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Novel tribenzylaminobenzolsulphonylimine based on their pyrazine and pyridazines: Synthesis, characterization, antidiabetic, anticancer, anticholinergic, and molecular docking studies

Gulnar Mamedova[a], Adila Mahmudova[a], Sabir Mamedov[a], Yavuz Erden[b], Parham Taslimi[c,*], Burak Tüzün[d], Recep Tas[c], Vagif Farzaliyev[a], Afsun Sujayev[a], Saleh H. Alwasel[e], İlhami Gulçin[f]

[a] Laboratory of Faine Organic Synthesis, Institute of Chemistry of Additives, Azerbaijan National Academy of Sciences, 1029 Baku, Azerbaijan
[b] Department of Molecular Biology and Genetics, Faculty of Science, Bartin University, 74100 Bartin, Turkey
[c] Department of Biotechnology, Faculty of Science, Bartin University, 74100 Bartin, Turkey
[d] Department of Chemistry, Faculty of Science, Cumhuriyet University, 58140 Sivas, Turkey
[e] Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia
[f] Department of Chemistry, Faculty of Sciences, Ataturk University, 25240 Erzurum, Turkey

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ABSTRACT

A new method of obtaining multifunctional pyrazoles by the reaction of 1,3-dipolar addition of tribenzylsulfonyliminochloride to polarophiles has been developed. This imine is obtained by reacting tribenzylamine with *N*-chlorobenzene sulfamide (chloramine-B). Regardless of the structure and composition of polarophiles, the cyclization reaction takes place in the presence of alkali in 6–8 h of boiling, which proves the activation of the methylene groups of tribenzylamine using the electron-withdrawing sulfonamide group. These novel derivatives were effective inhibitors of the α-glycosidase, butyrylcholinesterase (BChE), and acetylcholinesterase enzymes (AChE) with Ki values in the range of 0.45 ± 0.08–1.24 ± 0.27 μM for α-glycosidase, 6.04 ± 0.95–11.61 ± 2.84 μM for BChE, and 2.04 ± 0.24–4.23 ± 1.02 μM for AChE, respectively. The biological activities of the studied molecules against enzyme molecules were investigated by molecular docking calculations. The enzymes studied were AChE for ID 4M0E, BChE for ID 5NN0 BChE, and α-Glycosidase for ID 1XSI (α-Gly) respectively.

1. Introduction

Pyrazole sulfamides possess various types of biological activity. Thus, 1,2,3-benzoxondensated bicyclic sulfonylamide-azoles can be used as powerful selective and effective γ-secretase inhibitors [1]. Other functionally substituted pyrazoles have an analgesic effect and can be used against inflammation [2]. Sulfamides containing a carbamide moiety and a pyrazole heterocycle showed high antidiabetic efficacy [3].

The world safety formation of WHO, Alzheimer's ailment (AD) is the sixth driving cause for death overall ages in some countries. Now, with the time this number is enhancing. Recently, AD is supposed to be the cause of neuronal decay and neurodegeneration in the brain [4]. Various neurotic marks of AD have been distinguished and consideration of these characteristics has prompted a few theories in endeavors to clarify the basic reason for AD. Also, the correct cause and time allotment of chances prompting AD to stay unclear, and it likely includes an unpredictable cluster of components [5]. Acetylcholine (ACh) is a good neurotransmitter that under neurodegenerative situations helps in the modulation of function of memory while cholinesterases (ChEs) are a group of important enzymes that play a key role in the regulation of Ach and cholinergic signaling. BChE and AChE enzymes play a vital role in the regulation of ACh level, its synaptic hydrolysis and in terminating its neurotransmitter action. In order to re-establish the neurotransmitter level is done by using inhibitors of cholinesterase that function by suppressing the ChE enzymes, which results in both the level increase and duration of the neurotransmitter action [6–9].

Diabetes mellitus (DM) is a chronic metabolic disorder, which is characterized by an increase in blood glucose level (hyperglycemia) and causes disturbances in fat, protein and carbohydrate metabolism [10]. It causes decline in insulin secretion or its action or both. For the past few years the increase in the occurrence of diabetes has been

* Corresponding author.
E-mail addresses: ptaslimi@bartin.edu.tr, parham_taslimi_un@yahoo.com (P. Taslimi).

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observed. The prevalence of diabetic patients has increased from 171 million to 366 million since 2000 and it is expected to rise even more by 2030. In type 2 DM, starch hydrolysis by the pancreatic alpha-amylase and the absorption of glucose by the intestinal α-glycosidase like maltase and sucrase results in postprandial hyperglycemia (PPHG) [11–14]. Molecular docking studies were performed to compare the biological activities of the molecules obtained as a result of syntheses against enzyme molecules [15]. The optimized structures were obtained by using Gaussian package program. The resulting optimized structures were used in the docking server to calculate biological activities against enzyme molecules. The names of the protein molecules studied are acetylcholinesterase for ID 4M0E (AChE), butyrylcholinesterase for ID 5NN0 (BChE) α-glycosidase for ID 1XSI (α-Gly).

Cancer is a major health problem characterized by uncontrolled proliferation of cells in a tissue or organ and subsequent metastasis to other tissues or organs. This disease process can be caused by internal factors such as DNA damage, mutations, hormones and immune-related conditions, as well as by external factors such as ultraviolet and ionizing radiation, asbestos, cigarette smoke, viruses, bacteria and parasites [16]. Unfortunately, the treatment of this disease, which involves a rather complex process, is not available today. Although chemotherapy, radiotherapy and surgical methods are used primarily in cancer treatment, additional targeted applications (such as regulation of nutritional habits, sports and stress-free lifestyle and targeted drugs) are important for the success of treatment [17,18].

In this study, we performed the design, synthesis and evaluation of some of novel tribenzylaminobenzolsulphonylimine based on their pyrazine and pyridazines (I-X) which III, IV, IX, and X compounds were as effective α-glycosidase, AChE, and BChE inhibitors. Anticancer and molecular docking studies were performed on these compounds. Also, another goal of this work, is compared their inhibitory results with control compounds like acarbose and tacrine.

2. Experimental

2.1. General chemistry

The IR spectra were taken on a Nicolet İS-10 instrument, and the ^{1}H NMR spectra of the synthesized sulfamides were recorded on a Tesla-467 spectrophotometer with an operating frequency of 90 MHz. Mass spectroscopic analysis of 1-N1-methylphenyl-2-N2-phenylsulfonyl-4-hydroxyphenyl pyridazin-4-one was performed on an Agilent 5977 chromato-mass-mass spectrometer with a plasma-ionization detector. The value of the supplied sample is 1–2 L; the analysis time is 30 min. The analysis was carried out in capillary columns 50 × 0.25 mm in size, filled with a stationary liquid phase “Silikon HP-1”, with a programmed temperature range of 60–300 °C, with a speed of 1.0–2.0 mL/min, carrier gas-helium.

2.1.1. Tribenzylaminobenzenesulfonylimine chloride (I)

28.7 g (0.1 mol) of tribenzylamine were mixed with 100 mL of ethanol, acidified with hydrochloric acid to pH 5 and 29.4 g (0.11 mol) of chloramine-B were added 20 mL of water at 65–70 °C. The mixture was boiled for 1–2 h, the ethanol–water mixture was distilled off to half the volume, cooled to 0 °C until the crystals were completely precipitated, and then recrystallized from ethanol.

2.1.2. Pyrazoles (II-VI)

General method. 1 mmol of tribenzylamino-benzenesulfonylimine chloride (Com. I) was dissolved in 10–15 mL of absolute ethanol, 1 mmol of 1,2-dichloro-3-*N*-benzenesulfonylpropane, or acetylacetone, or 1-chloro-2,3-dihydropropane, or 2-bromopropionic acid, or chloroacetonitrile and a little more than the calculated amount of alkali (when reacting with acetylacetone-0.1 mmol of alkali). The reaction mass was boiled for 3–4 h, filtered hot, cooled, the precipitated crystals were filtered and recrystallized from 85% ethanol.

2.1.3. Pyridazines (VII-X)

The method of synthesis is similar to the method of obtaining pyrazoles. In the reaction of epichlorohydrin and 2-chloro-pyridine benzene sulfamide, 1.1 mmol of NaOH was taken, and in the synthesis of compound IX, 0.1 mmol of alkali.

