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Synthesis and biological evaluation of novel active arylidene derivatives of 5,6-dihydro-4*H*-cyclopenta[*b*]- and 4,5,6,7-tetrahydrobenzo[*b*]-thiophene-2-carboxylic acid

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Abstract A series of novel arylidene derivatives of 5,6-dihydro-4*H*-cyclopenta[*b*]-thiophene-2-carboxylic acid and 4,5,6,7-tetrahydrobenzo[*b*]-thiophene-2-carboxylic acid were synthesized by reacting benzylidene derivatives of chloro aldehyde with 2-mercaptoacetic acid. Benzylidene derivatives of chloro aldehyde were prepared from Vilsmeier reaction of 2-benzylidenecyclopentanone and 2-benzylidenecyclohexanone derivatives, obtained from condensation of various aromatic aldehydes with cyclopentanone and cyclohexanone. All synthesized compounds were characterized by nuclear magnetic resonance (NMR), infrared (IR), and mass spectroscopy and X-ray single-crystal analysis. The synthesized compounds were screened for their in vitro antimicrobial and antifungal activity. Good antimicrobial activity, especially against methicillin-resistant *Staphylococcus aureus*, was observed for most of the compounds tested. In particular, compound **9f** emerged as an effective antibacterial agent and may be a potential candidate for future drug discovery and development.

Keywords Vilsmeier reaction · Benzylidene derivatives of chloro aldehyde · 5,6-Dihydro-4*H*-cyclopenta[*b*]-thiophene-2-carboxylic acid derivatives · 4,5,6,7-Tetrahydrobenzo[*b*]-thiophene-2-carboxylic acid derivatives · Antimicrobial activity

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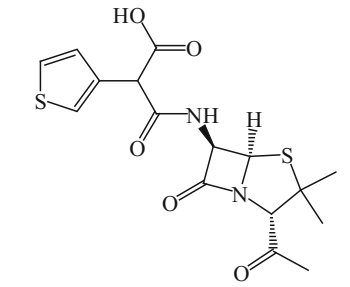
Introduction

Antimicrobial resistance has been considered to be one of the greatest threats to global health for several years. As a consequence of mutations in microbes and continued wide use of antibiotics in hospitals, the community, and agriculture, the rate of resistance to common antibiotics has increased in recent years [1]. Discovery of new antimicrobials with completely new scaffolds or modification of the structure of well-known antimicrobials are the two main approaches to address this problem [2]. Another strategy involves combining two or more pharmacophores from different antimicrobials to obtain molecules exhibiting powerful synergistic effects [3]. Heterocycles containing nitrogen and sulfur have been the subject of chemical and biological studies due to their versatile synthetic applicability and interesting pharmacological properties.

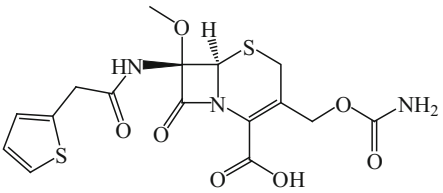
Thiophenes are an important class of heterocyclic compounds, being widely used as building blocks in many agrochemicals and pharmaceuticals, including antibacterial, antifungal, analgesic, antiinflammatory, antioxidant, local anesthetic, and antitumor agents; For example, ticarcillin, cefoxitin, cephalothin, and cephaloridine are thiophene-containing β-lactam antibiotics that are widely used for treating microbial infections, while tioconazole and sertaconazole are antifungal agents with thiophene moiety in their core structure (Fig. 1) [4]. Raloxifene is an oral selective estrogen receptor modulator (SERM) containing a thiophene ring, being used to treat osteoporosis in postmenopausal women [5].

Literature survey reveals that tetrahydrobenzothiophene derivatives have attracted continuous interest because of their various biological properties. Tetrahydrobenzothiophene derivatives were reported to have low micromolar inhibition of hepatitis C virus (HCV) nonstructural (NS)5B 4a full-length polymerase with selectivity against human DNA polymerase and calf thymus polymerase α [6]. They were also found to possess antiinflammatory and analgesic activities [7, 8]. A variety of tetrahydrobenzothiophene derivatives have been studied for their antibacterial [9, 10] and antitumor activity [11]. Replacement of aryl group with heterocyclic tetrahydrobenzothiophene has been proved to improve the potency and selectivity towards inhibition of Bruton's tyrosine kinase, which plays a significant role in the development of immunological disorders such as rheumatoid arthritis, lupus, multiple sclerosis, and B-cell malignancies [12].

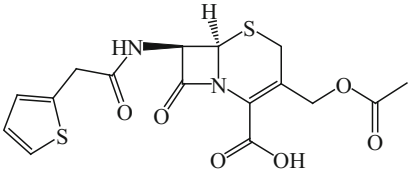
In view of this biological importance and in continuation of our studies on the chemistry of such compounds, we designed and synthesized new tetrahydrobenzothiophene-2-carboxylic acid derivatives with benzylidene moiety via aldol condensation of different *para*-substituted aromatic aldehydes and cyclic ketones in presence of base. Subsequently, the condensed product was subjected to Vilsmeier reaction, resulting in formation of chloro aldehyde derivatives. These chloro aldehydes were reacted with 2-mercapto acetic acid in presence of base to give our expected compounds. The synthesized compounds were characterized and evaluated for their antimicrobial activity against various microorganisms such as *Staphylococcus aureus* (methicillin-sensitive and methicillin-resistant strains, MSSA and MRSA, respectively), *Escherichia coli*, *Pseudomonas aeruginosa*, and



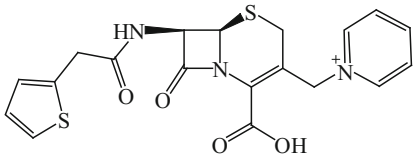
Ticarcillin



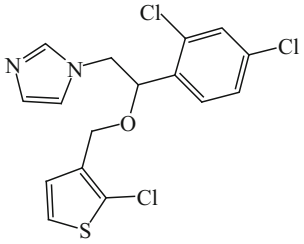
Cefoxitin



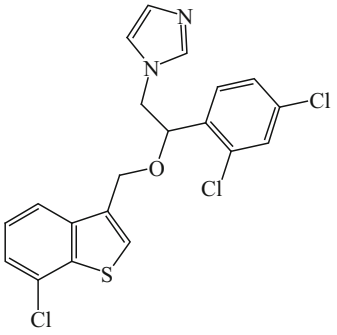
Cephalotin



Cephaloridine



Tioconazole



Sertaconazole

Fig. 1 Example thiophene-containing β-lactam antibiotics and antifungal agents

fungal strain *Candida albicans*, and the results were interpreted to determine their effect as antimicrobial agents.

