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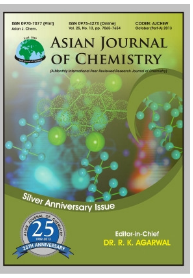

Antituberculosis and Antifungal Activities of Synthesized Benzoylthiourea Derivatives

MENG-MENG ZHAO*, XIU-YAN DONG, GANG LI, YU-HUA YANG, YU-JIE ZHANG and XIAO-QIN YANG

School of Chemical and Biological Engineering, Lanzhou Jiaotong University, Lanzhou 730070, P.R. China

*Corresponding author: E-mail: zhaomm80@163.com

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A series of benzoylthiourea derivatives have been synthesized from benzoyl isothiocyanate and phenyleneamine in CH_2Cl_2 medium under solid-liquid phase transfer catalysis conditions. Structures of these compounds have been characterized by elemental analyses as well as IR and ^{1}H NMR spectroscopy. All the compounds were tested for their antibacterial and antifungal activities, the results indicated that most of the compounds have antibacterial and antifungal activities close to the standard drugs, especially they were found to have remarkable antituberculosis and antifungal activities.

Key Words: Thiourea, Synthesis, Crystal structure, Antituberculosis and antifungal activity.

INTRODUCTION

Thiourea and its substituted derivatives have attracted intense attentions because of their unique applications such as versatile biological activities which is useful as herbicides, insecticides and plant-growth regulators[1-5]. Some thioureas are organic reaction catalyst in the metal-catalyzed asymmetric reduction of carbonyl compounds and carbonylative cyclization of *o*-hydroxyarylacetylenes[6-8]. A survey of the literature reveals that some work has been reported on benzoylthiourea, which has been found plenty of applications as a facile and simple ligand in the determination of traces of transition metals and as an available starting material in the preparation of a wide variety of metal complexes[9-15]. In a ^{13}C NMR study[16,17], the differences between the benzoyl (CO) chemical shift values of N-monosubstituted and N,N-disubstituted benzoyl-thioureas indicated the existence of an intramolecular hydrogen bond, namely between the benzoyl CO and the NH groups. This indication was also supported by the ^{1}H NMR spectrum[18-20]. Here we report the synthesis and antibacterial activities of benzoylthiourea derivatives.

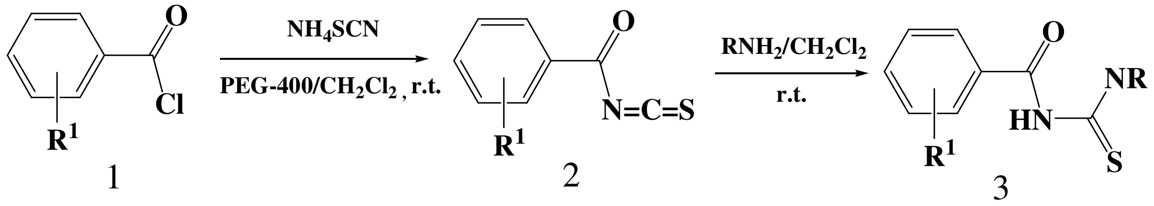
EXPERIMENTAL

Benzoyl chloride, *o*-chlorobenzoyl chloride, *p*-nitrobenzoyl chloride, 3-aminopyridine, 3-amino-2-chloropyridine and polyethylene glycol-400 were purchased and used without further purification. The other reagents and solvents were analytical grade reagents from Tianjin Chemical Reagent Factory. C, H and N analyses were carried out with a GmbH VariuoEL V3.00 automatic elemental analyzer. IR spectra in the range 4000-400 cm^{-1} were recorded on a VERTEX70 FT-IR spectrophotometer using KBr pellets. The ^{1}H NMR spectra were recorded on a Mercury-400BB spectrometer at room temperature using $CDCl_3$ as solvent. Melting points was measured by the use of a microscopic melting point apparatus made in Beijing Taike Instrument Limited Company and the thermometer was uncorrected.

The antimicrobial activity was determined using agar disc diffusion method by measuring the inhibition zone in mm. All organisms were obtained from the China Center of Industrial Culture Collection, including 3 bacterial stains *i.e.*, *Escherichia coli* ACCC11864 as gram-negative bacteria, *Staphylococcus aureus* ACCC01331 and *Mycobacterium tuberculosis* CVCC343 as gram-positive bacteria, 3 fungal stains: *Asperigillus flavus* ACCC30321, *Asperigillus fumigates* ACCC30367 and *Candida albicans* SCTCC100762. Ampicillin and fluconazole were served as standard antibacterial and antifungal agents, respectively.

