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ORIGINAL ARTICLE

Synthesis and inhibition profiles of N-benzyl- and N-allyl aniline derivatives against carbonic anhydrase and acetylcholinesterase – A molecular docking study

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Abstract The alkyl and aryl derivatives of aniline are important starting materials in fine organic synthesis. Allyl bromide and benzyl chloride were taken as substrates for the alkylation reaction and as a halide ion scavenger. Triethylamine was utilized at reflux condition of N,N-dimethylacetamide (DMA). Novel synthesized N-benzyl and N-allyl aniline derivatives (**1a-f**) were evaluated to be highly potent inhibitors for acetylcholinesterase (AChE) and carbonic anhydrases (hCAs). The half maximal inhibitory concentration (IC_{50}) of N-benzyl- and N-allyl aniline derivatives were calculated between 243.11 and 633.54 nM for hCA I, 296.32–518.37 nM for hCA II and 182.45–520.21 nM for AChE enzymes. On the other hand, K_i values are in the range of 149.24 ± 15.59 to 519.59 ± 102.27 nM for AChE, 202.12 ± 16.21 to 635.31 ± 45.33 nM for hCA I and 298.57 ± 94.13 to 511.18 ± 115.98 nM for hCA II isoenzyme. Additionally, *in silico* molecular docking computations were performed with Autodock Vina program to support the experimental *in vitro*

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studies for both hCAs and AChE inhibitors. The *in silico* molecular docking results demonstrated that the scores are in good agreement with the experimental results.

1. Introduction

Aniline derivatives are important starting materials in organic synthesis for producing drugs such as acetaminophen, phenacetin (Johansson, 1981), acetanilide (Brodie and Axelrod, 1948) and herbicides (Karasali and Maragou, 2016). N-alkyl anilines have been utilized in versatile applications including synthesis of dyes (Jurek et al., 2016), polymers (Tang et al., 2011), methylene diphenyl dicarbamate, which is a precursor for the production of industrially important methylene diphenyl diisocyanate as monomer for the synthesis of rigid polyurethane (Liu et al., 2007). Also, aromatic N-alkylamines and their derivatives were known as compounds with strong biological activities including antimicrobial agent (Kumar et al., 2009) and anticancer drug (Barmore et al., 1998).

Due to the importance stated above, N-alkyl amines have been synthesized with various notable methodologies previously. In this context, benzyl alcohol amination was catalyzed by copper powder taking an array of primary amines (Wu et al., 2019). Polymer-supported palladium-N-heterocyclic carbene was utilized for direct amination of carbonyl derivatives with primary and secondary amines (Bagal et al., 2012). Microwave-promoted mono-N-alkylation of aromatic amines was carried out in the water without a catalyst to achieve aromatic N-alkylamines with satisfactory yield (Marzaro et al., 2009). N-benzylation of aniline derivatives was conducted with efficient clay encapsulated ZnO nanoparticles as catalysts (Dhakshinamoorthy et al., 2011). Aniline derivatives were converted to Schiff's base then hydrogenated to yield N-alkylaniline moieties (Ayyangar et al., 1991).

Carbonic anhydrases (CAs) catalyze the reversible hydration of carbon dioxide and water to proton and bicarbonate (Boztas et al., 2015; Ozmen Ozgun et al., 2016; Nar et al., 2013). CAs have crucial roles in both multicellular and unicellular organisms such as tumorigenesis, calcification, bone resorption, gluconeogenesis, electrolyte secretions, respiration, and acid-base balance (Gul et al., 2016; Ozbey et al., 2016; Polat Köse and Gulcin, 2021). On the other hand, CA inhibitors have proven valuable and useful in several pathological disorders such as glaucoma, ulcers and osteoporosis (Erdemir et al., 2018). The best-known examples of these inhibitors are acetazolamide, brinzolamide, dorzolamide, tolsultazolamide, diclofenamide ethoxzolamide, zonisamide, and methazolamide (Koksal et al., 2019; Garibov et al., 2016). However, the side effects of these agents have led to critical analyzes of their metabolism and distribution in diverse organisms (Küçük and Gulcin, 2016). For CA isoenzymes, it has been essential to synthesize high biological value inhibitors that do not show any side effects (Ozgeris et al., 2016; Gulcin et al., 2016; Turkan et al., 2019).

Alzheimer's disease (AD) is one of the most common causes of death among older people in developed countries (Pedrood et al., 2021). AD is a brain disorder that gradually destroys memory and thinking skills and, ultimately, the ability to perform the simplest tasks in older people (Gulcin et al., 2017, 2018, 2020). Acetylcholinesterase (AChE) is a significant enzyme of the nervous system (Aktas et al., 2020; Bilginer et al., 2021). AChE hydrolysis acetylcholine (ACh) to choline and acetate to limit nerve impulses. ACh has important role in brain functions (Tugrak et al., 2020). AChE inhibitors to patients of AD will lighten ACh's level in their brains. Therefore, the effective treatment of AD has been focused on the development of AChE inhibitors (Bayrak et al., 2019; Yamali et al., 2020a,b).

In the present work, a series of N-Benzyl and N-Allyl aniline derivatives was synthesized and characterized by ^{13}C, ^{1}H NMR, and FT-IR. Their inhibition abilities were tested against some metabolic enzymes related to some global diseases including AD, idiopathic intracranial hypertension, mountain sickness, glaucoma, ulcers and osteoporosis. Relationship between efficiency and structure and of their biological activities and mechanism of their action were investigated.

