (d, J = 8.0, 1H), 7.50–7.61 (m, 4H), 7.34–7.44 (m, 4H), 7.30–7.31 (m, 1H), 7.24–7.28 (m, 1H), 7.20–7.22 (d, J = 8.0, 1H), 6.92–6.94 (m, 1H); ^{13}C NMR (100 MHz, CDCl3) δ 141.1, 140.0, 139.7, 139.5, 137.9, 137.6, 135.6, 133.21, 133.17, 130.3, 128.9, 128.2, 127.8, 127.6, 127.4, 126.9, 126.5, 126.3, 125.8, 125.4, 125.34, 125.28, 125.2, 124.0, 123.8, 123.7, 122.8, 122.1, 120.8, 120.5, 93.4, 87.6; IR (ATR) vmax (cm^{-1}): 636.34, 732.63, 759.06, 1435.42, 2199.58, 2923.58, 2980.52, 3055.86; HRMS calcd for $C_{32}H_{19}S_3$, 499.0649 $[M+H]^{+;}$ found 499.0575 $[M+H]^+$.

3-(3-(6-Methoxynaphthalen-2-yl)benzo[b]thiophen-2-yl)-2-(thiophen-2-yl)benzo[b]thiophene 15D was isolated in **58%** yield as a yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12–8.14 (d, J = 8.0, 1H), 7.85–7.91 (m, 2H), 7.69–7.71 (m, 1H), 7.52–7.64 (m, 4H), 7.46–7.49 (m, 1H), 7.35–7.41 (m, 2H), 7.20–7.27 (m, 3H), 7.10–7.13 (m, 1H), 7.04–7.05 (d, J = 4.0, 1H), 6.94–6.97 (m, 1H), 3.90 (s, 3H); 13C NMR (100 MHz, $CDCl_3$) δ 158.5, 140.9, 140.0, 139.9, 139.0, 138.1, 137.3, 135.8, 134.3, 131.5, 129.5, 129.2, 128.6, 127.7 (include 2C), 127.4, 126.8, 125.7, 125.3, 125.14, 125.10, 124.2, 123.89, 123.86, 122.7, 122.1, 120.2, 119.5, 118.2, 106.0, 96.0, 82.7, 55.6; IR (ATR) vmax (cm^{-1}): 699.57, 729.04, 766.36, 1029.85, 1196.55, 1391.35, 1600.36, 1627.59, 1732.15, 2202.73, 2926.61, 2956.69; HRMS calcd for $C_{33}H_{21}OS_3$, 529.0755 $[M+H]^{+;}$ 529.0755 $[M+H]^+$.

2.11 *Synthesis of 3-(1H-indole-2-yl)-2-(thiophen-2-yl)benzo[b]thiophene (**16**)*

To a solution of the 2-(2-aminobenzoethynyl)-2-(thiophen-2-yl) benzo[*b*]thiophene (**12J**) (99.3 mg, 0.3 mmol) in dichloromethane (10 mL) was added I_2 (226.8 mg, 0.9 mmol) under argon atmosphere. The mixture was stirred at room temperature for 1h, followed by quenching with sat. sodiumthiosulfate (30 mL) and extracted with DCM (20 mL × 3). The organic layer was dried over anhydrous $MgSO_4$ and filtered. Solvent was removed under vacuum, and the residue was purified by column chromatography over silica gel with Hexane/EtOAc (10/1) to afford 3-(1H-indole-2-yl)-2-(thiophen-2-yl) benzo[*b*]thiophene**16** as a white solid in **67%** yield. M.p.: 159.8–161.4 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.02 (b, 1H), 7.84–7.82 (m, 1H), 7.75–7.70 (m, 1H), 7.40–7.33 (m, 3H), 7.27–7.15 (m, 4H), 6.97–6.94 (m, 1H), 6.78–6.77 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.9, 138.2, 136.5, 135.8, 135.4, 130.4, 128.9, 127.6, 127.5, 127.4, 125.4, 125.2, 124.5, 123.6, 122.5, 122.1, 121.0, 120.3, 111.3, 104.8; IR (ATR) υ_{max} (cm^{-1}): 3444.54, 2917.97, 2850.71, 1729.02, 1419.21, 1231.16, 732.22, 699.57; HRMS calcd for $C_{20}H_{14}NS_2$, 332.0565 $[M+H]^{+;}$ found 332.0555 $[M+H]^+$.

