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Research Article

Focusing on the moderately active compound (MAC) in the design and development of strategies to optimize the apoptotic effect by molecular mechanics techniques

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ABSTRACT

Today, chemotherapeutic agents are mostly used to fight cancer in clinics. But even though they have selectivity for cancer cells, their mechanism of action could result in necrosis. Therefore, we aimed to suggest new design strategies using a moderately active compound (MAC) to get better activity and increase the apoptotic effect in this study. Although MAC, previously synthesized and evaluated for its anticancer properties, has been marked as a moderately active compound, it has let us develop new molecules using its molecular core supported by molecular docking and molecular dynamics simulation. The caspase-3 enzyme was subjected to density functional theory (DFT), docking, and molecular dynamics simulation studies, and the results were analyzed to better understand the structure-activity relationship (SAR); thus, new design strategies were proposed.

Keywords: Apoptosis, DFT, molecular dynamics simulation, thiazole, piperazine

1. INTRODUCTION

Drug research and development studies are a series of sensitive processes that involve time-consuming and expensive studies [1]. The main goal is, of course, to find an effective compound that can be used clinically. Therefore, many pharmaceutical and medicinal chemists first focus on determining potential compounds that can be applied to organisms [2-5]. This process can be performed in many different ways, such as the rule of five elimination [6], developing pharmacophore hypotheses [7], using the mimesis method [8], *etc*. Whatever method is used, eventually the designed compounds are synthesized and then tested for their activity. Also, considering recent studies [9, 10], eliminating compounds through the "rule of five" may sometimes miss some valuable compounds (drugs that violate the rule of five). Therefore, if the basic theory, "lock and key model" is working, the compound should be chosen and then should be evaluated/improved for the unfavorable parts of the compound.

In 2019, our study group designed and synthesized a series of thiazole-bearing piperazine derivatives using the mimesis method [11]. Anticancer activity of the synthesized compounds was evaluated against C6 rat glioma and A549 adenocarcinomic human alveolar basal epithelial cell lines. According to the results of the study, C6 cells were more sensitive to active compounds than A549 cells. After that, potential analogs were investigated for

their apoptotic effects against both cell lines. The results showed *N*-(4-(pyridin-4-yl)thiazol-2-yl)-2-(4-(pyrimidin-2-yl)piperazin-1-yl)acetamide (a moderately active compound, **MAC**) has a positive impact on apoptosis, especially on A549 cells. As mentioned in that study, the design of the compounds is based on dasatinib and imatinib. Therefore, **MAC** has been investigated deeply for its mechanism of action. Similar to the effects of dasatinib and imatinib, the caspase-3 enzyme should be stimulated to result in an apoptotic effect; hence, we focused on this enzyme and clarified the molecular mechanics.

Briefly, in this study, we aimed to offer new design strategies that can be useful approaches to designing and developing original compounds using molecular mechanics studies.

2. MATERIALS AND METHODS

2.1. DFT Studies

Theoretical analyses for our MAC were carried out using the Gaussian 09 W package [12] and GaussView 5.0 [13] molecular visualization tools. Using previously published procedures, the DFT study method was applied to MAC [14-16]). It is also critical to analyze charge transfer through intramolecular interactions. To explain this parameter, the energy levels of the examined compound's HOMO and LUMO orbitals must be time-dependent (TD). HOMO-LUMO energy values were used to compute the various chemical activity parameters.

2.2. Molecular Docking Study

Molecular docking studies were performed using an *in-silico* procedure to define the binding modes of active compounds in the allosteric regions of the enzyme X-ray crystal structures of caspase-3 (PDB ID: 1NMS) to understand how to stimulate the caspase-3 activity. The data were retrieved from the Protein Data Bank server (www.pdb.org, accessed 07 December 2021). The Schrödinger Maestro [17] interface was used for the molecular docking study, and the enzyme crystal was processed using the Protein Preparation Wizard protocol of the Schrödinger Suite 2020. Similar to previous studies [18, 19], **MAC** and dasatinib were prepared using the LigPrep module [20] to correctly assign the protonation states as well as the atom types. Bond orders were assigned, and hydrogen atoms were added to the structures. The grid generation was formed using the Glide module [21], and docking runs were performed in extra-precision docking mode (XP) for dasatinib. After obtaining the best pose for the dasatinib-caspase-3 complex, the ligand was removed, and the protein that remained was used as the generated grid map. The docking runs for **MAC** were performed in extra-precision docking mode (XP).

