Rec. Pharm. Biomed. Sci. 7 (1), 115-125, 2023

ORIGINAL ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Synthesis and Molecular Docking Investigations of Ring-Fused Benzimidazoles as Novel Acetylcholinesterase Inhibitors

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Received on: 01. 03. 2023

Revised on: 27. 03. 2023

Accepted on: 01. 04. 2023

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Abstract

Alzheimer's disease (AD) is a highly neurodegenerative brain disease and is the most prevalent type of dementia. AD affects parts of brain that are responsible for memory, language and thought. Cholinergic hypothesis proposed that deficiency of acetylcholine in certain zones of brain may be responsible for the progressive memory deterioration observed in patient with AD. Therefore, inhibition of acetylcholinesterase (AChE) may represent a hopeful target for treatment of AD. Herein, new series of ring-fused benzimidazoles (benzimidazo [1,2-c] quinazoline derivatives (**scheme 1**, compounds **2a-d**) and benzimidazo [2,1-c] triazole derivatives (**scheme 2**, compounds **7** and **8a-b**) as main scaffolds) were synthesized using benzene1,2-diamine as a starting material and characterized by mass spectroscopy, IR, ^{1}HNMR, ^{13}C NMR and elemental analysis. Molecular docking studies were performed on AChE and demonstrated the preferential binding interaction of synthesized compounds with AChE binding site. Therefore, the obtained results imply that ring-fused benzimidazoles may act as an auspicious lead compounds for further optimization and biological evaluation against AChE.

Keywords: Alzheimer disease, acetylcholinesterase, benzimidazoles, benzene1,2-diamine.

1. Introduction:

Alzheimer's disease (AD) is a neurodegenerative disease starting with mild memory loss, then symptoms developed gradually over many years and eventually become more severe to interfere with the most daily tasks (Hori, Watanabe et al. 2023). AD affects parts of brain that are responsible for memory, language and thought. AD comprises about 60-80 % of total cases of dementia all over the world (Femminella, Thayanandan et al. 2018). Recently more than 55 million people live with dementia globally and by 2050, AD and other dementia will cost the nations nearly $1 trillion acting as a considerable burden on health organization (Cubanski J 2022). Therefore, there is an insistent need for development of novel drugs used for potential treatment of AD. There are many hypotheses that tried to illustrate pathophysiology of Alzheimer's disease including β-amyloid hypothesis, cholinergic hypothesis and tau hypothesis (Soyer, Uysal et al. 2017). Amongst them, cholinergic hypothesis is the oldest one, which proposed that low level of acetylcholine in certain zones of the brain is responsible for memory deterioration associated with AD (Atanasova, Yordanov et al. 2015). Therefore, inhibiting acetylcholine degradation by cholinesterase enzymes can be a promising tactic for treatment of AD. Currently, there are just four acetylcholinesterase inhibitors approved by FDA for AD treatment: tacrine (withdrawn because of

hepatotoxicity), rivastigmine, donepezil and galantamine **(Figure 1)** (Atta, darwish et al. 2021) (Marucci, Buccioni et al. 2021). It's worth mentioning that the aim of our present study is to design and synthesis novel compounds act as acetylcholinesterase inhibitors and used for treatment of AD. Cholinesterase enzyme is a serine protease that catalyze the hydrolysis of acetylcholine neurotransmitter at synaptic cleft causing severe loss of cholinergic functioning (Hameed, Zehra et al. 2015). The binding site of AChE is located near the bottom of a narrow deep gorge and composed of various domains **(Figure 2)**.

