DOI: http://dx.doi.org/10.32571/ijct.1199221 E-ISSN: 2602-277X

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

The investigation of the effect of chalcones with benzoic acid on xanthine oxidase activity

Bedriye Seda KURŞUN AKTAR[1], Şevki ADEM[2], Emine Elçin ORUÇ-EMRE[3]

[1] *Malatya Turgut Özal University, Yeşilyurt Vocational School, Department of Hair Care and Beauty Services, Malatya, Türkiye*

[2] *Çankırı Karatekin University, Faculty of Sciences, Department of Chemistry, 18100 Çankırı, Türkiye*

[3] *Gaziantep University, Faculty of Art and Sciences, Department of Chemistry, Gaziantep, 27410, Türkiye*

Received: 3 November 2022; Revised: 5 December 2022; Accepted: 14 Aralık 2022

*Corresponding author e-mail: sedakursun@windowslive.com

Citation: Kurşun, Aktar, B. S.; Adem, Ş.; Oruç-Emre, E. E. *Int. J. Chem. Technol.* **2022**, 6 (2), 170-175.

ABSTRACT

Inhibitors of xanthine oxidase (XO) are effective and most major therapeutic drugs for the management of gout. Chalcone compounds are important in terms of biological activity and have great importance in enzyme studies in recent years. In the presented study, the effects of some chalcones on the enzyme were tested in vitro by the spectrophotometric method. Compounds showed an inhibitory effect between 7.21±0.07 and 13.78±0.13 µM IC50 values. The conformations and interactions of the compounds in the active site of the enzyme were determined by the molecular docking method using Molegro Virtual Docker software. Molecular modeling studies show that the B ring of chalcones has a significant contribution to the inhibition effect on the XO enzyme. The presented study suggests that chalcones may be a potential inhibitory group for XO.

Keywords: Chalcone, xanthine oxidase inhibitor, molecular docking.

Benzoik asitli kalkonlarin ksantin oksidaz inhibitörü üzerine etkisinin incelenmesi

ÖZ

Ksantin oksidaz (XO) inhibitörleri, gut tedavisinde çok önemli ve etkili terapötik ilaçlardır. Kalkon bileşikleri biyolojik aktivite açısından önemli olup, son yıllarda enzim çalışmalarında da büyük bir öneme sahiptir. Yapılan çalışmada, bazı kalkonların enzim üzerindeki etkileri spektrofotometrik yöntemlerle in vitro olarak test edildi. Bileşikler, 7.21±0.07 ve 13.78±0.13 uM IC50 değerleri arasında bir inhibitör etki gösterdi. Enzimin aktif bölgesindeki bileşiklerin konformasyonları ve etkileşimleri Molegro Virtual Docker yazılımı kullanılarak moleküler yerleştirme yöntemi ile belirlendi. Moleküler modelleme çalışmaları, kalkonların B halkasının, XO enzimi üzerindeki inhibisyon etkisine önemli bir katkısı olduğunu göstermektedir. Yapılan çalışma sonucunda, kalkonların XO için potansiyel bir inhibitör grup olabileceğini düşündürmektedir.

Anahtar kelimeler: Kalkon, ksantin oksidaz inhibitörü, molecular docking.

1. INTRODUCTION

Compounds with α-β unsaturated ketone structure containing 1,3-diarylprop-2-en-1-one structure are called chalcone. Chalcones can be isolated both naturally and synthetically. Chalcones show very different biological activities. In recent years, research on enzyme inhibition of chalcones has become very important. The chalcones have inhibitory effects against enzymes such as xanthine oxidase,[1] protein tyrosine kinase,[2] quinone reductase,[3] tyrosinase,[4] mammalian alpha-amylase[5], monoamine oxidase,[6] pyruvate kinase M2 (PKM2),[7] carbonic anhydrase I (CAI),[7] heme oxygenase,[8] carbonic anhydrase II (CAII),[7] aldose reductase, acetylcholinesterase (AChE),[9] α-amylase and α-glucosidase,[10] butyrylcholinesterase (BChE).[9] Xanthine oxidase inhibitor reduces uric acid production in humans and inhibits xanthine oxidase. Xanthine oxidase (XO) is a type of enzyme that is a form of xanthine oxidoreductase and produces reactive oxygen species. XO catalyzes the oxidation of hypoxanthine to xanthine and catalyzes the oxidation of xanthine to uric acid.

