

***In-vitro* and *in-silico* anticholinesterase, α -amylase and anti-urease inhibitory activities of *Zygophyllum simplex* L. ethanolic extract**

Original Article

Abstract:

In this study, we aimed to evaluate total phenolic and flavonoid contents (TPC & TFC), anti-AChE, anti-BChE, anti-urease and α -amylase activities of the ethanol extract (ZSE), obtained from the aerial parts of the Algerian *Zygophyllum simplex* L. Molecular docking was applied on the most abundant components identified previously from the plant around the world, which are six phenolic compounds, eleven flavonoids and five ursane-type saponins. Moderate values were observed for both TPC (29.674 ± 2.395 mg gallic acid/g extract) and TFC (35.843 ± 0.836 mg quercetin/g extract). ZSE displayed weak AChE (IC_{50} : 148.50 ± 1.09 μ g/mL) and α -amylase (IC_{50} : 101.63 ± 0.61 μ g/mL) inhibitory activity, while remarkable effects were observed for both anti-BChE (IC_{50} : 42.09 ± 3.88 μ g/mL) and anti-urease (IC_{50} : 63.61 ± 1.29 μ g/mL). In addition, molecular docking reveals human BChE (PDB ID 4EY4) and *Bacillus pasteurii* urease (PDB ID 4UBP), to be potential protein targets for *Z. simplex* components.

Key words:

Zygophyllum simplex, anticholinesterase, α -amylase, anti-urease, molecular docking

Apstrakt:

***In vitro* i *in silico* inhibitorna aktivnost etanolnog ekstrakta vrste *Zygophyllum simplex* L. u odnosu na acetilholinesterazu, α -amilazu i ureazu**

U ovoj studiji cilj je bio da se procene ukupni sadržaj fenolnih jedinjenja i flavonoida (TPC i TFC), kao i anti-AChE, anti-BChE, anti-ureazna i α -amilazna aktivnost etanolnog ekstrakta (ZSE) dobijenog iz nadzemnih delova alžirske vrste *Zygophyllum simplex* L. Molekularno dokovanje primenjeno je na najzastupljenije komponente prethodno identifikovane u ovoj biljci širom sveta, koje obuhvataju šest fenolnih jedinjenja, jedanaest flavonoida i pet saponina ursanskog tipa. Umerene vrednosti zabeležene su i za TPC ($29,674 \pm 2,395$ mg galne kiseline/g ekstrakta) i za TFC ($35,843 \pm 0,836$ mg kvercetina/g ekstrakta). ZSE je pokazao slabu inhibitornu aktivnost prema AChE (IC_{50} : $148,50 \pm 1,09$ μ g/mL) i α -amilazi (IC_{50} : $101,63 \pm 0,61$ μ g/mL), dok su izraženiji efekti uočeni kod inhibicije BChE (IC_{50} : $42,09 \pm 3,88$ μ g/mL) i ureaze (IC_{50} : $63,61 \pm 1,29$ μ g/mL). Pored toga, molekularno dokovanje ukazuje da humani BChE (PDB ID 4EY4) i ureaza bakterije *Bacillus pasteurii* (PDB ID 4UBP) predstavljaju potencijalne proteinske mete za komponente vrste *Z. simplex*.

Ključne reči:

Zygophyllum simplex, antiholinesterazna aktivnost, α -amilaza, antiureazna aktivnost, molekularno dokovanje

Introduction

Zygophyllum L., is the largest genus within the Zygophyllaceae family, comprise about 100 species, commonly found in desert and steppe habitats from the Mediterranean to central Asia, South Africa, and Australia (Amini-Chermahini et al., 2014). The use of many *Zygophyllum* species in the folk medicine of different civilizations have garnered attention, which has been highlighted by many studies revealing

their antidiabetic, antimicrobial, antitumor, and anti-inflammatory properties (Shawky et al., 2019). *Zygophyllum simplex* L. (Syn.: *Tetraena simplex* (L.)), is one of eight *Zygophyllum* species widespread in the Algerian desert (Mohammedi, 2020), reported to be used in folk medicine for treating gout, asthma, dry scaly patches, and cleaning the skin (Abdallah & Esmat, 2017). Triterpenoids and flavonoids represent the principal chemical constituents identified in *Z. simplex* extracts (Hassanean et al., 1992; Amin et

Boumediene Bentameur

Inorganic Materials Laboratory, Department of Chemistry, Faculty of Sciences, University of M'Sila, University Pole, Bordj Bou Arreridj Road, M'Sila 28000, Algeria
boumediene.bentameur@univ-msila.dz
(Corresponding author)

Imadeddine Kadi

Research Unit on Medicinal Plants (URPM, 30000, Laghouat), Biotechnology Research Center (CRBt, 25000, Constantine), Algeria

Zeyneb Benramdane

Inorganic Materials Laboratory, Department of Chemistry, Faculty of Sciences, University of M'Sila, University Pole, Bordj Bou Arreridj Road, M'Sila 28000, Algeria

Thamere Cheriet

Laboratory of Biological Engineering, Valorization and Innovation of Agro-Food Products, Institute of Applied Technical Sciences (ISTA), Ain M'lila, Larbi Ben M'hidi University, Oum El-Bouaghi 04000, Algeria
Unit for Valorization of Natural Resources, Bioactive Molecules, and Physicochemical and Biological Analysis (VARENBIMOL), University of Frères Mentouri Constantine, Algeria

Djamel Sarri

Department of Nature and Life Sciences, Faculty of Sciences, University of M'Sila, University Pole, Bordj Bou Arreridj Road, M'Sila 28000, Algeria

Received: December 12, 2025

Revised: March 22, 2026

Accepted: March 23, 2026



al., 2011; Hussein et al., 2011; Baky et al., 2020; Abdel Bar et al., 2025). Among these, isorhamnetin, quercetin, and their glycosylated derivatives emerge as the predominant metabolites, widely regarded as the primary contributors to the plant's antioxidant and anti-inflammatory activities (Kakrani et al., 2011; Abdallah & Esmat, 2017; Bourgou et al., 2017; Galal et al., 2025). Flavonoids more broadly are recognized for exerting dual antioxidant and anti-inflammatory effects, with documented relevance to conditions including neurodegeneration, diabetic complications, and gastric mucosal injury, mediated in part through inhibition of acetylcholinesterase (AChE), α -amylase, and urease. In this regard, quercetin and isorhamnetin have both been shown to inhibit AChE and attenuate neuroinflammation, positioning them as candidates for therapeutic interest in Alzheimer's disease research (Gong et al., 2020; Souza et al., 2025). Isorhamnetin additionally reverses scopolamine-induced increases in cholinesterase activity and decreases in brain-derived neurotrophic factor (BDNF) levels within the prefrontal cortex and hippocampus, thereby enhancing cholinergic signalling and synaptic plasticity (Olennikov et al., 2017). Quercetin inhibits α -amylase through direct strong binding that modifies its kinetic parameters (Huang et al., 2024), while isorhamnetin has been reported to protect against diabetes-associated cerebral changes by reducing astroglial activation, AChE activity, oxidative stress, apoptosis, and inflammatory signalling (Jamali-Raeufy et al., 2019). Quercetin, isorhamnetin, and their respective glycosides have further demonstrated moderate to strong anti-urease activity in several independent investigations (Ayouub et al., 2022; Goodarzi et al., 2024; Liu et al., 2025).

To our knowledge, no prior studies have reported the anticholinesterase, anti-urease, or α -amylase inhibitory activities of *Z. simplex* extracts, despite the plant's established richness in isorhamnetin, quercetin, and their glycosides. The present study therefore aimed to evaluate the *in vitro* inhibitory potential of the ethanol extract (ZSE) against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), urease, and α -amylase, complemented by molecular docking analyses of 22 previously identified constituents of the plant.

Materials and Methods

Identification of plant material

The aerial parts of *Z. simplex* were collected in December 2021 from the Algerian National Park „Tassili N'Ajjer” and identified by Dr Djamel Sarri on the basis of Quezel & Santa (1963). A voucher specimen has been deposited in the

Herbarium of the VARENBIOMOL Research Unit, Université des Frères Mentouri, under the flowing order n° 20/2021/ZSL.

Preparation of the plant extract

The dried powder (200 g) of the plant's aerial parts was extracted successively with 0.5 L of petroleum ether, chloroform and ethanol (3 h for each solvent) in a Soxhlet apparatus. The EtOH solution was filtered and then concentrated using a vacuum rotary evaporator at 40 °C to yield a crude ethanolic extract (ZSE: 4.746 g) which was then stored at -4 °C.

Total phenolic and flavonoid contents determination

The total phenolic content (TPC) of ZSE was determined according to the Folin-Ciocalteu spectrophotometric assay at 765 nm (Brahmi et al., 2012). 100 μ L of diluted sample was added to 2 mL of 2% Na₂CO₃ aqueous solution. After 2 min, 100 μ L of 50% F_C reagent was added. The final mixture was shaken and then incubated at room for 30 min in the dark at room temperature. The calibration curve was constructed using gallic acid (5-300 μ g/mL) as a standard and the results are expressed in mg gallic acid equivalents per gram extract (mg GAE/g extract).

The total flavonoid content (TFC) of ZSE was determined using a spectrophotometric method with aluminum chloride (Shraim et al., 2021). The sample contained 1 mL of ZSE methanolic solution (1 mg/mL) and 1 mL of 2% AlCl₃ solution dissolved in methanol. After incubation at room temperature for 15 min, quercetin was used to make the calibration curve (5-150 μ g/mL). The absorbance of the reaction mixture was measured at 430 nm, and the total flavonoid content was expressed in mg quercetin equivalents per gram of extract (mg QE/g extract).

Anticholinesterase inhibitory activities

Ellman's colorimetric method (Ellman et al., 1961; Elkolli et al., 2022), was utilized to evaluate acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory assays using a 96-well microplate reader (Perkin Elmer, EnSpire Multimode Plate Reader, USA). In this assay, 4 mg of the extract was dissolved in methanol and diluted to prepare seven concentrations ranging from 200 μ g/mL to 3.125 μ g/mL. Subsequently, 10 μ L of each sample was combined with 150 μ L of sodium phosphate buffer (0.1 M, pH 8.0) and 20 μ L of AChE or BChE (5.32×10^{-3} U). The mixture was incubated at 25 °C for 15 min. Following incubation, 10 μ L of DTNB (0.5 mM) and 10 μ L of acetylthiocholine iodide (ACI, 0.71 mM) or butyrylthiocholine

iodide (BuCl, 0.5 mM) were added to the reaction. Absorbance readings were taken at 412 nm at two time points (T_0 and T_1 5 min), and IC_{50} values ($\mu\text{g/mL}$) were calculated for each sample in triplicate.

Urease inhibitory activity

The urease inhibitory activity assay was performed following the method described by Weatherburn (Weatherburn, 1967). In brief, the reaction mixture consisted of 25 μL of enzyme, 10 μL of various concentrations of the sample, and 50 μL of urea, which were incubated at 30 °C for 15 min. Afterward, 45 μL of phenol reagent and 70 μL of basic reagent were added to the mixture, followed by a 50 min incubation at 30 °C. The absorbance was then recorded at 630 nm using a microplate reader (96-well format) (PerkinElmer, EnSpire Multimode Plate Reader, USA). The extent of urease inhibition was calculated and expressed as a percentage.

Alpha-amylase inhibitory activity

The α -amylase inhibition assay was conducted following the procedure described by Zengin et al., (2014). In summary, the tested extract was dissolved in methanol to achieve concentrations ranging from 4 mg/mL to 62.5 $\mu\text{g/mL}$. A volume of 25 μL from each concentration was combined with 50 μL of the enzyme (1U) and incubated at 25 °C for 10 min. Subsequently, 50 μL of a 0.1% substrate solution was added, and the mixture was incubated again at 37 °C for another 10 min. After this second incubation, 25 μL of hydrochloric acid (1 M) and 100 μL of potassium iodide (IKI) were added as a colorimetric reagent. A blank sample, containing all components except the enzyme, was also prepared. Absorbance readings were taken at 630 nm using a microplate reader (96-well format) (Perkin Elmer, EnSpire Multimode Plate Reader, USA). The degree of α -amylase inhibition was calculated and expressed as a percentage.

Statistical analysis

The results were expressed as mean $\pm$ standard error mean (S.E.M.). One-way ANOVA was applied for the analysis of anticonvulsant results, followed by Tukey's test, significance was considered at $p < 0.05$ using Graph Pad Prism 5.0.

Molecular docking study

For applying molecular docking study, we selected twenty-two compounds (Fig. S1) previously identified as the most abundant bioactive phytoconstituents from *Z. simplex* extracts (Hussein et al., 2011; Abdallah & Esmat, 2017; Baky et al., 2020; Bar et al., 2025). Their

3D-structures were generated and minimized using MMFF94x force field in builder tool executed in Molecular Operating Environment (MOE v2019.0102, Chemical Computing Group ULC) package aligned with fixing hydrogens, then saved as mol2 files.

The X-Ray diffraction structures of the targeted proteins: human AChE (PDB ID 4EY4), human BChE (PDB ID 4BDS), human α -amylase (PDB ID 2QV4) and *Bacillus pasteurii* urease enzyme (PDB ID 4UBP) were downloaded as PDB files from the protein data bank website (<https://www.rcsb.org>). As a first step, all water molecules and co-crystallized ligands were removed from the proteins structure using MOE software. Subsequently, they were subjected to MOE-QuickPrep option after setting potential at AMBER14 force field for energy minimization through fixing hydrogens and charges. MOE-Site finder option was also used to define the active site residues of each protein. Next, the prepared proteins were saved as PDB files. The device used in this study was with processor i5_2520M CPU 2.50 GHz and 4.00 GB of memory (RAM), Windows 10 Pro 2021 version (64-bits).

Both ligands, after adding Gastieger charges, and proteins were converted into PDBQT files (input) in AutoDockTools (v1.5.7) software (Morris et al., 2009), aligned with setting grid box parameters as outlined in **Tab. 1**. In the configuration settings prepared for Vina docking, the exhaustiveness and number of poses were set as '8' and '10', respectively. Therefore, the docking calculations were conducted using AutoDockVina (v1.1.2) (Trott & Olson, 2010). In order to validate the docking method, the native co-crystallized ligands tacrine and acarbose were re-docked into their targets 4BDS and 2QV4, respectively. Their RMSD values were quantified as 0.484 and 1.307 Å, respectively, with DockRMSD web server (<https://zhanggroup.org/DockRMSD>). However, tacrine and donepezil were docked and considered as references for the free 4EY4 protein (Costa et al., 2023). Meanwhile, Acetohydroxamic acid was re-docked into 4UBP protein and the obtained binding affinity in Vina was compared to that reported by Naseem et al., (2021).

