Supporting Information

Homology Modeling and Site-directed Mutagenesis to Identify Selective Inhibitors of Endothelin-Converting Enzyme-2

Khatuna Gagnidze[&], Sachchidanand[#^], Raphael Rozenfeld[&], Mihaly Mezei[#], Ming-Ming Zhou[#] and Lakshmi A. Devi*[,&]

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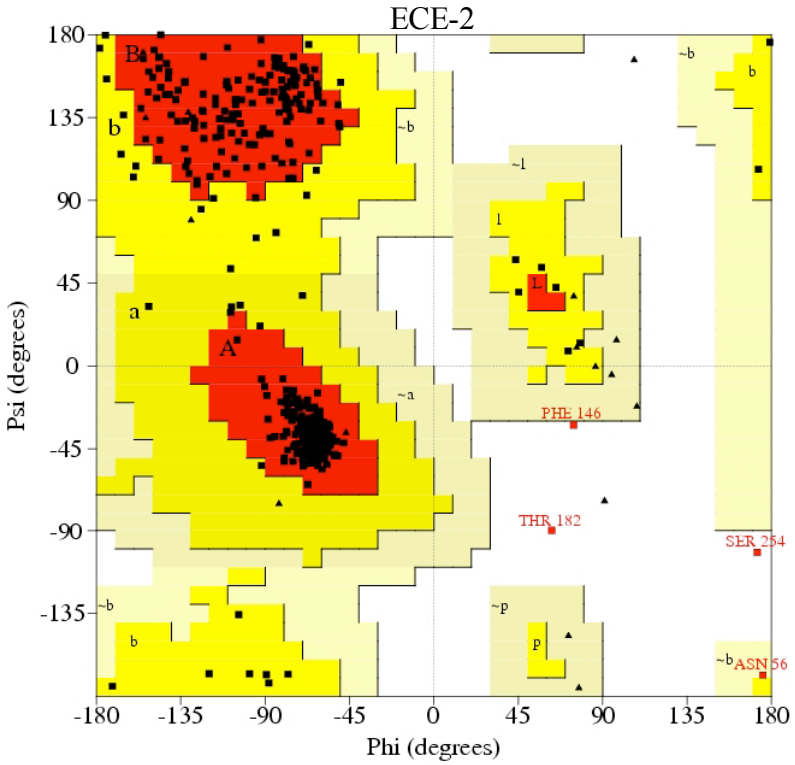

Plot statistics		
Residues in most favored regions {A, B, L]	563	93.4%
Residues in additional allowed regions [a, b, l, p]	36	6.0%
Residues in generously allowed regions [~a, ~b, ~l, ~p]	1	0.2%
Residues in disallowed regions	3	0.5%
Number of non-glycine and non-proline residues	603	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown in triangles)	42	
Number of proline residues	32	
Total number of residues	679	

Figure S1. Ramachandran plot of ECE-2 model.
The quality of the modeling was tested using its Ramachandran plot. This revealed that 93% of the residues were within the favorable limit. Among them all the residues in the active site are within the acceptable regions. The three residues, F146, T182 and S254, which are in disallowed region, are not in the binding site.

Table S1. Purification of ECE-2 activity

Purification steps	Total protein	Total activity	Yield	Specific activity	Purification
	mg	nmol/min	%	pmol/min/μg	Fold
Medium	22.29	25.24	100	1.13	1
Amicon YM100	3.13	6.64	26	2.12	1.89
DEAE-Sepharose	1.03	2.77	11	2.68	2.37
Talon affinity resin	0.06	0.72	2.85	12.79	11.32

Wild type and mutated ECE-2-containing conditioned media were centrifuged at 5000 x g for 30 min, and the supernatant was concentrated by passing the medium through Amicon ultrafiltration membrane, YM100 (Millipore Corp., Bedford, MA). For purification, the concentrated material was loaded onto a DEAE-Sepharose fast flow anion exchange column in 20mM Tris-Cl, pH 7.1. The column was washed with 10 column volumes of the same buffer and eluted with a linear gradient of 0-0.5M NaCl. The fractions were assayed for ECE-2 activity as described in the Experimental Section and for protein levels using the BCA reagent (Pierce Biotechnology, Inc., Rockford, IL). The peak of ECE-2 activity eluted around 0.25M NaCl. The fraction containing the highest activity was loaded onto a Talon™-Sepharose Co^{2+} affinity resin column equilibrated with Tris-Cl buffer, pH 7.1, containing 300mM NaCl. The enzyme bound to the resin was washed with the sodium acetate buffer, pH 5.9, containing 300mM NaCl and was eluted with the same buffer adjusted to pH 5.1.