2. Experimentals

2.1. General chemistry

4-Chloroaniline (98%), benzyl amine (98%), benzyl chloride (99%), DMA (99%), allyl bromide (99%), 2-chloroaniline (98%), 2,5-dichloroaniline (99%), 2,4-dichlorobenzaldehyde (98%) were purchased from Alfa Aesar. The compounds, which used for biological activities were purchased from Sigma-Aldrich. Triethylamine (99%) was supplied from Merck; IR spectral analysis was performed with Agilent Cary 630 FTIR. NMR spectra were recorded with Bruker 300 MHz NMR instrument.

2.1.1. Synthesis

0.12 mmol of chloroaniline, 0.018 mmol triethylamine, and 0.04 mmol allyl bromide and 10 mL DMA were added to 25 mL round bottom flask. The reaction was carried out at reflux condition for 7 h, then cooled to room temperature. The reaction mixture was transferred to 250 mL beaker, and treated with 30 mL brine solution. Then, the mixture was extracted with 200 mL ethyl acetate three times. Organic phase was collected with a separatory funnel and dried with anhydrous sodium sulfate. Ethyl acetate was evaporated with a rotary evaporator and the resultant mixture was purified with column chromatography using hexane/ethyl acetate (3/1) as an eluent. The products were elucidated with ^{13}C, ^{1}H NMR, and FT-IR.

N-allyl-4-chloroaniline (1a): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.62 (2H, d, J = 5.1 Hz), 3.74 (1H, s), 5.1 (1H, dd, J = 10.1, 1.2 Hz), 5.2 (1H, dd, J = 16.8, 1.3 Hz), 5.79–5.91 (1H, m), 6.41–6.50 (2H, m), 7.01–7.08 (2H, m); 13C NMR (75 MHz, CDCl3): δ 45.5, 113.8, 114.2, 121.8, 128.3, 134.7, 145.7; FT-IR (KBr, cm^{-1}): 1267, 1402, 1495, 1621, 2120, 2889, 2975, 3351, black liquid.

N-allyl-3-chloroaniline (1b): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.60 (2H, d, J = 5.0 Hz), 3.78 (1H, s), 5.2 (1H, dd, J = 10.1, 1.2 Hz), 5.3 (1H, dd, J = 16.8, 1.3 Hz), 5.89–5.93 (1H, m), 6.3–6.74 (3H, m), 7.11–7.18 (1H, m); 13C NMR (75 MHz, $CDCl_3$): δ 46.5, 115.8, 116.2, 121.4, 127.5, 134.6, 148.5; FT-IR (KBr, cm^{-1}): 1234, 1323, 1416, 1491, 1596, 2176, 2878, 2974, 3083, 3418, red liquid.

N-allyl-2,5-dichloroaniline (1c): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.60 (2H, d, J = 5.0 Hz), 3.78 (1H, s), 5.2 (1H, dd, J = 10.1, 1.2 Hz), 5.3 (1H, dd, J = 16.8, 1.3 Hz), 5.89–5.93 (1H, m), 6.9–6.8 (m, 2H), 7.3 (m, 1H), 13C NMR (75 MHz, CDCl3) δ 46.5, 110.5, 114.5, 124.3, 126.8, 127.8, 128.1, 139.3, 144.9, FT-IR (KBr, cm^{-1}): 1092, 1178, 1263, 1312, 1495, 1595, 2102, 2852, 2982, 3079, 3414, red liquid.

N-allyl-2-chloroaniline (1d): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.56 (2H, d, J = 5.0 Hz), 3.82 (1H, s), 5.23 (1H, dd, J = 10.1, 1.2 Hz), 5.32 (1H, dd, J = 16.8, 1.3 Hz), 5.88–5.92 (1H, m), δ 7.3–7.2 (m, 4H), 13C NMR (75 MHz, CDCl3) δ 46.5, 110.5, 114.5, 124.3, 126.8, 127.8, 128.1, 139.3, 144.9. FT-IR (KBr, cm^{-1}): 917, 1033, 1320, 1461, 1507, 1595, 2118, 2849, 2922, 3008, 3075, 3422, dark-red liquid.

N-benzyl-4-chloroaniline (1e): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.5 – 7.19 (m, 5H), 7.02 (d, J = 8.7 Hz, 2H), 6.45 (d, J = 8.7 Hz, 2H), 4.18 (s, 2H), 3.92 (s, 1H). 13C NMR (75 MHz, CDCl3) δ 47.54, 113.05, 121.3, 125.2, 126.12, 126.8, 127.5, 137.0, 144.6. FT-IR (KBr, cm^{-1}): 1077, 1047, 1379, 1453, 1513, 1603, 2161, 2914, 2866. 3027, 3366, dark-red liquid.

N-benzyl-3-chloroaniline (1f): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35–7.2 (m, 4H), 7.10–7.06 (m, 1H), 6.86 (t, J = 8.0 Hz, 1H), 6.5–6.43 (m, 1H), 6.40 (t, J = 2.0 Hz, 1H), 6.38 (d, J = 8.0 Hz, 1H), 4.12 (s, 2H), 4.05 (s, 1H). 13C NMR (75 MHz, CDCl3) δ 47.91, 110.23, 111.45, 116.35, 127.6, 128.62, 128.70, 130.20, 135.2, 137.69, 147.5. FT-IR (KBr, cm^{-1}):1074, 1323, 1484, 1595, 2855, 3027, 3064, 3392, orange liquid.