Experimental

General considerations

All chemicals and reagents used were of laboratory grade and procured from Sigma Aldrich. All solvents used were purchased from commercial suppliers and used

without further purification. Melting points were determined using a Büchi apparatus by open capillary tube method and are uncorrected. IR spectra were recorded using a PerkinElmer series 2000 Fourier-transform infrared (FTIR) spectrophotometer from KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance 400 spectrometer. Chemical shifts (δ) are reported in ppm relative to tetramethylsilane as internal standard. Coupling constants (J) are in hertz (Hz). Proton and carbon spectra were typically obtained at room temperature. Mass spectra were recorded on an PerkinElmer Sciex API 3000 ESI mass spectrometer. Precoated silica gel GF_{254} plates from Merck were used for thin-layer chromatography. Elemental analyses were carried out using a Thermo Finnigan Flash EA 1112 elemental analyzer.

Synthesis of 2-benzylidenecyclopentanone and 2-benzylidenecyclohexanone derivatives

These compounds were synthesized according to previously reported procedure [13–18].

General procedure for synthesis of compounds 6a–c and 7a–g

To dimethylformamide (45 ml), phosphorus oxychloride (0.12 mol) was added dropwise over a period of 15–30 min with stirring at 0–5 °C. Compound **4a–c** and **5a–g** (0.05 mol) dissolved in dimethylformamide (DMF, 70 ml) was added dropwise, followed by warming to room temperature, heating to temperature of 55–60 °C maintained for 4 h, cooling, and pouring slowly into 50 % solution of sodium acetate in water (100 ml) at 0–5 °C. The product was filtered, washed with water, followed by slurry washing with ethanol (100 ml) to obtain compounds **6a–c** and **7a–g**.

(*E*)-2-Chloro-3-(benzylidene)cyclopent-1-enecarbaldehyde (6a)

Pale-yellow powder; yield: 60 %; m.p. 99–101 °C; IR (KBr) cm^{-1}: 3435, 2942, 2845, 1659, 1566, 1448, 1246, 999, 880; ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.09 (s, 1H), 7.58–7.56 (d, $J = 7.6$ Hz, 2H), 7.46 (t, $J = 8.0$ Hz, 2H), 7.37 (t, $J = 7.6$ Hz, 1H), 7.03 (s, 1H), 3.03–2.99 (m, 2H), 2.69–2.68 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 188.2, 147.6, 143.5, 140.8, 136.2, 131.1, 129.4, 129.3, 128.8, 126.9, 27.5, 27.3; ESI–MS *m/z* calcd. 218.05; found: 219.1 $[M + H]^+$; anal. calcd. for $C_{13}H_{11}ClO$ (%): C, 71.40; H, 5.07; found (%): C, 71.38; H, 5.12.

(*E*)-2-Chloro-3-(4-fluorobenzylidene)cyclopent-1-enecarbaldehyde (6b)

Pale-yellow powder; yield: 63 %; m.p. 146–148 °C; IR (KBr) cm^{-1}: 3435, 2942, 2845, 1659, 1566, 1448, 1246, 999, 880; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.19 (s, 1H), 7.59–7.46 (m, 2H), 7.13–7.09 (m, 2H), 7.02 (s, 1H), 3.09–3.01 (m, 2H), 2.82–2.81 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 188.2, 163.4, 160.9 ($^{1}J_{19F–13C} = 246$ Hz), 147.5, 143.2, 143.2 ($^{4}J_{19F–13C} = 2.4$ Hz), 140.8, 132.8,

132.0, 131.9 ($^3J_{19F-13C}$ = 8.3 Hz), 125.7, 116.5, 116.3, 116.1 ($^2J_{19F-13C}$ = 21.4 Hz), 27.5, 27.1; ESI–MS *m/z* calcd. 236.04; found: 237.1 $[M + H]^+$; anal. calcd. for $C_{13}H_{10}ClFO$ (%): C, 65.97; H, 4.26; found (%): C, 65.92; H, 4.28.

(*E*)-2-Chloro-3-(4-chlorobenzylidene)cyclopent-1-enecarbaldehyde (6c)

Pale-yellow powder; yield: 65 %; m.p. 183–185 °C; IR (KBr) cm^{-1}: 3434, 3047, 2831, 1656, 1570, 1491, 1247, 1008, 888; ^1H NMR (400 MHz, $CDCl_3$): δ 10.19 (s, 1H), 7.44–7.37 (m, 4H), 7.01 (s, 1H), 3.02–3.00 (m, 2H), 2.82–2.79 (m, 2H); ESI–MS *m/z* calcd. 252.01; found: 253.1 $[M + H]^+$; anal. calcd. for $C_{13}H_{10}Cl_2O$ (%): C, 61.68; H, 3.98; found (%): C, 61.74; H, 3.94.

(*E*)-3-Benzylidene-2-chlorocyclohex-1-enecarbaldehyde (7a)

Pale-yellow powder; yield: 70 %; m.p. 87–90 °C; IR (KBr) cm^{-1}: 3434, 2949, 2875, 1663, 1560, 1440, 1255, 1001, 882; ^1H NMR (400 MHz, DMSO-d_6): δ 10.28 (s, 1H), 7.49 (s, 1H), 7.45–7.41 (m, 4H), 7.38–7.36 (m, 1H), 2.77–2.74 (m, 2H), 2.40–2.37 (m, 2H), 1.65–1.62 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 191.6, 146.0, 136.1, 134.7, 134.6, 133.3, 130.3, 128.9, 128.8, 28.3, 24.8, 21.2; ESI–MS *m/z* calcd. 232.07; found: 233.1 $[M + H]^+$; anal. calcd. for $C_{14}H_{13}ClO$,(%) C, 72.26; H, 5.63; found (%): C, 72.22; H, 5.68.

(*E*)-2-Chloro-3-(4-fluorobenzylidene)cyclohex-1-enecarbaldehyde (7b)

Yellow powder; yield: 68 %; m.p. 96–98 °C; IR (KBr) cm^{-1}: 3435, 2943, 2879, 1657, 1560, 1447, 1252, 999, 887; ^1H NMR (400 MHz, DMSO-d_6): δ 10.26 (s, 1H), 7.51–7.49 (m, 2H), 7.46 (s, 1H), 7.28–7.23 (m, 1H), 2.74–2.71 (m, 2H), 2.39–2.36 (m, 2H), 1.66–1.60 (m, 2H); ESI–MS *m/z* calcd. 250.06; found: 251.1 $[M + H]^+$; anal. calcd. for $C_{14}H_{12}FO$ (%): C, 67.07; H, 4.82; found (%): C, 67.02; H, 4.78.