DOI: http://dx.doi.org/10.32571/ijct.1199221 E-ISSN: 2602-277X

Xanthine oxidase is used for the treatment of hyperuricemia and gout, as it is an enzyme responsible for the catabolism of purines and their conversion to uric acid. The XO inhibitor drug, which has been used commercially for many years, is the purine analog called allopurinol.[11] However, new drug candidates were needed because of the many side effects of allopurinol such as hypersensitivity, gastrointestinal problems, and renal toxicity. Because XO inhibitors, especially in the treatment of gout and hyperuricemia; It has also been associated with diabetes, hypertension, and other cardiovascular diseases.

2. MATERIALS AND METHODS

Materials

Enzyme, substrate, inhibitor and other reagents used in enzyme activity studies were purchased from Sigma-Aldrich. Structures of the analysed compounds 1,[12] 2,[12] 3,[13] 4,[13] and 5[12] are given in Table 1.

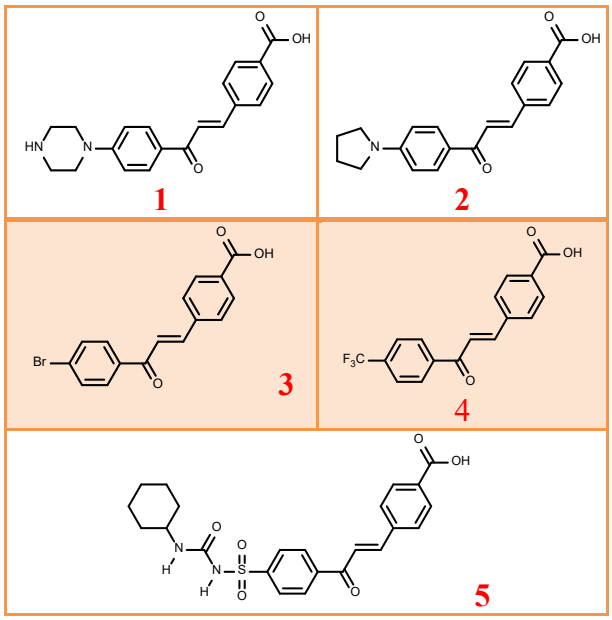

Table 1 The structures of the analysed compounds (1-5).

2.1. Inhibitory effects of Xanthine oxidase by Chalcones

The Xanthine oxidase inhibition experiment was performed according to the slightly changed methods in the previous reports.[14] Desired concentrations of enzyme and substrate were arranged by potassium phosphate buffer (pH 7.5, 50 mM), and the tested compounds were dissolved in DMSO at 1 mg/mL, then diluted with pure water ten times.

The enzyme solution (10 µL), 500 mM potassium phosphate buffer (50 µL), and different concentrations of chalcones (100 µL), and were added to the 96-well plate and incubated at 37 °C for 15 min. Then, hypoxanthine as substrate (60 µL, 3 mM) was added to each well and allowed to be incubated at 37 °C for 20 min. Finally, the alteration in the absorbance within 7 minutes was measured at 295 nm by using a multiple-reader spectrophotometer. Allopurinol was used as a positive control. The percent inhibition of xanthine oxidase activity was computed by comparing the inhibitor absorbance values with the control value without an inhibitor. IC_{50} values were obtained from the curve of concentration plots of molecules versus % activity.