Analysis and visualization. To investigate the amino acids involved in the active site pocket, hydrogen bonding, and hydrophobic interactions, both BIOVIA Discovery Studio (2024 client) and UCSF Chimera (v1.16) were used to analyse and generate 2D and 3D illustrations of ligand–protein complexes (output).

Results and Discussion

The evaluation of total phenolics and flavonoids

content’s yielded moderate values (29.674±2.395 mg gallic acid/g extract and 35.843±0.836 mg quercetin/g extract, respectively). Our findings are in accordance with the data in literature indicating that flavonoids level was always reported to be higher than phenolic compounds in *Zygophyllum* species (Hassanean et al., 1992; Hussein et al. 2011).

The examination of ZSE’s AChE and BChE inhibitory activities (**Tab. 1**) revealed the observation of potent BChE inhibition with an IC₅₀ value of 42.09±3.88 µg/mL, compared to the reference drug, galantamin (34.75±1.99 µg/mL), however, the AChE assay showed weak AChE inhibition (IC₅₀: 148.50±1.09 µg/mL). Our findings suggested that ZSE could be particularly effective in later stages of Alzheimer diseases when BChE activity predominated (Elsawalhy et al., 2024), which is in accordance with AChE and BChE properties data, previously reported for some *Zygophyllum* species (Orhan et al., 2004; Kchaou et al., 2016). Reposts suggested that many plant-derived products exert neuroprotective effects through mechanisms such as anti-inflammatory one (Ojetunde, 2024), which might be one of the reasons for the observed BChE inhibition effect of *Z. simplex* extract, previously reported for exerting potent anti-inflammatory properties for both extracts and pure compounds (Abdel Bar et al., 2025). Otherwise, the presence of some potent anti-BChE agents in *Z. simplex* extracts, in particular isorhamnetin (**7**), quercetin (**13**), and their derivatives (**8-11**, **14-16**), together with myricitrin (**12**) and luteolin 7-*O*-glucoside (**17**) (**Fig. S1**) (Szwajgier, 2015; Khan et al., 2018; Sezen Karaoğlu et al., 2023), might explain why ZSE remarkably inhibited BChE (**Fig. 1**).

Previous study demonstrated how four quinovic acid (**18**) sulfated derivatives, isolated from *Z. fabago* L., exert strong anti-urease activity (Khan et al., 2014). In our study, moderate inhibition (63.61 µg/mL) was observed for ZSE, previously reported to contain quinovic acid and its derivatives (**18-22**) (Baky et al., 2020), reported for exerting remarkable anti-urease activity (Nazeer et al., 2002). Moreover, molecular docking and structure–activity relationship analysis of some flavonoids including myricitrin (**12**), quercetin (**13**), and luteolin 7-*O*-glucoside (**17**), suggested that 3-OH, 5-OH, and 3',4'-dihydroxyl groups are typical structural character for strong urease inhibitory activity (Xiao et al., 2012). Besides **12**, **13**, and **17**, our extract contains other flavonoids, particularly **14-16**. This may explain the shown effect by ZSE.

On the other hand, some previous studies reported moderate or weak antidiabetic activity of

Table 1. Percentage of AChE, BChE, anti-urease and α-amylase inhibition properties of ethanolic extract from *Zygophyllum simplex*

	% Inhibition of AChE							IC ₅₀ (µg/mL)
	3.125 µg	6.25 µg	12.5 µg	25 µg	50 µg	100 µg	200 µg	400 µg
ZSE	11.29±1.91	16.46±1.11	22.50±2.21	29.17±2.25	36.75±1.26	44.45±0.79	56.43±0.65	Nt
Gal*	35.93±2.28	43.77±0.00	68.50±0.31	80.69±0.41	85.78±1.63	91.80±0.20	94.77±0.34	Nt
% Inhibition of BehE								
ZSE	21.36±1.65	27.23±1.42	36.26±1.33	44.16±1.33	54.01±1.29	64.37±0.86	72.38±1.60	Nt
Gal*	3.26±0.62	6.93±0.62	24.03±2.94	45.13±2.60	63.87±2.85	73.57±0.77	78.95±0.58	Nt
% Inhibition of Urease								
ZSE	Nt	25.52±0.95	33.76±1.84	41.32±1.04	48.64±0.58	56.82±0.47	65.65±1.67	74.61±1.04
Thiourea*	Nt	19.85±2.74	55.64±4.24	94.17±0.15	98.42±0.19	98.49±0.41	98.90±0.05	Nt
% Inhibition of α-amylase								
ZSE	Nt	16.90±1.09	22.77±2.16	35.30±1.12	41.58±0.47	51.33±1.02	63.53±0.60	71.28±1.19
								35.19±1.67

Gal: Galantamine, the values are expressed as means ± S.D. of three parallel measurements. (*) Reference compound. Nt: not tested

some *Zygophyllum* species (Mnafgui et al., 2014; Boumaza et al., 2016), which is the case of *Z. simplex*, showing weak α -amylase potential (IC_{50} 101.63 ± 0.61 μ g/mL).

Molecular docking was applied for the known inhibitors tacrine, donepezil, acarbose, acetohydroxamic acid, and thiourea (see **Tab. S1**, **Fig. S2**), together with twenty-two prepared ligands (molecules from *Z. simplex*, **Fig. S1**) in the active site of AChE (PDB ID 4BDS), BChE (PDB ID 4EY4), urease (PDB ID 4UBP), and α -amylase (PDB ID 2QV4) enzymes (see **Tab. S2**, **Figs. S3–S18**). The docked compounds (7–14, 16–22) for BChE, showed similar or higher scores (-8.9 – -11.3 Kcal/mol) than tacrine (-8.3 Kcal/mol). The aromatic rings of compounds 1–8, 13, and 17 were involved in π - π interactions with TRP 82 (3.77–5.69 Å), while the ones of 2, 8, 11, 14, 16, 18, 19, and 21 were interacted by π -alkyl bonds with ALA 328 (3.60–5.05 Å), similar to the ones shown by tacrine. Moreover, the OH groups of compounds 5, 8–12, 14–17, and 21 formed at least three hydrogen bonds with residues (2.07–3.09 Å) (see **Tab. S3**), while the aromatic rings of 7, 13, and 17 were the only ones to interact with ASP 70 by π -anion bond (4.61–4.69 Å). These interactions might be the reason for ZSE remarkable anti-BChE.

The docked inhibitors tacrine (-8.7 Kcal/mol) and donepezil (-9.6 Kcal/mol) were involved in π - π interactions with some AChE residues (TRP 286, TYR 341 and TRP 86, TRP 286, TYR 337, respectively), while hydrogen bond interactions were formed with ASP 74 and PHE 295. Similar interactions were observed for compounds 7, 9, 13, and 17 (1.98–4.43 Å). Moreover, the OH groups of 1–7, 10, 13, 15, 18, 21, and 22 showed at least three hydrogen bonds (1.89–2.9 Å) with different residues (see **Tab. S3**).

For the urease enzyme, the oxygen atoms in compounds 1–4 (**Fig. 2**; -5.3 – -6.3 Kcal/mol) were involved in metal acceptor interactions with NI 798, NI 799 (2.30–2.77 Å), and hydrogen bonds with different residues (2.06–2.71 Å), similar to the ones showed by acetohydroxamic

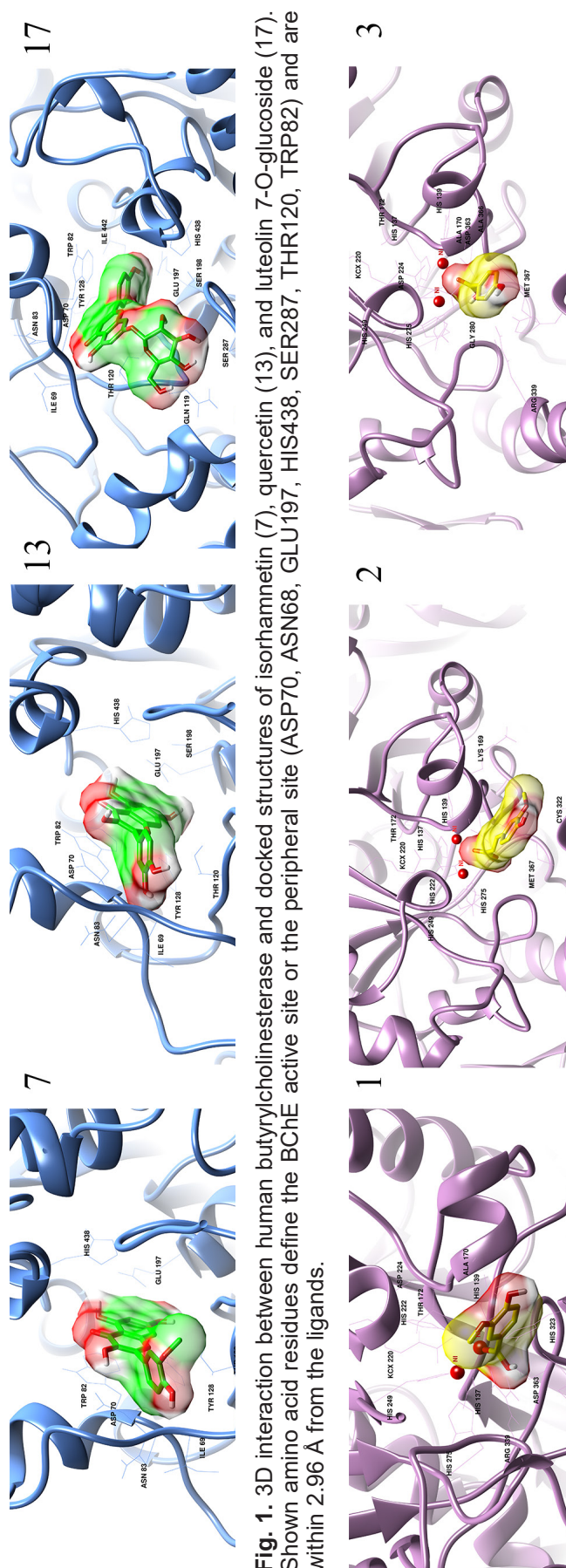


Fig. 1. 3D interaction between human butyrylcholinesterase and docked structures of isorhamnetin (7), quercetin (13), and luteolin 7-O-glucoside (17). Shown amino acid residues define the BChE active site or the peripheral site (ASP70, ASN68, GLU197, HIS438, SER287, THR120, TRP82) and are within 2.96 Å from the ligands.

Fig. 2. 3D interaction between *Bacillus pasteurii* urease and docked structures of vanillic acid (1), ferulic acid (2), and 4'-hydroxy benzoic acid (3). Shown amino acid residues define the urease active site or the peripheral site (ALA170, ALA366, ASP363, CYS322, GLU223, GLY280, HIS222, HIS249, HIS323, NI798, NI799) and are within 2.42 Å from the ligands.

acid, whereas ligands **1–4**, **6**, **8**, **11–14**, **16**, **17** (–5.3 – –8.5 Kcal/mol) were involved in hydrogen bond interactions (1.97–3.02 Å) with residues similar to the ones observed for thiourea. Moreover, aromatic rings of **8**, **11**, **13**, **14**, **16**, **17** formed π -anion (3.25–4.47 Å) and/or π -sulfur (4.86–5.84 Å) bonds with residues. On the other hand, acarbose (–10.1 Kcal/mol), which is an α -amylase inhibitor, was involved in conventional hydrogen interactions with thirteen active site residues (1.97 Å). Only compound **22** (–9.2 Kcal/mol) showed similar hydrogen interactions (2.02–2.96 Å), which explain the weak observed effect of ZSE. The obtained data indicated that many of the docked ligands from *Z. simplex* showed similar scores compared to the inhibitors, offering higher affinity and strong interactions, which suggest BChE (PDB ID 4EY4) and *B. pasteurii* urease (PDB ID 4UBP) to be potential protein targets for *Z. simplex* components.

Supplementary material

Tables S1–S4 and, Figures S1–S18 can be found in the supplementary material file.

Conclusions

Zygophyllum simplex is a medicinal plant known for the presence of some bioactive compounds such as flavonoids and saponins. Our findings revealed the remarkable anticholinesterase and anti-urease properties of the ethanol extract, supported by molecular docking data of the most abundant compounds, explaining the observed effects. Our results suggest that *Z. simplex* may be considered as a potential source of anti-BChE and anti-urease agents.

References

- Abdallah, H.M. & Esmat, A. (2017). Antioxidant and anti-inflammatory activities of the major phenolics from *Zygophyllum simplex* L. *Journal of Ethnopharmacology*, 205, 51–56. <https://doi.org/10.1016/j.jep.2017.04.022>
- Abdel Bar, F.M., Alonazi, R., Ibrahim, S., Elekhawwy, E., Alqarni, M.H., Foudah, A.I., & Samra, R.M. (2025). Lipoidal fractions of *Zygophyllum simplex* exhibit antibacterial and wound-healing activities in a *Pseudomonas aeruginosa*-infected burn model. *Scientific African*, 29, e02875. <https://doi.org/10.1016/j.sciaf.2025.e02875>
- Amini-Chermahini, F., Ebrahimi, M., Farajpour, M., & Taj Bordbar, Z. (2014). Karyotype analysis and new chromosome number reports in *Zygophyllum* species. *Caryologia*, 67(4), 321–324. <https://doi.org/10.1080/00087114.2014.980098>
- Amin, E., El-Hawary, S.S., Fathy, M.M., Mohammed, R., & Khan, I.A. (2011). A Phytochemical Study of *Zygophyllum simplex* L. *Planta medica*, 77(05), P_40. <https://doi.org/10.1055/s-0031-1273569>
- Ayoub, I.M., Abdel-Aziz, M.M., Elhady, S.S., Bagalagel, A.A., Malatani, R.T., & Elkady, W. M. (2022). Valorization of *Pimenta racemosa* essential oils and extracts: GC-MS and LC-MS phytochemical profiling and evaluation of *Helicobacter pylori* inhibitory activity. *Molecules*, 27(22), 7965. <https://doi.org/10.3390/molecules27227965>
- Baky, M.H., Gabr, N.M., Shawky, E.M., Elgindi, M.R., & Mekky, R.H. (2020). A Rare Triterpenoidal saponin isolated and identified from *Tetraena simplex* (L.) Beier & Thulin (Syn. *Zygophyllum simplex* L.). *Chemistry Select*, 5(6), 1907–1911. <https://doi.org/10.1002/slct.201903454>
- Bar, F.M.A., Alonazi, R., Elekhawwy, E., Samra, R.M., Alqarni, M.H., Badreldin, H., & Magdy, G. (2025). HPLC-PDA and *in vivo* anti-inflammatory potential of isorhamnetin-3-O- β -D-glucoside from *Zygophyllum simplex* L. *Journal of Ethnopharmacology*, 338, 119089. <https://doi.org/10.1016/j.jep.2024.119089>
- Boumaza, A., Ferdi, S., Sbayou, H., Touhami, F.K., Belmahi, M.H., & Benlatreche, C. (2016). Therapeutic effect of *Zygophyllum cornutum* on metabolic disturbances, oxidative stress in heart tissue and histological changes in myocardium of streptozotocin-induced diabetic rats. *Journal of Life Sciences*, 10, 192–197. <https://doi.org/10.17265/1934-7391/2016.04.004>
- Bourgou, S., Megdiche, W., & Ksouri, R. (2017). The halophytic genus *Zygophyllum* and *Nitraria* from North Africa: A phytochemical and pharmacological overview. In: Neffati, M., Najjaa, H., Máthé, Á. (Eds.) *Medicinal and Aromatic Plants of the World-Africa Volume 3* (pp. 345–356). Netherlands, Dodrecht: Springer. https://doi.org/10.1007/978-94-024-1120-1_13
- Brahmi, D., Ayed, Y., Hfaiedh, M., Bouaziz, C., Mansour, H.B., Zourgui, L., & Bacha, H. (2012). Protective effect of cactus cladode extract against cisplatin induced oxidative stress, genotoxicity and apoptosis in balb/c mice: combination with phytochemical composition. *BMC Complementary and Alternative Medicine*, 12(1), 111. <https://doi.org/10.1186/1472-6882-12-111>
- Costa, L.D., Silva, C.F., Pinto, D.C., Silva, A.M., Pereira, F., Faustino, M.A.F., & Tomé, A.C. (2023). Discovery of thiazolo [5, 4-c] isoquinoline

based compounds as acetylcholinesterase inhibitors through computational target prediction, molecular docking and bioassay. *Journal of Molecular Structure*, 1291, 136088. <https://doi.org/10.1016/j.molstruc.2023.136088>

