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Research Article

Design, Synthesis, Characterization and Preliminary Evaluation of New 1H-benzo[d]imidazole-1yl-derivatives as Acetylcholine Esterase Inhibitors

Shuhad Yaseen[1]*, Shahlaa Zuhair Abdul-Majeed[2], Sarah Ashour Hamood[3]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Al-Rafidain University College, Baghdad 10052, Iraq;* [2]*College of Pharmacy, Al-Esraa University, Baghdad, Iraq;* [3]*Biomedical Engineering Department, Engineering College, Al-Esraa University, Baghdad, Iraq*

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Abstract

Background: Alzheimer disease (AD) is the most common type of dementia, which is still a problem that everyone must deal with. In a continuous effort to find effective treatments, the new candidates for AD therapy have the capacity to scavenge excessive levels of free radicals and inhibit acetylcholinesterase (AChE). ***Objectives***: This study focuses on synthesizing and biologically evaluating novel hybrid compounds (1-3) as acetylcholine esterase inhibitors. ***Methods***: The benzimidazole has been added and then coupled with coumaric acid, cinnamic acid, and lipoic acid as conjugates, which are expected to have dual action as acetylcholinesterase inhibitors and antioxidants. The synthesis of benzimidazole derivatives (1-3) was accomplished and then characterized using ^{1}H-NMR and elemental analysis. Additionally, their characteristics were assessed in vitro against the AChE enzyme. ***Results***: The new compounds produced a potent inhibitory activity that may serve as a lead molecule for the synthesis of novel anti-AD molecules. Compound-1 has an inhibition percentage that is close to that of the authorized medication galantamine (95.386%), whereas compound-3 has the lowest inhibition percentage (88.647%). ***Conclusions***: A very good yield was achieved during the synthesis of the benzimidazole derivatives (1-3) from the starting material. They can serve as potential candidates for acetylcholine esterase inhibitors.

Keywords: AChE inhibitors, Benzo[d]imidazole, Characterization, Design, Synthesis.

تصميم وتخليق وتوصيف وتقييم أولي لمشتقات *H1*-بنزو[د]إيميدازول-1يل الجديدة كمثبطات لإنزيم أستيل كولين أستريز

الخلاصة

الخلفية: النوع الأكثر شيوعًا من الخرف، والذي لا يزال يمثل مشكلة يجب على الجميع التعامل معها. يتمتع هؤلاء المرشحون الجدد لعلاج مرض الزهايمر (*AD*) بالقدرة على التخلص من المستويات المفرطة من الجذور الحرة وتثبيط إنزيم الأسيتيل كولينستراز (*AChE*). في جهد متواصل لإيجاد علاجات فعالة لمرض الزهايمر. **الأهداف**: تركز هذه الدراسة على تخليق وتقييم المركبات الهجينة الجديدة (1-3) كمثبطات للأستيل كولين استريز. **الطرق**: تمت إضافة البنزيميدازول ثم اقترانه بحمض الكوماريك وحمض السيناميك وحمض الليبويك كمقترنات والتي من المتوقع أن يكون لها تأثير مزدوج كمثبطات أستيل كولينستريز ومضادات الأكسدة. تم تصنيع مشتقات البنزيميدازول (1-3) ثم تم تشخيصها باستخدام *H-NMR1* والتحليل العنصري وبنتيجة جيدة جداً. بالإضافة إلى ذلك، تم تقييم خصائصها في المختبر مقابل إنزيم *AChE*. **النتائج**: أنتجت المركبات الجديدة نشاطا مثبطا قويا يمكن أن يكون بمثابة جزيء رصاص لتخليق المضاد الجديد (*AD*). المركب (1) لديه نسبة تثبيط قريبة جداً من الدواء المعتمد جالانتامين (95.386%)، بينما المركب (3) لديه أقل نسبة تثبيط (88.647%). **الاستنتاج**: تم تحقيق عائد جيد جداً أثناء تحضير مشتقات البنزيميدازول (1-3) من المادة الأولية. يمكن أن تكون بمثابة مرشح محتمل كمثبطات أستيل كولين.

