Design, Synthesis and Biological Evaluation of 3-(4-Halophenyl)-3-oxopropanal and Their Derivatives as Novel Antibacterial Agents

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Received January 15, 2010; accepted June 6, 2010; published online June 11, 2010

In continuing our program aimed to search for potent drugs for bacterial infections, a series of 3-(4-halophenyl)-3-oxopropanal and their derivatives were designed, synthesized and their antibacterial activities *in vitro* against both Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* were evaluated. Compounds 7, 8, 13—16, 21 and 22 had moderate antibacterial activities against *Staphylococcus aureus* (minimal inhibitory concentration (MIC) $<16\,\mu g/ml$), suggesting that the introduction of mono-methoxyamine or ethoxyamine moiety might play an important role in determining the potent antibacterial activities. Furthermore, the antibacterial activities of select compounds 7, 15 and 16 against the clinically important pathogenic bacteria-methicillin-resistant *Staphylococcus aureus* (MRSA) were also investigated. Results showed that these compounds exhibited more potent activities than the well-known antibacterial agents Houttuynin and Levofloxacin.

Key words synthesis; 3-(4-halophenyl)-3-oxopropanal; derivative; antibacterial activity; structure–activity relationship analysis

The increasing incidence of bacterial resistance to currently available antibacterial agents is a growing global health problem. Therefore, modern medicine is engaged in an eager quest for new antibiotics, which can treat the infections caused by constantly emerging antibiotic resistant bacteria.[1—3] So far, many efforts have been spent in the search for effective and safe antibacterial drugs, and a large number of naturally occurring and synthetic antibiotics have already been reported. However, most of them are not potent enough to put into practical use due to their weak individual activities or safety concerns. For example, due to undesirable side effects, Grepafloxacin and Ttrovafloxacin were withdrawn from the market and their use is now prohibited in several countries.[4—6] Obviously, this is still needed to search and develop novel antibacterial drugs with better activities together with lower side effects.

Houttuynin, *n*-nonyl-β-oxo-aldehyde (Fig. 1), was originally isolated as one of the main active constituents from *Houttuynina cordata* THUNB, which has been used as antibiotic drug for a long time in China.[7] Recently studies have showed that Houttuyfonate homologues (HOU-C*n*) derived from Houttuynin could improve the immune ability of mice and inhibit the growth of *Staphylococcus aureus* and *Bacillus subtilis* efficiently.[8] In addition, it was worthy noted that, sodium houttuyfonate (Fig. 1), the addition compound of sodium bisulfite and Houttuynin, has been used as an antimicrobial medicine in clinical practice because of better activity and lower side effect than Houttuynin.[9—11] These results indicated that the modification of aldehyde group of Houttuynin might facilitate the antibacterial activity.

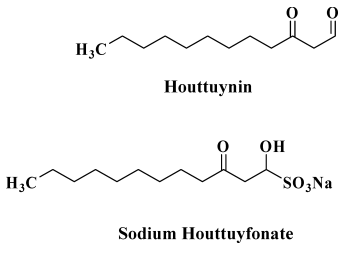

Fig. 1. The Chemical Structure of Houttuynin and Sodium Houttuyfonate

More recently, our group described that 3-(4-alkylphenyl)-3-oxopropane derivatives showed moderate antibacterial activities against Gram-positive pathogen *Staphylococcus aureus* (ATTC-25923). The preliminary structure–activity relationship (SAR) analysis showed that the introduction of appropriate substituent into position 4 of phenyl ring enhanced antibacterial activities of these compounds.[12] In addition, some literatures also reported that the introduction of halogen could significantly increased the antibacterial activity.[13]

Taking advantage of above information, in the present investigation, a series of 3-(4-halophenyl)-3-oxopropanal and their derivatives were designed, synthesized and their antibacterial activities against Gram-negative and Gram-positive bacteria *in vitro* were evaluated. Furthermore, the SAR was also discussed. The purpose of this study was to investigate the antibacterial effect of 3-(4-halophenyl)-3-oxopropanal derivatives, with the ultimate aim of developing novel potent antibacterial drugs with better activities and lower side effects.

Experimental

Chemistry Most chemical reagents were purchased from Darui Chemical Co. (Shanghai, China), some 1-(4-halophenyl)ethanones were preparation by the Friedel–Crafts reaction according to the methods described by Wang and Liang[14] tetrahydrofuran (THF) and toluene were dried over molecular sieve. The other commercially available reagents and solvents were used without further purification. All reactions were monitored by TLC (Merck Kieselgel 60, F254). Melting points (mp) were determined with Shanghai Precision & Scientific Instrument WRS-1B melting point apparatus and are uncorrected. NMR spectra were recorded on Varian Mercury-Plus300 spectrometers at 25 °C using $CDCl_3$ or DMSO-d_6 as a solvent. All chemical shifts (δ) are quoted in ppm downfield from tetramethylsilicane (TMS) and coupling constants (J) are given in Hz. Abbreviations used in the splitting pattern were an follows: s=singlet, d=doublet, t=triplet, q=quintet, m=multiplet, b=broad. Infrared (IR) spectra were recorded on a Perkin-Elmer Infracord 221 spectrometer and reported in cm^{-1}. LC-MS spectra

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were recorded using the Shimadzu LCMS-2010A. Elemental analyses were performed on a Vario EL instrument.

