

Article

# Synthesis of *N*-(6-Arylbenzo[d]thiazole-2-acetamide Derivatives and Their Biological Activities: An Experimental and Computational Approach

Yasmeen Gull <sup>1,2</sup>, Nasir Rasool <sup>1,\*</sup>, Mnaza Noreen <sup>1</sup>, Ataf Ali Altaf <sup>3</sup>, Syed Ghulam Musharraf <sup>4</sup>, Muhammad Zubair <sup>1</sup>, Faiz-UI-Hassan Nasim <sup>5</sup>, Asma Yaqoob <sup>5</sup>, Vincenzo DeFeo <sup>6,\*</sup> and Muhammad Zia-UI-Haq <sup>7</sup>

<sup>1</sup> Department of Chemistry, Faculty of Science and Technology, Government College University Faisalabad, Faisalabad 38000, Pakistan; yasmeenchem1@yahoo.com (Y.G.); mnazanoreen@yahoo.com (M.N.); nr\_308@hotmail.com (M.Z.)

<sup>2</sup> Department of Chemistry, Faculty of Science, University of Sargodhah, Bhakkar Campus, Bhakkar 30000, Pakistan

<sup>3</sup> Department of Chemistry, Faculty of Science, University of Gujrat, Hafiz Hayat Campus, Gujrat 50700, Pakistan; atafali\_altaf@yahoo.com

<sup>4</sup> International Center for Chemical and Biological Sciences, Hussain Ebrahim Jamal Research Institute of Chemistry, University of Karachi, Karachi 75270, Pakistan; musharraf1977@yahoo.com

<sup>5</sup> Department of Chemistry, Faculty of Science, Islamia University of Bahawalpur, Bahawalpur 63000, Pakistan; faiznasim@hotmail.com (F.-H.N.); asmayaqoobctn@gmail.com (A.Y.)

<sup>6</sup> Department of Pharmaceutical and Biomedical Sciences, University of Salerno, Via Ponte don Melillo, Fisciano (Salerno) I-84084, Italy

<sup>7</sup> Offices of Research, Innovation and Commercialization, Lahore College for Women University, Lahore 54600, Pakistan; ahirzia@gmail.com

\* Correspondence: nasirrasool@gcuf.edu.pk (N.R.); defeo@unisa.it (V.D.F.); Tel.: +92-332-749-1790 (N.R.); +39-089-959-751 (V.D.F.); Fax: +92-41-920-1032 (N.R.); +39-089-969-602 (V.D.F.)

Academic Editor: Maxim L. Kuznetsov

Received: 31 December 2015 ; Accepted: 1 February 2016 ; Published: 25 February 2016

**Abstract:** A new series of *N*-(6-arylbenzo[d]thiazol-2-yl)acetamides were synthesized by C-C coupling methodology in the presence of Pd(0) using various aryl boronic pinacol ester/acids. The newly synthesized compounds were evaluated for various biological activities like antioxidant, haemolytic, antibacterial and urease inhibition. In bioassays these compounds were found to have moderate to good activities. Among the tested biological activities screened these compounds displayed the most significant activity for urease inhibition. In urease inhibition, all compounds were found more active than the standard used. The compound *N*-(6-(*p*-tolyl)benzo[d]thiazol-2-yl)acetamide was found to be the most active. To understand this urease inhibition, molecular docking studies were performed. The *in silico* studies showed that these acetamide derivatives bind to the non-metallic active site of the urease enzyme. Structure-activity studies revealed that H-bonding of compounds with the enzyme is important for its inhibition.

**Keywords:** Suzuki cross coupling; Pd(0) catalyst; benzothiazole; nitric oxide scavenging activity; antiurease activity; haemolytic activity

## 1. Introduction

Benzothiazoles consist of a benzene ring fused with a thiazole ring. Various benzothiazole derivatives serve as drugs, dyes and industrial chemicals [1–3]. Benzothiazole and its derivatives such as esters have also been reported as active against Gram-positive and Gram-negative bacteria

such as *Staphylococcus epidermidis*, *Escherichia coli*, *Enterobacter* and yeast (*Candida albicans*) [4]. Benzothiazole derivatives have also found to possess anticancer, antifungal and antibacterial activities [5,6]. 2-Aminobenzothiazole and a number of other aminobenzothiazole derivatives have been reported as muscle relaxants [7,8]. A literature survey performed for the current study revealed that 6-substituted-2-aminobenzothiazole derivatives such as 6-methyl, 6-methoxy, 6-ethoxy and 6-isopropoxy show antibacterial, anti-inflammatory and analgesic properties [9]. Various other derivatives are found to be cytotoxic against various tumors [10,11].

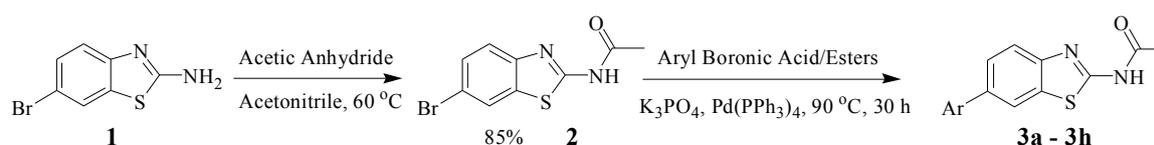
Suzuki cross coupling reactions are remarkable methods for C-C bond formation, utilized for the synthesis of agrochemicals, advanced materials and pharmaceuticals at both the industrial and laboratory scale [12–17].

The purpose of this study was to synthesize novel *N*-(6-arylbenzo[d]thiazol-2-yl)acetamides employing the Pd(0)-catalyzed Suzuki cross coupling methodology. This article describes our optimized experiments for the synthesis of *N*-protected 6-bromobenzothiazoles. The biological activities of these newly synthesized molecules were studied with the intention to explore their potential as future drugs. We investigated urease inhibition, nitric oxide scavenging, haemolytic and antibacterial activities. Molecular docking studies were performed to determine how they bind to the urease enzyme. To the best of our knowledge, all the studies reported in the current manuscript have not been reported in the literature to date.

## 2. Results and Discussion

### 2.1. Chemistry

Majo *et al.* reported low to moderate yielding one-step Suzuki cross coupling reactions using various boronic acids/ester with 2-bromobenzothiazole under thermal conditions [18]. We have previously reported Pd(0)-catalyzed reactions of 2-amino-6-bromobenzothiazole with different arylboronic pinacol esters/arylboronic acids using Suzuki cross coupling methodology with moderate yields [19]. We have not been able to achieve better yields as the amino moiety present in the benzothiazole molecule is basic and nucleophilic. In the current study, we have tried to enhance the yield of the synthesized molecules. In order to achieve high yields, the amino group was protected via acylation, which led to substantially enhanced yields of the products **3a–3h** (Figure 1) compared to unprotected benzothiazole derivatives as reported in literature [19].



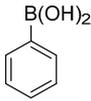
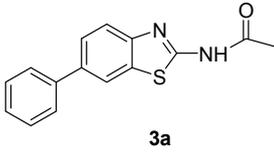
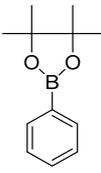
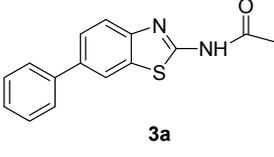
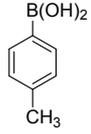
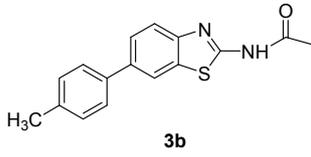
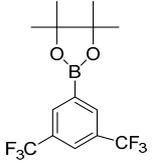
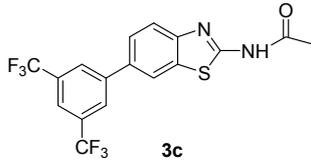
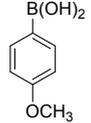
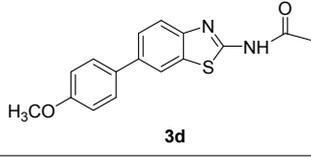
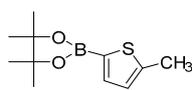
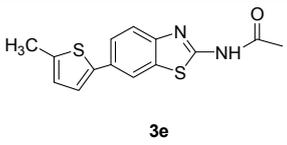
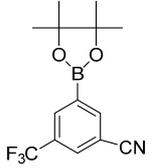
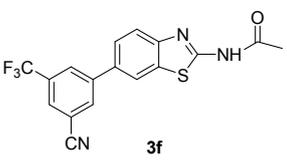
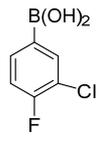
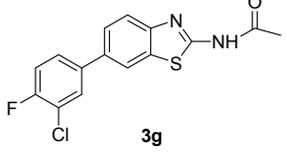
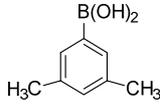
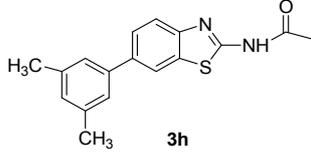
**Figure 1.** Synthesis of *N*-(6-bromobenzo[d]thiazol-2-yl)acetamide (**2**) and *N*-(6-aryl-benzo[d]thiazol-2-yl)acetamides **3a–3h**.