2.2. Molecular Docking

Molecular docking was accomplished by the MolegroVirtual Docker for enzyme ligand docking.[15] The XO crystal structures (PDB IDs. 3NVY/ Quercetin, 7D6O/ Oxypurinol (www.rcsb.org/structure/7D6O), 3NRZ/Hypoxanthine, 3NVW/ Guanine) were retrieved from RCSB Protein Data Bank.[16-18] The XO structures were optimized in the Protein Preparation tool to obtain the chemically proper configuration by repairing structural errors in amino acids. Inhibitor structures were modeled in ChemDraw, and optimized and prepared three-dimensional (3D) sdf structure using the MarvinSketch. The docking site was described as the area occupied by the co-crystal ligands plus a 13 Å wide zone in its immediate proximity, yielding a docking region that was wide enough to set each of the docked chalcones. The docking operation was repeated 10 times for each compound. The best poses were visualized and analysed using Discovery Studio 2021 Client program (v17.2, Accelrys, San Diego, CA).

3. RESULTS AND DISCUSSION

3.1. Biological Activity

Xanthine oxidase inhibitors use as a drug to treat hyperuricemia and gout disease.[19,20] XO inhibitors from many different groups have been reported in the literature.[21] The derivatives of some chalcones were reported as strong xanthine oxidase inhibitors.[22-24] In this study, we tested some chalcones to determine their inhibitor potentials against this enzyme activity at 1-25 µM concentrations. Also, we performed docking studies to predict the accuracy of binding poses of compounds with enzyme active sites. Results tabulated in Table 2.

As shown in Table 2, among the chalcone compounds examined here compounds **1** and **2** had the greatest effect with 7.21±0.07 and 7.54±0.09 µM IC_{50} values, respectively. Compounds **3**, **4**, and **5** showed a strong inhibitory effect on XO, and the IC_{50} of these compounds were 8.82± 0.12, 9.80± 0.10, and 13.78± 0.13 µM , respectively (Figure 1). Allopurinol was used as a control to compare, its IC_{50} values were determined as 6.18± 0.10. The compounds demonstrated an inhibition effect against the enzyme at values very close to the standard.

DOI: http://dx.doi.org/10.32571/ijct.1199221 E-ISSN: 2602-277X

The name of compounds/Molecular Weight (g/mol)	IC_{50} values µM	PDB ID and References ligands							
		3NVY/ Quercetin		7D6O/ Oxypurinol		3NRZ/Hypoxanthine		3NVW/ Guanine	
		MolDock Score	LE	MolDock Score	LE	MolDock Score	LE	MolDock Score	LE
1 /335.376	7.21±0.07	-142.45	-5.70	-176.96	-7.08	-176.21	-7.05	-179.97	-7.20
2 /320.362	7.54±0.09	-145.96	-6.08	-181.03	-7.54	-173.23	-7.22	-179.63	-7.48
3 /330.153	8.82± 0.12	-151.53	-7.58	-155.09	-7.75	-152.52	-7.63	-155.03	-7.75
4 /319.255	9.80± 0.10	-162.30	-7.06	-170.20	-7.40	-168.54	-7.33	-168.16	-7.31
5 /434.337	13.78± 0.13	-170.70	-5.33	-217.79	-6.81	-210.66	-6.58	-224.05	-7.00
Resolution		2.00 Å		1.99 Å		1.80 Å		1.60 Å	

Table 2 The results obtained from in vitro and in silico studies against xanthine oxidase.

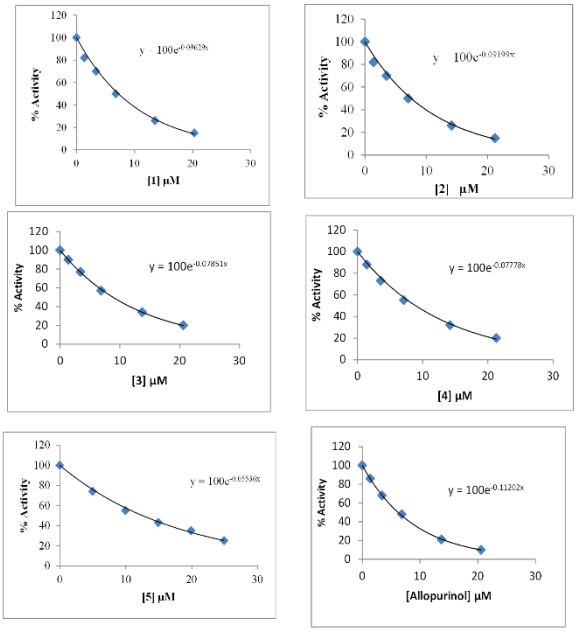

Figure 1 Inhibition rate graphs of chalcones on xanthine oxidase activity *in vitro*.

