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Molecular docking analysis of natural compounds as Human neutrophil elastase (HNE) inhibitors

Radhakrishnan Narayanaswamy[1], Lam Kok Wai[2] and Intan Safinar Ismail[1,3*]

[1]Laboratory of Natural Products, Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia
[2]Faculty of Pharmacy, Universiti Kebangsaan Malaysia (UKM), Jalan Raja Muda Abdul Aziz, Kuala Lumpur, Malaysia
[3]Department of Chemistry, Faculty of Science, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia

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ABSTRACT

In recent years regulation of the enzymatic activity of human neutrophil elastase (HNE) has been the main focus of investigation, due to its potential therapeutic application in medicinal field. In the present study, the docking behaviour of human neutrophil elastase (HNE) with 14 different ligands namely Chrotacumines-A, B, C, Grandols-B, D, G, Rohitukine, Quercitin, Ellagic acid, Artoindonesianin-F, Origanol-A Thymoquinone, Embelin and Vilangin was evaluated along with their putative binding sites using Discovery Studio Version 3.1. In addition, molecular descriptors analysis using Molinspiration online tool was also carried out. The molecular physicochemical analysis revealed that Quercitin, Artoindonesianin-F & Origanol-A violated the five rules of thumb. With regard to drug-likeness property, Thymoquinone exhibited better score compared to all other ligands. Docking studies and binding free energy calculations revealed that Vilangin has maximum interaction energy (-50.1 kcal/mol) and Thymoquinone with the least interaction energy (-18.1 kcal/mol) as compared to the other investigated ligands. Quercitrin is the only ligand showed interaction with Ser 195 amino acid residue. Therefore, it is strongly suggested that the present study outcomes might provide new insight in understanding these 14 ligands, as potential candidates for human neutrophil elastase (HNE) inhibitory activity.

Key words: Molecular physicochemical properties, Molecular docking, Chrotacumines, Grandols, Rohitukine, Embelin.

INTRODUCTION

Elastases belong to family of serine proteases that possess the ability to cleave or hydrolytic the extracellular matrix protein, notably elastin, which is widely distributed in vertebrate tissue especially abundant in the lung, arteries, skin and ligaments of human beings [1]. Human neutrophil elastase (HNE) is a proteolytic enzyme involved in the response to inflammatory stimuli [2] and it is present in azurophilic granules of neutrophils. In generally, HNE extracellular activity is regulated under normal physiological conditions by endogenous inhibitors such as α1-proteinase inhibitor (α1PI) and α-macroglobulin. However, during several pathological conditions such as acute respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF) and as well as other inflammatory associate disorders such as atherosclerosis, psoriasis and dermatitis, elevated HNE activity is commonly reported. In recent years, HNE has also been implicated in the progression of non-small cell lung cancer progression [3]. Hence, a number of clinical observations indicated that HNE represents a good therapeutic target for the treatment of inflammatory diseases and might also be valuable therapeutic agents in lung cancer [4].

Although human neutrophil elastase (HNE) was identified as a therapeutic target for COPD more than thirty years ago, Sivelestat is the one and only HNE inhibitor from Ono Pharmaceutical. This drug has been approved for clinical use but limited use in Japan only while its development in the USA was terminated in 2003 [5]. Similarly, Midesteine, ICI200880, ZD-8321 and CE-1037 were few other elastase inhibitors discontinued from pre-clinical or phase II/III trials for various reasons [6].

The exponentially increasing availability of three dimensional structures for many macromolecular drug targets and rapid advancement in computational chemistry and bioinformatics, both *in vitro* and *in silico* serve a new fertile platform for the development as well as exploring modern computational methods [7]. When the structure of the macromolecular target is known, then the design of the computational library can be customized to suit the geometry of the binding site [8-9]. Moreover, identifying binding sites and protein-ligand interactions using bioinformatics tools before venturing into wet laboratory studies saves the energy and time considerably. Therefore, in the present study 14 different selected ligands, which among them were in-house isolated compounds, are Chrotacumine-A, B, C, Grandol-B, D, G, Rohitukine, Quercitin, Ellagic acid, Artoindonesianin-F, Origanol-A Thymoquinone, Embelin and Vilangin were evaluated on the docking behaviour of Human neutrophil elastase (HNE). Investigation was also done on HNE putative binding sites using Discovery Studio Version 3.1.

EXPERIMENTAL SECTION

Ligand preparation

Chemical structures of ligands namely Chrotacumine-A [Chemspider ID 24678919], Chrotacumine-B [Chemspider ID 24657802], Rohitukine [Chemspider ID 4533914], Quercitrin [CID no: 5280459], Ellagic acid [CID no: 5281855], Thymoquinone [CID no: 10281] Embelin [CID no: 3218] and Vilangin [CID no: 417182] were retrieved from Chemspider [10] and Pubchem compound database [11] respectively. Unavailable three dimensional structures of Chrotacumine-C, Grandol-B, D, G, Artoindonesianin-F and Origanol-A were generated using ACD [12].

