Antimicrobial activity and molecular docking studies of some imidazo[2,1-b]thiazole derivatives

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Abstract. The synthesis of bioactive molecules is of major importance in the pharmaceutical industry. In this context, the study was conducted to determine the antimicrobial and antifungal potential of four chalcone-based imidazo[2,1-b]thiazole derivatives already synthesized by our research group. The synthetized compounds obtained in good yield were evaluated for their antibacterial and antifungal activities against *E. coli, Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Fusarium*, the results obtained are compared with the standard. Molecular docking studies were utilized to forecast the potential of these molecules as antimicrobial agents, the results obtained in vitro antibacterial were well confirmed by this method.
Keywords. Imidazothiazole, Antibacterial, Antifungal, Molecular Docking

1 Introduction

Nitrogen, oxygen, or sulfur-based heterocycles constitute a group of products characterized by intriguing chemical and biological properties.
According to the literature, compounds based on Imidazo[2,1-b] thiazole exhibit various biological activities, including antiviral,[1] anticancer,[2, 3] anti-inflammatory,[4] antimicrobial activities [5]. L-tetramisole or levamisole structure and their analogues remains the most used in therapy for for their immunomodulatory and antihelminthic properties.[6]

Chalcones are therefore very widely described in the literature and show multiple pharmacological potentials, and also demonstrate many anti-infectious properties: anti-retroviral[7], anti-HIV[8, 9], anti-polio[10, 11], antitubercular[12], anti-filariasis[13, 14], antimalarial[15, 16], antiparasitic[17], antibacterial[18, 19] or antifungal[20, 21]. Various properties can also be cited: osteogenic[22], antispasmodic[23], anti-gout[24], antihistamine[25], antiulcer[26], immunosuppressive[27].
Morover, the imidazothiazole – chalcone derivatives have been prepared and studied, Numerous derivatives of this fused ring system have been examined for their potential biological activity [2, 28-30].
Based on the above observations, and as part of our research program on the development of new compounds containing the imidazothiazole motif of biological interest, we have synthesized and characterized new fused imidazothiazole-chalcone derivatives.[2, 3, 30]
In this work, we tested the antibacterial activity of three imidazothiazole-chalcone derivatives previously synthesized and published by our research team[2] against *E. coli*, *P. aeruginosa*, and *S. aureus*, as well as their antifungal activity against *Fusarium*.. The biological activity of the synthesized compounds was Compared with standard drugs. Molecular docking studies are conducted on target proteins to comprehend the various potential interactions between protein and ligand.

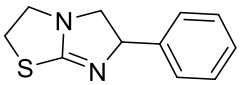

Fig.1. Structure de Lévamasole ou L-tétramisole

2 Materials and methods

2.1 Synthesis

The synthesis of these imidazothiazole derivatives and the supplementary data have been reported by Dadou et al.[2] The molecular structures are shown in Fig.2:

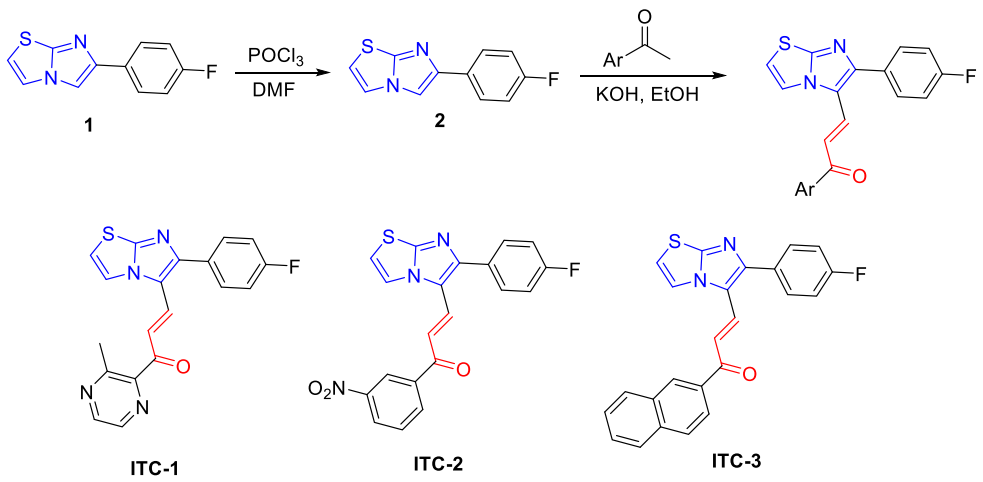

Fig.2. Synthetic route for the preparation of ITC-1, ITC-2 and ITC-3.

