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Molecular docking, drug-likeness, and ADMET study of 1-benzyl-3-benzoylurea and its analogs against VEGFR-2

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Abstract. *In silico* study was performed to predict the possibility of 1-benzyl-3-benzoylurea and 22 analogs as anticancer drug candidates, via VEGFR2 inhibition. Molecular docking studies against VEGFR2 receptor revealed that all of designed compounds have better score than the lead compound, of which three analogs (p-nitro, p-methoxy, and p-ethyl) were considered optimal among other compounds (< -90 kcal mol^{-1}). However, this result was not comparable to lenvatinib, which acts as native ligand of the receptor (-118.62 kcal mol^{-1}). Docking poses analysis showed that 1-benzyl-3-benzoylurea analogs failed to completely occupy VEGFR2 binding site. Therefore, it is argued that this has caused the non-optimal docking score of designed compounds. Furthermore, these compounds passed five different drug-likeness criteria successfully and were predicted to be orally bioavailable in rat. Ultimately, most of the analogs were predicted to have good ADMET characteristics, notably in terms of GI absorption and the absence of P-gp interaction, and low toxicity in rat. This study can be used as a starting point to validate this model by synthesis, *in vitro* and *in vivo* assay to validate the activity of 1-benzyl-3-benzoylurea and its analogs as potential anticancer candidate.
Keywords: Anticancer candidate, arylurea derivatives, *in silico* study, VEGFR-2 inhibitor.

1. Introduction

Cancer is a terminology which is commonly used to describe the abnormality in cell growth and division [1]. In 2012, the number of population with cancer has reached 14.1×10^6 people, with 8.2×10^6 mortality [2]. In Indonesia itself, the prevalence of cancer is 1.4 % of total population [3]. Hydroxyurea has been used for decades and still possesses valuable therapeutic activity against several type of cancer. This hydrophilic compund is distributed evenly in the human body fluid. Under physiologic condition (pH 7.4), the majority ofhydroxyurea will undergo ionization, thus reducing its ability to penetrate biological membrane. This condition will significantly diminished its biological activity [4].

Several analog of urea based compounds have been widely developed, one of which is arylurea. Numerous studies suggested that this modification showed potential anticancer activity. Furthermore, preliminary study has proven the anticancer activity of 1-benzyl-3-benzoylurea, one of the analog of benzoylurea. This compound inhibits the growth of MCF-7 cell culture in breast cancer better than hydroxyurea [5]. Based on the finding, it can be concluded that 1-benzyl-3-benzoylurea is potential to be further developed as anticancer agent. This premise is supported by the number of anticancer drugs

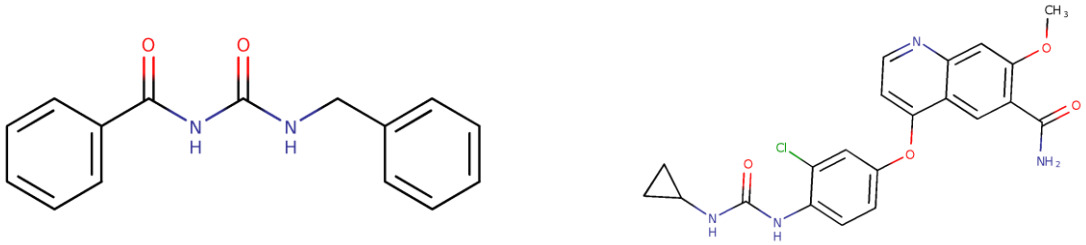
in the market which contain arylurea functional group, such as sorafenib and lenvatinib. These two compounds are known to act as VEGFR-2 inhibitor [6, Topliss in 7] (figure 1).

This research aimed to explore the possibility to improve the anticancer activity of 1-benzyl-3-benzoylurea analogs using *in silico* method. Several novel compounds were designed and predicted their activity using molecular docking against VEGFR-2. Furthermore, the drug-likeness and ADMET properties of those compounds were also evaluated.

2. Materials and methods

2.1. Preparation of 1-benzyl-3-benzoylurea analogs

Total of 23 novel analogs of 1-benzyl-3-benzoylurea were designed by implementing Topliss scheme [Topliss in 7], of which the steric and electronic properties of every substituent were taken into account. These compounds were drawn in 2D format (MarvinSketch 16.8.1) (http://www.chemaxon.com) and then transformed into 3D structure using semiempirical method of AM1 [8] available in MOPAC2016 (http://openmopac.net/MOPAC2016.html).



