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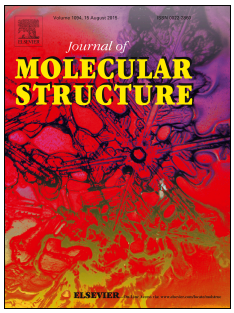

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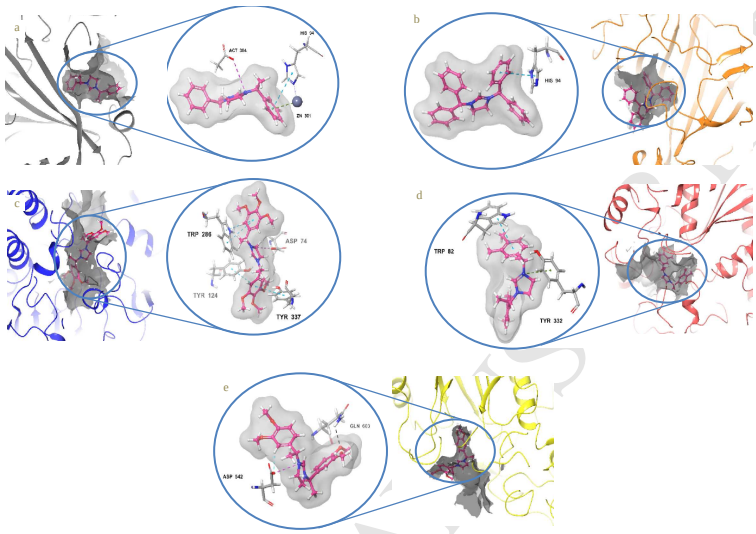

In this study, a serie of novel imidazolinium chloride salts bearing wing tip groups synthesised and characterized. Also, their inhibition effects were tested against carbonic anhydrase I and II isoenzymes and acetylcholinesterase; butyrylcholinesterase and α-glycosidase enzymes.

Imidazolinium Chloride Salts Bearing Wing Tip Groups: Synthesis, Molecular Docking and Metabolic Enzymes Inhibition

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ABSTRACT

A series of symmetrical imidazolinium chloride salts bearing secondary N-alkyl substituents were synthesized in good yield by the reaction of N,N'-dialkylethane-1,2-diamines and $HC(OEt)_3$ in the presence of NH_4Cl. These salts were characterized by spectroscopic methods. All compounds were tested as enzyme inhibitory agents. These novel symmetrical imidazolinium chloride salts derivatives **(3a-h)** effectively inhibited the cytosolic hCA I and hCA II, BChE, α-glycosidase and AChE with K_i values in the range of 18.41-121.73 nM for hCA I, 12.50-63.12 nM for hCA II, 3.72-34.58 nM for AChE, 5.50-32.36 nM for BChE, and 94.72-364.51 nM for α-glycosidase, respectively. CA II isoenzyme plays an crucial roles including acid-base balance homeostasis by excreting and secreting protons (H^+) due to the CO_2 hydration, HCO_3^- reabsorption mechanisms, and renal NH_4^+ output. Also, the molecular modeling are implementation for estimation of the binding proximity of symmetrical imidazolinium chloride salts bearing secondary wing tip groups and their inhibition mechanisms and kinetics in atomic levels at the catalytic domains.

Keywords: Carbonic anhydrase; butyrylcholinesterase; acetylcholinesterase; α-glycosidase; molecular modeling

1.INTRODUCTION

Azolium salts are the quaternary ammonium compounds, which had considerable attention in recent years because of their different applications [1,2]. These salts are an important class of carbene precursors, which have played an important role in the development of N-heterocyclic carbene (NHC) chemistry. The synthesis of N-heterocyclic carbenes is usually made by the deprotonation form of the azolium salts. Removal of the C2-proton of azolium salts in approtic solvents using a suitable strong base generates the N-heterocyclic carbenes. NHCs are neutral, two-electron donor ligands, which have strong σ-donating and weak π-accepting properties, they form stable N-heterocyclic carbene complexes with a range of transition metals [3,4]. NHC complexes have a variety of numerous applications such as catalysis, material and medicinal sciences [5,6]. Also, azolium salts are known to exhibit a broad range of biological activities including antibacterial, antitumor and antimicrobial activities [7,8]. Recently, studies on enzyme inhibitory properties of imidazole and benzimidazole salts have been reported [9-11]. Therefore, the synthesis of these salts is important. In literature, various methods have been reported for the synthesis of the azolium salts [12,13]. The most widely used methods for the synthesis of saturated imidazolium salts are (i) the cyclization reaction of the corresponding secondary diamine with an orthoformate in the presence of HX, (ii) N-alkylation of an imidazoline with an appropriate alkyl halide, and (iii) reactions of a formamidine with ethyl dication equivalent [14-17]. A great number of symmetrical and unsymmetrical imidazolinium salts have been prepared using these methods. Although aromatic aldehydes have been generally used for the synthesis of five-, six- and seven-membered NHC precursors as starting materials. The usge of inexpensive aromatic ketones was very limited due to probably poor reactivity toward nucleophiles [18].

The α-carbonic anhydrase (CA, E.C.4.2.1.1) isozymes efficiently perform the interconversion among water (H_2O) and carbon dioxide (CO_2) to protons (H^+) and bicarbonate (HCO_3^-), with a wide number of isozymes being explained in mammals: sixteen number in human cells and primates, and sixteen number in other vertebrate's groups [19-21]. These catalysts are motivated by CA reaction and very slow at physiologic pH levels for the hydration and dehydration processes [22,23]. Additionally, the large CO_2 quantity occurs in electron transport chain and oxidative metabolism [24-26]. Indeed, CA isozymes are key enzymes in most living organisms. Their role is connected with tightly controlled processes including pH regulation [27,28].

Two enzymes rated among cholinesterases are known as AChE (E.C.3.1.1.7.) and BChE (E.C.3.1.1.8.) and presented in humans [29-31]. AChE is expressed in multiple cells. It is present even on erythrocytes surface. However, the majority of AChE activity is present in cholinergic nervous system where it terminates cholinergic neurotransmission via fast hydrolysis of neurotransmitter acetylcholine [32-34]. Due to presence of AChE on erythrocytes, it was formerly called as a blood cholinesterase in some sources. Compared with AChE, BChE is not expressed in situ whereas it is produced by livers and distributed through plasma into the body [35,36]. For the reason, BChE was in some sources called plasmatic, serum cholinesterase or pseudocholinesterase. The both enzymes are markers of different pathologies including poisoning with cholinesterases inhibitors for the both cholinesterases and liver parenchyma degradation for BChE [37-39]. α-Glycosidase is available in the small intestine and catalyses the final phase in the digestive mechanism of carbohydrate molecules to release absorbable monosaccharide molecules resulting in increased blood glucose levels. Inhibition action of this enzyme regulates the liberation of D-glucose from the complex carbohydrates [40-42].

In this work, a series of novel symmetrical imidazolinium chloride salts derivatives (**3a-h**), which synthesized from aromatic ketones and ethylenediamine as low-cost starting materials. Also their inhibition properties were investigated against some metabolic enzymes.