2.2 Antibacterial and antifungal activity

Evaluating the biological activity of ligand synthesis was realized by the diffusion disk by Müller-Hinton agar at a concentration of 10 mg/ml was prepared initially using DMSO solvent amount against different bacterial strain and mushroom.

2.2.1 Antibacterial Activity

The antibacterial activity of the new imidazothiazole derivatives ITC-1, ITC-2, and ITC-3 was evaluated against Gram-positive and Gram-negative bacterial strains, namely Staphylococcus aureus (Gram-positive) and Pseudomonas aeruginosa, Escherichia coli (Gram-negative), respectively. Additionally, the antifungal activity of these derivatives was examined against selected fungal strains. The strains were chosen based on their clinical relevance and common occurrence in infections, ensuring a comprehensive assessment of the derivatives' effectiveness across a range of pathogens. The used concentrations are: C1: 1000 µg/ml; C2: 500 µg/ml; C3: 200 µg/ml and C4: 50 µg/ml.
The test conducted followed the protocol recommended by the NCCLS (National Committee for Clinical Laboratory Standards) and outlined in AFNOR 2004: NF-U47-107.
A bacterial suspension was prepared in sterile physiological water for each strain using young colonies aged 18–24 hours.
The growth was then diluted with Mueller Hinton Broth (MHB) to a turbidity equivalent at the standard 0.5 McFarland density, growth of the microorganisms was then inoculated in Mueller Hinton Agar and poured into a petri dish.
Sterilized discs, each with a diameter of 6 mm and prepared from Whatman's No. 1 filter paper in the laboratory, were impregnated with various test compounds. These saturated discs were then placed onto the surface of the agar. Petri dishes were initially left at room temperature for 30 minutes to allow for the prediffusion of substances, before being transferred to an oven set at 37°C for 24 hours of incubation.
After the incubation at 37 ° C for 24 hours, the diameters of the inhibition zones round the disks were measured in mm, the purpose of which was to determine MIC.

2.2.2 Antifungal Activity

The antifungal activity of all synthetized compounds was tested against *Fusarium* using the concentrations: (C_1=1 mg/ml; C_2=0,2 mg/ml; C_3=0,05 mg/ml; C_4=0,01 mg/ml).
Each compound was added to potato dextrose agar (PDA) at different concentrations before fungal culturing. Subsequently, the fungal cultures were incubated at 28°C for 5 days. The inhibition percentage of each compound was calculated by comparing the mycelium diameter observed in its presence to that observed in the negative control. The IC_{50} value was determined through linear regression analysis, correlating the natural logarithm of the concentrations with the corresponding growth inhibition percentages[31].

2.3 Molecular docking

Docking studies were conducted to elucidate the ligand-protein interactions comprehensively. All calculations were carried out using MOE 2015.10 software installed on a system equipped with an 8.0 G Core Intel(R) Core (TM) i7-5600U processor. The complexes underwent energy minimization using the MMFF94 force field until the gradient convergence reached 0.05 Kcal/mol[32].

3 Results and discussion

3.1 Antibacterial Activity

The synthetized compounds were exhibited antibacterial activity, thus, from the obtained results presented in **Table 1**, we can be concluded that the compound **ITC-2** exhibits some antibacterial activity against *E. coli* with an MIC=500 µg/ml and *P. aeruginosa* with an MIC=200 µg/ml. for compound **ITC-1** shows some activity against *S. aureus* with an MIC=500 µg/ml**.** while some antibacterial activity antibacterial observed for compound ITC-3 against *E. coli* and *P. aeruginosa* with an MIC=500 µg/ml.