Synthesis. General Procedures for 3-(4-Halophenyl)-3-oxopropane (1—3)[15] 1-(4-Halophenyl)ethanone (0.05 mol) was dissolved in dry THF or toluene, and stirred in the ice bath. Sodium (0.075 mol) was added to the solution, the mixture was stirred for 30 min in the ice bath. Ethyl formate was then added dropwise to the reaction mixture, which was maintained at <5 °C, after the addition, the reaction mixture was stirred for another 2 h. The reaction mixture was warmed to room temperature and stirred overnight (about 15 h). Water (150 ml) was added to the slurry mixture, and the reaction was stirred for an additional 30 min and then portioned between organic layer and water. Water layer was extracted with two 50 ml portions of dichloromethane. These extracts were discarded. The aqueous phase was acidified with acetic acid or 5% hydrochloric acid, and extracted with dichloromethane (3×50 ml). This extract was washed with water and brine, dried over anhydrous sodium sulfate, and the solvent was removed by rotary evaporation under reduced pressure to give thick yellow oil, which was purified by crystallization from hexane to afford the 3-(4-halophenyl)-3-oxopropane or by chromatography on silica gel to give product.

3-(4-Fluorophenyl)-3-oxopropanal (Enolic, **1**): Yield 76%, yellow solid, mp 53—54 °C; IR (KBr): 1679, 1599, 1232, 845; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.20 (d, 1H, J=4.5 Hz, =CH), 7.92 (d, 2H, J=7.6 Hz, PhH), 7.17 (d, 2H, J=7.6 Hz, PhH), 6.18 (d, 1H, J=4.5 Hz, =CH); ^{13}C-NMR(75 MHz, $CDCl_3$) δ: 188.4, 172.2, 153.6, 132.2, 129.9, 114.8, 96.6; electrospray ionization-mass spectrometry (ESI-MS) *m/z*: 167 (M+1); *Anal.* Calcd for $C_9H_7FO_2$: C, 65.06; H, 4.25. Found: C, 65.23; H, 4.11.

3-(4-Bromophenyl)-3-oxopropanal (Enolic, **2**): Yield 64%, yellow solid, mp 79—80 °C; IR (KBr): 1665, 1585, 837; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.27 (d, 1H, J=4.2 Hz, =CH), 7.78 (d, 2H, J=7.6 Hz, PhH), 7.62 (d, 2H, J=7.6 Hz, PhH), 6.19 (d, 1H, J=4.2 Hz, =CH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 189.5, 172.6, 135.2, 132.2, 129.7, 127.8, 97.9; ESI-MS *m/z*: 228 (M+1); *Anal.* Calcd for $C_9H_7BrO_2$: C, 47.61; H, 3.11. Found: C, 47.96; H, 3.43.

3-(4-Iodophenyl)-3-oxopropanal (Enolic, **3**): Yield 58%, yellow oil; IR (KBr): 1675, 1591, 839; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.29 (d, 1H, J=4.2 Hz, =CH), 7.83 (d, 2H, J=8.4 Hz, PhH), 7.67 (d, 2H, J=8.4 Hz, PhH), 6.21 (d, 1H, J=4.2 Hz, =CH); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 189.5, 172.6, 138.2, 135.8, 129.5, 102.1, 98.5; ESI-MS *m/z*: 275 (M+1); *Anal.* Calcd for $C_9H_7IO_2$: C, 39.44; H, 2.57. Found: C, 39.63; H, 2.61.

General Procedure for the Preparation of Sodium 1-Hydroxy-3-(4-halophenyl)-3-oxopropane-1-sulfonate (4—6) 1-(4-Halophenyl)ethanone (0.05 mol) was dissolved in dry THF or toluene, and stirred in the ice bath. Sodium (0.075 mol) was added to the solution, the mixture was stirred for 30 min in the ice bath. Ethyl formate was then added dropwise to the reaction mixture, which was maintained at <5 °C, after the addition, the reaction mixture was stirred for another 2 h. The reaction mixture was warmed to room temperature and stirred overnight (about 15 h). The precipitates was separated by filtration, the corresponding enolic sodium was obtained. The treatment of enolic sodium with saturated sodium bisulfite and acetic acid at room temperature for 2 h, the precipitates was separated by filtration, which was purified by crystallization from 50% ethanol to afford the sodium 1-hydroxy-3-(4-halophenyl)-3-oxopropane-1-sulfonate.

Sodium 1-Hydroxy-3-(4-fluorophenyl)-3-oxopropane-1-sulfonate (**4**): Yield 56%; yellow solid, mp 190—192 °C; IR (KBr): 3239, 1679, 1600, 1260, 807; ^{1}H-NMR (300 MHz, D_2O) δ: 7.91 (d, 2H, J=8.4 Hz, PhH), 7.68 (d, 2H, J=8.4 Hz, PhH), 5.26 (t, 1H, J=4.2 Hz, CH), 3.40 (d, 2H, J=4.2 Hz, CH_2); ^{13}C-NMR (75 MHz, D_2O) δ: 192.4, 144.6, 132.7, 131.2, 116.3, 81.4, 39.8; ESI-MS *m/z*: 247 (M−23); *Anal.* Calcd for $C_9H_8FNaO_5S$: C, 40.00; H, 2.98. Found: C, 40.32; H, 3.16.

