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ORIGINAL PAPER

Novel phenolic Mannich base derivatives: synthesis, bioactivity, molecular docking, and ADME-Tox Studies

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Abstract

In this study, it was aimed to synthesize novel molecules containing potential biological active phenolic Mannich base moiety and evaluate the inhibition properties against α-glycosidase (α-Gly) and acetylcholinesterase (AChE). For this purpose, phenolic aldehydes (1–3) were synthesized from 4-hydroxy-3-methoxy benzaldehyde (vanillin) according to the Mannich Reaction. Five different carboxylic acid hydrazides (4a-e) were synthesized from esters obtained from carboxylic acids. Fifteen Schiff base derivatives (5a-e, 6a-e, and 7a-e) were synthesized from the condensation reaction of compounds 1–3 with 4a-e. In this work, a series of novel Schiff bases from Phenolic Mannich bases (5a-e, 6a-e, and 7a-e) were tested toward α-Gly and AChE enzymes. Compounds 5a-e, 6a-e, and 7a-e showed Kis in ranging of 341.36 ± 31.84–904.76 ± 93.56 nM on AChE and 176.27 ± 22.87—621.77 ± 69.98 nM on α-glycosidase. Finally, novel compounds were found using molecular docking method to calculate the biological activity of these bases against many enzymes. The enzymes used in these calculations are acetylcholinesterase and α-glycosidase, respectively. Molecule 6b is more effective and active than other molecules with a docking score parameter value of − 8.77 against AChE enzyme and 6d is more effective and active than other molecules with a docking score parameter value of − 4.94 against α-Gly enzyme. After calculating the biological activities of novel compounds, ADME/T analysis parameters were examined to calculate the future drug use properties.

Keywords Phenolic Mannich bases · Schiff bases · Enzyme inhibition · Molecular docking

Introduction

Mannich reaction is a condensation reaction between active hydrogen compounds, an aldehyde, and an amine, leading to the formation of Mannich bases. Mannich bases are generally classified as C-Mannich bases, N-Mannich bases, S-Mannich bases, and P-Mannich bases. Mannich bases have various practical applications such as paint, cosmetics, the improvement of natural macromolecular materials, analytical reagents, water treatment products, petroleum additives, synthetic polymer products, textiles, leather, and paper [1]. However, the most important application area of Mannich bases is pharmaceutical chemistry and this claim is supported by a significant number of papers published on this subject every year. The Mannich reaction can be used to improve the distribution of drugs in the human body. Hydrophilic property of the drug can be increased by inserting a polar group into the structure by Mannich reaction. In addition, if the appropriate amine reagent is used in the Mannich reaction, the lipophilic property of the drug can be increased [2]. In addition, aminomethylated drugs can act as prodrugs, by releasing the active part with deaminomethylation [3] or deamination [4] under controlled hydrolytic conditions. Phenolic Mannich bases are in the group of C-Mannich bases and represent a very important class of compounds due to the formation of the C–C bond and their biological activities. There are many studies in the literature that phenolic Mannich bases show important biological activities such as

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anticancer and cytotoxic activity [5–7], antibacterial activity [8, 9], antimycobacterial activity [10, 11], antifungal activity [12, 13], antiviral activity [14–16], anti-hepatitis-B activity [17, 18], anticonvulsant activity [19, 20], anti-inflammatory activity [21, 22], analgesic activity [23], antioxidant [24, 25], enzyme inhibition activity [26], regulation of blood pressure [27, 28], and platelet aggregation inhibitory activity [29].

Type-2 diabetes mellitus (T2DM) is one of the most popular metabolic diseases in the world and is defined by hyperglycemia [30, 31]. α-Glycosidase enzymes are in the nutritive system that hydrolyzes carbohydrate molecules into glucose molecules. One mechanism that has been developed to therapy of T2DM is inhibition of α-glycosidase enzymes using synthetic drugs [32, 33]. Acetylcholinesterase (AChE) is an enzyme present in cholinergic neurons, and its key role is the rapid breakdown of the neurotransmitter acetylcholine (ACh) released throughout neurotransmission. The level of the active site of AChE is a 20 Å length gorge that includes an anionic catalytic site and a peripheral anionic binding site. Additionally, AChE inhibitor compounds modulate ACh hydrolysis and also play an important role in diagnosing the cholinergic tone [34–37].

When many studies are examined today, it is seen that theoretical and experimental studies are carried out together. It is seen that the studies done in this way are more popular. When these studies are examined, it is seen that the results of experimental and theoretical calculations are very close to each other [38–43]. Therefore, the theoretical calculations made before the experimental procedures guide the experimental procedures. With theoretical calculations, it is possible to synthesize more effective and active molecules with their results. In this study, the best method to compare the biological activities of novel compounds against enzymes for theoretical calculations is molecular docking. In the calculations made by molecular docking method, the biological activity values of the molecules found as a result of calculations made against enzymes of compounds 5a-e, 6a-e, and 7a-e are estimated. It is possible to compare the biological activities of other molecules with the numerical values obtained as a result of the calculation [44, 45]. Many parameters are obtained by molecular docking calculations of novel molecules against enzymes. From these parameters obtained from molecular docking calculations, a lot of information about the biological activities of novel molecules is obtained [46]. After these calculations, ADME/T (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis of novel molecules was performed. ADME/T analysis aims to predict the effects and reactions of molecules that can be drugs on the human body [47, 48]. These molecules have many parameters with ADME/T calculations. These effects and responses are tried to be predicted with numerical values of these parameters. Each parameter calculated theoretically calculates the effects of drug molecules on different organs and tissues. It is theoretically known whether these drug molecules will be more effective by applying them to the skin, taking them orally, or as a vaccine.

In this study, it was aimed to synthesize new Schiff bases from Phenolic Mannich bases and test the enzyme inhibition activities against α-Gly and AChE and molecular docking studies.

Result and discussion

Chemistry

Phenolic aldehydes (1–3) were synthesized according to the Mannich Reaction. Carboxylic acid hydrazides (4a-e) were synthesized from esters obtained from suitable carboxylic acids according to Fischer Esterification. In the last step phenolic aldehydes (1–3) were reacted with hydrazides (4a-e) and target compounds (5a-e, 6a-e, and 7a-e) were obtained with good yields (79–93%). Structures of new compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, and HRMS spectroscopic methods.

In the IR spectra of the compounds 5a-e, 6a-e, and 7a-e, NH stretching bands are observed at 3273–3273 cm^{-1}. C = O and CH = N stretching bands are observed at 1670–1626 cm^{-1} and 1611–1583 cm^{-1}, respectively. NH, C = O, and CH = N stretching bands are the characteristic bands of the compounds and compatible with structures.

In the ^{1}H NMR spectra of the compounds 5a-e, 6a-e, and 7a-e, peaks of NH protons are seen as a singlet at δ 12.05–10.20 ppm. Peaks of N = CH protons are observed as singlet at δ 8.75–8.19 ppm. Aromatic proton peaks are observed at δ 8.43–6.79 ppm as singlet, doublet, triplet, and multiplet relative to their chemical environment. Peaks of OCH_3 and Ph-CH_2-N protons are seen as a singlet at δ 3.98–3.79 and δ 3.85–3.61 ppm, respectively. Aliphatic protons of morpholine, piperazine, and piperidine moieties are observed at δ 3.78–0.80 ppm as doublet, triplet, and multiplet relative to their chemical environment. In the ^{1}H NMR spectra of compounds containing phenoxyacetyl moiety (5d, 6d, and 7d), peaks of NH, N = CH, and Ph–O–CH_2 protons are seen as two singlets due to E/Z isomers and cis/trans conformers of the molecules [49–51].

In the ^{13}C NMR spectra of final molecules, peaks of C = O and CH = N carbons are seen at δ 166.8–160.7 ppm and δ 149.9–147.4 ppm, respectively. Peaks of aromatic carbons are observed at δ 168.8–108.1 ppm. Peaks of Ph–O–CH_2 and OCH_3 carbons are seen at δ 61.4–57.9 ppm and δ 57.5–55.5 ppm, respectively. And aliphatic carbons of morpholine, piperazine, and piperidine moieties are observed at δ 66.7–19.2 ppm.

Finally, in the HRMS spectra of target compounds, the found M + 1 molecular ion values are compatible with the calculated M + 1 molecular ion values. Spectral data of the compounds are fully compatible with the structure of the molecules.

