ISSN: 0974 - 7516 Volume 8 Issue 3

Trade Science Inc.

Organic CHEMISTRY

An Indian Journal

Full Paper

OCAIJ, 8(3), 2012 [103-111]

Synthesis and antimicrobial studies on some new 3-chloro-1-benzothiophene-2-carbonylchloride derivatives

Gadada Naganagowda*, Amorn Petsom

Research Center for Bioorganic Chemistry (RCBC), Department of Chemistry, Faculty of Science, Chulalongkorn University, 254 Phayathai Road, Bangkok 10330, (THAILAND)

E-mail : amorn.p@chula.ac.th; ngchula.pdf@gmail.com

Received: 20th July, 2011 ; Accepted: 20th August, 2011

ABSTRACT

2-(3-Chloro-1-benzothiophen-2-yl)-2H-benzotriazole 5 has been synthesized in a three step procedure starting from 3-chloro-N-(2-nitrophenyl)-1-benzothiophene-2-carboximidoylchloride via a 5-(3-chloro-1-benzothiophen-2-yl)-1-(2-nitrophenyl)-1H-tetrazole intermediate. 3-Chloro-N-(3-chloro-4-fluorophenyl)-1-benzothiophene-2-carboximidoylisothiocyanate was treated with substituted amines and phenols to get compounds 9a-g and 10a-d, separately. The structures of all the synthesized compounds were confirmed by spectral data and have been screened for antibacterial and antifungal activity.

KEYWORDS

Benzothiophene;
Benzotriazole;
Carboximidamide;
Antimicrobial activity.

INTRODUCTION

Heterocyclic compounds containing sulfur and nitrogen atoms are considered to be one of the most effective antimicrobial drugs used either as single agents or in combination for cancer therapy[1, 2]. Recent study showed that several benzotriazole and triazole derivatives represented an interesting class of heterocycles[3] and became the most rapidly expanding group of antifungal compounds with the advantage of low toxicity, high oral bioavailability and broad spectrum activity[4-6]. Moreover, a variety of benzotriazoles have been reported to inhibit the growth of some microorganisms and some benzotriazole derivatives show anti-inflammatory properties[7].

Multiple antiretroviral agents have been produced to block replication of the HIV-1 virus by blocking HIV reverse transcriptase[8, 9] or by blocking HIV protease[10]. Among the most important antiretroviral agents recently introduced are the non-nucleoside reverse transcriptase inhibitors (NNRTI), such as nevirapine[11] and delavirdine[12], which are able to reduce reverse transcriptase inhibition to subnanomolar concentrations. Several heterocyclic thioureas have been reported as a new class of potent NNRTIs such as phenethylthiazolylthiourea(PETT1) derivatives[13-16] (Figure-1).

[17]described the synthesis of a series of thiazolethioureas with alkyl, aryl, and heteroaryl substituents as newly identified NNRTIs of HIV, including mutant strains of HIV, that are effective in the treatment of multidrug-resistant HIV infection. Fathala and Pazdera have recently reported a new and efficient synthesis of novel quinazoline thioureas based on the Domino reaction[18]. On the other hand, benzothiophenes are of current interest due to their wide spectrum of pharmacological properties such as anti-allergic, anti-inflammatory, analgesic, antitubercular, antimicrobial, ocular hypotensive, and serotonine N-

Full Paper

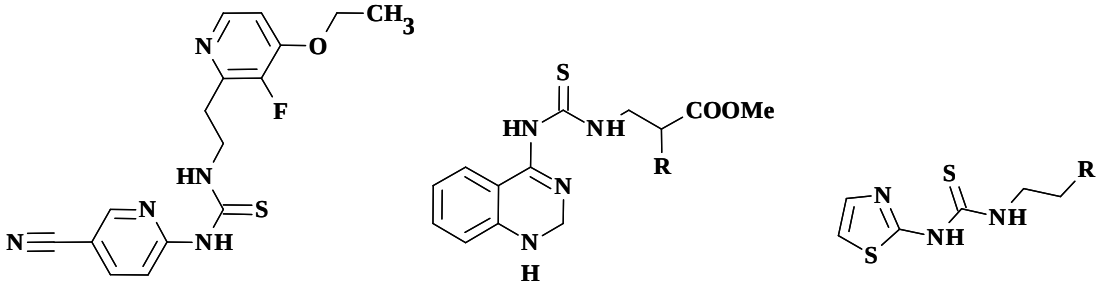
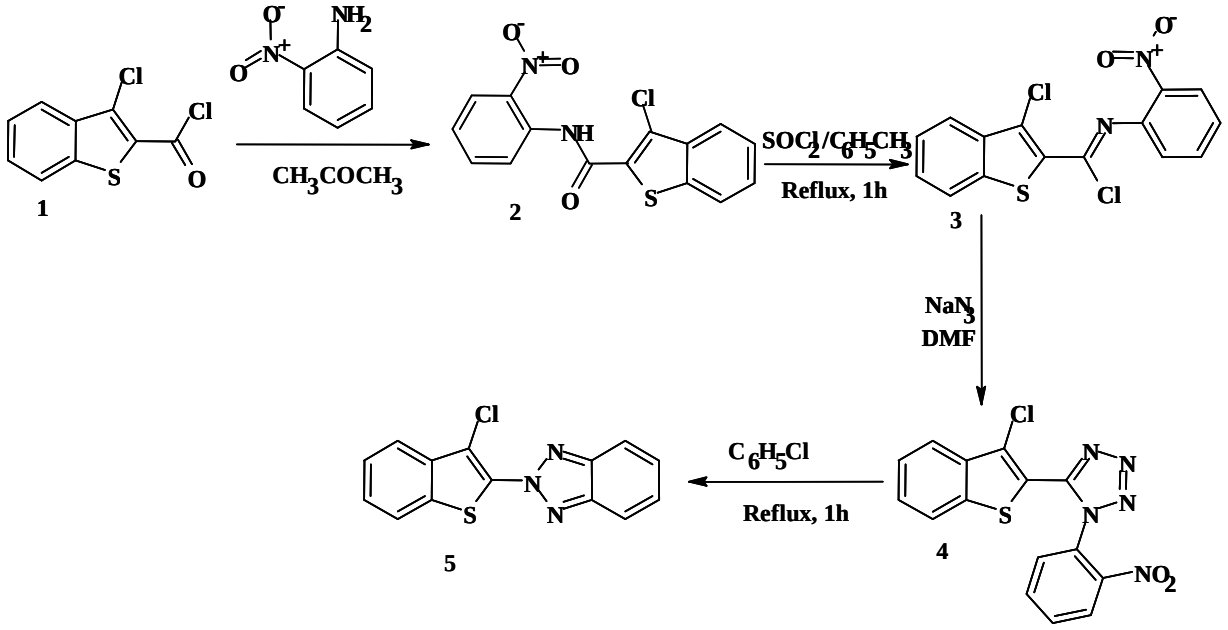