2.2. Enzymes inhibition studies

2.2.1. α-Glycosidase enzyme assay

Inhibitory effect of novel tribenzylaminobenzolsulphonylimine based on their pyrazine and pyridazines III, IV, IX, and X compounds on α-glycosidase enzyme activity was performed using *p*-nitrophenyl-D-glycopyranoside (*p*-NPG) substrate, according to the assay of Tao et al. [19]. Firstly, 200 μL of phosphate buffer was mixed with 40 μL of the homogenate solution in phosphate buffer (0.15 U/mL, pH 7.4). Also, 50 μL of *p*-NPG in phosphate buffer (5 mM, pH 7.4) after preincubation was added and again incubated at 30 °C. The absorbances were spectrophotometrically measured at 405 nm according to previous studies [20,21].

2.2.2. AChE/BChE activity determination and inhibition studies

The inhibitory efficacy of novel tribenzylaminobenzolsulphonylimine based on their pyrazine and pyridazines III, IV, IX, and X compounds on BChE/AChE activities was obtained conforming to the spectrophotometric procedure of Ellman et al. [22]. Acetylthiocholine iodide (AChI) and butrylcholine iodide (BChI) were used as substrates for both reactions. In this study, 5,5′-dithio-bis(2-nitro-benzoic)acid (DTNB) was used for the estimation of the both BChE/AChE enzymes activities. Briefly, 100 μL of buffer solution (pH 8.0, Tris/HCl, 1.0 M) and diverse concentration of sample solutions (30–300 μL) dissolved in deionized water were added to 50 μL of both BChE/AChE enzymes solutions (5.32×10^{-3} EU). Then the mixtures were incubated for 10 min at 20 °C. Finally, 50 μL of DTNB (0.5 mM and 25 mL) of both substrates BChI/AChI were added to incubated mixtures. Also, the reactions were initiated by the addition of 50 μL of BChI/AChI. Activities of BChE/AChE were evaluated spectrophotometrically at a wavelength of 412 nm [23–27].

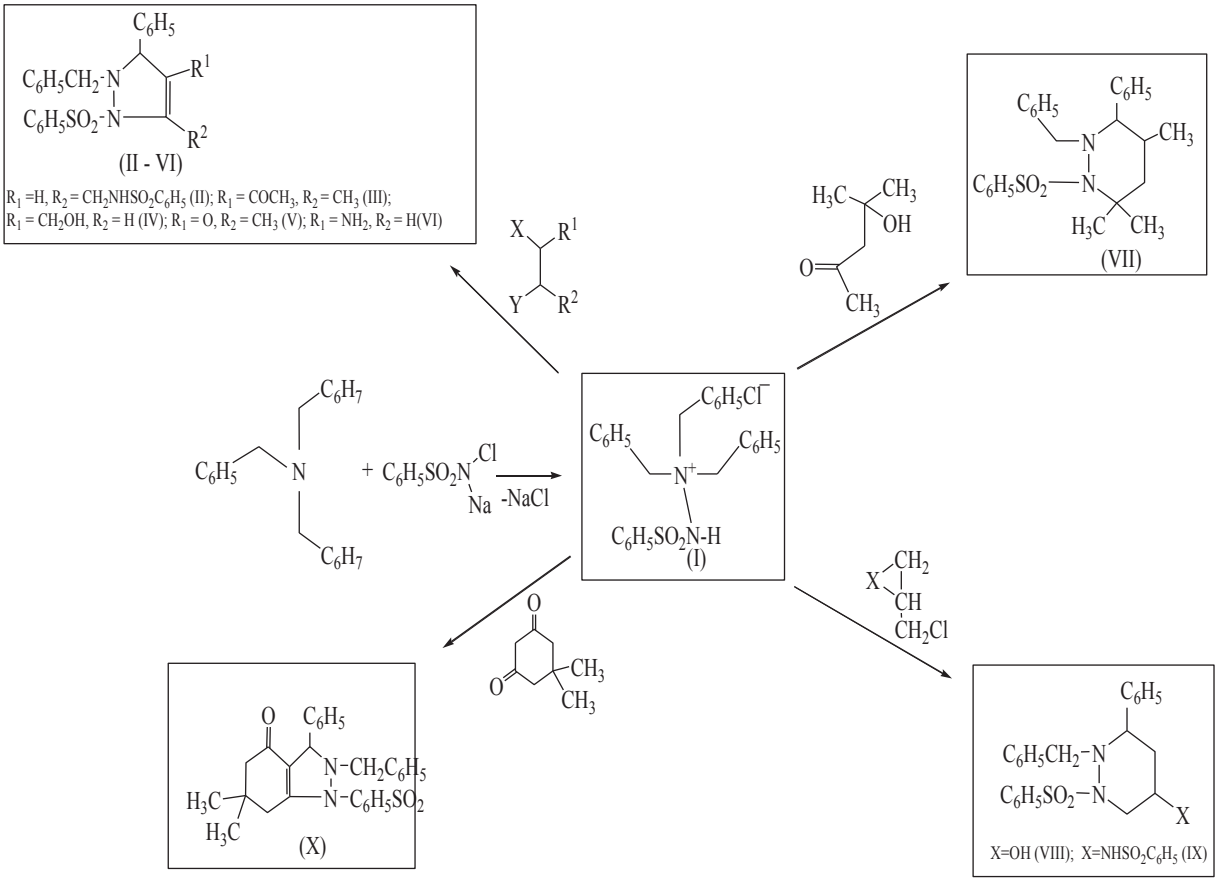
2.3. Docking studies

In this study, four molecules obtained as a result of syntheses were compared their biological activities against enzyme molecules. In this study, four molecules obtained as a result of syntheses were compared their biological activities against enzyme molecules. These molecules were optimized in the HF/6-31g++ basis set using the Gaussian Package program. Docking calculations were made using these optimized structures. The pH of human blood is 7.35. Therefore, all calculations made in molecular docking were made at the same pH as human pH. In this way, a great harmony was tried to be achieved with the calculations.

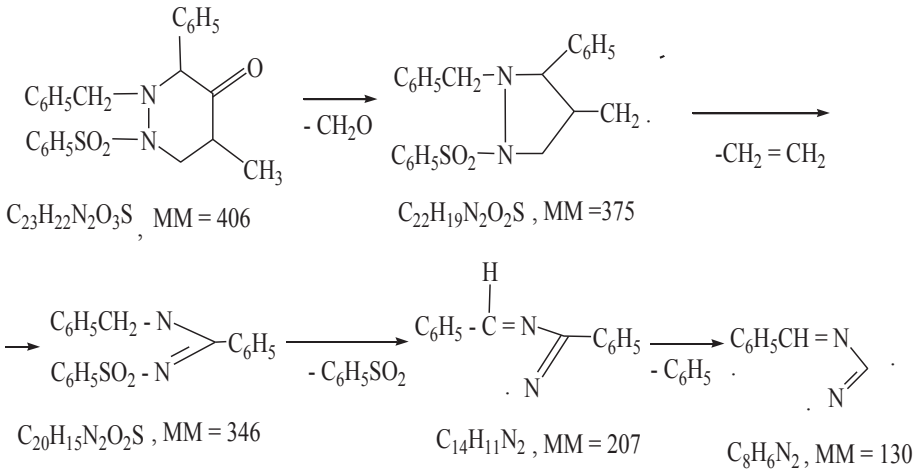
2.4. Anticancer methods

2.4.1. Proliferation of cells

Human prostate (PC-3, ATCC) and breast (MCF-7, ATCC) cancer cell lines were used to determine the anticancer properties of the test compounds. Cells were first extracted from liquid nitrogen and then cultured in 75 cm^2 culture flasks. Cultured PC-3 cells were prepared with RPMI-1640 (Sigma-Aldrich R8758, USA) medium (prepared by adding 10% FBS, 100 U/mL penicillin and 0.1 mg/mL streptomycin), and MCF-7 cells were DMEM (Gibco 41965039, UK) medium (prepared by adding 10% FBS, 100 U/mL penicillin and 0.1 mg/mL streptomycin, 10 μg/mL insulin). The media of the cells were changed twice a week and the cells were incubated at 37 °C (Thermo Forma II CO_2 Incubator, USA) with 5% CO_2 in all steps. Cells that were confluent at the base of the flask were removed from the flasks using trypsin-EDTA (Gibco



Scheme 1. The synthesis of novel tribenzylaminobenzolsulphonylimine based on their pyrazine and pyridazines (I-X).