* ***Corresponding author***: Shuhad Yaseen, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Al-Rafidain University College, Baghdad 10052, Iraq; Email: s.alsaji@yahoo.com

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INTRODUCTION

The enzymes acetylcholinesterase (AchE) and butyrylcholinesterase (BchE) facilitate the breakdown of acetylcholine into choline and acetic acid [1]. Consequently, acetylcholine shortages occur in brain regions such as the cortex and hippocampus, which are associated with highly developed psychological functions [2]. Alzheimer's disease (AD), a gradual and irreversible brain condition that disturbs the cholinergic system of the brain, is brought on by these enzymes. Memory loss, confusion, cognitive impairment, and difficulty thinking and solving problems are all potential effects of this disorder [3–5]. As we age, AD is the primary contributor to dementia. These enzymes influence the accumulation of neurotoxic beta-amyloid, leading to the apoptosis of neuronal cells. Targeting both acetylcholinesterase and butyrylcholinesterase is one strategy for treating Alzheimer's disease [6,7]. Acetylcholine has to be broken down at the catalytic site and the peripheral site on AchE in order for it to interact with beta-amyloid. The complex of AchE, which arises from its interactions with proteins, is responsible for producing neurotoxicity. The liver, kidneys, lungs, intestine, heart, and serum contain BchE, whereas the muscles, cholinergic neurons, and brain contain AchE. [8, 9]. When acetylcholine activity gradually declines in an Alzheimer's patient's brain, BchE function increases because AchE normally dominates in the brain. Consequently, there is a critical need for a medication that can stop the catalytic activity of both AchE and BchE [10,11]. The FDA has approved a number of medications in order to treat Alzheimer's illness, including galantamine, rivastigmine, and donepezil [12]. However, hepatotoxicity, inadequate activity, and gastrointestinal upset constrain the use and applicability of these medications [13, 14]. Researchers have shown significant interest in isolating these molecules to counteract the negative side effects of synthetic choline esterase inhibitors (ChEIs), and they have discovered several non-toxic bioactive (ChEIs) inhibitors from natural sources. [15,16]. While donepezil and galantamine are only active against AchE, tacrine and rivastigmine inhibit both AchE and BchE [17]. The benzimidazole nucleus has a wide range of functions, from antibacterial actions to application against the most life-threatening diseases in the world. Due to its affinity for many enzymes and protein receptors, it has become more significant in medicinal chemistry [18]. This work aims to design and synthesize a novel 1H-benzo[d]imidazole-1yl-deivative by coupling reaction with the antioxidants (*p*-coumaric acid, cinnamic acid and lipoic acid). Meanwhile, we report the enzymatic evaluation of the AChE inhibition for the new derivative.

METHODS

Chemicals and reagents

All chemicals and solvents were purchased from commercially available sources and were of analytical grade. p-coumaric acid, cinnamic acid, alpha-lipoic acid, THF anhydrous, triethylamine, thionyl chloride, benzimidazole, ethyl acetate, anhydrous $MgSO_4$, dichloromethane, methanol, n-hexane, and DTNP were purchased from Sigma-Aldrich (UK).

Instruments

1H-NMR bands (solvent DMSO-d6) were documented on a 500 MHZ spectrometer (Bruker, Germany) with TMS as an internal standard (Bruker, Germany). C.H.N. analyzers, Vario macro cube—the art of elemental analysis, Germany. Column chromatography consists of a solid stationary phase (silica) and a liquid mobile phase. Any mixture to be separated should be dissolved in the mobile phase before being added to the stationary phase from the top of the column. Each constituent is collected across the column at different rates, then collected as small fractions to be detected using TLC (Coskun, 2016). In this work, we used silica gel (high-purity grade, pore size 60 Å, 70–230 mesh, 63–200 µm) purchased from Sigma. 1H-NMR spectra were performed on the instrument Inova-Varian 500 MHz spectrometer frequency using DMSO as solvent at Tehran University. CHN analysis was performed at Tehran University. The examination was done via a variable macrocube, the art of elemental analysis.

