Supplementary Material

Legends:

Fig. S1. Enzymatic activity of ecarpholin S. The PLA_2 enzyme activity was measured by routine colorimetric assay (PLA_2 assay kit, Cayman Chemicals, Ann Arbor, MI). $ScPLA_2$ (Asp49-PLA_2) from *Echis carinatus sochureki* served as the control. The concentration of ecarpholin S is 1 mg/mL and the concentration of $scPLA_2$ is 0.01 mg/mL in the assay. The calculation equation of the PLA_2 activity is suggested by the manual of the kit according to the absorbance difference. All data points were acquired in triplicate and are represented by their means (points) and standard deviations (error bars).

Fig. S2. Induction of mouse footpad edema by ecarpholin S. The dose of ecarpholin S is 5, 10, 20, 50 µg/mouse. All data points were acquired in triplicate and are represented by their means (points) and standard deviations (error bars).

Fig. S3. Isothermal Titration Calorimetric (ITC) titration of ecarpholin S with $CaCl_2$. (A) Raw data. (B) Plot of the integrated heat versus the calcium chloride /protein molar ratio. Conditions: 0.1 mM ecarpholin S titrated with 5 µL injections of 10 mM $CaCl_2$. Both protein and $CaCl_2$ solutions are in 50 mM Tris-HCl at pH 7.0 and 30°C. From the heat change curve, only the dilution heat can be observed.

Fig. S4. Stereo diagram of superposition of the Ca2+-binding loop of PLA2s. Ecarpholin S (Red) and scPLA2 (PDB code 1OZ6, blue) are superimposed. Hydrogen bond is shown as dash line. Some residue sidechains are hidden for clear show. This figure was prepared by using the program Pymol (1).

Fig. S5. Stereo diagram of superposition of the catalytic sites of PLA_2s. Ecarpholin S (Red), scPLA2 (PDB code 1OZ6, blue) and Myotoxin II (PDB code 1CLP, yellow). This figure was prepared by using the program Pymol (1).

Fig. S6. **Surface charge distribution (red negative and blue positive) of apo ecarpholin S**. The electrostatic surface was calculated with APBS (2). This figure was prepared by using the program Pymol (1).

Fig. S7. Stereo diagram of comparison of suramin bound to ecarpholin S and Basp-II. One of monomers of ecarpholin S and Basp II (PDB code: 1Y4L) are superimposed. The monomers of ecarpholin S are shown as gray and the bound suramin molecules are shown as red. The monomers of Basp-II are shown as cyan and the bound suramin is shown as yellow. This figure was prepared by using the program Pymol (1).

Fig. S1.

