Synthesis, Characterization and Antimicrobial Evaluation with DFT Study of New Two-Amino-4-(4-Chlorophenyl) Thiazole Derivatives

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Abstract

2-amino-4-(4-chlorophenyl)-1,3-thiazole (1) was synthesized by refluxing thiourea with para-chloro phenacyl bromide in absolute methanol. The condensation of amine compound (1) with phenylisothiocyanate in the presence of pyridine will produce 1-(4-(4-chlorophenyl)thiazol-2-yl)-3-phenylthiourea (2), which is upon treatment with 2,4-dinitrophenyl hydrazine by conventional method, afforded 1-(4-(4-chlorophenyl) thiazol -2 – yl) - 3-phenylhydrazonamide, N’-(2,4-dinitrophenyl) (3). The characterization of the titled compounds were performed utilizing FTIR spectroscopy, 1HNMR and CHNS elemental analysis, and by measurements of their physical properties. The synthesized compounds had been screened for their, in vitro preliminary antimicrobial activity against three Gram-positive bacteria: (Staph. aureus, Micrococcus luteus and Bacillus subtilis) and three Gram-negative bacteria: (Pseud. aeruginosa, E.coli and Proteus mirabilis) and two fungal strains (Candida albicans and Candida glabrata), using a minimum inhibitory concentration (MIC) of 100 μg/ml of test compound, by well diffusion method. The derivatives showed moderate antibacterial activity against Gram-positive Staphylococcus aureus and Bacillus subtilis & high antifungal activity against Candida glabrata and Candida albicans. Computational study was performed to calculate some of thermodynamic parameters by using density functional theory (DFT).

Keywords: Antibacterial, 2-aminothiazole, Synthesis and DFT.

Introduction

Thiazoles are significantly important heterocyclic that represent interesting properties depending on the linked groups to the thiazole14. 2-aminothiazoles are a subclass of thiazole family that are of high interest due to their broad biological activity, fungicidal15, antioxidative16, analgesic17, bacitracid18 and anti-inflammatory12,19, antihiv20, antitubercular11. Thiazole containing N=C=S moiety have been used as antipyretics10 and antimalarial19, and as herbicidal16. Thiazoles have broadly been engaged as effective γ-secretase inhibitors to hinder Alzheimer’s disease18 and have a lot of derivations and because of its derivations type have a lot of application in various industries, medical science, and pharmacy. In 1887, Hantzsch et al. identified and established molecular structure of thiazoles. In 1889, the first derivation of thiazoles identified by Prop et al. and was named 2-amo thiazole12. After that until now, many derivations identified and reported13,14. Thiazole and related compounds are called 1, 3- azoles (nitrogen and one other heteroatom in a five- membered ring). They are isomeric with the 1, 2 – bazoles , the nitrogen, and sulfur compound being called isothiazole15.
compounds from readily available reagents is one of the major challenges in organic chemistry. Therefore to meet the facile results of these tough challenges thiazole nucleus was being considered. Among the wide variety of heterocycles that have been explored for developing pharmaceutically molecules. Our study focuses on derivatization of new molecules starting from the parent nucleus 2-amino-4-(4-chlorophenyl) 1,3-thiazole (1), by treatment with phenyl isothiocyanate, to yield (2), then refluxing it with 2,4-dinitrophenyl hydrazine to produce (3), subsequently evaluation of the produced compounds in vitro for their preliminary antimicrobial activity, also using DFT study to identify the changes in electronic structure responsible for pharmacological action.

**Material and Methods**

Chemicals used during the synthesis, supplied by hyper- chem/china. Completion of reactions and the purities of compounds were ascertained by thin-layer chromatography (TLC), using Silica gel G254 (type 60) pre-coated Aluminum sheets, Merck (Germany) exposed to UV-254nm light and the eluent used is n-hexane: ethyl acetate (6:0.4:0) for compound (1), n-hexane: ethyl acetate (8:0.2:0) for compound (2) and n-hexane: ethyl acetate (4.0:6.0:0) for compound (3). Melting points were measured by using Stuart SMP3 melting point apparatus in open capillary tubes, and are uncorrected. The infrared spectra were performed in KBr disc, (υ, cm$^{-1}$), using FTIR Spectrophotometer (Shimadzu, WQF-520, Japan) at the University of Baghdad College of Education for Pure Sciences Ibn Al-Haitham. The CHNS elemental microanalysis of the final synthesized products was done using (Elemental Vario MICRO cube instrument, Germany) in the University of Mustansiriya-College of Pharmacy.

