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Inhibition effects of isoproterenol, chlorpromazine, carbamazepine, tamoxifen drugs on glutathione S-transferase, cholinesterases enzymes and molecular docking studies

Fikret Türkan[a], Mehmet Harbi Calimli[b], Gülşah Saydan Kanberoğlu[c] and Muhammet Karaman[d]

[a]Health Services Vocational School, Igdir University, Igdir, Turkey; [b]Tuzluca Vocational School, Igdir University, Igdir, Turkey; [c]Department of Chemistry, Science Faculty, Van Yuzuncu Yıl University, Van, Turkey; [d]Department of Molecular Biology and Genetics, Faculty of Arts and Science, Kilis 7 Aralik University, Kilis, Turkey

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ABSTRACT

Nowadays, inhibition of acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and glutathione S-transferases (GSTs) have been a very crucial issue for pharmacological treatments of several disasters. Herein, we investigated inhibition effects of Tamoxifen (TAM), Isoprenaline (ISO), Chlorpromazines (CPZ) and Carbamazepine (CBZ) on GST, AChE, BChE and then molecular structures and active sides of the tested drugs by molecular docking process. The enzyme activity results showed that nearly the whole tested drugs inhibited GST, BChE, AChE efficiently. Chlorpromazine was found to be the best inhibitor for the GST enzyme and the Ki value of this drug was found to be 42.83 ± 8.52 nM. Besides, Isoproterenol drug with the Ki value of 51.80 ± 9.44 nM was found to be the most effective inhibitor on the AChE enzyme. Molecular docking studies showed that the receptor-binding sites of GST, AChE, and BChE were found to 1.069, 1.090, and 1.15 of Sitecore and 0.992, 1.113, and 1.217 of Dscore, respectively. The method was validated by doing validation studies and these validations revealed that re-docked ligands located a very closed position with co-crystallized ligand into the active site for all receptors. Calculation studies for determining the possible enzyme inhibition mechanism with the used drugs revealed that amino and aromatic ring in the structure of the drugs used are effective in inhibition reactions.

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Enzyme inhibition; molecular docking; isoproterenol; chlorpromazine; carbamazepine; tamoxifen

Introduction

Cholinesterases are important enzymes that are present in plasma, body fluids, non- cholinergic tissues, and cholinergic tissues(Köse & Gulcin, 2017). These enzymes are categorized into two groups that are acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) (Kuzu et al., 2019) . AChE is present as cholinergic synapses in the brain and neuromuscular junctions and serves as a preventer to terminate synaptic transmission, and additionally as a hydrolase, it hydrolysis the acetylcholine (as a neurotransmitter) (Akkaya et al., 2019; Kuzu et al., 2019). AChE functions to decrease the rate of acetylcholine (ACh) degradation rate that enhances the concentration of ACh in the brain. That event prevents the passing of cholinergic neurons because of the loss of acetylcholine, therefore, Alzheimer's occurs due to the decreasing functions of the cholinergic neurons (Taslimi et al., 2018). BChE is another important cholinesterase enzyme and actives more in some tissue including the heart, liver, lungs, and intestine compared to AChE (Brown et al., 2004). This enzyme, together with the ACHE enzyme, is an enzyme effective in the treatment of Alzheimer's, known as a type of dementia, which is one of the most common diseases of our time. Hitherto, several studies (Ellman et al., 1961) have been carried out about AChE and BChE enzymes and performed based on Ellman method (1961). Acetylcholinesterase iodate (AChI) and butyrylcholinesterase (BChE) are used as substrates in the Ellman method (1961) (Büyükokuroğ lu et al., 2001). In enzyme inhibition studies, the activities of AChE and BChE enzymes are measured using 2-nitro-benzoic acid. These compounds are used to determine the amount of colored material in the adsorption process (412 nm) (Bozdag et al., 2015). Glutathione S-transferases (GSTs) are very important enzymes for live life and toxicologically are key enzymes that detoxify (co-ordinate with glutathione) oxidative stress, environmental toxins, and carcinogens products (Arakawa, 2013). Many applications including nanotechnology, drug discovery and biotechnology are related to GSTs and these applications affect several cellular processes. GSTs present in protein constructions that are either homo or heterodimeric, these enzymes enable catalytic binding with various toxics compounds and glutathione that form a complex having a high dissolution rate in the aquatic medium (Dasari et al., 2018). GSTs also exhibit very important roles in cellular protection against toxic substances, radicals (Türkan et al., 2018). GST enzyme is an enzyme studied in inhibition studies. In particular, it prevents the interaction of anticancer agents with a tissue because it is over-expressed in cancerous tissues. For this reason, inhibition of this enzyme is extremely important (Türkan et al.,

CONTACT Mehmet Harbi Çalimli mharbi.calimli@igdir.edu.tr Health Services Vocational School, Igdır University, Iğdır 76000, Turkey
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2019). Likewise, inhibitors of AChE and BChE enzymes can be used in the treatment of Alzheimer's disease. Since this disease is very common in later ages, it is important to discover inhibition effects of these enzymes (Balcı et al., 2019). Antibiotic agents are substances that can be effective in bacterial death or development. Most antibiotics that can be found in nature are obtained by modifying the compounds by chemical means(Sisecioglu et al., 2011). Tamoxifen (TAM), acting as a non-steroidal modulator estrogen receptor, has been used in breast cancer treatments as a positive estrogen receptor for the last three decades. Thanks to acting as a positive estrogen receptor, TAM reduces breast cancer growth (Jordan, 2009), so it has been effectively used in breast cancer treatments. However, some safety concerns have been raised due to its side effects such as enhancing TAM resistance in 2-5 years treatments, 3 – fold increasing endometrial cancer, liver induction formation, etc (Gallicchio et al., 2004), (Mishra et al., 2011). Isoprenaline (ISO) or isoproterenol is a catecholamine drug and β-adrenergic agonists, which has been widely in treatments of diseases such as bronchial asthma, cardiac arrest, status asthmatic, allergic emergencies, glaucoma, ventricular bradycardia (Sakr et al., 2019), (Khezri et al., 2018). ISO activates beta-adrenoceptors and in this way, the reduction of peripheral vascular resistance and the increase of cardiac output is achieved that leading the relaxation of bronchial smooth muscles(Ali et al., 2019). Chlorpromazines (CPZ) is a phenothiazine and acts as a dopamine antagonist. CPZ has been found to be an inhibitor for possible proteins that are effective in the formation of many diseases like cancer, hereditary autism, schizophrenia, and prion. It has been detected as a possible inhibitor for S100B protein which has caused so many cancers (Wilder et al., 2010). Chlorpromazine, as a phenothiazine derivative (aliphatic), acts as a competitive for dopamine antagonists (Loch et al., 2018).