Furthermore, we also optimized other reaction parameters like catalyst loading, solvent, temperature and base used in the reactions producing **3a–3h**. Thus, we tried various solvents like toluene, dimethylformamide (DMF) and 1,4-dioxane at different temperatures (80–100 °C). It was noted (in Table 1) that the solvent has an effect on overall reaction yield. 1,4-Dioxane was found to be the best solvent because of its better solvation of the reactants. In our studies, these cross coupling reactions progressed efficiently, even in the presence of known sensitive groups such as CN, to give the desired products in very good yields. Finally after optimization, we investigated the coupling of **2** with different arylboronic pinacol esters/acids in the presence of Pd(PPh<sub>3</sub>)<sub>4</sub> as catalyst.

Product **3a** was prepared with 80% yield, when **2** was coupled with phenyl boronic acids under the set reaction conditions. The highest yield obtained in this series of reactions (85%) corresponded to product **3b**. Product **3h** was also obtained in excellent yield (83%) with this cross coupling method.

It was seen that the product **3c** with an electron withdrawing moiety showed a high yield (81%). Our studies showed that overall the acetylated 2-amino-6-bromobenzothiazole (**2**) gave good yields in these coupling reactions.

**Table 1.** Synthesis of *N*-(6-arylbenzo[d]thiazol-2-yl)acetamides **3a–3h**.

Entry	Arylborynic Pinacol Ester/Arylborynic Acid	Product	H <sub>2</sub> O/Solvent (1:4)	Yields %
1		 <b>3a</b>	Toluene 1,4-Dioxane	75 80
2		 <b>3a</b>	1,4-Dioxane	77
3		 <b>3b</b>	1,4-Dioxane	85
4		 <b>3c</b>	1,4-Dioxane	81
5		 <b>3d</b>	1,4-Dioxane	79
6		 <b>3e</b>	1,4-Dioxane	75
7		 <b>3f</b>	1,4-Dioxane	77
8		 <b>3g</b>	1,4-Dioxane	79
9		 <b>3h</b>	1,4-Dioxane	83

## 2.2. Biological Studies

### 2.2.1. Urease Inhibitory Activity

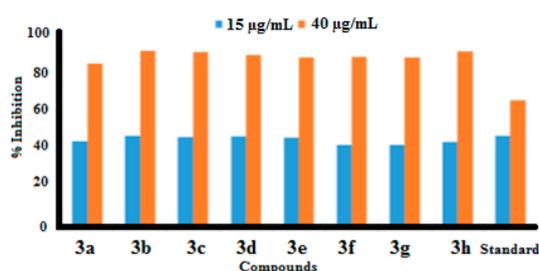
Urease is a nickel-containing metalloenzyme that catalyzes the hydrolysis of urea to form ammonia and carbamate, which further decomposes to yield a second equivalent of ammonia and carbon dioxide [20]. Bacterial ureases have been reported as an important virulence factor in the development of many harmful clinical conditions for human and animal health. Urease is directly involved in the formation of infectious stones and contributes to pathogenesis [21]. It is the major cause of pathogenesis induced by *Helicobacter pylori*, which plays an important role in peptic ulcers and may lead to stomach cancer. In recent years, a number of compounds have been proposed as urease inhibitors [22,23], which are considered interesting new targets for anti-ulcer drugs and for the treatment of infections caused by urease producing bacteria [24].

In the current study, urease inhibitory activity assays were performed following a previously reported protocol [25]. Thiourea was used as standard in the assay with an  $IC_{50}$  value of 23.1  $\mu\text{g/mL}$ . All of the synthesized benzothiazole derivatives were examined for their urease inhibitory activities at concentration of 15 and 40  $\mu\text{g/mL}$ . All of our synthesized compounds exhibit good to excellent urease inhibitory activities (Table 2, Figures 2 and 3).

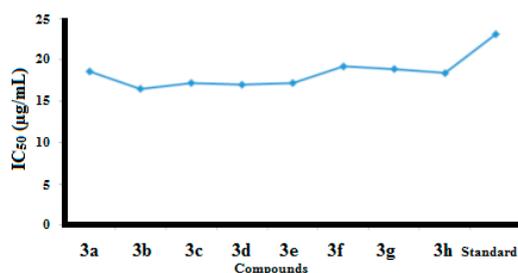
**Table 2.** Antiurease activity of *N*-(6-arylbenzo[d]thiazole-2-yl)acetamides 3a–3h at 15  $\mu\text{g/mL}$  and 40  $\mu\text{g/mL}$ .

Compound	% Inhibition at 15 $\mu\text{g/mL}$	% Inhibition at 40 $\mu\text{g/mL}$	$IC_{50}$ ( $\mu\text{g/mL}$ )
3a	44 $\pm$ 0.12	83.9 $\pm$ 0.12	18.6
3b	47 $\pm$ 0.11	90.51 $\pm$ 0.19	16.5
3c	46.09 $\pm$ 0.10	90.07 $\pm$ 0.20	17.2
3d	46.5 $\pm$ 0.15	88.5 $\pm$ 0.24	17
3e	46 $\pm$ 0.12	87 $\pm$ 0.26	17.2
3f	42 $\pm$ 0.12	87.5 $\pm$ 0.26	19.2
3g	42 $\pm$ 0.14	87 $\pm$ 0.26	18.9
3h	43.59 $\pm$ 0.13	90.33 $\pm$ 0.20	18.4
Standard	47 $\pm$ 0.31	65 $\pm$ 0.01	23.1

Each values is mean  $\pm$  Standard deviation of three parallel measurements.



**Figure 2.** The urease percentage inhibition values at 15  $\mu\text{g/mL}$  and 40  $\mu\text{g/mL}$ .



**Figure 3.**  $IC_{50}$  values of anti-urease activity.

Urease enzyme has an active binding site and it was believed that the newly synthesized benzothiazole derivatives have the capability to bind to these active sites of urease enzyme. In this way the hydrolysis of enzyme is stopped and activity of enzyme is inhibited. Compound **3b** showed the highest urease inhibition activity ( $90.51 \pm 0.19$ ) with an  $IC_{50}$  value of  $16.5 \mu\text{g/mL}$  at  $40 \mu\text{g/mL}$ .

Compounds **3h** and **3c** exhibited excellent urease inhibitory activities ( $90.07 \pm 0.20$  and  $90.33 \pm 0.20$  at  $40 \mu\text{g/mL}$ ) with  $IC_{50}$  values of  $17.2 \mu\text{g/mL}$  and  $18.4 \mu\text{g/mL}$ , respectively. Molecules **3a**, **3d**, **3e**, **3f** and **3g** exhibited good urease inhibition with  $IC_{50}$  values of 18.6, 17, 17.2, 19.2 and  $18.9 \mu\text{g/mL}$ , respectively. Notably we observed that the presence of electron donating methyl functional groups produced high urease inhibition. We also noted that different functional groups are responsible for variable antiurease activities of the compounds.

### 2.2.2. In Silico Studies with Urease

To understand the binding of the synthesized compounds with urease *in-silico* studies were performed. Only compounds with  $IC_{50}$  values were analyzed for docking studies using the freely available software AutoDock 4.2 and others as described in the Experimental Section. All the compounds were screened at different sites (A & B) of the enzyme. The nickel-containing catalytic site A is the most commonly tested site in the literature [26], while site B is less commonly targeted [20,27]. Our compounds bind more strongly to Site B than to site A. The docking studies results were compared with the experimental results and listed in Tables 3 and 4. Binding energy, inhibition constant and moldock scores are reported in Table 3, whereas the moldock H-binding energy and number of H-bonding interactions between the test compounds and enzyme are reported in Table 4.

**Table 3.** Experimental and docking comparative data.

Compound	* $IC_{50}$ ( $\mu\text{g/mL}$ )	Inhibition Constant	Binding Energy	Moldock Score
<b>3a</b>	18.6	267.93	−4.87	−83.39
<b>3b</b>	16.5	232.29	−4.96	−81.68
<b>3c</b>	17.2	232.56	−4.96	−68.81
<b>3d</b>	17.0	434.76	−4.59	−69.20
<b>3e</b>	17.2	278.10	−4.85	−87.00
<b>3f</b>	19.2	90.570	−5.52	−87.90
<b>3g</b>	18.9	153.46	−5.20	−79.91
<b>3h</b>	18.4	150.67	−5.21	−80.11

\* Experimentally measured *in vitro*.

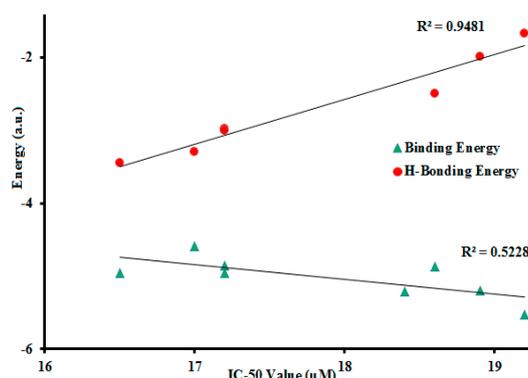
**Table 4.** H-bonding parameters calculated by Mol-Dock Molegero Molecular Viewer 2.5.

Compound	Number of H-bonds	H-Bonding Type (K-H-L) *	H-Bond Distance (K-L) (Å)	$IC_{50}$ ( $\mu\text{g/mL}$ )	H-Binding Energy
<b>3a</b>	1	O—H—N	2.957	18.6	−2.50
<b>3b</b>	2	N—H—N N—H—O	2.837 3.544	16.5	−3.45
<b>3c</b>	1	N—H—O	2.782	17.2	−2.98
<b>3d</b>	2	N—H—O N—H—N	3.023 3.23	17.0	−3.30
<b>3e</b>	2	N—H—O N—H—N	2.906 3.388	17.2	−3.01
<b>3f</b>	1	N—H—O	2.739	19.2	−1.67
<b>3g</b>	1	N—H—O	2.696	18.9	−1.99
<b>3h</b>	zero	—	—	18.4	—

\* K atom from the compound and L atom from the protein residue.