2.12 *Antimicrobial activity test*

Antimicrobial acitivities of the newly synthesized compounds were evaluated against reference strains kindly provided from Refik Saydam National Type Culture Collection (Turkey): three gram positive bacteria (*Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923), three gram negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853), an yeast (*Candida albicans* ATCC 10231) and a fungus (*Aspergillus niger* ATCC 16404). Initially, agar disk diffusion method was used to screen *in vitro* antimicrobial activities of the synthesized compounds[28] on the basis of Clinical and Laboratory Standards Institute (CLSI) recommendation. The results were recorded for each tested compound as the average diameter (in mm) of inhibition zones around the discs where no microbial growth occurred. The compounds that lead to significant zone diameters (> 10 mm) against the tested organisms were further analysed quantitatively by determining Minimal Inhibitory Concentration (MIC) values. MIC corresponds to the lowest concentration required to inhibit microbial growth. Broth microdilution method in cation-adjusted Mueller-Hinton Broth (MHB) (Oxoid) was performed in 96-well round-bottom microtiter plates according to the CLSI guidelines.[29] 100 µl of MHB was dispensed to each well. Then, the compounds dissolved in DMSO in a final concentration of 1024 µg/mL were added to first wells, and doubling dilution series were performed to get a concentration scale from 512 µg/mL to 0.125 µg/mL. The standards (amikacin for bacteria and flucanozole for fungi) were diluted in the same way. The sterility, growth and solvent controls (MHB without inoculum, bacteria in the absence of the compounds and dimethylsulfoxide (DMSO) diluted in the medium, respectively) were used, as well. Freshly prepared streak plated microorganisms were resuspended in 0.85% NaCl to 0.5 McFarland ($\cong$ $1-4 \times 10^8$ CFU/mL). 100 µL of this solution was tenfold diluted in MHB and next microbial inoculum was added to each well (as final concentration of $1-5 \times 10^5$ CFU/mL). Incubation was done at 37 °C for 16–20 h for bacteria and 26 °C for 24–48 h for fungi. All MIC determinations were carried out in triplicates. The MIC values of the tested compounds were detected visually by controlling the turbidity with respect to the controls.

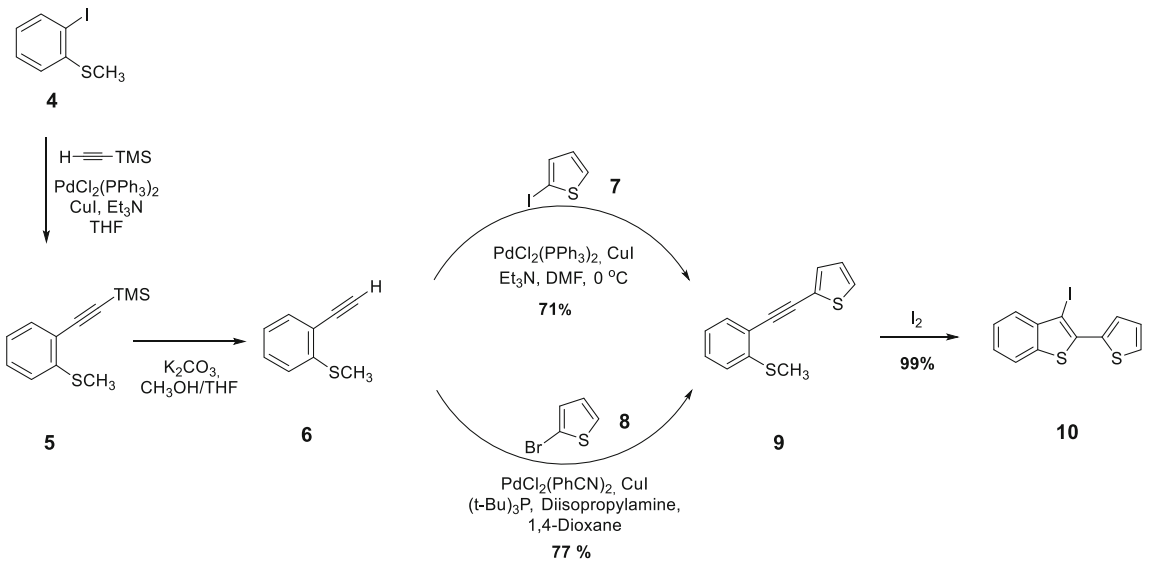
2.13 *Total antioxidant capacity test by ABTS assay*

