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Synthesis, structure–activity relationship and molecular docking of 3-oxoaurones and 3-thioaurones as acetylcholinesterase and butyrylcholinesterase inhibitors

Ehsan Ullah Mughal [a,*], Amina Sadiq [b], Shahzad Murtaza [a], Hummera Rafique [a], Muhammad Naveed Zafar [c], Tauqeer Riaz [a], Bilal Ahmad Khan [d], Abdul Hameed [e], Khalid Mohammed Khan [e]

[a] Department of Chemistry, University of Gujrat, Gujrat 50700, Pakistan
[b] Department of Chemistry, Govt. College Women University, Sialkot 51300, Pakistan
[c] Department of Chemistry, Quaid-i-Azam University, Islamabad 45320, Pakistan
[d] Department of Chemistry, University of Azad Jammu and Kashmir, Muzaffarabad, Pakistan
[e] H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan

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ABSTRACT

The present study describes efficient and facile syntheses of varyingly substituted 3-thioaurones from the corresponding 3-oxoaurones using Lawesson's reagent and phosphorous pentasulfide. In comparison, the latter methodology was proved more convenient, giving higher yields and required short and simple methodology. The structures of synthetic compounds were unambiguously elucidated by IR, MS and NMR spectroscopy. All synthetic compounds were screened for their inhibitory potential against in vitro acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes. Molecular docking studies were also performed in order to examine their binding interactions with AChE and BChE human proteins. Both studies revealed that some of these compounds were found to be good inhibitors against AChE and BChE.

1. Introduction

2-Benzylidenebenzofuran-3(2*H*)-one (aurone), is an interesting subclass of flavonoids, found from natural or synthetic sources. They have received less attention in comparison to the structurally similar and widely investigated other flavonoids such as flavones and flavonols. Nevertheless, they are equally important, and thus display an important role in the pigmentation of some flowers, fruits and particularly are responsible for giving bright yellow color of flowers.[1] They also display strong and wide variety of biological activities. For instance, they have been demonstrated antifungal,[2] insect antifeedant,[3] inhibition of tyrosinase,[4] antioxidants activities etc.[5–8] Surprisingly, the literature search gives a rather narrow number of reports on the preparation of 2-benzylidenebenzofuran-3-ones, despite having interesting biological properties. A very accepted way to prepare aurones was developed by Varma et al. which is based on the aldol-like condensation of benzofuran-3(2*H*)-ones with benzaldehydes.[9] This, and some other more or less classical methods for their synthesis were reviewed by Boumendjel et al.[10] Since then, a number of similar or refined methods have been reported in literature[11,12] in order to synthesize bioactive aurone derivatives. However, the corresponding 3-thio analogues of aurones have been less studied due to limited number of methods available for their preparation, although these sulfurated aurones may offer new biological activities and may also be used as precursors for the synthesis of other physiological active organic compounds. Keeping in view the above-mentioned biological importance of aurones and their derivatives, our continued interest in the chemistry of thio-analogues of flavonoids[13] and the less literature available on aurones encouraged us to introduce expedient and general methods for the synthesis of 3-thioaurones from 3-oxoaurones. To the best of our knowledge, there is no example available in literature for the preparation of 3-thioaurones. We, therefore, design a methodology to transform variously substituted aurones into the 3-thioaurones by using Lawesson's reagent (LR) as well as phosphorous pentasulfide (P_2S_5)/sodium hydrogen carbonate ($NaHCO_3$). Both of these reagents could efficiently convert substituted as well as unsubstituted aurones into their corresponding 3-thioanalogues. However, the latter

* Corresponding author. Tel.: +92 333 8464959.
E-mail address: ehsan.ullah@uog.edu.pk (E.U. Mughal).

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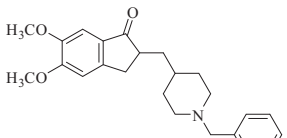
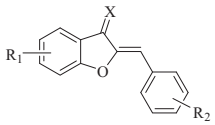
process was found to be more convenient, relatively cheaper and offers higher yields.