General procedure: Synthetic route to benzoylthiourea derivatives **3a-3f** is shown in Fig. 1, all the compound have been characterized by elemental analyses as well as IR and ^{1}H NMR spectroscopy.

Preparation and characterization of N-benzoyl-N'-(3-pyridyl)thiourea (3a): 1.41 g (0.01 mol) of benzoyl chloride was reacted with 1.14 g (0.015 mol) of ammonium thiocyanate in 15 mL of CH_2Cl_2 under solid-liquid phase transfer catalysis conditions, using 0.18 g of 3 % polyethylene glycol-400 as the catalyst, to give the corresponding benzoyl isothiocyanate, which was reacted with 0.85 g (0.01 mol) of 3-aminopyridine to give the title compound. The solid isolated was separated



3a: R^1=H, R=3-pyridine; 3b: R^1=2-Cl, R=3-pyridine;

3c: R^1=H, R=H_2; 3d: R^1=4-nitro, R=3-pyridine.

3e: R^1=H, R=2-chloro-3-pyridine;

3f: R^1=2-Cl, R=2-chloro-3-pyridine;

Fig. 1. Synthetic route to benzoylthiourea derivatives **3a-3f**

from the liquid phase by filtration, washed successively with CH_2Cl_2 and H_2O, respectively, The product was dried under reduced pressure and purified with recrystallization from chloroform to obtain white crystalline solid; Yield: 61.8 %. m.p. 431-432 K. Anal. calcd. (%) for $C_{13}H_{11}N_3OS$: C, 60.68; H, 4.31; N, 16.33. found (%): C, 60.73; H, 4.23; N, 16.05. Selected IR data (KBr pellet, ν_{max}, cm^{-1}): 3232, 3181 (NH), 1676 (C=O), 1156 (C=S). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) 7.37 (m, 1H, py), 7.58-7.94 (m, 5H, Ar-H), 8.34-8.37 (d, *J* = 6.0 Hz, 1H, py), 8.51 (s, 1H, py), 8.76 (s, 1H, py), 9.15 (s, 1H, HN), 12.69 (s, 1H, HN).

Preparation and characterization of N-(*o*-chloro)benzoyl-N'-(3-pyridyl)thiourea (3b): *o*-Chlorobenzoyl chloride (1.81 g, 0.01 mol) was reacted with ammonium thiocyanate (1.14 g, 0.015 mol) in CH_2Cl_2 (15 mL) under solid-liguid phase transfer catalysis, using 3 % polyethylene glycol-400 (0.18 g) as catalyst, to give the corresponding benzoyl isothiocyanate, which was reacted with 3-aminopyridine (0.86 g, 0.01 mol). The title compound **3b** precipitated immediately. The product was filtered, washed with water and CH_2Cl_2 and dried. Yield 76.6 %, m.p. 442-444 K. Anal. calcd. (%) for $C_{13}H_{10}N_3OSCl$: C, 53.52; H, 3.45; N, 14.40. found (%): C, 53.43; H, 3.46; N, 14.39. Selected IR data (KBr pellet, ν_{max}, cm^{-1}): 3428, 3256 (NH), 1687 (C=O), 1175 (C=S). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) 7.38 (dd, *J* = 8.5, 4.8 Hz, 1H, ArH), 7.47 (dd, J = 8.0, 5.6 Hz, 1H, ArH),7.52 (t, *J* = 10.6 Hz, 1H, ArH), 7.56 (t, *J* = 2.4 Hz, 1H, ArH), 7.81 (d, *J* = 8.8 Hz, 1H, PyH), 8.37 (dd, *J* = 8.0, 3.6 Hz, 1H, PyH), 8.55 (dd, *J* = 4.0, 1.2 Hz, 1H, PyH), 8.78 (d, *J* = 2.0 Hz, 1H, PyH), 9.41 (s, 1H, NH), 12.46 (s, 1H, NH).

