Thiourea Derivatives, Simple in Structure but Efficient Enzyme Inhibitors and Mercury Sensors

Faizan Ur Rahman 1, Maryam Bibi 2, Ezzat Khan 1,2,* 1, Abdul Bari Shah 3, Mian Muhammad 1, Muhammad Nawaz Tahir 4, Adnan Shahzad 5, Farhat Ullah 6, Muhammad Zahoor 7, Salman Alamery 8 and Gaber El-Saber Batiha 9

1 Department of Chemistry, University of Malakand, Lower Dir 18800, Pakistan; faizan.uom98@gmail.com (F.U.R.); mianchem@uom.edu.pk (M.M.)
2 Department of Chemistry, College of Science, University of Bahrain, Sakhir 32038, Bahrain; maryamdua55@gmail.com
3 Division of Applied Life Science (BK21 Plus), IALS, Gyeongsang National University, Jinju 52828, Korea; abs.uom28@gmail.com
4 Department of Physics, University of Sargodha, Sargodha 40100, Pakistan; dmntahir_uos@yahoo.com
5 Institute of Chemical Sciences, University of Swat, Swat 19150, Pakistan; adnanshahzad09@gmail.com
6 Department of Chemistry, College of Science, University of Malakand, Chakdara, Lower Dir 18800, Pakistan; farhataziz80@hotmail.com
7 Department of Biochemistry, University of Malakand, Lower Dir 18800, Pakistan; mohammadzahoorus@yahoo.com
8 Department of Biochemistry, College of Science, King Saud University, P.O. Box 22452, Riyadh 11451, Saudi Arabia; Salamery@ksu.edu.sa
9 Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour 22511, Egypt; gaberbatiha@gmail.com
* Correspondence: ekhan@uom.edu.pk or ekzhan@uob.edu.bh

Abstract: In this study six unsymmetrical thiourea derivatives, 1-isobutyl-3-cyclohexylthiourea (1), 1-tert-butyl-3-cyclohexylthiourea (2), 1-(3-chlorophenyl)-3-cyclohexylthiourea (3), 1-(1,1-dibutyl)-3-phenylthiourea (4), 1-(2-chlorophenyl)-3-phenylthiourea (5) and 1-(4-chlorophenyl)-3-phenylthiourea (6) were obtained in the laboratory under aerobic conditions. Compounds 3 and 4 are crystalline and their structure was determined for their single crystal. Compounds 3 is monoclinic system with space group P21/n while compound 4 is trigonal, space group R32:H. Compounds (1–6) were tested for their anti-cholinesterase activity against acetylcholinesterase and butyrylcholinesterase (hereafter abbreviated as, AChE and BChE, respectively). Potentials (all compounds) as sensing probes for determination of deadly toxic metal (mercury) using spectrofluorimetric technique were also investigated. Compound 3 exhibited better enzyme inhibition IC50 values of 50, and 60 µg/mL against AChE and BChE, respectively. The compound also showed moderate sensitivity during fluorescence studies.

Keywords: thiourea derivatives; enzyme inhibition; docking studies; mercury sensing; X-ray structure

1. Introduction

Thiourea derivatives play a pivotal role due to numerous applications in the field of nanoparticles, starting precursors in a large number of chemical reactions and therefore, remained focus of several review articles [1–5]. Efficient and easy accessibility of thiourea derivatives is an open challenge [6]. Several researchers have made thiourea derivatives under different conditions and many of them are now commercially available [7,8]. Thiourea derivatives such as cyclohexyl thiourea or phenylthiourea are attractive model compounds for the studies in solid-state chemistry due to their tendency for the formation of intra- and inter molecular hydrogen bonding wherein the N-H proton-donor groups and predominantly sulfur atoms are engaged. The presence of C=S groups in the same molecule...
make them more attractive for further chemistry [9]. These derivatives have an active role in pharmaceuticals as potential therapeutic agents against HIV, HCV [10–13], anticancer, anticonvulsant [14,15], antihyperlipidemic, antiallergic, antiparasitic, antiproliferative, antioxidant and antidiabetic and anticholinesterase [16,17]. Cholinesterases are various isolated enzymes which are classified as acetyl and butyryl associated with hydrolases enzymes [18,19]. Acetylcholinesterase (AChE) provides a signaling action following degradation of acetylcholine (ACh) [20,21], whereas butyrylcholinesterase (BChE) plays a pivotal role in the metabolism of different molecules. Hence, AChE inhibitors are considered to be one of the most efficient approaches for the treatment of Alzheimer’s disease (AD) by enhancing cholinergic objectives in AD patients. In a normal healthy brain, ACh metabolic degradation is also caused by BChE, hence BChE enhances progression of AD. Therefore, synchronous inhibition achieved by AChE and BChE may provide additional benefits for AD treatment [22]. Several thiourea derivatives such as isobutylphenylthiourea, and tert-butylphenylthiourea and their coordination complexes have recently been reported by our research group as enzyme inhibitors [23]. Several studies have been conducted with respect to thiourea derivatives which highlight the role of this versatile class of compounds in environmental applications particularly heavy metal sensing [24–30]. Heavy metals are expected to be one of the reasons of the AD and their timely control in the body of an individual is utmost necessary [31,32].

Fluorescence is one of the most important physical phenomena in which a particular chemical compound (thiourea for instance) emits electromagnetic radiations of longer wavelength after being excited by radiations with shorter wavelength. Fluorescence based detection, is a homogeneous system that allows direct measurement of binding in solution and has played a significant role at the forefront of the bio-analytical area, because of ultrahigh sensitivity and selectivity. One of the most important applications of fluorescence technology is the decreased size of a sample down to the single-molecule detection level, and this fact provides an opportunity for miniaturization and high throughput screening. In addition, fluorescence has enabled the elucidation of many biological processes, determination of structure and trace amounts of biological macromolecules. Fluorescence sensors employ a fluorescent signal for the detection of analytes. In order to detect different organics at an earlier stage of a disease development, a low detection limit of the organics is desirable [33]. Thiourea derivatives possess the ability to detect both anions and cations and are therefore attracting the attention to be versatile detectors in coming decades [5]. Thiourea derivatives are used as fluorescent detectors for heavy metal ions including Hg$^{2+}$ and various anions in aqueous media [4,34–36].