Synthesis of compound 1: 1-(1H-benzo[d]imidazol-1-yl)-3-(4-hydroxyphenyl) prop-2-en-1-one

To a solution of p-coumaric acid (0.7 g, 0.00146 mol) in anhydrous THF, triethylamine (0.6 g, 0.00437 mol) and thionyl chloride (0.42 g, 0.0058 mol) were added, respectively. The solution was mixed for 6 minutes; after that, a solution of benzimidazole (0.17 g, 0.00146 mol) in anhydrous THF was mixed, and the reaction was kept overnight at 25 ºC. The reaction was quenched with 10 ml of deionized water and then the reaction mixture was extracted with ethyl acetate and dried over anhydrous $MgSO_4$. The organic layer was combined and evaporated under reduced pressure. The resulting crude product was purified using column chromatography in the mobile phase (dichloromethane 9: methanol 1). The compound was obtained as white crystals (264 mg, 60% yield) [19] (Figure 1).

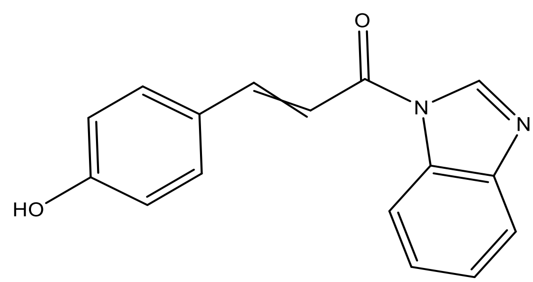

Figure 1: Compound 1

^{1}H-NMR (500 MHz, $CDCl_3$) 6.65 (d, 1H, trans-CH=CH), 6.70-7.06 (m, 3H, Ar.), 7.18 (s, 1H, Ar.), 7.22-7.37 (m, 4H, Ar.), 7.54 (d, 1H, trans-CH=CH), 8.10 (s, 1H, CH-N), 9.00 (S, 1H, OH). Anal. Calcd. for $C_{16}H_{12}N_2O_2$ (264.28): C, 72.72; H 4.58; N, 10.60; Found: C, 72.70; H, 4.59; N, 10.61. M.p.: 134ºC.

Synthesis of compound 2: 1-(1H-benzo[d]imidazol-1-yl)-3-phenylprop-2-en-1-one

Anhydrous THF was used to dissolve (0.2 g, 0.00146 mol) of cinnamic acid, and then (0.1 g, 0.00146 mol) of thionyl chloride and (0.6 g, 0.00438 mol) of tri-ethyl amine were added. In THF, anhydrous (0.17 g, 0.00146 mol.) benzimidazole was dissolved and then added to the reaction mixture after 5 minutes, and the mixture was quenched with deionized water after two hours. The substance was extracted with ethyl acetate, crystallized with methanol, and then collected by filtration to obtain 139 mg (56%) of the original amount as pure powder [19] (Figure 2).

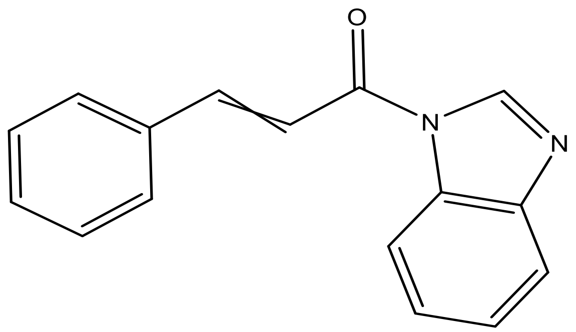

Figure 2: Compound 2

[1]H-NMR (500 MHz, $CDCl_3$) δ =6.65 (s, 2H, Ar.), 7.04 (d, 1H, Ar.), 7.18-7.63 (m, 7H, CH=CH and Ar.), 7.71 (d, 1H, Ar.), 8.12 (s, 1H, CH-N). Anal. Calcd. for $C_{16}H_{12}N_2O$ (248.29): C, 77.40; H, 4.87; N, 11.28; Found: C, 77.41; H, 4.87; N, 11.27. M.p.: 144°C.