**Chemical synthesis**

**General method for the Synthesis of 2-Amino-4-(4-chlorophenyl) thiazole (1)**

**Method 1 $^{18}$:**

A mixture of p-chloro phenacyl bromide (0.01 mol, 2.3 g) in 100 ml of absolute alcohol and thiourea (0.02 mol, 1.5 g) were taken in a round bottom flask and refluxed for 3 h. Then the reaction mixture was cooled and precipitated with cold water. The solid separated was collected by filtration and the solvent was removed under vacuum. The residue obtained was dried, recrystallized from ethanol.

2-amino-4-(4-chlor phenyl thiazole) (1) Off white powder, yield 95%, m.p 167-169°C, IR(KBr),(υ, cm$^{-1}$): (3438 and 3284) NH$_3$ str, of prim. amine, 3114 Ar(CH) str, 1535 Ar(C=Cl) str, 825 (C-Cl) str, 669 (C=S) str;

HNM R(300 MHz, DMSO-d$_6$ , $\delta$ = ppm): 7.98 (2H, d, Cl - 2Ar – H); 7.41 (2H, d, Cl-2Ar-H); 7.08 (1H, s, H$_2$-Thiazol); 7.06 (2H, s, NH$_2$).

**Method 2 $^{19}$:**

A mixture of p-chloro phenacyl bromide (0.0064 mol, 1.5 g), and thiourea (0.0064 mol, 0.489 g) in 10 ml of DMSO was stirred at room temperature until completion of the reaction. The time of the reaction was monitored by a stopwatch. The progress of the reaction was monitored by thin-layer chromatography. On completion of the reaction, the reaction mixture was poured into crushed ice. The precipitated product was filtered and dried and recrystallized from ethanol. The product was pure enough (single spot on TLC) for all practical purposes.

**General method for the synthesis of 1-(4-(4-chlorophenyl) thiazol-2-yl)-3-phenylthiourea (2) $^{20}$**

A mixture of (1), (0.01 mol, 2.1 g) in pyridine (20 mL) was added to phenyl isothiocyanate (0.01 mol, 1.35 g), and the reaction mixture refluxed for 6 h. It was then cooled, and the solvent removed in vacuo. The resulting residue was triturated with water, and the solid obtained was recrystallized from ethanol.

1-(4-(4-chlorophenyl)thiazol-2-yl)-3-phenylthiourea (2)

Yellow powder, yield 92%, m.p 255-260°C, IR(KBr) (υ, cm$^{-1}$): 3159 Ar( NH) str, 3113 Ar(C=CH) str, 1576 Ar(C=Cl) str, 1196 (C=N) str, 1658 (C=C) str, elemental micro analysis: calcd. for C$_6$H$_4$Cl$_2$N$_2$S$_2$: C, 55. 56; H, 3.50; N, 12.15; S, 18.54%. Found: C, 55.29; H, 3.363; N, 12.08; S, 18.196%.

**General method for the Synthesis of 1-(4-(4-chlorophenyl)thiazol-2-yl)-3-phenylhydrazonamide N'-(2,4-dinitrophenyl) (3) $^{21}$**

A mixture of (2) (0.001mol, 0.345g) and 2,4 di nitro phenyl hydrazine (0.001mol 1.98g) in absolute ethanol (30 ml) was refluxed for 7h. The product separated during reflux, was filtered off and recrystallized from ethanol as brown powder.

1-(4-(4-chlorophenyl)thiazol-2-yl)-3-phenylhydrazonamide N'-(2,4-dinitrophenyl) (3)

Brown powder, yield 78%, m.p 135-137°C, IR(KBr),(υ, cm$^{-1}$): 3313 (NH) str of sec. amine, 3026 Ar(CH) str, 1614(C=N) str, 1587 Ar(C=Cl) str, 1512 sym. NO$_2$ str, 1331 sym. NO$_2$ str, 1196 (C-N) str, 833 (C=C) str, 660(C=S) str.

