IN SILICO INVESTIGATION OF ECHINODERMATA SECONDARY METABOLITES AS HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1) REVERSE TRANSCRIPTASE INHIBITORS

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ABSTRACT

Objective: Human immunodeficiency virus (HIV) targets the immune system and weakens immune surveillance and defenses against infections, leading to acute immunodeficiency syndrome. Recent trends in drug discovery from natural sources emphasize investigations of compounds from marine ecosystems.

Methods: In this study, we compiled a database of chemical compounds from echinoderms and virtually screened for those that inhibit HIV-1 reverse transcriptase (RT). The database was generated from literature searches. Virtual screening analyses for inhibitors of HIV-1 RT were then performed using AutoDock software.

Results: Based on screening results, the top thirteen ranked compounds were nobilisidenol B, Ech_005, 17-deoxyholothurinogenin, 22,25-oxidoholothurinogenin, Ech_022, Ech_026, Ech_021, nobilisidenol A, Ech_025, 5α-cholest-8(14)-ene-3β,7α-diol, astropecten A, Ech_004, and phrygijasterol.

Conclusion: The present in silico screening analyses of compounds from marine ecosystems can be used to identify candidate compounds with high potential as drugs for the treatment of refractory HIV infections.

Keywords: Echinoderms, Human immunodeficiency virus, Reverse transcriptase inhibitor.

METHODS

 Ligand preparation

Two dimensional (2D) structures of echinoderms bioactive compounds were compiled by data mining numerous journals. Subsequently, three dimensional (3D) structures were created in MarvinSketch [15] using mmff94 force field and strict level optimization limits. The prepared ligands were saved in *.pdb files and were converted to *.pdbqt files.

 Macromolecule preparation

Macromolecules were downloaded from the Protein Data Bank using 3LP1 code [16]. Macromolecules were then prepared by separating from solvents, ligands, and other nonstandard residues using AutoDockTools [17]. We then optimized these by deleting water molecules, adding nonpolar hydrogen atoms, charges, and force fields, and by minimizing the structures using AutoDockTools. Prepared macromolecules were saved in *.pdb files and were then converted to *.pdbqt files.

 Docking parameter optimization

In silico docking was performed using AutoDock 4.0 [17], which was run automatically through PyRx [18], and the results were visualized using PyMOL [19] and LigandScout [20]. Molecular docking parameters were optimized by redocking nevirapine. Positive controls included nevirapine, delavirdine, efavirenz, rilpivirine, and etravirine, which are recommended by the United States Food and Drug Administration (FDA) for the treatment of HIV-1 [21].

Virtual screening

Virtual screening was performed with HIV-1 RT using the AutoDock function of PyRx. The parameters were as follows: Search space surrounding the binding pocket, 18.75 × 18.75 × 18.75 Å; maximum number of generations, 27,000; maximum number of energy of
Table 1: Virtual screening results of selected compounds to HIV-1 reverse transcriptase using AutoDock. Etravirine is listed as positive control