Carbamazepine (CBZ) is a drug that provides the balance of antiepileptic, anticonvulsant and effective agents used in the treatment of bipolar and epilepsy disorder. At the same time, carbamazepine can be administered in people with discomforts such as attention deficit, schizophrenia, hyperactivity (Daneshvar et al., 2016). (CBZ) is slowly absorbed when taken orally and reaches peak plasma concentration over a 4-8 h period. Hence, carbamazepine is seen in high concentrations in liver, kidney and brain tissues(Nkoom et al., 2019). To the best of our knowledge in this study, so far, no studies related to the inhibition of Tamoxifen (TAM), Isoprenaline (ISO), Chlorpromazine (CPZ) and Carbamazepine (CBZ) drugs on GST, BChE, and AChE enzymes have been conducted. Hereby, we investigated the inhibition effects of TAM, ISO, CPZ, and CBZ on GST, BChE, AChE and molecular docking that used to determine active sides in the composition of the drugs. Inhibition experimental studies revealed that TAM, ISO, CPZ, and CBZ are very good inhibitors on GST, BChE, AChE enzymes. By conducting molecular docking studies, we determined that amino and aromatic ring moieties in the investigated drug are effective agents on inhibitions of GST, BChE, and AChE. With these docking studies, we highlighted the inhibition mechanism of the drugs of isoproterenol, chlorpromazine, carbamazepine, and tamoxifen on some important enzymes such as GST, BChE, AChE.

Material and methods

Chemicals

AChE, BChE, GST, and solvents and other chemicals were purchased from Sigma and were of analytical degree used not further any purification. Distilled water was used in the cleaning of all glasswares TAM, ISO, CPZ, and CBZ were purchased from Signa Aldrich. All of these drugs were of analytical grade and used not further purifications.

GST activity

50% bacterial strains and 1-chloro-2, 4-dinitrobenzene (CDNB) compound as a substrate were used in the experiments done to determine GST activity. The results showed that GST has high activity in bacteria compared to fungi, algae, and protozoa. A phosphate buffer solution (pH 6.5), GST (20 mM) solution and CDNB (25 mM) solution were prepared to perform the GST activity. Each test was taken at 340 nm for 3 min with a spectrophotometer previous studies (Habig et al., 1974; Türkan et al., 2019).

BChE and AChE activity

Activity measurements of AChE and BChE enzymes were performed according to the partially modified Ellman method (Ellman et al., 1961) and previous studies (Taslimi et al., 2018; Türkan, Huyut, Taslimi et al., 2019). Butyrylthiocholine (BChI) iodide and acetylthiocholine iodide (AChI) were used as substrates in the BChE, AChE inhibitions reactions. The determination of BChE and AChE enzyme activities were performed using 2-nitro-benzoic (5,5-Dithiobis acid) at in vitro conditions. In order to detect the BChE and AChE enzyme activity, three solutions of 50 mL (5.32×10^{-3} M) BChE-AChE, 100 mL tris/HCl buffer solution containing (1 M, pH 8.0) and 10 mL drug solution were stirred and waited for 10 min at room temperature. 50 mL of 2-nitro-benzoic (5,5-Dithiobis acid) (0.5 mM) was added to the resulting mixture and then the hydrolysis reaction was initiated. The hydrolysis of substrates of BChI and AChI was obtained by using the spectrophotometric method. The yellow color change showed the reaction of the drugs with BChE and AChE enzymes as well as the formation of 2-nitro-benzoic (5,5-Dithiobis acid). The hydrolysis of BChI and AChI are determined at 412 nm wavelength by UV –Vis spectrophotometer (Türkan, Huyut, Taslimi et al., 2019).

Inhibition studies of BChE and AChE

In order to conduct the BChE and AChE inhibition studies, four 1 $mg.mL^{-1}$ stock solutions of each drug were prepared for further use. The drug solution to be tested was transferred into the reaction vessel to determine the inhibition effect on the enzymes. A control run is conducted in the

presence of the drug to be controlled its inhibition effects on the enzymes(Taslimi et al., 2018; Büyükokuroğlu et al., 2001). Ki constant values were determined using the BChI and AchI substrates solutions prepared with different concentrations. Kinetic parameters and V_{max} values of inhibition studies of BChE and AchE enzymes using the drugs were determined using Lineweaver–Burk plots (Taslimi et al., 2018). IC_{50} values of the inhibitions of BChE and AChE enzymes with the drugs were determined using activity percentages values versus the drugs used concentration graphics. Similar results have been detected in previous studies (Gocer et al., 2015; Gul et al., 2016; Nar et al., 2013).

Inhibition studies of glutathione S-transferase (GST)

The used drugs were tested to determine their inhibition effects on GST. For this purpose, a solution containing 200 mL sodium phosphate buffer solution (pH 7.2, 50 mM) and 20 ml of 2,4-dinitrochlorobenzene (%95, 25 mM) were prepared at room conditions. The inhibitions studies of the drugs on GST activity were performed as described elsewhere (Aslan et al., 2019). The GST enzyme solution was used as a control for the replacement of the potassium buffer solution. The graph is drawn with 1/V values against 1/S, Lineweaver-Burk and rate constants were used to calculate the kinetic studies of the inhibitor drugs on GST.