Bioactivity

The enzyme inhibitory effects of all the novel Schiff bases (5a-e, 6a-e, and 7a-e) were investigated against AChE and α-glycosidase as described in previous methods. The inhibitory activities were compared to standard compounds (Table 1 and Fig. 1). The following results were recorded:

These cholinergic enzyme inhibition results are reported in Table 1. Compounds 5a-e, 6a-e, and 7a-e had Kis in ranging from 341.36 ± 31.84 to 904.76 ± 93.56 nM for AChE (Table 1 and Fig. 1). In comparison, TAC had Ki of 787.25 ± 80.61 nM against indicated AChE enzyme. It could be seen from the table that all novel molecules demonstrated marked inhibitory effects against AChE with

Table 1 The enzyme inhibition results of compounds 5a-e, 6a-e, 7a-e against achethylcholinesterase (AChE) and α-glycosidase (α-Gly) enzymes

Compounds	IC_{50} (nM)			Ki (nM)		
	AChE	r^2	α-Gly	r^2	AChE	α-Gly
5a	1094.27	0.9781	411.28	0.9558	845.15 ± 56.01	476.13 ± 60.32
5b	502.88	0.9889	193.24	0.9972	441.88 ± 29.07	238.03 ± 26.95
5c	592.67	0.9610	244.06	0.9921	504.81 ± 50.01	278.03 ± 33.18
5d	632.51	0.9937	212.88	0.9328	553.94 ± 66.17	226.98 ± 37.92
5e	600.42	0.9614	200.74	0.9660	498.06 ± 43.06	254.14 ± 50.83
6a	835.02	0.9637	318.10	0.9031	779.21 ± 82.80	354.83 ± 26.96
6b	398.46	0.9041	201.52	0.9715	341.36 ± 31.84	214.04 ± 53.82
6c	445.90	0.9382	155.37	0.9183	401.96 ± 38.87	176.27 ± 22.87
6d	549.25	0.9901	192.66	0.9912	491.55 ± 49.77	200.15 ± 48.51
6e	513.86	0.9779	197.13	0.9551	455.15 ± 46.93	209.45 ± 26.17
7a	1015.74	0.9472	612.92	0.9621	904.76 ± 93.56	621.77 ± 69.98
7b	802.53	0.9284	504.06	0.9815	698.15 ± 72.08	557.13 ± 39.67
7c	841.38	0.9620	441.57	0.9228	735.85 ± 81.04	478.32 ± 67.90
7d	943.26	0.9399	341.48	0.9293	879.33 ± 90.02	359.62 ± 17.86
7e	813.44	0.9692	368.01	0.9815	711.47 ± 59.72	395.03 ± 47.01
TAC**	848.84	0.9668	–	–	787.25 ± 80.61	–
ACR***	–	–	513.41	0.9491	–	548.13 ± 62.38

*(They are control compounds)

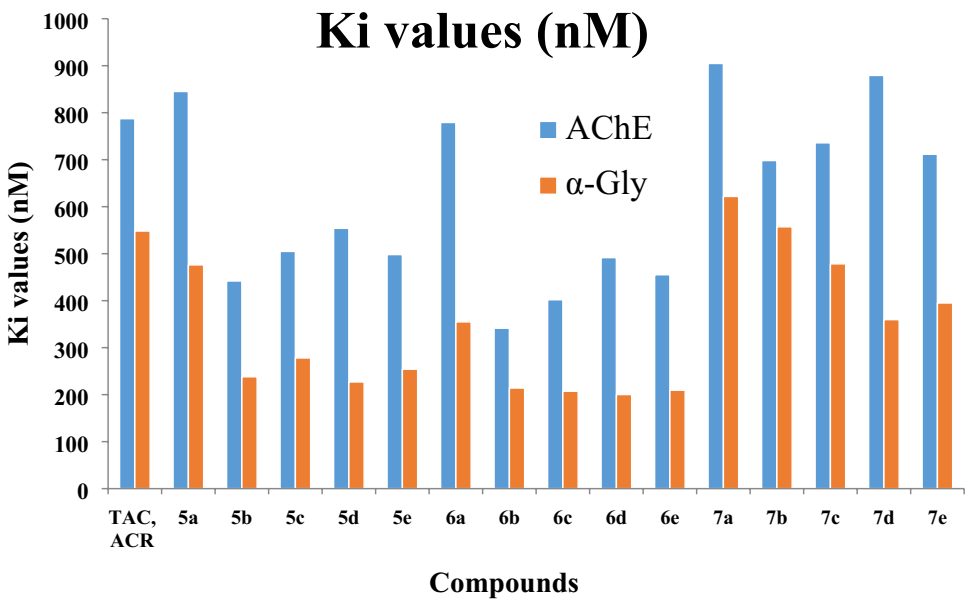

Fig. 1 Ki values of AChE and α-glycosidase enzymes

Ki values ranging in sub-nanomolar; however, compound 6b showed perfect inhibition effect against AChE (Ki: 341.36 ± 31.84 nM; Ki-TAC/Ki-6b: 2.28) (Table 1). For AChE, IC_{50} values of TAC as positive control and some novel compounds the following order: 6b (398.46 nM, r^2: 0.9041) < 6c (445.90 nM, r^2: 0.9382) < 5b (502.88 nM, r^2: 0.9882) < 6e (513.86 nM, r^2: 0.9779) < TAC (848.84 nM, r^2: 0.9668). Cholinergic neurotransmission plays a key role in impaired cognitive function in AD and adult-onset dementia disorders. Treatments to counteract amyloid-accumulation, tau hyperphosphorylation, and immunotherapy have been recommended but failed to produce effects and were therefore discontinued in phase II or III clinical trials. At present, enhancing cholinergic neurotransmission still represents the main approach to symptomatic treatment of cognitive and behavioral symptoms of mild and moderate stages of AD. In line with this therapeutic strategy, various molecules like linopirdine, an agent that increases hippocampus ACh release, muscarinic ACh receptor agonists like xanomeline, and AChE inhibitor compounds like tacrine and physostigmine were used [52–55].

Inhibitors of this enzyme delay the breakdown of carbohydrate molecules in the small intestine and diminish the postprandial blood glucose excursion; hence, inhibition of glycosidase enzyme has an important effect on polysaccharide metabolism, cellular interaction, glycoprotein processing, and widening opportunities for the discovery of new therapeutic factors against diseases like obesity, viral infection, diabetes, and metastatic cancer [56–58]. For α-glycosidase, IC_{50} values of ACR as positive control and some novel molecules the following order: 6d (192.66 nM, r^2: 0.9912) < 5b (193.24 nM, r^2: 0.9972) < 6c (195.37 nM, r^2: 0.9183) < 6b (201.52 nM, r^2: 0.9715) < ACR (513.41 nM, r^2: 0.9491). Indeed, AG as a glycosidase located in the brush border of the small intestine can selectively hydrolyze terminal (1 → 4)-linked α-glucose residues (disaccharides or starch) to release a single α-glucose molecule. Finally, for the α-glycosidase, compounds 5a-e, 6a-e, and 7a-e showed Kis between 176.27 ± 22.87–621.77 ± 69.98 nM (Table 1 and Fig. 1). The results demonstrated that compound 6d had effective α-glycosidase inhibition effects than that of acarbose (Ki: 548.13 ± 62.38 nM) as standard α-glycosidase inhibitor. Also, highly effective Kis were calculated for compound 6d (Ki: 176.27 ± 22.87 nM).

Molecular docking

The numerical values of many parameters obtained by the Schrödinger program used to calculate the biological activities of compounds 5a-e, 6a-e, 7a-e against enzymes are compared with the biological activity values of the molecules [80]. The numerical values of these parameters obtained for this comparison are used. The enzymes used to make this comparison are acetylcholinesterase (AChE) (pdb ID:4M0E) and α- glycosidase (α-Gly) (pdb ID:1R47). The parameters obtained from the interaction of synthesized compounds with these enzymes are given in Table 2.

As a result of molecular docking calculations, many parameters obtained for novel molecules are calculated. The most important parameter among these calculated parameters is the Docking score parameter of molecules. The numerical value of this parameter is a numerical expression of the interactions between molecules and enzyme. As a result of calculations, the molecule with the most negative numerical value of this parameter has higher biological activity than other molecules. It should be well known that the more interaction between any molecule and enzyme, the more the numerical value of this parameter decreases [46, 59]. Therefore, the most important factor affecting the biological activities of molecules is the interactions between compounds 5a-e, 6a-e, and 7a-e of enzymes. These interactions have many interactions such as hydrogen bonds, polar and hydrophobic interactions, π-π, and halogen [60–66]. These interactions are given in Figs. 2 and 3.

