Computer-aided Structure-based Design of 3,3'-Diallyl-[1,1'-biphenyl]-4,4'- diol Analogs of Eugenol as Potential Ligands for Estrogen Receptor Alpha

Enade Perdana Istyastono[1*], Yulia Anita[2], Andini Sundowo[2]

[1]*Faculty of Pharmacy, Sanata Dharma University, Campus 3 Paingan Maguwohardjo Sleman, Yogyakarta 55281, Indonesia*
[2]*Research Center for Chemistry, Indonesian Institute of Sciences, Komplek Puspiptek Serpong, Indonesia*

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Abstract:
The SBVS protocols to identify ligands for estrogen receptor alpha (ERα) were retrospectively validated and could serve as a virtual tool to screen potential ERα ligands. Subsequently, prospective virtual screen campaigns on eugenol analogs and their dimers were performed and have discovered 3,3'-diallyl-[1,1'-biphenyl]-4,4'-diol analogs of eugenol as the most potential ERα fragment to be further developed. This article presents the design of 3,3'-diallyl-[1,1'-biphenyl]-4,4'-diol analogs of eugenol based on the comparison between the docked poses of ZINC01914469 as a reference compound and 3,3'-diallyl-[1,1'-biphenyl]-4,4'-diol analogs of eugenol. The designed compounds were selected based on the docking score using the validated SBVS protocols. The docking scores of the selected compounds revealed that these compounds were statistically similar or better than ZINC01914469.

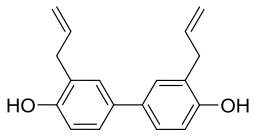
Key words: *Drug design, estrogen receptor alpha, fragment-based, molecular docking, structure-based, virtual screening*

Introduction

Prospective virtual screen campaigns on eugenol analogs and their dimers were performed by using a retrospectively validated structure-based virtual screening (SBVS) protocol [1] and discovered 3,3'-diallyl-[1,1'-biphenyl]-4,4'-diol analog of eugenol (**1**; Figure 1) as a potential fragment [2] for estrogen receptor alpha (ERα). The retrospective validation of the SBVS protocols to identify ligands for estrogen receptor alpha (ERα) resulted in the enrichment factor in 1% false positive ($EF_{1\%}$) value of 21.2, which is considered as acceptable [1, 3]. The retrospective SBVS compound employed a dataset of useful decoys (DUD) compiled by Huang *et al.*[3] and has selected a reference ligand to be compared in subsequent SBVS campaigns [1].

The reference ligand ZINC01914469 (**2**; Figure 1) [3, 4], an antagonist for ERα with IC50 value of 69.23 nM [5], was the compound ranked in the threshold line of the $EF_{1\%}$ value in the SBVS campaign [1]. Compound **1** was identified as the potential fragment to be developed further [1, 2], and subsequently the *in vitro* experiments has confirmed the prediction [1, 6].

As a confirmed fragment, compound **1** has plenty rooms for improvement to be developed as potent ligands for ERα by employing both growing and merging strategies [2]. The overlay of compounds **1** and **2** bound to the ERα binding pocked (Figure 2) resulted in the previous SBVS campaign [1] could serve as the starting point to perform computer-aided *de novo* structure-based designs. The designed compound should have similar or better ChemPLP scores [7] compared to the reference ligand **2** in a SBVS campaign to have better possibilities for the compound to be an ERα potent ligand.



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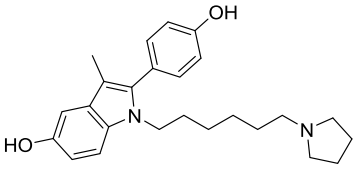

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Figure 1. 3,3'-diallyl-[1,1'-biphenyl]-4,4'-diol (**1**) [1] and ZINC01914469 (**2**) [4, 5].

The research presented in this article was aimed to employ the previously validated SBVS protocol to develop a potential ERα ligand by growing of compound **1**, an experimentally confirmed fragment for ERα [1].

***Corresponding author:** Enade Perdana Istyastono,
E-mail: enade@usd.ac.id

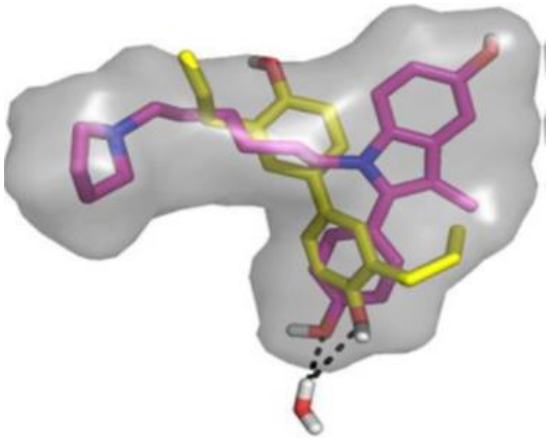


Figure 2. The docked poses superposition of ZINC01914469 (yellow carbon atoms) and 3,3'-diallyl-[1,1'-biphenyl]-4,4'-diol analog of eugenol (magenta carbon atoms). The conserved water molecule is also showed here for clarity. The hydrogen bonds are indicated by dashed black lines (Taken from [1]).

Inspired by Figure 2, several ligands were designed, and a potential ERα ligand could be identified. The ligand showed statistically similar ChemPLP scores to compound **2** as the reference compound.

Experimental

Materials

The same work station and softwares employed to perform the retrospective SBVS [1] were used in this research to virtually screen the designed compounds. The design processes were aided by visual inspection using molecular visualization software PyMOL 1.2r1 [8] and a virtual molecular builder BKChem 0.13.0[9]. The statistical analysis was performed in R computational statistics software 3.1.0 [10].

