Inhibition of Striatal-enriched Protein Tyrosine Phosphatase by Targeting Computationally Revealed Cryptic Pockets

Xuben Hou,[†,§] Jin-peng Sun,[‡] Lin Ge,[‡] Xiao Liang[†], Kangshuai Li,[‡] Yingkai Zhang,[§,⊥] and Hao Fang[†,*]

[†]Department of Medicinal Chemistry and Key Laboratory of Chemical Biology of Natural Products (MOE), School of Pharmaceutical Science, Shandong University, Jinan, Shandong, 250012, China

[§]Department of Chemistry, New York University, New York, New York 10003, United States

[‡]Key Laboratory Experimental Teratology of the Ministry of Education and Department of Biochemistry and Molecular Biology, School of Medicine, Shandong University, Jinan, Shandong 250012, China

[⊥]NYU-ECNU Center for Computational Chemistry, New York University−Shanghai, Shanghai 200122, China

Supporting Information
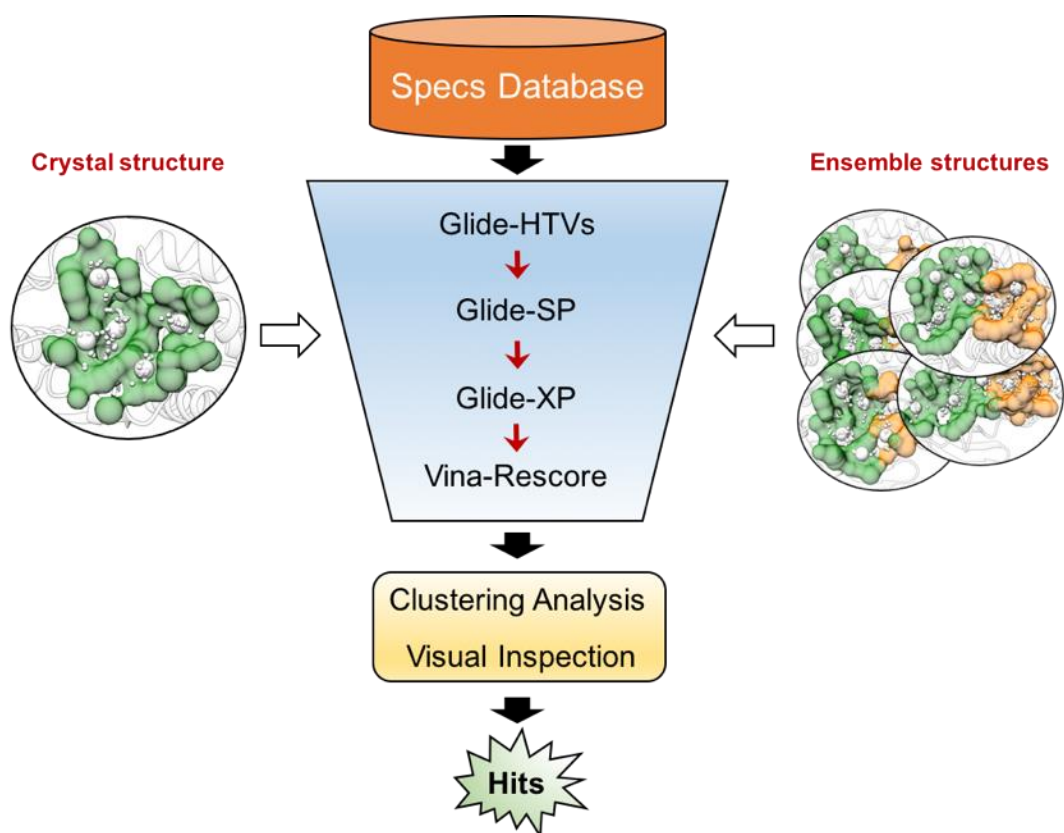
Corresponding author:

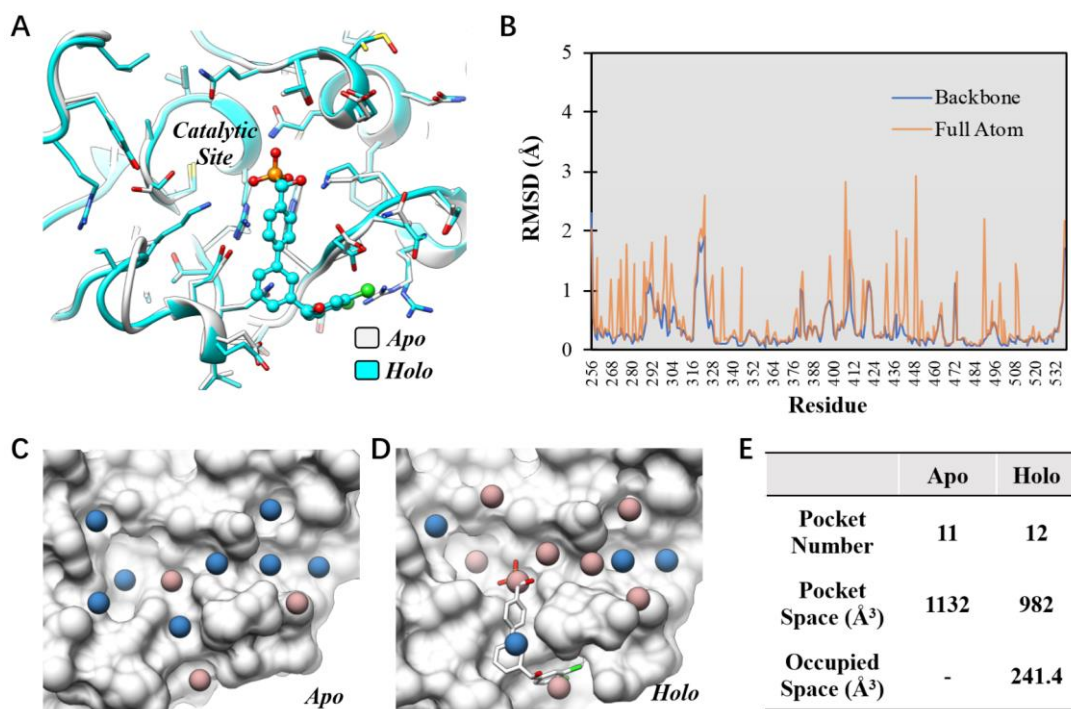* (H.F.) Email: haofangcn@sdu.edu.cn

27

28

29

30 **Figure S1.** Structure-based virtual screening of STEP inhibitors using either crystal structure

31 (PDB: 2BV5) and ensemble structures from Markov State Model (MSM).

32

33

34

Figure S2. Comparison of apo (PDB: 2BV5) and holo (PDB: 5OVX) crystal structures of STEP. (A) Structure alignment of apo and holo crystal structures. (B) Calculated per-residue RMSD values for backbone and full atom. (C-E) Pocket analysis of apo and holo crystal structures. All detected pockets are classified as auxiliary (blue sphere) and minor pockets (rosy brown sphere) by employing AlphaSpace pocket score as described before (ref). Blue spheres represent pockets with score >50 and rosy brown spheres represent pockets with score <50.
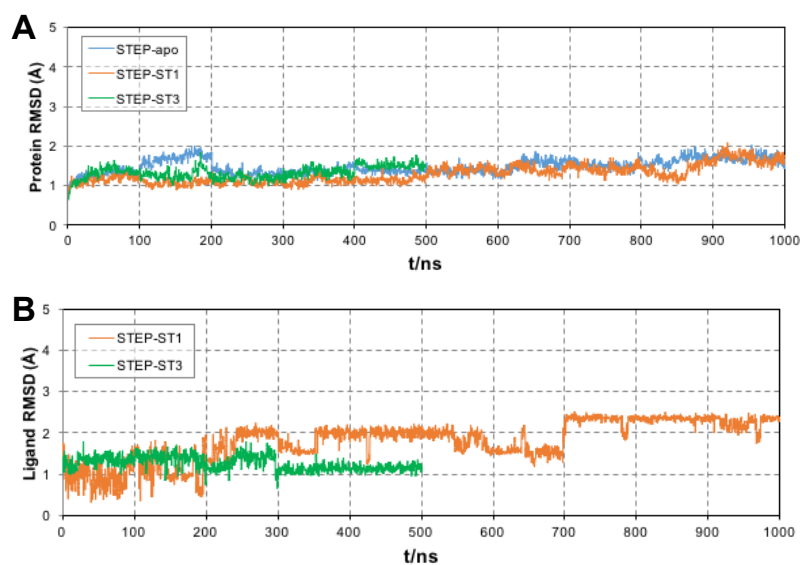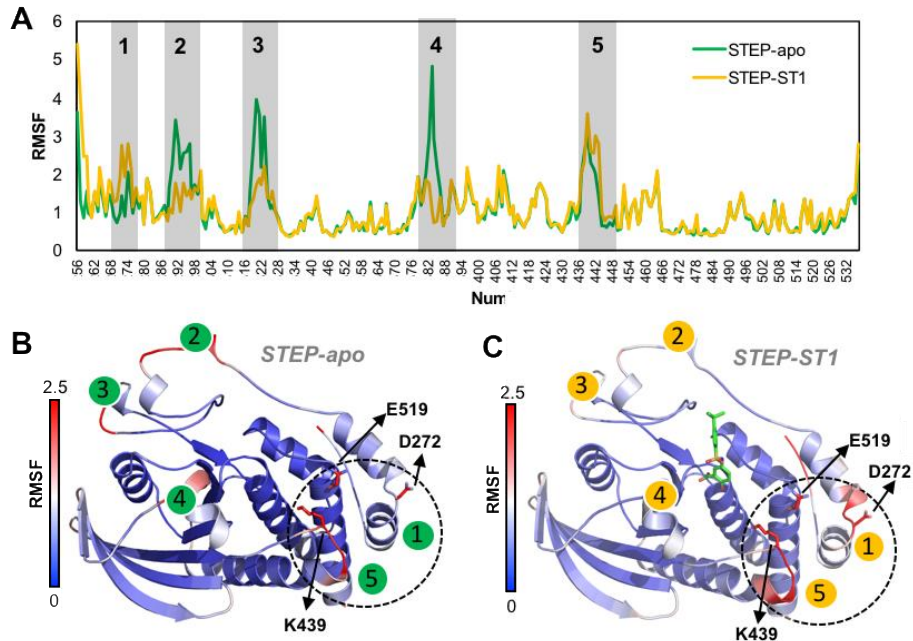