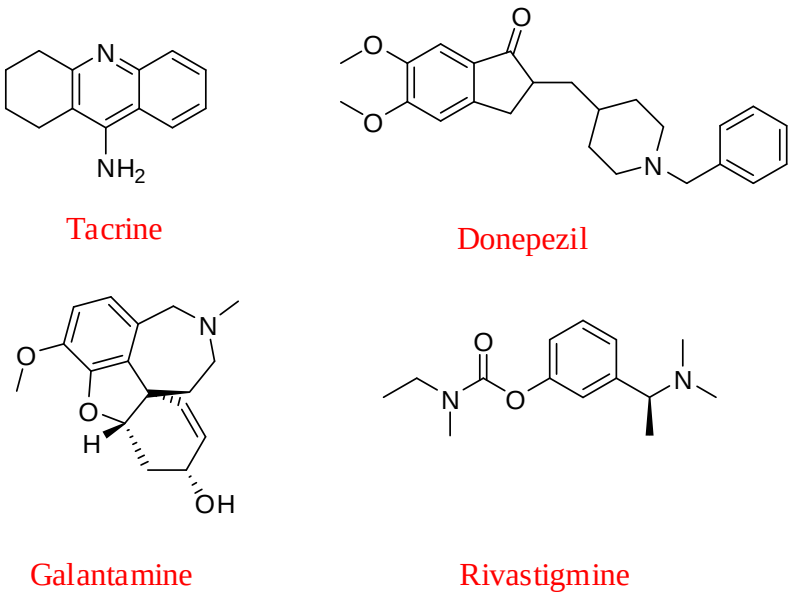


Figure 1: FDA approved cholinesterase inhibitors

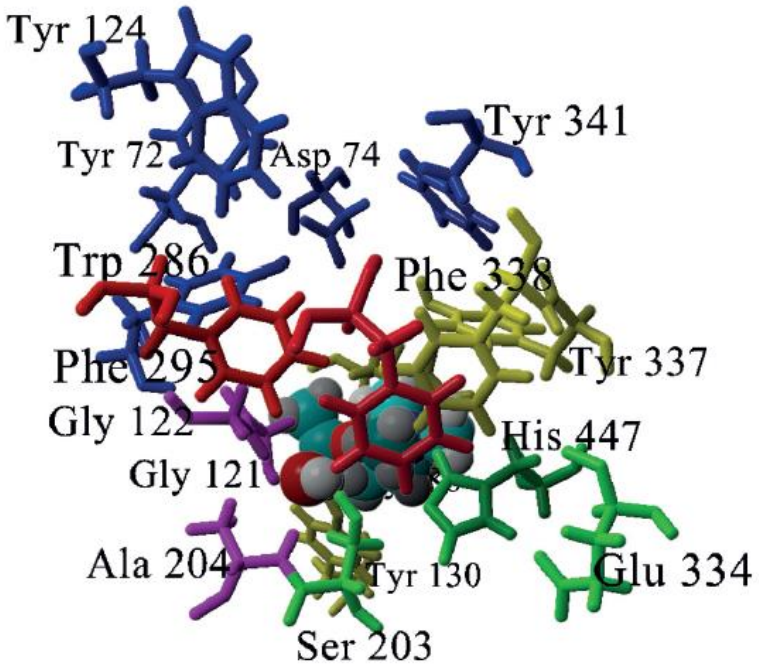


Figure 2: The binding site of human AChE consists of several domains: catalytic domain (in green), anionic domain (in yellow), acyl pocket (in red), oxyanion hole (in purple), peripheral anionic site (in blue). Bound ACh is given as balls (Atanasova, Yordanov et al. 2015).

Catalytic anionic site (CAS) lies at the bottom of the narrow gorge where the hydrolysis of acetylcholine occurs to choline and acetic acid. It consists of catalytic triad of Ser203, Glu334, and His447. Peripheral active site (PAS) lies at the entrance of the active site containing key amino acid residue like Asp74, Tyr124, Ser125, Trp286, Tyr337, and Tyr341. Furthermore, there are other functional sites including anionic subsite (contains aromatic residues like Trp86, Tyr130, Tyr337and Phe338), acyl pocket (contains bulky amino acid Phe295 and Phe297) and oxyanion hole (consists of Gly121, Gly122 and Ala204) (Atanasova, Yordanov et al. 2015).

Literature demonstrated that benzimidazole is a privilege heterocyclic pharmacophore in medicinal chemistry (Guo, Hou et al. 2021). Its scaffold is included in many natural compounds like histidine, purine and integral part of vitamin B12. Besides, benzimidazole and their derivatives have a remarkable role as precious therapeutic agents including anticancer, antihypertensive, antibacterial and analgesics (Kanwal, Saddique et al. 2018) (Djuidje, Durini et al. 2020).

Thus, in the present study, our endeavors have been devoted toward using benzimidazole-fused heterocycles as main scaffold for design and synthesis novel compounds as potent acetylcholinesterase inhibitors. Our initial efforts were focused on design and synthesize a novel series of benzimidazo [1,2-c] quinazoline derivatives **(scheme 1,** compounds **2a-d)** due to their resemblance with galantamine as tetracyclic compounds with extra nitrogen atoms (may involve in hydrogen bonding interaction) and phenyl group that may participate in π-π interactions with the active site of enzyme during inhibition **(Figure 3)**.

Inspired by structure of donepezil coupled with our docking studies, we have decided to synthesize novel series of benzimidazo [2,1-c] triazole derivatives **(scheme 2**, compound **7** and **8a-b**) comprising a linker and a terminal phenyl group either substituted or not in an attempt to enhance the binding affinity with acetylcholinesterase **(Figure 3)**.We have proposed that benzimidazo [2,1-c] triazole will fit into peripheral active site while piperazine ring will occupy catalytic active site allowing the terminal phenyl moiety to accommodate into the anionic site.

2. Results and Discussion

2.1. Chemistry

Scheme 1. Illustrates the synthesis of final compounds (**2a-d)** which achieved through the reaction of benzene1,2 diamine with anthranilic acid which gave compound **(1)** which then allowed to react with substituted benzoic acid to give the target compounds (**2a-d)**. The IR spectrum of compound (**2b)** showed C-Cl band at 752 cm^{-1} and IR spectrum of compound **2c** showed broad OH band at 3475cm^{-1} while IR spectrum of compound (**2d)** showed C-Br band at 663 cm^{-1}. ^{1}HNMR spectra of the synthesized compounds showed downfield multiplets of aromatic protons at range of 8.72-6.46 ppm. Additionally ^{1}HNMR spectrum of compound (**2c)** showed downfield singlet of proton of OH at 8.90 ppm.