Figure : 1

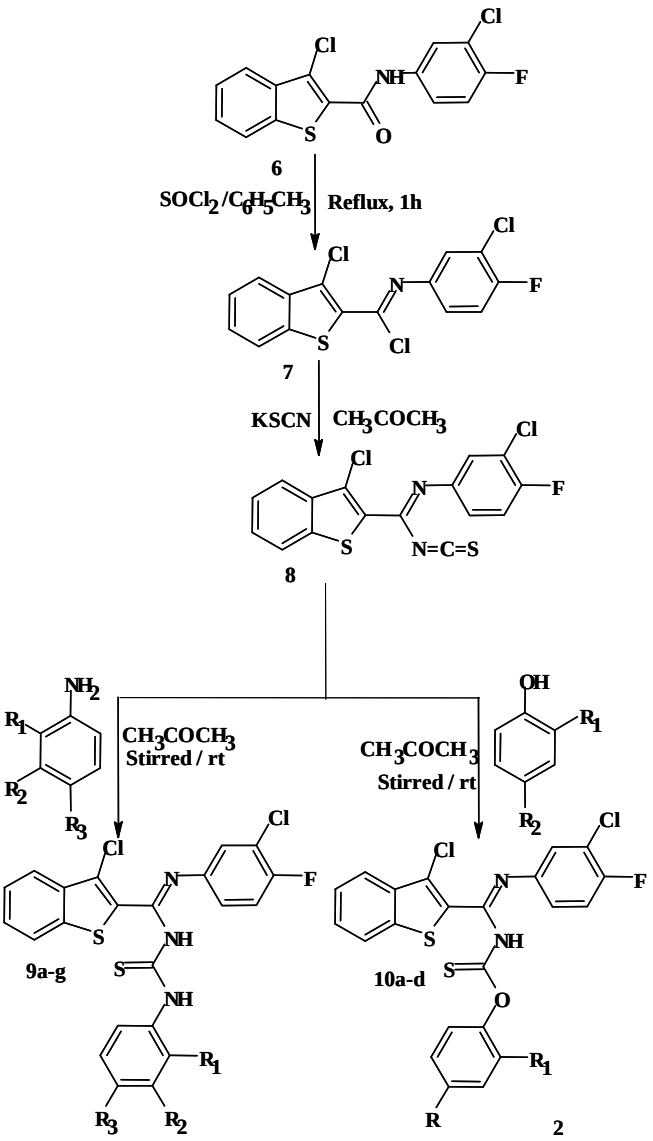
acetyltransferase inhibitors activities[19-23]. A drug based on the benzothiophene ring is raloxifene, approved by the U.S. Food and Drug Administration for the prevention and treatment of osteoporosis associated with postmenopausal women[24]. In view of these factors, it was thought important to synthesize a benzothiophene-incorporated biheterocycles to explore its biological efficacy.

RESULTS AND DISCUSSION

The preparation of the target carboxamide started from the amidation of 3-chlorobenzothiophene-2-carbonylchloride 1, which was prepared by the reaction of cinnamic acid with thionyl chloride in DMF and pyridine according to the reported method[25] and o-nitroaniline in dry acetone. The resulting carboxamide 2 exhibited N-H stretching absorption in its IR spectrum at 3215 cm^{-1} and C=O stretching at 1712 cm^{-1}. The ^{1}H-NMR spectrum of compound 2 exhibited a broad peak at 10.21 δ ppm due to -CONH proton (D_2O exchangeable) and a multiplet in between 8.09-7.63 δ ppm for eight aromatic protons. In addition, compound 2 exhibited a molecular ion peak at m/z 332.76 in its mass spectrum which confirmed for formation. Compound 2 was refluxed with thionyl chloride in toluene to get compound 3. Compound 3 exhibited a peaks at 672 cm^{-1} in its IR spectrum confirming the formation of a C-Cl bond. Compound 3 on cyclocondensation with sodium azide in DMF gave compound 4, which exhibited peaks at 1625 and 1429 cm^{-1} due to C=N and NO_2 stretching absorption frequencies, respectively. Compound 4 exhibited a molecular ion peak at m/z 357.77 in its mass spectrum



Scheme 1 : Synthesis of benzothiophen-2-yl-2*H*-benzotriazole 5.



Compound	R_1	R_2	R_3	Compound	R	R_2
9a	-F	-H	-H	10a	-H	-H
9b	-F	-H	-C1	10b	-H	$-CH_3$
9c	$-NO_2$	-H	-H	10c	$-NO_2$	-H
9d	-H	-C1	-H	10d	-H	$-NO_2$
9e	-C1	-H	-H			
9f	-Br	-H	-H			
9g	$-CF_3$	-H	-H			

Scheme 2 : Synthesis of benzothiophene-2-carboximidamide 9a-g and 10a-d.

which confirmed its formation. 3-Chloro-1-benzothiophen-2-yl)-1-(2-nitrophenyl)-1*H*-tetrazole was refluxed with nitrobenzene to get compound 5. The absence of an NO_2 absorption in its IR spectrum confirmed the formation of 5 (Scheme-1).

