

SUPPLEMENTARY MATERIALS

Dermatan sulfate and chondroitin sulfate from *Lophius litulon* alleviate
the allergy sensitized by Major royal jelly protein 1

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1. Preparation of MRJP1 polyclonal antibody

The protocol of experiment followed Niu with some modifications.¹ Four white rabbits were randomly divided into two groups: the immune group and the normal control group. In the immune group, the rabbits were intraperitoneally injected with 2 mg of MRJP1 (dissolved in 1 mL of saline and supplemented with 5 mg of aluminum hydroxide adjuvant). The normal control group was intraperitoneally injected with the same volume of saline (including 5 mg of aluminum hydroxide adjuvant). The injection time points were day 0, day 14, day 28, and day 35, respectively. The whole blood of rabbits was drawn one week after the last injection of MRJP1. The collected blood was placed at room temperature for one hour then centrifuged at 4 °C, 825 g, 10 min. The supernatant containing MRJP1 polyclonal antibody was separated and collected. The titer of the obtained antibody was 48000, which was measured by ELISA (Supplementary materials Table S1).

Table S1 Titer results of MRJP1 polyclonal antibody

Dilution ratio	Immune group OD ₄₅₀	Normal group OD ₄₅₀	Blank group OD ₄₅₀	P/N
1: 1000	2.79±0.033	0.36±0.010	0.095±0.004	10.19
1: 3000	2.65±0.060	0.36±0.010	0.095±0.004	9.67
1: 6000	2.26±0.017	0.36±0.010	0.095±0.004	8.18
1: 9000	2.00±0.053	0.36±0.010	0.095±0.004	7.22
1: 18000	1.86±0.055	0.36±0.010	0.095±0.004	6.66
1: 24000	1.59±0.056	0.36±0.010	0.095±0.004	5.66
1: 48000	1.07±0.059	0.36±0.010	0.095±0.004	3.77

Note: Blank group was PBS. P/N meant (Immune group-blank group) divided by (normal group-blank group). When the value of P/N was greater than or equal to 2.1, the corresponding dilution ratio was the MRJP1 polyclonal antibody titer. Therefore,

the titer of MRJP1 polyclonal antibody was 48000.

[1] N. Dandan, Isolation and determination of royal jelly main protein mrjp-1 and its growth promoting effect on cells [D], Zhejiang University, 2013.

2. The pre-experimental of MRJP1-sensitization mice model

According to the results, it showed all groups of MRJP1 allergen increased the production of total IgE and caused allergic symptoms. To better build the MRJP1-sensitized model, allergic dose should not only cause allergy, but also not cause death of mice. Therefore, the low-dose group (1mg) of MRJP1 allergen was chose for the following model dose of the hypoallergenic experiment.

Table S2 Changes of total IgE content and behavior in sensitized mice in pre-experiment

IgE (U/mL)	1 th 0d	2 th 5d	4 th 16d	5 th 22d
NC Group	5.35 ± 0.54	6.45 ± 0.91	normal	normal
L Group	21.9 ± 3.21**	23.62 ± 3.20**	Allergic symptoms	Allergic symptoms
M Group	32.92 ± 1.87**	20.15 ± 2.56**	Allergic symptoms	Allergic symptoms and two mice died
H Group	38.82 ± 3.46**	16.47 ± 2.18**	Allergic symptoms and one mouse died	Allergic symptoms
MC Group	6.45 ± 1.06	7.29 ± 1.05	Allergic symptoms	All mouse died

Note: NC stands for the normal control group; L, M and H stands for the low (1 mg), medium (5 mg) and high (10 mg) dose of MRJP1, respectively. MC stands for the model control group (Ovalbumin). ** indicates significant differences from the model control group value (** $p < 0.01$). 1th 0d means the first injection of allergen in mice.