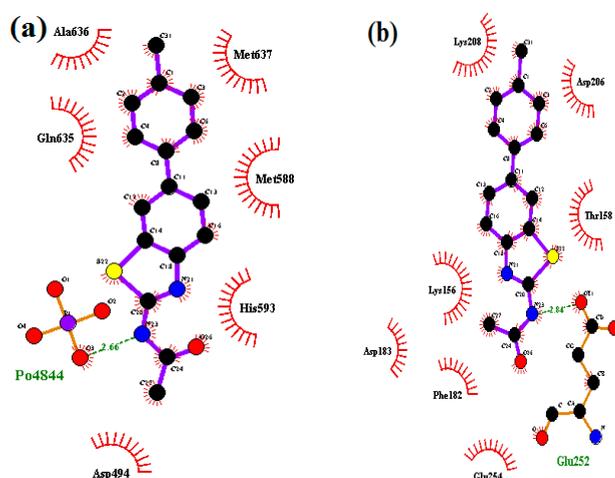
The number of H-bonding interactions was estimated from the most stable complex formed between the test compound and the enzyme. We found a linear correlation between the experimental  $IC_{50}$  values and the calculated binding energy, as shown in Figure 3. This linear correlation of results is

in agreement with the data reported in the literature [26,28]. More interestingly a better correlation was found between IC<sub>50</sub> values and the moldock H-binding energy (Figure 4).



**Figure 4.** Correlation between docking predicted energies (in arbitrary unit) and *in vitro* IC<sub>50</sub> values.

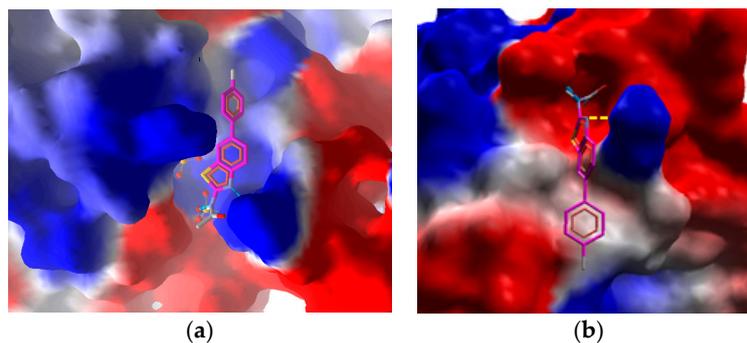
These results show that the H-bonding is more important, in urease inhibition mechanism, than other factors involved in this biological reaction. Compound **3b**, with the highest *in vitro* activity, is represented here for modeling analysis and its most active conformations are explained in the following paragraphs. As urease is a nickel-dependent enzyme, the active site A of the enzyme shows weak hydrophobic interaction with compound **3b** therefore, a drug with hydrophobic substituents would be able to bind strongly as it would project into the hydrophobic grooves of the enzyme and thus effectively inhibit its activity. The LIGPLOT interaction images show that compound **3b** has a total of seven interactions with enzyme site A (Figure 5a).



**Figure 5.** LIGPLOT images of compound **3b** with urease enzyme (a) at the catalytic site A; and (b) at the catalytic site B.

The amino acids His593, Met637, Ala636, Gln635, Met588 and Asp494 form hydrophobic interactions with compound **3b**. The hydrophobic interactions favor ligand binding with proteins having metal ions. Furthermore, the study showed hydrogen bonding (2.66 Å, N–H—O type) between compound **3b** and PO<sub>4</sub> group of the enzyme nickel catalytic site A.

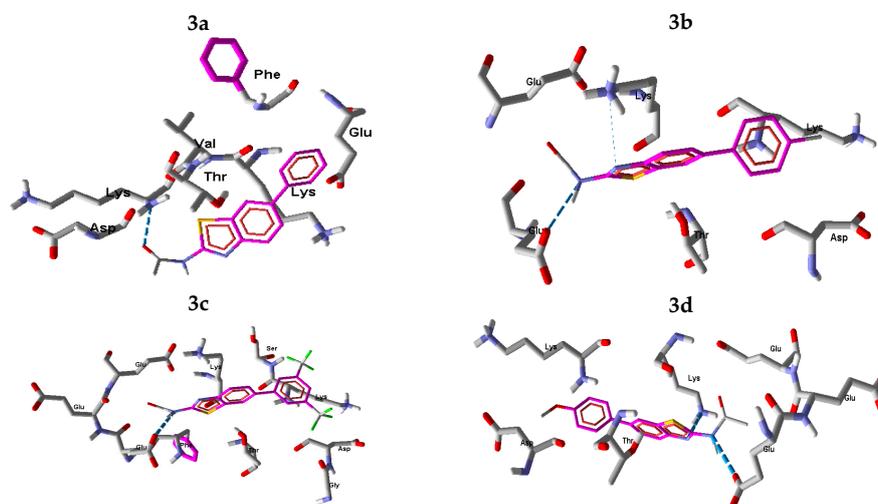
Figure 6a represents the most interacting conformation of **3b** in the active pocket (site A) of the enzyme at the electrostatic surface. Figure 6 is generated by Molegro Molecular viewer and analyzed by moldock score. The moldock analysis shows that there are two H-bonding interactions in both cases these are also reported in Table 4.



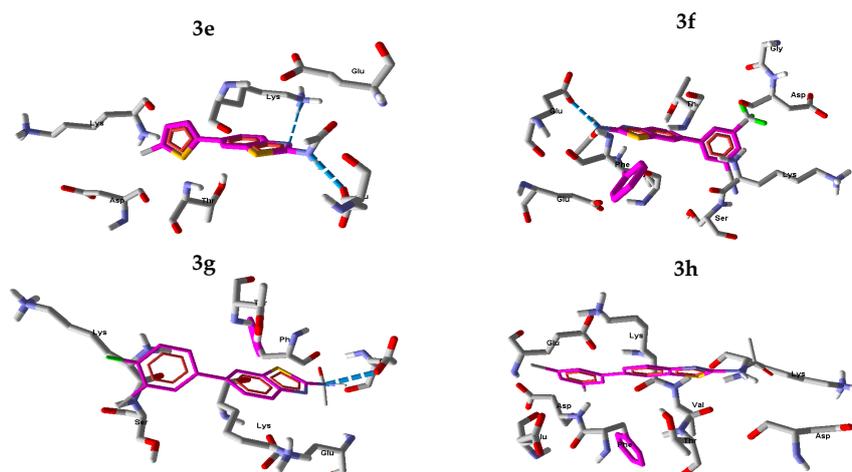
**Figure 6.** Compound **2c** in the Molegro molecular viewer generated electrostatic surface of urease enzyme (a) at the catalytic site A; and (b) at the catalytic site B. The yellow dashed line represents the H-bonding.

All of the synthesized compounds **3a–3h** show better interaction at site B. The LIGPLOT interaction diagram of compound **3b** illustrates that this inhibitor has better interactions with the protein. The LIGPLOT interaction images show that the compound **3b** has a total of eight interactions with enzyme site B (Figure 5b). The amino acids Lys208, Asp206, Thr158, Glu254, Phe182, Lys156 and Asp183 form cationic— $\pi$  interactions with compound **3b**, while Glu252 interacts via hydrogen bonding (2.84 Å, N—H—O type) with compound **3b**. Figure 5b presents the most interacting conformation of compound **3b** in the active pocket of the enzyme (site B). The diagram shows that the enzyme provides enough space for the accommodation of **3b** inside the pocket. The backbone dose sterically favors the **3b** molecule to interact with catalytic site.

As shown in Figure 4, a strong correlation with experimental results is found between the  $IC_{50}$  values and H-bonding data calculated by moldock in the Molegro docking software at site B of enzyme. In the moldock analysis for **3b** at site A two H-bonding interactions (N—H—O and N—H—N type) were observed between **3b** and PO<sub>4</sub>844, His593, respectively, while two strong H-bonding interactions between **3b** and enzyme residues Glu252 and Lys156 were observed at site B. The H-bonding distances and moldock scores for all compounds, at site B, are listed in Table 4. Figure 7 shows the H-bonding interactions of all of the compounds (except **3h**) with the active residue of urease enzyme at site B. Compound **3h** does not show any H-bonding interaction with the active site residue. In the linear correlation with *in vitro*  $IC_{50}$ , stronger H-bonding is found for compounds with lower  $IC_{50}$  value. All the compounds have H-bonding distances in the 2.696 Å–3.544 Å range and H-bonding energies in the –1.674–3.45 a.u. range.



**Figure 7.** Cont.



**Figure 7.** Molegro molecular viewer generated sketches between compounds **3a–3h** and the active residue of urease at site B. The blue dashed lines represent the H-bonding.

### 2.2.3. Nitric Oxide Scavenging Percentage Assay

The literature contains reports on the antioxidant activities of 6-fluorobenzothiazole-substituted triazoles using DPPH assays [29]. A survey of the literature showed that benzothiazole molecules along with pyroline rings showed the highest antioxidant activities. Having a phenyl ring on the pyroline increased the antioxidant activity in the ferric ion reduction and DPPH solution methods [30]. Our newly synthesized *N*-protected benzothiazole derivatives exhibit nitric oxide scavenging activities. Ascorbic acid was used as a standard in the assay with  $38.5 \pm 0.16$  and  $84.1 \pm 0.12$  percent nitric oxide scavenging at  $20 \mu\text{g/mL}$  and at  $50 \mu\text{g/mL}$  with an  $\text{IC}_{50}$  value of  $50.43 \mu\text{g/mL}$ . Synthesized compounds with their calculated  $\text{IC}_{50}$  values are listed in Table 5 (Figures 8 and 9).

