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Understanding molecular mechanisms of *Rhodiola rosea* for the treatment of acute mountain sickness through computational approaches (a STROBE-compliant article)

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Abstract

Rhodiola rosea has been used in the treatment of acute mountain sickness (AMS) for a long time, but the mechanism of its action is not still completely clear. In this paper, the therapeutic mechanism of *R rosea* for AMS was investigated by analysis of the relationship between *R rosea* compositions and hypoxia-inducible factor 1 (HIF-1) degradation pathway.

System biology and network biology, computational approaches were used to explore the molecular mechanisms of traditional Chinese medicine (TCM).

Our results showed that chemical compositions of *R rosea* could inhibit the targets of HIF-1 degradation pathway in multi-composition/multi-target ways.

We conclude that the 18 components with more than 2 targets and 5 targets (arrest-defective-1 [ARD1], forkhead transcription factor [FOXO4], osteosarcoma-9 [OS-9], prolyl hydroxylase 2 [PHD2], human double minute 2 [Hdm2]) deserve to be noticed, and PHD2, receptor for activated C-kinase1 (RACK1) and spermidine/spermine-N1-acetyltransferase-1 (SSAT1) may be the targets of active ingredients of rhodionin, rhodiosin, and rhodiolatuntoside, respectively.

Abbreviations: ADT = AutoDockTools, AMS = acute mountain sickness, ARD1 = arrest-defective-1, FOXO4 = orkhead transcription factor, GSK3β = glycogen synthase kinase 3β, Hdm2 = human double minute 2, HIF-1 = hypoxia-inducible factor 1, OS-9 = osteosarcoma-9, PHD2 = prolyl hydroxylase 2, pVHL = von Hippel–Lindau protein, RACK1 = receptor for activated C-kinase1, RMSD = root mean square deviation, SSAT1 = spermidine/spermine-N1-acetyltransferase-1, SSAT2 = spermidine/spermine-N1-acetyltransferase-2, TCM = traditional Chinese medicine.

Keywords: acute mountain sickness, computational approach, mechanism, *Rhodiola rosea*

1. Introduction

Acute Mountain Sickness (AMS), a kind of human pathological reaction to high altitude, is caused by acute exposure to low oxygen partial pressure at high altitude. It has a series of nonspecific symptoms, such as headache, dizziness, palpitation, disgust, fatigue, numbness in the extremities, convulsion, and so on.[1] AMS may occur when people ascend to the height above 2500 meters. Severely, it can develop into high altitude pulmonary edema (HAPE) or high altitude cerebral edema (HACE), which is likely to be life-threatening.[2] There is the largest plateau in China and many people settled on plateaus in the world.[3] In addition, lots of tourists, mountaineers and frontier guards enter into plateaus above 3000 meters every year. AMS has become an urgent problem to be solved, especially in executing tasks on highland. During the earthquake occurring in Yushu of Qinghai province of China in 2008, the morbidity of AMS reached as high as 81.5% in the emergency rescue workers, seriously affecting relief worker.[4]

Hypoxia-inducible factor 1 (HIF-1) is a transcription factor which is related to hypoxia.[5] HIF-1, a heterodimer, consists of a α subunit and a β subunit. Under normoxia, HIF-1α is continuously synthesized and rapidly degraded via the ubiquitin-proteasome pathway.[6] While under hypoxia, HIF-1α degradation is inhibited, then the accumulated HIF-1α binds with HIF-1β to form heterodimer HIF-1 with transcriptional activity, which could bind with hypoxia response elements (HREs) to activate transcription function of approximately 60 target genes, such as vascular endothelial growth factor (VEGF), inducible nitric oxide synthase (iNOS), erythropoietin (EPO) and so on.[7,8]

It has been reported that *Rhodiola rosea*, a traditional Tibetan medicine plant in China, can treat AMS through inhibiting HIF-1 degradation pathway.[7] As we know, Traditional Chinese medicine (TCM) has been used in treatment of diseases as a main means in China for thousands of years. Different from western medicine, TCM has the lower side effects[9] and exhibits synergistic effects of multi-compositions by multi-channels and multi-targets.[10] However, separation and extraction are time-consuming due to numerous chemical compositions in TCM, the assay about biological activity is expensive for each composition, and the common drug research methods are not suitable to synergistic effects, so the studies on TCM mechanism is limited.

