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Original Research Article

In silico studies on novel inhibitors of MERS-CoV: Structure-based pharmacophore modeling, database screening and molecular docking

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Abstract

Purpose: *To search for novel scaffolds as potential inhibitors of 3CLpro protease enzyme and as antiviral drugs.*
Methods: *NCI database was screened using structure-based pharmacophore modeling, database screening and molecular docking. Also, Lipininski's rule of 5 was applied in order to test the drug-likeness of the retrieved compound. Pharmacophore modelling and subsequent post-docking analyses were used for comparison of the binding mode of the retrieved hits with that of the x-ray inhibitor, R30, against MERS-CoV 3CLpro enzyme.*
Results: *Five compounds were identified as potential agents for the treatment of corona virus, MERS-CoV, which showed similar binding to MERS-CoV 3CLpro like that of the x-ray inhibitor, R30. As protease enzyme plays an indispensable role during virus life cycle, CoV 3CLpro has been reported as a highly validated drug target and it is considered viable for the design of broad spectrum inhibitors. The selected five hit compounds bind to MERS-CoV 3CLpro in a manner similar to that of the x-ray inhibitor, R30, and showed pharmacophore-fit and docking score values higher than those of R30, MERS-CoV 3CLpro-inhibitor.*
Conclusion: *The retrieved five hits are proposed as new scaffolds for further evaluation and optimization of their activity against MERS-CoV.*

Keywords: *MERS-CoV pharmacophore, Molecular docking, Protease enzyme, X-ray inhibitor*

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INTRODUCTION

In June 2012, the Middle East respiratory syndrome coronavirus (MERS-CoV) was first diagnosed from a Saudi Arabian patient who had died from progressive respiratory and renal failure [1]. Up to July 2015, it had infected at least 1401 people with a fatality rate surpassing 39 % [2]. Recent reports have confirmed the human-to-human transmission of MERS-CoV. Thus, prompt efforts are necessary to develop new antiviral drugs or therapy for treatment of MERS-CoV.

Protease enzyme is essential for viral replication by mediating the maturation of viral replicase

polyprotein by the viral papain-like protease (PLpro) and the 3-chymotrypsin-like protease (3CLpro) thus, these proteases become alternative targets for potential antiviral drugs. Since the 3CLpro and PLpro enzymes are unique in the virus, present only in the virus and not in the host cell, this therapeutic strategy appears to be much safer than the one that targets host cell proteins [3,4]. Viral proteases, including the papain-like protease (PLpro) and the main protease 3C-like protease (3CLpro), process the replication of the genomic RNA of MERS coronavirus.

Since 2012, there are no effective antiviral therapeutic against human coronaviruses, including MERS-CoV but only primary organ support for respiratory and renal failure were the only clinical management of the infection. Use of broad-spectrum antivirals including antiviral agents against influenza, has also been recommended [5]. Treatment with interferon has also been suggested as a promising therapeutic strategy against MERS-CoV [6]. In 2013, first generation of protease inhibitor antiviral drugs, such as N3 [3] and CE-10 [7] that were optimized for inhibition of SARS-CoV 3CLpro have been reported to block the activity of MERS-CoV 3CLpro. Some SARS-CoV 3CLpro peptidomimetics which contains a Michael acceptor, (i.e., α,β-unsaturated carbonyl) [1], or contains activated carbonyl functionality displayed inhibition against MERS-CoV 3CLpro.

In view of these findings there is still a need for novel potent MERS-CoV inhibitors. Traditional synthesis and screening of a series of new compounds is well known as cost and time consuming process. On the other hand, an alternative process can be used by applying *in silico* screening of small molecule databases for novel compounds. Dependent on the amount of information available, either ligand-based or structure-based molecular design can be applied to find lead molecules [8]. In this work we describe screening a set of compounds from National Cancer Institute (NCI) database against MERS-CoV 3CLpro protein, with bound ligand R30 [9], by utilizing a ligandScout program [10] and AutoDock Vina program [11].