It was found that acetyl-protected amino group products were more active in the nitric oxide scavenging assay, than the previously reported non-acetylated compounds [19]. Molecules **3a**, **2b**, **3e**, **3g** and **3h** were found to be the most active for nitric oxide scavenging activity, with percentage inhibitions of  $57.75 \pm 0.12$ ,  $69 \pm 0.12$ ,  $55 \pm 0.31$ ,  $51.25 \pm 0.15$  and  $60.5 \pm 0.1$  at  $40 \mu\text{g/mL}$  with  $\text{IC}_{50}$  values of  $32.7$ ,  $26.4$ ,  $37.1$ ,  $39.1$  and  $32.3$ , respectively. Compound **3f**, however, was found to be inactive in the nitric oxide scavenging assay. We are unable to account for this inactivity.

**Table 5.** Nitric oxide scavenging activity of *N*-(6-arylbenzo[d]thiazole-2-yl)acetamides **3a–3h** at  $20 \mu\text{g/mL}$  and  $40 \mu\text{g/mL}$ .

Compound	% Activity at $20 \mu\text{g/mL}$	% Activity at $40 \mu\text{g/mL}$	$\text{IC}_{50}$ ( $\mu\text{g/mL}$ )
<b>3a</b>	$36.25 \pm 0.12$	$57.75 \pm 0.12$	32.7
<b>3b</b>	$41 \pm 0.11$	$69 \pm 0.12$	26.4
<b>3c</b>	$10 \pm 0.18$	$41.75 \pm 0.20$	NC
<b>3d</b>	$14.25 \pm 0.17$	$46 \pm 0.2$	NC
<b>3e</b>	$20.25 \pm 0.15$	$55 \pm 0.31$	37.1
<b>3f</b>	0	0	NC
<b>3g</b>	$20.25 \pm 0.15$	$51.25 \pm 0.15$	39.1
<b>3h</b>	$33.5 \pm 0.13$	$60.5 \pm 0.1$	32.3
Standard	$38.5 \pm 0.16$	$84.1 \pm 0.12$	50.43

Each value is mean  $\pm$  Standard deviation of three parallel measurements. NC stands for not calculated due to less activity.

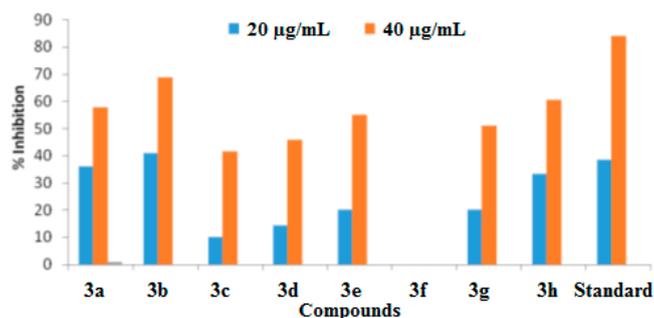


Figure 8. The percentage nitric oxide inhibition at 20 µg/mL and 40 µg/mL.

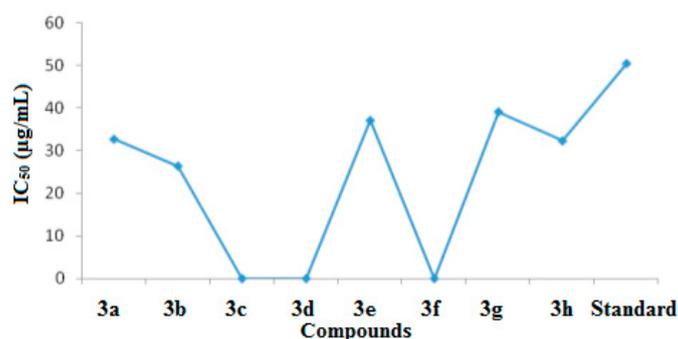


Figure 9. IC<sub>50</sub> values of nitric oxide scavenging activity.

#### 2.2.4. Haemolytic Activity

The haemolytic activity of benzothiazole derivatives has already been reported. A literature survey reveals that amino-substituted derivatives of benzothiazole have high cytotoxicities. Benzothiazole compounds with halogen substitutions show cytotoxicity towards cancer cell lines [31]. The haemolytic activity of the newly synthesized benzothiazole derivatives were studied against Triton X-100 by a reported method [32].

The newly synthesized benzothiazole derivatives exhibit moderate to high haemolytic activities (see in Table 6, Figure 10). Compound 3c exhibits the highest haemolytic activity ( $47.089 \pm 0.130$ ). Fluorinated analog 2d also displayed the highest toxicity among all the tested compounds. The antitumor activity of a compound might be considered as corresponding to the highest haemolytic activity. It was observed that substitution does not markedly affected the haemolytic activity of these newly synthesized *N*-protected benzothiazole derivatives. Compounds 2, 3b and 3d–3g showed good haemolytic activities. The lowest haemolytic activity was found for compound 3h. It was concluded that halogen substitution on *N*-protected benzothiazole molecules promotes haemolytic activity. These compounds have potential to be used as future anticancer agents.

Table 6. Haemolytic activity of newly synthesized *N*-(6-bromobenzo[d]thiazol-2-yl)acetamide (2) and *N*-(6-arylbenzo[d]thiazole-2-yl)acetamides (3a–3h).

Entry	% lysis of RBC	Entry	% lysis of RBC
2	$44.628 \pm 0.369$	3e	$44.425 \pm 0.181$
3a	$42.123 \pm 0.479$	3f	$44.063 \pm 0.314$
3b	$44.179 \pm 0.157$	3g	$43.614 \pm 0.157$
3c	$47.089 \pm 0.130$	3h	$40.661 \pm 0.216$
3d	$44.078 \pm 0.279$		
Standard			$99.78 \pm 0.912$

Each value is mean  $\pm$  Standard deviation of three parallel measurements.

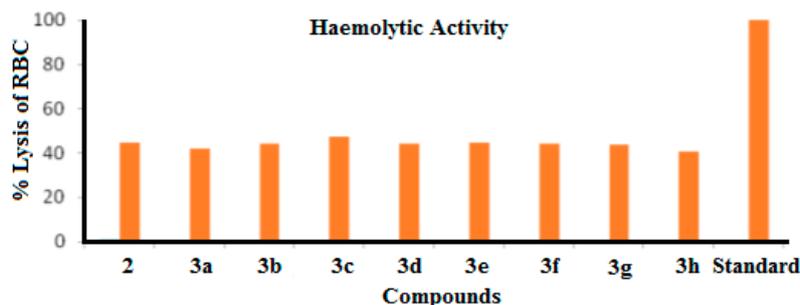


Figure 10. Haemolytic activity of the synthesized compounds.

### 2.2.5. Antibacterial Activity

The synthesized benzothiazole derivatives were examined for their anti-bacterial activity against two Gram positive-bacterial strains (*Bacillus subtilis*, *Staphylococcus aureus*) and four Gram-negative strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Salmonella typhae*) at concentrations of 40 and 80  $\mu\text{g/mL}$  (Tables 7 and 8). It was concluded that the potent antibacterial activities of these compounds might be due to electron withdrawing groups present on the aryl moiety in *N*-protected benzothiazole molecule. Similar observations are also reported by other groups which suggest that the presence of electron releasing and electron withdrawing groups substantially affects the antibacterial activity [33].

Table 7. Antibacterial activity (40  $\mu\text{g/mL}$ ) of *N*-(6-arylbenzo[d]thiazole-2-yl)acetamides 2b–2i.

Entry	% Activity at 40 $\mu\text{g/mL}$					
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. dysenteriae</i>	<i>S. typhae</i>	<i>E. coli</i>
3a	-	-	-	$0.94 \pm 0.45$	-	$37.52 \pm 0.38$
3b	-	-	-	-	-	$34.04 \pm 0.40$
3c	-	-	-	$0 \pm 0.45$	$6.0 \pm 0.47$	$49.05 \pm 0.32$
3d	-	-	-	$2.92 \pm 0.44$	-	$42.10 \pm 0.36$
3e	-	-	-	-	-	$37.82 \pm 0.38$
3f	-	-	$7.54 \pm 0.6$	$2.59 \pm 0.44$	$1.2 \pm 0.50$	$33.53 \pm 0.41$
3g	-	-	-	$1.45 \pm 0.44$	-	$45.93 \pm 0.3$
3h	-	-	-	$3.51 \pm 0.43$	-	$22.89 \pm 0.47$
Ampicillin	$23 \pm 0.1$	$29 \pm 0.61$	$25 \pm 0.12$	$35 \pm 0.32$	$29 \pm 0.61$	$19 \pm 0.31$

Each value is mean  $\pm$  standard deviation of three parallel measurements.

Table 8. Antibacterial activity (80  $\mu\text{g/mL}$ ) of *N*-(6-arylbenzo[d]thiazole-2-yl)acetamides 2b–2i.