2.13a *ABTS assay*: ABTS, 2, 2′- azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, potassium persulfate and ethanol were purchased from Sigma-Aldrich. All chemicals and solvents were analytical grade and were used as received. Antioxidant capacities of the synthesized chemicals were determined according to the modified method of Rea *et al.,*[30] Trolox was used as the antioxidant standard. ABTS stock solution, including ABTS (7 mM) and potassium persulphate (2, 45 mM), was prepared in distilled H_2O and incubated at room temperature for 12–16 h. Then, ABTS stock solution was diluted with ethanol to achieve an absorbance of 0.700 ± 0.02 at 734 nm to be used as working solution. The synthesized dibenzothiophenes and trolox were dissolved and diluted with pure ethanol. After that, trolox standard or our compounds were mixed with ABTS working solution (1:1) and incubated in the dark at 25 °C for 30 min with continuous moderate shaking. The reaction steps were also repeated for controls containing ethanol and ABTS. Absorbances were measured at 734 nm. All measurements were done triplicated under dim light. This procedure was repeated three times at different dates.

The percentage of Radical Scavenging Activity (%RSA) was calculated using the following equation:

$$\text{Radical Scavenging Activity (\%RSA)} = \frac{(\text{Control Absorbance} - \text{Sample Absorbance})}{(\text{Control Absorbance})} \times 100$$

The IC_{50} value is the concentration of compound that is able to reduce the absorbance value of the $ABTS^+$ radical cation solution to half of its original value. This value obtained from linear curve of RSA% *versus* different concentrations of tested compound. If the % RSA value could not exceed 50% to be used in the calculation of IC_{50} value, then % RSA value itself was used as a unit in the evaluation of the total antioxidant capacity after calculation of the ratio with the applied concentration of the molecules (% RSA/applied concentration, mL/mg).



Scheme 2. Synthesis of 3-iodo-2-(thiophen-2-yl) benzo[*b*] thiophene (**10**).