Disease-associated enzyme's inhibition by a small molecular drug has been emerged a promising strategy to cure human diseases. Alzheimer's disease (AD) is the most common type of dementia which affects millions of people worldwide. AD has been accounted in the loop of top ten death causing diseases, which stimulate medicinal chemists to develop new lead molecule as drug candidates. The various complications associated with AD are slow memory deterioration, language skills impairment and many other cognitive dysfunctions. Cholinesterases (ChEs) are considered to be a major focal point of pharmaceutical research for the treatment of some of the symptoms of Alzheimer's disease (AD). Two ChEs found in humans, which are known as acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), are consider as principal factors for AD complications. Both of these enzymes are present in cholinergic synapses in the central nervous system (CNS), in the parasympathic synapses in the periphery, and in the neuromuscular junction. However, AChE is selective for ACh hydrolysis and BChE hydrolyses acetylcholine and other choline esters as a non-specific cholinesterase.[14] These enzymes have unique binding pockets which are well-suited for interactions with small drug molecules. In consequence, the nature of the chemistry of enzyme catalysis makes ChEs amenable to inhibition by small molecular weight, drug-like molecules. Therefore, it is an urgent need to investigate and develop such drug structures which could efficiently inhibit ChEs in order to cure AD. Recently, aurone and structurally resembled compound such as benzofurans, indanones and coumarins were screened for their inhibition potential against AChE and BChE enzymes.[15–19]

The present research work is aimed at the syntheses of varyingly substituted 3-oxoaurones, 3-thioaurones and determination of their inhibitory potential against AChE and BChE enzymes. Molecular docking was also performed in order to study the binding affinities of the synthesized compounds for previously mentioned human proteins.



Donepezil



3-Oxo/Thio-aurones
X = O or S **(1-10)**

2. Materials and methods

2.1. General

Melting points were measured on an Electrothermal melting point apparatus and are uncorrected. The IR spectra were recorded on a Bio-red spectrophotometer using KBr discs. NMR spectra were measured on a Bruker DRX 500 instrument (^{1}H, 500 MHz, ^{13}C, 125.7 MHz). Mass spectra were recorded on a Fisons VG Autospec X double-focusing mass spectrometer. Accurate mass measurements were carried out with the Fisons VG sector-field instrument (EI) and a FT-ICR mass spectrometer. All chemicals were purchased from Alfa Aesar or Sigma Aldrich and used as delivered.

2.1.1. General method for synthesis of 3-thioaurones using P_2S_5

A solution of P_2S_5 (1.5 mmol) in anhydrous THF (5 mL) was added to a stirred solution of aurone (1.0 mmol) in THF (10 mL) followed by the addition of solid $NaHCO_3$ (6 equiv) to the same reaction mixture and stirring was continued for 1–2 h. After the completion of reaction (monitored by TLC), the reaction mixture was poured into water and the resultant solid was filtered, washed with water and crystallized from ethanol.

2.1.2. General method for synthesis of 3-thioaurones using Lawesson's reagent (LR):[13]

A mixture of aurone (1.0 mmol) and LR (1.5 mmol) was refluxed in anhydrous toluene (10 mL) under argon atmosphere. Initially, the reaction mixture was stirred at room temperature for 15 min and then 3 h at 90 °C. After the completion of reaction (TLC analysis), the solvent was removed under reduced pressure. The residue was then purified through silica gel column (*n*-hexane/dichloromethane; 7:3) to afford 3-thioaurones.

The spectroscopic data for all newly synthesized compounds is given below. However, the spectral data of 3-oxoaurones **2**, **4**[2] and **5**[20] have also been published in literature.

2.1.2.1. (*Z*)-2-Benzylidene-6-bromobenzofuran-3(2*H*)-one (1). This substance was obtained as yellow solid in 90% yield, mp 127–129 °C. IR (KBr): $\bar{\nu} = 1718$ (C=O), 1610 (C=C). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.95 (d, J = 8.0 Hz, 1H, ArH), 7.84 (d, J = 2.0 Hz, 1H, ArH), 7.68 (dd, J = 8.0, 2.0 Hz, 1H, ArH), 7.35–7.19 (m, 5H, ArH), 6.90 (s, 1H, benzylic). ^{13}C NMR (125 MHz, DMSO-d_6): δ 184.2, 166.1, 164.6, 162.4, 146.0, 137.0, 137.0, 133.7, 128.6, 125.0, 124.1, 122.0, 116.7, 113.1, 113.5; MS (ESI, +ve): m/z (%), 322 (100, $[M+Na]^+$), 324 (97); accurate mass (ESI, +ve) of $[M+Na]^+$: Calcd. for $C_{15}H_9BrO_2Na$ 322.9659; found 322.9640.

