RSC Medicinal Chemistry

Supplementary Data

Identification of a new class of proteasome inhibitors based on a naphthyl-azotricyclic-urea-phenyl scaffold

Duncan Allardyce, Priscilla Adu Mantey, Monika Szalecka, Robert Nkwo, Eriketi Z. Loizidou*

Middlesex University, Faculty of Science and Technology, Department of Natural Sciences, The Burroughs, London, NW4 4BT, United Kingdom

*Author to whom correspondence should be addressed:

Dr Eriketi Z. Loizidou, Middlesex University, Department of Natural Sciences, The Burroughs, NW4 4BT, London, UK; e.loizidou@mdx.ac.uk

Table S1. Molecular docking results from top 20 scoring ligands from initial screening with Autodock Vina and further analysis with Autodock 4.2 against the β5c active site of the proteasome. Argyrin B, a reversible inhibitor of the proteasome was used as control.

ZINC ID	**Autodock Vina** Binding energy (Kcal/mol)	**Autodock 4.2** Binding energy (Kcal/mol)			
		1st run	2nd run	3rd run	Mean
ZINC4270569	-11.3	-8.23	-8.22	-7.99	-8.15
ZINC5411973	-10.8	-8.44	-7.81	-7.36	-7.87
ZINC4098367	-10.7	-6.95	-7.41	-7.66	-7.34
ZINC4259483	-10.6	-9.39	-9.45	-9.09	-9.31
ZINC4201115	-10.4	-9.91	-9.95	-11.02	-10.29
ZINC4258888	-10.4	-11.30	-11.27	-11.30	-11.29
ZINC4259440	-10.3	-8.20	-8.18	-8.43	-8.27
ZINC4259794	-10.3	-10.20	-10.13	-9.5	-9.94
ZINC5433649	-10.3	-10.07	-10.09	-10.31	-10.16
ZINC4235436	-10.2	-8.92	-8.4	-9.11	-8.81
ZINC5413755	-10.2	-9.72	-9.32	-10.15	-9.73
ZINC4235418	-10.1	-6.98	-9.01	-9.24	-8.41
ZINC4259443	-10.1	-10.02	-10.11	-10.30	-10.14
ZINC4259861	-10.1	-9.89	-10.73	-9.81	-10.14
ZINC5396040	-10.1	-7.61	-6.8	-6.38	-6.93
ZINC5399419	-10.1	-9.16	-8.91	-9.29	-9.12
ZINC5398884	-10	-6.63	-7.09	-6.30	-6.67
ZINC5399237	-10	-8.33	-8.92	-10.89	-9.38
ZINC5413761	-10	-10.36	-10.43	-10.62	-10.47
ZINC5434032	-10	-8.57	-7.57	-7.55	-7.90
ARGYRIN B	-9.4	-8.09	-8.10	-8.09	-8.10

Table S2. Summary of estimated binding energies and inhibition constants at the active sites of the constitutive proteasome (β1c, β2c and β5c) and immunoproteasome (β1i, β2i and β5i) as determined from molecular modeling.

Compound	**Estimated average binding energy (Kcal/mol)**					
	β1c	**β1i**	**β2c**	**β2i**	**β5c**	**β5i**
C1	-12.08	-12.11	-13.89	-11.08	-11.43	-11.34
C2	-11.24	-10.53	-11.70	-11.02	-11.13	-10.98
C3	-11.13	-10.87	-11.95	-11.17	-11.18	-10.62
C4	-11.16	-11.17	-10.94	-11.05	-10.81	-10.94
C5	-9.68	-9.43	-10.10	-9.55	-10.09	-9.42
C6	-11.65	-10.30	-11.20	-10.10	-8.82	-10.26
C7	-9.60	-9.99	-9.64	-9.09	-10.11	-10.05
C8	-9.94	-9.73	-10.86	-9.92	-8.60	-9.27
C9	-10.16	-11.0	-10.91	-11.08	-9.38	-9.92
	Estimated average inhibition constant (nM)					
	β1c	**β1i**	**β2c**	**β2i**	**β5c**	**β5i**
C1	1.40	1.33	0.07	7.60	4.21	4.89
C2	5.79	11.43	2.66	8.35	6.93	8.90
C3	6.92	10.81	1.77	6.45	6.34	16.55
C4	6.65	6.48	9.65	7.90	11.86	9.64
C5	80.37	122.2	68.52	99.22	40.18	187.07
C6	2.90	28.32	6.21	39.22	343.73	333.63
C7	92.01	47.76	106.97	216.91	38.52	125.83
C8	51.33	73.68	10.95	56.13	494.94	2830
C9	35.78	8.65	10.05	7.66	220.37	169.09