3. Results and Discussion

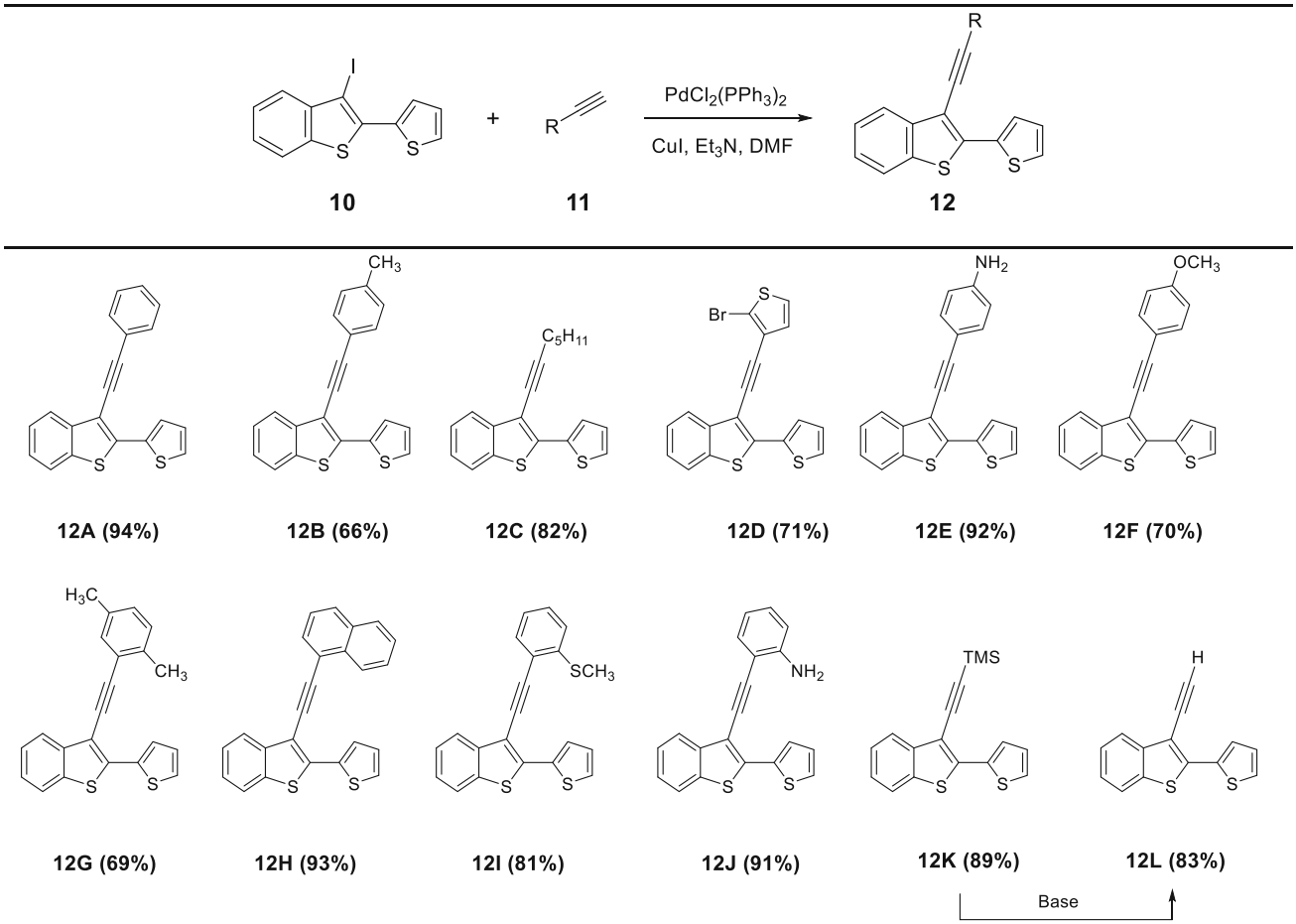
3.1 *Synthesis*

Initially, palladium catalyzed Sonogashira Coupling reaction between 2-iodothioanisole **4** and trimethylsilylacetylene was performed for the preparation of 2-trimethylsilanylethynylthioanisole **5** under mild reaction conditions. After the isolation of compound **4**, the trimethylsilyl group was removed by desilylation with potassium carbonate in methanol for the formation of necessary terminal alkyne as a 2-ethynylthioanisole **5**. When compound **5** was allowed to react with 2-bromothiophene in the presence of $PdCl_2(PhCN)_2$, CuI, $(t\text{-}Bu)_3P$ in Dioxane at room temperature for 12 h, desired alkyne **9** was obtained in 77% yield. Interestingly, when 2-iodothiophene was applied for the synthesis of **9** in the presence of Pd-catalyst at room temperature, only self-coupling product of terminal alkyne was obtained instead of **9**. The same reaction condition was reapplied with 2-iodothiophenes at 0 °C, so that the desired compound **9** was isolated in 72% yield.