Scheme 2. Involves synthesis of target compounds **(7)** and (**8a-b)** through first reaction of benzene1,2 diamine with carbon disulfide to give 1H-benzo [d] imidazole-2-thiol **(3)** followed by reaction with hydrazine hydrate to afford compound **(4).** The IR spectrum of compound **(4)** revealed characteristic biforked band corresponding to NH_2 at 3321 cm^{-1} and also NH bands at 3448 and 3506 cm^{-1}. Then cycliztion of compound **(4)** with trimethyl orthoformate afforded compound **(5)** which reacted with chloro-acetyl chloride to give compound **(6)**. IR spectrum of compound **(6)** revealed the C-Cl band at 740 cm^{-1} and sharp band at 1708 cm^{-1} belongs to (C=O) group. Finally refluxing of compound **(6)** with benzyl piperazine, 4-chloroaniline or 4-bromoaniline afforded target compounds **(7)** and (**8a-b)** respectively. The IR spectra of the synthesized compounds showed bands of (C=O) at range of 1643-1743 cm^{-1} and IR spectra of compounds **(8a)** and **(8b)** revealed the bands of C-Cl and C-Br at 756 cm^{-1} and 613 cm^{-1} respectively. ^{1}HNMR spectrum of compound **(7)** showed the characteristic two upfield sharp singlets peaks of $2CH_2$ at 4.29 ppm and 3.23ppm while triplets of piperazine ring appeared at 3.19 ppm($2CH_2$) and 2.66 ppm ($2CH_2$). Also, ^{13}C NMR spectrum showed the piperazine ring peaks at 52.91 (2C), 48.71 (2C) and $2CH_2$ peaks at 60.99 and 45.49 ppm. Additionally, ^{1}H NMR spectra of compounds **(8a-b)** showed the upfield sharp singlets belong to CH_2 at 5.67 ppm and 4.59 ppm respectively which also appeared clearly at ^{13}C spectra at 47.93 ppm and 61.71 ppm respectively.

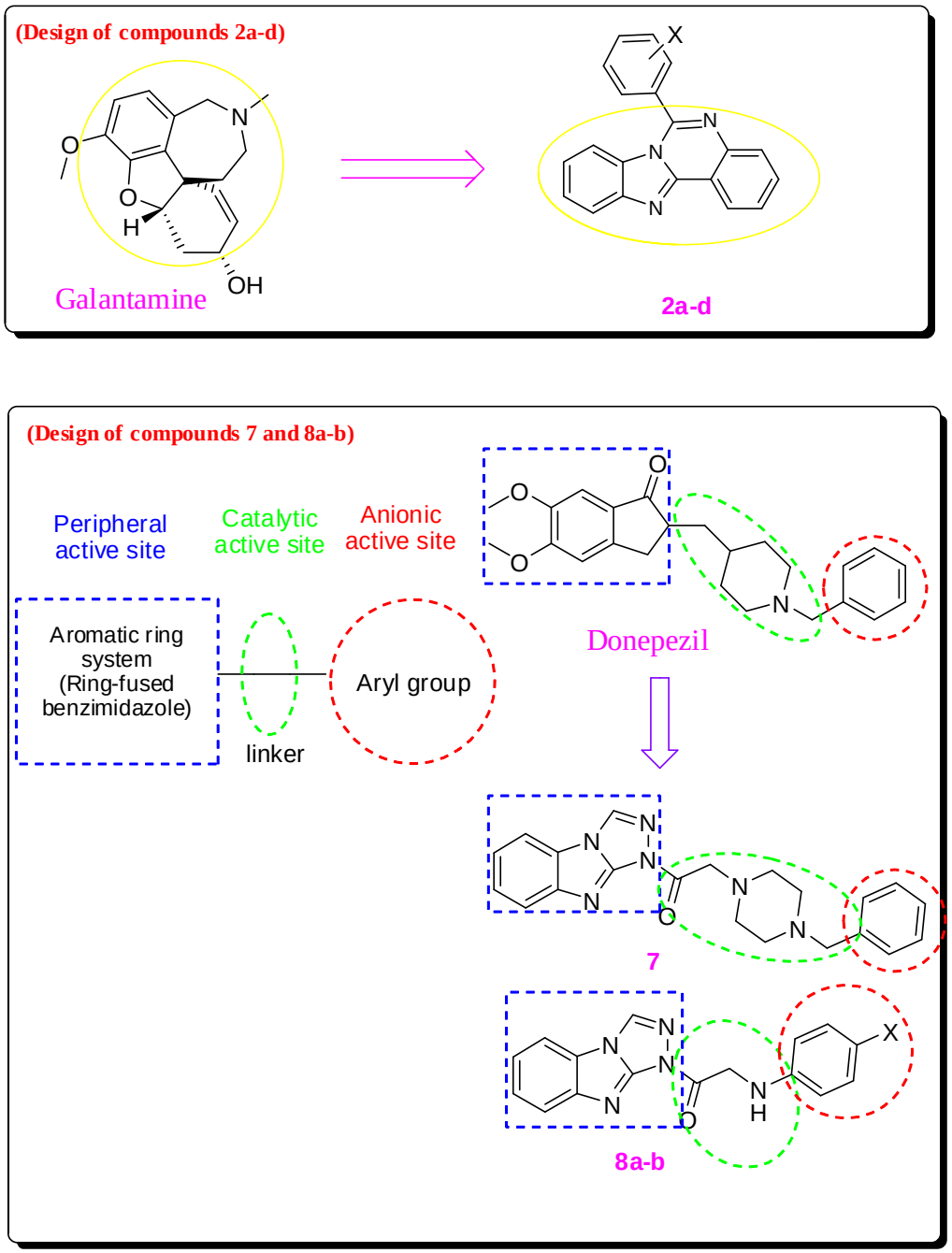


Figure 3: Design of target compounds 2 a-d , 7 and 8 a-b

2.2. Molecular docking

All the connections formed between the docked compounds and the active residues of AChE displayed negative binding energies. This means that the process of the docking molecules binding was favorable from a thermodynamic perspective. Analyzing the binging mode of co-crystalized ligand (donepezil) revealed that the benzene ring involved in π- π stacking interaction with Trp86, indanone ring oriented to the peripheral binding site and formed π-π stacking interaction with Trp286 residue while carbonyl group of indanone involved in a hydrogen bonding interaction with Phe295 residue in acyl pocket **(Figure 4)** (Cheung, Rudolph et al. 2012).

