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Synthesis and antifungal activities of phenylenedithioureas

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Abstract—A total of 20 new phenylenedithiourea derivatives was synthesized by reaction of phenylenediisothiocyanates with aromatic amines as aminobenzoic, aminosalicylic acid and their derivatives. Their chemical structures were confirmed by elemental analysis, IR spectrometry and ^{1}H NMR. The compounds were screened for in vitro antifungal, antibacterial activities and some of them have strong antifungal activities comparable to the activity observed for ketoconazole.

Compounds containing thiourea group are present in many drugs such as antithyroid, and anaesthetic. Monothiourea derivatives which are obtained by the condensation of isothiocyanate with esteramines have shown strong antifungal activities, especially against *Candida* and *Aspergillus* in several studies.[1,2] Plaunotol and its thioureas derivatives presented antibacterial activities against *Helicobater pylori* as urease inhibitors.[3,8] Anti-HIV activity of thioureas were also reported in recent studies.[4,5] However, the synthesis and antifungal, antibacterial activities of phenylenedithiourea classes have not been reported.

Thioureas are prepared by the reaction between isothiocyanates and amines. Isothiocyanate derivatives are synthesized by the thiocarbamate method, for example reaction between amines and carbon disulfide CS_2 with presence of alkali at low temperature (0–5 °C). This is a nucleophile addition reaction (Ad_N) which depends on electron density of amine group (NH_2). In phenylenediamines the electron pairs of nitrogen conjugate with aromatic nucleus, so the reactivity of amino groups decrease. However, in 1,4-phenylenediamine and 1,3-phenylenediamine, there are interactions between the electron pairs of amino groups. These interactions themselves are the causes of increasing the electron densities of amino groups in 1,4-phenylene-diamine and 1,3-phenylenediamine so 1,3- and 1,4-phenylene-bis-dithiocarbamate are easily obtained in the reaction of 1,3- and 1,4-phenylenediamine with carbon disulfide CS_2. Meanwhile, dimer macrolide are created instead of phenylene-bis-dithiocarbamate as 1,2-phenylenediamine reacts with carbon disulfide CS_2. Phenylenediisothiocyanates are prepared by treatment of phenylene-bis-dithiocarbamate with lead nitrate $Pb(NO_3)_2$ then removes element of hydrogen sulfide.

Hereby, we report the study on synthesis of 1,4-, 1,3-, 1,2-phenylenedithiourea derivatives by condensation of isothiocyanates with 4-aminobenzoic, 4-aminosalicylic acid and their ester derivatives, and testing for antifungal, antibacterial activities of these compounds. By using such amino aromatic carboxylic acids, we want to combine antimicrobial effect of these amino acids with the biological activities of thiourea group.

1. Chemistry

Starting from aniline and nitrobenzene, phenylenediamines **1a–c** were synthesized. 1,3-, 1,4-phenylene-bis-dithiocyanate **2a–b** were synthesized in 2 steps with intermediate of dithiocarbamate. 1,3-, 1,4-phenylenediamine react with carbon disulfide CS_2 at low temperature (0–5 °C) giving 1,3-, 1,4-phenylene-bis-dithiocarbamate. In the presence of lead nitrate $Pb(NO_3)_2$, 1,3-,1,4-phenylene-bis-dithiocarbamates formed to dithiocyanate **2a–b**. Reaction between 1,3-phenylenediisothiocyanates **2a** with 4-amino-benzoic acid, 4-aminosalicylic acid and their ester derivatives at room temperature give (1,3-phenylene)-bis-[3-(4′-carboxy-phenyl)thiourea], (1,3-phenylene)-bis-[3-(2′-hydroxy-4′-carboxy-phenyl)-

