Quantitative Structure–Activity Relationship and Molecular Docking of Artemisinin Derivatives to Vascular Endothelial Growth Factor Receptor 1

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Abstract. *Background/Aim: The antimalarial drug artemisinin has been shown to exert anticancer activity through anti-angiogenic effects. For further drug development, it may be useful to have derivatives with improved anti-angiogenic properties. Material and Methods: We performed molecular docking of 52 artemisinin derivatives to vascular endothelial growth factor receptors (VEGFR1, VEGFR2), and VEGFA ligand using Autodock4 and AutodockTools-1.5.7.rc1 using the Lamarckian genetic algorithm. Quantitative structure-activity relationship (QSAR) analyses of the compounds prepared by Corina Molecular Networks were performed using the Molecular Operating Environment MOE 2012.10. Results: A statistically significant inverse relationship was obtained between in silico binding energies to VEGFR1 and anti-angiogenic activity in vivo of a test-set of artemisinin derivatives (R=–0.843; p=0.035). This served as a control experiment to validate molecular docking predicting anti-angiogenc effects. Furthermore, 52 artemisinin derivatives were docked to VEGFR1 and in selected examples also to VEGFR2 and VEGFA. Higher binding affinities were calculated for receptors than for the ligand. The best binding affinities to VEGFR1 were found for an artemisinin dimer, 10-dihydroartemisinyl-2-propylpentanoate, and dihydroartemisinin α-hemisuccinate sodium salt. QSAR analyses revealed significant relationships between VEGFR1 binding energies and defined molecular descriptors of 35 artemisinins assigned to the training set (R=0.0848, p<0.0001) and 17 derivatives assigned to the test set (R=0.761, p<0.001). Conclusion: Molecular docking and QSAR calculations can be used to identify novel artemisinin derivatives with anti-angiogenic effects.*

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Although cancer therapy has improved tremendously, cure from cancer is still not a reality for many patients. In the 1990s, our group and others discovered the profound anticancer activity of artemisinin-type compounds (1-3), which were firstly established as antimalarial drugs to treat *Plasmodium falciparum* infection. Artemisinin is a sesquiterpene isolated from *Artemisia annua* L. and has been used in Chinese medicine for the treatment of fever for more than 2000 years (4). As most anticancer drugs have severe side-effects, artemisinins raised considerable attention as candidates for safe and efficient cancer therapy. Artemisinins were found to have anticancer activity against cancer cells *in vitro* and human xenograft tumors in nude mice (5-10). *A. annua* preparations and artesunate have also been investigated against spontaneously-occurring veterinary tumors (11, 12). In addition to the compassionate use of artemisinins in patients with cancer (13), artesunate showed clinical activity towards cervical carcinoma and colonic carcinoma in clinical phase II trials (14, 15).

Among different modes of action claimed to explain the anticancer activity of this class of compounds (16), anti-angiogenic activity is well-documented (17-20). Angiogenesis is a crucial process in cancer biology, supplying tumors with nutrition and oxygen. Therefore, anti-angiogenic therapies have been developed for cancer therapy (21, 22).

The aim of the present study was to explore the anti-angiogenic potential of artemisinin-type compounds. We first investigated a series of artemisinin derivatives known to exert anti-angiogenic effects *in vivo* (23). Bioinformatical docking experiments of these artemisinin derivatives to vascular endothelial growth factor receptor 1 (VEGFR1) were performed to confirm that molecular docking predicts anti-angiogenic activity *in vivo*. We then mined the PubChem Compound and Substance databases for artemisinin derivatives. Fifty-two chemical structures were identified, which were then docked to VEGFR1, VEGFR2 and VEGF. These results were subjected to quantitative structure-activity relationship (QSAR) calculations to determine chemical descriptors for the anti-angiogenic activity of artemisinin derivatives.

Material and Methods

Molecular docking. Fifty-two artemisinin derivatives were selected from PubChem Databases (http://www.ncbi.nlm.nih.gov/pccompound/ and /pcsubstance/). Canonical SMILES notations for each compound were retrieved and PDB structure files created by CORINA Online (https://www.molecular-networks.com/online_demos/corina_demo). Protein structures were obtained from Protein Data Bank (http://www.rcsb.org/pdb) for: VEGFR1 tyrosine kinase domain (PDB ID: 3HNG), VEGFR2 tyrosine kinase domain (PDB ID: 3U6J), VEGFA ligand (PDP ID: 4KZN). The protocol for molecular docking (24) and the pharmacophore selection for VEGFR1 and VEGFR2 tyrosine kinase domains were reported by us (25). A grid box was then constructed to define docking spaces in VEGFR1 and VEGFR2 according to its pharmacophores. Blind docking was performed for VEGFA by covering the whole protein. Docking parameters were set to 250 runs and 2,500,000 energy evaluations for each cycle. Docking was performed three times independently by Autodock4 (*The Scripps Research Institute, CA, USA*) and with AutodockTools-1.5.7rc1 (26) using the Lamarckian genetic algorithm. Lowest binding energies and predicted inhibition constants were obtained from docking log files. The mean and SD of binding energies and predicted inhibition constants were calculated from three independent dockings. Compounds were ranked in the order of binding energies. Selected dockings were visualized with Visual Molecular Dynamics software (University of Illinois, USA).

