International Journal of Molecular and Clinical Microbiology 2 (2013) 310-314

International Journal of
Molecular and Clinical Microbiology

Synthesis, Characterization and Antibacterial Activity Studies of Some N-Acyl-N'-aryl Thiourea Derivatives

Khalil Pourshamsian

Department of Chemistry, Tonekabon branch, Islamic Azad University, Tonekabon, Iran

ARTICLE INFO

Article history:
Received 14 May 2013
Accepted 21 November 2013
Available online 1 December 2013

Keywords:
Green chemistry, Thiourea derivatives, Antibacterial activity

ABSTRACT

Some N¬-acyl-N'-aryl thiourea derivatives 4(a-f) have been prepared by the reaction of acyl halides ammonium thiocyanate and aryl amines. The structures of synthesized compounds have been characterized by IR, 1HNMR spectral studies. The synthesized compounds 5(a-f) have been screened for antibacterial activity. The effect of the structure of the investigated compounds on the antibacterial activity is discussed.

1. Introduction

Thioureas are important compounds as building blocks in synthesis of heterocycles (Arslan et al., 2002; Mansuroglu et al., 2008; Ozer et al., 2009). Compounds containing carbonyl and thiocarbonyl groups occupy an important position among organic reagents as potential donor ligands for transition metal ions (Binzet et al., 2006; Ugur et al., 2006; Emen et al., 2005; Arslan et al., 2006). Among these thiourea derivatives are potentially very versatile ligands (Henderson et al., 2002), able to coordinate a range of metal centers as neutral ligands (Sacht et al., 2000), monoanios (Lipowska et al., 1996) or dianions (Zuckerman et al., 2000). The Nitrogen, Oxygen and Sulfur donor atoms of thiourea derivatives provide a multitude of bonding possibilities (Henderson et al., 1992). Thiourea and their metal complexes display a wide range of biological activity including antibacterial, antifungal (Yuan et al., 2001), antitubercular (Zhang et al., 2004), antithroid (Zhang et al., 1998), antihelmintic (Zhou et al., 2004), rodenticidal, insecticidal and herbicidal and plant-growth regulator properties (Eweis et al., 2006).

In view of this, we focused our attention to synthesis, characterization and antibacterial activity of new thiourea derivatives. Thus, here we report the synthesis of six novel high substituted *N*-acyl-*N*'-aryl thiourea and antibacterial activity of them against standard bacteria (two different standard strains of *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Streptococcus pyogenes, Bacillus cereus, Esherichia coli, Pesudomonas aeruginosa, Enterobacter cloacae, Proteus vulgaris* and *Enterobacter aerogenes*). The results obtained are shown in scheme 1 and table 1.

2. Material and Methods

2.1. Synthesis

All the melting points were determined in open capillaries Electrothermal-9100s apparatus and are uncorrected. The reactions were

*Corresponding author: Dr. Pourshamsian
E-mail address: kshams49@gmail.com

monitored on TLC. The IR spectra were recorded in KBr pellets on a Shimadzu IR-460 spectrophotometer. ^{1}HNMR spectra were recorded on a Bruker DRX-400 AVANCE instrument in $CDCl_3$ at 400 MHz respect to δ in ppm and *J* in Hz. All substrate and reagent purchased from Merck Company and used without further purification.

Typical procedure for preparation of compounds 4(a-f): To a mixture of acetone (10 mL) and 0.078g (1 mmol) aqueous solution of ammonium thiocyanate was added acyl halides derivatives (1 mmol) and stirred at room temperature for 30 min. A solution of primary aryl amines (1 mmol) in acetone (5 mL) was added and stirring continued for another 15 min. The progress of reaction was monitored by TLC using n- hexane: ethylacetate 1:1 $^{V}/_{V}$ as eluent). After completion the reaction, cold water (50 mL) was added to the mixture and the solid precipitate were filtered and washed with cold ethanol. The product was recrystallized from ethanol. After drying in oven, the structure of products characterized by physical a spectroscopic data. The physical spectroscopic data of synthesized compounds were given in continue.