Elkolli, M., Chafai, N., Chafaa, S., Kadi, I., Bensouici, C., & Hellal, A. (2022). New phosphinic and phosphonic acids: Synthesis, antidiabetic, anti-Alzheimer, antioxidant activity, DFT study and SARS-CoV-2 inhibition. *Journal of Molecular Structure*, 1268, 133701. <https://doi.org/10.1016/j.molstruc.2022.133701>

Ellman, G.L., Courtney, K.D., Andres Jr, 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](https://doi.org/10.1016/0006-2952(61)90145-9)

Elsawalhy, M., Abdel-Rahman, A.A.H., Basiony, E.A., Ellithy, S.A., Hassan, A.A., Abou-Amra, E.S., Ismail, A., Almhizia, A.A., Al-Omar, M.A., Naglah, A.M., & Hassan, N.A. (2024). Novel dual acetyl- and butyrylcholinesterase inhibitors based on the pyridyl-pyridazine moiety for the potential treatment of Alzheimer's disease. *Pharmaceuticals*, 17(10), 1407. <https://doi.org/10.3390/ph17101407>

Galal, N.M., Al Touby, S.S., Alrashdi, Y.B.A., & Hossain, M.A. (2025). Evaluation of toxicity and antioxidant activities of various crude extracts of leaves and stems of *Zygophyllum simplex*. *Advances in Biomarker Sciences and Technology*, 7, 1-7. <https://doi.org/10.1016/j.abst.2024.11.003>

Goodarzi, N.N., Asforooshani, M.K., Shahbazi, B., Rahimi, N.R., & Badmasti, F. (2024). Identification of novel drug targets for *Helicobacter pylori*: Structure-based virtual screening of potential inhibitors against DAH7PS protein involved in the shikimate pathway. *Frontiers in Bioinformatics*, 4, 1482338. <https://doi.org/10.3389/fbinf.2024.1482338>

Gong, G., Guan, Y.-Y., Zhang, Z.-L., Rahman, K., Wang, S.-J., Zhou, S., Luan, X., & Zhang, H. (2020). Isorhamnetin: A review of pharmacological effects. *Biomedicine & Pharmacotherapy*, 128, 110301. <https://doi.org/10.1016/j.biopha.2020.110301>

Hassanean, H.A., & Desoky, E.K. (1992). An acylated isorhamnetin glucoside from *Zygophyllum simplex*. *Phytochemistry*, 31(9), 3293-3294. [https://doi.org/10.1016/0031-9422\(92\)83503-q](https://doi.org/10.1016/0031-9422(92)83503-q)

Huang, M., Xiao, Q., Li, Y., Ahmad, M., Tang, J., Liao, Q., & Tan, C. (2024). Inhibition of α -amylase activity by quercetin via multi-spectroscopic and molecular docking approaches. *Food Bioscience*, 61,

104951. <https://doi.org/10.1016/j.fbio.2024.104951>

Hussein, S.R., Marzouk, M.M., Ibrahim, L.F., Kawashty, S.A., & Saleh, N.A. (2011). Flavonoids of *Zygophyllum album* L. and *Zygophyllum simplex* L. (Zygophyllaceae). *Biochemical Systematics and Ecology*, 39(4), 778. <https://doi.org/10.1016/j.bse.2011.07.009>

Jamali-Raeufy, N., Baluchnejadmojarad, T., Roghani, M., Keimasi, S., Goudarzi, M. (2019). Isorhamnetin exerts neuroprotective effects in STZ-induced diabetic rats via attenuation of oxidative stress, inflammation and apoptosis. *Journal of Chemical Neuroanatomy*, 102, 101709. <https://doi.org/10.1016/j.jchemneu.2019.101709>

Kakrani, H.K.N., Kakrani, P.H., & Saluja, A.K. (2011). Evaluation of analgesic and anti-inflammatory activity of ethyl acetate extract of *Zygophyllum simplex* Linn. herb. *International Journal of Research in Phytochemistry & Pharmacology*, 1(3), 180-183. <https://scienztech.org/index.php/ijrpp/article/view/776>

Kchaou, M., Salah, H.B., Mhiri, R., & Allouche, N. (2016). Anti-oxidant and anti-acetylcholinesterase activities of *Zygophyllum album*. *Bangladesh Journal of Pharmacology*, 11(1), 54-62. <https://doi.org/10.3329/bjp.v11i1.25463>

Khan, H., Amin, S., Kamal, M.A., & Patel, S. (2018). Flavonoids as acetylcholinesterase inhibitors: Current therapeutic standing and future prospects. *Biomedicine & Pharmacotherapy*, 101, 860-870. <https://doi.org/10.1016/j.biopha.2018.03.007>

Khan, S.S., Khan, A., Khan, A., Wadood, A., Farooq, U., Ahmed, A., Zahoor, A., Ahmad, V.U., Sener, B., & Erdemoglu, N. (2014). Urease inhibitory activity of ursane type sulfated saponins from the aerial parts of *Zygophyllum fabago* Linn. *Phytomedicine*, 21(3), 379-382. <https://doi.org/10.1016/j.phymed.2013.09.009>

Liu, H., Xie, K., Rui, Y., Wang, S. (2025). Inhibitory effects and mechanisms of flavonoids in sea buckthornon (*Hippophae rhamnoides* L.) on *Helicobacter pylori*. *Foods*, 14(23), 3995. <https://doi.org/10.3390/foods14233995>

Mnafgui, K., Kchaou, M., Hamden, K., Derbali, F., Slama, S., Nasri, M., Hichem, & Salah, B., Allouche, N., & Elfeki, A. (2014). Inhibition of carbohydrate and lipid digestive enzymes activities by *Zygophyllum album* extracts: effect on blood and pancreas inflammatory biomarkers in alloxan-induced diabetic rats. *Journal of physiology and biochemistry*, 70(1), 93-106. <https://doi.org/10.1007/s13105-013-0284-1>

- Mohammedi, Z.** (2020). Phytochemical, antidiabetic and therapeutic properties of *Zygophyllum*. *Herbal Medicines Journal*, 5(4). <https://doi.org/10.22087/hmj.v5i4.813>
- Morris, G.M., Huey, R., Lindstrom, W., Sanner, M.F., Belew, R.K., Goodsell, D.S., & Olson, A.J.** (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of computational chemistry*, 30(16), 2785-2791. <https://doi.org/https://doi.org/10.1002/jcc.21256>
- Naseem, S., Ashraf, M., Khan, S., Rafiq, M., Kashif, M., Rahman, J., Rauf, M.K., Halim, S.A., Uddin, J., Khan, A., Al-Harrasi, A., & Shafiq, Z.** (2021). Exploring biologically active hybrid pharmacophore N-substituted hydrazine-carbothioamides for urease inhibition: *In vitro* and *in silico* approach. *International Journal of Biological Macromolecules*, 182, 534-544. <https://doi.org/10.1016/j.ijbiomac.2021.04.036>
- Nazeer, M.S., Pasha, T.N., Abbas, S., & Ali, Z.** (2002). Effect of yucca saponin on urease activity and development of ascites in broiler chickens. *International Journal of Poultry Science*, 1(6), 174-178. <https://doi.org/10.3923/ijps.2002.174.178>
- Olennikov, D.N., Kashchenko, N.I., Chirikova, N.K., Akobirshoeva, A., Zilfikarov, I.N., & Vennos, C.** (2017). Isorhamnetin and quercetin derivatives as anti-acetylcholinesterase principles of marigold (*Calendula officinalis*) flowers and preparations. *International Journal of Molecular Sciences*, 18(8), 1685. <https://doi.org/10.3390/ijms18081685>
- Ojetunde, A.O.** (2024). The neuroprotective and therapeutic effects of medicinal plants and natural products against aluminium chloride-induced alzheimer's disease. *Recent Update. Biology, Medicine, & Natural Product Chemistry*, 13(1), 7-33. <https://doi.org/10.14421/biomedich.2024.131.7-33>
- Orhan, I., Şener, B., Choudhary, M.I., & Khalid, A.** (2004). Acetylcholinesterase and butyrylcholinesterase inhibitory activity of some Turkish medicinal plants. *Journal of Ethnopharmacology*, 91(1), 57-60. <https://doi.org/10.1016/j.jep.2003.11.016>
- Quezel, P. & Santa, S.** (1963). *Nouvelle flore de l'Algérie et des régions désertiques méridionales. Tome II*. Eds. du Centre Nat. de la Recherche Scientifique.
- Sezen Karaoğlu, E., Hancı, H., Koca, M., & Kazaz, C.** (2023). Some bioactivities of isolated apigenin-7-O-glucoside and luteolin-7-O-glucoside. *Applied Sciences*, 13(3), 1503. <https://doi.org/10.3390/app13031503>
- Shawky, E., Gabr, N., El-gindi, M., & Mekky, R.** (2019). A comprehensive review on genus *Zygophyllum*. *Journal of Advanced Pharmacy Research*, 3(1), 1-16. <https://doi.org/10.21608/aprh.2019.5699.1066>
- Shraim, A.M., Ahmed, T.A., Rahman, M.M., & Hijji, Y.M.** (2021). Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. *LWT-Food Science and Technology*, 150, 111932. <https://doi.org/10.1016/j.lwt.2021.111932>
- Souza, F.N., Oliveira, N.K.S., de Lima, H.B., Silva, A.G., Cruz, R.A.S., Oliveira, F.R., Federico, L.B., & Hage-Melim, L.I.S.** (2025). Therapeutic potential of quercetin in the treatment of Alzheimer's disease: *In silico*, *in vitro* and *in vivo* approach. *Applied Sciences*, 15(19), 10340. <https://doi.org/10.3390/app151910340>
- Szwajgier, D.** (2015). Anticholinesterase activity of selected phenolic acids and flavonoids—interaction testing in model solutions. *Annals of Agricultural and Environmental Medicine*, 22(4), 690-694. <https://doi.org/10.5604/12321966.1185777>
- Trott, O. & Olson, A.J.** (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of computational chemistry*, 31(2), 455-461. <https://doi.org/10.1002/jcc.21334>
- Weatherburn, M.** (1967). Phenol-hypochlorite reaction for determination of ammonia. *Analytical chemistry*, 39(8), 971-974. <https://doi.org/10.1021/ac60252a045>
- Xiao, Z.P., Wang, X.D., Peng, Z.Y., Huang, S., Yang, P., Li, Q. S., Zhou, L.H., Hu, X.J., Wu, L.J., Zhou, Y., & Zhu, H.L.** (2012). Molecular docking, kinetics study, and structure-activity relationship analysis of quercetin and its analogous as *Helicobacter pylori* urease inhibitors. *Journal of Agricultural and Food Chemistry*, 60(42), 10572–10577. https://doi.org/10.1021/JF303393N/ASSET/IMAGES/MEDIUM/JF-2012-03393N_0005.GIF
- Zengin, G., Sarikurkcu, C., Aktumsek, A., Ceylan, R., & Ceylan, O.** (2014). A comprehensive study on phytochemical characterization of *Haplophyllum myrtifolium* Boiss. endemic to Turkey and its inhibitory potential against key enzymes involved in Alzheimer, skin diseases and type II diabetes. *Industrial Crops and Products*, 53, 244-251. <https://doi.org/10.1016/j.indcrop.2013.12.043>

Supplementary material

Table S1. Docking scores of the reference inhibitors of AChE, BChE, urease and α -amylase

	Docking Score (Kcal/mol)			
	AChE (PDB ID 4BDS)	BChE (PDB ID 4EY4)	Urease (PDB ID 4UBP)	α -amylase (PDB ID 2QV4)
Tacrine	-8.7	-8.3	–	–
Donepezil	-9.6	–	–	–
Acetohydroxamic acid	–	–	-3.8	–
Theourea	–	–	-3.2	–
Acarbose	–	–	–	-10.1

Table S2. Molecular docking calculation of the selected molecules from *Z. simplex* with AChE, BChE, anti-urease and α -amylase enzymes

Compound	Score (Kcal/mol)			
	AChE (4BDS)	BChE (4EY4)	Urease (4UBP)	α -amylase (2QV4)
Vanillic acid (1)	-6.6	-6	-6.3	-5.5
Ferulic acid (2)	-6.7	-6.7	-6.1	-6.7
4'-Hydroxy benzoic acid (3)	-6.2	-5.8	-5.6	-5.5
4'-Hydroxy acetophenone (4)	-6.2	-5.8	-5.3	-5.7
<i>p</i> -Hydroxy acetophenone glucoside (5)	-7.6	-7.8	-7.1	-7.2
Androsin (6)	-7.8	-7.9	-6.8	-7.8
Isorhamnetin (7)	-8.9	-8.9	-7.7	-9
Isorhamnetin 3- <i>O</i> -glucoside (8)	-8.1	-9.2	-8.3	-8.1
Isorhamnetin 3,7-diglucoside (9)	-9.3	-9.5	-8.3	-8.7
Isorhamnetin 3- <i>O</i> -(6'-malonyl)glucoside (10)	-8.2	-9.6	-7.7	-8.8
Isorhamnetin 3-rutinoside (11)	-8.8	-10.4	-8.2	-8.3
Myricitrin (12)	-8.9	-9.7	-8.5	-8.5
Quercetin (13)	-9.6	-9.3	-7.9	-9.2
Quercetin 3- <i>O</i> -glucoside (14)	-8.5	-9.4	-8.5	-8.5
Quercetin 3- <i>O</i> -(6"-malonyl) glucoside (15)	-7.8	-10.4	-7.7	-9
Quercetin 3- α -L-rhamnoside (16)	-10	-9.9	-8.4	-9.7
Luteolin 7- <i>O</i> -glucoside (17)	-9.1	-9.3	-8.4	-8.4
Quinovic acid (18)	-8.9	-10.6	-7	-8.7
3-methoxyquinovic acid (19)	-9	-10.6	-6.9	-8.5
Quinovic acid 3- α -L-rhamnoside (20)	-8.9	-11.3	-7.6	-10
3- <i>O</i> - β -D-glucopyranosyl quinovic acid (21)	-9.8	-10.9	-7.4	-9.4
3- <i>O</i> - β -D-glucosyl-(1 $\rightarrow$ 2)-D-glucosyl quinovic acid-28- <i>O</i> - β -D-glucopyranosyl ester (22)	-8.3	-9	-8.1	-9.2