In order to further analyse the conformation and orientation between compounds, and xanthine oxidase, molecular docking was performed using MolegroVirtual Docker software. First, to evaluate the binding patterns of some substrates and inhibitors to the active site of the enzyme, interactions in their crystal structures were visualized with the Discovery Studio 2021 Client program (Figure 2). Then, the co-crystalline ligands were placed in the centers of the grids, and docking was performed against the active region. As seen in Table 2, the results of the others are similar, except for the crystal structure to which the quercetin ligand is attached. Docking results with the crystal structure 3NVW with the best resolution were selected for detailed interaction analysis. Figure 3 shows the docking site, the conformations of active site amino acids and ligands, and the overlap of ligands. The tested compounds displayed similar binding diagrams due to the parallel orientations of the ligands. The docking score of compounds **1**, **2**, **3**, **4**, and **5** are -176.21, -173.23, -152.52, -168.54 and -210.66 MolDock Score. When the *in vitro* and in silico results are evaluated together, there is a difference between the experimental results of compound **5** and the in silico results. It may be normal for it to have a higher MolDock Score because it has a higher molecular weight and contains more functional groups. However, another factor in evaluating the docking results is the Ligand Efficiency value obtained by the ratio of the docking score to the number of heavy atoms in the molecule. When the results are evaluated according to this value, it is seen that the in silico results generally support the in vitro results.

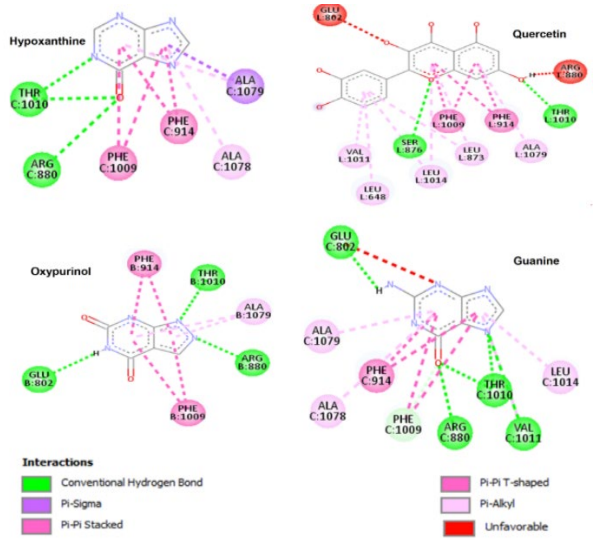

Figure 2. Dimensional view of the interaction diagrams of reference ligands with xanthine oxidase.