The AD is greatly influenced by heavy metal ions concentration and can be controlled either by inhibition of specific enzymes or quenching of responsible metal ions. In the current paper, we present the dual function of thiourea derivatives for the same purpose to control AD in an efficient way. Literature review reveals that solid state structure of these compounds is not yet reported. We hereby report solid state structure of compounds 3 and 4, enzyme inhibition and fluorescence potentials of thiourea derivatives which are already in field (Figure 1). It is notable that most of the compounds are potent inhibitors with respect to the standard, galantamine. Molecular docking study is also carried out for compound 3 and 4 in order to investigate their inhibitory action. The synthesized compounds were also investigated as a sensing probe for determination of mercury with the intent to use these biologically active molecules in removal technology in order to eliminate complications of bioacceptance and accessibility. The fluorescent properties of compounds are very interesting and the compound 2 was employed in the determination of mercury, as sensing probe. These studies invite further research in the field of single molecule detectors for determination of heavy metal ions of biological concerns in controlling a variety of diseases.
Compounds 1–6 are structurally very simple; they are accessible in the market and can also be obtained in laboratory under ambient conditions [37]. Compounds structurally analogous to 1–6 are good corrosion inhibitors in acidic medium [38] and show excited results in affording metal sulfides for useful applications [39]. Compound 5 has been used as starting precursors for the preparation of benzothiazole derivatives under catalyst free conditions [40]. There are several other reports wherein simple thiourea molecules after certain modifications have shown efficiency as bioactive compounds [41]. Compound 6 has already been used for its inhibitory activity against melanin B16 cells and mushroom tyrosinase and its synthesis has been reviewed [6,42]. The IC<sub>50</sub> value as melanin B16 inhibitor was promising, 3.4 µM, while it exhibited moderate potency as mushroom tyrosinase inhibitor. The enzyme inhibition (AChE and BChE) and metal sensing capability of compounds 1–6 (Figure 1) have not been reported so far. Since they are already in the field of bioorganic chemistry therefore these studies will help exploring their multidimensionality applications within a single system or organism whatever the case may be.

### 2.1. Structural Description of Compound 3

Compound 3 was crystalline in nature and good quality crystals were obtained at room temperature, which allowed for collection of X-ray diffraction data. The structure of compound 3 is depicted in Figure 2, along with a dimer unit held together with the help of H-bonding. The refinements and crystal structure solution parameters are summarized in Table 1, Bond angles and lengths of structural importance are summarized in Table 2, and the experimental data are compared with theoretical. Both data sets were in close agreement with each other. The surrounding of C7 is trigonal planar with expected bond lengths and angles and the data also revealed the sp<sup>2</sup> hybridized environment of the carbon. The C=S bond length was 1.695 Å, which fall within the expected limit for the said derivative [43]. The C7-N1 and C7-N2 distances were 1.351 and 1.326 Å, where the effect of substituent on the N was very clear, the lone pair of N1 was delocalized towards Ph ring while that of N2 was attracted towards C7 imparting partial double bond characters to N2-C7 bond (Table 2). This difference in delocalization of electron pair on both the N atoms provided insights to identify suitable H-bonding sites. The 3-CIPh-N1 was comparatively electron rich and is suitable donor, the hydrogen bonding was thus established making a dimer as given in Figure 2. Short ranged interactions were not observed in molecules of compound 3. See Table 3 for data pertaining to hydrogen bond parameters.
Figure 2. X-ray structure of Compound 3 (left), ellipsoids are drawn at 50% probability level, H atoms are omitted, selected carbon atoms and all non-carbon atoms are numbered. Dimer of compound 3 (right) stabilized through N1H–S1 interactions. Other secondary interactions in molecules were not detected. For data related to structural determination and refinements and selected structural parameters (bond lengths and angles) see Tables 1 and 2, respectively.

Table 1. Structure solution and refinements parameters of compound 3 and 4.

|                          | Compound 3               | Compound 4               |
|--------------------------|--------------------------|--------------------------|
| CCDC No *                | 2050647                  | 2050648                  |
| Chemical formula         | C_{13}H_{17}ClN_{2}S     | C_{15}H_{24}N_{2}S       |
| M_r                      | 268.79                   | 264.42                   |
| Temperature (K)          | 296                      | 296                      |
| Crystal system, space group | Monoclinic, P2_1/n    | Trigonal, R^3: H         |
| a, b, c (Å)              | 5.5080 (4), 22.1068 (17), | 25.550 (2), 25.550 (2), |
| β (°)                    | 11.4404 (8)              | 90.00                    |
| V (Å^3)                  | 1372.01 (17)             | 7448.8(13)               |
| Z                        | 4                        | 18                       |
| Radiation type           | Mo Kα                    | Mo Kα                    |
| μ (mm^-1)                | 0.41                     | 0.18                     |
| Crystal size (mm)        | 0.40 × 0.32 × 0.26       | 0.44 × 0.38 × 0.36       |
| Diffractometer           | Bruker Kappa APEXII CCD  |                          |
| Absorption correction    | Multi-scan               | Multi-scan               |
| T_{min}, T_{max}         | 0.815, 0.925             | 0.875, 0.955             |
| No. of measured,         |                          |                          |
| independent and observed |                          |                          |
| [I > 2σ(I)] reflections  | 9399, 3283, 2340         | 12267, 3614, 1852        |
| R_{int}                  | 0.048                    | 0.074                    |
| (sin θ/λ)_{max} (Å^-1)   | 0.660                    | 0.641                    |
| R[F^2 > 2σ(F^2)], wR(F^2), S | 0.048, 0.135, 1.02  | 0.056, 0.168, 1.02      |
| No. of reflections       | 3283                     | 3614                     |
| No. of parameters/restraints | 154/0          | 188/214                  |
| H-atom treatment         | H-atom parameters constrained |
| Δρ_{max}, Δρ_{min} (e Å^-3) | 0.34, −0.32          | 0.18, −0.16             |

Crystallographic information files pertaining to compounds 3 and 4 can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; Fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).
Table 2. Experimental and theoretical (B3LYP/3-21G level) selected structural parameters, bond lengths and angles of compounds 3 and 4.

| Compound | Atoms | Bond length | Exp. | Cal. | Bond angle | Exp. | Cal. |
|----------|-------|-------------|------|------|------------|------|------|
| 3        | S1-C7 | 1.695(19)   | 1.730| C7-N1-C1 | 127.52(17) | 128.41|
|          | N1-C7 | 1.351(2)    | 1.383| C7-N1-C1 | 127.52(17) | 128.41|
|          | N1-C1 | 1.423(2)    | 1.434| N2-C7-N1 | 117.68(17) | 117.29|
|          | N2-C7 | 1.326(2)    | 1.342| N2-C7-N1 | 117.68(17) | 117.29|
|          | N2-C8 | 1.464(2)    | 1.483| N2-C7-N1 | 117.68(17) | 117.29|
| 4        | S1-C7 | 1.686(2)    | 1.731| C7-N1-C1 | 127.84(19) | 128.23|
|          | N1-C7 | 1.352(3)    | 1.390| N2-C7-N1 | 115.86(18) | 116.10|
|          | N1-C1 | 1.426(3)    | 1.421| N2-C7-N1 | 115.86(18) | 116.10|
|          | N2-C7 | 1.352(3)    | 1.390| N2-C7-N1 | 115.86(18) | 116.10|