In Scheme-2, A mixture of compound 1 and 3-chloro-4-fluoroaniline in dry acetone gave compound 6. The ^{1}H-NMR spectrum of compound 6 exhibited a multiplet in the region between 8.68-7.98 δ ppm due to seven aromatic protons and a singlet at 10.24 δ ppm which corresponds to the one proton of the CONH group. Finally, compound 6 produced a molecular ion peak at m/z 340.19 which is in accordance with the structure. Benzothiophene-2-carboxamide was refluxed with thionyl chloride in toluene to give carboximidoylchloride 7. The absence of a CONH peak in its ^{1}H-NMR spectrum is consistent with its formation. Compound 8 was slowly added to 4-fluoroaniline to get compound 9a. The IR spectrum of compound 9a showed absorption bands at 3120, 1620 and 1320 cm^{-1} due to N-H, C=N and C=S stretching absorption frequencies, respectively. The ^{1}H-NMR spectrum exhibited a multiplet in the region from 8.09-7.69 δ ppm due to eleven aromatic protons, two singlets at 12.63 and 2.05 δ ppm corresponding to two protons of the NH groups. As an additional proof, the mass spectrum of compound 9a exhibited a molecular ion peak at m/z 492.39. Compound 8 was stirred with phenol to get compound 10a. As anticipated the IR spectrum of compound 10a exhibited peaks at 3123, 1623 and 1328 cm^{-1} corresponding to NH, C=N and C=S stretching absorption frequencies, respectively. The ^{1}H-NMR spectrum of compound 10a exhibited a multiplet in the region between 8.68-7.38 δ ppm due to twelve aromatic protons and singlet at 2.09 δ ppm which corresponds to the one proton of the NH group. Finally, compound 10a produced a molecular ion peak at m/z 475.38 which is in accordance with the structure.

BIOLOGICAL EVALUATION

Antibacterial activity

A Cup plate method using Hi-Media agar medium was employed to study the antibacterial activity of the synthesized compounds against two Gram-positive bacteria, *Staphylococcus aureus*-ATCC 25923 and *Bacillus subtilis*-ATCC 6633 and Gram-negative bacteria, *Pseudomonas aeruginosa*-ATCC 10145 and *Escherichia coli*-ATCC 35218. Preparation of nutrient broth, subculture, base layer medium, agar medium and

peptone water is done as per the standard procedure[26]. Each test compound (50 mg) was dissolved in dimethyl formamide (50 mL, 1000 µg/mL) to obtain a sample solution. Sample volume for all the compounds was fixed at 0.1 mL. The cups were made by scooping out agar medium with a sterilized cork borer in a petri dish, which was previously inoculated with the microorganisms. The solution of each test compound (0.1 mL) was added to the cups and petri dishes and were subsequently incubated at 37°C for 48 h. Chloramphenicol and Streptomycin were used as reference drugs and dimethyl formamide as a control. The zone of inhibition produced by each compound was measured in mm. As shown in the TABLE 1, the tested compounds showed slightly to moderate antibacterial activity compared to standard drugs against each microorganism.

Antifungal activity

The antifungal activity of the synthesized compounds was tested against four different fungi, *i.e. Candida albicans, Crysosporium pannical, Aspergillus niger* and *Rhizopus oryzae* by a filter paper disc technique[27]. The concentration of test compounds was 1000 µg/mL. After treatment for 48 h the zone of inhibition produced by each compound was measured in mm. Griseofulvin was used as the standard antifungal agent and dimethyl formamide as a control. The results of antifungal activity are depicted in TABLE 2. Tested compounds showed slight to moderate antifungal activity.

TABLE 1 : Antibacterial activity of the tested compounds

Compound	Zone of inhibition (mm)			
	Gram positive bacteria		Gram negative bacteria	
	S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
2	13	14	14	14
3	12	15	13	14
4	11	15	13	13
5	14	15	15	11
6	10	14	15	11
7	15	13	13	15
9a	13	15	15	13
9c	13	14	12	14
9d	13	16	12	15
9f	14	12	10	11
9g	14	14	14	12
10a	16	13	15	15
10b	15	15	13	13
10c	13	11	11	11
Control(DMF)	00	00	00	00
Chloramphenicol	24	25	20	22
Streptomycin	23	21	18	20

TABLE 2 : Antifungal activity of the tested compounds

Compound	Zone of inhibition (mm)			
	C. albicans	*C. pannical*	*A.niger*	*R. oryzae*
2	12	17	15	15
3	12	11	11	11
4	11	15	15	15
5	15	10	10	10
6	10	17	17	17
7	15	15	11	12
9a	14	11	11	11
9c	14	13	14	14
9d	16	12	11	13
9f	12	11	12	14
9g	12	15	14	11
10a	13	10	13	13
10b	12	17	15	15
10c	15	12	12	15
Control(DMF)	00	00	00	00
Griseofulvin	24	25	23	22

EXPERIMENTAL

All chemicals were analytical grade, purchased from commercial suppliers and used as received without further purification. Melting points were determined in open capillary and were uncorrected. FT-IR spectra were recorded on a Nicolet Fourier Transform Infrared Spectrophotometer: Impact 410 (Nicolet Instrument Technologies, Inc. WI, USA). Infrared spectra were recorded between 400 cm^{-1} to 4,000 cm^{-1} in transmittance mode. ^{1}H-NMR and ^{13}C-NMR were obtained in DMSO-d_6 at 400 MHz for ^{1}H nuclei and 100 MHz for ^{13}C nuclei (Varian Company, USA). All chemical shifts were reported in parts per million (ppm) using residual proton or carbon signal in deuterated solvents as internal references. Mass spectra were obtained using matrix-assisted laser desorption ionization mass spectrometry (MALDI-TOF) by using dithranol as a matrix. Elemental analysis (C, H, N and S) was per-

formed on Perkin Elmer 240 analyzer. The purity of the compound was checked by TLC on silica gel and further purification was performed through column chromatography (silica gel, 60–120 mesh).