(*E*)-2-Chloro-3-(4-chlorobenzylidene)cyclohex-1-enecarbaldehyde (7c)

Greenish-yellow powder; yield: 66 %; m.p. 76–79 °C; IR (KBr) cm^{-1}: 3435, 2954, 2861, 1659, 1558, 1488, 1250, 1012, 884; ^1H NMR (400 MHz, DMSO-d_6): δ 10.28 (s, 1H), 7.55–7.49 (m, 4H), 7.46 (s, 1H), 2.75–2.72 (m, 2H), 2.40–2.37 (m, 2H), 1.67–1.61 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 191.6, 145.7, 135.3, 135.0, 134.9, 133.4, 132.0, 131.9, 128.9, 28.2, 24.8, 21.2; ESI–MS *m/z* calcd. 266.03; found: 267.0 $[M + H]^+$; anal. calcd. for $C_{14}H_{12}Cl_2O$ (%): C, 62.94; H, 4.53; found (%): C, 62.88; H, 4.58.

(*E*)-3-(4-Bromobenzylidene)-2-chlorocyclohex-1-enecarbaldehyde (7d)

Pale-yellow powder; yield: 66 %; m.p. 82–85 °C; IR (KBr) cm^{-1}: 3435, 2953, 2860, 1658, 1557, 1485, 1252, 1004, 889; ^1H NMR (400 MHz, DMSO-d_6): δ 10.29 (s, 1H), 7.65–7.61 (m, 2H), 7.43–7.41 (m, 3H), 2.80–2.73 (m, 2H), 2.40–2.37 (m, 2H), 1.66–1.63 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 191.1, 145.2, 134.8,

134.6, 131.8, 131.5, 131.4, 121.6, 27.8, 24.3, 20.7; ESI–MS *m/z* calcd. 309.98; found: 311.0 $[M + H]^+$; anal. calcd. for $C_{14}H_{12}BrClO$ (%): C, 53.96; H, 3.88; found (%): C, 53.92; H, 3.86.

(*E*)-2-Chloro-3-(4-methylbenzylidene)cyclohex-1-enecarbaldehyde (7e)

Yellow powder; yield: 65 %; m.p. 72–75 °C; IR (KBr) cm^{-1}: 3435, 2947, 2862, 1664, 1559, 1442, 1249, 996, 883; ^1H NMR (400 MHz, DMSO-d_6): δ 10.28 (s, 1H), 7.46 (s, 1H), 7.38–7.36 (m, 2H), 7.26–7.24 (m, 2H), 2.78–2.75 (m, 2H), 2.39–2.37 (m, 2H), 2.33 (s, 3H), 1.67–1.61 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 191.1, 145.7, 138.0, 133.9, 133.3, 132.9, 132.8, 129.9, 129.1, 27.9, 24.3, 20.9, 20.7; ESI–MS *m/z* calcd. 246.08; found: 247.1 $[M + H]^+$; anal. calcd. for $C_{15}H_{15}ClO$ (%): C, 73.02; H, 6.13; found (%): C, 73.12; H, 6.08.

(*E*)-2-Chloro-3-(4-isopropylbenzylidene)cyclohex-1-enecarbaldehyde (7f)

Yellow powder; yield: 64 %; m.p. 72–75 °C; IR (KBr) cm^{-1}: 3433, 2921, 2862, 1657, 1559, 1441, 1248, 996, 883; ^1H NMR (400 MHz, DMSO-d_6): δ 10.28 (s, 1H), 7.46 (s, 1H), 7.37–7.35 (m, 2H), 7.25–7.19 (m, 2H), 3.09 (m, 1H), 2.76–2.71 (m, 2H), 2.38–2.36 (m, 2H), 1.64–1.62 (m, 2H), 1.28–1.27 (d, $J = 6.1$ Hz, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 191.0, 145.8, 138.0, 133.8 133.3, 132.9, 129.9, 129.1, 128.9, 27.9, 24.3, 20.9, 20.8, 20.7; ESI–MS *m/z* calcd. 274.11; found: 275 $[M + H]^+$; anal. calcd. for $C_{17}H_{19}ClO$ (%): C, 74.31; H, 6.97; found (%): C, 4.26; H, 6.98.

(*E*)-2-Chloro-3-(4-methoxybenzylidene)cyclohex-1-enecarbaldehyde (7g)

Pale-yellow powder; yield: 68 %; m.p. 77–79 °C; IR (KBr) cm^{-1}: 3435, 2935, 2839, 1657, 1557, 1440, 1255, 995, 893; ^1H NMR (400 MHz, DMSO-d_6): δ 10.27 (s, 1H), 7.46–7.44 (m, 3H), 7.02–6.99 (m, 2H), 3.80 (s, 3H), 2.78–2.75 (m, 2H), 2.38–2.37 (m, 2H), 1.66–1.63 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 191.4, 159.8, 146.5, 133.9 133.3, 132.5, 132.2, 128.5, 114.8, 55.7, 28.5, 24.7, 21.2; ESI–MS *m/z* calcd. 262.08; found: 263.1 $[M + H]^+$; anal. calcd. for $C_{15}H_{15}ClO_2$,(%); C, 68.57; H, 5.75; found (%): C, 68.52; H, 5.72.

General procedure for synthesis of compounds 8a–c and 9a–g

Compound **6a–c** and **7a–g** (0.0042 mol) was added at room temperature to solution of potassium hydroxide (0.017 mol) and 2-mercapto acetic acid (0.0064 mol) in methanol:water (40:10 ml). The mixture was refluxed for 4 h and cooled to room temperature. The reaction mixture was slowly acidified with conc. HCl over 30–45 min at 25–30 °C. The product was filtered, washed with water, and dried. The pure product was isolated by column chromatography over silica gel (60–120 mesh) using ethyl acetate/hexane mixture.

(*E*)-6-Benzylidene-5,6-dihydro-4*H*-cyclopenta[*b*]-thiophene-2-carboxylic acid (8a)

Pale-brown powder; yield: 65 %; m.p. 204–206 °C; IR (KBr) cm^{-1}: 3429, 2925, 2853, 1668, 1530, 1447, 1280, 1168, 1039, 863; ^1H NMR (400 MHz, DMSO-d_6): δ 7.57 (s, 1H), 7.49–7.48 (m, 2H), 7.39–7.38 (m, 2H), 7.27–7.23 (m, 1H), 6.74 (s, 1H), 2.97–2.94 (m, 2H), 0.81–0.78 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.5, 151.9, 151.3, 139.7, 137.6, 129.6, 129.1, 128.6, 127.2, 120.5, 34.3, 27.6; ESI–MS *m/z* calcd. 256.01; found: 255.1 $[M - H]^-$; anal. calcd. for $C_{15}H_{12}O_2S$ (%): C, 70.29; H, 4.72; found (%): C, 70.34; H, 4.76.