2.3. Molecular Dynamics Study (MDS)

MDS have been considered an important computational tool for evaluating the time-dependent stability of the ligand-receptor complex. In this study, MDS for 100 ns was carried out to ensure the stability of the identified hits from the docking results, and then the interaction types and strengths were analyzed. The method was applied similarly to our previous studies [14, 22-24]. Using the standard force field (OPLS3e) of Schrodinger's Suite with a transferable intermolecular potential with 3 points (TIP3P) water model followed by energy minimization of the complex in the Desmond application [25]. The neutralization of the system was achieved using Na^+ and Cl^- ions, and 150 mM NaCl was added to the dynamics condition. The molecular dynamics simulation was performed following the completion of the system setup. The radius of gyration (Rg), root mean square fluctuation (RMSF), and root mean square deviation (RMSD) values were calculated by the Desmond application.

3. RESULTS AND DISCUSSION

3.1. DFT Studies

The variables that affect the reactivity of various biomolecules based on DFT have piqued the interest of many researchers in recent years. Several critical factors, such as the properties, stability, and composition of **MAC** compounds, have been determined using global reactivity data based on DFT. The best molecular structures produced by

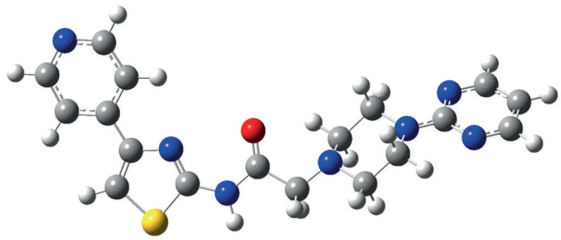
theoretical methods can be used to describe the three-dimensional structure of the investigated substances. When the B3LYP/6-31G (d,p) basis range was used, no imaginary frequency was found after using DFT to optimize the structure of **MAC**. **Figure 1** displays **MAC**'s optimized structures.

Certain highly significant chemical properties, such as optical polarization, chemical softness and strength, molecular electronic transport, and so on, may be computed using the energy difference between HOMO and LUMO [26]. Table 1 shows several reactive characteristics of the MAC molecule. The studied molecule is highly stable, as evidenced by the negative energy levels of the HOMO and LUMO orbitals (**Figure 2**). The most prominent ionization potential (I) and electron affinity (A) values associated with HOMO and LUMO energy, respectively, are found in the MAC structure, which has a low I value and a high A value.

The ability of an atom to attract other atoms' shared electrons, also known as electron density, is known as electronegativity (χ). The more electrons an atom or substituent group attracts, the higher its corresponding electronegativity. It can be shown that MAC has a lower electron affinity (0.1305 eV) and higher electronegativity (0.0519 eV). The MAC has a low η value and a high S value in terms of chemical hardness-softness values that are useful in determining intramolecular charge transfer of molecular structures.

The molecular electrostatic potential (MEP) estimates ligand binding and hydrogen bonds with biomolecules and validates the charge distribution (positive and negative) of 3D molecules [27]. The MEP mapping findings for **MAC** are shown in **Figure 3**.

In the MEP scheme, red represents a partial negative charge brought on by an electron-rich zone, blue represents a partial positive charge brought on by an electron-deficient zone, yellow represents a moderately electron-rich zone, and green represents a neutral zone [28]. The MEP mapping indicates that the oxygen and nitrogen atoms in MAC have the highest negative potential.



Total Energy: -1554.05459719 a.u.

Figure 1. Optimized molecular structures and total energy values of MAC

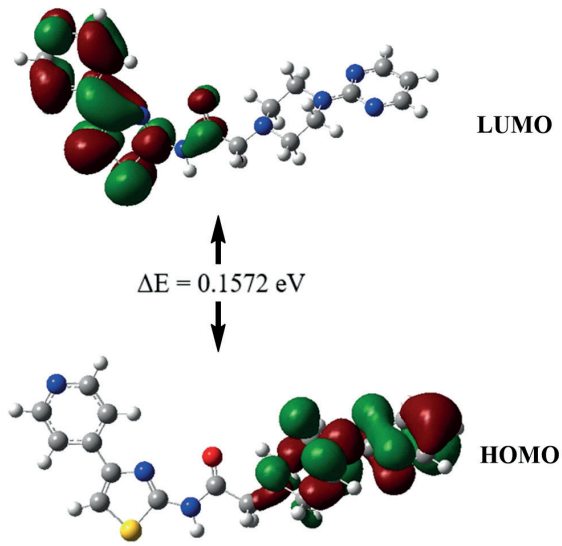


Figure 2. HOMO-LUMO diagrams of the compound MAC

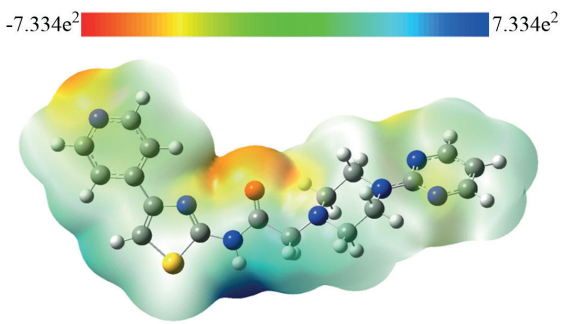


Figure 3. Molecular electrostatic potential (MEP) surfaces presentation of the MAC

