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REGULAR ARTICLE

Synthesis of novel *bis*-allyloxy and hydroxypropoxy derivatives of 4, 5-diaryl thiophene-2-carboxylic acid and their biological evaluation

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Abstract. In our earlier studies, we have shown that the introduction of amino moieties at carboxylic acid of 4,5-diarylthiophene-2-carboxylic acid significantly improved the anti-inflammatory activity of the compound against the standard drug diclofenac sodium. In the present study, we have synthesized new derivatives of 4,5-diarylthiophene-2-carboxylic acid by modifying the hydroxyl group of the phenyl ring and carboxylic acid group of the thiophene ring. A series of novel 4,5-diarylthiophene-2-carboxylic acid derivatives containing *bis*-allyloxy and hydroxypropoxy with methyl or ethyl ester moieties were synthesized, characterized and subsequently evaluated for anti-inflammatory and antioxidant property. Among the novel compounds, the inhibition of bovine serum albumin denaturation assay revealed that the compound 4,5-*bis*(4-(3-hydroxypropoxy)phenyl)thiophene-2-carboxylic acid (**15**) and ethyl ester (**13**) having anti-inflammatory activity better than the standard drug diclofenac sodium. The antioxidant screening showing 4,5-*bis*(4-(allyloxy)phenyl)thiophene-2-carboxylic acid (**10**), 4,5-*bis*(4-(3-hydroxypropoxy)phenyl)thiophene-2-carboxylic acid methyl ester (**11**) and 4,5-*bis*(4-(3-hydroxypropoxy)phenyl)thiophene-2-carboxylic acid ethyl ester (**13**) exhibited a slightly moderate antioxidant activity than standard ascorbic acid. Molecular docking analysis was performed for the synthesized compounds with the cyclooxygenase-2 (COX-2) receptor (PDB 1D: 1PXX). Docking studies revealed that all the synthesised compounds exhibit greater binding affinity than the standard drug. Particularly, the compound ethyl 4,5-*bis*(4-(allyloxy)phenyl)thiophene-2-carboxylate (**8**) and allyl 4,5-*bis*(4-(allyloxy)phenyl)thiophene-2-carboxylate (**9**) having high free energy binding of −10.40 and −10.48 Kcal/mol, respectively.

Keywords. *Bis*-allyloxy derivatives; hydroxypropoxy derivatives; 4,5-diarylthiophene-2-carboxylic acid; anti-inflammatory; antioxidant; molecular docking.

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are therapeutic agents commonly used in the treatment of inflammation, pain and fever. The therapeutic effect of these substances involves inhibition of cyclooxygenases (COX) thereby preventing prostaglandin (PGs) and thromboxane A2 formation from arachidonic acid.[1] There are two isoforms, COX-1 and COX-2, where inhibition of COX-1 is responsible, in part, for gastrointestinal and renal side effects, whereas selective COX-2 inhibitors, have been designed, synthesized and clinically introduced as gastrointestinal sparing NSAIDs. Hence, the beneficial effect of selective inhibition of COX-2 over COX-1 has triggered therapeutic interest on the former, resulting in the evolution of drugs with reduced side effect to the patients.

The general structure of COX-2 selective inhibitors consist of two aryl groups attached to neighboring atoms of a central ring, one of the aryl groups is *para*-substituted with either a methyl sulfonyl (SO_2CH_3) group, e.g., etoricoxib and rofecoxib or a sulfonamide (SO_2NH_2), e.g., celecoxib and parecoxib.[2] The central

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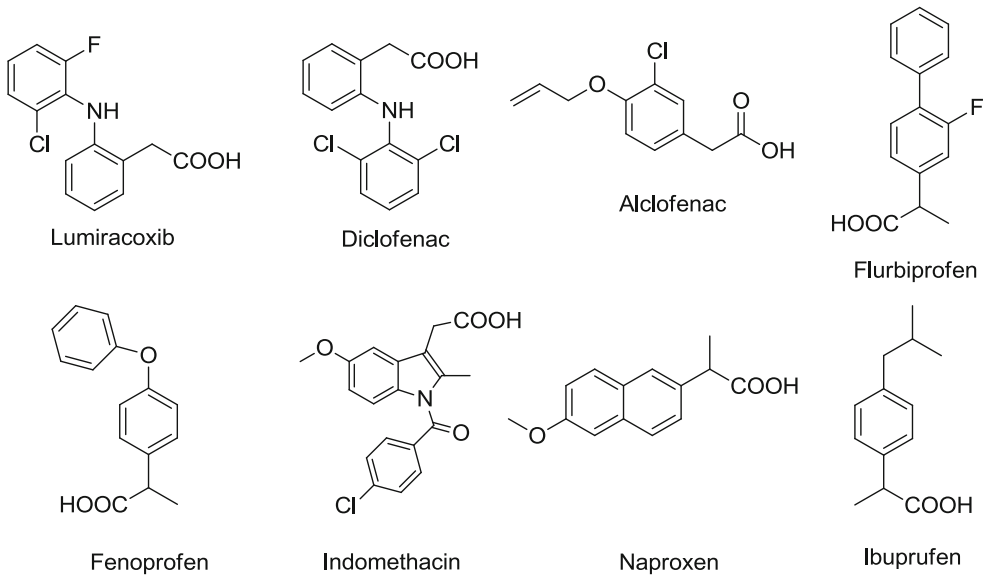


Figure 1. Some of the non-steroidal anti-inflammatory drugs.

rings of compounds are thiophene, pyrazole, furanone, isoxazole, and cyclopentene. There are also examples of cycloalkyl, alkoxy or phenoxy moieties in the non-sulfonyl containing 'aryl' ring.[3–5] One of the important heterocyclic systems which attract progressive interest of many researchers is thiophene, because of plethora of biological, pharmacological and industrial importance. Thiophene containing drugs exhibit a variety of biological activities which include antimicrobials, antipsychotics,[6] sedatives,[7–9] antidepressants (e.g., Duloxetine and Trazodone) and antihypertensive (e.g., Tiamenidine) drugs. Thiophene derivatives are also well known for their pronounced anti-inflammatory activities as evident from the approval of Lornoxicam, Tenoxicam, Tiaprofenic acid and Tenidap by the US Food and Drug Administration (FDA). Recently, several new Thiophene-2-carboxylic acid derivatives have been reported as potent HCV NS5B polymerase inhibitors,[10] 5-LOX/COX-inhibitor,[11] and with anti-inflammatory activities.[12] The introduction of carboxylic acid into a biologically active compound not only impacts the water solubility of the compound but also establishes relatively strong electrostatic interactions or hydrogen-bond bridges with the COX enzymes, conferring both binding affinity and specificity to the drug–target as in the case of flurbiprofen.[13] Also, carboxylic acid moiety is critical for their biological activity of several NSAID's like aspirin, ibuprofen, naproxen, indomethacin, diclofenac, and lumiracoxib (Figure 1). In addition, esters and amides of indomethacin indeed are more COX2 selective and possess potent anti-inflammatory effects without being ulcerogenic.[14] Another analogue of diclofenac with a carboxylic moiety and a selective COX2 inhibitor lumiracoxib was reported with its carboxylate group forming hydrogen bonding interactions with Ser530 and Tyr385 at the top of the active site.[15] These findings have encouraged us to replace the amide moiety with carboxylic acid group of the previously reported potent 4,5-diarylthiophene-2-carboxamide derivatives.[16] In view of these observations and in continuance of our effort to develop novel thiophene analogues,[16] it was thought worthwhile to synthesize new allyloxy and hydroxypropoxy derivatives of 4,5-diarylthiophene-2-carboxylic acid and to evaluate them for their anti-inflammatory and antioxidant properties.

2. Experimental

2.1 *Materials and methods*

All the chemicals and reagents used were lab grade material procured from Alfa aesar, India. All the solvents used were purchased from commercial suppliers and were used without further purification. The melting points were determined using Buchi apparatus by the open capillary tube method and were uncorrected. The IR spectra were recorded in Perkin-Elmer series 2000 FTIR spectrophotometer using KBr pellet. ^{1}H NMR and ^{13}C NMR spectra were obtained in CDCl3, DMSO-d_6 on a Bruker spectrometer at 400 and 100 MHz, respectively. The chemical shifts are reported in ppm (δ) relative to tetramethylsilane as internal standard, coupling constants (J) are in hertz (Hz). Spin multiplicities are given as s (singlet), d (doublet), t (triplet), dd (doublet of doublet), bs (broad signal) and m (multiplet). Residual proton and carbon solvent signal for CDCl3, δ_H7.26 ppm, δ_C77.0 ppm, δ_6-DMSO, δ_H2.50 ppm, δ_C40.0 ppm. Proton and carbon spectra

were typically obtained at room temperature. Mass spectra were recorded on ESI—Perkin Elmer Sciex, API 3000 mass spectrometer. Pre-coated silica gel GF254 plates from Merck were used for thin layer chromatography (TLC). The elemental analyses were recorded in Thermo Finnigan Flash EA 1112 elemental analyser.