2.MATERIALS AND METHODS

All preparative reactions for the imidazolinium salts (**3a-h**) were performed under Ar in flame-dried glassware using standard Schlenk-type flasks. All reagents were purchased from Sigma Aldrich and Fluka. All ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ with tetramethylsilane as an internal reference using a Varian AS 400 Merkur spectrometer operating at 400 MHz (^{1}H), 100 MHz (^{13}C). Chemical shifts (δ) are given in ppm relative to TMS, coupling constants (*J*) in hertz. NMR multiplicities are abbreviated as follows: quint = quintet, m = multiplied, s = singlet, d = doublet, t = triplet signal. FT-IR spectra were recorded as KBr pellets in the range 400-4000 cm^{-1} on Perkin Elmer Spectrum 100. Melting points (m.p.) were determined in open capillary tubes with an Electrothermal-9200 melting point apparatus and uncorrected. Elemental analyses were carried out at Inonu University Research Center.

2.1.General preparation of diimines, 1

Ethylenediamine (5 mmol) was slowly added to a solution of the appropriate aromatic ketone (10 mmol), which was previously dissolved in toluene (30 mL) over a period of 2 min. The resulting mixture was refluxed for 12 h. After reaction completed, the solvent was removed under vacuum to dryness. The residue was recrystallized in dichloromethane or ethyl alcohol / diethyl ether.

2.2. General preparation of N,N'-dialkylethane-1,2-diamines, 2

$NaBH_4$ (15 mmol) was added slowly to a solution of the appropriate diimine (10 mmol) in methyl alcohol (30 mL) at room temperature over a period of 30 min. The reaction mixture was stirred during 12 h and then refluxed for 1 h. After cooling, the mixture was treated with 1.0 N HCl, and organic phase was extracted with CH_2Cl_2 (3 × 30 mL). After drying over $MgSO_4$ and evaporation, the residue was recrystallized from toluene / *n*-hexane.

2.3. General preparation of symmetrical 1,3-dialkylimidazolinium salts, 3

A mixture of N,N'-dialkylethylenediamines (6.2 mmol), NH_4Cl (6.2 mmol) and $HC(OEt)_3$ (10 mL) was heated for 12 h at 110 ^oC. After cooling, colorless crystals were formed. The crystals were filtered, washed with diethyl ether (3 × 15 mL) and dried under vacuum. The obtained salt was recrystallized from ethyl alcohol / diethyl ether.

2.4.1. 1,3-Di(1-phenylethyl)imidazolinium chloride, 3a

This compound was prepared according to published procedures [18].

2.4.2. 1,3-Di[1-(4-methylphenyl)ethyl]imidazolinium chloride, 3b

Yield: 1.86 g, 88%, m.p. 185-186 ^oC. IR: $\nu_{(NCN)}$ = 1634 cm^{-1}. Anal. Calc. for $C_{21}H_{27}N_2Cl$: C, 73.58 H, 7.88; N, 8.18. Found: C, 73.60; H, 7.85; N, 8.20%. ^1H NMR ($CDCl_3$) δ: 1.79 (d, 6H, *J* = 7.0 Hz, $CH(CH_3)C_6H_4$-CH_3), 5.24 - 5.32 (m, 2H, $CH(CH_3)C_6H_4$-CH_3), 2.33 (s, 6H, $CH(CH_3)C_6H_4$-CH_3), 3.50 - 3.76 (m, 4H, NCH_2CH_2N), 7.18 (d, 4H, *J* = 7.8 Hz, Ar-*H*) and 7.30 (d, 4H, *J* = 8.1 Hz, Ar-*H*), 10.57 (s, 1H, NC*H*N). ^{13}C NMR ($CDCl_3$) δ: 18.9 ($CH(CH_3)C_6H_4$-CH_3), 21.1 ($CH(CH_3)C_6H_4$-CH_3), 45.5 (NCH_2CH_2N), 57.4 ($CH(CH_3)C_6H_4$-CH_3), 127.0, 129.8, 134.4 and 138.8 (Ar-*C*), 156.8 (N*C*HN).

2.4.3. 1,3-Di[1-(3,4-dimethylphenyl)ethyl]imidazolinium chloride, 3c

Yield: 1.85 g, 81%, m.p. 191-193 ^{o}C. IR: $\nu_{(NCN)}$ = 1629 cm^{-1}. Anal. Calc. for $C_{23}H_{31}N_2Cl$: C, 74.49; H, 8.37; N, 7.56. Found: C, 74.51; H, 8.35; N, 7.54%. ^{1}H NMR ($CDCl_3$) δ: 1.79 (d, 6H, *J* = 6.9 Hz, CH(C*H*$_3$)C$_6$H$_3$-(CH$_3$)-3,4), 5.19 - 5.26 (m, 2H, C*H*(CH$_3$)C$_6$H$_3$-(CH$_3$)$_2$-3,4), 2.24 (s, 6H, CH(CH$_3$)C$_6$H$_3$-(C*H*$_3$)$_2$-*3*,4), 2.26 (s, 6H, CH(CH$_3$)C$_6$H$_3$-(C*H*$_3$)$_2$-3,*4*), 3.51-3.74 (m, 4H, NC*H*$_2$C*H*$_2$N), 7.13 - 7.15 (m, 6H, Ar-*H*), 10.41 (s, 1H, NC*H*N). ^{13}C NMR ($CDCl_3$) δ: 19.1 (CH(*C*H$_3$)C$_6$H$_3$-(CH$_3$)$_2$-3,4), 19.4 (CH(CH$_3$)C$_6$H$_3$-(*C*H$_3$)$_2$-*3*,4), 19.8 (CH(CH$_3$)C$_6$H$_3$-(*C*H$_3$)$_2$-3,*4*), 45.3 (NCH$_2$*C*H$_2$N), 57.5 (*C*H(CH$_3$)C$_6$H$_3$-(CH$_3$)$_2$-3,4), 124.4, 128.2, 128.3, 130.3, 134.8, 134.9, 137.4, 137.5 and 137.6 (Ar-*C*), 156.6 (N*C*HN).

2.4.4. 1,3-Di[1-(3,4-dimethoxyphenyl)ethyl]imidazolinium chloride, 3d

Yield: 2.04 g, 76%, m.p. 161-163^{o}C. IR: $\nu_{(NCN)}$ = 1628 cm^{-1}. Anal. Calc. for $C_{23}H_{31}N_2O_4Cl$: C, 63.52; H, 7.13; N, 6.44. Found: C, 63.54; H, 7.12; N, 6.45%. ^{1}H NMR ($CDCl_3$) δ: 1.78 (d, 6H, *J* = 6.9 Hz, CH(C*H*$_3$)C$_6$H$_3$(OMe)$_2$-3,4), 3.49 - 3,69 (m, 4H, NC*H*$_2$C*H*$_2$N), 3.83 (s, 6H, CH(CH$_3$)C$_6$H$_3$(O*Me*)$_2$-*3*,4), 3.91 (s, 6H, CH(CH$_3$)C$_6$H$_3$(O*Me*)$_2$-3,*4*), 5.18 (quart, 2H, *J* = 6.9 Hz, C*H*(CH$_3$)C$_6$H$_3$(OMe)$_2$-3,4), 6.77 - 7.19 (m, 6H, Ar-*H*), 10.68 (s, 1H, NC*H*N). ^{13}C NMR ($CDCl_3$) δ: 18.8 and 18.9 (CH(*C*H$_3$)C$_6$H$_3$(OMe)$_2$-3,4), 45.1 and 45.2 (NCH$_2$*C*H$_2$N), 55.9 CH(CH$_3$)C$_6$H$_3$(O*Me*)$_2$-*3*,4), 56.6 CH(CH$_3$)C$_6$H$_3$(O*Me*)$_2$-3,*4*), 57.6 (*C*H(CH$_3$)C$_6$H$_3$(OMe)$_2$-3,4), 110.7, 110.8, 110.9, 111.0, 119.0, 129.8, 129.9, 149.4, 149.6 and 149.7 (Ar-*C*), 156.8 (N*C*HN).