Scheme 2. The fragmentation reaction of pyrazine (V).

25300054, UK) and stained with 0.4% trypan blue followed by cell counts under an inverted microscope (Optec BDS400, China). Experimental studies were initiated when cell viability was 90% or more [28].

2.4.2. Cell viability (MTT assay)

The effect of test compounds on viability in human prostate and breast cancer cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide (MTT) method, which is widely used in the assessment of cell viability [29,30]. Cells of which viability levels were determined were cultured in 96-well plates with 15×10^3 cells per well. The plates were incubated for one day in CO_2 medium and then the test compounds were treated with concentrations in ranging of 1–100 mM prepared in DMSO for 24 h. After this time, 0.5 mg/mL MTT (Sigma-Aldrich M2128, USA) working solution was prepared to determine the changes in cell viability and 100 mL of the prepared MTT solution was added to each well and incubated for 3 h in a CO_2 incubator. At the end of incubation, the optical densities of the cells in the plates were read in the microplate reader (Thermo MultiskanGo, USA) at a wavelength of 570 nm [31]. Control wells with cells and medium only were measured the mean absorbance values obtained were taken as 100% viable cells. Absorbance values from wells treated with vehicle control and test compounds were proportional to control absorbance and percent viability calculated [32,33]. The effect of compounds on

Table 1
Physical and chemical characteristics and data of IR spectra of the compounds (I-X).

№	Yield (%)	$T_{melt.}$ (°C)	Brutto formula	Elemental analysis, Found % Calculated		Data of IR spectroscopy (cm^{-1})
				N	S	
I	80.9	85–87	$C_{27}H_{27}N_2O_2SCl$	5.49 5.85	–	–
II	70.8	115–117	$C_{29}H_{29}N_3O_4S_2$	7.27 7.68	11.37 11.70	SO_2N: 1450, 1160
III	74.2	102–104	$C_{25}H_{23}N_2O_3S$	6.79 6.5	7.12 7.4	C=O: 1695, SO_2N: 1451, 1150
IV	69.4	106–108	$C_{23}H_{24}N_2O_3S$	7.02 6.86	7.68 7.84	C – OH: 3381, SO_2N: 1454, 1120
V	71.2	163–165	$C_{23}H_{22}N_2O_3S$	7.23 6.9	7.56 7.88	C=O: 1659, SO_2N: 1453, 1119.6
VI	73.9	82–84	$C_{22}H_{21}N_3O_2S$	10.57 10.74	7.92 8.18	–
VII	59.8	185–187	$C_{26}H_{28}N_2O_2S$	6.69 6.48	7.87 7.41	–
VIII	67.9	113–115	$C_{23}H_{23}N_2O_3S$	7.29 7.68	11.46 11.70	C-OH: 3392, SO_2N: 1461, 1156
IX	69.8	112–114	$C_{29}H_{29}N_3O_4S_2$	7.79 8.16	5.92 6.21	C-NH: 3348, SO_2N: 1450–1465, 1150–1155
X	68.3	183–185	$C_{28}H_{28}N_2O_3S$	5.63 5.98	6.59 6.84	C=O: 1709.5, SO_2N: 1454, 1175

Table 2
α-Glycosidase, acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) enzymes inhibition effects of novel tribenzylaminobenzolsulphonylimine based on their pyrazine and pyridazines III, IV, IX, and X compounds.

Compounds	IC_{50} (μM)						K_i (μM)		
	AChE	r^2	BChE	r^2	α-Gly	r^2	AChE	BChE	α-Gly
III	2.81	0.9683	11.83	0.9588	0.34	0.9693	2.04 ± 0.24	8.94 ± 1.40	0.64 ± 0.14
IV	4.84	0.9890	15.62	0.9027	0.27	0.9206	4.23 ± 1.02	11.61 ± 2.84	0.45 ± 0.08
IX	3.72	0.9526	8.41	0.9382	0.73	0.9642	2.58 ± 0.86	6.04 ± 0.95	0.96 ± 0.16
X	3.04	0.9732	13.55	0.9741	0.88	0.9889	2.26 ± 0.45	10.37 ± 1.48	1.24 ± 0.27
TAC**	6.87	0.9914	18.30	0.9424	–	–	5.88 ± 1.14	14.70 ± 2.55	–
ACR***	–	–	–	–	22.80	–	–	–	12.60 ± 0.78

** Tacrine (TAC) was used as a control for AChE and BChE enzymes.
*** Acarbose (ACR) was used as a control for α-glycosidase enzyme.

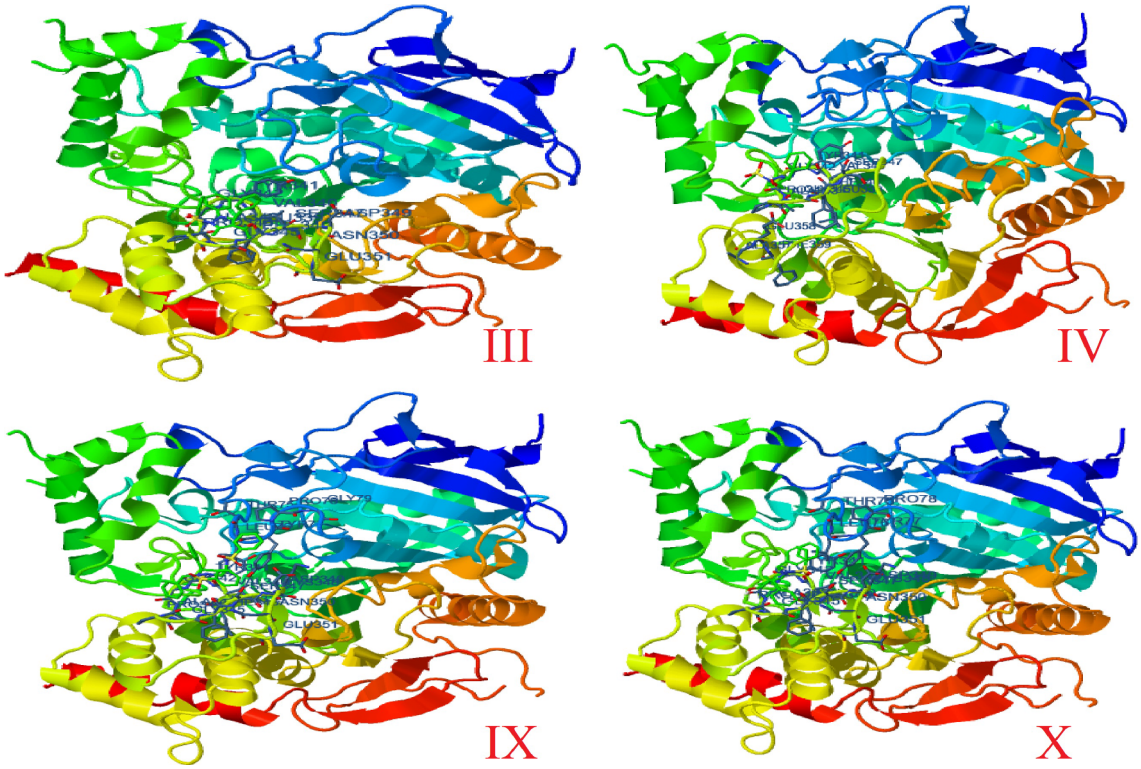


Fig. 1. Showing of the interaction of acetylcholinesterase (AChE) enzyme with the molecules.

cell viability was analyzed in comparison to control and solvent. These experiments were repeated 5 times independently on different days.

2.4.3. Statistical analysis

Statistical analyzes were performed using GraphPad Prism 5 package program. Homogeneity of the variances was evaluated by Bartlett tests test. One-way ANOVA was used to determine the differences between the groups and Bonferroni test was used for multiple comparisons. Quantitative data were expressed as mean ± standard error deviation (mean ± SD) and $p < 0.05$ was considered significant [34].