Many more parameters were obtained from the interaction of compounds 5a-e, 6a-e, and 7a-e with enzymes. These parameters explain the interaction between molecule and enzyme from different angles. Glide hbond, Glide evdw, and Glide ecoul parameters of novel compounds provide information about the chemical interactions between molecules and enzymes [46, 59]. These interactions are hydrogen bonding, van der Waals, and Coulomb interactions. On the other hand, Glide emodel, Glide energy, Glide einternal, and Glide posenum parameters of novel Schiff bases provide information about the interaction pose that occurs in the interaction between molecules and enzymes [67, 68]. Another parameter obtained from molecular docking calculations is Glide Ligand Efficiency, which is a numerical value of the efficiency of novel Schiff bases against enzymes [69, 70].

After the studied molecules interact with cancer proteins, ADME/T analysis was performed to use the molecules as advanced drugs. With this analysis, the behavior of molecules in human metabolism is tried to be predicted theoretically. With this analysis, how it is absorbed, functioning and excretion process by human tissues and organs is examined. Among the parameters to be examined for this analysis, the two most important parameters are RuleOfFive [71, 72] and RuleOfThree [73]. These parameters consist of a combination of many parameters. The numerical values of these parameters are required to be minimum zero and maximum three for RuleOfThree and four for RuleOfFive (Table 3).

Table 2 Numerical values of the docking parameters of molecule against enzymes

AChE	Docking score	Glide ligand efficiency	Glide hbond	Glide evdw	Glide ecoul	Glide emodel	Glide energy	Glide einternal	Glide posenum
5a	–	–	–	–	–	–	–	–	–
5b	–	–	–	–	–	–	–	–	–
5c	−7.84	−0.26	−0.13	−29.94	−10.73	−58.21	−40.68	8.63	183
5d	−7.66	−0.27	−0.46	−37.55	−13.55	−77.20	−51.11	11.79	394
5e	−8.24	−0.32	−0.40	−35.03	−14.26	−80.78	−49.29	10.06	71
6a	−8.24	−0.25	0.00	−32.92	−19.06	−78.60	−51.97	14.69	377
6b	−8.77	−0.26	−0.36	−44.94	−15.27	−86.88	−60.21	20.99	330
6c	−8.03	−0.22	0.00	−37.76	−16.62	−82.73	−54.39	7.48	60
6d	−4.78	−0.14	−0.30	−44.16	−9.09	−63.13	−53.25	7.89	221
6e	–	–	–	–	–	–	–	–	–
7a	−8.64	−0.34	−0.32	−35.14	−11.38	−84.47	−46.52	8.64	73
7b	–	–	–	–	–	–	–	–	–
7c	–	–	–	–	–	–	–	–	–
7d	6.71	−0.23	−0.58	−40.46	−8.24	−70.77	−48.69	11.73	116
7e	–	–	–	–	–	–	–	–	–
α-Gly	Docking score	Glide ligand efficiency	Glide hbond	Glide evdw	Glide ecoul	Glide emodel	Glide energy	Glide einternal	Glide posenum
5a	−4.29	−0.20	−0.01	−21.59	−16.00	−55.62	−37.59	4.73	365
5b	−4.59	−0.16	−0.47	−28.61	−12.00	−51.08	−40.61	5.03	334
5c	−3.55	−0.12	−0.02	−31.36	−6.13	−43.31	−37.49	7.18	84
5d	−2.96	−0.11	−0.28	−26.30	−7.83	−35.60	−34.13	8.30	233
5e	−2.97	−0.11	−0.06	−26.50	−5.91	−35.83	−32.41	8.02	109
6a	−4.58	−0.14	0.00	−27.28	−14.04	−53.20	−41.32	11.34	353
6b	−4.38	−0.13	−0.16	−33.05	−10.90	−54.68	−43.95	13.88	245
6c	–	–	–	–	–	–	–	–	–
6d	−4.94	−0.16	−0.20	−34.88	−10.80	−55.42	−45.68	7.28	397
6e	−2.88	−0.09	0.00	−31.93	−5.71	−41.33	−37.64	13.25	208
7a	−2.00	−0.07	−0.21	−25.05	−4.40	−35.95	−29.45	2.03	5
7b	−4.50	−0.16	−0.16	−30.80	−11.00	−55.72	−41.81	5.87	318
7c	–	–	–	–	–	–	–	–	–
7d	−2.30	−0.08	−0.22	−25.85	−7.93	−36.50	−33.78	5.49	371
7e	–	–	–	–	–	–	–	–	–

Conclusion

Biological activity values of compounds 5a-e, 6a-e, and 7a-e against acetylcholinesterase (AChE) and α- glycosidase (α-Gly) were calculated. This novel Schiff bases derived from phenolic Mannich bases were recorded to have anticholinergic and antidiabetic properties; also, they are appropriate for future significant drug searches. Afterward, after examining the interaction of these compounds against enzymes, ADME/T analysis was made and the properties of drugs were examined. As a result of the docking calculations of the molecules, it has been seen that 6b is more effective and active than other molecules with a docking score parameter value of − 8.77 against AChE enzyme and 6d is more effective and active than other molecules with a docking score parameter value of − 4.94 against α-Gly enzyme. The presence of more electron pairs on heteroatoms in molecules and the interaction of the regions with higher electron density with enzymes increase the activity. After this examination, numerical values of parameters of new molecules can be used in future in vivo and in vitro studies for the discovery of new drug candidates.