(*E*)-6-(4-Fluorobenzylidene)-5,6-dihydro-4*H*-cyclopenta[*b*]-thiophene-2-carboxylic acid (8b)

Brown powder; yield: 67 %; m.p. 222–224 °C; IR (KBr) cm^{-1}: 3436, 2918, 2852, 1649, 1531, 1438, 1222, 1171, 1072, 868; ^1H NMR (400 MHz, DMSO-d_6): δ 7.56 (s, 1H), 7.53–7.50 (m, 2H), 7.23–7.19 (m, 2H), 6.74 (s, 1H), 3.32–3.29 (m, 2H), 2.96–2.93 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.5, 162.5, 160.0, 151.8, 151.2, 139.4, 139.3, 137.6, 134.2, 134.1, 130.6, 130.5, 129.5, 119.4, 116.0, 115.8, 34.2, 27.5; ESI–MS *m/z* calcd. 274.05; found: 273.1 $[M - H]^-$; anal. calcd. for $C_{15}H_{11}FO_2S$ (%): C, 65.68; H, 4.04; found (%): C, 65.66; H, 4.08.

(*E*)-6-(4-Chlorobenzylidene)-5,6-dihydro-4*H*-cyclopenta[*b*]-thiophene-2-carboxylic acid (8c)

Brown powder; yield: 66 %; m.p. 250–252 °C; IR (KBr) cm^{-1}: 3436, 2921, 2854, 1667, 1532, 1449, 1285, 1168, 1009, 864; ^1H NMR (400 MHz, DMSO-d_6): δ 7.56 (s, 1H), 7.50–7.48 (m, 2H), 7.43–7.41 (m, 2H), 6.73 (s, 1H), 3.27 (m, 2H), 2.96–2.93 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.5, 151.7, 151.6, 140.6, 137.9, 136.5, 131.5, 130.2, 129.5, 129.0, 119.2, 34.4, 27.6; ESI–MS *m/z* calcd. 290.02; found: 289.1 $[M - H]^-$; anal. calcd. for $C_{15}H_{11}ClO_2S$ (%): C, 61.96; H, 3.81; found (%): C, 61.94; H, 3.84.

(*E*)-7-Benzylidene-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-2-carboxylic acid (9a)

Yellow powder; yield: 68 %; m.p. 191–194 °C; IR (KBr) cm^{-1}: 3434, 2936, 2857, 1680, 1539, 1450, 1264, 1166, 764; ^1H NMR (400 MHz, DMSO-d_6): δ 7.46 (s, 1H), 7.42–7.37 (m, 4H), 7.29–7.26 (m, 1H), 6.88 (s, 1H), 2.75 (m, 2H), 2.51 (m, 2H), 1.78 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.7, 144.0, 140.1, 136.7, 134.4, 133.2, 128.9, 128.8, 127.4, 123.8, 27.3, 25.8, 23.5; ESI–MS *m/z* calcd. 270.07; found: 269.1 $[M - H]^-$; anal. calcd. for $C_{16}H_{14}O_2S$ (%): C, 71.08; H, 5.22; found (%): C, 71.14; H, 5.24.

(*E*)-7-(4-Fluorobenzylidene)-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-2-carboxylic acid (9b)

Yellow powder; yield: 66 %; m.p. 222–225 °C; IR (KBr) cm^{-1}: 3435, 2944, 2829, 1637, 1545, 1448, 1271, 1161, 1014, 870; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.48–7.43 (m, 3H), 7.23–7.19 (m, 2H), 6.87 (s, 1H), 2.73–2.67 (m, 4H), 1.79–1.76 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.4, 162.7, 160.3, 144.3, 140.2, 134.8, 133.2, 133.1, 132.9, 131.7, 131.6, 131.3, 122.9, 115.8, 115.6, 27.2, 25.7, 23.5; ESI–MS *m*/*z* calcd. 288.06; found: 287.1 $[M - H]^-$; anal. calcd. for $C_{16}H_{13}FO_2S$ (%): C, 66.65; H, 4.54; found (%): C, 66.70; H, 4.58.

(*E*)-7-(4-Chlorobenzylidene)-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-2-carboxylic acid (9c)

Yellow powder; yield: 65 %; m.p. 213–216 °C. IR (KBr) cm^{-1}: 3435, 2938, 2859, 1666, 1540, 1449, 1265, 1165, 1012, 867; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.48 (s, 1H), 7.42 (m, 4H), 6.86 (s, 1H), 2.71–2.68 (m, 4H), 1.78–1.76 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.4, 144.1, 140.5, 135.6, 134.7, 133.8, 131.9, 131.6, 131.4 128.8, 122.7, 27.2, 25.7, 23.5; ESI–MS *m*/*z* calcd. 304.03; found: 303.1 $[M - H]^-$; anal. calcd. for $C_{16}H_{13}ClO_2S$ (%): C, 63.05; H, 4.30; found (%): C, 63.12; H, 4.26.

(*E*)-7-(4-Bromobenzylidene)-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-2-carboxylic acid (9d)

Yellow powder; yield: 65 %; m.p. 205–208 °C; IR (KBr) cm^{-1}: 3435, 2940, 2841, 1668, 1541, 1450, 1266, 1167, 1008, 872; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.60–7.56 (m, 2H), 7.47 (s, 1H), 7.38–7.34 (m, 4H), 6.84 (s, 1H), 2.74–2.68 (m, 4H), 1.80–1.77 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.1, 143.4, 140.1 135.5, 134.0, 133.5, 131.3, 131.2, 122.2, 120.1, 26.8, 25.3 23.0; ESI–MS *m*/*z* calcd. 347.98; found: 347.0 $[_{79}M^{Br} - H]^-$; 349.0 $[_{81}M^{Br} - H]^-$; anal. calcd. for $C_{16}H_{13}BrO_2S$ (%): C, 55.03; H, 3.75; found (%): C, 55.16; H, 3.70.