(a) (b)

Figure 1. Chemical structure of 1-Benzyl-3Benzoylurea (a) and lenvatinib (b).

2.2. Preparation of protein

VEGFR-2 protein was obtained from Brookhaven Protein Data Bank (PDB) (https://www.rcsb.org/pdb/) with PDB ID of 3WZD. This protein was chosen since it is complexed with lenvatinib, an arylurea analog [6]. Hydrogen atom was then added, followed by the assignment of Amber ff14SB partial charge [9]. Correction of sidechain residue was also performed by employeeing the most recent Dunbrack rotamer library [10]. The purpose of this step was to reconstruct the missing information of amino acid sidechain. Lenvatinib was also prepared by adding hydrogen atom. The whole process was performed using Chimera 1.13 [11].

2.3. Molecular docking

Molegro Virtual Docker 5.0 (Molegro ApS, 2013) was applied to evaluate the interaction of designed compounds with VEGFR-2.

2.4. ADMET and drug-likeness evaluation

Drug candidates should possess favorable ADME properties and ideally non-toxic. Therefore, the designed compounds were evaluated of their ADME profile, including drug-likeness, partition coefficient, solubility, and several other parameters using SwissADME [12] module provided in SIB (Swiss Institute of Bioinformatics) webserver (https://www.sib.swiss). Furthermore, the toxicity aspect of designed compound was also predicted using webserver ProTox [13] (https://tox.charite.de).

3. Results

3.1. Molecular docking

Molecular docking was performed to evaluate the interaction mode of designed compounds against VEGFR-2 and to assess the magnitude of interaction between them. Similar study have been performed [14] using similar receptor, albeit complexed with different arylurea analog [15]. This study

was conducted in order to verify the previous result using slightly modified method. Docking method needs to be validated by redocking the native ligand to the protein prior to the usage [16]. Based on redocking result of lenvatinib to its protein [6], RMSD result of 0.7 was obtained. This implies that the docking method used is able to predict the ligand orientation accurately. Docking method selected in this study was simplex evolution as pose generator which was evaluated using grid based MolDock score [17]. The findings showed that no designed compounds performed better than native ligand, in terms of docking score (table 1).

Docking pose analysis of three best performing analogs (Compound 11, 22, and 23) showed the dissimilarity of binding mode compared to Lenvatinib. Similarity is only observed in the urea moiety of both compound, which is with Glu 885 and Asp 1046. Lenvatinib as the newly discovered VEGFR-2 inhibitor classified as type V inhibitor, interact in a similar way with Sorafenib as the older, type II VEGFR-2 inhibitor [6, Topliss in 7]. Therefore, the pharmacophore motive for Sorafenib-like, type II VEGFR-2 inhibitor [18] could also be applicable for type V inhibitor. However, docking pose of all designed compounds showed occupation only in the RDP (Regulatory Domain Pocket) area. While type II inhibitor and Lenvatinib also occupy ATP binding domain. This made the docking score of 1-benzyl-3benzoylurea analogs are higher than lenvatinib and sorafenib [14] (figure 2).

3.2. ADMET and drug-likeness evaluation

SwissADME provides detail and extensive physicochemical profile, ADME, and medicinal chemistry property of a compound. Regarding the physicochemical aspect, two parameters (partition coefficient and solubility) are considered to play important roles. Based on predicted LogP value, it is concluded that all of designed compounds lies within the range value of 1.6 to 3.6. Eventhough LogP value does not always correspond to certain ADME aspect, this parameter could depict the probability of a compund as drug candidate, where in this case all 23 compunds possess such quality to be considered as drug-like [19]. SwissADME LogP value was calculated from five different algorithm, therefore it is assumed that the value represents real condition [12]. On the other hand, solubility prediction was carried out using three different method with the output of LogS value. Generally, the designed compounds are predicted to have optimum water solubility, even if there was a slightly different result among the method. The most obvious difference was observed in SILICOS-IT (http://silicos-it.be.s3-website-eu-west-1.amazonaws.com/software/filter-it/1.0.2/filter-it.html) method since it employs fragment-based approach in LogS calculation, while the other methods [20, 21] based on complete molecular topology (table 2).

