Synthesis, Characterization and Antibacterial Activity of New 1,2- and 1,4-Bis(*N′*-Substituted Thioureido)benzene Derivatives

Aamer Saeed[a,*], Naeem Abbas[a], Zaman Ashraf[b] and Michael Bolte[c]

[a]*Department of Chemistry, Quaid-I-Azam University, Islamabad 45320, Pakistan.*
[b]*Department of Chemistry, Allama Iqbal Open University, Islamabad, Pakistan.*
[c]*Institut für Anorganische Chemie, J.W.-Goethe-Universität, Max-von-Laue-Str. 7, D-60438 Frankfurt/Main, Germany.*

Received 31 December 2012, revised 20 May 2013, accepted 19 August 2013.

ABSTRACT

Synthesis of two series of 1,2- and 1,4-bis(thioureido)benzene derivatives was accomplished by the treatment of corresponding alkanoyl/aroyl chlorides with potassium thiocyanate in dry acetone to afford the respective isothiocyanates as intermediates. The latter were treated *in situ* with 1,2- and 1,4-diaminobenzene, respectively, to afford the title compounds in high yields. A total of sixteen new compounds are reported herein. The structures of the products were confirmed by spectroscopic techniques (IR, ^{1}H and ^{13}C NMR, mass spectrometry), elemental analysis and in case of 1d, by X-ray diffraction technique. All the synthesized compounds were also subjected to antibacterial bioevaluation against ten different Gram-positive and Gram-negative bacterial strains using levofloxacin as the standard drug and were shown to possess promising activities.

KEYWORDS

Bis(thioureido)benzene, antibacterial activity, crystal structure.

1. Introduction

Thioureas are leading precursors for the synthesis of several heterocyclic systems, covering the whole field of pharmacy along with other industrial applications.[1,2] Their pharmaceutical importance was demonstrated from literature as Hedgehog Inhibiting Activity[3] inhibitors of trans-membrane-anchored carbonic anhydrase,[4] and antifungal and antibacterial agents.[5] Optically active thioureas and their oxidative cyclization benzothiazol product derivatives were shown to be antitumor agents.[6] The thiourea-derived drug isoxyl is clinically used against tuberculosis. Phetsuksiri *et. al*. have studied the mechanism of action of this drug on *Mycobacterium tuberculosis*.[7] The catalytic activity of thiourea in the Morita-Baylis-Hillman reaction was also studied[8] and further extended to bis(thiourea) catalysis.[9] Tautomeric equilibrium of iminothiol/thiourea derivatives was studied along with their DNA interaction.[10]

Cyclic voltammetric data of bis(substituted thiourea)ferrocene derivatives was collected and reported.[11] Metal complexes of bis- and tris-thioureas as non-linear optical material have been investigated by the Raman spectroscopy.[12] Bis-urea and thiourea containing azo-moieties were shown to exhibit solution phase anion sensing and binding properties.[13] Bis-(aryl)thioureas were found to be potent and selective inhibitors of te cytomegalovirus (CMV) in cultured HFF cells.[14] Thiourea derivatives were used as phase change materials for thermal energy storage[15] and 1,1′-(naphthalene-1,8-diyl)-3,3′-dibenzoyl-bisthiourea as an anion-binding receptor.[16] In view of the aforementioned biological and synthetic significance and our previous interest[17–9] in various aspects of the chemistry of thioureas, the aim of the present work was the synthesis of new bis-thiourea derivatives, their conversion into various heterocycles and detailed bioevaluation.

* To whom correspondence should be addressed. E-mail: aamersaeed@yahoo.com

2. Results and Discussion

Freshly dried acetone was used as a solvent for the preparation of a clear solution potassium thiocyanate to which dropwise addition of a suitable alkanoyl/aroyl chloride was carried out. The corresponding alkanoyl/aroyl isothiocyantes intermediates thus obtained *in situ* and were treated with 1,2- or 1,4-diaminobenzene, respectively, to afford the corresponding series of 1,2- and 1,4-bis(thioureido)benzenes (Scheme 1). All the synthesized compounds were purified by recrystallization from aqueous ethanol and characterized by spectroscopic techniques and in one case by the X-ray diffraction technique.