3.2. Molecular Docking Study

For the caspase-3 enzyme, Arg64, Ser120, His121, Gly122, Gln161, Cys163, Tyr204, Ser205, Trp206, Arg207, Asn208, Ser209, Ser249, and Phe250 amino

Table 1. Some reactivity parameters of the MAC.

Compounds	E_{HOMO} (eV)	E_{LUMO} (eV)	ΔE (eV)	I (eV)	A (eV)	χ (eV)	η (eV)	S (eV−1)	μ (eV)	ω (eV)
MAC	-0.2091	-0.0519	0.1572	0.2091	0.0519	0.1305	0.0786	6.3613	-0.1305	0.1083

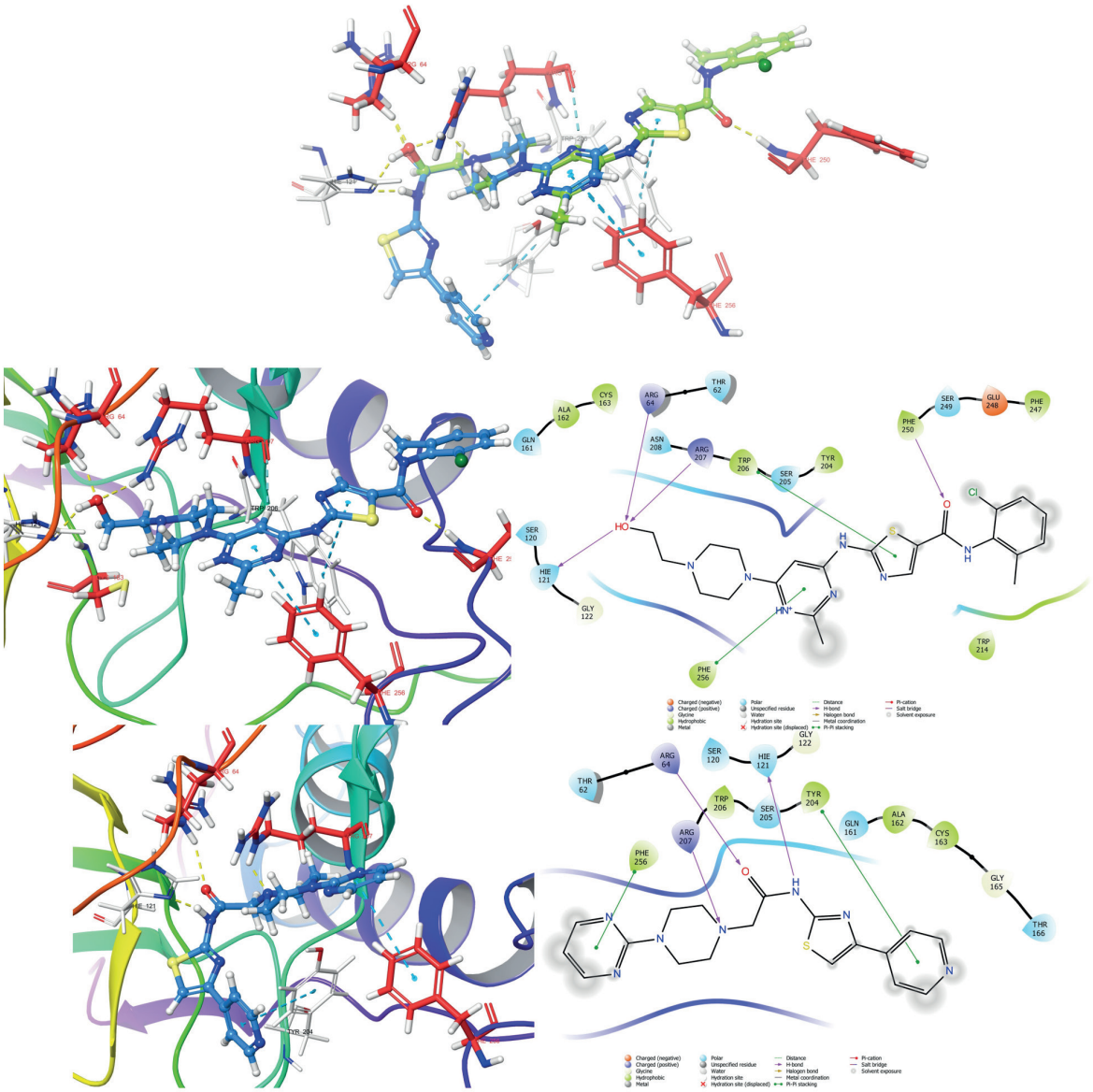


Figure 4. The best docking poses for dasatinib and active compound. **A:** Superimposed of both molecules (green carbons for dasatinib and blue carbons for active compound); **B:** Dasatinib and caspase-3 complex; **C:** MAC and caspase-3 enzyme complex.

acids were identified as binding region amino acids to stimulate the enzyme activity allosterically [29].

According to results (**Figure 4**), dasatinib interacted with Arg64 (H-bond), His121 (H-bond), Trp206 (π-π stacking), Arg207 (H-bond and aromatic H-bonds), Phe250 (H-bond), and Phe256 (π-π stacking) amino acids, while **MAC** interacted with Arg64 (H-bond), His121 (H-bond), Tyr204 (π-π stacking), Arg207 (H-bond), and Phe256 (π-π stacking) amino acids. Both interactions were found to be very similar to

each other. However, the difference between the molecules is dasatinib tailing to the hydrophobic pocket of the enzyme, in which it can interact with Phe250 amino acid. Meanwhile, because the MAC curled into the polar pocket, the H-bond interaction between its acetamide moiety and the Arg64 and Arg207 residues was allowed to occur.

As a result, the following statements can be suggested to the pharmaceutical chemist who will design and synthesize the new caspase-3 stimulants:

The presence of nitrogen-rich structures is valuable for activity, and the presence of aza-cyclic structures and protonable nitrogen significantly increases the activity. Because this structure can form hydrogen bonds with the amino acid Arg207.

The presence of one sp^3 carbon distance between the acetamide structure and the protonable nitrogen structure allows it to form hydrogen bonds with both the arginine loop amino acids (Arg64 and Arg207), and the peripheral loop amino acids (His121).

Interaction with hydrophobic cycle amino acids (Phe256) requires the presence of an aromatic structure linked to a heterocyclic structure with protonable nitrogen. Furthermore, the 3- to 5-carbon-long linkage that can be substituted at the 3rd position of this aromatic structure, as well as this linkage, should be suitable for hydrogen bonding. Finally, the heteroaromatic structure connected to this linkage will significantly affect the intensity of the activity.