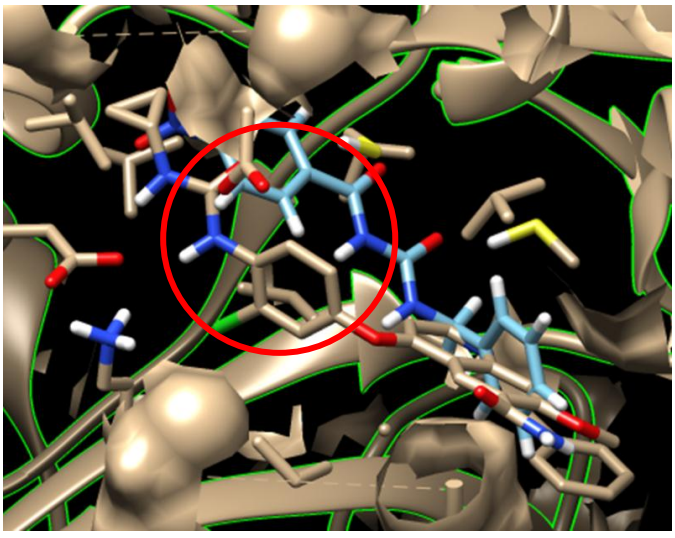
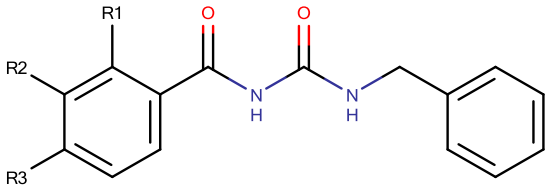


Figure 2. Docking pose superposition of one of 1-Benzyl-3-Benzoylurea analogs (compound 23) (light blue) with Lenvatinib (gray). It showed a dissimilarity in binding mode between those two compounds, notably in urea moiety (highlighted).

Table 1. Docking score of 1-Benzyl-3-Benzoylurea and its analogs against VEGFR-2.



Code	Compound	Substituents	Docking Score (kcal mol^{-1})
1	1-benzyl-3-benzoylurea	R1=H;R2=H;R3=H	-73.95
2	1-benzyl-3-(2-chloro)-benzoylurea	R1=Cl;R2=H;R3=H	-76.92
3	1-benzyl-3-(3- chloro)-benzoylurea	R1=H;R2=Cl;R3=H	-80.38
4	1-benzyl-3-(4-chloro)-benzoylurea	R1=H;R2=H;R3=Cl	-81.71
5	1-benzyl-3-(2,4-dichloro)-benzoylurea	R1=Cl;R2=H;R3=Cl	-79.14
6	1-benzyl-3-(3,4-dichloro)-benzoylurea	R1=H;R2=Cl;R3=Cl	-79.83
7	1-benzyl-3-(4-chloromethyl)-benzoylurea	R1=H;R2=H;R3=CH_2Cl	-75.48
8	1-benzyl-3-(3-chloromethyl)-benzoylurea	R1=H;R2=CH_2Cl;R3=H	-87.19
9	1-benzyl-3-(2- chloromethyl)-benzoylurea	R1=CH_2Cl;R2=H;R3=H	-79.72
10	1-benzyl-3-(4-methyl)-benzoylurea	R1=H;R2=H;R3=CH_3	-74.15
11	1-benzyl-3-(4-ethyl)-benzoylurea	R1=H;R2=H;R3=C_2H_5	-90.73
12	1-benzyl-3-(3-ethyl)-benzoylurea	R1=H;R2=C_2H_5;R3=H	-78.99
13	1-benzyl-3-(2-ethyl)-benzoylurea	R1=C_2H_5;R2=H;R3=H	-78.6
14	1-benzyl-3-(4-propyl)- benzoylurea	R1=H;R2=H;R3=C_3H_7	-83.51
15	1-benzyl-3-(4-t-butyl)-benzoylurea	R1=H;R2=H;R3=$C(CH_3)_3$	-69.76
16	1-benzyl-3-(4-fluoro)-benzoylurea	R1=H;R2=H;R3=F	-82.21
17	1-benzyl-3-(2-trifluoromethyl)-benzoylurea	R1=CF_3;R2=H;R3=H	-81.13

Continue to the next page.

