

In silico QSAR of 1-benzoyl-3-benzylurea lead and its analogue compounds as anticancer by VEGFR-2 inhibition

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ABSTRACT: VEGFR-2 plays a role in proangiogenic activity. An *in-silico* study was conducted on 1-benzoyl-3-benzylurea lead and its analogue compounds as anticancer by VEGFR-2 (PDB code: 4ASD) inhibition. The purpose of this study is to find Quantitative Structure Activity Relationship (QSAR) by designing novel compounds and predicting their bioavailability and toxicity. Structural modification was carried out by substituting some substituent with certain physicochemical properties (lipophilic, electronic, and steric) into benzoyl group. The prediction of bioavailability (F) and toxicity (LD₅₀) were performed by ACD-I/Lab. The prediction of activity (Rerank Score/RS) was carried out by Molegro Virtual Docker (MVD) 5.0. The result of regression from 1-benzyl-3-benzoylurea lead and its analogue compounds by IBM® SPSS® Statistic 20 shows that there are nonlinear relationships between modification of physicochemical properties with bioavailability prediction ($F > 70\% \text{ oral} = -1.548 \text{ ClogP} + 0.198 \text{ ClogP}^2 + 0.125 \text{ pKa} - 0.168 \text{ CMR} + 3.502$) and modification of physicochemical properties with activity prediction ($\text{Rerank Score} = 1.802 \text{ Es} + 5.421 \text{ ClogP}^2 - 44.744 \text{ ClogP} - 11.152$). Also, there is a linear relationship between modification of physicochemical properties and toxicity prediction ($\text{LD}_{-50} \text{ Mouse} = -7.422 \text{ Mw} - 117.197 \text{ pKa} + 260.565 \pi + 4342.379$ and $\text{LD}_{-50} \text{ Rat} = 691.028 \text{ CMR} - 21.453 \text{ Etot} - 430.187 \pi - 4775.208$). These quantitative equations can be used as foundations for further structural modification to discover a novel potential anticancer drug with better bioavailability, activity, and minimum toxicity.

Key Words: QSAR, 1-benzoyl-3-benzylurea lead and its analogue compounds, VEGFR-2 inhibition, *in-silico*.

1 INTRODUCTION

Angiogenesis is a normal and vital process in growth and development, as well as in wound healing and in the formation of granulation tissue. However, it is also a fundamental step in the transition of tumors from a benign state to a malignant one, leading to the use of angiogenesis inhibitors in the treatment of cancer.

An angiogenesis inhibitor is a substance that inhibits the growth of new blood vessels (angiogenesis). Some angiogenesis inhibitors are endogenous and a normal part of the body's control and others are obtained exogenously through pharmaceutical drugs or diet.

Angiogenesis inhibitors were once thought to have potential as a "silver bullet" treatment applicable to many types of cancer, but the limitations of anti-angiogenic therapy have been shown in prac-

tice. Therefore, a novel effective angiogenesis inhibitor is urgently needed.

VEGF (Vascular endothelial growth factor) signaling is critical for blood vessel formation and is involved in all stages of angiogenesis, its inhibition is an attractive therapy target in a wide range of tumor types, and disruption of the VEGF signal has become one of the dominant strategies for the angiogenesis-related treatment of cancer (Avendano,2015).

Inhibiting angiogenesis requires treatment with anti-angiogenic factors or drugs which prevent pro-angiogenic factors to bind with their receptors or to block their actions. All members of the VEGF family stimulate cellular responses by being bound to the tyrosine kinase receptors (the VEGFRs) on the cell surface, causing them to dimerize and activating them through transphosphorylation. Inhibition of the VEGF pathway has become the focus of angiogenesis research as approximately 60% of malignant tumors express high concentrations of VEGF. Strategies to inhibit the VEGF pathway directed against

VEGF or VEGFR. VEGFR-2, a type II transmembrane TK receptor, expressed on endothelial cells and on circulating bone marrow-derived endothelial progenitor cells, is the principal mediator of the VEGF-induced angiogenic signaling. VEGFR-2 is also a novel target. Biological and pre-clinical evidence suggests that the blockage of VEGFR-2 could be a promising strategy to inhibit tumor-induced angiogenesis (Fontanella C et al., 2014).