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With the development of system biology and network biology, systematic docking and drug-target network analysis approach compensate for the limitation above. In recent 10 years, it has been widely applied to study molecular mechanism of TCMs.[11,12] Molecular docking method can explore the binding mode and binding strength between targets and compounds at molecular level.[13] AutoDock Vina program,[14] the most commonly used software for molecular docking, has been successfully applied in drug design and approved to be effective.[15] Target-composition interaction network constructed by network biology method is a powerful tool for analyzing the relationship between targets and TCM compositions.[12,16] The combination of the two methods can contribute to more comprehensive understanding of TCM function.

To estimate the interaction between HIF-1 degradation pathway and a single composition of *R rosea*, we first collected 187 effective molecules from *R rosea*, and then docked all these molecules with the targets that could decrease HIF-1α degradation using AutoDock Vina software. Finally, we built a compound-target network using Cytoscape program[17] commonly used for biological research.[18] From the interaction network, we analyzed the potential target of compositions of *R rosea* and provided a reference for the therapeutic mechanism of *R rosea*.

2. Materials and methods

All study methods were approved by ethics committee of General Hospital of Chinese People's Armed Police Force.

2.1. Data preparation

A total of 187 compounds were collected from Panossian's and Zhou's articles[19,20] and were used to build compound database of *R rosea*. First, the structures of the 187 compounds were drawn using ChemDraw module of ChemBioOffice software and saved as 2D cdx format. Then, these compositions were converted into 3D conformation using chem3D module and were optimized with MM2 force field to get 3D mol2 format. Finally, we used AutoDockTools (ADT)[21] to prepare ligands before docking simulation, including adding hydrogens and Gasteiger partial charges.

It is worth noting that the structure of pyridrde which is named compound 184 in Zhou's article,[20] is different from that in other papers. Herein, we used 2-Benzhydrylpiperidine (right side in Table 1) as pyridrde with substitution of compound 184, and the other compounds, as well as numbers, are the same as those of Zhou's.

2.2. Selection of targets

As reported,[7] Rhodiola genus plants could treat AMS by inhibiting HIF-1 degradation pathway. In this study, we focused on HIF-1α degradation pathway-related targets. HIF-1α degradation pathways mainly include von Hippel–-Lindau protein (pVHL) -dependent pathway and pVHL-independent pathway.

Under normoxia, in pVHL-dependent pathway, residues PRO402 and PRO564 of HIF-1α can be identified and hydroxylated by prolyl hydroxylase 2 (PHD2), then the hydroxylated HIF-1α binds with pVHL enriching elongin-C/elongin-B/cullin-2 E3 ubiguitin ligase, and is finally degraded by 26S proteasome.[22] Under hypoxia, in the O2/pVHL/PHD pathway, arrest-defective-1 (ARD1), osteosarcoma-9 (OS-9), and spermidine/spermine-N1-acetyltransferase-2 (SSAT2) all can promote HIF-1α degradation by acetylating HIF-1α,[23] binding with HIF-1α/PHD2 or HIF-1α/PHD3 complexes[24] and binding with pVHL/E3 ligase,[25] respectively.

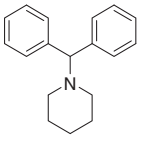
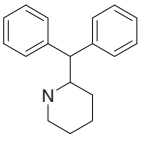
In pVHL-independent pathway, receptor for activated C-kinase1 (RACK1) can enrich elongin-C/elongin-B/cullin-2 E3 ligase to induce HIF-1α degradation.[26] Spermidine/spermine-N1-acetyltransferase-1 (SSAT1)[27] can stabilize the reaction between HIF-1α and RACK1. In addition, glycogen synthase kinase 3β (GSK3β) can promote HIF-1α degradation by phosphorylation.[28]

Besides above targets, E3 ligase, human double minute 2 (Hdm2),[29] and forkhead transcription factor (FOXO4)[30] have been proved to be also conducive to HIF-1α degradation.

