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Research Article

Evaluation of Novel *N*-(Dibenzylcarbamothioyl)benzamide Derivatives as Antibacterial Agents by Using DFT and Drug-Likeness Assessment

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Isomers of monothioureas, **2a**–**2d**, derived from the reaction of disubstituted benzoyl isothiocyanate and dibenzylamine were synthesised and characterised by using elementary analysis CHNS and IR, ^{1}H NMR, and ^{13}C NMR spectroscopies. The compounds were screened for their *in vitro* antibacterial activity by using selected Gram-positive bacteria, and moderate inhibition activity was displayed for compound **2b** with the value of inhibition zone 11 ± 0.8 mm at a concentration of 50 mg/ml. The outcomes of Lipinski's rule of five assessment appeared to be in agreement with all compounds as they adhered to most of the rules, in which they can be preliminarily classified as active drug-like. The frontier molecular orbitals (HOMO and LUMO) for halogen-substituted 3,4-dichloro (**2a**) and 3,4-difluoro (**2b**) were also determined by applying the computational method of density functional theory (DFT) to determine their relationship as a molecular descriptor in antibacterial activities. The value of LUMO energy for compound **2b** (1.8229 eV) is lower than that of compound **2a** (1.8492 eV) which indicates higher antibacterial activities.

1. Introduction

In recent times, the occurrence of microbial infections has tremendously raised in many countries around the world due to antimicrobial resistance [1]. This phenomenon has led to the design of novel antimicrobial as well as antibiotic divergent from the current classes of compounds [2]. Specifically, the growth of the new classes of antibacterial agents and modification to the known drugs must be conducted in such a way that would induce them to preserve their physiological action, but a reduction in their resistance towards the agents [3]. Thiourea is known as a versatile compound that has been intensely synthesised due to its ability to undergo structural modification [4]. Having two units of reactive primary amine group has led thiourea to be a suitable precursor for the synthesis of many derivatives in new compounds [5]. Oxygen, nitrogen, and sulphur atoms of thiourea derivatives provide a multitude of bonding possibilities that may contribute to a broad spectrum of thiourea applications in the pharmaceutical field, such as antiparasitic, anticancer, antioxidant, antibacterial, antifungal, antimalarial, and anti-HIV [6–8]. Furthermore, Mandava et al. explained in his research that the amide and indole groups at the moiety of thiourea showed considerable interaction with active site amino acid of ribosyltranferase [9]. In the literature survey and to the best of our knowledge, the disubstituted carbonyl thiourea derived from the secondary amines is still in scarcity [10].

In this study, the synthesis and characterisation of novel disubstituted carbonyl monothioureas (Figure 1) are reported after conducting related antibacterial screening. The

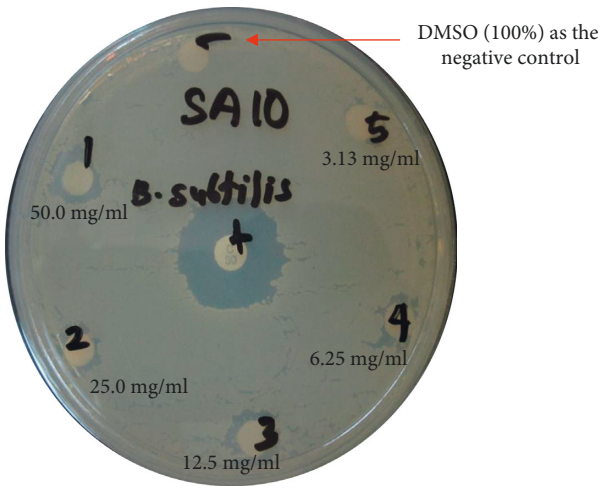


FIGURE 1: Antibacterial activity of compound **2b** towards bacterium *Bacillus subtilis*.

frontier molecular orbitals (HOMO and LUMO) were also determined via the computational method of DFT to determine their relationship as a molecular descriptor in antibacterial activities.

