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Research Article

Resorcinol derivatives as human acetylcholinesterase inhibitor: An *In Vitro* and *In Silico* study

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ABSTRACT

Inhibitors of Acetylcholinesterase (Acetylcholine acetylhydrolase, AChE, E.C.3.1.1.7) are highly significant in the therapy of a chronic illness such as Alzheimer's disease (AD) due to the deep relationship with memory and acetylcholine. So investigation of natural AChE inhibitors having minimal side effects has become important. In this paper human erythrocytes AChE enzyme (0.032 EU mg^{-1} protein) was partially isolated by using DE-52 anion exchange chromatography. Then, primer effects of resorcinol derivatives on the enzyme activity were studied and IC_{50} values were found in the range of 2.74-363.61 µM. Besides, inhibition profiles were elucidated by molecular docking and the highest inhibition potency was observed in 4-hexylresorcinol with the free binding energy of -6.16 kcal mol^{-1}. In conclusion, it was found that 4-hexylresorcinol had the highest inhibitory potential on human AChE. So, this compound may be used in drug design in memory-lost diseases.

Keywords: Acetylcholinesterase, resorcinol, inhibition, molecular docking.

İnsan asetilkolinesteraz inhibitörü olarak resorsinol türevleri: Bir *In Vitro* ve *In Silico* çalışma

ÖZ

Asetilkolinesteraz inhibitörleri (Asetilkolin asetilhidrolaz, AChE, E.C.3.1.1.7), hafıza ve asetilkolin ile derin ilişkisi nedeniyle Alzheimer hastalığı (AD) gibi kronik bir hastalığın tedavisinde oldukça önemlidir. Bu nedenle minimal yan etkiye sahip doğal AChE inhibitörlerinin araştırılması önem kazanmıştır. Bu çalışmada insan eritrosit AChE enzimi (0.032 EU mg^{-1} protein) DE-52 anyon değiştirme kromatografisi kullanılarak kısmen izole edilmiştir. Daha sonra resorsinol türevlerinin enzim aktivitesi üzerindeki primer etkileri incelenmiş ve IC_{50} değerleri 2.74-363.61 µM aralığında bulunmuştur. Ayrıca inhibisyon profilleri moleküler docking ile aydınlatılmış ve en yüksek inhibisyon gücü -6.16 kcal mol^{-1} serbest bağlanma enerjisi ile 4-heksilresorsinolde gözlenmiştir. Sonuç olarak, 4-heksilresorsinolün insan AChE üzerinde en yüksek inhibitör potansiyele sahip olduğu bulunmuştur. Bu nedenle, bu bileşiğin hafıza kaybı olan hastalıklarda ilaç tasarımında rehberlik edebileceği düşünülmektedir.

Anahtar Kelimeler: Asetilkolinesteraz, resorsinol, inhibisyon, moleküler docking.

1. INTRODUCTION

Alzheimer's disease (AD), the cause of about 60-70% of dementia cases, is a chronic neurodegenerative disease, and its incidence accelerates exponentially with age. AD, which initially shows symptoms of short-term memory loss and mental dysfunction, as it progresses it leads to mood swings, loss of motivation, inability to manage personal care and behaviour, physical dysfunction and eventually death. Approximately 24 million people suffered from AD in 2018, and it is estimated that this number will quadruple in 2050.[1-3] Although the pathogenesis of AD has not been fully clarified, there are some hypothesis on pathophysiology of AD such as cholinergic hypothesis, β-amyloid (AP) peptide theory, irregularity on energy metabolism, and oxidative stress.[4-7] One of the most common studied of these is cholinergic hypothesis and this hypothesis is generally based on the treatment of AD. The cholinergic system plays a role in the transport of impulses between central

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and peripheral nervous system cells, and the decline of cholinergic neurons and loss of neurotransmission have been reported to be the main causes of decreased cognitive function in patients with AD. The cholinergic hypothesis explains the main cause of AD with a decrease in the synthesis of acetyl choline (ACh).[8] ACh, an important neurotransmitter involved in signal transduction, is synthesized in the cholinergic neurons of the brain. It has been reported that ACh decreases in some neurodegenerative diseases.[9-12]

In a healthy brain, in the reaction catalysed by Acetylcholinesterase (AChE, E.C. 3.1.17) approximately 80% of ACh hydrolyses to acetate ions and choline.[11, 13] This enzyme is mainly localized in the synaptic cavities of the central and peripheral nervous system and red cell membranes. It plays a key role in nerve conduction as it terminates the stimuli in cholinergic pathway with ACh hydrolysis.[11, 14-15] It has been declared that inhibition of AChE can significantly reduce behavioural disorders of patients with AD. Therefore, the cholinergic hypothesis based on inhibiting the activity of acetylcholinesterase (AChE) is used in development of therapeutic strategies[16] and this leaded to the discovery of the first 4 drugs (tacrine, donepezil, rivastigmine, and galantamine) certified by FDA.