**Figure S3.** RMSD values for STEP proteins and inhibitors from MD simulations.

Figure S4. Comparison of the protein flexibility of STEP with and without inhibitor binding. (A) Per-residue RMSF calculated from MD simulations of STEP-apo (green) and STEP-**ST1** (yellow) systems. Conformational dynamics of STEP-apo structure (B) and STEP-ST1 complex (C) colored by their per-residue RMSF values. The crystal structure PDB:2BV5 serves as a template for comparison. The binding pose of ST1 was derived from molecular docking using the crystal structure. The color scale shows the calculated RMSF in Å.
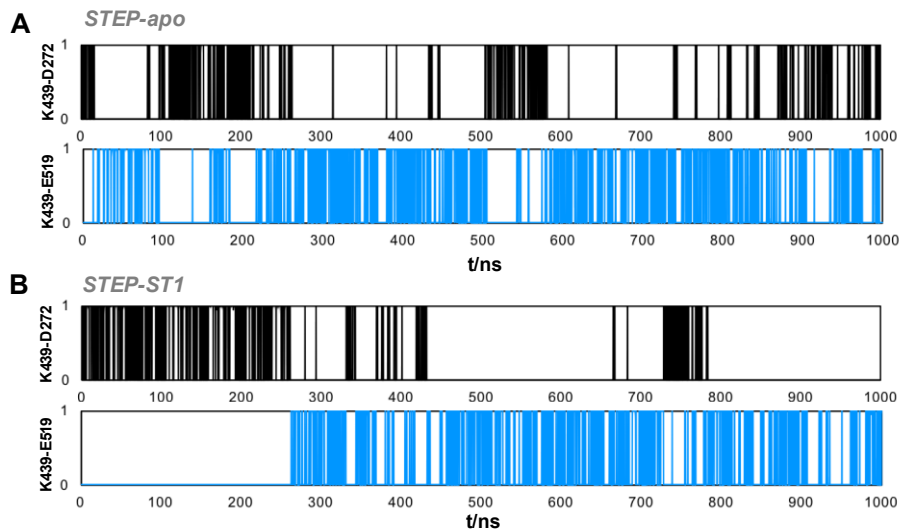
Figure S5. Formation of salt-bridge interactions between K439-D272 (black) and K439-E519 (blue) during 1-μs MD simulation on STEP-apo and STEP-ST1 systems.