(*E*)-7-(4-Methylbenzylidene)-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-2-carboxylic acid (9e)

Brown powder; yield: 66 %; m.p. 178–180 °C; IR (KBr) cm^{-1}: 3435, 2942, 2860, 1675, 1542, 1449, 1268, 1170, 1020, 875; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.59 (s, 1H), 7.31–7.29 (m, 2H), 7.21–7.19 (m, 4H), 6.84 (s, 1H), 2.73 (m, 2H), 2.68 (m, 2H), 2.31 (s, 3H), 1.77 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.0, 144.0, 139.4, 136.4, 134.1, 133.4, 131.9, 129.2, 128.9, 123.5, 26.9, 25.3, 23.1, 20.8; ESI–MS *m*/*z* calcd. 284.09; found: 283.2 $[M - H]^-$; anal. calcd. for $C_{17}H_{16}O_2S$ (%): C, 71.80; H, 5.67; found (%): C, 71.78; H, 5.64.

(*E*)-7-(4-Isopropylbenzylidene)-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-2-carboxylic acid (9f)

Yellow powder; yield: 65 %; m.p. 215–217 °C; IR (KBr) cm^{-1}: 3434, 2925, 2854, 1673, 1541, 1447, 1266, 1168, 1019, 874; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58 (s, 1H), 7.28–7.26 (m, 1H), 7.19–7.17 (m, 3H), 6.93 (s, 1H), 2.80–2.74 (m, 4H), 2.36 (m, 4H), 1.87 (m, 2H), 1.25 (m, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 162.9, 144.2, 139.4, 136.4, 134.3, 133.4, 131.9, 130.7, 129.2, 128.9, 123.5, 26.9, 25.3, 23.1, 20.8; ESI–MS *m*/*z* calcd. 312.12; found: 311.0 $[M - H]^-$; anal. calcd. for $C_{19}H_{20}O_2S$ (%): C, 73.04; H, 6.45; found (%): C, 73.10; H, 6.48.

(*E*)-7-(4-Methoxybenzylidene)-4,5,6,7-tetrahydrobenzo[*b*]-thiophene-2-carboxylic acid (9g)

Pale-yellow powder; yield: 70 %; m.p. 219–221 °C; IR (KBr) cm^{-1}: 3435, 2935, 2835, 1669, 1539, 1445, 1254, 1167, 1030, 872; ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.47 (s, 1H), 7.37–7.35 (m, 2H), 6.96–6.94 (m, 2H), 6.83 (s, 1H), 3.77 (s, 3H), 2.73–2.66 (m, 4H), 1.78–1.75 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 163.4, 158.8, 144.9, 139.5, 134.8, 131.2, 131.1, 130.7, 129.2, 123.8, 114.3, 55.6, 27.3, 25.7, 23.5; ESI–MS *m*/*z* calcd. 300.08; found: 299.1 $[M - H]^-$; anal. calcd. for $C_{17}H_{16}O_3S$ (%): C, 67.98; H, 5.37; found (%): C, 67.92; H, 5.34.

Biology

Antimicrobial activity

The synthesized compounds were screened for antimicrobial activity by agar well diffusion method [19] using bacterial strains *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) and fungal strain *Candida albicans* (ATCC 90028). Compounds which showed activity against *S. aureus* ATCC 29213 were further tested against methicillin-resistant *S. aureus* strains ATCC 33591 and NRS 100.

Ampicillin and ciprofloxacin, and fluconazole were used as standard antibacterial and antifungal substances, respectively. Dimethyl sulfoxide (DMSO) was used as negative control. Müller–Hinton agar (MHA) medium was used for bacteria, and Sabouraud dextrose agar medium for fungal strains.

Müller–Hinton agar plates were inoculated with overnight-grown bacterial cultures previously adjusted to 0.5 McFarland standard turbidity and diluted to 1:10 using sterile saline solution by following Clinical and Laboratory Standards Institute (CLSI) recommendations. Sabouraud dextrose agar plates were inoculated with overnight-grown fungal culture previously adjusted to 0.8 McFarland standard turbidity and diluted to 1:10 using sterile saline solution by following CLSI recommendations. For agar well diffusion, wells were formed on Müller–Hinton agar plates for bacterial cultures and Sabouraud dextrose agar plates for fungal cultures, and the test compounds were added to the wells. Standard antibiotics used for quality control were also added to respective wells. All plates were incubated at

37° C for 18–24 h, and the zone of inhibition was measured to determine the antimicrobial activity.

The test compounds were dissolved in dimethylsulfoxide (DMSO), then the antimicrobial effect of the synthesized compounds was evaluated. The wells were filled with 50 µl of test compound at concentration of 200 µg/ml. The plates were incubated at 37 °C for 48 h. Antimicrobial activity was evaluated by measuring the zone of growth inhibition of bacteria surrounding the wells after 24 and 48 h. Ampicillin (10 µg/ml), ciprofloxacin (5 µg/ml), and fluconazole (25 µg/ml) served as antimicrobial controls. DMSO was taken as negative control, which did not produce any significant zone of inhibition.

Determination of minimum inhibitory concentration

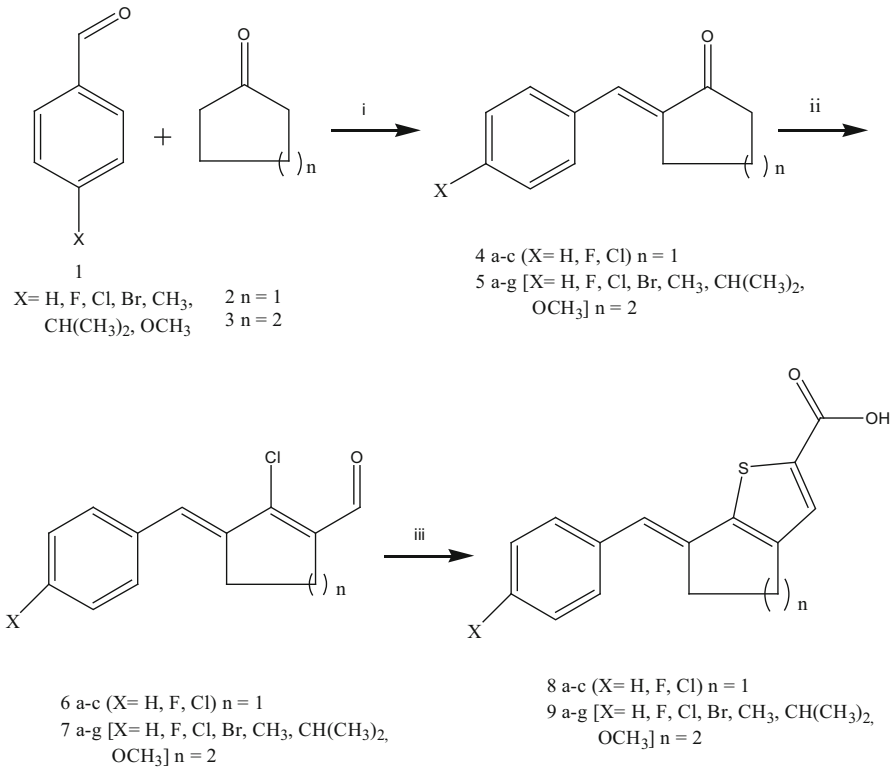
The minimum inhibitory concentration (MIC) of the compounds against the tested bacteria was determined by broth microdilution method [20]. The MIC is the lowest concentration of an antimicrobial compound that inhibits visible growth of a microorganism after overnight incubation. Stock solutions of the compounds were prepared in DMSO at concentration of 2.56 mg/ml. Stock solution was diluted 1:10 in cation-adjusted Müller–Hinton broth, and 100 µl of the diluted solution was added to the first well of a 96-well microplate. The compounds were then diluted in doubling dilutions to obtain descending concentrations (128, 64, 32, 16, 8, 4, 2, and 1 µg/ml) of the compound. The references, linezolid and vancomycin, were included in the assay as positive controls. MIC determinations were performed in triplicate, and the results averaged (Table 2).

