Neprilysin inhibitor from herbal compounds as the latest adjuvant treatment of chronic heart failure

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Abstract: Neprilysin (NEP) is an endopeptidase that metabolizes vasoactive peptides such as natriuretic peptides. Angiotensin Receptor-Neprilysin Inhibitor (ARNI) is an alternative therapy for chronic heart failure (CHF) which is better than angiotensin receptor antagonist therapy alone. This study aimed to identify herbal compounds as in silico NEP inhibitor for adjuvant treatment of CHF. In this study, structure of NEP was obtained from Protein Data Bank (5JMY) and sacubitril as a standard ligand was obtained from PubChem database (9811834). Indonesian herbal compounds were derived from the HerbalDB database that met criteria of Lipinski’s rule. Binding affinity and sites were determined using the AutoDock Vina software. Interaction of herbal compounds and NEP were visualized using the PyMol software. Indonesian herbal compounds with the same binding site at Arg₁₀² dan Arg₁₁₀ amino acids with sacubitril (-6.73 ± 0.06 Kcal/mol) was NSC93241 (-7.07 ± 0.05 Kcal/mol). From 517 herbal compounds, NSC93241 had similar conformation to the standard ligand. NSC93241 has similar molecular formula and molecular weight to herbal plant (Ruscus aculeatus Linn or Butcher’s broom). NSC93241 potentially becomes an NEP inhibitor in silico for adjuvant treatment of CHF. Further investigation is required for evaluation of the antagonist effect of this compound towards NEP.

1. Introduction
Chronic heart failure (CHF) can be defined as a condition that the heart is unable to provide blood needs into entire the human body [1,2], which results from complex pathophysiology through activations of sympathetic nervous system (SNS) and renin-angiotensin-aldosterone system (RAAS) [3]. CHF causes hyperactivation of the SNS that can increase peripheral vascular resistances, ventricular arrhythmias, and cardiac dysfunction [4]. CHF also activates the natriuretic peptides (NPs) family system, which consists of atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), and C-type natriuretic peptide (CNP), to prevent cardiac hypertrophy and fibrosis due to increased excretion of natrium and water and vasodilation [5,6]. However, BNP expression is reduced in proportion to the severity of CHF. Neprilysin (NEP) is the peptide enzyme that is responsible for the degradation of the NPs [7].

Neprilysin (NEP) is a neutral endopeptidase that degrades vasoactive peptides such as NPs, bradykinin, adrenomedullin, endothelin-1, and other peptides [8]. Thus, NEP has become the target for drug development to treat CHF and sacubitril is the latest generation of NEP inhibitor. Sacubitril has...
many beneficial effects in CHF patients if combined to angiotensin converting enzyme inhibitor or angiotensin receptor blocker (ARB) [3]. A combination of ARB (valsartan) and sacubitril reduces 20% heart failure hospitalization [9]. However, long term and continuous uses of sacubitril have not been established yet [10]. Therefore, natural compounds from herbal plants probably become an alternative NEP inhibitor because Indonesia has various plant species that show pharmacological properties [11]. Thus, our study focused on finding phytochemicals from Indonesia’s herbal plants, which had inhibitory effect against NEP using in silico method.

2. Methods
This biocomputational study was done using public data bases and used virtual screening with a molecular docking method.

2.1. Protein preparation
NEP protein was prepared using AutoDock Vina Program 1.1.2 version, which was freely downloaded from http://vina.scripps.edu/download.html. Three dimensional (3D) structure of NEP was obtained from Protein Data Bank (http://www.rcsb.org/pdb/) with access code 5JMY. Before running the molecular docking, water was removed from the NEP protein and then hydrogen atoms were added to the NEP protein in order to increase polarity in the binding pocket. The modified NEP protein was saved in *mol2 and *sdf formats. After that, a grid box was made by selection of some amino acids in the NEP active site that surrounded the binding pocket. Hence, two binding sites of sacubitril to the NEP were located in the center of grid box.

2.2. Standard ligand preparation
Sacubtril was used as a standard ligand for inhibition of NEP protein and its 3D structure was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/) with access code 9811834. Sacubitril was molecularly validated to NEP protein using the Autodock Vina 1.1.2 software three times to obtain binding scores (Table 1). Visualization of sacubitril-NEP interactions was done using the Pymol software version 1.7 (https://pymol.org/2/).