DOI: http://dx.doi.org/10.32571/ijct.1199221 E-ISSN: 2602-277X

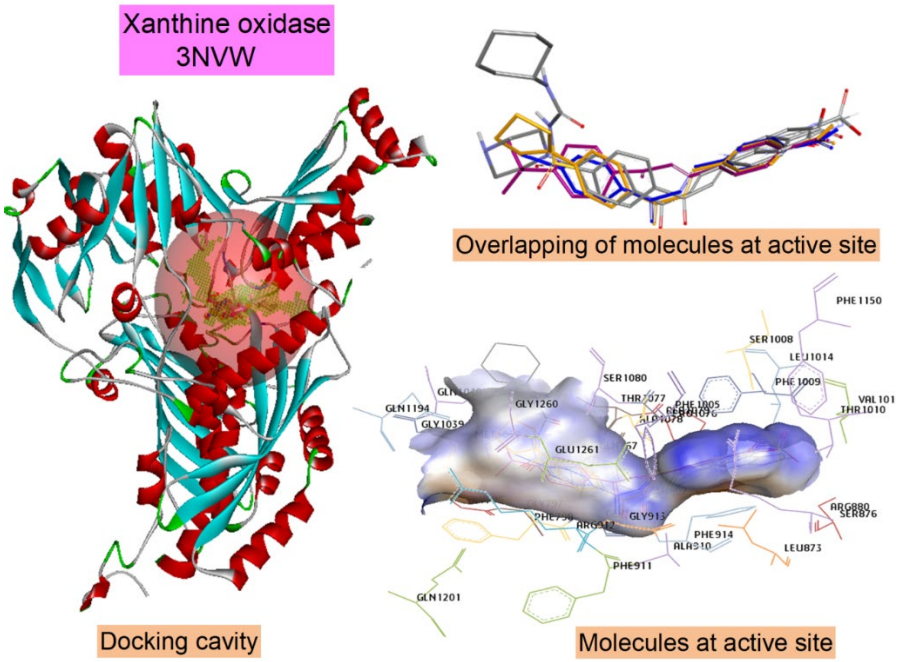

Figure 3 The cavity (active site) of xanthine oxidase, ligands with amino acid residues at the active site, and overlapping of chalcones.

It is observed that compound **1** formed hydrogen bonding with THR1010 amino acid. GLY799 formed a carbon-hydrogen bond with the O of propenal on the chalcone. On the other hand, the sulfur atom present in Methionine1038 residue interacted with the A ring of chalcone to form a Pi-Sulfur interaction. MET1038, ALA 1078, and ARG912 residues performed Alkyl interactions with cyclic structures on the Pi-fur molecule (Figure 4).

The oxygen of carboxylic acid moiety in the B ring of ligand **2** could form three hydrogen bonds with the backbone donor hydrogen bond of Thr1010, the side chain donor hydrogen bond of Arg880 and the side chain donor hydrogen bond of Thr1010. This compound formed the hydrophobic interactions with ALA1079, ALA1078, PHE914, ARG912 and MET1038 via piperazine and benzene groups (Figure 5).

The compound **3** established interactions with residues MET1038, ARG912, GLY799 PHE914, ARG880, THR1010, ALA1079, ALA1078, and PHE914. The carboxyl substituent of the B ring formed hydrogen bonds with ARG880 and THR1010. Benzene groups of compound Br-Far showed hydrophobic interactions with

ARG912, PHE914, ALA1079, and ALA1078. Also, a Pi-Sulfur interaction between MET1038 and A ring was observed. Brome moiety established an alkyl interaction with MET1038.

As seen in Figure, ARG880, THR1010 and GLU802 residues play a vital role in the binding of the substrate and inhibitors to the active pocket of the XO enzyme. The carboxyl group attached to the B benzene ring of the molecules makes strong hydrogen bonds with ARG880 and THR1010 in the enzyme's active site. Also, modifications to other parts of the molecule did not strongly affect the inhibitory potential of the molecule towards the enzyme. The inhibition effect of hydroxylated chalcones against the enzyme was tested previously. The article reported that molecules without the -OH group on the B ring did not affect the enzyme activity. The -OH groups in various positions on the B ring enhanced the inhibition effect of the molecules against the enzyme.[22] Similar results are seen in the results of another study. Also, the addition of -OCH-substituted groups on the B ring reduced or eliminated the effect of the molecules on the enzyme activity.[23-25]

The previously reported studies and the data obtained in this study may be said to be one of the main fragments of the B ring in the inhibition of the XO enzyme by chalcone-type molecules. In addition, heterocyclic structures participate in Pi-Pi Stached and Pi-Pi T-Tshaped hydroboical interactions. PHE914 and PHE1009 are the most important amino acid residues contributing to such interactions. Similar to the substrate and inhibitors, PHE1009, ALA1079, and PHE914 were evaluated to be the vital residues in the molecular interaction between chalcones and xanthine oxidase.