In the end, we selected these targets including pVHL, PHD, ARD1, OS-9, SSAT2, RACK1, SSAT1, GSK3β, Hdm2, and FOXO4 as docking simulation.

Table 1

Structure of Pyridrde.

Pyridrde in Zhou's[21]	Pyridrde in this study
	

Note: reference 21.

2.3. Molecular docking

To prepare for docking simulation, water molecules, solvent molecules, and substrate ligands were removed using Pymol software; hydrogen and partial charges were added using ADT, and the ligand binding site for each protein was defined as either the location of the co-crystallized ligand or the site suggested by experimentalists.[31,32] The relevant parameters are shown in Table 2.

Prior to molecular docking, except the targets including RACK1 without ligand and DNA-binding FOXO4, crystal recovery test was performed in other 8 targets to ensure the reliability of method and parameter. AutoDock Vina software[14] was employed to dock ligands with protein active site. Evaluation criterion was set to 2 angstrom or less by calculating root mean square deviation (RMSD) between docked ligand conformation and co-crystallized ligand conformation. RMSD value and AutoDock Vina score are shown in Table 2.

Except for OS-9 and SSAT1, RMSDs of other 6 targets were less than or about 2 angstrom, which was regarded as successful recovery. Sugar ring of OS-9 and purine ring of SSAT1 were thought to be the major responsibility for simulation failure. It can be seen in Figure 1 that sugar ring and purine ring were mostly outside the pocket and there were fewer interactions to maintain their orientations, so it was difficult to recover their locations. Except for the ring outside pocket, others in pocket could recover both location and major interaction (Fig. 1) in OS-9 and SSAT1. For above reason, the parameters of OS-9 and SSAT1 were still used in the following docking simulation.

For the docking simulation, AutoDock Vina software[14] was employed to dock the 187 compounds with the structures of 10 proteins, respectively. The macromolecules were treated as rigid, while the ligands were flexible. The box parameters are shown in Table 2, and other parameters were set as default.

Table 2

Parameter and result in recovery.

Target name	PDB Code	Active site parameter	RMSD(Å)	Vina score
ARD1	4KVX	14.89/17.51/29.11; 30/30/26	2.04	−7.8
GSK3β	4ACC	20.23/ 18.34/ 8.09; 30/24/24	1.06	−7.5
Hdm2	4HBM	16.92/ 14.3/ 16.3; 24/24/24	0.59	−9.3
OS-9	3AIH	23.57/ 21.42/ −0.28; 26/ 26/24	6.83	−5.7
PHD2	4BQW	−37.03/22.73/1.8; 26/24/24	0.22	−8.3
pVHL	4BKS	−10.01/30.49/3.98; 22/24/28	1.30	−8.3
SSAT1	2FXF	6.95/ −2.11/ 38.17; 30/ 30/34	5.16	−9.3
SSAT2	2BEI	−9.39/47.9/12.95; 24/24/28	2.00	−7.6
FOXO4	3L2C	−5.08/ 18.11/ −13.51; 32/30/32	–	–
RACK1	4AOW	69.49/17.1/0.33; 30/30/32	–	–

Active site parameters include box center (X_center/Y_center/Z_center) and box size (X_size/Y_size/Z_size). RACK1 with no ligand and FOXO4 with DNA ligand were excluded to carry on recovery test, which had "–" mark in RMSD and score. ARD1 = arrest-defective-1, FOXO4 = forkhead transcription factor, GSK3β = glycogen synthase kinase 3β, Hdm2 = human double minute 2, OS-9 = osteosarcoma-9, PDB = Protein Data Bank, PHD2 = prolyl hydroxylase 2, pVHL = von Hippel–Lindau protein, RACK1 = receptor for activated C-kinase1, RMSD = root mean square deviation, SSAT1 = spermidine/spermine-N1-acetyltransferase-1, SSAT2 = spermidine/spermine-N1-acetyltransferase-2.