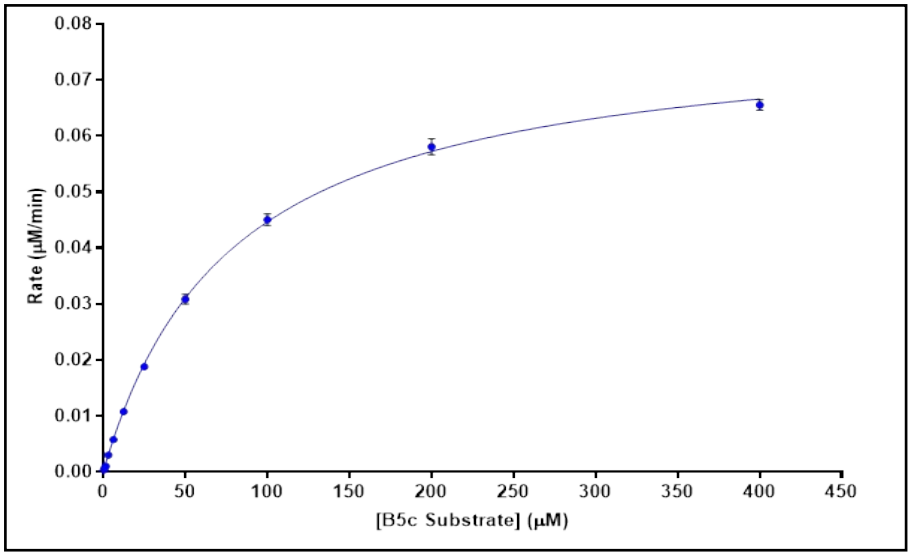

Figure S1. Michaelis Menten kinetics SNLR plot of Suc-LLVY-AMC liberation (SEM error bars) at β5c. Enzyme concentration was constant at 1 nM and a range of substrate concentrations were used. Km was determined using Michaelis-Menten least squares fit according to model Y = Vmax*X/(Km + X), which showed Km = 78.4 µM, 95% CI 72.3-85.0 µM, (n=3).

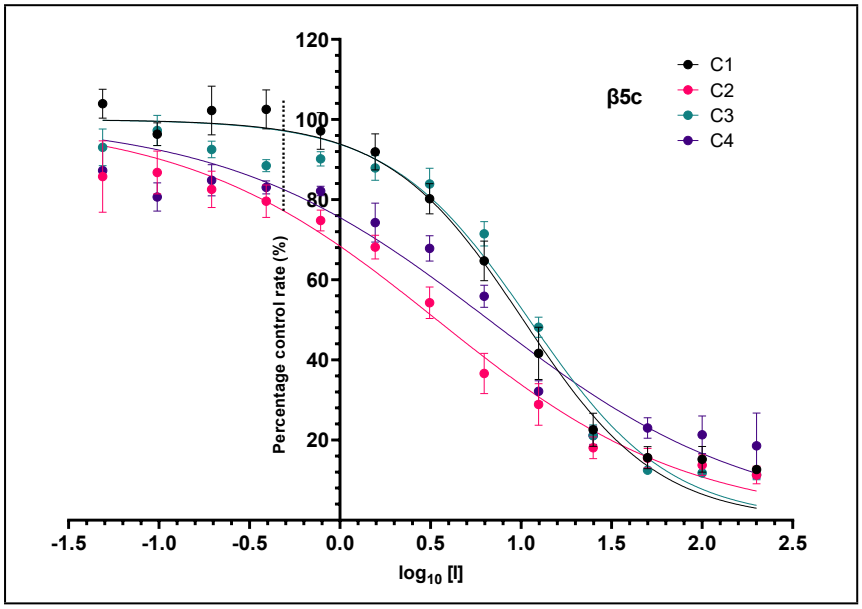

Figure S2. IC_{50} plots at β5c site. Logarithmic concentration of C1-4 against percentage control, initial rate velocity. Tested at 1nM proteasome enzyme concentration, [S] = K_m (K_m values: β5c = 78.4 µM) and compound concentration within 0.01-200 µM (using 1:1 serial dilution).