(4a) 3-Benzyl-1-(2-fluorobenzoyl)-thiourea

Pale yellow powder; yield: 0.53 g (90%); mp 81-82°C. IR (KBr): 3413, 3208 (NH); 1670 (C=O). ^{1}H-NMR: 4.94 (*d*, $^{3}J_{HH}$ = 6.0, CH_2); 6.99-7.70 (*m*, 7CH arom); 7.81-8.41 (*m*, CH arom); 9.62 (*d*, $^{5}J_{HF}$ = 12.2, NH); 10.97 (*t*, $^{3}J_{HH}$ = 6.0, NH). ^{13}C-NMR: 50.3 (CH_2); 117.3 (*d*, $^{2}J_{CF}$ = 24.0, CH); 119.5 (*d*, $^{2}J_{CF}$ = 20.5, C); 121.2 (CH); 125.8 (*d*, $^{4}J_{CF}$ = 3.2, CH); 128.3, 129.3 (2 CH); 132.4 (*d*, $^{3}J_{CF}$ = 9.9, CH); 136.1 (*d*, $^{3}J_{CF}$ = 9.7, CH); 136.6 (CH); 160.9 (*d*, $^{1}J_{CF}$ = 249.0, C-F); 163.3 (*d*, $^{3}J_{CF}$ = 4.1, C=O); 180.3 (C=S). EI-MS: 288 (7, M^{+}), 123 (34), 105 (56), 91 (100), 32 (13), 28 (30), 19 (27). Anal. calcd. for $C_{15}H_{13}FN_2OS$ (288.35): C, 62.48; H, 6.59; N, 9.72; found: C, 62.68; H, 6.42; N, 9.84.

(4b)3-(4-Ethylphenyl)-1-(2-fluorobenzoyl)-thiourea

Pale yellow powder; yield: 0.48 g (79%); mp 79-81°C. IR (KBr): 3422 (NH); 1668 (C=O). ^{1}H-NMR: 1.27 (*t*, $^{3}J_{HH}$ = 7.0, Me); 2.69 (*d*, $^{3}J_{HH}$ = 7.0, CH_2); 7.23-8.14 (8 H, *m*, arom); 9.66 (*d*, $^{5}J_{HF}$ = 15.0, NH); 12.46 (*s*, NH). ^{13}C-NMR: 15.8 (Me); 28.9 (CH_2); 117.2 (*d*, $^{2}J_{CF}$ = 21.1, CH); 119.5 (*d*, $^{2}J_{CF}$ = 20.7, CH); 124.5, 125.8 (2 CH); 128.7 (*d*, $^{4}J_{CF}$ = 3.3, CH); 132.5 (*d*, $^{3}J_{CF}$ = 9.6, CH); 135.6(CH); 136.2 (*d*, $^{3}J_{CF}$ = 9.6, CH); 143.6 (CH); 161.0 (*d*, $^{1}J_{CF}$ = 264.7, C-F); 163.4 (*d*, $^{3}J_{CF}$ = 3.9, C=O); 178.5 (C=S). EI-MS: 302 (9, M^{+}), 123 (28), 106 (43), 105 (76), 91 (100), 32 (31), 28 (17), 19 (10). Anal. calcd. for $C_{16}H_{15}FN_2OS$ (302.37): C, 63.56; H, 5.00; N, 9.26; found: C, 63.69; H, 5.19; N, 9.37.