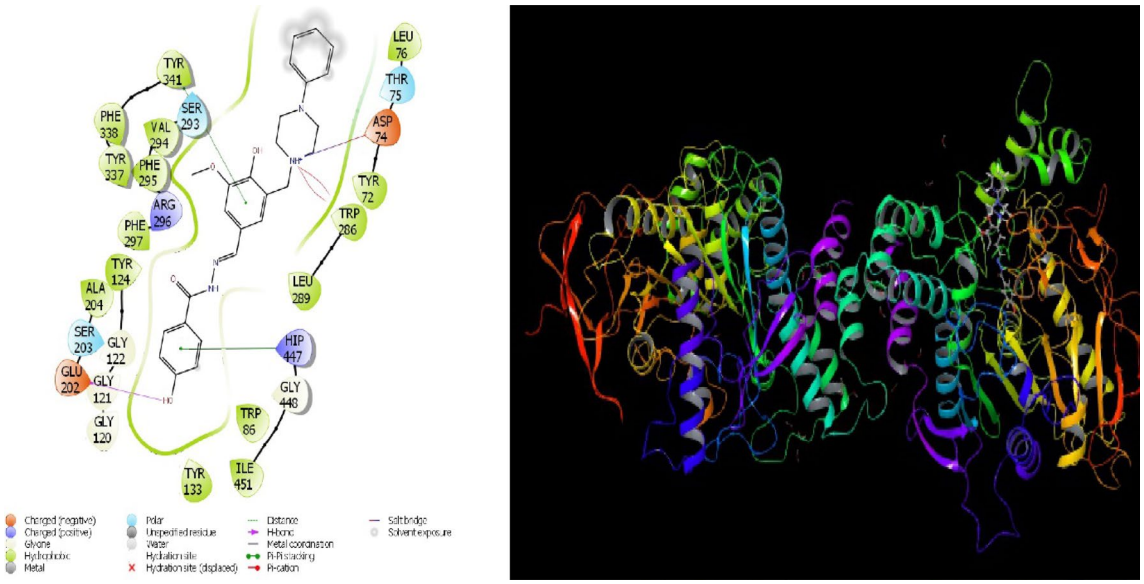

Fig. 2 Presentation interactions of molecule 6b with AChE enzyme

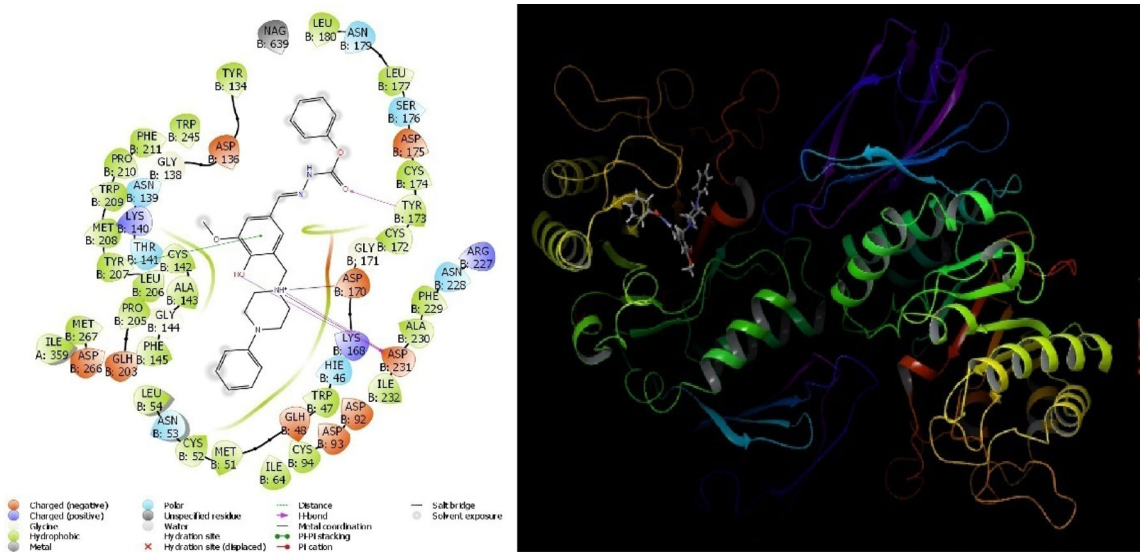

Fig. 3 Presentation interactions of molecule 6d with α-Gly enzyme

Experimental

Chemistry

The chemicals used in this study were supplied from Sigma-Aldrich (Germany). Melting points were determined on WRS-2A Microprocessor Melting-point Apparatus and are uncorrected. IR spectra of compounds were recorded using ALPHA-P BRUKER FT-IR Spectrophotometer.^{1}H-NMR spectra were recorded on Bruker (400 MHz) spectrometer. ^{13}C-NMR spectra were recorded on Bruker (100 MHz) spectrometer. Chemical shifts are reported as δ in ppm relative to tetramethylsilane (TMS) (δ 0.00 singlets) in deuterated dimethylsulfoxide (DMSO-d_6) and deuterated chloroform ($CDCl_3$). HRMS spectra were recorded on Agilent 6530 Accurate-Mass spectrometer, and acetonitrile was used as the solvent.

General procedure for synthesis compounds 1–3

To a solution of paraformaldehyde (15 mmol) in ethanol (20 mL), secondary amine (12 mmol) was added and the mixture was refluxed for an hour. 4-Hydroxy-3-methoxy

Table 3 ADME properties of molecule

	5a	5b	5c	5d	5e	6a	6b	6c	Referance range
mol_MW	293	385	414	385	375	445	461	490	130–725
dipole (D)	6.5	5.1	12.3	5.3	8.1	6.8	5.0	12.6	1.0–12.5
SASA	577	691	717	733	668	794	808	834	300–1000
FOSA	336	310	310	318	310	258	258	258	0–750
FISA	178	185	231	143	138	115	170	215	7–330
PISA	64	197	177	271	175	422	380	361	0–450
WPSA	0.0	0.0	0.0	0.0	44.8	0.0	0.0	0.0	0–175
volume (A^3)	987	1244	1295	1268	1205	1451	1476	1528	500–2000
donorHB	2	3	2	2	2	2	3	2	0–6
accptHB	7.7	8.45	8.7	8.2	7.7	7	7.75	8	2.0–20.0
glob (Sphere = 1)	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.75–0.95
QPpolrz (A^3)	29.4	39.7	41.6	42.1	38.6	50.8	50.8	52.7	13.0–70.0
QPlogPC16	9.5	13.2	13.6	13.5	12.4	16.1	16.6	17.1	4.0–18.0
QPlogPoct	17.0	22.3	23.1	21.3	20.5	24.1	25.9	26.6	8.0–35.0
QPlogPw	11.7	14.7	13.7	13.5	12.2	13.3	15.4	14.5	4.0–45.0
QPlogPo/w	0.6	1.8	1.8	2.5	2.4	4.5	3.7	3.8	−2.0–6.5
QPlogS	−1.9	−3.1	−3.5	−4.1	−3.3	−5.5	−5.2	−5.7	−6.5–0.5
CIQPlogS	−1.6	−3.4	−3.9	−3.6	−3.5	−5.5	−5.5	−6.0	−6.5–0.5
QPlogHERG	−5.3	−6.3	−6.3	−7.2	−6.0	−7.8	−7.7	−7.7	*
QPPCaco (nm/sec)	51	44	16	109	122	202	60	22	**
QPlogBB	−1.2	−1.5	−2.0	−1.1	−0.8	−0.8	−1.5	−2.0	−3.0–1.2
QPPMDCK (nm/sec)	22	19	6	50	99	97	26	9	**
QPlogKp	−6.0	−5.4	−6.3	−4.5	−4.7	−3.4	−4.5	−5.4	Kp in cm/hr
IP (ev)	9.5	9.4	9.7	9.5	9.4	9.0	9.0	9.1	7.9–10.5
EA (eV)	0.2	0.1	2.3	0.1	0.6	0.1	0.0	2.2	−0.9–1.7
#metab	5	6	6	5	6	4	5	5	1–8
QPlogKhsa	−0.4	−0.1	0.0	0.1	0.1	0.8	0.6	0.7	−1.5–1.5
Human Oral Absor	3	2	2	3	3	3	3	2	–
Per. Human Oral Absor	61	67	59	78	78	94	81	73	***
PSA	106	116	140	104	92	89	112	136	7–200
RuleOfFive	0	0	0	0	0	0	0	0	Maximum is 4
RuleOfThree	0	0	1	0	0	0	0	2	Maximum is 3
Jm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	–

	6d	6e	7a	7b	7c	7d	7e	Referance range
mol_MW	461	451	381	397	426	397	387	130–725
dipole (D)	5.2	8.0	7.6	5.9	13.4	6.1	8.9	1.0–12.5
SASA	848	783	713	727	753	767	702	300–1000
FOSA	266	258	365	365	365	373	365	0–750
FISA	129	123	108	164	209	122	117	7–330
PISA	453	357	240	197	178	271	175	0–450
WPSA	0.0	44.8	0.0	0.0	0.0	0.0	44.8	0–175
volume (A^3)	1501	1438	1292	1316	1368	1341	1278	500–2000
donorHB	2	2	2	3	2	2	2	0–6
accptHB	7.5	7	6	6.75	7	6.5	6	2.0–20.0
glob (Sphere = 1)	0.7	0.8	0.8	0.8	0.8	0.8	0.8	0.75–0.95
QPpolrz (A^3)	53.1	49.7	42.7	42.6	44.5	45.0	41.5	13.0–70.0
QPlogPC16	17.1	15.8	13.2	13.7	14.2	14.1	12.9	4.0–18.0
QPlogPoct	24.9	24.0	20.6	22.3	23.3	21.4	20.6	8.0–35.0
QPlogPw	14.3	12.9	10.9	13.0	12.0	11.8	10.5	4.0–45.0

Table 3 (continued)

	6d	6e	7a	7b	7c	7d	7e	Referance range
QPlogPo/w	4.5	4.4	3.7	3.0	3.0	3.8	3.8	−2.0–6.5
QPlogS	−6.3	−5.5	−4.5	−4.2	−4.6	−5.2	−4.4	−6.5–0.5
CIQPlogS	−5.7	−5.6	−4.2	−4.2	−4.7	−4.4	−4.3	−6.5–0.5
QPlogHERG	−8.5	−7.4	−6.6	−6.4	−6.5	−7.3	−6.1	*
QPPCaco (nm/sec)	149	167	233	69	26	172	192	**
QPlogBB	−1.1	−0.7	−0.6	−1.3	−1.8	−0.9	−0.6	−3.0–1.2
QPPMDCK (nm/sec)	70	139	113	30	10	81	162	**
QPlogKp	−3.6	−3.8	−4.0	−5.0	−5.9	−4.1	−4.4	Kp in cm/hr
IP (ev)	9.0	9.0	9.4	9.4	9.6	9.4	9.4	7.9–10.5
EA (eV)	0.0	0.6	0.0	0.0	2.2	0.0	0.5	−0.9–1.7
#metab	4	5	4	5	5	4	5	1–8
QPlogKhsa	0.8	0.8	0.6	0.4	0.5	0.6	0.5	−1.5–1.5
Human Oral Absor	1	3	3	3	2	3	3	–
Per. Human Oral Absor	92	93	91	77	70	89	90	***
PSA	100	87	83	106	130	93	81	7–200
RuleOfFive	0	0	0	0	0	0	0	Maximum is 4
RuleOfThree	1	0	0	0	0	0	0	Maximum is 3
Jm	0.0	0.0	0.0	0.0	0.0	0.0	0.0	–

*Corcern below −5, **a < 25 is poor and a > 500 is great, ***b < 25 is poor and b > 80 is high

benzaldehyde (vanillin) (10 mmol) was added to this mixture and refluxed for 4 h. Reaction progress was monitored by TLC (hexane: ethyl acetate–7:3). After completion, half of the solvent was removed under reduced pressure and the mixture was left in the freezer overnight and the formed solid was filtered off. The crude product was recrystallized from ethanol (Fig. 4).