Table S3. List of hydrogen bond interactions between ligands from *Z. simplex* and human acetyl- and butyryl-cholinesterase receptors

N°	AChE		BChE	
	Interactions ^a	H– length (Å)	Interactions ^a	H– length (Å)
1	TYR 124, PHE295, ARG 296, TYR 341	2.57–2.87	HIS 438	2.26
2	TYR 124, TYR 337, ASP 74	2.08–2.53	TYR 332	2.57
3	PHE 295, ARG 296, TYR 341	2.59–2.69	–	–
4	TYR 124, PHE 295, ARG 296	2.29–2.83	–	–
5	TYR 124, HIS 447, SER 203, GLY 122	1.89–2.67	ASP 70, TRP 430, TYR 128, TYR 332, TYR 440	1.95–2.94
6	TYR 124, HIS 287, SER 293	2.21–2.67	GLY 117, SER 198	2.49–3.06
7	SER 293, PHE 295, ARG 296, GLN 291	1.97–2.74	HIS 438	2.68
8	TYR 72, SER 293	1.99–2.69	ASP 70, ASN 83, LEU 286, SER 287	2.21–2.71
9	PHE 295	2.17	ASP70, GLU 197, THR 120	2.37–2.90
10	TYR 72, PHE 295, SER 293, GLN 291, ARG 296	2.12–2.90	ASP 70, HIS 438, TRP 82	2.24–2.68
11	GLU 292, ARG 296	2.64–2.65	GLY 115, GLY 117, TRP 82	2.38–3.09
12	PHE 295, SER 293	1.87–2.21	TRP 82, TYR 128, GLU 197	2.18–2.39
13	SER 293, ASP 74, TYR 124, PHE 295, ARG 296	2.10–2.79	GLU 197, HIS 438	2.68–2.72
14	SER 293	2.27	TRP 82, SER 198, SER 287, GLY 78	2.43–2.65
15	TYR 124, PHE 295, ARG 296, TYR 72, SER 293, GLN 292	2.19–2.83	GLY 117, THR 120, ALA 199, SER 287, HIS 438, GLY 78, TYR 440	2.06–2.78
16	SER 293, TYR 124	1.80–2.95	TYR 332, GLU 197, SER 287	2.50–2.76
17	GLY 122, PHE 295	2.55–2.68	ASN 68, GLU 197, SER 287	2.04–2.96
18	GLN 369, PRO 235, ARG 296	2.22–2.93	ASP 70	2.33
19	GLN 369, ARG 296	2.270–2.272	ASP 70	2.36
20	HIS 287, ASP 74	2.60–2.70	ASP 70	2.5
21	ARG 296, ASN 533, ASN 233, GLU 313	1.88–2.39	TYR 332, THR 284, SER 287	2.62–2.83
22	ASN 233, GLY 234, THR 238	2.19–2.41	GLU 197, GLU 276, GLU 276, SER 287	1.98–2.95

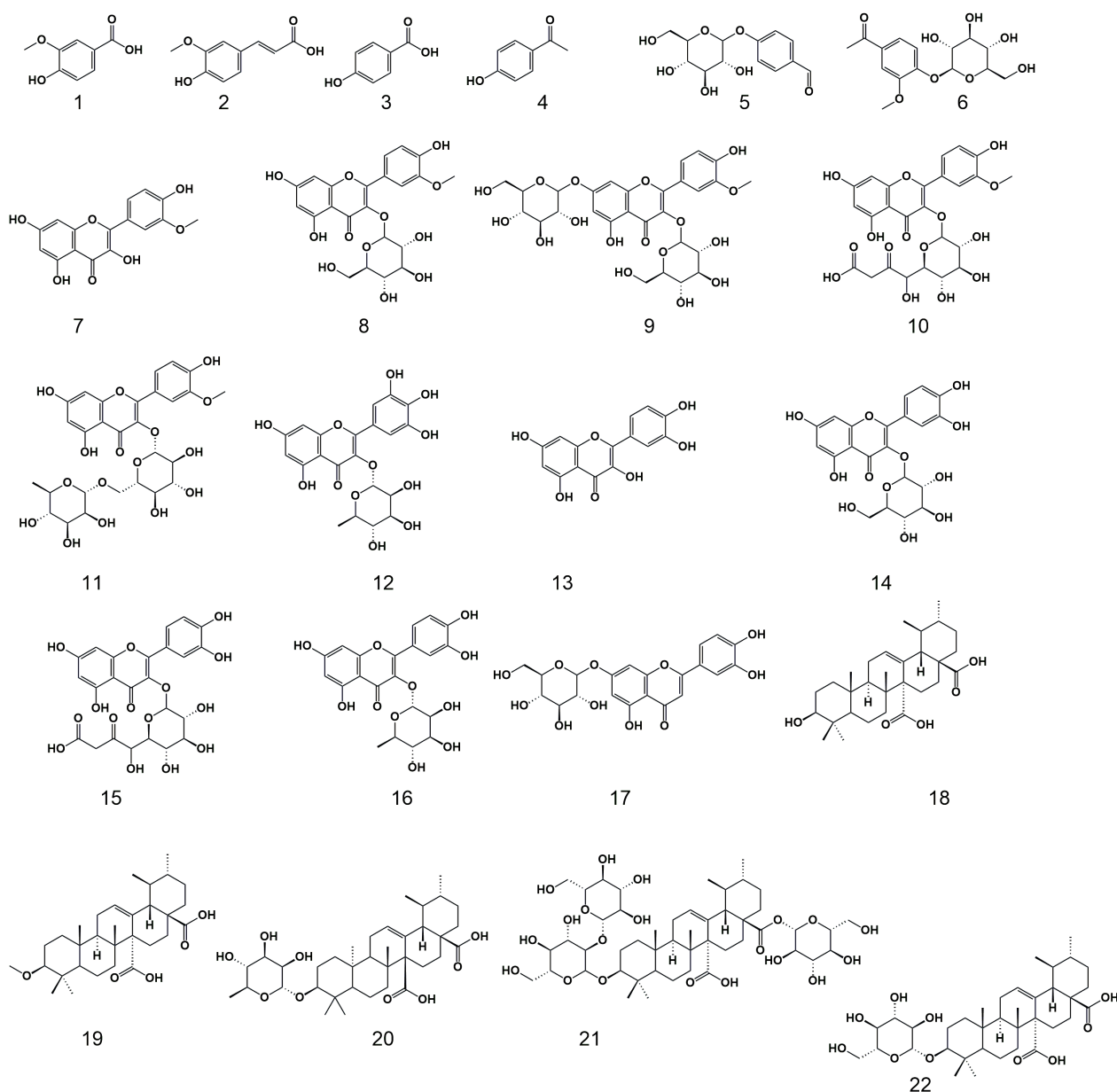


Fig. S1. The selected phenolic compounds (1-6), flavonoids (7-17), and ursane type saponins (18-22) for molecular docking calculations, previously identified in *Zygophyllum simplex* extracts

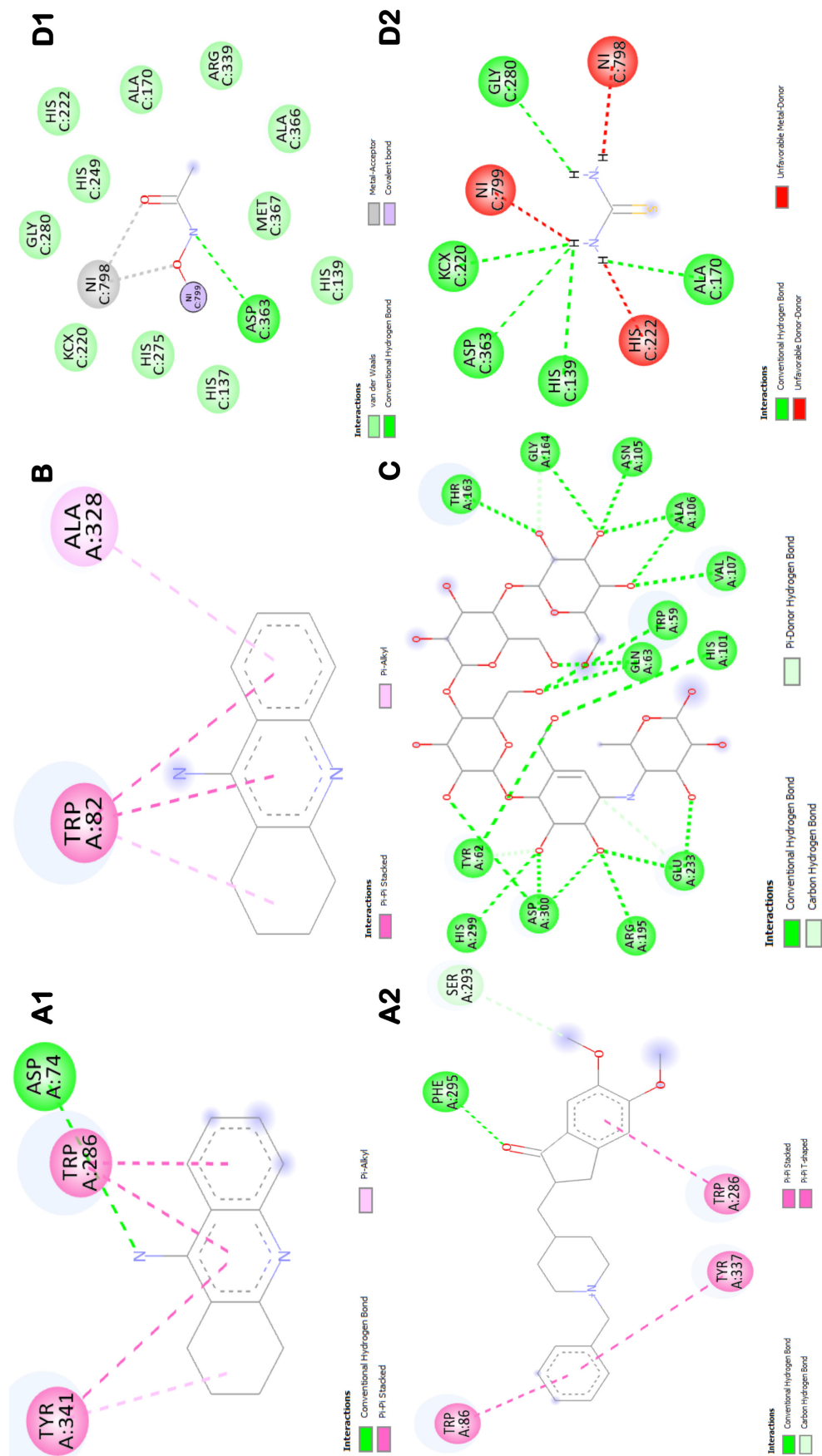


Fig. S2. Overlay of the co-crystallized ligands in the active sites of AChE (**A1**: Tacrine, **A2**: donepezil), BChE (**B**: Tacrine), α-amylase (**C**: Acraabose), and urease (**D1**: Acetohydroxamic acid, **D2**: Theourea) enzymes after re-docking (PDB ID 4BDS, 4EY4, 2QV4, 4UBP)