2. Experimental

2.1. Physical Measurement. All synthesis processes were carried out by using the conventional method of reflux, and no preventative measure was taken to exclude air or moisture. Chemical and solvents were purchased from Sigma-Aldrich or Merck and directly used without further purification. Infrared spectra were recorded by using FTIR Perkin Elmer Model Spectrum GX in the range of 400–4000 cm^{-1}. The ^1H NMR and ^{13}C NMR spectra of the samples were verified by using a spectrometer NMR model Joel EX 400 MHz in d_6-$DMSO_4$.

2.2. General Synthesis of Thiourea Derivatives. A mixture of disubstituted 3,4-difluorobenzoyl chloride (**1a**) (0.317 g, 0.0018 mol) and NH_4SCN (0.1370 g, 0.0018 mol) in 10 ml acetone was stirred at room temperature for 15 minutes. The mixture was filtered and directly uses "in situ" into a round bottom flask containing dibenzylamine (0.355 g, 0.0018 mol). The solution was refluxed for 3 hours [11]. The solution was filtered and poured into a beaker containing ice cubes to form a precipitate, which was then filtered, and the precipitate was proceeded for characterisation. The procedure was repeated for the synthesis of **2b**–**2d** using 3,4-dichlorobenzoyl chloride (**1b**) (0.377 g, 0.0018 mol), 2-chloro-4-fluorobenzoyl chloride (**1c**) (0.0.612 g, 0.0018 mol), and 2-chloro-5-fluorobenzoyl chloride (**1d**) (0.3430 g, 0.0018 mol).

*2.2.1. 3,4-Difluoro-N-(dibenzylcarbamothioyl)benzamide (**2a**).* Percentage yield, 74%; Mp: 365.7–366.3°C, (found: C, 63.08; H, 4.42; N, 6.54%; $C_{22}H_{18}F_2N_2OS$ requires C, 66.65; H, 4.58; N, 6.54%); IR (KBr pellets) ν (cm^{-1}): 3170, 1690, 1186. ^1H NMR (DMSO-d_6, 600 MHz) δ (ppm): 4.68, 5.24 (s, 4H, 2 × C-H_2); 7.19–7.90 (*m*, 8H, Ar-*H*); 11.08 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 150 MHz) δ (ppm): 56.2, 55.2 (2 × CH_2), 118.2–153.5 (Ar-C), 162.8 (C=O), 183.4 (C=S).

*2.2.2. 3,4-Dichloro-N-(dibenzylcarbamothioyl)benzamide (**2b**).* Percentage of yield, 65%; Mp: 365.4–366.5°C, (found: C, 62.80; H, 4.77; N, 7.91%; $C_{22}H_{18}Cl_2N_2OS$ requires C, 61.54; H, 4.22; N, 6.53%); IR (KBr pellets) ν (cm^{-1}): 3171, 1697, 1185. ^1H NMR (DMSO-d_6, 600 MHz): δ 4.71, 5.24 (2 × s, 4H, CH_2); δ 7.05–7.87 (8 × m, 8H, Ar); δ 8.54 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 56.9, 55.9 (2 × CH_2), δ 126.9–135.6 (Ar), δ 162.4 (C=O), δ 180.6 (C=S) [12].

*2.2.3. 2-Chloro-4-fluoro-N-(dibenzylcarbamothioyl)benzamide (**2c**).* Percentage of yield, 70%; Mp: 366.4–367.3°C, (found: C, 64.03; H, 3.71; N, 7.94%; $C_{22}H_{18}Cl_2N_2OS$ requires C, 64.00; H, 4.40; N, 6.79%); IR (KBr pellets) ν (cm^{-1}): 3271, 1702, 1189. ^1H NMR (DMSO-d_6, 600 MHz): δ 4.83, 5.20 (2 × s, 4H, CH_2); δ 7.22–7.94 (8 × m, 8H, Ar); δ 11.29 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 55.9, 55.2 (2 × CH_2), δ 114.9–162.2 (Ar), δ 163.8 (C=O), δ 182.4 (C=S).