Sodium 1-Hydroxy-3-(4-bromophenyl)-3-oxopropane-1-sulfonate (**5**): Yield 33%; yellow solid, mp 204—205 °C; IR (KBr): 3226, 2913, 1679, 1582, 1402, 820; ^{1}H-NMR (300 MHz, D_2O) δ: 7.85 (d, 2H, J=8.7 Hz, PhH), 7.73 (d, 2H, J=8.7 Hz, PhH), 5.71 (t, 1H, J=4.2 Hz, CH), 3.27 (d, 2H, J=4.2 Hz, CH_2); ^{13}C-NMR (75 MHz, D_2O) δ: 192.8, 135.2, 132.3, 130.1, 129.5, 81.2, 39.6; ESI-MS *m/z*: 308 (M−23); *Anal.* Calcd for $C_9H_8BrNaO_5S$: C, 32.65; H, 2.44. Found: C, 32.84; H, 2.81.

Sodium 1-Hydroxy-3-(4-iodophenyl)-3-oxopropane-1-sulfonate (**6**): Yield 45%; yellow solid, mp 238—239 °C; IR (KBr): 3235, 2912, 1678, 1589, 815; ^{1}H-NMR (300 MHz, D_2O) δ: 7.74 (d, 2H, J=7.8 Hz, PhH), 7.68 (d, 2H, J=7.8 Hz, PhH), 5.42 (t, 1H, J=4.5 Hz, CH), 3.18 (d, 2H, J=4.5 Hz, CH_2); ^{13}C-NMR (75 MHz, D_2O) δ: 193.2, 138.2, 135.4, 130.0, 102.1, 81.2, 36.9; ESI-MS *m/z*: 355 (M−23); *Anal.* Calcd for $C_9H_8INaO_5S$: C, 28.59; H, 2.13. Found: C, 28.22; H, 2.43.

General Procedure for the Preparation of Schiff Bases of 3-(4-Halophenyl)-3-oxopropanal (7—24)[16—18] A Procedure: The corresponding 3-(4-halophenyl)-3-oxopropanal (5 mmol) was dissolved in anhydrous ethanol (10 ml). Semicarbazide, thiosemicarbazide (5 mmol) was added to the solution. The mixture was stirred for 5—8 h under refluxing conditions. The reaction mixture was cooled to room temperature, the precipitate was separated by filtration.

B Procedure: The corresponding 3-(4-halophenyl)-3-oxopropanal (5 mmol) was dissolved in anhydrous ethanol (10 ml), methoxyamine or ethoxyamine (5 mmol or 10 mmol) was added to the reaction mixture. The reaction carried out at room temperature for 6—9 h, and the reaction process was monitored by TLC. After completion of the reaction, the reaction mixture was concentrated *in vacuo*, diluted with dichloromethane, washed with water, dried over anhydrous sodium sulfate, concentrated *in vacuo*. The residue was workup by chromatography on silica gel to give product.

3-(4-Fluorophenyl)-3-oxopropanal Oxime-methyl Ether (**7**): Yield 80%; yellow solid, mp 65—67 °C; IR (KBr) ν_{max}, cm^{-1}: 2947, 2911, 1669, 1598, 1509, 1215, 829; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.01 (d, 2H, J=8.4 Hz, PhH), 7.66 (t, 1H, J=6.0 Hz, CH), 7.18 (d, 2H, J=8.4 Hz, PhH), 4.01 (dd, 2H, J=6.0, 6.9 Hz, CH_2), 3.87 (s, 3H, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 194.2, 164.5, 144.9, 132.7, 131.2, 116.3, 61.9, 39.4; ESI-MS *m/z*: 196 (M+1); *Anal.* Calcd for $C_{10}H_{10}FNO_2$: C, 61.53; H, 5.16; N, 7.18. Found: C, 61.59; H, 5.51; N, 7.57.

3-(4-Fluorophenyl)-3-oxopropanal Oxime-ethyl Ether (**8**): Yield 68%; yellow solid, mp 55—56 °C; IR (KBr) ν_{max}, cm^{-1}: 2983, 2892, 1676, 1597, 1390, 1050, 830; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.01 (d, 2H, J=8.5 Hz, PhH), 7.66 (t, 1H, J=4.8 Hz, CH), 7.17 (d, 2H, J=8.5 Hz, PhH), 4.20—4.10 (m, 2H, CH_2), 4.01 (dd, 2H, J=4.8, 6.0 Hz, CH_2), 1.31(t, 3H, J=6.6 Hz, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 194.2, 164.4, 144.5, 132.8, 131.2, 116.2, 69.7, 39.6, 14.8; ESI-MS *m/z*: 210 (M+1); *Anal.* Calcd for $C_{11}H_{12}FNO_2$: C, 63.15; H, 5.78; N, 6.69. Found: C, 62.89; H, 5.63; N, 6.92.

3-(4-Fluorophenyl)-3-oxopropanal Semicarbazone (**9**): Yield 80%; yellow solid, mp 166—167 °C; IR (KBr) ν_{max}, cm^{-1}: 3342, 3336, 1685, 1583, 1451, 1068, 825; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.59 (bs, 1H, NH), 8.06 (d, 2H, J=6.8 Hz, PhH), 7.85 (bs, 2H, NH_2), 7.67 (t, 1H, J=5.4 Hz, CH), 7.33 (d, 2H, J=6.8 Hz, PhH), 3.95 (d, 2H, J=5.4 Hz, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 194.3, 158.2, 154.6, 144.3, 132.6, 130.8, 115.7, 35.8; ESI-MS *m/z*: 224 (M+1); *Anal.* Calcd for $C_{10}H_{10}FN_3O_2$: C, 53.81; H, 4.52; N, 18.83. Found: C, 53.89; H, 5.13; N, 18.92.

