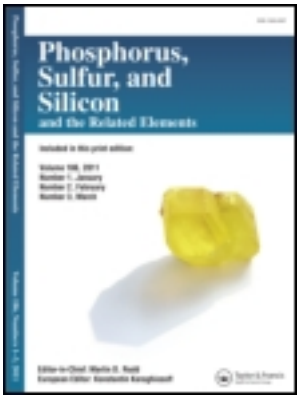

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SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF ACETOXYBENZOYL THIOUREAS WITH ARYL AND AMINO ACID SIDE CHAINS

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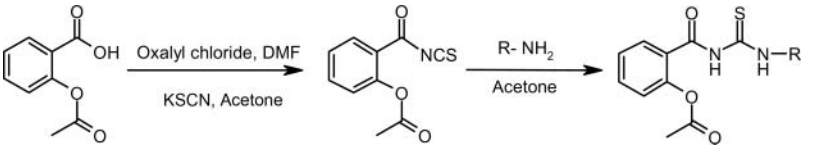
GRAPHICAL ABSTRACT



Abstract *A series of acetoxybenzoylthioureas derivatives with aryl and amino acid ester side chains were prepared by reaction of acetoxybenzoyl isothiocyanate, an acyloxy benzyl ester-based derivative of aspirin, with aryl amines or amino-functionalized amino acids with overall yields of 46–73%. The products that display a thiourea segment as a linker showed improved antibacterial properties in comparison with aspirin. The structures of the synthesized compounds were characterized by infra red spectroscopy, ^{13}C nuclear magnetic resonance (NMR), and ^{1}H NMR spectroscopy. The compounds were screened for their antibacterial activity by using gram-negative bacteria (E. coli ATCC 8739). [2-(phenylcarbamothioylcarbamoyl)phenyl] acetate showed the highest antibacterial activity against E. coli compared with other synthesized compounds.*

Supplemental materials are available for this article. Go to the publisher's online edition of Phosphorus, Sulfur, and Silicon and the Related Elements *to view the free supplemental file.*

Keywords Thiourea; aspirin; amino acid; antibacterial activity

INTRODUCTION

Aspirin has been widely used as an analgesic and anti-inflammatory drug. It also plays an important role in the prevention of cardiovascular diseases and cancer. Modification

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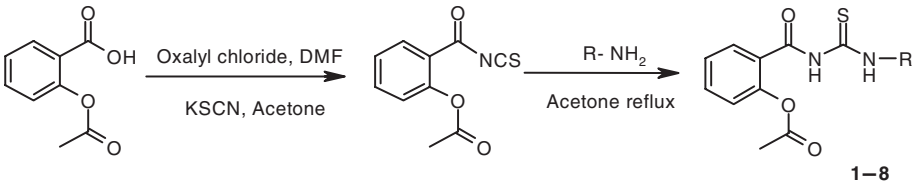
of aspirin has been reported to inhibit the growth of various human cancer cell lines originating from colon, lung, liver, pancreas, and breast.[1] Due to its significant biological properties, extensive effort has been exerted to improve the pharmacological properties of aspirin.[2,3]

It is well known that thioureas play important roles in many biological processes,[4] which stimulated our interest in the synthesis of a series of compounds containing aspirin associated with thiourea moiety. Presently, the search for new thiourea derivatives with reduced undesirable side effects and improved oral bioavailability has intensified. For example, 3-benzoyl-1-butyl-1-methyl-thiourea was reported to have antifungal activities and successfully inhibited the growth of *Penicillium digitatum* and *Saccharomyces cerevisiae*.[5]

In this paper, we report the synthesis of acetoxybenzoyl thioureas with aryl and amino acid side chains (**1–8**) by reaction of acetoxybenzoyl isothiocyanates with aniline,[6] aminophenol,[6] glycine, beta-alanine, alpha-alanine, phenylalanine, methionine, and aspartic acid, respectively. The preparation of thiourea derivatives **1** and **2** via 2-acetoxybenzoyl isothiocyanate has been independently described in a recent Chinese patent.[6] The antibacterial activities of synthesized thiourea derivatives toward *E. coli* ATCC 8739 have also been characterized.

RESULTS AND DISCUSSION

The acetoxybenzoyl thiourea derivatives **1–8** exhibiting aryl and an amino acid ester side chains were prepared by first reacting aspirin with oxalyl chloride under nitrogen atmosphere with dimethylformamide as catalyst to yield 2-(chlorocarbonyl)phenyl acetate. The thiourea derivatives of aspirin were prepared by reacting acyl chloride with potassium thiocyanate under basic conditions, followed by the reaction of the acetoxybenzoyl isothiocyanate with an aryl amine or an amino-functionalized amino acid to afford **1–8** with overall yields of 46–73%. The low yields of thiourea might be due to the possible formation of by-products such as urethanes.[7] The preparation of compounds **1–8** is depicted in Scheme 1.