QSAR. 3D structures of 52 artemisinin derivatives were prepared by Corina Molecular Networks, all compounds were saved in MOL file format and uploaded in the Molecular Operating Environment (MOE 2012.10, Chemical Computing Group Inc., Montreal, Canada). Binding energies for each compound were obtained from three independent defined molecular dockings to VEGFR1 (Table I). We divided compounds randomly using a random number generator algorithm into a training set model (35 compounds) and test set (17 compounds) to evaluate the model (Table I). Seven descriptors including ionization potential, water accessible surface area, number of H-bond donor acceptor atoms, number of rotatable single bonds, log octanol/water partition coefficient, dipole moment and molecular weight were calculated for each compound in order to obtain predictor variables (27). The training set was analyzed by partial least squares regression for the correlation plot. Correlation coefficients were then calculated for both training and test sets.

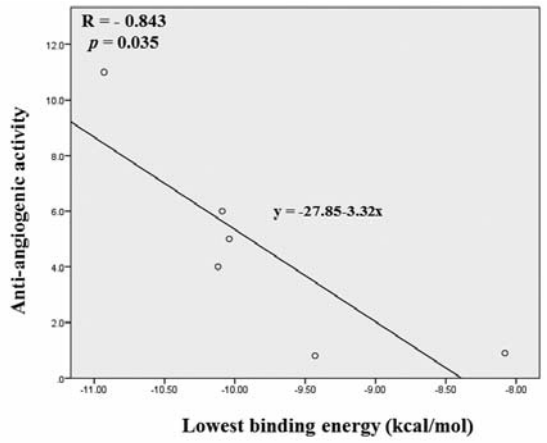

Figure 1. *Correlation between lowest binding energy and in vivo anti-angiogenic activity of artemisinin derivatives (Pearson correlation test). The anti-angiogenic activity of these derivatives was recently described (23).*

Results

Molecular docking. We used a panel of recently synthesized compounds, calculated their energies for binding to VEGFR1, and correlated these to the published degrees of anti-angiogenic inhibition in a Zebrafish (*Danio rerio*) model *in vivo* (23). A statistically significant correlation was obtained (Figure 1), indicating that VEGFR1 binding is sufficient to explain the anti-angiogenic effects of these compounds.

After this control experiment, we investigated the binding energies of 52 artemisinin derivatives taken from the PubChem databases and determined whether artemisinin derivatives interact more strongly with the receptors (VEGFR1, VEGFR2) than a ligand (VEGFA). Indeed, strong interactions were found for VEGFR1 (−11.23 kcal/mol to −6.50 kcal/mol) (Table I). The three most interactive compounds (artemisinin dimer, 10-dihydroartemisinyl-2-propylpentanoate, dihydroartemisinin α-hemisuccinate sodium salt) and artesunate as control compound were also investigated for their interaction with VEGFR2 and VEGFA. Comparable binding energies were found for VEGFR2 (Table II), but lower values for VEGFA (Table III). Docking of selected compounds to VEGFR1 showed that they bound to the same site on VEGFR1 (Figure 2). These results indicate that artemisinin derivatives target VEGFR1 and VEGFR2 rather than VEGFA.

QSAR. We used the binding energies obtained from VEGFR1 docking to establish a predictive QSAR model by the PLS method. The summation of all descriptor variables was correlated with binding energies for all compounds in training and test sets (Figure 3). The predicted activity for our training set model fit best to binding energies with R=0.848 and $p<0.0001$. The predictive R-value for the test set was slightly lower than that for the training set at

Table I. *Defined molecular docking of artemisinin derivatives on VEGFR-1 (PDB ID: 3HNG) and randomization for QSAR calculations.*