2.4.5. 1,3-Di[1-(3,4,5-trimethoxyphenyl)ethyl]imidazolinium chloride, 3e

This compound was prepared according to previous procedures [18].

2.4.6. 1,3-Dibenzhydrylimidazolinium chloride, 3f

This compound was prepared according to previous procedures [18].

2.4.7. 1,3-Di[1-phenyl-1-(4-methylphenyl)methyl]imidazolinium chloride, 3g

Yield: 2.51 g, 87%, m.p. 86-88 ^{o}C. IR: $\nu_{(NCN)}$ = 1638 cm^{-1}. Anal. Calc. for $C_{31}H_{31}N_2Cl$: C, 79.74; H, 6.65; N, 6.00. Found: C, 79.75; H, 6.64; N, 6.01%. ^{1}H NMR ($CDCl_3$) δ: 4.04 (s, 4H, NC*H*$_2$C*H*$_2$N), 6.37 (s, 2H, C*H*-Ar), 7.15 and 7.25 (d, 8H, *J* = 8.0 Hz, C$_6$*H*$_4$-CH$_3$), 7.30 - 7.37 (m, 10H, C$_6$*H*$_5$), 2.31 (s, 6H, C$_6$H$_4$-C*H*$_3$), 8.11 (s, 1H, NC*H*N). ^{13}C NMR ($CDCl_3$) δ: 48.7 (NCH$_2$*C*H$_2$N), 66.0 (*C*H-Ar), 21.1 (C$_6$H$_4$-*C*H$_3$), 128.3, 128.4, 128.5, 128.8, 129.2, 129.8, 132.6, 135.8 and 138.9 (Ar-*C*), 158.4 (N*C*HN).

2.4.8. 1,3-Di[1,1-di(4-methylphenyl)methyl]imidazolinium chloride, 3h

Yield: 2.41 g, 79%, m.p. 219-221 ^{o}C. IR: $\nu_{(NCN)}$ = 1658 cm^{-1}. Anal. Calc. for $C_{33}H_{35}N_2Cl$: C, 80.08; H, 7.08; N, 5.66. Found: C, 80.10; H, 7.06; N, 5.67%. ^{1}H NMR ($CDCl_3$) δ: 4.03 (s, 4H, NC*H*$_2$C*H*$_2$N), 6.32 (s, 2H, C*H*-Ar), 7.15 and 7.24 (d, 16H, *J* = 8.0 Hz, C_6H_4-CH_3), 2.31 (s, 12H, C_6H_4-C*H*$_3$), 8.15 (s, 1H, NC*H*N). ^{13}C NMR ($CDCl_3$) δ: 48.5 (N*C*H$_2$*C*H$_2$N), 65.8 (*C*H-Ar), 21.1 (C_6H_4-*C*H$_3$), 128.3, 129.8, 132.8 and 138.7 (Ar-*C*), 158.4 (N*C*HN).

2.5. Biochemical studies

CA inhibitory effects of novel symmetrical imidazolinium chloride salts derivatives (**3a-h**) were measured according to Verpoorte et al. [43] conforming to previous studies [44,45] and measured at 348 nm spectrophotometrically using p-nitrophenylacetate (PNA) substrate. AChE and BChE inhibitory effects of novel symmetrical imidazolinium chloride salts derivatives (**3a-h**) were determined according to Ellman et al. [46] conforming to previous studies [47,48] and measured at 412 nm spectrophotometrically using acetylthiocholine iodide as a substrate. 5,5'-Dithio-bis(2-nitrobenzoic) acid compound was used for activities measurements of both cholinergic enzymes. Inhibitory effect of novel symmetrical imidazolinium chloride salts derivatives (**3a-h**) against α-glycosidase activity was realized by p-Nitrophenyl-D-glycopyranoside (p-NPG) substrate [49]. For this purpose, an aliquot of phosphate buffer (200 µL, pH 7.4) was mixed with 40 µL of the homogenate solution, which prepared in same buffer. Then, an aliquot of p-NPG (50 µL) in phosphate buffer (pH 7.45, mM) was added to mixture and incubated at 30ºC again. α-Glycosidase activity was spectrophotometrically recorded at 405 nm according to previous studies [50,51].

2.6. Docking studies

x-Ray crystal structures of AChE, BChE, hCA I, hCA II and α-glycosidase (PDB code: 4WR7, 5AML, 4M0E, 5NN0 and 3L4U, respectively) were taken from RCSB Protein Data Bank with resolution of 1.5 Å, 1.36 Å, 2 Å, 2.1 Å and 1.9 Å, respectively. Molecular docking studies were performed using Small Drug Discovery Suites package (Schrödinger 2017-2, LLC, USA). The 3D crystal structures were repaired and prepared via protein preparation wizard in Maestro 11.4. Bond order and charges were assigned and then all missing hydrogen atoms were added to protein structure. Missing side chains were filled using Prime module of the program. Amino acids were ionized by setting physiological pH with the help of Propka

software. Water molecules that were formed less than 3 contacts with the protein or ligand were removed. Finally, energy minimization has also been performed using OPLC force field. After protein preparation, top-ranked potential protein binding sites and single binding site were identified using SiteMap module in Maestro 11.4 [52].

3D structures of synthesized compounds were produced with Maestro 11.4. by sketching 2D structures. 3D structure of ligands was created using LigPrep module of Schrodinger. In order to obtain correct molecular geometries and protonation state at pH 7.0 ± 2.0, Epik module and OPLS-2005 force field were used [52].

Molecular docking was performed to identify binding affinity and interactions between synthesized compounds and receptors. Briefly, grid box was generated by selecting crystallized inhibitors at the binding site using the Receptor Grid Generation platform before the docking process. Docking calculations were set as Extra Precision (XP) by keeping the ligand flexible. After the docking process, the types of interactions and interacted residues for best-scored compound results were analyzed. Docking validation was performed with re-docking procedure by extracting inhibitor complexed in the crystal structure of receptor [52].