Thiourea	R	MIC (ppm)
1	$-C_6H_5$	87
2	$-C_6H_4OH$	>200
3	$-CH_2COOH$	200
4	$-CH_2CH_2COOH$	210
5	$-CH(CH_3)COOH$	145
6	$-CH(CH_2C_6H_5)COOH$	>200
7	$-CH(CH_2CH_2SCH_3)COOH$	195
8	$-CH(CH_2COOH)COOH$	>200

Scheme 1 Synthesis of thiourea derivatives **1–8**.

The Fourier transform-infra red (FT-IR) spectra showed bands at 3269–3289 cm^{-1} and 1159–1231 cm^{-1}, which were attributed to ν(NH) and ν(C=S). The peaks at 1602–1608 $cm^{-1}$and 1659–1732 cm^{-1} were attributed to ν(C—N) and ν(C=O), respectively. Disappearance of the NCS peak at 2000 cm^{-1} and appearance of a NH peak at 3269–3289 cm^{-1} proved that the reaction was complete. The expected normal carbonyl absorption at 1659–1672 cm^{-1} (C=O) suggested a possible hydrogen bond formation between the hydrogen atom of the NH group and the oxygen atom of the carbonyl group.[8]

The chemical structures of all synthesized compounds were further confirmed by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy, which exhibited the signals corresponding to the structures. The presence of aromatic protons was observed in the range of 6.70–8.38 ppm in the ^{1}H NMR spectra, whereas CONH and CSNH peaks were observed at 11.38–11.65 ppm and 8.91–11.40 ppm, respectively. The CONH signal was shifted to higher frequency due to the downfield effect.[8] The ^{13}C NMR spectrum showed the presence of CONH at 168.46–172.88 ppm, whereas CSNH was observed at 178.87–206.55 ppm. The resonance at 115.79–157.48 ppm was attributed to aromatic carbons.

The inhibition activity of compounds **1–8** against *E. coli* ATCC 8739 is shown in Figure 1. Compound **1** showed complete inhibition against *E. coli* at all concentrations compared to that of aspirin, which has no antibacterial activity (Figure 1, compound **1**). Compounds **2–8**, on the other hand, showed varying degrees of antibacterial activity against the tested bacteria.

Different effects of the synthesized acetoxybenzoyl thiourea derivatives at various concentrations are further shown by their minimum inhibitory concentrations (MIC). The MIC value of these compounds were determined by extrapolating the concentration at the zero growth rate of *E. coli* ($\mu = 0$)[9] based on the specific growth rate of *E. coli* ATCC 8739. The series for MIC observed was 87, >200, 200, 210, 145, >220, 195, and >200 ppm (as shown in Scheme 1). Compound **1** exhibited the best bacteriostatic activities followed by compounds **5** and **7**. Other compounds showed MIC values of 200 and above due to very weak antibacterial activities.

These results indicated that C=S, NH, and C=O groups in all synthesized compounds play an important role with respect to bacteriostatic activities, as these important groups can be protonated under acidic condition, reacted with the carboxyl and phosphate groups of the bacterial surface, and thus show antibacterial activity.[10] Lipophilicity, which correlates well with the bioactivity of chemicals, is a very important molecular descriptor, and different lipophilic behavior of compounds plays an important role in their biological activity mechanisms. Compounds with phenyl groups showed more lipophilic character compared with the compounds without phenyl groups.[11]

Compounds **5** and **7** with one phenyl group exhibit lower antibacterial activity whereas compound **1** with two phenyl groups showed higher antibacterial activity. Due to their lower lipophilicity, compounds **4** and **7** did not penetrate into the microorganisms as easily as compound **1** with two phenyl groups.[11] Increasing the length or bulkiness of the side chain afforded varied effects and comparable results.[12] Although compound **6** possesses two phenyl groups, it does not show as potent antibacterial properties as compound **1**. This might be due to the presence of bulky groups in the structure, which led to a dramatic decrease in activity. The occurrence of steric hindrance prevents the compounds from reaching the active site.[12]