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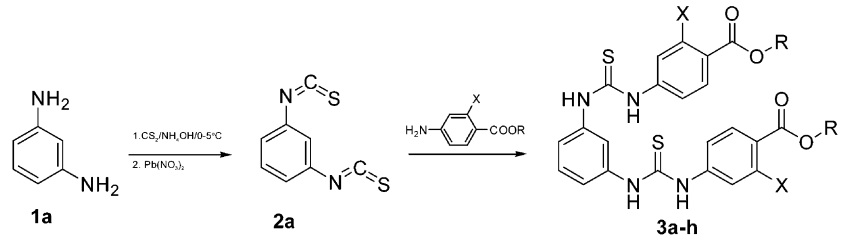
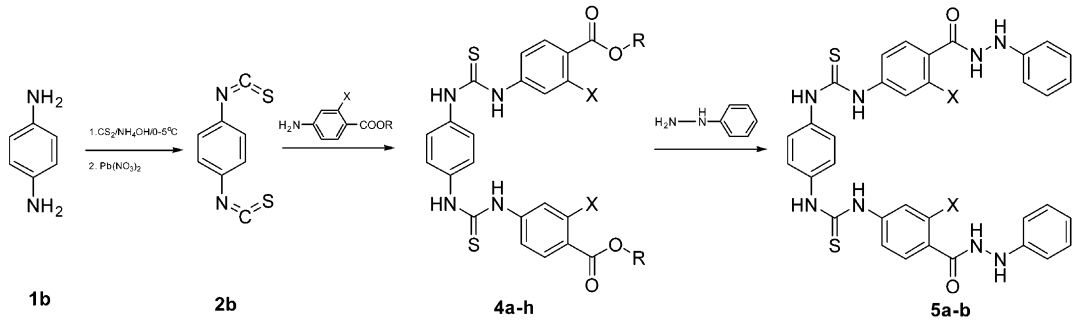
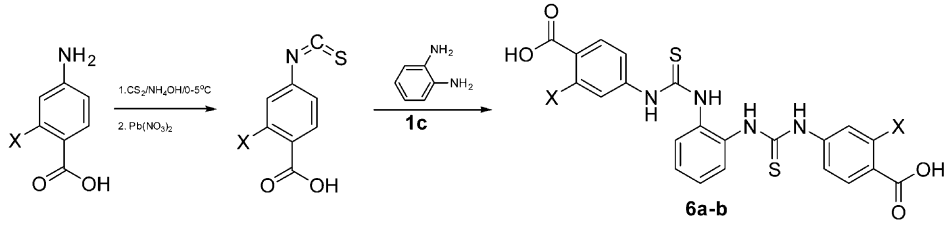


Scheme 1. Synthesis of 1,3-phenylenedithioureas.



Scheme 2. Synthesis of 1,4-phenylenedithiourea derivatives.



Scheme 3. Synthesis of 1,2-phenylenedithioureas.

thiourea] and their derivatives **3a–h** in high yield (90–93%). Depending on the ester group of amines, EtOH, MeOH or *i*-PrOH are employed as solvent for these reaction. The process of synthesis is showed on Scheme 1.

1,4-Phenylenedithiourea derivatives **4a–h** were synthesized from **2b** and 4-aminobenzoic, 4-aminosalicylic acid and ester derivatives shown in Scheme 2. (1,4-phenylene)-bis[3-(4′-carbomethoxy-phenyl)thiourea] **4b** or (1,4-phenylene)-bis[3-(2′-hydroxy-4′-carbomethoxy-phenyl)thiourea] **4f** heating with solution of phenyl-hydrazine in methanol are obtained (1,4-phenylene)-bis-[3-(4′-(*N*′-phenyl-hydrazino-carbonyl)-phenyl)thiourea] **5a** or (1,4-phenylene)-bis-[3-(2′-hydroxy-4′-(*N*′-phenyl-hydrazino-carbonyl)-phenyl)thiourea] **5b** in 83–85% yields.

Thiocarbamate method has not applied for 1,2-phenylene-diamine **1c** to synthesize 1,2-phenylenedithioureas because of the formation of cyclic dithiourea (dimer) as **1c** reacted with carbon disulfide CS_2. Consequently, 1,2-phenylene-dithioureas **6a–b** were synthesized by condensation of 4-isothiocyanatobenzoic acid or 4-isothiocyanatosalicylic acid and 1,2-phenylenediamine **1c**. 4-isothiocyanato-benzoic, 4-isothiocyanatosalicylic acid were also prepared by thiocarbamate method from 4-aminobenzoic, 4-aminosalicylic acid. The synthesized process is showed in Scheme 3.

