In Silico Profiling of Binding Affinities of Hybrid Molecules of Oseltamivir Carboxamides Cross-linked with Hydroxamic Acid as possible Anti-influenza Agents

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Abstract:

Influenza is an infectious respiratory disease caused by the influenza virus and is a persistent and significant global public health concern. This infection displays a tendency for recurring outbreaks during specific seasons and occasional widespread epidemics. A number of drugs and vaccines have been approved and recommended to treat this infection.

Oseltamivir is an FDA-Approved anti-influenza drug acts by inhibiting influenza Neuramidase enzyme and used as therapeutic management and prophylaxis of influenza types A and B and by this way preventing virus budding and release and impeding the dissemination of the virus and mitigating the intensity and duration of influenza infection. Oseltamivir is administered as a prodrug in the form of an ethyl ester and in the body hepatic esterases convert it to the active oseltamivir carboxylate. An approach of synthesizing Oseltamivir carboxamides with certain amino acids (series one) and Oseltamivir-amino acid-Hydroxamic acid conjugates, as hybrid molecules (series two), these may enhance efficacy, penetration into various organs, including lung tissues, and manage resistance of Oseltamivir. These two series of compounds were subjected to molecular docking using GOLD Suite (version 5.7.1) on Neuramidase. These hybrids have recorded slightly higher PLP fitness, binding affinities represented as docking scores (59.14-72.23 Kcal/mol) based on the lowest docking scores on the target enzyme compared to Oseltamivir acid (56.24 Kcal/mol).

Key words: Oseltamivir, Amino acids, Hydroxamic acid, Molecular hybridization.

تحليل الربط الحاسوبي للتفاعلات التشبيكية للجزيئات المختلطة من كاربوكساميدات أوزيلتاميفير المترابطة مع حمض الهيدروكساميك كوكلاء محتملين لمكافحة الإنفلونزا

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الخلاصة:

الإنفلونزا هي مرض تنفسي معدي يسببه فيروس الإنفلونزا، وهو قضية صحية عامة عالمية مستمرة وهامة. يظهر هذا العدوى تصاعداً متكرراً خلال فصول معينة وفي بعض الأحيان تفشيات واسعة المدى. تمت الموافقة على عدد من الأدوية واللقاحات

وتوصي بها لعلاج هذه العدوى. أوزيلتاميفير هو دواء معتمد من قبل إدارة الغذاء والدواء الأمريكية لعلاج الإنفلونزا، حيث يعمل عن طريق تثبيط إنزيم نيوراميداز الإنفلونزا، ويُستخدم في الإدارة العلاجية والوقائية لأنواع الإنفلونزا A وB، وبهذه الطريقة يمنع تكوين الفيروس وإطلاقه ويحجب انتشار الفيروس ويخفف من شدة ومدة الإصابة بالإنفلونزا. يُعطى أوزيلتاميفير في شكل استر الإيثيل، وفي الجسم، تحوله الإنزيمات الكبدية إلى أوزيلتاميفير الكاربوكسيلات الفعّال.
يُعتبر نهج تخليق كاربوكساميدات أوزيلتاميفير مع بعض الأحماض الأمينية المحددة (السلسلة الأولى) وتكامل أوزيلتاميفير مع حمض الأمين وحمض الهيدروكساميك كجزيئات هجينة (السلسلة الثانية)، ويُفترض أن يعززوا الفعالية والاختراق إلى أعضاء متنوعة، بما في ذلك أنسجة الرئة، ويقلل مقاومة أوزيلتاميفير. تمت خضوع هاتين السلسلتين من المركبات إلى عمليات النمذجة الجزيئية باستخدام GOLD Suite (الإصدار 5.7.1) على إنزيم النيوراميداز. سُجلت هذه الهجن PLPأعلى قليلاً، وتمثل القوى الرابطة على شكل درجات الربط (59.14-72.23 كيلوكالوري/مول) استناداً إلى أدنى درجات الربط على الإنزيم المستهدف مقارنة بحمض الأوزيلتاميفير (56.24 كيلوكالوري/مول).