One of proved VEGFR-2 inhibitor on the market today is Sorafenib tosylate (Fig.1). In vitro study, sorafenib tosylate inhibits both wild-type and V599E mutant B-Raf activity with IC₅₀ of 22 nM and 38 nM, respectively. Sorafenib tosylate also potently inhibits mVEGFR-2 (Flk-1), mVEGFR-3, mPDGFR β , Flt3, and c-Kit with IC₅₀ of 15 nM, 20 nM, 57 nM, 58 nM, and 68 nM, respectively (Lu et al., 2013)

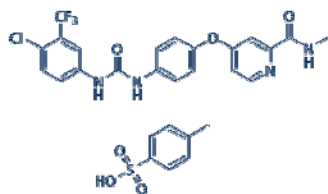


Figure 1. Sorafenib tosylate

Suhud et al. (2015) has proven that a lead compound 1-benzoyl-3-benzylurea (Fig.2) *in-silico* inhibits Raf-kinase (PDB code 1-UWH) with Rerank Score -90,5615 Kcal/mol and *in-vitro* against MCF-7 cell line with IC₅₀ 384,87 μ M.

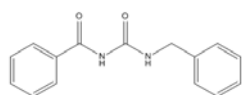


Figure 2. 1-Benzoyl-3-benzylurea

Both of sorafenib and 1-benzoyl-3-benzylurea have the same urea functional group. In order to find a novel effective angiogenesis inhibitor, the recent study on structural modification 1-benzoyl-3-benzylurea was conducted. All compounds will interfere with the binding of VEGF to VEGFR-2, inhibiting VEGF-induced signal, and block cancer growth. Prediction of activity as VEGFR-2 inhibitor would be performed by MVD 5.0 molecular docking. Prediction of bioavailability and toxicity will be performed by ACD-I/ Lab.

2 METHODS

Structural modification was carried out by substituting 22 substituents with certain physicochemical properties (lipophilic, electronic, and steric) into benzoyl group of the lead compound 1-benzoyl-3-benzylurea:

2-chloro; 3-chloro; 4-chloro; 2,4-dichloro; 3,4-dichloro; 4-chlorometil; 3-chloromethyl; 2-chloromethyl; 4-methyl; 4-ethyl; 3-ethyl; 2-ethyl; 4-propyl; 4-t-butyl; 4-fluoro; 2-trifluoromethyl; 3-trifluoromethyl; 4-trifluoromethyl; 4-bromo; 4-bromomethyl; 4-nitro; 4-methoxy.

2.1 Molecular docking

Computational method in term of *in-silico* activity test is started with searching *Protein Data Bank /PDB database* (Yanuar, 2012). Molecular docking was done to 1-benzoyl-3-benzylurea lead and its analogue compounds, also the reference hydroxyurea and 5-fluorouracil by *Molegro Virtual Docker(MVD) 2011.5*. Two dimation (2D) and 3D structures were performed by *ChemBioDraw Ultra 12.0* 2010 from *CambridgeSoft*[®].

2.2 Test parameter

Prediction of some physicochemical properties, bio-availability and toxicity were performed by ACD/I-Lab Prediction Engine from Advances Chemistry Development, Inc. free access on <https://ilab.acdlabs.com/iLab2/>. Prediction of activity is performed by Molegro Virtual Docker 2011.5. Quantitative Structure- Bioavailability/ Activity/ Toxicity were analyzed using IBM[®] SPSS[®] versi 20 from IBM Corp.

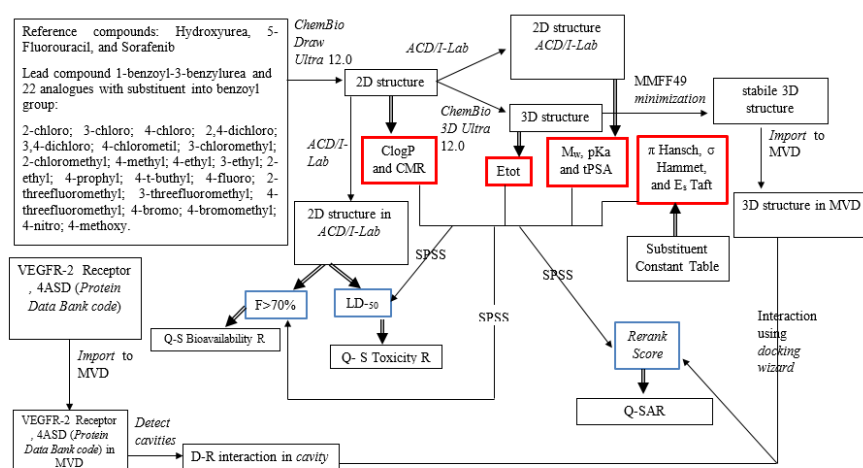


Figure 3. Research Procedure

3 RESULTS AND DISCUSSION

Computational (*in silico*) methods has been done to bioavailability, activity and toxicity test. These *in silico* methods include databases, quantitative structure- bioavailability/ activity and toxicity relationships using computer and software as tools. Such methods are frequently used in the discovery and optimization of novel compounds with affinity to a target. The aim of these *in silico* methods for pharmacology in terms of the targets addressed (Ekins S *et al.*,2007)).