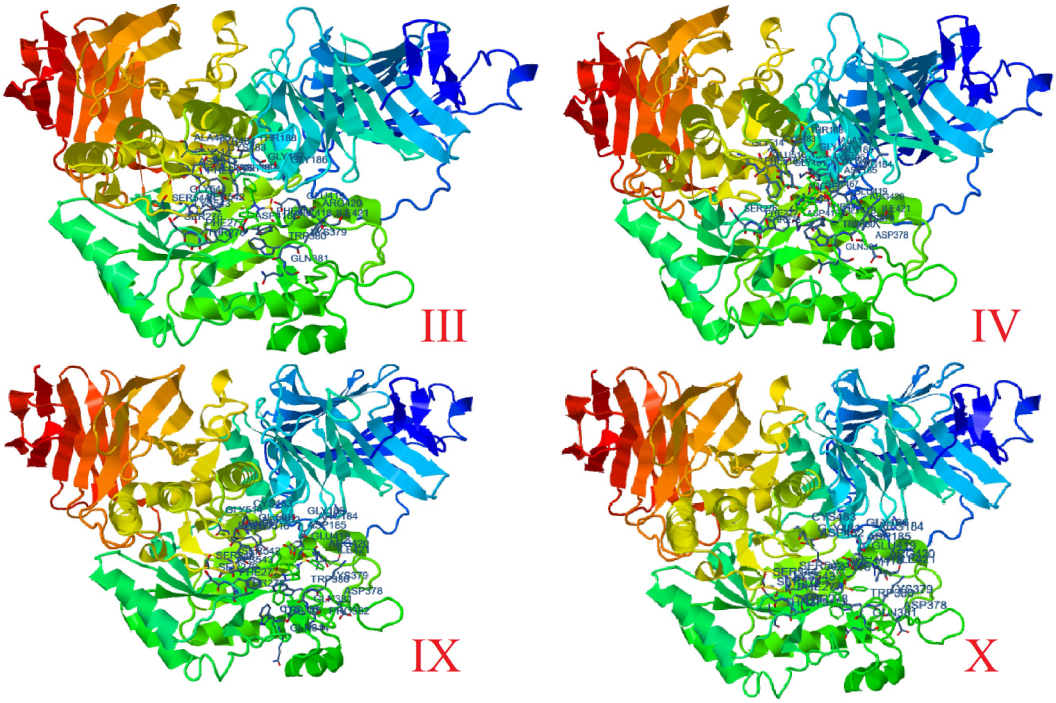


Fig. 2. Showing of the interaction of butyrylcholinesterase (BChE) enzyme with the molecules.

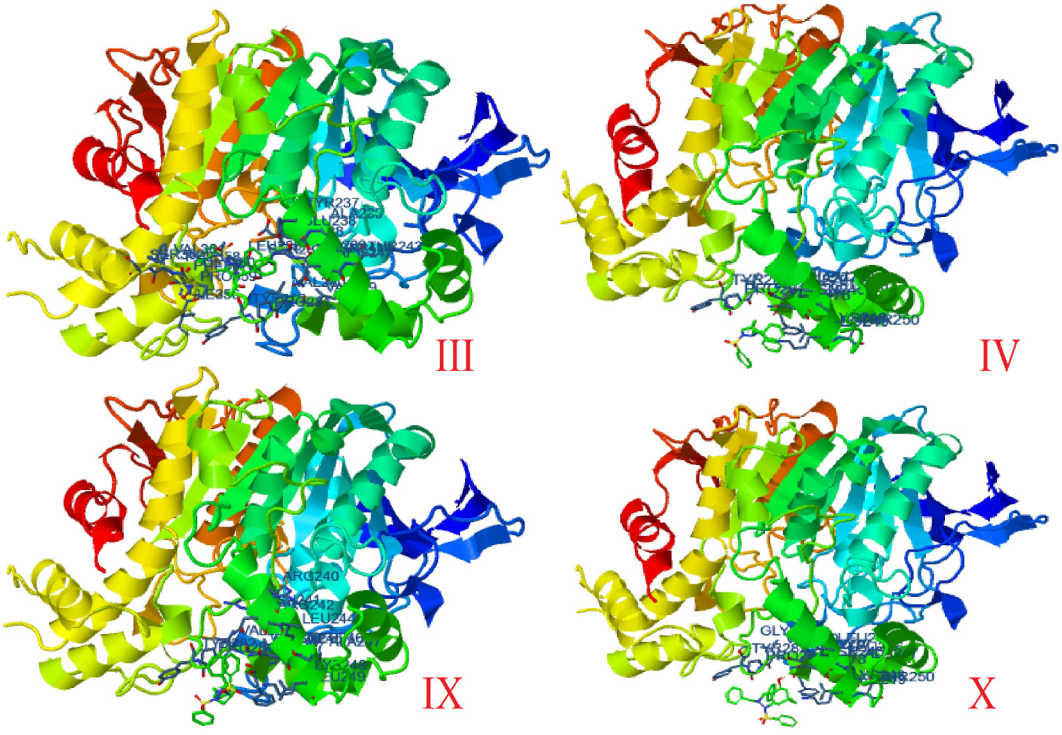


Fig. 3. Showing of the interaction of α-Glycosidase (α-Gly) enzyme with the molecules.

3. Results and discussion

3.1. Chemistry

The main method for the synthesis of pyrazoles is the heterocyclization of functionally substituted hydrazines with 1,3-polarophiles [35]. For the synthesis of diazolsulfonamide derivatives [36], a three-component reaction of acylhydrazine, isocyanate, and sulfonyl chloride was carried out in the presence of phosphazine. When the reaction of heterocyclization of tolyl isocyanate with 2-(4-R-benzoylmethyl)-3-(fura-2-yl) acrylhydrazide was performed, pyrazoles were obtained [37]. The sulfamide moiety is not involved in this reaction. 2-Aminophenyl-1H-pyrazole was used as a cleavable, bidendental, directing group for copper-initiated, aerobic, oxidative amidation of C (sp2-H) bond [38]. However, the synthesis of hetarylsulfamides containing various heterocycles and polyheterocyclic compounds has not been studied enough by the reaction of quaternary ammonium salts with polarophiles. Methods have been developed for the synthesis of

Table 3
Molecular docking energy data for studies molecule for 3 enzymes.

	III	IV	IX	X
AChE				
Est. Free Energy of Binding/*kcal/mol*	−4.11	−4.05	−5.72	−4.32
Est. İnhibition Constant. Ki/*mM*	967.98	1.08	63.73	682.12
vdW + Hbond + desolv Energy/*kcal/mol*	−5.37	−5.85	−6.07	−5.64
Electrostatic Energy/*kcal/mol*	−0.06	−0.05	−0.01	+0.02
Total Intermolec. Energy/*kcal/mol*	−5.43	−5.90	−6.09	−5.62
Frequency	%20	%20	%10	%30
Interact. Surface	498.28	535.98	636.48	560.45
BChE				
Est. Free Energy of Binding/*kcal/mol*	−2.91	−2.99	−0.90	−2.97
Est. İnhibition Constant. Ki/*mM*	7.30	6.45	217.41	6.63
vdW + Hbond + desolv Energy/*kcal/mol*	−5.29	−4.28	−3.19	−4.83
Electrostatic Energy/*kcal/mol*	+0.17	−0.04	−0.33	−0.01
Total Intermolec. Energy/*kcal/mol*	−5.13	−4.32	−3.52	−4.84
Frequency	%10	%10	%10	%10
Interact. Surface	874.18	482.71	511.65	535.68
α-Gly				
Est. Free Energy of Binding/*kcal/mol*	−3.79	−3.37	−1.27	−3.78
Est. İnhibition Constant. Ki/*mM*	1.67	3.39	117.39	1.69
vdW + Hbond + desolv Energy/*kcal/mol*	−4.98	−4.95	−4.11	−5.33
Electrostatic Energy/*kcal/mol*	−0.02	−0.10	−0.23	−0.10
Total Intermolec. Energy/*kcal/mol*	−5.00	−5.05	−4.34	−5.43
Frequency	%30	%10	%20	%10
Interact. Surface	765.76	712.81	804.41	818.60

pyridine-*N*-imines obtained by the reaction of pyridine with hydrazine sulfonate [39], aminosulfonic acids [40]. A new method for the synthesis of *N*-imines has been developed [41]. Pyridine-*N*-imines are also obtained by the reaction of pyridine with *N*-monochloramines [42]. The reaction of heterocyclization of pyridine-*N*-imine with alkenes and alkynes was studied [43]. Pyridine-*N*-imines are easily cyclized even with dipolarophiles, forming pyrazolo-pyridines [44]. In the literature, there are no data on the synthesis of *N*-imines using other tertiary amines and their study in dipolar addition reactions. We investigated the reaction of addition of tribenzylamine to chloramine-B.