2. Biological activities

The in vitro antimicrobial activity of the synthesized compounds were investigated against several representative pathogenic bacteria and fungi. The Minimum Inhibitory Concentrations (MICs) were determined by agar plate dilution method. Tryptic Soy Agar and Sabouraud Dextrose Agar were employed for bacterial and fungal growth, respectively. Stock solutions of tested compounds were prepared in dimethyl sulfoxide (DMSO). Inocula containing approximately 10^7 CFUs/mL of bacteria and 10^6 CFUs/mL of fungi were prepared from broth cultures in log phase growth. Bacterial and fungal plates were made in triplicate and incubated at 37 °C within 16–24 h for bacteria, at 30 °C about 24–48 h for yeast, about 72 h for moulds and about 168 h for dermatophytes. The MIC value was defined as lowest concentration of the antifungal, antibacterial agent at which there showed optically clear and experiments were repeated at least three times.

Antifungal activities of phenylenedithiourea compounds were investigated against eight pathogenous fungi as

Table 1. Structure and in vitro antifungal, antibacterial activities of phenylenedithiourea derivatives (MIC, μg/mL)

Compd	R	X	MIC (μg/mL)									
			Fungi tested						Bacteria tested			
			Ca	*Tm*	*Ta*	*Mg*	*Mc*	*Mn*	*Sa*	*Sf*	*Pa*	*Ec*
3a	H	H	>512	64	64	64	32	64	256	512	512	>512
3b	Me	H	>512	64	64	128	64	128	512	>512	>512	512
3c	Et	H	>512	64	256	64	256	256	512	>512	>512	512
3d	*i*-Pro	H	>512	32	64	64	16	8	512	>512	>512	512
3e	H	OH	>512	32	32	64	8	64	256	>512	>512	512
3f	Me	OH	512	64	128	64	32	32	256	>512	>512	512
3g	Et	OH	>512	32	32	32	64	32	256	>512	>512	512
3h	*i*-Pro	OH	>512	16	32	32	32	32	256	>512	>512	512
4a	H	H	512	32	128	32	128	128	>512	512	512	>512
4b	Me	H	>512	32	128	256	128	32	>512	512	512	>512
4c	Et	H	>512	512	256	>512	128	128	>512	512	512	>512
4d	*i*-Pro	H	>512	64	64	32	64	64	>512	512	512	>512
4e	H	OH	256	128	128	128	128	64	>512	512	512	>512
4f	Me	OH	>512	64	32	16	64	32	>512	512	512	>512
4g	Et	OH	>512	>512	>512	256	256	128	>512	512	512	>512
4h	*i*-Pro	OH	>512	>512	128	256	128	128	>512	512	512	>512
5a	–	H	>512	16	128	64	16	8	128	512	512	512
5b	–	OH	512	32	32	128	64	64	512	512	512	256
6a	–	H	>512	32	16	32	32	16	>512	512	512	>512
6b	–	OH	512	8	32	16	32	32	>512	512	512	>512
KZ	–	–	128	16	16	16	8	16	NT	NT	NT	NT
CP	–	–	NT	NT	NT	NT	NT	NT	128	16	16	16

MIC represents the mean from dose–response curves of at least three experiments. MIC values were read after 16–24 h for bacteria, 24–48 h for *Candida albicans;* 72 h for *Aspergillus* species and 168 h for dermatophytes. Fungi- *Ca*: *Candida albicans* ATCC 10231, *Tm*: *Trichophyton mentagrophytes*, *Ta*: *Trichophyton ajelloi*, *Mg*: *Microsporum gypseum*, *Mn*: *Microsporum nanum*, *Mc*: *Microsporum cookei*; Bacteria- *Sa*: *Staphylococcus aureus* ATCC 29213, *Sf*: *Streptococcus faecalis* ATCC 29212, *Pa*: *Pseudomonas aeruginosae* ATCC 27853, *Ec*: *Escherichia coli* ATCC 25853. MICs on *Aspergillus niger*, *Aspergillus flavus* ≥512 μg/mL are not showed in this table. KZ: ketoconazole; CP: chloramphenicole; NT: non tested.