Table 3. Hydrogen-bond geometries (Å) of compounds 3 and 4.

| Compound | D—H···A | D—H | H···A | D···A | D—H···A | Symmetry Codes |
|----------|---------|------|-------|-------|---------|----------------|
| 3        | N1—H1   | 0.86 | 2.53  | 3.343 | 157.4   | −x, −y, −z + 2 |
|          | C2—H2   | 0.93 | 2.96  | 3.878 | 167.6   | −x, −y, −z + 1 |
|          | C9—H9B  | 0.97 | 2.91  | 3.483 | 118.9   | -              |
|          | N1—H1   | 0.86 | 2.70  | 3.484 | 151.5   | x−y + 2/3, x + 1/3, −z + 4/3 |
|          | C8A—H8A | 0.97 | 2.96  | 3.840 | 151.5   | −x + y − 1/3, −x + 1/3, z + 1/3 |
|          | C8A—H8B | 0.97 | 2.75  | 3.711 | 172.2   | −x−y + 2/3, x + 1/3, −z + 4/3 |
|          | C8B—H8C | 0.97 | 2.95  | 3.84  | 152.3   | −x + y − 1/3, −x + 1/3, z + 1/3 |
|          | C8B—H8D | 0.97 | 2.62  | 3.53  | 155.3   | x−y + 2/3, x + 1/3, −z + 4/3 |
|          | C9B—H9C | 0.97 | 2.65  | 3.179 | 114.8   | -              |
|          | C12B—H12D | 0.97 | 2.56  | 3.00  | 107.6   | -              |

2.2. Structural Description of Compound 4

Molecular structure of thiourea derivative 4 is given in Figure 3 (left), structure determination and refinements were carried out as summarized in Table 1, and selected bond lengths and angles are summarized in Table 2. The bond angles and bond lengths around the central carbon, C7 were very similar to compound 3. The sum of all bond angles is 360° indicating that C7 was sp² hybridized with characteristic trigonal planar geometry. The C7=S1 bond length was 1.686 Å, which is slightly shorter than the same bond in compound 3. The C7-N1 and C7-N2 distances were 1.352 and 1.344 Å were slightly different from each other revealing the effect of substituent on N atom. The difference was small as compared to compound 3, indicating that lone pair of electrons at both N atoms were almost equally involved in delocalization through the NCN fragment. The NH and CH₂ hydrogen atoms in molecules played an important role in stabilizing the supramolecular structure in a 3D manner. The H8B and NH1 were attached to S1 of one molecule with H–S separation distance of 2.748 and 2.704 Å, respectively and the H8A to S atom of the other molecule (separation distance 2.960 Å). The difference in separation distance was obvious and it can be clearly observed that the former interaction combined molecule in hexameric ring form and the latter one helped to keep chains together in a 3D fashion. Non-covalent interactions between molecules of a hexameric ring made an R1²(7) type loop in a cyclic arrangement and further extended in a 3D manner as discussed earlier. Different views of supramolecular structure of compound 4 are shown in Figure 4, and the hydrogen bonding data are presented in Table 3.
Figure 3. Single crystal structure (left hand) of compound 4, ellipsoids are drawn at 50% probability level, H-atoms except NH and CH$_2$ are omitted and selected atoms have been numbered. A cyclic hexamer (right hand) is shown, molecules are linked with the help of S–H interactions making a macrocyclic ring containing six thiourea molecules (See Table 2 for selected structural parameters).

Figure 4. Different view of supramolecular structure of compound 4, single layer of mutually connected hexameric units (top), mutually connected layers (middle and bottom), the molecular architecture extends in a 3D manner.
Optimized structure of compound 3 and 4, are given in Figure 5, a comparison of experimental and theoretical bond parameters showed a close relationship, with negligible variation. The data suggested that gas phase and solid-state structure did not vary from each other.

![Figure 5](image)

**Figure 5.** Optimized structures of compounds 3 and 4, selected hydrogen atoms are omitted.

### 2.3. Enzyme Inhibition Studies

The effectiveness of the synthesized compounds 1–6 as inhibitors of the selected cholinesterase was tested as discussed in experimental section, vide supra. The observed activities are summarized in Table 4. The IC₅₀ value of most of the compounds was high > 100 µg/mL, except two compounds. Compound 3 was the most potent with excellent activity 50 and 60 µg/mL against AChE and BChE, respectively. Compound 4 also exhibited very close IC₅₀ values 58 and 63 µg/mL, respectively. The IC₅₀ value observed for the standard was 15 µg/mL for inhibition of both the enzymes in a respective manner. Further derivatization of compound 3, can be useful in future studies to achieve the target properties. The efficiency of this compound can be linked to the intermolecular interactions as discussed above. Lack of secondary interactions in other molecules like 4 is the probable reason of poor biological efficiency.

| No | Conc. (µg/mL) | %AChE Inhibition | IC₅₀ (µg/mL) | %BChE Inhibition | IC₅₀ (µg/mL) |
|----|---------------|------------------|--------------|------------------|--------------|
| 1  | 1000          | 69.47 ± 0.22     | 89           | 74.4 ± 0.68      | 84           |
|    | 500           | 63.94 ± 0.45     |              | 66.2 ± 0.73      |              |
|    | 250           | 57.61 ± 1.70     |              | 61.0 ± 0.33      |              |
|    | 125           | 53.64 ± 0.16     |              | 56.4 ± 0.63      |              |
|    | 62.5          | 46.52 ± 0.38     |              | 46.9 ± 0.42      |              |
|    | 1000          | 63.34 ± 0.98     | 300          | 65.17 ± 0.72     | 185          |
|    | 500           | 56.32 ± 1.06     |              | 57.85 ± 0.97     |              |
|    | 125           | 44.70 ± 1.25     |              | 46.73 ± 0.78     |              |
|    | 62.5          | 38.74 ± 0.68     |              | 41.34 ± 1.01     |              |
|    | 1000          | 73.39 ± 0.60     |              | 76.7 ± 0.66      |              |
|    | 500           | 67.39 ± 0.49     | 50           | 71.3 ± 1.11      |              |
|    | 125           | 61.36 ± 0.49     |              | 65.5 ± 1.04      |              |
|    | 62.5          | 57.34 ± 0.55     |              | 57.2 ± 0.57      |              |
|    | 1000          | 51.90 ± 1.16     |              | 49.9 ± 0.65      |              |
Table 4. Cont.