Target protein identification and preparation

The three dimensional structure of the HNE (PDB ID: 1H1B) was obtained from the Research collaborator for structural bioinformatics (RCSB) Protein data bank [13]. The proteins were pre-processed separately by deleting the ligand as well as the crystallographically observed water molecules (water without Hydrogen bonds).

Molecular descriptors calculation

Molinspiration online database was used to calculate thirteen descriptors [14], which are logP, polar surface area, molecular weight, number of atoms, number of O or N, number of OH or NH, number of rotatable bonds, volume, drug likeness includes G protein coupled receptors (GPCR) ligand, ion channel modulator, kinase inhibitor and nuclear receptor ligand, and number of violations to Lipinski's rule, for all ligands selected except Embelin and Vilangin.

Docking studies

Docking studies were carried out on the crystal structure of HNE retrieved from Protein Data Bank using the CDOCKER protocol under the protein-ligand interaction section in Discovery Studio® 3.1 (Accelrys, San Diego, USA). In general, CDOCKER is a grid-based molecular docking method that employs CHARMM force fields. This protein was firstly held rigid while the ligands were allowed to flex during the refinement. Two hundred random ligand conformations were then generated from the initial ligand structure through high temperature molecular dynamics, followed by random rotations, refinement by grid-based (GRID 1) simulated annealing, and a final grid-based or full force field minimisation [15]. In this experiment, the ligand was heated to a temperature of 700 K in 2000 steps. The cooling steps were set to 5000 steps with 300 K cooling temperature. The grid extension was set to 10 Å. Hydrogen atoms were added to the structure and all ionisable residues were set at their default protonation state at a neutral pH. For each ligand, ten ligand binding poses were ranked according to their CDOCKER energies, and the predicted binding interactions were analysed.

RESULTS AND DISCUSSION

Molecular physicochemical and the drug-likeness are the two most significant properties to be considered for a compound to become a successful drug candidate. It is also important for drug development where a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity, as well as drug-like properties as described by Lipinski's rule [16]. LogP (Octanol-water partition coefficient) is used as significant tool in both quantitative structure-activity relationship (QSAR) studies and rational drug design as a measure of molecular hydrophobicity. The hydrophobicity nature of drug will affect drug absorption, bioavailability, hydrophobic drug-receptor interactions and metabolism of molecules, as well as its toxicity. LogP value of less than

5 and molecular weight in the range between160 to 480 g/ mol are preferred for drug-likeness property as reported by Tambunan and Wulandari (2010) [17]. With regard to the preferred number of N, O (hydrogen bond acceptors), and OH and NH (hydrogen bond donors) which is 10 and/or less than 10, and 5 and/or less than 5, respectively, are compliance with the Lipinski's rules number three and four. As for the rule number five, the number of rotatable bonds (rotb) is favored to be 15 and/or less than 15. Violations of zero will be the main target compound wherein observed for Chrotacumine-A, B, C, Grandol-B, D, G, Rohitukine, Ellagic acid and Thymoquinone. This suggested that these compounds complied very well with the set five rules. However, Quercitin, Artoindonesianin-F, Origanol-A showed two, one and two violations respectively, as shown in Table 1.

Table 1. Molecular descriptors analysis of 12 ligands using Molinspiration online software tool

Ligand	Log A [a]	TPSA[b]	Natoms [c]	MW[d]	noN [e]	nOH NH [f]	Nviolations [g]	Nrotb [h]	Volume [i]
Chrotacumine-A	3.46	89.2	30	407	7	1	0	3	353.2
Chrotacumine-B	3.06	100.2	28	387	7	2	0	4	352.2
Chrotacumine-C	3.18	127.9	36	499	10	2	0	7	439.7
Grandol-B	4.29	77.7	31	432	4	3	0	5	441.3
Grandol-D	4.23	74.5	31	430	4	2	0	5	435.1
Grandol-G	3.29	94.8	32	446	5	3	0	5	442.8
Rohitukine	1.12	94.1	22	305	6	3	0	1	271.7
Quercitrin	0.64	190.2	32	448	11	7	2	3	363.9
Ellagic acid	0.94	141.3	22	302	8	4	0	0	221.7
Artoindonesianin-F*	5.25	80.9	23	312	4	4	1	4	292.3
Origanol-A*	-0.25	186.3	31	438	11	7	2	7	364.4
Thymoquinone	1.9	34.1	12	164	2	0	0	1	161.1

[a]_ Octanol-Water partition coefficient, [b]_ Polar surface area, [c]_ Number of non hydrogen atoms, [d]_ Molecular weight, [e]- Number of hydrogen bond acceptors [O and N atoms], [f]_ Number of hydrogen bond donors [OH and NHgroups], [g]_ Number of Rule of 5 violations, [h]_ Number of rotatable bonds & [i]_ Molecular volume. [*]- Patent filed for tyrosinase inhibitory activity by Cavinkare Pvt Ltd, Chennai, India [18-19].