4-Hydroxy-3-methoxy-5-(morpholinomethyl)benzaldehyde (1)

White solid; yield: 85%, mp: 99–100 °C [74]. IR (ATR, cm^{-1}) ν_{max}2945, 2866, 2829, 2733, 1647, 1592, 1270, 1120, 868, 705; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 9.79 (s, 1H), 7.36 (m, 1H), 7.19 (m, 1H) 3.95 (s, 3H), 3.82 (s, 2H), 3.78 (m, 4H), 2.63 (m, 4H); ^{13}C-NMR (100 MHz, $CDCl_3$)δ 190.6, 153.5, 148.6, 128.5, 125.5, 120.3, 109.8, 66.6 (2C), 61.1, 56.0, 52.7 (2C).

4-Hydroxy-3-methoxy-5-((4-phenylpiperazin-1-yl)methyl) benzaldehyde (2)

White solid; yield: 90%, mp: 156 °C (lit: 156–157 °C) [75]. IR (ATR, cm^{-1}) ν_{max}2959, 2938, 2827, 2737, 1677, 1586, 1315, 1235, 1141, 760, 691;^{1}H-NMR (400 MHz, $CDCl_3$) δ10.90 (brs, 1H), 9.81 (s, 1H), 7.39–7.38 (m, 1H), 7.32–7.27

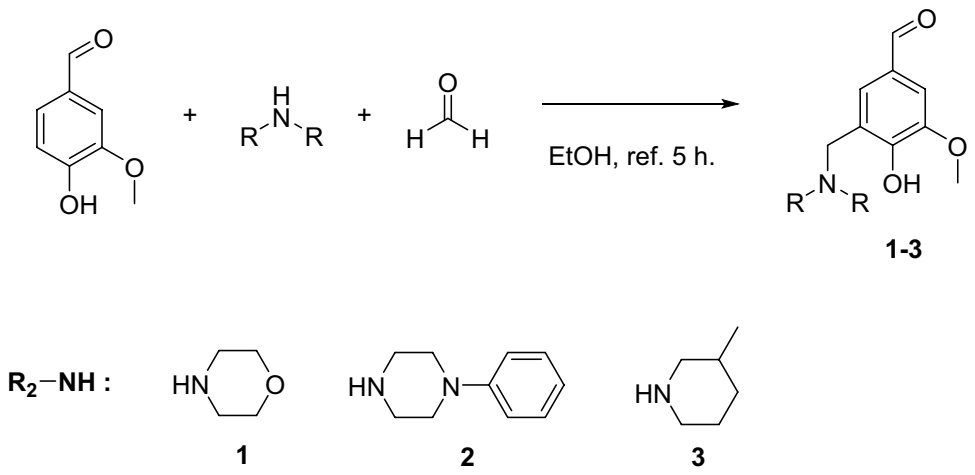

Fig. 4 Synthesis of compounds 1–3

(m, 2H), 7.22–7.17 (m, 1H) 6.95–6.86 (m, 3H), 3.96 (s, 3H), 3.89 (s, 2H), 3.28 (m, 4H), 2.80 (m,4H); ^{13}C-NMR (100 MHz, $CDCl_3$)δ 190.6, 153.7, 150.7, 148.7, 129.2 (2C), 128.4, 125.5, 120.6, 120.4, 116.5 (2C), 109.9, 60.8, 56.0, 52.5 (2C), 49.2 (2C).

4-Hydroxy-3-methoxy-5-((3-methylpiperidin-1-yl)methyl) benzaldehyde (3)

Light brown solid; yield: 88%, mp: 142–144 °C; IR (ATR, cm^{-1}) ν_{max}2946, 2922, 2853, 2748, 1651, 1592, 1271, 1147, 864, 707;^{1}H-NMR (400 MHz, $CDCl_3$) δ11.55 (s, 1H), 9.76 (s, 1H), 7.33 (m, 1H), 7.15 (m, 1H) 3.94 (s, 3H), 3.78 (m, 2H), 2.96–2.90 (m, 2H), 2.11 (t, J = 10.5 Hz, 1H), 1.83–1.58 (m, 5H), 0.98–0.95 (m, 1H), 0.89 (d, J = 6.3 Hz, 3H),^{13}C-NMR (100 MHz, $CDCl_3$)δ 190.6, 154.8, 148.6, 127.8, 125.4, 120.8, 109.5, 61.2, 60.7, 55.9, 53.2, 32.2, 31.0, 25.0, 19.2.

General procedure for synthesis compounds 4a-e

Concentrated H_2SO_4 (1 mL) was added to the solution of suitable carboxylic acids (10 mmol) in methanol (20 mL) and refluxed for 2 h. The mixture was cooled to room temperature, and saturated $NaHCO_3$ solution (50 mL) was added slowly. The aqueous layer was extracted with dichloromethane (3 × 15 mL). Organic layer was dried with sodium sulfate and the solvent was removed by evaporation under reduced pressure. The crude product was dissolved ethanol (20 ml) and hydrazinium hydroxide (5 ml, 80%) was added to this mixture. It was refluxed for 4 h and left in the freezer overnight. Formed crystals were filtered off, dried and recrystallized from ethanol (Fig. 5).

General procedure for synthesis compounds 5a-e, 6a-e, and 7a-e

Suitable aldehyde (1–3) (10 mmol) and hydrazide (4a-e) (10 mmol) were dissolved in absolute ethanol (20 mL) and 4–5 drops of acetic acid was added. Reaction mixture was refluxed for 2–3 h. Compounds 5a-e and 6a-e precipitated during the reaction. For compounds 7a-e, half of the solvent was removed under reduced pressure and the mixture was left in the freezer overnight and the formed solid was filtered off. The crude product was recrystallized from ethanol (Fig. 6).

N′-(4-hydroxy-3-methoxy-5-(morpholinomethyl) benzylidene)benzohydrazide(5a)

White solid, yield: 84%, mp:231–233 °C, IR (ATR, cm^{-1}) ν_{max}3183, 3062, 2951, 1639, 1601, 1303, 1116, 858, 690;^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 10.20 (s, 1H), 8.30 (s, 1H), 7.92 (d, 2H, J = 8.0 Hz), 7.50 (t, 1H, J = 7.2 Hz),7.40 (t, 1H, J = 7.4 Hz), 7.24 (s, 1H), 6.91 (s, 1H), 3,81 (s, 3H), 3.72 (m, 4H), 3.62 (s, 2H), 2,52 (m, 4H). ^{13}C-NMR (100 MHz, $CDCl_3$,δ/ppm): δ164.5, 149.5, 149.2, 148.2, 133.2, 131.9, 128.6 (2C), 127.6 (2C), 125.0, 121.9, 120.4, 109.1, 66.6 (2C), 61.0, 55.9, 52.7 (2C). HRMS (Q-TOF) *m/z* calcd for $C_{20}H_{23}N_3O_4$: 369.1689, found: 370.1745 $[M+H]^+$.

N′-(4-hydroxy-3-methoxy-5-(morpholinomethyl)benzylide ne)-4-hydroxybenzohydrazide(5b)

White solid, yield: 88%, mp:249–250 °C, IR (ATR, cm^{-1}) ν_{max}3193, 3069, 2937, 1649, 1607, 1304, 1108, 852, 698;^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ 11.51 (s, 1H), 8.31 (s, 1H), 7.80 (d, 2H, J = 8.0 Hz), 7.22 (s, 1H), 7.07 (s, 1H), 6.86 (d, 2H, J = 8.0 Hz), 3,83 (s, 3H), 3.63 (s, 2H), 3.61 (m, 4H), 2.45 (m, 4H). ^{13}C-NMR (100 MHz, DMSO-d_6, δ/ppm): δ162.6, 160.5, 147.9, 147.7, 147.4, 129.6 (2C), 125.1, 124.0, 122.5 (2C), 121.9, 115.0, 108.1, 66.1 (2C), 58.2, 55.6, 52.7 (2C). HRMS (Q-TOF) *m/z* calcd for $C_{20}H_{23}N_3O_5$: 385.1638, found: 386.1695 $[M+H]^+$.