VEGFR-2 appears to mediate almost all of the known cellular responses to VEGF (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4260048/> downloaded on 04/16/2017)

VEGFR2 is considered to be one of the most important regulators of angiogenesis, and is a key target in anti-cancer treatment (Avendano, 2015; Endo *et al.*, 2003; <http://jcp.bmj.com> downloaded on 09/12/2016,).

Inhibition of VEGFR-2 thus blocked all VEGF-induced endothelial cellular responses tested, and became a potentially targeted therapy as antiangiogenesis (Cervello, *et al.*, 2012; Endo *et al.*,2003)

VEGFR-2 with PDB code (Protein Data Bank) 4ASD was chosen because of sorafenib as a ligand. Sorafenib (Nexavar), approved by Food and Drug Administration (FDA) for the treatment of advanced renal cell carcinoma (RCC) in 2005 and unresectable hepatocellular cell carcinoma (HCC) in 2007, is the first orally bioavailable, multi-receptor tyrosine kinase inhibitor. This diaryl urea small molecule inhibits several kinases involved in tumor proliferation and tumor angiogenesis including Raf, vascular endothelial growth factor receptor

(VEGFR), and platelet derived growth factor receptor (PDGFR), (Lu *et al.*, 2013; Avendano, 2015).

Sorafenib is also used as a reference beside of hydroxyurea and 5-fluorouracil which were established as anticancer. The same urea functional group in these compounds becomes the reason to dock hydroxyurea, 5-fluorouracil, 1-benzoyl-3-benzylurea lead and its analogue compounds as anti-cancer by VEGFR-2 inhibition. The docking result is presented in Table 1.

Table 1. Docking results of all compounds

No.	Compound	Rerank Score (kcal/mol)
1	1-benzoyl-3-benzylurea	-96,9887
2	1-(2-chlorobenzoyl)-3-benzylurea	-97,0605
3	1-(3-chlorobenzoyl)-3-benzylurea	-100,64
4	1-(4-chlorobenzoyl)-3-benzylurea	-99,0598
5	1-(2,4-dichlorobenzoyl)-3-benzylurea	-98,7292
6	1-(3,4-dichlorobenzoyl)-3-benzylurea	-104,613
7	1-(4-chloromethylbenzoyl)-3-benzylurea	-102,067
8	1-(3-chloromethylbenzoyl)-3-benzylurea	-106,427
9	1-(2-chloromethylbenzoyl)-3-benzylurea	-102,834
10	1-(4-methylbenzoyl)-3-benzylurea	-100,401
11	1-(4-ethylbenzoyl)-3-benzylurea	-101,873
12	1-(3-ethylbenzoyl)-3-benzylurea	-109,53
13	1-(2-ethylbenzoyl)-3-benzylurea	-104,537
14	1-(4-propylbenzoyl)-3-benzylurea	-105,938
15	1-(4- <i>t</i> -butylbenzoyl)-3-benzylurea	-100,14
16	1-(4-fluorobenzoyl)-3-benzylurea	-98,4593
17	1-(2-trifluoromethylbenzoyl)-3-benzylurea	-93,2305
18	1-(3-trifluoromethylbenzoyl)-3-benzylurea	-111,711
19	1-(4-trifluoromethylbenzoyl)-3-benzylurea	-104,119
20	1-(4-bromobenzoyl)-3-benzylurea	-99,6173
21	1-(4-bromomethylbenzoyl)-3-benzylurea	-100,269
22	1-(4-nitrobenzoyl)-3-benzylurea	-104,774
23	1-(4-methoxybenzoyl)-3-benzylurea	-98,6492
24	Hydroxyurea	-41,5724
25	5-Fluorouracil	-60,7791
26	Sorafenib	-136,297