3-(4-Fluorophenyl)-3-oxopropanal Thiosemicarbazone (**10**): Yield 82%; yellow solid, mp 176—178 °C; IR (KBr) ν_{max}, cm^{-1}: 3439, 3260, 1674, 1585, 1408, 827; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.29 (bs, 1H, NH), 8.07 (d, 2H, J=8.4 Hz, PhH), 7.85 (bs, 2H, NH_2), 7.67 (t, 1H, J=5.7 Hz, CH), 7.35 (d, 2H, J=8.4 Hz, PhH), 4.04 (d, 2H, J=5.7 Hz, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 195.9, 178.5, 155.6, 142.3, 133.5, 131.8, 116.4, 42.8; ESI-MS *m/z*: 240 (M+1); *Anal.* Calcd for $C_{10}H_{10}FN_3OS$: C, 50.20; H, 4.21; N, 17.56. Found: C, 50.56; H, 4.08; N, 17.72.

3-(4-Fluorophenyl)-3-oxopropanal Dioxime-methyl Ether (**11**): Yield 56%; yellow oil; IR (KBr) ν_{max}, cm^{-1}: 2940, 2903, 1605, 1512, 1429, 840; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.67 (d, 2H, J=8.7 Hz, PhH), 7.66 (t, 1H, J=5.2 Hz, CH), 7.08 (d, 2H, J=8.7 Hz, PhH), 4.01 (s, 3H, CH_3), 3.93 (s, 3H, CH_3), 3.76 (dd, 2H, J=5.2, 6.2 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 158.3, 153.2, 146.0, 128.5, 127.6, 116.6, 62.4, 62.3, 30.6; ESI-MS *m/z*: 225 (M+1); *Anal.* Calcd for $C_{11}H_{13}FN_2O_2$: C, 58.92; H, 5.84; N, 12.49. Found: C, 58.87; H, 5.62; N, 12.58.

3-(4-Fluorophenyl)-3-oxopropanal Dioxime-ethyl Ether (**12**): Yield 62%; yellow oil; IR (KBr) ν_{max}, cm^{-1}: 2987, 2891, 1592, 1384, 1051, 832; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.68 (d, 2H, J=8.4 Hz, PhH), 7.46 (t, 1H, J=5.2 Hz, CH), 7.07 (d, 2H, J=8.4 Hz, PhH), 4.29—4.09 (m, 4H, $2CH_2$), 3.78 (dd, 2H, J=5.2, 6.1 Hz, CH_2), 1.21—1.36 (m, 6H, $2CH_3$); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 162.7, 152.3, 145.5, 128.5, 128.2, 115.8, 70.4, 69.5, 28.8, 15.1, 14.8; ESI-MS *m/z*: 253 (M+1); *Anal.* Calcd for $C_{13}H_{17}FN_2O_2$: C, 61.89; H, 6.79; N, 11.10. Found: C, 61.97; H, 6.62; N, 11.23.

3-(4-Chlorophenyl)-3-oxopropanal Oxime-methyl Ether (**13**): Yield 52%; yellow oil; IR (KBr) ν_{max}, cm^{-1}: 2971, 2884, 1670, 1584, 1346, 1052, 819; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.68 (d, 2H, J=8.4 Hz, PhH), 7.49 (d, 2H, J=8.4 Hz, PhH), 7.40 (t, 1H, J=4.8 Hz, CH), 3.94 (s, 3H, CH_3), 3.69 (dd, 2H, J=4.8,6.5 Hz, CH_2); ESI-MS *m/z*: 212 (M+1); *Anal.* Calcd for $C_{10}H_{10}ClNO_2$: C, 56.75; H, 4.76; N, 6.62. Found: C, 56.53; H, 4.63; N, 6.45.

3-(4-Chlorophenyl)-3-oxopropanal Oxime-ethyl Ether (**14**): Yield 56%; yellow crystals, mp 94—95 °C; IR (KBr) ν_{max}, cm^{-1}: 2936, 2815, 1718, 1597, 1484, 1394, 1052, 832; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.99 (d, 2H, J=8.4 Hz, PhH), 7.61 (d, 2H, J=8.4 Hz, PhH), 7.54 (t, 1H, J=4.3 Hz, CH), 4.10 (q, 2H, J=7.0 Hz, CH_2), 4.08 (dd, 2H, J=4.3, 6.0 Hz, CH_2), 1.20 (t, 3H, J=7.0 Hz, CH_3); ESI-MS *m/z*: 226 (M+1); *Anal.* Calcd for $C_{11}H_{12}ClNO_2$: C, 58.54; H, 5.36; N, 6.21. Found: C, 58.50; H, 5.24; N, 6.35.

3-(4-Bromophenyl)-3-oxopropanal Oxime-methyl Ether (**15**): Yield 80%; yellow solid, mp 68—70 °C; IR (KBr) ν_{max}, cm^{-1}: 2935, 1674, 1579, 1391, 1042, 807; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.82 (d, 2H, J=7.8 Hz, PhH), 7.63 (d, 2H, J=7.8 Hz, PhH), 7.19 (t, 1H, J=5.0 Hz, CH), 3.63 (dd, 2H, J=5.0, 6.2 Hz, CH_2), 3.93 (s, 3H, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 192.7, 142.3, 133.8, 131.0, 128.7, 127.9, 61.0, 34.1; ESI-MS *m/z*: 257 (M+1); *Anal.* Calcd for $C_{10}H_{10}BrNO_2$: C, 46.90; H, 3.94; N, 5.47. Found: C, 47.22; H, 4.28; N, 5.41.