N′-(4-hydroxy-3-methoxy-5-(morpholinomethyl)benzylide ne)-3-nitrobenzohydrazide(5c)

Yellow solid, yield: 90%, mp:235–237 °C [76]. IR (ATR, cm^{-1}) ν_{max}3176, 3080, 2945, 1644, 1611, 1532, 1348, 1308, 1115, 858, 703; ^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ 12.04 (s, 1H), 8.75 (s, 1H), 8.43 (d, 1H, J = 8.0 Hz), 8.36 (s, 1H), 7.84 (t, 1H, J = 8.0 Hz), 7.26

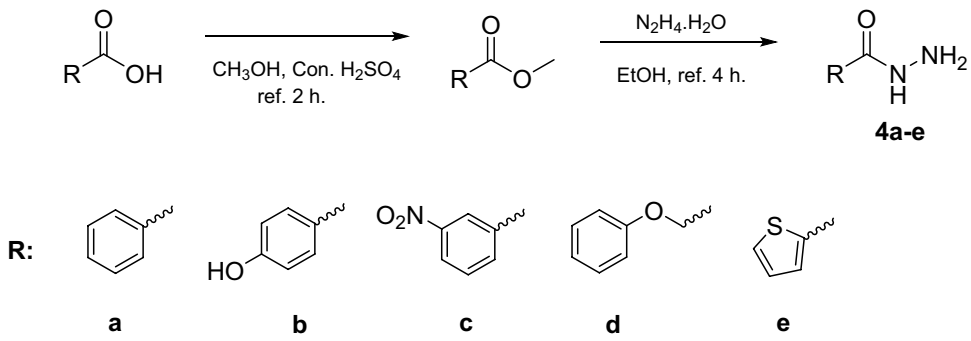

Fig. 5 Synthesis of compounds 4a-e

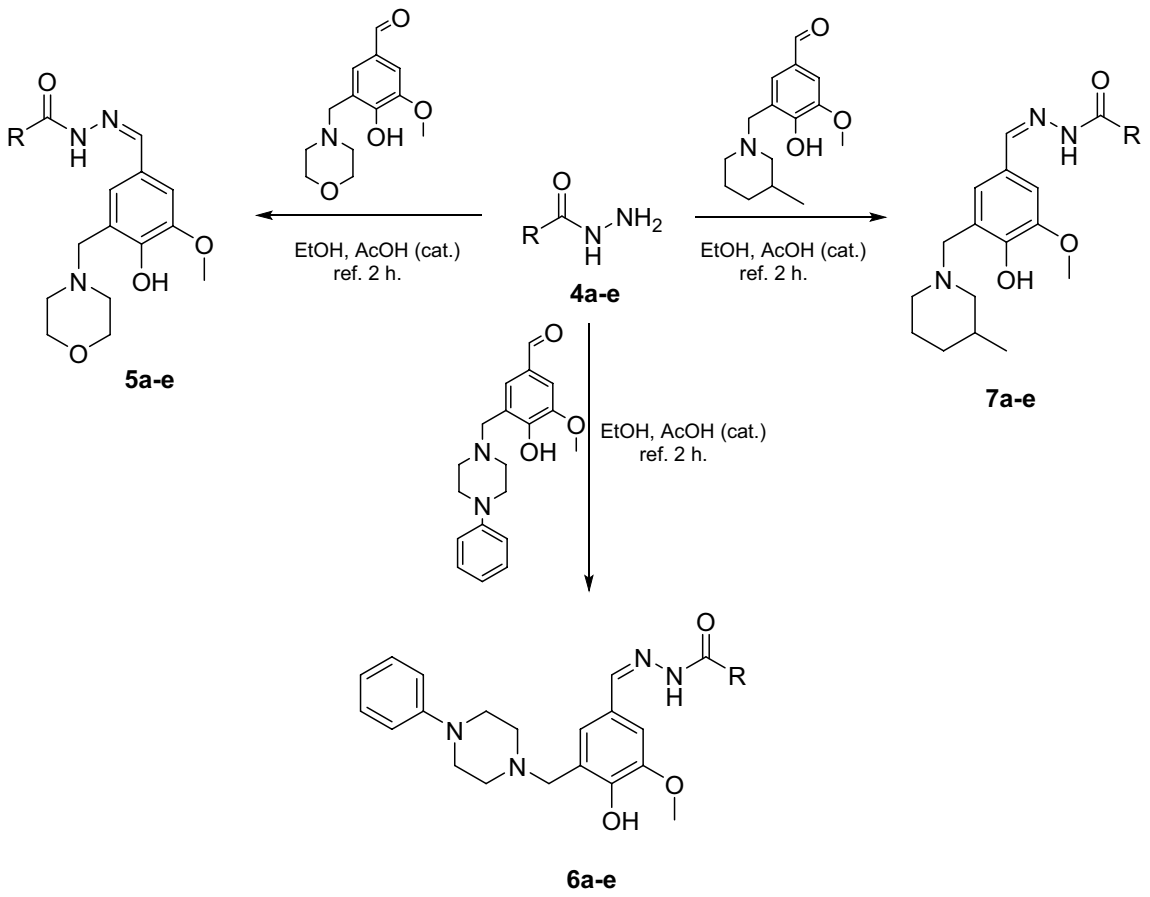

Fig. 6 Synthesis of compounds 5a-e, 6a-e, and 7a-e

(s, 1H), 7.12 (s, 1H), 3,85 (s, 3H), 3.65 (s, 2H), 3.61 (m, 4H), 2.46 (m, 4H).:^{13}C-NMR (100 MHz, DMSO-d_6, δ/ppm): δ160.7, 149.3, 148.4, 147.8, 147.7, 134.9, 134.0, 130.2, 126.2, 124.6, 122.5, 122.3, 122.2, 108.2, 66.1 (2C), 58.2, 55.6, 52.7 (2C). HRMS (Q-TOF) *m/z* calcd for $C_{20}H_{22}N_4O_6$: 414.1539, found: 415.1599 $[M+H]^+$.

N′-(4-hydroxy-3-methoxy-5-(morpholinomethyl)benzylidene)-2-phenoxyacetohydrazide(5d)

White solid, yield: 89%, mp:205–206 °C, IR (ATR, cm^{-1}) ν_{max}3183, 3036, 2951, 1661, 1591, 1298, 1114, 832, 690;^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ 11.48 and 11.44 (2 s, 1H), 8.20 and 7.90 (2 s, 1H), 7.34–7.27 (m, 2H), 7.21 (d, 1H, $J=8.0$ Hz), 7.07 (s, 1H), 7.00 (d, 1H, $J=8.0$ Hz), 6.96–6.91 (m, 2H), 5.14 and 4.65 (2 s, 2H),3,81 (s, 3H), 3.63 (s, 2H), 3.60 (m, 4H), 2.44 (m, 4H). ^{13}C-NMR (100 MHz, DMSO-d_6, δ/ppm): δ168.8, 163.9, 158.2, 157.7, 148.3, 147.9, 144.2, 129.5, 129.4, 124.6, 122.1, 121.4, 120.6, 114.6, 108.4, 66.1 (2C), 64.6, 58.3, 55.6, 52.6 (2C). HRMS (Q-TOF) *m/z* calcd for $C_{21}H_{25}N_3O_5$: 399.1794, found: 400.1846 $[M+H]^+$.

N′-(4-hydroxy-3-methoxy-5-(morpholinomethyl) benzylidene)thiophene-2-carbohydrazide(5e)

Beige solid, yield: 87%, mp:217–219 °C, IR (ATR, cm^{-1}) ν_{max}3169, 3043, 2945, 1626, 1584, 1300, 1115, 838, 711;^{1}H-NMR (400 MHz, $CDCl_3$,δ/ppm): δ 10.87 (s, 1H), 8.26 (s, 1H), 7.93 (s, 1H), 7.71 (d, 1H, $J=4.0$ Hz), 7.43 (s, 1H), 7.19 (s, 1H), 6.98 (s, 1H), 3,98 (s, 3H), 3.78 (m, 6H), 2.62 (m, 4H). ^{13}C-NMR (100 MHz, $CDCl_3$,δ/ppm): δ163.1, 149.4, 148.4, 145.2, 135.5, 134.5, 132.7, 126.5, 125.3, 121.9, 120.6, 109.2, 66.7 (2C), 61.4, 55.9, 52.8 (2C). HRMS (Q-TOF) *m/z* calcd for $C_{18}H_{21}N_3O_4S$: 375.1253, found: 376.1305 $[M+H]^+$.

N′-(4-hydroxy-3-methoxy-5-((4-phenylpiperazin-1-yl) methyl)benzylidene)benzohydrazide(6a)

White solid, yield: 93%, mp:249–251 °C, IR (ATR, cm^{-1}) ν_{max}3198, 3057, 2949, 1649, 1594, 1312, 1110, 686;^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ 11.74 (s, 1H), 8.37 (s, 1H), 7.92 (d, 2H, $J=8.0$ Hz), 7.59–7.53 (m, 3H), 7.27–7.20 (m, 3H), 7.12 (s, 1H), 6.94 (d, 2H, $J=8.0$ Hz),

6.79 (t, 1H, $J = 7.2$ Hz) 3,85 (s, 3H), 3.72 (s, 2H), 3.17 (m, 4H), 2,62 (m, 4H). ^{13}C-NMR (100 MHz, DMSO-d_6, δ/ppm): δ162.9, 150.8, 148.3, 147.8, 133.6, 131.6, 128.9 (3C), 128.4 (2C), 127.5 (2C), 124.9, 122.7, 122.0, 119.0, 115.5 (2C), 108.2, 57.9, 55.6, 52.1 (2C), 48.2 (2C). HRMS (Q-TOF) *m/z* calcd for $C_{26}H_{28}N_4O_3$: 444.2161, found: 445.2220 $[M+H]^+$.

