In Silico Screening of Selected Natural Product CompoundsAgainst Human Neutrophil Elastase

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Abstract: Human neutrophil elastase (HNE) is one of the key proteases present in the neutrophil and is involved in the pathogenesis of different inflammatory disorders. In recent years, the investigation for potential HNE inhibitors are increasing and one of the strategies in drug discovery is the use of *in silico* drug screening of the reported isolated compounds from natural products. In this study, the docking behavior of HNE with a series of compounds isolated from herbs that inhibit lung inflammation was investigated. iGEMDOCK v.2.1. (Graphical Environment for Recognizing Pharmacological Interactions and Virtual Screening) was used for the docking, virtual screening, and post-screening analysis of pharmacological interactions between the enzyme HNE and various lead compounds as the ligand. And based on the results, Compound 22 ([(2R,3R, 4S, 6S0-3,4,5-trihydroxy-6{[(2R)-5-hydroxy-2-(4-hydroxyphenyl0-4-oxo-2,3-dihydro-1-benzopyran-7-yl]oxy}oxan-2-yl]methyl(2E)-3-4-hydroxyphenyl)prop-2-enoate has the highest inhibition potential according to the calculated Gibbs free energy. The finding of this study is the first to be reported based on the current knowledge of the authors. It is recommended that further analysis of the test compound must be performed both *in vitro* and *in vivo* to validate its bioactivity.

Keywords: *Neutrophil Elastase; Natural product compounds; In silico screening; lung inflammation; psoriasis*

1. INTRODUCTION

Human neutrophil elastase (HNE, EC:3.4.21.37), also known as bone marrow serine protease, elastase-2, or human leukocyte elastase is a 30 kDa serine protease with four disulfide bridges utilizing a catalytic triad of Ser195, His57, and Asp102 that is stored in the azurophilic granules of neutrophils which belong to the chymotrypsin family (UniProt Consortium European Bioinformatics Institute Protein Information Resource SIB Swiss Institute of Bioinformatics, 2019). HNE has various physiological processing functions, such as blood coagulation, apoptosis, and inflammation. Specifically, intracellular HNE functions by modifying natural killer cells, monocytes and granulocytes; whereas the extracellular HNE assists in neutrophil migration to inflammation sites through the degradation of host proteins such as extracellular matrix proteins (Feng et al., 2013) which is controlled by endogenous inhibitors called serpins such as α_1-antitrypsin (α_1-AT), secretory leukocyte proteinase inhibitor, α_2- macroglobulin, and elafin (Feng et al., 2013; UniProt Consortium European Bioinformatics Institute Protein Information Resource SIB Swiss Institute of Bioinformatics, 2019). However, proteases and oxygen radicals can inactivate these inhibitors which can cause an imbalance between the HNE and its regulatory inhibitors resulting in the development of pulmonary diseases, such as chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), acute respiratory distress syndrome (ARDS), acute lung injury (ALI), as well as inflammatory disorders, such as psoriasis, dermatitis, atherosclerosis, and rheumatoid arthritis. Thus, promising therapeutic agents for the treatment of diseases involving excessive HNE activity can be developed in order to modulate its proteolytic activity. Currently, only two drugs (UniProt Consortium European Bioinformatics Institute Protein Information Resource SIB Swiss Institute of Bioinformatics, 2019) are available for clinical use – the Prolastin, a peptide drug used for the treatment of α_1-AT deficiency, and Sivelestat sodium hydrate, a low molecular weight non-peptide selective HNE inhibitor with an IC_{50} value of 44nM. It has been shown that the mechanism of action of these compounds were competitive and pseudo-irreversible HNE inhibitors, with an appreciable selectivity for HNE versus other proteases (Feng et al., 2013).