2.3. Molecular docking of herbal compounds with NEP protein
Indonesian herbal compounds used in this molecular docking study were registered in a HerbalDB database that was developed by Department of Pharmacy, Universitas Indonesia. Molecular structures of selected Indonesian herbal compounds were downloaded from a public database (PubChem) and 517 compounds were matched to Lipinski’s rule of five criteria. Data of all selected compounds were converted in to *pdbqt format file using the Pyrx 0.8 program (https://pyrx.sourceforge.io/downloads). All herbal compounds were molecularly docked with NEP protein using the AutoDock Vina 1.1.2 program to analyze their binding scores. Molecular interaction between herbal compounds and NEP protein was then visualized using the Pymol 1.7 program. herbal compound-NEP binding complexes had lower binding scores than sacubitril and had similar binding sites to sacubitril were considered as a new candidate of NEP inhibitor.

3. Results and discussion
Table 1, Figure 1 and Figure 2 showed binding affinity and binding sites of NEP and its inhibitor (sacubitril). Sacubitril had -6.73± 0.06 Kcal/mol the average of binding affinity and interacted with NEP protein at Ser\(^{101}\), Arg\(^{102}\), Asp\(^{107}\), and Arg\(^{110}\) residues. Moreover, O atoms in sacubitril bound to hydrogen atoms of NEP protein. From a previous study, binding affinity and binding sites between sacubitril and NEP in silico have not been reported yet, but it found amino acids in the active sites of NEP that interacted with sacubitrilat (sacubitril’s active metabolite) [7]. Our finding also indicated that sacubitril interacted with the NEP protein through hydrogen bonds. In drug design and development, hydrogen bond is very important not only in inhibition of targeted proteins (enzyme/receptor) but also in binding affinity [12]. Binding affinity in molecular docking programs is
calculated by summing up all molecular interactions such as hydrophobic, hydrogen, van der Walls, electrostatic, and solvation effect. Therefore, stronger molecular interaction will have lower binding affinity and more stable ligand binding complex [13, 14].

### Table 1. Validation of sacubitril bound to NEP protein.

| Compound (ID PubChem) | Binding Affinity (Kcal/mol) | Binding Site |
|-----------------------|-----------------------------|--------------|
|                       | 1  | 2  | 3  | Mean ± SD | |
| Sacubitril (9811834)  | -6.7 | -6.7 | -6.8 | -6.73 ± 0.06 | Ser101, Asp107, Arg102, dan Arg110 |

**Figure 1.** Overlay of sacubitril-NEP binding complexes in cartoon. Dark blue and light blue colour indicated sacubitril and NEP respectively. Binding sites of sacubitril to NEP were Arg102 and Arg110 residues (red colour) showed in black arrows.

**Figure 2.** Sacubitril-NEP binding complexes was visualized using Pymol 1.7 software. Red circles showed interaction between sacubitril (green sticks) and NEP (light blue sticks) at Ser101, Arg102, Arg110, and Asp107 residues. Black dots were atom interaction between oxygen (red) and hydrogen (white).

From 517 compounds which met Lipinski’s criteria, only six compounds had lower binding affinity than sacubitril (Table 2). The lowest molecular weight was found in eurycomalactone but five other compounds had higher molecular weight than sacubitril. All compounds had H bond donor and acceptor, similar to sacubitril. In regard to liphopilicity, AC1L4N48, eurycomalactone, and withanolide D were lower than sacubitril. According to Doak et al, a new potential drug has to meet the Lipinski’s criteria [15]. Our results indicated that all herbal compounds potentially became new NEP inhibitors.

Compared to sacubitril, docking scores of all compounds were lower than -6.73 ± 0.06 Kcal/mol and the lowest docking scores was observed in AC1L4N48 (-8.13 ± 0.38 Kcal/mol) (Table 3). In drug development, lower binding affinity is required for stability of interaction between ligand and targeted proteins [16] and enhances the ability of ligand to inhibit targeted proteins [14]. Therefore, all herbal compounds in our study can be selected as a new NEP inhibitor.