Under the influence of the electron-withdrawing sulfamide fragment, the protons of the methylene groups of the benzyl are activated and, in the presence of alkali, in a proton solvent, synchronously heterocyclic with polarophiles. The formation of a pyrazole or pyridazine heterocycle depends on the composition and structure of polarophiles. When tribenzylaminobenzenesulfonylimine chloride (I) reacts with polarophiles, pyrazoles or dihydropyrazoles are formed.

Compounds II, IV, V are dihydropyrazoles. Probably in the reaction of imine I with chlorinated polarophiles, such as 1,2-dichloro-3-benzenesulfamidopropane, 1-chloro-2,3-dihydroxypropane, 2-bromopropionic acid and chloroacetonitrile heterocyclization begins with the substitution of a sulfide by hydrogen by a hydrogen sulfide moiety, followed by cyclization through a methylene group of benzyl. Despite the 1.3-dipolar acetylacetone, the heterocyclization reaction proceeds with the formation of a five-membered cycle. Probably, carbanion is

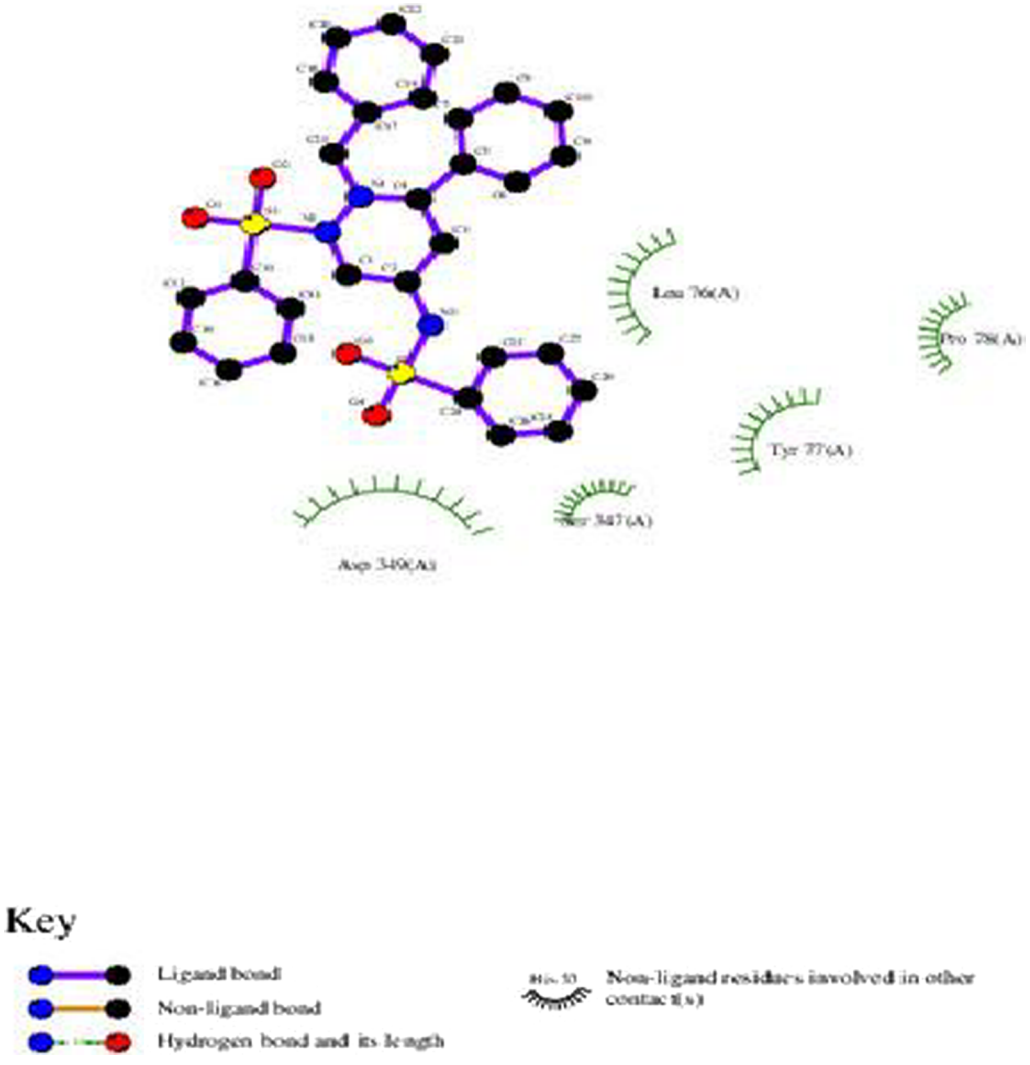


Fig. 4. Showing of acetylcholinesterase (AChE) enzyme and molecule IX interactions.

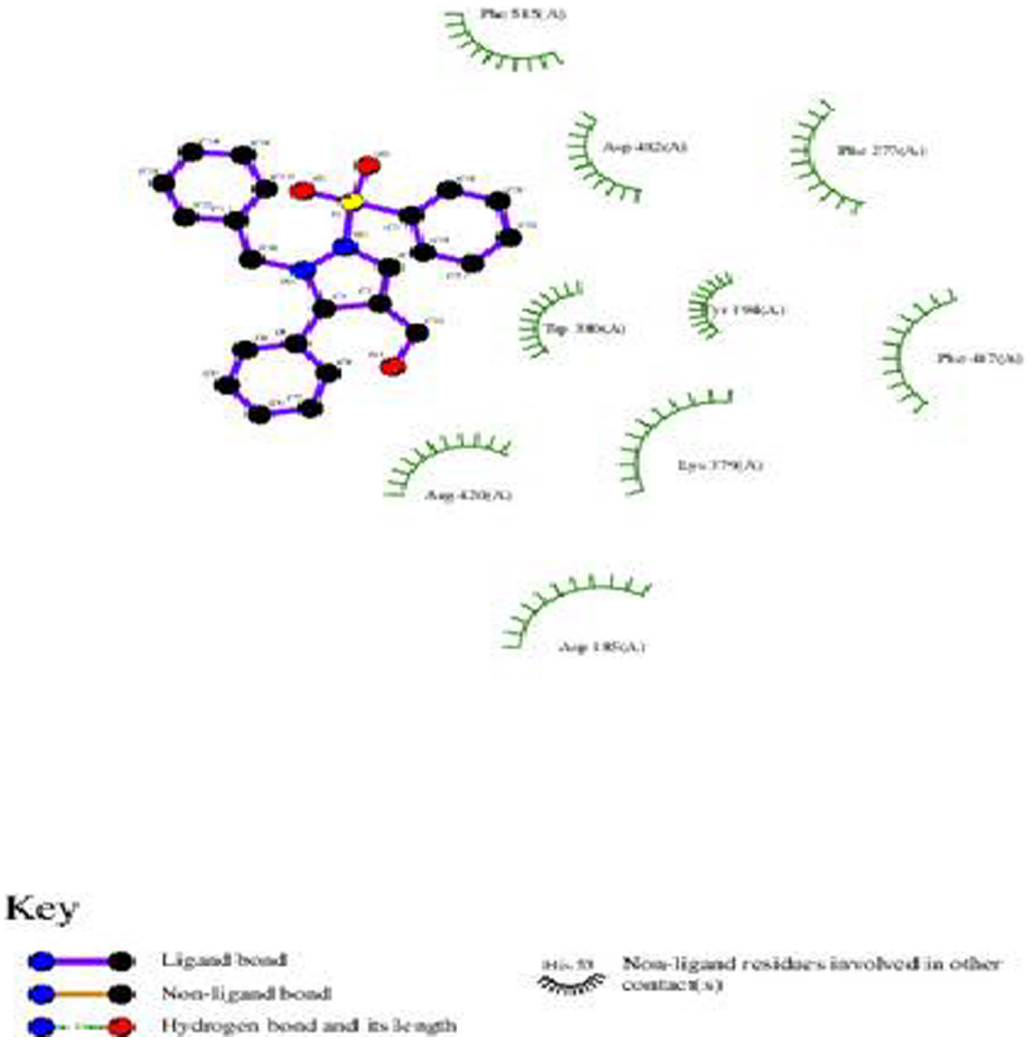


Fig. 5. Showing of butyrylcholinesterase (BChE) enzyme and molecule IV interactions.