| Rank | Name                          | IUPAC name                                                                                                                                                                                                 | Bond Energy/∆G (kcal/mol) | Structure reference |
|------|-------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------|---------------------|
| 1    | Nobilisidenol B               | (2S,5R,6S,9R,13S,16S,18R)-5,16-dihydroxy-2,6,13,17,17-pentamethyl-6-[(1E)-4-methylpent-1-en-1-yl]-7-oxapentacyclo[10.8.0.0²,9.0.0⁵,9.0.13,18]icos-11-en-8-one | -11.15                    | Blunt et al., 2007 [22] |
| 2    | Ech_005                       | [(2S,5S,7S,8S,10S,11S,14R,15R)-8-hydroxy-2,15-dimethyl-14-[(2R)-6-methyl-4-oxoheptan-2-yl]tetracyclo[8.7.0.0²,7.0.0⁴,7.0.11]icos-1(17)-en-5-yl] oxidanesulfonic acid | -11.14                    | Blunt et al., 2003 [23] |
| 3    | 17-Deoxyholothurinogenin      | (2S,5S,6S,9S,13S,18R)-16-hydroxy-2,6,13,17,17,18-hexamethyl-6-[(2S)-2,5,5-trimethyloxolan-2-yl]-7-oxapentacyclo[10.8.0.0²,9.0.0⁵,9.0.13,18]icosa-1(20),11-dien-8-one | -11.12                    | Bhakuni and Rawat, 2005 [24] |
| 4    | 22,25-Oxidoholothurinogenin   | (2S,5R,6S,9R,13S,18R)-5,16-dihydroxy-2,6,13,17,17,18-hexamethyl-6-((2S)-2,5,5-trimethyloxolan-2-yl)-7-oxapentacyclo[10.8.0.0²,9.0.0⁵,9.0.13,18]icosa-1(20),11-dien-8-one | -10.97                    | Bhakuni and Rawat, 2005 [24] |
| 5    | Ech_022                       | sodium [(4E,6R)-3-methyl-6-[(1S,2R,5S,7R,8R,10R,11S,12S,14R,15R)-5,7,8,12-tetrahydroxy-2,15-dimethtyltetracyclo[8.7.0.0²,7.0.0⁴,7.0.11]icos-10-ene-5,7,11-diol] | -9.76                     | Blunt et al., 2007 [22] |
| 6    | Ech_026                       | (3β,5α,6β,7α,24S)-cholestane-3,6,8,15,24-pentol                                                                                                                                             | -9.58                     | Blunt et al., 2007 [22] |
| 7    | Ech_021                       | sodium [1S,2R,5S,7R,8R,10R,11S,12S,14R,15R]-14-[[2R,3E,6S]-6-hydroxy-5,6-dimethyl-7-(sulfonatoxy)hept-3-en-2-yl]-2,15-dimethtyltetracyclo[8.7.0.0²,7.0.0⁴,7.0.11]icos-5,7,8,12-tetrol | -9.37                     | Blunt et al., 2007 [22] |
| 8    | Nobilisidenol A               | (2S,5R,6S,9R,13S,16S,18R)-5,16-dihydroxy-2,6,13,17,17,18-hexamethyl-6-((2S)-2,5,5-trimethyloxolan-2-yl)-7-oxapentacyclo[10.8.0.0²,9.0.0⁵,9.0.13,18]icosa-1(20),11-dien-8-one | -9.3                        | Blunt et al., 2007 [22] |
| 9    | Ech_025                       | (3β,5α,6β,7α,24S)-cholestane-3,6,8,15,24-pentol                                                                                                                                                  | -9.17                     | Blunt et al., 2007 [22] |
| 10   | 5α-Cholest-8(14)-ene-3β,7α-diol| (1R,2S,SS,7R,9R,14R,15R)-2,15-dimethtyl-14-[(2R)-6-methylheptan-2-yl]tetracyclo[8.7.0.0²,7.0.0⁴,7.0.11]heptadec-10-ene-5,9-diol | -9.09                     | Thao et al., 2014 [25] |
| 11   | Astropecten A                 | (1R,2R,5S,6R,9R,13S,15R,16R)-1,13-dihydroxy-6,10-dimethyl-5-[(2R)-6-methylheptan-2-yl]tetracyclo[7.7.0.0²,7.0.0⁴,7.0.11]heptadec-10-ene-5,9-diol | -8.98                     | Thao et al., 2014 [25] |
| 12   | Ech_004                       | [(2S,5S,7S,8S,10S,11S,12R,13R,14R,15R)-8-hydroxy-2,15-dimethyl-14-[(2R)-6-methyl-4-oxohexan-5-yl]tetracyclo[8.7.0.0²,7.0.0⁴,7.0.11]icos-10-ene-5,7,11-diol | -8.91                     | Blunt et al., 2003 [23] |
| 13   | Phrygiasterol                 | (1R,2S,SS,7S,8S,10S,11S,12R,13R,14R,15R)-14-[(2R)-4-(1R,2R)-2-(hydroxyethyl) cyclopropyl]butan-2-yl]-2,15-dimethtyltetracyclo[8.7.0.0²,7.0.0⁴,7.0.11]heptadec-5,8,10,12,13-pentol | -8.86                     | Blunt et al., 2007 [22] |
| 14   | Etravirine                    | 4-[6-amino-5-bromo-2-(4-cyanomethyl) pyrimidin-4-yl] oxo-3,5-dimethylbenzonitrile                                                                                                                                 | -8.82                     | PubChem [26] |