Results and discussion

Chemistry

We synthesized a novel series of benzylidene derivatives of dihydro-cyclopenta[*b*]-thiophene-2-carboxylic acid and tetrahydrobenzothiophene-2-carboxylic acid as shown in Scheme 1. In an effort to prepare benzylidene derivatives of dihydro-cyclopenta[*b*]-thiophene-2-carboxylic acid and tetrahydrobenzothiophene-2-carboxylic acid, our initial experiments were directed towards reaction of aldehyde moiety with dihydro-cyclopenta[*b*]-thiophene-2-carboxylic acid and tetrahydrobenzothiophene-2-carboxylic acid, which failed to afford the desired product. A recent study revealed formation of **7a** and **7b** as byproducts of vinylogous aldol reaction [21] when a cyclic β-chloro enal was reacted with an aldehyde in presence of tetrabutylammonium fluoride in tetrahydrofuran at 25 °C.

In this work we synthesized benzylidene derivatives of chloro aldehyde through Vilsmeier reaction. Initially, cyclohexanone was condensed with aldehydes in presence of sodium hydroxide in water at 90–95 °C through reported procedure to get **4a**–**c** and **5a**–**g** [13–18]. These condensation products were subjected to Vilsmeier reaction using dimethylformamide (DMF) and phosphorus oxychloride ($POCl_3$) to yield chloro aldehydes **6a**–**c** and **7a**–**g** in 60–70 % yield [22, 23]. The



Scheme 1 Reagents: (i) sodium hydroxide, (ii) DMF, $POCl_3$, (iii) 2-mercapto acetic acid, KOH

chloro aldehyde was then reacted and cyclized with mercapto acetic acid in presence of potassium hydroxide in methanol under reflux condition to give benzylidene derivatives of tetrahydrobenzothiophene-2-carboxylic acid in 65–70 % yield [24] and further purified by using column chromatography. A possible synthetic mechanism of the thiophene carboxylic acid derivatives is given in Fig. 2.

The structure of the synthesized intermediate compounds **6a–c** and **7a–g** was confirmed by various spectral techniques such as NMR, mass, and IR. The IR spectra of the compounds revealed a band at around $\sim$1658 cm^{-1}, corresponding to carbonyl group of aldehyde group in cyclopentyl moiety, and at $\sim$1663 cm^{-1} for cyclohexyl moiety. In the ^1H NMR spectra, singlets at around δ 10.19 ppm and δ 10.28 ppm correspond to –CH proton of aldehyde group of cyclopentyl and cyclohexyl moiety, respectively. Singlets at around δ 7.02 and 7.49 ppm correspond to benzylidene proton of cyclopentyl and cyclohexyl moiety. The multiplet at around δ 3.03–2.99 ppm and δ 2.82–2.79 ppm corresponds to methylene protons of cyclopentyl group. The multiplet at around δ 2.75–2.71 ppm, δ 2.39–2.31 ppm, and δ 1.65–1.60 ppm corresponds to methylene protons of cyclohexyl group. The ^{13}C NMR spectra recorded in DMSO-d_6 showed a signal at δ 188.2 ppm corresponding to carbonyl carbon of aldehyde group in cyclopentyl moiety. Signals at around δ 27.4 ppm and δ 27.3 ppm confirm methylene carbons of cyclopentyl group. The ^{13}C

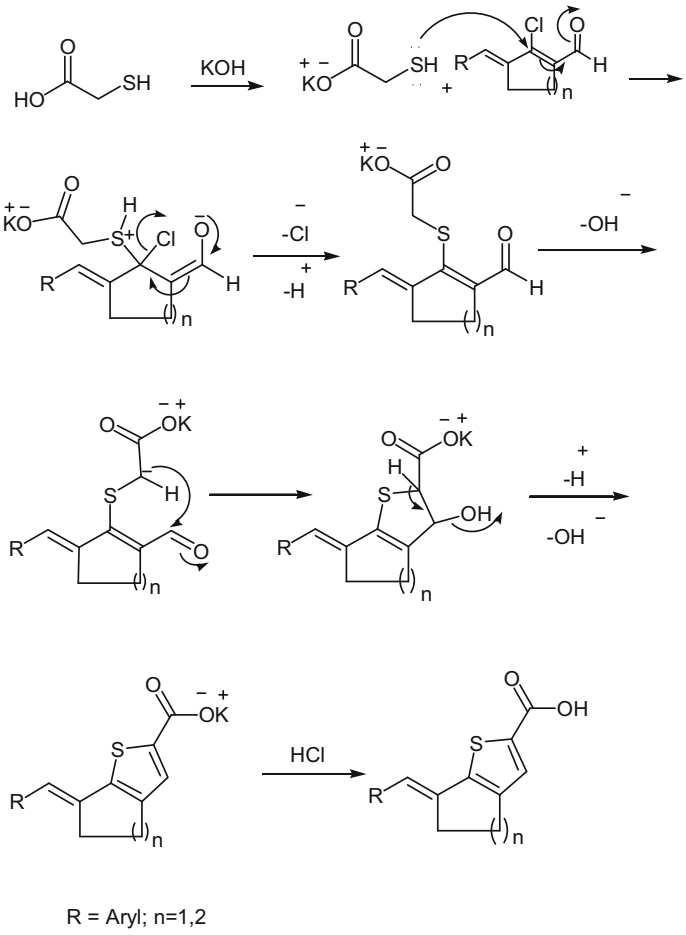


Fig. 2 Plausible reaction mechanism for synthesis of thiophene 2-carboxylic acid derivatives