Table 1. Diameter of zone of inhibition and the MIC of tested compounds against the three bacterium E.coli, P.aeruginosa and S.aureus

Compound	Conc. Of compound (µg/ml)	Bacterium					
		E.coli		P.aeruginosa		S.aureus	
		ID* (mm)	MIC (µg/ml)	ID* (mm)	MIC (µg/ml)	ID* (mm)	MIC (µg/ml)
ITC-1	1000	-	-	-	-	11	500
	500	-		-		7	
	200	-		-		-	
	50	-		-		-	
ITC-2	1000	10	500	12	200	-	-
	500	7		8		-	
	200	-		5		-	
	50	-		-		-	
ITC-3	1000	12	500	9	500	-	-
	500	7		6		-	
	200	-		-		-	
	50	-		-		-	
Ciprofloxacin				23	25		
Amoxicillin		22	20			22	19
Negative control		-	-	-	-	-	-
DMSO		-	-	-	-	-	-

*: ID = Inhibitory Diameter ;

3.2 Antifungal Activity

The synthetized compounds ITC-1, ITC-2 and ITC-3 were assayed for their antifungal activities against *Fusarium* using the agar diffusion technique method. The findings from the in vitro evaluation of the antifungal activity of the compounds are documented in Table 2. From the antifungal activity data Table 2, it is observed the compound ITC-3 are the most active with an IC_{50}=37 µg/ml compared to the other two compounds.

Table 2. Screening Test of Antifungal activity of the compounds

Compound	Concentration (µg/ml)				IC50(µg/ml)
	10	50	200	1000	
ITC-1	18	46	79	100	74
ITC-2	11	40	71	100	106
ITC-3	29	57	91	100	37
Negative control	-	-	-	-	-
DMSO	-	-	-	-	-

3.3 Molecular docking results

The synthesized compounds ITC-1, ITC-2 and ITC-3 docked into the binding pocket of the active site of Thymidylate kinase (PDB Code: 3UWO). In the present study, a molecular docking was carried out to predict the likely targets of compounds ITC-1, ITC-2 and ITC-3 against Thymidylate kinase. Molecular mooring results with S-score values (kcal / mol), RMSD and different types of ligand interactions are given in the following table Table 3. The docking reliability was confirmed through the re-docking of the co-crystallized ligand (0DF) into the binding site of Pseudomonas aeruginosa Thymidylate kinase. A score of -7.81 kcal/mol was computed for 0DF, and the predicted pose closely resembled the experimental pose observed in the original crystal structure, with an RMSD of 0.242 Å (Fig. 3). The predicted pose of the ligand 0DF demonstrated hydrogen-bonding interactions with amino acid residues.[30]

Table 3. Docking results of compounds ITC-1, ITC-2 and ITC-3 into the active site of thymidylate kinase.

Compound	S(score) kcal/mol	hydrogen bond between atoms of compound and amino acid			RMSD (Å)
		Atom of ligand	Amino acid	distance (Å)	
0DF	-7.81	NH	GLN 105	2.71	0.242
		N	GLN 105	3.34	
		-C=O	THR 101	2.59	
		-C=O	ARG 74	2.77	
ITC-1	-7.085	S	ASP 153	3.91	0.808
		N (Pyrazine)	GLN 105	3.47	
ITC-2	-6.458	S	GLU 12	3.55	1.954
		-C=O	GLN 105	3.44	
		O (NO_2)	ARG 74	2.66	
		O (NO_2)	THR 101	2.76	
ITC-3	-7.177	-C=O	TYR 104	2.89	1.051