Entry	% Activity at 80 $\mu\text{g/mL}$					
	<i>B. subtilis</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. dysenteriae</i>	<i>S. typhae</i>	<i>E. coli</i>
3a	$6.08 \pm 0.571$	$15.25 \pm 0.5$	-	$8.47 \pm 0.44$	$18.5 \pm 0.58$	$57.97 \pm 0.25$
3b	-	-	-	$6.57 \pm 0.45$	$17.7 \pm 0.5$	$56.13 \pm 0.32$
3c	-	-	-	-	$26.2 \pm 0.53$	$50.49 \pm 0.30$
3d	$7.31 \pm 0.5635$	-	-	$5.92 \pm 0.46$	$28.86 \pm 0.5$	$51 \pm 0.30$
3e	$12.66 \pm 0.531$	$3.75 \pm 0.65$	-	-	$19.2 \pm 0.5$	$50.8 \pm 0.30$
3f	$4.27 \pm 0.582$	$8.89 \pm 0.5$	$2.96 \pm 0.6$	$10.93 \pm 0.43$	$25 \pm 0.54$	$55 \pm 0.32$
3g	-	-	-	$11.51 \pm 0.42$	$21.05 \pm 0.5$	$57.84 \pm 0.25$
3h	$5.67 \pm 0.5735$	-	-	$7.48 \pm 0.45$	$17.43 \pm 0.5$	$53.96 \pm 0.40$
Ampicillin	$50.5 \pm 0.31$	$52.9 \pm 0.29$	$52 \pm 0.26$	$56 \pm 0.26$	$42.9 \pm 0.29$	$45.9 \pm 0.21$

Each value is mean  $\pm$  standard deviation of three parallel measurements.

The results show that the benzothiazole compounds 3a–3h exhibit higher activities than the standard against some species. Functional group changes in the benzothiazole molecule led to differences in activity. The newly synthesized compounds were found to be inactive against *Bacillus*

*subtilis* and *Staphylococcus aureus*. Only compound **3f** showed activity against *Pseudomonas aeruginosa* with a very small value ( $7.54 \pm 0.6$ ). These newly synthesized compounds do not exhibit considerable antibacterial activity and the highest value ( $3.51 \pm 0.43$ ) was observed for compound **3h** against *Shigella dysenteriae* at concentration of 40  $\mu\text{g}/\text{mL}$ . These new benzothiazole molecules showed weak activities against *Salmonella typhae* at 40  $\mu\text{g}/\text{mL}$ . It was found that all newly synthesized benzothiazole compounds gave good to very good activity against *E. coli* at 40  $\mu\text{g}/\text{mL}$ . Compounds **3c** and **3ge** exhibited very good activities against the *E. coli* strain with values of  $49.05 \pm 0.32$  and  $45.93 \pm 0.3$ , respectively. These differences in activities may be attributed to the presence of electron loving atoms/groups on the aryl moiety of these *N*-protected benzothiazole derivatives.

The synthesized compounds were also checked for antibacterial activities at 80  $\mu\text{g}/\text{mL}$  and compared against ampicillin. It was shown that these compounds showed moderate activities against *Bacillus subtilis* with the highest value ( $12.66 \pm 0.531$ ) corresponding to compound **3e**. The authors concluded that these benzothiazole derivatives showed non-significant activity against *Staphylococcus aureus*. In addition, these compounds were to be found active against *Shigella dysenteriae* and *Salmonella typhae* at 80  $\mu\text{g}/\text{mL}$ . These compounds displayed very good activity against *E. coli* at 80  $\mu\text{g}/\text{mL}$ . The benzothiazole derivatives were discovered to be the most potent against the *E. coli* strain. Compounds **3a**, **3b** and **3g** proved to be the most potent at the concentration of 80  $\mu\text{g}/\text{mL}$  with the highest antibacterial activities with values of  $57.97 \pm 0.25$ ,  $57.84 \pm 0.25$  and  $56.13 \pm 0.32$ , respectively. The results of this study revealed that electron withdrawing group substitution on the aryl moiety on the benzothiazole molecule enhanced the antimicrobial activity of the compounds.

### 3. Experimental Section

#### 3.1. General Information

All reagents and chemicals were brought from Alfa-Aesar Chemical Co. (Ward Hill, MA, USA) and Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Solvents  $\text{CDCl}_3$  and  $\text{CD}_3\text{OD}$  were used for  $^{13}\text{C}$ -NMR and  $^1\text{H}$ -NMR spectra on an Aspect AM-400 instrument at 400/100 MHz (Bruker, Billerica, MA, USA). The coupling constant was determined in Hz and chemical shift in  $\delta$  in ppm. A JMS-HX-110 spectrometer (JEOL, Peabody, MA, USA) was used for EI-MS spectra. Melting points of benzothiazole compounds were measured on a B-540 melting point apparatus (Büchi, New Castle, DE, USA). Column chromatography with silica gel (mesh size 70 to 230 and 230 to 400) was used for compound purification. TLC (silica gel 60 PF 254 cards, Merck, Kenilworth, NJ, USA) was used for reaction monitoring. Plates were visualized using a UV lamp (254 to 365 nm) (Spectronics Corporation, Westbury, NY, USA).

#### 3.2. Procedure for the Preparation of *N*-(6-arylbenzo[d]thiazole-2-yl)acetamides **3a–3h**

The preparation of products **3a–3h** was carried out under a nitrogen atmosphere. Compound **2** (synthesized by literature reported method [19]) (2.183 mmol) and 5 mol %  $\text{Pd}(\text{PPh}_3)_4$  in 1,4-dioxane (20 mL) was mixed and stirred for 30 min. After 30 min  $\text{K}_3\text{PO}_4$  (4.366 mmol), aryl boronic pinacol esters/aryl boronic acids (2.401 mmol), and  $\text{H}_2\text{O}$  (1.5 mL) was added under inert atmospheric conditions. The mixture was stirred for 30 h at 95  $^\circ\text{C}$  and cooled down to room temperature. Ethyl acetate was used for work up and the organic layer was separated and dried under vacuum. For purification purposes, column chromatography was done. The desired product was obtained by using ethylacetate and *n*-hexane (20% and 80% respectively) as eluents. The desired products were characterized by various spectroscopic techniques [34].

*N*-(6-Phenylbenzo[d]thiazol-2-yl)acetamide(**3a**).m.p. 203–205  $^\circ\text{C}$ ;  $^1\text{H}$ -NMR ( $\text{CDCl}_3 + \text{CD}_3\text{OD}$ )  $\delta$  9.46 (s, 1H), 7.92 (s, 1H), 7.67–7.50 (m, 4H), 7.46–7.31 (m, 3H), 2.31 (s, 3H);  $^{13}\text{C}$ -NMR ( $\text{CDCl}_3 + \text{CD}_3\text{OD}$ )  $\delta$  168.3, 159.2, 151.9, 145.6, 132.4, 130.1, 129.8 (2C), 128.4, 128.4 (2C), 124.2, 121.7, 116.8, 23.7; EIMS (*m/z* + ion mode): 269.32 [ $\text{M} + \text{H}^+$ ] 269.08; Anal Calcd for  $\text{C}_{15}\text{H}_{12}\text{N}_2\text{OS}$ : C, 67.13; H, 4.53; N, 10.43; found C, 67.23; H, 4.54; N, 10.37.

*N*-(6-(*p*-Tolyl)benzo[*d*]thiazol-2-yl)acetamide (**3b**). m.p. 205–207 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub> + CD<sub>3</sub>OD) δ 9.33 (s, 1H), 8.11 (d, *J* = 10 Hz, 3H), 7.61 (d, *J* = 10.4 Hz, 1H), 7.31–7.18 (m, 3H), 2.41 (s, 3H), 2.35 (s, 3H); <sup>13</sup>C-NMR (CDCl<sub>3</sub> + CD<sub>3</sub>OD) δ 168.2, 159.3, 148.4, 146.8, 133.1, 131.3, 129.6 (2C), 128.4 (2C), 126.1, 124.4, 121.2, 116.8, 22.9, 21.6; EIMS (*m/z* + ion mode): [M + H]<sup>+</sup> = 283.08; [M – COCH<sub>3</sub>]<sup>−</sup> = 241.01. Anal Calcd for C<sub>16</sub>H<sub>14</sub>N<sub>2</sub>OS: C, 68.07; H, 5.01; N, 9.93 found C, 68.12; H, 5.04; N, 9.81.

*N*-(6-(3,5-bis(Trifluoromethyl)phenyl)benzo[*d*]thiazol-2-yl)acetamide (**3c**). m.p. 203–205 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub> + CD<sub>3</sub>OD) δ 9.41 (s, 1H), 7.92 (s, 1H), 7.66–7.61 (m, 2H), 7.55–7.43 (m, 3H), 2.27 (s, 3H); <sup>13</sup>C-NMR (CDCl<sub>3</sub> + CD<sub>3</sub>OD) δ 168.7, 159.4, 153.7, 146.3, 137.1, 132.1 (2C), 129.3, 128.4 (2C), 124.1 (2C), 123.3, 121.4, 120.12, 118.6, 25.2; EIMS (*m/z* + ion mode): 405.32, [M + H]<sup>+</sup>; 405.16. Anal Calcd for C<sub>17</sub>H<sub>10</sub>F<sub>6</sub>N<sub>2</sub>OS: C, 50.51; H, 2.48; N, 6.92 found C, 50.44; H, 2.52; N, 6.82.