In recent years, regulation of the enzymatic activity of HNE has gained a lot of attention worldwide due to its potential therapeutic application in medicinal field. Potential source of HNE inhibitors are natural compounds such as curcumin which reportedly inhibit HNE activity *in vitro*. In the present study, the docking behavior of HNE with a series of compounds isolated from herbs that inhibit lung inflammation was investigated. iGEMDOCK (Graphical Environment for Recognizing Pharmacological Interactions and Virtual Screening) was used for the docking, virtual screening, and post-screening analysis of pharmacological interactions between the enzyme HNE and various lead compounds as the ligand.

2. METHODOLOGY

2.1 Protein structure preparation

Human neutrophil elastase (HNE) crystal structure complex with 1/2SLPI was retrieved from Protein Data Bank (PDB ID: 2z7f) at 1.3A root mean square deviation resolution as illustrated in Figure 1.

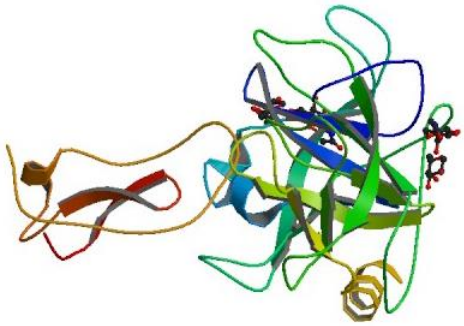


Figure 1. Biological Assembly of Human Neutrophil Elastase (HNE) retrieved from protein data bank (PDB ID: 2z7f)

2.2. Ligand preparation

The ligands derived from various natural products were retrieved from various journals. The mol format of the compounds was used in the docking study.

2.3 Molecular docking and physicochemical analysis of the natural product compounds:

Docking analysis was performed using iGEMDOCK version 2.1 molecular docking software. The top five compounds with the lowest binding energy were considered as the optimum candidate and the physicochemical properties of the five compounds were analyzed using Marvin Sketch followed by Lipinski's rule.

3. RESULTS AND DISCUSSION

Out of 25 natural product compounds screened for potential HNE inhibitor (Table 1), Compounds 3, 13, 20, 22, and 24 are the top five natural product compounds with the highest negative Gibbs free energy (Table 1) which implicate the potential of these agents as new HNE inhibitors. Based on the calculated docking scores of the poses, Compound 22 showed effectiveness in terms of anchoring at the active site channel of the enzyme located at H-S-Val-62, H-M, Val-65, H-S-Val-82, V-S-Val-62, V-M-Arg-63, V-S-Arg-63, V-M-Val-65, V-S-Val-65, V-M-Val-66, V-M-Val-82, V-M-

Phe-83, V-S-Phe-83, V-M-Ala-84, V-S-Ala-84, V-M-Ala-85, and V-S-ASN-109 (Figure 2).

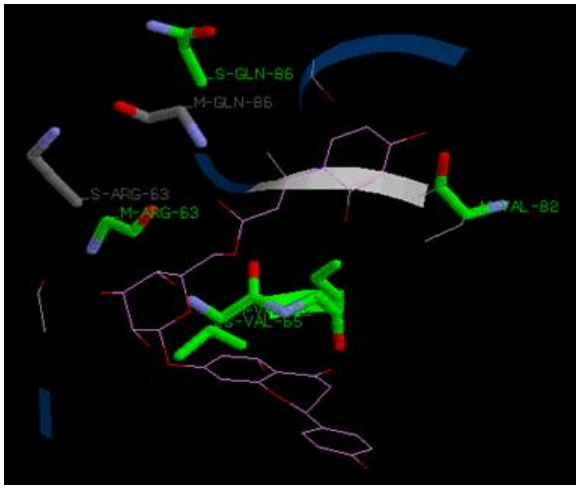


Figure 2. Docking pose of Compound 22 in HNE pocket.