Preparation and characterization of N-benzoylthiourea (3c): Benzoyl chloride (0.71g, 0.005 mol) was reacted with ammonium thiocyanate (0.57 g, 0.0075 mol) in CH_2Cl_2 (10 mL) solution under soild-liquid phase transfer catalysis, using polyethylene glycol-400 (0.09 g) as the catalyst, to give the corresponding benzoyl isothiocyanate. The resulting mixture was evaporated to dryness using a rotatory evaporator and the residue was extracted with chloroform, to give the title compound **3c**. Yield, 85.2 %. m.p. 386-388 K. Anal. calcd. (%) for $C_8H_8N_2OS$: C, 53.31; H,4.47; N, 15.54. found (%): C, 53.23; H, 4.58; N, 15.32. Selected IR data (KBr pellet, ν_{max}, cm^{-1}): 3311, 3225 (NH), 1681 (C=O), 1112 (C=S). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.54-7.88 (m, 5H, Ar-H), 9.99 (s, 1H, HN), 10.07 (s, 2H, H_2N).

Preparation and characterization of N-(*p*-nitro)benzoyl-N'-(3-pyridyl)thiourea (3d): The *p*-nitrobenzoyl chloride (0.93 g, 0.005 mol) was reacted with ammonium thiocyanate (0.57 g, 0.0075 mol) in CH_2Cl_2 (15 mL) solution under soild-liquid phase transfer catalysis, using polyethylene glycol-400 (0.09 g) as the catalyst, to give the corresponding *p*-nitrobenzoyl isothiocyanate, which was reacted with 3-aminopyridine (0.425 g, 0.005 mol), to give the title compound **3d**. Yield 68.6 %, m.p. 443-445 K. Anal. calcd. (%) for $C_{13}H_{10}N_4O_3S$: C, 51.65; H, 3.33; N, 15.88. found (%): C, 51.47; H, 3.45; N, 15.69. Selected IR data (KBr pellet, ν_{max}, cm^{-1}): 3345, 3175 (NH), 1682 (C=O), 1172 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 7.50 (dd, *J* = 8.4, 4.8 Hz, 1H, PyH), 8.13 (t, *J* = 8.2 Hz, 1H, PyH), 8.22 (dd, *J* = 20.4, 8.8 Hz, 2H, ArH), 8.35 (dd, *J* = 20.4, 8.8 Hz, 2H, ArH), 8.46 (dd, *J* = 27, 6.6 Hz, 1H, PyH), 8.75 (s, 1H, PyH), 12.12 (s, 1H, NH), 12.32 (s, 1H, NH).

Preparation and characterization of N-benzoyl-N'-(2-chloro-3-pyridyl)thiourea (3e): Benzoyl chloride (0.07 g, 0.005 mol) was reacted with ammonium thiocyanate (0.38 g, 0.005 mol) in acetonitrile solution (25 mL) with constant stirring for 3 h at room temperature, to give the corresponding benzoyl isothiocyanate, which was added 3-amino-2-chloro-pyridine (0.64 g, 0.005 mol). After stirring for 20 h at room temperature, the precipitate was reduced pressure filtered, washed successively with acetonitrile and diethyl ether. The product was dried *in vacuo* and obtained needle-like crystalline solid. Yield, 41.1 %. m.p. 424-426 K. Anal. calcd. (%) for $C_{13}H_{10}ClN_3OS$: C, 53.52; H, 3.45; N, 14.40. found (%): C, 53.61; H, 3.51; N, 14.38. Selected IR data (KBr pellet, ν_{max}, cm^{-1}): 3230, 3185 (NH), 1672 (C=O), 1158 (C=S). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) 7.37 (m, 1H, py), 7.56-7.90 (m, 5H, Ar-H), 8.30-8.34 (d, *J* = 6.0 Hz, 1H, py), 8.76 (s, 1H, PyH), 9.15 (s, 1H, HN), 12.68 (s, 1H, HN).