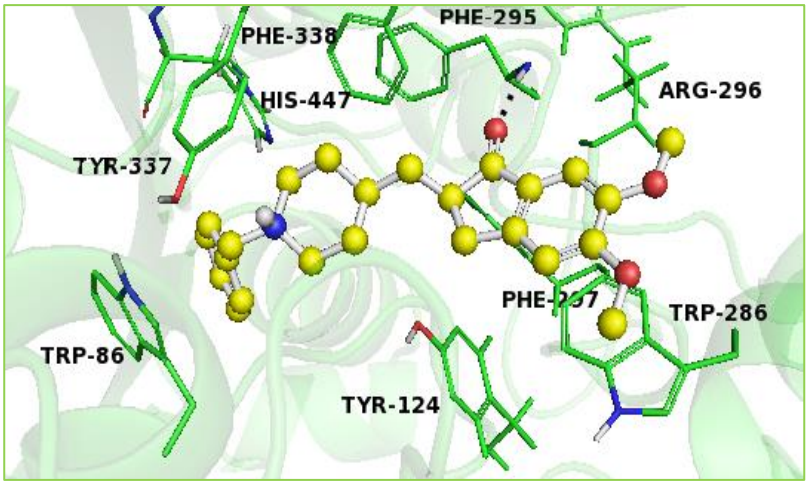


Figure 4: Binding of donepezil with AChE binding site (PDB: 4EY7). Hydrogen bond is represented as black dashed lines.

Structures of compounds **(2a-d) (scheme 1)** were based on using ring fused benzimidazole scaffold (benzimidazo [1,2-c] quinazoline) as main scaffold (tetracyclic ring system) with additional phenyl moiety or ortho / meta substituted phenyl with either chloride, bromide or hydroxyl substituent. Fortunately, compounds **(2a-d)** demonstrate good binding affinity with AChE enzyme binding site however compound **(2c**) exhibited the superior binding affinity amongst them. For instance, Benzimidazo [1,2-c] quinazoline scaffold of compound **(2c**) occupied the anionic domain and forms π-π stacking interaction with Trp86 residue. Furthermore, the substituted phenyl group accommodated near His447 in the catalytic site directing its hydroxyl substituent to interact perfectly with hydroxyl group of key amino acid Tyr124 via a significant hydrogen bonding interaction (2.4 A°) **(Figure 5).**

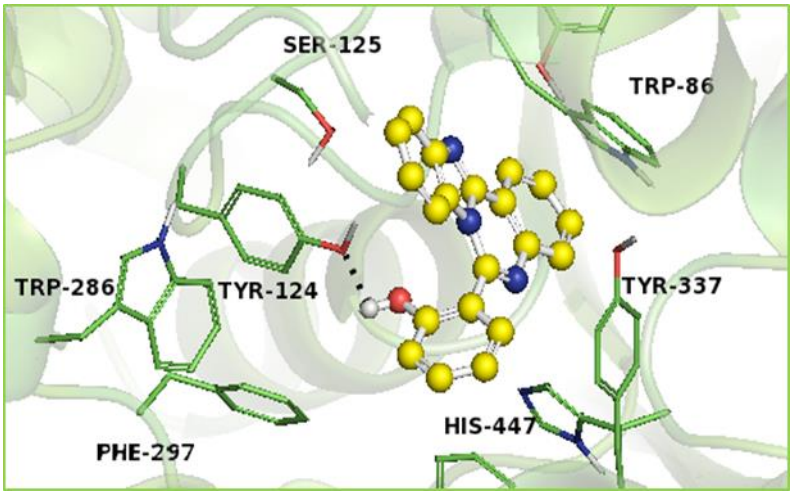


Figure 5: Binding of compound (2c) with AChE binding site (PDB: 4EY7). Hydrogen bond is represented as black dashed lines.

Through our journey to explore other potential acetylcholinesterase inhibitors, we decided to use benzimidazo [2,1-c] triazole instead of (benzimidazo [1,2-c] quinazoline) with a longer terminal side chain (either substituted phenylamine ethanone or benzylpiperazine ethanone) **(scheme 2).** Notably, the most compounds showed similar binding mode to the co-crystalized ligand where tricyclic ring system occupied PAS region and the side chains extended into the anionic binding site.

Compound (7) demonstrated outstanding binding interaction where tricyclic ring (benzimidazo [2,1-c] triazole) fitted deeply into the peripheral binding site and involved in π-π stacking interaction with Trp286 in an orientation that allowed piperazine nitrogen to accommodate near Hist447 of catalytic triad and its nitrogen to interact with the key Tyr124 amino acid via hydrogen bonding interaction. Moreover, the terminal phenyl moiety formed π-π stacking interaction with Trp86 in the anionic subsite **(Figure 6)**.