Table S3 Energy of molecular docking of *Ll* DS and *Ll* CS with MRJP1

Poses	Docking Interaction Energy (kcal/mol)	Poses	Docking Interaction Energy (kcal/mol)
DS-2	-46.7577	CS-2	-42.7632
DS-3	-46.6353	CS-3	-42.5389
DS-4	-46.2403	CS-4	-42.3861
DS-5	-45.2293	CS-5	-41.2363
DS-6	-44.6201	CS-6	-40.2308
DS-7	-44.5509	CS-7	-39.1874
DS-8	-44.353		
DS-9	-42.0262		
DS-10	-41.9041		

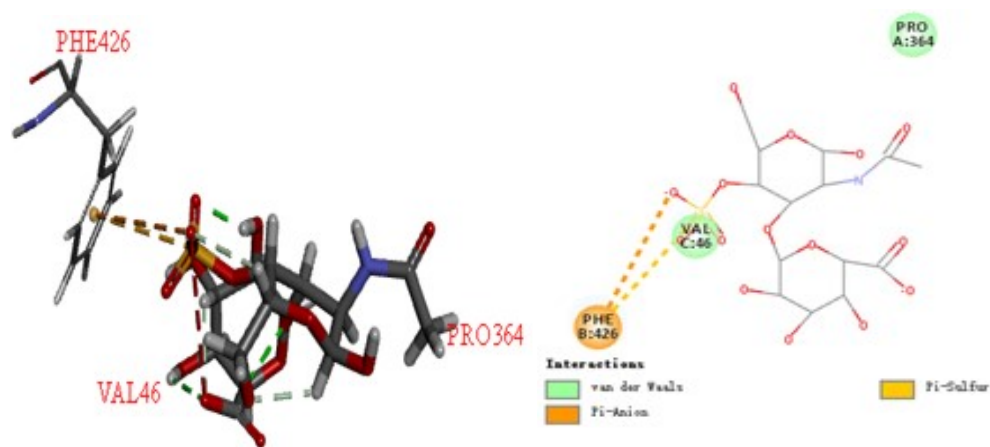


Fig S1. The interaction model of *Ll* DS with MRJP1 (pose 2)

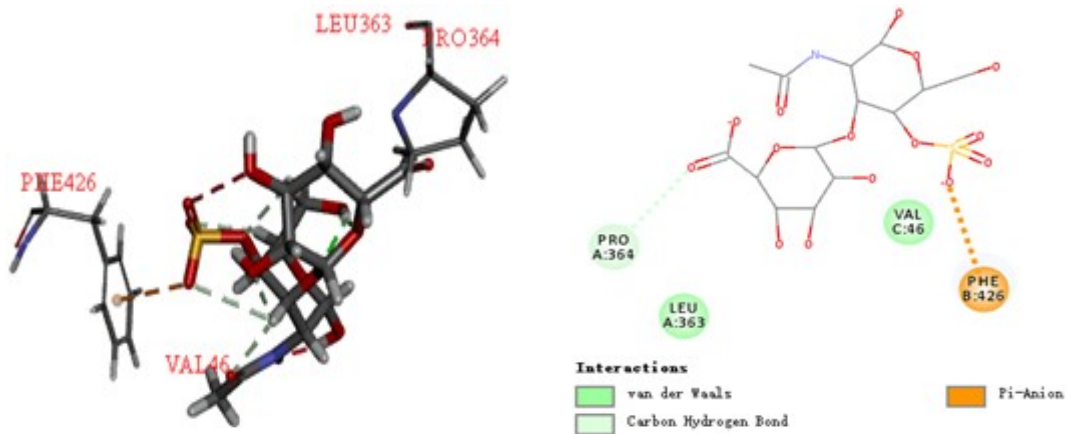


Fig S2. The interaction model of *Ll* DS with MRJP1 (pose 3)

