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ORIGINAL ARTICLE / ÖZGÜN MAKALE

MOLECULAR DOCKING STUDIES OF SOME TETRAHYDRONAPHTALENE-BENZIMIDAZOLE DERIVATIVES AND CORRELATION WITH THEIR CORRESPONDING ANTI-MRSA ACTIVITIES

YENİ TETRAHİDRONAFTALEN-BENZİMİDAZOL TÜREVİ BİLEŞİKLERİN MOLEKÜLER DOKİNG ÇALIŞMALARI VE ONLARIN ANTİ-MRSA AKTİVİTELERİNİN KARŞILAŞTIRILMASI

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ABSTRACT

Objective: *Methicillin- resistant S. aureus (MRSA) is a type of bacteria which is resistant to various types of antibiotics and causes mortality in hospital environment and community. To further investigate the inhibition activity of previously synthesized retinoidal compounds against MRSA, docking studies of these compounds with MRSA pyruvate kinase (PK) were made.*

Material and Method: *As a first step, ligand preparation procedure has been made. For optimization of compounds, Hyperchem Professional was used. Molecular Mechanics Force Field (MMFF) and semi-empirical methods have been implemented in this program. After converting the ligands to pdb files, charges and torsions were added via AutoDockTools 1.5.6. Macromolecule file for MRSA Pyruvate kinase (PDB ID:3T07) was procured from protein data bank. Appropriate chain for binding was chosen via UCSF Chimera. Polar hydrogens and Gasteiger charges were added to macromolecule via AutoDockTools 1.5.6. Gridbox has been predicted by protein- ligand complex which is currently present in protein data bank and prepared via same software. Docking process was made via AutoDock Vina. For MIC values of retinoidal compounds, previous study by Ates-Alagoz et al. has been used. In addition, some QSAR properties were calculated via Hyperchem Professional and were also interpreted.*

Result and Discussion: *Compounds 1, 4, 5, 6, 7 were selected for their PK inhibitor activities. Addition to their relatively lower MIC values, they also show similar binding modes to previously presented PK inhibitor candidates. Binding of compounds with His365 and Ile361 in both monomeric units of PK, creates a bridge that*

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links these units. In terms of QSAR, molecular volume below 1010 Å3 is favorable. Moreover, log P does not have an impact on activity. This binding mode and interactions with aminoacid residues may be the cause of their promising inhibition activity against MRSA Pyruvate kinase.

Keywords: *benzimidazoles, docking, bioinformatics, antibiotics, QSAR*

ÖZ

Amaç: *Metisiline dirençli S. aureus (MRSA), birçok antibiyotiğe karşı dirençli olup, özellikle hastane ortamında mortaliteye neden olmaktadır. Önceden sentezlenmiş olan retinoidal bileşiklerin MRSA üzerindeki inhibitor aktivitesini incelemek adına bu bileşiklerin QSAR özellikleri hesaplanmış ve MRSA Pirüvat kinaz (PK) ile doking çalışmaları gerçekleştirilmiştir.*

Gereç ve Yöntem: *Başlangıçta, ligand hazırlama gerçekleştirilmiştir. Bileşiklerin optimizasyonu için Hyperchem Professional kullanılmış, bu yazılımda Molecular Mechanics Force Field (MMFF) ve semi-empirik metod uygulanmıştır. Ligand dosyalarını pdb formatına dönüştürdükten sonra, yük ve torsiyon özellikleri AutoDockTools 1.5.6 ile eklenmiştir. MRSA pirüvat kinaz makromolekül dosyası (PDB ID: 3T07) protein data bank'tan alınmıştır. Bağlanma için uygun zincir UCSF Chimera ile belirlenmiştir. Makromoleküle polar hidrojenler ve Gasteiger yükleri AutoDockTools 1.5.6 ile eklenmiştir. Ligandın proteine bağlanma cebi, protein data bank'taki protein-ligand kompleksinden yola çıkılarak tespit edilmiştir. Retinoidal bileşiklerin MİK değerleri için Ates-Alagoz ve ark. tarafından yapılmış olan çalışmaya başvurulmuştur.*