Synthesis of compound 3: 1-(1H-benzo[d]imidazol-1-yl)-5-(1,2-dithiolan-3-yl) pentan-1-one

We addt 0.4 g (0.00231 moles) of alpha-lipoic acid in 10 ml of THF anhydrous and mix it. Then add 0.96 g (0.0069 moles) of triethylamine and 0.19 g (0.00276 moles) of thionyl chloride. After dissolving 0.27 g (0.00231 moles) of benzimidazole in THF for 5 minutes at room temperature and monitoring the reaction with TLC, 10 ml of deionized water quenched the reaction. Around two hours. We purified the product using the column chromatography process. Ethyl acetate: n-hexane served as the mobile phase (6:4) (0.63 g, 67% yield) of white solid powder [19] (Figure 3).

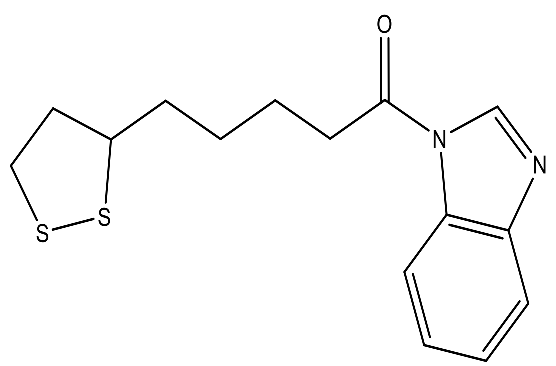

Figure 3: Compound 3

[1]H-NMR (500 MHz, CDCl3): δ = 1.11-2.17 (m, 11H, aliphatic CH_2 and dithiolane), 2.51 (s, 3H, CH_3), 2.69 (m, 2H, CH_2C=O),6.54-6.97 (m, 2H, Ar.), 7.04-7.25 (m, 2H, Ar.), 8.09 (s, 1H, CH-N). Anal. Calcd. for $C_{15}H_{18}N_2OS_2$ (306.44): C, 58.79; H, 5.92; N, 9.14; O, 5.22; S, 20.92; Found: C, 58.77; H, 5.93; N, 9.14. O, 5.24; S, 20.91.

Ellman's assay

The AChE activity of our synthesized compounds (1-3) was tested in vitro utilizing the well-known spectrophotometric method called Ellman's method, as shown in Scheme 1 [20]. In this procedure, we substitute acetylcholine with acetylthiocholine iodide, then react the thiocholine product with 5,5-dithiobis (2-nitrobenzoic acid) (DTNB, Ellman's reagent) to produce a yellow-colored anion, 5-thio-2-nitrobenzoic acid (TNB), which we detected using a spectrometer at 412 nm. [21]. Each test tube was filled with 1.7 mL of 50 mM Tris-HCl buffer solution and 20 µL of 10 mM DTNB to create a set of test tubes. Then, 10 µL of 6.67 UmL-1 AChE and 250 mL of drug sample at various concentrations (ranging from 25 to 400 µg/mL) were added. As a positive control, galantamine was made at the same serial concentration as the drug sample. All were incubated for fifteen minutes. at 37 °C. All samples were then given 10µ L of 10 mM acetylthiocholine iodide. Blank solutions were prepared using buffer rather than enzyme. For three minutes, the absorbance was measured at 412 nm every three seconds. The rate of change in absorbance over time (V = Abs/Δt) was used to quantify the percentage of acetylcholinesterase enzyme inhibition. The formula for calculating inhibition (%) is 100 - the difference between the sample absorbance and the blank absorbance, multiplied by 100 [22,23].

Statistical analysis

The Minitab® Version 18 statistical pack was used for the statistical analysis of the one-way ANOVA, two-way ANOVA, and Tukey *post hoc* test. The significance value was set at $p<0.05$.

RESULTS

The result of chemical synthesis for compounds 1–3 is shown in Figure 4.