In terms of binding sites, only NSC93241 compound that had similar binding sites to sacubitril at Ser101, Arg102, and Arg110 residues. Meanwhile, other compounds were able to interact with the NEP at different binding sites. It could be Arg102 for AC1L4N48 and castasterone and Arg110 for NSC640465, withanolide D, and eurycomalactone. Figure 3 and 4 showed that NSC93241 occupied the NEP binding pocket as similar as sacubitril except val541. NSC93241 bound to NEP at Arg102 and Arg110.
residues and had similar conformation to sacubitril. Based on Ehrt et al, the same binding sites of a new drug candidate against the standard drug is an important way to estimate its molecular function either competitive or non-competitive inhibition [17]. In our study, only NSC93241 has similar binding sites to sacubitril and becomes a NEP inhibitor.

### Table 2. Herbal compound Lipinski’s rule of five criteria.

| Compound (ID PubChem) | Molecular Formula | Lipinski’s Rule of Five |
|-----------------------|-------------------|-------------------------|
|                       | MW (<500) (Da)    | H Bond Donor (<5) | H Bond Acceptor (<10) | Liphopilicity (XLogP3-AA<5) |
| NSC93241 (261265)     | C_{27}H_{42}O_{4} | 430.629           | 2  | 4  | 4.1  |
| Sacubitril (9811834)  | C_{24}H_{29}O_{5} | 411.498           | 2  | 5  | 3.7  |
| AC1L4N48 (160143)     | C_{26}H_{40}O_{4} | 480.598           | 4  | 8  | 2.6  |
| NSC640465 (363978)    | C_{28}H_{40}O_{5} | 450.575           | 0  | 5  | 4.8  |
| Withanolide D (161671)| C_{23}H_{30}O_{6} | 470.606           | 2  | 6  | 3.1  |
| Castasterone (133534) | C_{28}H_{40}O_{5} | 464.687           | 4  | 5  | 4.7  |
| Eurycomalactone (441793)| C_{19}H_{24}O_{6} | 348.395           | 2  | 6  | 0.1  |

### Table 3. Result of docking between herbal compound with NEP.

| Compound (ID PubChem) | Binding Affinity (Kcal/mol) | Binding Site |
|-----------------------|----------------------------|--------------|
|                       | 1   | 2   | 3   | Mean ± SD |                     |
| NSC93241 (261265)     | -7.1| -7  | -7.1| -7.07 ± 0.05| Ser^{101}, Arg^{102}, Arg^{110}, and Val^{341} |
| Sacubitril (9811834)  | -6.7| -6.7| -6.8| -6.73 ± 0.06| Arg^{101}, Asp^{107}, Arg^{102} and Arg^{110} |
| AC1L4N48 (160143)     | -8.4| -8.4| -7.6| -8.13 ± 0.38| Arg^{102}, Asp^{542}, Ser^{101}, and Asp^{111} |
| NSC640465 (363978)    | -8.2| -6.4| -8.1| -7.57 ± 0.83| Arg^{110} and Val^{341} |
| Withanolide D (161671)| -6.7| -6.7| -9  | -7.47 ± 1.08| Arg^{110}, Asp^{542}, and Ser^{101} |
| Castasterone (133534) | -7.1| -7.1| -7.3| -7.23 ± 0.09| Arg^{102} and Ser^{101} |
| Eurycomalactone (441793)| -7.2| -7.2| -6.5| -6.97 ± 0.33| Arg^{110}, Glu^{114}, and Asn^{512} |
Figure 3. Overlay of sacubitril/NSC93241-NEP binding complexes in cartoon. Green and dark blue colour indicated NSC93241 and sacubitril compounds respectively whereas blue colour was NEP. Binding sites of both compounds to NEP were Arg$^{102}$ and Arg$^{110}$ residues (red colour) showed in black arrows.

Figure 4. Docking result of NSC93241-NEP binding complexes was visualized using the Pymol 1.7 software. Red circles showed interaction between NSC93241 (green sticks) and NEP (blue sticks) at Ser$^{101}$, Arg$^{102}$, Arg$^{110}$, and Val$^{541}$ residues. Black dots were atom interaction between oxygen (red) and hydrogen (white).

NSC93241 has similar molecular formula and MW to herbal plant (Ruscus aculeatus Linn or Butcher’s broom). Butcher’s broom extract has been used for treatment of vascular disorders such as hemorrhoids, atherosclerosis, varicose veins, and chronic venous insufficiency [18]. Therefore, extract of this plant is more likely able to inhibit NEP activity in future.

4. Conclusion
In conclusion, NSC93241 potentially becomes a new NEP inhibitor in silico, which is better than sacubitril for adjuvant treatment of CHF. To verify this bio-computational findings, in vitro study is required.

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