Ligand and receptor preparation for molecular docking studies

Ligands and crystal structures of receptors were prepared according to the method proposed in our previous study (Turkan et al., 2019). 2 D structures of drugs were downloaded from PubChem (Abo-Ashour et al., 2018). Correct molecular geometries and protonation state at pH 7.0 ± 2.0 of the 3 D structures of drugs were produced. The x-ray crystal structures of GST, AChE, and BChE receptors which have high resolution and best percentile rank were assigned and the crystal structures of receptors (PDB ID: 3DK9, 4M0E, and 5NN0, respectively) were downloaded from RCSB Protein Data Bank. Briefly, bond order and charges were assigned and then all missing hydrogen atoms were added to protein structure. Missing side chains were filled, amino acids were ionized by setting physiological pH, water molecules that were formed less than 3 contacts with the protein or ligand were removed. Lastly, the energy was minimized.

The top-ranked potential binding site of the receptors was predicted using the SiteMap module. Sitecore and Dscore of the predicted binding site were also calculated with the same module according to the method described by Abo-Ashour et al. (2018); Turkan et al., 2019). Docking validation and induced fit docking (IFD) studies were performed according to the method proposed in previous studies (Fattah et al., 2018). Briefly, the centroid of the residues was generated around the selected residues in the catalytic site. After that, side chains were automatically trimmed based on B-factor; the closest residues to the ligand were refined within 3.4 Å of ligand pose. Then, molecular docking studies were performed. In the docking validation studies, active site ligand of each receptor was re-docked into the active site of the receptor. Docking methodology was validated by comparing the position of re-docked ligand and co-crystallized ligand into the active site of the receptor.

Result and discussion

Inhibition effects of drugs examinated on enzimes of AChE, BChE, and GST

AChE and BChE enzymes abundantly present the brain, erythrocyte membrane, heart, intestine and kidney and effect highly life events (Bozdag et al., 2015). GSTs are toxicologically key enzymes that detoxify (co-ordinate with glutathione) oxidative stress, environmental toxins, and carcinogens products(Arakawa, 2013). The drugs investigated in this paper have some vital life events effect on living life as summarized below. TAM acts as a non-steroidal modulator estrogen receptor and used in treatments of breast cancer as a positive estrogen receptor for the last three decades. Isoproterenol activates beta-adrenoceptors and in this way, the reduction of peripheral vascular resistance and the increase of cardiac output is achieved that leading the relaxation of bronchial smooth muscles. CPZ has been detected as a possible inhibitor for S100B protein which has caused so many cancers. CPZ provides the balance of antiepileptic, anticonvulsant and effective agents used in the treatment of bipolar and epilepsy disorder. Inhibition effects of these considerable important drugs on the enzymes of AChE, BChE and GST were investigated and Ki and IC_{50} values obtained from the experiments of the inhibition effects of the drugs used on the enzymes of GST, BChE and AChE are given in Table 1.

Biological activity

To determine the biological activity of the drugs, we studied by taking nanomolar levels of drugs in the enzyme inhibition experiments. The obtained IC_{50} and Ki values for each drug with AChE, BChE, and GST enzymes are given in Table 1. As seen in Table 1, the tested drugs exhibited very efficient inhibition. Lineweaver-Burk plots given in Figure 1 were used to calculate K_i values and revealed inhibition types for inhibitors used. The graphs given in Figure 2 are can be used to compare inhibitions activies of tested drugs in the study. Chlorpromazine was found to be the best inhibition drug for the GST enzyme and its Ki value was calculated to be 42.83 ± 8.52 nM. In addition, Isoproterenol was found to be the best inhibitory drug for the AChE enzyme and the Ki value was calculated 51.80 ± 9.44 nM approximately. And finally, carbamazepine was found to be the best inhibitor of the enzyme BChE and the Ki value is 8.63 ± 0.98 respectively.

Molecular docking studies

We firstly predicted the receptor's top-ranked potential binding site and calculated Sitecore and Dscore understanding whether the binding sites were catalytic active site and

Table 1. Glutathione S-transferase, acetylcholinesterase (AChE) and butyrylcholinesterase enzyme inhibition values of some drugs.

	IC_{50} (nM)						K_i (nM)		
Compounds	GST	r^2	AChE	r^2	BChE	r^2	GST	AChE	BChE
Tamoxifen	93.63	0.934	220.67	0.9712	28.04	0.9622	63.94 ± 14.84	193.63 ± 28.86	14.53 ± 1.33
Chlorpromazine	32.98	0.981	43.91	0.9891	43.98	0.9701	42.83 ± 8.52	53.34 ± 5.64	31.82 ± 4.36
Carbamazepine	54.60	0.9612	63.16	0.9544	21.82	0.9742	58.03 ± 8.43	59.03 ± 11.08	8.63 ± 0.98
Isoproterenol	171.33	0.9767	311.13	0.9802	287.39	–	106.57 ± 16.28	51.80 ± 9.44	27.24 ± 4.98
Tacrine*	–	–	168.46	0.9482	108.24	0.9812	–	136.45 ± 21.16	45.31 ± 11.02

*Tacrine (TAC) was used as a positive control for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes.