3. Results

A total of 187 compounds were saved as pdbqt file. They were divided into 6 categories and are listed in Table 3.

All the scores of 187 compounds to the 10 proteins are listed in Table 4. To select compounds that could potentially inhibit the 10 proteins, we choose the top 5% of the compounds ranked by the lowest docking energy, respectively. If different compounds possessed the same score meeting with the top 5% criterion, these compounds all were regarded as positive results. Each target had 9 to 13 compounds with top 5% criterion (Table 4).

No. of compounds that have interaction with corresponding target proteins are listed in Table 4. The interaction relationships between all the 10 proteins and related compounds were merged in compound-target network using Cytoscape software[17] (Fig. 2).

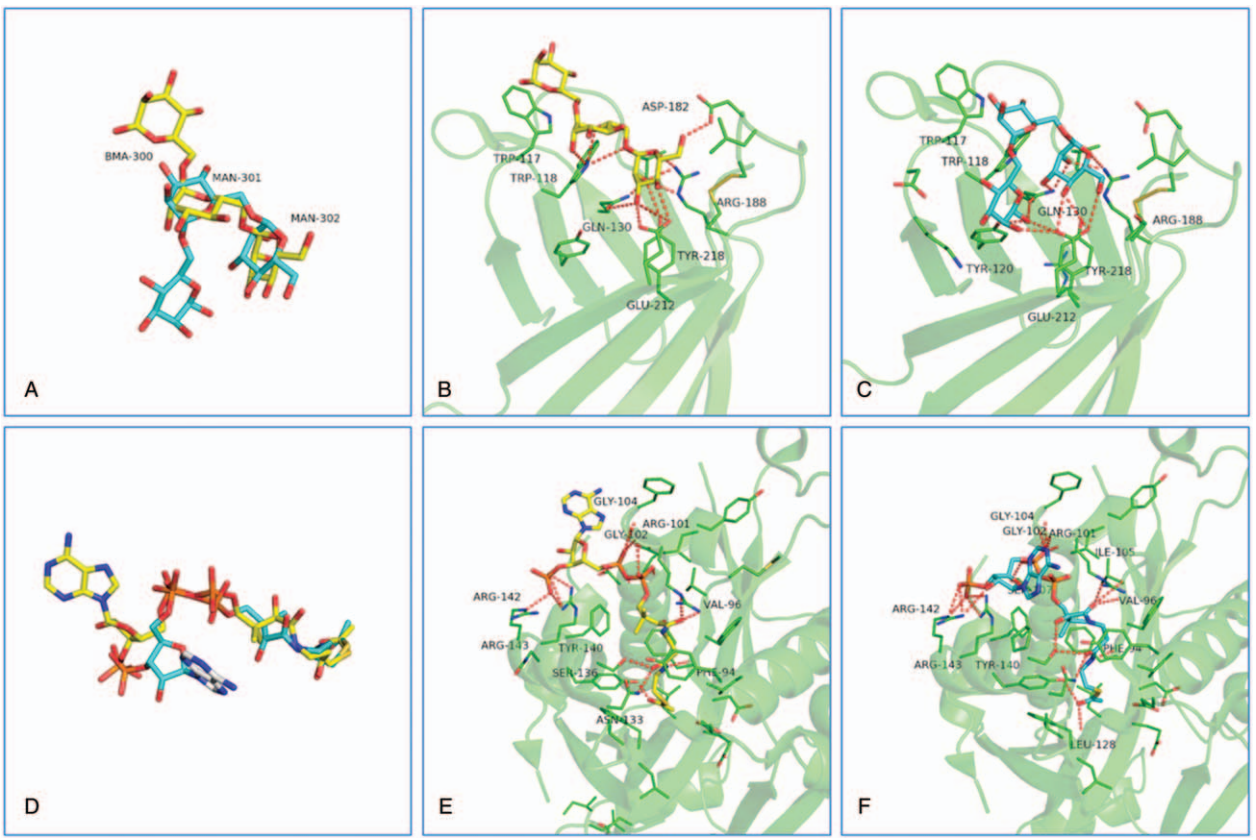


Figure 1. Recovery graphs of OS9 and SSAT1 targets. Graphs A-C indicate recovery of OS9 target (PDB code: 3AIH), while graphs D–F indicate SSAT1 target (PDB code: 2FXF). Crystal structure is colored green, ligand and docked conformation are represented as yellow and cyan sticks, respectively. H-bond are shown as red dashes, interaction residues are colored green sticks and labeled. OS9 = osteosarcoma-9, SSAT1 = spermidine/spermine-N1-acetyltransferase-1.

Table 3

Category and number of compounds.

Class	Category of compounds	No. of compounds	Counts
1	Flavonoids and flavonoid glycosides	1–50	50
2	Glycosides	51–136	86
3	Terpenoids	137–169	33
4	Sterols	170–173	4
5	Tannins	174–183	10
6	Other chemical constituents	184–187	4

4. Discussions

For each target, Table 4 lists the compound which was regarded as possible inhibitors calculated by AutoDock Vina. Of the 42 interaction compounds, 18 have connection with more than 2 targets, while 27 compounds (64%) had more than 1 target. The relationships of targets and compounds are shown in Figure 2, which suggests TCM's multi-target/multi-component strategy for disease treatment.

Compounds which either act on more than 4 proteins or are proved as an active ingredient[20,33,34] are listed in Table 5. In these compounds, 5 compositions (Rhodiosin,[35] Rhodiolatuntoside,[34] Rhodionin,[30,36] Crenulatin,[33] Pyridrde[20]) have been proved as active ingredients, and 4 of them (80%) are inhibitors for more than 2 targets.