2.1.2. AChE and hCAs activity assays

In the present work, AChE from electrical eel (*Electrophorus electricus*) was purchased from Sigma-Aldrich. *In vitro* inhibition effects of the novel synthesized N-benzyl and N-Allyl aniline derivatives (**1a-f**) and reference compound (TAC) on AChE activity were evaluated by the Ellman et al. (1961) as described previously (Yamali et al., 2018; Kazancı et al., 2021). The absorbances were spectrophotometrically measured at 412 nm using acetylthiocholine iodide (PubChem CID: 74629, Sigma 01480) as a substrate according to previous studies (Turan et al., 2016; Huseynova et al., 2018). On the other hand, both hCA isoenzymes were purified from human erythrocytes by Sepharose-4B-L-tyrosine-sulfanilamide affinity chromatography (Caglayan et al., 2019a,b, 2020). The inhibition effects of the N-Benzyl and N-allyl aniline derivatives (**1a-f**) and reference compound (AZA) versus, the esterase activity of the hCAs were determined by following the change in absorbance at 348 nm according to the assay defined by Verpoorte et al. (1967) as described in details (Burmaoğlu et al., 2019; Küçükoglu et al., 2019). hCAs activities were measured using p-nitrophenyl acetate substrate (PNA, PubChem CID:13243, Sigma N8130) (Taslimi et al., 2016a; Gul et al., 2017; Bicer et al., 2019). Protein quantity during the purification processes was determined according to Bradford's technique as described in prior studies (Koksal and Gulcin, 2008; Hisar et al., 2005b). Both isoenzyme purity was controlled by Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS_PAGE) (Taslimi et al., 2016b; Sujayev et al., 2016). All the measurements related to Ki values average of three analysis. One CA enzyme unit is given as the amount of CA, which had absorbance difference at 348 nm over a 3 min at 25 °C (Akbaba et al., 2014; Taslimi et al., 2017a, 2017b; Kocyigit et al., 2018).

2.1.3. AChE and hCAs kinetic assay

To investigate the *in vitro* inhibitory effects of the novel synthesized N-benzyl and N-allyl aniline derivatives (**1a-f**), kinetic studies were made with the variable compound and substrate concentrations. IC_{50} values were obtained from graphs plotted from enzyme activity corresponding to increasing inhibitor concentration (Erdemir et al., 2019). Ki values were obtained from Lineweaver-Burk curves (1934), which given in the previous studies (Kuzu et al., 2019; Ozmen Ozgun et al., 2019; Artunc et al., 2020). From the observed data, IC_{50} and K_i values for these derivatives were computed, and the types of inhibition of AChE and hCAs were determined as in previous studies (Eruygur et al., 2019; Caglayan et al., 2019b; Demir et al., 2019).

3. Results and discussion

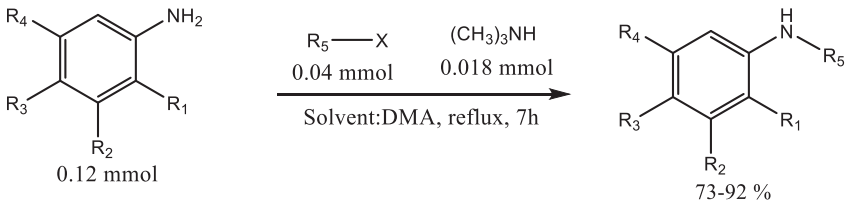
3.1. Chemistry

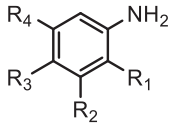
Vast utilization of N-alkyl anilines as building blocks for pharmaceutical and industrially important products inspired us to synthesize products according to the reaction in Scheme 1.

Allyl bromide and benzyl chloride were taken as substates for the alkylation reaction and as a halide ion scavenger, triethylamine was utilized at reflux condition of DMA.

3.2. Biological evaluation

The novel synthesized N-benzyl and N-allyl aniline derivatives, **1a-f**, were tested against cytosolic hCA I and hCA II isoenzymes and AChE enzyme. According to Table 1, it is depicted that all N-benzyl and N-allyl aniline derivatives effectively inhibited hCA I, hCA II and AChE enzymes. CA isozymes take part some biochemical and physiological processes (Scozzafava et al., 2015; Taslimi et al., 2017). They play an important role in some diseases such as cerebral edema, glaucoma, and epilepsy (Hisar et al., 2005a; Bilginer et al., 2019; Gunsel et al., 2021). Cytosolic CA I isoenzyme is the most abundant in erythrocytes, while another cytosolic isoenzyme (CA II) is highly expressed in most organs and contributes to many important physiological processes (Aktaş et al., 2017; Demir et al., 2018; Yigit et al., 2018). Recently, CA inhibitors have been commonly used as novel antiglaucoma, diuretics, antiobesity, anticancer and anti-infective medications (Bal et al., 2020; Hashmi et al., 2021). In the current study, all the novel synthesized N-benzyl and N-allyl aniline derivatives medium inhibited hCA I isoenzyme with IC_{50} values ranging from 243.11 to 633.54 nM and K_i values ranging from 202.12 ± 16.21 to 635.31 ± 45.33 nM. According to the results, both N-allyl and N-benzyl compounds, bound to the chlorine group in the *para-* position, increased hCA I inhibition. N-allyl group showed a better inhibition effect than N-benzyl group. A similar situation was observed with the binding of chlorine group to the *meta-* position. The positions of the chlorine group attached to the N-allyl aniline derivatives are increased following order of *para-* > *meta-* > *orto-* when ordered according to their inhibition effects for hCA I. A similar situation is encountered with the N-benzyl groups. When N-allyl-4-chloroaniline (**1a**) compared to N-allyl-2-chloroaniline **(1d),** it showed 3.14 times more inhibition (K_i: 202.12 ± 16.21 nM). The addition of the chlorine group to the *orto*-positions of the aniline group in the N-allyl-2-chloroaniline **(1d)** caused a 1.23-fold change in inhibition (N-allyl-2,5-dichloroaniline **(1c),** K_i: 511.18 ± 77.32 nM).



