Supporting Information

Design, Synthesis, Protein-ligand X-ray Structures and Biological Evaluation of a Series of Novel Macrocyclic HIV-1 Protease Inhibitors to Combat Drug-resistance

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Table 1. HPLC purity of inhibitors **14a-h** and **15a-g**

Inhibitor	Method A		Method B	
	R_t (min)	Purity (area %)	R_t (min)	Purity (area %)
14a-*E*	10.9	98	11.8	98
14a-*Z*	10.8	99	13.6	99
14b-*E*	11.2	98	13.1	97
14b-*Z*	11.3	96	13.3	96
14c-*E*	10.7	98	12.9	99
14c-*Z*	10.4	97	12.7	97
14d	9.3	98	11.6	97
14e	9.8	99	12.0	98
14f	9.0	99	11.1	97
14g	8.3	98	10.4	96
14h	6.6	99	9.7	97
15a	12.7	98	14.3	98
15b	12.0	99	13.6	99
15c	13.2	97	10.5	97
15d	10.1	98	12.2	99
15e	9.3	99	11.4	99
15f	8.4	99	10.7	97
15g	6.7	99	10.0	98

HPLC conditions:

- Method A = Zorbax XDB-C_{18} (150 x 4.6 mm, 5 micron); Flow = 1.5 mL/min; Gradient T = 0-1 min (25:75 MeCN:H_2O); T = 15-17 min (10:90% MeCN:H_2O), T = 17.1-20 min (25:75 MeCN:H_2O); T = 25 °C; λ = 215 nm.
- Method B = Zorbax XDB-C_{18} (150 x 4.6 mm, 5 micron); Flow = 1.5 mL/min; Gradient T = 0-1 min (30:70 MeOH:H_2O); T = 15-17 min (95:5 MeOH:H_2O), T = 17.1-20 min (30:70 MeOH:H_2O); T = 25 °C; λ = 215 nm.

Table 2. HPLC purity of inhibitors **19a-c** through **22a-c**.

Inhibitor	R_t (min)	Purity (area %)
19a	14.2	99.5
20a	13.4	100.0
21a	13.5	99.6
22a	13.9	97.2
19b/19c	14.1	99.6
20b	12.9	100.0
21b	13.3	99.6
22b	13.4	97.2
19c	14.1	99.6
20c	12.9	100.0
21c	13.1	99.7
22c	13.5	97.1

HPLC conditions: Zorbax XDB-C_{18} (150 x 4.6 mm, 5 micron); Flow = 1.5 mL/min; Gradient T = 0-1 min (30:70 MeOH:H_2O); T = 15-18 min (100% MeOH), T = 18.1-21 min (30:70 MeOH:H_2O); T = 35 °C; λ = 215 nm.