4
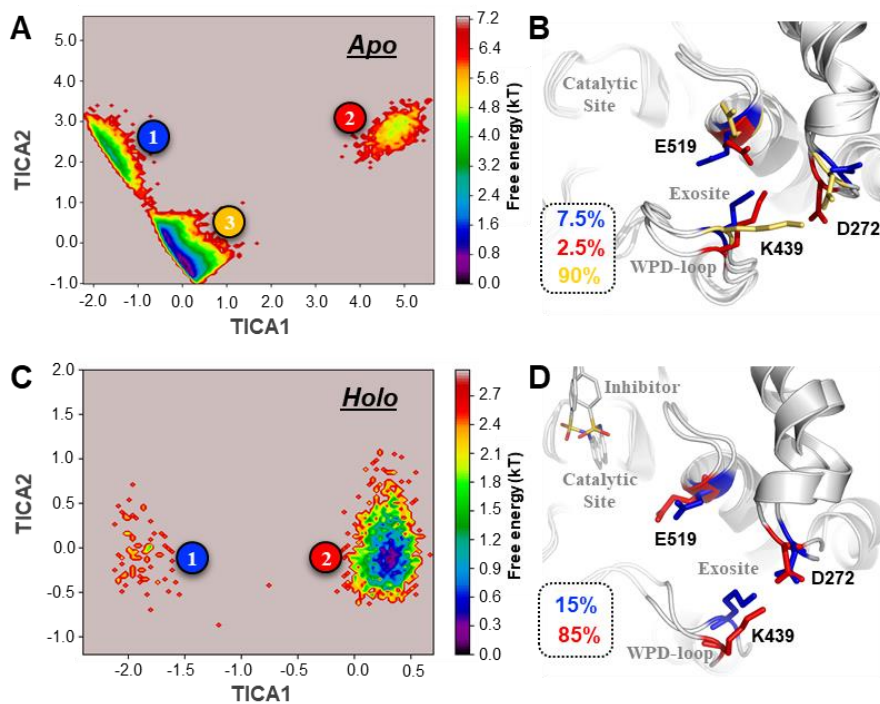
Figure S6. Free-energy landscapes of (A) the STEP-apo system and (C) the STEP-**ST1** system obtained via MSM analysis along the first and second TICA coordinates. Representative MD snapshots of macrostates from both (B) the STEP-apo system and (D) the STEP-**ST1** system. The three residues (D272, K439 and E519) used for TICA construction are shown as stick models. The location of each macrostate in the free-energy landscapes (left) is illustrated using the same color as the residues in the representative MD snapshots (right).
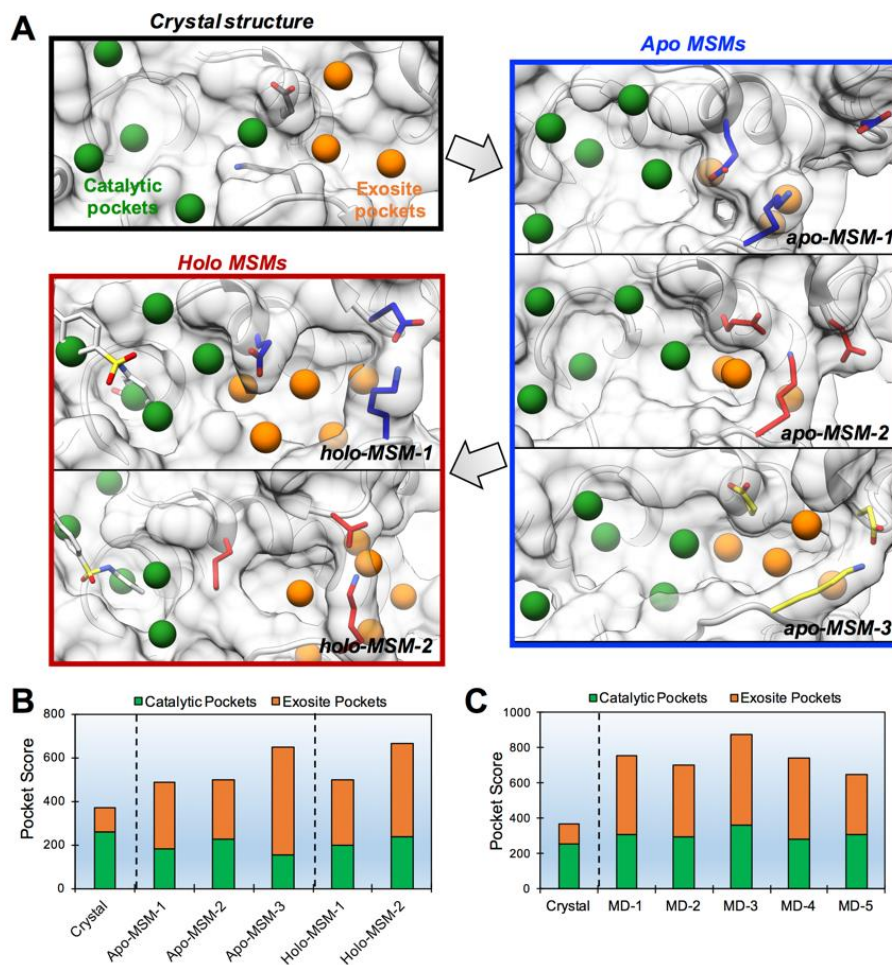
Figure S7. (A) Pocket analysis of apo crystal structure and representative MD snapshots from selected MSMs of STEP-apo and STEP-holo systems. Catalytic pockets and exosite pockets are presented using green and orange spheres. Key residues used for MSM construction and the inhibitor **ST1** are shown as stick models. (B) Comparison of pocket scores for catalytic pocket and exosite pocket in crystal structures and selected MSMs. (C) Comparison of pocket scores of catalytic pockets and exosite pockets from crystal structure and five selected MD snapshots from MSMs.
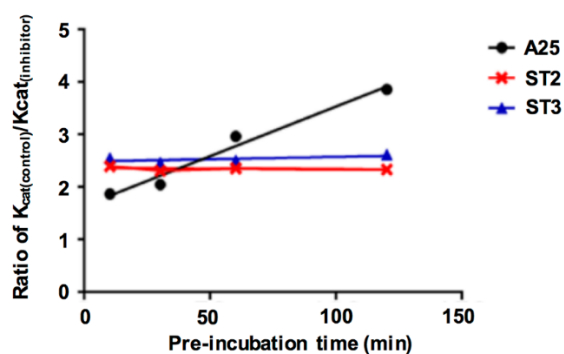
Figure S8. The time-depended ratios of K_{cat}(control)/K_{cat}(inhibitor) for LYP inhibitor (**A25**) and STEP inhibtiors (**ST2** and **ST3**).

78

79 **Table S1**. 32 Hit compounds from apo structure-based virtual screening.

Specs ID	Structure	Specs ID	Structure
AQ-344/13518277		AQ-390/42132976	
AK-777/36504012		AR-422/41345504	
AK-968/15363155		AK-918/15000007	
AC-907/34130062		AS-871/43489763	
AE-907/30536043		AB-323/25048464	
AE-641/30108041		AH-262/34398043	
AN-465/43266004		AE-641/00770041	
AR-422/43115151		AF-399/40714286	
AI-204/43372118		AN-329/43219660	
AK-918/41371592		AG-690/15434541	
AO-476/41610193		AS-871/43476422	
AS-871/43476127		AG-205/13459129	
AE-641/00198040		AE-641/06279003	

7

AE-406/41056893		AI-204/31726003	
AL-281/15328110		AI-204/31682033	
AK-918/37074019		AP-906/41640126	**(ST1)**
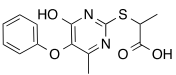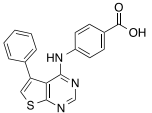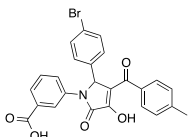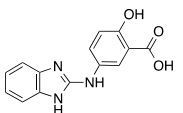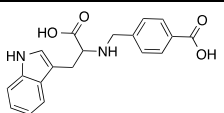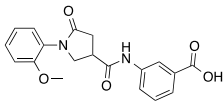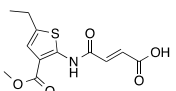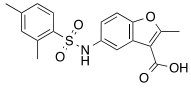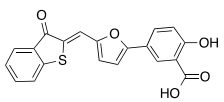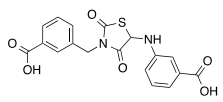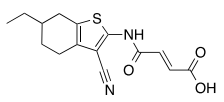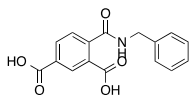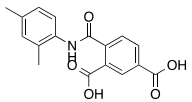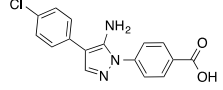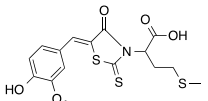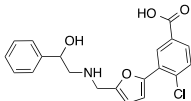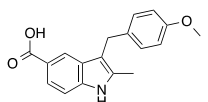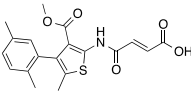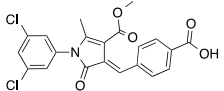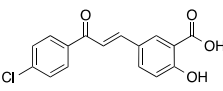
80