Sonuç ve Tartışma: *1, 4, 5, 6, 7 numaralı bileşikler PK inhibitor aktiviteleri en yüksek adaylar olarak seçilmiştir. Bu bileşikler, nispeten düşük olan MİK değerlerine ek olarak önceden bildirilen PK inhibitörü aday bileşiklere benzer bağlanma şekilleri göstermiştir. PK'nın iki monomerik ünitesinde yer alan His365 ve Ile361 ile etkileşme, bu üniteler arasında bir köprü oluşturmaktadır. Yapılan QSAR hesaplamasına göre, en aktif olan bileşiklerde 1010 Å3'dan düşük moleküler hacim değerleri görülmektedir. Buna ek olarak, log P'nin aktivite üzerinde bir etkiye sahip olmadığı görülmüştür. Bu bağlanma şekli ve etkileşimler, söz konusu bileşiklerin MRSA Pirüvat kinaz'daki umut verici inhibitor aktivitelerinin nedeni olabilir.*

Anahtar Kelimeler: *Benzimidazol, doking, biyoinformatik, antibiyotikler, QSAR*

INTRODUCTON

Therapy protocols against infections caused especially by *S. aureus* have become rather challenging due to increased resistance [1]. Among other pathogens, it is the most encountered one in both hospital environment and community. In this case, in addition to higher incidence, this type of bacteria also inflicts higher mortality rates on patients [2].

Pyruvate kinase is an enzyme at the final-stage of glycolysis and catalyzes the transfer of phosphoryl group from phosphoenolpyruvate (PEP) to adenosine diphosphate (ADP), thus generates adenosine triphosphate (ATP) and pyruvate [3]. This reaction is a major control point in the regulation of glycolytic flux [4]. Same enzyme also takes part in numerous metabolic pathways as an intersection [5]. Consequently, PK plays a key role in not only energy generation, but also in control of metabolic flux distribution [6-9]. Inhibiting this enzyme disrupts energy production and metabolic stability of bacteria. To implement efficient inhibitors against this enzyme, various classes of compounds have been evaluated. Retinoidal compounds shows promise as anti-MRSA agents [10].

MATERIAL AND METHOD

The 2D structures of the compounds were drawn on ChemDraw Ultra 12.0, minimized on Hyperchem Professional [11] with MMFF and semi-empirical method. Then these files were converted to pdb files using Avogadro software [12]. Subsequently, charges and torsion were added to ligand files with AutoDockTools 1.5.6 [13]. The 3D crystal structure of the MRSA pyruvate kinase (PDB ID:3T07; resolution 3.3 Å) was retrieved from protein data bank [14]. RMSD values of protein in complex with cis-3,4-dihydrohamacanthin B are 0.011 for bond lengths (Å) and 1.413 for bond angles (º). Furthermore, monomeric structure was conserved between holo and apo structure (RMSD = 0.56 Å). [15] UCSF Chimera [16] was used for deleting waters, choosing the appropriate chain for binding. After this process, polar hydrogens and Gasteiger charges were added using AutoDockTools 1.5.6. Grid box was also prepared via this software. Prepared ligands were docked with Autodock Vina [17], interaction diagrams and binding modes were created using Discovery Studio Visualizer [18].

RESULTS AND DISCUSSION

In addition to their docking study, molecular descriptor calculation of these compounds have been made. Compounds 1, 4, 5, 6, 7 which show favorable inhibition against MRSA PK also have lower molecular volumes (below 1010 Å3). Volume may effect the compound's permeability into cell, as well as their binding to the cavity between two monomeric units of PK. Most potent of tested compounds 7 has the highest log P but interestingly does not fit Lipinski's rule of five. All of the listed compounds show suitable log P values to this rule. Therefore, Log P may not play an essential role in binding.

Table 1. Calculated QSAR properties via Hyperchem Professional.