NMR spectra recorded in DMSO-d_6 showed a signal at δ 191.4 ppm, corresponding to carbonyl carbon of aldehyde group in cyclohexyl moiety. Signals at around δ 28.3 ppm, δ 24.3 ppm, and δ 20.9 ppm confirmed methylene carbons of cyclohexyl group. Mass spectra acquired in positive and negative electrospray ionization (ESI) mode confirmed the molecular mass of the compounds. In addition to the above spectral evidence, the structure of synthesized compound **7a** was further confirmed by single-crystal X-ray diffraction unequivocally. The crystal data were deposited at the Cambridge Crystallographic Data Centre (CCDC) with no. 1449251. Similarly, the structures of **7b** and **7e** were assigned by single-crystal X-ray diffraction results, deposited at CCDC with nos. 1405840 and 1405845, respectively. Based on the single-crystal data, the structure of the obtained compounds was confirmed to be the *E*-isomer. Oak Ridge thermal ellipsoid plot (ORTEP) diagrams for the above crystalline compounds are shown in Fig. 3a–c.

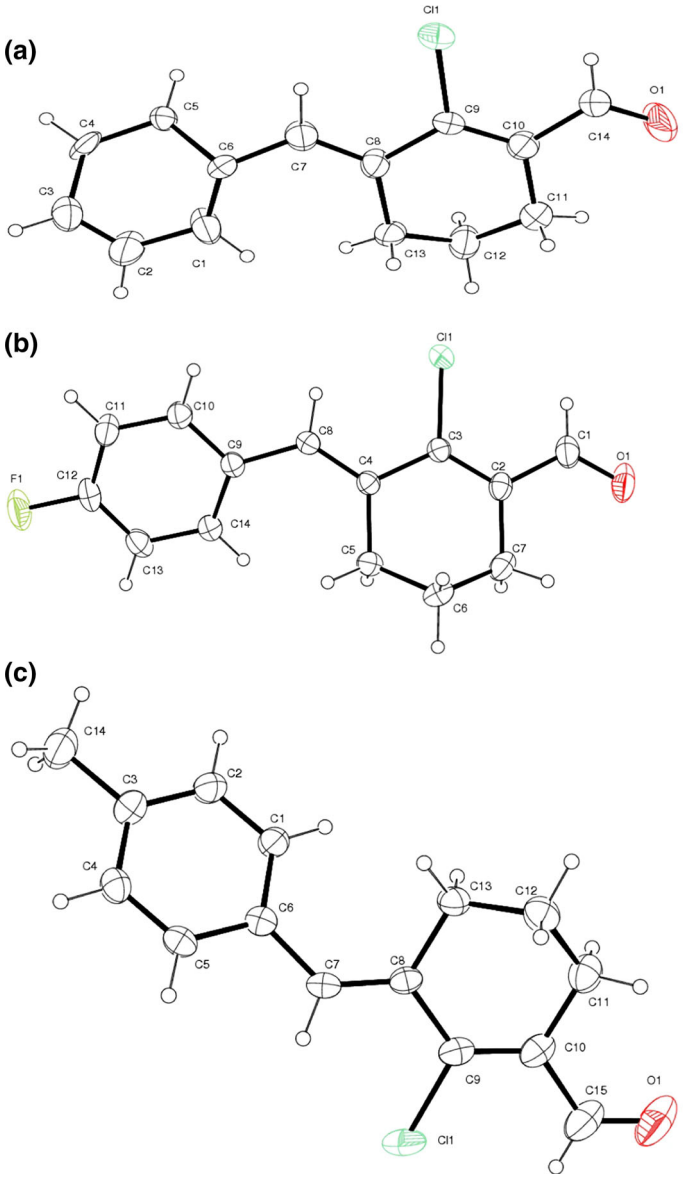


Fig. 3 ORTEP diagrams of **a** (*E*)-3-benzylidene-2-chlorocyclohex-1-enecarbaldehyde (compound **7a**, CCDC 1449251), **b** (*E*)-2-chloro-3-(4-fluorobenzylidene)cyclohex-1-enecarbaldehyde (compound **7b**, CCDC 1405840), and **c** (*E*)-2-chloro-3-(4-methylbenzylidene)cyclohex-1-enecarbaldehyde (compound **7e**, CCDC 1405845)

The structure of synthesized compounds **8a–c** and **9a–g** was confirmed by various spectral techniques such as NMR, mass, and IR data. The IR spectra revealed a band at around 3400–3450 cm^{-1}, corresponding to –OH stretching of the acid group. The band at around $\sim$1669 cm^{-1} corresponds to carbonyl group of

carboxylic acid group. In the ^{1}H NMR spectra, the singlets at around δ 7.56 and δ 7.48 ppm correspond to benzylidene proton of cyclopentyl and cyclohexyl moiety. Singlets observed at around δ 6.74 and 6.84 ppm correspond to thiophene ring proton of cyclopentyl and cyclohexyl moiety, respectively. The multiplet at around δ 3.32–3.29 ppm and δ 2.96–2.93 ppm corresponds to methylene protons of cyclopentyl group, and that at δ 1.78–1.76 ppm and δ 2.73–2.68 to methylene protons of cyclohexyl group. The ^{13}C NMR spectra recorded in DMSO-d_6 showed a signal at δ 163.5 and δ 163.4 ppm, corresponding to carbonyl carbon of the acid group. The signals at δ 34.3 and 27.3 ppm confirmed methylene carbons of cyclopentyl group. The signals at δ 27.2, 25.3, and 23.1 ppm confirmed methylene carbons of cyclohexyl group. Mass spectra acquired in positive and negative ESI mode confirmed the molecular mass of the compounds.