Iodine-mediated electrophilic cyclization reactions have been used for the regioselectively synthesis of five or six-membered heterocyclic compounds.[31,32] Recently, Zora *et al.,* have found a novel methodology for the synthesis of iodo-substituted-pyrazoles from corresponding acetylenic hydrazone derivatives via electrophilic cyclization under mild reaction conditions.[33] Previous studies showed that CH_2Cl_2 (DCM) and chloroform were among the most employed solvents for those kinds of cyclization, and different iodine reagents such as molecular iodine, monoiodochloride (ICl), PhSeCl, etc. were used as electrophiles for electrophilic cyclization reactions.[34] Molecular iodine is chosen being as a mild and nontoxic electrophile which catalyzed various organic reactions with high efficiency and selectivity. When **9** was reacted with molecular iodine in DCM for 2 h at room temperature, our expected product 3-iodo-2-(thiophen-2-yl)benzo[*b*]thiophene (**10**) was obtained in good yield (99%) (Scheme 2).

After isolation of structure **10**, a variety of 3-alkynylic benzothiophene derivatives were synthesized by using Pd-catalyzed Sonogashira Cross-Coupling reactions.[35] As seen in Table 1, when **10** was allowed to react with phenyl acetylene in the presence of $Pd(PPh_2)_2Cl_2$ and CuI in DMF/Et_3N at room temperature for overnight, **12A** was obtained in 94% yield (Table 1). When the same reaction condition was applied for the synthesis of **12B**, the isolated yield was found as 66%. When the effect of aliphatic compound was tested by using heptanyl, the 82% yield of **12C** was reached. Notably, **12D** was obtained in moderate yield (71%). Moreover, amin-substituted **12E** and **12J** were synthesized in high yields 92% and 91%, respectively, and polyaromatic substituted-**12H** was prepared in 93% yield. When the same reaction was performed with ethynyltrimethylsilane, **12K** was formed with 89% yield. Then, **12K** underwent desilylation reaction with potassium carbonate in methanol to give the new benzothiophene substituted terminal alkyne **12L** which might be used as a novel precursor for the formation of biologically active compounds. As a result of the first part of study, Sonogashira cross coupling reactions were found to be general for a wide range of terminal alkynes and tolerated the presence of aliphatic,

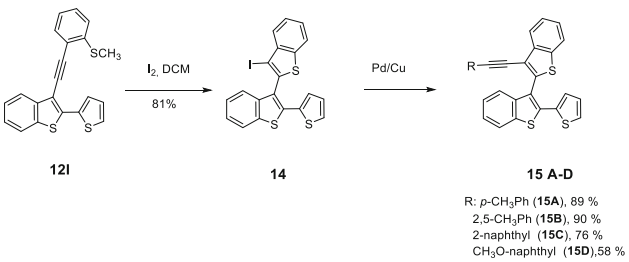
Table 1. Synthesis of 3-(substituted-ethynyl)-2-(thiophen-2-yl) benzo[*b*]thiophene (**12**) derivatives.



aromatic, polyaromatic and heteroaromatic moieties with electron-withdrawing and electron-donating substituents (Table 1).

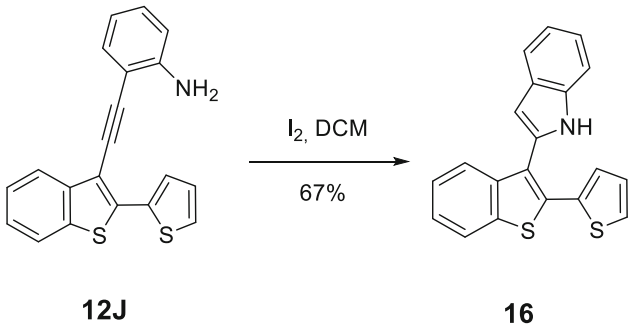
After preparation of 3-(substituted-ethynyl)-2-(thiophen-2-yl) benzo[*b*]thiophenes (**12A–L**), the synthesis of corresponding iodo substituted-thienodibenzothiophene (**13**) was examined by using iodine-catalyzed electrophilic cyclization reaction (**S. I.**). Initially, **12A** was allowed to react with 1 equiv. of molecular iodide in DCM at room temperature for 24 h, all starting compound was recovered. When excess amount of iodide was used in the same reaction conditions, there was no formation of products.