Moving on the advantages of AChEIs in the treatment of AD, in the current study, it was aimed to investigate the effects of resorcinol derivatives (Figure 1) on the activity of AChE. Besides, the interactions between compounds and the enzyme were clarified by the molecular docking method in order to understand the inhibition mechanism more clearly. In some research, alkylresorcinols have been reported to have some biological activities, such as antioxidant, anticancer, antimicrobial, antiparasitic, antifungal activities, and enzyme inhibition potency.[17-18]

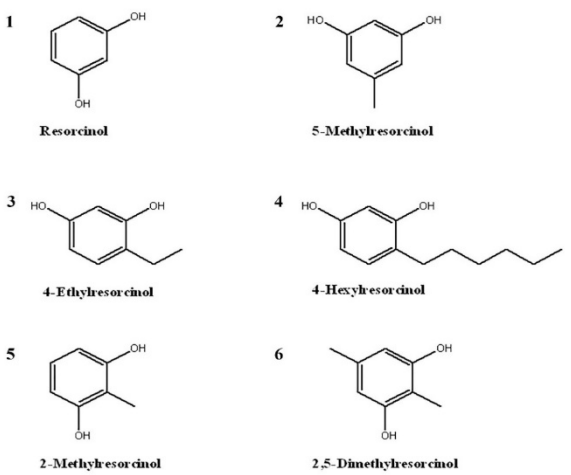

Figure 1. The chemical structures of resorcinol derivatives used in this study.

2. MATERIALS AND METHODS

2.1. Chemicals

All chemicals used in the isolation and activity determination of enzyme were procured from E. Merk AG. The erythrocyte of waste human blood was taken from the Turkish Red Crescent, Erzurum, Turkey.

2.2. Enzyme assay and Quantitative Protein Determination

AChE activity was measured spectrophotometrically at 436 nm according to Worek et al.'s method, modified from Ellman procedure which is more sensitive than Ellman's method.[19] In this method, during the reaction, the yellow-colored anion 2-nitro-5 thiobenzoate (TNB) formed from the 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) in the presence of the acetylthiocholine iodide. At 436 nm increased absorbance is recorded and used in activity calculations. The protein amount of enzyme samples was analysed according to the Bradford method at 595 nm by using bovine serum albumin (BSA, 1 mg ml^{-1}) as standard protein.[20]

2.3. Partial purification of acetylcholinesterase from human erythrocytes by DE-52 anion exchange chromatography

Erythrocytes were haemolysis by stirring with ice water in a ratio of 1:5. Than the haemolysed erythrocyte sample was centrifuged at 5 000 g for 15 minutes and pH of it was increased to 7.8 with 0.05 M K_2HPO_4 solution and AChE was isolated by DE-52 anion exchange chromatography, equilibrated with phosphate buffer at pH 7.5, as reported in Guller and co-workerset.[21] The AChE was eluted with the increasing salt gradient in the equilibration buffer. All these operations were carried out at about +4°C.

2.4. Inhibition studies

For examining the effects of resorcinols, activity determinations were performed at the different amount of compounds and at least five various inhibitor concentrations were chosen. Enzyme activity measured without resorcinols was taken as 100%. Activities determined in the presence of each compound calculated as activity%. Then activity%- [resorcinol derivatives] graphs were drawn using Microsoft Office 2016 Excel, and concentrations of compound that inhibited 50% of the activity (IC_{50}) were calculated from these graphs.[21-22]

2.5. Analysis of bindings models

The crystal structure of the acetylcholinesterase enzyme (PDB code: 4EY7)[23] was downloaded from the RSCB Protein Data Bank (PDB) with 2.0 Å resolution. Molecular structures of the derivatives were gotten by

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ChemDraw Professional 15.0 and Avogadro software *.pdb file. Receptor protein and ligands were prepared by using Autodock4.2 tool.[24] Finally, the grid box dimension (80 × 80 × 80 Å), grid spacing (0.375 Å), and grid boxes centres (x= -2.980, y= -40.109, z= 30.750) were adjusted to provide optimum binding poses. The Lamarckian genetic algorithm was used to determine the appropriate binding positions of ligands. The binding positions and interactions of ligand-receptor complexes were viewed using Discovery Studio Visualizer.