METHODS

Preparation of the protein structure and ligand structures

The National Cancer Institute (NCI) database, a free database for virtual screening, contains about 250,250 compounds in 3D formats. The x-ray crystal structure of MERS-CoV 3CLpro bound by the ligand, N-{4-[(1H-benzotriazol-1-ylacetyl)(thiophen-3-ylmethyl)amino]phenyl}propanemide (R30), was obtained from the Protein Data Bank (PDB code: 4YLU) [9]. The undertaken molecular modelling works were done on PC Windows 7 Home Premium Intel(R) Core(TM)2 Duo, 1.83GHz.

Structure-based pharmacophore building

The spatial arrangement of atoms or functional groups, called a pharmacophore, suggests how a ligand molecule can interact inside the binding site of a target protein.

The pharmacophore theory is based on the fact that ligand molecules that have the same biological binding site, should have common pharmacophore [12]. Based on the available information of ligands and the target macromolecule, there are two pharmacophore modelling approaches. The ligand-based approach is carried out by extracting common spatial arrangement of the chemical features of a set of known ligands postulated to have same binding mode to a specific target protein. While, the structure based approach demands necessitates the 3D structure of a target molecule or a macromolecule–ligand complex. Using LigandScout program [10], the latter approach was utilized to build a pharmacophore model making use of the x-ray crystal structure of MERS-CoV 3CLpro bound with inhibitor compound R30 (PDB code: 4YLU) [9]. In this study, the pharmacophore model is obtained through the investigation of the complementary chemical features of the binding site and their 3D-arrangement around the bound ligand.

Docking protocol

The coordinates of the x-ray structure of the MERS-CoV 3CLpro (4YLU) bound to its ligand (R30) were got from the Protein Data Bank (PDB) [9]. The x-ray ligand was redocked into its binding pocket to examine whether the docked pose could have same orientation as the x-ray ligand indicating the validity of the parameters in the docking simulation and in reproducing the x-ray crystal structure. The AutoDock Vina program was used for molecular docking-based virtual screening, VSDK.

Virtual screening

The coordinate files of ligands datasets in the NCI database are filtered by Lipinski's rule. Only the molecules which in accordance with Lipinski's rule can be considered as hits, retrieved from NCI database and grouped in a

chemical library. The obtained library was subjected to virtual screening using structure-based pharmacophore model resulting in a reduced size chemical library. The candidates in the obtained library were ranked based on docking score using AutoDock Vina program. Top ranked candidates were suggested for experimental screening as potential therapeutic agents for treatment of MERS-CoV.

RESULTS

Lipinski's rule of 5 was used for filtering the NCI database (250,250 entries) and identified a reduced diversity subset including 3120 small molecules that were subjected to further screening, using macromolecule-ligand-complex-based pharmacophore model (Figure 1 and Figure 2) and using docking study (Table 1, Figure 3).

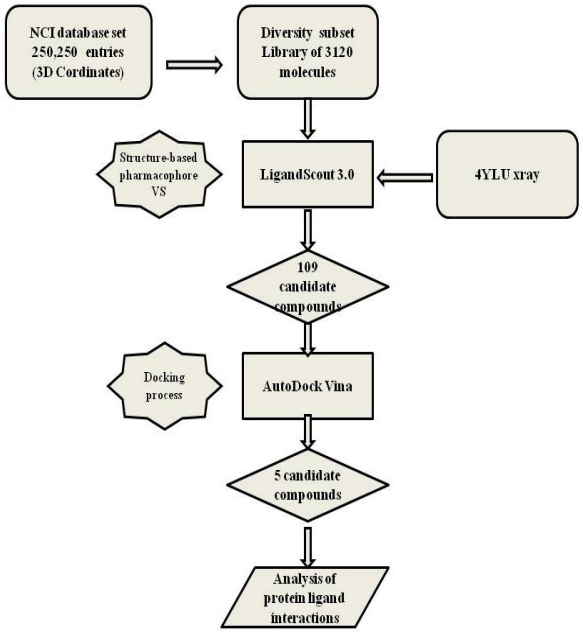

Figure 1: Schematic representation of the structure-based virtual screening work-flow

Docking study was undertaken in order to screen the hits retrieved from virtual screening using the pharmacophore query. The target macromolecule file (PDB ID: 4YLU) was obtained from PDB (www.rcsb.org) and explored the binding mode of peptide-like-inhibitor, N-{4-[(1H-benzotriazol-1-ylacetyl)(thiophen-3-ylmethyl)amino]phenyl}propanamide, R30, in the binding pocket (Figure 4).