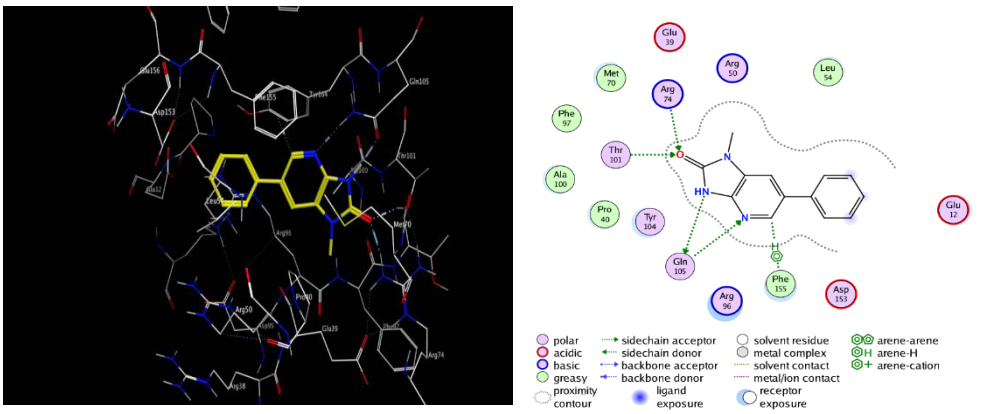

Fig. 3. The docking pose of 0DF in the active site of thymidylate kinase (PDB code: 3UWO) is depicted in both 3D (left) and 2D (right) representations

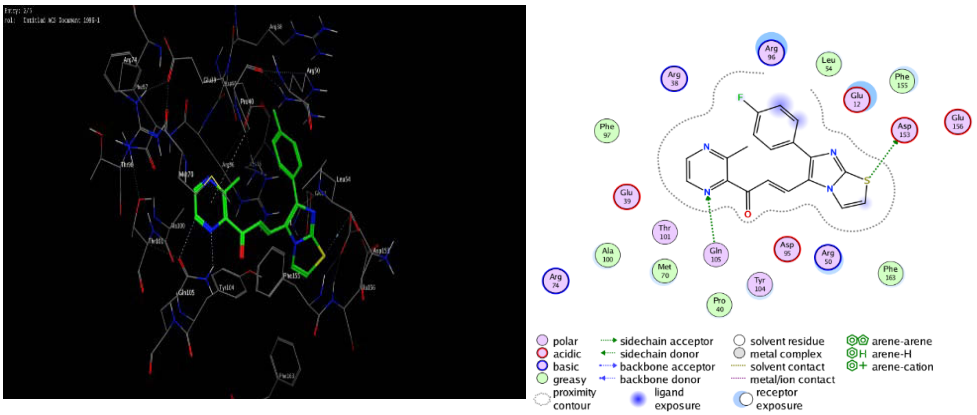

Fig. 4. The docking pose of compound ITC-1 in the active site of thymidylate kinase (PDB code: 3UWO) is depicted in both 3D (left) and 2D (right) representations.

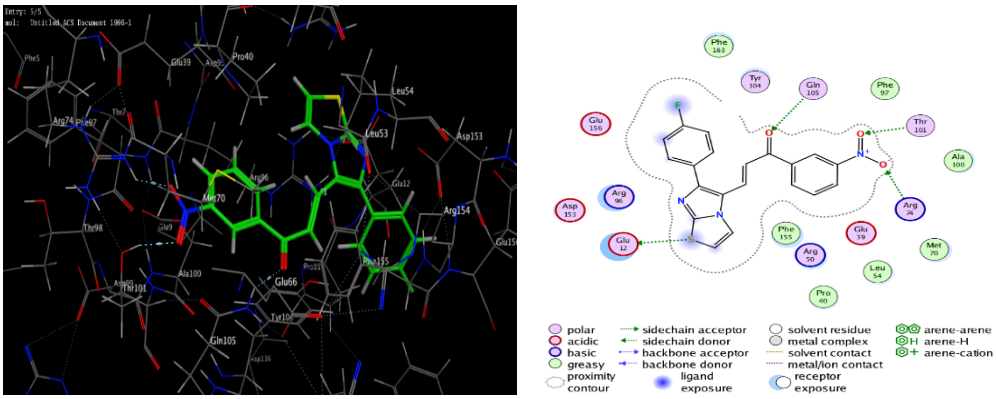

Fig. 5. The docking pose of compound ITC-2 in the active site of thymidylate kinase (PDB code: 3UWO) is depicted in both 3D (left) and 2D (right) representations.