Preparation of 3-chloro-1-benzothiophene-2-carbonylchloride (1)

Compound 1 was prepared according to the literature procedure[25], mp. 112-114^{0}C (Lit. mp. 110-112^{0}C)

Preparation of 3-chloro-N-(2-nitrophenyl)-1-benzothiophene-2-carboxamide (2)

Compound 1 (2.31 g, 0.01 mol) was treated with o-nitroaniline (1.38 g, 0.01 mol) in dry acetone (25 mL) and stirred for 1 h at room temperature, the precipitate was filtered, washed with water, dried and recrystallized from 1,4-dioxane to get pure compound 2.

Yield 65%; mp 221-223^{0}C; IR υ (cm^{-1}): 3215 (N-H), 1712 (C=O), 1572 (C=C), 1423 (NO_2), 1074 (=C-Cl), 685 (C-S-C); ^{1}H-NMR δ (ppm): 10.21 (s, 1H, CONH), 8.09-7.63 (m, 8H, Ar-CH); ^{13}C-NMR δ (ppm): 161.8, 142.4, 141.6, 135.9, 133.4, 131.2, 131.1, 129.9, 126.7, 125.3, 125.2, 124.4, 124.3, 122.8, 118.4; MS, m/z: 332.76 (M^+). Anal. calcd. for $C_{15}H_9ClN_2O_3S$: C, 54.14; H, 2.73; N, 8.42; S, 9.64; found: C, 54.08; H, 2.70; N, 8.38; S, 9.59%.

Preparation of 3-chloro-N-(2-nitrophenyl)-1-benzothiophene-2-carboximidoylchloride (3)

A mixture of compound 2 (3.32 g, 0.01 mol) and thionyl chloride (1.18 g, 1.02 mL, 0.01 mol) in toluene (20 mL) was refluxed for 1 h, after which the hot solution was filtered and the solvent removed under reduced pressure to get compound 3 as light yellow color solid.

Yield 60%; mp 267-269^{0}C; IR υ (cm^{-1}): 1620 (C=N), 1420 (NO_2), 672 (C-Cl); ^{1}H-NMR δ (ppm): 8.19-7.81 (m, 8H, Ar-CH); ^{13}C-NMR δ (ppm): 146.7, 139.6, 135.9, 135.6, 135.5, 131.7, 125.9, 124.4, 124.3, 123.2, 122.8, 120.6, 118.0, 114.4, 73.8; MS, m/z: 351.20 (M^+). Anal. calcd. for $C_{15}H_8Cl_2N_2O_2S$: C, 51.30; H, 2.30; N, 7.98; S, 9.13; found: C, 51.26; H, 2.25; N, 7.93; S, 9.10%.

Preparation of 5-(3-chloro-1-benzothiophen-2-yl)-1-(2-nitrophenyl)-1H-tetrazole (4)

Compound 3 (3.51 g, 0.01 mol) was dissolved in dry DMF (20 mL) and added dropwise to a vigorously stirred mixture of sodium azide (0.65 g, 0.01 mol) and dry DMF (10 mL) over a period of 45 min, the temperature being kept below 25ºC throughout. The suspension thus obtained was heated at 80ºC for 1 h and then cooled. Next, enough water to dissolve any inorganic salts present and cause turbidity was added and the mixture stored refrigerated at 5ºC for one day. The crystals thus formed were filtered off and washed with water to obtain compound 4.

Yield 74%; mp 272-274^{0}C; IR υ (cm^{-1}): 1625 (C=N), 1429 (NO_2); ^{1}H-NMR δ (ppm): 8.33-7.78 (m, 8H, Ar-CH); ^{13}C-NMR δ (ppm): 146.2, 136.1, 134.1, 130.3, 130.1, 129.3, 125.1, 124.4, 124.3, 123.2, 122.8, 122.3, 122.3, 141.7; MS, m/z: 357.77 (M^+). Anal. calcd. for $C_{15}H_8ClN_5O_2S$: C, 50.36; H, 2.25; N, 19.57; S, 8.96; found: C, 50.30; H, 2.21; N, 19.54; S, 8.92%.

Preparation of 2-(3-chloro-1-benzothiophen-2-yl)-2H-benzotriazole (5)

Compound 4 (2g) and freshly distilled nitrobenzene (20 mL) were heated together under vigorous reflux for 1 h. After the reaction mixture was cooled, the nitrobenzene was removed by vacuum distillation and the residue purified by column chromatography [silica-gel, petroleum ether/ethyl acetate (95:5)] yielded pure 5.

Yield 71%; mp 296-298^{0}C; IR υ (cm^{-1}): 1623 (C=N); ^{1}H-NMR δ (ppm): 8.38-8.01 (m, 8H, Ar-CH); ^{13}C-NMR δ (ppm): 143.0, 143.0, 141.8, 136.1, 126.2, 126.2, 124.4, 124.3, 123.2, 122.8, 122.4, 122.4; MS, m/z: 285.75 (M^+). Anal. calcd. for $C_{14}H_8ClN_3S$: C, 58.84; H, 2.82; N, 14.71; S, 11.22; found: C, 58.82; H, 2.78; N, 14.69; S, 11.16%.

Preparation of 3-chloro-N - (3 - chloro - 4 - fluorophenyl)-1-benzothiophene-2-carboxamide (6)

A mixture of compound 1 (2.31 g, 0.01 mol) and 3-chloro-4-fluoroaniline (1.37 g, 0.01 mol) in dry acetone (20 mL) was refluxed for 1 h. After the completion of the reaction (TLC-monitoring), the reaction mixture was cooled down to room temperature and then poured into crushed ice. The precipitate was filtered, dried and recrystallized from 1,4-dioxane to get compound 6 as a light yellow solid.