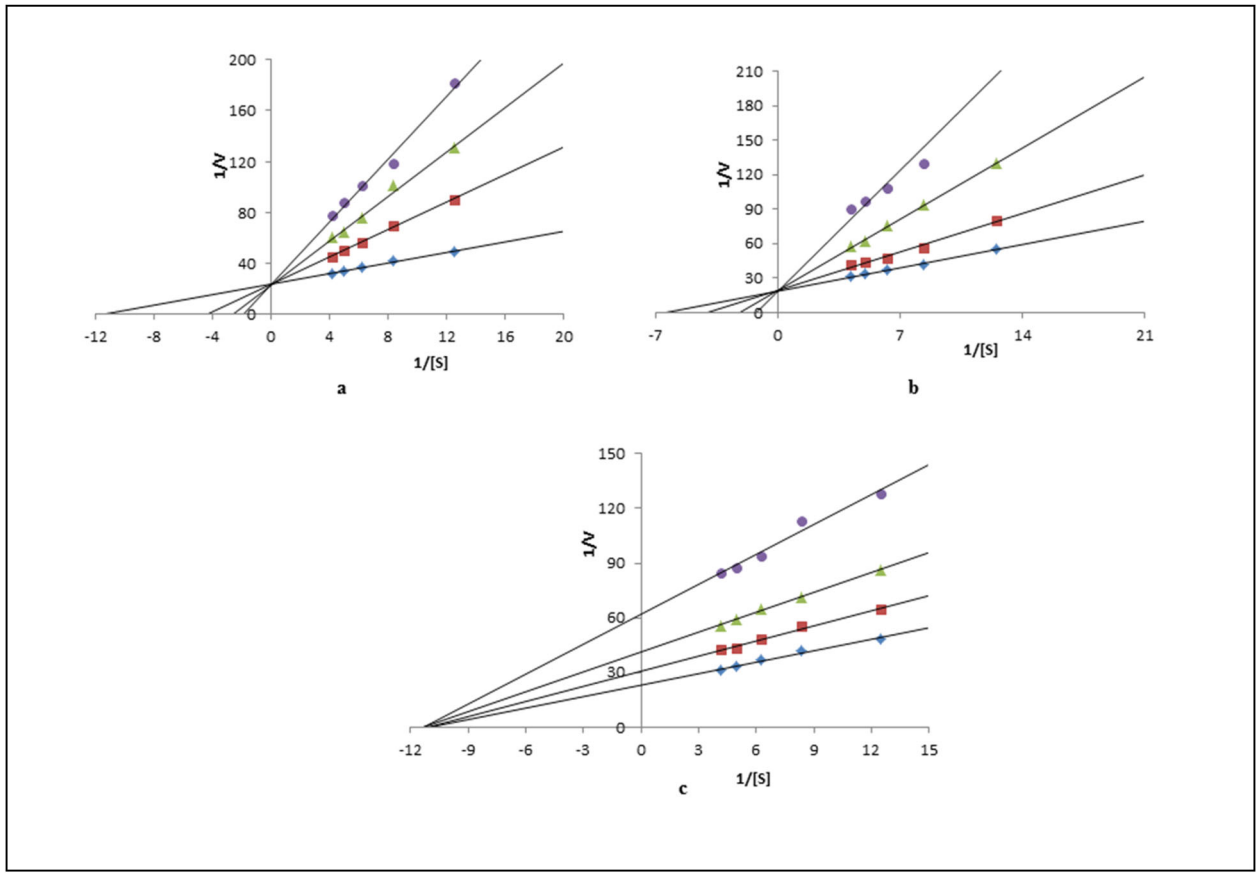


Figure 1. Chlorpromazine the graphs of drugs which exhibited the best inhibitions effects for enzymes studied, (a) for GST, (b) Isoproterenol for AChE, (c) Carbamazepine for BChE.

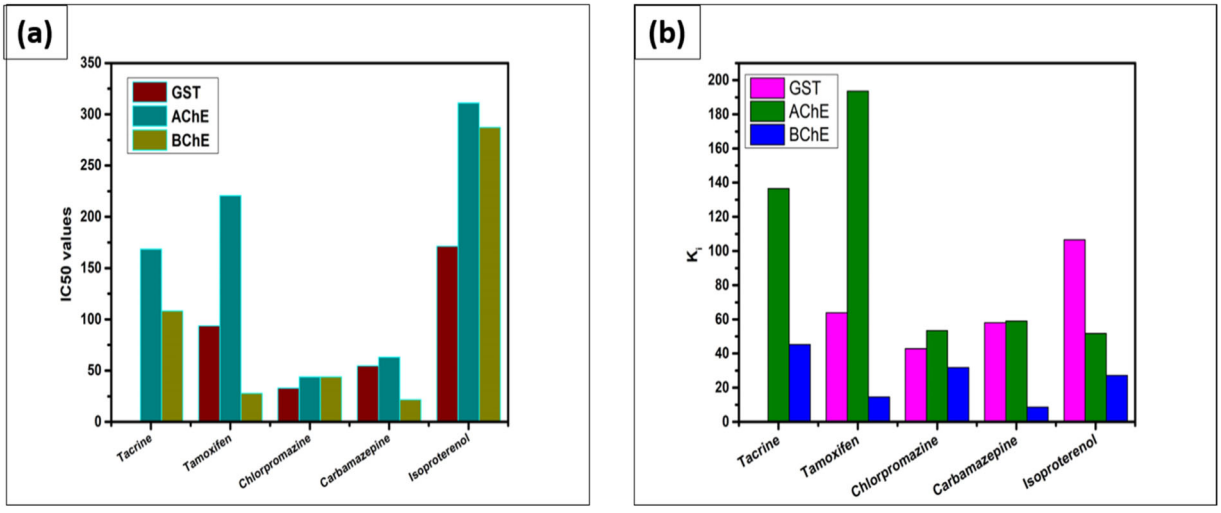


Figure 2. (a) The comparision of IC_{50} values obtained for different drugs on GST, AChE and BChE. (b) The comparision of Ki values obtained for different drugs on GST, AChE and BChE.