2.2 *Synthesis*

2.2a *Synthesis of 2,3-bis(4-methoxy phenyl)-3-chloro-2-prop-2-ene aldehyde (**2**)*: Phosphorous oxychloride ($POCl_3$) (7.66 g, 0.05 mole) was added drop wise over a period of 15–30 min with stirring at 0−5°C to 20 mL of dimethylformamide. The mass was maintained at 0−5°C over 30 min and a solution of desoxyanisoin (10 g, 0.04 mole) in dimethylformamide (50 mL) at 0−5°C was added under stirring. The reaction mass was heated to 70−75°C and maintained for 4 h. The progress of the reaction was monitored using TLC (toluene). After completion, the reaction mixture was cooled and poured slowly into 25% solution of sodium acetate in water (100 mL). The product was filtered and washed with water, followed by slurry wash with ethanol (100 mL) which after drying gave 9.1 g (78% yield) of the title compound **2**. White powder, M.p.: 145−147 °C. [Lit.[12]; 158°C]. IR(KBr) cm^{-1}: 2932, 1680, 1513, 1442, 1080, 813, 772. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.66 (s, 1H), 7.52 (d, J = 7.8 Hz, 2H), 7.25–7.22 (m, 2H), 6.99–6.91 (m, 4H), 3.89 (s, 3H), 3.85 (s, 3H). ^{13}C NMR (100 MHz, CDCl3): δ 190.5, 162.1, 159.7, 155.1, 139.8, 132.4, 132.1, 131.1, 130.6, 128.4, 126.7, 114.2, 113.9, 55.8, 55.5. ESI-MS *m/z* Calculated 302.1. Found: 303.1 $[M + H]^+$.

2.2b *Synthesis of 4,5-bis(4-methoxyphenyl)thiophene-2-carboxylic acid (**3**)*: 2,3-*Bis*(4-methoxy phenyl)-3-chloro-2-prop-2-ene aldehyde **2** (5 g, 0.0165 mol) was added to a solution of potassium hydroxide (4 g, 0.714 mol) and 2-mercapto acetic acid (3.1 g, 0.034 mol) in methanol: water (40 mL:10 mL) mixture at room temperature. The mixture was refluxed for 4 h, and reaction was monitored using TLC (hexane: ethyl acetate 3:7). After completion, the reaction mixture was cooled to room temperature and slowly acidified with concentrated HCl over 30–45 min at 25−30°C. The product **3** was filtered, washed with water and dried to get compound **3**. 4 g (71% yield). Yellow powder, M.p.: 211−213°C. [Lit.[12]; 215°C]. IR(KBr) cm^{-1}: 2933, 2542, 1668, 1546, 1449, 1247, 1033, 827. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.7 (bs, 1H), 7.87 (s, 1H), 7.27 (d, J = 8.6 Hz, 2H), 7.20 (d, J = 8.6 Hz, 2H), 6.85–6.81 (m, 4H), 3.83 (s, 3H), 3.81 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.0, 159.9, 159.1, 147.0, 138.4, 137.9, 130.6, 130.3, 129.5 128.1, 125.9, 114.3, 114.1, 55.5, 55.4. ESI-MS *m/z* Calculated 340.1. Found: 339.1 $[M - H]^-$.

2.2c *Synthesis of 4,5-bis(4-hydroxyphenyl)thiophene-2-carboxylic acid (**4**)*: 4,5-*Bis*(4-methoxyphenyl) thiophene-2-carboxylic acid **3** (6 g, 0.0176 mol) was added to the mixture of aluminium chloride (9.38 g, 0.0704 mol) in chlorobenzene (60 mL) at 25–30°C. The reaction mixture was heated to 95–105°C and maintained for 3 h. The progress of the reaction was monitored using TLC (hexane:ethyl acetate 3:7). After completion of the reaction, aqueous HCl (1:1, 50 mL) was added to the mass at 25−30°C. The product was extracted with ethyl acetate (50 mL × 2) and the organic layer was washed with water. Ethyl acetate was evaporated under vacuum and the residue was triturated with dichloromethane (100 mL), filtered to get compound **4**. 4.85 g (88% yield). Light green powder, M.p.: 240−243°C. IR(KBr)cm^{-1}: 3428, 3318, 1639, 1545, 1442, 1255, 1069, 829. ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6): δ 8.48 (bs, 1H), 8.19 (bs, 1H), 7.75 (s, 1H), 7.17 (d, J = 8.5 Hz, 2H), 7.11 (d, J = 8.4 Hz, 2H), 6.77–6.75 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$+DMSO-d_6): δ 164.3, 157.5, 156.4, 144.9, 137.8, 136.2, 130.9, 130.4, 130.0, 127.1, 124.9, 115.6, 115.5. ESI-MS *m/z* Calculated 312.0. Found: 313.0 $[M + H]^+$.

2.2d *Synthesis of methyl 4,5-bis(4-hydroxyphenyl) thiophene-2-carboxylate (**5**)*: 4,5-*Bis*(4-hydroxyphenyl) thiophene-2-carboxylic acid (3.12 g, 0.01 mol) dissolved in methanol (78 mL) and concentrated sulphuric acid (1.0 g, 0.01 mol) was added slowly and refluxed for 8 h. The reaction was monitored by using TLC (hexane: ethyl acetate 3:7). After the reaction was completed, the reaction mixture was cooled and concentrated. To this residue 20 mL of water added, the product was extracted with ethyl acetate (2 × 50 mL). The organic phase was separated, washed with water, 10% sodium carbonate solution followed by water and evaporated under vacuum. The residue was triturated with heptane (20 mL), filtered to get compound **5**. 2.8 g (86% yield). The product obtained did not require any further purification for next step.

Off-white powder, M.p.: 211−213°C. IR (KBr)cm^{-1}: 3325, 2949, 1664, 1547, 1441, 1227, 1079, 834, 756. ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6) δ 8.12 (s, 1H), 7.76 (s, 2H), 7.17 (d, 2H, J = 8.44 Hz), 7.10 (d, 2H, J = 8.4 Hz), 6.77–6.75 (m, 4H), 3.89 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ 162.9, 157.5, 156.4, 145.4, 137.9, 136.5, 130.4, 130.1, 129.5, 127.0, 124.8, 115.7, 115.6, 52.12. ESI-MS *m/z* Calculated 326.1. Found: 327.1 $[M+H]^+$. Anal. Calculated for $C_{18}H_{14}O_4S$: C, 66.24; H, 4.32%. Found: C, 66.28; H, 4.30%.

2.2e *Synthesis of ethyl 4,5-bis(4-hydroxyphenyl) thiophene-2-carboxylate (**6**)*: 4,5-*Bis*(4-hydroxyphenyl) thiophene-2-carboxylic acid (3.12 g, 0.01 mol) dissolved in ethanol (78 mL) and concentrated sulphuric acid (1.0 g, 0.01 mol) was added slowly and refluxed for 8 h. The reaction was monitored by using TLC (hexane:ethyl acetate 3:7). After the reaction was completed, the reaction mixture was cooled and concentrated. To this residue water (20 mL) was added and the product was extracted with ethyl acetate (2 × 50 mL). The organic phase was separated, washed with water, 10% sodium carbonate solution followed by water and evaporated under vacuum. The residue was triturated with heptane (20 mL), filtered to get compound **6**. 3.0 g (88% yield). The

product obtained did not require any further purification for next step.

Yellow powder, M.p.: 202−204°C. IR (KBr) cm^{-1}: 3317, 2937, 1656, 1547, 1442, 1228, 1077, 833, 756. ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO-d_6) δ 9.19(s, 1H), 8.99 (s, 1H), 7.71(s, 1H), 7.14 (d, 2H, J = 8.56 Hz), 7.08 (d, 2H, J = 8.52 Hz), 6.76–6.73 (m, 4H), 4.37 (q, 2H, J = 7.08 Hz), 1.39 (t, 3H, J = 7.12 Hz). ^{13}C NMR (100 MHz, $CDCl_3$ + DMSO-d_6) δ 160.9, 156.8, 155.7, 143.8, 136.8, 134.9, 129.2, 128.9, 125.4, 123.4, 123.2, 114.6, 114.5, 59.86, 13.36. ESI-MS *m/z* Calculated 340.1. Found: 341.1 $[M + H]^+$. Anal. Calculated for $C_{19}H_{16}O_4S$: C, 67.04; H, 4.74%. Found: C, 67.08; H, 4.72%.