| No | Conc. (µg/mL) | %AChE Inhibition | IC₅₀ (µg/mL) | %BChE Inhibition | IC₅₀ (µg/mL) |
|----|---------------|-------------------|-------------|------------------|-------------|
| 4  | 1000          | 93.58 ± 1.12      | 58          | 98.00 ± 0.00     | 63          |
|    | 500           | 85.40 ± 0.20      |             | 94.40 ± 0.50     |             |
|    | 250           | 77.85 ± 2.26      |             | 89.80 ± 1.50     |             |
|    | 125           | 71.80 ± 1.50      |             | 77.40 ± 1.70     |             |
|    | 62.5          | 65.49 ± 0.47      |             | 53.50 ± 0.90     |             |
|    | 31.5          | 59.01 ± 0.88      |             | 47.30 ± 0.70     |             |
|    | 1000          | 66.79 ± 0.63      |             | 71.62 ± 0.74     |             |
|    | 500           | 59.67 ± 0.61      |             | 63.86 ± 0.60     |             |
| 5  | 250           | 41.69 ± 0.77      | 345         | 44.48 ± 0.64     | 310         |
|    | 125           | 35.54 ± 0.50      |             | 37.54 ± 0.50     |             |
|    | 62.5          | 29.00 ± 0.30      |             | 31.74 ± 0.61     |             |
|    | 1000          | 69.58 ± 1.12      |             | 71.33 ± 0.49     |             |
|    | 500           | 61.65 ± 1.34      |             | 63.03 ± 0.23     |             |
| 6  | 250           | 47.90 ± 0.96      | 285         | 49.00 ± 0.58     | 265         |
|    | 125           | 39.03 ± 0.48      |             | 42.67 ± 0.89     |             |
|    | 62.5          | 31.90 ± 0.48      |             | 33.00 ± 1.15     |             |
|    | 1000          | 83.19 ± 0.73      |             | 89.64 ± 0.62     |             |
|    | 500           | 77.54 ± 0.91      |             | 81.83 ± 0.37     |             |
|    | 250           | 71.93 ± 0.14      | 15          | 74.29 ± 0.73     | 15          |
|    | 125           | 65.72 ± 0.49      |             | 67.92 ± 0.98     |             |
|    | 62.5          | 61.67 ± 0.94      |             | 64.93 ± 0.87     |             |

**Galantamine**

The complete analysis of co-crystalized thiourea derivative 3 and AChE inhibitor showed that tyr-124, Ser-203 and Tyr-337 are the most potent residues showing binding interactions with inhibitor compound 3 with in the active pocket of protein. Our synthesized compound 3 forms strong hydrogen bonding with Tyr-124 with calculated length 2.4 Å and with binding score of −10.01 kJ/mol. In addition to strong hydrogen bonding, some weak polar interactions and hydrophobic interactions were also observed with Tyr-337, Tyr-341 and Ser-203 given in Figure 6a. The compound 4 forms hydrogen bond with Trp-532 with a separation distance of 2.7 Å with binding score of −8.031 kJ/mol and hydrophobic interactions with His-405 and Pro-537 given Figure 6b. The binding energy shows that compound 3 exhibited strong binding interactions as compared with compound 4, thus supporting the experimental inhibition data of structurally analogous compounds [44].

During the docking studies of compound 3 and 4 with BChE, it was observed that 3 established interactions with Asn-83, Asn-85 and His-77, while compound 4 was efficient in establishing interactions with Tyr-128 and Trp-82. These interactions are responsible to make a compound more potent against BChE. The co-crystalized compound 3 formed strong hydrogen bonding with Asn-83 and His-126 with a distance of 2.2 and 3.0 Å, respectively and the binding score of −8.04 kJ/mol. In addition to this, compound 3 also established weak hydrophobic interactions with same residues of BChE given in Figure 6c, while compound 4 formed weak hydrogen bonding with Tyr-456 (2.4 Å) and weak interaction with Lys-427 residues (3.2 Å) with binding score of −6.98 kJ/mol [45].
The comparison of binding energy shows that compound 3 was also a potent inhibitor of BChE as compared to compound 4, thus our calculations support the experimental data, Figure 6d.

![Figure 6. 3D binding interaction modes of compounds 3 and 4 as inhibitor of AChE and BChE. (a) Interaction posing of compound 3 with AChE; (b) interaction of compound 4 with AChE; (c) interaction of compound 3 with BChE and (d) interaction of compound 4 with BChE.](image)

2.4. Fluorescence and UV-Visible Characteristics of the Compounds 1–3, 5 and 6

The UV-visible absorption spectra of compounds were measured for a 2 ppm solution in the range 200–800 nm. The absorption maxima of all the tested compounds appeared between 200–400 nm as shown in Figure S1, Supplementary Materials. The methanol solution of compounds exhibited $\lambda_{max}$ 296, 294, 298, 294 and 295 nm, respectively. The fluorescence characteristics of the synthesized compounds (1–3, 5, 6) were investigated against the solvent blank by scanning the $\lambda_{ax}$ and $\lambda_{em}$ in the range of 250–750 nm. The fluorescence spectra of compounds are shown in Figure 7 (for two selected compounds) and respective data are tabulated in Table 5. It was observed that compound 3, 5 and 6 showed excellent fluorescence intensity, compound 3 at low sensitivity level while compounds 1 and 2 show excellent instrumental sensitivity. The large stokes shift (>300 nm) for compounds 3 and 5 could possibly be attributed to the intramolecular charge transfer characteristic (ICT) due to the electron-accepting nature of the moiety because of its well-defined dipole moment. Compounds which undergo ICT, exhibited different properties both in their ground and excited states, which was caused by an electronic rearrangement after photo excitation. This temporary dipole alternation led to larger changes in dipole moments and to the relaxation of the structure. The energy difference between the Frank–Condor state and the ICT state was the predominant reason for the large Stokes shift, which is ideal in fluorescence methods [46,47]. The fluorescence behavior of these compounds shows that all the compounds at concentration as low as 10 $\mu$g mL$^{-1}$ could be used for fabrication of fluorescence-based sensing tools for detection of environmental pollutants especially pesticides, toxic heavy metals and drugs. The analyte molecules could interact effectively with the compounds causing fluorescence enhancement or fluorescence quenching. The
interaction could be easily employed for development of sensitive analytical methods for
determination of the subject analytes which is the future perspective of this work.