DOI: http://dx.doi.org/10.32571/ijct.1199221 E-ISSN: 2602-277X

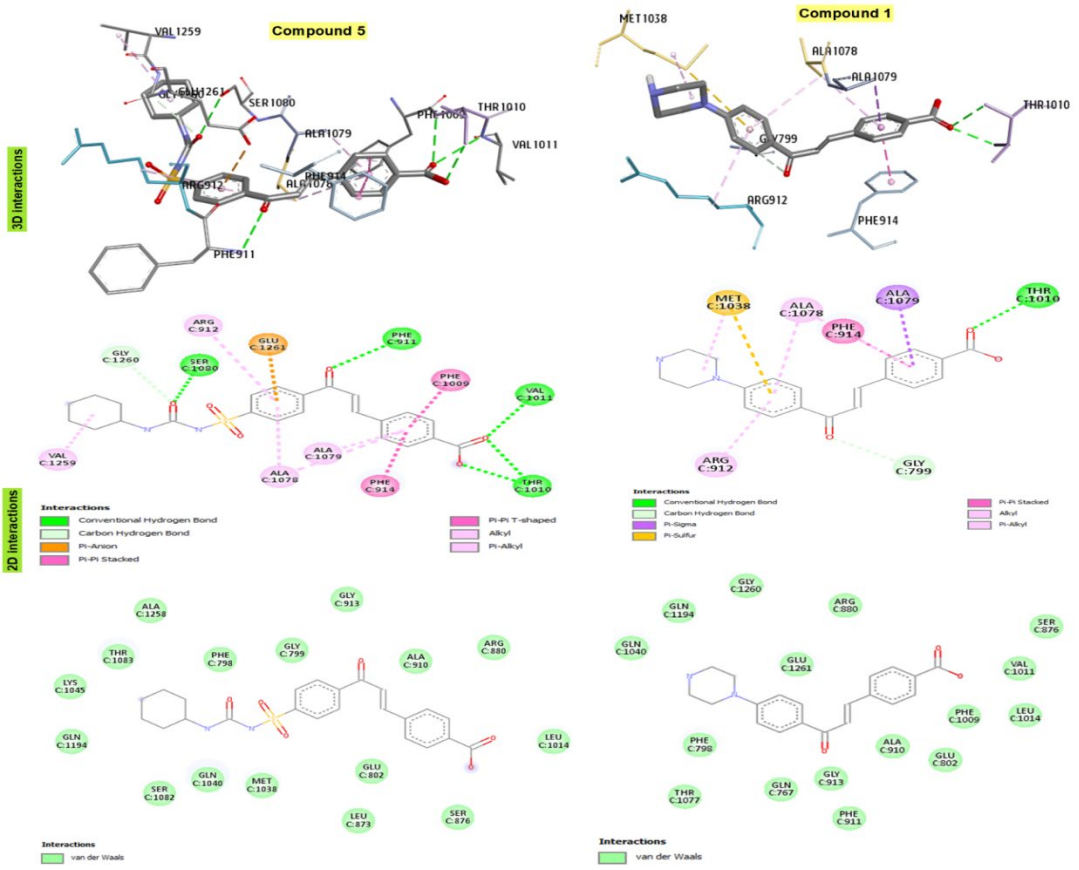

Figure 4 Three and two-dimensional view of the interaction diagram of chalcones with xanthine oxidase.