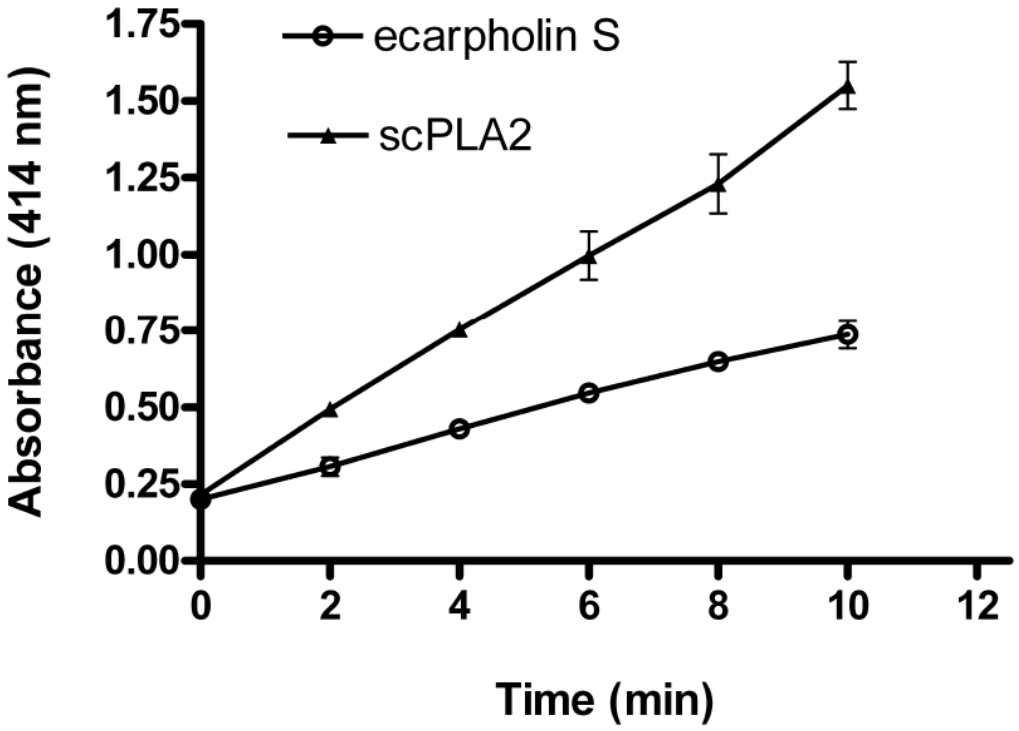

ecarpholin S
scPLA2
Absorbance (414 nm)
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0
2
4
6
8
10
12
Time (min)

Fig. S2.

Fig. S3.

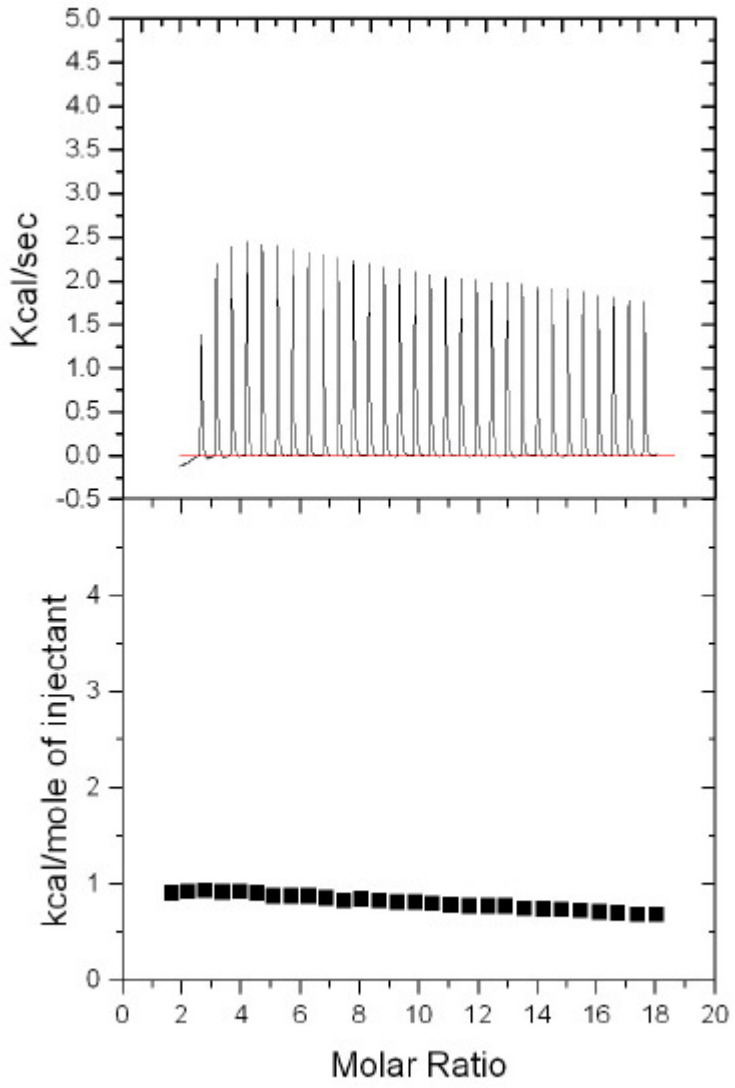

Kcal/sec
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
kcal/mole of injectant
4
3
2
1
0
0
2
4
6
8
10
12
14
16
18
20
Molar Ratio