(4c)1-(2-Fluorobenzoyl)-3-(2-methylphenyl)-thiourea

Pale yellow powder; yield: 0.53 g (92%); mp 68-70°C. IR (KBr): 3413, 3225 (NH); 1678 (C=O); 1155 (C=S). ^{1}H-NMR: 2.39 (*s*, Me); 7.23-7.34 (*m*, 4 CH arom); 7.39 (*m*, CH arom); 7.62-7.79 (*m*, 2 CH arom); 8.15 (*m*, CH arom); 9.75 (*d*, $^{5}J_{HF}$ = 13.6, NH); 12.21 (*s*, NH). ^{13}C-NMR: 18.4 (Me); 117.2 (*d*, $^{2}J_{CF}$ = 24.2, CH); 119.4 (*d*, $^{2}J_{CF}$ = 19.8, C); 125.9 (*d*, $^{4}J_{CF}$ = 3.4, CH); 126.6 (CH); 126.9 (CH); 128.1 (CH); 131.2 (CH); 132.6 (*d*, $^{3}J_{CF}$ = 9.4, CH); 133.7 (CH); 136.3 (*d*, $^{3}J_{CF}$ = 9.7, CH); 136.8 (CH); 161.0 (*d*, $^{1}J_{CF}$ = 249.4, C-F); 163.5 (*d*, $^{3}J_{CF}$ = 3.3, C=O); 179.5 (C=S). EI-MS: 288 (5, M^{+}), 188 (34), 123 (65), 105 (100), 32 (19), 28 (32), 19 (21). Anal. calcd. for $C_{15}H_{13}FN_2OS$ (288.35): C, 62.48; H, 6.59; N, 9.72; found: C, 62.66; H, 6.39; N, 9.79.

(4d) 3-Benzyl-1-(4-chlorobenzoyl)-thiourea

Pale yellow powder; yield: 0.48 g (90%); mp 214-216°C. IR (KBr): 3260 (NH); 1634 (C=O); 1167 (C=S). ^{1}H-NMR: 4.93 (*d*, $^{3}J_{HH}$ = 6.0, CH_2); 7.33-7.40 (*m*, C_6H_5); 7.50 (2 H, *d*, $^{3}J_{HH}$ = 7.7, C_6H_4); 7.79 (2 H, *d*, $^{3}J_{HH}$ = 7.7, C_6H_4); 9.06 (*s*, NH); 10.96 (*t*, $^{3}J_{HH}$ = 6.0, NH). ^{13}C-NMR: 50.3 (CH_2); 128.4, 129.27, 129.3, 129.9, 130.5, 136.4, 140.6 (C_6H_4, C_6H_5); 166.1 (C=O); 180.3 (C=S). EI-MS: 304 (4, M^{+}), 140 (18), 139 (23), 105 (78), 91 (100), 32 (21), 28 (17). Anal. calcd.

for $C_{15}H_{13}ClN_2OS$ (304.80): C, 59.11; H, 4.30; N, 9.19; found: C, 59.38; H, 4.42; N, 9.35.

(4e)1-(2-Fluorobenzoyl)-3-methylphenyl-thiourea

Pale yellow powder; yield: 0.52 g (96%); mp 89-91°C. IR (KBr): 3414, 3368 (NH); 1675 (C=O); 1143 (C=S). ^{1}H-NMR: 7.30-7.80 (8 H, *m*, CH arom); 8.13-8.15 (1 H, *m*, CH arom); 9.65 (*d*, $^5J_{HF}$ = 13.5, NH); 12.50 (*s*, NH). ^{13}C-NMR: 117.2 (*d*, $^2J_{CF}$ = 24.1, CH); 119.4 (*d*, $^2J_{CF}$ = 9.9, C); 124.5 (CH); 125.9 (*d*, $^4J_{CF}$ = 3.1, CH); 127.3 (CH); 129.3 (CH); 132.6 (*d*, $^3J_{CF}$ = 9.4, CH); 136.3 (*d*, $^3J_{CF}$ = 9.7, CH); 138.0 (CH); 161.0 (*d*, $^1J_{CF}$ = 248.1, C-F); 163.5 (*d*, $^3J_{CF}$ = 3.4, C=O); 178.5 (C=S). EI-MS: 274 (6, M^+), 197 (58), 123 (100), 105 (87), 32 (17), 28 (24), 19 (22). Anal. calcd. for $C_{14}H_{11}FN_2OS$ (274.32): C, 61.30; H, 4.04; N, 10.21; found: C, 61.41; H, 4.09; N, 10.34.