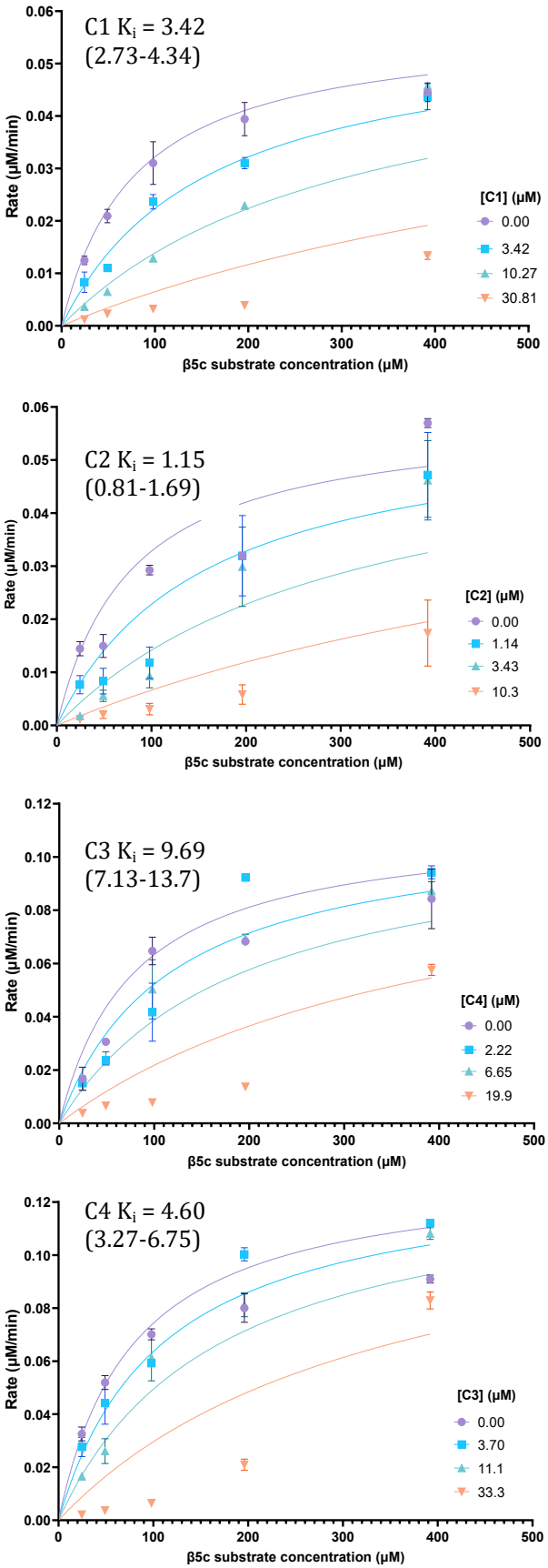

Figure S3. Kinetic enzyme inhibition studies o C1-4 at β5c site. Ki estimation from competitive fit SNLR analysis at β5c: C1 = 3.42μM, C2 = 1.15μM, C3 = 9.69μM, C4 =

4.60μM reported alongside 95% CI. Inhibitor concentrations [I] at ranges relative to IC50: x3, x1, x0.033.