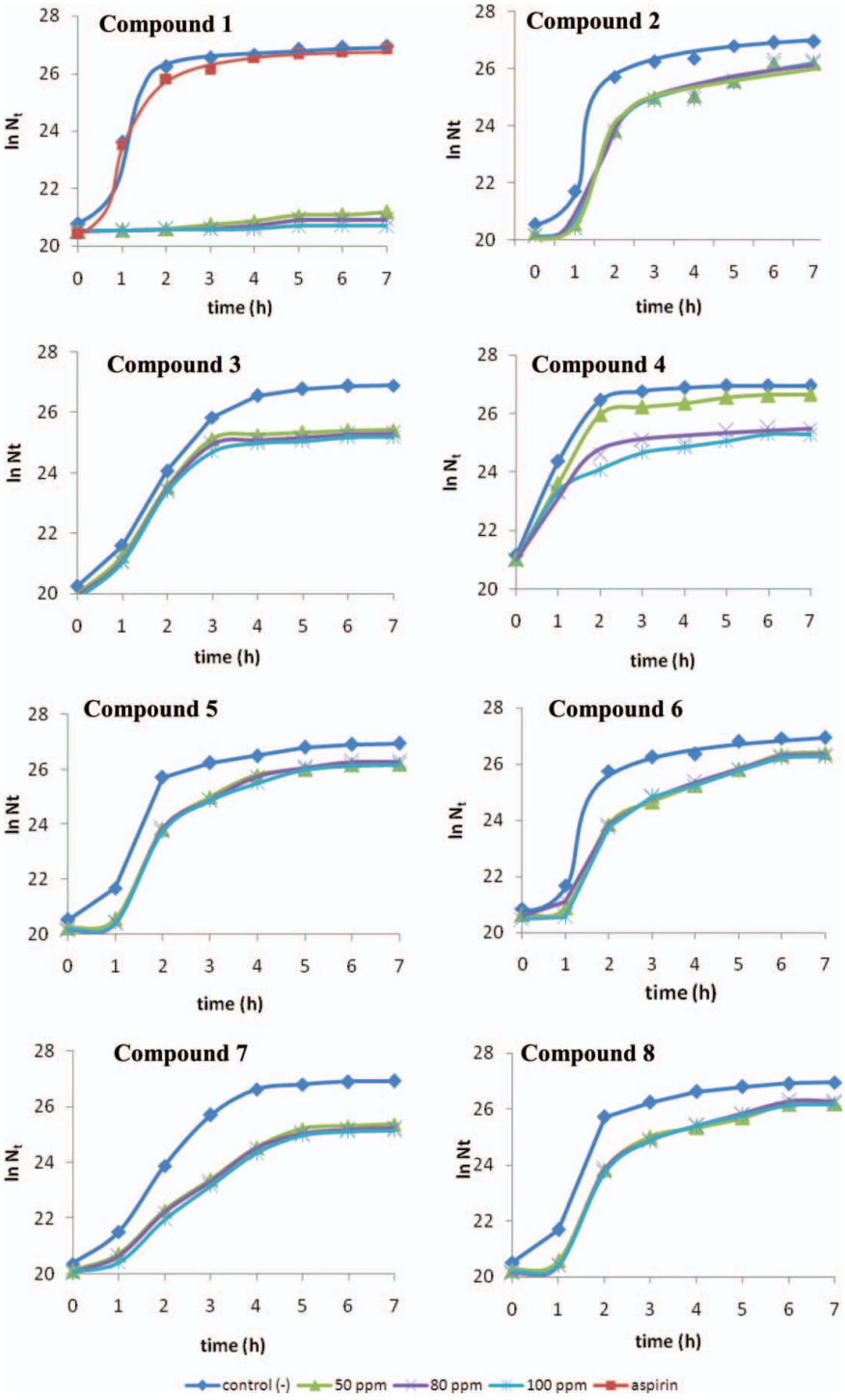


Figure 1 Inhibition activity of aspirin and **1–8** against *E. coli* shown as ln N_t for *E. coli* growth versus time.

CONCLUSION

A series of acetoxybenzoyl thioureas with aryl or amino acid ester side chains (**1–8**) has been synthesized and screened for their antibacterial activities. The acetoxybenzoyl thioureas showed antibacterial activity against gram-negative bacteria in contrast to the standard aspirin. Compounds containing C=S, C=O, and NH groups have been demonstrated to possess antibacterial properties. It is well established that the C=S, C=O, and NH groups induce antibacterial activity by reacting with the carboxyl and phosphate groups of the bacterial surface.

EXPERIMENTAL

Aspirin, oxalyl dichloride, aniline, aminophenol, glycine, beta-alanine, alpha-alanine, phenylalanine, methionine, and aspartic acid were obtained from Merck and used without further purification. All other reagents and solvents were used as received.