Fig. S4

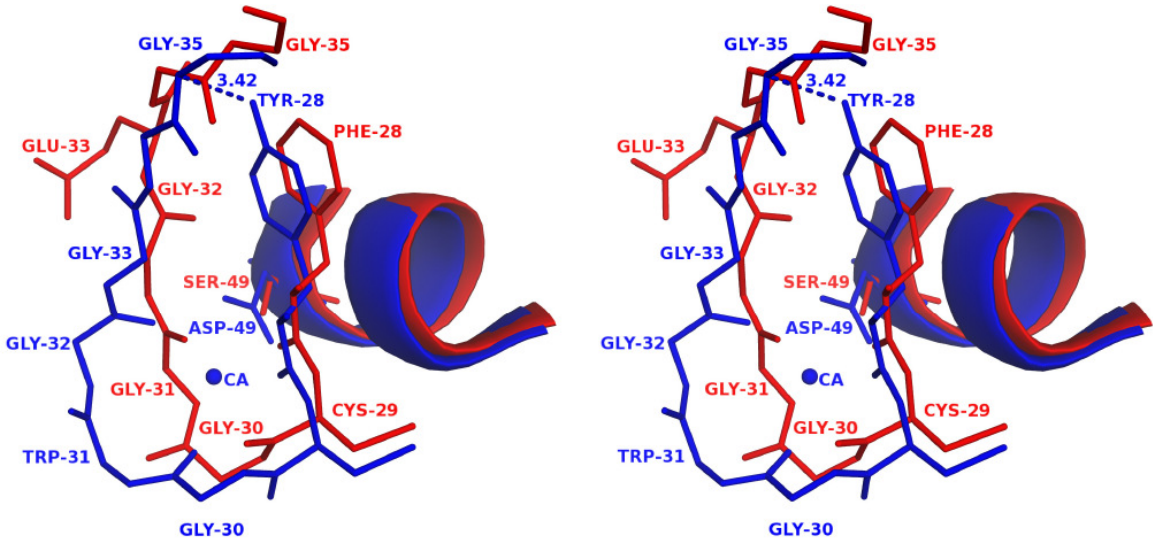

GLY-35
GLY-35
3.42
TYR-28
PHE-28
GLU-33
GLY-32
GLY-33
SER-49
ASP-49
GLY-32
CA
GLY-31
CYS-29
GLY-30
TRP-31
GLY-30
GLY-35
GLY-35
3.42
TYR-28
PHE-28
GLU-33
GLY-32
GLY-33
SER-49
ASP-49
GLY-32
CA
GLY-31
CYS-29
GLY-30
TRP-31
GLY-30