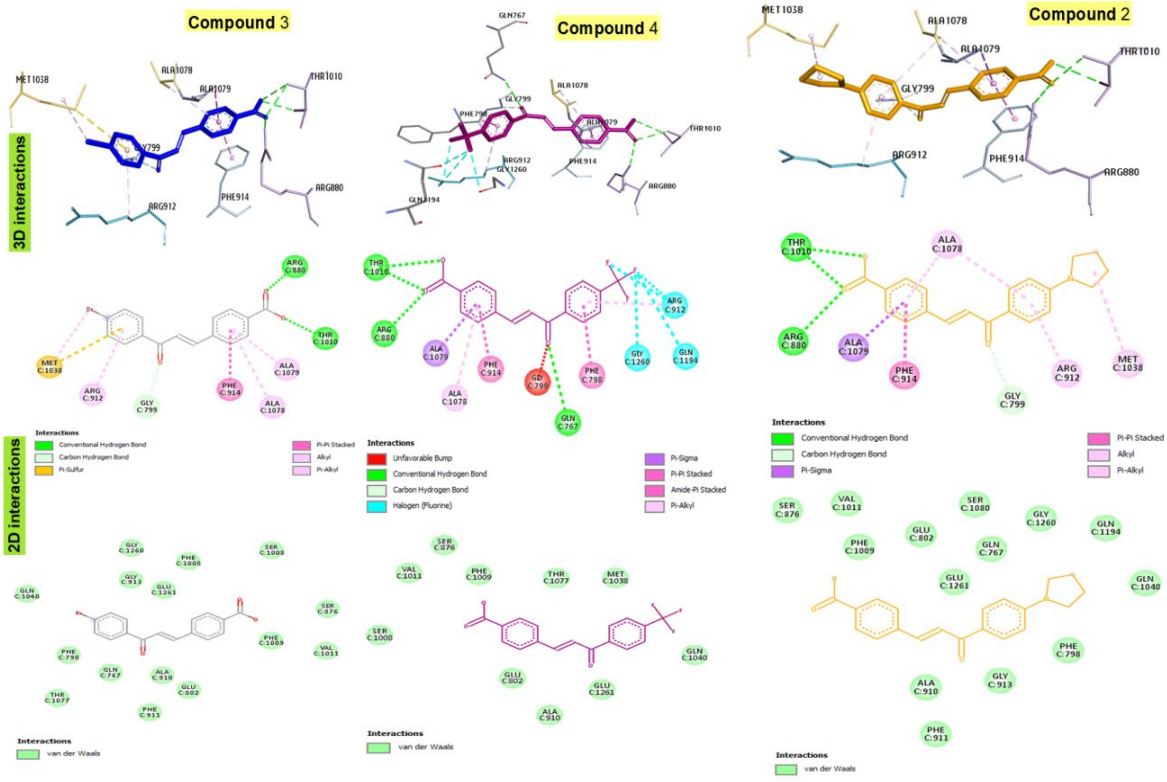

Figure 5 Three and two-dimensional view of the interaction diagrams of chalcones with xanthine oxidase.

DOI: http://dx.doi.org/10.32571/ijct.1199221 E-ISSN: 2602-277X

4. CONCLUSIONS

Some chalcone evaluated their xanthine oxidase inhibitor activity. Compounds 1 and 2 had the greatest effect with 7.21±0.07 and 7.54±0.09 µM IC50 values, respectively.

Conflict of interests

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

REFERENCES

1. Burmaoglu, S.; Ozcan, S.; Balcioglu, S.; Gencel, M.; Noma, S. A. A.; Essiz, S.; Ateş, B.; Algul, O. Bioorg. Chem. **2019**, 91, 103149.

2. Nerya, O.; Musa, R.; Khatib, S.; Tamir, S.; & Vaya, J. Phytochemistry, **2004**, 65(10), 1389- 1395.

3. Miranda, C. L.; Aponso, G. L. M.; Stevens, J. F.; Deinzer, M. L.; Buhler, D. R. Cancer Lett. **2000**, 149(1-2), 21-29.

4. Chaves, O. A.; Barros, L. S.; Oliveira, M. C.; Sant'Anna, C. M. R.; Ferreira, A. B.; Silva, F. A.; Sobrinho, D.C.; Netto-Ferreira, J. C. J. Fluor. Chem. **2017**, 199, 30-38.

5. Najafian, M.; Ebrahim-Habibi, A.; Hezareh, N.; Yaghmaei, P.; Parivar, K.; Larijani, B. Mol. Biol. Rep. **2011**, 38(3), 1617-1620.

6. Chimenti, F.; Fioravanti, R.; Bolasco, A.; Chimenti, P.; Secci, D.; Rossi, F.; Yanez, M.; Orallo, F.; Ortuso, F.; Alcaro, S. J. Med. Chem. **2009**, 52(9), 2818-2824.