81

82 **Table S2**. 32 Hit compounds from ensemble structure-based virtual screening.

Specs ID	Structure	Specs ID	Structure
AP-853/43386858	**(ST8)**	AO-022/43452506	**(ST5)**
AK-968/40709324		AP-853/43445418	
AE-848/14138703	**(ST6)**	AF-399/15285005	
AQ-149/13890403		AN-989/41838787	
AO-022/40415417		AG-205/13052034	**(ST2)**
AI-204/33265041		AE-406/41057026	**(ST10)**

8

AG-205/34704054		AP-906/41027680 **(ST12)**	
AO-022/43452907		AQ-086/43457605	
AN-465/43411092		AK-918/42815229	
AK-968/15364107		AQ-390/42861800 **(ST4)**	
AG-205/33667015 **(ST3)**		AP-893/40872493	
AK-968/12515454 **(ST11)**		AP-263/12245698	
AP-263/12245694		AG-670/42920055	
AF-399/37181008		AN-465/43421754	
AQ-086/43383935 **(ST7)**		AK-968/15363412 **(ST9)**	
AO-081/41131569		AP-185/43377268	

83

84

Table S3. Specs ID for **ST2** analogs and **ST3** analogs.

Compound	Specs ID	Compound	Specs ID
ST2-1	AG-690/13153056	**ST3-1**	AG-690/11835134
ST2-2	AG-690/12869899	**ST3-2**	AG-205/11234130
ST2-3	AG-690/13153051	**ST3-3**	AN-023/13438050
ST2-4	AG-690/13153079	**ST3-4**	AG-205/37007075
ST2-5	AG-690/37099003	**ST3-5**	AO-081/40681146

Table S4. Inhibitory activities of selected compounds against wide type STEP and F523A mutant.

Protein	Inhibitory activity (μM)			
	ST2	ST2-5	ST3	ST3-5
WT	9.7±2.6	7.7±1.5	10.7±0.9	7.5±1.2
F523A	31.3±8.5	>100	99.7±10.9	81.9±9.2

Video Captions

Video S1. MD simulation of STEP-apo system. STEP protein is shown as white surface with catalytic site and exosite site colored in green and orange yellow, respectively.
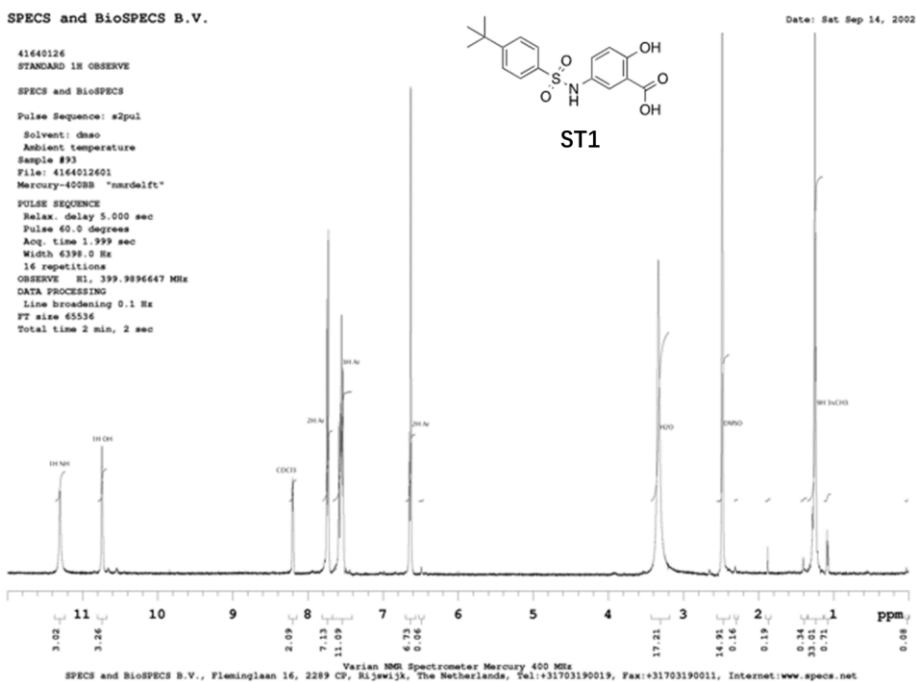
Video S2. MD simulation of STEP-**ST3** system. STEP protein is shown as white surface with catalytic site and exosite site colored in green and orange yellow, respectively. Inhibitor **ST3** are shown as spheres and colored according to atom type.