Table 3. High resolution mass spectrometry data for all inhibitors

Inhibitor	Molecular Formula	Ion	Calculated	Found	Inhibitor	Molecular Formula	Ion	Calculated	Found
14a-*E*	$C_{34}H_{46}N_2O_9S$	$[M+Na]^+$	681.2822	681.2815	**15e**	$C_{30}H_{40}N_2O_9S$	$[M+Na]^+$	605.2533	605.2526
14a-*Z*	$C_{34}H_{46}N_2O_9S$	$[M+Na]^+$	681.2822	681.2819	**15f**	$C_{29}H_{38}N_2O_9S$	$[M+H]^+$	591.2376	591.2381
14b-*E*	$C_{33}H_{44}N_2O_9S$	$[M+Na]^+$	667.2665	667.2660	**15g**	$C_{28}H_{36}N_2O_9S$	$[M+H]^+$	577.2220	577.2222
14b-*Z*	$C_{33}H_{44}N_2NaO_9S$	$[M+Na]^+$	667.2665	667.2667	**19a**	$C_{37}H_{52}N_2O_9S$	$[M+H]^+$	701.3472	701.3473
14c-*E*	$C_{32}H_{42}N_2O_9S$	$[M+H]^+$	631.2689	631.2698	**20a**	$C_{35}H_{48}N_2O_9S$	$[M+Na]^+$	695.2978	695.2970
14c-*Z*	$C_{32}H_{42}N_2O_9S$	$[M+H]^+$	631.2689	631.2706	**21a**	$C_{35}H_{48}N_2O_9S$	$[M+Na]^+$	695.2978	695.2989
14d	$C_{31}H_{40}N_2O_9S$	$[M+H]^+$	617.2533	617.2534	**22a**	$C_{35}H_{50}N_2O_9S$	$[M+H]^+$	675.3315	675.3318
14e	$C_{31}H_{40}N_2O_9S$	$[M+Na]^+$	639.2352	639.2345	**19b/19c**	$C_{36}H_{50}N_2O_9S$	$[M+Na]^+$	709.3135	709.3131
14f	$C_{30}H_{38}N_2O_9S$	$[M+H]^+$	603.2376	603.2369	**20b**	$C_{34}H_{46}N_2O_9S$	$[M+Na]^+$	681.2822	681.2829
14g	$C_{29}H_{36}N_2O_9S$	$[M+Na]^+$	611.2039	611.2040	**21b**	$C_{34}H_{46}N_2O_9S$	$[M+Na]^+$	681.2822	681.2825
14h	$C_{28}H_{34}N_2O_9S$	$[M+Na]^+$	597.1883	597.1887	**22b**	$C_{34}H_{48}N_2O_9S$	$[M+Na]^+$	683.2978	683.2987
15a	$C_{34}H_{48}N_2O_9S$	$[M+Na]^+$	683.2978	683.2984	**19c**	$C_{36}H_{50}N_2O_9S$	$[M+Na]^+$	709.3135	709.3139
15b	$C_{33}H_{46}N_2O_9S$	$[M+Na]^+$	669.2822	669.2828	**20c**	$C_{34}H_{46}N_2O_9S$	$[M+Na]^+$	681.2822	681.2819
15c	$C_{32}H_{44}N_2O_9S$	$[M+Na]^+$	655.2668	655.2667	**21c**	$C_{34}H_{46}N_2O_9S$	$[M+Na]^+$	681.2822	681.2812
15d	$C_{31}H_{42}N_2O_9S$	$[M+Na]^+$	641.2509	641.2512	**22c**	$C_{34}H_{48}N_2O_9S$	$[M+Na]^+$	683.2978	683.2976

Table 4. Data collection and refinement statistics for inhibitor **14c**

	14c
Space group	$P2_12_12$
Unit cell dimensions: (Å)	
a	58.76
b	86.33
c	45.98
Resolution range (Å)	50-1.17
Unique reflections	72,239
R_{merge} (%) overall (final shell)	10.8 (45.7)
I/σ(I) overall (final shell)	14.7 (2.4)
Completeness (%) overall (final shell)	90.9 (52.6)
Data range for refinement (Å)	10-1.17
R (%)	16.0
R_{free} (%)	19.4
No. of solvent atoms (total occupancies)	181 (166.8)
RMS deviation from ideality	
Bonds (Å)	0.014
Angle distance (Å)	0.034
Average B-factors ($Å^2$)	
Main-chain atoms	14.5
Side-chain atoms	18.8
Inhibitor	12.1
Solvent	26.7
Residual density (max/min) ($eÅ^{-3}$)	0.31/-0.26
Relative occupancy of inhibitor 1	0.5/0.5
RMS deviation (Å) with PR-DRV (2IEN)	0.33

Table 5. Crystallographic Data Collection and Refinement Statistics for inhibitor **2**