formed first in the presence of alkali. With the participation of hydrogen of the sulfamide group, water is split off and the resulting unsaturated ketone is added to the methylene group of the benzyl with a ring closure. Unlike acetylacetone, 2-pentanone-4-methyl-4-hydroxy (diacetone alcohol) with imine forms pyridazine (VII). During the reaction of heterocyclization of tribenzylphenylsulfonylimine (I) with 1-chloromethylbenzenesulfonyl aziridine and epichlorohydrin, synchronous heterocyclization occurs with the formation of tetrahydropyridazines. The reaction of the imine (I) with dimedone takes place synchronously and the benzpyrazole is obtained. All of the reactions shown in Scheme 1:

In the ^{1}H NMR spectrum of 1-N'-phenylmethylene-N'-phenylsulfonylamide-4-methylene-phenylsulfamide-5-phenylpyrazole (compound II), the protons of the methylene group of pyrazole in the form of a singlet are in the region of 1.5 ppm, 2CH of protons of pyrazole in the form of the doublet is in the region of 2.95 ppm The protons CH2-N- under the action of the electron-withdrawing sulfonide fragment fall into a weaker region-3.6 ppm near the DMSO peak. Hydrogen N—H in the form of a doublet appears at 5.2 ppm. The protons of the four phenyl groups are manifested together in the region of 7.2–7.7 ppm. In the ^{1}H NMR spectrum N'-methylphenyl-2-N"- phenylsulfamide-3-methyl-4-oxo-5-phenylpyrazole (V) in DMSO solution protons of methyl groups appear in the region of 2.0 ppm The protons CH_2-N, 2H are in the region of 3.9 ppm, and the protons of the three aromatic rings appear together in the region of 7.202–7.392. Integral intensity corresponds to the number of protons in a molecule. The mass spectrum of compound (V) was taken. Based on the obtained peaks, the obtained radicals are fragmented. As can be seen, the fragmentation of this pyrazine proceeds according to the Scheme 2:

Data NMR spectroscopy and fragmentation of the mass spectrum confirm the expected structure of the synthesized compounds. In the ^{1}H NMR spectrum of compound (VIII), the protons of the 2CH_2- and 2CH-groups appear together with DMSO in the region of 3.06–3.8 ppm. The proton of the amino groups in the form of a doublet is in the region of 5.46 ppm. The unresolved protons of the four aromatic rings manifest themselves in the region of 7.02–7.56 ppm. Physico-chemical characteristics of the compounds (I-X) are given in Table 1.

3.2. Enzymes inhibition results

The cholinesterase inhibitors (ChEIs) are the first production of drugs for the therapy of myasthenia gravis, AD, glaucoma, and also are used for the therapy of many other neuromuscular disturbances [45,46]. It has a restriction, with the increment in the level of Ach molecule, symptoms of these diseases increase. Indeed, the search for novel ChEIs is highly appreciated for further advancement in the treatment of such diseases [47,48]. The inhibitory effects of the novel tribenzylaminobenzolsulphonylimine based on their pyrazine and pyridazines III, IV, IX, and X compounds on AChE are shown in Table 2. The AChE inhibition profiles of the compounds evaluated here were quite interesting. Overall, the novel tribenzylaminobenzolsulphonylimine based on their pyrazine and pyridazines III, IV, IX, and X

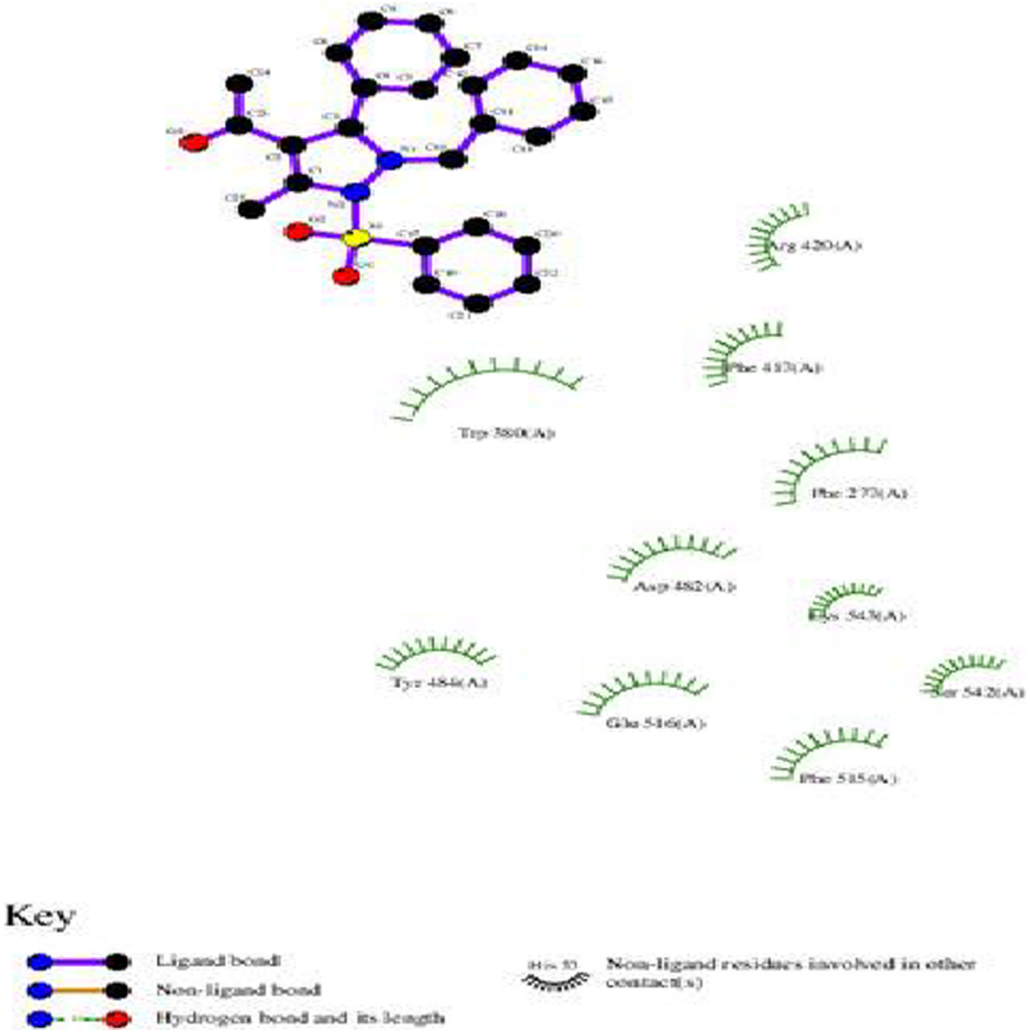


Fig. 6. Showing of α-glycosidase (α-Gly) enzyme and molecule III interactions.

compounds had excellent inhibitory activity with K_i values ranging from 2.04 ± 0.24 μM to 4.23 ± 1.02 μM. Furthermore, tacrine, used as a standard AChE inhibitor in this study, demonstrated K_i value of 5.88 ± 1.14 μM toward AChE. As these results show, the inhibition of AChE of novel tribenzylaminobenzolsulphonylimine based on their pyrazine and pyridazines III, IV, IX, and X compounds is much better than standard drugs. The compounds of III, and X showed excellent inhibition profile against AChE with K_i values of 2.04 ± 0.24 and 2.26 ± 0.45 μM, respectively (Table 2). Recently, researchers have been trying to comprehend AD by addressing some therapeutic aims, like the tau phosphorylation, Aβ aggregation process, oxidative stress, secretase enzymes modulation, AChE inhibition etc. Still, currently available drugs for palliative treatment of AD are ChEIs, like tacrine, donepezil, galantamine, and rivastigmine and the *N*-methyl-d-aspartate antagonist memantine. Tacrine as quinoline derivative was the first drug approved by the Drug Administration (FDA) to treat AD in 1991 [49–53]. For the BChE enzyme, the novel tribenzylaminobenzolsulphonylimine based on their pyrazine and pyridazines III, IV, IX, and X compounds had IC_{50} values in the range of 8.41–15.62 and K_i values in the range of 6.04 ± 0.95–11.61 ± 2.84 μM (Table 2).