2.2f *Synthesis of methyl 4,5-bis(4-(allyloxy)phenyl) thiophene-2-carboxylate (**7**)*: Allyl bromide (2.5 g, 0.02 mol) was added to the mixture of methyl 4,5-*bis*(4-hydroxyphenyl)thiophene-2-carboxylate (2.5 g, 0.008 mol), potassium carbonate (2.77 g, 0.02 mol) in acetonitrile (50 mL). The reaction mixture was refluxed for 4 h. The reaction was monitored by using TLC (hexane: ethyl acetate 1:1). After the completion of the reaction, the reaction mixture was cooled and filtered to remove insolubles. The organic mass was evaporated under vacuum and to this residue water (20 mL) was added and the product was extracted with ethyl acetate (2 × 50 mL). The organic phase was separated, washed with water, and evaporated under vacuum. The residue was triturated with hexane (20 mL), cooled to 10–15°C and filtered to get compound **7**. 2.7 g (87% yield). The product obtained was used for next step without further purification. Pale brown powder, M.p.: 66−68°C. IR (KBr) cm^{-1}: 3390, 2946, 2862, 1704, 1651, 1543, 1440, 1242, 1073, 833, 754. ^{1}H NMR (400Mz, $CDCl_3$) δ 7.78 (s, 1H), 7.24 (d, 2H, J = 6.76 Hz), 7.18 (d, 2H, J = 6.76 Hz), 6.85–6.82 (m, 4H), 6.07–6.02 (m, 2H), 5.44–5.39 (m, 2H), 5.31–5.28 (m, 2H), 4.54–4.52 (m, 4H), 3.89 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.9, 158.8, 157.9, 145.0, 137.9, 136.4, 133.3, 133.1, 130.5, 130.3, 130.2, 128.3, 126.1, 118.0, 117.87, 114.9, 114.8, 68.9, 68.8, 52.25. ESI-MS m/z Calculated 406.1. Found: 407.1 $[M+H]^+$. Anal. Calculated for $C_{24}H_{22}O_4S$: C, 70.91; H, 5.46%. Found: C, 70.95; H, 5.48%.

2.2g *Synthesis of ethyl 4,5-bis(4-(allyloxy)phenyl) thiophene-2-carboxylate (**8**)*: Allyl bromide (2.5 g 0.02 mol) was added to the mixture of ethyl 4,5-*bis*(4-hydroxyphenyl)thiophene-2-carboxylate (2.7 g, 0.008 mol), potassium carbonate (2.77 g, 0.02 mol) in acetonitrile (50 mL). The reaction mixture was refluxed for 4 h. The reaction was monitored by using TLC (hexane:ethyl acetate 1:1). After the reaction was completed, the reaction mixture was cooled and filtered to remove insolubles. The organic mass was evaporated under vacuum and to this residue water (20 mL) was added and the product was extracted with ethyl acetate (2 × 50 mL). The organic phase was separated, washed with water, and evaporated under vacuum. The residue was triturated with hexane (20 mL), cooled to 10–15°C filtered to get compound **8**. 2.94 g (88% yield). The product obtained was used for next step without further purification.

Yellow powder, M.p.: 65−67°C IR (KBr) cm^{-1}: 3429, 2925, 2862, 1696, 1650, 1546, 1441, 1247, 1073, 827, 755. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.24 (d, 2H, J = 8.76 Hz), 7.18 (d, 2H, J = 8.68 Hz), 6.85–6.82 (m, 4H), 6.11–6.00 (m, 2H), 5.44–5.39 (m, 2H), 5.31–5.28 (m, 2H), 4.54–4.52 (d, 4H, J = 5.32 Hz), 4.39–4.33 (q, 2H, J = 7.12 Hz), 1.40 (t, 3H, J = 7.12 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.4, 158.7, 157.9, 144.8, 137.8, 136.1, 133.2, 133.1, 133.0, 132.9, 130.7, 130.4, 130.1, 128.3, 126.1, 117.9, 117.8, 114.8, 114.7, 114.7, 114.6, 68.8, 68.7, 61.2, 14.4. ESI-MS *m/z* Calculated 420.1. Found: 421.3 $[M + H]^+$. Anal. Calculated for $C_{25}H_{24}O_4S$: C, 71.40; H, 5.75%. Found: C, 71.44; H, 5.78%.

2.2h *Synthesis of allyl 4,5-bis(4-(allyloxy)phenyl) thiophene-2-carboxylate (**9**)*: Allyl bromide (4.2 g, 0.035 mol) was added to the mixture of ethyl 4,5-*bis*(4-hydroxyphenyl)thiophene-2-carboxylic acid (3 g, 0.01 mol), potassium carbonate (4.8 g, 0.035 mol) in acetonitrile (75 mL). The reaction mixture was refluxed for 4 h. The reaction was monitored by using TLC (hexane:ethyl acetate 1:1). After the reaction was completed, the reaction mixture was cooled and filtered to remove insolubles. The organic mass was evaporated under vacuum and to this residue water (20 mL) was added and the product was extracted with ethyl acetate (2 × 50 mL). The organic phase was separated, washed with water, and evaporated under vacuum to get compound **9**. 3.4 g (82% yield). Brown liquid, IR (KBr) cm^{-1}: 2924, 2862, 1709, 1547, 1443, 1243, 1069, 831, 753. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (s, 1H), 7.16 (d, 2H, J = 8.4 Hz), 7.10 (d, 2H, J = 8.4 Hz), 6.77–6.74 (m, 4H), 6.01–5.91 (m, 3H), 5.36–5.31 (m, 3H), 5.22–5.20 (m, 3H), 4.73 (d, 2H, J = 5.32 Hz), 4.45 (d, 4H, J = 5 Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.7, 162.1, 158.9, 158.0, 145.3, 138.0, 136.5, 133.3, 133.1, 132.2, 130.6, 130.4, 130.2, 128.4, 126.2, 118.5, 118.0, 117.9, 114.9, 114.8, 68.9, 65.7, 31.1. ESI-MS *m/z* Calculated 432.1. Found: 433.2 $[M + H]^+$. Anal. Calculated for $C_{26}H_{24}O_4S$: C, 72.20; H, 5.59%. Found: C, 72.26; H, 5.56%.

2.2i *4,5-Bis(4-(allyloxy)phenyl)thiophene-2-carboxylic acid (**10**)*: Ethyl 4,5-*bis*(4-(allyloxy)phenyl)thiophene-2-carboxylate (0.6 g, 0.0014 mol) was added at room temperature to a solution of sodium hydroxide (0.54 g, 0.0135 mol) in ethanol:water (8 mL:2 mL). The mixture was refluxed for 2 h. The reaction was monitored by using TLC (hexane: ethyl acetate 2:8). After completion of the reaction, the mixture was cooled and the impurities were extracted with toluene. The aqueous phase was acidified with hydrochloric acid and the product was extracted with ethyl acetate (20 mL). The organic phase was washed with water, dried and evaporated to get compound **10**. (0.42 g, 90% yield). Off-white powder, M.p.: 110−111°C. IR (KBr) cm^{-1}: 2919, 2856, 2537, 1672, 1544, 1444, 1247, 1074, 830, 757. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.71 (s, 1H), 7.22–7.17 (m, 4H), 6.95–6.89 (m, 4H), 6.05–6.01 (m, 2H), 5.42–5.38 (m, 2H), 5.28-5.25 (m,

2H), 4.56 (s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.8, 158.4, 157.5, 143.6, 137.6, 135.5, 133.7, 133.5, 131.8, 130.2, 129.9, 127.5, 125.4, 117.8, 117.6, 115.1, 114.8, 68.3, 68.2, 59.8, 20.8, 14.1. ESI-MS *m/z* Calculated 392.1. Found: 391.4 $[M-H]^-$. 393.2 $[M + H]^+$, Anal. Calculated for $C_{23}H_{20}O_4S$: C, 70.39; H, 5.14%. Found: C, 70.42; H, 5.16%.