7. Aktar, B. S. K.; Oruç-Emre, E. E.; Demirtaş, İ.; Yağlıoğlu, A. Ş.; İyidoğan, A. K.; Güler, Ç.; Adem, Ş. Turk. J. Chem. **2018**, 42(2), 482-492.

8. Lee, S.H.; Seo, G.S.; Kim, H.S.; Woo, S.W.; Ko, G.; Sohn, D.H. Biochem. Pharmacol. **2006**, 72(10), 1322-1333.

9. Kurşun-Aktar, B. S.; Sıcak, Y.; Tok Taşkın, T.; Oruç-Emre, E. E.; Şahin-Yağlıoğlu, A.; Karaküçük-İyidoğan, A.; Öztürk, M.; Demirtaş, İ. J. Mol. Struct. **2020**, 1211, 128059.

10. Mphahlele, M. J.; Zamisa, S. J.; & El-Gogary, T. M. J. Mol. Struct. **2021**, 1245, 131090.

11. Ardan, T.; Kovačeva, J.; Čejková, J. Acta histochemica, **2004**,106(1), 69-75.

12. Aktar, B.S.K.; Sıcak, Y.; Oruç-Emre, E.E. Int. J. Chem. Technol. **2022**,6(1), 7-14.

13. Tao, X. X.; Duan, Y. T.; Chen, L. W.; Tang, D. J.; Yang, M. R.; Wang, P. F.; Xu, C.; Zhu, H. L. Bioorg. Med. Chem Lett. **2016**, 677-683.

14. Wang, Y.; Zhang, S.; Zhang, Y.; Yao, F.; Zhao, G.; Wang, J.; Liu, L.; Yang, Y.; Li, X.; Sun, Y.; Hu, Y.; Bai, Z.; Wang, P.; Li, R.; Xu, X. ACS Food Sci. Technol. **2021**, 1: 2182-91.

15. Thomsen, R.; Christensen, M. H. J. Med. Chem. **2006**, 49(11), 3315-3321.

16. Cao, H.; Hall, J.; Hille, R. Biochemistry. **2014**, 53(3), 533-541.

17. Cao, H.; Pauff, J. M.; Hille, R. J. Biol. Chem. **2010**, 285(36), 28044-28053.

18. Cao, H.; Pauff, J. M.; Hille, R. J. Nat. Prod. **2014**, 77(7), 1693-1699.

19. Borges, F.; Fernandes, E.; Roleira, F. Curr. Med. Chem. **2002**, 9(2), 195-217.

20. Pacher, P.A L.; Nivorozhkin, A.; Szabó, C. Pharmacol. Rev. **2006**, 58(1), 87-114.

21. Kaur, G.; Singh, A.; Arora, G.; Monga, A.; Jassal, A. K.; Uppal, J.; Bendi, P. M. S.; Bora, K. S. Chem. Biol. Drug Des. **2022**, 100(3), 443-468.

22. Hofmann, E.; Webster, J.; Do, T.; Kline, R.; Snider, L., Hauser, Q.; Higginbottom, G.; Campbell, A.; Ma, L.; Paula, S. Bioorg. Med. Chem. **2016**, 24(4), 578-587.

23. Bui, T. H.; Nguyen, N. T.; Dang, P. H.; Nguyen, H. X.; Nguyen, M. T. T. SpringerPlus. **2016**, 5(1), 1-8.

24. Niu, Y.; Zhu, H.; Liu, J.; Fan, H.; Sun, L.; Lu, W.; Liu, X.; Li, L. Chem.-Biol. Interact. **2011**, 189(3), 161-166.

25. Choi, W.; Villegas, V.; Istre, H.; Heppler, B.; Gonzalez, N.; Brusman, N.; Snider, L.; Hogle, E.; Tucker, J.; Oñate, A.; Oñate, S.; Ma, Li.; Paula, S. **2019**, Bioorg.Chem. 86, 686-695.