3.RESULTS AND DISCUSSION

3.1.Synthesis of imidazolinium salts

The symmetrical 1,3-dialkylimidazolinium chloride salts (**3a-h)** were prepared according to literature procedure (Scheme 1). Ethylenediamine was reacted with the appropriate aromatic ketones (2 eq.) in ethanol to give the corresponding diimines **1**. Subsequent reduction of **1** with $NaBH_4$ in methyl alcohol at room temperature, followed by their cyclization with triethyl ortoformate in the presence of NH_4Cl led to the expected imidazolinium salts **3a-h**. These salts were isolated as colourless solids in 76-88% yields and completely characterized using FTIR, elemental analysis, ^1H and ^{13}C NMR spectroscopies. Their melting points were determined. All results support the proposed structure. The ^1H NMR spectra of imidazolinium salts **3** show the characteristic resonance signals of NC*H*N protons as singleds at 10.57 for **3b**, 10.41 for **3c**, 10.68 for **3d**, 8.11 for **3g** and 8.15 ppm for **3h,** respectively. The ^{13}C NMR spectra of imidazolinium salts **3** show characteristic chemical resonances at 156.8 for **3b** and **3d**, 156.6 for **3c** and 158.4 ppm for **3g** and **3h,** corresponding to the N*C*HN carbons. The IR spectra of **3** also exhibit characteristic $\upsilon_{(NCN)}$ band of imidazolinium ring at 1634, 1629, 1628, 1638 and 1658 cm^{-1} for **3b**, **3c**, **3d**, **3g** and **3h**, respectively. The elemental analysis data of imidazolinium salts are in agreement with the theoretical requirements of their structures.

Scheme 1

3.2.Metabolic enzymes inhibition results

The activation or inhibition properties of many CA isozymes in tissues including brain and kidney are crucial for biochemical applications. The diuretic effect of the CAIs is well recorded in recent. They lead to the progress of diuretics belonging to multiple classes including thiazides and acetazolamide compound [53]. Many drugs had strong CA inhibition effects resulting in diuretic effects against several mammalian CA isoenzymes are extensively utilized clinically. Among them, furosemide, thiazides, metolazone, quinethazone, indapamide and chlorthalidone countable. Their applications were dealt with widely in a previous study [54]. Most of the recent work relevant to the renal applications of the CA isoforms and their inhibition effects deal predominantly with aspects relevant to the decrease of drug toxicity effect by suing CAIs [55]. Novel symmetrical imidazolinium chloride salts derivatives (**3a-h**) were tested for evaluation of inhibition effects towards the hCA I and hCA II isoenzymes, AChE, BChE and α-glycosidase enzymes. The chemical structure of novel symmetrical imidazolinium chloride salts derivatives (**3a-h**) was shown in Scheme 1. Their inhibition data related to AChE, BChE, α-glycosidase, and hCA I, and hCA II are given in Table 1. The following results are concluded in this Table. The hCA I isoenzyme was inhibited by these compounds with Ki values between 18.41±3.76 and 121.73±52.55 µM. In addition, compounds **3a**, and **3g** demonstrated as the most effective hCA I inhibition profile with Ki values of 18.41±3.76 and 28.39±10.22 µM, respectively. Acetazolamide (AZA), which is positive control and used a clinical drug, demonstrated a Ki value of 127.63±14.77 µM. The results clearly showed that the investigated molecules shown better inhibitory effects when compared to AZA (Figure 1 and Table 1). The results demonstrated that hCA II isoenzyme was impressively inhibited by the novel symmetrical imidazolinium chloride salts derivatives (**3a-h**). These compounds had strong hCA II inhibition with Ki values in ranging of 12.50±6.50 - 63.12 ± 26.98 nM. Ki values of novel molecules are better than that of AZA (Ki: 91.37 ± 10.62 µM). All the evaluated novel molecules showed effective inhibition properties against hCA II. However, the compounds **3f**, and **3a** showed the best inhibition profiles against hCA II with Ki values of 12.50±6.50 and 25.68±10.40 µM, respecticelly (Table 1).

Table 1

Both AChE and BChE are a structurally similar proteins belonging to the esterase-lipase family. Their inhibitors are promising for the therapy as they are activating cholinergic system as well [56]. The current drugs are donepezil, galantamine, rivastigmine and memantine and used in most countries for therapy of Alzheimer's disease (AD). Among them, memantine is a non-competitive antagonist for N-methyl-D-aspartic acid receptors [57,58]. The other drugs, (donepezil, galantamine and rivastigmine) are used as inhibitors of cholinesterases. Donepezil is marketed under different trade [59]. It is a reversible inhibitor for both cholinergic enzymes. The inhibitory effects of novel synthesized compounds on AChE enzyme are shown in Table 1. In this work, AChE was also extremely inhibited by novel symmetrical imidazolinium chloride salts derivatives (**3a-h**) at the low nanomolar inhibition (K_is: 3.72±0.70-34.58±4.79 nM, Table 1). However, the most effective AChE inhibition was recorded by novel compound of **3e** (Ki: 3.72 ± 0.70 nM, Figure 1). Also, the other compounds had highly efficient inhibition effects against AChE. Additionally, 1,2,3,4-tetrahydroacridin-9-amine (Tacrine) that acts as cholinesterase inhibitor used for AD therapy showed Ki value of 118.93 ± 9.07 nM against AChE. Finally, novel symmetrical imidazolinium chloride salts derivatives (**3a-h**) inhibited another cholinergic enzyme (BChE). Ki values were found in range of 5.50 ± 1.25-32.36 ± 7.70 nM obtained from Lineweaver-Burk plots (Table 1). Whereas, tacrine, as the first cholinesterase inhibitor and used for the management of AD symptoms since 1993, had Ki value of 97.26±16.48 nM against BChE. Also, donepezil showed lower AChE inhibition activity (IC_{50}: 55.0 nM). As seen in Table 2, IC_{50} values of new synthesized compounds are between 48.34-290.04 nM against AChE with range of 47.91-108.37 nM for BChE (Figure 1 and Table 1).

The modern antidiabetic drugs are available in both oral and injectable forms. Among them, α-glycosidase inhibitors (AGIs) are the main therapeutic agents to treat type II diabetes [60,61]. In diabetic patients, AGIs help to retard the metabolism of carbohydrates and regulate the elevation of high postprandial blood glucose level. Acarbose, miglitol and voglibose are some of the currently used AGI drugs. Although AGIs prevent the hypoglycemic condition and also control the micro- and macrovascular complications, side effects like gastrointestinal irritation, diarrhea, flatulence, and abdominal discomfort occur with them [62,63]. For this metabolic enzyme, the novel symmetrical imidazolinium chloride salts derivatives (**3a-h**) had IC_{50} values of 100.37-303.63 µM. Ki values were found in the range of 94.72±17.53-364.51±104.23 µM for novel synthesized compounds (Figure 1 and Table 1). It is obvious that all novel symmetrical imidazolinium chloride salts derivatives (**3a-h**) had efficient α-

glycosidase inhibition profile when compared to acarbose (IC_{50}: 22.80 μM) as a positive control α-glycosidase [59,60]. On the other hand, the most effective Ki value was obtained by 1,3-di[1-(3,4-dimethoxyphenyl)ethyl]imidazolinium chloride, **3d** and 1,3-di[1-(3,4-dimethylphenyl)ethyl]imidazolinium chloride, **3c**, with Ki values of 94.72 ± 17.53 and 109.38 ± 24.71 μM, respectively.