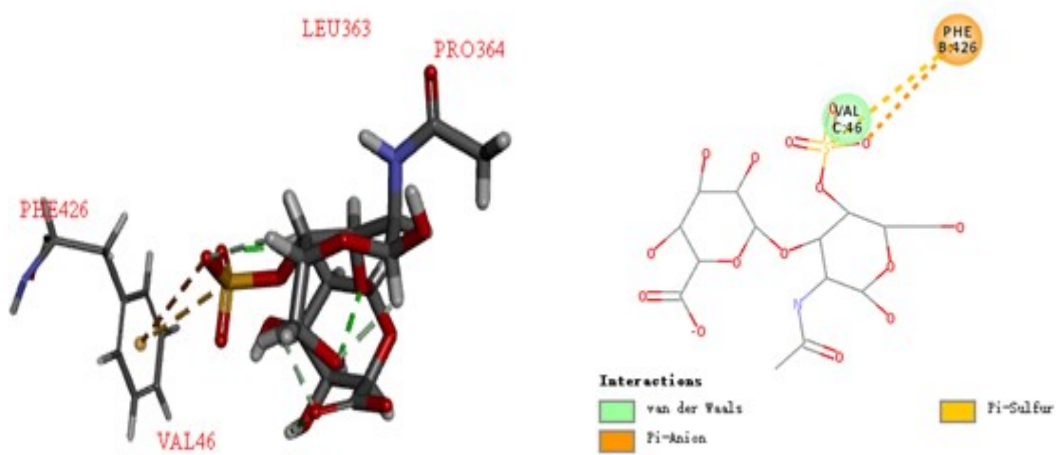


Fig S3. The interaction model of *Ll* DS with MRJP1 (pose 4)

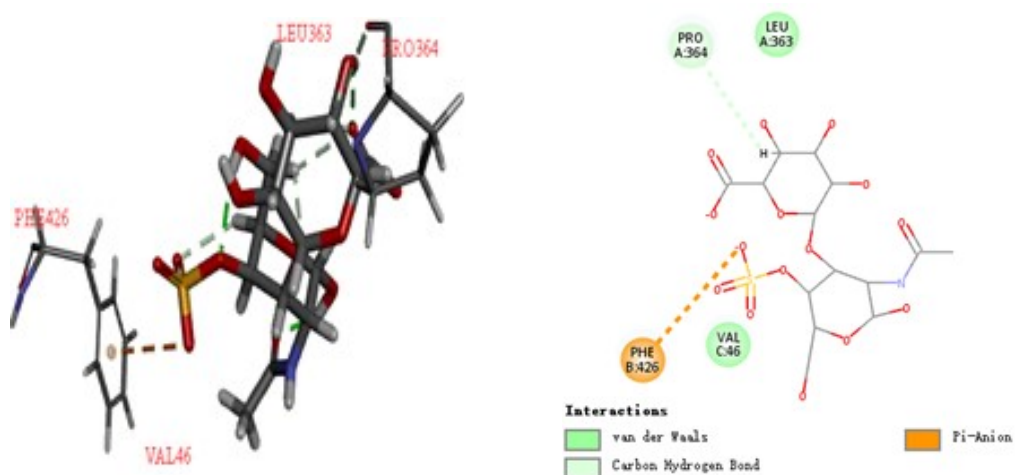


Fig S4. The interaction model of *Ll* DS with MRJP1 (pose 5)