الكلمات المفتاحية: أوزيلتاميفير, الأحماض الأمينية, حمض الهيدروكساميك, كجزيئات هجينه.

Introduction

Influenza, an infectious respiratory disease caused by the influenza virus, is a persistent and significant global public health concern [1]. The viral infection, which displays a tendency for recurring outbreaks during specific seasons and occasional widespread epidemics, has a significant impact on society and the economy, while also posing an ongoing risk to human well-being [2]. The etiological agents, which are usually classed as Influenza A, B, and C, exhibit a notable propensity for genetic change [3,4]. This characteristic enables the virus to avoid immune responses and necessitates the frequent updating of vaccinations [5].

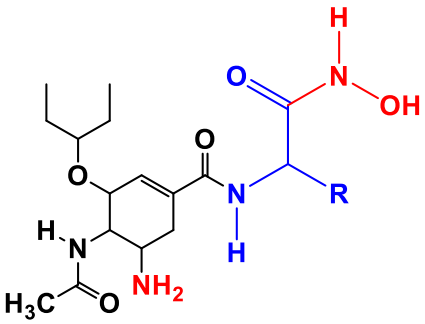
Neuraminidase is the most attractive target for the development of novel medications because it is an essential enzyme for viral replication [6,7]. The glycoside hydrolase neuraminidase breaks down sialic acid residues in glycoproteins and glycolipids found on the surface of host cells [8]. The discharge of freshly generated virions from infected cells and the virus's ability to infect other host cells depend on this mechanism [9]. N1 through N9, the nine subtypes of Neuraminidase NA, are present in influenza A and B viruses [10]. Cavity 150 and cavity 430 are two hydrophobic pockets located near the active site of neuraminidase [11]. They are formed by residues from the 150 loop and 430 loop, respectively [12]. These two cavities play an important role in the binding of oseltamivir to neuraminidase [13]. Cavity 150 is a relatively small cavity, lined by residues such as Tyr151, Leu222, Trp147, and Leu152 [14]. Cavity 430 is a slightly larger cavity, lined by residues such as Tyr430, Leu222, Trp429, and Leu433 [15]. The modification of the amino group of oseltamivir has been shown to improve its efficacy against drug-resistant strains of influenza [16]. Several studies have investigated the modification of the amino group of oseltamivir, including the substitution with acyl guanidine carboxylate derivatives and modification of the functional groups of oseltamivir [17,18]. These modifications have shown improved activity against drug-resistant strains of influenza [19]. The structure, function, mechanism, and inhibition by natural products of neuraminidase inhibitors will all be covered in this report's medical chemistry section [20]

Experimental work

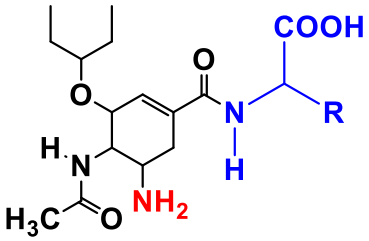
Preparation of the investigated hybrid molecules

Oseltamivir carboxamides with aliphatic and aromatic amino acids (series one) were prepared using the ester aminolysis method, as adopted for this work. Oseltamivir carboxamides were reacted with hydroxylamine. HCl using Zinc dust catalysis method [21] to afford Oseltamivir carboxamides cross-linked with hydroxamic

acid, as the hybrid molecules (series two). The chemical structures of the new hybrids and their SMILES (Simplified Molecular-Input Line-Entry Systems) notations were constructed using Chemdraw ultra-10.0. The chemical structures of the newly synthesized hybrids were shown on Figure 1.



Series 2



Series 1

Compounds of **Series 1**, **S1**: R= -CH-(CH3)2 (Valine), **S2**: R= -CH2-OH (Serine), **S3**: R= Phenol (Tyrosine), **S4**: Phenyl (Phenylalanine).
Compounds of Series **2**, **S5-S8** (Hydroxamate derivatives).