3-(4-Bromophenyl)-3-oxopropanal Oxime-ethyl Ether (**16**): Yield 69%; yellow solid, mp 68—69 °C; IR (KBr) ν_{max}, cm^{-1}: 2978, 2891, 1673, 1579, 1391, 1059, 813; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.84 (d, 2H, J=8.4 Hz, PhH), 7.60 (d, 2H, J=8.4 Hz, PhH), 7.18 (t, 1H, J=4.9 Hz, CH), 4.21—4.12 (m, 2H, CH_2), 4.00 (dd, 2H, J=4.9, 6.0 Hz, CH_2), 1.30 (t, 3H, J=7.6 Hz, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 194.8, 144.3, 135.0, 132.3, 130.0, 129.1, 69.7, 39.7, 14.8; ESI-MS *m/z*: 271 (M+1); *Anal.* Calcd for $C_{11}H_{12}BrNO_2$: C, 48.91; H, 4.48; N, 5.19. Found: C, 48.98; H, 4.56; N, 5.02.

3-(4-Bromophenyl)-3-oxopropanal Semicarbazone (**17**): Yield 49%; yellow solid, mp 170—172 °C; IR (KBr) ν_{max}, cm^{-1}: 3440, 3339, 1686, 1589, 1455, 1070, 821; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 9.96 (bs, 1H, NH), 7.89 (d, 2H, J=7.4 Hz, PhH), 7.51 (bs, H, NH_2) 7.32 (t, 1H, J=5.4 Hz, CH), 7.26 (d, 2H, J=7.4 Hz, PhH), 6.24 (s, H, NH_2), 3.92 (d, J=5.4 Hz, 2H, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 194.7, 157.2, 154.7, 136.3, 132.1, 130.9, 127.6, 36.8; ESI-MS *m/z*: 285 (M+1); *Anal.* Calcd for $C_{10}H_{10}BrN_3O_2$: C, 42.27; H, 3.55; N, 14.79. Found: C, 42.36; H, 3.68; N, 14.85.

3-(4-Bromophenyl)-3-oxopropanal Thiosemicarbazone (**18**): Yield 72%; yellow solid, mp 182—183 °C; IR (KBr) ν_{max}, cm^{-1}: 3369, 3266, 1675, 1644, 1582, 1161, 800; ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 11.2 (bs, 1H, NH), 7.92 (d, 2H, J=7.1 Hz, PhH), 7.66 (d, 2H, J=7.1 Hz, PhH), 7.60 (bs, H, NH_2), 7.59 (t, 1H, J=5.7 Hz, CH), 7.55 (bs, H, NH_2), 3.98 (d, 2H, J=5.7 Hz, CH_2); ^{13}C-NMR (75 MHz, DMSO-d_6) δ: 196.6, 178.5, 154.6, 142.1, 132.5, 130.8, 128.4, 42.8; ESI-MS *m/z*: 301 (M+1); *Anal.* Calcd for $C_{10}H_{10}BrN_3OS$: C, 40.01; H, 3.36; N, 14.00. Found: C, 40.41; H, 3.42; N, 14.15.

3-(4-Bromophenyl)-3-oxopropanal Dioxime-methyl Ether (**19**): Yield 58%; yellow oil; IR (KBr) ν_{max}, cm^{-1}: 2937, 2901, 1591, 1427, 1049, 828; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.57 (d, 2H, J=8.1 Hz, PhH), 7.49 (d, 2H, J=8.1 Hz, PhH), 7.43 (t, 1H, J=4.8 Hz, CH), 4.00 (ds, 6H, $2CH_3$), 3.74 (d, 2H, J=4.8, 6.1 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 152.5, 145.2, 134.1, 131.8, 128.1, 123.9, 62.7, 61.8, 28.2; ESI-MS *m/z*: 286 (M+1); *Anal.* Calcd for $C_{11}H_{13}BrN_2O_2$: C, 46.33; H, 4.60; N, 9.82. Found: C, 46.45; H, 4.68; N, 9.66.

3-(4-Bromophenyl)-3-oxopropanal Dioxime-ethyl Ether (**20**): Yield 56%; yellow oil; IR (KBr) ν_{max}, cm^{-1}: 2985, 2893, 1590, 1381, 1055, 831; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.51 (d, 2H, J=7.8 Hz, PhH), 7.06 (d, 2H, J=7.8 Hz, PhH), 6.89 (t, 1H, J=5.0 Hz, CH), 4.25—4.10 (m, 4H, $2CH_2$), 3.96 (dd, 2H, J=5.0, 6.2 Hz, CH_2), 1.38—1.22 (m, 6H, $2CH_3$); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 156.9, 145.9, 133.8, 132.0, 128.1, 123.7, 69.7, 69.2, 28.1, 14.9, 14.3; ESI-MS *m/z*: 314 (M+1); *Anal.* Calcd for $C_{13}H_{17}BrN_2O_2$: C, 49.85; H, 5.47; N, 8.94. Found: C, 50.17; H, 5.91; N, 8.71.