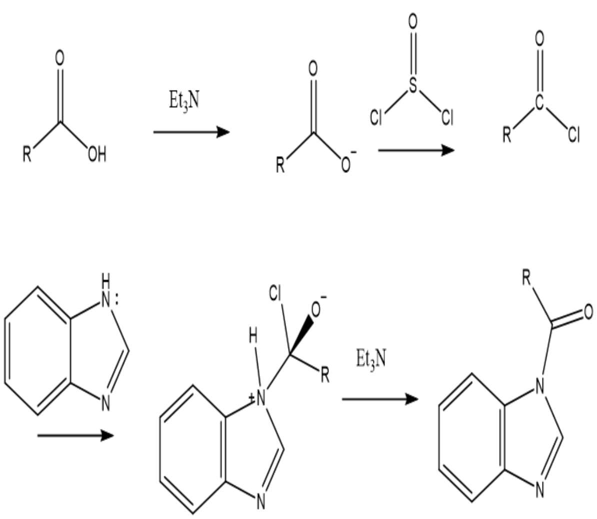

Figure 4: Synthesis of designed compounds 1-3.

The percentage of inhibition for the derivatives 1-3 of 1H-benzo[d]imidazol-1-yl against AChE enzyme results is shown in Table 1. A Tukey *post hoc* test has been used to indicate the statistical analysis results in Table 1.

Table 1: Inhibition effect of compounds 1-3 for AChE enzyme activity.

Conc. (µg/ml)	Comp.1 Inhibition (%)	Comp. 2 Inhibition (%)	Comp. 3 Inhibition (%)	Galantamine Inhibition (%)
400	95.386±1.25[a]	91.373±0.31[a]	88.647±0.57[a]	97.863±0.180[a]
200	87.777±1.70[b]	84.442±0.82[b]	77.298±0.66*[b]	95.062±0.092[a]
100	79.819±1.67[c]	74.132±1.44[c]	59.731±0.89*[c]	89.989±0.010[b]
50	76.719±1.61[c]	70.650±0.95[c]	43.456±0.85*[d]	79.512±0.102[c]
25	52.611±0.91*[d]	44.884±0.97*[d]	38.123±0.70*[d]	62.442±0.062[d]

Values were expressed as mean±SE.* significantly different compared to galantamine at the same concentration (p<0.05); values with different superscripts (a,b,c,d) among different concentrations within the same compound are significantly different (p<0.05). ANOVA: p=0.0001 between different compounds and between their concentrations.

The half-maximal inhibitory concentration (IC50), as demonstrated in Table 2, shows the difference between compounds using a one-way ANOVA (p= 0.000) with galantamine. A Tukey posttest comparison has been used to indicate the statistical analysis results in Table 2.

Table 2: IC_{50} for the derivatives 1-3 and positive control

Compounds	IC_{50} of AChE inhibition (µg/ml)
1	13.008±0.43[a]
2	22.367±1.89[b]
3	53.623±2.25[c]
Galantamine	9.169±0.48[d]

Values were expressed as mean±SE. ANOVA: p=0.0001. Values with different superscripts (a,b,c,d) among different compounds are significantly different significant difference between compounds (Tukey *post hoc* test).

DISCUSSION

The synthesis of compounds 1-3 was achieved successfully. The novel compounds were achieved after optimization of the reaction conditions, where the yield of the reaction was enhanced by increasing the number of equivalents of thionyl chloride ($SOCl_2$) from 1.5 to 4. The target compounds were characterized using ^{1}H-NMR and elemental microanalysis (CHNS). We also experienced that the outcome of the reaction is strongly dependent on the order of reagent addition. In fact, if benzimidazole is preliminarily added to thionyl chloride and Et_3N is added subsequently, the reaction yield is lowered and after 5 minutes, the reaction is not yet complete. The percentage of inhibition for the derivatives 1-3 of 1H-benzo[d]imidazol-1-yl against the AChE enzyme was conducted utilizing Ellman's approach to assess their performance; results are shown in Table 1. Compound 1 had very potent inhibition (95.386±1.25) and comparable action to galantamine (97.863±0.180) at a 400 µg/ml concentration. Additionally, compounds 2 and 3 showed promise as strong AChE inhibitors (91.373±0.31) and (88.647±0.57), respectively. statistical analysis revealed that there is a significant difference between products (p=0.000) and their concentration (p=0.000). Increasing the concentration resulted in an increase in inhibition, while galantamine and compound 1 showed superior effects over the other two compounds. The Tukey posttest showed that there is no statistically significant difference between galantamine and compound 1 at all concentrations and that at high concentrations (400 µg/ml), there is no statistically significant difference between the four compounds. The half-maximal inhibitory concentration (IC50), as demonstrated in Table 2, for compounds 1-3 revealed that compound 1 had the highest AChE inhibitor activity, with an IC_{50} value of 13.008±0.43 when compared to the control galantamine. Overall, there is a significant difference between compounds (one-way ANOVA, p=0.000) with galantamine and compound 1 showing superior inhibition at lower concentrations. The Tukey posttest comparison revealed that the difference is significant among all, despite the slight variation between galantamine and compound 3.