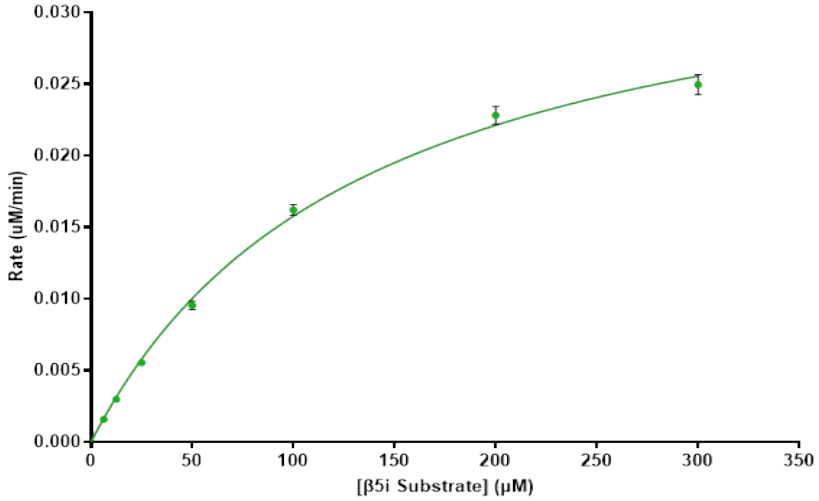

Figure S4. Michaelis Menten kinetics SNLR plot of Suc-LLVY-AMC liberation (SEM error bars) at β5i. Enzyme concentration was constant at 3 nM and a range of substrate concentrations were used. Km was determined using Michaelis-Menten least squares fit according to model Y = Vmax*X/(Km + X), which showed Km = 135 μM, 95% CI 117.5-155.8 μM, n=3.

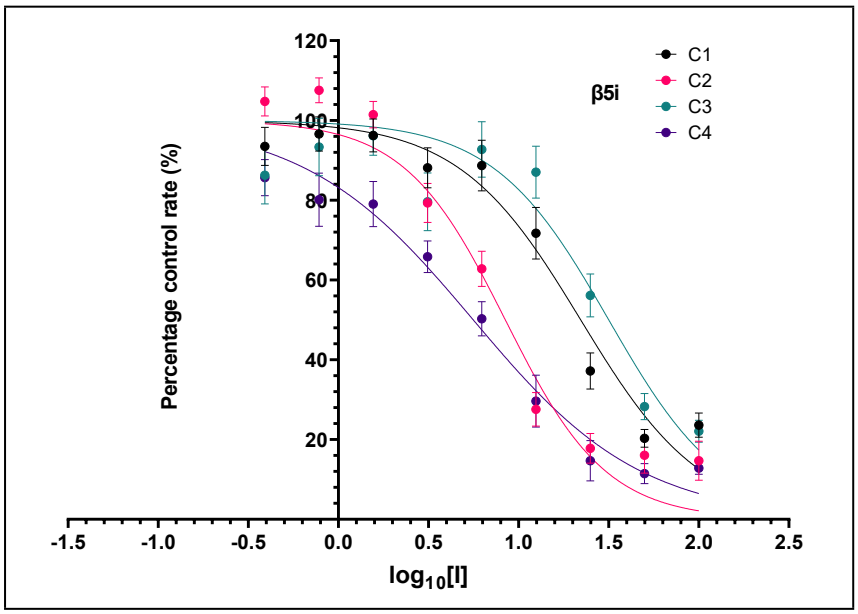

Figure S5. IC_{50} plots at β5i site. Logarithmic concentration of C1-4 against percentage control, initial rate velocity. Tested at 3nM immunoproteasome enzyme concentration, [S] = K_m (K_m values: β5i = 135.0 μM) and compound concentration within 0.4-200 μM (using 1:1 serial dilution).