3-(4-Iodophenyl)-3-oxopropanal Oxime-methyl Ether (**21**): Yield 59%; yellow solid, mp 103—104 °C; IR (KBr) ν_{max}, cm^{-1}: 2929, 1673, 1575, 1389, 1044, 800; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.86 (d, 2H, J=7.8 Hz, PhH), 7.69 (d, 2H, J=7.8 Hz, PhH), 7.63 (t, 1H, J=4.8 Hz, CH), 4.00 (dd, 2H, J=4.8, 6.0 Hz, CH_2), 3.93 (s, 3H, CH_3); ^{13}C-NMR(75 MHz, $CDCl_3$) δ: 194.6, 143.5, 138.4, 135.6, 129.8, 102.0, 62.2, 35.3; ESI-MS *m/z*: 304 (M+1); *Anal.* Calcd for $C_{10}H_{10}INO_2$: C, 39.63; H, 3.33; N, 4.62. Found: C, 39.71; H, 3.38; N, 4.55.

3-(4-Iodophenyl)-3-oxopropanal Oxime-ethyl Ether (**22**): Yield 61%; yellow solid, mp 105—106 °C; IR (KBr) ν_{max}, cm^{-1}: 2973, 2936, 1672, 1580, 1391, 1052, 809; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.86 (d, 2H, J=8.1 Hz, PhH), 7.70 (d, 2H, J=8.1 Hz, PhH), 7.63 (t, 1H, J=4.9 Hz, CH), 4.26—4.03 (m, 2H, CH_2), 3.99 (dd, 2H, J=4.9, 6.0 Hz, CH_2), 1.30 (t, 3H, J=6.9 Hz, CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 195.1, 144.3, 138.3, 135.5, 129.8, 102.0, 69.7, 39.6, 14.9; ESI-MS *m/z*: 318 (M+1); *Anal.* Calcd for $C_{11}H_{12}INO_2$: C, 41.66; H, 3.81; N, 4.42. Found: C, 41.57; H, 3.82; N, 4.28.

3-(4-Iodophenyl)-3-oxopropanal Dioxime-methyl Ether (**23**): Yield 54%; yellow oil; IR (KBr) ν_{max}, cm^{-1}: 2937, 2899, 2818, 1683, 1586, 1329, 1047, 827; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.71 (d, 2H, J=8.1 Hz, PhH), 7.43 (d, 2H, J=8.1 Hz, PhH), 7.37, 6.67 (t, 1H, J=5.0 Hz, CH), 4.01 (s, 3H, CH_3), 3.92 (s, 3H, CH_3), 3.73 (dd, 2H, J=5.0, 6.1 Hz, CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 152.7, 145.3, 137.8, 134.7, 128.2, 95.9, 62.7, 61.8, 28.1; ESI-MS *m/z*: 333 (M+1); *Anal.* Calcd for $C_{11}H_{13}IN_2O_2$: C, 39.78; H, 3.95; N, 8.43. Found: C, 40.12; H, 4.29; N, 8.22.

3-(4-Iodophenyl)-3-oxopropanal Dioxime-ethyl Ether (**24**): Yield 51%; yellow oil; IR (KBr) ν_{max}, cm^{-1}: 2971, 2927, 1671, 1580, 1406, 1054, 824; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 7.70 (d, 2H, J=7.8 Hz, PhH), 7.43 (d, 2H, J=7.8 Hz, PhH), 7.40 (t, 1H, J=4.9 Hz, CH), 4.29—4.13 (m, 4H, $2CH_2$), 3.76 (dd, 2H, J=4.9, 6.0 Hz, CH_2), 1.35—1.21 (m, 6H, $2CH_3$); ^{13}C-NMR (75 MHz, $CDCl_3$) δ: 155.2, 144.9, 138.1, 135.2, 129.6, 98.0, 69.7, 69.2, 28.8, 14.9, 14.5; ESI-MS *m/z*: 361 (M+1); *Anal.* Calcd for $C_{13}H_{17}IN_2O_2$: C, 43.35; H, 4.76; N, 7.78. Found: C, 43.68; H, 4.97; N, 7.43.

***In Vitro* Antibacterial Tests** All of the compounds were evaluated for their antibacterial activity using conventional agar-dilution method. Twofold serial dilutions of the compounds and reference drugs were prepared in Mueller–Hinton agar. Drugs (6.4 mg) were dissolved in dimethylsulfoxide (DMSO, 1 ml) and the solution was diluted with water (9 ml) without precipitation. Further progressive double dilution with melted Mueller–Hinton agar was performed to obtain the required concentrations of 4, 2, 1, 0.5, 0.25, 0.125, 0.06, 0.03, and 0.015 mg/ml. Petri dishes were incubated with 1—5×10^4 colony forming units (cfu) and incubated at 37 °C for 18 h. The minimal inhibitory concentration (MIC) was the lowest concentration of the test compound, which resulted in no visible growth on the plate. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment. For the minimal bactericidal concentration assay (MBC) assay, aliquots (10 μl) of each culture without visible growth were transferred to 90 μl of Mueller–Hinton broth. Incubation, reading and interpretation were performed according to MIC specifications.[19]