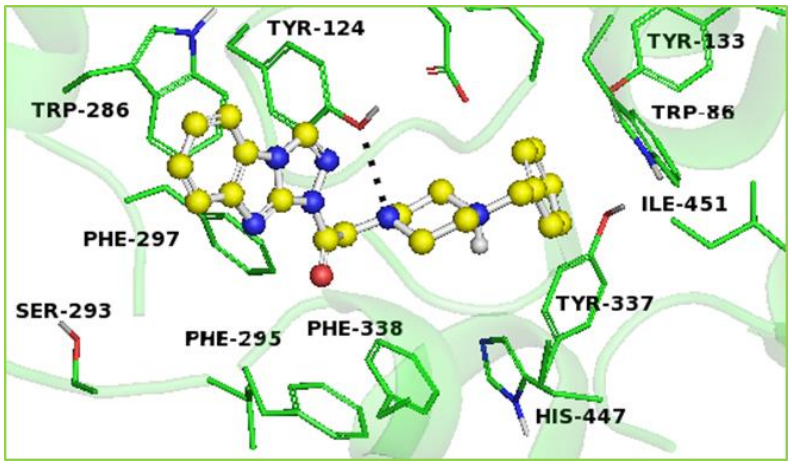


Figure 6: Binding of compound (7) with AChE binding site (PDB: 4EY7). Hydrogen bond is represented as black dashed lines.

On the other hand, docking of **compound (8a)** showed similar binding mode where tricyclic ring accommodated into PAS and involved in π-π stacking interaction with Tyr341, carbonyl moiety formed a significant hydrogen bonding interaction with Tyr124 residue. Furthermore phenyl fitted into anionic site and involved in π-π stacking interaction with Trp86 while chloride substituent formed an additional interaction with amino acid Glu202 **(Figure 7)**.

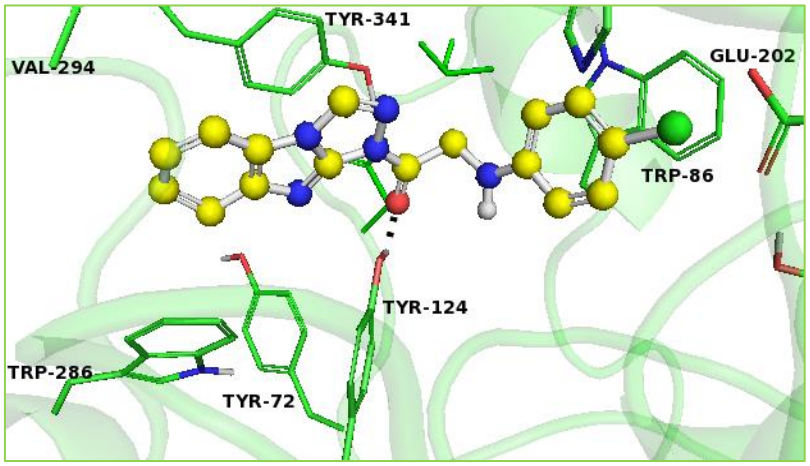
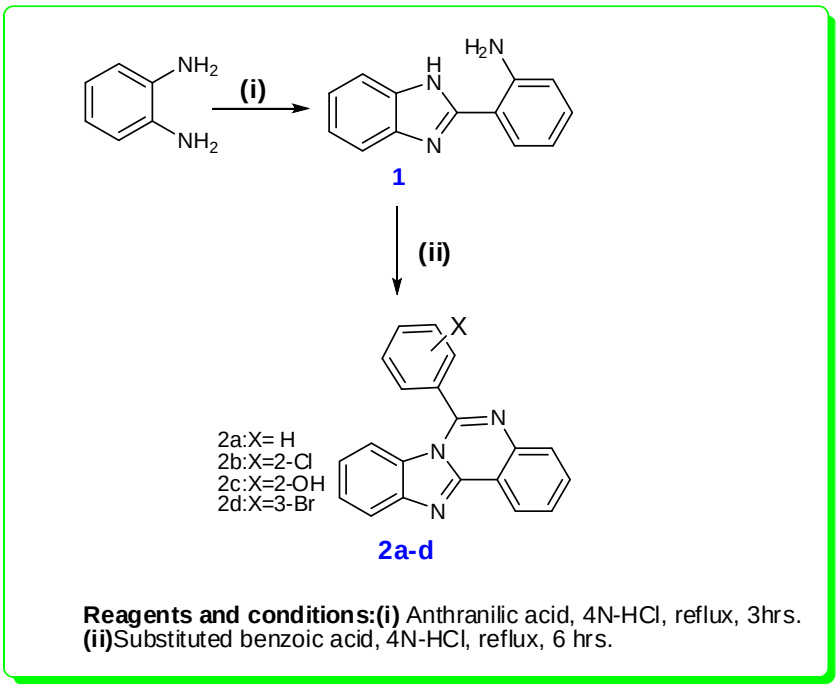


Figure 7: Binding of compound (8a) with AChE binding site (PDB: 4EY7). Hydrogen bond is represented as black dashed lines.