N′-(4-hydroxy-3-methoxy-5-((4-phenylpiperazin-1-yl) methyl)-4-hydroxybenzylidene)benzohydrazide(6b)

White solid, yield: 91%, mp:254–256 °C, IR (ATR, cm^{-1}) ν_{max}3273, 3061, 2939, 1646, 1598, 1308, 1114, 756;^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ 11.52 (s, 1H), 8.33 (s, 1H), 7.81 (d, 2H, $J = 8.2$ Hz), 7.27–7.20 (m, 3H), 7.09 (s, 1H), 6.94 (d, 2H, $J = 8.0$ Hz), 6.79 (t, 1H, $J = 7.2$ Hz), 3,84 (s, 3H), 3.71 (s, 2H), 3.16 (m, 4H), 2,62 (m, 4H). ^{13}C-NMR (100 MHz, DMSO-d_6, δ/ppm): δ162.5, 160.5, 150.8, 148.1, 147.7, 147.4, 129.6, 128.9 (3C), 125.1, 124.0, 122.6, 121.8, 119.0, 115.5 (3C), 114.9, 108.1, 58.0, 55.6, 52.1 (2C), 48.2 (2C). HRMS (Q-TOF) *m/z* calcd for $C_{26}H_{28}N_4O_4$: 460.2111, found: 461.2167 $[M+H]^+$.

N′-(4-hydroxy-3-methoxy-5-((4-phenylpiperazin-1-yl) methyl)benzylidene)-3-nitrobenzohydrazide(6c)

Yellow solid, yield: 93%, mp:223–224 °C [76]. IR (ATR, cm^{-1}) ν_{max}3194, 3075, 2946, 1641, 1599, 1531, 1348, 1306, 688;^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ 12.04 (s, 1H), 8.75 (s, 1H), 8.44 (d, 1H, $J = 7.8$ Hz), 8.37 (d, 2H, $J = 9.8$ Hz),7.84 (t, 1H, $J = 8.0$ Hz),7.28–7.15 (m, 4H), 6.94 (d, 2H, $J = 8.0$ Hz), 6.79 (t, 1H, $J = 7.2$ Hz), 3,86 (s, 3H), 3.73 (s, 2H), 3.17 (m, 4H), 2,63 (m, 4H). ^{13}C-NMR (100 MHz, DMSO-d_6, δ/ppm): δ160.7, 150.8, 149.3, 148.5, 147.8, 134.9, 134.1, 130.3, 128.9 (3C), 126.2, 124.7, 122.7, 122.2, 119.0, 115.5 (3C), 108.3, 57.9, 55.7, 52.1 (2C), 48.2 (2C). HRMS (Q-TOF) *m/z* calcd for $C_{26}H_{27}N_5O_5$: 489.2012, found: 490.2080 $[M+H]^+$.

N′-(4-hydroxy-3-methoxy-5-((4-phenylpiperazin-1-yl) methyl)benzylidene)-2-phenoxyacetohydrazide(6d)

White solid, yield: 90%, mp:210–212 °C, IR (ATR, cm^{-1}) ν_{max}3174, 3035, 2973, 1657, 1593, 1314, 1226, 749, 686;^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ 11.50 and 11.46 (2 s, 1H), 8.23 and 7.92 (2 s, 1H), 7.34–7.19 (m, 5H), 7.10 (s, 1H), 7.00 (d, 1H, $J = 8.2$ Hz), 6.93 (m, 4H), 6.79 (t, 1H, $J = 7.0$ Hz), 5.14 and 4.65 (2 s, 2H), 3,82 (s, 3H), 3.70 (s, 2H), 3.15 (m, 4H), 2.61 (m, 4H). ^{13}C-NMR (100 MHz, DMSO-d_6, δ/ppm): δ168.8, 163.9, 158.2, 157.7, 150.8, 148.3, 147.7, 144.2, 129.5, 128.9, 124.6, 122.6, 121.2, 120.6, 119.0, 115.5, 114.6, 108.5, 66.4, 64.6, 57.9, 55.6, 52.1 (2C), 48.2 (2C). HRMS (Q-TOF) *m/z* calcd for $C_{27}H_{30}N_4O_4$: 474.2267, found: 475.2339 $[M+H]^+$.

N′-(4-hydroxy-3-methoxy-5-((4-phenylpiperazin-1-yl) methyl)benzylidene)thiophene-2-carbohydrazide(6e)

Beige solid, yield: 92%, mp:222–224 °C, IR (ATR, cm^{-1}) ν_{max}3216,3187, 3061, 2946, 1627, 1583, 1298, 842, 713;^{1}H-NMR (400 MHz, $CDCl_3$, δ/ppm): δ 10.44 (s, 1H), 8.25 (s, 1H), 7.89 (s, 1H), 7.70 (s, 1H), 7.44 (s, 1H), 7.30–7.25 (m, 2H), 7.19 (s, 1H), 6.99 (s, 1H), 6.91–6.88 (m, 3H), 3,98 (s, 3H), 3.84 (s, 2H), 3.26 (m, 4H), 2,78 (m, 4H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ/ppm): δ162.9, 159.7, 150.8, 149.6, 148.5, 145.0, 135.5, 134.5, 129.3 (2C), 126.5, 125.1, 121.9, 120.9, 120.4, 116.5 (2C), 109.2, 61.0, 56.0. 52.5 (2C), 49.1 (2C). HRMS (Q-TOF) *m/z* calcd for $C_{24}H_{26}N_4O_3S$: 450.1726, found: 451.1794 $[M+H]^+$.

N′-(4-hydroxy-3-methoxy-5-((3-methylpiperidin-1-yl) methyl)benzylidene)benzohydrazide(7a)

Beige solid, yield: 79%, mp:199–201 °C, IR (ATR, cm^{-1}) ν_{max}3230, 3066, 2929, 1643, 1603, 1298, 692;^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ 11.79 (s, 1H), 8.37 (s, 1H), 7.94 (d, 2H, $J = 8.0$ Hz), 7.58–7.52 (m, 4H), 7.25 (s, 1H), 7.01 (s, 1H), 3,81 (s, 3H), 3.65 (s, 2H), 2.78 (m, 2H), 1.97 (t, 1H, $J = 10.40$ Hz), 1.70–1.43 (m, 5H), 0,89–0,80 (m, 4H). ^{13}C-NMR (100 MHz, DMSO-d_6, δ/ppm):δ162.9, 149.2, 148.4, 147.7 133.6, 131.6, 128.4 (2C), 127.5 (2C), 124.7, 122.2, 121.5, 108.2, 60.2, 59.6, 55.5, 52.6, 32.0, 30.6, 24.8, 19.2. HRMS (Q-TOF) *m/z* calcd for $C_{22}H_{27}N_3O_3$: 381.2052, found: 382.2111 $[M+H]^+$.

N′-(4-hydroxy-3-methoxy-5-((3-methylpiperidin-1-yl) methyl)benzylidene)-4-hydroxybenzohydrazide(7b)

Beige solid, yield: 81%, mp:180–182 °C, IR (ATR, cm^{-1}) ν_{max}3177, 3010, 2932, 1641, 1600, 1276, 1169, 848;^{1}H-NMR (400 MHz, CH_3OD, δ/ppm): δ 8.20 (s, 1H), 7.84 (d, 2H, $J = 8.6$ Hz), 7.60 (s, 1H), 7.02 (s, 1H), 6.89 (d, 2H, $J = 8.6$ Hz), 3,91 (s, 3H), 3.85 (s, 2H), 3.06–3.00 (m, 2H), 2.30 (t, 1H, $J = 11.2$ Hz), 2.05–1.99 (m, 1H), 1.79–1.59 (m, 4H), 1.04–0.98 (m, 1H), 0,91 (d, 3H, $J = 6.4$ Hz).^{13}C-NMR (100 MHz, CH_3OD, δ/ppm): δ166.8, 162.8, 151.9, 150.2, 149.9, 130.8 (2C), 126.2, 124.8, 124.6, 121.3 (2C), 116.4, 110.1, 61.2, 60.4, 57.5, 53.9, 32.9, 31.9, 25.6, 19.6. HRMS (Q-TOF) *m/z* calcd for $C_{22}H_{27}N_3O_4$: 397.2002, found: 398.2066 $[M+H]^+$.