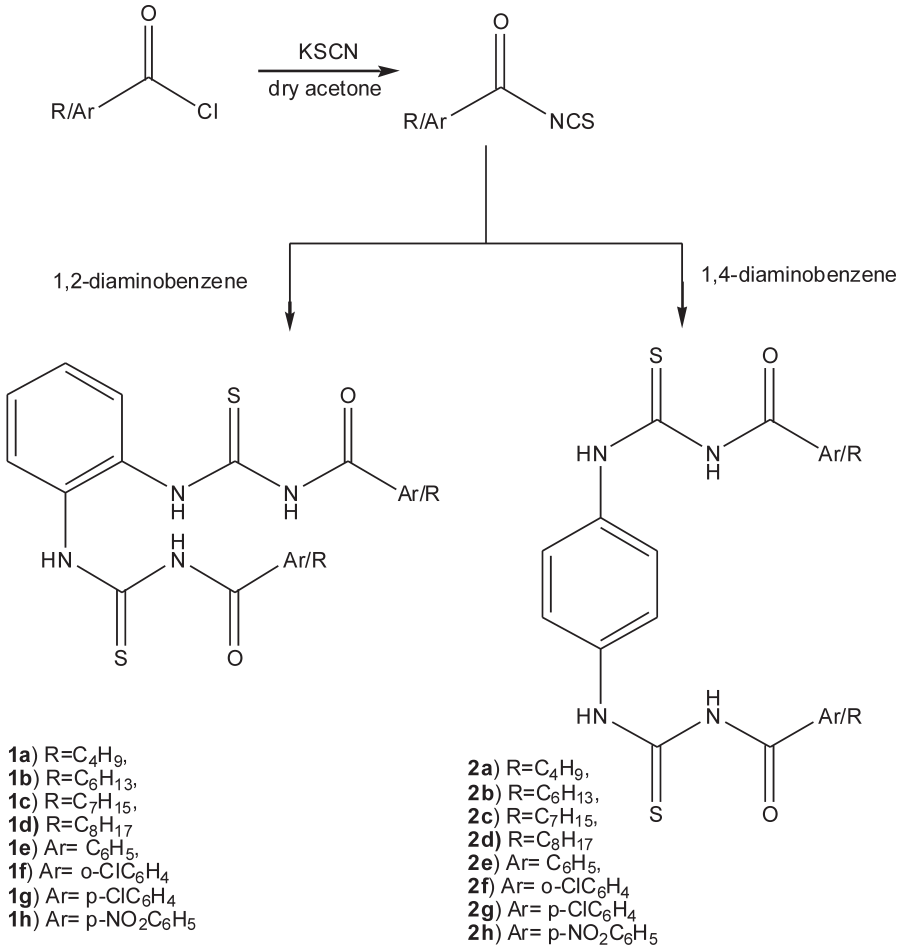
Considering compound **1c** as a typical 1,2-bis(thioureido)benzene derivative, the structure was supported by FTIR spectroscopy with appearance of absorption bands at 3251, 3149 cm^{-1} for free and associated NH groups along with absorption bands at 1687 cm^{-1} for carbonyl carbon and stretching absorption for the thiocarbonyl carbon at 1255 cm^{-1}. ^{1}H NMR indicated the presence of two NH as broad singlets at 12.38 and 10.35 ppm while aromatic protons appeared in range of 7.99–7.33 ppm while alkyl protons observed in range of 2.56–0.92 ppm.

In the ^{13}C NMR spectrum the thiocarbonyl and carbonyl group appeared at 180.5 and 174.9 ppm, respectively, the aromatic carbons at 133.4, 126.8 126.3 ppm and the alkyl carbons at 36.2, 31.0, 24.3, 22.1 and 13.3 ppm. In GCMS the base peak appeared at 99 a.m.u. due to the benzoyl cation.

The structure of **1d** was unequivocally confirmed by single crystal X-ray analysis (Fig. 1). Single crystals suitable for X-ray diffraction studies were obtained by slow evaporation of ethanol.

The conformation of the **1d** is stabilized by two intramolecular N-H...O hydrogen bonds. Two methylene chains adopt an all-trans conformation.

Figure 2 shows the packing diagram with a view of the bc-plane. The molecules are connected by N-H...S hydrogen



1a) R=C_4H_9,
1b) R=C_6H_{13},
1c) R=C_7H_{15},
1d) R=C_8H_{17}
1e) Ar= C_6H_5,
1f) Ar= o-ClC_6H_4
1g) Ar= p-ClC_6H_4
1h) Ar= p-$NO_2C_6H_5$

2a) R=C_4H_9,
2b) R=C_6H_{13},
2c) R=C_7H_{15},
2d) R=C_8H_{17}
2e) Ar= C_6H_5,
2f) Ar= o-ClC_6H_4
2g) Ar= p-ClC_6H_4
2h) Ar= p-$NO_2C_6H_5$

Scheme 1
Synthesis of 1,2-and 1,4-bis(N′-substituted thyoureido)benzene derivatives.

bonds to zigzag chains running along [1–1 0]. The details of the structure determination are compiled in Table 1.

Considering compound **1k** as a typical 1,4-bis(thioureido)benzene, the appearance of IR stretches at 3258 cm^{-1} and 3134 cm^{-1} for free and associated NH groups, at 1693 cm^{-1} for the carbonyl group and the thiocarbonyl group at 1251 cm^{-1} were observed. ^{1}H NMR indicated broad singlets at 12.56 and 11.47 ppm for the two NH groups while magnetically equivalent aromatic protons appeared as a single peak at 7.67 ppm and alkyl protons observed in the range of 2.47–0.87 ppm. In ^{13}C NMR spectrum the thiocarbonyl carbon appeared at 179.2 ppm and the carbonyl carbon at 176.0 ppm, while the aromatic carbons were observed at 135.9 and 124.7 ppm and the alkyl carbons in the range of 36.1–14.2 ppm.

2.1. Antibacterial Activity

Table 2 shows the antibacterial activity data of all synthesized compounds against ten different bacterial strains. The activity of the synthesized compounds is presented in zone of inhibition of bacterial growth in mm (Table 2). The % zone inhibition is based

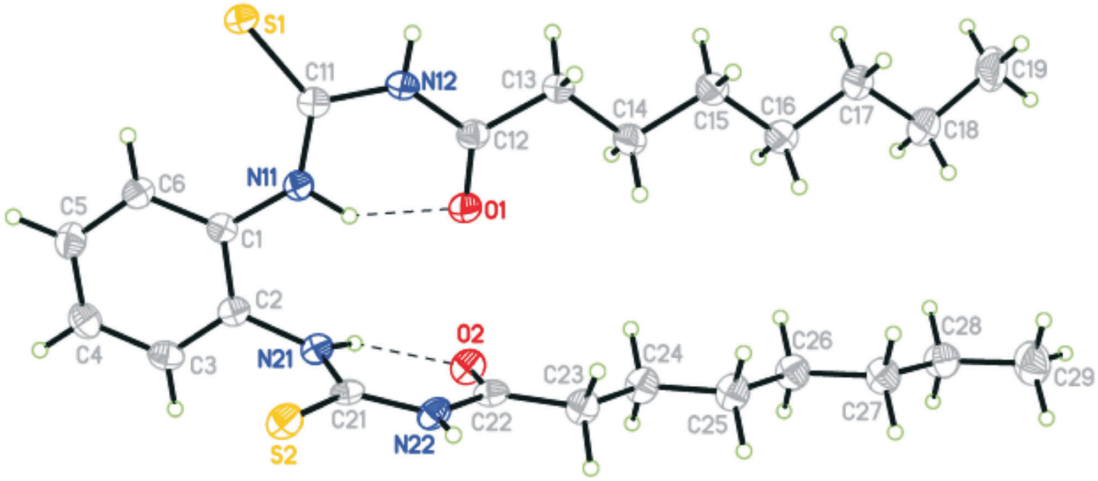


Figure 1 Molecular structure with displacement ellipsoids at the 50 % probability level.