Conclusion

A very good yield was achieved during the synthesis of the benzimidazole derivatives (1-3) from the starting material. ^{1}H-NMR and elemental analysis were used to characterize these substances. The synthesized compounds also showed strong inhibitory activity against the AChE enzyme in vitro, which suggests that they could be used as lead compounds for new AD medications. Compound (1) has an inhibition percentage that is very close to that of the standard drug galantamine (95.386%), while compound (3) has the lowest inhibition percentage (88.647%).

Conflict of interests

No conflict of interests was declared by the authors.

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The authors did not receive any source of fund.

Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

REFERENCES

1. Türkan F. Investigation of the toxicological and inhibitory effects of some benzimidazole agents on acetylcholinesterase and butyrylcholinesterase enzymes. *Arch Physiol Biochem*. 2021;127(2):97-101. doi: 10.1080/13813455.2019.1618341.
2. Maness EB, Burk JA, McKenna JT, Schiffino FL, Strecker RE, McCoy JG. Role of the locus coeruleus and basal forebrain in arousal and attention. *Brain Res Bull*. 2022;188:47-58. doi: 10.1016/j.brainresbull.2022.07.014.
3. Chen ZR, Huang JB, Yang SL, Hong FF. Role of cholinergic signaling in Alzheimer's disease. *Molecules*. 2022;27(6):1816. doi: 10.3390/molecules27061816.
4. Bosia M, Cook F, Bigai G, Martini F, Fregna L, Leave C, et al, (Eds.), Organic mental disorders and psychiatric issues in the elderly. In: Fundamentals of Psychiatry for Health Care Professionals, Springer; 2022, p. 297-331. doi: 10.1111/jar.13217.
5. Melgarejo da Rosa M, Cassimiro de Amorim L, Victor de Oliveira Alves J, Fidélis da Silva Aguiar I, Granja da Silva

Oliveira F, Vanusa da Silva M. The promising role of natural products in Alzheimer's disease. *Brain Disord*. 2022;7:100049. doi: 10.1016/j.dscb.2022.100049.