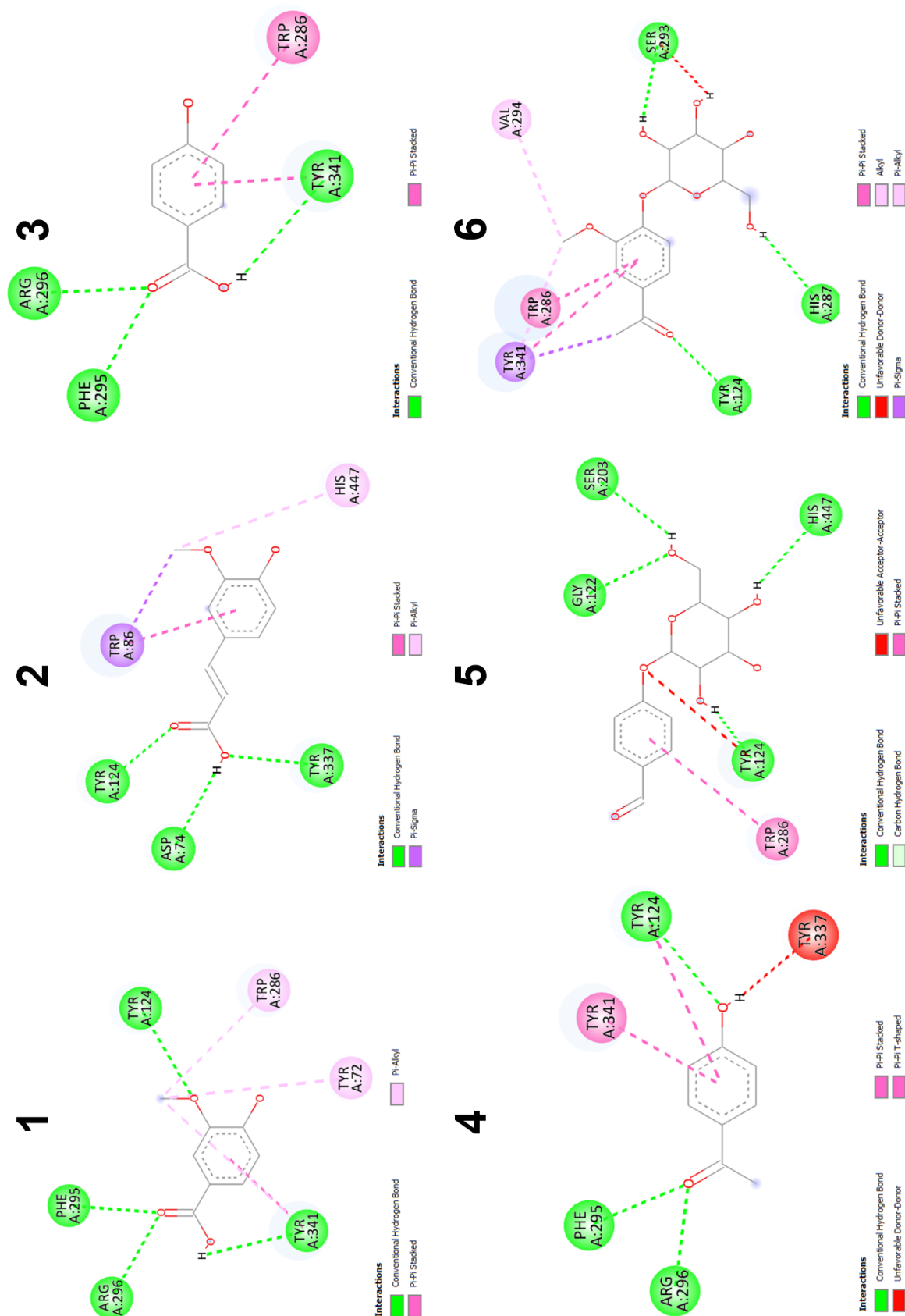


Fig. S3. 2D interactions between compounds 1–6 and AChE enzyme

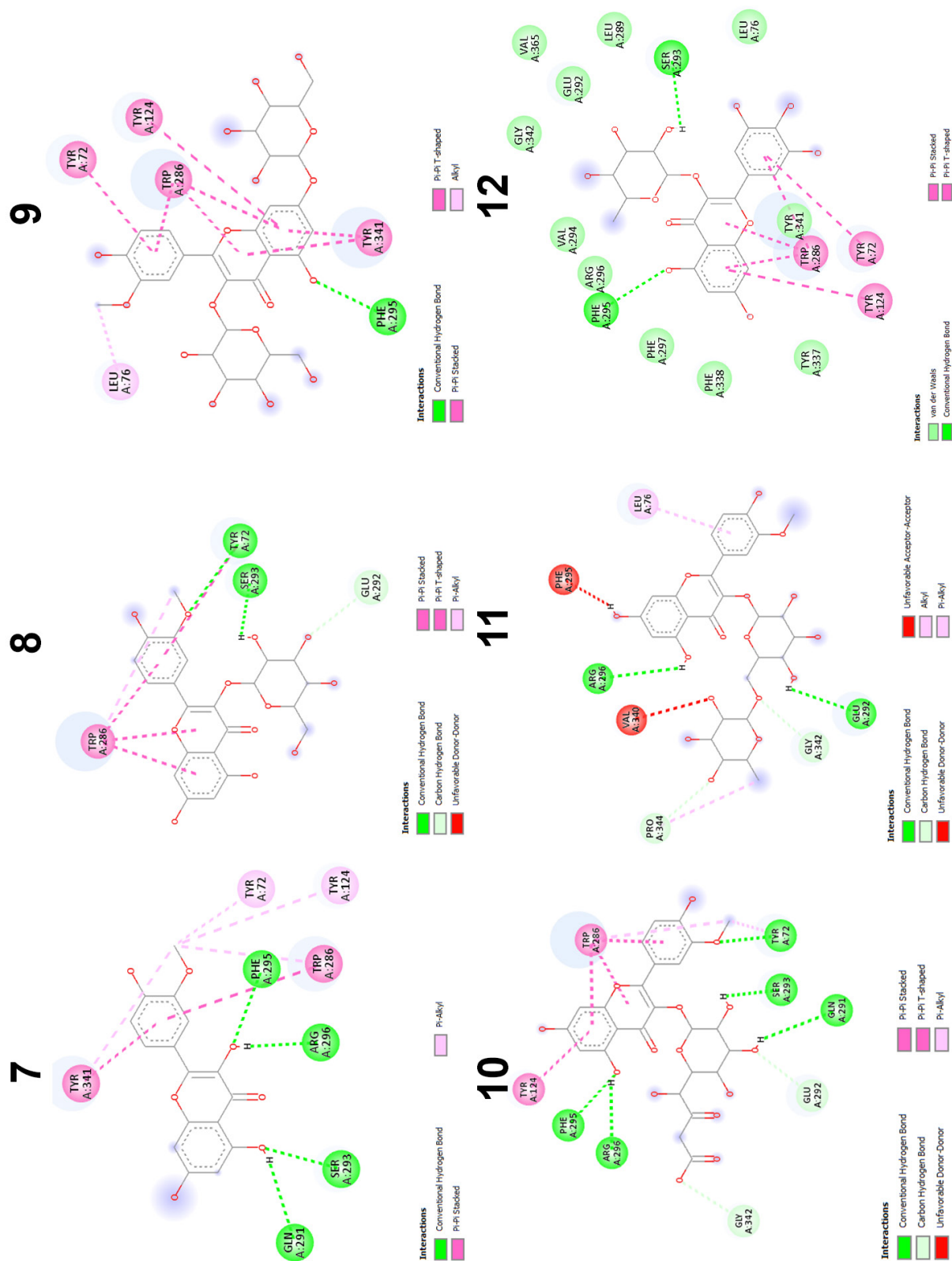


Fig. S4. 2D interactions between compounds 7-12 and AChE enzyme

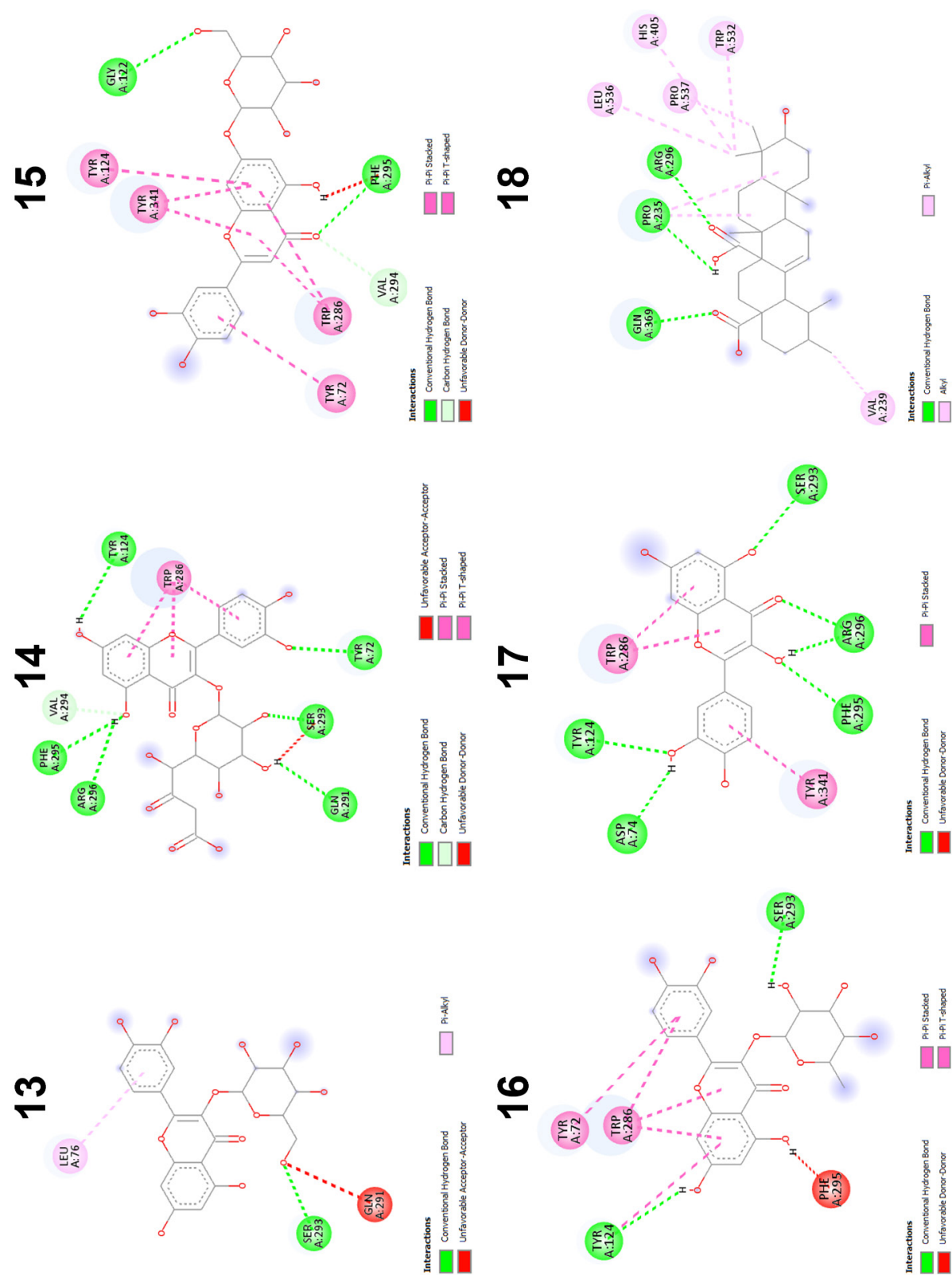


Fig. S5. 2D interactions between compounds 12–18 and AChE enzyme