3.3. Molecular Dynamics Study (MDS)

After determining the best poses of dasatinib and **MAC**, further analysis was carried out using MDS to check the complex stability and understand the structure-activity relationship (SAR) under environmental changes. The stability diagrams (**Figure 5**) point out that the values of both complexes are in the acceptable range. Two Rg plots indicated that there were no drastic changes during the simulation. RMSD values of **MAC** are under 3 Å, while RMSD values of dasatinib sometimes cross the line of 3 Å, yet it can be concluded that both complexes protect their stability. The RMSF plots displayed that the interactions with loop amino acids (green line in the white area) have a positive impact, such as decreasing the fluctuation intensity, hence, it can be said that both molecules stabilized their own ligand-protein complex. Although the interactions with Phe247-Pro263 loop amino acids differed between the two complexes, these interactions had no direct impact on the complex's stability because the major impact is revealed by Asn52-Gly66 loops and Ser198-Ser213 loops.

After approving the stability of the complex, the interaction types, their continuity, and their interaction strength were evaluated (**Figure 6**).

Dasatinib was interested in Arg64, Ser120, His121, Gln161, Tyr204, Trp206, Arg207, Asn208, Trp214, Phe250, Ser251, Asp253 and Phe256 residues (>0.2 interaction fraction). These interactions were observed as H-bond, water-mediated H-bond, aromatic H-bonds, hydrophobic, and ionic interactions (**Figure 6** and **video1**). The interaction continuity was between dasatinib and Arg207 (H-bond strength: 39% and 49%), Phe250 (H-bond strength: 88%), and Phe256 (π-π stacking strength: 36%; π-cation strength: 30%) amino acids. Interestingly, when connections with Tyr204 and Phe256 were observed, interactions with the Asn52-Gly66 loop region were lost, which points out that even if Arg64 and the other amino acids of this loop region support the stability of the complex, the connections with this loop may not be necessary to observe the activity. Thus, we suggest that it's enough to interact with at least one arginine amino acid to observe caspase-3 activity, although the recognition site is constituted by both arginine amino acids (Arg64 and Arg207) [30].