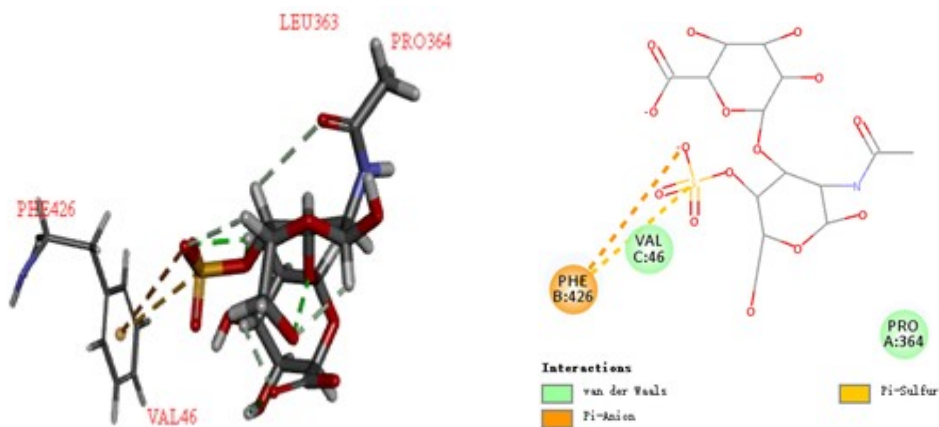


Fig S5. The interaction model of *Ll* DS with MRJP1 (pose 6)

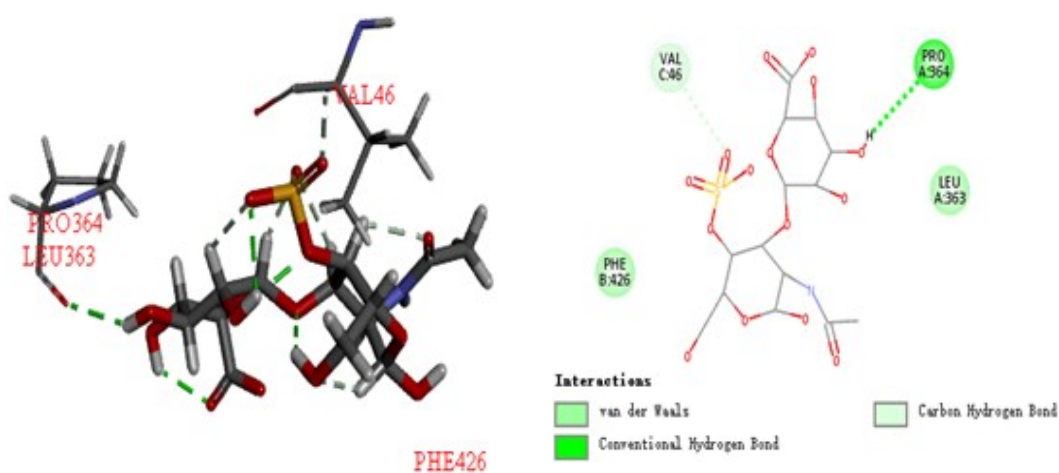


Fig S6. The interaction model of *Ll* DS with MRJP1 (pose 7)

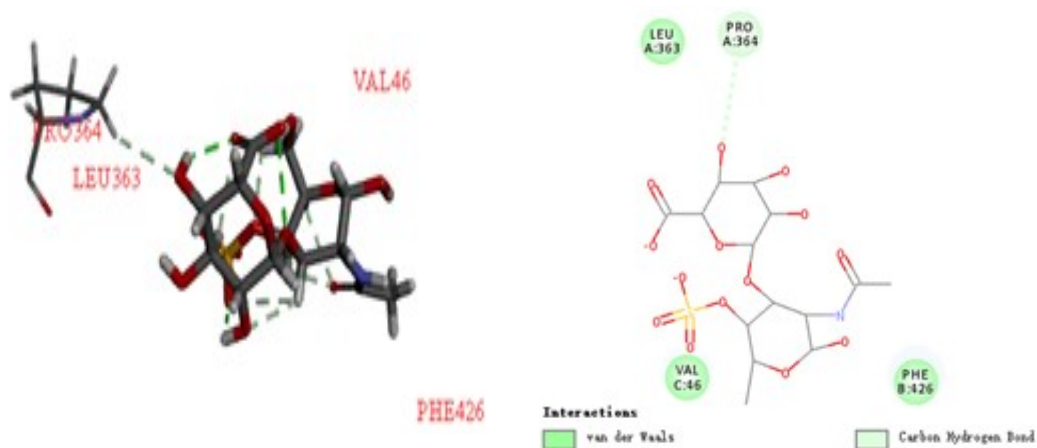


Fig S7. The interaction model of *Ll* DS with MRJP1 (pose 8)