*N*-(6-(4-Methoxyphenyl)benzo[*d*]thiazol-2-yl)acetamide (**3d**). m.p.: 218–220 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub> + CD<sub>3</sub>OD) δ 9.41 (s, 1H), 8.01 (s, 1H), 7.66 (d, *J* = 8, 2H), 7.57–7.47 (m, 2H), 7.08 (d, *J* = 8, 2H), 3.57 (s, 3H), 1.91 (s, 3H). <sup>13</sup>C-NMR (CDCl<sub>3</sub> + CD<sub>3</sub>OD) δ 168.2, 158.8, 151.3, 149.5, 145.3, 132.2, 131.8 (2C), 129.5, 123.7, 121.3, 116.6, 115.5 (2C), 49.2, 22.5; EIMS (*m/z* – ion mode): [M – H]<sup>−</sup> = 297.25; [M – COCH<sub>3</sub>]<sup>−</sup> = 255.34; [M – OCH<sub>3</sub> and COCH<sub>3</sub>]<sup>−</sup> = 227.01; Anal Calcd for C<sub>16</sub>H<sub>14</sub>N<sub>2</sub>O<sub>2</sub>S<sub>2</sub>: C, 64.42; H, 4.73; N, 9.38 found C, 64.51; H, 4.71; N, 9.42.

*N*-(6-(5-Methylthiophen-2-yl)benzo[*d*]thiazol-2-yl)acetamide (**3e**). m.p.: 192–194 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub> + CD<sub>3</sub>OD) δ 9.41 (s, 1H), 7.90 (s, 1H), 7.67–7.35 (m, 4H), 2.34 (s, 3H), 2.11 (s, 3H); <sup>13</sup>C-NMR (CDCl<sub>3</sub> + CD<sub>3</sub>OD) δ 166.2, 159.6, 148.7, 145.2, 132.4, 132.3, 129.5, 128.7, 128.3, 123.6, 121.8, 116.8, 22.7, 14.03; EIMS (*m/z* – ion mode): [M – H]<sup>−</sup> = 286.93; [M – COCH<sub>3</sub>]<sup>−</sup> = 224.93; [M – CH<sub>3</sub>]<sup>−</sup> = 271.02; Anal Calcd for C<sub>14</sub>H<sub>12</sub>N<sub>2</sub>OS<sub>2</sub>: C, 58.32; H, 4.20; N, 9.72 found C, 58.41; H, 4.12; N, 9.79.

*N*-(6-(3-Cyano-5-(trifluoromethyl)phenyl)benzo[*d*]thiazol-2-yl)acetamide (**3f**). m.p.: 212–213 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub> + CD<sub>3</sub>OD) δ 9.48 (s, 1H), 7.92 (d, *J* = 1.2, 1H), 7.72–7.41 (m, 4H), 7.36 (s, 1H), 2.27 (s, 3H); <sup>13</sup>C-NMR (CDCl<sub>3</sub> + CD<sub>3</sub>OD) δ 167.7, 158.8, 148.7, 146.4, 132.3, 132.0, 132.0, 131.8, 129.6, 128.4, 124.2, 123.3, 121.8, 120.3, 117.7, 114.8, 22.5; EIMS (*m/z* – ion mode): 360.33 [M – H]<sup>−</sup> 360.01; Anal Calcd for C<sub>17</sub>H<sub>11</sub>F<sub>3</sub>N<sub>3</sub>OS: C, 56.52; H, 2.78; N, 11.62 found C, 56.60; H, 2.77; N, 11.57.

*N*-(6-(3-Chloro-4-fluorophenyl)benzo[*d*]thiazol-2-yl)acetamide (**3g**). m.p.: 166–167 °C; <sup>1</sup>H-NMR (CDCl<sub>3</sub> + CD<sub>3</sub>OD) δ 9.32 (s, 1H), 8.11 (d, *J* = 10 Hz, 1H), 7.90 (s, 1H), 7.80–7.41 (m, 4H), 2.30 (s, 3H); <sup>13</sup>C-NMR (CDCl<sub>3</sub> + CD<sub>3</sub>OD) δ 168.5, 156.3, 151.2, 147.8, 137.3, 135.1, 131.0, 129.7, 129.6, 124.6, 124.0, 121.6, 117.5, 116.3, 22.5; EIMS (*m/z* + ion mode): [M + H]<sup>+</sup> = 321.16; [M – Cl and F-benzene and COCH<sub>3</sub>]<sup>+</sup> = 150.07; Anal Calcd for C<sub>15</sub>H<sub>12</sub>ClFN<sub>2</sub>OS: C, 56.16; H, 3.13; N, 8.72 found C, 56.30; H, 3.15; N, 8.76.

*N*-(6-(3,5-Dimethylphenyl)benzo[*d*]thiazol-2-yl)acetamide (**3h**). m.p. 205–207 °C; <sup>1</sup>H-NMR (acetone-*d*<sub>6</sub>) δ 9.41 (s, 1H), 8.14 (d, *J* = 2 Hz, 1H), 7.63 (d, *J* = 1.5, 1H), 7.72–7.53 (m, 4H), 2.31 (s, 6H), 2.28 (s, 3H); <sup>13</sup>C-NMR (acetone-*d*<sub>6</sub>) δ 167.2, 158.0, 147.7, 140.3 (2C), 133.0, 129.9, 129.5, 128.3, 127.4 (2C), 123.7, 121.3, 119.2, 23.1, 22.2 (2C); EIMS (*m/z* – ion mode): [M – H]<sup>−</sup> = 294.91; [M – COCH<sub>3</sub> – 2OCH<sub>3</sub> and benzene]<sup>−</sup> = 149.24; [M – 2OCH<sub>3</sub> and benzene]<sup>−</sup> = 190.6; Anal Calcd for C<sub>17</sub>H<sub>16</sub>N<sub>2</sub>OS: C, 68.88; H, 5.43; N, 9.44 found C, 68.83; H, 5.46; N, 9.34.

### 3.3. Procedure for Urease Inhibition Activity

Urease inhibitory assay of newly synthesized compounds **3a–3h** were determined as follows: Enzyme (1 unit) in phosphate buffer (200 μL, pH 7) was combined with a particular stock solution (20 μL, a test compound or thiourea) and phosphate buffer (230 μL). The solution was incubated for 5 min at 25 °C. After incubation period 400 μL of urea stock solution (20 mM) was added to the solution. Calibration solution was synthesized without urea solution. For the action of urease, test tubes were incubated for 10 min at 40 °C. The solution of phenol hypochlorite reagent (1150 μL) was added. These tubes were incubated for 25 min at 56 °C. Absorbance of the blue colored compound

was noted at 625 nm after 5 min of cooling. Then percentage urease inhibition was determined. While EZ-fit kinetic data base was used to obtain  $IC_{50}$  values [25,35].

### 3.4. Molecular Docking Study

The PDB structure of 3LA4 was retrieved for docking purposes as a complex co-crystallized with inhibitor 2-amino-3-(2-(2-hydroxyethyl)disulfanyl)propan-1-ol and (5-amino-6-hydroxyhexyl)carbamic acid at the nickel-containing catalytic site [36]. Then, the amino acid chain was retained and the water molecules and co-crystallized ligands were removed and subsequently the missing atom types were repaired using Modeller 9.11 (University of California San Francisco, San Francisco, CA, USA). Afterwards, the polar hydrogen was added to the receptor and the resulting protein was subjected to minimization using OPLS 2005 force field. The prepared protein was saved in pdbqt format using Autodock Tools 1.5.4 [21,37–39]. The ligand coordinates were generated using MarvinSketch 5.8.3, 2012 (ChemAxon LLC, Cambridge, MA, USA) [40], which was converted to 3D structure using Openbabel version (2.3.1). Finally the pdbqt formats (The input format of docking software) of the ligands were prepared with Autodock Tools 1.5.4 using default parameters. AutodockVina ver. 1.1.1 (The Scripps Research Institute, La Jolla CA, USA) was used for docking calculations with default parameters except for exhaustiveness that was set to 80. For all the docking calculations, a grid box size of  $40 \times 40 \times 40$ , centered at the geometrical center of co-crystallized ligands was used. Co-crystallized ligands were attached at two different sites, one near the nickel catalytic site (A) and the other site where the inhibitor 2-amino-3-(2-(2-hydroxyethyl)disulfanyl)propan-1-ol was attached (Site B) [20]. The coordinates  $x, y, z$  for the center of grid box were (Site A)  $-39.86, -45.06, 72.52$  and (Site B)  $-75.03, 20.84, 81.83$  respectively. To validate our docking procedure, the co-crystallized ligands were re-docked into their respective site of the enzyme and the reasonable RMSD value of 1.947 Å was obtained. Finally, the conformations with the most favorable free energy of binding were selected for analyzing the interactions between urease and its inhibitor. All of the 3D models are generated using the Molegro Molecular Viewer 2.5 (CLC bio company, Aarhus N, Denmark) [41] and LigPlot + (The European Bioinformatics Institute, Hinxton, Cambridge, UK) [42] software.

### 3.5. Nitric Oxide Scavenging Activity

The activity of newly synthesized benzothiazole derivatives was determined using the Garrat method. Griess reported the Garrat method which is followed by a diazotization reaction [43]. Under acidic conditions, this method utilizes sodium nitroprusside as the source of nitric oxide, sulfanilamide and *N*-1-naphthylethylenediamine dihydrochloride to detect  $NO_2^-$ , produced at the expense of nitric oxide. A known amount of tested compounds **3a–3h** was dissolved in sodium nitroprusside solution (20 mM, 100  $\mu$ L) and then the volume was made up to 1000  $\mu$ L with phosphate buffer (200 mM, pH 7.4). This solution was incubated for 2 h at 37 °C and Griess reagent (100  $\mu$ L) was added. This solution was stored for 20 min at room temperature. At 528 nm, optical density of this colored solution was observed. For positive control, ascorbic acid was used. Negative control was used to form the standard curve [43].