Figure 7. Fluorescence spectra of 3 and 5 as two representative examples.

Table 5. Fluorescence characteristics of compounds 1–3, 5 and 6.

| Compound | Concentration (µg mL⁻¹) | Scan Range (nm) | λ_ex (nm) | λ_em (nm) | Mode of Sensitivity | Fluorescence Intensity |
|----------|------------------------|-----------------|----------|----------|--------------------|-----------------------|
| 1        | 10                     | 350–700         | 312      | 613      | High               | 276.483               |
| 2        | 220–750                | 304.0           | 328.0    |          | High               | 16.189                |
| 3        | 220–650                | 329             | 661      |          | Low                | 212.958               |
| 5        | 220–600                | 337             | 662      |          | Low                | 180.234               |
| 6        | 220–600                | 311             | 624      |          | Low                | 275.143               |

2.5. Application of Compounds 1–3, 5, 6 as Sensing Probe for Determination of Mercury

In order to know the interaction between Hg(II) and the subject compounds, variable
concentration of Hg(II) solutions was added to known concentration of each compound
and FI at the respective excitation and emission of each compound was measured at low
and high sensitivity mode. The results are given in Table 6 and for two selected compounds
3 and 5 are shown in Figure 8. As evident from the data, FI of the mixtures increased
linearly with increase in the concentration of Hg(II) up to the maximum FI level. The reason behind this interaction of the compounds with Hg(II) was based on the soft and hard acid base concept [36]. Mercury (Hg(II)) is a soft center with high polarizability and sulfur in the thiourea molecule is soft center too. The only compound that does not show this sort of enhancement interaction with Hg(II) is 6. Though 6 also bore a sulfur atom the probable reason of negligible interactions could be due to inappropriate cavity size and orientation for binding of Hg(II) ions. It happened due to diminishing of photoinduced electron transfer phenomenon between receptor and fluorophore [36]. This fluorescence enhancement could be successfully applied for trace level determination of Hg(II) in complex environmental samples including water, soil, vegetables and fruits. The same could also be applied for remediation of Hg(II) ion in real biological samples in order to develop efficient reagent/medicine to combat AD.

Figure 8. Representative fluorescence spectra of 3 and 5 as a function of concentration of Hg(II) ion.
Table 6. Hg(II) sensing studies of compounds 1–3, 5 and 6. FI in the Table stands for fluorescence intensity.

| Compound | Concentration (ppm) | λ_ex/λ_em(nm) | Fluorescence Intensity | Concentration of Hg(II) ppm | Fluorescence Intensity |
|----------|---------------------|--------------|------------------------|----------------------------|------------------------|
|          |                     |              |                        |                            |                        |
| 3        | 10                  | 329/661      | FI = 212.958           | 0.1                        | 608.132                |
|          |                     | FL = 121.958 | Curve                  | 0.2                        | 901.543                |
|          |                     |              |                        | 0.3                        | 1015.790               |
| 1        | 10                  | 312/613      | FI = 276.483           | 0.1                        | 460.580                |
|          |                     | FL = 276.483 | Curve                  | 0.2                        | 646.740                |
|          |                     |              |                        | 0.3                        | 876.296                |
|          |                     |              |                        | 0.5                        | 1015.790               |
| 2        | 0.01                | 264/284      | FI = 16.189            | 0.01                       | 60.193                 |
|          |                     | FL = 16.189  | Curve                  | 0.02                       | 81.624                 |
|          |                     |              |                        | 0.03                       | 117.136                |
|          |                     |              |                        | 0.04                       | 139.836                |
| 5        | 5.0                 | 337/662      | FI = 180               | 0.05                       | 197                    |
|          |                     | FL = 180     | Curve                  | 0.1                        | 220                    |
|          |                     |              |                        | 0.2                        | 400                    |
|          |                     |              |                        | 0.5                        | 550                    |
| 6        | 10                  | 311/624      | FI = 275               | 0.05                       | No interaction         |
|          |                     | FL = 275     | Curve                  | 0.1                        |                        |
|          |                     |              |                        | 0.2                        |                        |
|          |                     |              |                        | 1.0                        |                        |

3. Materials and Methods

3.1. General Consideration

All reactions were carried out under aerobic atmosphere and no special precautions were taken to exclude air or moisture during experiments. All chemicals were purchased from Sigma Aldrich, TCI or Across Organics and were used without further purification. Thiourea derivatives described in this study are also commercially available in the marked, we reproduced them in the laboratory, they were obtained as crystalline material. The formation of compounds was confirmed with the help of available spectroscopic techniques, such as ATR-FTIR spectrometer and 1H- and 13C NMR spectra (in order to assist readers these data is provided in supplementary file). Single crystal X-ray analysis was carried out at room temperature by using a Bruker Kappa APEXII CCD diffractometer. Absorption of solution for determination of enzyme inhibition was measured by Thermoelectron corporation, USA and florescence studies were carried out using fluorescence spectrophotometer model RF-5301 Schimadzu, Japan, for their potential use in sensing environmental pollutants. Thiourea derivatives were prepared by reactions as previously reported [44,48].

3.2. Anticholinesterase Evaluation

Anticholinesterase activity of compounds 1–6 was evaluated by Ellman’s assay using two enzymes, AChE and BChE and following our previously reported protocol without further modifications [49–51]. The enzyme AChE is derived from Electric eel and BChE from equine serum [52]. Buffer of phosphate, 0.1 M at pH 8.0 was prepared to whom varied concentration (31.625–1000 µg/mL) of the respective compound was added. The solution of AChE was diluted to 0.03 U/mL from 518 U/mg, similarly BChE from 7–16 U/mg to 0.01 U/mL at the given pH condition. Distilled water was used as solvent, for preparation of 0.5 mM acetylthiocholine iodide (ATchI), 0.2273 mM 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) and 0.5 mM butyrylthiocholine iodide (BTchI). All solutions were stored at 8 °C in a refrigerator prior to performing the experiment.

Enzyme solution (5 µL), corresponding compound (205 µL), and DTNB reagent (5 µL) were mixed in a cuvette. The solution was incubated for 15 min at 30 °C followed by addition of 5 µL of the substrate solution. Absorbance of the incubated sample was
measured at 412 nm by a double-beam spectrophotometer (Thermo Electron Corporation, USA) for 4 min along with the reaction time at 30 °C. The well-established Galanthamine was used as positive control and all experiments were performed in triplicate. The percent enzyme activity and enzyme inhibition by control and test samples were calculated from the rate of absorption with respect to change in time \( V = \frac{\Delta \text{Abs}}{\Delta t} \) using the same procedure as reported previously [49–51].