Entry		R_5—X	Products	Yield (%)[a]
1	R_1, R_2, R_4 = H, R_3 = Cl		**1a**	92
2	R_1, R_3, R_4 = H, R_2 = Cl	R_5 = Allyl	**1b**	88
3	R_2, R_3 = H, R_1, R_4 = Cl	X = Br	**1c**	78
4	R_2, R_3, R_4 = H, R_1 = Cl		**1d**	81
5	R_1, R_2, R_4 = H, R_3 = Cl	R_5 = Benzyl	**1e**	76
6	R_1, R_3, R_4 = H, R_2 = Cl	X = Cl	**1f**	73

Scheme 1 Synthesis of N-benzyl-, and N-allyl aniline derivatives.

Table 1 Inhibition data of AChE, hCA I and hCA II enzymes with the novel synthesized N-benzyl and N-Allyl aniline derivatives (**1a-f**).

Compounds	IC_{50} (nM)						K_i (nM)		
	hCA I	r^2	hCA II	r^2	AChE	r^2	hCA I	hCA II	AChE
1a	243.11	0.9975	404.44	0.9965	182.45	0.9822	202.12 ± 16.21	389.11 ± 88.76	149.24 ± 15.59
1b	487.45	0.9746	296.32	0.9783	520.21	0.9729	492.11 ± 60.13	305.45 ± 97.75	519.59 ± 102.27
1c	544.67	0.9787	518.37	0.9785	367.77	0.9775	516.33 ± 77.32	511.18 ± 115.98	359.55 ± 91.49
1d	633.54	0.9917	500.55	0.9817	405.55	0.9719	635.31 ± 45.33	502.37 ± 97.67	192.64 ± 8.13
1e	370.43	0.9919	304.21	0.9952	489.98	0.9883	299.11 ± 44.55	299.57 ± 94.13	424.69 ± 98.56
1f	514.23	0.9799	494.33	0.9830	446.32	0.9724	502.33 ± 102.37	489.55 ± 23.66	468.26 ± 198.21
AZA	491.22	0.9783	435.78	0.9873	–	–	237.77 ± 54.54	189.44 ± 26.76	–
TAC	–	–	–	–	371.27	0.9867	–	–	342.82 ± 65.38

N-benzyl and N-allyl aniline derivatives had medium inhibition against dominant cytosolic hCA II isoenzyme with IC_{50} values ranging from 296.32 to 518.37 nM and K_i values ranging from 299.57 ± 94.13 to 511.18 ± 115.98 nM. N-benzyl-4-chloroaniline (**1e**) showed the best inhibition effect among N-benzyl and N-allyl aniline derivatives. As seen in results given for hCA I, the chlorine group bound to N-benzyl aniline in the *para-* position caused it to show more effective inhibition than the *meta-* position. Unlike hCA I in N-allyl groups, chlorine group in the *meta-* position attached to aniline showed more effective inhibition. The positions of the chlorine group attached to the N-allyl aniline are *meta-* > *para-* > *orto-* positions when ordered according to their inhibition effects for hCA II isoenzyme. Unlike hCA I, the addition of the chlorine group to the *orto-* position of the aniline group in the **(1d)** caused a decrease inhibition effect.

AD is accompanied by an abnormality in the cholinergic neurotransmission of the central nervous system and gives rise to emotional trouble (Lolak et al., 2020; Kızıltas et al., 2021a; Bingolet al., 2021; Atmaca et al., 2021). In the cholinergic mechanism, AChE possessed a significant role and its inhibition improved the cognitive functions (Akocak et al., 2021; Kızıltas et al., 2021b; Riaz et al., 2021). The discovery of novel AChE inhibitors sounds an important strategy to introduce novel drug candidates against AD and Parkinson disease. In this study, it was found that novel synthesized N-Benzyl and N-Allyl aniline derivatives had effective inhibition profile toward AChE with IC_{50} values ranging from 182.45 to 520.21 nM and K_i values ranging from 149.24 ± 15.59 to 519.59 ± 102.27 nM. In newly synthesized molecules, N-benzyl-4-chloroaniline (**1e**) demonstrated the best inhibition effect against AChE as a main cholinergic enzyme. When the hCA I and hCA II enzyme inhibition results are examined, it is seen that the chlorine group attached to the aniline ring in the 2nd position reduces the inhibition effect of the enzymes. However, this situation was not observed for AChE enzyme. The chlorine group attached to the aniline ring in the second position made a 2.70-fold difference when compared to the

inhibitor showing the lowest inhibitory effect. The positions of the chlorine group attached to the N-allyl groups are 4th position > 2th position > 3th position when ordered according to their inhibition effects for AChE. N-allyl-3-chloroaniline (**1b**) showed the lowest inhibition effect on AChE (K_i: 519.59 ± 10 2.27 nM).

3.3. Computational details

In this research, *in silico* docking studies were carried out by AutoDock Vina program (Trott and Olson, 2010). Additionally, the optimized structures of the novel synthesized N-benzyl and N-allyl aniline derivatives (**1a-f**) for molecular docking were determined with DFT/B3LYP theory and 6-311 + + G(d,p) basis set by Gaussian 09 W package program (Frisch et al., 2009).