Figure 1

3.3.Docking studies

We performed docking studies to identify the best pose of the compounds, which interacts with the active site residues of the receptors and possible inhibition mechanism after *in vitro* experimental results. Besides, AZA was used as a reference inhibitor compound for hCA I and hCA II receptors, TAC was used as a reference inhibitor compound for cholinergic receptors and ACR was used as a reference inhibitor compound for α-glycosidase receptor. The reference inhibitors were similarly docked into catalytic active site of receptors. In order to analyse the interactions between best-scored compounds and receptors, pose viewer module was used. Molecular docking studies showed that all synthesised compounds fit well into the catalytic active sites of BChE, AChE, α-glycosidase, hCA I, and hCA II enzymes along with high binding affinity towards the enzyme. The results of Glide docking are summarized in Table 2. We detected that *in vitro* end *in silico* result be compatible on a large scale. In present study, we focus on explanation for interactions and binding mechanisms between ligands and receptors and we considered glide score, H-bonds and non-bonded interactions for docking results analysis. The docking process was confirmed with re-docking procedure. In the validation, the docking accuracy showed that co-crystallized and docked ligands are well docked into the receptors as seen in Figure 2.

Table 2 and Figure 2

Docking studies exhibited that all compounds could easily fit into the catalytic active site of all the receptors. However, we have illustrated only best pose of compounds exhibited best inhibition effect in results of *in vitro* study for each receptor. According to results of *in vitro* study, compound **3a** was most active inhibitor and was also one of compounds with best docking score towards hCA I isoenzyme. The compound formed π-π interaction with Hie94 residue and also interacted through π-cation with Zn301, which is cofactor of the enzyme into the catalytic active site of hCA I isoenzyme. Additionally, the compound formed salt bridge

with Act304, which is ion at protein crystal structure. We detected similarity between inhibition mechanisms of compound **3a** and **AZA,** a standard inhibitor for hCAs. Two compounds interacted with Zn301 and Act304 into the catalytic active site of hCA I isoenzyme. We also detected that very similar hydrophobic and polar amino acid residues as seen Figure 3a and 5f surrounded best-poses of compounds 3a and AZA. Compound **3a** exhibited very similar interactions as formerly reported inhibitors [63,64].

Although compound **3g** was identified as best scored compound towards hCA II with docking study. We observed that compound **3f** was the most active inhibitor of hCA II *in vitro* experiment. Therefore, interaction and binding mechanism of compound **3f** were analyzed. The compound formed π-π interaction with His94 residue. In previously study, it was revealed that the residue is located into the catalytic active site of hCA II and plays a critical role inhibiting the enzyme [65-67]. **AZA** formed hydrogen bond with Asn67 and Thr199 amino acid residues and moreover formed a salt bridge with Zn265 atom into the catalytic active site of hCA II (Figures 3b and 3g).

According to results of docking study, all of synthesised compounds exhibited good binding affinity ranges from -7.414 to - 12.435 kcal/mol the catalytic active site of AChE receptor in comparison with binding affinity of **TAC** standard inhibitor. Compound **3e** was identified as most active compound against AChE enzyme. Docking score of the compound was more negative than docking score of **TAC**. Therefore, we analyzed interaction and binding mechanism of **3e** with active site residues of AChE. The compound formed hydrogen bond with Tyr124, Trp286, and Tyr337 residues. Furthermore, the compound formed salt bridge with Asp74 residue. **3e** was surrounded by a number of hydrophobic amino acid residues and binding pocket of **3e** and **TAC** is very similar due to hydrophobic amino acid residues including Try72, Trp286, Phe295, Phe297, Tyr341, Tyr337 and Phe338. **3e** exhibited very similar interaction likes previously reported inhibitors [68-70].

Compound **3c** has both most active compound and highest binding affinity against BChE enzyme. The compound also exhibited excellent binding affinity against BChE enzyme in comparison with binding affinity of **TAC,** standard inhibitor. The compound formed π-π interaction with Tpr82 residue and π-cation interaction Try332 residue into the catalytic active site of BChE (Figure 3d). It has observed that the interactions play a critical role in the enzyme activity and inhibition as previously studies [71]. Besides, **TAC** formed π-π

interaction with Tpr82 residue and also was surrounded with greatly similar hydrophobic residues as seen Figure 3i.

We detected that compound **3b** was best-scored compound against α-Gly with docking study. According result of *in vitro* study, compound **3d** was determined most active compound for the enzyme. Therefore, we analysed best pose of compound **3d** to understand inhibiting mechanism of the compound. The compound formed hydrogen bond with Gly603 residue and salt bridge with Asp542 residue (Figure 3e). Asp542 residue was one of residues interacted of **ACR**. Moreover, compound **3d** and **ACR** located similar binding pocket and were surrounded with similar residues as seen Figure 3e and 4j. Our finding is similar with the result, which given in literature [72]. They have detected that (R)-2-ethylhexyl 2*H*-1,2,3-triazole-4-carboxylate and (E)-2-(prop-1-enyl)-*N*-methylquinolinium-4-olate extracted from the stems of *Paramignya trimera* inhibited human α-glycosidase by interacting through hydrogen bond with Asp542 residue of the enzyme [72]. The results show that Asp542 is a key residue inhibiting the enzyme.

Figure 3

Superimposed pose of compounds, which are most active inhibitor, showed that the compounds well placed into the catalytic active site of the receptors as seen in Figure 4. In this figure, the detailed binding mode of the compounds has also shown. The binding mode of compounds revealed that this compounds interacted with the key residues into the catalytic active site of the receptors.

Figure 4

4.CONCLUSION

In summary, the symmetrical imidazolinium chloride salts were successfully prepared in good yields by the treatment of N,N'-dialkylethane-1,2-diamines with $HC(OEt)_3$ in the presence of NH_4Cl and characterized by spectroscopic methods, and microanalysis techniques. The hCA I inhibitors are clinically utilized predominantly as anti-epileptics and diuretics. However, the novel synthesized compounds can be used in clinical applications in the management of some disease after some further evaluations because of their inhibition effects against some metabolic enzymes including carbonic anhydrase I and II isoenzymes, acetylcholinesterase; butyrylcholinesterase and α-glycosidase. Also, the molecular modeling are implementation for estimation of the binding proximity of symmetrical imidazolinium chloride salts bearing

secondary wing tip groups and their inhibition mechanisms and kinetics in atomic levels at the catalytic domains.

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Table 1. Human carbonic anhydrase isoenzymes I, and II, α-Glycosidase, AChE and BChE enzymes inhibition values of novel symmetrical imidazolinium chloride salts derivatives (**3a-h**)