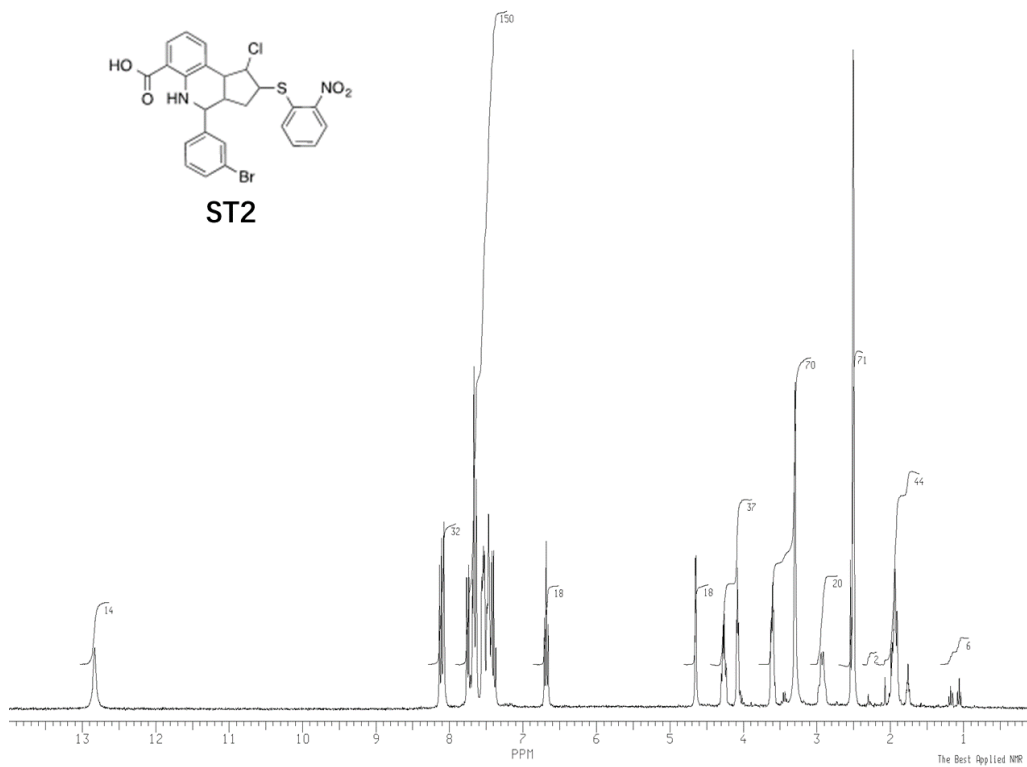## 102 ¹H-NMR for representative compounds