Randomized assignment	Compound	Lowest binding energy (kcal/mol)	pKi (μM)
Training set	Artemisinin dimer	–11.23±0.13	0.01±<0.00
Test set	10-Dihydroartemisinyl-2 - propylpentanoate	–10.86±0.07	0.01±<0.00
Training set	Dihydroartemisinin alpha-hemisuccinate sodium salt	–10.77±0.02	0.01±<0.00
Training set	10-Dihydroartemisinyl 2, 2-dichloroacetate	–10.14±0.02	0.04±<0.00
Test set	10-Dihydroartemisinyl butyrate	–10.09±0.02	0.04±<0.00
Training set	10-Dihydroartemisinyl isobutyrate	–10.03±0.01	0.04±0.01
Training set	Dihydroartemisinin, 10-O-(t-butyloxy)-	–9.98±0.01	0.05±<0.00
Training set	10 β-(p-fluorophenoxy) dihydroartemisinin	–9.65±0.01	0.08±0.01
Training set	10 β-(p-bromophenoxy) dihydroartemisinin	–9.64±0.02	0.09±<0.00
Test set	Sodium artesunate	–9.53±0.07	0.10±0.01
Test set	Dihydroartemisinin, 2-nitro-5-[carbosymethoxy]benzyl ether	–9.46±0.11	0.12±0.01
Test set	10-Dihydroartemisinyl acetate	–9.41±0.03	0.13±0.01
Test set	β n-Propylether of 11-epi-dihydroartemisinin	–9.36±0.02	0.14±0.01
Test set	Dihydroartemisinin, 9-deoxy-9-[2-(isopropylaminocarbonyl) ethyl]-	–9.15±0.01	0.19±0.01
Test set	Artemether-d3	–8.84±0.01	0.33±<0.00
Training set	Artemether	–8.84±0.01	0.33±0.01
Training set	Artelinic acid.	–8.81±0.03	0.35±0.02
Test set	Dihydroxyartemisinin-d3	–8.80±<0.00	0.36±<0.00
Training set	Dihydroxyartemisinin 13C, d4	–8.80±<0.00	0.36±<0.00
Test set	Artemisinin dimer hemisuccinate	–8.71±0.37	0.46±0.24
Training set	Dihydroartemisinin, 6-deshydro-5-deshydroxy -3-desoxy	–8.67±<0.00	0.44±<0.00
Training set	Artemisinin	–8.66±<0.00	0.45±<0.00
Training set	9-Epiartemisinin	–8.66±<0.00	0.45±<0.00
Training set	9(l)-Hydroxy-10(R) - [m-chlorobenzoyl] dihydroartemisinin	–8.63±0.03	0.47±0.02
Test set	Arteether	–8.60±0.01	0.50±0.01
Training set	Dihydroxyartemisinin glucouronide	–8.59±0.05	0.51±0.05
Training set	Artemisitene	–8.53±<0.00	0.56±<0.00
Training set	9b-Hydroxyartemisinin	–8.47±<0.00	0.62±<0.00
Training set	Deoxyarteether	–8.37±0.01	0.72±0.01
Training set	14-Acetoxyarteether	–8.26±0.11	0.90±0.17
Training set	9-Nor-8-n-butyl-10-deoxy-dihydroartemisinin	–8.16±0.01	1.05±0.02
Test set	Artemisinin, 9-demethyl-9-propyl	–8.13±0.01	1.09±<0.00
Test set	Artesunate-d4	–8.13±0.07	1.10±0.13
Test set	Artesunate	–8.12±0.04	1.13±0.06
Training set	Artesunate-d3	–8.09±0.08	1.18±0.14
Training set	Dihydroartemisinin, 5-deshydroxy-6-deshydro	–7.97±<0.00	1.43±0.01
Test set	Anhydrodihydroartemisinin	–7.97±<0.00	1.43±<0.00
Test set	10-Deoxy-10-n-propyl-dihydroartemisinin	–7.94±0.02	1.52±0.04
Test set	3-Hydroxydeoxyartemisinin	–7.91±0.01	1.61±0.02
Training set	Deoxyartemisinin-d3	–7.80±0.01	1.90 ±0.01
Training set	13-Methoxy carbonyl artemisinin	–7.70±0.01	2.28±0.02
Training set	Artemisinin dimer sulfonate dimethyl carbomate	–7.68±<0.00	2.30±<0.00
Training set	Dihydroartemisinin, 3-desoxy-	–7.64±<0.00	2.50±<0.00
Training set	Artemisinin B	–7.60±0.03	2.68±0.15
Training set	Dihydroxyartemisinin	–7.58±<0.00	2.79±<0.00
Training set	Artemisinin G	–7.57±0.01	2.83±0.02
Training set	Deoxyartemisinin	–7.48±<0.00	3.27±0.01
Test set	Artemisic acid	–7.40±<0.01	3.73±0.01
Training set	3-Hydroxy artemether	–7.17±<0.00	5.54±0.03
Training set	3-Hydroxydeoxy dihydroxyartemisinin	–7.09±<0.00	6.33±0.02
Training set	Dihydroxyartemisinin furano acetate	–6.83±0.01	9.82±0.11
Training set	Artemether tetrafuran acetate	–6.50±0.01	17.36±0.39

R=0.761 (p=0.001). Furthermore, we correlated our model with anti-angiogenic activity in a Zebrafish (*Danio rerio*) of recently synthesized compounds, we obtained R-value of 0.57 (data not shown) (23).