6. Sharifi-Rad J, Rapposelli S, Sestito S, Herrera-Bravo J, Arancibia-Diaz A, Salazar LA, et al. Multi-target mechanisms of phytochemicals in Alzheimer's disease: Effects on oxidative stress, neuroinflammation and protein aggregation. *J Personalized Med*. 2022;12(9):1515. doi: 10.3390/jpm12091515.
7. Rekha K, Neerja V, Shailja S, Taha A, Mohannad A, Alexander N, et al. Ameliorative effects of phytomedicines on Alzheimer's patients. *Curr Alzheimer Res*. 2022;19(6):420-439. doi: 10.2174/1567205019666220610155608.
8. Anurag Tk, Amit K, Rajnish K, Taher D. Osteric binding sites of Aβ peptides on the acetylcholine synthesizing enzyme ChAT as deduced by in silico molecular modeling. *Int J Mol Sci*. 2022;23(11):6073. doi: 10.3390/ijms23116073.
9. Garrett D, Naoya T, Kazuya T, Shun-ichi I, Sotiris S, Masaaki F. Structure of gas phase monohydrated nicotine: Implications for nicotine's native structure in the acetylcholine binding protein. *J Am Chem Soc*. 2022;144(37):16698-16702. doi: 10.1021/jacs.2c04064.
10. Javad S, Simona R, Simona S, Jesús H, Alejandra A, Luis A, et al. Advancements in the development of multi-target directed ligands for the treatment of Alzheimer's disease. *Bioorg Med Chem*. 2022:116742. doi: 10.1016/j.bmc.2022.116742.
11. Shohag S, Akhter S, Islam S, Sarker T, Sifat MK, Rahman MM, et al. Perspectives on the molecular mediators of oxidative stress and antioxidant strategies in the context of neuroprotection and neurolongevity: An extensive review. *Oxid Med Cell Longev*. 2022;2022:7743705. doi: 10.1155/2022/7743705.
12. Tarana U, Rampratap M, Muste H, Pawan K, Asim A. Recent updates in development of small molecules as potential clinical candidates for Alzheimer's disease: A review. *Chem Biol Drug Design*. 2022;100(5):674-681. doi: 10.1111/cbdd.14133.
13. Srinivas R, Salwa S, Navya A, Shirleen M, Lalit K. Recent advances in nanoformulation development of Ritonavir, a key protease inhibitor used in the treatment of HIV-AIDS. *Expert Opin Drug Deliv*. 2022;19(9):1133-1148. doi: 10.1080/17425247.2022.2121817.
14. Xing N, Meng X, Wang S. Isobavachalcone: A comprehensive review of its plant sources, pharmacokinetics, toxicity, pharmacological activities and related molecular mechanisms. *Phytother Res*. 2022;36(8):3120-3142. doi: 10.1002/ptr.7520.
15. Villablanca E, Selin K, Hedin C. Mechanisms of mucosal healing: treating inflammatory bowel disease without immunosuppression. *Nature Rev Gastroenterol Hepatol*. 2022:1-15. doi: 10.1038/s41575-022-00604-y.
16. Bischoff S. European guideline on obesity care in patients with gastrointestinal and liver diseases Joint ESPEN/UEG guideline. *Clin Nutr*. 2022;41(10):2364-2405. doi: 10.1016/j.clnu.2022.07.003.
17. Nutho B, Yanarojana S, Supavilai P, Structural dynamics and susceptibility of anti-Alzheimer's drugs donepezil and galantamine against human acetylcholinesterase.
18. Kumar A, Nimsarkar P, Singh S, Systems pharmacology aiding benzimidazole scaffold as potential lead compounds against leishmaniasis for functional therapeutics. *Life Sci*. 2022:120960. doi: 10.1016/j.lfs.2022.120960.
19. Leggio A, Belsito EL, De Luca G, Di Gioia ML, Leotta V, Romio E, et al. One-pot synthesis of amides from carboxylic acids activated using thionyl chloride. *Rsc Advances*. 2016;6(41):34468-34475.
20. Ömer Ş, Ümmühan Ö, Nurgül S, Şevki A, Zeynel S, Synthesis, characterization, molecular docking and in vitro screening of new metal complexes with coumarin Schiff base as anticholine esterase and antipancreatic cholesterol esterase agents. *J Biomol Struct Dyn*. 2022;40(10):4460-4474. doi:10.1080/07391102.2020.1858163.
21. Ali-Shtayeh MS, Jamous RM, Zaitoun SY, Qasem IB, In-vitro screening of acetylcholinesterase inhibitory activity of extracts from Palestinian indigenous flora in relation to the treatment of Alzheimer's disease. *Funct Food Health Dis*. 2014;4(9):381-400. doi: 10.31989/ffhd. v4i9.149.
22. Tan L, Guo S, Ma F, Chang C, Gómez-Betancur I, In vitro inhibition of acetylcholinesterase, alphaglucosidase, and xanthine oxidase by bacteria extracts from coral reef in Hainan, South China Sea. *J Marine Sci Engineer*. 2018;6(2):33. doi: 10.3390/jmse6020033.
23. Ferreira J, Santos S, Pereira H, In vitro screening for acetylcholinesterase inhibition and antioxidant activity of Quercus suber cork and corkback extracts. *Evidence-Based Complement Altern Med*. 2020; 2020:3825629. doi: 10.1155/2020/3825629.