Space group	$P2_12_12_1$
Unit cell dimensions: (Å)	
a	51.9
b	59.0
c	62.6
Resolution range (Å)	20-1.7
Unique reflections	20,809
R_{merge} (%) overall (final shell 1.76-1.70 Å)	5.2 (39.6)
I/σ(I) overall (final shell 1.76-1.70 Å)	17.7 (2.8)
Completeness (%) overall (final shell)	95.5 (92.4)
Data range for refinement (Å)	20-1.70
R_{work} (%)	21.6
R_{free} (%)	28.9
No. of solvent atoms	100
RMS deviation from ideality	
Bonds (Å)	0.009
Angle (degree)	1.5
Average B-factors ($Å^2$)	
Protein	28.3
Inhibitor	19.6
Solvent	41.0

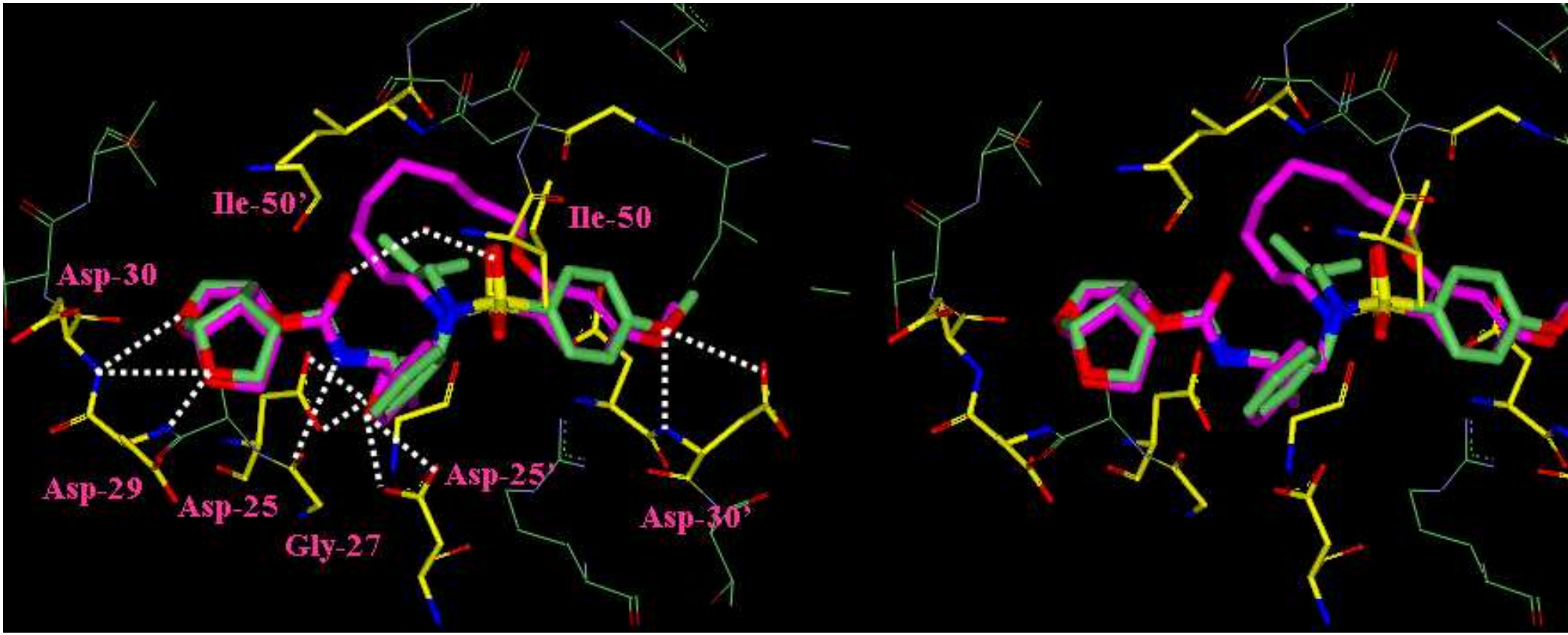

Figure 2. An overlay of energy-minimized macrocyclic inhibitor **15c** (magenta) with the X-ray structure of inhibitor **2** (green)-bound HIV-1 protease.