Table 1. Continued.

Code	Compound	Substituents	Docking Score (kcal mol^{-1})
18	1-benzyl-3-(3-trifluoromethyl)-benzoylurea	R1=H;R2=CF_3;R3=H	-87.26
19	1-benzyl-3-(4-trifluoromethyl)-benzoylurea	R1=H;R2=H;R3=CF_3	-79.78
20	1-benzyl-3-(4-bromo)-benzoylurea	R1=H;R2=H;R3=Br	-76.53
21	1-benzyl-3-(4- bromomethyl)-benzoylurea	R1=H;R2=H;R3=CH_2Br	-74.34
22	1-benzyl-3-(4- nitro)-benzoylurea	R1=H;R2=H;R3=NO_2	-95.2
23	1-benzyl-3-(4- methoxy)-benzoylurea	R1=H;R2=H;R3=OCH_3	-90.91
	Lenvatinib		-118.62

ADME prediction showed that the analog compounds possess several favorable ADME properties. Based on ratio of WlogP to tPSA [22], all of the compound was predicted to have good absorption in GIT and 22 of which could penetrate blood-brain barrier. Analog with nitro substituent possesses the lowest partition coefficient, hence too hydrophyllic to penetrate blood-brain barrier. Surprisingly, there is no single compound which has a tendency to act as P-gp substrate. P-glycoprotein, a macromolecule found to be overexpressed in multi-drug resistant cancer, has an important role in transporting xenobiotics out from cell [23]. Therefore, this finding indicates that 1-benzyl-3-benzoylurea scaffold could be used as potential anticancer agent. Furthermore, SwissADME also enable one to predict the possible occurrence of CYP450-mediated biotransformation. The result showed that the majority of compound interacts with at least one of the five isoforms, where two of the analogs (4-propyl and 4-bromomethyl) would interact with four out of five isoforms. On the contrary, three of the analogs (4-fluoro, 4-nitro, and 4-methoxy) along with the parent compound possibly would not interact with any of the CYP450 isoforms. It is also concluded that 1A2 and 2C19 are the major isoform which would become the target of most of the analogs, while no interaction is predicted to happen with 3A4 (figure 3).

Drug-likeness is a key criteria in screening drug candidates at the earlier phase of drug discovery and development. This parameter can be described as a mean to correlate physicochemical aspect of a compound with its biopharmaceutical aspect in human body, especially its influence in bioavailability of per oral route [24]. Based on five commonly used drug-likeness criteria applied in the webserver [12], 1-benzyl-3-benzoylurea with its 22 analogs are considered to pass all of them, thus can be categorized as drug-like compounds. In addition, evaluation was also carried out using ABS criteria [25], where all of the compounds obtained the value of 0.55. This criteria is based on the probability value of a compound to possess optimum profile of bioavailability and permeability, where value of 0.55 implies the obedience of Lipinski rule of five [26] and 55 % probability of rat bioavailability value higher than 10 %.

In addition, detection of structural alert [27] was performed in order to identify whether the designed compound possess problematic functional group, which could lead to a toxicity, mutagenicity, or metabolic instability. It showed that five of designed compounds possess indesirable moiety (alkyl halide and nitroarene). Studies have indicated the reactivity of compounds containing such functional groups, which lead to carcinogenic, mutagenic, and hepatotoxic effect [28]. This

criteria could be used as preliminary alert to give more attention to the activity and toxicity in developing 1-benzyl-3-benzoylurea scaffold [29].

Table 2. Water solubility prediction of 1-Benzyl-3-Benzoylurea and its analogs.