In our previous report, it has been shown the molecular physicochemical and the drug-likeness properties of two ligands which are embelin and Vilangin [20]. With regard to drug-likeness property, thymoquinone has exhibited a better score compared to all other ligands as shown in the Table 2.

Table 2. Drug-likeness property analysis of 12 ligands using Mol inspiration online software tool

Ligand	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Chrotacumine-A	-0.18	-0.34	-0.13	-0.09	-0.08	-0.01
Chrotacumine-B	-0.10	-0.25	-0.20	0.20	-0.10	0.19
Chrotacumine-C	-0.14	-0.30	-0.05	-0.10	-0.11	-0.03
Grandol-B	0.09	-0.13	-0.51	0.92	0.14	0.65
Grandol-D	0.11	0.26	-0.60	0.93	0.19	0.63
Grandol-G	0.09	0.22	-0.56	0.87	0.21	0.52
Rohitukine	0.04	-0.26	0.15	0.04	-0.08	0.17
Quercitrin	-0.01	-0.08	0.08	0.17	-0.06	0.37
Ellagic acid	-0.29	-0.27	-0.01	0.11	-0.18	0.17
Artoindonesianin-F	0.05	-0.02	-0.04	0.23	-0.07	0.15
Origanol-A	0.04	0.02	-0.11	0.04	0.03	0.26
Thymoquinone	-1.40	-0.31	-1.27	-1.47	-1.44	-0.40

Table 3 shows the docking studies and binding free energy calculations in which Vilangin exhibited the maximum interaction energy (-50.1 kcal/mol). However, it did not exhibit any interaction with any of active site amino acid residues (Table 3 and Figure 1). In contrast, thymoquinone showed very least interaction energy (-18.1 kcal/mol) compared to all other ligands and furthermore exhibited interaction with Arg A147 th amino acid residue.

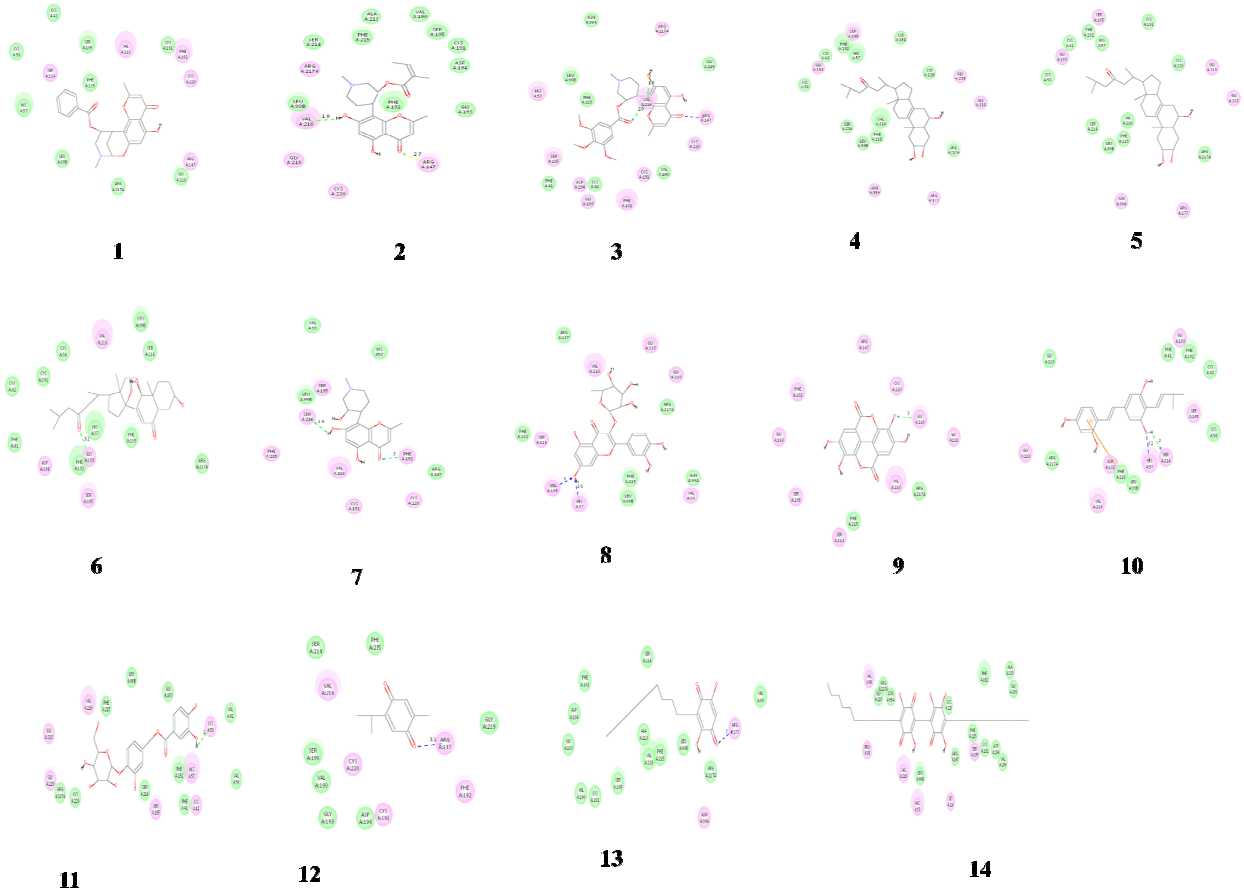
Human neutrophil elastase (HNE) is a 30kD molecular weight glycoprotein and synthesized as zymogen, which becomes active form after post-translation modification [21]. It has specificity towards small hydrophobic amino acids. The potent catalytic activity is facilitated by a catalytic triad that is conserved among all serine proteinase, which consists of His, Asp and Ser residues forming a charge relay system. During proteolysis, the side chain of the peptide is located in the S1 specificity pocket. Its backbone carbonyl is placed in the 'oxy anion hole' and forms hydrogen bonds with the amino group of Gly193 and Ser 195 residues, thus stabilizing the charge transition state [22].