Preparation and characterization of N-(*o*-chloro)benzoyl-N'-(2-chloro-3-pyridyl)thiourea (3f): *o*-Chlorobenzoyl chloride (0.9 g, 0.005 mol) was reacted with ammonium thiocyanate (0.57 g, 0.0075 mol) in CH_2Cl_2 (10 mL) under solid-liguid

TABLE-1
RESULTS OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES

Compound	Diameter of zone of inhibition (mm)					
	M. tuberculosis	*S. aureus*	*E. coli*	*C. albicans*	*A. flavus*	*A. fumigatus*
3a	8.9 ± 0.3	5.1 ± 0.15	–	8.9 ± 0.2	10.4±0.35	8.8±0.25
3b	10.5 ± 0.4	8.7 ± 0.25	10.2 ± 0.4	13.6±0.15	12.2±0.5	11.7±0.35
3c	–	–	4.6±0.1	7.0 ±0.2	–	6.1±0.15
3d	9.2±0.35	7.6±0.1	–	10.5±0.45	11±0.5	9.4±0.35
3e	9.8±0.25	8.5±0.3	9.1±0.4	11.3±0.3	14.1±0.45	9.9±0.25
3f	12.1±0.45	12.5±0.25	13.2±0.1	14.2±0.6	16.8±0.7	13.2±0.25
Ampicillin	12.3±0.6	13.6±0.5	15.9±0.7	–	–	–
Fluconazole	–	–	–	16.9±0.55	17.4±0.6	16.2±0.65

–: Indicates no activity.

phase transfer catalysis, using 3 % polyethylene glycol-400 (0.18 g) as catalyst, to give the corresponding benzoyl isothiocyanate, which was reacted with 3-amino-2-chloropyridine (0.65 g, 0.005 mol). After stirring for 10 h at room temperature, the precipitate was reduced pressure filtered, washed successively with water and CH_2Cl_2 and dried. Yield 76.1 %, m.p. 401-402 K. Anal. calcd. (%) for $C_{13}H_9Cl_2N_3OS$: C, 47.87; H, 2.78; N, 12.88. found (%): C, 47.98; H, 2.65; N, 12.72. Selected IR data (KBr pellet, ν_{max}, cm^{-1}): 3420, 3252 (NH), 1680 (C=O), 1172 (C=S). ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm) 7.39 (dd, *J* = 8.4, 4.8 Hz, 1H, ArH), 7.46 (dd, *J* = 8.0, 5.6 Hz, 1H, ArH),7.51 (t, *J* = 10.6 Hz, 1H, ArH), 7.55 (t, *J* = 2.4 Hz, 1H, ArH), 7.80 (d, *J* = 8.8 Hz, 1H, PyH), 8.36 (dd, *J* = 8.0, 3.6 Hz, 1H, PyH), 8.77 (d, *J* = 2.0 Hz, 1H, PyH), 9.40 (s, 1H, NH), 12.45 (s, 1H, NH).

Antimicrobial activity: The culture media used were nutrient agar and sabourd's medium for bacteria and fungus strains, respectively. The hot nutrient agar and sabourd's medium solution was poured into sterilized petridishes and allowed to attain room temperature. Seed layer medium which contains the previously grown subculture was lawned into the Petri dishes. Cups were made using sterile borer of 5 mm diameter. To these cups 0.5mL of the drug solution (50 µg/mL), standard solution were added and allowed to cool for 1 h to facilitate diffusion. The plate was incubated at 37 ºC for 48 h. Zone of inhibition around wells were measured. All tests were repeated three times to confirm the results. The results are presented in Table-1.

RESULTS AND DISCUSSION

A series of benzoylthiourea derivatives **3a-3f** have been synthesized with good yields and the structures are confirmed by elemental analyses, IR spectra and ^{1}H NMR data.

Most of the synthesized compounds have shown good antimicrobial activities as compared to their standard drugs but the antifungal activity is more prominent. Among them, because of the two chloride group, Compound **3f** showed the highest antibacterial and antifungal activities, it possess antibacterial and antifungal activities slightly lower than that of standard Ampicillin and Fluconazole. Compounds **3b** and **3e** also possess higher antibacterial and antifungal activities because of one chloride group. At the concentration of 50 µg/mL, compounds **3a** and **3d** were both have no inhibition on gram negative bacteria just like the experiment of *E. coli*. Compound **3c** showed the lowest antibacterial and antifungal activities, it also has no inhibition on gram-positive bacteria and some fungus like the experiments of *S. aureus* and *A. flavus*, it only have slightly antibacterial and antifungal activities compared with that of the other bacteria and fungus have been tested. Finally, except compound **3c**, other compounds all have the abilities to inhibited the bacteria of *M. tuberculosis*. These results indicated the potential of these molecules as antimicrobial and screening against large clinical isolates is suggested for further work.

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