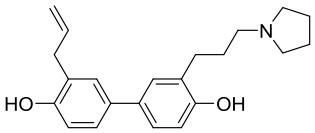
Computational Methods

Based on visual inspections on the superposition of compounds **1** and **2** bound to the ERα binding pocked (Figure 2) by employing PyMOL 1.2r1[8], an analog of compound **1** was built and exported to a .mol file using BKChem0.13.0 [9] and virtually screened three times by employing the retrospectively validated SBVS protocol [1].

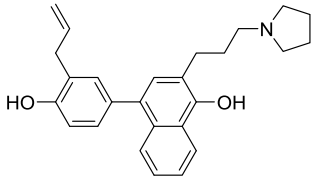
The ChemPLP scores obtained through the virtual screening (VS) were then subsequently analyzed using one-tailed t-test with confidence level of 95% to check whether the scores were higher than the ChemPLP scores of compound **2**. If the scores were higher, the proceses steps were repeated starting from the visual inspections on the docking poses of the analog to design another analog of compound **1** until the statistical analysis showed that the ChemPLP scores of the designed compound were similar or less than the ChemPLP scores of compound **2**.

Results and Discussion

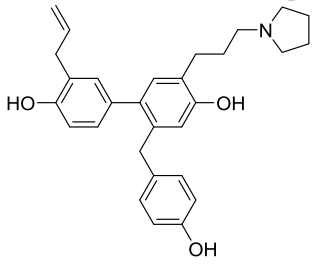
The research aimed to design a potential ligand for ERα based on a confirmed fragment compound 1 by employing a validated SBVS protocol to identify ERα ligands [1]. The designed compounds (compounds 3-6) and their ChemPLP values are presented in Figure 3. Compound 5 was the potential ERα ligand virtually identified in this research.



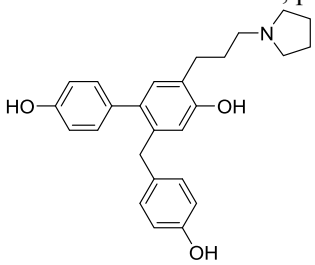
3; ChemPLP score = –104.563 ± 2.057; p-value = 0.005



4; ChemPLP score = –99.363 ± 1.266; p-value = 0.002



5; ChemPLP score = –125.338 ± 1.818; p-value = 0.955



6; ChemPLP score = –117.142 ± 0.892; p-value = 0.435

Figure 3. The ChemPLP scores of Analogs of compound **1** obtained through virtual screening and their p-values.

As shown in figure 4, only polar hydrogen atoms (presented in white) residues (presented in sticks mode, carbon atoms are in green) with hydrogen bond interaction (presented in black dashes), ionic interaction (presented in red dashes) and aromatic interaction to the ligand, and a conserved water molecule are presented for the sake of clarity. Nitrogen and oxygen atoms are presented in blue and red, respectively.

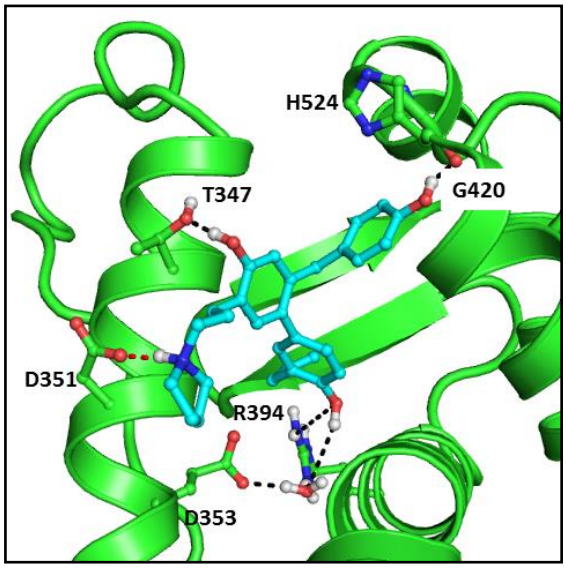


Figure 4. The docking pose of compound 5 (Ball and sticks mode; Carbon atoms are in cyan) in the ERα (Cartoon mode; Carbon atoms are in green) binding pocket [11].

By analyzing Figure 2, compound **3** was designed by substitution of a 1-propene moiety from compound **1** to 1-propylpyrrolidine. Compound **3** showed a significantly better ChemPLP score compared to compound **1** [1]. An additional benzene ring to compound **3** (compound **4**) showed a slightly higher ChemPLP score, but an additional *p*-cresol from moiety (compound **5**) could significantly decrease the ChemPLP score. Notably, the ChemPLP score of compound **5** is statistically similar or better (p-value ≥ 0.050) compared to the ChemPLP score of the reference ligand compound **2**. Elimination of the 1-propene moiety from compound **5** (compound **6**) showed a slightly higher ChemPLP score, but the ChemPLP score is still statistically similar or better compared to the ChemPLP score of the reference ligand compound **2**. It is therefore suggested to have a room for affinity improvement by substitution of the moiety to other non-polar moieties to obtain better hydrophobic interaction to ERα.

Visual inspection of the binding pose of compound **5** in ERα binding pocket (Figure 4) could provide insight for further structure-based design of ERα ligands. The ligand has all interaction possessed by 4-hydroxy-tamoxifen (**7**) [11] with an additional hydrogen bond to the backbone of G420. Compound **5** should be synthesized and further investigated in *in vitro* experiments to be confirmed as a ligand for ERα.

Conclusions

Compound **5** (Figure 3) is a potential analog of 3,3'-Diallyl-[1,1'-biphenyl]-4,4'-diol analog of eugenol to be developed further as a potent ERα ligand.

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