Discussion

In the present investigation, we found a significant correlation between anti-angiogenic effects and VEGFR1 docking for a series of artemisinin derivatives with proven *in vivo* anti-angiogenic effects recently reported by us (23). Therefore, we conclude that molecular docking is a valid strategy to search for anti-angiogenic compounds. In another set of artemisinin derivatives, comparably high binding energies were found for VEGFR1 and VEGFR2, but lower ones for VEGFA, indicating that artemisinins rather bind to the receptors than to the ligand. The two receptors can form hetero- and homodimers upon activation by the ligand (28). It can be hypothesized that binding of artemisinins to the receptors may inhibit VEGF binding and receptor activation.

The VEGFR1 binding energies for 52 artemisinin-related structures were subjected to QSAR analyses to describe physiochemical properties and structural properties of the compounds necessary for VEGFR1 binding. The high

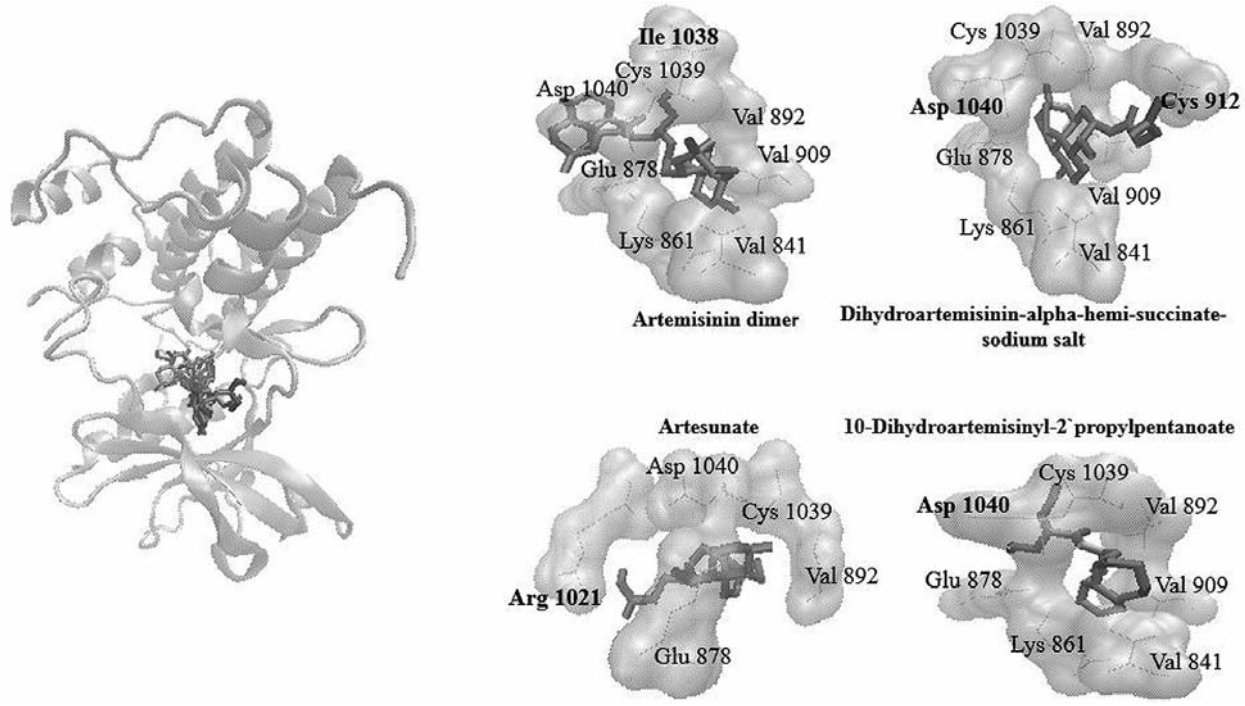

Figure 2. *Molecular docking of artemisinin dimer, dihydroartemisinin-α-hemisuccinate sodium salt, artesunate and 10-dihydroartemisinyl-2'propylpentanoate (compounds were depicted in bond representation) to VEGFR1 tyrosine kinase domain (PDB ID: 3HNG, black cartoon representation, interacting amino acids were depicted in surface representation).*