Measurements: Melting points were determined by the open tube capillary method and are uncorrected. Infra red (IR) spectra (ν/cm^{-1}) were recorded as KBr pellets on a Perkin Elmer 1605 FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL ECA 500 spectrometer at 500MHz (^{1}H) and 126 MHz (^{13}C) with the chemical shifts δ (ppm) reported relative to acetone-d_6 and DMSO-d_6 as standards.

General Procedure for the Preparation of Aspirin–Thiourea Derivatives

Oxalyl chloride (2.5 mmol) was added to aspirin (2.5 mmol) in 20 mL of dichloromethane. Few drops of dimethylformamide (DMF) were added to initiate the reaction. The solution was stirred for 1 h at room temperature under N_2. The mixture was added into potassium thiocyanate (KSCN) (2.5 mmol) in dry acetone (20 mL) at room temperature to form a precipitate. The mixture was filtered and the solid was removed. A solution of amine (2.5 mmol) in dry acetone (20 mL) was added to the filtrate. The reaction mixture was refluxed for 6 h, cooled to room temperature, and filtered. The filtrate was poured into a beaker with ice to form a solid, which was recrystallized in EtOH/CH_3CN (1:1) to give **1–8**.

N-(2-acetoxybenzoyl)-N′-phenylthiourea **(1)**:[6] Compound **1** was obtained as a light brown solid. Yield: 0.57 g (73%); mp 172 °C–175 °C; ν 3269 (NH), 3072 (Ar), 1732 (C=O ester), 1659 (C=O carbonyl), 1604 (C−N), 1201 (C=S). δ_H (DMSO-d_6) 2.08 (3H, s, CH_3), 7.05–8.00 (9H, m, Ar−H), 11.68 (1H, s, NH), 12.65 (1H, s, NH). δ_C (acetone-d_6) 30.11, 117.65, 118.11, 121.67, 124.52, 127.04, 129.49, 132.37, 136.28, 139.21, 157.42, 166.35, 171.55, and 179.49.

N-(2-acetoxybenzoyl)-N′-(4-hydroxyphenyl)thiourea **(2)**:[6] Compound **2** was obtained as a dark brown solid. Yield: 0.50 g (60%); mp 169 °C–178 °C; ν 3276 (NH), 1608 (C−N), 1208 (C=S), 3453 (OH), 1659 (C=O amide). δ_H (acetone-d_6) 1.32 (3H, s, CH_3), 6.70–8.08 (8H, m, Ar−H), 8.91 (1H, m, NH), 11.38 (1H, s, NH). δ_C (acetone-d_6) 23.93, 115.79, 115.91, 118.07, 121.53, 121.66, 126.23, 126.31, 132.29, 136.15, 154.17, and 156.60.

[N′-(2-acetoxybenzoyl)thioureido]-ethanoic acid **(3)**: Compound **3** was obtained as a yellow solid. Yield: 0.43 g (57.5%); mp 148 °C–154 °C; ν 3261 (NH), 1602 (C−N), 1194 (C=S), 3347 (OH), 1716 (C=O ester), 1677 (C=O amide). δ_H (DMSO-d_6) 2.28 (3H, s, CH_3), 4.21 (2H, d, J 4.0, CH_2), 7.26–7.67 (4H, m, Ar−H), 10.83 (1H, s, NH), 11.40

(1H, s, NH). δ_C (acetone-d_6) 29.49, 47.28, 124.52, 126.64, 127.10, 131.34, 134.52, 149.52, 165.74, 169.10, and 181.16.

3-[N′-(2-acetoxybenzoyl)thioureido]propanoic acid **(4)**: Compound **4** was obtained as a yellow solid. Yield: 0.42 g (57%); mp 185 °C–188 °C; ν 3289 (NH), 3199 (CH), 1703 (C═O ester), 1665 (C═O carbonyl), 1606 (C—N), 1222 (C═S). δ_H (DMSO-d_6) 2.08 (3H, s, CH_3), 2.64 (2H, t, *J* 6.6, CH_2), 3.44 (1H, s, OH), 3.82 (2H, t, *J* 6.3, CH_2), 7.01–7.92 (4H, m, Ar—H), 10.92 (1H, t, *J* 5.7, NH), 11.48 (1H, s, NH). δ_C (DMSO-d_6) 30.65, 32.31, 40.70, 116.49, 117.26, 120.14, 131.11, 135.20, 156.63, 164.53, 172.86, and 179.32.

