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Discovery of loperamide as an antagonist of angiopoietin1 and angiopoietin2 by virtual screening

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ABSTRACT

The angiopoietin–Tie2 binding and related signal transduction pathways are crucial for vascular angiogenesis, blood vessel integrity and maturation. In this study, we preformed a virtual screening of small molecules targeting to Tie2. The binding site was selected at the extracellular ligand binding region of Tie2, rather than its conventional endocellular ATP binding region. It was found that loperamide, a widely-used antidiarrhea drug, was among the top hits. The binding between loperamide and Tie2 was confirmed by surface plasmon resonance (SPR) assay. Loperamide competitively inhibited the binding of both angiopoietin1 and angiopoietin2. These results indicate that loperamide is an antagonist of angiopoietin1 and angiopoietin2.

Angiogenesis, the formation of new capillary blood vessels from existent micro vessels, is essential for the growth of most primary tumors and their subsequent metastasis.[1] Thirty years ago, Dr. Judah Folkman suggested that inhibition of angiogenesis could be a means of treating cancer.[2] Based on this theory, there have been many antiangiogenic agents developed. Some of these agents, such as Bevacizumab (Avastin, Genentech/Roche), Sorafenib (Nexavar, Bayer) and Sunitinib (Sutent, Pfizer), have been approved for human use. Meanwhile, dozens of other angiogenesis inhibitors are under clinical evaluations.[1]

The angiopoietin/Tie2 pathway plays crucial roles during angiogenesis, blood vessel maturation, and vascular endothelial integrity.[3] In the absence of VEGF, angiopoietin2 acts as an antagonist of angiopoietin1 and destabilizes vessels, ultimately leading to vessel regression.[4] In the presence of VEGF, angiopoietin2 facilitates vascular sprouting.[4] Disruption of Tie2 function in transgenic mice resulted in embryonic lethality because of defects in vascular development.[5] Similar vascular defects occurred after disrupting the functions of angiopoietins.[6]

Some agents that target the angiopoietin/Tie2 pathway have been reported, which include small molecules,[7–14] antibody,[15] nucleic acid fragment,[16] and extracellular domain of Tie2.[17] Some of the small molecules could inhibit both Tie2 and VEGF receptor.[8,11–13] However, these small molecules all target to the endocellular ATP binding region of Tie2. It is known that cancer cell resistance is one of the major reasons for chemotherapeutic failure of cancer patients. Multi-drug resistance (MDR) is the result of over expression of membrane bound proteins, such as P-glycoprotein (P-gp) and multi-drug resistance-associated protein (MRP), which could efflux drugs from cells, thus decreasing the intracellular concentrations of the drugs.[18]

In this study, molecular docking, one of the computer-aided drug design (CADD) methods, was used for virtual screening of small molecules targeting to Tie2. The extracellular ligand binding region of Tie2, rather than its endocellular ATP binding region, was selected as the docking site. By such a strategy, the obtained lead compounds could bind to Tie2 without the transmembrane process. Meanwhile, they may interfere with the binding of angiopoietins.

The DOCK6.1 suite of programs and the drug-like subset of ZINC database (http://zinc.docking.org/), both of them provided by UCSF DOCK team, were used for the virtual screening. The crystal structure of the extracellular ligand-binding region of Tie2 in complex with angiopoietin2, which was retrieved from protein data bank (PDB) with an entry 2GY7, was utilized for the preparation of the receptor structure. The docking was carried out according to DOCK6 user manual (http://dock.compbio.ucsf.edu/DOCK_6/dock6_manual.htm). The primary docking was performed with grid score to write out the topmost pose. Amber score was then used for rescoring based on the topmost pose. Such a protocol could curtail the computational cost while maintaining the accuracy. The main advantage of amber score is that both the ligand and the protein binding site can be flexible, allowing small structural rearrangements to reproduce the so-called 'induced fit'.

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Table 1
Summary of properties of the lead compounds

Compound	ZINC number	CAS	Molecular weight	Grid score (kcal/mol)	Amber score (kcal/mol)
Loperamide	00537928	34552-83-5	513.5	−40.3	−23.0
Cetirizine	01530910	83881-52-1	388.9	−44.0	−16.6
Alpha-aminobenzylpenicillin	01607283	69-53-4	349.4	−42.1	−13.2
Fmoc-D-2-fluorophenylalanine	04208792	198545-46-9	404.4	−40.8	−11.1