Elemental analysis: calcd. for C$_8$H$_7$Cl$_2$N$_3$O$_2$: C, 51.82; H, 3.16; N, 19.23; S, 6.29%. Found: C, 51.050; H, 3.11; N, 18.93; S, 5.98%.
IR spectrum of compound 1

$^1$HNMR of compound 1
Scheme 1. Synthesis of 2-amino thiazole derivatives (2 and 3)

Antimicrobial screening
The antimicrobial activities of the synthesized derivatives were measured using well diffusion technique with a comparison to cefotaxime sodium (cefot.) and sulfamethoxazole (sulf.) as standard antibacterial agents, and miconazole as standard antifungal agent, using dimethylsulfoxide (DMSO) as solvent and as a control, and it was run in the Ministry of Health/ National Center for Drug Control and Research (NCDCR) / Baghdad, and in University of Baghdad/ College of Education for Pure Sciences Ibn Al-Haitham /central service laboratory. The synthesized compounds had been screened for their in vitro preliminary antimicrobial activity against three Gram-positive bacteria: (Staph. aureus, Micrococcus luteus and Bacillus subtilis), three Gram-negative bacteria (Pseud. aeruginosa, E.coli, and Proteus mirabilis) and two fungi (Candida albicans and Candida glabrata), using a minimum inhibitory concentration (MIC) of
100 µg/ml of reference compounds (2) and (3) in DMSO as shown in Table 1.

**Results and Discussion**

**Chemistry**

The 2-Amino-4-(4-chlorophenyl)thiazole (1) synthesis, was carried out according to Hantzsch method by reaction of phenacyl bromide with thiourea in the presence of absolute methanol for 3 hours. They are characterized by FTIR, due to the appearance of primary amine (NH\(_2\)) stretching at 3438 and 3284 cm\(^{-1}\) and then reaction of (1) with phenylisothiocyanate in the presence of pyridine to produce (2) which was characterized by FTIR, a band of (C=S) stretching displayed at 1254 cm\(^{-1}\), and NH stretching band at 3159 cm\(^{-1}\), and finally condensation of (2) with 2,4 dinitrophenylhydrazine using absolute ethanol as a solvent, to afford (3) which showed the characteristic NO\(_2\) (asymmetrical and symmetrical) stretching at 1512 and 1331 cm\(^{-1}\), respectively, also peak attributed to (C=N) band recorded at 1614 cm\(^{-1}\).

**Synthesis of compound (1)**

The method of parent nucleus synthesis was described by Hantzsch, in 1887 the cyclization of α-halo carbonyl compounds by a great variety of reactants containing the N-C-S fragment of the ring is still the widely used method of synthesis of thiazoles, as shown in the following scheme (2) \(^22\).

**Synthesis of compound (2)**

The condensation of parent nucleus (1), with phenyl isothiocyanate, afforded the corresponding 1- (4-(4-chlorophenyl)thiazol-2-yl) -3-phenylthiourea derivative (2), the unshared pair of electron of NH\(_2\) of (1) would attack the carbon (C = S) of thio carbonyl group \(^23\) as shown in the following scheme 3.
Synthesis of compound (3)

The reactivity of phenyl isothiocyanate is, determined by two active centers the nucleophilic nitrogen atom, and thiocarbonyl groups, which make them capable of participating in diverse type of addition and cyclization reactions. The unshared pair of electron of NH₂ group of 2,4-dinitro phenyl hydrazine would attack thiocarbonyl carbon(C=S) of (2) and in the presence of acidic hydrogen of ethanol, H₂S gas would be evolved, and the tatumeric structure of (3), would also formed (24), as shown in the scheme 4.

Scheme 4. Mechanism of Synthesis of (3)

Antimicrobial Activity

From the results illustrated in table 1, showed that the new synthesized derivative (2 and 3) have moderate activity against Gram-positive bacteria Staph. aureus and Bacillus subtilis also, both compounds have no antibacterial activity against Gram-negative bacteria. It is evident that the tested compounds demonstrated the most potent antifungal activity against Candida albicans and Candida glabrata.
Table 1. The antibacterial and antifungal activity of the tested compounds.

| Comp. No. | Conc. µg/ml | S. aureus Conc. µg/ml (G⁺⁺⁺) | Micrococcus luteus Conc. µg/ml(G⁺⁺⁺) | Bacillus subtilis Conc. µg/ml (G⁺⁺⁺) | Proteus mirabilis Conc. µg/ml (G⁺⁺⁺) | Pseudomonas aeruginosann Conc. µg/ml (G⁺⁺⁺) | E. coli Conc. µg/ml (G++) | Candida glabrata Conc. µg/ml | Candida albicans Conc. µg/ml |
|-----------|-------------|-------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|------------------------------------------|---------------------|-----------------------------|-----------------------------|
| 2         | 100         | 9.3                           | 8.0                                 | 10.15                               | -                                   | -                                         | -                   | 17                          | 15.5                        |
| 3         | 100         | 10.4                          | -                                   | 10.37                               | -                                   | -                                         | -                   | 16                          | 18.6                        |
| Cefot.    | 100         | 44.5                          | 34.4                                | 36.05                               | 33.5                                | 35.5                                      | 56.7                | -                           | -                           |
| sulf.     | 100         | 23                            | 19.06                               | 25.15                               | 19.5                                | 18.9                                      | 35.6                | -                           | -                           |
| Miconazole| 100         | -                             | -                                   | -                                   | -                                   | -                                         | -                   | 17.0                        | 16.9                        |
| DMSO      | 100         | -                             | -                                   | -                                   | -                                   | -                                         | -                   | -                           | -                           |