103 Compound ST1

104

105

106 Compound ST2

107

108

11

109

110 **Compound ST3**

ST3

AG-205/33667015
9000033667015

INTEGRAL
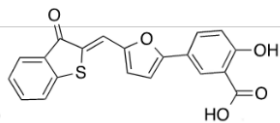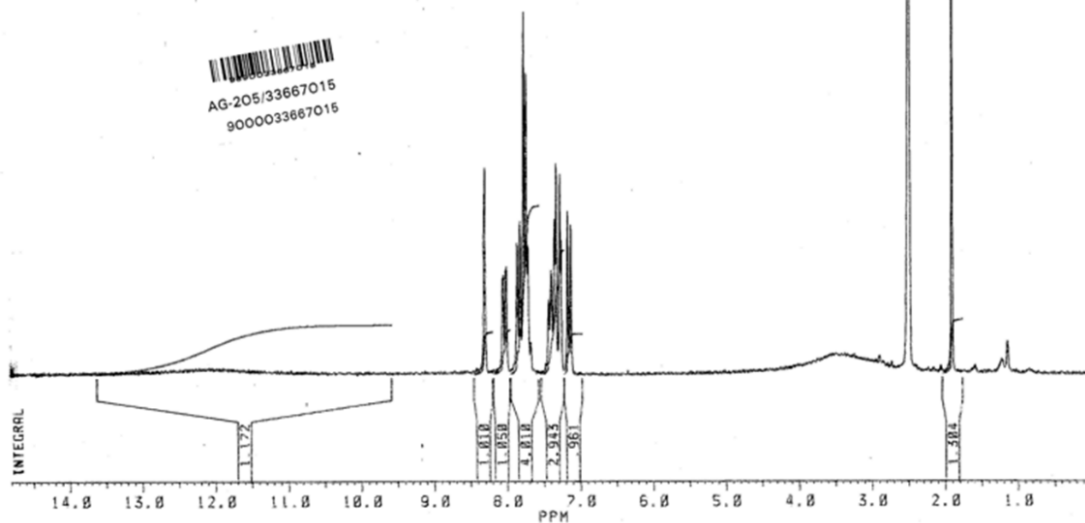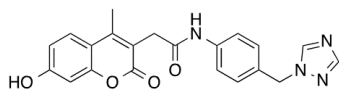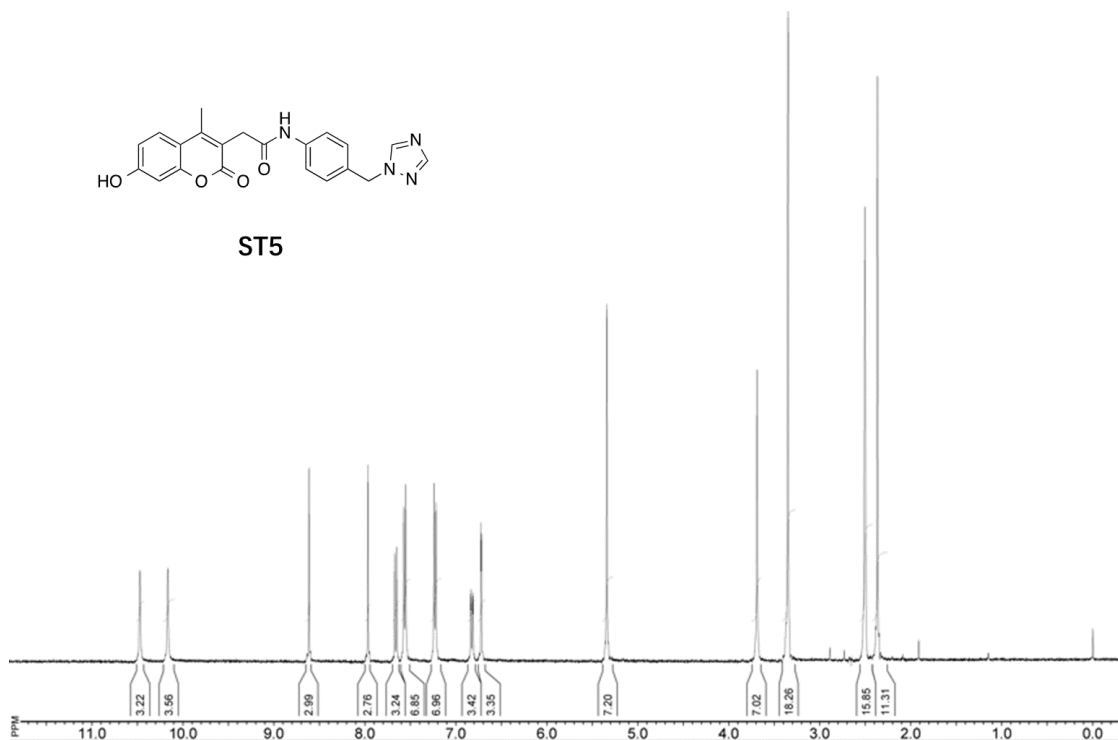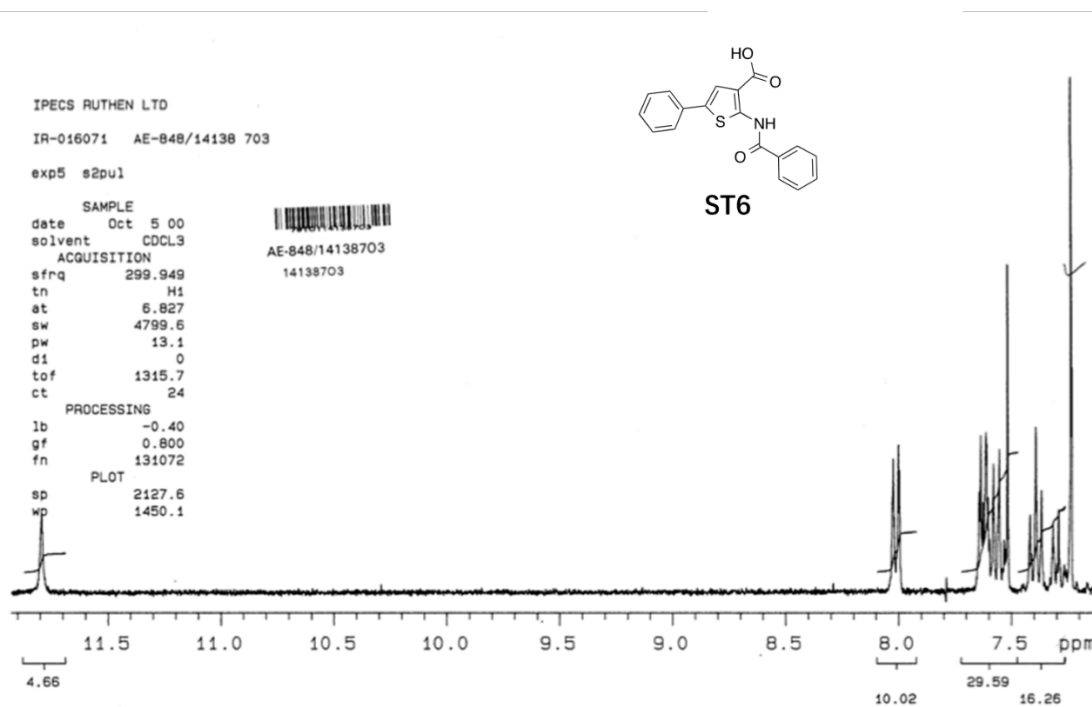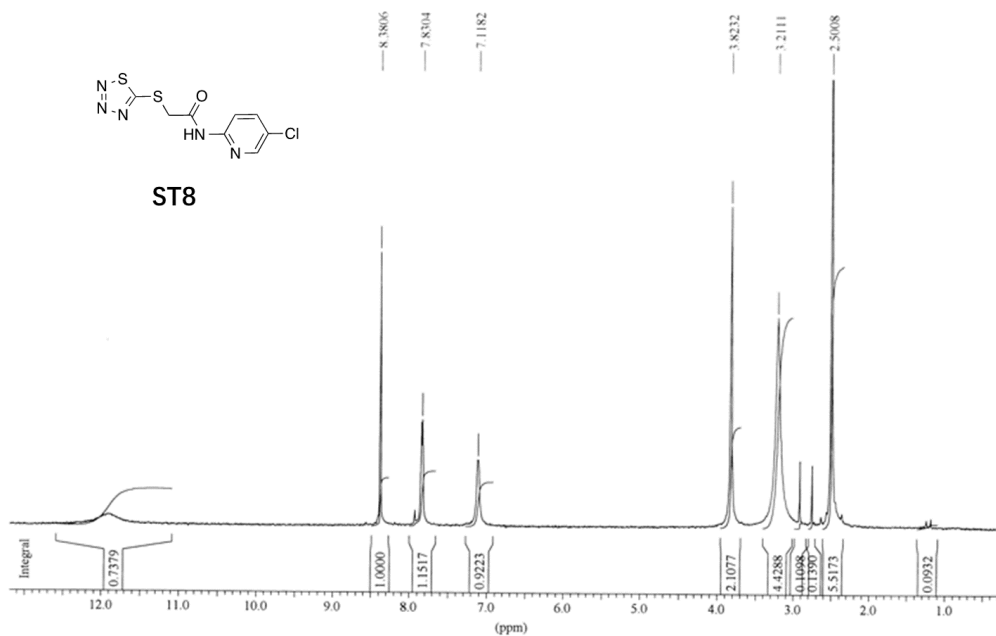
111
112