2.2j *Methyl 4,5-bis(4-(3-hydroxypropoxy)phenyl)thiophene-2-carboxylate (**11**)*: Methyl 4,5-*bis*(4-(allyloxy) phenyl)thiophene-2-carboxylate (2 g, 0.005 mol) dissolved in tetrahydrofuran (50 mL) and cooled to 0°C. Borane 1 M solution in THF (11.8 mL) was added and stirred at 0°C for 1 h. The reaction mixture was slowly warmed to room temperature and maintained for 4 h. The reaction mixture was quenched with water (1 mL) followed by addition of 2 N sodium hydroxide solution (2 mL), hydrogen peroxide (40% w/w, 2 mL) solution and stirred for 1 h at room temperature. The product was extracted with ethyl acetate (100 mL). The organic phase was separated and washed with 20% w/w sodium chloride solution followed by water, dried and evaporated under vacuum. The reaction produced a mixture of compounds, the major (80%) component of the mixture being methyl 4,5-*bis*(4-(3-hydroxypropoxy)phenyl) thiophene-2-carboxylate (**11**) (R_f = 0.25, SiO_2, 30% ethyl acetate in hexanes), and the minor (20%) methyl 5(4)-(4-(2-hydroxypropoxy)phenyl)-4(5)-(4-(3-hydroxypropoxy) phenyl) thiophene-2-carboxylate (**12**) (R_f = 0.4, SiO_2, 30% ethyl acetate in hexanes). The product was isolated by column chromatography by eluting with 6:4 v/v hexane–ethyl acetate to get compound **11** (1.2 g, 55% yield) and **12** (0.3 g, 14% yield). Yellow liquid, IR (KBr) cm^{-1}: 3423, 2928, 2877, 1712, 1547, 1446, 1247, 1063, 832, 755. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.23 (d, 2H, J = 8.76 Hz), 7.17 (d, 2H, J = 8.72 Hz), 6.83–6.80 (m, 4H), 4.12 (t, 4H, J = 5.88 Hz), 3.89 (s, 3H), 3.87–3.84 (m, 4H), 2.06-*1.97 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.0, 159.1, 158.2, 145.2, 138.0, 136.5, 130.6, 130.3, 130.2, 128.4, 126.2, 114.7, 114.6, 65.7, 65.6, 60.5, 60.3, 52.3, 32.1, 29.8. ESI-MS *m/z* Calculated 442.2. Found: 487.2 $[M + HCOO]^-$. Anal. Calculated for $C_{24}H_{26}O_6S$: C, 65.14; H, 5.92%. Found: C, 65.16; H, 5.96%.

2.2k *Methyl 5(4)-(4-(2-hydroxypropoxy)phenyl)-4(5)-(4-(3-hydroxypropoxy)phenyl)thiophene-2-carboxylate (**12**)*: Yellow liquid, IR (KBr) cm^{-1}: 3400, 2925, 2872, 1710, 1546, 1446, 1246, 1076, 830, 754. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.25–7.22 (m, 2H), 7.19–7.16 (m, 2H), 6.84–6.82 (m, 4H), 4.20 (brs, 1H), 4.14 (t, 2H, J = 8.76 Hz), 3.96–3.94 (m, 1H), 3.90 (s, 3H), 3.89–3.87 (m, 2 H), 3.82–3.79 (t, 1H, J = 8.32 Hz), 2.28–2.07 (m, 1H), 2.06–2.04 (m, 2H), 1.70–1.65 (m, 1H), 1.30 (d, 3H) J = 6.36 Hz). ^{13}C NMR (100 MHz, $CDCl_3$)δ 163.0, 158.8, 158.2, 145.2, 138.1, 136.6, 130.7, 130.5, 130.4, 130.3, 128.4, 126.2, 114.8, 114.7, 73.4, 66.4, 65.8, 60.6, 52.4, 32.1, 18.92. ESI-MS *m/z* Calculated 442.2. Found: 487.3 $[M + HCOO]^-$. Anal. Calculated for $C_{24}H_{26}O_6S$: C, 65.14; H, 5.92%. Found: C, 65.18; H, 5.94%.

2.2l *Ethyl 4,5-bis(4-(3-hydroxypropoxy)phenyl)thiophene-2-carboxylate (**13**)*: Ethyl 4,5-*bis*(4-(allyloxy) phenyl)thiophene-2-carboxylate (2.1 g, 0.005 mol) was dissolved in tetrahydrofuran (50 mL) and cooled to 0°C. Borane 1 M solution in THF (11.8 mL) was added and stirred at 0°C for 1 h. The reaction mixture was slowly warmed to room temperature and maintained for 4 h. The reaction mixture was quenched with water (1 mL) followed by addition of 2 N sodium hydroxide solution (2 mL), hydrogen peroxide (40% w/w, 2 mL) solution and stirred for 1 h at room temperature. The product was extracted with ethyl acetate (100 mL) and the organic phase was separated, washed with 20% w/w sodium chloride solution followed by water, dried and evaporated under vacuum. The reaction produced a mixture of compounds, the major (80%) component of the mixture being ethyl 4,5-*bis*(4-(3-hydroxypropoxy)phenyl)thiophene-2-carboxylate (**13**) (R_f = 0.25, SiO_2, 30% ethyl acetate in hexanes), and the minor (20%) ethyl 5(4)-(4-(2-hydroxypropoxy)phenyl)-4(5)-(4-(3-hydroxypropoxy) phenyl)thiophene-2-carboxylate (**14**) (R_f = 0.4, SiO_2, 30% ethyl acetate in hexanes). The product was isolated by column chromatography by eluting with 6:4 v/v hexane–ethyl acetate to get compound **13** (1.3 g, 57% yield) and **14** (0.4 g, 17% yield). Yellow liquid, IR (KBr) cm^{-1}: 3413, 2926, 2874, 1704, 1547, 1443, 1246, 1061, 830, 754. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.78 (s, 1H), 7.25 (d, 2H, J = 8.8 Hz), 7.18 (d, 2H, J = 8.72 Hz), 6.85–6.82 (m, 4H), 4.40 (q, 2H, J = 7.12 Hz), 3.89 (m, 4H), 2.85 (s, 2H), 2.08–1.98 (m, 4H), 1.44–1.38(m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.6, 159.1, 158.2, 144.9, 137.9, 136.3, 130.9, 130.6, 130.3, 128.5, 126.2, 114.7, 114.6, 65.8, 65.7, 61.3, 60.4, 60.3, 32.2, 32.1, 14.5. ESI-MS *m/z* 501.3 $[M + HCOO]^-$. ESI-MS *m/z* Calculated 456.2. Found: 501.3 $[M + HCOO]^-$. Anal. Calculated for $C_{25}H_{28}O_6S$: C, 65.77; H, 6.18%. Found: C, 65.74; H, 6.16%.

2.2m *Ethyl 5(4)-(4-(2-hydroxypropoxy)phenyl)-4(5)-(4-(3-hydroxypropoxy)phenyl)thiophene-2-carboxylate (**14**)*: Yellow liquid, IR (KBr) cm^{-1}: 3421, 2926, 2873, 1705, 1547, 1443, 1247, 1072, 830, 754. ^{1}H NMR (400 MHz, $CDCl_3$)δ 7.70 (s, 1H), 7.17–7.14 (m, 2H), 7.10–7.08 (m, 2H), 6.77–6.73 (m, 4H), 4.31–4.27 (m, 2H), 4.14–4.10 (m, 1H), 4.06 (t, 2H, J = 5.28 Hz), 3.88–3.84 (m, 1H), 3.81–3.77 (m, 2 H), 3.74 (t, 1H, J = 8.28 Hz), 1.99–1.96 (m, 2H), 1.35–1.33(m, 3H), 1.22–1.20 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.4, 157.9, 157.0, 143.9, 136.9, 135.0, 129.8, 129.5, 129.2, 127.6, 125.4, 113.7, 113.5, 72.2, 65.2, 64.6, 60.2, 59.4, 30.9, 17.6, 13.4. ESI-MS *m/z* Calculated 456.2. Found: 501.2 $[M + HCOO]^-$. Anal. Calculated for $C_{25}H_{28}O_6S$: C, 65.77; H, 6.18%. Found: C, 65.72; H, 6.14%.