3.4. Molecular docking analysis

Molecular docking is the basis of drug design; the calculations had a great importance in pharmacology. Therefore, docking is still a widely used, reliable and short time-consuming method for determining the binding position and protein–ligand interactions (Tokalı et al., 2021; Genç Bilgiçli et al., 2020; Karimov et al., 2020). In this part, the *in silico* molecular docking interactions of **1a-f** series within the both hCA I and II isoenzymes and AChE receptors were investigated by the AutoDock Vina program. Firstly, each molecule or ligand (**1a-f**) was optimized in gas phase with Gaussian 09 W package program by Density Functional Theory (DFT) method/B3LYP functional and 6-311 + + G (d, p) basis set, and the structures was determined and shown in Fig. 1 (according to the Gaussian 09W numbering format) and the pdb forms of the ligands were recorded.

Then starting from the experimental method, the targets was selected as hCA I/PDB: 2CAB (Kannan et al., 1984), hCA II/PDB: 5AML (Ivanova et al., 2015) and AChE/PDB: 1EVE (Kryger et al., 1999), and the 3D-pdb forms of the receptors were retrieved from RCSB (Protein Data Bank) (https://www.rcsb.org/). Here, the hetero atoms within the three targets were removed, the polar hydrogen charges were added and re-recorded as pdb form, these preparations were made via Discover Studio Visualizer 4.0 (DSV 4.0) software (http://www.3dsbiovia.com/). In order to perform the docking procedure more efficient, the active sites/residues of the proteins were determined as follows: HIS119, HIS96 and HIS94 for hCA I/PDB: 2CAB; THR200, THR199, LEU198, PHE131, VAL121, HIS119, HIS96, HIS94, GLN92, ASN67, ASN62 for hCA II/PDB: 5AML; and HIS440, PHE330, GLU327, TRP279, SER200, TRP84 for AChE/PDB: 1EVE. Therefor the grid parameters were selected as 124 × 96 × 96 $Å^3$ x, y, z dimensions, 0.375 Å space and 38.015, −10.534, 14.088 x, y, z centers for hCAI/PDB: 2CAB, 46x70x52 $Å^3$ x, y, z dimensions, 0.375 Å space and −7.11, 3.083, 12.314 x, y, z centers for hCA II/PDB: 5AML and 70x56x82 $Å^3$ x, y, z dimensions, 0.375 Å space and 6.326, 61.577, 59.577 x, y, z centers for AChE/PDB: 1EVE. As a result of the research, it was found that docking method was successful or adequate both in determining the active site cavities of the three receptor and in determining the ligand conformation in these cavities, ten conformations were determined for each receptor. The obtained molecular docking scores (binding energy values) were ranked in kcal/mol in Table 2 and Figs. 2–4 and S1-S15 (Supporting Information) for every structure.

When the results were evaluated, it was observed that the **1a** molecule (to be highly potent inhibitors) could inhibit the interactions with hCA I/PDB: 2CAB, hCA II/PDB: 5AML and AChE/PDB: 1EVE quite well with energies such as −9.1, −8.8 and −9.3 kcal/mol, respectively. For this reason, the evaluations of the interactions were performed by considering only the **1a** + 2CAB, **1a** + 5AML, **1a** + 1EVE. For **1a** + 2CAB docking mechanism was given in Fig. 2. As seen from the Fig. 2 (a-3D and b-2D), conventional hydrogen bond was observed between H12 and GLN92 residue with the 4.20 Å bond length. The π-sigma interaction was found LEU198

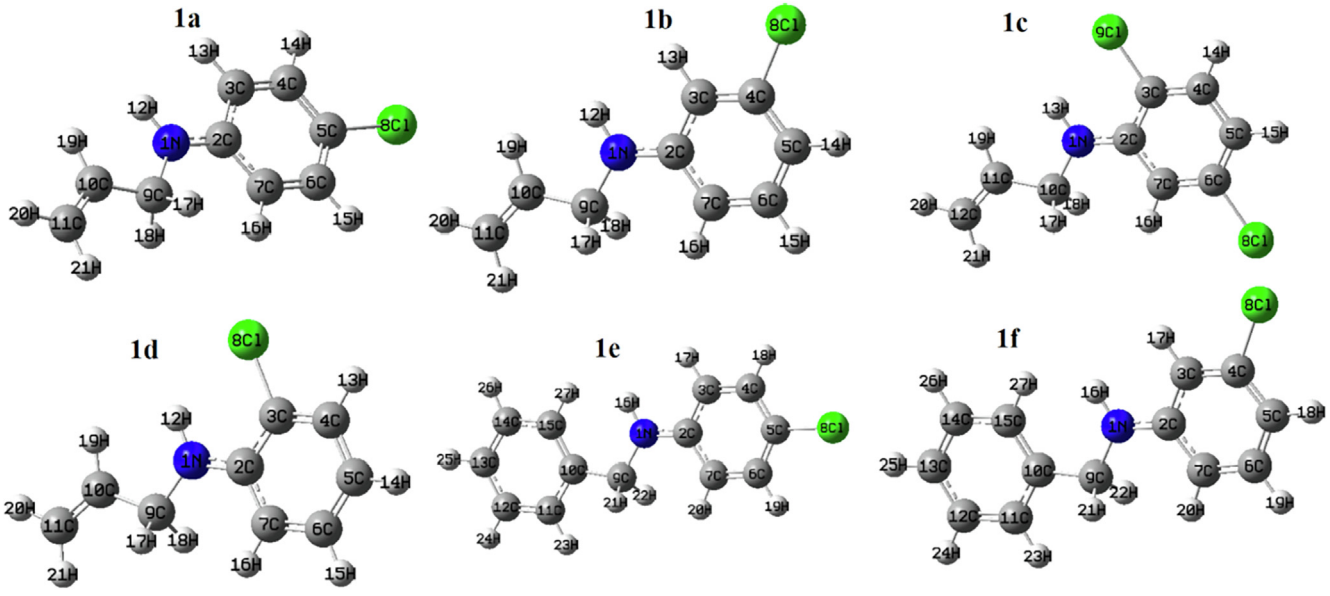


Fig. 1 The optimized structures of novel synthesized N-benzyl and N-allyl aniline derivatives (**1a-f**).