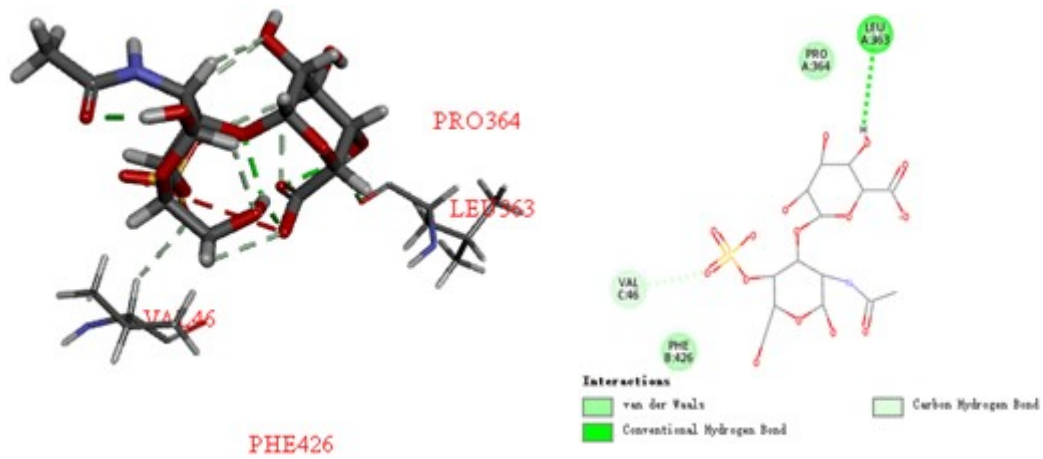


Fig S8. The interaction model of *LI* DS with MRJP1 (pose 9)

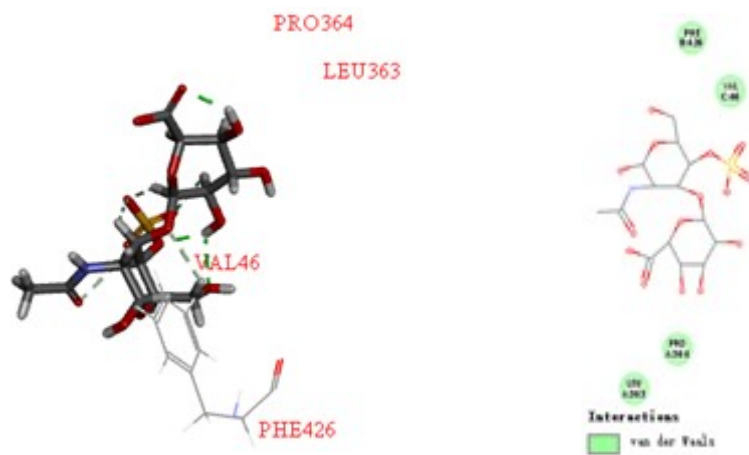


Fig S9. The interaction model of *LI* DS with MRJP1 (pose 10)