When **12I** and **12J** were allowed to cyclization reaction in the presence of molecular iodine, **14** and **16** were obtained in 81% and 67% yields, respectively (Schemes 3 and 4). Although, Larock *et al.,* reported the synthesis of 3-benzofuran-substituted-2-phenyl-benzothiophene via electrophilic cyclization reactions, there is not any information on indoles or benzothiophene-susbtituted benzothiophene structures



Scheme 3. Synthesis of 3-(2-thioanisolethynyl)-2-(thiophen-2-yl)benzo[*b*]thiophene (**12I**) and 3-(arylethynyl)-2′-(thiophen-2-yl)-2,3′-bibenzo[b]thiophene (**15 A–D**).

in literature.[36] The methodology proposed in current study gave the opportunity to the synthesis of novel heteroaromatic compounds including two benzothiophenes and benzothiophenes-indoles. Interestingly, the cyclization reaction of **12J** produced the only indole (**16**) instead of iodine-substituted corresponding product (Scheme 4). Moreover, **15A–D** were synthesized



12J **16**

Scheme 4. Synthesis of 2-(2-(thiophen-2-yl) benzo[*b*] thiophen-3-yl)-1H-indole.

from **14** by using palladium catalyzed Sonogashira cross coupling reactions (Scheme 4).

3.2 *Evaluation of antimicrobial activities of the newly synthesized compounds*

In the present study, antimicrobial activities of new benzothiophene derivatives were tested against indicator microorganisms by using agar disc diffusion method (Table S2, SI). When the zone inhibition values were compared to interpretive standards (CLSI), the compounds (**9**) (against*C. albicans* ATCC 10231), (**10**) (against *B. subtilis* ATCC 6633, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, *C. albicans* ATCC 10231 and *A. niger* ATCC 16404), (**6**), (**12E**), (**12J**) and (**12L**) (against *S. aureus* ATCC 25923), and (**12K**) (against *A. niger* ATCC 16404) were found to have considerable antimicrobial activities and their MIC values were determined by microdilution method (Table S3, Supplementary Information). According to the inhibition zone and MIC data, 2-trimethylsilanylethynylthioanisole (**5**) was not found to be effective against tested microorganisms. On the other hand, structure **6** gave slightly higher inhibition zone value corresponding to a weak antibacterial activity (MIC of 256 μg/mL) against *S. aureus* ATCC 25923, a gram positive bacterium. Compound **9** displayed a weak antifungal activity only for *C. albicans* ATCC 10231. Compound **10**, obtained from electrophilic cyclization, showed good antibacterial and antifungal activities against *B. subtilis* ATCC 6633 and *A. niger* ATCC 16404 with MIC values of 42,7 and 128 μg/ml, respectively; and, expanded its activity spectrum mildly against *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603 and *C. albicans* ATCC 10231 (MIC values of 250 μg/mL). The isolated molecules (**12A–L**) exhibited the highest antimicrobial activity against *S. aureus* ATCC 25923 and *A. niger* ATCC 16404. **12E**, **12J** and **12L** were found to be the most potent antibacterial compound against *S. aureus* ATCC 25923 with MIC values of 4–5, 3, 16 and 4 μg/mL, respectively. The highest antifungal activity against *A. niger* ATCC 16404 (MIC: 64 μg/ml) was observed for compound **12K**. As a summary, our novel benzothiophenes seem to be significantly effective on gram positive bacteria, *S. aureus* ATCC 25923 and *B. subtilis* ATCC 6633; and fungi, *C. albicans* ATCC 10231 and *A. niger* ATCC 16404. The compounds **12E**, **12L** and **12J** with potent antibacterial activities against *S. aureus* might be new candidates for the development of a new class of antimicrobial agents in treatment of multidrug resistant strains. Similarly, compound **10** and **12K** might be further modified to design potent antifungal agents against current fungal diseases.