yeast (*Candida albicans* ATCC 10231), dermatophytes *(Microsporum gypseum, Microsporum nanum, Microsporum cookei, Trichophyton mentagrophytes, Trichophyton ajelloi)* and moulds *(Aspergillus niger, Aspergillus flavus)*. Most derivatives showed significant in vitro antifungal activities against tested fungi. The MICs of antifungal activities are showed on Table 1. The compounds **3d**,[10] **3e**, **3h**,[11] **5a**,[12] **6a–b** exhibited to have strong antifungal activities with low MICs values included in the range of 8–64 μg/mL. These compounds also showed to have a broad antifungal spectrum compared to the reference drug ketoconazole. The synthesized compounds have very weak effects on *Candida albicans*. Phenylenedithiourea compounds do not show the effect on the growth of moulds *Aspergillus niger*, *Aspergillus flavus* with MIC over 512 μg/mL.

Four bacterial strains including gram-negative rods (*Escherichia coli* ATCC25853, *Pseudomonas aeruginosae* ATCC 27853), gram-positive cocci (*Staphylococcus aureus* ATCC29213, *Streptococcus faecalis* ATCC29212) were used in antimicrobial assay. The antibacterial activities were shown in Table 1. The compounds have weak effects on bacteria. MIC values for the Gram-negative and Gram-positive bacteria were larger than 256 μg/mL. Compounds **5a** show a light effect on *Staphylococcus aureus* ATCC 29213 with MIC = 128 μg/mL.

In terms of structure–activity relationships (SARs), the potent antifungal activities are descending from the 1,2-phenylenedithiourea **6a–b** to 1,3-phenylenedithiourea **3a–h** and 1,4-phenylenedithiourea **4a–h**. In general, benzoyl derivatives exhibited stronger antifungal activities than salicyl derivatives. In addition, SARs have observed from alkyl moiety of ester group. The *iso*-propyl moiety of phenylenethiourea improves antifungal activities than methyl moiety. Ethyl moiety showed less effect than methyl moiety on the fungistatic potency. Hydrazide-substituted compounds indicate increasing on the inhibitions of both fungi and bacteria (**5a**, **5b** vs **4a–h**). Displacing ester group with hydrazide group maybe make compounds more stable in live cell. Compound **5a** expresses an antimicrobial potency on both fungi and Gram-positive bacteria. Compounds **3d**, **3e**, **3h**, **5a**, **6a–b** show the antifungal potency compared to ketoconazole with a wide broad antifungal spectrum.

FlexS program in Sybyl 6.9 software (Tripos Inc.) were employed to examine flexible ligand superposition of phenylenedithiourea compounds.[9] Compound **3h** was kept rigid and the others were treated as flexible. The flexible superposition of all compounds were shown the relative

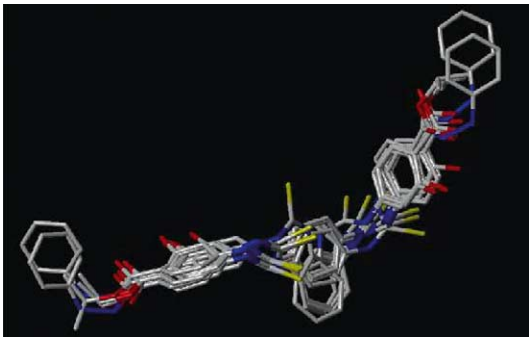


Figure 1. FlexS superposition of 20 phenylenedithiourea compounds.

geometry and their similarity (Fig. 1). The total FlexS docking score (Total-Score) were obtained from −787.4 for compound **4a** to −1298.9 for compound **3d**. Despite the difference of thiourea moiety position on the phenyl ring (1,2-, 1,3-, 1,4-phenylenedithiourea), the flexible alignment show the similarity of both pharmacophore and chemical composition of all compounds. The FlexS *in silico* result expresses a corresponding with in vitro antifungal activities by the ranking of structures. 1,3- and 1,2-phenylenedithiourea analogues get the high rank on the total FlexS docking score. 1,4-phenylenedithiourea derivatives have low rank on FlexS superposition and 4 compounds **4c–d**, **4g–h** were failed in FlexS flexible alignment procedure when compound **6a** was setup as reference structure. Structure of compounds **3d**, **3e**, **3h**, **5a**, **6a–b** are placed high rank in flexible superposition ranking.