Compounds	IC_{50} (nM)										K_i (nM)				
	hCA I	**r^2**	**hCA II**	**r^2**	**AChE**	**r^2**	**BChE**	**r^2**	**α-Gly**	**r^2**	**hCA I**	**hCA II**	**AChE**	**BChE**	**α-Gly**
3a	11.43	0.9844	13.54	0.9692	113.56	0.9832	49.78	0.9922	248.12	0.9614	18.41±3.76	25.68±10.40	23.64±10.23	26.50±4.10	267.13±63.12
3b	17.02	0.9631	24.10	0.9402	210.68	0.9812	61.61	0.9881	293.05	0.9889	40.84±10.20	62.44±25.34	7.65±0.88	24.03±11.80	303.73±50.23
3c	15.15	0.9693	20.34	0.9671	255.41	0.9931	86.58	0.9723	123.74	0.9720	54.13±17.02	40.70±14.93	12.60±0.60	5.50±1.25	109.38±24.71
3d	16.16	0.9702	28.72	0.9631	226.55	0.9871	63.63	0.9851	100.37	0.9924	39.97±5.57	53.67±14.83	7.60±0.92	17.10±2.49	94.72±17.53
3e	19.19	0.9291	31.17	0.9321	174.60	0.9781	59.45	0.9753	303.63	0.9691	48.90±4.50	63.12 ±26.98	3.72±0.70	12.33 ±0.98	348.20±73.51
3f	18.47	0.9862	24.38	0.9441	118.47	0.9651	108.37	0.9641	347.71	0.9377	121.73±52.55	12.50±6.50	21.42±3.48	7.05±2.39	364.51±104.23
3g	30.59	0.9841	20.56	0.9501	290.04	0.9761	49.06	0.9681	265.72	0.9633	28.39±10.22	40.50±10.90	7.02±1.47	32.36±7.70	239.76±94.32
3h	20.49	0.9872	28.43	0.9333	48.34	0.9842	47.91	0.9772	217.54	0.9521	52.67±6.34	43.56±11.60	34.58±4.79	14.29±0.95	263.98±66.14
AZA*	134.14	0.9811	103.11	0.9709	-	-	-	-	-	-	127.63±14.77	91.37±10.62	-	-	-
TAC**	-	-	-	-	156.82	0.9691	128.02	0.9508	-	-	-	-	118.93±9.07	97.26±16.48	-
ACR***	-	-	-	-	-	-	-	-	2800	0.9634	-	-	-	-	12600±780

*Acetazolamide (AZA) was used as a standard inhibitor for both hCA I, and II isoenzymes.
** Tacrine (TAC) was used as a standard inhibitor for AChE and BChE enzymes.
***Acarbose (ACR) was used as positive control for α-glycosidase enzyme, which obtained from reference 73.

Table 2. Glide and IFD scores (kcal/mol) of the pyrazoline derivatives in the catalytic sites of hCA I, hCA II, AChE, BChE, and α-Gly enzymes

Compounds	XP GScore				
	hCA I	**hCA II**	**AChE**	**BChE**	**α-Gly**
3a	-4.777	-4.377	-10.190	-9.703	-6.000
3b	-4.570	-3.807	-10.452	-10.282	-6.233
3c	-3.386	-3.914	-10.343	-11.501	-5.929
3d	-4.438	-3.803	-11.139	-9.664	-5.147
3e	-4.374	-3.601	-9.560	-10.524	-4.269
3f	-4.408	-4.631	-7.414	-6.587	-3.136
3g	-4.946	-6.213	-12.435	-10.182	-4.411
3h	-3.980	-1.920	-7.892	-8.928	-3.719
AZA*	-8.331	-8.172	-	-	-
TAC**	-	-	-8.978	-8.440	-
ACR***	-	-	-	-	-16.933

*Acetazolamide (AZA) was used as a standard inhibitor for both hCA I, and II isoenzymes.

** Tacrine (TAC) was used as a standard inhibitor for AChE and BChE enzymes.

***Acarbose (ACR) was used as positive control for α-glycosidase enzyme, which taken of reference 73.

FIGURE LEGENDS

Figure 1. Ki values of novel compounds on metabolic enzymes

Figure 2. Docking validation. Receptors are depicted in the ribbon model which hCA I with grey color, hCA II with orange color, AChE with blue color, BChE with faded red, and α-Gly with yellow color. The poses of co-crystallized ligands are represented in grey color ball and stick modeling while that of docked ligands is represented in magenta color ball and stick modeling.

Figure 3. 2D receptor–ligand interaction profile; (a) **3a**-hCA I, (b) **3f**-hCA II, (c) **3e**-AChE, (d) **3c**-BChE, (e) **3d**-α-Gly, (f) AZA-hCA I, (g) AZA-hCA II, (h) TAC-AChE, (i) TAC-BChE, and (j) ACR-α-glycosidase.

Figure 4. The lowest energy conformation of compounds into hCA I, hCA II, AChE, BChE, and α-glycosidase receptors. (a) Best-pose of **3a** into catalytic active site of hCA I, (b) Best-pose of **3f** into catalytic active site of hCA II, (c) Best-pose of **3e** into catalytic active site of AChE, (d) Best-pose of **3c** into catalytic active site of BChE, and (e) Best-pose of **3d** into catalytic active site of α-glycosidase. Receptors are depicted in the ribbon model which hCA I with grey color, hCA II with orange color, AChE with blue color, BChE with faded red, and α-glycosidase with yellow color. Catalytic active sites are represented as solid surface. Compounds are represented in ball and stick modeling which magenta color carbon and residues are represented in ball and stick modeling which grey color carbon.