(4f) 3-Benzyl-1-(4-nitrobenzoyl)-thiourea

Pale yellow powder; yield: 0.57 g (91%); mp 222-224°C. IR (KBr): 3168, 3116 (NH); 1671 (C=O); 1447 (NO_2). ^{1}H-NMR: 4.93 (*d*, $^3J_{HH}$ = 6.0, CH_2); 7.40-7.41 (*m*, C_6H_5); 8.04 (2 H, *d*, $^3J_{HH}$ = 8.0, C_6H_4); 8.37 (2 H, *d*, $^3J_{HH}$ = 8.0, C_6H_4); 9.20 (*s*, NH); 10.85 (*t*, $^3J_{HH}$ = 6.0, NH). ^{13}C-NMR: 50.4 (CH_2); 124.7, 128.3, 128.5, 129.2, 129.4, 136.2, 137.6, 151.1 (C_6H_4, C_6H_5); 165.3 (C=O); 179.8 (C=S). EI-MS: 315 (7, M^+), 145 (100), 91 (43), 76 (56), 32 (18), 28 (23). Anal. calcd. for $C_{15}H_{13}N_3O_3S$ (315.35): C, 57.13; H, 4.16; N, 13.32; found: C, 57.37; H, 4.25; N, 13.44.

2.2. Antimicrobial activity

The compounds were screened for their *in vitro* antibacterial activities. Antibacterial activities were determined by the broth micro dilution procedure and principles of the Clinical and Laboratory Standards Institute (CLSI) (NCCLS, 1997). Minimal inhibitory concentrations (MIC) for each compound were investigated against standard bacterial strains; *S.aureus* (ATCC 25923), *S.epidermidis* (ATCC 12228), *E.faecalis* (ATCC 29212), *S.pyogenes* (Clinical isolate), *B.cereus* (Clinical isolate), *E.coli* (ATCC 25922), *P.aeruginosa* (ATCC 27853), *E.cloacae* (ATCC 13047), *P.vulgaris* (ATCC 13315) and *E.aerogenes* (Clinical isolate) obtained from Faculty of Medicine, Tehran University. Bacteria were suspended directly into a small volume of 0.9% saline and further diluted until turbidity matched the Mc Farland standard no: 0.5 petridishes containing Muller-Hinton agar for bacteria were impregnated with these microbial suspensions. The stock solution was prepared in dimethyl sulfoxide (DMSO), which had no effect on the microorganisms in the concentrations studied. All of the dilutions were done with distillated water. The concentrations of tested compounds were 400, 200, 100, 50, 25, 12.5, 6.25, 3.12 $^{\mu g}/_{mL}$. DMSO was used as negative control. Amikacin, gentamycin were used as reference drugs Gram positive anti-bacterial activity and Gram negative antibacterial activity, respectively. All the inoculated plates were incubated at 35°C and results were evaluated after 18-20h. The lowest concentration of the compounds that prevented visible growth was considered to be minimal inhibitor concentrations (MIC_S).

3. Results

The syntheses (scheme 1) involve the reaction of acyl halides, ammonium thiocyanate and aromatic primary amines in acetone (scheme 1). The newly synthesized compounds were characterized by FT-IR, and ^{1}HNMR spectral data.

Six of the newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Streptococcus pyogenes, Bacillus cereus, Esherichia coli, Pesudomonas aeruginosa, Enterobacter cloacae, Proteus vulgaris* and *Enterobacter aerogenes* organisms. Broth micro dilution procedure was used for determination of the preliminary antibacterial activities. The Gram positive antibacterial agent, amikacin, the Gram negative antibacterial agent, gentamycin were used as controls. The *in vitro* antibacterial properties against a number of

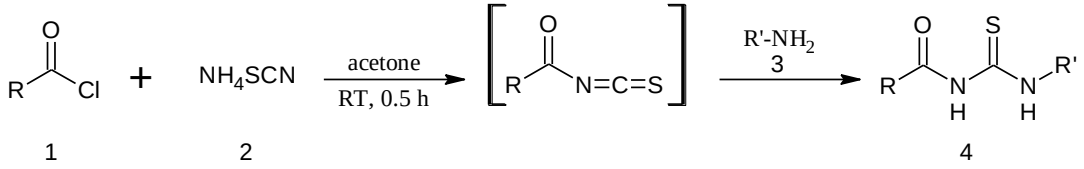
Gram positive and Gram negative bacteria of synthesized thiourea derivative 4(a-f) are presented in Tables 1-2, respectively.