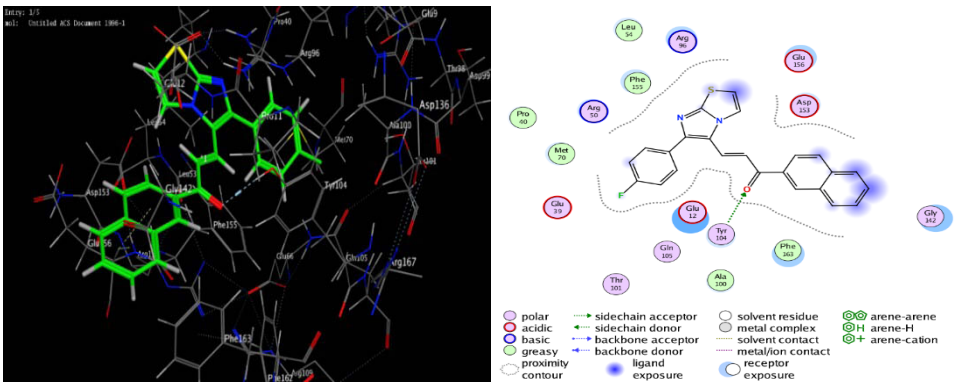

Fig. 6. The docking pose of compound ITC-3 in the active site of thymidylate kinase (PDB code: 3UWO) is depicted in both 3D (left) and 2D (right) representations.

Docking of our compounds in the thymidylate kinase receptor (PDB code: 3UWO), showed that the three compounds ITC-1, ITC-2, and ITC-3 interact with the receptor indicated by different types of interactions, with a score of -7.085, -6.458 and -7.177 kcal / mol respectively. The RMSD values for the three compounds are 0.808, 1.954, and 1.051 Å, respectively.

4 Conclusion

During this work, we present the biological evaluations of the compounds previously obtained against three bacterial strains (P. aeruginosa, E. coli and S. aureus) and one fungal strain (F. oxysporum). Some activities antibacterial have been observed especially for the two compounds ITC-2, and a conspicuous antifungal activity was observed especially for compound ITC-3. Molecular docking studies further aid in comprehending the diverse interactions between the ligands and the active site of the Pseudomonas aeruginosa Thymidylate kinase enzyme.

References

[1] I. Conti, R. Morigi, A. Locatelli, M. Rambaldi, G. Bua, G. Gallinella, A. Leoni, Synthesis of 3-(Imidazo [2, 1-b] thiazol-6-yl)-2H-chromen-2-one Derivatives and Study of Their Antiviral Activity against Parvovirus B19, Molecules, 24 (2019) 1037.

[2] S. Dadou, A. Altay, M. Koudad, B. Türkmenoğlu, E. Yeniceri, S. Çağlar, M. Allali, A. Oussaid, N. Benchat, K. Karrouchi, Design, synthesis, anticancer evaluation and molecular docking studies of new imidazo [2, 1-b] thiazole-based chalcones, Medicinal Chemistry Research, 31 (2022) 1369-1383.

[3] S. Dadou, A. Altay, C. Baydere, E.H. Anouar, B. Türkmenoğlu, M. Koudad, N. Dege, A. Oussaid, N. Benchat, K. Karrouchi, Chalcone-based imidazo [2, 1-b] thiazole derivatives: synthesis, crystal structure, potent anticancer activity, and computational studies, Journal of Biomolecular Structure and Dynamics, DOI (2023) 1-16.

[4] N.S. Shetty, I.A.M. Khazi, C.-J. Ahn, Synthesis, anthelmintic and anti-inflammatory activities of some novel imidazothiazole sulfides and sulfones, Bulletin of the Korean Chemical Society, 31 (2010) 2337-2340.

[5] B. Singh, A. Maheshwari, G. Dak, K. Sharma, G. Talesara, Studies of antimicrobial activities of some 4-thiazolidinone fused pyrimidines,[1, 5]-benzodiazepines and their oxygen substituted hydroxylamine derivatives, Indian journal of pharmaceutical sciences, 72 (2010) 607.

[6] A.H. Raeymaekers, F.T. Allewijn, J. Vandenberk, P.J. Demoen, T.T. Van Offenwert, P.A. Janssen, Novel Broad-Spectrum Anthelmintics. Tetramisole1 and Related Derivatives of 6-Arylimidazo [2, 1-b] thiazole, Journal of Medicinal Chemistry, 9 (1966) 545-551.