Scheme 1. Synthesis of target compounds **(2a-d).**

3. Materials and Methods

3.1. Instruments

Melting points were uncorrected and measured in Stuart melting point apparatus SMP10 (UK). Infrared (IR) were measured using KBr discs on a Shimadzu Spectrophotometer (λ_{max} in cm^{-1}). (^{1}H-NMR) was performed using the residual solvent signal (internal standard) with a Varian AS 400. Chemical shifts are detected in δ values (ppm) relative to tetramethylsilane (TMS). Abbreviations used in ^{1}H-NMR analysis are as follows: d=doublet, m=multiplet, q=quartet, s=singlet, t=triplet. Electron impact mass spectra (EI-MS) were recorded on DI Analysis Shimadzu QP-2010 Plus mass spectrometer. Elemental analysis was performed in the Micro-analytical center, Cairo University, Egypt. All Reactions were monitored by thin layer chromatography (TLC) on Merck silica gel 60_{F254}.

3.2. Experimental

3.2.1. Chemistry

Synthesis of compounds **1-8** was shown in schemes 1 and **2**. Intermediates **1,3,4,5** and **6** are prepared according to reported procedures. (Aboelmagd, Ali et al. 2013), (Elgawish, Nafie et al. 2022), (Monge, Parrado et al. 1987), (Ozkay, Tunalı et al. 2011), (Reddy and Reddy 2010).

General procedure for synthesis of (2a-d)

An equimolar mixture of 2-(1H-benzo[d]imidazol-2-yl) aniline **(1)** (0.009 mol) and substituted benzoic acid (0.009 mol) in 4N-HCl (50 ml) were refluxed for 6 hrs. The reaction mixture then cooled, poured into ice water followed by neutralization with NH_3 solution to pH 9.00. The separated solid then filtered, washed with cold water and was recrystallized from ethanol. (Deng, Dong et al. 2012)

6-phenyl-benzimidazo[1,2-*c*] quinazoline (2a)

Off white powder (yield 20%), M.p. = 190-193˚C. IR (cm^{-1}): 1539(C=N), 1581(C=N).
Mass spectrum: m/z (%): 295 (M^+, 9.06%), 271 (7.85%), 256 (8.04%), 228 (11.35%), 204 (25.41%), 171 (19.18%), 145 (63.60%), 130 (63.75%), 91 (41.73%), 77 (base peak, 100%), 49(83.88%).^{1}H NMR (400 MHz, DMSO) δ 8.71-7.17 (m, 13H, Ar-H). ^{13}C NMR (100 MHz, DMSO) δ 165.78, 160.68, 158.67, 157.64, 153.44, 136.85, 134.43, 132.98, 130.21 (2C), 129.72, 129.49 (2C), 125.26 (2C), 122.64, 122.10, 119.89, 117.59, 112.53. Anal.Calcd. for $C_{20}H_{13}N_3$: C, 81.34; H, 4.44; N, 14.23. Found: C, 81.56; H, 4.74; N, 14.51.

6-(2-Chlorophenyl)-benzimidazo[1,2-*c*]quinazoline (2b)

Grey powder (yield 70%), M.p. = 185-187˚C. IR (cm^{-1}): 752 (C-Cl), 1554 (C=N), 1616 (C=N). Mass spectrum: m/z (%): 331 (M^++2, 6.22%), 329 (M^+, 15.03%), 295 (10.29%), 257 (18.34%), 235 (15.64%), 220 (30.16%), 205 (18.34%), 192 (22.28%), 167 (17.56%), 128 (14.04%), 107 (22.16%), 92 (94.17%), 77 (base peak, 100%). ^{1}H NMR (400 MHz, DMSO) δ 8.72-7.17 (m, 12H, Ar-H). ^{13}C NMR (100 MHz, DMSO) δ 172.02, 159.54, 158.15, 154.25, 154.05, 140.31, 135.48, 134.71, 131.01, 130.26, 130.12 (2C), 129.56, 129.25, 128.86, 126.04, 123.67, 119.93, 116.86, 113.72. Anal.Calcd. for $C_{20}H_{12}ClN_3$: C, 72.84; H, 3.67; N, 12.74. Found: C, 72.62; H, 3.92; N, 12.68.

2-Benzimidazo[1,2-c]quinazolin-6-yl-phenol (2c)