Meanwhile, **MAC** interacted with Met61, Thr62, Ser63, Arg64, Ser120, His121, Gly122, Glu123, Cys163, Tyr204, Ser205, Trp206, and Arg207 residues. These are mostly water-mediated H-bonds, as well as H-bonds, aromatic H-bonds, and hydrophobic interactions (**Figure 6** and **video2**). The interaction continuity was observed between **MAC**

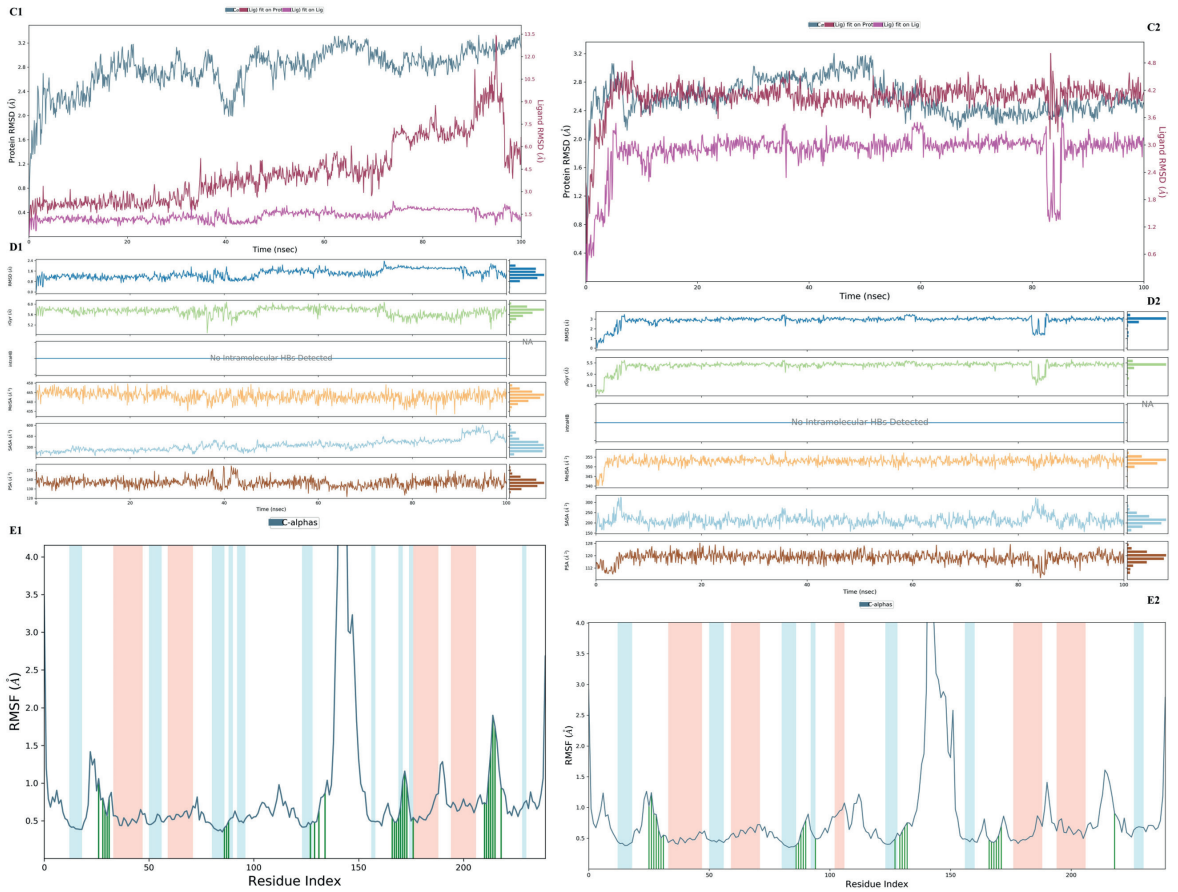


Figure 5. The stability diagrams of the complexes of caspase-3-dasatinib (**C1**, **D1** and **E1**) and MAC (**C2**, **D2** and **E2**); **C**,**D**: Ligand properties (RMSD, Rg, MolSA, SASA, PSA); **E**: RMSF, respectively.

and Gly122 (water-mediated H-bond strength: 57%), Glu123 (water-mediated H-bond strength: 52%), Ser205 (water-mediated H-bond strength: 59%), Trp206 (π-π stacking strength: 34%), and Arg207 (H-bond strength: 91%; water-mediated H-bond strength: 60% and 45%) residues (cutoff: 20%). Interestingly, even if the bond strength of **MAC**-Cys163 amino acids was not enough for the diagram, it is remarkable since it showed parallelism with the interactions of **MAC**-Arg207. Cys163 amino acid is an important amino acid, as the caspase-3 enzyme is a member of the cysteine-aspartic acid protease family. The functional effect of the enzyme starts here. Thus, we suggest that the moiety of the **MAC** [*N*-(thiazole-2-yl)acetamide] that interacts with Cys163 is a pharmacophore group (**video2**).