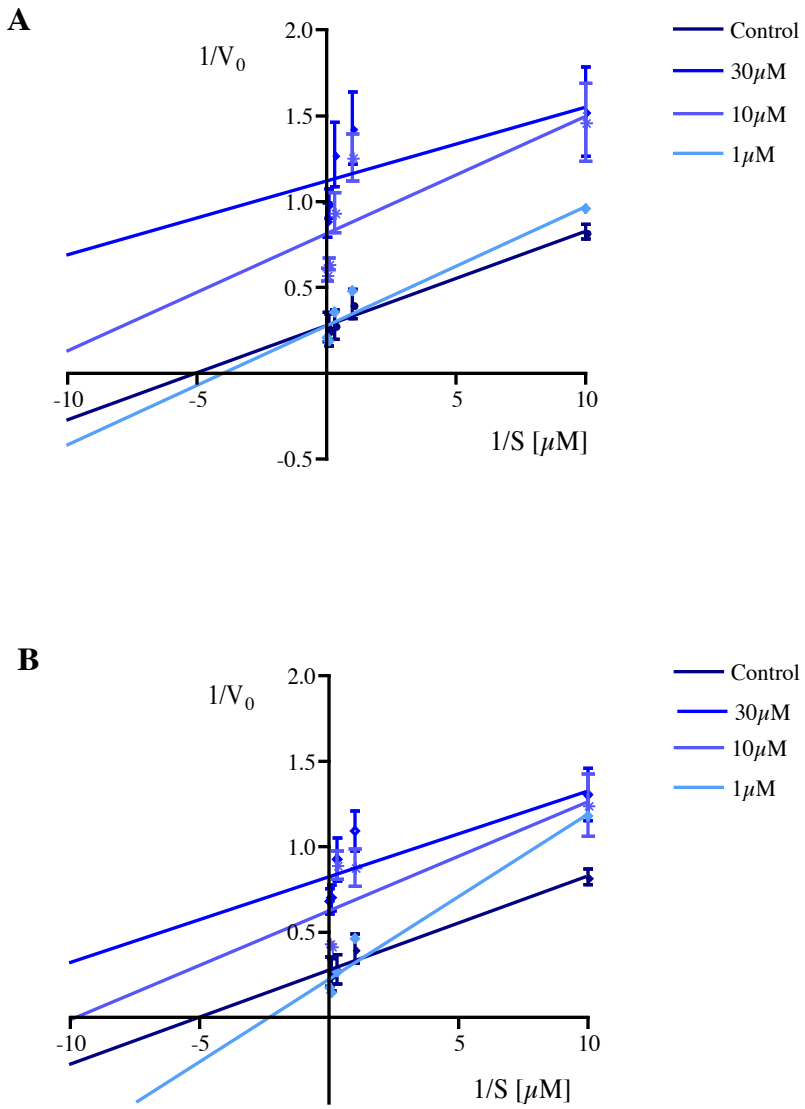

Figure S2. Characterization of inhibitory activity of compounds **1** (A) and **2** (B). The initial rate of McaBk2 hydrolysis (V_0) at final concentrations of 0.1-30μM was determined by measuring the appearance of product under initial rate conditions in the absence and presence of increasing concentrations of inhibitor compound. Lineweaver-Burk plots were generated by plotting the reciprocal of substrate concentration (1/[S]) as a function of reciprocal of initial rate of substrate hydrolysis ($1/V_0$) for each concentration of **1** (A) and **2** (B) and the kinetic parameters (K_m and V_{max}) were determined.