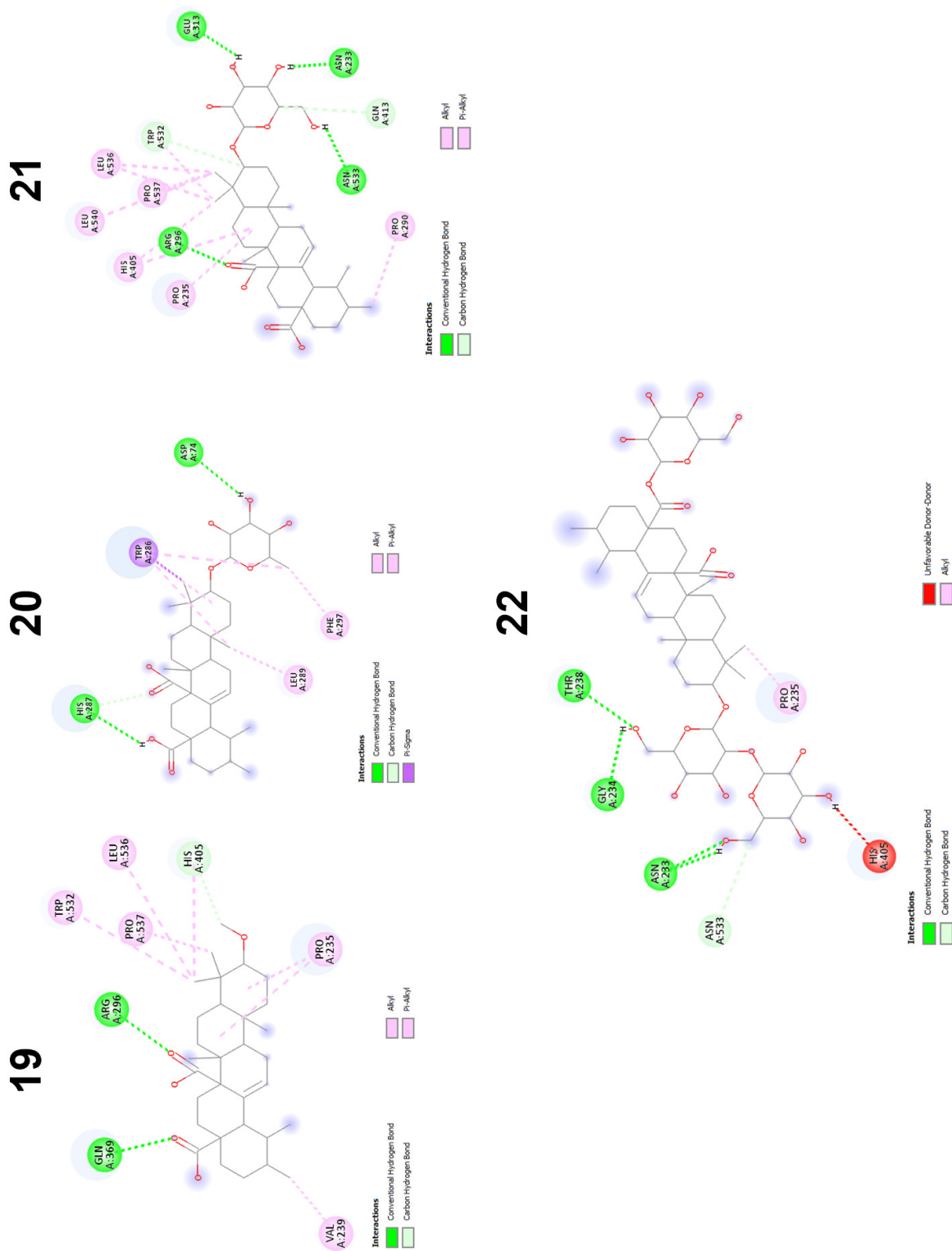


Fig. S6. 2D interactions between compounds 19–22 and AChE enzyme

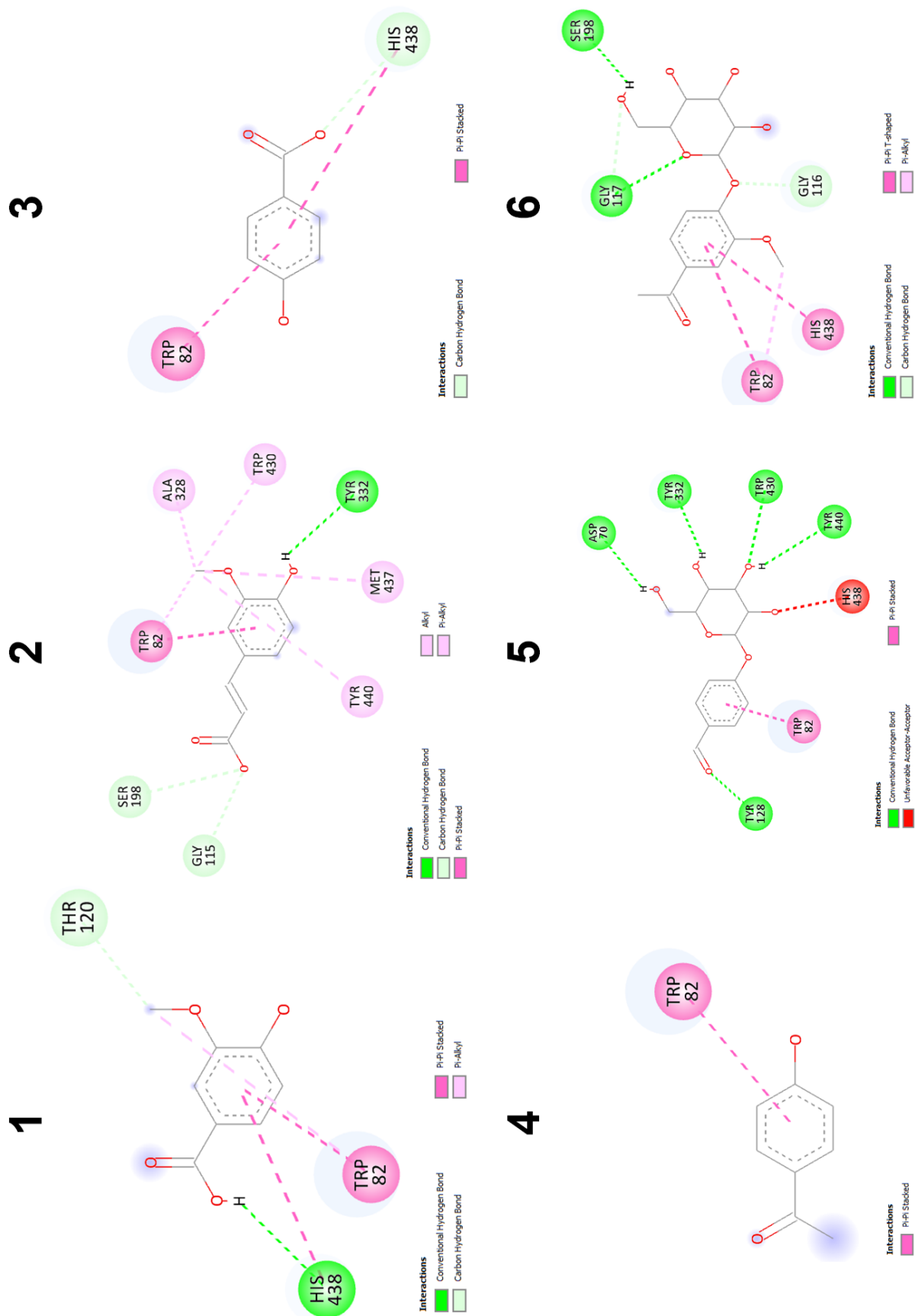


Fig. S7. 2D interactions between compounds 1–6 and BChE enzyme

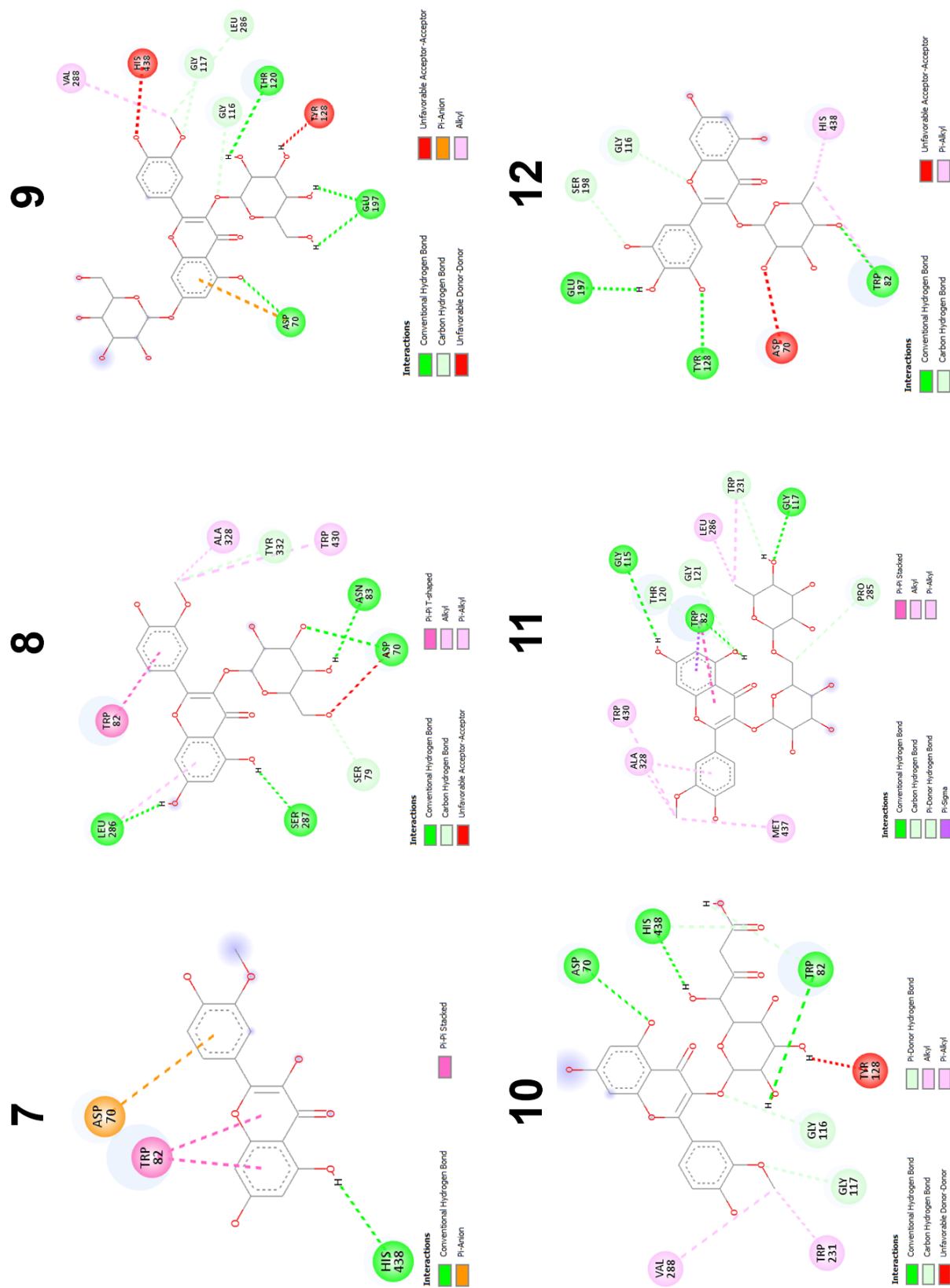
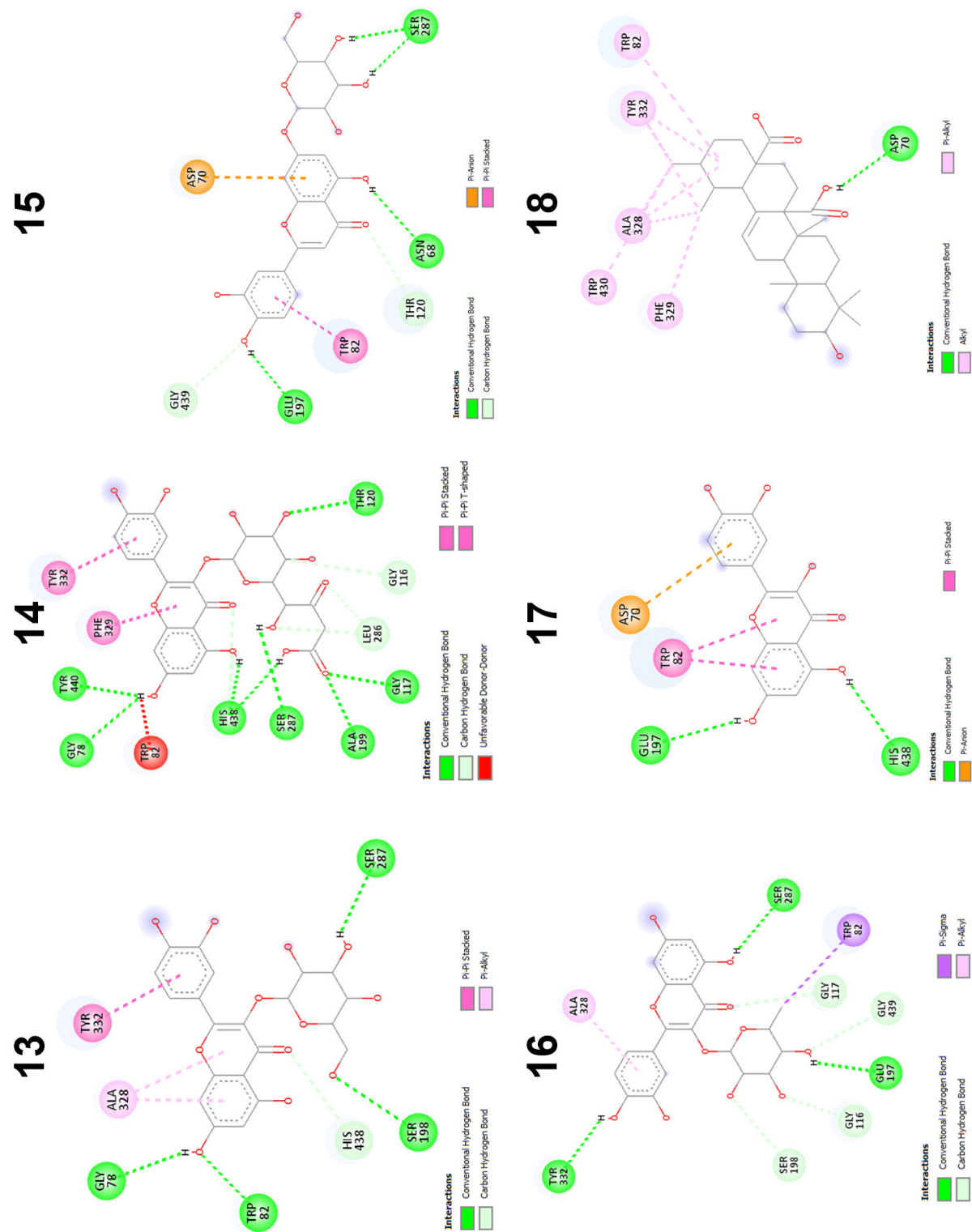