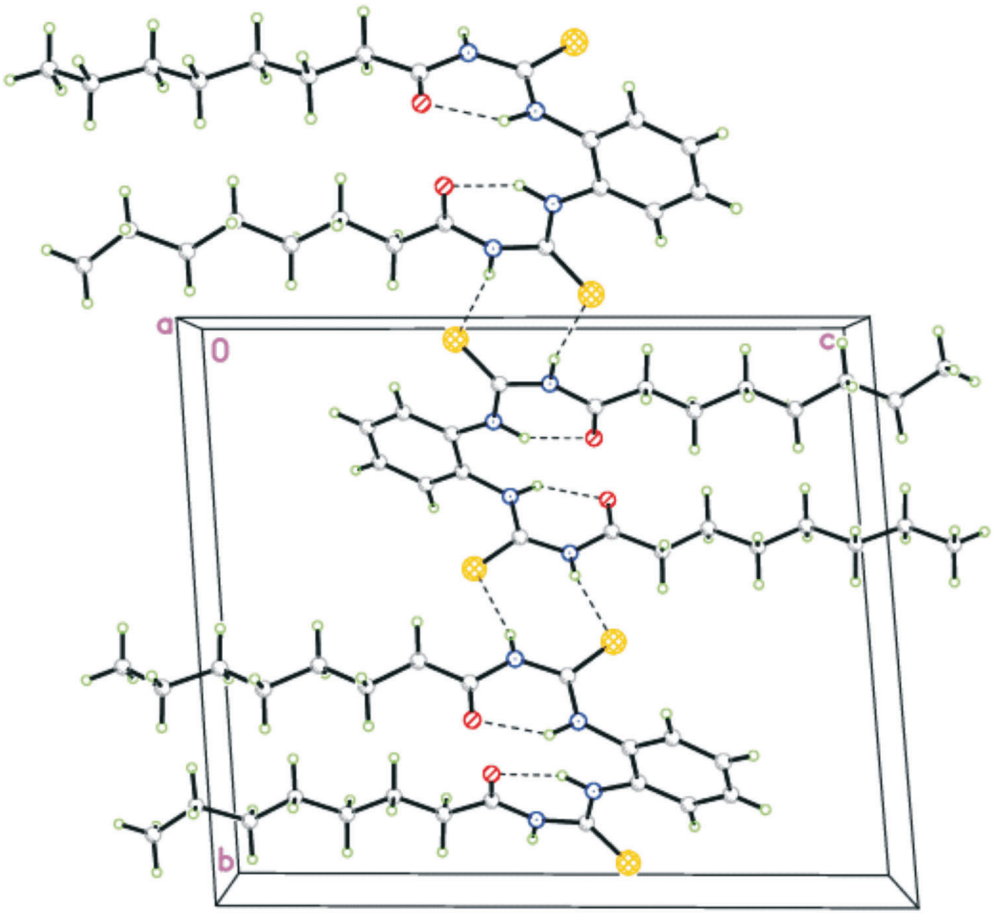


Figure 2 Packing diagram with view onto the bc-plane. Hydrogen bonds are drawn as dashed lines.

on the activity of the reference drug (see the experimental section for the exact formula). Among all of the tested compounds, **1c** showed excellent activity against *Bacillus subtilis* with maximum zone of inhibition of 95 % and 76 % against *Staphylococcus aureus* and *Pseudomonas putida*. Compound **1g** exhibited maximum inhibition against *Bacillus subtilis* of 90 %. Compound **2f** exhibited good activity against a number of bacterial strains with zones of inhibition: 83 %, 80 %, 73 % and 64 % against *Shigella flexineri, Escherichia coli, Pasteurella multocida* and *Klebsiella pneumonae*, respectively. Similarly compound **2g** showed good activity against *Escherichia coli* (80 %), *Shigella flexineri* (70 %) and *Pasteurella multocida* (66 %). Compound **1** was found to be active against *Staphylococcus aureus*, *Pasteurella multocida* and *Pseudomonas aeruginosa* with zone inhibition of 84 %, 66 % and 53 %, respectively. 1,4-Bis(*N'*-substituted thioureido)benzene derivatives (**2a, 2b, 2c** and **2e**) showed very poor or no zone of inhibition. The results of antibacterial bioscreening indicate that activities depend on the length of the hydrocarbon side chain (lipo/hydrophilicity) in case of aliphatic derivatives and on the electron-donating or withdrawing nature and position of substituent in case of the aromatic compounds.

3. Experimental

Melting points of all synthesized compounds were determined in an open capillary using a digital Gallenkamp (SANYO) model MPD BM 3.5 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were determined with a 300 MHz Bruker AM-300 spectrophotometer. FTIR spectra were measured with a FTS 3000 MX spectrophotometer and mass spectra (EI, 70 eV) with a GC-MS instrument (Agilent Technologies 1200 series USA).