*2.2.4. 2-Chloro-5-fluoro-N-(dibenzylcarbamothioyl)benzamide (**2d**).* Percentage of yield, 63%; Mp: 366.3–367.5°C, (found: C, 64.75; H, 4.84; N, 6.91%; $C_{22}H_{18}Cl_2N_2OS$ requires C, 64.00; H, 4.40; N, 6.79%); IR (KBr pellets) ν (cm^{-1}): 3179, 1708, 1190. ^1H NMR (DMSO-d_6, 600 MHz): δ 4.81, 5.20 (2 × s, 4H, CH_2); δ 7.14–7.42 (8 × m, 8H, Ar); δ 8.70 (s, 1H, NH). ^{13}C NMR (DMSO-d_6, 150 MHz): δ 56.3, 55.9 (2 × CH_2), δ 117.7–134.6 (Ar), δ 161.9 (C=O), δ 180.4 (C=S) [13].

2.3. Computational Method. Computational-assisted geometry optimisation, conformational analysis, and total energy calculation were carried out in the gas phase with Gaussian 09 suite of program using Beck's three-parameter hybrid method, B3LYP, with 6-31G (d, p) basis set to gain better comprehension regarding the molecular structure of the thiourea derivatives [14, 15].

2.4. Antibacterial Screening. Antibacterial screening was carried out using the disc diffusion technique with strains of bacteria from Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, and *Bacillus subtilis*). The control positive was chloramphenicol (50 mg/ml), while DMSO (100% of concentration) was the control negative. The bacteria were cultured in nutrient broth and left for 18–24 hours to grow. The nutrient agar and nutrient broth were in aseptic condition to prevent the bacteria from air, which may affect the results. The thioureas were dissolved in DMSO with 5 different concentrations: 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, and 3.125 mg/ml. Then, the single colony of bacteria was picked from the nutrient agar and placed into the nutrient broth. By using a micropipette, 5 μl of each compound with different concentrations was dropped onto the filter paper (6 mm

Table 1: Antibacterial screening of compounds **2a**, **2b**, **2c**, and **2d**.

Compound	Gram-positive bacteria (mm)/concentration of sample (mg/mL)		
	Staphylococcus aureus	*Enterococcus faecalis*	*Bacillus subtilis*
2a	—	—	—
2b	11 ± 0.8 (50 mg/ml)	—	8 ± 0.4 (50 mg/ml)
2c	—	—	—
2d	—	—	—
Positive control (chloramphenicol)	30.1 ± 0.0 (50 mg/ml)	20.0 ± 0.0 (50 mg/ml)	32.8 ± 0.0 (50 mg/ml)

"—" denotes no activity.

diameter). The inhibition result of the bacteria was noted after 18–24 hours of incubation at 37°C–48°C. The bacteria tests were performed in triplicate, and the results are shown in Table 1 [16].

3. Results and Discussion

The compounds **2a**–**2d** were synthesised in good yield (63–74%) by the reaction between disubstituted benzoyl chlorides (**1a**–**1d**) and ammonium thiocyanate to give the disubstituted benzoyl isothiocyanate as the intermediate which is later to be reacted with dibenzylamine (Scheme 1). The formations of products were confirmed by NMR, IR, and elemental analysis.

3.1. Antibacterial Analysis. The activity of compounds **2a**–**2d** against bacteria was observed after 24 hours of inhibition at 37°C–42°C. At concentration of 25.0, 12.5, 6.25, and 3.13 mg/ml, the compound showed no inhibition activity except for concentration of 50 mg/ml. Gram-positive bacteria of *Staphylococcus aureus* and *Bacillus subtilis* except *Enterococcus faecalis* exhibited positive results towards compound **2b**, which contradicted to compounds **2a**, **2c**, and **2d**, as no inhibition was recorded, as shown in Table 1. Figure 1 illustrates the antibacterial activity of compound **2b** towards bacteria *Bacillus subtilis*. 100% concentration of DMSO was used as a negative control and shows no clear expansion which indicates no inhibition of bacterial activity.