Yield 83%; mp 303-307^{0}C; IR υ (cm^{-1}): 3212 (N-

H), 1713 (C=O), 890 (C-F); ^{1}H-NMR δ (ppm): 10.24 (s, 1H, CONH), 8.64-7.98 (m, 7H, Ar-CH); ^{13}C-NMR δ (ppm): 154.4, 141.6, 135.9, 134.5, 133.4, 129.9, 126.7, 124.4, 124.3, 124.2, 123.6, 122.8, 120.9, 113.6, 61.6; MS, m/z: 340.19 (M^+). Anal. calcd. for $C_{15}H_8Cl_2FNOS$: C, 52.96; H, 2.37; N, 4.12; S, 9.43; found: C, 52.90; H, 2.34; N, 4.04; S, 9.41%.

Preparation of 3 - chloro - N - (3 - chloro - 4 - fluorophenyl)-1-benzothiophene-2-carboximi doylchloride (7)

A mixture of 3-chloro-*N*-(2-nitrophenyl)-1-benzothiophene-2-carboxamide (3.58 g, 0.01 mol) and thionyl chloride (1.18 g, 1.03 mL, 0.01 mol) in toluene (20 mL) was refluxed for 1 h, after which the hot solution was filtered and the solvent removed under reduced pressure to give carboximidoyl chloride 7.

Yield 74%; mp 287-289^{0}C; IR υ (cm^{-1}): 1634 (C=N), 894 (C-F); ^{1}H-NMR δ (ppm): 8.16-7.45 (m, 7H, Ar-CH); ^{13}C-NMR δ (ppm): 164, 157.3, 146.0, 131.6, 126.7, 125.9, 124.4, 124.3, 124.2, 122.8, 122, 122.0, 122.0, 119.9, 118.2; MS, m/z: 358.64 (M^+). Anal. calcd. for $C_{15}H_7Cl_3FNS$: C, 50.23; H, 1.97; N, 3.91; S, 8.94; found: C, 50.18; H, 1.94; N, 3.89; S, 8.89%.

Preparation of 3 - chloro - N - (3 - chloro - 4 - fluorophenyl) - 1 -benzothiophene-2-carboxim idoylisothiocyanate (8)

Potassium thiocyanate (0.76 g, 0.01 mol) in dry acetone (10 mL) was slowly added dropwise at 0^{0}C to 3-chloro-1-benzothiophene-2-carbonylchloride 1 (2.31 g, 0.01 mol) in dry acetone (10 mL) with continuous stirring and then filtered to get carboximidoyl isothiocyanate 8 as a light yellowish liquid.

General procedure for synthesis of compounds 9a-g.

Exemplary detail for 3-chloro-N'-(3-chloro-4-fluorophenyl)-N-[(4-fluorophenyl)carbamothioyl]-1-benzothiophene-2-carboximidamide (9a)

Potassium thiocyanate (0.76 g, 0.01 mol) in dry acetone (10 mL) was slowly added dropwise at 0^{0}C to an 3-chloro-1-benzothiophene-2-carbonylchloride 1 (2.31 g, 0.01 mol) in dry acetone (10 mL) with continuous stirring and then filtered. The filtrate was slowly added at room temperature to 4-fluoroaniline (0.94 g, 0.75 mL, 0.01 mol) in dry acetone (10 mL) with continuous stirring thereafter, two-third of the solvent was distilled under vacuum. The concentrate was left overnight in a refrigerator. The solid product was filtered off, washed with water and recrystallised from methanol and purified through column chromatography by using petroleum ether and ethyl acetate (5:95) as an eluent to get pure 9a. Compounds 9b-g were prepared in a similar manner.

Yield 60%; mp 261-263^{0}C; IR υ (cm^{-1}): 3130 (N-H), 1620 (C=N), 1320 (C=S), 770 (C-F), 680 (C-Cl); ^{1}H-NMR δ (ppm): 12.63 (s, 1H, NH), 8.09-7.69 (m, 11H, Ar-CH), 2.05 (s, 1H, NH); ^{13}C-NMR δ (ppm): 177.4, 163.2, 146.1, 157.3, 134.1, 131.6, 131.0, 131.0, 126.7, 125.9, 124.4, 124.3, 124.2, 122.8, 122, 122.0, 122.0, 118.2, 115.5, 115.5; MS, m/z: 492.39 (M^+). Anal. calcd. for $C_{22}H_{13}Cl_2F_2N_3S_2$: C, 53.66; H, 2.66; N, 8.53; S, 13.02; found: C, 53.61; H, 2.60; N, 8.50; S, 13.00%.

3-Chloro-N'-(3-chloro-4-fluorophenyl)-N-[(3-chloro-4-fluorophenyl)carbamothioyl]-1-benzothiophene-2-carboximidamide (9b).

Yield 62%; mp 255-257^{0}C; IR υ (cm^{-1}): 3133 (N-H), 1623 (C=N), 1321 (C=S), 774 (C-F), 683 (C-Cl); ^{1}H-NMR δ (ppm): 12.58 (s, 1H, NH), 8.12-7.77 (m, 12H, Ar-CH), 2.03 (s, 1H, NH); ^{13}C-NMR δ (ppm): 177.1, 157.3, 154.8, 146.0, 134.1, 131.6, 129.1, 128.5, 126.7, 125.9, 124.4, 124.3, 124.2, 122.8, 122.8, 122.0, 122.0, 121.1, 118.2, 113.7; MS, m/z: 526.83 (M^+). Anal. calcd. for $C_{22}H_{12}Cl_3F_2N_3S_2$: C, 50.16; H, 2.30; N, 7.98; S, 12.17; found: C, 50.13; H, 2.22; N, 7.90; S, 12.14%.

3-Chloro-N'-(3-chloro-4-fluorophenyl)-N-[(4-nitrophenyl)carbamothioyl]-1-benzothiophene-2-carboximidamide (9c).