3.3. Molecular Docking Study

Molecular docking was carried out to determine the binding interaction mode of the synthesized compounds for the competitive and noncompetitive inhibition with the mark enzymes, AChE and BChE [53,54]. The crystal structure of the said enzymes was obtained from protein databank. Proteins were theoretically prepared by using auto dock mgl tools [55] and was optimized by removing all water molecules, heteroatoms and co-factors, for simplicity in calculations. The structures of the synthesized compound 3 and 4 were obtain from CIFs using Avogadro and respective CCDC software. Protein-ligands images were developed by using pymol [56,57].

3.4. Fluorescence Measurements

Spectrofluorophotometer (RF-5301 PC, Shimadzu, Japan) having 150 W Xenon lamp as excitation source with 1.0 cm quartz cell was used throughout the experimental work for fluorescence measurements. The emission and excitation slit width was 4 nm for fluorometric operation. The thiourea derivatives (1–3, 5, 6) of variable concentration were prepared in acetone and incubated for 60 min at room temperature. All the tested thiourea derivatives, were found to show excellent fluorescence behavior at low as well as at high sensitivity levels. These compounds were employed to fabricate a sensing probe for determination of mercury in water samples.

4. Conclusions

Thiourea derivatives 1–6 were reproduced in the laboratory and structure of two compounds 3 and 4 was determined with the help of X-ray diffraction for single crystal. All compounds were tested for their enzyme inhibitory efficiency where compounds 3 was found more efficient. The activity of this compound (IC\(_{50}\) 50 and 60 µg/mL against AChE and BChE, respectively) is very close to the efficiency of the standard drug and is worth studying in future investigations. The experimental data particularly inhibitory efficiency of compound 3, were validated through its docking studies and both data sets were found in close agreement with each other. Further investigations to know their sensing capacity against heavy metals ions, such as Hg(II) were carried out and the results are promising, compound 3 came out to be among other compounds with better sensitivity level. This study reveals that thiourea derivatives possess manifold useful characteristics and may be able to control AD by inhibiting AChE, BChE or controlling the concentration of heavy metal ion. These compounds are better sensors to determine specific metals in real biological samples.

Supplementary Materials: Figure S1: UV absorption spectra of compounds 1–3, 5 and 6; Table S1: UV-Visible spectroscopic data of compounds 1–3, 5 and 6 and experimental procedure for preparation of compounds, their FTIR and NMR characterization.

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**References**

1. Hegde, S.; Joshi, S.; Mukherjee, T.; Kapoor, S. Photochemical synthesis of gold nanoparticles in N,N'-dimethylformamide via thiourea-derivatized polyoxometalate. *Res. Chem. Intermed.* 2014, 40, 1125–1133. [CrossRef]

2. Zhou, W.; Peng, K.; Tao, F.-M. Theoretical mechanism for the oxidation of thiourea by hydrogen peroxide in gas state. *J. Mol. Struct. THEOCHEM* 2007, 821, 116–124. [CrossRef]

3. Bai, W.; Ji, J.; Huang, Q.; Wei, W. Synthesis and evaluation of new thiourea derivatives as antitumor and antiangiogenic agents. *Tetrahedron Lett.* 2020, 61, 152366. [CrossRef]

4. Khan, E.; Khan, S.; Gul, Z.; Muhammad, M. Medicinal importance, coordination chemistry with selected metals (cu, Ag, au) and Chemosensing of Thiourea derivatives. A review. *Crit. Rev. Anal. Chem. 2020.* [CrossRef]

5. Bregović, V.B.; Basarić, N.; Milinarić-Majerski, K. Anion binding with urea and thiourea derivatives. *Coord. Chem. Rev.* 2015, 295, 80–124. [CrossRef]

6. Thanigaimalai, P.; Lee, K.-C.; Sharma, V.K.; Joo, C.; Cho, W.-J.; Roh, E.; Kim, Y.; Jung, S.-H. Structural requirement of phenylthiourea analogs for their inhibitory activity of melanogenesis and tyrosinase. *Bioorganic Med. Chem. Lett.* 2011, 21, 6824–6828. [CrossRef]

7. Taylor, E.C.; Ravindranathan, R. Reaction of Anthranilonitrile and N-Methylanthranilonitrile with Phenyl Isocyanate and Phenyl phosphine complexes containing seleno- and thiosemicarbazonato ligands. *Dalton Trans.* 2012, 41, 11667–11674. [CrossRef]

8. Maddani, M.R.; Prabhu, K.R. A concise synthesis of substituted thiourea derivatives in aqueous medium. *J. Org. Chem. 1962,* 27, 2622–2627. [CrossRef]

9. Binzet, G.; Kavak, G.; Külcü, N.; Özbey, S.; Flörke, U.; Arslan, H. Synthesis and characterization of novel thiourea derivatives and their nickel and copper complexes. *J. Chem.* 2013, 2013, 536562. [CrossRef]

10. Molter, A.; Rust, J.; Lehmann, C.W.; Deepa, G.; Chiba, P.; Mohr, F. Synthesis, structures and anti-malaria activity of some gold (I) phosphine complexes containing seleno-and thiosemicarbazonato ligands. *Dalton Trans.* 2011, 40, 9810–9820. [CrossRef]

11. Meng, G.; Wang, M.; Dong, M.; Zheng, A.; Shi, J.; De Clercq, E.; Balzarini, J. Synthesis and Anti Hiv-1 Reverse Transcriptase Evaluation of a Series of N-Mono Substituted Thio-urea Derivatives. *Int. J. AIDS Res.* 2015, 2, 19–27.