3. RESULT AND DISCUSSION

The role of acetylcholine (ACh), a neurotransmitter, is to ensure the neural impulses reach the muscles or related tissues after being released into the synaptic space by the nerve cells. In diseases related to memory loss, ACh reported to break down in a very short time. Therefore, it is used in the diagnosis and treatment of Alzheimer's disease (AD) today.[25] It was determined that ACh levels in normal-aged control groups' brain are different from normal-aged AD patients' brain around 50%.[25-27] When ACh is hydrolysed by Acetylcholinesterase (AChE), its role in neurotransmission ends. It has been determined that the transmission among nerves is strengthened by inhibition of AChE.[28] Besides, it has been seen that anticholinesterase drugs provide a significant regression in behavioural disorders in patients with memory-related diseases.[29] Considering the above-mentioned importance of AChE inhibition in treatment of memory lost diseases, in this study primer effects of resorcinols on enzyme activity were investigated. Also, inhibition mechanisms were clarified by molecular docking. Firstly, 0.032 EU mg^{-1} AChE was isolated from human erythrocytes by DE-52 anion exchange chromatography as described in method section. Then, *in vitro* inhibition studies of compounds on enzyme activity were performed spectrophotometrically. Activity%-[inhibitor] graphs were plotted (Figure 2). From the equations of these exponential graphs, the concentrations of compounds that inhibits the activity by half was calculated.

As seen from the Table1, IC_{50} values of compounds found in range between 2.74-363.61 µM. Most effective inhibitor was determined as 4-hexylresorcinol (**4**) with the IC_{50} value of 2.74 µM. The IC_{50} value of tacrine, used as a standard inhibitor, was found to be 0.013 µM. The inhibition potency of the studied compounds was found to be lower than the standard inhibitor (IC_{50} values were higher). The previous researcher described that so many compounds such as N-(4-methoxyphenethyl)-N-(substituted)-4-methylbenzenesulfonamides, carbamate substituted coumarin derivatives, salicylanilide and 4-chlorophenol-based N-monosubstituted carbamates, tacrine hybrids with carbohydrate derivatives, bivalent β-carboline derivatives, N-arylisomaleimides, and benzylidenemalononitrile derivatives, carbazole-coumarin hybrids, azoderivatives.[30-38]

Table 1. Results of *in vitro* and molecular docking studies of resorcinol derivatives on hAChE.

Compounds	IC_{50} (µM)	R^2	Estimated Free Energy of Binding (kcal/mol)	Estimated K_i constant (µM)
1	363.16	0.93	-4.96	231.63
2	345	0.98	-5.09	187.20
3	36.31	0.95	-5.52	89.32
4	2.74	0.98	-6.16	30.33
5	3.2	0.94	-4.93	243.77
6	57.5	0.92	-5.26	139.53
Tacrin	0.013	0.96	-7.49	3.26

IC_{50} values of N-(4-methoxyphenethyl)-N-(substituted)-4-methylbenzene sulfonamides on human erythrocytes AChE were found in range of 0.0751- 22.549 µM.[30,39] determined that donepezil-flavonoid hybrids inhibited human erythrocytes AChE with IC_{50} values of 0.021-7.49 µM. It was seen that inhibition potencies of compounds **4** and **5** in the current study were almost similar those of the studies conducted by Abbasi and co workers[30] and Valencia and co workers.[39] When compared with resorcinol, that the inhibitory effect of 4-hexylresorcinol (**4**), derived from C 4, was found 132.5 times higher. Güller and co workers[38] reported that 4-hexylresorcinol inhibited the human glutathione reductase (GR) higher than other derivatives. Studies examining the enzyme inhibition effect of resorcinols showed that; resorcinols derived from the 4th and 5th positions exhibited significant inhibition effect on cytochrome P450 and GR.[18,40]