Generally, in addition to the above suggestions, the following statements can be suggested to the medicinal chemist who will design and synthesize new caspase-3 stimulants:

The *N*-(thiazol-2-yl)acetamide moiety is a pharmacophore structure, thus, this structure should be protected.

4-Pyridinyl substitution on the thiazole ring is remarkable, however, the pyridine ring can be substituted with a nucleophilic group (such as NH_2) to increase the activity. In fact, it also supports forming H-bonds. However, the carbonyl group on the pyridine ring may also be appropriate to interact with histamine and glycine amino acids, two members of the ß-sheet region of the enzyme.

The piperazine moiety is an essential group to observe caspase-3 activity since the proton able nitrogen atom interacts with important arginine amino acids (Arg64, Arg207).

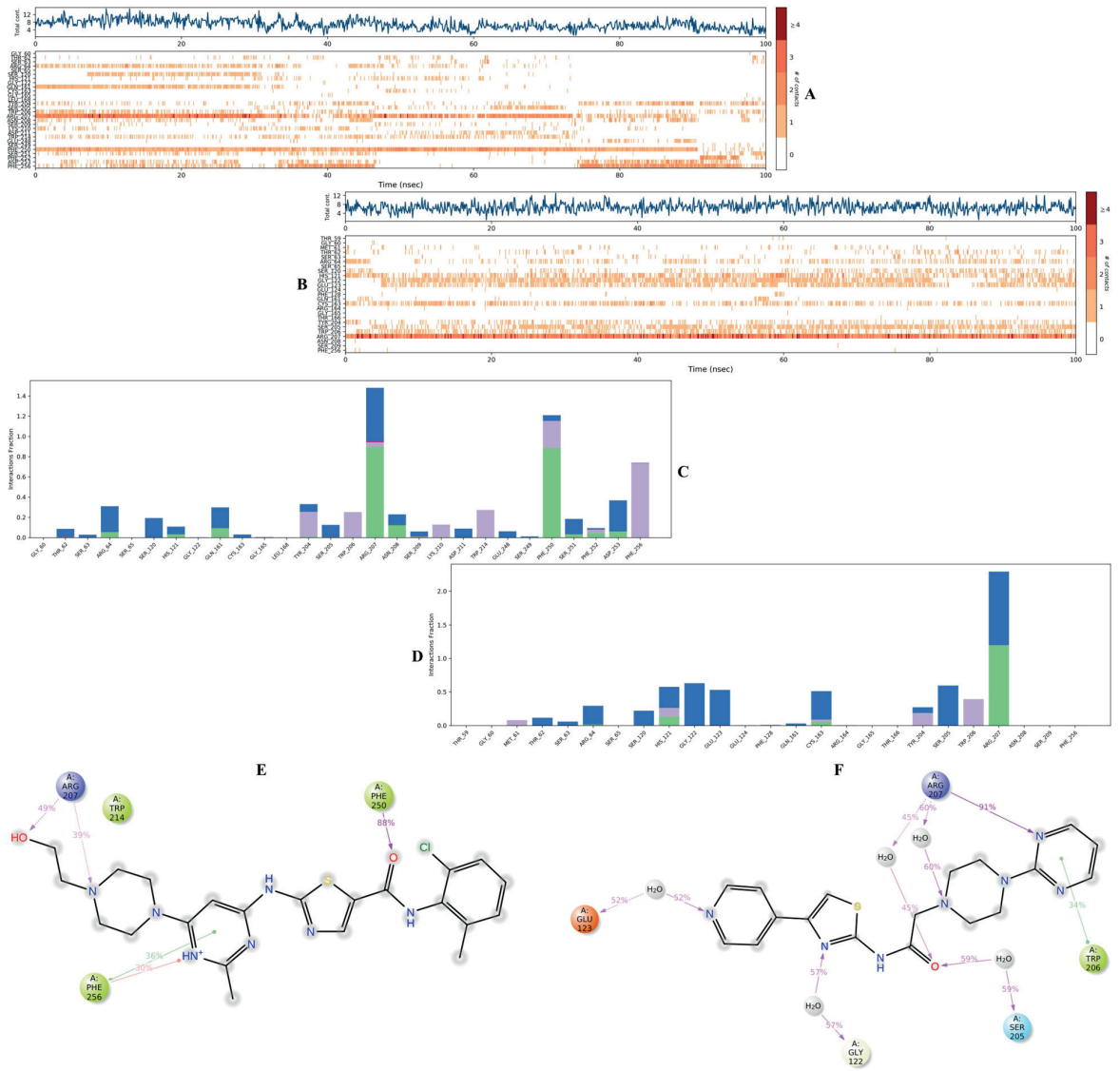


Figure 6. The interaction diagrams of the complexes of caspase-3-dasatinib (left) and MAC (right). **A**,**B**: The plot of total bond number-amino acid fraction during the simulation time; **C**,**D**: Types of interactions with the amino acids and their fraction graphic; **E**,**F**: The bond strength (cutoff=20%), respectively.

4. CONCLUSION

In this study, new design strategies were offered using a moderately active compound to get better activity and increase the apoptotic effect. For this purpose, a thiazole-piperazine derivative that had previously been synthesized and evaluated for its anticancer properties was used. The DFT, docking, and molecular dynamics simulation studies were performed on the caspase-3 enzyme, and the results were evaluated to understand the structure-activity relationship (SAR) and enable the explicate of structural modifications, thereby leading to the suggestion of new design strategies. As a result, the **MAC** has enabled us to develop new molecules based on its molecular core, supported by molecular mechanics.

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Author contribution

Concept: AEE, DN; Design: AEE, DN; Supervision: LY; Materials: AEE, DN; Data Collection and/or Processing: AEE, DN; Analysis and/or Interpretation: AEE, DN; Literature Search: AEE, DN; Writing: AEE, DN; Critical Reviews: LY.

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Conflict of interest

The authors declared that there is no conflict of interest.

REFERENCES

1. Lagorce D, Sperandio O, Galons H, Miteva MA, Villoutreix BO. FAF-Drugs2: free ADME/tox filtering tool to assist drug discovery and chemical biology projects. BMC Bioinformatics. 2008;9:396. https://doi.org/10.1186/1471-2105-9-396
2. Acar Cevik U, Saglik BN, Osmaniye D, et al. Synthesis and docking study of benzimidazole-triazolothiadiazine hybrids as aromatase inhibitors. Arch Pharm (Weinheim). 2020;353(5):e2000008. https://doi.org/10.1002/ardp.202000008
3. Saglik BN, Sen AM, Evren AE, et al. Synthesis, investigation of biological effects and in silico studies of new benzimidazole derivatives as aromatase inhibitors. Z Naturforsch C J Biosci. 2020;75(9-10):353-362. https://doi.org/10.1515/znc-2020-0104