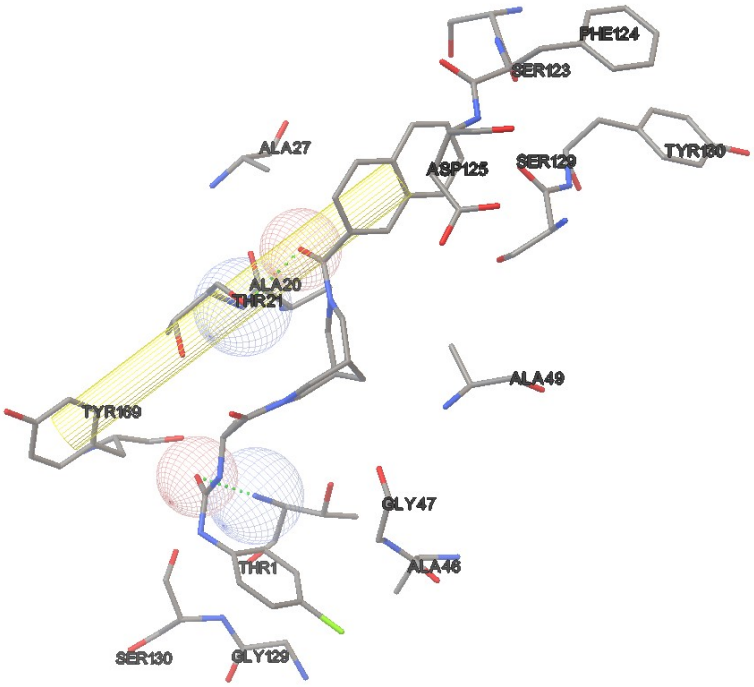

Figure S6. Autodock image of best docked conformation of C2 within β5c showing all interactions. Hydrogen bonding interactions are shown as spheres as well as dotted green lines and pi-pi stacking interactions as yellow cylinders.

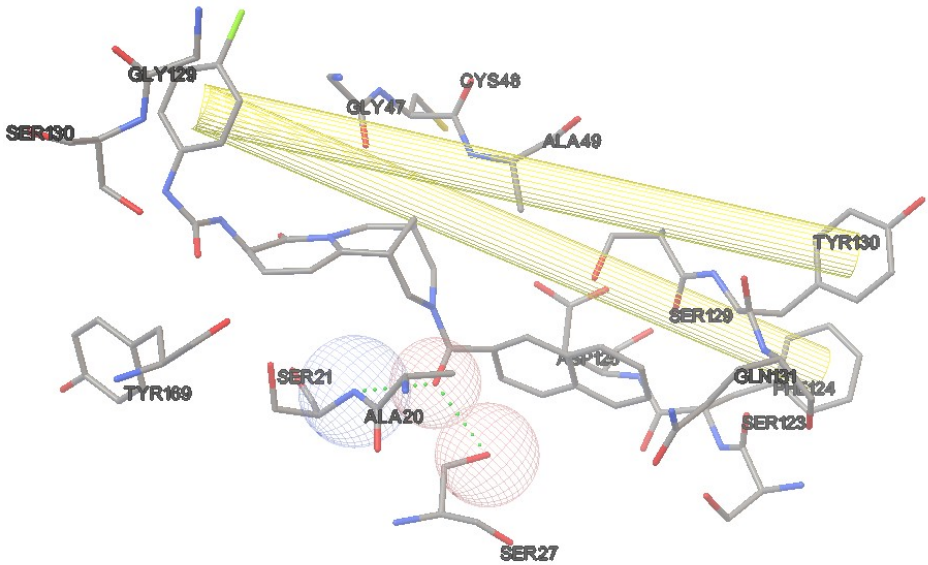

Figure S7. Autodock image of best docked conformation of C2 within β5i showing all interactions. Hydrogen bonding interactions are shown as spheres as well as dotted green lines and pi-pi stacking interactions as yellow cylinders.