Table 2 Molecular docking scores of PSA series.

Compounds	hCA I/PDB: 2CAB			hCA II/PDB: 5AML			AChE /PDB: 1EVE		
	Binding Energy (kcal/mol)	K_i (nM)	N.H.B	Energy (kcal/mol)	K_i (nM)	N.H.B	Energy (kcal/mol)	K_i (nM)	N.H.B
1a	−9.1	213.625	1	−8.8	354.448	1	−9.3	152.423	0
1b	−8.6	496.769	0	−8.9	299.401	0	−8.6	496.769	0
1c	−8.5	588.105	1	−8.5	588.105	1	−8.8	354.448	0
1d	−8.4	696.234	0	−8.6	496.769	1	−9.0	252.902	0
1e	−8.9	299.401	2	−8.9	299.401	1	−8.7	419.618	0
1f	−8.6	496.769	2	−8.5	588.105	0	−8.7	419.618	0

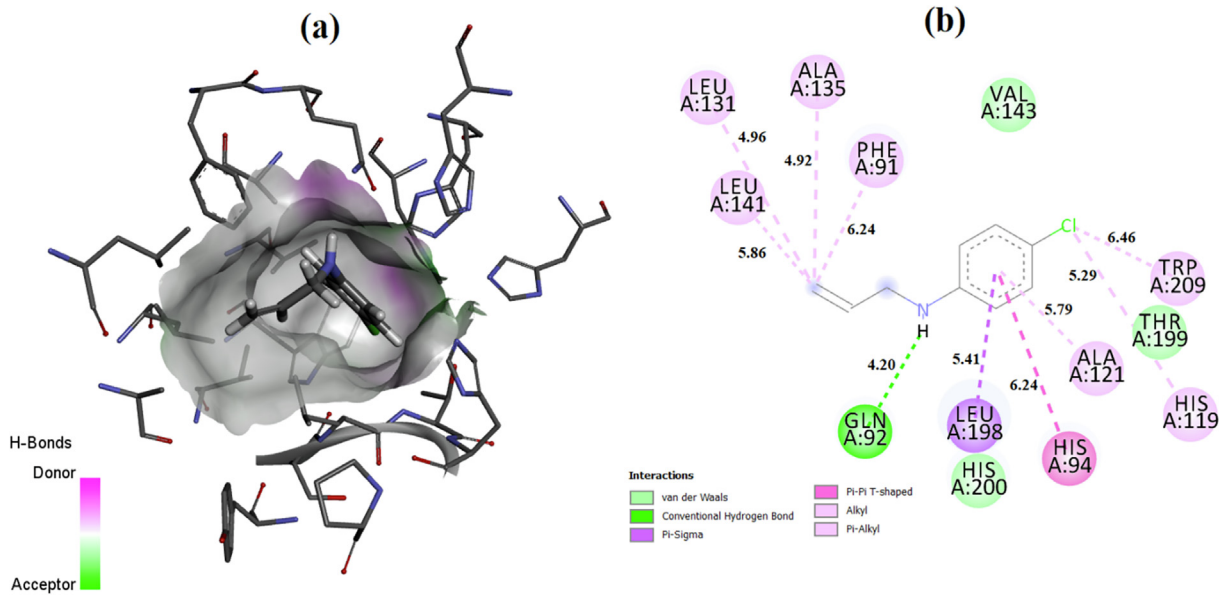


Fig. 2 (a) 3D and (b) 2D molecular docking results of the hCA I/PDB:2CAB + **1a.**