Table 3. The interaction energy analysis of 14 ligands with that of HNE using Discovery Studio® 3.1

Ligand name	cDocker interaction energy* (kcal/mol)	Interaction amino acid residue	Bond distance (Å)
Chrotacumine-A	-31.0	Nil	Nil
Chrotacumine-B	-35.0	Val A216 & Arg A147	1.9 & 2.7
Chrotacumine-C	-42.4	Val A216 & Arg A147	1.9, 2.9 & NA**
Grandol-B	-39.0	Nil	Nil
Grandol-D	-38.0	Nil	Nil
Grandol-G	-36.0	Gly A193	3.2
Rohitukine	-27.0	Ser A214 & Phe A192	1.9 & 3.0
Quercitrin	-40.0	Ser A195 & His A57	3.0 & 2.9
Ellagic acid	-28.0	Gly A219	3.0
Artoindonesianin-F	-33.0	Ser A214 & His A57	2.0 & 3.2
Origanol-A	-41.2	Cys A58	2.0
Thymoquinone	-18.1	Arg A147	3.1
Embelin	-33.0	Arg A177	3.0
Vilangin	-50.1	Nil	Nil

*_ *Calculated interaction energy for the highest ranked, docking pose.* **- *NA- Not analysed*

Figure 1. The interaction analysis of the 14 ligands with that of HNE



Where 1-3 - Chrotacumine-A, B, C; 4-6 - Grandol-B, D, G; 7-Rohitukine; 8-Quericitin; 9-Ellagic acid; 10-Artoindonesianin-F; 11-Origanol-A; 12-Thymoquinone; 13-Embelin & 14-Vilangin.

In the present study among the 14 ligands studied, only Quercitrin showed interaction with Ser 195 amino acid residue (Table 3 and Figure1). Quercitrin and Thymoquinone have been reported to exhibit HNE inhibitory activity [1]. Ellagic acid has been reported to exhibit Porcine pancreatic elastase (PPE) inhibitory activity [23]. However, until the present there is no report available with regard to their docking studies. Even for compounds Embelin [24-25] and Rohitukine [26] which had well been known for their anti-inflammatory activity, there is no available reported investigation for their HNE inhibitory activity.

CONCLUSION

The regulation of the enzymatic activity of Human neutrophil elastase (HNE) has been the prime focus of investigation due to its potential therapeutic application in medicinal field. Understanding and inhibiting HNE indeed would be significant in therapeutically point of view owing to its clear role in acute respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF) and as well as other inflammatory associate disorders such as atherosclerosis, psoriasis and dermatitis. Hence, it is strongly believed that the results of this present study might provide new insight in understanding these 14 ligands as potential candidates for HNE inhibitory agents. Furthermore, the present molecular docking studies could contribute for further development and understanding of HNE inhibitors for the prevention of inflammatory associate disorders.

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