In this way, TCM offers an efficient treatment for diseases, which can be explained by molecular docking and network method to some extent. As some active compounds have been successfully selected, the method is regarded as an effective approach for exploring TCM's. Moreover, the identified 18 multi-target compounds with more than 2 targets are worth being noticed. However, these need validation for future experimental studies.

However, the common component, salidroside, was not identified, while 5-reported active ingredients were screened out from the 187 compounds. This may be that salidroside is likely to play a role in another pathway, such as HIF-1α synthetic pathway.[37] Due to fewer study about this pathway, we didn't include it in this study. In the following research, we will focus attention on HIF-1α synthetic pathway to explore the mechanism of salidroside.

Corresponding to the 10 targets, the compounds with approved active ingredients are listed in Table 6. From Table 6,

Table 4

Relationship between targets and compositions with top 5%.

Targets	PDB Code	Top 1% Score	Top 5% Score	No. of Compound
ARD1	4KVX	−10.6	−9.9	169,45,5,6,16,4,3,182,29
GSK3B	4ACC	−10.2	−9.5	26,12,166,27,17,13,41,187,169,45
Hdm2	4HBM	−8.8	−8.1	169,184,45,173,49,26,27,106,170,187,168
OS-9	3AIH	−8.0	−7.0	45,29,181,182,16,14,20,31,187,10,15
PHD2	4BQW	−9.9	−9.1	15,17,42,31,6,9,29,11,16,187,45
pVHL	4BKS	−8.3	−7.6	29,26,167,106,181,168,31,183,5
SSAT1	2FXF	−10.4	−9.7	171,4,169,57,62,18,3,182,56,64,17,45,9
SSAT2	2BEI	−9.6	−9.0	57,169,17,166,11,9,31,15,6,49,42
FOXO4	3L2C	−7.8	−7.3	45,16,5,29,27,181,4,3,18
RACK1	4AOW	−11.1	−10.2	191,169,16,29,168,45,5,165,167,33,170

ARD1 = arrest-defective-1, FOXO4 = forkhead transcription factor, GSK3β = glycogen synthase kinase 3β, Hdm2 = human double minute 2, OS-9 = osteosarcoma-9, PDB = Protein Data Bank, PHD2 = prolyl hydroxylase 2, pVHL = von Hippel–Lindau protein, RACK1 = receptor for activated C-kinase1, SSAT1 = spermidine/spermine-N1-acetyltransferase-1, SSAT2 = spermidine/spermine-N1-acetyltransferase-2.