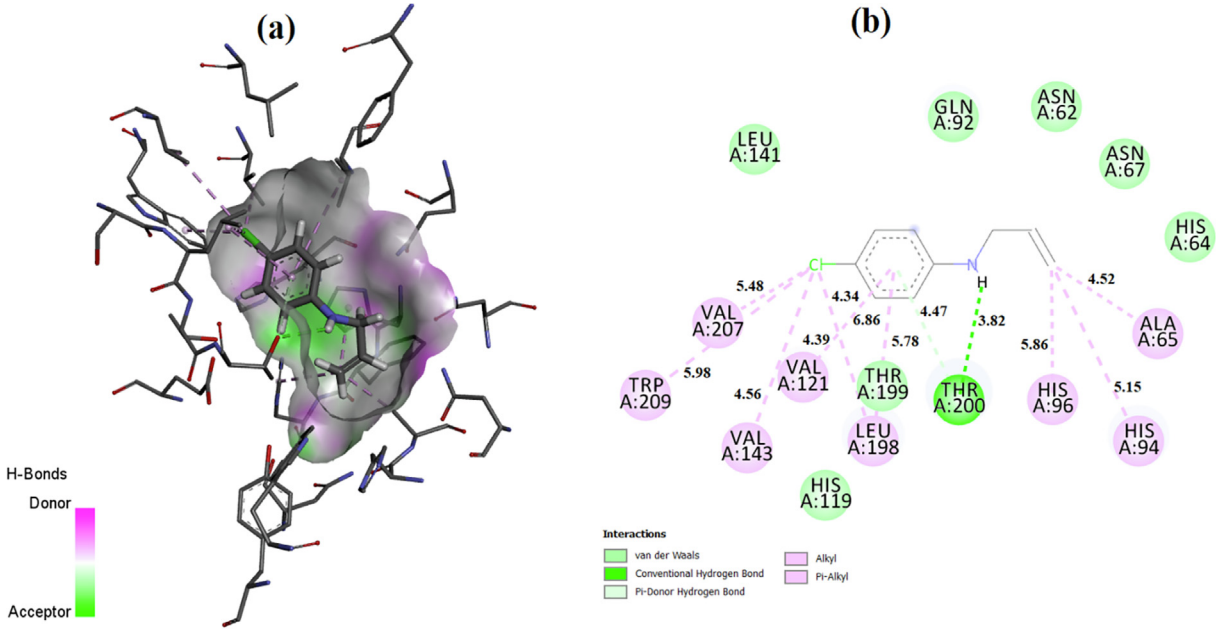


Fig. 3 (a) 3D and (b) 2D molecular docking results of the hCA II/PDB:5AML + **1a.**

residue and the center of phenyl ring with 5.41 Å bond length. The alkyl and π-alkyl interactions were observed between ALA121 residue and the center of phenyl ring and between TRP209 residue and Cl8 atom with 5.79 and 6.46 Å bond lengths, respectively. Additionally, alkyl or π-alkyl interactions were found between LEU141, LEU131, ALA135, PHE91 residues and $C_{11}H_{20,21}$ group with 5.86, 4.96, 4.92, 6.24Å bond lengths, respectively. Finally, π-π-T shaped and π-alkyl interactions were determined between HIS94 active residue and the center of phenyl ring and between HIS119 active residue

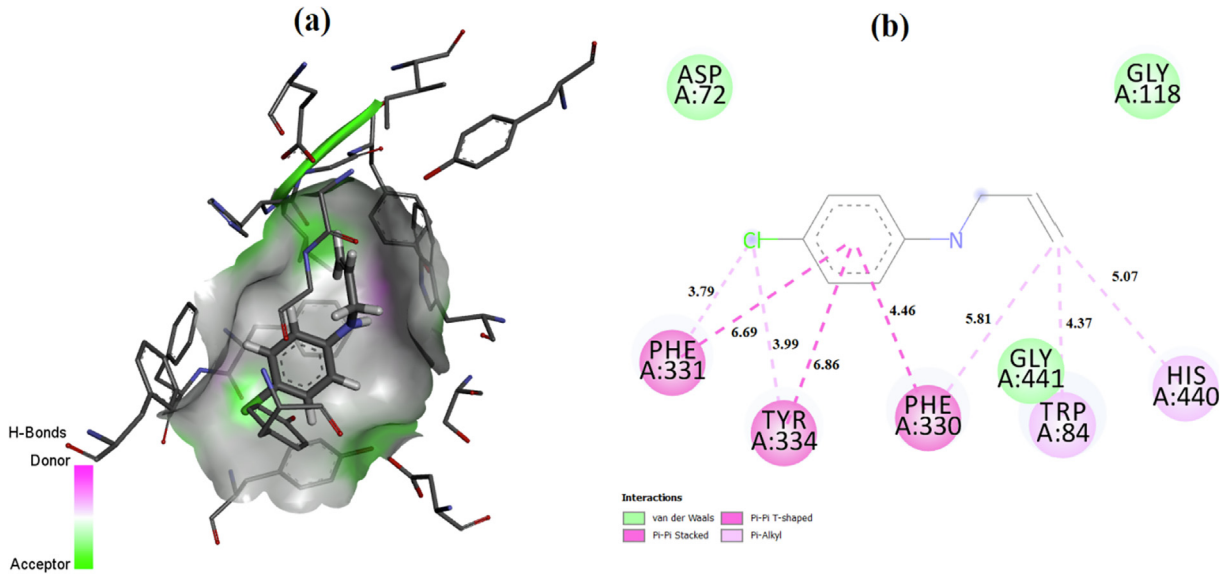


Fig. 4 (a) 3D and (b) 2D molecular docking results of the AChE/PDB:1EVE + **1a.**