3.1. Crystal Structure Determination of 1d

X-ray data were collected on a STOE IPDS-II diffractometer with graphite-monochromated MoK_α radiation. An empirical absorption correction with the PLATON program[20] was performed. The structure was solved by direct methods and refined with full-matrix least-squares on F^2 using the program SHELXL97.[21] H-atoms bonded to carbon atoms were placed on ideal positions and refined with fixed isotropic displacement parameters using a riding model. H-atoms bonded to N were freely refined.

3.2. General Procedure for Synthesis of Bis(*N'*-substituted Thioureido)benzenes

To a solution of KSCN (1.0 mmol) in dry acetone (50 mL) was dropwise added alkanoyl/aroyl chloride (1.0 mmol) with vigorous stirring during 30 min. A solution of 1,2-phenylene diamine/1,4-phenylene diamine (0.5 mmol) in dry acetone (60 mL) was dropwise added and the reaction mixture was refluxed for 2–4 h and then poured onto ice-cold water. Filtration of solids obtained followed by washing with cold water and crystallization from ethanol afforded the title compounds. A total of 16 new compounds are reported.

1,2-Bis(N'-butanoylthioureido)benzene (**1a**)

Yield: 68 %, R_f: 0.67 (Solvent for R_f petroleum ether: Ethyl acetate, 1:1), m.p. 209–210 °C, IR: 3256 (N-H), 3139 (N-H), 2956 (CH), 1697 (C=O), 1269 (C=S) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.26 (s, 2H, CO-NH-CS), 11.51 (s, 2H, CS-NH-C), 7.84–7.81 (m, 2H, ArH), 7.35–7.32 (m, 2H, ArH), 2.42 (t, 4H, J = 7.1 Hz, $2CH_2$), 1.60–1.53 (m, 4H, $2CH_2$), 0.91–0.87 (t, 6H, J = 6.9 Hz, $2CH_3$), ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.6, 175.5, 133.6, 127.4, 127.0, 37.9,

Table 1 Crystal data and structure refinement for **1d.**

Empirical formula	$C_{24}H_{38}N_4O_2S_2$
Formula weight	478.70
Temperature	173(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P -1
Unit cell dimensions	a = 5.2123(4) Å a = 85.665(6) ° b = 14.5145(12) Å b = 88.703(6) ° c = 17.1538(13) Å g = 86.550(6) °
Volume	1291.48(18) Å^3
Density (calculated)	1.231 mg m^{-3}
Absorption coefficient	0.233 mm^{-1}
F(000)	516
Crystal size	0.28 × 0.23 × 0.11 mm^3
Theta range for data collection	3.55 to 25.58 °
Index ranges	–6≤h≤6, –17≤k≤14, –20≤l≤20
Reflections collected	13407
Independent reflections	4803 [R(int) = 0.0547]
Completeness to theta = 25.00 °	99.4 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.9748 and 0.9375
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	4803/0/306
Goodness-of-fit on F^2	1.067
Final R indices [I>2sigma(I)]	R1 = 0.0381, wR2 = 0.0999
R indices (all data)	R1 = 0.0462, wR2 = 0.1030
Extinction coefficient	0.013(2)
Largest diff. peak and hole	0.251 and –0.195 e.Å^{-3}
CCDC No.	CCDC 782145

18.2, 13.8. EIMS *m/z* (%): 366 [M$^+$] (14), 71 (100 %), 87 (50). Anal. calcd. for $C_{16}H_{22}N_4O_2S_2$: C, 52.43, H, 6.05, N, 15.29, S, 17.50 %, found: C, 52.31, H, 6.19, N, 15.41, S, 17.62 %.

1,2-Bis(N′-pentanoyl thioureido)benzene (**1b**)

Yield: 72 %, R_f: 0.74 (Solvent for R_f petroleum ether: Ethyl acetate, 1:1), m.p. 178–180 °C, IR: 3244 (N-H), 3140 (N-H), 2958 (CH), 1690 (C=O), 1257 (C=S) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.02 (s, 2H, CO-NH-CS), 11.49 (s, 2H, CS-NH-C), 7.85–7.51 (m, 2H, ArH), 7.32–7.16 (m, 2H, ArH), 2.42 (t, 4H, J = 7.0 Hz, 2CH$_2$), 1.66–1.42 (m, 8H, 4CH$_2$), 0.92–0.87 (t, 6H, J = 6.9 Hz, 2CH$_3$), ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.5, 175.6, 130.6, 128.3, 126.1, 37.4, 18.2, 15.6, 13.8. EIMS *m/z* (%): 394 [M$^+$] (17), 85 (100 %), 101 (49). Anal. calcd. for $C_{18}H_{26}N_4O_2S_2$: C, 54.79, H, 6.64, N, 14.20, S, 16.25 %. found: C, 54.12, H, 6.87, N, 14.42, S, 16.10 %.

1,2-Bis(N′-hexanoyl thioureido)benzene (**1c**)

Yield: 64 %, R_f: 0.76 (Solvent for R_f petroleum ether: Ethyl acetate, 1:1), m.p. 138–140 °C, IR: 3251 (N-H), 3149 (N-H), 2955 (CH), 1687 (C=O), 1255 (C=S) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.38 (s, 2H, CO-NH-CS), 10.35 (s, 2H, CS-NH-C), 7.99–7.96 (m, 2H, ArH), 7.37–7.33 (m, 2H, ArH), 2.56 (t, 4H, J = 7.2, 2CH$_2$), 1.75–1.65 (m, 4H, 2CH$_2$), 1.42–1.34 (m, 8H, 4CH$_2$), 0.92 (t, J = 6.8, 6H, 2CH$_3$), ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.5, 174.9, 133.4, 126.8, 126.3, 36.2, 31.0, 24.38, 22.1, 13.3. EIMS *m/z* (%): 422 [M$^+$] (16), 99 (100 %), 115 (32). Anal. Calcd. for $C_{20}H_{30}N_4O_2S_2$: C, 56.84, H, 7.16, N, 13.26, S, 15.17 %. found: C, 56.42, H, 7.01, N, 13.47, S, 15.32 %.