Table II. *Defined molecular docking of selected artemisinin derivatives on VEGFR-2 (PDB ID: 3U6J). Only three compound that showed lowest binding energy were re-docked on VEGFR2, artesunate were taken as a control.*

Compound	Lowest binding energy (kcal/mol)	pKi (μM)
Artemisinin dimer	–11.48±0.14	0.004±<0.001
Dihydroartemisinin alpha-hemisuccinate sodium salt	–10.36±0.02	0.03±<0.001
10-dihydroartemisyl-2-propylpentanoate	–10.34±0.14	0.03±0.01
Artesunate	–9.32±0.02	0.14±0.01

Table III. *Molecular docking of selected artemisinin derivatives on VEGF-A (PDB ID: 4KZN) (blind docking approach). The compounds showed lower affinity to the ligand than its receptor.*

Compound	Lowest binding energy (kcal/mol)	pKi (μM)
Artemisinin dimer	–8.33±0.09	0.79±0.11
Dihydroartemisinin alpha-hemisuccinate sodium salt	–7.35±0.19	4.24±1.39
Artesunate	–6.75±0.04	11.35±0.83
10-dihydroarteminiyl-2-propylpentanoate	–5.59±0.03	79.60±3.63

correlations for the training and test sets (R=0.848 and 0.761, respectively) reflected the high predictive power of our model.

It has been stated that artemisinins exert their antitumor activity *via* their endoperoxide moiety, which is activated by reduced ferrous iron, leading to the formation of highly toxic radicals (23, 29). If we inspect the structures of the best derivatives in terms of predicted activity, they all possess an endoperoxide bridge, and interestingly, the artemisinin dimer which bound best to VEGFR1 has two endoperoxide bridges. For 10-dihydroartemisinylbutyrate, the long alkyl chain together with the endoperoxide bridge may lead to high VEGFR1 binding. Structural similarity of 10-dihydroartemisinylbutyrate to the known VEGFR inhibitor artesunate (18) underscores our hypothesis. On the contrary, the derivatives that lack an endoperoxide bridge, such as artemisinin B and artemisic acid, had lower predicted activity. This observation further supports our hypothesis.

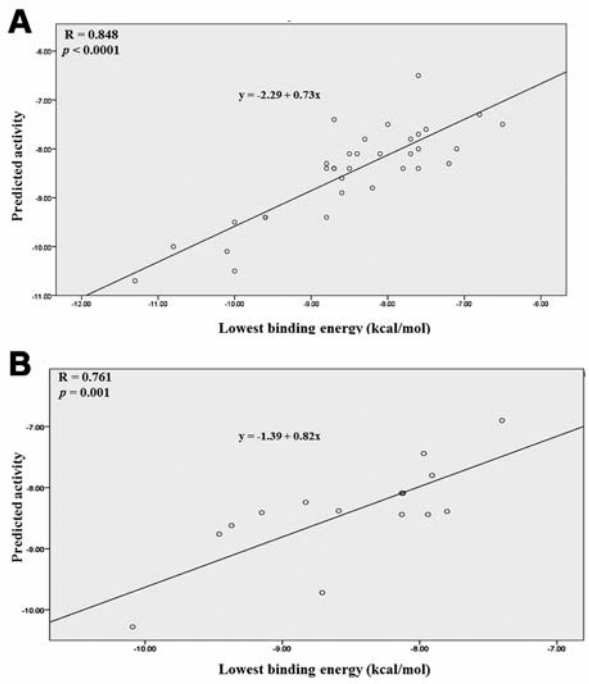

Figure 3. *QSAR calculations of binding energies of artemisinin derivatives versus predicted activity in the training set (A) and in the test set (B).*

Natural products are frequently multi-target compounds (30). This is also true for artemisinins. In addition to inhibition of angiogenesis, they induce DNA lesions (31), bind to specific target proteins, *e.g.* translationally controlled tumor protein (32), and inhibit epidermal growth factor receptor-related signaling (33). The fact that we predicted artemisinin compounds with high binding energy to VEGFR1 might enable use of this strategy to increase the specificity of natural lead compounds for a specific therapeutic target. Hence, compounds may be identified with higher selectivity for the target of choice using molecular docking and QSAR analysis. This approach warrants more detailed investigations.

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Conflicts of Interest

The Authors declare they have no conflicts of interest in regard to this study.

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