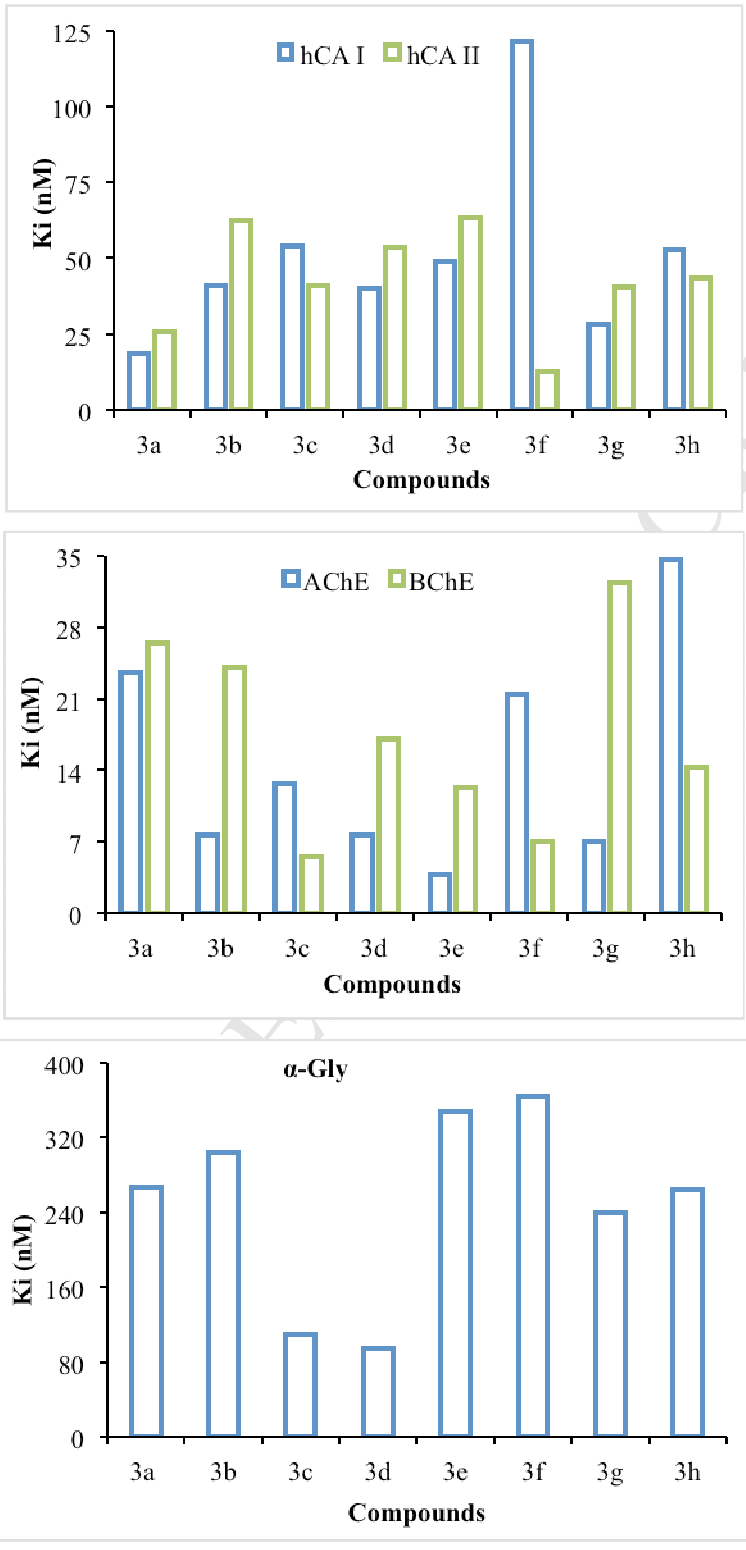

hCA I
hCA II
Ki (nM)
125
100
75
50
25
0
3a
3b
3c
3d
3e
3f
3g
3h
Compounds
AChE
BChE
Ki (nM)
35
28
21
14
7
0
3a
3b
3c
3d
3e
3f
3g
3h
Compounds
α-Gly
Ki (nM)
400
320
240
160
80
0
3a
3b
3c
3d
3e
3f
3g
3h
Compounds