1,2-Bis(N′-octanoyl thioureido)benzene (**1d**)

Yield: 61 %, R_f: 0.79 (Solvent for R_f petroleum ether: Ethyl acetate, 1:1), m.p. 137–138 °C, IR: 3268 (N-H), 3167 (N-H), 2959 (CH), 1695 (C=O), 1262 (C=S) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.26 (s, 2H, CO-NH-CS), 11.50 (s, 2H, CS-NH-C), 7.86–7.83 (m, 2H, ArH), 7.36–7.31 (m, 2H, ArH), 2.51 (t, 4H, J = 7.5 Hz, 2CH$_2$), 1.56–1.51 (m, 4H, 2CH$_2$), 1.39–1.20 (m, 16H, 8CH$_2$), 0.86 (t, J = 6.5 Hz, 6H, 2CH$_3$), ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.6, 175.6, 133.5, 127.3, 126.9, 36.1, 31.3, 30.1, 28.9, 24.8, 22.5, 14.4. EIMS *m/z* (%): 478 [M$^+$] (10), 127 (100 %), 143 (35). Anal. Calcd. for $C_{24}H_{38}N_4O_2S_2$: C, 60.21, H, 8.00, N, 11.70, S, 13.40 %. found: C, 60.31, H, 8.36, N, 11.88, S, 13.66 %.

1,2-Bis(N′-benzoyl thioureido)benzene (**1e**)

Yield: 69 %, R_f: 0.68 (Solvent for R_f petroleum ether: Ethyl acetate, 1:1), m.p. 156–158 °C, IR: 3234 (N-H), 3142 (N-H), 1672 (C=O), 1244 (C=S) cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.63 (s, 2H, CO-NH-CS), 10.48 (s, 2H, CS-NH-C), 8.06–8.00 (m, 6H, ArH), 7.70–7.65 (m, 2H, ArH), 7.56–7.51 (m, 4H, ArH), 7.44–7.41 (m, 2H, ArH), ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.5, 167.9, 133.6, 133.3, 132.2, 128.6, 128.3, 127.2, 126.5. EIMS *m/z* (%): 434 [M$^+$] (15), 105 (100 %), 121 (40). Anal. Calcd. for $C_{22}H_{18}N_4O_2S_2$: C, 60.81, H, 4.18, N, 12.89, S, 14.76 %. found: C, 60.45, H, 4.26, N, 12.77, S, 14.69 %.

Table 2 Antibacterial activity of compounds (**1a–h, 2a–h**).

Compound	*P.m.*	*B.s.*	*E.c.*	*S.a.*	*P.p.*	*P.a.*	*S.t.*	*M.l.*	*S.f.*	*K.p.*
1a	10	12	–	13	16	–	–	16	17	–
1b	18	–	11	17	21	13	08	15	19	07
1c	21	19	15	19	23	15	–	17	11	–
1d	08	–	–	04	11	13	–	–	17	11
1e	13	07	–	15	–	06	–	16	21	15
1f	10	05	–	11	–	14	05	–	–	–
1g	15	18	06	18	10	–	–	09	19	–
1h	20	11	16	21	13	15	–	06	–	–
2a	–	05	–	–	06	11	–	–	–	–
2b	10	–	05	–	–	–	–	07	10	–
2c	–	07	–	05	–	06	–	–	–	07
2d	05	–	07	–	–	04	–	–	–	–
2e	18	–	20	11	06	–	–	05	21	–
2f	22	12	24	15	10	15	18	15	25	16
2g	20	10	24	11	08	12	15	13	21	14
2h	07	–	05	–	06	–	10	–	08	–
Levofloxacin	30	20	30	25	30	28	30	25	30	25

Activity is presented in millimetres (mm),
(–) = No activity measured

1,2-Bis(N′-(2-chlorobenzoyl) thioureido)benzene (**1f**)

Yield: 72 %, R_f: 0.58 (Solvent for R_f petroleum ether: Ethyl acetate, 1:1), m.p. 200–202 °C, IR: 3250 (N-H), 3142 (N-H), 1670 (C=O), 1240 (C=S) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 12.22 (s, 2H, CO-NH-CS), 12.14 (s, 2H, CS-NH-C), 7.95–7.92 (m, 2H, ArH), 7.56–7.49 (m, 6H, ArH), 7.42–7.37 (m, 4H, ArH), ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.4, 168.1, 134.7, 133.7, 132.6, 130.5, 130.0, 129.6, 127.6, 127.4, 127.1. EIMS *m/z* (%): 503 [M^+] (10), 138 (100 %), 155 (27). Anal. Calcd. for $C_{22}H_{16}Cl_2N_4O_2S_2$: C, 52.49, H, 3.20, N, 11.13, S, 12.74 %. found: C, 52.20, H, 3.11, N, 11.09, S, 12.88 %.

1,2-Bis(N′-(4-chlorobenzoyl)thioureido)benzene (**1g**)

Yield: 66 %, R_f: 0.71 (Solvent for R_f petroleum ether: Ethyl acetate, 1:1), m.p. 210–212 °C, IR: 3230 (N-H), 3152 (N-H), 1670 (C=O), 1251 (C=S) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 12.32 (s, 2H, CO-NH-CS), 12.11 (s, 2H, CS-NH-C), 7.87–7.80 (m, 2H, ArH), 7.62–7.54 (m, 6H, ArH), 7.42–7.36 (m, 4H, ArH), ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.5, 168.1, 134.8, 133.7, 131.5, 130.9, 130.2, 128.6, 127.1. EIMS *m/z* (%): 503 [M^+] (11), 138 (100 %), 155 (29). Anal. Calcd. for $C_{22}H_{16}Cl_2N_4O_2S_2$: C, 52.49, H, 3.20, N, 11.13, S, 12.74 %. found: C, 52.67, H, 3.29, N, 11.25, S, 12.45 %.