Compounds	LogP	Water Solubility		
	(Consensus LogP)	LogS (ESOL)	LogS (Ali)	LogS (SILICOS-IT)
1	2.16	Soluble	Soluble	Moderately Soluble
2	2.79	Soluble	Moderately Soluble	Moderately Soluble
3	2.74	Soluble	Moderately Soluble	Moderately Soluble
4	2.73	Soluble	Moderately Soluble	Moderately Soluble
5	3.32	Moderately Soluble	Moderately Soluble	Poorly Soluble
6	3.26	Moderately Soluble	Moderately Soluble	Poorly Soluble
7	2.8	Soluble	Moderately Soluble	Poorly Soluble
8	2.77	Soluble	Moderately Soluble	Poorly Soluble
9	2.78	Soluble	Moderately Soluble	Poorly Soluble
10	2.59	Soluble	Moderately Soluble	Moderately Soluble
11	2.86	Soluble	Moderately Soluble	Poorly Soluble
12	2.86	Soluble	Moderately Soluble	Poorly Soluble
13	2.89	Soluble	Moderately Soluble	Poorly Soluble
14	3.25	Moderately Soluble	Moderately Soluble	Poorly Soluble
15	3.55	Moderately Soluble	Moderately Soluble	Poorly Soluble
16	2.55	Soluble	Soluble	Moderately Soluble
17	3.24	Moderately Soluble	Moderately Soluble	Poorly Soluble
18	3.29	Moderately Soluble	Moderately Soluble	Poorly Soluble
19	3.27	Moderately Soluble	Moderately Soluble	Poorly Soluble
20	2.79	Moderately Soluble	Moderately Soluble	Poorly Soluble
21	2.92	Moderately Soluble	Moderately Soluble	Poorly Soluble
22	1.62	Soluble	Moderately Soluble	Moderately Soluble
23	2.23	Soluble	Soluble	Moderately Soluble

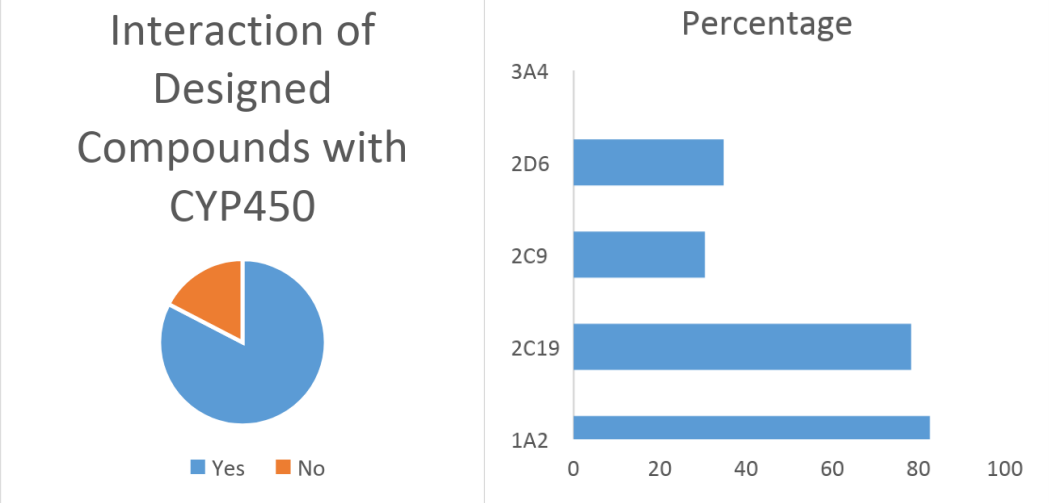


Figure 3. Prediction of interaction of designed compounds with several CYP450 isoforms.

Ultimately, prediction of toxicity using ProTox [13] showed that the designed compound are predicted to have oral LD50 value ranging from 818 mg kg^{-1} to 3 000 mg kg^{-1} in rat model, with analogs of p-methyl and p-trifluoromethyl bearing the lowest value and four analogs (o-chloro, o-chloromethyl, m-chloromethyl, and o-trifluoromethyl) bearing the highest one. It was also observed that the substituent position in aromatic could significantly affect the predicted value of LD50 as was shown in the case of trifluoromethyl (*vide supra*).

4. Conclusion

Molecular docking study showed that three of 1-benzyl-3-benzoylurea analog (p-nitro, p-methoxy, and p-ethyl) possess better docking score among the other analogs with the value around -90 kcal mol^{-1}. However, there is no single compound which performs better than Lenvatinib (-118.62 kcal mol^{-1}) due to the inability of all designed compound to completely occupy the VEGFR-2 binding site. This hypothesis would be used as a foundation which needs to be proven via *in vitro* assay in the future. Furthermore, it can be implied that the majority of compound have good physicochemical profile with several other ADMET properties, notably in terms of P-gp interaction. Nevertheless, the identification of several analogs with problematic moiety, should be noted in order to develop more safe and potent anticancer agent based on 1-benzyl-3-benzoylurea scaffold.

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