[7] S.U.F. Rizvi, H.L. Siddiqui, M. Johns, M. Detorio, R.F. Schinazi, Anti-HIV-1 and cytotoxicity studies of piperidyl-thienyl chalcones and their 2-pyrazoline derivatives, Medicinal Chemistry Research, 21 (2012) 3741-3749.

[8] H. Sharma, S. Patil, T.W. Sanchez, N. Neamati, R.F. Schinazi, J.K. Buolamwini, Synthesis, biological evaluation and 3D-QSAR studies of 3-keto salicylic acid chalcones and related amides as novel HIV-1 integrase inhibitors, Bioorganic & medicinal chemistry, 19 (2011) 2030-2045.

[9] S. Cheenpracha, C. Karalai, C. Ponglimanont, S. Subhadhirasakul, S. Tewtrakul, Anti-HIV-1 protease activity of compounds from Boesenbergia pandurata, Bioorganic & medicinal chemistry, 14 (2006) 1710-1714.

[10] R.A. Reddy, V.K. Reddy, B. Mahesh, B. Ram, B. Balram, SYNTHESIS OF 4-ETHYNYL CHALCONES AS POTENT ANTIBACTERIAL AGENTS, DOI (2015).

[11] D.K. Mahapatra, S.K. Bharti, V. Asati, Chalcone scaffolds as anti-infective agents: Structural and molecular target perspectives, European journal of medicinal chemistry, 101 (2015) 496-524.

[12] L.D. Chiaradia, P.G.A. Martins, M.N.S. Cordeiro, R.V.C. Guido, G. Ecco, A.D. Andricopulo, R.A. Yunes, J. Vernal, R.J. Nunes, H.n. Terenzi, Synthesis, biological evaluation, and molecular modeling of chalcone derivatives as potent inhibitors of Mycobacterium tuberculosis protein tyrosine phosphatases (PtpA and PtpB), Journal of medicinal chemistry, 55 (2011) 390-402.

[13] K.V. Sashidhara, K.B. Rao, V. Kushwaha, R.K. Modukuri, R. Verma, P. Murthy, Synthesis and antifilarial activity of chalcone–thiazole derivatives against a human

lymphatic filarial parasite, Brugia malayi, European journal of medicinal chemistry, 81 (2014) 473-480.

[14] S.K. Awasthi, N. Mishra, S.K. Dixit, A. Singh, M. Yadav, S.S. Yadav, S. Rathaur, Antifilarial activity of 1, 3-diarylpropen-1-one: effect on glutathione-S-transferase, a phase II detoxification enzyme, The American journal of tropical medicine and hygiene, 80 (2009) 764-768.

[15] V. Tomar, G. Bhattacharjee, S. Rajakumar, K. Srivastava, S. Puri, Synthesis of new chalcone derivatives containing acridinyl moiety with potential antimalarial activity, European journal of medicinal chemistry, 45 (2010) 745-751.

[16] S. Srivastava, S. Joshi, A.R. Singh, S. Yadav, A. Saxena, V. Ram, S. Chandra, J. Saxena, Oxygenated chalcones and bischalcones as a new class of inhibitors of DNA topoisomerase II of malarial parasites, Medicinal Chemistry Research, 17 (2008) 234-244.

[17] M. Chen, S.B. Christensen, J. Blom, E. Lemmich, L. Nadelmann, K. Fich, T.G. Theander, A. Kharazmi, Licochalcone A, a novel antiparasitic agent with potent activity against human pathogenic protozoan species of Leishmania, Antimicrobial agents and chemotherapy, 37 (1993) 2550-2556.

[18] M.I. Abdullah, A. Mahmood, M. Madni, S. Masood, M. Kashif, Synthesis, characterization, theoretical, anti-bacterial and molecular docking studies of quinoline based chalcones as a DNA gyrase inhibitor, Bioorganic chemistry, 54 (2014) 31-37.

[19] P.M. Sivakumar, S. Ganesan, P. Veluchamy, M. Doble, Novel chalcones and 1, 3, 5-triphenyl-2-pyrazoline derivatives as antibacterial agents, Chemical biology & drug design, 76 (2010) 407-411.