Figure 1. The chemical structures of the newly designed Oseltamivir carboxamides and the hybrid molecules

Molecular docking

Molecular docking has been carried out using GOLD Suite (version 5.7.1) and the PLP Fitness (kcal/mol) as the docking scores function representing the energy required for binding to receptor. The chemical structure of Neuramidase was retrieved from protein data bank (PDB: 3CLO). The ΔG (kcal/mol) and the amino acids that are involved in the interaction of the hybrid molecules and Oseltamivir to the target enzyme Neuramidase of influenza N1 type 3CLO were listed on **Table 1**. The successful candidates were those that recorded high binding affinities based on the high PLP fitness scores (Kcal/mole).

Computational methods for the characterization of the investigated hybrid molecules

ADME program

The SwissADME server [22] was used to predict the ADME parameters (Absorption, Distribution, Metabolism and Excretion) and the other physicochemical properties of the newly synthesized hybrids. ChemAxon's Marvin JS was used to draw the chemical structures of all compounds and their SMILES notations. The BOILED EGG approach was used to assess the possibility of passive gastrointestinal absorption and brain penetration, and the polarity and lipophilicity of the investigated small molecules [23].

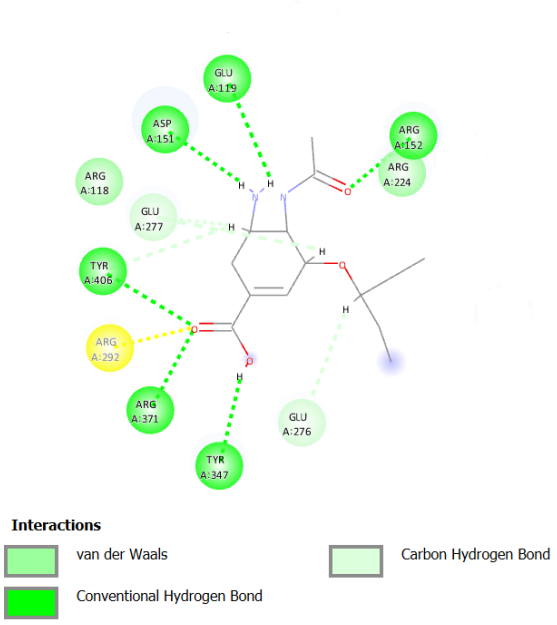
Results and Discussion

The docking procedure was optimized using GOLD (Genetic Optimization for Ligand Docking) software. The Protein Data Bank of neuraminidase of influenza N1 type (PDB: ID 3CL0), provides access to the 3.0-A resolution crystal structure of this enzyme.

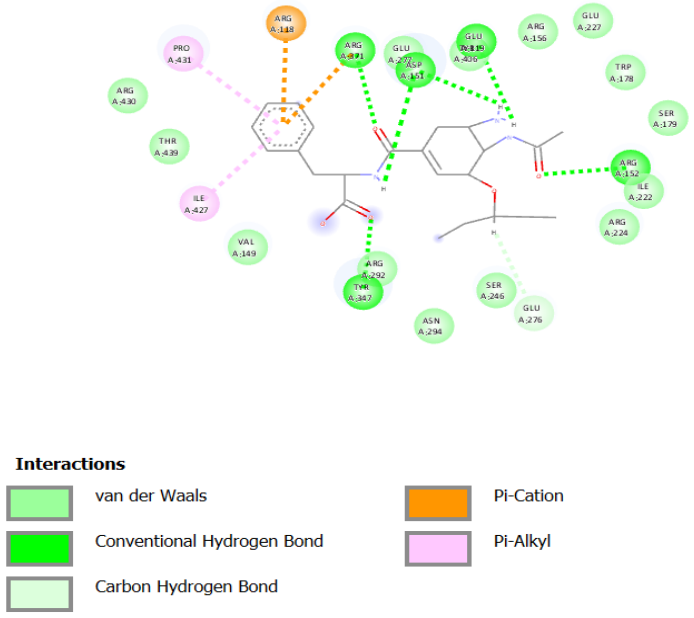
All the hybrid molecules have recorded PLP fitness scores higher than Oseltamivir acid