Biology

Antimicrobial activity

Synthesized compounds **8a–c** and **9a–g** were tested for their antimicrobial activity by agar well diffusion method against *S. aureus* (ATCC 29213), methicillin-resistant *S. aureus* (ATCC 33591, NRS 100), *E. coli* (ATCC 25922), and *P. aeruginosa* (ATCC 27853), and *C. albicans* (ATCC 90028) for testing antifungal activity. All synthesized compounds **8a–c** and **9a–g** displayed variable degree of antimicrobial activity against *S. aureus* (ATCC 29213) strain, as indicated in Fig. 4. None of these compounds were active against Gram-negative bacterial strains such as *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) or fungal strain

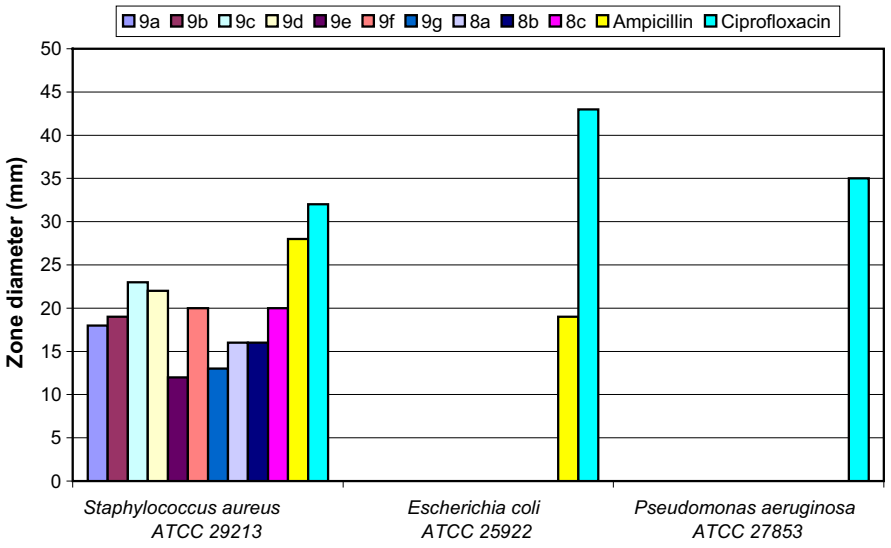


Fig. 4 Sensitivity pattern of bacterial strains to benzylidene derivatives of thiophene-2-carboxylic acid

C. albicans (ATCC 90028). The zones of inhibition (in mm) of the synthesized compounds are summarized in Table 1.

As the synthesized compounds showed marked activity against the *Staphylococcus* strain, further experiments were planned to determine the minimum inhibitory concentration (MIC) for these compounds against methicillin-sensitive and methicillin-resistant *S. aureus* strains. The MIC value of the compounds against the tested bacteria was determined by broth microdilution method, and the values are given in Table 2. The most active compound against ATCC 29213 *S. aureus* strain was the $–CH(CH_3)_2$ derivative (**9f**) with MIC of 16 μg/ml, while the lowest activity was observed for the $–CH_3$ and $–OCH_3$ derivatives (**9e**, **g**) with MIC of 128 μg/ml. Among the halogen substituents, chloro and bromo substitution (**8c**, **9b**–**d**) had beneficial effect in inhibiting growth of ATCC 29213 *S. aureus* strain, with MIC values falling below 64 μg/ml, whereas fluoro substitution of the cyclopentyl derivative (compound **8b**) abolished the antibacterial potency (MIC = 128 μg/ml). The MICs of the compounds against the methicillin-resistant strains (ATCC 33591 and NRS 100) of *S. aureus* were determined according to the same procedure employed for the microbiological assay against the sensitive standard strain (ATCC 29213). The halo-substituted cyclohexyl and cyclopentyl derivatives showed activity against the multidrug-resistant strains of *S. aureus* in the order

Table 1 Zone of inhibition (mm) of arylidene derivatives of 5,6-dihydro-4*H*-cyclopenta[*b*]- and 4,5,6,7-tetrahydrobenzo[*b*]-thiophene-2-carboxylic acid **8a**–**c** and **9a**–**g** (μg/ml)

Compound	*Staphylococcus aureus* ATCC 29213	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*C. albicans* ATCC 90028
	Zone diameter (mm)			
9a	18	NZ	NZ	NZ
9b	19	NZ	NZ	NZ
9c	23	NZ	NZ	NZ
9d	22	NZ	NZ	NZ
9e	12	NZ	NZ	NZ
9f	20	NZ	NZ	NZ
9g	13	NZ	NZ	NZ
8a	16	NZ	NZ	NZ
8b	16	NZ	NZ	NZ
8c	20	NZ	NZ	NZ
Ampicillin	28	19	–	NA
Ciprofloxacin	32	43	35	NA
Fluconazole	NA	NA	NA	25
DMSO	NZ	NZ	NZ	NZ

NZ no zone; controls: ampicillin and ciprofloxacin (standard antibacterial agents), and fluconazole (s-tandard antifungal agent)

Table 2 Antibacterial activity (MIC, μg/ml) of benzylidene derivatives of thiophene-2-carboxylic acid tested in broth microdilution assay

Compound	MSSA	MRSA	
	Staphylococcus aureus ATCC 29213	*Staphylococcus aureus* ATCC 33591	*Staphylococcus aureus* NRS 100
9a	64	64	64
9b	64	64	64
9c	64	32	32
9d	32	32	32
9e	128	128	128
9f	16	32	16
9g	128	128	128
8a	>128	>128	>128
8b	128	128	128
8c	32	32	32
Linezolid	2	2	1
Vancomycin	1	2	1

Br > Cl > F. It was observed that the most active compound against ATCC 33591 and NRS 100 *S. aureus* strains was **9f** with MIC = 16–32 μg/ml. These results are in conformity with those previously determined for the ATCC 29213 strain.

In general, benzylidene substituents chosen in this work increased the antibacterial property of the thiophene carboxylic acid to a larger extent. The presence of the weakly electron-donating isopropyl group attached to the benzylidene moiety (**9f**) exerted a positive influence on the antibacterial activity. However, this influence was not seen with the smaller alkyl group substituent (**9e**). Substitution by the strong electron-donating methoxy group (**9g**) resulted in an analogue that was inactive against the tested *Staphylococcus aureus* strains. On the other hand, weakly deactivating halogens on the benzylidene moiety also had positive influence on the antibacterial activity, with potency decreasing in the order Br > Cl > F. It is clear that electronic and steric effects probably play the most important role in determining the antibacterial activity.

Conclusions

A new series of arylidene derivatives of 5,6-dihydro-4*H*-cyclopenta[*b*]- and 4,5,6,7-tetrahydrobenzo[*b*]-thiophene-2-carboxylic acid (**8a–c**, **9a–g**) were synthesized and characterized by using ^{1}H and ^{13}C NMR, IR, and mass spectroscopy. Antimicrobial evaluation of all the compounds revealed that compounds **9a–d**, **f** and **8c** showed comparable inhibitory active against *S. aureus* of both methicillin-susceptible and methicillin-resistant strains. Thus, the results of this study identify **9f** as the most bioactive compound of this series, since it exhibited excellent bactericidal activities. More importantly, the similar antibacterial spectra seen for both methicillin-

sensitive and methicillin-resistant *S. aureus* strains indicate that this microorganism does not yet have mechanisms of resistance to this class of compounds. Such novel thiophene carboxylic acid analogues could represent an alternative approach for development of drugs for treatment of infections caused by methicillin-resistant *S. aureus* strains.

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