DM as metabolic disturbance syndrome is caused by the loss of islet function with the symptoms of polyphagia, polyuria, polydipsia, and etc. More studies defined that the major harm of diabetes lies not in its own but in its serious complications which led to high mortality, like heart cerebrovascular sclerosis, blindness, extremity gangrene, kidney failure and so on. After years of research, considerable drugs have been developed to treat diabetes, like miglitol, acarbose, and voglibose, which were competitive-glycosidase inhibitors commonly utilized in clinical with structures similar to glucose and oligosaccharides [54–56]. Although these drugs have a certain therapeutic effect on diabetes, they may also cause gastrointestinal adverse reactions, such as abdominal pain, diarrhea, bloating, and etc. Therefore, it is urgent to develop new therapeutic agents for diabetes mellitus, as well as for the prevention or treatment of comorbidities [57–62]. For the α-glycosidase enzyme, the novel tribenzylaminobenzolsulphonylimine based on their pyrazine and pyridazines III, IV, IX, and X compounds had IC_{50} values in the range of 0.27–0.88 μM and K_i values in the range of 0.45 ± 0.08–1.24 ± 0.27 μM (Table 2). The results obviously showed that all novel tribenzylaminobenzolsulphonylimine based on their pyrazine and pyridazines III, IV, IX, and X compounds demonstrated efficient α-glycosidase inhibitory effects than that of acarbose (IC_{50}: 22.80 μM, Ki: 12.60 μM) [63,64] as a standard α-glycosidase inhibitor. However, the most effective K_i values were obtained by compounds of IV and III, with K_i values of 0.45 ± 0.08 and 0.64 ± 0.14 μM, respectively.

3.3. Docking studies

Many methods are used to compare the biological activities of the studied molecules [65]. Molecular docking is the most widely used method to compare the biological activity of molecules against enzyme molecules. In docking studies, enzymes that interact with molecules are

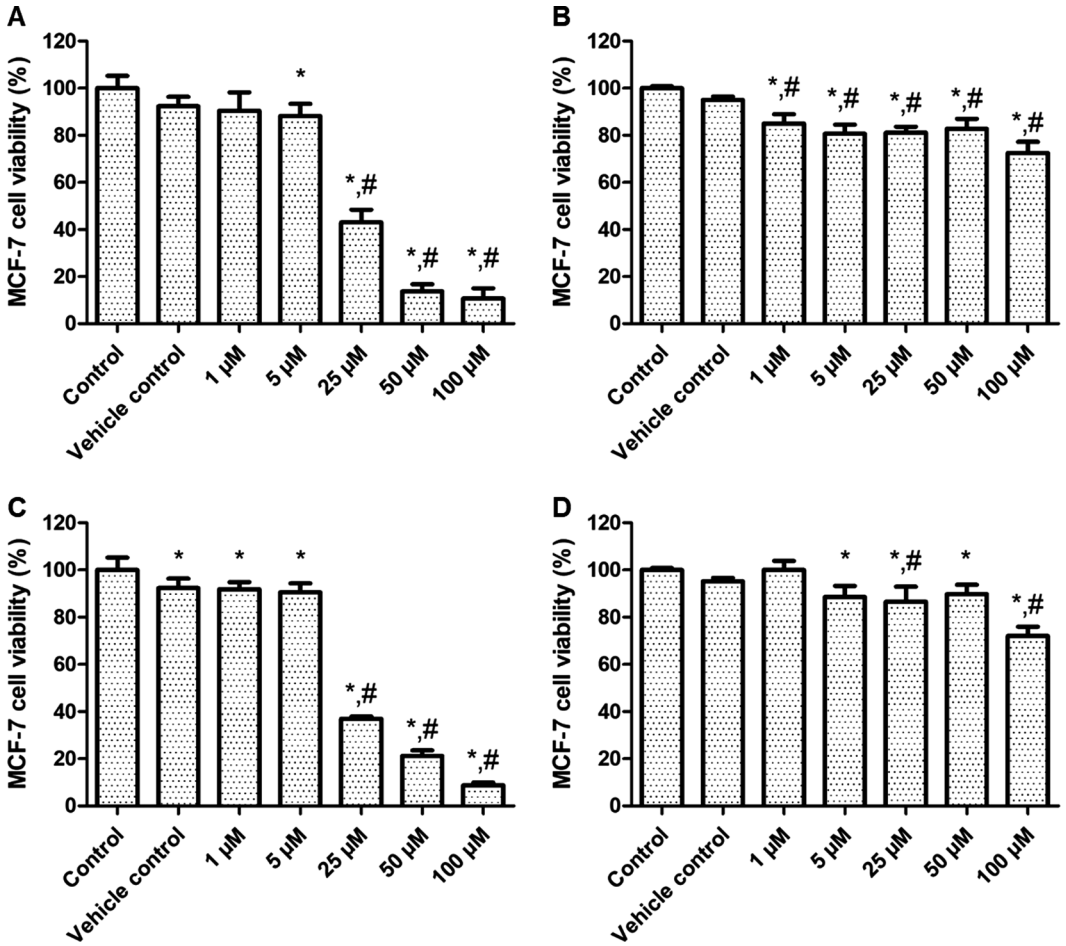


Fig. 7. The relative cell viability of MCF-7 cells after a 24 h treatment with compounds of IX (A), III (B), IV (C) and X (D). The changes on the cell viability (%) caused by test compounds are compared with the control and vehicle control data. Data are shown as the mean ± SD. * $p < 0.05$ *vs* Control and # $p < 0.05$ *vs* Vehicle control.

composed of more than one protein. The interaction of these proteins with molecules at different points has been studied. This was examined both experimentally and theoretically. The results obtained from these methods were compared with each other. The interaction of the molecules studied with the enzymes is shown in Figs. 1–3. All calculations in molecular docking were performed at pH 7.3. The most important reason is that the enzymes studied are most active at this pH. This pH value provides a great harmony between experimental and theoretical values.

In the docking calculations, many parameters are obtained for the interaction of molecules with enzymes. The parameters obtained from the interaction of enzymes and molecules and the numerical value of these parameters are given in Tables 2–4. The first parameter obtained in the docking study Est. Free Energy of Binding, which has the numerical value of this parameter, respectively: −4.11, −4.05, −5.72, −4.32 for AChE enzyme, for AChE, −2.91, −2.99, −0.90, −2.97 for BChE enzyme and −3.79, −3.37, −1.27, −3.78 for α-glycosidase. The first parameter obtained from the interaction of enzymes with molecules is used to compare the biological activities of molecules [66]. If it is desired to list biological activities according to the first parameter, it is seen that it is molecule IX according to AChE enzyme, molecule IV according to BChE enzyme and molecule X according to α-glycosidase enzyme. The molecules with the highest biological activity appear to be similar to the experimental data. Second parameter is Est. Inhibition constant, which is the numerical values of this parameter as follow: 967.98, 1.08, 63.73, 682.12 for AChE, 7.30, 6.45, 217.41, 6.63 for BChE enzyme and 1.67, 3.39, 117.39, 1.69 for α-glycosidase. This parameter shows whether the molecule studied inhibits the enzyme. It has been shown that the enzyme formed by proteins can interact with the substrate. When looking at the numerical values of the molecules studied, if one of the molecules has a numerical value greater than the other molecules, an extra molecule is required to prevent the activity of the enzyme [67–69]. When the obtained results are examined, it is seen that the numerical values of BChE and α-glycosidase enzymes are compatible with experimental values. The third parameter is vdW + Hbond + desolv Energy, whose numerical values are listed as follows: −5.37, −5.85, −6.07, −5.64 for AChE, −5.29, −4.28, −3.19, −4.83 for BChE enzyme and −4.98, −4.95, −4.11, −5.33 for α-Gly. This parameter is one of the most important parameters for molecular docking calculations. This parameter is used to determine the position of the enzymes made up of the molecules and proteins studied. If this value has a positive value for a molecule, it indicates that it does not bind well with the enzyme [6]. When the obtained values are examined, it is seen that the most negative values obtained from the interaction of molecules with enzymes are obtained from molecule IX for AChE enzyme. This was found to be consistent with experimental values. The last parameter examined is Electrostatic Energy, the numerical values obtained for this parameter are listed as follows: −0.06, −0.05, −0.01, +0.02 for AChE, +0.17, −0.04, −0.33, −0.01 for BChE enzyme and −0.02, −0.10, −0.23, −0.10 for α-glycosidase. When these values are examined, if the numerical value of this parameter has a positive value, it shows that there is no chemical interaction between the molecule and the enzymes formed by protein molecules. When the obtained values are examined, it is seen that all values are