According to the antibacterial studies, the efficacy against Gram positive bacterial is higher than Gram negative bacteria. The investigated compound antibacterial activity values in this research were higher than that reported of other thiourea derivatives the main difference in the thiourea derivatives reported in this paper is the presence of the Fluro moiety. 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.

Compound with benzyl groups have more lipophilic character as other compounds.

All compounds inhibited the growth of bacteria with MIC values ranging between 6.25 and 400 $^{\mu g}/_{mL}$. Regarding the activity of the thiourea derivatives against bacteria, the results revealed that compounds 4d and 4f exhibited brood spectrum of antibacterial profile against the rested organisms.

Although MIC values for some compounds are good unfortunately, the antibacterial activity values of all tested compounds are lower than the reference compounds, thus these compounds cannot be suggested for clinical use.



1 2 4

4	R	R'	mp (°C)	Yield (%)
a	2-F-C_6H_4	Bn	81-82	90
b	2-F-C_6H_4	4-Et-C_6H_4	79-81	79
c	2-F-C_6H_4	2-Me-C_6H_4	68-70	82
d	4-Cl-C_6H_4	Bn	214-216	79
e	4-F-C_6H_4	Ph	89-91	96
f	4-NO_2-C_6H_4	Bn	222-224	91

Scheme 1: Synthesis of the compounds and results

Table 1. MIC values ($^{Mg}/_{mL}$) of the compounds 4(a-f) aginst the tested Gram positive bacteria.

Compound	*S. aureus* (ATCC 25923)	*S. epidermidis* (ATCC 12228)	*E. faecalis* (ATCC 29212)	*S. pyogenes* (Clinical isolate)	*B. cereus* (Clinical isolate)
4a	200	200	200	200	200
4b	300	400	200	200	100
4c	100	200	100	200	100
4d	25	12.5	6.25	25	12.5
4e	50	100	50	100	100
4f	25	12.5	12.5	12.5	6.25
Amikacin	2	0.5	4	1	2

Table 2. MIC values ($^{Mg}/_{mL}$) of the compounds 4(a-f) aginst the tested Gram negative bacteria.

Compound	*E. coli* (ATCC 25922)	*P. aeruginosa* (ATCC 27853)	*E. cloacae* (ATCC 13047)	*P. vulgaria* (ATCC 13315)	*E. aerogenes* (Clinical isolate)
4a	300	300	200	200	300
4b	300	400	300	200	200
4c	200	400	300	200	200
4d	6.25	12.5	12.5	12.5	6.25
4e	100	100	50	100	100
4f	12.5	25	12.5	6.25	12.5
Gentamycin	0.5	1	2	2	0.5

Conclusion

In conclusion, the objective of the present study was to synthesize and investigate the antibacterial activities of some new thiourea derivatives as potent antibacterial agents. The obtained results clearly revealed that some of the newly synthesized compounds exhibited better antibacterial activity.

Acknowledgment

The author is so grateful to the Laboratory complex of I. A. U. Tonekabon branch for valuable technical assistance.

Refereces

Arslan, H., Florke, U., Kulcu, N., Emen, M.F., 2006. Crystal structure and thermal behaviour of copper (II) and zinc(II) complexes with N-pyrrolidine-*N*'-(2-chloro-benzoyl)thiourea. J. Coord. Chem. 59, 223-228.

Arslan, H., Kulcu, N., Florke, U., 2003. Synthesis and characterization of copper (II), nickel (II) and cobalt (II) complexes with novel thiourea derivatives. Transit. Metal Chem. 28, 816-819.

Binzet, G., Arslan, H., Florke, U., Kulcu, N., Duran, N., 2006. Synthesis, characterization and antimicrobial activities of transition metal complexes of N, N-dialkyl-N'-(2-chloro-benzoyl)thiourea derivatives. J. Coord. Chem. 59, 1395-1406.

Emen, M.F., Arslan, H., Kulcu, N., Florke, U., Duran, N., 2005. Synthesis, characterization and antimicrobial activities of some metal complexes with *N*'-(2-chloro-benzoyl) thiourea ligands: The crystal structure of fac-[CoL3] and cis-[PdL_2]. Pol. J. Chem. 79, 1615-1626.