3. Experimental

Melting points (mp) were determined by using a Gallenkamp apparatus. physiochemical parameters UV and IR were determined on UV spectrometry 3191 PC-Shimazdu and IR spectrometry on FTIR 8101-Shimazdu. NMR spectra were recorded in the given solvent with Bruker AC200 spectrometer. Chemical shifts are reported as (δ values in parts per million). The splitting pattern abbreviations are as follows: m (multiplet), s (singlet), d (doublet), t (triplet). All reported products showed ^{1}H NMR spectra in agreement with the assigned structures. Elemental analyses were found suitable with calculated of elemental from structure.

3.1. General procedure for synthesis of 1,4-phenylenedithioureas and 1,3-phenylenedithioureas

In round-bottomed flask, place 0.01mol of 1,4-phenylene-diisothiocyanate **2a** (or 1,3-phenylenediisothiocyanate **2b**), 0.02 mol of amine and 30mL EtOH (or MeOH, *iso*-propanol depend on the ester group of amines). Heat the mixture on water bath at 50–60 °C for 15 min and leave at room temperature for 24 h. Filter and collect the precipitate and recrystallize from EtOH (or MeOH) afforded compounds **3a–h, 4a–h**.

3.2. General procedure for synthesis of hydrazide derivatives of 1,4-phenylenedithiourea

In a round-bottomed flask, place 0.01 mol of **4b** or **4f** 0.022 mol of phenylhydrazine and 30 mL MeOH, heating reflux the mixture on water bath at 60–70 °C for 3 h, and leave the mixture at room temperature for 8 h. Remove excessive phenylhydrazine by using HCl 10%. Filter and collect the precipitate and recrystallize it from mixture EtOH–H_2O 1:1, dry at 60 °C to have **5a–b.**

3.3. General procedure for synthesis of 1,2-phenylenedithioureas

Place 0.01mol of 1,2-phenylenediamine, 0.02mol of 4-isothiocyanatobenzoic acid or 4-isothiocyanatosalicylic acid and 20mL EtOH. Heat the mixture on water bath at 50–60 °C for 15 min. Keep the mixture at room temperature for 24 h. The precipitate was filtered and recrystallized from EtOH to afford **6a–b**.

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10. Analytical data for compound **3d**: mp (°C) 154–155; UV (nm) 301.0; IR (KBr) (cm^{-1}) 1721, 1604, 1335, 1109; ^{1}H NMR (δ ppm) 1.32 (*d*, $J_{CH3-CH}=7$, 12H, CH_3), 3.78 (*m*, 2H, CH), 7.66 (*m*, 12H, ArH), 10.07 (*s*, 4H, NH). Anal. calcd for $C_{28}H_{30}N_4O_4S_2$: N, 10.17; S, 11.65. Found: N, 10.14; S, 11.61.
11. Analytical data for compound **3h**: mp (°C) 180–181; UV (nm) 316.2; IR (KBr) (cm^{-1}) 1312, 1343, 1219, 837; ^{1}H NMR (δ ppm) 1.33 (*d*, $J_{CH3-CH}=7$, 12H, CH_3), 3.88 (*m*, 2H, CH), 7.44 (*m*, 10H, ArH), 10.10 (*s*, 4H, NH), 10.60 (*s*, 2H, ArOH). Anal. calcd for $C_{28}H_{30}N_4O_6S_2$: N, 9.62; S, 11.01. Found: N, 9.46; S, 11.00.
12. Analytical data for compound **5a**: mp (°C) 205–206; UV (nm) 301.2; IR (KBr) (cm^{-1}) 3266, 1518, 1113, 841; ^{1}H NMR (δ ppm) 7.65 (*m*, 22H, ArH), 10.09 (*s*, 8H, NH). Anal. calcd for $C_{34}H_{30}N_8O_2S_2$: N, 17.32; S, 9.92. Found: N, 16.94; S, 9.72.