and that might be due to the better scaffold of the new compounds. Moreover, compounds **S4, S7** and **S8** have recorded the maximum binding ability to neuraminidase enzyme (**Table 1**). Compounds of **series 1** recorded comparable results of docking on neuramidase type 3CLO to that of Oseltamivir acid, although, slightly better binding affinities (**Table 1**), particularly, compound **S4**, which included a phenylalanine moiety. Apparently, an aromatic moiety may contribute to the binding affinity in a much better way than the aliphatic moieties. However, this case was not noticed with compound **S3**, which contain a tyrosine moiety. This may be due to the presence of a p-phenolic hydroxyl group leading to formation of weak hydrogen bonding rather that hydrophobic binding. The interaction of Oseltamivir and Oseltamivir carboxamide with phenylalanine and Oseltamivir carboxamide linked to hydroxamate with neuramidase type 3CLO were illustrated on **Figure 2**. The hydrogen bonding and the hydrophobic interaction were clearly shown with their counterpart's functionalities on the target site.

Oseltamivir acid



Oseltamivir carboxamide with L-Phenylalanine



Oseltamivir carboxamide with L-Phenylalanine-Hydroxamate

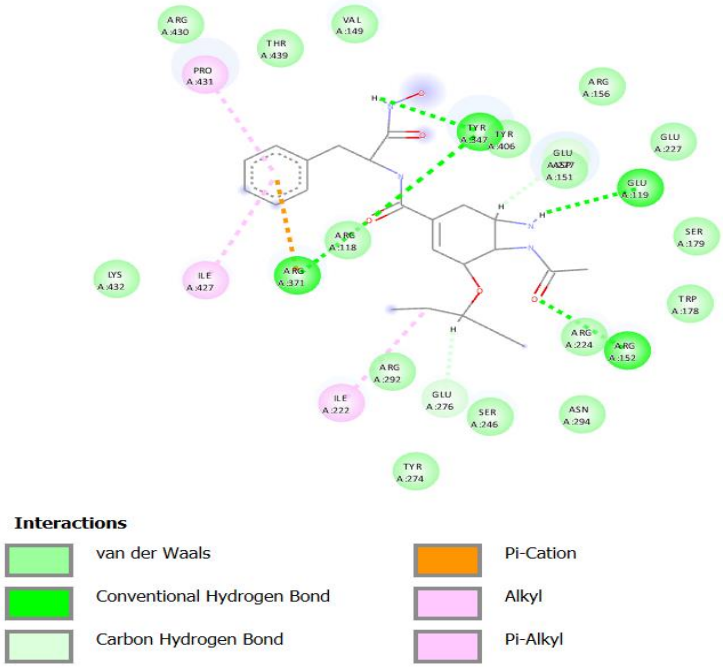


Figure 2. Interaction of Oseltamivir and Oseltamivir-linked to L-phenylalanine and Oseltamivir-L- Phenylalanine hydroxamate on neuramidase type 3CLO. Dark green represents the hydrogen bonding, while the light green represents the Van der Waals bonding and the faint green represent the carbon hydrogen bonding.

Table 1. Docking scores of Oseltamivir carboxamides and the hybrid molecules on neuramidase of influenza virus N1 (type 3CLO).