DISCUSSION

Figure 1 shows the schematic representation of screening workflow. Lipinski's Rule of Five describes the molecular characteristics crucial for *in vivo* drug's pharmacokinetics, involving their absorption, distribution, metabolism, and elimination (ADME). The rule determines whether a biologically-active molecule would be a likely orally active drug or not. First, Lipinski's rule of 5 was used for filtering the NCI database (250,250 entries) and identified a reduced diversity subset including 3120 small molecule. We screened these 3120 compounds using macromolecule-ligand-complex-based pharmacophore modelling. Using the 3D structure of the MERS-CoV 3CLpro-ligand complex (4YLU.pdb), LigandScout program analyse the spatial arrangement of the matching chemical characteristics of the binding site and design a pharmacophore model with the selected features. The resulting pharmacophore model is composed of four features including two hydrophobes, one hydrogen acceptor, and one hydrogen donor, features, as shown in Figure 2.

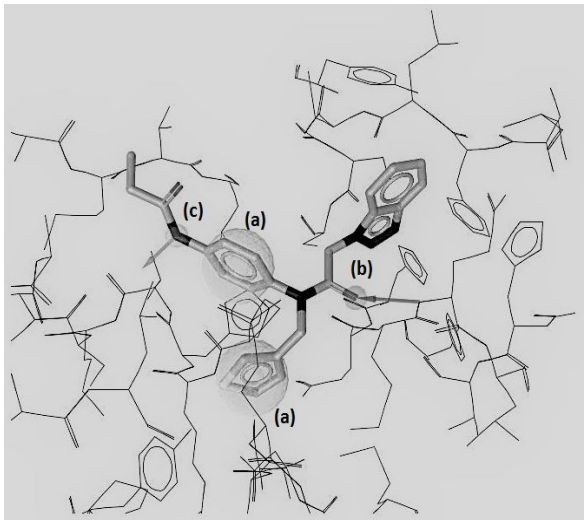

Figure 2: The macromolecule-ligand-complex-based pharmacophore model shows two hydrophobes (a), one hydrogen acceptor (b) and one hydrogen donor (c)

Table 1: Binding energy (Kcal/mol) and hydrogen bonds of the selected hit compounds

Compound	Binding energy (kcal mol^{-1})	No. of H-bonds	Interacting residues
NSC648199	-8.7	2	Gln167, Phe143
NSC159375	-8.3	1	Glu169
NSC29007	-8.1	1	Gly146
NSC335985	-8.1	2	Ser147, Glu169
NSC337571	-7.8	0	
R30 (x-ray ligand)	-7.6	3	Tyr54, His166, Glu169