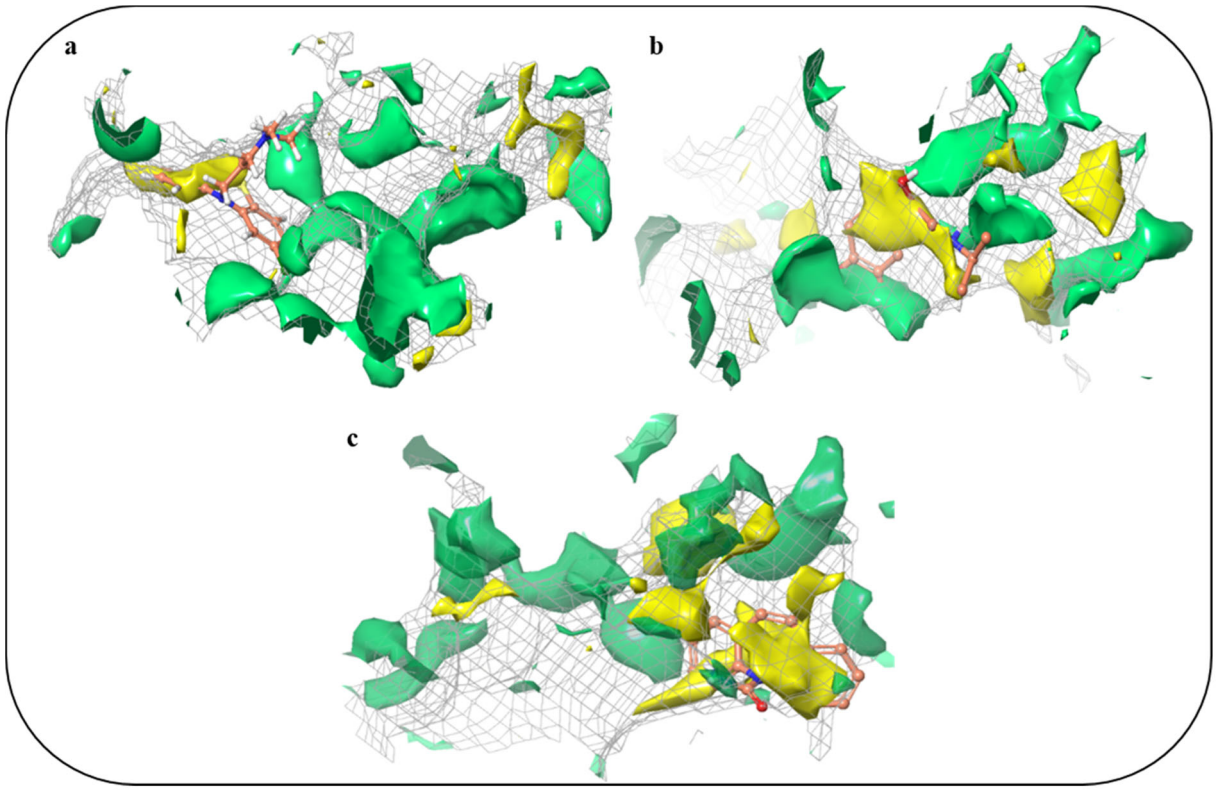


Figure 3. Interaction of catalytic active site surface and ligands. (a) Chlorpromazine-hGST, (b) Isoproterenol-AChE, and (c) Carbamazepine-BChE. Catalytic active site was represented as grey mesh, hydrophilic site was represented as green surface, and hydrophobic site is represented as yellow surface.

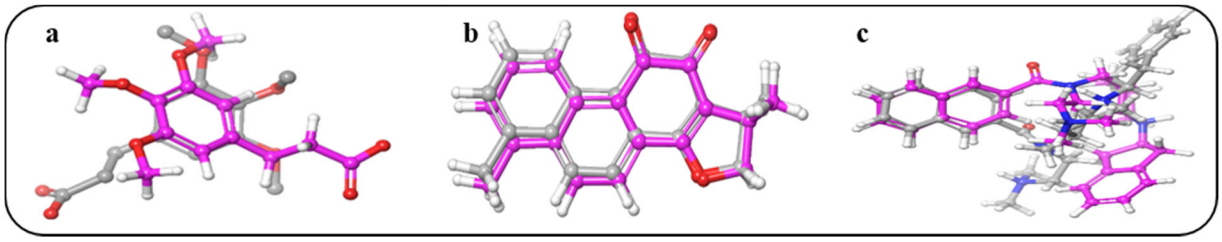


Figure 4. Docking validation. The best-posed co-crystallized ligands are represented in grey color ball and stick modeling and the best-posed docked ligands are represented in purple color ball and stick modeling for (a) 3LF, (b)1YL, and (c) 92H ligands.

Table 2. Binding affinities (kcal/mol) of the drugs on the receptors.

Drugs	GST	AChE	BChE
Chlorpromazine	−6.137	–	–
Isoproterenol	–	−9.793	–
Carbamazepine	–	–	−8.121

druggable sites. In accordance with the calculations, the GST, AChE, and BChE receptor's binding sites have 1.069, 1.090, and 1.15 of Sitecore and 0.992, 1.113, and 1.217 of Dscore, respectively. The binding sites were accepted as a catalytic active site due to their SiteScores. Since the catalytic active site of the receptors has a value of more than 1, the catalytic active sites exhibited druggable site properties. We also used to SiteMap results expressing how ligands were surrounded by active sites including hydrophilic, and hydrophobic in Figure 3. In previous studies showed that ligands exhibit a high binding affinity to enzymes. The results of molecular docking conducted herein are compatible with the findings of the literature about docking studies (Bal et al., 2020; Taslimi et al., 2019)

We validated the docking methodology with docking validation studies and expressed validation results in Figure 4. These results showed that re-docked ligands located a very closed position with co-crystallized ligand into the active site for all receptors. Drugs that exhibited the best inhibitory effect on the receptors were docked into the catalytic active site of the receptors with validated docking methodology. Then, the docking results were analyzed on the basis of binding energies, binding pose, and interactions of the ligand-receptor complex. We presented the calculated binding energies of the ligand-receptor complexes in Table 2. The binding energies have shown that most active drugs exhibited potent binding affinity against the receptors.

We consider that pose with most negative binding energy was as best-pose of the drugs complexed with the receptors. 2 D interaction diagram and detailed 3 D binding mode of best-posed drugs were illustrated in Figure 5 and interactions between the best-posed drug and catalytic active site residues were analyzed.