2.2n *4,5-Bis(4-(3-hydroxypropoxy)phenyl)thiophene-2-carboxylic acid (**15**)*: Methyl 4,5-*bis*(4-(3-hydroxypropoxy)phenyl)thiophene-2-carboxylate (0.5 g, 0.001 mol) was added at room temperature to a solution of sodium hydroxide (0.5 g, 0.0125 mol) in ethanol:water

(8 mL:2 mL). The mixture was refluxed for 2 h. The reaction was monitored by TLC using hexane:ethyl acetate (2 mL:8 mL) as a solvent system. After the reaction was completed, the reaction mixture was cooled and the impurities were extracted with toluene. The aqueous phase was acidified with hydrochloric acid and the product was extracted with ethyl acetate (20 mL). The organic phase was washed with water, dried and evaporated to get compound **15**. (0.44 g, 91% yield). Pale brown powder, M.p.: 136−138°C. IR (KBr) cm^{-1}: 3385, 2925, 2854, 2602, 1686, 1547, 1442, 1248, 1086, 832, 757. ^{1}H NMR (400MHz, DMSO-d_6) δ 7.71 (s, 1H), 7.23 (d, 2H, J = 8.64 Hz), 7.17 (d, 2H, J = 8.6 Hz), 6.93 (d, 2H, J = 8.76 Hz), 6.90 (d, 2H, J = 8.72 Hz), 4.05 (quartet, 4H, J = 6.0 Hz), 3.58(t, 4H, J = 6.08 Hz), 1.90 (quintet, 4H, J = 6.12 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.8, 158.9, 158.0, 143.6, 137.6, 131.7, 130.2, 129.9, 127.3, 125.1, 114.8, 114.5, 114.3, 64.6, 64.5, 57.2, 32.1, 32.0. ESI-MS *m/z* Calculated 428.1. Found: 427.2 $[M-H]^-$. Anal. Calculated for $C_{23}H_{24}O_6S$: C, 64.47; H, 5.65%. Found: C, 64.49; H, 5.62%.

2.2o *5(4)-(4-(2-Hydroxypropoxy)phenyl)-4(5)-(4-(3-hydroxypropoxy)phenyl)thiophene-2-carboxylic acid **(16)***: Methyl 5(4)-(4-(2-hydroxypropoxy)phenyl)-4(5)-(4-(3-hydroxypropoxy)phenyl)thiophene-2-carboxylate (0.5 g, 0.001 mol) was added at room temperature to a solution of sodium hydroxide (0.5 g, 0.0125 mol) in ethanol:water (8 mL:2 mL). The mixture was refluxed for 2 h. The reaction was monitored by using TLC (hexane:ethyl acetate 2:8). After the reaction was completed, the reaction mixture was cooled and the impurities were extracted with toluene. The aqueous phase was acidified with hydrochloric acid and the product was extracted with ethyl acetate (20 mL). The organic phase was washed with water, dried and evaporated to get compound **16**. (0.42 g, 87% yield). Yellow powder, M.p.: 150−152°C. IR (KBr) cm^{-1}: 3394, 2923, 2853, 1682, 1545, 1445, 1246, 1060, 830, 754. ^{1}H NMR (400 MHz, DMSO-d6) δ 12.9 (brs, 1H), 7.71 (s, 1H), 7.22–7.17 (m, 4H), 6.93–6.87 (m, 4H), 4.90 (s, 1H), 4.58 (2,1H), 4.05–4.01 (m, 2H), 3.97–3.93 (m, 1H), 3.85–3.76 (m, 2H), 3.57–3.55 (m, 2 H), 1.89–1.83 (m, 2H), 1.16 (t, 3H, J = 6.24 Hz). ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.8, 158.9, 158.0, 143.6, 137.6, 131.8, 130.2, 129.9, 127.3, 127.2, 125.2, 125.1, 115.1, 114.9, 114.8, 114.5, 114.2, 64.8, 64.5, 57.2, 32.1, 20.1. ESI-MS *m/z* Calculated 428.1. Found: 427.2 $[M-H]^-$. Anal. Calculated for $C_{23}H_{24}O_6S$: C, 64.47; H, 5.65%. Found: C, 64.45; H, 5.68%.

2.3 *In vitro anti-inflammatory activity (anti-denaturation assay)*

The *in vitro* anti-inflammatory activity of synthesized compounds were studied using bovine serum albumin denaturation method.[17,18] In brief, increasing concentrations of the test or reference compound were incubated with 0.5% w/v of bovine serum albumin at 37°C for 20 min and the temperature was increased to keep the samples at 57°C for 30 min. After cooling to room temperature, the turbidity was measured using UV-Visible spectrophotometer at 660 nm following addition of phosphate buffered saline. The control represents 100% protein denaturation. The results were compared with reference drug diclofenac sodium. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\begin{aligned}\text{Percentage Inhibition} = 100 \\ - [(\text{optical density of test solution} \\ - \text{optical density of product control}) \\ \div (\text{optical density of test control})] \times 100.\end{aligned}$$

2.4 *In vitro anti-oxidant activity*

The antioxidant activity of the test drug was determined using the 1,1-diphenyl-2 picrylhydrazyl (DPPH) free radical scavenging assay.[19] The test drug was mixed with 95% methanol to prepare the stock solution in required concentration (100 µg/mL). From the stock solution, 10 µg/mL, 20 µg/mL, 40 µg/mL, 60 µg/mL and 100 µg/mL concentration of test drug was prepared. Ascorbic acid was used as standard and was prepared in same concentration as that of the test drug by using methanol as solvent. Final reaction mixture containing 1 mL of 0.3 mmol DPPH methanol solution was added to 2.5 mL of sample solution of different concentrations and allowed to react at room temperature after 15 min incubation period at 37°C. Absorbance was read out at 517 nm. Control reading was observed without adding test drug. % scavenging = [(Absorbance of control − Absorbance of test sample) ÷ (Absorbance of control)] × 100.

2.5 *Molecular docking studies*

Molecular docking was performed for the synthesised compounds in order to investigate their possible binding mode in the cyclooxygenase-2 (COX-2) receptor. Docking was performed using the Auto Dock Tools (ADT) version 1.5.6 and Auto Dock version 4.2.5.1 docking program.[20,21] The structure of COX-2 was downloaded from the Protein Data Bank (PDB ID: 1PXX).[22] The co-crystallized ligand and water molecules present in the 1PXX structure were removed. Then, polar hydrogen atoms were added, and lower occupancy residue structures were deleted. ADT was used to replace any incomplete side chains. Gasteiger charges were added to each atom and merged the non-polar hydrogen atoms to the protein structure. The hydrogen bond distance between donor and acceptor atoms were defined as 1.9 Å with a tolerance of 0.5 Å and the threshold for acceptor-hydrogen-donor angle was set to not less than 120°. For further studies in ADT, the structures were saved in PDBQT file format. A grid box centred on 27.131, 24.348 and 14.747 with the dimension of 60 × 60 × 60 $Å^3$ and 0.375 Å spacing was created around the binding site of co-crystallised ligand using ADT. Grid energy calculations were carried out with the centre of the box was set at co-crystallised ligand centre. Default docking parameters were used and twenty docked conformations for each compound were generated. Genetic algorithm was used to estimate the energy of the binding interactions. PyMOL

was used to visualise the binding modes and interactions of the docked compounds with amino acid residues in the active site of COX-2 receptor.

3. Results and Discussion

3.1 *Chemistry*

In the present work, we synthesized a new series of allyloxy and hydroxypropoxy derivatives of 4,5-diaryl thiophene-2-carboxylic acid, as shown in Scheme 1. The first step in the Scheme 1 is the Vilsmeier reaction[23,24] of desoxyanisoin **1** with dimethylformamide (DMF) and phosphorous oxychloride ($POCl_3$) to give compound **2** in 78% yield after recrystallization from ethanol.[12] Compound **2** was condensed and cyclized with 2-mercaptoacetic acid (thioglycolic acid) in the presence of potassium hydroxide to afford compound **3**[25] in 71% yield. Treatment of compound **3** with aluminium chloride in chlorobenzene[16,26] at 95–105°C over 3 h under stirring is vital for the demethylation of compound **3** to get compound **4** in good yield (88%). The obtained compound **4** did not require any further purification.

The compound **4** obtained was converted to the corresponding ester **5** and **6** by using alcohol in presence of sulphuric acid. Esterification of compound **4** with methanol or ethanol and sulphuric acid under reflux condition over 8 h gave 86% and 88% yield of compound **5** and**6**, respectively. The allyation of compound **4**, **5** and **6** with ally bromide and potassium carbonate[27] in acetonitrile under reflux condition gave 87%, 88% and 82%, yield of compound **7, 8** and **9**, respectively.

The allyl carboxylic acid ester **7** or **8** or **9** was hydrolysed with sodium hydroxide to get compound **10** in 90% yield. Similarly compound **14** was hydrolysed with sodium hydroxide to get carboxylic acid compound **16** in 88% yield.