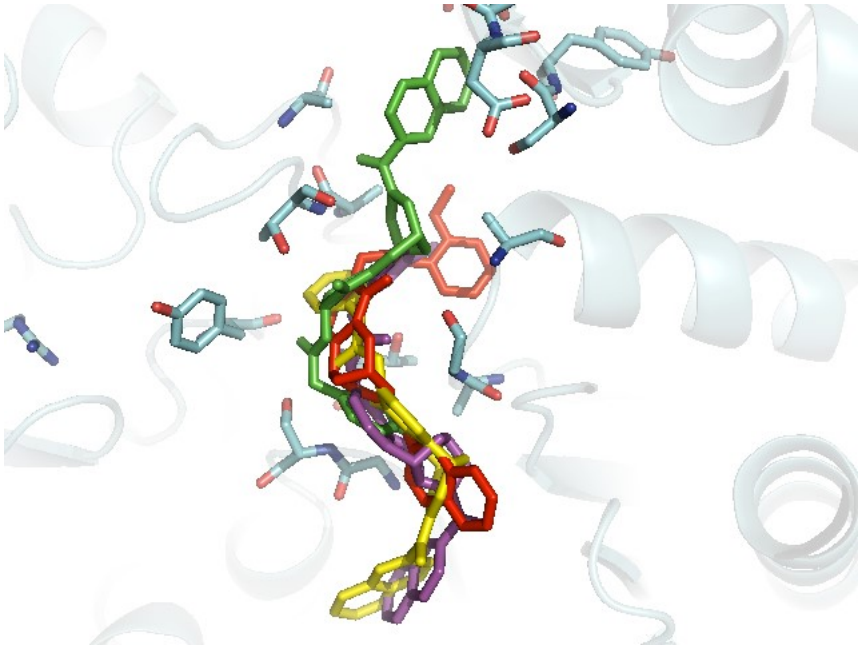

Figure S8. Overlay of C1 (magenta), C2 (green), C3 (yellow), C4 (red) best docked conformations within the β5c active site.

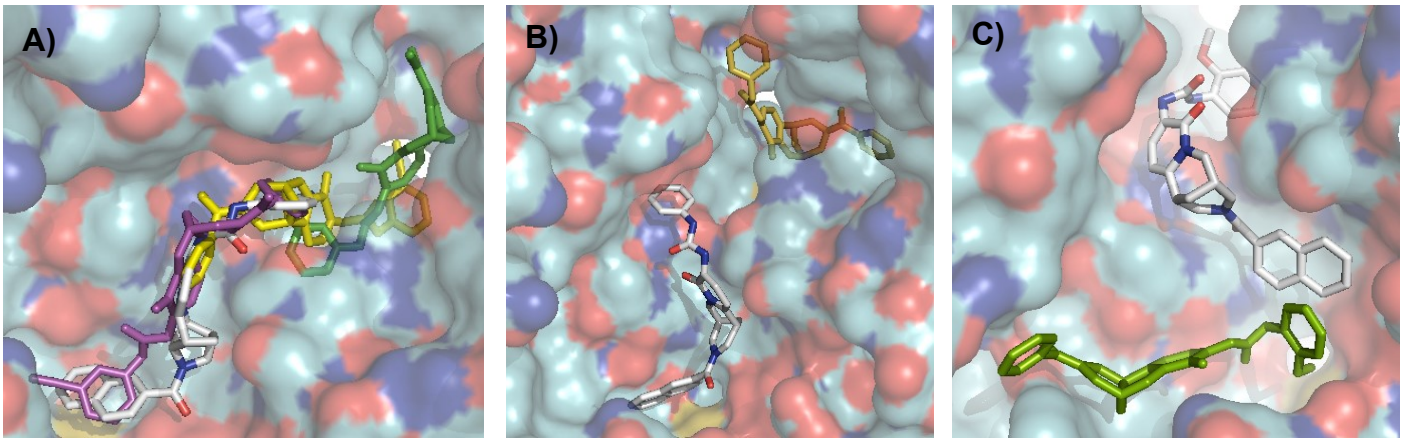

Figure S9. Overlay of A) C1 (grey), 5 (green), 6 (yellow), 9 (magenta), B) C3 (grey) and C7 (yellow) and C) C4 (grey) and C8 (lime green) within β5c.