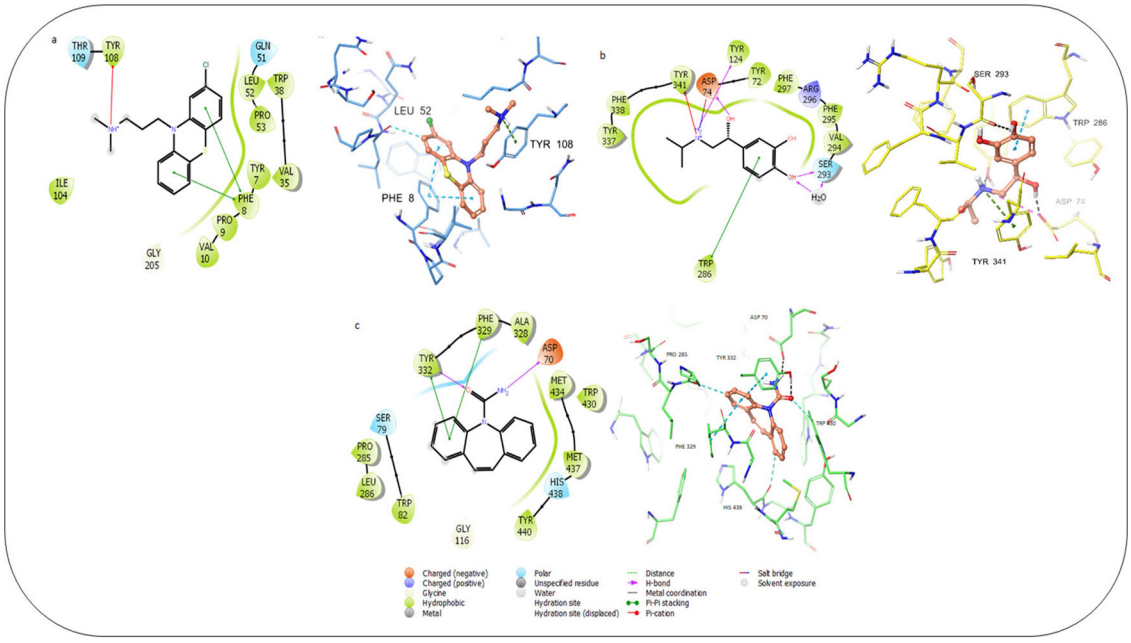


Figure 5. 2 D ligand-receptor interaction profile and detailed binding mode of best-posed ligands into the receptors. (a) Chlorpromazine-hGST, (b) Isoproterenol-AChE, and (c) Carbamazepine-BChE. All drugs were represented faded red-orange ball and stick modeling. The residues of GST, AChE, and BChE receptors are represented in thick tube modeling with blue, yellow and green color, respectively.

Phenothiazine aromatic rings of chlorpromazine formed π-π interaction with Phe8 residues and dimethylamine moiety formed π-cation interaction with Tyr108 residues of the hGST receptor. When the detailed binding mode is examined, it formed aromatic hydrogen bound with Leu52 residue (Figure 5a). Isopropyl amino moiety of isoproterenol formed a salt bridge, hydrogen bond, and π-cation interaction with Asp74, Tyr124, and Tyr341 residues, respectively. The aromatic ring interacted through π-π interaction with Trp286 residues. 1-hydroxyl and aromatic ring hydroxyl formed a hydrogen bond with Asp 74 and Ser293 residues, respectively (Figure 5b). A carbon oxide moiety of carbamazepine formed a hydrogen bond with Asp 70 and Tyr 332 and benzo moiety of carbamazepine π-π interactions with Phe 329 and Tyr 332 residues of BChE receptors. When it was examined detailed binding mode, the same moieties interacted through π-cation with Pro285, Trp430, and His438 residues (Figure 5c).

When residues surrounding drugs were examined in the catalytic active site, chlorpromazine was surrounded with hydrophobic active site residues of the GST receptor (Figure 3a). Isoproterenol was also surrounded by hydrophobic active site residues, but aromatic ring hydroxyl of isoproterenol closely interacted with hydrophilic residues of AChE receptor (Figure 3b). Benzo moiety of carbamazepine was surrounded by hydrophobic active site residues of the BChE receptor. Contrary to this, hydrophilic active site residues of the BChE receptor surrounded the carbon oxide moiety of carbamazepine (Figure 3c).

Conclusions

Summary, the tested TAM, ISO, CPZ, and CBZ drugs were detected to have well inhibition effects on GST, BChE, AChE enzymes with a nanomolar level of the drugs. All the used drugs inhibited efficiently the tested enzymes. CPZ inhibited GST with 42.83 ± 8.52 Ki nM value. ISO inhibited AChE with 51.80 ± 9.44 mM. CBZ inhibited very efficiently BChE with 8.63 ± 0.98 nM Ki value. The inhibition experimental results showed that these drugs are very promising inhibitors against the enzymes. Additionally, our molecular docking studies' results show that an amino and aromatic ring moiety of drugs is an important inhibition of all three enzymes. Our results have provided more insight inhibition mechanism of the drugs on the receptors.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Mehmet Harbi Calimli http://orcid.org/0000-0001-9756-191X

References

Abo-Ashour, M. F., Eldehna, W. M., Nocentini, A., Ibrahim, H. S., Bua, S., Abou-Seri, S. M., & Supuran, C. T. (2018). Novel hydrazido benzenesulfonamides-isatin conjugates: Synthesis, carbonic anhydrase inhibitory activity and molecular modeling studies. *European Journal of Medicinal Chemistry*, *157*, 28–36. https://doi.org/10.1016/j.ejmech.2018.07.054

Akkaya, R., Koçyiğit, Ü. M., Daştan, T., Taştan, S. D., Taslimi, P., Gürses, F., & Gülçin, İ. (2019). The effects of wireless on carbonic anhydrase and acetylcholinesterase enzyme activities in some tissues related to respiratory, excretory and reproductive systems of rats. *Cumhuriyet Medical Journal*, *41*(1), 1–9. https://doi.org/10.7197/223.vi.515249

Ali, A., Redfors, B., Lundgren, J., Alkhoury, J., Oras, J., Gan, L. M., & Omerovic, E. (2019). Effects of pretreatment with cardiostimulants and beta-blockers on isoprenaline-induced takotsubo-like cardiac dysfunction in rats. *International Journal of Cardiology*, *281*, 99–104. https://doi.org/10.1016/j.ijcard.2018.12.045

Arakawa, S. (2013). Utilization of glutathione S-transferase Mu 1 - and Theta 1 -null mice as animal models for absorption, distribution, metabolism, excretion and toxicity studies. *Expert Opinion on Drug Metabolism & Toxicology*, *9*(6), 725–736. https://doi.org/10.1517/17425255.2013.780027