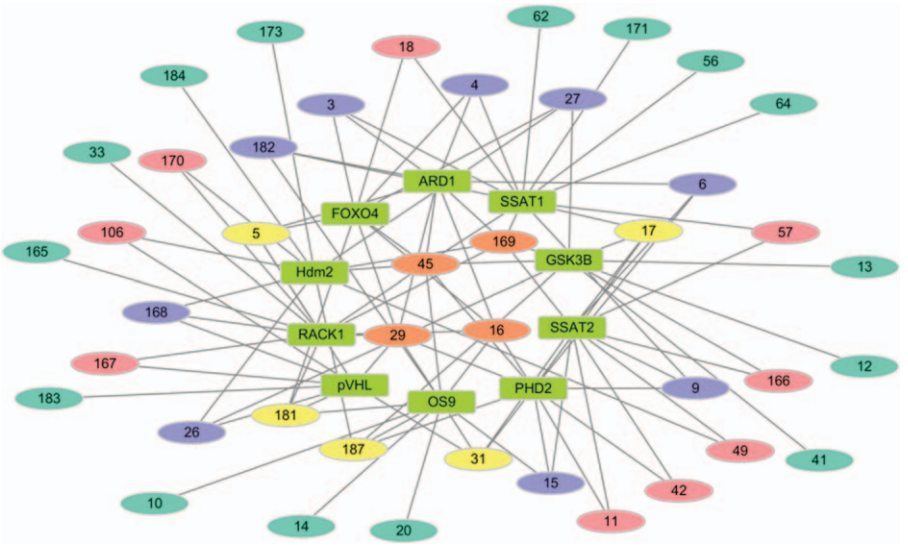
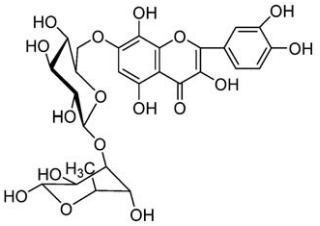
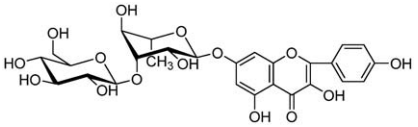
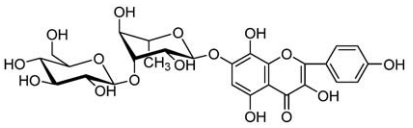
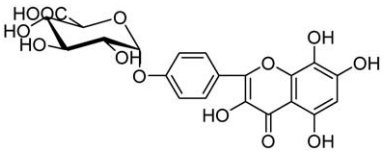
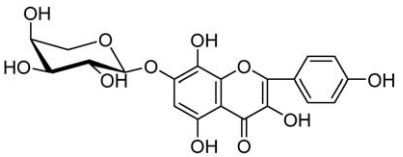
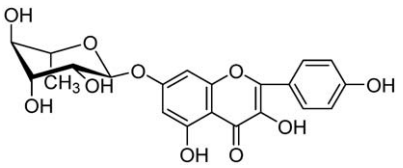
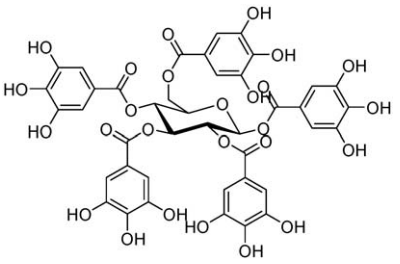
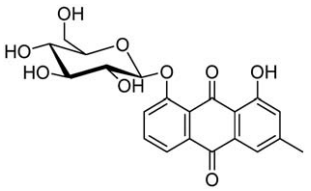
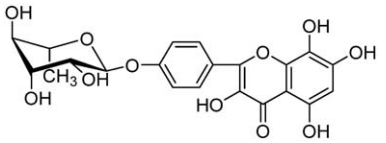
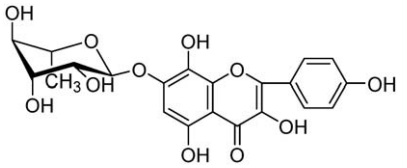
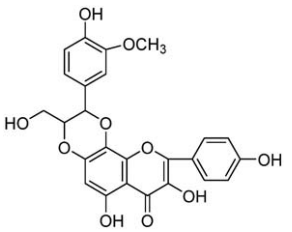
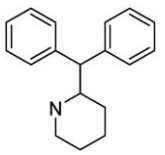