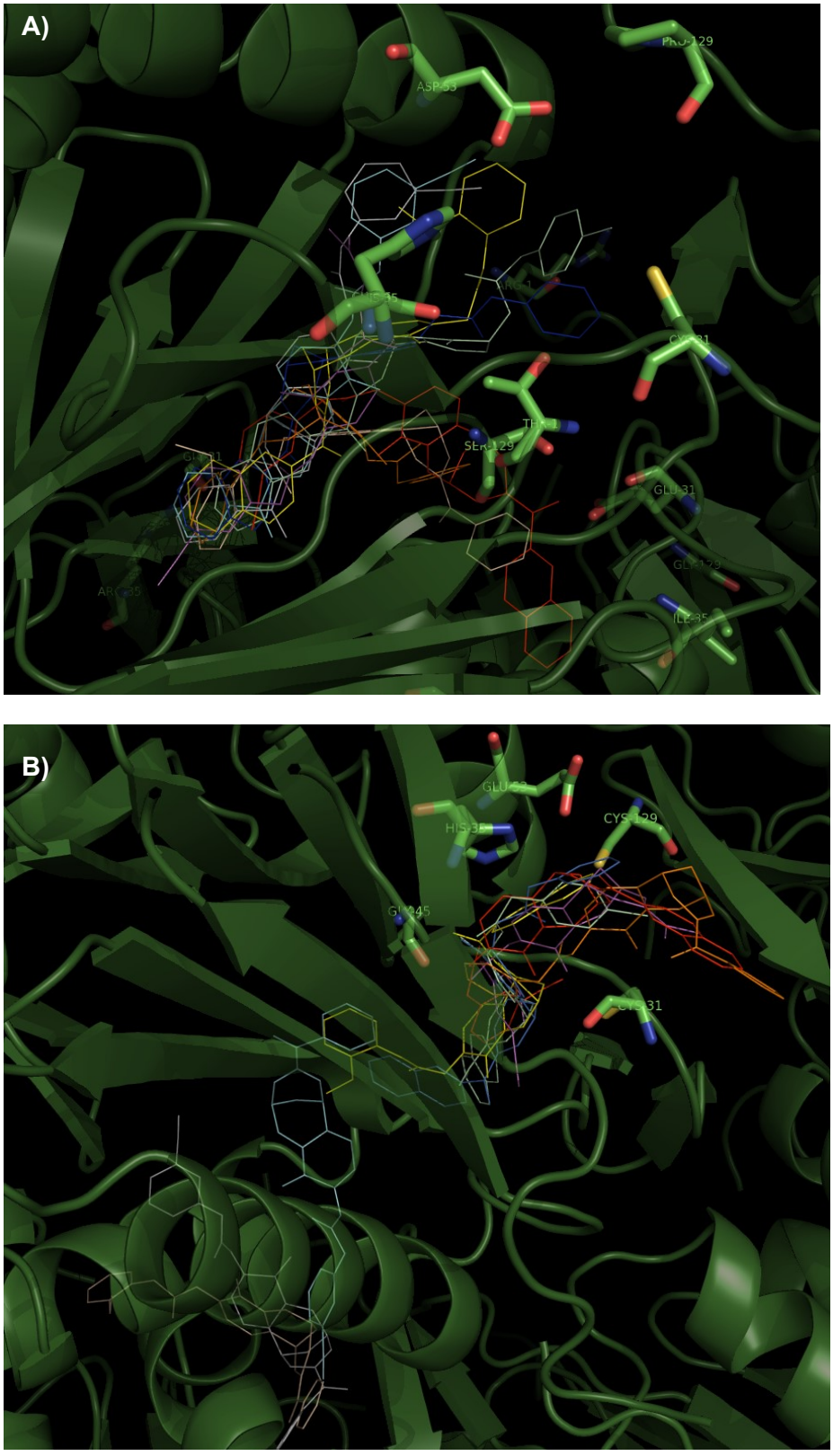

Figure S10. Overlay of best docked conformations of C1-9 at the A) β2c and B) β2i active sites. S1 pocket amino acids G45, C31, H35, D53 (β2), E53 (β2i), C129 are shown as green stick representations. C1-9 are shown as line representations C1 (red), C2 (green), C3 (blue), C4 (yellow), C5 (magenta), C6 (cyan), C7 (orange), C8 (wheat), C9 (grey).