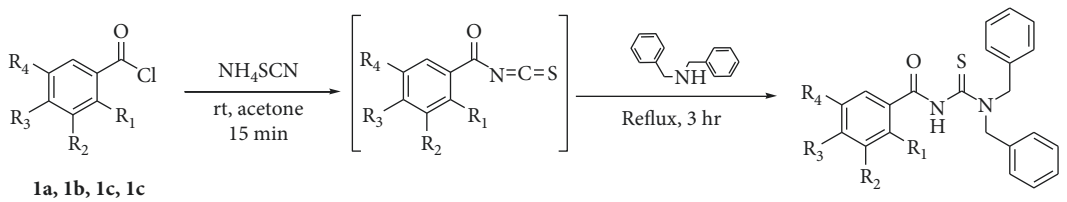
Overall, the synthesised compound **2b** displayed a significant inhibition activity compared to compounds **2a**, **2c**, and **2d**. It was clear from the study that the disubstituted halogen compound (3,4-chloro, **2b**) on the phenyl group showed comparatively potent activity than the 3,4-fluoro (**2a**), 2-chloro-4-fluoro (**2c**), and 2-chloro-5-fluoro (**2d**) substituted. This is due to the size of chlorine atom that is bigger than that of fluorine, thus exerting a significant impact on the London dispersion force and also the lipophilicity [17, 18]. In general, the addition of halogen substituents (in particular, Cl, Br, and I) increases the lipophilicity of a molecule. Larger and heavier atoms or molecules demonstrate stronger dispersion forces than smaller and lighter ones. As the London dispersion force increases, the lipophilicity interaction increases and as a result, penetration of the compounds into the bacterial membrane is eased [19]. Furthermore, the position of the chlorine atom at the meta-para (**2b**) position generally correlates well but not for ortho-substituted ones (**2c** and **2d**) due to the *ortho* steric effect and *ortho* polar effects which resulted to no antibacterial activity for compounds **2c** and **2d** that in contrast to **2b** [20].

Besides, in reference to Lipinski's rule of five regarding drug-likeness, all of the compounds appeared to adhere to most of the rules with only one violation, where lipophilicity (log P) value exceeded 5 (Table 2). This, however, concludes that compounds **2a**–**2d** can be classified as drug-like [21]. The evaluation of Lipinski's rule on the designated compound is crucial as the preliminary study on the ability of the compound to act as a drug, which then can be further carried out and tested in various related analyses. In detail, thiourea **2b** displayed the highest lipophilicity value (6.6), when compared to **2a**, **2c**, and **2d** which is 5.8 that corresponded to the result of antibacterial analysis. The higher the lipophilicity, the higher is the inhibition of the compound towards the bacteria. Further structural modification, including changing of substituents group, might be necessary for the other compounds **2a**, **2c**, and **2d** to refine their ability as antibacterial agents for them to display exceptional criteria as a drug from Lipinski's rule evaluation.

In addition, this antibacterial study is also supported by the computational calculation of density functional theory (DFT) at level theory of hybrid functional B3LYP/6-31G (d, p) basis set method to determine the band gap energy of HOMO-LUMO and the value of LUMO energy. The comparative study was conducted between (3,4-dichloro) **2a** and (3,4-di-fluoro) **2b** which have similar position for halogen substituted. The results reported in Table 3 show that the value of the LUMO energy for compound **2b** (1.8229 eV) is lower than that of **2a** (1.8492 eV), which is in agreement to the antibacterial result. Compound **2b** exhibited higher antibacterial activity, when compared to compound **2a,** as tabulated in Table 1. This is related to the lowest energy value of LUMO for compound **2b**, which contributed to its higher antibacterial activity as well as the size of the halogen (Cl) substituent [17, 22]. The collected data from the frontier molecular orbitals (HOMO-LUMO) analysis of thioureas **2a** and **2b** are recorded in Table 3 and illustrated in Figure 2.