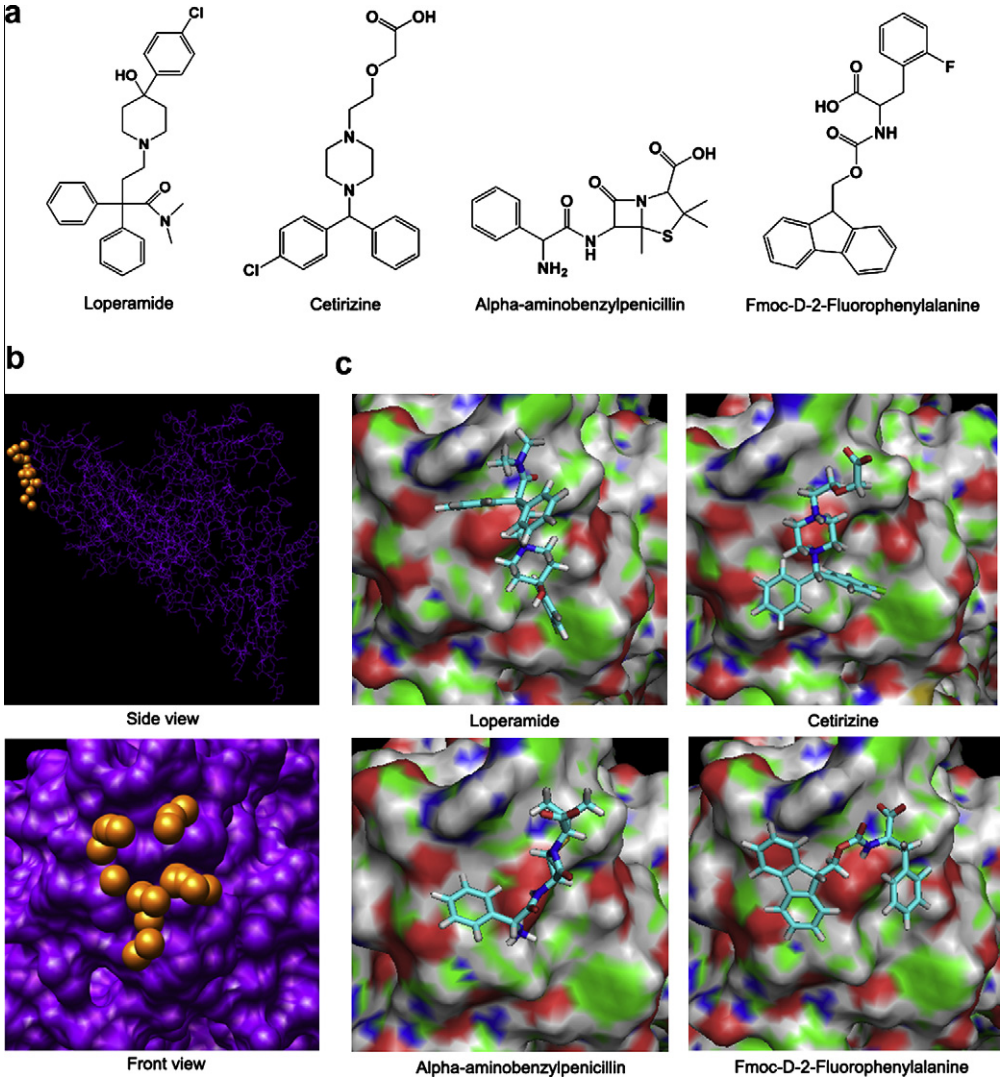

Figure 1. Chemical structures of the leads and their binding models against Tie2 in the binding site. (a) Chemical structures of the leads. (b) The shape of the binding site from deferent angles. The binding site was shown as yellow balls. (c) The binding models of the leads. The compounds are rendered in sticks and colored by atom types while the receptor is shown as the surface.

In primary docking, to ensure the sufficient fit of the compounds, maximum orientations and bumps were set to be 2000 and 12, respectively. As a result, 2000 compounds with grid scores less than −40 kcal/mol were screened out from the database containing 1,920,000 compounds and were then subjected to amber score. The default parameters were used in amber score and the top 200 compounds with the energy scores smaller than −10 kcal/mol were selected for analysis. It was found that four of them were in clinical use, while the others were new chemical entities without relevant references in American Chemical Abstracts (CA). The four clinically-used small molecular drugs, that is, loperamide, cetirizine, alpha-aminobenzylpenicillin, and Fmoc-D-2-fluorophenylalanine, were selected as leads for subsequent biological assays. The properties and structures of them were shown in Table 1 and Figure 1a, respectively. The grid scores for loperamide, cetirizine, alpha-aminobenzylpenicillin, and Fmoc-D-2-fluorophenylalanine were −40.3, −44.0, −42.1, and −40.8 kcal/mol, respectively. The amber scores for them were −23.0, −16.6, −13.2, and −11.1 kcal/mol, respectively. Loperamide had the worst grid score but best amber score, which demonstrated the importance of rescoring.

In this study, the binding site located at the extracellular ligand binding region of Tie2 with a flat shape (Fig. 1b). The binding models of the compounds against Tie2 in the binding site were shown