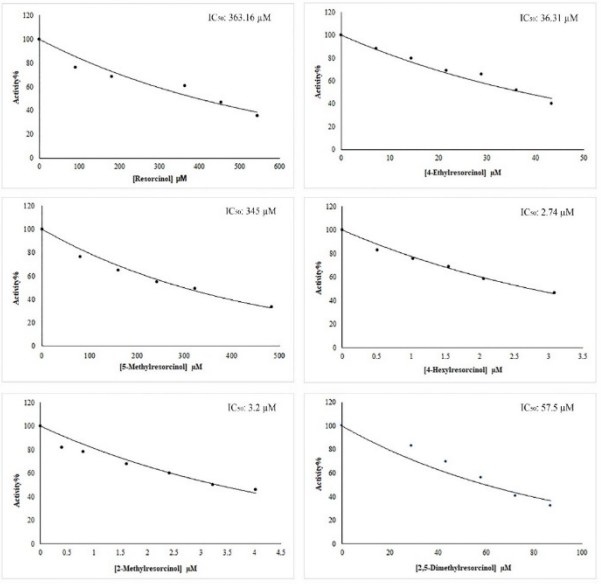

Figure 2. Activity%-[inhibitor] graphs of resorcinol derivatives.

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After *in vitro* inhibition, molecular docking studies were performed by AuthoDock 4.2 to clarify the inhibition mechanism. Estimated Binding Energies were predicted in a computer-aided manner and summarized in Table. Results of molecular docking studies were found to be consistent with *in vitro* inhibition results. The lowest binding energy was estimated for Compound **4** with -6.16 kcal mol^{-1}. However, all of the compounds had lower inhibition effects than the standard inhibitor. Free energy of binding was predicted for tacrine as -7.49 kcal mol^{-1}. When the inhibition mechanism inspected closer, it was seen that Try337 residue of hAChE receptor showed pi-sigma interaction with tacrine (Figure 3). Benzene moieties of compound had pi-alky interactions with Val294, Phe338, and Tyr341 residues. Compound formed pi-pi stacked interaction with phenly group of Phe338 and pi-donor hydrogen bond with Phe295. Besides, tacrine had several van der Waals interactions with Gly122, Tyr124, Trp286, Ser293, Arg296, and Phe297 amino acids.

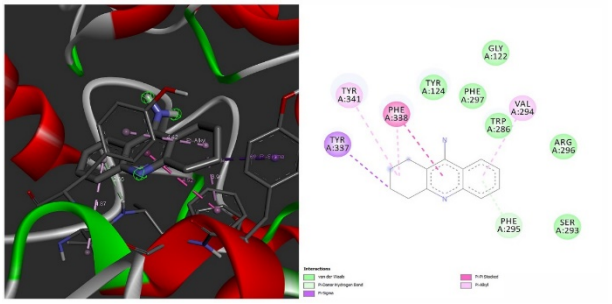

Figure 3. Potential binding modes and 2D ligand-receptor interaction diagrams of tacrine with hAChE receptor.

Compound **4** showed pi-sigma interaction with AChE in the same manner with tacrine (Figure 4). Tyr72, Asp74, and Tyr124 residues showed hydrogen bonds with –OH group of compound. It was observed that there was a pi-pi stacked interaction between the benzene ring of the molecule and the phenyl ring of Tyr124. Compound **4** had several van der Waals interaction with active site residues.

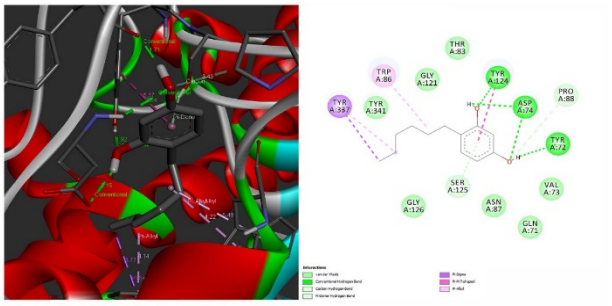

Figure 4. Potential binding modes and 2D ligand-receptor interaction diagrams of the most effective compound, 4-hexylresorcinol with hAChE receptor.

The interactions of compound **4** with hAChE receptor were found to be similar to those for the best inhibitors in the studies of Zhang and co workers[41] and Fernandes and co workers.[42]

4. CONCLUSIONS

Consequently, in this study, inhibition potential of resorcinol derivatives on human erythrocyte AChE were investigated and mechanism were clarified. The inhibition of AChE is widely used in the treatment of illnesses of which symptoms are memory loss. As a result of *in vitro* inhibition studies, it was seen that most effective inhibitor of hAChE is compound **4** (4-hexylresorcinol) with the IC_{50} of 2.74 µM. Also, molecular docking studies predict docking score as -6.16 kcal mol^{-1}. The results of both the *in vitro* and *in silico* approaches were seen to support each other. The results of the current paper may contribute to the studies regarding the design of new therapeutics targeting the activity of hAChE.