2.1.2.2. (*Z*)-2-(4-Isobutylbenzylidene)benzofuran-3(2*H*)-one (3). This compound was obtained as yellow sticky material in 83% yield. mp 121–123 °C IR (KBr): $\bar{\nu} = 1717$ (C=O), 1608 (C=C). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.98 (dd, J = 8.1, 2.5 Hz, 1H, ArH), 7.90–7.50 (m, 3H, ArH), 7.30 (d, J = 7.5 Hz, 2H, ArH), 7.08 (d, J = 7.5 Hz, 2H, ArH), 6.97 (s, 1H, C=C–H), 2.52 (d, J = 7.5 Hz, 2H, CH_2), 1.88 (m, 1H, CH), 0.90 (d, J = 7.4 Hz, 6H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 185.2, 168.2, 162.5, 150.5,139.2, 135.0, 130.7, 126.2, 125.3, 125.0, 123.0, 119.0, 117.0, 115.0, 113.6, 45.0, 30.0, 23.0; MS (EI, 70 eV): m/z (%), 278 (35, $[M]^{+\cdot}$), 235 (100), 221 (22), 178 (7), 121 (8), 117 (23),; accurate mass (EI-MS) of $[M]^{+\cdot}$: Calcd. for $C_{19}H_{18}O_2$ 278.1306; found 278.1309.

2.1.2.3. (*Z*)-2-Benzylidene-6-bromobenzofuran-3(2*H*)-thione (6). Pale yellow solid; mp 213–215 °C. IR (KBr): $\bar{\nu} = 1249$ (C=S), 1609 (C=C). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.93 (d, J = 8.0 Hz, 1H, ArH), 7.82 (d, J = 2.0 Hz, 1H, ArH), 7.67 (dd, J = 8.0, 2.5 Hz, 1H, ArH), 7.32–7.15 (m, 5H, ArH), 6.92 (s, 1H, benzylic). ^{13}C NMR (125 MHz, DMSO-d_6): δ 208.1, 166.0, 164.4, 162.4, 145.5, 137.0, 136.2, 133.5, 128.5, 123.0, 123.5, 121.5, 116.21, 113.0, 113.2; MS (EI, 70 eV): m/z (%), 315 (100, $[M]^{+\cdot}$), 317 (98, $[M+2]^+$), 268 (58), 251 (30), 203 (15), 167 (35), 149 (90); accurate mass (EI-MS) of $[M]^{+\cdot}$: Calcd. for $C_{15}H_9BrOS$ 317.2004; found 317.2008.

2.1.2.4. (*Z*)-2-(4-Methoxybenzylidene)benzofuran-3(2*H*)-thione (7). Dark yellow solid; mp 143–145 °C. IR (KBr): $\bar{\nu} = 1249$ (C=S), 1608 (C=C). ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.00 (d, J = 7.5 Hz, 2H, ArH), 7.81 (d, J = 7.5 Hz, 2H, ArH), 7.57 (m, 2H, ArH), 7.33 (m, 1H, ArH), 7.10 (m, 1H, ArH), 6.99 (s, 1H, C=C–H), 3.85 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 208.1, 167.2, 160.5, 149.5, 138.2, 136.0, 129.0, 125.2, 125.0, 124.9, 124.0, 118.0, 116.5, 114.0, 113.1, 56.0; MS (EI, 70 eV): m/z (%), 268 (100, $[M]^{+\cdot}$), 253 (21), 240 (7), 197 (15), 92 (6), 77 (10), 53 (4); accurate mass (EI-MS) of $[M]^{+\cdot}$: Calcd. for $C_{16}H_{12}O_2S$ 268.3303; found 268.3306.