Figure 2. Interaction network between compounds and targets. Targets are shown as green square, while compounds are shown as circle; interaction number for each compound is represented as different colors (cyan: 1; pink: 2; purple: 3; yellow: 4; and orange: >4).

Table 5

Compound-target interactions for the compounds with either more than 4 proteins or approved active ingredient.

No.	Compound name	Compound structure	Target name
45	Rhodioflavonoside		ARD1,FOXO4, GSK3B,Hdm2, OS9,PHD2, RACK1, SSTA1
29	Crenuloside		ARD1,FOXO4, pVHL,OS9, PHD2, RACK1,
169	Glutin-5-en-one		ARD1 GSK3B, Hdm2, RACK1, SSTA1,SSAT2
16	Rhodiosin*		ARD1,FOXO4, OS9,PHD2, RACK1
5	Alginin		ARD1,FOXO4, pVHL, RACK1
17	Gelolin		GSK3B, PHD2, SSTA1,SSAT2
31	Kaempferol-7-O-α-L-rhamnoside		OS9,PHD2, pVHL, SSAT2
181	1,2,3,6-tetra-O-Galloyl-β-D-glucopyranoside		FOXO4, OS9, pVHL,RACK1,

No.	Compound name	Compound structure	Target name
187	chrysophanol-8-O-β-glucopyranosyl		GSK3B, Hdm2, OS9, PHD2
4	Rhodiolatuntoside*		ARD1, SSAT1, FOXO4
15	Rhodionin*		OS9, PHD2, SSAT2
26	Crenulatin*		GSK3B, Hdm2, pVHL
184	Pyridrde*		Hdm2

* indicates the compounds that have been proved as active ingredients. ARD1 = arrest-defective-1, FOXO4 = forkhead transcription factor, GSK3β = glycogen synthase kinase 3β, Hdm2 = human double minute 2, OS-9 = osteosarcoma-9, PHD2 = prolyl hydroxylase 2, pVHL = von Hippel–Lindau protein, RACK1 = receptor for activated C-kinase1, SSAT1 = spermidine/spermine-N1-acetyltransferase-1, SSAT2 = spermidine/spermine-N1-acetyltransferase-2.

we could see that targets ARD1, FOXO4, OS-9, PHD2, and Hdm2 existed in 2 different active compounds. Of the 5 targets, 3 targets (ARD1, OS-9, and PHD2) are located in pVHL-dependent pathway, while the remaining 2 targets (FOXO4 and Hdm2) belong to neither pVHL-dependent nor pVHL-independent pathway. We speculate that pVHL-dependent pathway is more important for HIF-1α degradation and also is a main mechanism for treatment of AMS. In addition, different from other targets, Hdm2 is a special target, because the 2 different compositions (Flavonoids and Other chemical constituent) (Table 3) all contain Hdm2 target.

Among all predicted interaction between target and compound, 3 target-compound interactions (PHD2-Rhodionin, RACK1-Rhodiosin, SSAT1-Rhodiolatuntoside) are worth being

Table 6

Compond-target interactions for the compounds with approved active ingredient.

Target	ARD1	FOXO4	OS-9e	PHD2	Hdm2	SSAT1	RACK1	SSAT2	GSK3B	pVHL
No. of compounds	4, 16	4, 16	15, 16	15, 16	26, 184	4	16	15	26	26

ARD1 = arrest-defective-1, FOXO4 = forkhead transcription factor, GSK3β = glycogen synthase kinase 3β, Hdm2 = human double minute 2, OS-9 = osteosarcoma-9, PHD2 = prolyl hydroxylase 2, pVHL = von Hippel–Lindau protein, RACK1 = receptor for activated C-kinase1, SSAT1 = spermidine/spermine-N1-acetyltransferase-1, SSAT2 = spermidine/spermine-N1-acetyltransferase-2.