3.3 *Assessment of antioxidant capacities of the newly synthesized compounds*

Trolox is a water soluble vitamin E analogue[37] and those analogues are used in food industry and medicine because of being good chain-breaking antioxidants which prevent peroxidation of lipids and also being adventageous stabilizers of biological membranes via the restriction of components' mobilities in membranes. In this manner, a new compound with higher antioxidant capacity than that of trolox is a good candidate for the development of better ingredients in those industrial areas.

Antioxidant capacities of newly synthesized dibenzothiophenes were tested by a universal consent method of Trolox Equivalent Antioxidant Capacity Assay (TEAC) as defined before and the results indicated that among all derivatives, amine-substituted derivative of 3-(substituted-ethynyl)-2-(thiophen-2-yl) benzo[b] thiophene (**12E**) and the product of the cyclization of a homologues of this analog in the presence of iodine (**16**) had the highest antioxidant capacities as compared to trolox (Table S4, Supplementary Information).

Almost one of third of the newly synthesized dibenzothiophene derivatives exerted strong radical scavenging activities *in vitro* (TEAC assay) and IC_{50} values could be calculated. Compound **16** was determined to be 2.5 times more potent than trolox (Table S5, Supplementary Information) and it made this derivative more powerful not only against vitamin E derivatives like trolox but also against many other phenolic compounds such as ascorbic acid, chlorogenic acid, phloridzin,[38] and caffeic acid[39] found in food supplies and medicines. Additionaly, the derivative **12E**, also, exhibited a slightly higher TEAC value than trolox which made it a significant antioxidant, too.

In summary, except the highly outstanding derivatives **16** and **12E**, the others that could be used in the

development of better antioxidant compounds, but still having lower TEAC values than trolox standard (Table S5, Supplementary Information), showed a sorting as: **12J** > **12D** > **9** > 6.

4. Conclusions

In this study, novel benzothiophene derivatives were designed, synthesized and investigated for some of their biological properties. Our compounds have special structures of molecules that possessed interesting biological properties and pharmaceutical potentials. It was found that 2-(thiophen-2-yl) benzo[*b*]thiophene (**12E**), 3-ethynyl-2-(thiophen-2-yl) benzo[*b*]thiophene (**12L**) and 3-(2-aminobenzoethynyl)-2-(thiophen-2-yl) benzo[*b*]thiophene (**12J**) have high antibacterial activities against *S. aureus* ATCC 25923 which is a very dangerous temporizer pathogenic bacterium colonized on one of every five human living on the world. Moreover, 3-iodo-2-(thiophen-2-yl) benzo[*b*]thiophene (**10**) and 3-(trimethylsilylethynyl)-2-(thiophen-2-yl) benzo[*b*] thiophene (**12K**) could be used for the treatment of fungal diseases caused by *A. niger* ATCC 16404. The derivatives **16** and **12E** displayed quite high antioxidant capacities with TEAC values of 2.5 and 1.1, respectively. Finally, the synthesized novel compounds possessed interesting positions which could work as keys for further verifying reactions to increase the number of new derivatives in future studies.

Supplementary Information (SI)

Characterization of the new benzo[*b*]thiophene products including Scheme 2, Table S2, S3, S4 and S5, ^{1}H NMR, ^{13}C NMR, and FTIR spectra, as well as mass characterization data. Supplementary Information is available on www.ias.ac.in/chemsci.

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