2.1.2.5. (*Z*)-2-(4-Isobutylbenzylidene)benzofuran-3(2*H*)-thione (8). Red solid; mp 192–194 °C. IR (KBr): $\bar{\nu} = 1254$ (C=S), 1598 (C=C). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.92 (d, J = 8.0, 1H, ArH), 7.80 (m, 1H, ArH), 7.57 (m, 2H, ArH), 7.32 (d, J = 7.5, 2H, ArH), 7.20 (d, J = 7.5, 2H, ArH), 6.96 (s, 1H, C=C–H), 2.49 (d, J = 7.5, 2H, CH_2), 1.82 (m, 1H, CH), 0.86 (d, J = 7.5 Hz, 6H, CH_3); ^{13}C NMR (125 MHz, DMSO-d_6): δ 208.12, 167.2, 160.5, 149.5, 138.2, 136.0, 129.0, 125.2, 125.0, 124.9, 124.0, 118.0, 116.5, 114.0, 113.1, 44.5, 29.0, 23.0; MS (EI, 70 eV): *m*/*z* (%), 294 (65, $[M]^{+\cdot}$), 251 (100), 223 (14), 195 (2), 165 (6), 152 (4), 125 (3); accurate mass (EI-MS) of $[M]^{+\cdot}$: Calcd. for $C_{19}H_{18}OS$ 294.4106; found 294.4101.

2.1.2.6. (*Z*)-2-(Benzylidene)benzofuran-3(2*H*)-thione (9). Orange solid; mp 94–96 °C. IR (KBr): $\bar{\nu} = 1226$ (C=S), 1608 (C=C). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.92 (m, 2H, ArH), 7.80 (m, 2H, ArH), 7.57 (m, 2H, ArH), 7.32 (m, 1H, ArH), 7.20 (m, 2H, ArH), 6.96 (s, 1H, C=C–H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 208.1, 167.2, 160.5, 149.5, 138.2, 135.8, 128.7, 125.2, 125.0, 124.9, 124.5, 118.0, 116.5, 114.0, 113.1; MS (EI, 70 eV): *m*/*z* (%), 238 (45, $[M]^{+\cdot}$), 237 (100), 205 (9), 207 (8), 176 (9), 165 (11), 77 (8); accurate mass (EI-MS) of $[M]^{+\cdot}$: Calcd. for $C_{15}H_{10}OS$ 238.3043; found 238.3054.

2.1.2.7. (*Z*)-2-(4-Fluorobenzylidene)benzofuran-3(2*H*)-thione (10). Orange red solid; mp 76–78 °C. IR (KBr): $\bar{\nu} = 1222$ (C=S), 1606 (C=C). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.97 (d, J = 7.5 Hz, 2H, ArH), 7.85 (d, J = 7.5 Hz, 2H, ArH), 7.60 (m, 2H, ArH), 7.36 (m, 1H, ArH), 7.22 (m, 1H, ArH), 6.97 (s, 1H, CH); ^{13}C NMR (125 MHz, DMSO-d_6): δ 208.4, 167.2, 160.5, 150.0, 138.2, 136.0, 128.1, 125.0, 125.2, 125.1, 124.0, 118.3, 117.0, 114.0, 113.1; MS (EI, 70 eV): *m*/*z* (%), 256 (100, $[M]^{+\cdot}$), 228 (17), 199 (32), 170 (11), 123 (14), 95 (17), 76 (10); accurate mass (EI-MS) of $[M]^{+\cdot}$: Calcd. for $C_{15}H_9FOS$ 256.2948; found 256.2955.

2.2. Enzyme inhibition assay

Enzyme inhibition studies were based on the method adopted by Ryan[21] and Ellman[22] with slight modifications. 100 μL of each sample solution (0.001 M/1 mM in DMSO) was placed into a small test tube followed by the addition of 50 μL corresponding enzyme (i.e. AChE/BChE). Then reaction mixture was allowed to stand for 10 min so that enzyme and sample could mix well. After incubation, the buffer solution (500 μL) with concentration of 50 mM and pH 7.7 was added followed by the addition of 50 μL substrate (i.e. acetylthiocholine iodide (AChI) and butyrylthiocholine chloride (BChCl)) and DTNB [5,5′-dithiobis(2-nitrobenzoic acid)] (50 μL) in it. The resulting solution was incubated for 20 min at 37 °C. Finally, spectrophotometric absorbances were measured at 400 nm (for AChE) and 412 nm (for BChE) by using UV–Vis spectrophotometer and percentage inhibition was calculated by following formula:

$$\text{Inhibition}\ (\%) = (B - A)/B \times 100$$

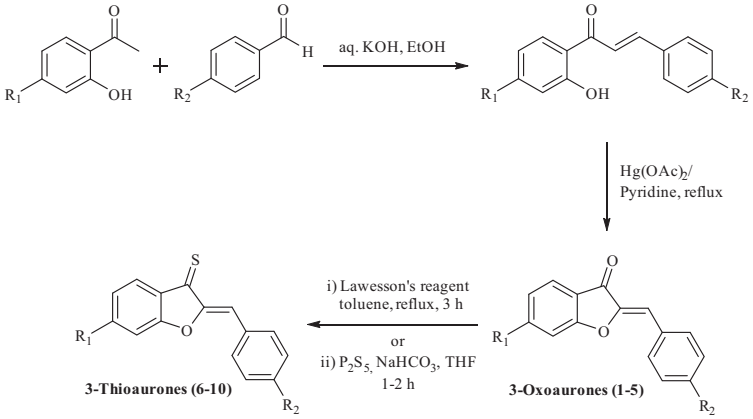
Here A = absorbance of the enzyme with test sample and B = absorbance of the enzyme without test sample. Each experiment was repeated in triplicate and mean values were calculated. Inhibition was also estimated by calculating the IC_{50} values. IC_{50} is the concentration at which a compound shows 50% inhibition of the enzyme. To calculate IC_{50} values, each selected sample (1 mM in DMSO) was assayed at five concentrations (20 μL, 40 μL, 60 μL, 80 μL, 100 μL). IC_{50} values were obtained from dose–effect curves by linear regression.

2.3. Molecular docking

Molecular docking analysis was performed in order to investigate the interaction of synthetic compounds with AChE and BChE. The protein was exported from Protein Data Bank. Molecular structures of the envisioned compounds were drawn by ACD/ChemSketch and were 3D optimized by using Chem 3D Pro 12.0 and was saved as SYBYL mol2 file format. Docking of Protein was done by using AutoDock Tool v1.5.6. In this docking process, hundred different possess were produced and selected one of the best pose after visualizing each pose by using Discovery Studio Visualizer v4.0.[23]

3. Results and discussion

It is reported that P_2S_5 could be efficiently used as thiation agent particularly for the conversion of flavones, chromones and



3-Oxoaurones	3-Thioaurones	Substituents R_1	Substituents R_2
1	**6**	Br	H
2	**7**	H	OMe
3	**8**	H	*i*Bu
4	**9**	H	H
5	**10**	H	F

Scheme 1. Synthesis of 3-oxoaurones and 3-thioaurones.

Table 1
A comparison of obtained yields by using P_2S_5 and LR

Aurone	3-Thioaurone	R_1	R_2	Yield (%) by P_2S_5	Yield (%) by LR
1	**6**	Br	H	73	70
2	**7**	H	OCH_3	88	79
3	**8**	H	^{i}Bu	92	80
4	**9**	H	H	90	79
5	**10**	H	F	78	72

related structures into their thio analogues.[24] Interestingly, when we applied this reagent in the presence of sodium bicarbonate in anhydrous tetrahydrofuran (THF) to the varyingly substituted and unsubstituted aurones, an exciting reaction took place to produce 3-thioaruones **6**–**10** from their oxo-analogues. Furthermore, sulfuration of aurones **1**–**5** was also achieved by treating with Lawesson's reagent in refluxing anhydrous toluene.[13] Both reagents worked efficiently in order to furnish 3-thioaurones **6**–**10** in moderate to good yields and have no influence on substitution pattern of aurones **1**–**5** (Scheme 1). However, the former methodology was found to be relatively cheap, high yielding and has simple workup. A comparison of yields by both the reagent is given in Table 1.

Aurones **1**–**5** were synthesized according to the literature procedure[1,12b] and then allowed to react with $P_2S_5/NaHCO_3$/THF and LR/toluene to give 3-thioaurones **6**–**10**. The structures of synthetic compounds were elucidated by IR, mass spectrometry and NMR spectroscopy. For example, in IR spectrum, the disappearance of the C=O signal and appearance of C=S new signal around 1200–1250 cm^{-1} unequivocally confirm the replacement of oxygen by a sulfur atom. This result was further supported by the C=S signal observed in their ^{13}C NMR spectra between 200–208 ppm. The molecular masses of the compounds were confirmed by Electron Ionization mass spectrometry (EIMS). Their mass spectra showed the presence of molecular ion peaks as base peaks and give characteristics fragmentation pattern of aurones. All spectral data (^{1}H and ^{13}C and EIMS spectra) are in good agreement with the skeleton of 3-oxoaurones **1** & **3** and 3-thioaurones **6**–**10**.