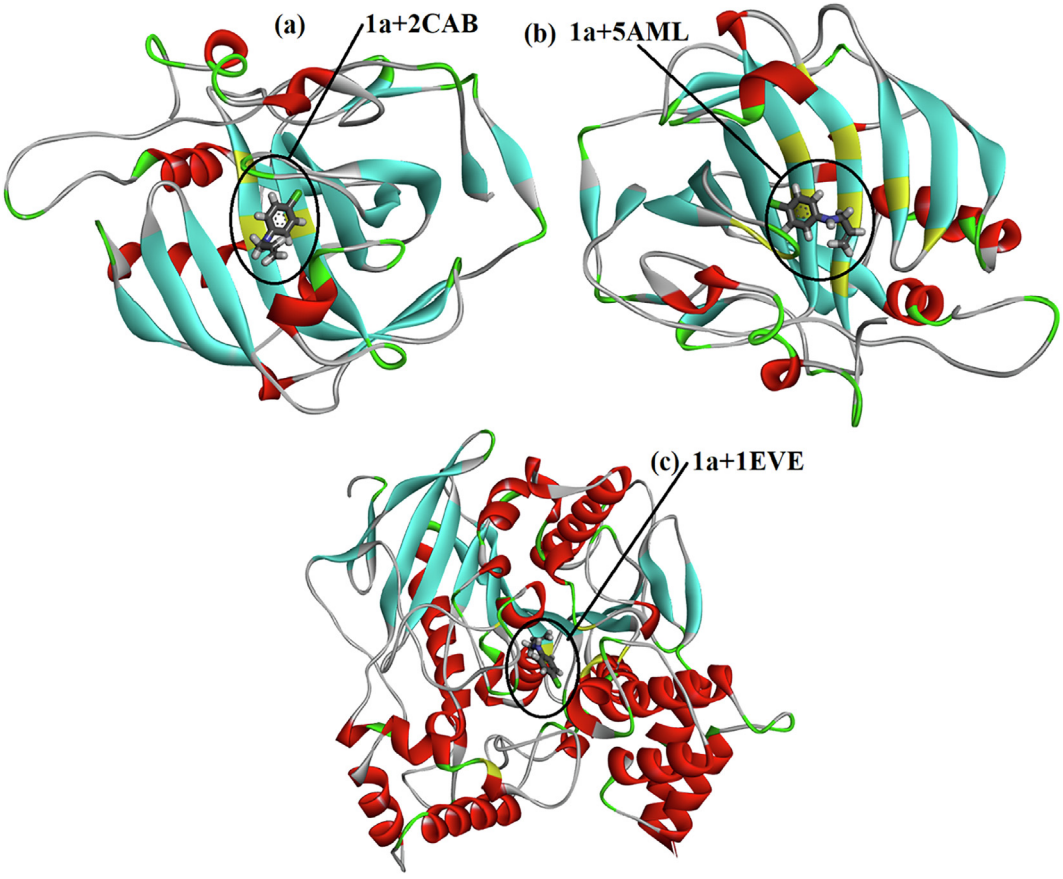


Fig. 5 The three-dimensional structure of 2CAB, 5AML and 1EVE proteins with the compound **1a.**

and 8Cl atom with 6.24 and 5.29 Å bond lengths, respectively. The other **1b-f** molecular docking graphics were presented in Figures S1-S5 (Supporting Information).

For **1a** + 5AML interaction was shown in Fig. 3. From the Fig. 3 (a-3D and b-2D), conventional hydrogen bond was determined between H12 and THR200 with the 3.82 Å bond length. The alkyl or π-alkyl interactions were found between VAL207, TRP209, VAL143, active-LEU198 residues and Cl8 atom with 5.48, 5.98, 4.56 and 4.34 Å bond lengths, respectively; between VAL121, active-LEU198 residues and the center of phenyl ring with 6.86 and 5.78 Å bond lengths, respectively; between active-HIS96, active-HIS94, ALA65 residues and $C_{11}H_{20,21}$ group with 5.86, 5.15 and 4.52 Å bond lengths, respectively. The other **1b-f** + 5AML molecular docking graphics were presented in Figs. S6-S10 (Supporting Information).

For **1a** + 1EVE interaction was shown as Fig. 4. From the Fig. 4 (a-3D and b-2D), conventional hydrogen bond could not be determined. The π-alkyl interactions were observed between PHE331, TYR334 residues and Cl8 atom with 3.79 and 3.99 Å bond lengths, respectively; between active-PHE330, TRP84, HIS440 residues and $C_{11}H_{20,21}$ group with 5.81, 4.37 and 5.07 Å bond lengths, respectively. Finally, π-π stacked or π-π T shaped interactions were observed between PHE331, TYR334, active-PHE330 and the center of phenyl ring with 6.69, 6.86, 4.46 Å bond lengths, respectively. The other **1b-f** + 1EVE molecular docking graphics were presented in Figs. S11-S15 (Supporting Information).

Ki values of the molecular docking interactions within the Table 2 were found and presented with the help of Ki = exp (ΔG/RT) equation, in which ΔG: binding energy, R: gas constant = 1.9872036 × 10–3 kcal/mol and T: room temperature (298.15 K). When the results obtained are evaluated in general, newly synthesized N-Benzyl and N-Allyl aniline derivatives (**1a-f**) were determined to be highly potent inhibitors for carbonic anhydrases (hCA I and II) and acetylcholinesterase (AChE). Here, K_i values were in the range of 213.625 to 696.234 nM, 299.401 to 588.105 nM, and 152.423 to 496.769 nM for hCA I/PDB: 2CAB, hCA II/PDB: 5AML and AChE/PDB: 1EVE, respectively. Finally, when all the results were compiled, it was seen that the **1a** molecule has the potential to inhibit all three enzymes, and especially it can inhibit the AChE enzyme quite strongly. In addition, the proximity of the **1a** molecule to the active sites in enzymes and their internal positions were shown in Fig. 5. In this study, the supporting the theoretical results with experimental results is promising for future drug design.

4. Conclusions

In this study, six N-allyl and N-benzyl aniline derivatives were synthesized from medium to quantitative yield with a new method. The synthesized N-benzyl and N-Allyl aniline derivatives had effective inhibition profiles against AChE with IC_{50} values ranging from 182.45 to 520.21 nM and K_i values ranging from 149.24 ± 15.59 to 519.59 ± 102.27 nM. Of these molecules, N-benzyl-4-chloroaniline (**1e**) exhibited the best inhibition effect on AChE. When the hCA I and hCA II isoenzymes inhibition results are examined, it is seen that the chlorine group attached to the aniline ring in the *orto-* position reduces the inhibition effect of both isoenzymes. However, this situation was not seen for AChE enzyme. The chlorine group attached to the aniline ring in the *orto-* position made a 2.70-fold difference compared to the molecule, which showing the lowest inhibitory effect. The positions of the chlorine group attached to the N-allyl aniline are *para-* > *orto-* > *meta-* positions when ordered according to their inhibition effects for AChE. N-allyl-3-chloroaniline (**1b**) showed the lowest inhibition effect on AChE (K_i: 519.59 ± 102.27 nM). In this context, it is thought that these compounds will make important contributions to the design of drugs to be used for treatment and related applications in the future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2021.103645.

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