Yield 66%; mp 214-216^{0}C; IR υ (cm^{-1}): 3138 (N-H), 1621 (C=N), 1316 (C=S), 773 (C-F), 687 (C-Cl); ^{1}H-NMR δ (ppm): 12.50 (s, 1H, NH), 8.09-7.65 (m, 11H, Ar-CH), 2.08 (s, 1H, NH); ^{13}C-NMR δ (ppm): 177.8, 157.3, 146.0, 144.6, 143.9, 131.6, 126.7, 125.9, 124.8, 124.8, 124.4, 124.3, 124.2, 124.2, 124.2, 122.8, 122.7, 122.2, 122.2, 118.2; MS, m/z: 519.39 (M^+). Anal. calcd. for $C_{22}H_{13}Cl_2FN_4O_2S_2$: C, 50.87; H, 2.52; N, 10.79; S, 12.35; found: C, 50.83;

H, 2.48; N, 10.76; S, 12.31%.

3-Chloro-N'-(3-chloro-4-fluorophenyl)-N-[(2-chlorophenyl)carbamothioyl]-1-benzothiophene-2-carboximidamide (9d).

Yield 55%; mp 228-230^{0}C; IR υ (cm^{-1}): 3136 (N-H), 1622 (C=N), 1315 (C=S), 772 (C-F), 686 (C-Cl); ^{1}H-NMR δ (ppm): 12.48 (s, 1H, NH), 8.19-7.09 (m, 11H, Ar-CH), 2.06 (s, 1H, NH); ^{13}C-NMR δ (ppm): 177.7, 157.3, 146.0, 136.6, 133.7, 131.6, 131.1, 131.1, 129.1, 129.1, 126.7, 125.9, 124.4, 124.3, 124.2, 122.8, 122.6, 122.3, 122.3, 118.2; MS, m/z: 508.84 (M$^+$). Anal. calcd. for $C_{22}H_{13}Cl_3FN_3S_2$: C, 51.93; H, 2.58; N, 8.26; S, 12.60; found: C, 51.89; H, 2.55; N, 8.21; S, 12.58%.

3-Chloro-N'-(3-chloro-4-fluorophenyl)-N-[(4-chlorophenyl)carbamothioyl]-1-benzothiophene-2-carboximidamide (9e).

Yield 60%; mp 296-298^{0}C; IR υ (cm^{-1}): 3128 (N-H), 1618 (C=N), 1308 (C=S), 771 (C-F), 683 (C-Cl); ^{1}H-NMR δ (ppm): 12.44 (s, 1H, NH), 8.45-7.35 (m, 11H, Ar-CH), 2.13 (s, 1H, NH); ^{13}C-NMR δ (ppm): 177.5, 157.3, 146.0, 136.6, 133.7, 131.6, 131.1, 131.1, 129.1, 129.1, 126.7, 125.9, 124.4, 124.3, 124.2, 122.8, 122.3, 122.3, 122.0, 118.2; MS, m/z: 508.84 (M$^+$). Anal. calcd. for $C_{22}H_{13}Cl_3FN_3S_2$: C, 51.93; H, 2.58; N, 8.26; S, 12.60; found: C, 51.90; H, 2.54; N, 8.22; S, 12.58%.

N-[(4-bromophenyl)carbamothioyl]-3-chloro-N'-(3-chloro-4-fluorophenyl)-1-benzothiophene-2-carboximidamide (9f).

Yield 65%; mp 291-293^{0}C; IR υ (cm^{-1}): 3125 (N-H), 1616 (C=N), 1317 (C=S), 777 (C-F), 689 (C-Cl); ^{1}H-NMR δ (ppm): 12.59 (s, 1H, NH), 8.34-7.91 (m, 11H, Ar-CH), 2.09 (s, 1H, NH); ^{13}C-NMR δ (ppm): 177.9, 157.3, 146.0, 137.5, 131.9, 131.9, 131.7, 131.7, 131.6, 126.7, 125.9, 124.4, 124.3, 124.2, 122.9, 122.8, 122.8, 122.7, 122.7, 118.7; MS, m/z: 553.29 (M$^+$). Anal. calcd. for $C_{22}H_{13}BrCl_2FN_3S_2$: C, 47.76; H, 2.37; N, 7.59; S, 11.59; found: C, 47.71; H, 2.36; N, 7.55; S, 11.56%.

3-Chloro-N'-(3-chloro-4-fluorophenyl)-N-{[4-trifluoromethyl)phenyl]carbamothioyl}-1-benzothiophene-2-carboximidamide (9g).

Yield 60%; mp 209-211^{0}C; IR υ (cm^{-1}): 3123 (N-H), 1619 (C=N), 1314 (C=S), 770 (C-F), 681 (C-Cl); ^{1}H-NMR δ (ppm): 12.53 (s, 1H, NH), 8.69-7.44 (m, 11H, Ar-CH), 2.10 (s, 1H, NH); ^{13}C-NMR δ (ppm): 177.4, 157.2, 146.1, 141.8, 132.5, 131.6, 126.8, 126.8, 126.7, 125.9, 125.4, 125.4, 124.4, 124.3, 124.2, 124.1, 122.8, 122.5, 122.3, 122.3, 119.4, 118.5; MS, m/z: 542.39 (M$^+$). Anal. calcd. for $C_{23}H_{13}Cl_2F_4N_3S_2$: C, 50.93; H, 2.42; N, 7.75; S, 11.82; found: C, 50.89; H, 2.39; N, 7.72; S, 11.78%.

General procedure for synthesis of compounds 10a-d.