Table 1
Protein-ligand Binding Docking Position and Energy Values

Ligand	*Total Energy*	*Van Der Waals*	*Hydrogen Bond*	*Average Con Pair*
Compound 1	-62.5032	-58.8761	-3.62705	15.6957
Compound 2	-70.4923	-59.9923	-10.5	14.2308
Compound 3	-75.1716	-59.3572	-15.8145	14.3077
Compound 4	-53.0446	-50.5446	-2.5	18.75
Compound 5	-51.2515	-43.9401	-7.3115	14.381
Compound 6	-64.9138	-64.9138	0	15.1538
Compound 7	-55.636	-41.6492	-13.9868	18.9375
Compound 8	-45.4404	-45.4404	0	19.1111
Compound 9	-69.4953	-57.551	-11.9443	17.5
Compound 10	-39.1176	-39.1176	0	19.1667
Compound 11	-61.6212	-48.9337	-12.6875	15.1364
Compound 12	-61.2361	-54.419	-6.81711	15.2857
Compound 13	-75.4748	-70.4864	-4.9884	13.5
Compound 14	-57.8549	-40.5167	-17.3383	18.5
Compound 15	-54.0468	-34.2542	-19.7925	13.2903
Compound 16	-39.3398	-28.8525	-10.4874	18.4706
Compound 17	-53.9477	-45.1964	-8.75133	20.1
Compound 18	-55.0187	-43.0821	-11.9366	18.8421
Compound 19	-59.0318	-55.5318	-3.5	19.7368
Compound 20	-77.1056	-73.6056	-3.5	16.2069
Compound 21	-57.3182	-40.8671	-16.451	20.25
Compound 22	-91.833	-77.111	-14.722	12.9762
Compound 23	-67.9255	-53.9132	-14.0123	15.3913
Compound 24	-78.9632	-59.8395	-19.1237	14.7667
Compound 25	-67.1427	-57.6537	-9.48906	16.8182

The top five HNE inhibitors (Compounds 3, 13, 30, 22, and 24) were selected as indicated by italicization. The analyses of the top five HNE inhibitors were limited due to unavailability of publication describing the other potential application of these compounds. As shown in Table 2, Compound 22 has the highest HLB value (hydrophilic/lipophilic) which explains that this compound is a water-soluble agent and the route of administration/preparation must be considered for its intended site of action. HNE is one of the key proteases present in the neutrophil and is involved in the pathogenesis of different inflammatory disorders such as renal glomerulus, skin, joints, intestinal mucosa ischemia/perfusion (Baylac & Racine, 2004; Bode, Meyer, & Powers, 1989). The role of the enzyme also depends on its location, intracellular HNE is responsible for the foreign protein degradation engulfed by leukocytes during phagocytosis while extracellular HNE targets the elastin- the main components of the lungs, blood vessels and other organs (Ellis & Luscombe, 1994). The diverse actions of the enzyme made it a potential target for development of drug with possible anti-inflammatory activity in lung disease, such as H1N1 and SARS virus infections and induce hyperproliferation (Feng et al., 2013; Feng, Rogalski, Meyer-Hoffert, Proksch, & Wiedow, 2002).

According to Lipinski's rule of five, Compound 22 has a probable poor oral absorption or permeation to the cells due to its high molecular weight that exceed 500 and number of hydrogen bond acceptor that exceed 10 counts (Table 4). In addition, compound 22 also violated the parameters set by Ghose, Viswanadhan, and Wendoloski (1999) (Table 3) in order to consider a test compound as a potential anti-inflammatory agent. Compound 22 also violated the prescribed number of hydrogen bond and molecular weight in the BBB rules of Pardridge; that is hydrogen bond must be 8-10, molecular weight must be from 400 to 500, and there must be no acidic groups (Di & Kerns, 2008).

A similar drug likeness rule was published by Ghose, Viswanadhan, and Wendoloski (1999) in which the authors defined the drug-like properties for the CMC (Comprehensive Medicinal Chemistry) database (Table 5). The study states that the qualifying range (covering more than 50% of the compounds of the calculated log P) is 1.3 to 4.1. For molecular weight, the qualifying range is 230 – 390; for molar refractivity, the qualifying range is 70 – 110; and for the total number of atoms, the qualifying range is 30 - 55. In addition, the following range must be met to qualify the test compound as medicinal agent (Table 3).