Fig. S8. 2D interactions between compounds 7-12 and BChE enzyme



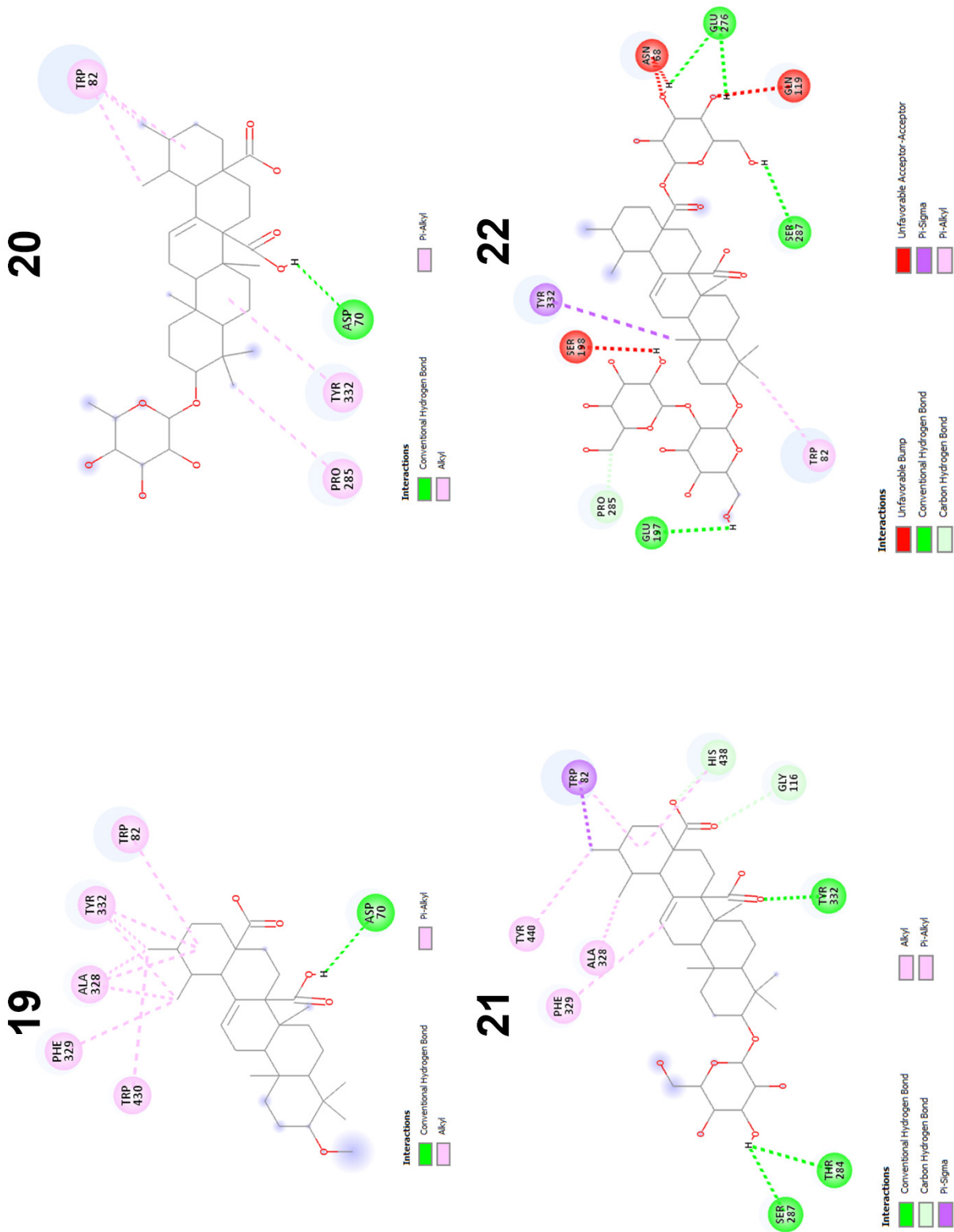


Fig. S10. 2D interactions between compounds 19–22 and BChE enzyme

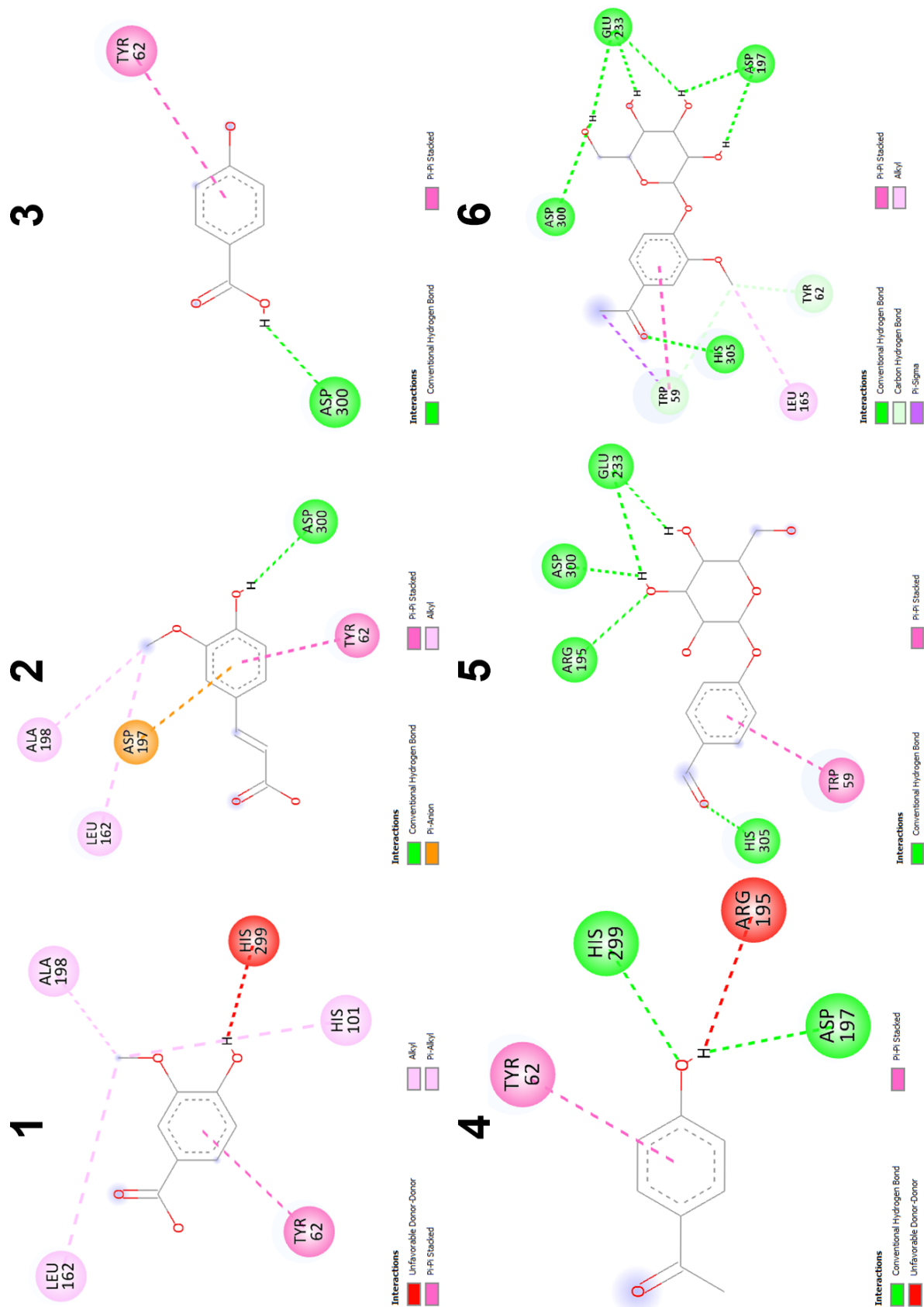


Fig. S11. 2D interactions between compounds 1-6 and α-amylase enzyme

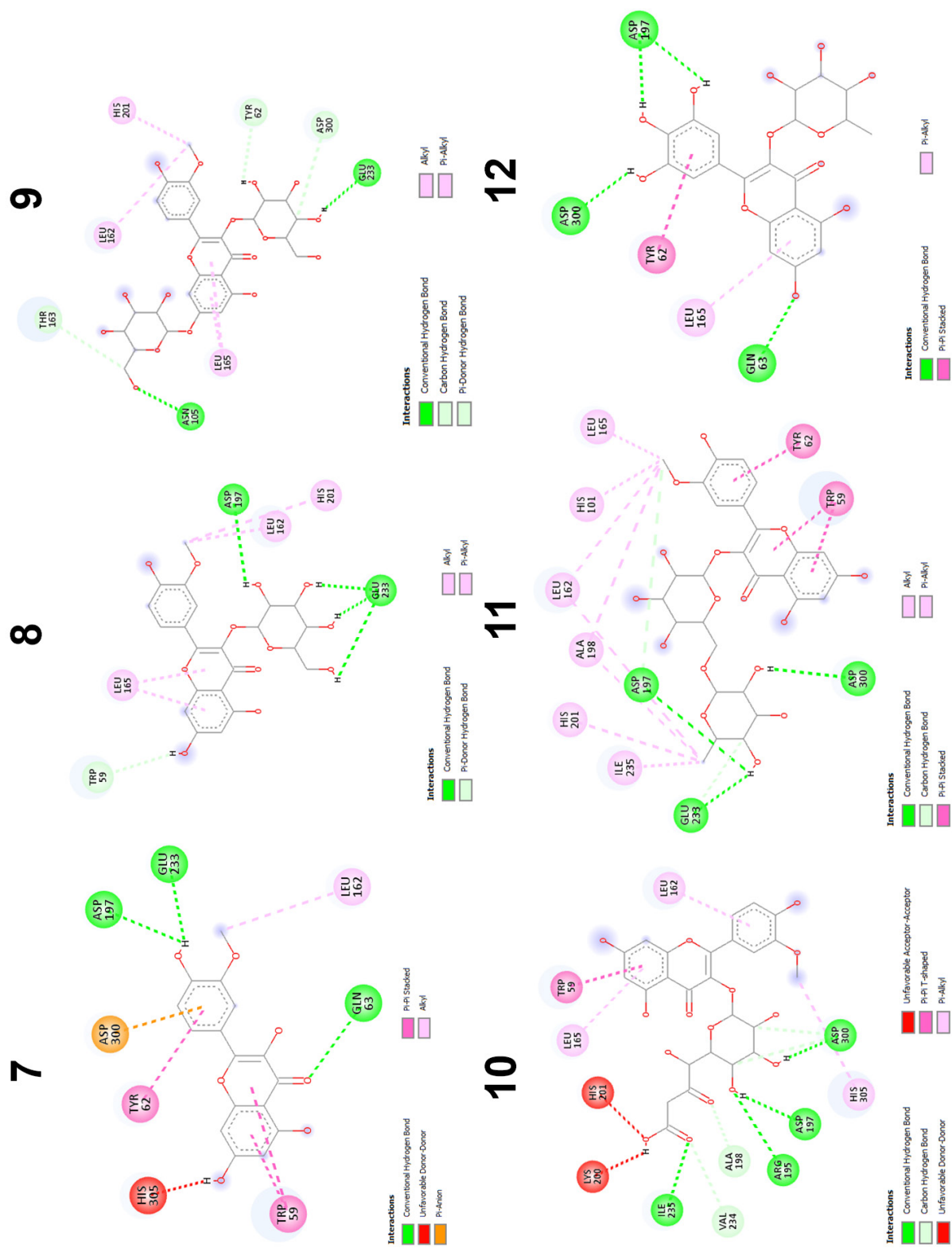


Fig. S12. 2D interactions between compounds 7–12 and α-amylase enzyme.