1,2-Bis(N′-(4-nitrobenzoyl)thioureido)benzene (**1h**)

Yield: 58 %, R_f: 0.54 (Solvent for R_f petroleum ether: Ethyl acetate, 1:1), m.p. 179–181 °C, IR: 3260 (N-H), 3148 (N-H), 1669 (C=O), 1254 (C=S) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 12.30 (s, 2H, CO-NH-CS), 11.24 (s, 2H, CS-NH-C), 7.94–7.83 (m, 4H, ArH), 7.55–7.49 (m, 4H, ArH), 7.37–7.31 (m, 4H, ArH), ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.6, 168.3, 139.7, 138.8, 133.9, 130.5, 129.7, 124.6, 123.4. EIMS *m/z* (%): 524 [M^+] (11), 150 (100 %), 166 (24). Anal. Calcd. for $C_{22}H_{16}N_6O_6S_2$: C, 50.38, H, 3.07, N, 16.02, S, 12.23 %. found: C, 50.48, H, 3.19, N, 16.29, S, 12.34 %.

1,4-Bis(N′-butanoyl thioureido)benzene (**2a**)

Yield: 75 %, R_f: 0.52 (Solvent for R_f petroleum ether: Ethyl acetate, 1:1), m.p. 180–181 °C, IR: 3261 (N-H), 3151 (N-H), 2959 (CH), 1691 (C=O), 1250 (C=S) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 12.39 (s, 2H, CO-NH-CS), 11.01 (s, 2H, CS-NH-C), 7.59 (s, 4H, ArH), 2.36 (t, 4H, J = 6.99 Hz, $2CH_2$), 1.52–1.50 (m, 4H, $2CH_2$), 0.90–0.79 (t, 6H, J = 6.9 Hz, $2CH_3$), ^{13}C NMR (75 MHz, $CDCl_3$): δ 179.8, 176.6, 134.4, 124.4, 38.6, 18.8, 13.1. EIMS *m/z* (%): 366 [M^+] (15), 71 (100 %), 87 (55). Anal. calcd. for $C_{16}H_{22}N_4O_2S_2$: C, 52.43, H, 6.05, N, 15.29, S, 17.50 %. found: C, 52.29, H, 6.21, N, 15.01, S, 17.41 %.

1,4-Bis(N′-pentanoyl thioureido)benzene (**2b**)

Yield: 71 %, R_f: 0.77 (Solvent for R_f petroleum ether: Ethyl acetate, 1:1), m.p. 215–217 °C, IR: 3254 (N-H), 3164 (N-H), 2953 (CH), 1677 (C=O), 1261 (C=S) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 11.98 (s, 2H, CO-NH-CS), 11.36 (s, 2H, CS-NH-C), 7.92 (s, 4H, ArH), 2.56 (t, 4H, J = 7.0 Hz, $2CH_2$), 1.74–1.39 (m, 8H, $4CH_2$), 0.93–0.87 (t, 6H, J = 6.9 Hz, $2CH_3$), ^{13}C NMR (75 MHz, $CDCl_3$): δ 180.4, 175.4, 134.6, 123.1, 37.5, 18.4, 15.9, 12.2. EIMS *m/z* (%): 394 [M^+] (20), 85 (100 %), 101 (41). Anal. calcd. for $C_{18}H_{26}N_4O_2S_2$: C, 54.79, H, 6.64, N, 14.20, S, 16.25 %. found: C, 54.42, H, 6.96, N, 14.31, S, 16.36 %.

1,4-Bis(N′-hexanoyl thioureido)benzene (**2c**)

Yield: 74 %, R_f: 0.79 (Solvent for R_f petroleum ether: Ethyl acetate, 1:1), m.p. 168–169 °C, IR: 3258 (N-H), 3134 (N-H), 2938 (CH), 1693 (C=O), 1251 (C=S) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 12.56 (s, 2H, CO-NH-CS), 11.47 (s, 2H, CS-NH-C), 7.67 (s, 4H, ArH), 2.47 (t, 4H, J = 7.5, $2CH_2$), 1.59–1.54 (m, 4H, $2CH_2$), 1.29–1.27 (m, 8H, $4CH_2$), 0.87 (t, 6H, J = 6.3, $2CH_3$), ^{13}C NMR (75 MHz, $CDCl_3$): δ 179.2, 176.0, 135.9, 124.7, 36.1, 31.1, 24.4, 22.3, 14.2. EIMS *m/z* (%): 422 [M^+] (15), 99 (100 %), 115 (35). Anal. Calcd. for $C_{20}H_{30}N_4O_2S_2$: C, 56.84, H, 7.16, N, 13.26, S, 15.17 %. found: C, 56.57, H, 7.09, N, 13.11, S, 15.14 %.

1,4-Bis(N′-octanoyl thioureido)benzene (**2d**)

Yield: 69 %, R_f: 0.80 (Solvent for R_f petroleum ether: Ethyl acetate, 1:1), m.p. 170–172 °C, IR: 3242 (N-H), 3155 (N-H), 2959 (CH), 1686 (C=O), 1261 (C=S) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 12.56 (s, 2H, CO-NH-CS), 11.46 (s, 2H, CS-NH-C), 7.66 (s, 4H, ArH), 2.45 (t, 4H, J = 7.5, $2CH_2$), 1.58–1.53 (m, 4H, $2CH_2$), 1.31–1.25 (m, 16H, $8CH_2$), 0.86 (t, J = 6.4, 6H, $2CH_3$), ^{13}C NMR (75 MHz, $CDCl_3$): δ 179.1, 176.0, 135.9, 124.7, 36.2, 31.5, 28.8, 24.7, 22.5, 21.1, 14.4. EIMS *m/z* (%): 478 [M^+] (11), 127 (100 %), 143 (37). Anal. Calcd. for $C_{24}H_{38}N_4O_2S_2$: C, 60.21, H, 8.00, N, 11.70, S, 13.40 %. found: C, 60.33, H, 8.24, N, 11.69, S, 13.12 %.