HIV-1: Human immunodeficiency virus type 1, IUPAC: International Union of Pure and Applied Chemistry
RESULTS AND DISCUSSION

Macromolecule preparation

We initially prepared macromolecules by searching the protein database and deleting nonstandard residues. The nonstandard residues nevirapine ligand, manganese ion (II), and LP8 were then separated from structures as identifier molecules. Hydrogen atoms that are not generally identified in crystal structures were then added to manipulate hydrogen bonding with added ligands. AutoDock force field was then applied with Gasteiger charges as a common default for docking in AutoDock applications. We then performed energy minimization to identify geometrical conformations with the least optimal energy so that the structures could be considered stable as docking targets. The coordinates of the nevirapine binding site were determined as a positive control and the binding site was defined as x = 10.350, y = 14.076, and z = 18.252. The grid was set at 50 × 50 × 50 units at 0.375 Å. This coordinate determination for the receptor target corresponded with previous research [28].

Ligand database preparation

The ligand database that was developed in this experiment comprised ligand names from the International Union of Pure and Applied Chemistry (IUPAC), 2D and 3D structures, species names from which the compounds were extracted/isolated, and pharmacological actions (if any) and references. Data from various journals were obtained from the PubChem Open Chemistry Database [26], the ChemSpider Search and Share Chemistry [29] and other resources in *.sdf and *.mol formats. Hydrogen atoms were added because the software scoring function requires polar and nonpolar hydrogen atoms. Gasteiger charges and AutoDock force fields were added because they are generally used in AutoDock software. Some of the compounds that were compiled from journals already have names, whereas others have only IUPAC names. To facilitate docking and analysis, ligand nomenclature for unnamed compounds was standardized as Ech XXX, where XXX are three-digit numbers of compounds that were sorted based on literature reviews.

Analysis of virtual screening results

Virtual screening was performed for 127 compounds that were identified by data mining of several journals. Virtual screening tests of RT inhibitory activities of ligands were sorted and ranked based on ΔG values. Compounds with the lowest ΔG values were placed in the first rank. The results are listed in Table 1.

Based on our screening results, the top thirteen ranked compounds were nobilisidenol B, Ech_005, 17-deoxocholothurinogenin, 22,25-oxidoholothurinogenin, Ech_022, Ech_026, Ech_021, nobilisidenol A, Ech_025, 5α-cholest-8(14)-ene-3β,7α-diol, astrosexide, A, Ech_004, and phrygiasterol. These compounds were selected for further analysis based on energy bond values that were superior to the positive control [30]. These distances were measured using PyMOL and showed that HB1 is a medium H-bond and HB2 is a weak H-bond. Moreover, hydrophobic interactions were identified between nobilisidenol B and the amino-acid residues llel80, Leu100, Tyr181, Val106, and Val79.

Interactions between the positive control and the thirteen compounds from echinoderms and amino-acid residues are shown in Table 3. Among these, Lys101 is considered important because it has the most interactions of all 3LP1 amino-acid residues, which participates in hydrogen and ionic bonds with some compounds from echinoderms more than with other amino-acid residues. In a book published in 2006, Skowron and Ogden wrote about a number of these specific hydrogen bonds and hydrophobic interactions. These are key properties for stabilizing ligands in open conformations of protein structures. Specifically, hydrogen bonds are formed through interactions between hydrogen atoms that are covalently bound to electron-negative donor atoms with electron-negative acceptor atoms. These interactions are important for proteins and occur predominantly between NH and O groups of main α-helical chains. Hydrogen bonds between macromolecules and their ligands (proteins, nucleic acids, substrates, effectors, and inhibitors) contribute directionality and specificity of interactions and serve as fundamental mediators of molecular recognition [30-33].