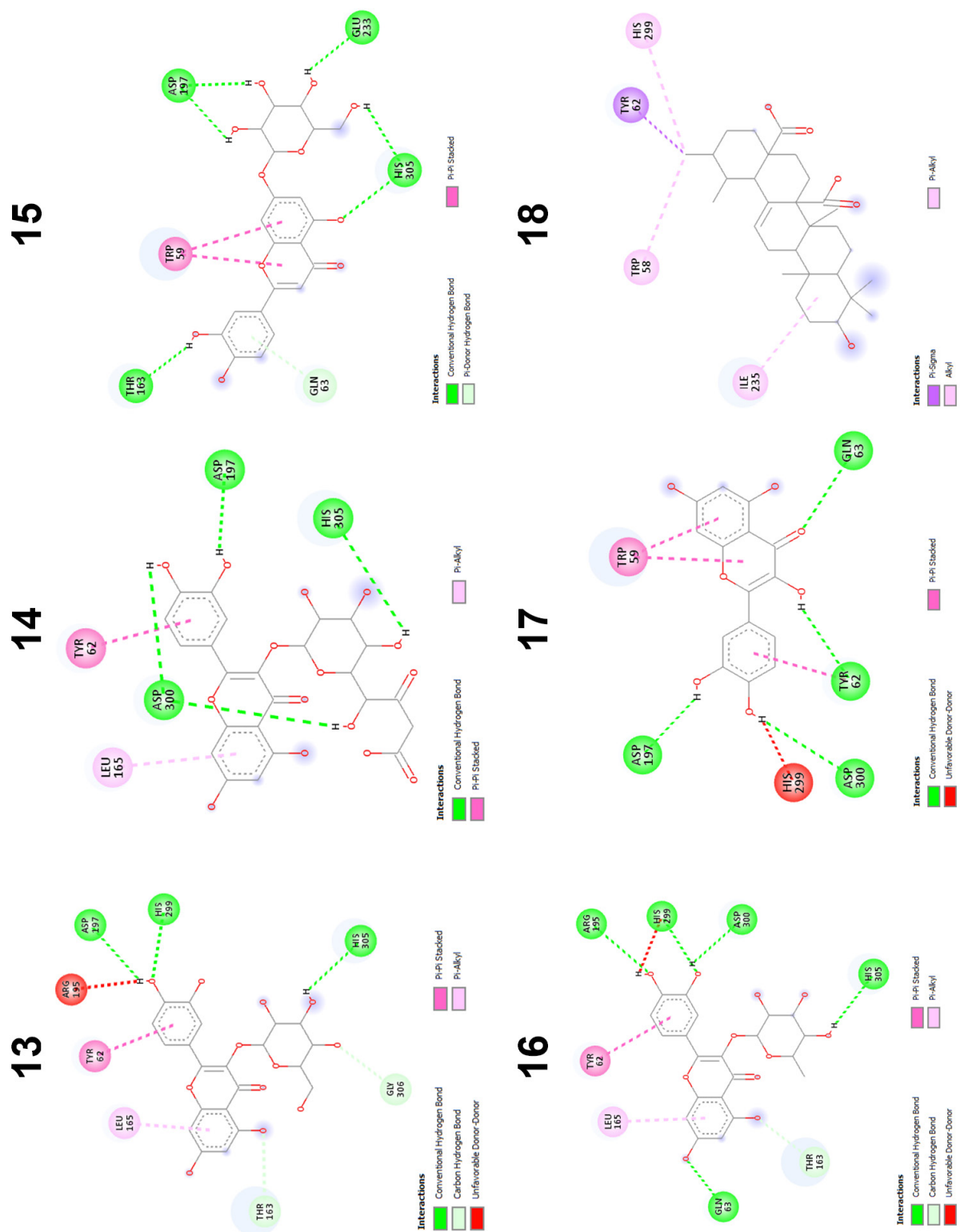


Fig. S13. 2D interactions between compounds 13–18 and α -amylase enzyme

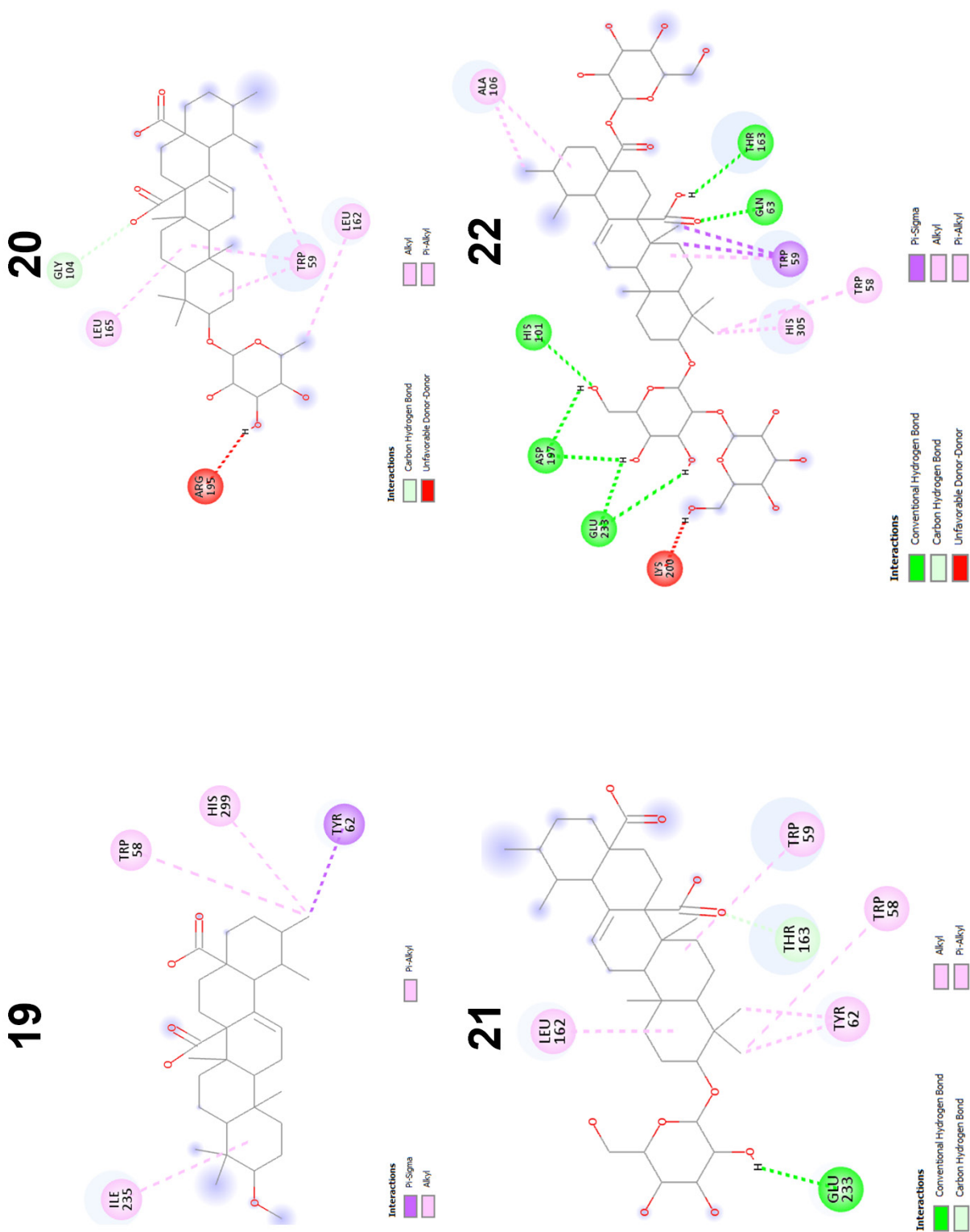


Fig. S14. 2D interactions between compounds 19-22 and α-amylase enzyme

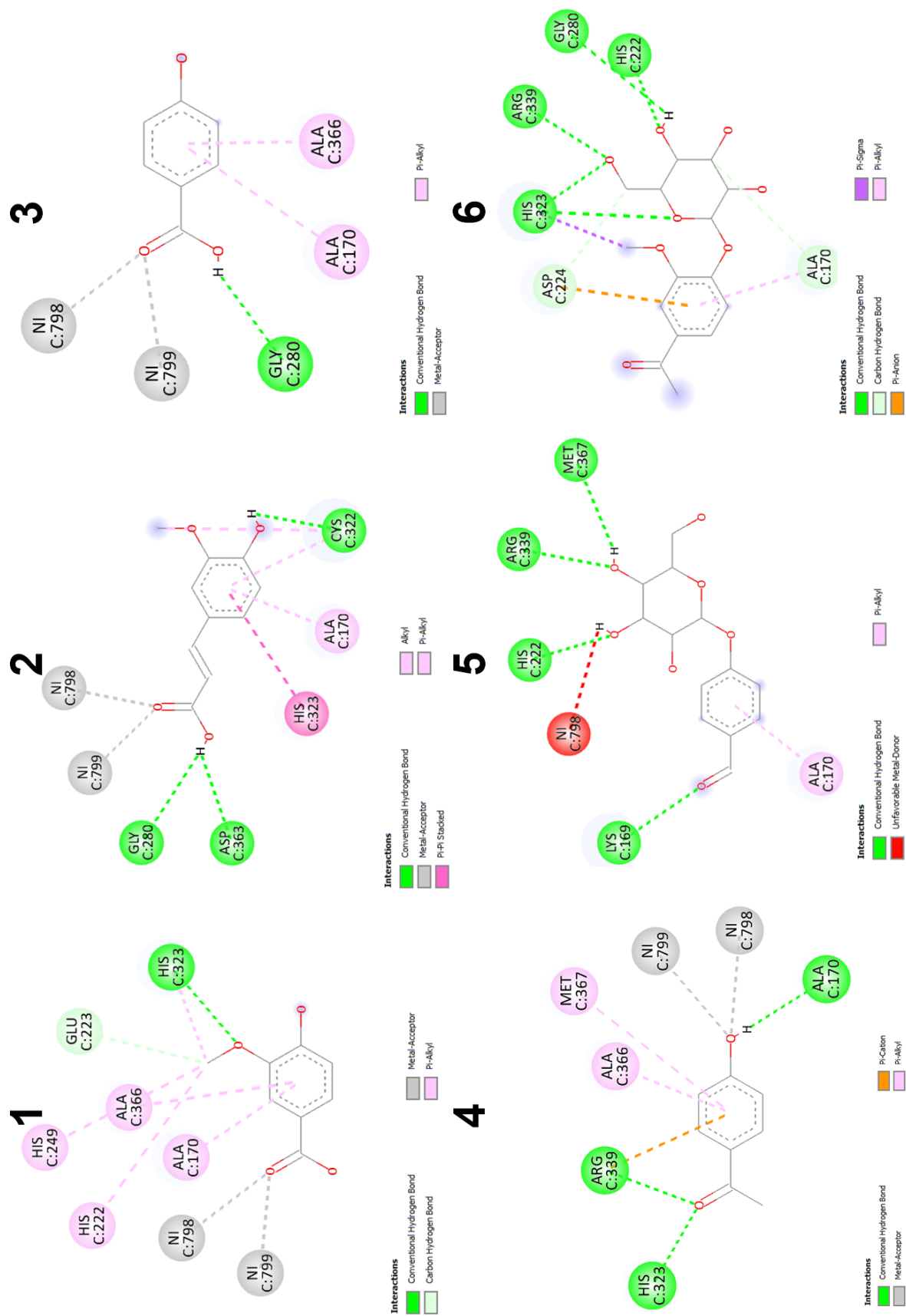


Fig. S15. 2D interactions between compounds 1–6 and urease enzyme

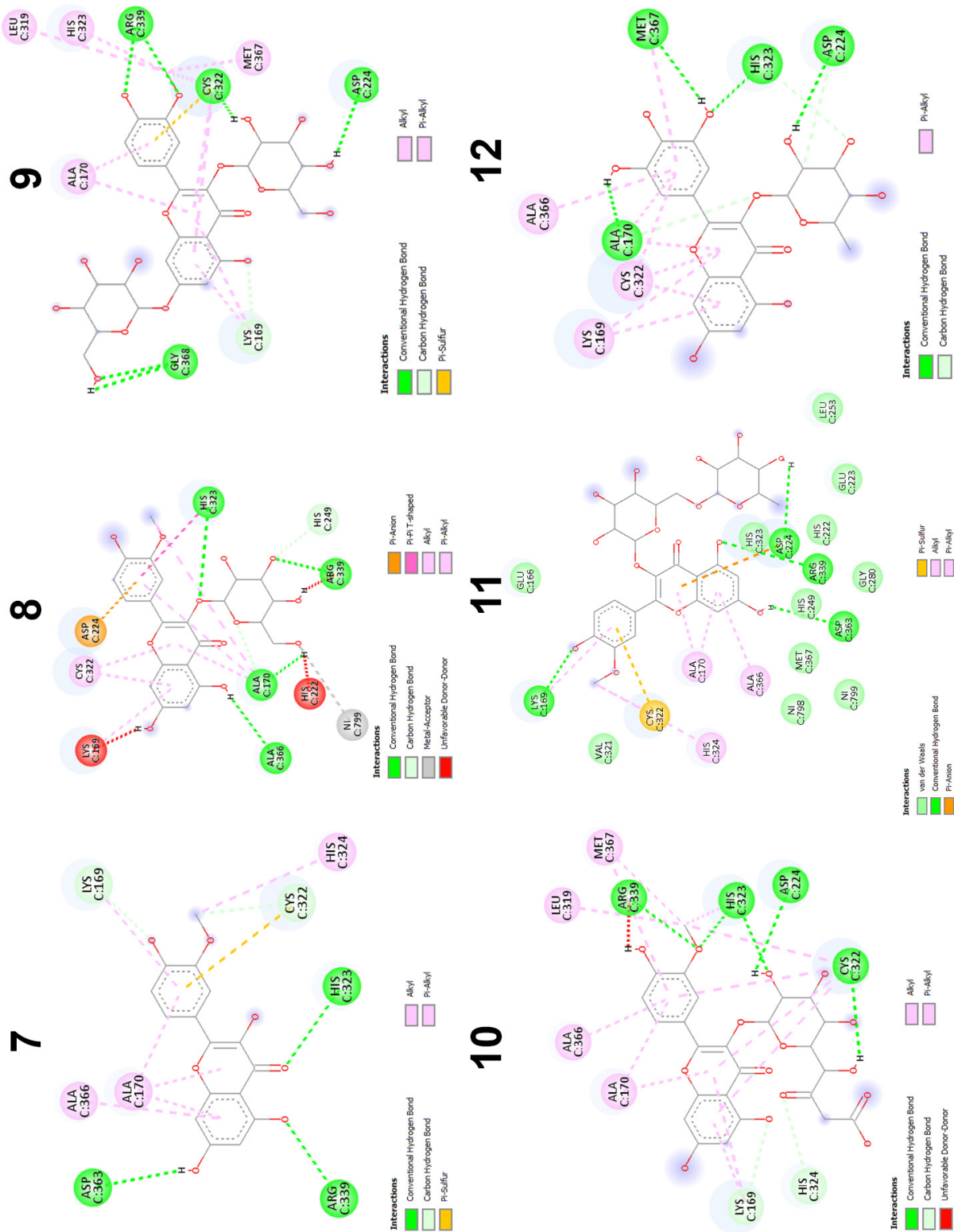


Fig. S16. 2D interactions between compounds 7–12 and urease enzyme

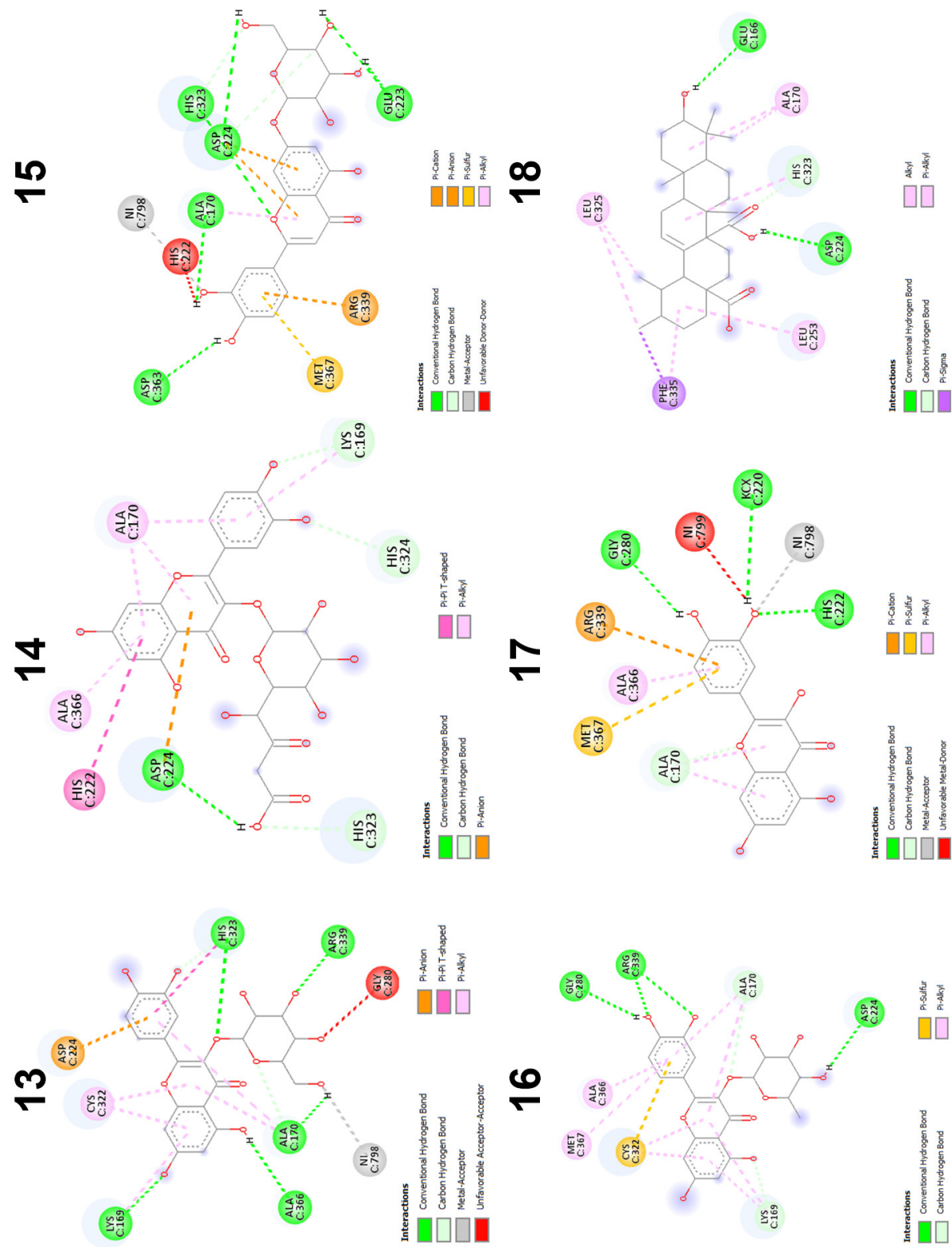


Fig. S17. 2D interactions between compounds 13–18 and urease enzyme

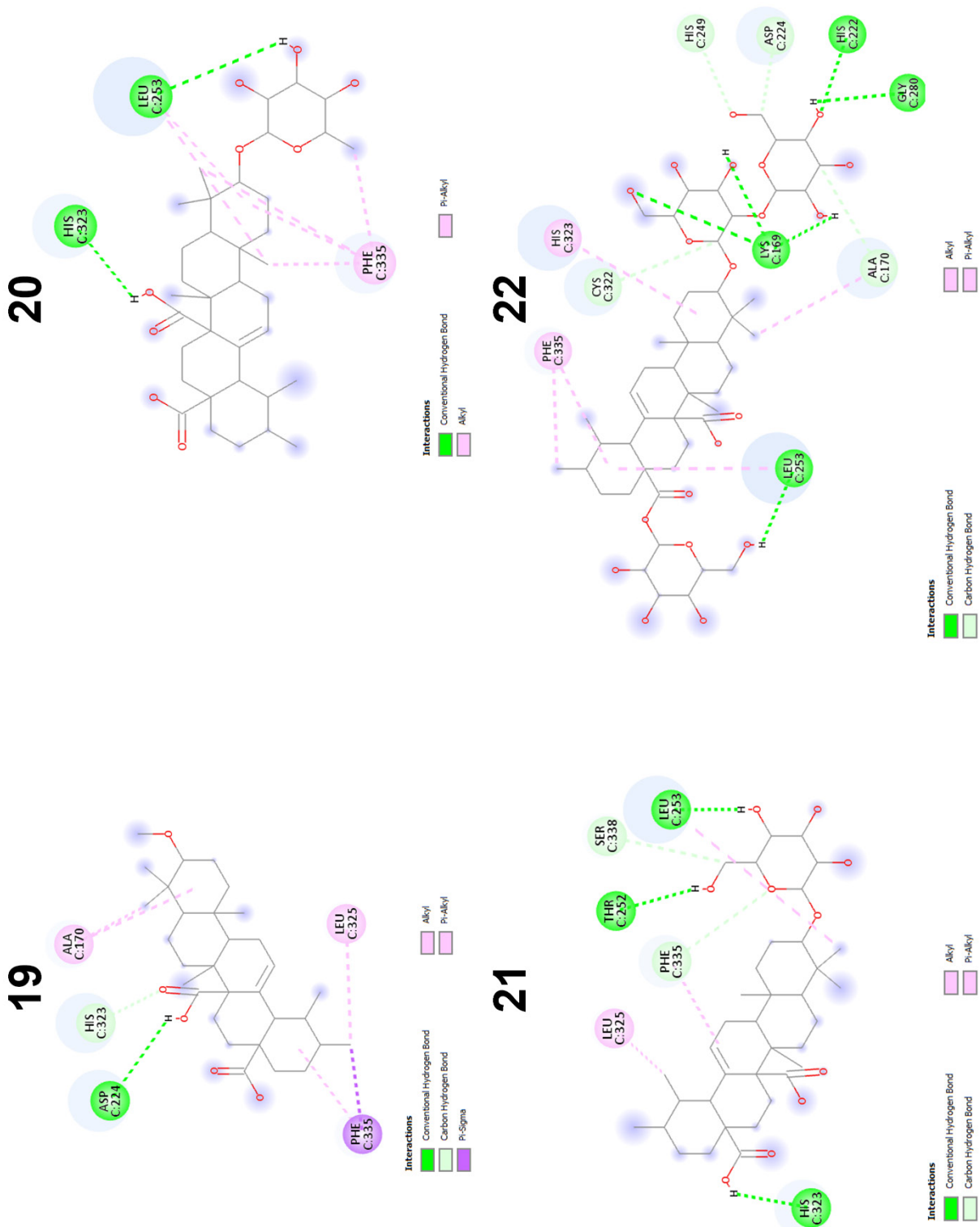


Fig. S18. 2D interactions between compounds 19–22 and α-amylase enzyme