4. Osmaniye D, Karaca Ş, Kurban B, et al. Design, synthesis, molecular docking and molecular dynamics studies of novel triazolothiadiazine derivatives containing furan or thiophene rings as anticancer agents. Bioorg Chem. 2022;122:105709. https://doi.org/10.1016/j.bioorg.2022.105709
5. Dawbaa S, Evren AE, Cantürk Z, Yurttaş L. Synthesis of new thiazole derivatives and evaluation of their antimicrobial and cytotoxic activities. Phosphorus Sulfur Silicon Relat Elem. 2021;196(12):1093-1102. https://doi.org/10.1080/10426507.2021.1972299
6. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 1997;23(1-3):3-25. https://doi.org/10.1016/s0169-409x(96)00423-1
7. Wani RR, Chaudhari HK. Docking and 3D QSAR Studies on Substituted Cyclobutylphenyl Quinoline Derivatives as Inhibitors of Bacterial DNA Gyrase. Curr Comput Aided Drug Des. 2018;14(4):322-337. https://doi.org/10.2174/1573409914666180516114700
8. Nuha D, Evren AE, Yılmaz Cankılıç M, Yurttaş L. Design and synthesis of novel 2,4,5-thiazole derivatives as 6-APA mimics and antimicrobial activity evaluation. Phosphorus Sulfur Silicon Relat Elem. 2021;196(10):954-960. https://doi.org/10.1080/10426507.2021.1946537
9. Doak BC, Zheng J, Dobritzsch D, Kihlberg J. How Beyond Rule of 5 Drugs and Clinical Candidates Bind to Their Targets. J Med Chem. 2016;59(6):2312-2327. https://doi.org/10.1021/acs.jmedchem.5b01286
10. Shultz MD. Two Decades under the Influence of the Rule of Five and the Changing Properties of Approved Oral Drugs. J Med Chem. 2019;62(4):1701-1714. https://doi.org/10.1021/acs.jmedchem.8b00686
11. Evren AE, Yurttaş L, Eksellı B, Akalın-Cıftcı G. Novel Tri-substituted Thiazoles Bearing Piperazine Ring: Synthesis and Evaluation of their Anticancer Activity. Lett Drug Des Discov. 2019;16(5):547-555. https://doi.org/10.2174/1570180815666180731122118
12. Frisch M, Trucks G, Schlegel H, et al. Gaussian09, Revision A. Wallingford CT Inc.; 2009. p. 150-166.
13. Dennington R, Keith T, Millam J. GaussView, version 5. 2009.
14. Nuha D, Evren AE, Ciyanci ZS, Temel HE, Akalin Ciftci G, Yurttas L. Synthesis, density functional theory calculation, molecular docking studies, and evaluation of novel 5-nitrothiophene derivatives for anticancer activity. Arch Pharm (Weinheim). 2022;355(9):e2200105. https://doi.org/10.1002/ardp.202200105
15. Nuha D, Evren AE, Kapusiz Ö, et al. Design, synthesis, and antimicrobial activity of novel coumarin derivatives: An in-silico and in-vitro study. J Mol Struct. 2023;1272:134166. https://doi.org/10.1016/j.molstruc.2022.134166
16. Dawbaa S, Nuha D, Evren AE, CankiliÇ MY, YurttaŞ L, Turan G. New Oxadiazole/Triazole Derivatives with Antimicrobial and Antioxidant Properties. J Mol Struct. 2023:135213. https://doi.org/10.1016/j.molstruc.2023.135213
17. Schrödinger Release. 2020-3, Maestro. Schrödinger, LLC, New York, NY, USA; 2020.
18. Yurttaş L, Evren AE, Özkay Y. Synthesis and In Silico Evaluation of Some New 2,4-Disubstituted Thiazole Derivatives. European Journal of Life Sciences. 2022;1(1):8-19. https://doi.org/10.55971/ejls.1089425
19. Evren AE. Bazi yeni kinolin türevlerinin sentezi, antimikrobiyal ve DNA giraz inhibitörü etkilerinin araştırılması [master's thesis]. Eskişehir, Türkiye: Anadolu Üniversitesi; 2019.