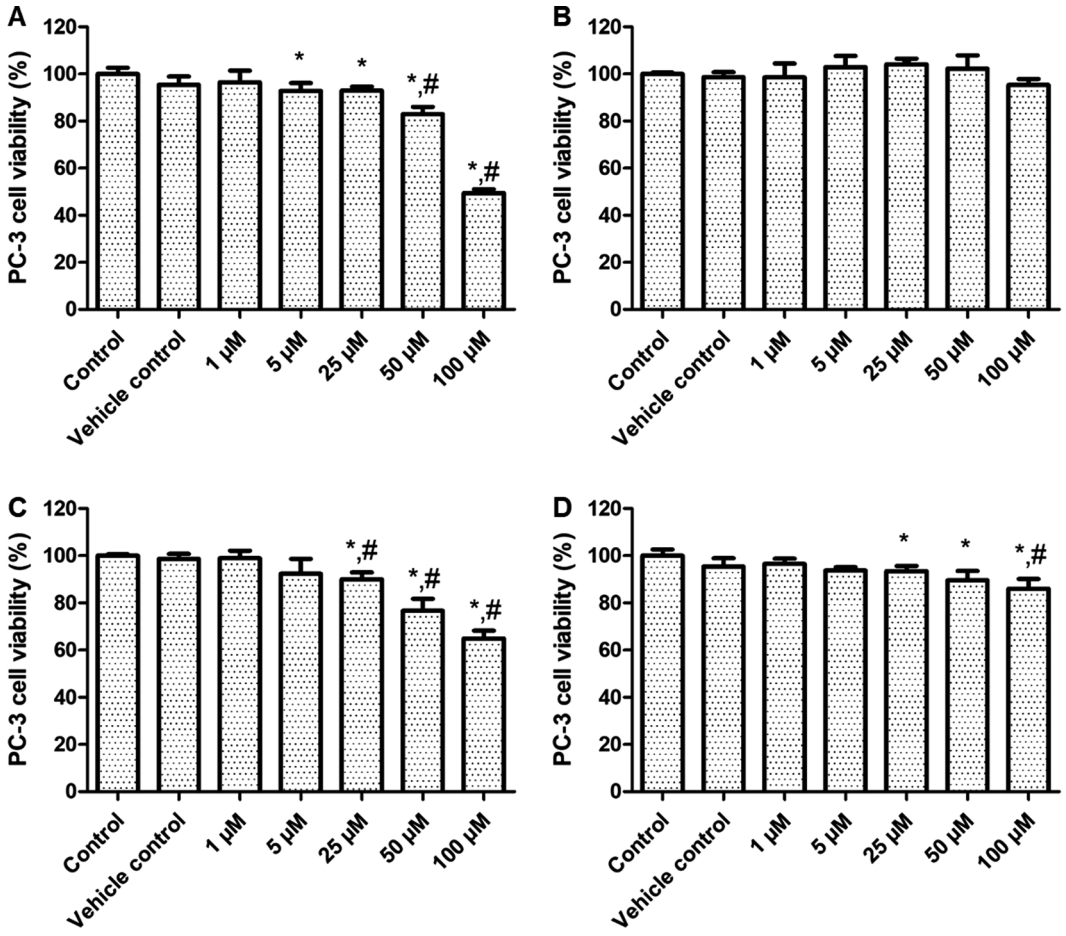


Fig. 8. The relative cell viability of PC-3 cells after a 24 h treatment with compounds of IX (A), III (B), IV (C) and X (D). The changes on the cell viability (%) caused by test compounds are compared with the control and vehicle control data. Data are shown as the mean ± SD. * $p < 0.05$ *vs* Control and # $p < 0.05$ *vs* Vehicle control.

negative in general. This shows that there is a chemical interaction between the enzymes formed by proteins and molecules (Table 3).

Enzymes are composed of many proteins, many of which have been seen to interact with the molecules studied. These interactions have been shown to affect the biological activities of molecules [70–72]. For this, the most important part of docking studies is the part where these interactions are examined. As the interaction between enzymes and molecules increases, the biological activity of the molecules increases. Accordingly, the interactions between the molecule IX and the enzyme AChE are examined in Fig. 4. The enzyme AChE is composed of several proteins, the names of which are TYR77, SER347, and ASP349. The distance between the TYR77 protein and the N3 atom in molecule IX is 3.88 atomic units. The distance between the SER347 protein and the O4 atom in molecule IX is 3.73 atomic units. The distance between the ASP349 protein and the O4 atom in molecule IX is 3.37 atomic units. However, the interactions between the molecule IV and the enzyme BChE are examined in Fig. 5. The enzyme BChE is composed of several proteins, the names of which are ASP185, TYR194, and ASP482. The distance between the ASP185 protein and the O3 atom in molecule IV is 3.76 atomic units and the H22 atom in molecule IV is 3.06 atomic units. The distance between the TYR194 protein and the O3 atom in molecule IV is 3.75 atomic units. The distance between the ASP482 protein and the O3 atom in molecule IV is 3.35 atomic units and the H22 atom in molecule IV is 3.76 atomic units. On the other hand, the interactions between the molecule IV and the enzyme α-Gly are examined in Fig. 6. The enzyme α-Gly is composed of several proteins, the names of which are GLU238. The distance between the GLU238 protein and the O2 atom in molecule III is 3.52 atomic units.

From the light of the results given in the previous explanations, interactions between molecules and enzymes formed by proteins are mostly seen with heteroatoms. In docking studies, it was seen that chemical interactions affect biological activity of molecules. As the interactions between molecules and enzymes increase, the biological activity of the molecules increases. This has been shown to affect the distance between the molecule and the enzyme.

3.4. Anticancer results

Viability changes in human breast and prostate cancer cell lines treated with test compounds for 24 h are shown in Figs. 1 and 2, respectively. High concentrations of all compounds applied to MCF-7 cells significantly reduced cell viability ($p < 0.05$). In addition, other concentrations (especially 25 and 50 μM) were found to affect different levels of viability ($p < 0.05$). In PC-3 cells, high concentrations of compounds (except III compound) caused decrease in cell viability ($p < 0.05$). As shown in Figs. 1 and 2, the cytotoxic effect of the compounds on MCF-7 and PC-3 cells is similar. Doses of compounds IX, IV and X at 50 and 100 μM showed similar effects in both cell lines. On the other hand, it was determined that III showed effect on cell viability only in MCF-7 cells (Fig. 7) and no significant effect on viability in PC-3 cells (Fig. 8). Breast cancer is one of the most common cancers worldwide, causing approximately 570.000 deaths in 2015. Every year more than 1.5 million women (25% of women with cancer) are diagnosed with breast cancer every year in the world [73,74]. The development of breast cancer involves a process involving multiple cell types and disruption of different cellular processes. Breast cancer is a

metastatic cancer and can often pass to distant organs such as bone, liver, lung, and brain if untreated. Early diagnosis of the disease can lead to good prognosis and high survival [75].

4. Conclusions

This study showed that an increase in the intensity of the reaction center of the molecule due to the repulsion of the sulfamide group and the aromatic core leads to an increase in the reaction rate. The creation of sulfamide-containing active dipoles makes it possible to synchronously attach them to polarophiles according to the [3 + 2] or [4 + 2] principle, which makes it easy to synthesize five- and six-membered heterocyclics. Some novel synthesized molecules effectively inhibited some metabolic enzymes such as α-glycosidase, BChE and AChE enzymes at the micromolar levels. As we characterized above, novel derivatives can be acceptable candidate drugs. Additionally, T2DM is a metabolic problem created by high blood glucose content and can reason other health disturbances, like high blood pressure, weakness, nephropathy retinopathy, neuropathy, gangrene, cardiovascular disease, and other dysfunctions. Also, these novel molecules can be selective inhibitor cholinesterases and α-glycosidase enzymes. Docking studies showed that experimental and theoretical calculations were in great harmony. As a result of the experimental processes, the activities of molecules against the enzymes studied were compared. As a result of this comparison, the molecules with the highest biological activity were found to be similar in experimental and theoretical processes. The results showed that the molecule with the highest biological activity against the enzyme AChE is molecule IX. Other enzyme study, the results showed that the molecule with the highest biological activity against the enzyme BChE is molecule IV. Finally, the results showed that the molecule with the highest biological activity against the enzyme α-glycosidase is molecule III.

Declaration of Competing Interest

The authors declare that no conflicts of interests.

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