1,4-Bis(N′-benzoyl thioureido)benzene (**2e**)

Yield: 70 %, R_f: 0.57 (Solvent for R_f petroleum ether: Ethyl acetate, 1:1), m.p. 223–225 °C, IR: 3235 (N-H), 3167 (N-H), 1655 (C=O), 1261 (C=S) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 12.59 (s, 2H, CO-NH-CS), 10.88 (s, 2H, CS-NH-C), 7.99–7.78 (m, 6H, ArH), 7.67 (s, 4H, ArH), 7.38–7.21 (m, 4H, ArH), ^{13}C NMR (75 MHz, $CDCl_3$): δ 178.3, 166.1, 134.5, 131.2, 129.8, 127.2, 125.6, 123.7. EIMS *m/z* (%): 434 [M^+] (14), 105 (100 %), 121 (46). Anal. Calcd. for $C_{22}H_{18}N_4O_2S_2$: C, 60.81, H, 4.18, N, 12.89, S, 14.76 %. found: C, 60.69, H, 4.37, N, 12.50, S, 14.91 %.

1,4-Bis(N′-(2-chloro benzoyl) thioureido)benzene (**2f**)

Yield: 64 %, R_f: 0.69 (Solvent for R_f petroleum ether: Ethyl acetate, 1:1), m.p. 175–177 °C, IR: 3230 (N-H), 3136 (N-H), 1667 (C=O), 1257 (C=S) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 12.22 (s, 2H, CO-NH-CS), 12.14 (s, 2H, CS-NH-C), 7.84–7.79 (m, 2H, ArH), 7.74 (m, 4H, ArH), 7.67 (s, 4H, ArH), 7.33–7.28 (m, 2H, ArH), ^{13}C NMR (75 MHz, $CDCl_3$): δ 181.6, 166.4, 134.5, 133.4, 131.8, 130.2, 128.6, 126.6, 125.9, 124.0. EIMS *m/z* (%): 503 [M^+] (14), 138 (100 %), 155 (20). Anal. Calcd. for $C_{22}H_{16}Cl_2N_4O_2S_2$: C, 52.49, H, 3.20, N, 11.13, S, 12.74 %. found: C, 52.25, H, 3.40, N, 11.21, S, 12.91 %.

1,4-Bis(N′-(4-chloro benzoyl) thioureido)benzene (**2g**)

Yield: 67 %, R_f: 0.71 (Solvent for R_f petroleum ether: Ethyl acetate, 1:1), m.p. 230–232 °C, IR: 3257 (N-H), 3142 (N-H), 1665 (C=O), 1249 (C=S) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 12.02 (s, 2H, CO-NH-CS), 11.31 (s, 2H, CS-NH-C), 7.84 (d, 4H, J = 7.5 Hz, ArH), 7.61 (s, 4H, ArH), 7.50 (d, 4H, J=7.5 Hz, ArH), ^{13}C NMR (75 MHz, $CDCl_3$): δ 179.6, 168.1, 133.7, 131.7, 130.5, 132.9, 127.0, 124.1. EIMS *m/z* (%): 503 [M^+] (13), 138 (100 %), 155 (21). Anal. Calcd. for $C_{22}H_{16}Cl_2N_4O_2S_2$: C, 52.49, H, 3.20, N, 11.13, S, 12.74 %. found: C, 52.39, H, 3.07, N, 11.00, S, 12.51 %.

1,4-Bis(N′-(4-nitro benzoyl) thioureido)benzene (**2h**)

Yield: 61 %, R_f: 0.68 (Solvent for R_f petroleum ether: Ethyl acetate, 1:1), m.p. 234–236 °C, IR: 3242 (N-H), 3149 (N-H), 1658 (C = O), 1241 (C=S) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 12.25 (s, 2H, CO-NH-CS), 11.45 (s, 2H, CS-NH-C), 7.94 (d, 4H, J = 7.6 Hz, ArH), 7.65 (s, 4H, ArH), 7.56 (d, 4H, J = 7.5 Hz, ArH), ^{13}C NMR (75 MHz, $CDCl_3$): δ 179.9, 166.4, 139.8, 138.0, 133.5, 131.5, 128.9, 124.6. EIMS *m/z* (%): 524 [M^+] (14), 150 (100 %), 166 (17) Anal. Calcd. for $C_{22}H_{16}N_6O_6S_2$: C, 50.38, H, 3.07, N, 16.02, S, 12.23 %. found: C, 50.59, H, 3.21, N, 16.21, S, 12.12 %.