Aslan, H. E., Demir, Y., Özaslan, M. S., Türkan, F., Beydemir, Ş., & Küfrevioğlu, Ö. I. (2019). The behavior of some chalcones on acetylcholinesterase and carbonic anhydrase activity. *Drug and Chemical Toxicology*, *42*(6), 634–640. https://doi.org/10.1080/01480545.2018.1463242

Bal, S., Kaya, R., Gök, Y., Taslimi, P., Aktaş, A., Karaman, M., & Gülçin, İ. (2020). Novel 2-methylimidazolium salts: Synthesis, characterization, molecular docking, and carbonic anhydrase and acetylcholinesterase inhibitory properties. *Bioorganic Chemistry*, *94*, 103468. https://doi.org/10.1016/j.bioorg.2019.103468

Balcı, N., Türkan, F., Şakiroğlu, H., Aygün, A., & Şen, F. (2019). Purification and characterization of glutathione S-transferase from blueberry fruits (Vaccinium arctostaphylos L.) and investigated of some pesticide inhibition effects on enzyme activity. *Heliyon*, *5*(4), e01422. https://doi.org/10.1016/j.heliyon.2019.e01422

Bozdag, M., Carta, F., Vullo, D., Akdemir, A., Isik, S., Lanzi, C., Scozzafava, A., Masini, E., & Supuran, C. T. (2015). Synthesis of a new series of dithiocarbamates with effective human carbonic anhydrase inhibitory activity and antiglaucoma action. *Bioorganic & Medicinal Chemistry*, *23*(10), 2368–2376. https://doi.org/10.1016/j.bmc.2015.03.068

Brown, M., Davies, I. M., Moffat, C. F., Redshaw, J., & Craft, J. A. (2004). Characterisation of choline esterases and their tissue and subcellular distribution in mussel (Mytilus edulis). *Marine Environmental Research*, *57*(3), 155–169. https://doi.org/10.1016/S0141-1136(03)00067-9

Büyükokuroğlu, M. Elın., gülçİn, İİ., Oktay, Mİr., & küfrevİoğlu, Ö. İ. (2001). In vitro antioxidant properties of dantrolene sodium. *Pharmacological Research*, *44*(6), 491–494. https://doi.org/10.1006/phrs.2001.0890

Daneshvar, L., Rounaghi, G., E'shaghi, Z., Chamsaz, M., & Tarahomi, S. (2016). Electrochemical determination of carbamazepin in the presence of paracetamol using a carbon ionic liquid paste electrode modified with a three-dimensional graphene/MWCNT hybrid composite film. *Journal of Molecular Liquids*, *215*, 316–322. https://doi.org/10.1016/j.molliq.2015.12.059

Dasari, S., Ganjayi, M. S., Yellanurkonda, P., Basha, S., & Meriga, B. (2018). Role of glutathione S-transferases in detoxification of a polycyclic aromatic hydrocarbon, methylcholanthrene. *Chemico-Biological Interactions*, *294*(January), 81–90. https://doi.org/10.1016/j.cbi.2018.08.023

Ellman, G. L., Courtney, K. D., Andres, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, *7*(2), 88–95. https://doi.org/10.1016/0006-2952(61)90145-9

Fattah, T. A., Saeed, A., Channar, P. A., Larik, F. A., Hassan, M., Raza, H., Abbas, Q., & Seo, S. Y. (2018). Synthesis and molecular docking studies of (E)-4-(Substituted-benzylideneamino)-2H-Chromen-2-one Derivatives: Entry to new carbonic anhydrase class of inhibitors. *Drug Research*, *68*(07), 378–386. https://doi.org/10.1055/s-0043-123998

Gallicchio, L., Tkaczuk, K., Lord, G., Danton, M., Lewis, L. M., Lim, C. K., & Flaws, J. A. (2004). Medication use, tamoxifen (TAM), and TAM metabolite concentrations in women with breast cancer. *Cancer Letters*, *211*(1), 57–67. https://doi.org/10.1016/j.canlet.2004.04.001

Gocer, H., Topal, F., Topal, M., Küçük, M., Teke, D., Gülçin, İ., Alwasel, S. H., & Supuran, C. T. (2015). Acetylcholinesterase and carbonic anhydrase isoenzymes I and II inhibition profiles of taxifolin. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *31*(3), 1–7. https://doi.org/10.3109/14756366.2015.1036051

Gul, H. I., Tugrak, M., Sakagami, H., Taslimi, P., Gulcin, I., & Supuran, C. T. (2016). Synthesis and bioactivity studies on new 4-(3-(4-Substitutedphenyl)-3a,4-dihydro-3 *H* -indeno[1,2-c]pyrazol-2-yl) benzenesulfonamides. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *31*(6), 1619–1624. https://doi.org/10.3109/14756366.2016.1160077

Habig, W. H., Pabst, M. J., & Jakoby, W. B. (1974). Glutathione S transferases. The first enzymatic step in mercapturic acid formation. *The Journal of Biological Chemistry*, *249*(22), 7130–7139.

Jordan, V. C. (2009). Tamoxifen (ICI46,474) as a targeted therapy to treat and prevent breast cancer. *British Journal of Pharmacology*, *147*(S1), S269–S276. https://doi.org/10.1038/sj.bjp.0706399

Khezri, S., Bahram, M., & Samadi, N. (2018). Hydrogen bonding recognition and colorimetric detection of isoprenaline using 2-amino-5-mercapto-1,3,4-thiadiazol functionalized gold nanoparticles. *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, *189*, 522–527. https://doi.org/10.1016/j.saa.2017.06.063

Köse, L. P., & Gulcin, I. (2017). Inhibition effects of some lignans on carbonic anhydrase, acetylcholinesterase and butyrylcholinesterase enzymes. *Records of Natural Products*, *11*(6), 558–561. https://doi.org/10.25135/rnp.71.17.04.074

Kuzu, B., Tan, M., Taslimi, P., Gülçin, İ., Taşpınar, M., & Menges, N. (2019). Mono- or di-substituted imidazole derivatives for inhibition of acetylcholine and butyrylcholine esterases. *Bioorganic Chemistry*, *86*, 187–196. https://doi.org/10.1016/j.bioorg.2019.01.044