Eweis, M., Elkholy, S.S., Elsabee, M.Z., 2006. Antifungal efficacy of chitosan and its thiourea derivatives upon the growth of some sugar-beet pathogens. Int. J. Biol. Macromol. 38, 1-8.

Henderson, W., Kemmitt, R.D.W., Mason, S., Moore, M.R., Russell, D.R., 1992. Thia- diaza trimethyl enemethane and N,N',P-Tri phenyl phosphonothioic Diamide Complexes of Platinum (Ii). J. Chem. Soc., Dalton Trans. 1, 59-66.

Henderson, W., Nicholson, B.K., Dinger, M.B., Bennett, R.L., 2002. Thiourea monoanion and dianion complexes of rhodium (III) and ruthenium (II). Inorg. Chim. Acta. 338, 210-218.

Lipowska, M., Hayes, B.L., Hansen, L., Taylor, A., Marzilli, L.G., 1996. Rhenium (V) oxo complexes of novel N_2S_2 dithiourea (DTU) chelate ligands: Synthesis and structural characterization. Inorg. Chem. 35, 4227-4231.

Mansuroglu, D.S., Arslan, H., Florke, U., Kulcu, N., 2008. Synthesis and characterization of nickel and copper complexes with 2,2-diphenyl-N-(alkyl(aryl)carbamothioyl) acetamide: The crystal structures of HL1 and cis-[Ni(L-1)(2)]. J. Coord. Chem. 61, 3134-3146.

National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, Approved Standard M7-A4. NCCLS: Viallanova, PA, USA, (1997).

Ozer, C.K., Arslan, H., VanDerveer, D., Binzet, G., 2009. Synthesis and characterization of N-(alky(aryl)carbamothioyl)cyclohexanecarboxamide derivatives and their Ni(II) and Cu(II) complexes. J. Coord. Chem. 62, 266-276.

Sacht, C., Datt, M.S., Otto, S., Roodt, A., 2000. Synthesis, characterisation and coordination chemistry of novel chiral N,N-dialkyl-N-menthyloxycarbonylthioureas. Crystal and molecular structures of N,N-diethyl-N-(-)-(3R)-menthyloxycarbonylthiourea and cis-(S,S)-N-(+)-(3R)-menthyloxy carbonyl-N'-morpholino thiourea or N-benzoyl-N',N'-diethylthiourea]. J. Chem. Soc. Dalton Trans. 24, 4579-4586.

Ugur, D., Arslan, H., Kulcu, N., 2006. Synthesis, characterization and thermal behavior of 1,1-dialkyl- 3-(4-(3,3-dialkylthioureidocarbonyl) benzoyl)thiourea and its Cu(II), Ni(II), and Co(II) complexes. Russ. J. Coord. Chem. 32, 669-675.

Yuan, F., Wang, T., Gimeno, C., Laguna, A., Jones, P.G., 2001. Synthesis and characterisation of copper complexes with N-ferrocenoyl-N '(alkyl) thioureas. Inorg. Chim. Acta. 324, 309- 317.

Zhang, Y.M., Wei, T.B., Wang, X.C., Yang, S.Y., 1998. Synthesis and biological activity of N-aroyl-N'-carboxyalkyl thiourea derivatives. *Indian J. Chem. Sect B.* 37, 604-606.

Zhang, Y.M., Wei, T.B., Xian, L., Gao, L.M., 2004. An efficient synthesis of polymethylene-bis-aroyl thiourea derivatives under the condition of phase-transfer catalysis. Phosphorus Sulfur Silicon Relat. Elem. 179, 2007-2013.

Zhou, W.Q., Li, B.L., Zhu, L.M., Ding, J.G., Yong, Z., Lu, L., Yang, X.J., 2004. Structural and spectral studies on N-(4-chloro) benzoyl-N'-(4-tolyl) thiourea. J. Mol. Str. 690, 145-150.

Zuckerman, R.L., Bergman, R.G., 2000. Structural factors that influence the course of overall [2+2] cycloaddition reactions between midozirconocene complexes and heterocumulenes.Organometallics. 19, 4795-4809.