3.3. Pharmacological Assays

In vitro evaluation of antibacterial activity of the compounds was carried out by agar well diffusion assay[20] against ten different Gram-positive and Gram-negative bacteria (*Pasteurella multocida (P.m.), Bacillus subtilis (B.s.), Escherichia coli (E.c.), Staphylococcus aureus (S.a.), Pseudomonas putida (P.p.), Pseudomonas aeruginosa (P.a.), Salmonella typhi (S.t.), Micrococcus luteus (M.l.),*

Shigella flexineri (S.f.) and *Klebsiella pneumonae (K.p.)*). Antibacterial activity was determined by using the Mueller Hinton Agar (MHA).[n] The fresh inoculums of these bacteria were prepared and diluted by sterilized normal saline. The turbidity of these cultures was adjusted by using 0.5 Mc-Farland. A homogeneous bacterial lawn was developed by sterile cotton swabs. The inoculated plates were bored by 6 mm sized borer to make the wells. The sample dilutions were prepared by dissolving each sample (1.0 mg) in 1.0 mL of DMSO used as negative control in this bioassay. The equimolar concentration of Levofloxacin (1.0 mg mL^{-1}), a broad spectrum antibiotic (positive control) was prepared. These plates were incubated at 37 °C for 24 hours. Antibacterial activity of the compounds was determined by measuring the diameter of zone of inhibition (mm, ± standard deviation) and presented by subtracting the activity of the negative control in Table 2. The % zone inhibition is therefore defined as:

$$\%\ \text{zone inhibition} = \frac{\text{zone of inhibition by compound}}{\text{zone of inhibition by standard drug}} \times 100\ .$$

4. Conclusion

Two novel series of 1,2- and 1,4-bis(*N′*-substituted thioureido) benzenes (a total of 16 new compounds) were synthesized by treatment with 1,2- and 1,4-diaminobenzene with respective isothiocyanates produced *in situ*. The X-ray single crystal diffraction analysis of compound **1d** shows that the conformation is stabilized by two intramolecular N-H...O hydrogen bonds and the two methylene chains adopt an all-trans conformation. The results of antibacterial screening indicated that compound **1c** from 1,2-bis(*N′*-substituted thioureido)benzene series showed excellent activity against *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas putida*, respectively. Whereas amongst 1,4-bis(*N′*-substituted thioureido)benzene series, compound **2f** exhibited good activity against *Shigella flexineri*, *Escherichia coli*, *Pasteurella multocida* and *Klebsiella pneumonae* compared with levofloxacin, the standard drug.

Acknowledgement

N.A. gratefully acknowledges a research scholarship from HEC Islamabad under the HEC Indigenous PhD Scholarship 5000 Scheme.

References

1 V.V. Kachhadia, M.R. Patel and H.S. Joshi, *J. Serb. Chem. Soc.*, 2005, **70**, 153–161.
2 D. Wilson, M.A. Arada, S. Alegret and M. del Valle, *J. Hazard. Mater.,* 2010, **181**, 140–146.
3 A. Solinas, H. Faure, H. Roudaut, E. Traiffort, A. Schoenfelder, A. Mann, F. Manetti, M. Taddei and M. Ruat, *J. Med. Chem.,* 2012, **55**, 1559–1571.
4 J. Moeker, K. Teruya, S. Rossit, B.L. Wilkinson, M. Lopez, L.F. Bornaghi, A. Innocenti, C.T. Supuran and S.-A.Poulsen, *Bioorg. Med. Chem*., 2012, **20**, 2392–2404.
5 N.A. Mohamed and N.A.A. El-Ghany, *Int. J. Biol. Macromol*., 2012, **50**, 1280–1285.
6 S.N. Manjyula, N.M. Noolvi, K.V. Parihar, S.A.M. Reddy, V. Ramani, A.K. Gada, G. Singh, N.G. Kutty and C.M. Rao, *Eur. J. Med. Chem*., 2009, **44**, 2923–2929.
7 B. Phetsuksiri, M. Jackson, H. Scherman, M. McNeil, G.S. Besra, A.R. Baulard, R.A. Slayden, A.E. DeBarber, C.E. Barry, M.S. Baird, D.C. Crick and P.J. Brennan, *J. Biol. Chem.*, 2003, **278**, 53123–53130.
8 Y. Sohtome, N.Takemura, R. Takagi, Y. Hashimoto and K. Nagasawa, *Tetrahedron*, 2008, **64**, 9423–9429.
9 Y. Nakayama, T. Gotanda and K. Ito, *Tetrahedron Lett.*, 2011, **52**, 6234–6237.
10 M. Breton, M. Bessodes, S. Bouaziz, J. Herscovici, D. Scherman and D. Mignet, *Biophys. Chem.*, 2009, **145**, 7–16.
11 Y.-F. Yuan, S.-M. Ye, L.-Y. Zhang, B. Wang and J.-T. Wang, *Polyhedron*, 1997, **16**, 1713–1718.
12 R.G. Kumari, V. Ramakrishnan, M.L. Carolin, J. Kumar, A. Sarua and M. Kuball, *Spectrochim. Acta, Part A*, 2009, **73**, 263–267.
13 B. Garg, T. Bisht and S.M.S. Chauhan, *Sens. Actuators, B*, 2012, **168**, 318–328.
14 C. Alkan, Y. Tek and D. Kahraman, *Turk. J. Chem*. 2011, **35**, 769–777.
15 F. Aydin, N. Tunoglu, D. Aykac, N.B. Arslan and C. Kazak, *Turk. J. Chem*., 2012, **36**, 764–777.
16 A. Saeed, U. Shaheen, A. Hameed and S.Z. Haider Naqvi, *J. Fluorine Chem.,* 2009, **130**, 1028–1034.
17 A. Saeed, M.F. Erben and U. Flörke, *J. Mol. Struc.,* 2010, **982**, 91–99.
18 A. Saeed, A. Mumtaz and H. Ishida, *J. Sulfur Chem.,* 2011, **32**, 45–54.
19 A. Saeed, M.F. Erben and M. Bolte, *Spectrochimica Acta A,* 2013, **102**, 408–413.
20 M.I. Okeke, C.U. Iroegbu, E.N. Eze, A.S. Okoli and C.O. Esimone, *J. Ethnopharm*., 2001, **78**, 119.
21 A.L. Spek, *Acta Cryst.,* 2009, D**65**, 148–155
22 G.M. Sheldrick, *Acta Cryst.,* 2008, A**64**, 112–122).