	Surface Area (Approximate) ($Å^2$)	**Surface Area (grid)** ($Å^2$)	**Volume** ($Å^3$)	**Hydration Energy**(kcal/mol)	**Log P**	**Refractivity** ($Å^3$)	**Polarizability** ($Å^3$)	**Mass** (amu)
1	502.41	590.01	1009.91	-7.37	2.15	109.15	39.90	348.44
2	550.64	628.13	1070.98	-2.78	2.18	113.92	41.74	362.47
3	583.49	662.28	1127.72	-2.15	2.52	118.67	43.57	376.50
4	489.02	570.98	976.03	-2.10	2.54	107.87	39.27	338.88
5	451.99	548.21	931.44	-2.43	2.76	103.15	37.35	304.43
6	495.70	575.25	985.21	-1.25	2.92	107.43	39.18	318.46
7	508.62	584.57	995.92	-7.32	6.11	108.87	39.19	349.43
8	491.33	577.54	988.79	-6.20	0.02	108.87	39.19	349.43
9	578.05	643.98	1108.89	-0.53	2.43	118.82	43.57	376.50
10	529.73	608.39	1047.36	-5.10	2.39	114.05	41.74	362.47
11	622.64	687.87	1215.48	0.53	3.11	128.31	47.24	404.55
12	541.45	616.94	1095.15	-4.69	2.74	118.80	43.57	376.50
13	664.58	741.29	1334.09	-1.24	3.22	152.28	55.16	487.04

In the docking pattern for MRSA pyruvate kinase which was previously presented by El Sayed et al. [1] H-bonds and steric interactions with Thr353, Ser354, Ile361, Ser362, His365, Thr366 and Asn369 are present. A link between two monomeric units of PK may play a vital role in inhibition of the enzyme. Benzimidazole and tetrahydronaphtalene structures in main scaffold are responsible for hydrophobic interactions. Compound 1 forms alkyl and pi interactions with His365 (A, B) and Ile361 (A, B) residues in both monomeric sub-units of pyruvate kinase. Additionally, carboxyl group in its benzimidazole ring causes a H-bond with Ala358. In a similar manner, compound 4 interacts with His365 and Ile361 on both sides. Chlorine group which has taken the place of carboxyl causes hydrophobic interactions. Nonsubstituted compound 5, other than hydrophobic interaction with benzimidazole and Ala358, shows similar pattern with His365 and Ile361. Most potent derivative compound 7 which has the lowest MIC, solely forms steric interactions with His365 and Ile361.

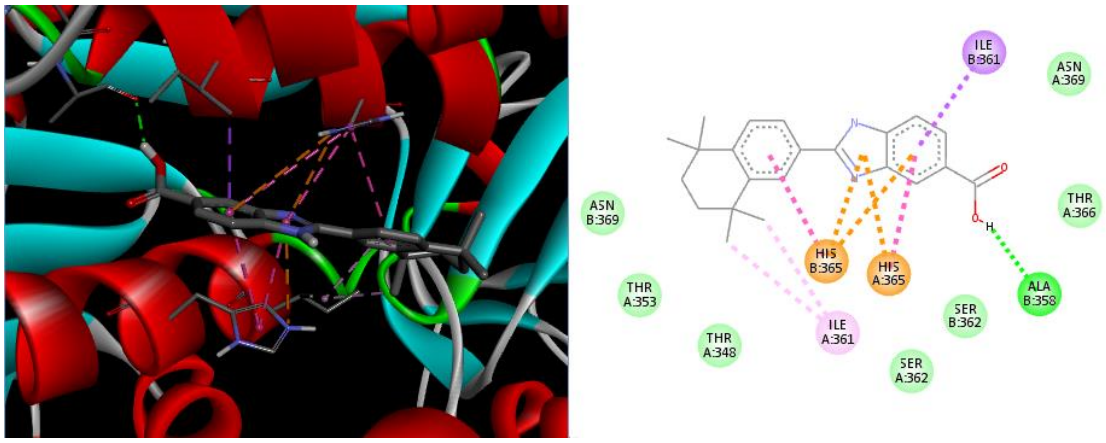

Figure 1. Binding mode of compound **1** with *MRSA* Pyruvate kinase. Hydrogen bonding interactions are shown in green, light pink being alkyl interaction whereas pink and magenta are Pi interactions.