[20] K. Lahtchev, D. Batovska, P. St P, V. Ubiyvovk, A. Sibirny, Antifungal activity of chalcones: A mechanistic study using various yeast strains, European journal of medicinal chemistry, 43 (2008) 2220-2228.

[21] P. Boeck, P.C. Leal, R.A. Yunes, V.C. Filho, S. López, M. Sortino, A. Escalante, R.L. Furlán, S. Zacchino, Antifungal Activity and Studies on Mode of Action of Novel Xanthoxyline-Derived Chalcones, Archiv der Pharmazie: An International Journal Pharmaceutical and Medicinal Chemistry, 338 (2005) 87-95.

[22] X.R. Ortolan, B.P. Fenner, T.J. Mezadri, D.R. Tames, R. Corrêa, F. de Campos Buzzi, Osteogenic potential of a chalcone in a critical-size defect in rat calvaria bone, Journal of Cranio-Maxillofacial Surgery, 42 (2014) 520-524.

[23] Y. Sato, J.-X. He, H. Nagai, T. Tani, T. Akao, Isoliquiritigenin, one of the antispasmodic principles of Glycyrrhiza ularensis roots, acts in the lower part of intestine, Biological and Pharmaceutical Bulletin, 30 (2007) 145-149.

[24] D.W. Kim, M.J. Curtis-Long, H.J. Yuk, Y. Wang, Y.H. Song, S.H. Jeong, K.H. Park, Quantitative analysis of phenolic metabolites from different parts of Angelica keiskei by HPLC–ESI MS/MS and their xanthine oxidase inhibition, Food chemistry, 153 (2014) 20-27.

[25] T. Yamamoto, M. Yoshimura, F. Yamaguchi, T. Kouchi, R. Tsuji, M. Saito, A. Obata, M. Kikuchi, Anti-allergic activity of naringenin chalcone from a tomato skin extract, Bioscience, biotechnology, and biochemistry, 68 (2004) 1706-1711.

[26] K.V. Sashidhara, S.R. Avula, V. Mishra, G.R. Palnati, L.R. Singh, N. Singh, Y.S. Chhonker, P. Swami, R. Bhatta, Identification of quinoline-chalcone hybrids as potential antiulcer agents, European journal of medicinal chemistry, 89 (2015) 638-653.

[27] Y. Luo, R. Song, Y. Li, S. Zhang, Z.-J. Liu, J. Fu, H.-L. Zhu, Design, synthesis, and biological evaluation of chalcone oxime derivatives as potential immunosuppressive agents, Bioorganic & medicinal chemistry letters, 22 (2012) 3039-3043.

[28] A. Kamal, D. Dastagiri, M.J. Ramaiah, J.S. Reddy, E.V. Bharathi, C. Srinivas, S. Pushpavalli, D. Pal, M. Pal-Bhadra, Synthesis of imidazothiazole–chalcone derivatives as anticancer and apoptosis inducing agents, ChemMedChem, 5 (2010) 1937-1947.

[29] M. Koudad, C. El Hamouti, A. Elaatiaoui, S. Dadou, A. Oussaid, F. Abrigach, G. Pilet, N. Benchat, M. Allali, Synthesis, crystal structure, antimicrobial activity and docking studies of new imidazothiazole derivatives, Journal of the Iranian Chemical Society, 17 (2020) 297-306.

[30] M. Koudad, C. El Hamouti, A. Elaatiaoui, S. Dadou, A. Oussaid, F. Abrigach, G. Pilet, N. Benchat, M. Allali, Synthesis, crystal structure, antimicrobial activity and docking studies of new imidazothiazole derivatives, Journal of the Iranian Chemical Society, 17 (2020) 297-306.

[31] J.L. Watts, Clinical, L.S. Institute, Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals: approved standard, National Committee for Clinical Laboratory Standards2008.

[32] S. Vilar, G. Cozza, S. Moro, Medicinal chemistry and the molecular operating environment (MOE): application of QSAR and molecular docking to drug discovery, Current topics in medicinal chemistry, 8 (2008) 1555-1572.