ACKNOWLEDGMENTS

There is no clinical application so, no animals or humans were used in this study. Human blood used for enzyme isolation was the waste blood that brought from the Turkish Red Crescent (Erzurum branch).

Conflict of interest

I declares that there is no a conflict of interest with any person, institute, company, etc.

REFERENCES

1. Blennow, K.; de Leon, M. J.; Zetterberg, H. *Lancet (London, England)* **2006,** *368* (9533), 387-403.

2. Burns, A.; Iliffe, S. *BMJ (Clinical researched)* **2009,** *338:b158*.

3. Reitz, C.; Mayeux, R. *Biochemical pharmacology* **2014,** *88* (4), 640-651.

4. Baptista, F. I.; Henriques, A. G.; Silva, A. M.; Wiltfang, J.; da Cruz e Silva, O. A. *ACS chemical neuroscience* **2014,** *5* (2), 83-92.

5. Du, X.; Wang, X.; Geng, M. *Translational neurodegeneration* **2018,** *7* (1), 1-7.

6. Hardy, J.; Selkoe, D. J. *science* **2002,** *297* (5580), 353-356.

7. Tang, B. L.; Kumar, R. *Ann Acad Med Singapore* **2008,** *37* (5), 406-5.

DOI: http://dx.doi.org/10.32571/ijct.944620 E-ISSN: 2602-277X

8. Coyle, J. T.; Price, D. L.; Delong, M. R. *Science* **1983,** *219* (4589), 1184-1190.

9. Blokland, A., *Brain Research Reviews* **1995,** *21* (3), 285-300.

10. Francis, P. T.; Palmer, A. M.; Snape, M.; Wilcock, G. K. *Journal of Neurology, Neurosurgery & Psychiatry* **1999,** *66* (2), 137-147.

11. H Ferreira-Vieira, T.; M Guimaraes, I.; R Silva, F.; M Ribeiro, F. *Current neuropharmacology* **2016,** *14* (1), 101-115.

12. Pope, C. N.; Brimijoin, S. *Biochemical pharmacology* **2018,** *153*, 205-216.

13. Greig, N. H.; Utsuki, T.; Yu, Q.-s.; Zhu, X.; Holloway, H. W.; Perry, T.; Lee, B.; Ingram, D. K.; Lahiri, D. K. *Current medical research and opinion* **2001,** *17* (3), 159-165.

14. Ahmad, B.; Mukarram Shah, S.; Khan, H.; Hassan Shah, S. *Journal of enzyme inhibition and medicinal chemistry* **2007,** *22* (6), 730-732.

15. Colovic, M. B.; Krstic, D. Z.; Lazarevic-Pasti, T. D.; Bondzic, A. M.; Vasic, V. M. *Current neuropharmacology* **2013,** *11* (3), 315-335.

16. Sun, Y.; Lai, M. S.; Lu, C. J.; Chen, R. C. *European journal of neurology* **2008,** *15* (3), 278-283.

17. Kozubek, A.; Tyman, J. H. P. *Chem Rev* **1999,** *99* (1), 1-25.

18. Srimai, V.; Ramesh, M.; Parameshwar, K. S.; Parthasarathy, T. *Medicinal Chemistry Research* **2013,** *22* (11), 5314-5323.

19. Worek, F.; Mast, U.; Kiderlen, D.; Diepold, C.; Eyer, P. *Clinica Chimica Acta* **1999,** *288* (1-2), 73-90.

20. Bradford, M. M. *Anal Biochem* **1976,** *72* (1-2), 248-254.

21. Guller, U.; Guller, P.; Ciftci, M. *Altern Ther Health Med* **2020**.

22. Adem, S.; Akkemik, E.; Aksit, H.; Guller, P.; Tufekci, A. R.; Demirtas, I.; Ciftci, M. *Medicinal Chemistry Research* **2019,** *28* (5), 711-722.

23. Cheung, J.; Rudolph, M. J.; Burshteyn, F.; Cassidy, M. S.; Gary, E. N.; Love, J.; Franklin, M. C.; Height, J. J. *Journal of medicinal chemistry* **2012,** *55* (22), 10282-10286.

24. Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J, *J Comput Chem* **2009,** *30* (16), 2785-2791.

25. Geula, C.; Mesulam, M. M. *Cholinergic Systems in Alzheimer's Disease, Alzheimer Disease*. 2 ed.; Lippincott, Williams and Wilkins: Philadelphia, PA, 1999.

26. Dhanasekaran, S.; Perumal, P.; Palayan, M. *Journal of Applied Pharmaceutical Science* **2015,** *5* (02), 012-016.

27. Forette, F. B. Alzheimer Hastalığında İlaç Geliştirilmesi: Tarihçesine Bakış ve Geleceğine İlişkin Öngörüler. In *Alzheimer Hastalığının Farmakoterapisi*, Gauthier, S., Ed. Yelkovan Yayıncılık: İstanbul, 2000; pp 1-5.

28. Bores, G. M.; Huger, F. P.; Petko, W.; Mutlib, A. E.; Camacho, F.; Rush, D. K.; Selk, D. E.; Wolf, V.; Kosley, R.; Davis, L. *Journal of Pharmacology and Experimental Therapeutics* **1996,** *277* (2), 728-738.

29. Giacobini, E. Cholinesterase Inhibitors. In *Enzymes of the cholinesterase family*, Springer: 1995; pp 463-469.

30. Abbasi, M. A.; Hassan, M.; Siddiqui, S. Z.; Shah, S. A. A.; Raza, H.; Seo, S. Y. *PeerJ* **2018,** *6*, e4962.

31. Biscussi, B.; Sequeira, M. A.; Richmond, V.; Mañez, P. A.; Murray, A. P. *Journal of Photochemistry and Photobiology A: Chemistry* **2021**, 113375.

32. Song, M.-q.; Min, W.; Wang, J.; Si, X.-X.; Wang, X.-J.; Liu, Y.-W.; Shi, D.-H. *Journal of Molecular Structure* **2021,** *1229*, 129784.

33. Zhao, Y.; Ye, F.; Xu, J.; Liao, Q.; Chen, L.; Zhang, W.; Sun, H.; Liu, W.; Feng, F.; Qu, W. *Bioorganic & medicinal chemistry* **2018,** *26* (13), 3812-3824.

34. Lopes, J. P. B.; Silva, L.; da Costa Franarin, G.; Ceschi, M. A.; Lüdtke, D. S.; Dantas, R. F.; de Salles, C. M. C.; Silva-Jr, F. P.; Senger, M. R.; Guedes, I. A. *Bioorganic & medicinal chemistry* **2018,** *26* (20), 5566-5577.

35. Krátký, M.; Štěpánková, Š.; Vorčáková, K.; Vinšová, J. *Bioorganic chemistry* **2018,** *80*, 668-673.

36. Guevara, J. A.; Trujillo, J. G.; Quintana, D.; Jiménez, H. A.; Arellano, M. G.; Bahena, J. R.; Tamay, F.; Ciprés, F. J. *Medicinal Chemistry Research* **2018,** *27* (3), 989-1003.

37. Kurt, B. Z.; Gazioglu, I.; Kandas, N. O.; Sonmez, F. *ChemistrySelect* **2018,** *3* (14), 3978-3983.

DOI: http://dx.doi.org/10.32571/ijct.944620 **E-ISSN: 2602-277X**

38. Güller, P.; Dağalan, Z.; Güller, U.; Çalışır, U.; Nişancı, B. *Journal of Molecular Structure* **2021,** *1239*, 130498.

39. Valencia, M. E.; Herrera-Arozamena, C.; de Andrés, L.; Pérez, C.; Morales-García, J. A.; Pérez-Castillo, A.; Ramos, E.; Romero, A.; Viña, D.; Yáñez, M. *European journal of medicinal chemistry* **2018,** *156*, 534-553.

40. Güller, P. *Journal of Molecular Structure* **2021,** *1228*, 129790.

41. Zhang, X.-Z.; Xu, Y.; Jian, M.-M.; Yang, K.; Ma, Z.-Y. *Medicinal Chemistry Research* **2019,** *28* (10), 1683-1693.

42. Fernandes, T. B.; Cunha, M. R.; Sakata, R. P.; Candido, T. M.; Baby, A. R.; Tavares, M. T.; Barbosa, E. G.; Almeida, W. P.; Parise-Filho, R. *Archiv der Pharmazie* **2017,** *350* (11), 1700163.