Exemplary detail for phenyl-{(Z)-(3-chloro-1-benzothiophen-2-yl)-[(3-chloro-4-fluorophenyl) imino] methyl} carbamothioate (10a)

Potassium thiocyanate (0.76 g, 0.01 mol) in dry acetone (10 mL) was slowly added dropwise at 0^{0}C to 3-chloro-1-benzothiophene-2-carbonylchloride 1 (2.31 g, 0.01 mol) in dry acetone (10 mL) with continuous stirring and then filtered. The filtrate was slowly added at room temperature to phenol (0.94 g, 0.75 mL, 0.01mol) in acetone (10 mL) with continuous stirring thereafter, two-third of the solvent was distilled under vacuum. The concentrate was left overnight in a refrigerator. The solid product was filtered off, washed with water and recrystallized from methanol and purified through column chromatography by using petroleum ether and ethyl acetate (95:5) as an eluent to get pure compound 10a. Compounds 10b-d were prepared by a similar methodology.

Yield 79%; mp 212-214^{0}C; IR υ (cm^{-1}): 3123 (N-H), 1623 (C=N), 1328 (C=S), 773 (C-F), 684 (C-Cl); ^{1}H-NMR δ (ppm): 8.68-7.38 (m, 12H, Ar-CH), 2.09 (s, 1H, NH); ^{13}C-NMR δ (ppm): 157.3, 155.5, 146.0, 145.0, 131.6, 130.1, 130.1, 126.7, 125.9, 124.4, 124.3, 124.2, 122.8, 122.7, 122.3, 122.0, 119.9, 118.2, 115.8, 115.8; MS, m/z: 475.38 (M$^+$). Anal. calcd. for $C_{22}H_{13}Cl_2FN_2OS_2$: C, 55.58; H, 2.76; N, 5.89; S, 13.49; found: C, 55.57; H, 2.73; N, 5.85; S, 13.43%.

4-Methylphenyl) - {(Z) - (3 - chloro - 1 - benzothiophen - 2 - yl) [(3 - chloro - 4 - fluorophenyl) imino] methyl}carbamothioate (10b).

Yield 72%; mp 219-221^{0}C; IR υ (cm^{-1}): 3126 (N-H), 1626 (C=N), 1326 (C=S), 778 (C-F), 688 (C-

Cl); ^{1}H-NMR δ (ppm): 8.35-7.37 (m, 11H, Ar-CH), 2.08 (s, 1H, NH), 3.32 (s, 3H, CH_3); ^{13}C-NMR δ (ppm): 146.0, 152.5, 157.3, 145.0, 131.6, 131.0, 130.4, 130.4, 126.7, 125.9, 124.4, 124.3, 124.2, 122.8, 122.8, 122.2, 119.9, 118.2, 115.3, 115.3, 21.1; MS, m/z: 489.41 (M^+). Anal. calcd. For $C_{23}H_{15}Cl_2FN_2OS_2$: C, 56.44; H, 3.09; N, 5.72; S, 13.10; found: C, 56.38; H, 3.06; N, 5.70; S, 13.08%.

2- Nitrophenyl)-{(Z) - (3 - chloro - 1 - benzothiophen - 2 - yl)[(3 - chloro - 4 - fluorophenyl)imino] methyl}carbamothioate (10c).

Yield 66%; mp 222-224^{0}C; IR υ (cm^{-1}): 3138 (N-H), 1619 (C=N), 1325 (C=S), 768 (C-F), 679 (C-Cl); ^{1}H-NMR δ (ppm): 8.30-7.13 (m, 11H, Ar-CH), 2.09 (s, 1H, NH); ^{13}C-NMR δ (ppm): 164.8, 157.3, 146.0, 145.0, 140.5, 131.6, 126.7, 126.3, 126.3, 125.9, 124.4, 124.3, 124.2, 122.8, 122.7, 122.3, 119.9, 118.2, 116.3, 116.3; MS, m/z: 520.38 (M^+). Anal. calcd. for $C_{22}H_{12}Cl_2FN_3O_3S_2$: C, 50.78; H, 2.32; N, 8.07; S, 12.32; found: C, 50.73; H, 2.28; N, 8.30; S, 12.28%.

4-Nitrophenyl) - {(Z) - (3-chloro-1-benzothiophen-2-yl)[(3-chloro-4-fluorophenyl)imino] methyl}carbamothioate (10d).

Yield 68%; mp 219-221^{0}C; IR υ (cm^{-1}): 3126 (N-H), 1618 (C=N), 1331 (C=S), 767 (C-F), 683 (C-Cl); ^{1}H-NMR δ (ppm): 8.79-7.33 (m, 11H, Ar-CH), 2.10 (s, 1H, NH); ^{13}C-NMR δ (ppm): 161.3, 157.2, 146.0, 145.0, 140.5, 131.6, 126.7, 126.3, 126.3, 125.9, 124.4, 124.3, 124.2, 122.8, 122.2, 122.3, 119.9, 118.2, 116.6, 116.6; MS, m/z: 520.38 (M^+). Anal. calcd. for $C_{22}H_{12}Cl_2FN_3O_3S_2$: C, 50.78; H, 2.32; N, 8.07; S, 12.32; found: C, 50.73; H, 2.28; N, 8.02; S, 12.29%.

CONCLUSION

In conclusion, a new series of benzothiophene-containing heterocycles were synthesized, fully characterized and evaluated for their antibacterial and antifungal activities. The newly synthesized heterocyclics exhibited moderate antibacterial activity against *S. aureus, B. subtilis, P. aeruginosa* and *E. coli* and significant antifungal activity against *C. albicans, C. pannical, A. niger* and *R. oryzae.* It can be concluded that these classes of compounds certainly holds great promise towards good active leads in medicinal chemistry. A further study to acquire more information concerning pharmacological activity is in progress.

ACKNOWLEDGEMENT

This work was supported by the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education Commission (project no. AM1079). The post doctoral fellowship grant from the Ratchadapisakesompote Endownment Fund, Chulalongkorn University (to G.N.) was gratefully acknowledged.