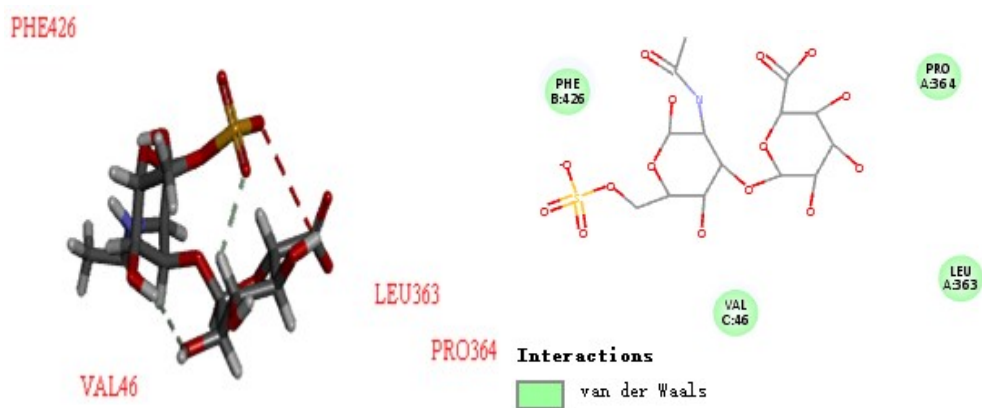


Fig S10. The interaction model of *LI* CS with MRJP1 (pose 2)

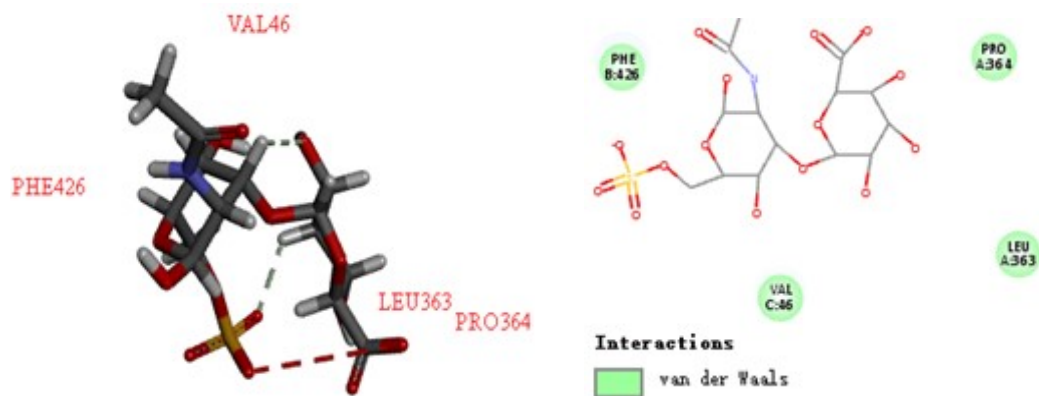


Fig S11. The interaction model of *Ll* CS with MRJP1 (pose 3)

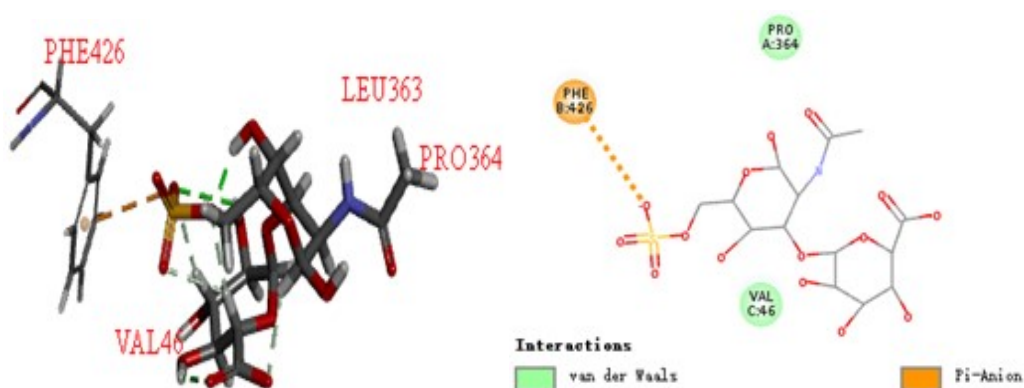


Fig S12. The interaction model of *Ll* CS with MRJP1 (pose 4)