N′-(4-hydroxy-3-methoxy-5-((3-methylpiperidin-1-yl) methyl)benzylidene)-3-nitrobenzohydrazide (7c)

Beige solid, yield: 80%, mp:230–231 °C, IR (ATR, cm^{-1}) ν_{max}3241, 3084, 2943, 1642, 1585, 1528, 1349, 1301, 765;^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ 12.05 (brs, 1H), 8.75 (s, 1H), 8.43 (d, 2H, $J = 7.8$ Hz), 8.36 (d, 1H,

$J = 6.8$ Hz), 7.83 (t, 1H, $J = 7.8$ Hz),7.24 (s, 1H),7.03 (s, 1H), 3.82 (s, 3H), 3.67 (s, 2H), 2.80 (m, 2H), 2.02–1.97 (m, 1H), 1.73–1.45 (m, 5H), 0.90–0.82 (m, 4H). ^{13}C-NMR (100 MHz, DMSO-d_6, δ/ppm):δ160.7, 149.4, 149.3, 147.7, 134.9, 134.0 (2C), 130.2, 126.1, 124.4, 122.3, 122.2, 121.7, 108.3, 60.2, 59.5, 55.5, 52.6, 32.0, 30.7, 24.8, 19.2. HRMS (Q-TOF) *m/z* calcd for $C_{22}H_{26}N_4O_5$: 426.1903, found: 427.1971 $[M+H]^+$.

N′-(4-hydroxy-3-methoxy-5-((3-methylpiperidin-1-yl) methyl)benzylidene)-2-phenoxyacetohydrazide(7d)

Beige solid, yield: 82%, mp:172–173 °C, IR (ATR, cm^{-1}) ν_{max}3198, 3060, 2925, 167, 1593, 1223, 1119, 745, 686;^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ 11.47 and 11.46 (2 s, 1H), 8.19 and 7.89 (2 s, 1H), 7.34–7.27 (m, 2H), 7.20 (s, 1H), 7.03–7.91 (m, 4H),5.13 and 4.64 (2 s, 2H), 3.79 (s, 3H), 3.71 (s, 2H), 2.83 (m, 2H), 2.07 (m, 1H), 1.81–1.46 (m, 5H), 0,93–0.91 (m, 1H), 0.84 (d, 3H, $J = 6.2$ Hz). ^{13}C-NMR (100 MHz, DMSO-d_6, δ/ppm):δ168.8, 163.9, 158.2, 157.7, 149.1, 148.9, 148.3, 147.7, 144.2, 129.5, 129.3,124.4, 121.8, 121.2, 121.0, 120.6, 114.6, 114.4, 108.7, 108.5, 66.4, 64.6, 60.0, 55.5, 52.5, 31.8, 30.5, 24.6, 19.2. HRMS (Q-TOF) *m/z* calcd for $C_{23}H_{29}N_3O_4$: 411.2158, found: 412.2222 $[M+H]^+$.

N′-(4-hydroxy-3-methoxy-5-((3-methylpiperidin-1-yl) methyl)benzylidene)thiophene-2-carbohydrazide(7e)

Beige solid, yield: 80%, mp:185–186 °C, IR (ATR, cm^{-1}) ν_{max}3225, 3069, 2926, 1633, 1603, 1294, 842, 705;^{1}H-NMR (400 MHz, DMSO-d_6, δ/ppm): δ 11.75 (s, 1H), 8.31 (s, 1H), 8.05–7.86 (m, 2H), 7.36 (s, 1H), 7.22 (m, 2H), 7.06–7.02 (m, 1H), 3,84 (s, 3H), 3.67 (s, 2H), 2.80–2.78 (m, 2H), 2.00 (t, 1H, $J = 10.8$ Hz), 1.76–1.45 (m, 5H), 0.93–0.88 (m, 1H), 0.82 (d, 3H, $J = 6.4$ Hz). ^{13}C-NMR (100 MHz, DMSO-d_6, δ/ppm):δ161.0, 157.5, 149.2, 147.8, 144.2, 134.9, 133.0, 131.5, 128.7, 126.5, 124.6, 121.4, 108.4, 60.2, 59.5, 55.5, 52.6, 32.0, 30.7, 24.8, 19.2. HRMS (Q-TOF) *m/z* calcd for $C_{20}H_{25}N_3O_3S$: 387.1617, found: 388.1679 $[M+H]^+$.

Enzyme inhibition studies

The effect of these compounds on the acetylcholinesterase enzyme was investigated according to the Ellman [77] method. For this purpose, IC50 and Ki values were found and inhibition types were determined. The basis of this method can be explained as follows: Cholinesterases catalyze the breakdown of acetylcholine into thiocholine and acetate. It forms yellow colored 5-thio-2-nitrobenzoic acid, which is formed by the reaction of thiocholine and DTNB, which is released as a product. The resulting compound color gave an absorbance at 412 nm. The absorbance of the sample and blank cuvettes was measured at a wavelength of 412 nm and for 5 min [78–81]. Additionally, α-glycosidase enzyme activity was determined using p-NPG as substrate according to the procedure of Tao et al.; [82] Samples were prepared by dissolving 20 mg in 2 mL (EtOH:H_2O). If all enzyme inhibition was achieved, multiple solutions were prepared in phosphate buffer. First, 75 µL of phosphate buffer was mixed with phosphate buffer (0.15 U/mL, pH 7.4) and 20 µL of enzyme solution in 5 µL of sample. It was then pre-incubated at 35 °C for 10 min before adding p-NPG to the start of the reaction. Also, after pre-incubation, 20 µL of p-NPG in phosphate buffer (5 mM, pH = 7.4) was added and incubation was carried out again at 35 °C. IC_{50} and Ki values were calculated by curve fitting the data. Acarbose compound was used as a positive control. Absorbances were measured spectrophotometrically at 405 nm [83–85]. One unit of α-glycosidase is the amount of enzyme (pH: 7.4) that catalyzes 1.0 mol of substrate hydrolysis per minute [86].

The inhibitory effect of novel Schiff bases from Phenolic Mannich bases (5a-e, 6a-e, and 7a-e) on AChE activity was performed according to spectrophotometric method of Ellman [77] as described previously [78–81]. α-Glycosidase inhibition effect of novel compounds (5a-e, 6a-e, and 7a-e) was evaluated according to the method of Tao et al. [82] The absorbances of samples were recorded at 405 nm as previously described [83–86].

Docking studies

One of the most important methods used to calculate the theoretical biological activities of molecules against enzymes is molecular docking. In the calculations made by molecular docking method, many parameters are obtained from the interactions of molecules with enzymes to compare biological activities. These parameters are very important parameters to explain the theoretical biological activities of molecules [45]. AChE and α-Gly were used to calculate the biological activity of Schiff bases from Phenolic Mannich bases (5a-e, 6a-e, 7a-e). In this study, the crystal structure of acetylcholinesterase (AChE) (pdb ID:4M0E) and α-glycosidase (α-Gly) (pdb ID:1R47) [87] was used for docking study of compounds 5a-e, 6a-e, 7a-e. Molecular docking calculations to calculate the biological activity of compounds 5a-e, 6a-e, 7a-e were performed using Maestro Molecular modeling platform (version 12.2) by Schrödinger. Proteins and new molecules should be prepared for calculations using the Maestro Molecular modeling platform (version 12.2) by Schrödinger program. In docking calculations, different processes are performed for novel molecules and enzymes at each stage. It was primarily used from Gaussian software program [88] to obtain optimized structures of novel compounds. Using these optimized structures, all calculations were made with the Maestro Molecular modeling platform (version 12.2) by Schrödinger, LLC [89]. The Maestro Molecular modeling platform (version 12.2)

by Schrödinger comes together from many modules. In the first module between these modules, the protein preparation module [90, 91] is used to prepare the enzymes formed by proteins for calculations. In the next module, the LigPrep module [92, 93] was used to prepare compounds 5a-e, 6a-e, 7a-e for calculations. In the next module, The Glide ligand docking module [94] was used for enzymes to interact with novel molecules. In all modules used, OPLS3e method was used in all calculations for docking calculations of novel compounds and proteins. After docking calculations, ADME/T analysis (absorption, distribution, metabolism, excretion, and toxicity) was performed to examine the properties of novel compounds for future drug use. The Qikprop module [95] of the Schrödinger software was used for ADME/T analysis.

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Declarations

Conflicts of interest The authors declare that they have no conflicts of interest to disclose.

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