REFERENCES

[1] K.Katarzyna, N.Zelika, Z.Justyna; Bioorg.Med. Chem., **12**, 2617-2628 **(2004)**.
[2] H.Q.Li, C.Xu, H.S.Li, Z.P.Xiao, L.Shi, H.L.Zhu; J.Med.Chem., **2**, 1361-1368 **(2007)**.
[3] G.Biagi, I.Giorgi, O.Livi, V.Scartoni, P.L.Barili; Calderone.Farmaco., **56**, 827-832 **(2001)**.
[4] G.Scapin, S.B.Patel, J.W.Becker, Q.Wang, C.Desponts, D.Waddleton, K.Skorey, W.Cromlish, C.Bayly, M.Therien, J.Y.Gauthier, C.S.Li, C.K.Lau, C.Ramachandran, B.P.Kennedy; Asante-Appiah,E.Biochemistry., **42**, 11451-11458 **(2003)**.
[5] C.Antonio, P.Michele, B.Gianpiero, B.Bernardetta, M.Marta, L.Roberta; Farmaco., 59637-59638 **(2004)**.
[6] L.Z.Xu, F.F.Jian, H.R.Gao, C.Y.Chong; Chin.J. Chem., **21**, 1615-1623 **(2003)**.
[7] M.B.Bushuev, A.V.Virovets, Y.Garcia, C.Gieck, L.A.Sheludyakova, V.N.Ikorskii, W.Tremel, P.Gütlich, L.G.Lavrenova; Polyhedron., **21**, 797-806 **(2002)**.
[8] K.Morris; Lancet., **350**, 1683-1689 **(1997)**.
[9] Y.El Safadi, V.Vivet-Boudou, R.Marquet; Appl.Microbiol.Biotechnol., **75**, 723-729 **(2007)**.
[10] A.K.Patick, K.E.Potts; Clin.Microbiol.Rev., **11**, 614-623 **(1998)**.
[11] E.De Clercq; Med.Res.Rev., **16**, 125-129 **(1996)**.
[12] D.L.Romero; R.A.Olmsted, T.J.Poel, R.A.Morge, C.Biles, B.J.Keiser, L.A.Kopta, J.M.Frils, J.D.Hosley, K.J.Stefanski, D.G.Wishka, D.B.Evans, J.Morris, R.G.Stehle, S.K.Sharma, Y.Yagi, R.L.Voorman, W.J.Adams, W.G.Tarpley; J.Med.Chem., **39**, 3769-3774 **(1996)**.

[13] C.Ahgren, K.Backro, F.W.Bell, A.S.Cantrell, M.Clemens, J.Colacino, M.J.B.Deeter, J.A.Engelhardt, S.R.Jaskunas, N.G.Johansson, C.L.Jordan, J.S.Kasher, M.D.Kinnick, P.Lind, C.Lopez, J.M.Morin, M.A.Muesing, R.Noreen, B.Oberg, C.J.Paget, J.A.Palkowitz, C.Parrish, P.Pranc, M.K.Rippy, C.Rydergard, C.Sahlberg, S.Swanson, R.Ternansky, J.Unge, T.R.T.Vasileff, L.Vrang, S.J.West, H.Zhang, X.X.Zhou; Antimicrob.Agents Chemother., **39**, 1329-1336 **(1995)**.

[14] G.Heinisch, B.Matuszczak, S.Pachler, D.Rakowitz; Antivir.Chem.Chemother., **8**, 443-448 **(1997)**.

[15] J.Ren, J.Diprose, J.Warren, R.M.Esnouf, L.E.Bird, S.Ikemizu, M.Slater, J.Milton, J.Balzarini, D.I.Stuart, D.K.Stammers; J.Biol.Chem., 275, 5633-5639 **(2000)**.

[16] C.Ahgren, K.Backro, F.W.Bell, S.Cantrell, M.Clemens, J.M.Colacino, M.J.Deeter, J.A.Engelhardt, M.Hogberg, S.R.Jaskunas; Antimicrob.Agents Chemother., **39**, 1329-1341 **(1995)**.

[17] F.M.Uckun, T.K.Venkatachalam; U.S.Patent, 6960606, **(2005)**.

[18] W.Fathalla, P.Pazdera; ARKIVOC, 236, **(2007)**.

[19] D.T.Connor, W.A.Cetenko, M.D.Mullican, R.J.Sorenson, P.C.Unangst, R.J.Weikert, R.L.Adolphson, J.A.Kennedy, D.O.Thueson, C.D.Wright, M.C.Conroy; J.Med.Chem., **35**, 958-964 **(1992)**.

[20] G.Joachim, M.Werner, M.Albrecht; (a) Ger.Offen., **391**, **(1973)**. (b) Chem.Abstr., **80**, 59857q, **(1974)**.

[21] M.Descamps, V.Etienne; (a) Ger.Offen., **2**, **(1974)**. (b) Chem.Abstr., **80**, 59856p **(1974)**.

[22] M.Thaker, V.V.Kachhadia, H.S.Joshi; Indian J.Chem., **42B**, 1544-1552 **(2003)**.

[23] S.L.Graham, K.L.Shepard, P.S.Anderson, J.J.Baldwin, D.B.Best, M.E.Christy, M.B.Freedman, P.Gautheron, C.N.Habecker, J.M.Hoffman, P.A.Lyle, S.R.Michelson, G.S.Ponticello, C.M.Robb, H.Schwam, A.M.Smith, R.L.Smith, J.M.Sondey, K.M.Strohmaier, M.F.Sugrue, S.L.Varga; J.Med.Chem., **32**, 2548-2556 **(1989)**.

[24] C.Mesangeau, S.Yous, P.Chavatte, G.Ferry, V.Audinot, J.A.Butin, P.Delagrange, C.Bennejean, P.Rauard, D.J.Lesieur; Enz.Inh.Med.Chem., **18**, 119-124 **(2003)**.

[25] S.Parkey, N.J.Castle; Heterocycl.Chem., **23**, 1571-1579 **(1986)**.

[26] British Pharmacopoeia, Vol.IV, Appendix XIV, A300 **(2005)**.

[27] J.G.Vincent, H.W. Vincent; Proc. Soc. Exp. Biol.Med., **55**, 162-169 **(1944)**.