Code	Compound	PLP fitness	Amino acids that are involved in interaction
---	**Oseltamivir acid**	**56.24**	**Glu119, Arg224, Arg292, Tyr347, Arg371, Tyr406**
S1	Oseltamivir-Valine	**59.14**	Arg118, **Glu119**, Arg152, **Arg224**, **Tyr347**
S2	Oseltamivir-Serine	**60.93**	Arg118, **Glu119**, Asp151, Arg152, **Arg224**, Glu277, **Tyr347**
S3	Oseltamivir-Tyrosine	**60.73**	Arg118, **Glu119**, Asp151, Arg152, Trp178, Ile222, **Arg224**, Tyr274, Glu277, **Tyr347**
S4	Oseltamivir-Phenylalanine	**72.23**	Arg118, **Glu119**, Arg152, **Arg224**, **Tyr347**, **Arg371**
S5	Oseltamivir-Valine-Hydroxamate	**61.81**	Arg118, **Glu119**, Asp151, Arg152, Ile222, Ser246, **Arg347, Tyr371**
S6	Oseltamivir-Serine-Hydroxamate	**61.38**	Arg118, **Glu119**, Asp151, Arg152, Ile222, Ser246, Glu277, **Tyr347, Arg371**
S7	Oseltamivir-Tyrosine-Hydroxamate	**64.31**	Arg118, **Glu119**, Val149, Asp151, Arg152, Ile222, Ser246, **Tyr347, Arg371**
S8	Oseltamivir-Phenylalanine-Hydroxamate	**70.18**	Arg118, **Glu119**, Asp151, Arg152, Ile222, **Arg224**, Ser246, **Tyr347, Arg371**

The investigated compounds were tested against Lipinski rule violations [21] and the results are listed on **Table 2**. All the compounds were clear of any violations except compounds **S7** and **S8**, which have shown one violation and exceeding the H-bond donor by one group.

Table 2: Lipinski parameters for Oseltamivir acid and the hybrid molecules.

Code	Compound	Lipinski violations	H-bond donor	H-bond acceptor
---	Oseltamivir acid		4	6
S1	Oseltamivir-Valine	Yes; 0 violation	4	6
S2	Oseltamivir-Serine	Yes; 0 violation	5	7
S3	Oseltamivir-Tyrosine	Yes; 0 violation	5	7
S4	Oseltamivir-Phenylalanine	Yes; 0 violation	5	6
S5	Oseltamivir-Valine-Hydroxamate	Yes; 0 violation	5	6
S6	Oseltamivir-Serine-Hydroxamate	Yes; 0 violation	6	7
S7	Oseltamivir-Tyrosine-Hydroxamate	Yes; 1 violation: NH or OH>5	6	7
S8	Oseltamivir-Phenylalanine-Hydroxamate	Yes; 1 violation: NH or OH>6	2	5

The investigated compounds have shown low indication of absorption through the gastrointestinal tract, except compounds **S1** and **S2**, which showed possible high absorption, when compared with Oseltamivir, as listed on **Table 3**. All the hybrid molecules **S5-S8** (hydroxamate-containing compounds) recorded low indication of oral absorption. Compounds of series **1** and series **2** (**S1-S8**) recorded very low log Kp values in comparison to that of Oseltamivir.

Table 3. Pharmacokinetic properties of Oseltamivir acid and the hybrid molecules.

Code	Compound	GI absorption	log Kp (cm/s)
---	Oseltamivir	High	-7.42
S1	Oseltamivir-Valine	High	-9.31
S2	Oseltamivir-Serine	High	-9.46
S3	Oseltamivir-Tyrosine	Low	-10.82
S4	Oseltamivir-Phenylalanine	Low	-9.66
S5	Oseltamivir-Valine-Hydroxamate	Low	-8.31
S6	Oseltamivir-Serine-Hydroxamate	Low	-8.46
S7	Oseltamivir-Tyrosine-Hydroxamate	Low	-9.81
S8	Oseltamivir-Phenylalanine-Hydroxamate	Low	-8.66

Conclusion

Briefly, two series of Oseltamivir acid-based compounds were considered and evaluated using virtual screening. All these compounds had higher PLP fitness scores than Oseltamivir acid. The use of aliphatic or aromatic amino acids did make much difference in binding affinities. The compounds did not show any violations of Lipinski rule, except compounds **S7** and **S8**. The GI absorption was predicted to be of higher values for compounds **S1** and **S2**, while, the other compounds recorded low values.

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