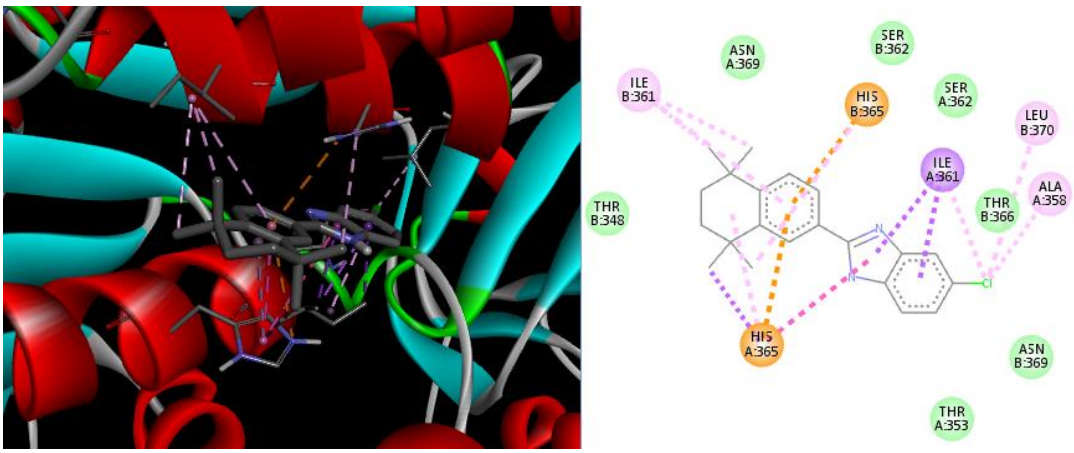

Figure 2. Binding mode of compound **4** with *MRSA* Pyruvate kinase. Hydrogen bonding interactions are shown in green, light pink being alkyl interaction whereas pink and magenta are Pi interactions.