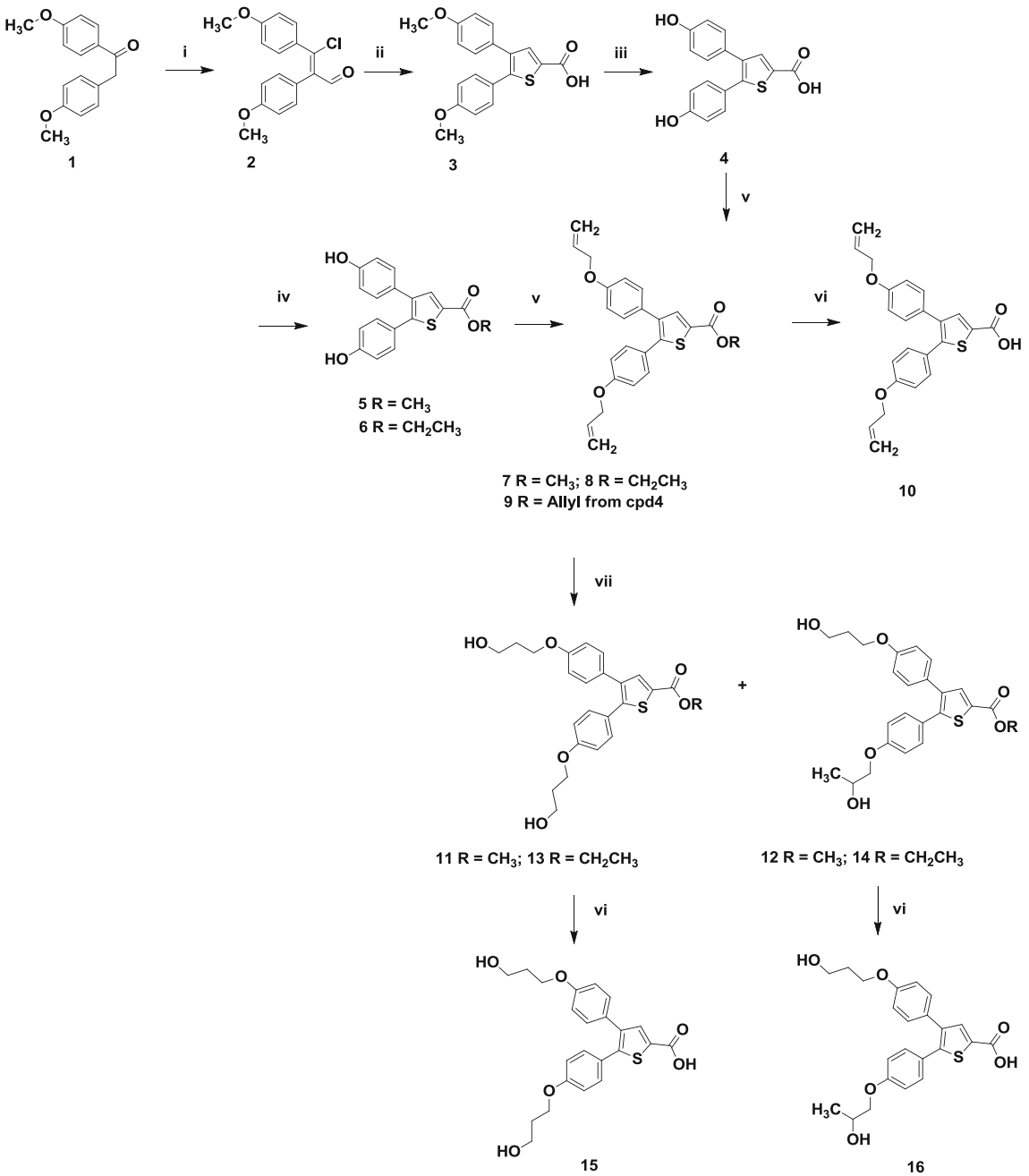
The allyl carboxylic acid ester **7** was converted to alcohol by hydroboration followed by oxidation with hydrogen peroxide and sodium hydroxide solution.[27] The reaction produced a mixture of compounds, the major (80%) component of the mixture being methyl 4,5-*bis*(4-(3-hydroxypropoxy)phenyl)thiophene-2-carboxylate compound **11** and the minor (20%) methyl 5(4)-(4-(2-hydroxypropoxy)phenyl)-4(5)-(4-(3-hydroxypropoxy)phenyl)thiophene-2-carboxylate compound **12**. The product was isolated by column chromatography eluting with 6:4 v/v hexane–ethyl acetate to afford the product **11** (55% yield) and **12** (14% yield).

Similarly compound **8** was converted to alcohol compound **13** (57% yield) and **14** (17% yield). The carboxylic acid ester **11** or **13** was hydrolysed with sodium hydroxide in aqueous ethanol under reflux condition to get compound **15** in 88% yields. Similarly compound **12** and **14** was hydrolysed with sodium hydroxide in aqueous ethanol under reflux condition to get compound **16** in 88% yield.

3.2 *Characterization*

The structure of the synthesized intermediate compound **7** was confirmed by various spectral techniques such as NMR, Mass and IR data. IR spectrum of the compound **7** revealed that the bands between ~2950 cm^{-1} and ~2850 cm^{-1} correspond to the asymmetric and symmetric stretching of methylene groups and the band at around ~1704 cm^{-1} corresponds to the carbonyl group of the ester moiety. The ^1H NMR spectrum recorded in $CDCl_3$ showed twenty-two protons. The singlet observed at δ 7.78 ppm corresponds to the aryl proton of the thiophene moiety. The multiplet at around δ 4.54–4.52 ppm corresponds to the methylene protons attached to phenoxy ring system. The singlet observed at δ 3.89 ppm corresponds to the methyl proton of the ester group. The ^{13}C NMR spectrum recorded in $CDCl_3$ showed twenty-four signals, in which the signal observed at δ 162.9 ppm correspond to carbonyl carbon of the ester moiety. The two signals at δ 114.9 ppm and at δ 114.8 ppm correspond to the four aryl carbons at the ortho position to hydroxyl derivative of the phenyl rings. The signals at δ 68.9 and δ 68.8 ppm confirmed the methylene carbons of allyloxy group. The signal at δ 52.3 ppm confirmed the methyl carbon of ester group. Mass spectrum acquired in positive ionization ESI mode, showed a signal at 407.1 Da corresponding to $[M + H]^+$, which confirmed the molecular mass of the compound. In addition to the above spectral evidences discussed, the structure of synthesized compound **7** was further confirmed unequivocally with single-crystal X-ray diffraction data. The crystal data was deposited at CCDC, and the CCDC No. is 1406560. The ORTEP diagram of above crystal compound is shown in Figure 2.

The structure of the synthesized compound **15** was confirmed by various spectral techniques such as NMR, Mass and IR data. IR spectrum of the compound **15** revealed that the broad band observed at around 3360–3300 cm^{-1} corresponds to the carboxylic acid stretching coupled with the hydroxyl stretching of the propyl group. The bands observed at ~2925 cm^{-1} and ~2854 cm^{-1} correspond to the asymmetric and symmetric stretching of methylene groups. The band at around ~1686 cm^{-1} corresponds to the carbonyl group of the acid moiety. The ^1H NMR spectrum recorded in DMSO-d_6 showed twenty-four protons in which the protons of hydroxyl and carboxylic groups weren't



Scheme 1. Synthesis of 4,5-diarylthiophene-2-carboxylic acid derivatives. Reagents and conditions: (i) DMF and $POCl_3$, at 70−75°C for 4 h; (ii) 2-mercapto acetic acid, KOH, methanol and water, at reflux for 4 h; (iii) $AlCl_3$ and chlorobenzene, at 95−105°C for 3 h; (iv) methanol or ethanol, sulphuric acid, at reflux for 8 h; (v) allyl bromide, K_2CO_3, acetonitrile reflux for 4 h; (vi) NaOH, ethanol and water, at reflux for 2 h; (vii) Borane in THF at 0°C 1 h, H_2O_2/NaOH at RT 1 h.

observed. The singlet observed at δ 7.71 ppm corresponds to the aryl proton of the thiophene ring system. The quartet at δ 4.05 ppm corresponds to four methylene protons attached to two hydroxy groups, the triplet at δ 3.58 ppm corresponds to four methylene protons attached to two phenoxy group. The ^{13}C NMR spectrum recorded in DMSO-d_6 showed twenty-three signals, in which the signal observed at δ 162.9 ppm correspond to carbonyl carbon of the acid group. The four signals at δ 114.9, δ 114.8, δ 114.7 and δ114.5 ppm correspond to the four aryl carbons of the phenoxy ring. The signal at δ 57.5 ppm corresponds to the methylene groups of the hydroxy propyl side chain. Mass spectrum acquired in negative ionization ESI mode showed the signal at 427.2 Da which corresponds to [M − H] confirmed the molecular mass of the compound.