(-)= No activity, (+) = slightly active (Inhibition Zone in between 5-10 mm), (++) = moderately active (Inhibition Zone in between 10-15 mm), (+++) = highly active (Inhibition Zone More Than 15 mm).

**Computational Studies**

**Density function theory (DFT) study**

In order to explore the theoretical-experimental consistency, quantum chemical calculations were performed with complete geometry optimizations using standard Spartan 10 software. Geometry optimization was carried out by B3LYP/6-31G* level of theory. The chemical reactivity descriptors calculated using DFT are: total energy (E), log p and electronegativity (μ).

On the basis of frontier molecular orbitals, chemical hardness corresponds to the gap between the HOMO and LUMO, where ELUMO and EHOMO are the LUMO and HOMO energies. electronegativity (μ) is defined as the negative of electronegativity of a molecule. Physically, (μ) describes the escaping tendency of electrons from an equilibrium system. It is a measure of the stabilization in energy after a system accepts additional amount of electronic charge from the environment.

**Results and Discussion**

**Structural and electronic properties**

DFT calculations were performed for compound 1-3. Optimized molecular structures of the most stable form are shown in Figure 1.
It can be seen that the energy gaps between HOMO and LUMO for compound 1 is 6.915, compound 2 is –5.85, compound 3 is 1.425. The lower value in the HOMO and LUMO energy gap explains the eventual charge transfer interaction taking place within the molecules. The larger the HOMO–LUMO energy gap, more stable/less reactive the molecule, for example, compound 1. The values of (μ) for compounds 1, 2 and 3 are presented in (Table 1 row 5). The trend in electronegativity for compounds 1-3 are: 2 > 3 > 1 . The greater the electronic chemical potential, the less stable or more reactive is the compound. Therefore, compound 2 is more reactive than compounds 3 and 1, respectively. Therefore, compound 2 is the most reactive, and 3 and 1 are the least reactive.

**Electrostatic potential charges and related quantum chemical properties**

The distribution of the electronic density (electrostatic potential charges), related quantum chemical parameters; HOMO/LUMO gap (Table 1, row 4), the trends in energy gap 1> 3> 2 . On the other hand log p provides a measure of a molecule’s lipophilicity, where high log p-value indicates high lipophilicity, while low value suggests low lipophilicity. The partition coefficients of the compounds (log p), (Table 1, row 6) were calculated for observed compounds 1-3. The results indicated that compounds having the highest lipophilicity were 3 > 2 > 1 with corresponding values 6.66, 6.04 and 3.61, respectively. These values and properties are very useful and can be used in order to evaluate chemical properties and possibilities for interaction of compounds with biological macromolecules (receptors, enzymes) (27).

| Compound (3) | Compound (2) | Compound (1) | Parameters |
|--------------|--------------|--------------|------------|
| Total Energy kcal/mol | 1.4619 | -4.2637 | 23.7835 |
| HOMO ev | -8.052 | -5.51 | -6.283 |
| LUMO ev | -1.137 | 0.34 | -4.858 |
| Energy gap ev | 1.425 | -5.85 | 6.915 |
| Electronegative μ/ eV | -4.59 | -2.59 | -3.85 |
| Log p | 3.61 | 6.04 | 6.66 |
Conclusion
A new derivatives of 2-amino thiazole were successfully synthesized using conventional method and tested for their preliminary antimicrobial activity, using well diffusion method. The synthesized compounds displayed slight antibacterial activities against Gram-positive types, while demonstrated no antibacterial activities against Gram-negative bacteria, and high antifungal activity against tested fungal species.

The quantum chemistry calculations using the Density Function Theory (DFT) method was performed to study stability of compounds 1-3. The results showed that the compound 1 is more stable than compound 3, as was indicated via calculations of the larger the HOMO–LUMO energy gap parameter (28).

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