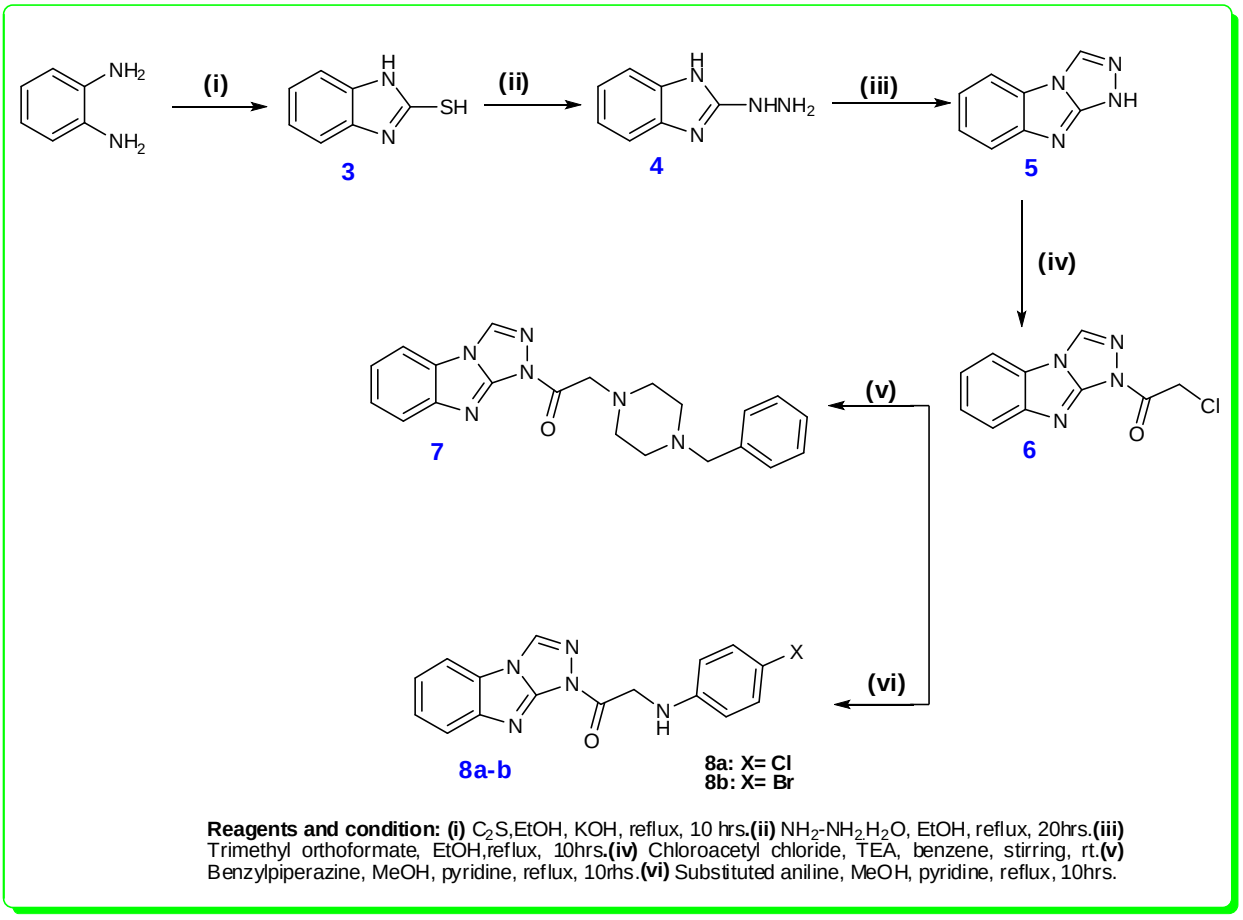
White powder (yield 50%), M.p. = 140-144˚C. IR (cm^{-1}): 1585 (C=N), 1616 (C=N), 3475 (OH). Mass spectrum: m/z(%): 311 (M^+, 4.27%), 256 (6.34%), 231 (2.25%), 193 (6.29%), 167 (5.52%), 155 (10.40%), 137 (63.66%), 119 (base peak, 100%), 92 (86.37%), 45 (92.96%). ^{1}H NMR (400 MHz, DMSO) δ 8.90 (s, 1H, OH), 8.25- 7.05 (m, 12H, Ar-H). ^{13}C NMR (100 MHz, DMSO) δ 165.74, 161.11, 157.62, 153.42, 149.97, 136.80, 134.39, 132.94, 130.18 (2C), 129.66, 129.46, 125.25, 122.62, 122.07, 119.85, 117.57, 117.07, 115.86, 112.49. Anal.Calcd. for $C_{20}H_{13}N_3O$: C, 77.16; H, 4.21; N, 13.50. Found: C, 77.46; H, 4.45; N, 13.81.

6-(3-Bromophenyl)-benzimidazo[1,2-c]quinazoline (2d)

Grey powder (yield 68%), M.p. = 160-162˚C. IR (cm^{-1}): 663 (C-Br), 1562 (C=N), 1612 (C=N). Mass spectrum: m/z(%): 375(M^++2, 2.69%), 373 (M^+, 3.55%), 294 (1.91%), 259 (22.48%), 241 (34.85%), 223 (9.67%), 206 (5.83%), 178 (11.14%), 153 (13.28%), 131 (base peak, 100%), 104 (33.87%), 93 (45.48%), 77 (47.43%).^{1}H NMR (400 MHz, DMSO) δ 8.04 (s, 1H, Ar-H), 7.92- 6.46 (m, 11H, Ar-H). ^{13}C NMR (100 MHz, DMSO) δ 170.14, 167.40, 151.77, 150.29, 137.27, 134.54, 133.76, 132.14, 131.60, 131.47, 130.70 (2C), 129.80, 128.44, 122.01, 121.77, 116.96, 116.62, 114.87, 110.86. Anal.Calcd. for $C_{20}H_{12}BrN_3$: C, 64.19; H, 3.23; N, 11.23. Found: C, 64.40; H, 3.39; N, 11.52.