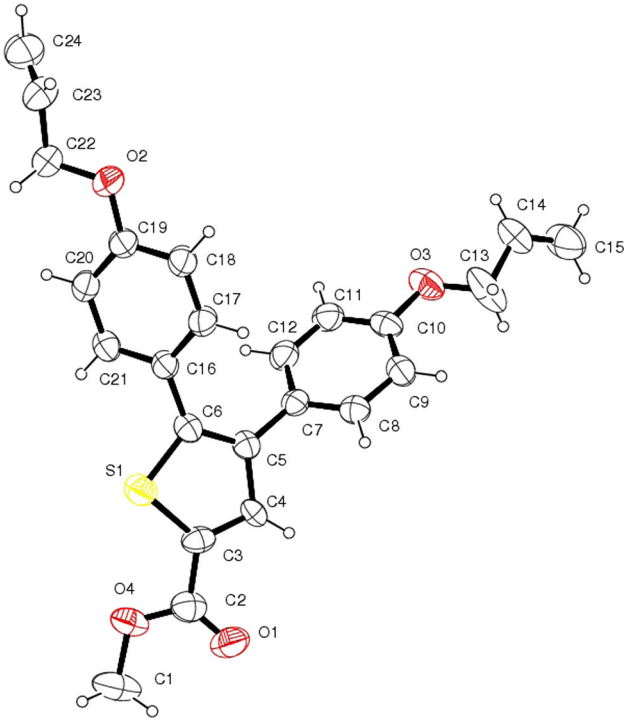


Figure 2. ORTEP diagram of compound **7** (methyl 4,5-*bis*(4-(allyloxy)phenyl)thiophene-2-carboxylate, CCDC 1406560).

3.3 *Biological evaluation: structure activity relationship (SAR)*

3.3a *In vitro anti-inflammatory activity*: In our earlier studies, we have identified that the introduction of amino moieties at carboxylic acid of thiophene ring[16] significantly enhanced the anti-inflammatory activity spectrum of 4,5-diarylthiophene-2-carboxylic acid analogues. In the present study, we developed and synthesized some newer derivatives of compound **4** by introducing hydroxyl substituents to the phenyl ring and esterification of carboxylic acid of thiophene ring. The anti-inflammatory activity of novel substituted 4,5-diarylthiophene-2-carboxylic acid derivatives were evaluated using inhibition of bovine serum albumin denaturation method and compared with standard drug diclofenac sodium. The results are summarized in Table 1.

Most of the derivatives exhibited anti-inflammatory activity that is comparable to diclofenac sodium. The free acid **4** is less potent; however, derivatisation of the hydroxy group to allyl with bisallyloxy substituted phenyl moiety (**10**) resulted in analogues having anti-inflammatory activity comparable to the standard drug. Although, bisallyloxy substituted phenyl moiety with methyl (**7**), ethyl (**8**) and allyl (**9**) esters of acid group resulted in less active analogues. In contrast, the compound 4,5-*bis*(4-(3-hydroxypropoxy)phenyl)thiophene-2-carboxylic acid (**15**) and its methyl (**11**), or ethyl ester (**13**) exhibited better anti-inflammatory activity in comparison to the standard drug whereas their byproducts 5(4)-(4-(2-hydroxypropoxy)phenyl)-4(5)-(4-(3-hydroxypropoxy)phenyl)thiophene-2-carboxylic acid (**16**) and its methyl (**12**) or ethyl ester (**14**) showed moderate activity.

Taken together, these results clearly suggest that allyloxy/hydroxypropoxy substitution on the phenyl group of 4,5-diarylthiophene-2-carboxylic acid derivatives is advantageous in improving or retaining the anti-inflammatory spectrum. Further, an important SAR observation relevant to the nature of substituents to the diaryl ring indicates that symmetricity of substituents is

Table 1. In vitro anti-inflammatory activity of compounds **4, 7–16** by inhibition of protein denaturation method (Bovine serum albumin).

Entry	Compounds	Activity (% inhibition of protein denaturation)				
		25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL
1	**4**	4.86	9.84	16.47	28.13	30.33
2	**7**	10.42	18.85	26.04	35.41	43.75
3	**8**	5.21	12.5	17.71	21.88	29.17
4	**9**	14.58	20.83	29.17	35.42	41.67
5	**10**	34.38	42.71	51.05	62.5	70.83
6	**11**	22.92	31.25	45.83	55.21	60.42
7	**12**	19.79	29.17	38.54	46.88	52.08
8	**13**	35.42	40.63	50.00	62.50	75.00
9	**14**	14.58	23.96	31.25	46.88	54.17
10	**15**	21.88	34.38	43.75	57.3	70.63
11	**16**	8.33	15.63	22.92	31.29	45.83
12	Std	32.29	40.65	51.04	60.41	66.66

Std: Diclofenac sodium

Table 2. Antioxidant activity of compounds **4, 7–16** by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay.

Entry	Compounds	% Inhibition					Antioxidant IC50 value
		10 μg/mL	20 μg/mL	40 μg/mL	60 μg/mL	100 μg/mL	
1	**4**	3.59	7.61	14.16	19.34	23.78	208.64
2	**7**	2.78	3.17	9.83	12.68	19.45	255.54
3	**8**	6.76	15.01	17.34	27.17	30.97	164.96
4	**9**	1.27	8.77	13.53	18.39	23.78	204.97
5	**10**	24.1	30.87	39.22	46.93	53.28	80.95
6	**11**	21.25	28.86	37.42	43.66	50.63	89.48
7	**12**	9.62	15.12	20.82	27.91	34.88	148.87
8	**13**	27.27	39.32	45.77	52.75	65.22	56.16
9	**14**	13.95	19.45	24.84	28.86	37.53	146.81
10	**15**	9.73	13.11	23.57	32.03	37.42	129.19
11	**16**	12.05	24.63	29.07	35.41	41.23	120.77
12	Std	41.01	61.84	73.25	86.89	92.39	5.754

Std: Ascorbic acid

required for optimal anti-inflammatory as in case of **11** and **15** whereas when the symmetricity is lost (**12**, **14** and **16**) a reduction in activity was observed. This indicates that lengthier substituents to the diaryl ring are well tolerated resulting in substantial increase of anti-inflammatory potency.

The 4,5-diarylthiophene-2-carboxylic acid **4** or its carboxyamide analogue reported in our earlier work[16] possess very little anti-inflammatory property in comparison to diclofenac. On the other hand, derivatization of the acid to ester or the amide with *p*-halo substituted phenyl moiety resulted in analogues possessing activity comparable to diclofenac. The lipophilic substituent, namely the hydroxypropyl moiety to the diaryl ring as in **13** and **15** appears to be the most important substituent with regard to enhancing the activity which was not seen with substitution to the diaryl ring with a methoxy group.[16] Taken together, the SAR analysis with the carboxylic acid derivatives mentioned herein and with the carboxamide derivatives reported earlier[16] indicates that a bulkier lipophilic substitution to the diaryl ring and with simple esters of the carboxylate group might provide a lipophilic-hydrophilic balance to the 4,5-diarylthiophene skeleton which led to an increase in anti-inflammatory spectrum. In conclusion, this study has identified some important substituents and its arrangement on the diaryl ring which is crucial for the anti-inflammatory property of 4,5-diarylthiophene-2-carboxylic acid.

3.4 *Antioxidant activity*

The antioxidant activity of the test drug was determined using the 1,1-diphenyl-2 picrylhydrazyl (DPPH) free radical scavenging assay. The free radical scavenging activity of the synthesized compounds was assessed through their ability to quench the DPPH using ascorbic acid as a standard. The potencies for the antioxidant activity of compounds **4**, **7–16** to the reference drug are shown in Table 2. In general, all the synthesized compounds were less potent than the reference compound. Among the synthesized compounds, compound **10**, **11** and **13** exhibited slightly moderate antioxidant activity when compared to the standard.

3.4a *Molecular docking studies*: Molecular docking analysis was performed for the synthesised compounds with the cyclooxygenase-2 (COX-2) receptor, which is an important target for the anti-inflammatory activity.[28] COX-2 is an attractive target for medicinal chemists because of its very high expression in inflamed tissues as well as many tumors.[29,30] The compounds were docked using Auto Dock Tools (ADT) version 1.5.6 and Auto Dock version 4.2.5.1 docking program[20,21] into the crystal structure of COX-2 receptor (PDB ID: 1PXX).[22]

To verify the reproducibility of the docking calculation, the co-crystallised ligand was extracted from the complex and submitted for one-ligand run calculation. Docking of co-crystallised ligand with bound X-ray conformation for 1PXX exhibits root-mean-square deviation (RMSD) value of 0.58 Å. This result signifies that this method is valid enough to be used for docking studies of other compounds (Figure 3A).