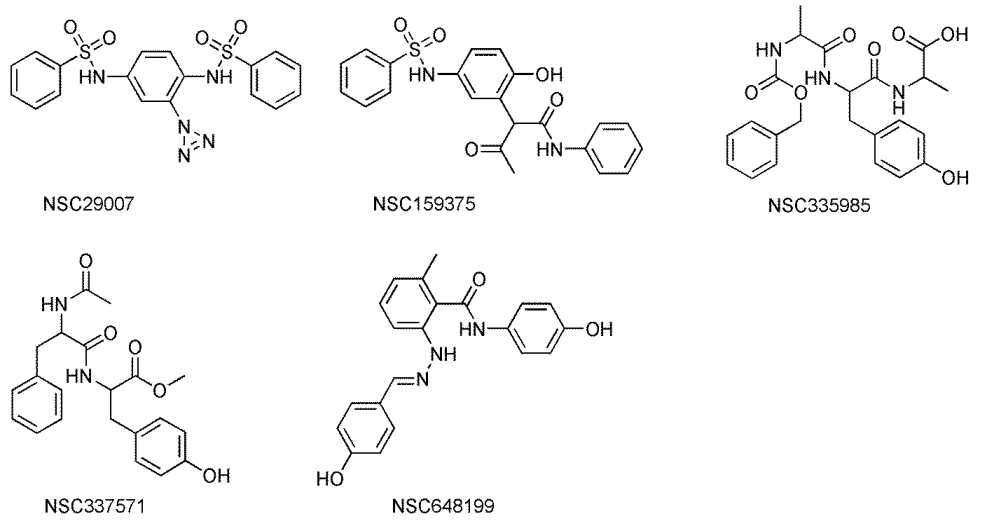

Figure 3: 2D chemical structures of the selected hit compounds

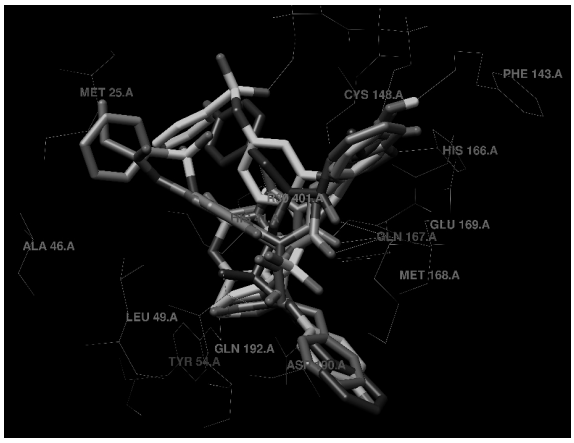

Figure 4: The 3D diagrams of interaction between the MERS-CoV 3CLpro and the five hit compounds overlaid on R30 x-ray ligand (colored black). The dotted lines show the hydrogen bond between the compounds and the binding.

Based on the pharmacophore fit value, the top 109 ligands were suggested for further screening using AutoDock Vina program. The AutoDock Vina algorithm searches different orientations of the compounds inside the binding site of receptor. Rely on the binding energy scores, five ligands were selected, NSC159375, NSC29007, NSC337571, NSC335985, NSC648199 (Table 1, Figure 3) were having lower energy scores than that R30 (xray ligand) which reveal promising binding affinity towards the active site of MERS-CoV 3CLpro. These ligands might act as potential inhibitors for the MERS-CoV 3CLpro and were suggested for experimental screening.

Docking study was undertaken using AutoDock Vina in order to screen the hits retrieved from virtual screening using the structure-based pharmacophore model. Also, the possible interactions between the top-score ranked hits and the active site of the MERS-CoV 3CLpro were compared with the binding mode of the known 4YLU peptide-like-inhibitor, N-{4-[(1H-benzotriazol-1-ylacetyl)(thiophen-3-ylmethyl)amino]phenyl}propanamide, R30. R30 has a tricyclic structure and is oriented in its binding site that is lined with the side chains of Met25, His41, Ala46, Leu49, Tyr54, Phe143, Cys148, His166, Met168, Glu169, Asp190, Gln192 (Figure 4, Table 1).

One of the nitrogen atoms of the benzotriazole moiety form hydrogen bond to the imidazole-nitrogen of His166, while the carbonyl oxygen of the acetyl moiety from a hydrogen bond to the amide-nitrogen of Glu169. Replication of the binding mode of the bound inhibitor, R30, by the docking algorithm used in the virtual screen would indicate that it is well calibrated for this class of receptor.

CONCLUSION

A computational approach using pharmacophore modelling and docking study has successfully applied to screen NCI database against MERS-CoV 3CLpro structure in order to discover new inhibitors of MERS-CoV. Using the structure-based screening approach, five compounds are identified as potential agents for the treatment of corona virus, MERS-CoV. Binding mode analyses reveal that the way these compounds bind to MERS-CoV 3CLpro is the same as that of the x-ray inhibitor, R30. The results suggest that these molecules can possibly be developed as novel lead compounds in anti-MERS-CoV drug design.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

This work was done by the author(s) named in this article and all responsibilities relating to the content of this article will be borne by the authors. Awwad A Radwan conceived and designed the study, Awwad A Radwan and Fares K Alanazi gathered and evaluated the data, Awwad A Radwan and Fares K Alanazi wrote read and approved the manuscript for publication.

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