12. Dong, Y.; Venkatachalam, T.; Narla, R.K.; Trieu, V.N.; Sudbeck, E.A.; Uckun, F.M. Antioxidant function of phenethyl-5-bromopyridyl thiourea compounds with potent anti-HIV activity. *Bioorganic Med. Chem. Lett.* 2000, 10, 87–90. [CrossRef]

13. Kang, I.-J.; Wang, L.-W.; Lee, C.-C.; Lee, Y.-C.; Chao, Y.-S.; Hsu, T.-A.; Chern, J.-H. Design, synthesis, and anti-HCV activity of thiourea compounds. *Bioorganic Med. Chem. Lett.* 2009, 19, 1950–1955. [CrossRef]

14. Khatri, N.; Lather, V.; Madan, A. Diverse classification models for anti-hepatitis C virus activity of thiourea derivatives. *Chemosom. Intell. Lab. Syst.* 2015, 140, 13–21. [CrossRef]

15. Shakeel, A.; Altaf, A.A.; Qureshi, A.M.; Badshah, A. Thiourea derivatives in drug design and medicinal chemistry: A short review. *J. Drug Des. Med. Chem.* 2016, 2, 10. [CrossRef]

16. Vedavathi, P.; Sudhamani, H.; Raju, C.N. Synthesis and antimicrobial activity of new urea and thiourea derivatives of (2′-(1H-tetrazol-5-yl) biphenyl-4-yl) methanamine. *Res. Chem. Intermed.* 2017, 43, 3251–3263. [CrossRef]

17. Rehman, T.U.; Khan, I.U.; Riaz, S. Novel substituted 3-phenyl 1-(4-(5-bromopyridin-3-yl)-6-phenylpyrimidin-2-yl)-thiourea compounds as key small organic molecules for the potential treatment of type II diabetes mellitus: In vitro studies against yeast α-glucosidase. *Med. Chem. Res.* 2017, 26, 1098–1106. [CrossRef]

18. Darvesh, S.; Hopkins, D.A.; Geula, C. Neurobiology of butyrylcholinesterase. *Nat. Rev. Neurosci.* 2003, 4, 131–138. [CrossRef][PubMed]

19. Mattes, C.; Bradley, R.; Slaughter, E.; Browne, S. Cocaine and butyrylcholinesterase (BChE): Determination of enzymatic parameters. *Life Sci.* 1996, 58, PL257–PL261. [CrossRef]

20. Shen, Y.; Zhang, J.; Sheng, R.; Dong, X.; He, Q.; Yang, B.; Hu, Y. Synthesis and biological evaluation of novel flavonoid derivatives as dual binding acetylcholinesterase inhibitors. *J. Enzym. Inhib. Med. Chem.* 2009, 24, 372–380. [CrossRef]

21. Želik, P.; Lukešová, A.; Voloshko, L.N.; Štys, D.; Kopecký, J. Screening for acetylcholinesterase inhibitory activity in cyanobacteria of the genus Nostoc. *J. Enzym. Inhib. Med. Chem.* 2009, 24, 531–536. [CrossRef][PubMed]
22. Lane, R.M.; Pottkin, S.G.; Enz, A. Targeting acetylcholinesterase and butyrylcholinesterase in dementia. *Int. J. Neuropsychopharmacol.* 2006, 9, 101–124. [CrossRef] [PubMed]

23. Rahman, F.U.; Bibi, M.; Altaf, A.A.; Tahir, M.N.; Ullah, F.; Zia Ur, R.; Khan, E. Zn, Cd and Hg complexes with unsymmetric thiourea derivatives; syntheses, free radical scavenging and enzyme inhibition essay. *J. Mol. Struct.* 2020, 1211, 128096. [CrossRef]

24. Shade, C.W.; Hudson, R.J.M. Determination of MeHg in Environmental Sample Matrices Using Hg—Thiourea Complex Ion Chromatography with On-line Cold Vapor Generation and Atomic Fluorescence Spectrometric Detection. *Environ. Sci. Technol.* 2005, 39, 4974–4982. [CrossRef] [PubMed]

25. An, F.-Q.; Wang, Y.; Xue, X.-Y.; Hu, T.-P.; Gao, J.-F.; Gao, B.-J. Design and application of thiourea modified D301 resin for the effective removal of toxic heavy metal ions. *Chem. Eng. Res. Des.* 2018, 130, 78–86. [CrossRef]

26. Rahman, M.M.; Ahmed, J.; Asiri, A.M. Thiourea sensor development based on hydrothermally prepared CMO nanoparticles for environmental safety. *Biosens. Bioelectron.* 2018, 99, 586–592. [CrossRef]

27. El-Korashy, S.A.; Elwakeel, K.Z.; El-Hafeiz, A.A. Fabrication of bentonite/thiourea-formaldehyde composite material for Pb(II), Mn(VII) and Cr(VI) sorption: A combined basic study and industrial application. *J. Clean. Prod.* 2016, 137, 40–50. [CrossRef]

28. Loto, C.; Loto, R.; Popoola, A. Corrosion inhibition of thiourea and thiadiazole derivatives: A review. *J. Mater. Environ. Sci.* 2012, 3, 885–894.

29. Rahman, F.U.; Bibi, M.; Elwakeel, K.Z.; Ahmed, J. Comparison study of Ag(I) and Au(III) loaded on magnetic thiourea-formaldehyde as disinfectants for water pathogenic microorganism’s deactivation. *J. Environ. Chem. Eng.* 2016, 4, 4380–4390. [CrossRef]

30. Yusof, M.S.M.; Jusoh, R.T.H.; Kairuil, W.M.; Yamin, B.M. Synthesis and characterisation a series of N-(3,4-dichlorophenyl)-N′-(2,3 and 4-methylbenzoyl)thiourea derivatives. *J. Mol. Struct.* 2010, 975, 280–284. [CrossRef]

31. Saxe, S.R.; Wekstein, M.W.; Kryscio, R.J.; Henry, R.G.; Cornett, C.R.; Snowdon, D.A.; Grant, F.T.; Schmitt, F.A.; Donegan, S.J.; Wekstein, D.R.; et al. Alzheimer’s disease, dental amalgam and mercury. *J. Am. Dent. Assoc.* 1999, 130, 191–199. [CrossRef] [PubMed]

32. El-Liethy, M.A.; Elwakeel, K.Z.; Ahmed, M.S. Fluorescence studies of Ag(I) and Au(III) loaded on magnetic thiourea-formaldehyde as disinfectants for water pathogenic microorganism’s deactivation. *J. Environ. Chem. Eng.* 2016, 4, 4380–4390. [CrossRef]

33. Wang, X.; Yang, C.; Yan, M.; Ge, S.; Yu, J. A novel fluorescence probe based on p-acid-Br and its application in thiourea detection. *RSC Adv.* 2016, 6, 45001–45008. [CrossRef]

34. Mishra, J.; Kaur, H.; Ganguli, A.K.; Kaur, N. Fluorescent chemosensor based on urea/thiourea moiety for sensing of Hg (II) ions in an aqueous medium with high sensitivity and selectivity: A comparative account on effect of molecular architecture on chemosensing. *J. Mol. Struct.* 2018, 1161, 34–43. [CrossRef] [PubMed]

35. Ngah, F.A.A.; Zakariah, E.I.; Hassan, N.I.; Yamin, B.; Sapari, S.; Hasbullah, S.A. Synthesis of thiourea derivatives and binding behavior towards the mercury ion. *Malays. J. Anal. Sci.* 2017, 21, 1226–1234.