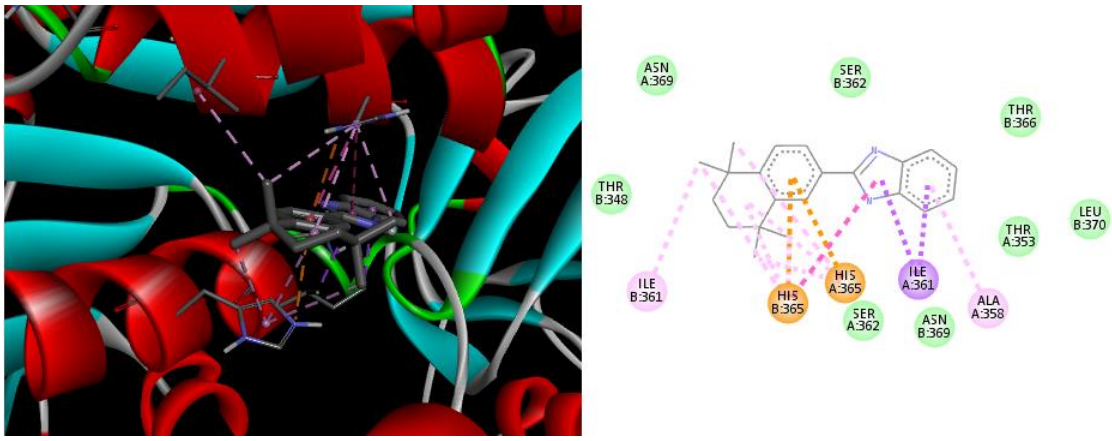

Figure 3. Binding mode of compound **5.**

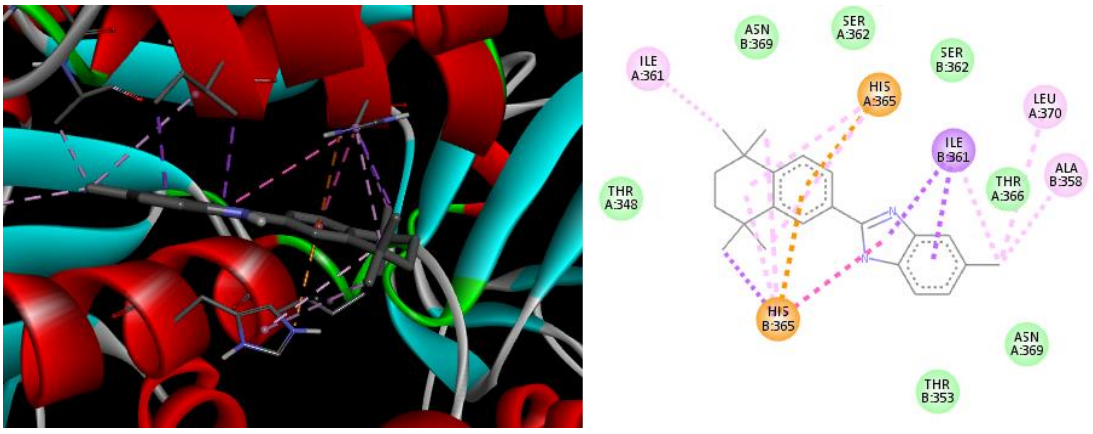

Figure 4. Binding mode of compound **6.**

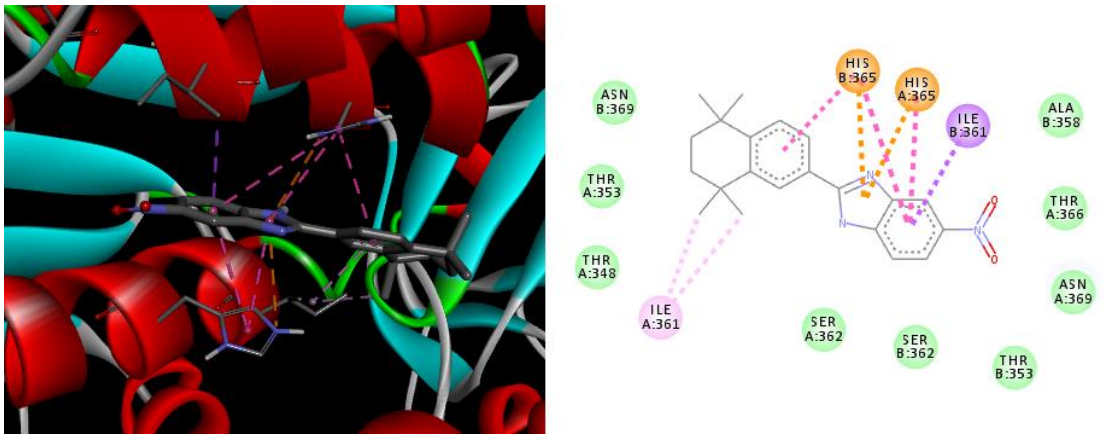

Figure 5. Binding mode of compound **7.**

Table 2. Calculated binding energy values of cis-3,4-dihydrohamacanthin B, and tetrahydronaphtalene-benzimidazole derivatives.

	Binding energy (kcal/mol)
cis-3,4-dihydrohamacanthin B	-9.0
1	-8.6
4	-8.1
5	-8.0
6	-8.4
7	-8.3

According to these results, suitable candidates can be developed. Compounds to be developed as inhibitors against MRSA PK, need to have molecular volumes below 1010 $Å^3$ and they have to interact with previously presented amino acids in the literature, namely His365 and Ile361 in both monomeric units. Partition coefficient, however have no significant influence on inhibiting this enzyme. As seen on table, tested compounds exhibit distinct values below the ligand from complex. By doing so, they may very well be potent inhibitors. In our future studies, we intend to develop new compounds in accordance with this guideline.

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