113 **Compound ST5**

ST5

114
115

116

117 **Compound ST6**

118

119 **Compound ST8**

120
121
122
123
124

125   **Compound ST9**

ST9

AK-968/15363412   Bruker AC-300 SF=300.13 MHz   SI=16K, SW=5376, PW=3   AQ=1.33, RD=3.0, NS=16   SR=4787.697, TE=318K

Moscow, 12 September 2000   OPR:Moiseev S.V.I.;   SOLV:DMSO-d6+CCl4;   PREP:UNE-258;

126
127

128   **Compound ST10**

3940120493
STANDARD 1H OBSERVE

Pulse Sequence: s2pul

Solvent: CDCl3
Temp. 30.0 C / 303.1 K
Sample #69
File: 394012049301
Mercury-400BB "nmrdelft"

PULSE SEQUENCE
Relax. delay 5.000 sec
Pulse 60.2 degrees
Acq. time 1.999 sec
Width 7987.2 Hz
16 repetitions
OBSERVE   H1, 399.9877910 MHz
DECOUPLE C13, 100.5847929 MHz
Power 26 dB
off during acquisition
DATA PROCESSING
Line broadening 0.1 Hz
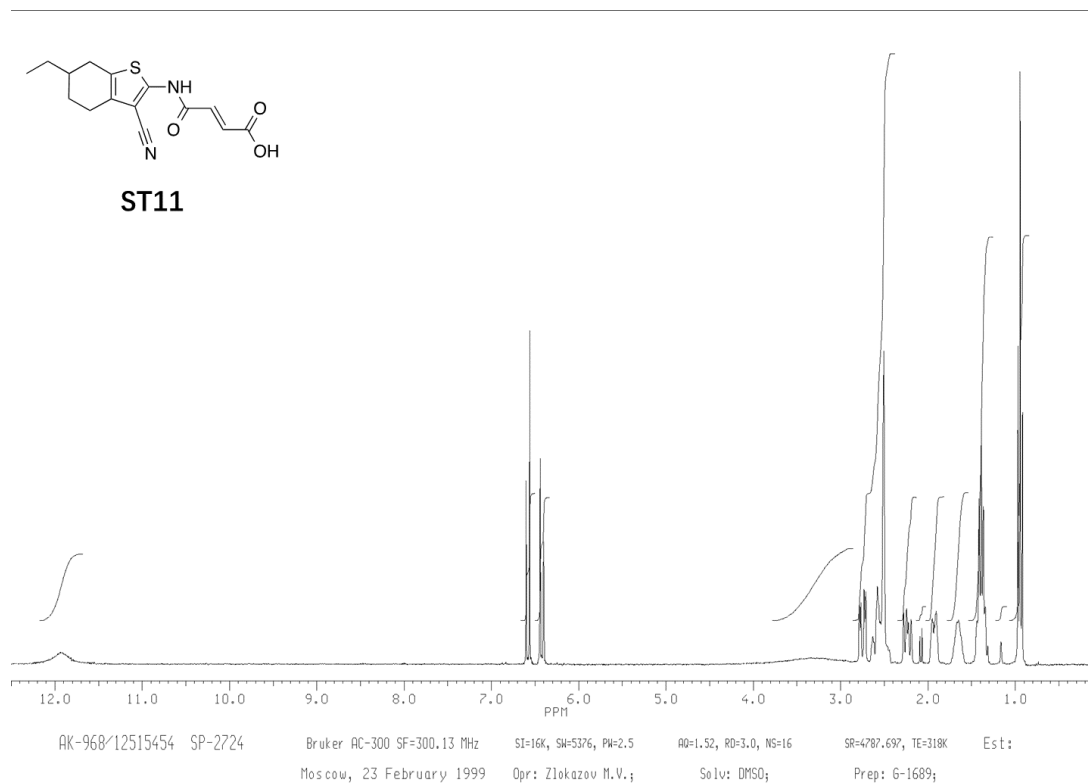Gauss apodization 0.200 sec
FT size 65536
Total time 2 min, 12 sec

ST10

129
130
131

132

133   **Compound ST11**

ST11

134