20. Schrödinger Release. 2020-3, LigPrep. Schrödinger, LLC, New York, NY, USA; 2020.

21. Schrödinger Release. 2020-3, Glide. Schrödinger, LLC, New York, NY, USA; 2020.

22. Turan Yucel N, Evren AE, Kandemir U, Can OD. Antidepressant-like effect of tofisopam in mice: A behavioural, molecular docking and MD simulation study. J Psychopharmacol. 2022;36(7):819-835. https://doi.org/10.1177/02698811221095528

23. Dawbaa S, Evren AE, Saglik BN, Gundogdu-Karaburun N, Karaburun AC. Biological activity evaluation of novel monoamine oxidase inhibitory compounds targeting Parkinson disease. Future Med Chem. 2022;14(22):1663-1679. https://doi.org/10.4155/fmc-2022-0167

24. Evren AE, Nuha D, Dawbaa S, Saglik BN, Yurttas L. Synthesis of novel thiazolyl hydrazone derivatives as potent dual monoamine oxidase-aromatase inhibitors. Eur J Med Chem. 2022;229:114097. https://doi.org/10.1016/j.ejmech.2021.114097

25. Schrödinger Release. 2020-3, Desmond. Schrödinger, LLC, New York, NY, USA; 2020.

26. Fukui K. Role of frontier orbitals in chemical reactions. Science. 1982;218(4574):747-754. https://doi.org/10.1126/science.218.4574.747

27. Politzer P, Murray JS. The fundamental nature and role of the electrostatic potential in atoms and molecules. Theoretical Chemistry Accounts: Theory, Computation, and Modeling (Theoretica Chimica Acta). 2002;108(3):134-142. https://doi.org/10.1007/s00214-002-0363-9

28. Luque FJ, Orozco M, Bhadane PK, Gadre SR. SCRF calculation of the effect of water on the topology of the molecular electrostatic potential. The Journal of Physical Chemistry. 2002;97(37):9380-9384. https://doi.org/10.1021/j100139a021

29. Cade C, Swartz P, MacKenzie SH, Clark AC. Modifying caspase-3 activity by altering allosteric networks. Biochemistry. 2014;53(48):7582-7595. https://doi.org/10.1021/bi500874k

30. Luo M, Lu Z, Sun H, et al. Nuclear entry of active caspase-3 is facilitated by its p3-recognition-based specific cleavage activity. Cell Res. 2010;20(2):211-222. https://doi.org/10.1038/cr.2010.9