### 3.6. Haemolytic Activity

Haemolytic activity of newly synthesized benzothiazole derivatives **3a–3h** was determined using a reported method [44]. Solutions of compounds were prepared at concentrations of 1 mg/mL in 10% DMSO with 90% water Heparinized human fresh blood (3 mL) was used that was homogeneously mixed and added into a 15 mL sterile Falcon tube. It was centrifuged for 5 min and the supernatant was removed. Chilled sterile isotonic phosphate buffer saline solution (5 mL, 7.4 pH) at 4 °C was used three times. Washed red blood cells were suspended in chilled RBS (20 mL). A haemocytometer was used for counting erythrocytes. For each assay  $7.068 \times 10^8$  red blood cells per mL count were maintained and then diluted blood cells (180  $\mu$ L) were added to the test compound (20  $\mu$ L) and suspended in Eppendorf tubes. It was incubated for 35 min at 37 °C then the tubes were kept in an ice bath for 5 min

and centrifuged again for 5 min. After centrifugation, the obtained supernatant was collected carefully and diluted with 900  $\mu\text{L}$  of chilled PBS. All these tubes were kept in ice bath and solution (200  $\mu\text{L}$ ) was added into 96 well plates from each Eppendorf tube. For each assay, Triton X-100 (0.1%) was taken as positive control. For negative control, phosphate buffer was used. A microplate reader was used for determining the absorbance at 576 nm [32].

### 3.7. Antibacterial Activity

These newly benzothiazole derivatives were tested for their antibacterial activities against two Gram positive strains (*Bacillus subtilis*, *Staphylococcus aureus*) and four Gram negative strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae* and *Salmonella typhae*) using a reported protocol [45]. Streptomycin was used as positive control. The 96 well plate method was optimized for measuring the antibacterial activities of these compounds. In each well, sterilized broth (175  $\mu\text{L}$ ) was added and inoculated with glycerol stock (5  $\mu\text{L}$ ) of a specific bacterial strain. The initial absorbance was observed between 0.12–0.19. The bacteria were allowed to grow in an incubator overnight. After a certain waiting time (12 h), test sample (20  $\mu\text{L}$ ) was added to the pre-determined wells. Concentration of test sample was 20  $\mu\text{g}$ /well. Total volume was 200  $\mu\text{L}$  in each well. These plates were incubated for 16–24 h at 37 °C. Absorbance was observed at 630 nm by using an ELISA plate reader. The difference in absorbance values were observed and were used as an index of bacterial growth. The following formula was used to calculate percentage inhibition:

$$\text{Percentage Inhibition} = (\text{O.D of + ve control} - \text{O.D of sample} \times 100) / \text{O.D of + ve control} \quad (1)$$

## 4. Conclusions

This study reports C-C coupling reactions of **2** with various arylboronic pinacol ester/aryl boronic acids using palladium catalyst. These new products **3a–3h** were prepared in moderate to good yields. These Suzuki coupling benzothiazole derivatives were checked for their biological (urease inhibitory, nitric oxide scavenging, haemolytic and antibacterial) activities. The urease inhibition results showed that product **3b** was an excellent urease inhibitor. Products with electron releasing groups on the aryl moiety of the benzothiazole molecule showed the highest inhibition of urease activity. Molecular docking studies of the urease inhibitory activity showed that the H-bonding ability present in these *N*-protected benzothiazoles prevents the catalytic activity of the enzyme. Nitric oxide scavenging assays were done for these compounds. Compound **3b** also exhibited highest nitric oxide scavenging activity. All newly synthesized compounds showed haemolytic activity. It was found that electron withdrawing substitution on the aryl produced the highest haemolytic activity. The newly synthesized benzothiazole derivatives **3a–3h** showed excellent antibacterial activities against *E. coli*.

**Acknowledgments:** This study reported herein is part of Ph.D. thesis work of Yasmeen Gull. Financial assistance for this study was provided by the Higher Education Commission (HEC), Pakistan and HEC Scholarship awarded to Ms. Yasmeen Gull.

**Author Contributions:** Conceived and designed the experiments: Y.G., N.R., M.N., A.A.A. Performed the experiments: (Synthesis and bioassay—Y.G., M.N.); (Enzyme inhibition—A.Y.); (Docking and other computational—A.A.A.). Analyzed the data: Y.G., A.A.A., M.N., M.Z., S.G.M. Contributed to manuscript preparation N.R., F.-H.N., M.Z., V.D.F.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Gajdos, P.; Magdolen, P.; Zahradnik, P.; Foltinova, P. New conjugated benzothiazole-*N*-oxides: Synthesis and biological activity. *Molecules* **2009**, *14*, 5382–5388. [[CrossRef](#)] [[PubMed](#)]
2. Mishra, A.; Behera, R.K.; Behera, P.K.; Mishra, B.K.; Behera, G.B. Cyanines during the 1990s: A review. *Chem. Rev.* **2000**, *100*, 1973–2012. [[CrossRef](#)] [[PubMed](#)]

3. Michaelidou, A.S.; Hadjipavlou-Litina, D. Nonsteroidal anti-inflammatory drugs (NSAIDs): A comparative QSAR study. *Chem. Rev.* **2005**, *105*, 3235–3271. [[CrossRef](#)] [[PubMed](#)]
4. Yadav, P.S.; Devprakash; Senthikumar, G.P. Benzothiazole: Different method of synthesis and diverse biological activities. *Int. J. Pharm. Sci. Drug Res.* **2011**, *3*, 1–7.
5. Aiello, S.; Wells, G.; Stone, E.L.; Kadri, H.; Bazzi, R.; Bell, D.R.; Stevens, M.F.; Matthews, C.S.; Bradshaw, T.D.; Westwell, A.D. Synthesis and biological properties of benzothiazole, benzoxazole, and chromen-4-one analogues of the potent antitumor agent 2-(3,4-dimethoxyphenyl)-5-fluorobenzothiazole (PMX 610, NSC 721648). *J. Med. Chem.* **2008**, *51*, 5135–5139. [[CrossRef](#)] [[PubMed](#)]
6. Cho, Y.; Ioerger, T.R.; Sacchetti, J.C. Discovery of novel nitrobenzothiazole inhibitors for mycobacterium tuberculosis ATP phosphoribosyl transferase (HisG) through virtual screening. *J. Med. Chem.* **2008**, *51*, 5984–5992. [[CrossRef](#)] [[PubMed](#)]
7. Bondock, S.; Fadaly, W.; Metwally. Recent trends in the chemistry of 2-aminobenzothiazoles. *J. Sulfur Chem.* **2009**, *30*, 74–107. [[CrossRef](#)]
8. Saeed, A.; Rafique, H.; Rasheed, S. Synthesis and antibacterial activity of some new 1-Aroyl-3-(substituted-2-benzothiazolyl)thioureas. *Pharm. Chem. J.* **2008**, *48*, 191–195. [[CrossRef](#)]
9. Bele, D.S.; Singhvi, J. Synthesis of some mannich bases of 6-substituted-2-aminobenzothiazole as analgesic. *Res. J. Pharm. Technol.* **2014**, *7*, 316–321.
10. Hutchinson, I.; Chua, M.; Bradshaw, T.D.; Matthews, C.S.; Stevens, F.G.; Westwell, A. Antitumor benzothiazoles, part 20: 3'-Cyano and 3'-alkynyl-substituted 2-(4'aminophenyl) benzothiazoles as new potent and selective analogues. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 471–474. [[CrossRef](#)]
11. Trapani, G.; Franco, M.; Latrofa, A.; Reho, A.; Liso, G. Synthesis *in vitro* and *in vivo* cytotoxicity, and prediction of the intestinal absorption of substituted 2-ethoxycarbonyl-imidazo[2,1-*b*]benzothiazoles. *Eur. J. Pharm. Sci.* **2001**, *14*, 209–216. [[CrossRef](#)]
12. Kamal, M.; Dawood. Microwave-assisted Suzuki-Miyaura and Heck-Mizoroki cross-coupling reactions of aryl chlorides and bromides in water using stable benzothiazole-based palladium(II) precatalysts. *Tetrahedron* **2007**, *63*, 9642–9651.
13. Ali, S.; Rasool, N.; Ullah, A.; Nasim, F.; Yaqoob, A.; Zubair, M.; Rashid, U.; Riaz, M. Design and synthesis of arylthiophene-2-Carbaldehydes via suzuki-miyaura reactions and their biological evaluation. *Molecules* **2013**, *18*, 14711–14725. [[CrossRef](#)] [[PubMed](#)]
14. Rizwan, K.; Zubair, M.; Rasool, N.; Ali, S.; Zahoor, A.F.; Rana, U.A.; Khan, S.; Shahid, M.; Zia-Ul-Haq, M.; Jaafar, H.Z. Regioselective synthesis of 2-(bromomethyl)-5-aryl-thiophene derivatives via palladium (0) catalyzed suzuki cross-coupling reactions: As antithrombotic and haemolytically active molecules. *Chem. Cent. J.* **2014**, *8*. [[CrossRef](#)]
15. Noreen, M.; Rasool, N.; Khatib, M.E.; Molander, G.A. Arylation and heteroarylation of thienylsulfonamides with organotrifluoroborates. *J. Org. Chem.* **2014**, *79*, 7243–7249. [[CrossRef](#)] [[PubMed](#)]
16. Ikram, H.M.; Rasool, N.; Ahmad, G.; Chotana, G.A.; Musharraf, S.G.; Zubair, M.; Rana, U.A.; Zia-Ul-Haq, M.; Jaafar, H.Z. Selective C-arylation of 2,5-dibromo-3-hexylthiophene via suzuki cross coupling reaction and their pharmacological aspects. *Molecules* **2015**, *20*, 5202–5214. [[CrossRef](#)] [[PubMed](#)]
17. Rasheed, T.; Rasool, N.; Noreen, M.; Gull, Y.; Zubair, M.; Ullah, A.; Rana, U.A. Palladium (0) catalyzed Suzuki cross-coupling reactions of 2,4-dibromothiophene: Selectivity, characterization and biological applications. *J. Sulfur Chem.* **2015**, *36*, 240–250. [[CrossRef](#)]
18. Majo, V.J.; Prabhakaran, J.; Mann, J.J.; Kumar, J.S.D. An efficient palladium catalyzed synthesis of 2-arylbenzothiazoles. *Tetrahedron Lett.* **2003**, *44*, 8535–8537. [[CrossRef](#)]
19. Gull, Y.; Rasool, N.; Noreen, M.; Nasim, F.; Yaqoob, A.; Kousar, S.; Rashid, U.; Bukhari, I.H.; Zubair, M.; Islam, M.S. Efficient synthesis of 2-amino-6-Arylbenzothiazoles via Pd(0) suzuki cross coupling reactions: Potent urease enzyme inhibition and nitric oxide scavenging activities of the products. *Molecules* **2013**, *18*, 8845–8857. [[CrossRef](#)] [[PubMed](#)]
20. Benini, S.; Rypniewski, W.R.; Wilson, K.S.; Miletto, S.; Ciurli, S.; Mangani, S. A new proposal for urease mechanism based on the crystal structures of the native and inhibited enzyme from *Bacillus pasteurii*: Why urea hydrolysis costs two nickels. *Structure* **1999**, *7*, 205–216. [[CrossRef](#)]
21. Frishman, D.; Argos, P. Knowledge-based protein secondary structure assignment. *Prot. Struct. Funct. Bioinform.* **1995**, *23*, 566–579. [[CrossRef](#)] [[PubMed](#)]