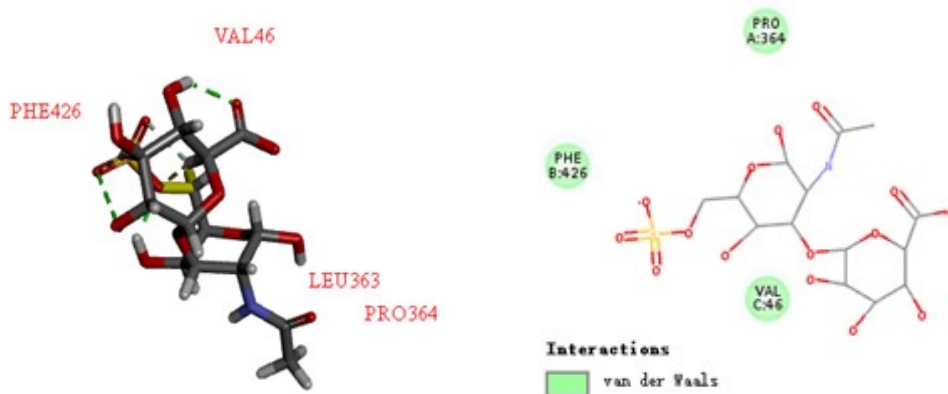


Fig S13. The interaction model of *Ll* CS with MRJP1 (pose 5)

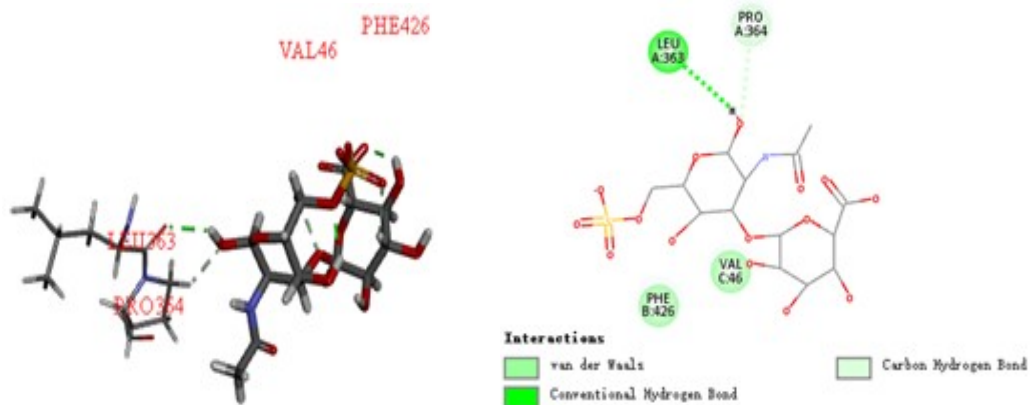


Fig S14. The interaction model of *LI* CS with MRJP1 (pose 6)

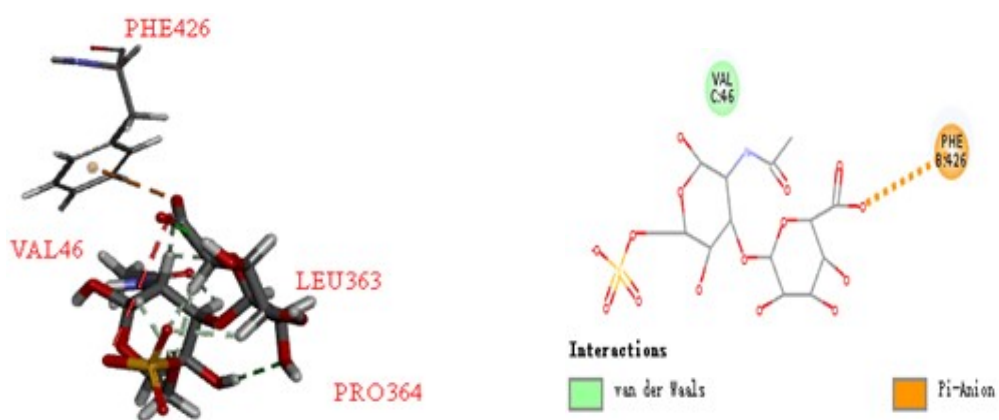


Fig S15. The interaction model of *LI* CS with MRJP1 (pose 7)