Fig. S5

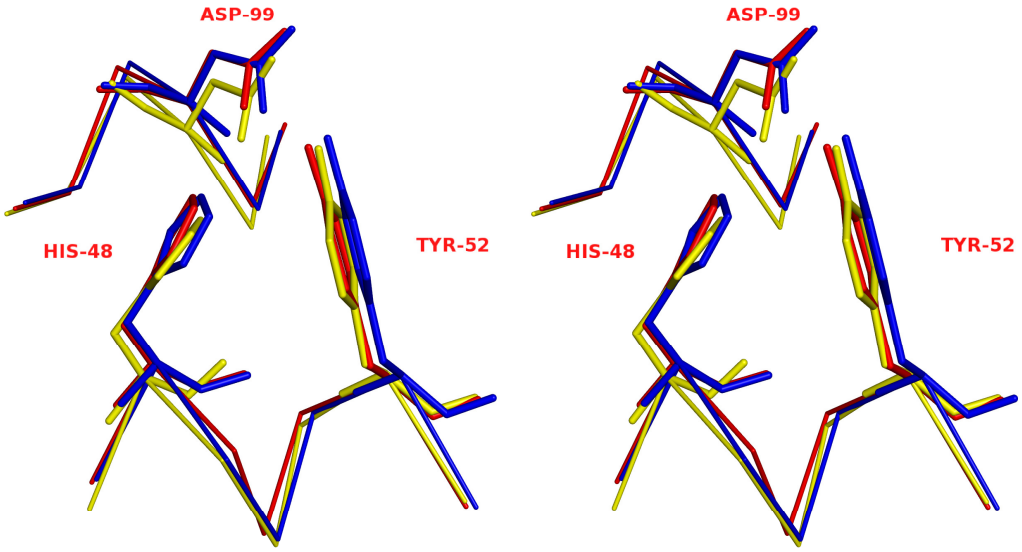


Fig. S6

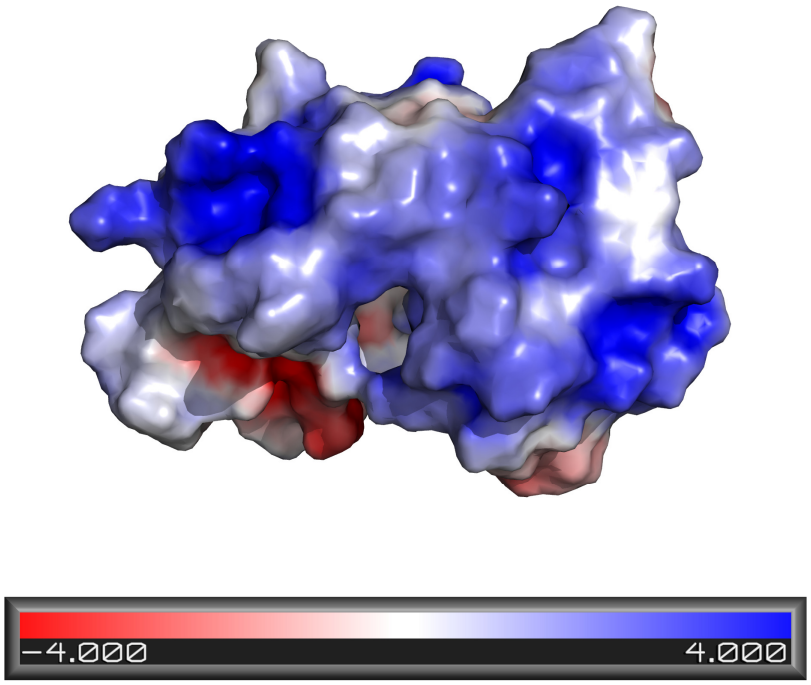
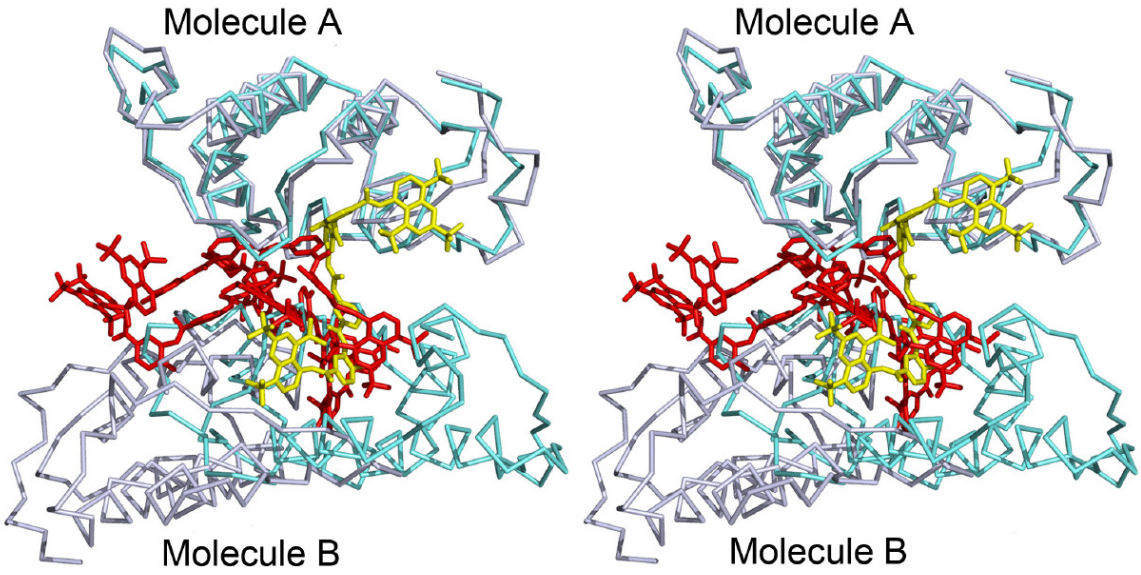


Fig. S7



1. Delano, W. S. 2002. The PyMOL Molecular Graphics System. In http://www.pymol.org
2. Baker, N. A., D. Sept, S. Joseph, M. J. Holst, and J. A. McCammon. 2001. Electrostatics of nanosystems: application to microtubules and the ribosome. Proceedings of the National Academy of Sciences of the United States of America 98:10037-10041.