22. Jamil, M.; Zubair, M.; Farid, M.A.; Altaf, A.A.; Rasool, N.; Nasim, F.U.H.; Ashraf, M.; Rashid, M.A.; Ejaz, S.A.; Yaqoob, A.; *et al.* Study of Antioxidant, Cytotoxic, and Enzyme Inhibition Activities of Some Symmetrical  $N^3,N^{3'}$ -Bis(disubstituted)isophthalyl-bis(thioureas) and  $N^3,N^3,N^{3'},N^{3'}$ -Tetrakis (disubstituted)isophthalyl-bis(thioureas) and Their Cu(II) and Ni(II) Complexes. *J. Chem. Soc. Pak.* **2014**, *36*, 491–497.
23. Jamil, M.; Zubair, M.; Altaf, A.A.; Farid, M.A.; Hussain, M.T.; Rasool, N.; Bukhari, I.H.; Ahmad, V.U. Synthesis, Characterization and Antibacterial Activity of Some Novel Symmetrical  $N$ -3, $N$ -3'-Bis (disubstituted) isophthalyl-bis (thioureas) and  $N$ -3, $N$ -3, $N$ -3', $N$ -3'-Tetrakis (disubstituted) isophthalyl-bis (thiourea) and Their Cu (II) and Ni (II) Complexes. *J. Chem. Soc. Pak.* **2014**, *35*, 737–743.
24. Onoda, Y.; Iwasaki, H.; Magaribuchi, T.; Tamaki, H. Effects of the new anti-ulcer agent 12-sulfodehydroabietic acid monosodium salt on healing of acetic acid-induced gastric ulcers in rats. *Arzneim. Drug Res.* **1991**, *41*, 546–548.
25. Serwar, M.; Akhtar, T.; Hameed, S.; Khan, K.M. Synthesis urease inhibition and antimicrobial activities of some chiral 5-aryle-4-(1-phenylpropyl)-2H-1,2,4-triazole-3(4H)- thiones. *ARKIVOC* **2009**, *7*, 210–221.
26. Mao, W.J.; Lv, P.C.; Shi, L.; Li, H.Q.; Zhu, H.L. Synthesis, molecular docking and biological evaluation of metronidazole derivatives as potent *Helicobacter pylori* urease inhibitors. *Bioorg. Med. Chem.* **2009**, *17*, 7531–7536. [[CrossRef](#)] [[PubMed](#)]
27. Aslam, M.A.S.; Mahmood, S.U.; Shahid, M.; Saeed, A.; Iqbal, J. Synthesis, biological assay *in vitro* and molecular docking studies of new schiff base derivatives as potential urease inhibitors. *Eur. J. Med. Chem.* **2011**, *46*, 5473–5479. [[CrossRef](#)] [[PubMed](#)]
28. Gul, R.; Rauf, M.; Badshah, A.; Azam, S.S.; Tahir, M.N.; Khan, A. Ferrocene-based guanidine derivatives: *In vitro* antimicrobial, DNA binding and docking supported urease inhibition studies. *Eur. J. Med. Chem.* **2014**, *85*, 438–449. [[CrossRef](#)] [[PubMed](#)]
29. Naresh, P.; Pattanaik, P.; Rajeshwar, B. Synthetic characterization and antioxidant screening of some novel 6-fluorobenzothiazole substituted [1,2,4] triazole analogues. *Int. J. Pharm. Sci.* **2013**, *3*, 170–174.
30. Hazra, K.; Nargund, L.; Rashmi, P.; Chandra, J.N.S.; Nandha, B. Synthesis and antioxidant activity of some novel fluorobenzothiazolopyrazoline. *Der. Chem. Sin.* **2011**, *2*, 149–157.
31. Devmurari, V.P.; Shivan, P.; Goyani, M.B.; Jivani, N.P. Synthesis and anticancer activity of some novel 2-substitued benzothiazole. *Int. J. Chem. Sci.* **2010**, *8*, 663–675.
32. Powell, W.A.; Catranis, C.M.; Maynard, C.A. Design of self-processing antimicrobial peptides for plant protection. *Lett. Appl. Microbiol.* **2000**, *31*, 163–168. [[CrossRef](#)] [[PubMed](#)]
33. Saeed, S.; Rashid, N.; Jones, P.G.; Ali, M.; Hussain, R. Synthesis, characterization and biological evaluation of some thiourea derivatives bearing benzothiazole moiety as potential antimicrobial and anticancer agents. *Eur. J. Med. Chem.* **2010**, *45*, 1323–1331. [[CrossRef](#)] [[PubMed](#)]
34. Miyaura, N.; Suzuki. Palladium-catalyzed cross-coupling reactions of organoboron compounds. *Chem. Rev.* **1995**, *95*, 2457–2483. [[CrossRef](#)]
35. Pervez, H.; Rmzan, M.; Yaqub, M.; Nasim, F.H.; Khan, K. Synthesis and biological evaluation of some new  $N$ -4-aryl substituted 5-chloroisatin-3-thiosemicarbazones. *Med. Chem.* **2012**, *8*, 505–514. [[CrossRef](#)] [[PubMed](#)]
36. Balasubramanian, A.; Ponnuraj, K. Crystal structure of the first plant urease from jack bean: 83 Years of journey from its first crystal to molecular structure. *J. Mol. Biol.* **2010**, *400*, 274–283. [[CrossRef](#)] [[PubMed](#)]
37. Srinivasan, R.; Rose, G.D. A physical basis for protein secondary structure. *Proc. Nat. Acad. Sci. USA* **1999**, *96*, 14258–14263. [[CrossRef](#)] [[PubMed](#)]
38. Baker, N.A.; Sept, D.; Joseph, S.; Holst, M.J.; Mccammon, J.A. Electrostatics of nanosystems: Application to microtubules and the ribosome. *Proc. Nat. Acad. Sci. USA* **2010**, *98*, 10037–10041. [[CrossRef](#)] [[PubMed](#)]
39. Harris, R.; Olson, A.J.; Goodsell, D.S. Automated prediction of ligand-binding sites in proteins. *Struct. Funct. Bioinform.* **2003**, *70*, 1506–1517. [[CrossRef](#)] [[PubMed](#)]
40. ChemAxon. Available online: <http://www.chemaxon.com> (accessed on 31 December 2015).
41. CLC Drug Discovery Workbench. Available online: <http://www.molegro.com/mmv-product.php> (accessed on 31 December 2015).
42. Laskowski, R.A.; Swindells, M.B. LigPlot+: Multiple ligand-protein interaction diagrams for drug discovery. *J. Chem. Inf. Mod.* **2011**, *51*, 2778–2786. [[CrossRef](#)] [[PubMed](#)]
43. Garrat, D. *The Quantitative Analysis of Drugs*, 3rd ed.; Chapman and Hall Ltd. Tokyo: Tokyo, Japan, 1996; pp. 456–458.

44. Riaz, M.; Rasool, N.; Bukhari, I.H.; Shahid, M.; Zubair, M.; Rizwan, K.; Rashid, U. *In vitro* antimicrobial, antioxidant, cytotoxicity and GC-MS analysis of *Mazus goodenifolius*. *Molecules* **2011**, *17*, 14275–14287. [[CrossRef](#)] [[PubMed](#)]
45. Rehman, A.U.; Awais, U.R.; Muhammad, A.B.; Hira, K.; Parsa, D. Synthesis and biological screening of *N*-substituted derivatives of *N*-benzyl-4-chlorobenzenesulfonamide. *Asian J. Pharm. Health Sci.* **2012**, *2*, 384–389.

**Sample Availability:** Samples of all the newly synthesized compounds are available from the authors.



© 2016 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons by Attribution (CC-BY) license (<http://creativecommons.org/licenses/by/4.0/>).