Rerank score -111,711 kcal/mol indicates that 1-(3-trifluoromethylbenzoyl)-3-benzylurea is the most stable D-R interaction and is predicted to have the best activity as VEGFR 2 inhibitor. Theoretically, trifluoromethyl (-CF₃) changes the electronic distribution because of its most electronegativity. Electronegativity is based on an arbitrary scale, with fluorine being the most electronegative (EN4.0). Fluorine attracts electrons strongly. Group with electronic effect induced D-R interaction and reduced electronic density (Thomas, 2003; Mc.Murry, 2011). Hydroxyurea and 5-fluorouracil showed *rerank score* -41,5724 Kcal/mol and -60,7791 kcal/mol that mean less stable D-R interaction compared to all tested compounds. Lipophilic groups like benzyl and benzoyl could stabilize D-R interaction leading to increasing activity. Moreover, other substituents with variety in lipophilic, electronic, and steric properties into benzoyl group seem to increase activity. Unfortunately, all tested compounds have higher *rerank score* (in range -93,2305 to -111,711 Kcal/mol) compared to sorafenib (-136,297 Kcal/mol). It was probably influenced by the difference of aminoacids site bonding to sorafenib and all tested compounds. Sorafenib bound Asp 1046 on one site of -CO group and also bound Glu 885 on two sites of -NH from urea pharmacophore. On the

other hand, almost all of the tested compounds bound Asp 1046 on one site of -CO group and only bound Glu 885 on one site of -NH from urea pharmacophore. Based on these results, all tested compounds have higher VEGFR-2 inhibitor activity compared to hydroxyurea and 5-fluorouracil but lower than sorafenib. That is necessary to develop activity with equations below:

- Non linear relationships between modification of physicochemical properties and bioavailability prediction

$$F > 70\% \text{ oral} = -1.548 \text{ ClogP} + 0.198 \text{ ClogP}^2 + 0.125 \text{ pKa} - 0.168 \text{ CMR} + 3.502$$

(n = 23; r = 0,717; SE = 0,093351; F = 4,757; sig = 0,009)

There is a nonlinear significant relationship between physicochemical properties and bioavailability. ClogP dominantly influenced bioavailability. Increasing ClogP will be followed by increasing in bioavailability until a certain point. After that, the bioavailability will decrease if ClogP is increased.

- Nonlinear relationships between modification of physicochemical properties and activity prediction

Rerank Score = 1.802 Es + 5.421 ClogP² - 44.744 ClogP - 11.152

(n = 23; r = 0,622; SE = 3,5801997; F = 4,004; sig = 0,023)

There is also a non linear significant relationship between physicochemical properties and activity. ClogP take the main role in activity compared to Es. Increasing ClogP will be followed by increasing in activity until a certain point. After that, the activity will decrease if ClogP is increased.

- A linear relationship between modification of physicochemical properties and toxicity prediction

LD₋₅₀ Mouse = -7.422 Mw - 117.197 pKa + 260.565 π + 4342.379 (n = 23; r = 0,793; SE = 140,87733; F = 10,062; sig = 0,000) There is a linear significant relationship between physicochemical properties and toxicity. π take the main role in reducing toxicity in mouse. Increasing π will be followed by increasing LD₋₅₀ Mouse.

Thus mean the toxicity will decrease.

LD₋₅₀ Rat = 691.028 CMR - 21.453 Etot - 430.187 π - 4775.208). (n = 23; r = 0,733; SE = 288,67963; F = 7,353; sig = 0,002)

There is also a linear significant relationship between physicochemical properties and toxicity in rat. CMR take the main role in reducing toxicity in rat. Increasing CMR will be followed by increasing LD₋₅₀ Rat. Thus mean the toxicity will decrease.

These quantitative equations can be used as foundations for further structural modification to discover a novel potential anticancer drug with better bioavailability, activity, and minimum toxicity.

4 CONCLUSION

There are nonlinear relationships between modification of physicochemical properties with bioavailability prediction ($F > 70\%$ oral = $-1.548 \text{ ClogP} + 0.198 \text{ ClogP}^2 + 0.125 \text{ pKa} - 0.168 \text{ CMR} + 3.502$) and modification of physicochemical properties with activity prediction (Rerank Score = $1.802 \text{ Es} + 5.421 \text{ ClogP}^2 - 44.744 \text{ ClogP} - 11.152$). Also, there is a linear relationship between modification of physicochemical properties and toxicity prediction (LD₋₅₀ Mouse = $-7.422 \text{ Mw} - 117.197 \text{ pKa} + 260.565 \pi + 4342.379$ and LD₋₅₀ Rat = $691.028 \text{ CMR} - 21.453 \text{ Etot} - 430.187 \pi - 4775.208$). These quantitative equations can be used as foundations for further structural modification to discover a novel potential anticancer drug with better bioavailability, activity, and minimum toxicity.

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Comment [S1]: ????????

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