Loch, J. I., Bonarek, P., Tworzydło, M., Łazińska, I., Szydłowska, J., Lipowska, J., Rzęsikowska, K., & Lewiński, K. (2018). The engineered β-lactoglobulin with complementarity to the chlorpromazine chiral conformers. *International Journal of Biological Macromolecules*, *114*, 85–96. https://doi.org/10.1016/j.ijbiomac.2018.03.074

Mishra, R., Bhadauria, S., Murthy, P. K., & Murthy, P. S. R. (2011). Glycine soya diet synergistically enhances the suppressive effect of tamoxifen and inhibits its tamoxifen-promoted hepatocarcinogenesis in 7,12-dimethylbenz[α]anthracene-induced rat mammary tumor model. *Food and Chemical Toxicology*, *49*(2), 434–440. https://doi.org/10.1016/j.fct.2010.11.020

Nar, M., Çetinkaya, Y., Gülçin, İ., & Menzek, A. (2013). (3,4-Dihydroxyphenyl)(2,3,4-trihydroxyphenyl)methanone and its derivatives as carbonic anhydrase isoenzymes inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry*, *28*(2), 402–406. https://doi.org/10.3109/14756366.2012.670807

Nkoom, M., Lu, G., Liu, J., Yang, H., & Dong, H. (2019). Bioconcentration of the antiepileptic drug carbamazepine and its physiological and biochemical effects on Daphnia magna. *Ecotoxicology and Environmental Safety*, *172*, 11–18. https://doi.org/10.1016/j.ecoenv.2019.01.061

Sakr, M., Hanafi, R., Fouad, M., Al-Easa, H., & El-Moghazy, S. (2019). Design and optimization of a luminescent Samarium complex of isoprenaline: A chemometric approach based on Factorial design and Box-Behnken response surface methodology. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, *208*, 114–123. https://doi.org/10.1016/j.saa.2018.09.061

Sisecioglu, M., Uguz, M. T., Cankaya, M., Özdemir, H., & Gülçin, I. (2011). Effects of ceftazidime pentahydrate, prednisolone, amikacin sulfate, ceftriaxone sodium and teicoplanin on bovine milk lactoperoxidase activity. *International Journal of Pharmacology*, *7*(1), 79–83. https://doi.org/10.3923/ijp.2011.79.83

Taslimi, P., Caglayan, C., Farzaliyev, V., Nabiyev, O., Sujayev, A., Turkan, F., Kaya, R., & Gulçin, İ. (2018). Synthesis and discovery of potent carbonic anhydrase, acetylcholinesterase, butyrylcholinesterase, and α-glycosidase enzymes inhibitors: The novel N,N′-bis-cyanomethylamine and alkoxymethylamine derivatives. *Journal of Biochemical and Molecular Toxicology*, *32*(4), e22042. https://doi.org/10.1002/jbt.22042

Taslimi, P., Sujayev, A., Turkan, F., Garibov, E., Huyut, Z., Farzaliyev, V., Mamedova, S., & Gulçin, İ. (2018). Synthesis and investigation of the conversion reactions of pyrimidine-thiones with nucleophilic reagent and evaluation of their acetylcholinesterase, carbonic anhydrase inhibition, and antioxidant activities. *Journal of Biochemical and Molecular Toxicology*, *32*(2), e22019. https://doi.org/10.1002/jbt.22019

Taslimi, P., Türkan, F., Cetin, A., Burhan, H., Karaman, M., Bildirici, I., Gulçin, İ., & Şen, F. (2019). Pyrazole[3,4-d]pyridazine derivatives: Molecular docking and explore of acetylcholinesterase and carbonic anhydrase enzymes inhibitors as anticholinergics potentials. *Bioorganic Chemistry*, *92*, 103213. https://doi.org/10.1016/j.bioorg.2019.103213

Türkan, F., Aygun, A., Şakiroğlu, H., & Şen, F. (2019). Glutathione S-Transferase: purification and characterization of from Cherry Laurel (Prunus laurocerasus L.) and the investigation in vitro effects of some metal ions and organic compounds on. *BioNanoScience*, *9*(3), 683–691. https://doi.org/10.1007/s12668-019-00636-w

Türkan, F., Huyut, Z., Demir, Y., Ertaş, F., & Beydemir, Ş. (2019). The effects of some cephalosporins on acetylcholinesterase and glutathione S-transferase: An in vivo and in vitro study. *Archives of Physiology and Biochemistry*, *125*(3), 235–243. https://doi.org/10.1080/13813455.2018.1452037

Türkan, F., Huyut, Z., Taslimi, P., & Gülçin, İ. (2019). The effects of some antibiotics from cephalosporin groups on the acetylcholinesterase and butyrylcholinesterase enzymes activities in different tissues of rats. *Archives of Physiology and Biochemistry*, *125*(1), 12–18. https://doi.org/10.1080/13813455.2018.1427766

Türkan, F., Huyut, Z., Taslimi, P., Huyut, M. T., & Gülçin, İ. (2018). Investigation of the effects of cephalosporin antibiotics on glutathione S-transferase activity in different tissues of rats in vivo conditions in order to drug development research. *Drug and Chemical Toxicology*, *545*, 1–6. https://doi.org/10.1080/01480545.2018.1497644

Turkan, F., Cetin, A., Taslimi, P., Karaman, M., & Gulçin, İ. (2019). Synthesis, biological evaluation and molecular docking of novel pyrazole derivatives as potent carbonic anhydrase and acetylcholinesterase inhibitors. *Bioorganic Chemistry*, *86*, 420–427. https://doi.org/10.1016/j.bioorg.2019.02.013

Wilder, P. T., Charpentier, T. H., Liriano, M. A., Gianni, K., Varney, K. M., Pozharski, E., Coop, A., Toth, E. A., Mackerell, A. D., & Weber, D. J. (2010). In vitro screening and structural characterization of inhibitors of the S100B-p53 interaction. *International Journal of High Throughput Screening*, *2010*(1), 109–126. https://doi.org/10.2147/IJHTS.S8210