1-Subsitituted-1H-[1,2,4]triazolo[4,3-a] benzimidazole (7 and 8a-b)

Pyridine (0.008 mol) and methanolic solution of appropriate piperazine or substituted aniline (0.008 mol) were added to a solution of 1-(2-chloroacetyl)-1H- [1,2,4] triazolo [4,3-a] benzimidazole **(6)** (0.008 mol) in methanol (HPLC) (100 mL). The mixture was refluxed for 10 hrs. Then the extra solvent was eliminated by distillation under reduced pressure by using a rotatory vacuum evaporator. The residue obtained by cooling was filtered and washed with cold distilled water. The product then recrystallized from ethanol. (Rajak, Kharya et al. 2008)



Scheme 2. Synthesis of target compounds **(7)** and **(8a-b)**

1-[2-[4-(Benzyl)-1-piperazinyl] acetyl]-1H- [1,2,4] triazolo [4,3-a] benzimidazole (7)

Reddish brown powder (yield 30%), M.p. = 125-128˚C. IR (cm^{-1}): 1543 (C=N), 1581 (C=N), 1643 (C=O). Mass spectrum: m/z (%): 374 (M^+, 10.21%), 298 (3.98%), 282 (3.06%), 269 (1.24%), 226 (30.37%), 199 (5.02%), 185 (8.39%), 157 (27.06%), 129 (13.37%), 98 (65.79%), 69 (base peak, 100%).^{1}HNMR (400 MHz, DMSO) δ 7.94-6.55 (m, 9H ,Ar-H), 6.02 (s, 1H, triazole), 4.29 (s, 2H, CH_2), 3.23 (s, 2H, CH_2), 3.19 (t, 4H, piperazine), 2.66 (t, 4H, piperazine). ^{13}C NMR (101 MHz, DMSO) δ 167.96, 151.40, 151.28, 145.78, 138.88, 129.46 (2C), 129.38 (2C), 119.80, 119.34, 116.30 (2C), 115.86 (2C), 60.99, 52.91 (2C), 48.71 (2C), 45.49. Anal.Calcd. for $C_{21}H_{22}N_6O$: C, 67.36; H, 5.92; N, 22.44. Found: C, 67.49; H, 5.99; N, 22.74.

1-[2-[(4-Chlorophenyl) amino] acetyl]-1H- [1,2,4] triazolo[4,3-a] benzimidazole (8a)

Grey powder (yield 50%), M.p. = 115-118˚C. IR (cm^{-1}): 756 (C-Cl), 1590 (C=N), 1621 (C=N), 1743 (C=O), 3471 (NH). Mass spectrum: m/z(%): 327 (M^++2, 1.11%), 325 (M^+, 3.62%), 291 (6.39%), 243 (15.91%), 199 (2%), 187 (26.27%), 164 (49.02%), 150 (base peak, 100%), 131 (62.09%), 90 (69.19%), 63 (71.68%). ^{1}H NMR (400 MHz, DMSO) δ 8.21-7.48 (m, 9H, Ar-H), 5.67 (s, 2H, CH_2). ^{13} C NMR (101 MHz, DMSO) δ 170.88, 151.43, 147.53, 142.85, 128.93(2C), 128.25, 125.56, 125.06, 120.17 (2C), 115.41, 114.26 (2C), 110.42, 47.93. Anal.Calcd. for $C_{16}H_{12}ClN_5O$: C, 58.99; H, 3.71; N, 21.50. Found: C, 58.72; H, 3.99; N, 21.81.

1-[2-[(4-Bromophenyl) amino] acetyl]-1H- [1,2,4] triazolo[4,3-a] benzimidazole (8b)

White powder (yield 62%). M.p. = 172-175˚C. IR (cm^{-1}): 613 (C-Br), 1500 (C=N), 1735 (C=O), 3363 (NH). Mass spectrum: m/z (%): 371 (M^++2, 0.77%), 369 (M^+, 0.89%), 287 (9.04%), 207 (6.62%), 187 (6.29%), 150 (36.69%), 118 (base peak, 100%), 91 (67.18%), 65 (60.68%). ^{1}H NMR (400 MHz, DMSO) δ 9.16(s, 1H, NH), 8.26- 7.45 (m, 9H, Ar-H), 4.59 (s, 2H, CH_2). ^{13}C NMR (101 MHz, DMSO) δ 166.45, 158.06, 148.80, 131.90, 126.74 (2C), 124.30, 122.31, 122.27 (2C), 122.16, 121.15 (2C), 121.01, 110.23, 61.71. Anal.Calcd. for $C_{16}H_{12}BrN_5O$: C, 51.91; H, 3.27; N, 18.92. Found: C, 51.64; H, 3.52; N, 18.68.

3.2.2 Molecular Modeling Experiment

To investigate how the compounds might attach to AChE, we carried out molecular docking investigations using the Molecular Operating Environment (MOE) software created by the Chemical Computing Group based in Montreal, Canada. Initially, structures of our synthesized compounds were sketched by Chem draw program and then protonated with help of MOE. The MMFF94x force field was utilized to execute energy minimization on the compounds. The crystal structure of acetylcholinesterase (PDB ID: 4ey7) was obtained from the protein data bank and the water molecules were removed. Following this, hydrogen atoms were added to the protein and the enzyme was subjected to energy minimization. The active site of enzyme was recognized according to the co-crystallized ligand receptor complex. The best pose was selected according to the docking score and visualized using the PyMOL Molecular Graphics System v2.3.

4. Conclusion

Acetylcholinesterase is a promising and potential target for treatment Alzheimer's disease. Our novel synthesized compounds that based on using ring-fused benzimidazoles either benzimidazo [1,2-c] quinazoline or benzimidazo [2,1-c] triazole as main scaffolds demonstrated a preferential binding affinity with acetylcholinesterase binding site which may predict a considerable biological activity against acetylcholinesterase as potent inhibitors. Thus, in the near future, biological evaluation will be carried out on the synthesized compounds against acetylcholinesterase enzyme.

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