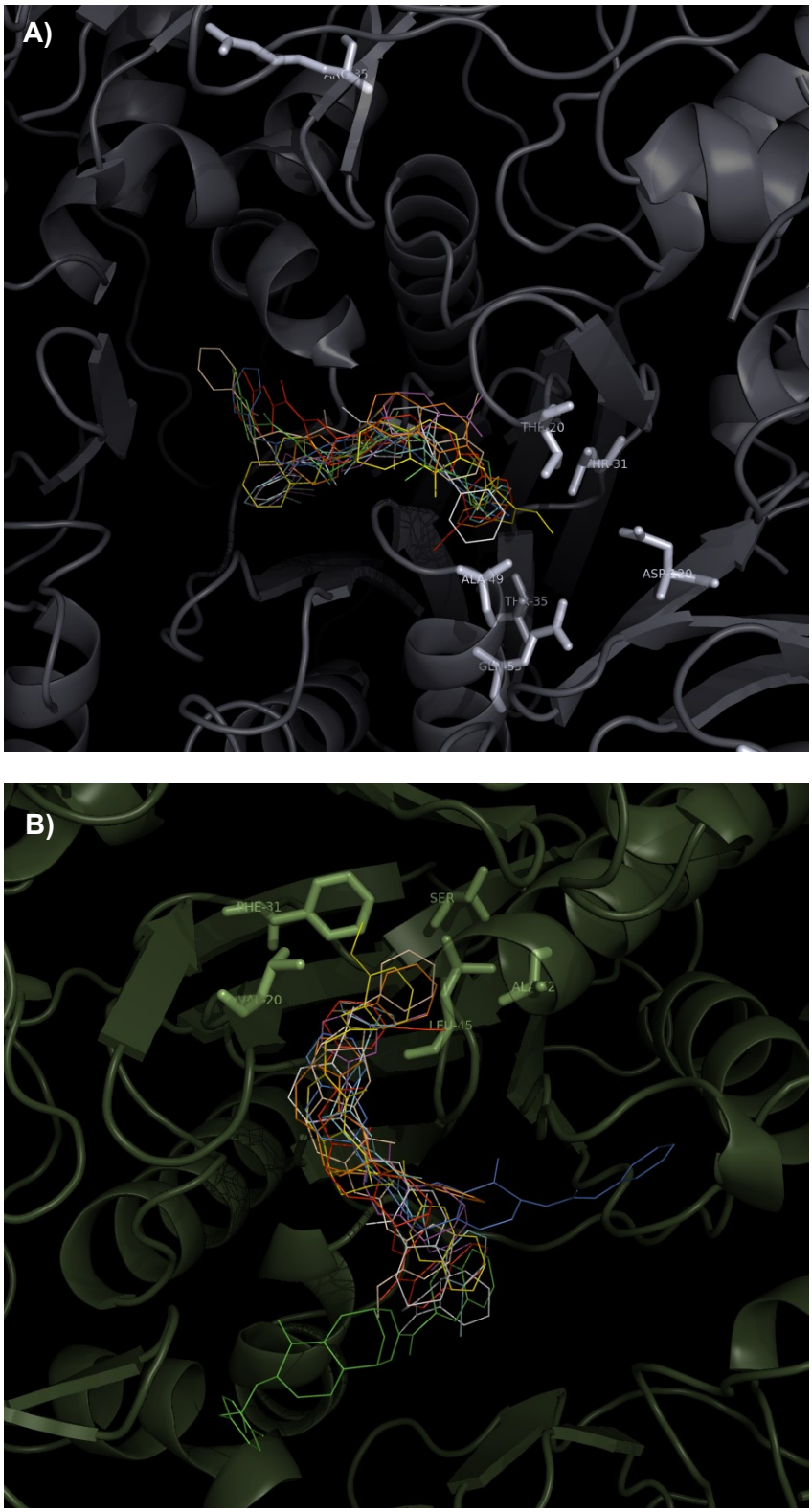

Figure S11. Overlay of best docked conformations of C1-9 at the A) β1c and B) β1i active sites. S1 pocket amino acids T20, T31, T35, R45, A49, Q53, D120 (β1), V20, F31, L45, A52 (β1i) are shown as stick representations. C1-9 are shown as line representations C1 (red), C2 (green), C3 (blue), C4 (yellow), C5 (magenta), C6 (cyan), C7 (orange), C8 (wheat), C9 (grey).