Docking simulation of all the synthesised compounds was performed using the same protocol of validation study. For each of the test molecules, dockings were achieved by taken into 2.5 million energy evaluations. The conformation of docked ligand with COX-2 recep-

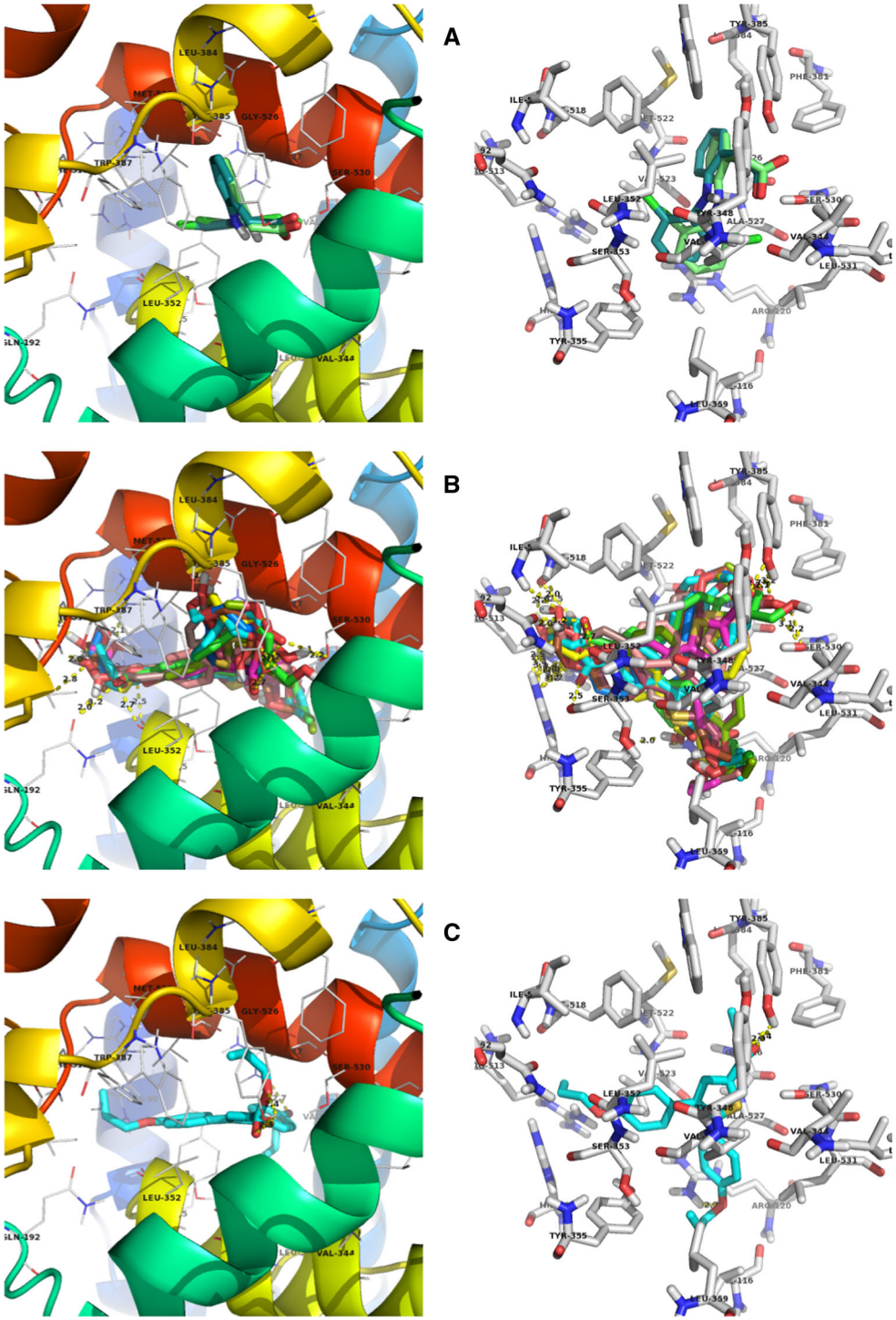


Figure 3. Molecular docking simulation with COX-2 receptor (1PXX). (**A**) Method validation using crystallised and docked ligand diclofenac; (**B**) docking simulation of all the compounds in the active site; and (**C**) docking simulation of the most binding energy compound **9**.

tor was analysed in terms of energy, hydrogen bonding, hydrophobic and $\pi - \pi$ interaction. The final coordinates of the ligand and receptor were saved after the clear analysis of ligand–receptor interactions. Ligand and receptor interactions were investigated using PyMol software. The free energy of binding (FEB) of all compounds were estimated and given in the Table 3.

The docking studies demonstrate that the synthesised compounds have good free energy of binding with

Table 3. Free energy of binding (FEB) of all the synthesised compounds.

Sl. No.	Compound	Free Energy of Binding (kcal/mol)[a]
		COX-2 (PDB ID: 1PXX)
1	**4**	−8.97
2	**7**	−9.95
3	**8**	−10.40
4	**9**	−10.48
5	**10**	−9.80
6	**11**	−9.45
7	**12**	−9.25
8	**13**	−8.90
9	**14**	−8.85
10	**15**	−8.98
11	**16**	−8.75
12	Diclofenac	−8.15

[a]Calculated using Autodock4

COX-2 receptor. Compounds display free energy of binding value from −8.75 to −10.48 kcal/mol. When compared with the standard drug diclofenac sodium, all the synthesised compounds exhibit greater binding affinity. Docking revealed that all the synthesised compounds shows various interactions such as hydrophobic, hydrophilic, π − π interaction and hydrogen bonding with 25 binding site amino acids namely HIS-90, VAL-116, ARG-120, GLN-192, VAL-344, TYR-348, VAL-349, LEU-352, SER-353, TYR-355, LEU-359, PHE-381, LEU-384, TYR-385, TRP-387, ARG-513, ILE-517, PHE-518, MET-522, VAL-523, GLY-526, ALA-527, SER-530, LEU-531 and LEU-534. The docking conformation of all the compounds with COX-2 receptor is shown in Figure 3B.

Among all the compounds docked, compounds **8** and **9** exhibit very high binding with COX-2 receptor. Compound **9** shows the binding affinity of −10.48 kcal/mol with three hydrogen bonds with two active site amino acids, namely ARG-120 and TYR-385. In Compound **9**, ester carbonyl and oxygen interact with the O-H of TYR-385 and forms two hydrogen bonds with the bond length of 2.3 Å and 2.4 Å, respectively. One of the phenoxy oxygen interacts with the N-H of ARG-120 and forms a hydrogen bond with the bond length of 2.7 Å. In addition to the polar interactions, hydrophobic interaction was observed with the VAL-116, VAL-349, LEU-352, LEU-356, LEU-384, VAL-523, ILE-517, GLY-526 and ALA-527 amino acids. Furthermore, phenyl rings of the compound **9** display π − π interaction with the phenyl rings of the TYR-355 and PHE-518. The docking conformation of compound **9** with COX-2 receptor is shown in Figure 3C.

4. Conclusions

In summary, a new series of bishydroxypropoxy substituted 4, 5-diarylthiophene-2-carboxylic acid derivatives were synthesized, characterized and evaluated for their *in vitro* anti-inflammatory activity. The *in vitro* anti-inflammatory activity revealed that the compound 4,5-*bis*(4-(3-hydroxypropoxy) phenyl)thiophene-2-carboxylic acid (**15**) and ethyl ester (**13**) having anti-inflammatory activity better than the standard drug diclofenac sodium. The antioxidant screening showed 4,5-*bis*(4-(allyloxy) phenyl) thiophene-2-carboxylic acid (**10**), 4,5-*bis*(4-(3-hydroxypropoxy)phenyl)thiophene-2-carboxylic acid methyl ester (**11**) and 4,5-*bis*(4-(3-hydroxypropoxy)phenyl)thiophene-2-carboxylic acid ethyl ester (**13**) exhibited a slightly moderate antioxidant activity than the standard ascorbic acid. Docking studies with COX-2 enzyme revealed that all the synthesised compounds exhibit greater binding affinity than the standard drug. In particular, the compounds ethyl 4,5-*bis*(4-(allyloxy)phenyl)thiophene-2-carboxylate (**8**) and allyl 4,5-*bis*(4-(allyloxy)phenyl)thiophene-2-carboxylate (**9**) have high free energy binding of −10.40 and −10.48 Kcal/mol, respectively. Further studies are in progress to improve the biological activities of bishydroxypropoxy substituted 4,5-diarylthiophene-2-carboxylic acid derivatives.

Supplementary information (SI)

The characterization of the compounds **2**–**16** using ^{1}H NMR, ^{13}C NMR, IR and Mass spectral data (Figures S1–S60) are given in the Supplementary Information, which is available at www.ias.ac.in/chemsci.

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