3.1. Enzyme inhibition assays

In vitro, the compounds **1**–**10** were screened for their enzyme inhibition potential against acetylcholinesterase and butyrylcholinesterase by using spectrophotometric method. The results of enzyme inhibition activities are presented in Table 2. Their IC_{50} values were also calculated and donepezil was used as a positive control.[21,22]

It is evident from the results that compounds **2**, **3** and **9** showed inhibition activities against AChE whereas, compounds whereas **3** and **10** against BChE. Their IC_{50} values are given in Table 2. It is noteworthy that the (*Z*)-2-(4-isobutylbenzylidene)benzofuran-3(2*H*)-one **3** was found to be the dual inhibitor of AChE (IC_{50} = 0.98 - μM) and BChE (IC_{50} = 1.02 μM) which indicated that this compound may be used as inhibitor for both enzymes at the same time and may cure over expression of two enzymes. It is isobutylbenzylidene derivative of aurone and fine tuning by chemical modification may results in single inhibitor of both enzymes. However, compound (*Z*)-2-(4-methoxybenzylidene)benzofuran-3(2*H*)-one **2** (IC_{50} = 1.26 μM) found to have considerable selective activity against AChE and may serve as lead compound for the development of powerful inhibitor for AChE. In general, 3-oxoaurones **1**–**5** are appeared to be more potent as compared to 3-thioaurones **6**–**10**. The replacement of oxygen with sulfur caused decreased in the activities such compounds **2**, and **3** when transformed **6** and **7** they completely lost the AChE activity. However, weak activities of compounds **9** and **10** against AChE and BChE, respectively clearly indicates that the sulfur analogs may not be ignored they may be useful, if there is an appropriate substituent installed on it. Therefore, we cannot rule out the sulfur containing analogs as AChE and BChE inhibitors. In case of sulfur containing compounds the sulfur atom has also influence to control the activity; however position and nature of the substituent appeared to be more influential in the inhibition activities. The difference in activities could be rationalized on the basis of enzymes nature and substitution and their position on the 3-oxoaurones or 3-thioaurones molecules. This could be attributed to enhanced binding interaction between non-polar molecular parts and hydrophobic pocket of the enzymes. These findings could further be supported by molecular docking analysis. In the light of our current study, we are planning to continue the synthesis of more 3-oxoaurone and-thioaurone molecules along with some chemical modifications on compounds **2**, **3**, **9**, and **10** to explore this class of compound as a potent class of AChE and BChE inhibitors.

3.2. Molecular docking

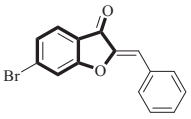
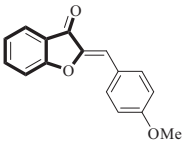
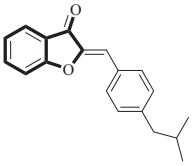
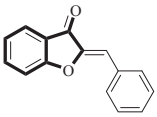
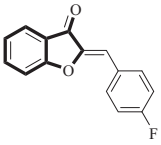
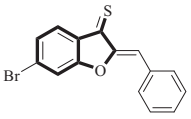
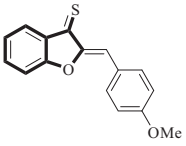
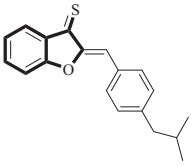
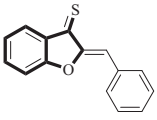
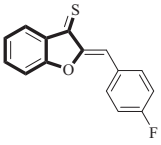
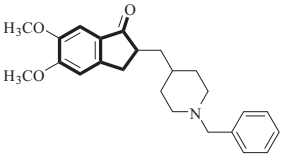
Molecular docking analyses of the series of compounds **1**–**10** were accomplished into the receptor site of the crystal structures of AChE and BChE in order to elucidate the probable mechanism by which the title compounds could induce enzyme inhibition activities and to figure out the ligand–protein interaction at molecular level for establishing structure–activity relationships. The lowest possible binding energies of the synthetic compounds **1**–**10** obtained after docking studies are shown in Table 3.

Inhibitors were analyzed on the bases of their affinity energy (Table 3) and type of interactions they made with protein residues. As more amino acids of active site are hydrophobic in nature so they show more non-ionic interactions. The visualization of the most potent compound **2** inside AChE enzyme revealed several important interactions. In the active pocket of AChE this compound forms a hydrogen bonding interaction with amino acid residues THR83, TYR72, GLN71 and ASN87. This also forms a π-anion interaction with amino acid ASP74. The catalytic amino acid TYR337 and TRP86 were also observed to form π–π stacking interactions with aryl ring of the envisioned compound. The putative binding mode of this can be found in Figure 1.