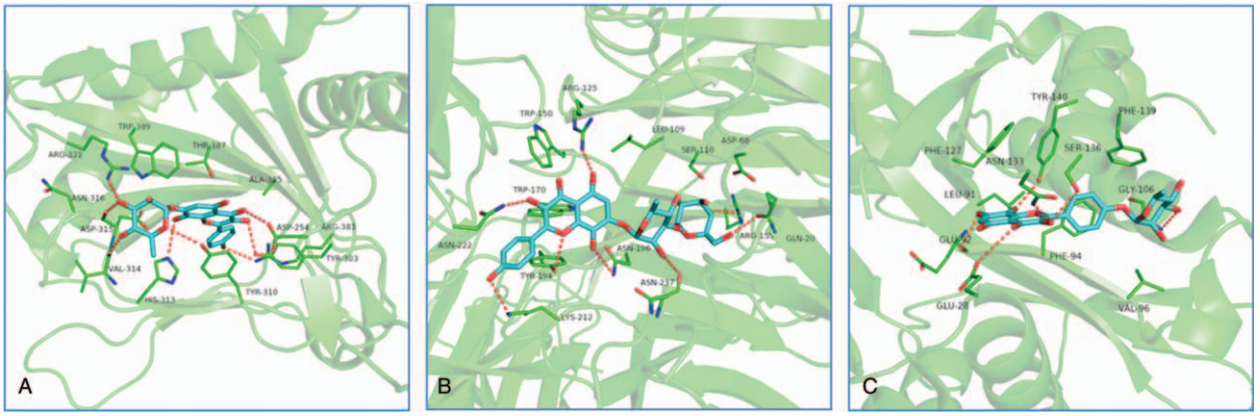


Figure 3. Predicted binding mode of targets and compounds. A: PHD2-Rhodionin; B: RACK1-Rhodiosin; C: SSAT1-Rhodiolatuntoside. PHD2 = prolyl hydroxylase 2, RACK1 = receptor for activated C-kinase1, SSAT1 = spermidine/spermine-N1-acetyltransferase-1.

noticed due to their highest score in the 187 target-compound pairs. Based on this, we speculate that targets of the 3 active compounds [(Rhodionin (No.15), Rhodiosin (No.16), and Rhodiolatuntoside (No.4)] are PHD2, RACK1, and SSAT1, respectively. The predicted binding modes of them are shown in Figure 3 which indicates moderate hydrogen bond interaction, stacking interaction and van der Waals interaction in each binding graph. Further experiment will be needed to confirm their bindings.

4.1. Limitation

This study had several limitations. First, this study did not cover entire proteome which might have been affected by *R rosea*. Second, the result is purely computational. Third, target proteins were selected based on the previous research about HIF-1 which requires biochemical or cell biological experiment to confirm the result conducted in this study.

5. Conclusions

We used molecular docking and network method to study the therapeutic mechanism of *R rosea* on AMS specifically investigated the relationship between the active compositions of *R rosea* and HIF-1α degradation pathway. Our results show that *R rosea* can inhibit HIF-1α degradation pathway through at least 5 constituents (Rhodiosin,[35] Rhodiolatuntoside,[34] Rhodionin,[30,36] Crenulatin,[33] and Pyridrde[20]). In addition, 5 targets (ARD1, FOXO4, OS-9, PHD2, Hdm2) of HIF-1α degradation pathway are worth being noticed. Our data also suggest that PHD2, RACK1 and SSAT1 are likely to be the targets of the 3 active compounds [(Rhodionin (No.15), Rhodiosin (No.16), and Rhodiolatuntoside (No.4)], respectively. Our results remain to be confirmed by further experiments.

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