In 2009, Eugene E. Kwan classified hydrogen bonds with donor-acceptor distances of 2.2–2.5 Å as “strong, mostly covalent,” of 2.5–3.2 Å as “medium, mostly electrostatic,” and of 3.2–4.0 Å as “weak, electrostatic” [33]. This hydrogen bonds classification is defined in Table 2.

Analysis and visualization of interactions between compounds from echinoderms and macromolecule targets using AutoDock

2D and 3D structures of docking results were modeled using LigandScout. As shown in Fig. 1, the highest-ranked compound nobilisidenol B was docked to 3LP1. This triterpene was isolated from the sea cucumber Holothuria nobilis [22].

The LigandScout analysis and visualization of 2D and 3D structures identified two hydrogen bonds. In one of these, nobilisidenol B provides a hydrogen bond donor and an acceptor from the same amino-acid residue Tyr318. The nobilisidenol B functional group that acted as both H-bond donor (HB1) and acceptor (HB2) comprises hydroxyl groups of the phenantherene core, with distances of 3.2 Å and 2.8 Å, respectively. These distances were measured using PyMOL and showed that HB1 is a medium H-bond and HB2 is a weak H-bond. Moreover, hydrophobic interactions were identified between nobilisidenol B and the amino-acid residues llel80, Leu100, Tyr181, Val106, and Val79.

Table 1: Classification of hydrogen bond strength

| Indicator | Strong | Medium | Weak |
|-----------|--------|--------|------|
| Bond energy (kcal/mol) | 14–40 | 4–14 | 0–4 |
| Interaction type | Mostly covalent | Mostly electrostatic | Electrostatic |
| A—B (Å) | 2.2–2.5 | 2.5–3.2 | 3.2–4.0 |
| H—B (Å) | 1.2–1.5 | 1.5–2.2 | 2.2–3.2 |
| Bond angle (°) | 175–180 | 130–180 | 90–150 |

Source: Kwan, E. E., 2009 [33]

Fig. 1: Visualization of interactions between nobilisidenol B and 3LP1

Source: LigandScout
Table 3: Analysis of interactions between compounds from echinoderms and HIV-1 reverse transcriptase using LigandScout and PyMOL

| Compound                        | Interactions with HIV-RT             |
|--------------------------------|-------------------------------------|
| Nevirapine                     | 2.1 Å (OH...N); 2.6 Å (NI)           |
| 17-Deoxyholothurinogenin        | 2.1 Å (OH...N); 2.8 Å (O...HN)       |
| 22,25-Oxidoholothurinogenin    | 2.1 Å (OH...N); 3.6 Å (OH...N)       |
| Astropecten A                  | 2.1 Å (OH...N); 2.2 Å (OH...N)       |
| Ech_004                        | 2.1 Å (OH...N); 2.3 Å (OH...N)       |
| Ech_005                        | 2.2 Å (OH...N)                       |
| Ech_021                        | 2.2 Å (OH...N)                       |
| Ech_022                        | 2.5 Å (OH...N); 2.65 Å (NI)          |
| Phrygiasterol                  | 2.4 Å (OH...N)                       |

Interactions between Lys101 and SO$_{4}^{2−}$ ions deserve special attention because they have not been recognized in the previous studies of NNRTI binding sites. Accordingly, no NNRTI drugs that were approved by the FDA have reported ionic bonding interactions with Lys101. Therefore, due to SO$_{4}^{2−}$ groups that participate in ionic interactions with Lys101, the echinoderm compounds Ech_005, Ech_021, and Ech_022 are promising candidate NNRTI drugs, along with the other top thirteen ranked compounds.

CONCLUSION

Database and literature searches of chemical compounds from echinoderms revealed 127 candidate compounds. Subsequent virtual screening for RT inhibitory activities using AutoDock identified nobilisidenol B, Ech_005, 17-deoxyholothurinogenin, 22,25-Oxidoholothurinogenin, Ech_022, Ech_026, Ech_021, nobilisidenol A, Ech_025, 5α-cholest-8(14)-ene-3β-diol, astropecten A, Ech_004, and phrygiasterol as high potential candidate anti-HIV drugs.

CONFLICTS OF INTEREST STATEMENT

The authors do not have any conflicts of interest to declare.

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