Similarly, the visualization of the other most potent compound **3** inside AChE enzyme exhibited enormous important interactions. In the active pocket of AChE the compound **3** forms hydrogen bonding interaction with amino acid residues like SER125 and TYR337. It also forms pi-anion interaction with amino acid ASP74. The catalytic amino acid GLY121 and GLY122 were also observed to form amide-pi staking interactions with phenyl ring of this compound. It has also been seen that the compound **2** makes pi-alkyl interactions with amino acids ALA204, TRP236, PHE295, PHE297 and HIS447. The putative binding mode of the compound **3** can be found in Figure 2.

In analogy to previous discussion, it is manifested from the Figure 3 that the highly potent compound **3** inside BChE enzyme exhibited several valuable interactions. For example, in the active pocket of BChE the compound **3** forms hydrogen bonding interaction with amino acid residues GLY116, GLY117, ALA199 and

Table 2
Structures and IC_{50} of the synthetic compounds **1**–**10** against AChE and BChE

Compound No.	Structure	AChE IC_{50} ± SEM[a] (μM)	BChE IC_{50} ± SEM[a] (μM)
1		NA[b]	NA[b]
2		1.26	NA[b]
3		0.98	1.02
4		NA[b]	NA[b]
5		NA[b]	NA[b]
6		NA[b]	NA[b]
7		NA[b]	NA[b]
8		NA[b]	NA[b]
9		6.39	NA[b]
10		NA[b]	5.27
Donepezil[St]		0.09[c]	0.13[c]

[St] Standard inhibitor for AChE and BChE.
[a] IC_{50} values (mean ± standard error of mean).
[b] NA: Not active.
[c] Standard IC_{50} values for AChE and BChE.

Table 3
Binding energies of the selective poses against human AChE and BChE

Compound	*h*AChE lowest binding energy ΔG in KJ mol^{-1}	*h*BChE lowest binding energy ΔG in KJ mol^{-1}
1	−8.72	−8.42
2	−8.75	−7.79
3	−8.40	−8.97
4	−8.31	−7.91
5	−8.24	−7.81
6	−9.14	−8.85
7	−8.73	−7.81
8	−10.20	−8.39
9	−8.79	−8.15
10	−8.53	−8.03
Standard	−10.82 (HUW)	−6.90 (THA)

SER198. This inhibitor also forms π–π interactions with amino acid TRP82. π–Alkyl interaction has also been observed with amino acid TRP82, TRP430, TRP440 and LEU286. π–π T-shaped interaction was observed with TRP82, TRP231, PHE398 and HIS438. The catalytic amino acid ALA328 and MET437 form interaction with alkyl group. With all these interactions this inhibitor shows best orientation with minimum energy of −8.97 kJ/mol.

4. Conclusion

In summary, we have developed a simple, versatile and rapid methodology to synthesize 3-thioaurones from 3-oxoaurones using $P_2S_5/NaHCO_3$ and Lawesson's reagent. Both reagents offer high functional groups tolerance. However, the former methodology avoids tedious workup, and thus provides a convenient and an efficient access to 3-thio derivatives of substituted and unsubstituted 3-oxoaurones. The synthetic compounds bearing heterocyclic moiety were screened for their inhibitory potential against AChE and BChE. The results revealed that the compounds **2**, **3**, **9**, and **10** show weak to good inhibitory potential, in particular, the aurone **3** was identified as dual inhibitor for both enzymes. Herein, the molecular docking was used to get insights into the interaction of synthesized inhibitory compounds with AChE and BChE