36. Mishra, J.; Kaur, H.; Ganguli, A.K.; Kaur, N. Fluorescent chemosensor based on urea/thiourea moiety for sensing of Hg (II) ions in an aqueous medium with high sensitivity and selectivity: A comparative account on effect of molecular architecture on chemosensing. *J. Mol. Struct.* 2018, 1161, 34–43. [CrossRef] [PubMed]

37. Gan, S.-F.; Wan, J.-P.; Pan, Y.-J.; Sun, C.-R. Highly efficient and catalyst-free synthesis of substituted thioureas in water. *Mol. Divers.* 2011, 15, 809–815. [CrossRef]

38. Shetty, P. Corrosion inhibition behaviour of thiourea derivatives in acid media against mild steel deterioration: An overview. *Surf. Eng. Appl. Electrochem.* 2017, 53, 587–591. [CrossRef]

39. Owen, J.S.; Hendricks, M.P.; Campos, M.P.; Cleveland, G.T.; Jen-La PLANTE, I.; Hamachi, L.S. Methods of Producing Metal Complexes of Thioureas. *Int. J. Mol. Sci.* 2018, 19, 1226–1234.

40. Wang, R.; Yang, W.-j.; Yue, L.; Pan, W.; Zeng, H.-y. DDQ-Promoted C–S Bond Formation: Synthesis of 2-Aminobenzothiazole Derivatives under Transition-Metal-, Ligand-, and Base-Free Conditions. *Synlett* 2012, 530–538. [CrossRef]

41. Saeed, A.; Zaib, S.; Ashraf, S.; Iftikhar, J.; Muddassar, M.; Zhang, K.Y.; Iqbal, J. Synthesis, cholinesterase inhibition and molecular modelling studies of coumarin linked thiourea derivatives. *Bioorganic Chem.* 2015, 63, 58–63. [CrossRef] [PubMed]

42. Czaplińska, B.; Malarz, K.; Mrozek-Wilczkiewicz, A.; Słodek, A.; Korzec, M.; Musiol, R. Theoretical and Experimental Investigations of Large Stokes Shift Fluorophores Based on a Quinoline Scaffold. *Molecules* 2020, 25, 2488. [CrossRef] [PubMed]

43. Yang, G.; Li, S.; Wang, S.; Hu, R.; Feng, J.; Li, Y.; Qian, Y. Novel fluorescent probes based on intramolecular charge-and proton-transfer compounds. *Pure Appl. Chem.* 2013, 85, 1465–1478. [CrossRef]

44. Khan, U.A.; Badshah, A.; Tahir, M.N.; Khan, E. Gold (I), silver (I) and copper (I) complexes of 2, 4, 6-trimethylphenyl-3-benzoylthiourea; synthesis and biological applications. *Polyhedron* 2020, 181, 114485. [CrossRef]
49. Khan, E.; Khan, A.; Gul, Z.; Ullah, F.; Tahir, M.N.; Khalid, M.; Asif, H.M.; Asim, S.; Braga, A.A.C. Molecular salts of terephthalic acids with 2-aminopyridine and 2-aminothiazole derivatives as potential antioxidant agents; Base-Acid-Base type architectures. *J. Mol. Struct.* **2020**, *1200*, 127126. [CrossRef]

50. Gul, Z.; Din, N.U.; Khan, E.; Ullah, F.; Tahir, M.N. Synthesis, molecular structure, anti-microbial, anti-oxidant and enzyme inhibition activities of 2-amino-6-methylbenzothiazole and its Cu (II) and Ag (I) complexes. *J. Mol. Struct.* **2020**, *1199*, 126956. [CrossRef]

51. Khan, E.; Ahmad, T.; Gul, Z.; Ullah, F.; Tahir, M.N.; Noor, A. Methyl-substituted 2-aminothiazole–based cobalt (II) and silver (I) complexes: Synthesis, X-ray structures, and biological activities. *Turk. J. Chem.* **2019**, *43*, 857–868. [CrossRef]

52. Dingova, D.; Leroy, J.; Check, A.; Garaj, V.; Krejci, E.; Hrabovska, A. Optimal detection of cholinesterase activity in biological samples: Modifications to the standard Ellman’s assay. *Anal. Biochem.* **2014**, *462*, 67–75. [CrossRef] [PubMed]

53. Ahmad, H.; Ahmad, S.; Khan, E.; Shahzad, A.; Ali, M.; Tahir, M.N.; Shaheen, F.; Ahmad, M. Isolation, crystal structure determination and cholinesterase inhibitory potential of isotalatizidine hydrate from Delphinium denudatum. *Pharm. Biol.* **2017**, *55*, 680–686. [CrossRef]

54. Ahmad, S.; Ahmad, H.; Khan, H.U.; Shahzad, A.; Khan, E.; Ali Shah, S.A.; Ali, M.; Wadud, A.; Ghufran, M.; Naz, H.; et al. Crystal structure, phytochemical study and enzyme inhibition activity of Ajaconine and Delectinine. *J. Mol. Struct.* **2016**, *1123*, 441–448. [CrossRef]

55. Qiao, L.; Huang, J.; Hu, W.; Zhang, Y.; Guo, J.; Cao, W.; Miao, K.; Qin, B.; Song, J. Synthesis, characterization, and in vitro evaluation and in silico molecular docking of thiourea derivatives incorporating 4-(trifluoromethyl) phenyl moiety. *J. Mol. Struct.* **2017**, *1139*, 149–159. [CrossRef]

56. Chigurupati, S.; Selvaraj, M.; Mani, V.; Selvarajan, K.K.; Mohammad, J.I.; Kaveti, B.; Bera, H.; Palanimuthu, V.R.; Teh, L.K.; Salleh, M.Z. Identification of novel acetylcholinesterase inhibitors: Indolopyrazoline derivatives and molecular docking studies. *Bioorganic Chem.* **2016**, *67*, 9–17. [CrossRef] [PubMed]

57. Sonmez, F.; Zengin Kurt, B.; Gazioglu, I.; Basile, L.; Dag, A.; Cappello, V.; Ginex, T.; Kucukislamoglu, M.; Guccione, S. Design, synthesis and docking study of novel coumarin ligands as potential selective acetylcholinesterase inhibitors. *J. Enzym. Inhib. Med. Chem.* **2017**, *32*, 285–297. [CrossRef] [PubMed]