Figure 1

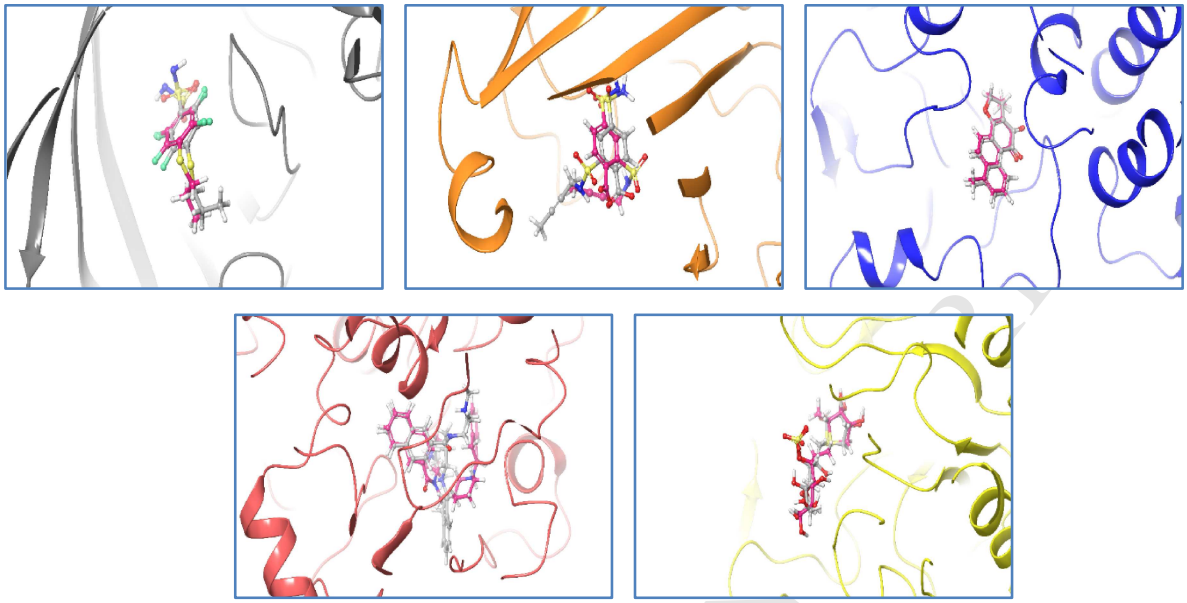


Figure 2

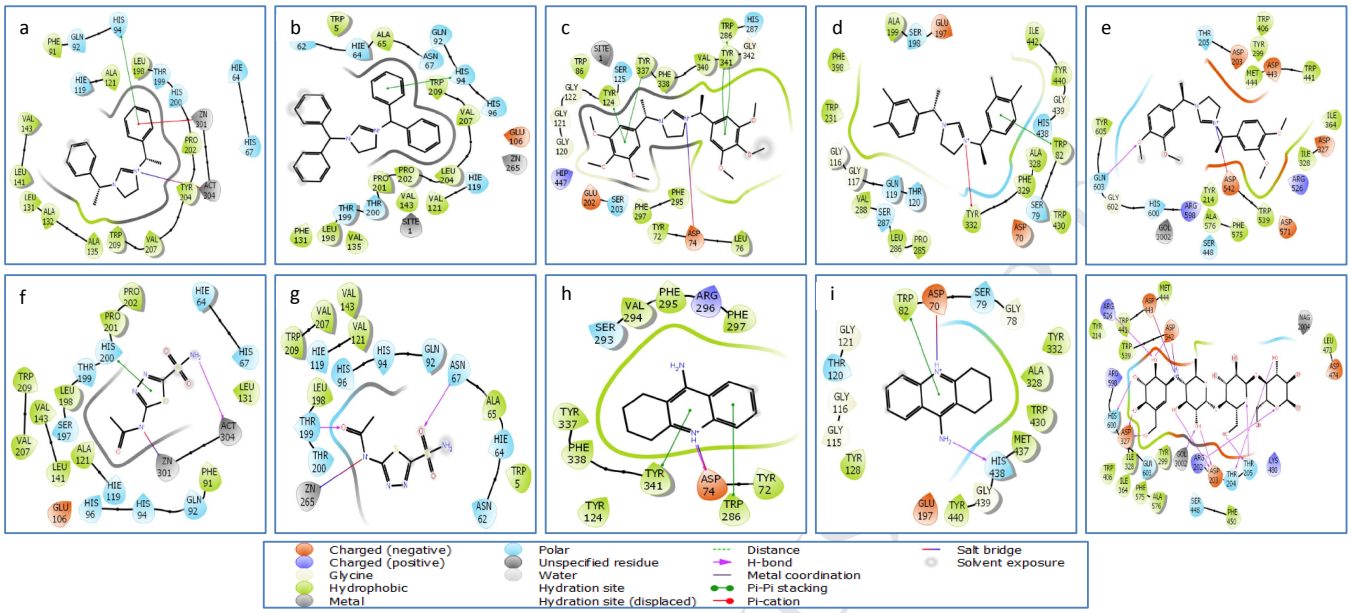


Figure 3

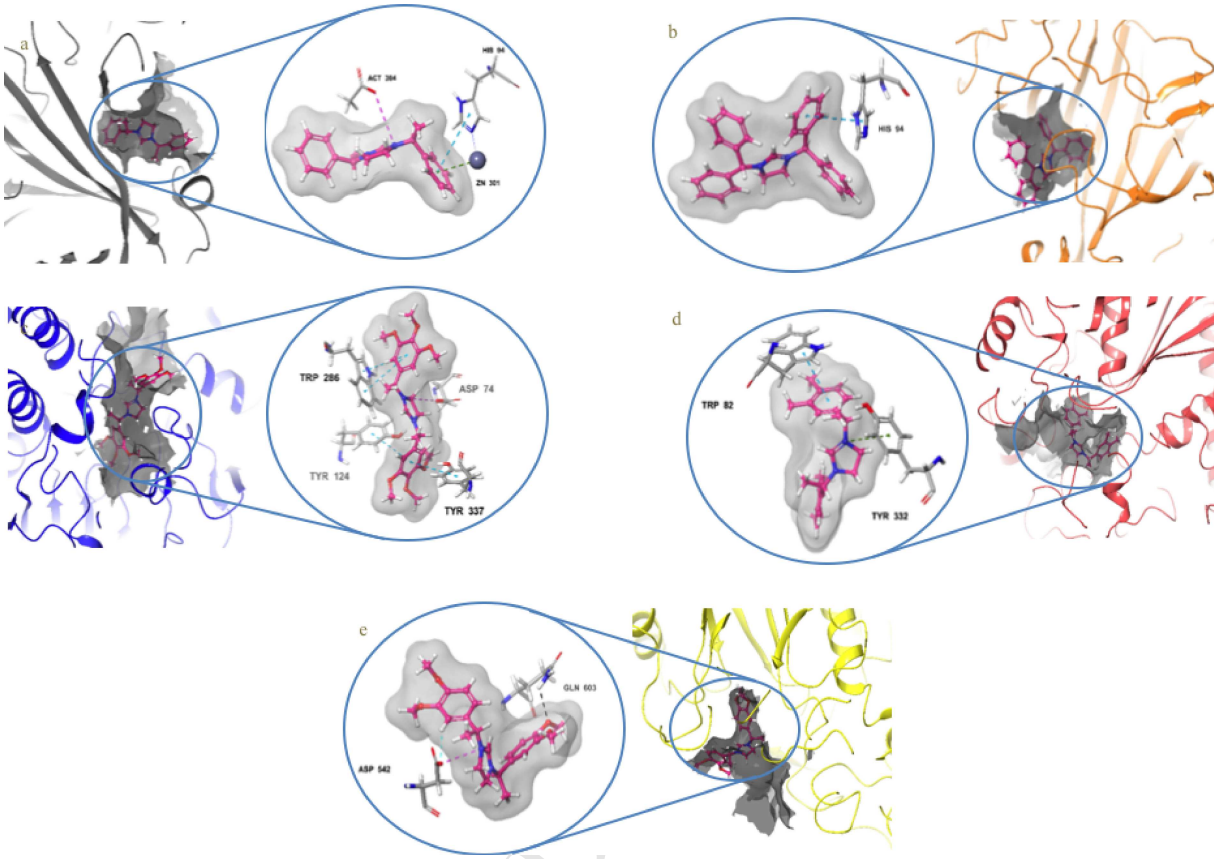
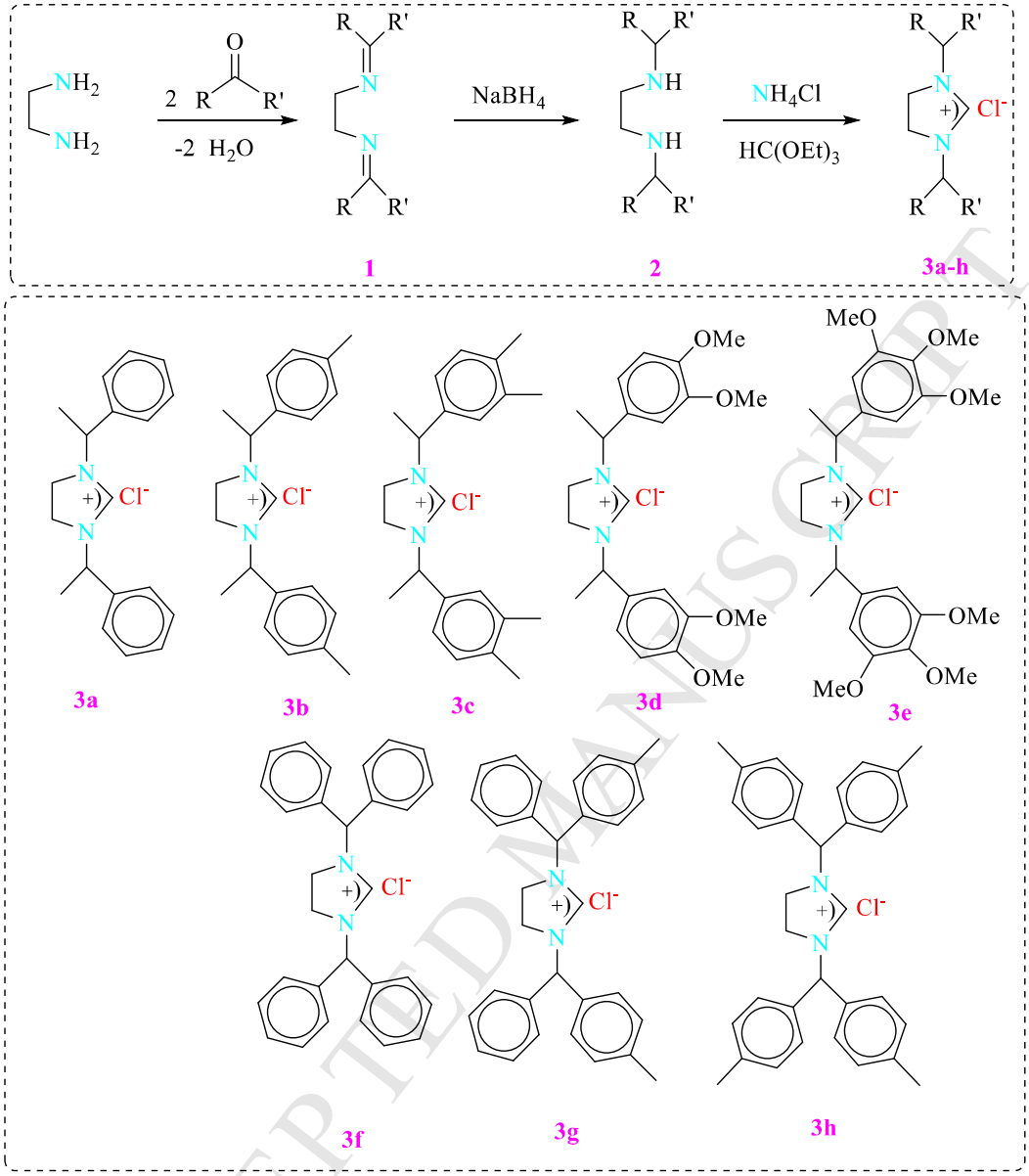


Figure 4



Scheme 1

Highlight

- A series of symmetrical imidazolinium chloride salts bearing secondary N-alkyl substituents were synthesized.
- These novel compounds have been characterized by ^{1}H and ^{13}C NMR and FTIR spectroscopies.
- Their inhibition effects against hCA I, hCA II, AChE, BChE and α-glycosidase were determined.
- The molecular modeling was obtained for the best inhibitors and enzymes.