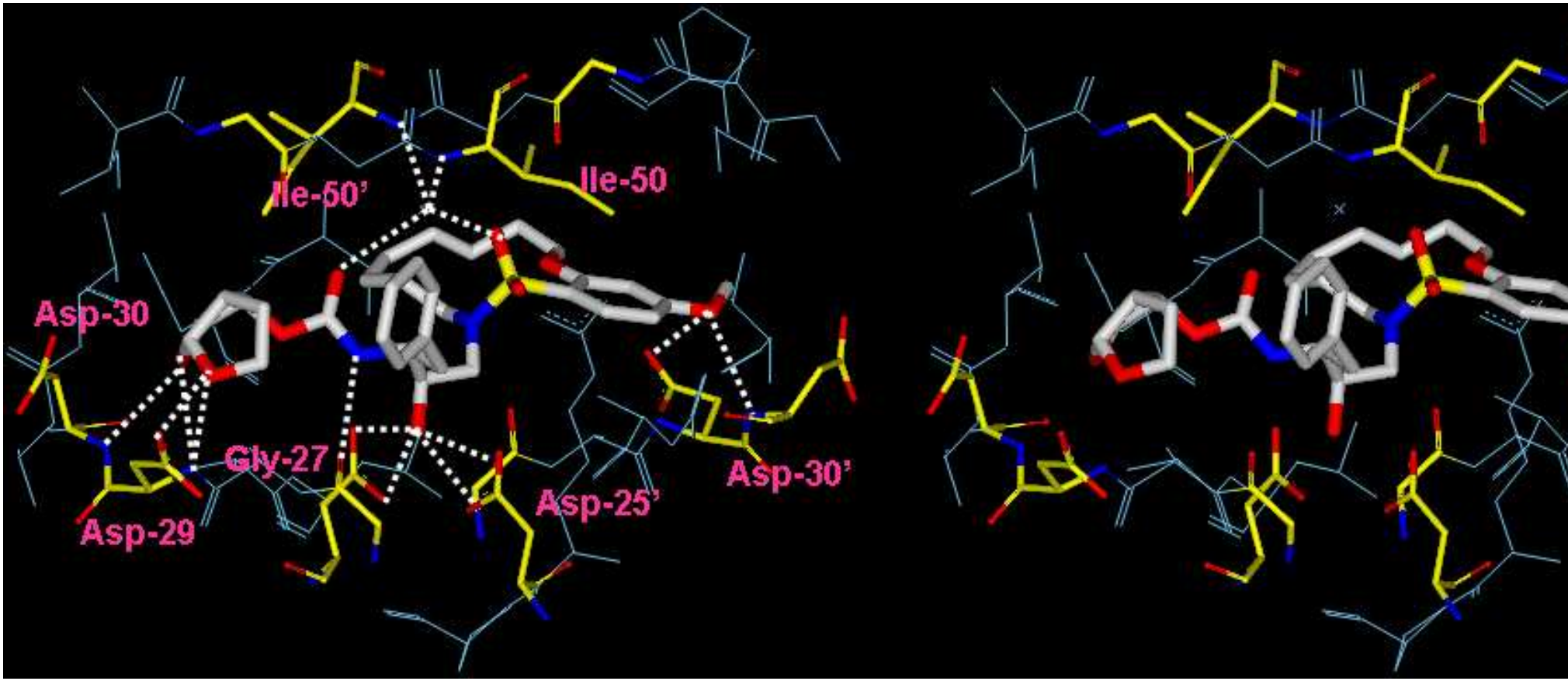

Figure 3. A stereoview of the X-ray structure of macrocyclic inhibitor **14c** (light gray)-bound HIV-1 protease. All strong hydrogen bonding interactions are shown as dotted lines.