Table 2
Physicochemical Properties of the Top 5 HNE Inhibitor

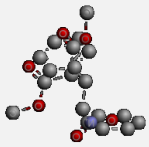
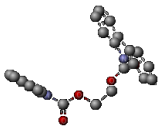
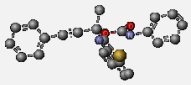
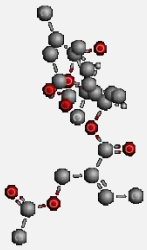
Ligand Structure	*IUPAC Name*	*Estimated LogP*	*HLB value (Griffin)*	*H-bond count*	*H-bond sites*
Compound 3 	1,3-dimethyl-2-[(1R,2S)-2-nitro-1,2-diphenylethyl]propane dioate	3.34	7.25	4	9
Compound 13 	2-(9H-carbazole-9-carbonyloxy)ethyl 9H-carbazole-9-carboxylate	5.89	2.30	1	1
Compound 20 	(2R)-2-(1,3-benzothiazol-2-yl)-4-phenylbut-3-yn-2-yl N-phenylcarbamate	6.61	0.85	1	1
Compound 22 	[(2R,3R, 4S, 6S0-3,4,5-trihydroxy-6{[(2R)-5-hydroxy-2-(4-hydroxyphenyl0-4-oxo-2,3-dihydro-1-benzopyran-7-yl]oxy}oxan-2-yl]methyl (2E)-3-4-hydroxyphenyl)prop-2-enoate	3.30	17.10	6	6
Compound 24 	(1R, 2Z, 4R, 8R, 9R, 11S)-1-hydroxy-2,11-dimethyl-7-methylidene-6-oxo-5,14-dioxytricyclo[9.2.1.0] tetradeca-2,12-dien-9-yl(2Z)-[(acetoxy)methyl]but-2-enote	2.48	10.75	5	10

Table 3
Qualifying Range in Comprehensive Medicinal Chemistry Database

Drug class	*Estimated Log P (80%)*	*AMR (80%)*	*Molecular Weight (80%)*	*Number of Atoms (80%)*
Anti-Inflammatory	1.4 ~4.5	59 ~ 119	212 ~ 447	24 ~ 59
Anti-Depressant	1.4 ~ 4.9	62 ~ 114	210 ~ 380	32 ~ 56
Anti-Psychotic	2.3 ~ 5.2	85 ~ 131	274 ~ 464	40 ~ 63
Anti-Hypertensive	-0.5 ~ 4.5	54 ~ 128	206 ~ 506	28 ~ 66
Anti-Hypnotic	0.5 ~ 3.9	43 ~ 97	162 ~ 360	20 ~ 45
Anti-Neoplastic	-1.5 ~ 4.7	43 ~ 128	180 ~ 475	21 ~ 63

Table 4
Lipinski's Rule Calculation of the Top 5 HNE Inhibitors

Ligand Number	*Molecular Weight*	*Hydrogen Bond Acceptor*	*Hydrogen Bond Donor*
3	337.97	4	0
13	427.99	6	0
20	379.97	4	0
22	551.94	12	0
24	394.98	8	0

Table 5
CMC-50 like Rule and BBB-likeness of top 5 HNE Inhibitors

Ligand Number	*Estimated LogP*	*AMR*	*Number of Atoms*	*Number of Acidic Group*	*Number of Hydrogen Bond*
3	2.76	97.84	26	0	4
13	2.90	143.26	34	0	6
20	3.96	129.01	29	0	4
22	-0.87	156.54	42	0	12
24	1.76	106.26	33	0	8

4. CONCLUSION

Compound 22 was a potential HLE inhibitor based on the *in silico* screening; however, further studies must be done to validate this result by conducting an *in vitro* analysis and toxicity study of the test compound. This is the first investigation demonstrating the molecular docking mechanism of Compound 22.

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