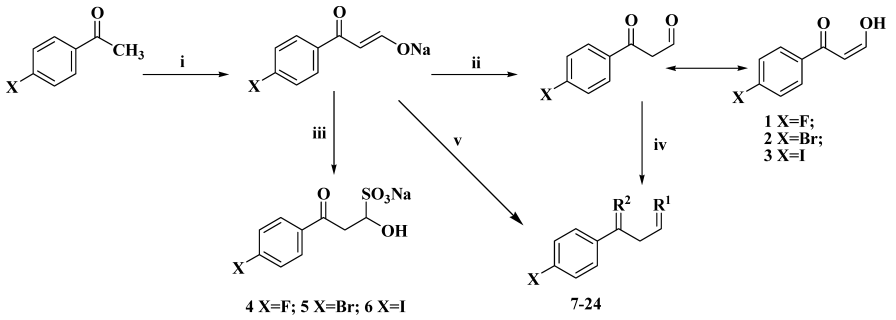
Results and Discussion

Synthetic Chemistry The synthesis of of 3-(4-halophenyl)-3-oxopropanal and their derivatives are shown in Chart 1. In brief, the reaction of 4′-haloacetophenone with 2.0 eq of ethyl formate and 1.5 eq of metal sodium in dry toluene gave the corresponding intermediate sodium salt of 1-(4-halophenyl)-3-hydroxyprop-2-en-1-one (Chart 1-i). This intermediate sodium salt was acidified with 5% hydrochloric acid or acetic acid to give the 3-(4-halophenyl)-3-oxopropanal (**1**—**3**) in 62—78% overall yield (Chart 1-ii).[15] The corresponding sodium salt was treated with saturated sodium bisulfite at room temperature to give sodium 1-hydroxy-3-(4-halophenyl)-3-oxopropane-1-sulfonate (**4**—**6**) (Chart 1-iii). Reaction of 3-(4-halophenyl)-3-oxopropanal (**1**—**3**) with methoxyamine, ethoxyamine, semicarbazide, and thiosemicarbazide, respectively, in EtOH gave the corresponding compound (**7**—**24**) in 71—86% overall yield (Chart 1-iv).[16—18]

Biology The antibacterial activities *in vitro* of all the obtained compounds were tested against both Gram-positive bacteria *Staphylococcus aureus* (ATTC-25923) and Gram-negative bacteria *Escherichia coli* (ATTC-25922) and *Pseudomonas aeruginosa* (ATTC-27853). It was reported as the minimum inhibitory concentration (MIC) in μg/ml that was determined by the agar dilution method as recommended by the NCCLS (National Committee for Clinical Laboratory Standards).[19] The obtained MIC values, together with the data of the reference antibacterial agents Levofloxacin, Clarithromycin and Houttuynin for comparison are summarized in Table 1.

As shown in Table 1, Compounds **7**, **8**, **13**—**16**, **21** and **22** had moderate antibacterial activities against *Staphylococcus aureus* (MIC$<$16 μg/ml). Especially, compound **21** was found to be most active antibacterial agent with MIC of 1.0 μg/ml against *Staphylococcus aureus*. However, of all the synthesized samples, only compounds **15** and **21** showed the weak and same activities against *Escherichia coli* and *Pseudomonas aeruginosa* (MIC=64 μg/ml). The results indicated that Gram-positive bacteria were more sensitive to newly prepared compounds than Gram-negative bacteria.

From the results of the antibacterial activity of the synthe-



7. X=F, R^1=$NOCH_3$, R^2=O; 8. X=F, R^1=NOEt, R^2=O;
9. X=F, R^1=$NNHCONH_2$, R^2=O; 10. X=F, R^1=$NNHCSNH_2$, R^2=O;
11. X=F, R^1=R^2=$NOCH_3$; 12. X=F, R^1=R_2=NOEt;
13. X=Cl, R^1=$NOCH_3$, R^2=O; 14. X=Cl, R^1=NOEt, R^2=O
15. X=Br, R^1=$NOCH_3$, R^2=O; 16. X=Br, R^1=NOEt, R^2=O;
17. X=Br, R^1=$NNHCONH_2$, R^2=O; 18. X=Br, R^1=$NNHCSNH_2$, R^2=O;
19. X=Br, R^1=R^2=$NOCH_3$; 20. X=Br, R^1=R^2=NOEt;
21. X=I, R^1=$NOCH_3$, R^2=O; 22. X=I, R^1=NOEt, R^2=O;
23. X=I, R^1=R^2=$NOCH_3$; 24. X=I, R^1=R^2=NOEt

Reagents: (i) ethyl formate/Na/toluene, (ii) 5% HCl or acetic acid, (iii) saturating sodium bisulfite solution, (iv) $NH_2NHCSNH_2$, $NH_2NHCONH_2$, NH_2OCH_3, $NH_2OCH_2CH_3$/EtOH, (v) NH_2OCH_3·HCl or $NH_2OCH_2CH_3$·HCl/EtOH.

Chart 1. Synthesis of 3-(4-Halophenyl)-3-oxopropanal and Their Derivatives

Table 1. Antibacterial Activity of 3-(4-Halophenyl)-3-oxopropanal and Their Derivatives in Comparison with Clarithromycin, Levofloxacin and Houttuynine

Compds.	MIC (μg/ml)		
	Staphylococcal aureus (ATTC-25923)	*Escherichia coli* (ATTC-25922)	*Pseudomonas aeruginosa* (ATTC-27853)
1	>64	>64	>64
2	>64	>64	>64
3	>64	>64	>64
4	>64	>64	>64
5	>64	>64	>64
6	>64	>64	>64
7	16	>64	>64
8	8	>64	>64
9	>64	>64	>64
10	>64	>64	>64
11	>64	>64	>64
12	>64	>64	>64
13	8	>64	>64
14	8	>64	>64
15	2	64	64
16	8	>64	>64
17	>64	>64	>64
18	>64	>64	>64
19	64	>64	>64
20	>64	>64	>64
21	1	64	64
22	4	>64	>64
23	32	>64	>64
24	64	>64	>64
Clarithromycin	0.12	32	>64
Levofloxacin	0.25	0.025	0.5
Houttuynin	32	—	—

sized 3-(4-halophenyl)-3-oxopropanal and their derivatives, the following SAR could be derived:

(1) In general, it was observed that most of the compounds with halogen substituted phenyl rings at position 4 showed better antibacterial activity than the ones with a non-substituted phenyl ring.[12] This further supported our previous conclusion which reported that the introduction of appropriate substituents into position 4 of phenyl ring enhanced antibacterial activities of these compounds.

(2) Compounds **21**—**24**, having iodine substitution at position 4 of phenyl ring, showed more profound antibacterial activity than other halogen substituted homologous ones. The results suggested that the antibacterial activity of 3-(4-halophenyl)-3-oxopropanal and their derivatives might be associated with the electronic character of the halogen atom on the 4-position of benzene. In the present investigation, the halogen substitution for improving the antibacterial activity strength followed the order: I>Br>Cl>F.

(3) Among all the compounds investigated, the oxime-methyl ether (**7**, **13**, **15**, **21**) and oxime-ethyl ether derivatives (**8**, **14**, **16**, **22**) exhibited potent antibacterial activities with MIC values ranging from 1 to 16 μg/ml. However, sodium 1-hydroxy-3-(4-halophenyl)-3-oxopropane-1-sulfonates (**4**—**6**) and the semicarbazones and thiosemicarbazone derivatives failed to exhibit the antibacterial activity at the concentration of 64 μg/ml. It implicated that the *O*-methyl and *O*-ethyl oxime moiety might play an important role in improving the potent antibacterial activities.

(4) When an additional *O*-methyl or *O*-ethyl oxime moiety was introduced to the active compounds **7**, **8**, **13**—**16**, **21** and **22**, the obtained corresponding dioxime-methyl ether or ethyl ether derivatives were nearly inactive at the concentration of 16 μg/ml. The results suggested that the presence of ketone carbonyl moiety might be absolutely necessarily for determining the antibacterial activity of these compounds. The above SAR results were summarized in Fig. 2.

Furthermore, three compounds (**7**, **15**, **16**) were selected to test the antibacterial activities *in vitro* against the clinically important pathogenic bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA) and the results were summarized in Tables 2 and 3. As shown in Table 2, compounds **7**, **15** and **16** displayed more potent antibacterial activities against MRSA than the Houttuynin and Levofloxacin, suggesting that further development of such compounds may be of interest.

Table 2. MIC of Compounds **7**, **15** and **16** against Strains of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Compds.	MIC (μg/ml)					MIC_{50}	MIC_{90}
	MRSA1	MRSA2	MRSA3	MRSA4	MRSA5		
7	8	8	8	8	4	8	8
15	4	8	8	4	8	8	8
16	8	8	8	4	8	8	16
Levofloxacin	8	8	16	16	>16	16	>16
Houttuynin	16	16	16	16	16	16	16

Table 3. MBC of Compounds **7**, **15** and **16** against Strains of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Compds.	MBC (μg/ml)					MIC_{50}	MIC_{90}
	MRSA1	MRSA2	MRSA3	MRSA4	MRSA5		
7	512	>512	512	>512	512	512	>512
15	128	128	128	256	128	128	128
16	64	128	256	128	64	128	256
Levofloxacin	>16	>16	>16	>16	>16	>16	>16
Houttuynin	32	128	64	32	64	64	128

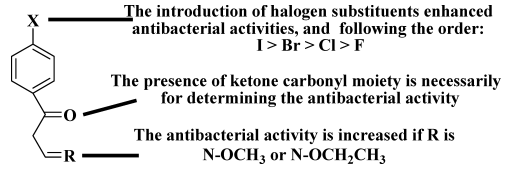


Fig. 2. SAR for the Antibacterial Activity of 3-(4-Halophenyl)-3-oxopropanal and Their Derivatives

Conclusion

The present investigation for the first time reported that 3-(4-halophenyl)-3-oxopropanal and their derivatives had potent antibacterial activities, and Gram-positive pathogens were more susceptible to these compounds. Of all the investigated compounds, compound **24** was found to be most active antibacterial agent with MIC of 1.0 μg/ml against *Staphylococcus aureus*. SAR analysis indicated that (1) the introduction of appropriate substituents into position 4 of phenyl ring enhanced antibacterial activities. (2) The antibacterial activity of these compounds might be associated with the electronic character of the halogen atom on the 4-position of benzene. (3) The *O*-methyl and *O*-ethyl oxime moiety might play an important role in determining the potent antibacterial activities. (4) The presence of ketone carbonyl moiety might be absolutely necessarily for improving the antibacterial activity of these compounds. Furthermore, the antibacterial activities of select compounds **7**, **15** and **16** against the clinically important pathogenic bacteria-methicillin-resistant *Staphylococcus aureus* (MRSA) were also investigated. Results showed that these compounds exhibited more potent activities than the well-known antibacterial agents Houttuynin and Levofloxacin, suggesting that further development of such compounds may be of interest.

Acknowledgment This work was supported by the Foundation of Education Department of Hunan Province, China (09B091).

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