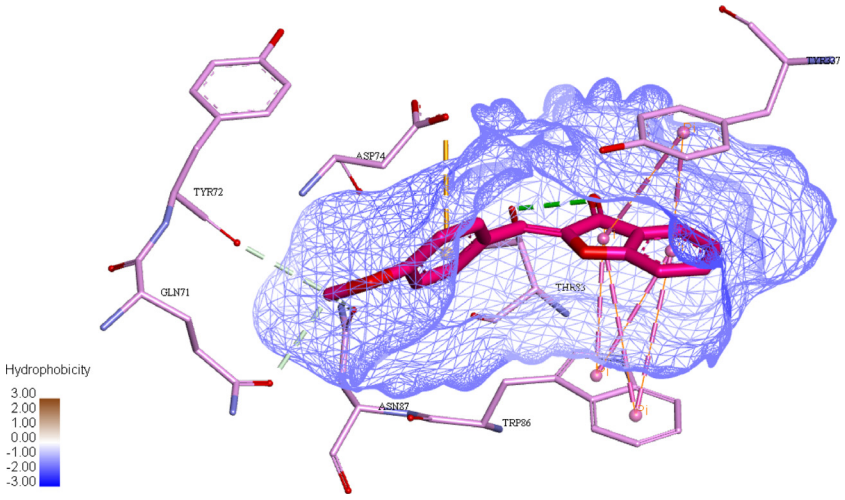


Figure 1. Putative binding of compound **2** inside AChE active pocket, hydrogen bonding interactions are shown as green dashed lines and π–π staking interaction are shown as solid yellow lines while π-anion interaction are shown as orange dashed lines.

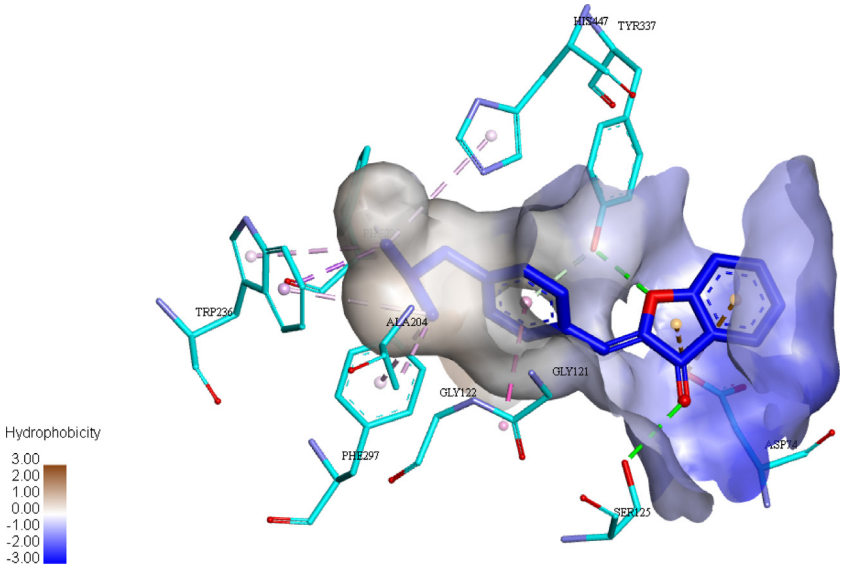


Figure 2. Most probable binding mode of compound **3** inside AChE enzyme.

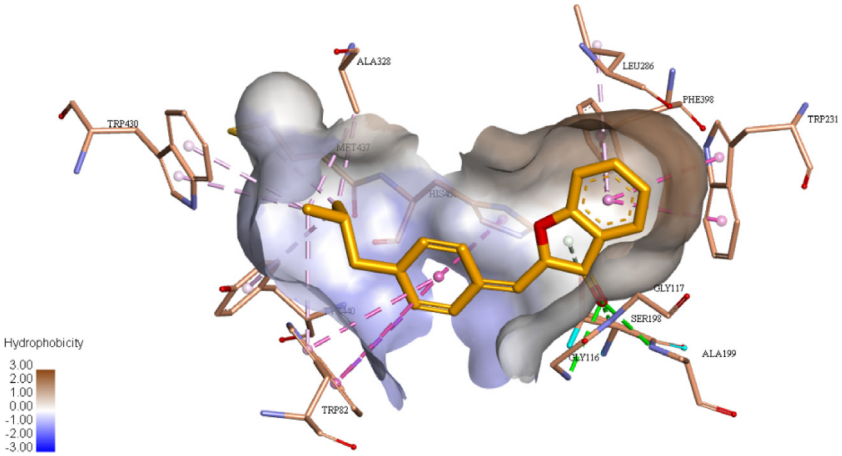


Figure 3. Most probable binding mode of compound **3** inside BChE enzyme.

enzymes. The studies performed have important implications for the design of AChE and BChE inhibitors. It could be worth exploring to make further structural modifications of existing scaffolds in order to enhance the inhibitory potential of the presented series of 3-oxoaurone and-thioaurone derivatives.

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