2-[N′-(2-acetoxybenzoyl)thioureido]propanoic acid **(5)**: Compound **5** was obtained as a pale yellow solid. Yield: 0.41 g (53%); mp 173 °C–178 °C; ν 3278 (NH), 1605 (C—N), 1181 (C═S), 3406 (OH), 1701 (C═O ester) 1662 (C═O amide). δ_H (DMSO-d_6) 1.36 (3H, d, *J* 7.1, CH_3), 1.82 (3H, s, CH_3), 4.82 (1H, m, CH), 6.99–8.14 (4H, m, Ar—H), 11.18 (1H, d, *J* 7.5, NH), 11.65 (1H, s, NH). δ_C (DMSO-d_6) 17.27, 39.66, 53.32, 116.39, 117.42, 120.02, 131.13, 135.33, 157.10, 165.02, 172.88, and 178.91.

2-[N′-(2-acetoxybenzoyl)thioureido]-3-phenylpropanoic acid **(6)**: Compound **6** was obtained as a light yellow solid. Yield: 0.50 g (51%); mp 158 °C–160 °C; ν 3272 (NH), 3160 (C-H), 3028 (Ar), 1704 (C═O ester), 1666 (C═O carbonyl), 1604 (C—N), 1226 (C═S). δ_H (DMSO-d_6) 2.08 (3H, s, CH_3), 2.24 (2H, d, *J* 4.5, CH_2), 3.46 (1H, s, OH), 5.04 (1H, t, *J* 5.0, CH), 7.00–7.91 (9H, m, Ar—H), 11.07 (1H, d, *J* 5.0, NH), 11.60 (1H, s, NH). δ_C (DMSO-d_6) 22.33, 36.05, 58.77, 116.30, 117.35, 120.19, 126.94, 128.44, 129.27, 131.19, 135.43, 136.15, 156.82, 164.90, 171.32, and 179.28.

2-[N′-(2-acetoxybenzoyl)thioureido]-4-(methylthio)butanoic acid **(7)**: Compound **7** was obtained as a yellow solid. Yield: 0.57 g (59%); mp 155 °C–158 °C; ν 3277 (NH), 3079 (CH), 1720 (C═O ester), 1672 (C═O carbonyl), 1604 (C—N), 1231 (C═S), 715 (C-S). δ_H (DMSO-d_6) 2.06 (3H, s, CH_3), 2.14 (2H, m, CH_2), 2.23 (2H, m, CH_2), 2.54 (3H, s, CH_3), 3.57 (1H, s, OH), 4.97 (1H, t, *J* 5.0, CH), 7.02–7.96 (4H, m, Ar—H), 11.17 (1H, d, *J* 10.0, NH), 11.60 (1H, s, NH). δ_C (DMSO-d_6) 14.70, 29.21, 29.71, 30.55, 56.75, 116.39, 117.34, 120.22, 131.20, 135.41, 156.80, 164.99, 171.81, and 179.54.

2-[N′-(2-acetoxybenzoyl)thiourido]butanedioic acid **(8)**: Compound **8** was obtained as yellow crystals. Yield: 0.40 g (46%); mp 133 °C–140 °C; ν 3269 (NH), 1604 (C—N), 1159 (C═S), 3448 (OH), 1715 (C═O ester), 1649 (C═O amide), 1206 (C-O-C). δ_H (DMSO-d_6) 1.94 (3H, s, CH_3), 2.39 (2H, d, *J* 10.0, CH_2), 2.99 (1H, s, CH), 6.95–8.38 (4H, m, Ar—H), 11.40 (1H, d, *J* 7.3, NH), 11.56 (1H, s, NH). δ_C (DMSO-d_6) 25.78, 30.64, 39.67, 117.22, 117.46, 118.42, 119.81, 128.17, 130.32, 130.87, 134.13, 134.62, 157.05, 161.19, 161.35, 172.00, 172.21, and 206.55.

Antibacterial Studies

The antibacterial activities of the synthesized compounds against *E. coli* ATCC 8739 were investigated by the turbidimetric kinetic method. The inoculums was prepared by allowing *E. coli* to grow on media containing nutrient broth at 37 °C with permanent stirring at 250 rpm for 18 h. Inoculums (0.2 mL) were inoculated with 10 mL of culture medium (with increasing concentration of the compounds dissolved in DMSO). The mixture was shaken at 180 rpm at 37 °C. Inoculums with DMSO solvent were used as controls. Aliquots of each replicate were taken at every 1-h interval for 7 h. The transmittance (T) was registered in a UV-visible spectrophotometer (Optima SP-300). The antibacterial activities were determined by a graph as ln Nt, define as transmittance value, which was related to the number colony forming units (CFU) mL^{-1} for *E. coli* versus time.[9]

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