4. Conclusion

The compounds **2a**–**2d** were successfully synthesised and fully characterised via spectroscopic analyses. From the antibacterial study, only thiourea **2b** exhibited moderate inhibition activity against selected Gram-positive bacteria, which are 11 ± 0.8 mm (50 mg/ml) towards bacteria *Staphylococcus aureus* and 8 ± 0.4 mm (50 mg/ml) towards bacteria *Bacillus subtilis*, in comparison to chloramphenicol as the control positive. Theoretical calculation unveiled that the antibacterial activity has some relationship with the LUMO energy value. The lower the LUMO value, the



1a = R_1:H, R_2:F, R_3:F, R_4:H
1b = R_1:H, R_2:Cl, R_3:Cl, R_4:H
1c = R_1:Cl, R_2:H, R_3:F, R_4:H
1d = R_1:Cl, R_2:H, R_3:H, R_4:F

Scheme 1: Reaction scheme to synthesis thiourea derivatives **2a**, **2b**, **2c**, and **2d**.

Table 2: Evaluation of Lipinski's rule of five for compounds **2a**, **2b**, **2c**, and **2d**.

Lipinski rule/compounds	**2a**	**2b**	**2c**	**2d**
1. Log P (lipophilicity) < 5	5.8	6.6	5.8	5.8
2. Hydrogen bond donor < 5	1	1	1	1
3. Hydrogen bond acceptor < 10	2	2	2	2
4. Molecular weight < 500 g/mol	396.46	429.36	412.91	412.91

Table 3: List of HOMO-LUMO energy of compounds **2a** and **2b**.

Compounds	HOMO (eV)	LUMO (eV)	HOMO-LUMO (eV)
2a	5.8295	1.8492	3.980
2b	5.9541	1.8229	4.131

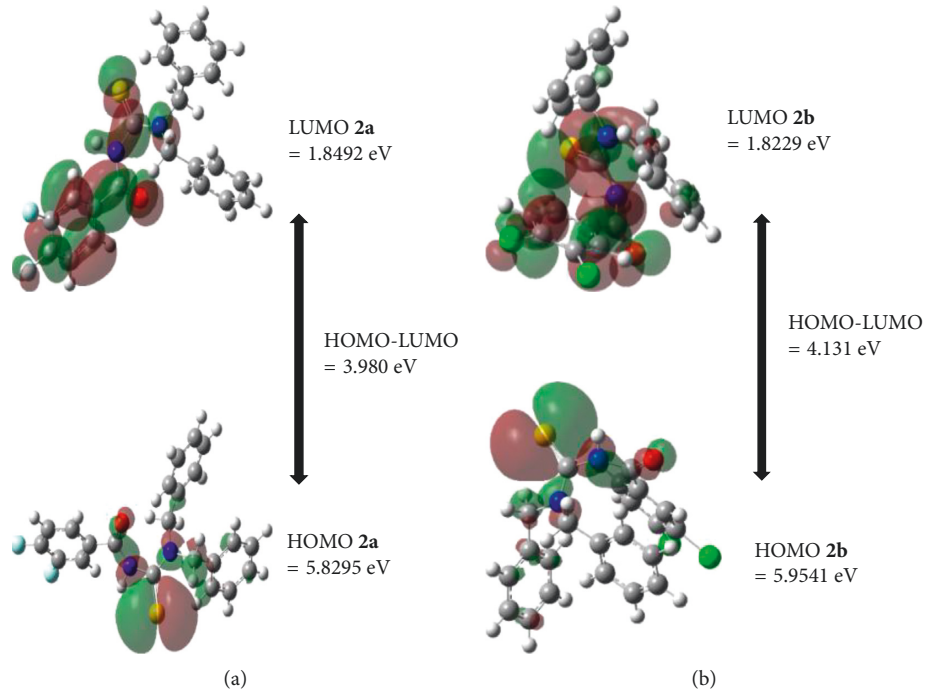


Figure 2: Frontier molecular orbitals (HOMO and LUMO) of thioureas **2a** and **2b**.

higher the antibacterial activity. Assessment from Lipinski's rule of five depicted that all of the valuable compounds can be classified as drug-like, thus suggesting an exceptional starting point for the compounds to be explored in future development. This study offers good insight towards selective applications in the antibacterial field

as it highlights the remarkable characteristics of a good antibacterial agent. Therefore, further detailed study, including modification of the substituents and the fragments of the compounds as antibacterial agents, is deemed necessary.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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