Supplementary Information

Synthesis and biological evaluation of potential small molecule inhibitors of Tumor Necrosis Factor

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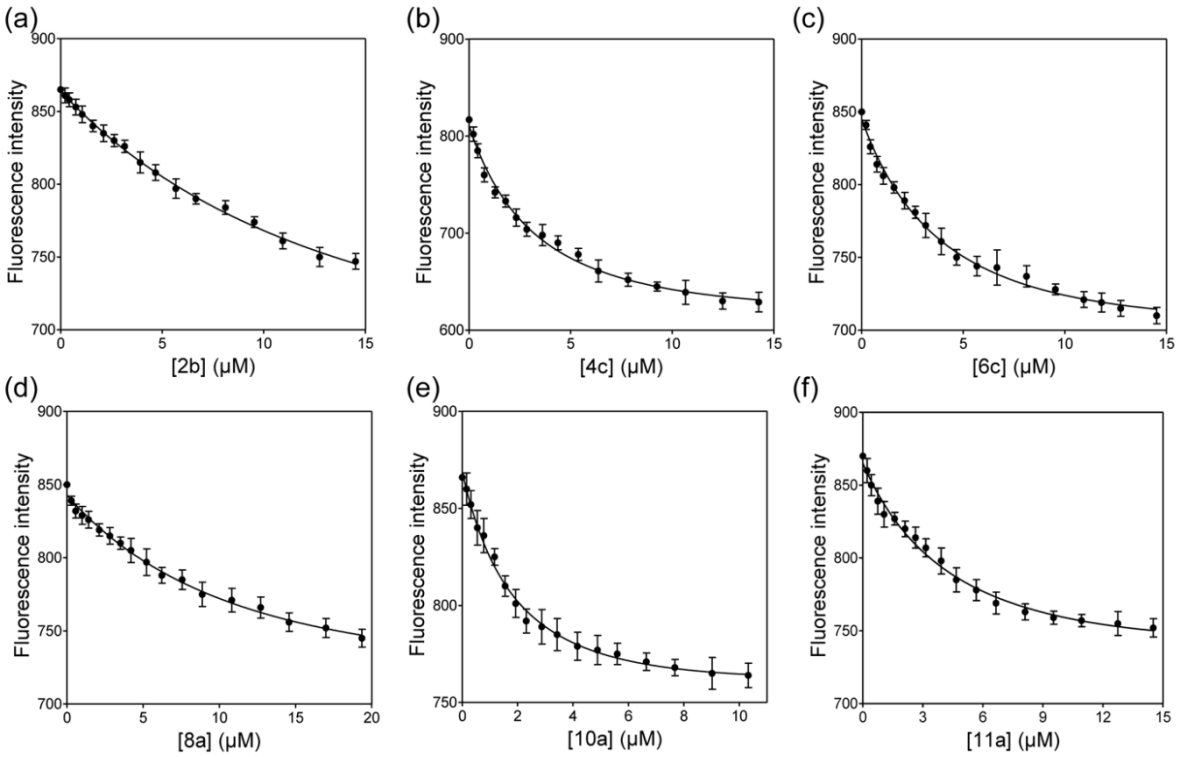

Figure S1. Examples of dissociation constant (K_d) determination of TNF with some of the novel synthesized SPD-304 analogs. Representative plots obtained by monitoring the changes of fluorescence intensity of TNF by increasing the concentration of the compounds **2b** (a); **4c** (b); **6c** (c); **8a** (d); **10a** (e) and **11a** (f) respectively. In each case data were corrected using a blank sample containing tyrosine with the same fluorescence signal. Experiments were performed in 10 mM citrate-phosphate (pH 6.5) containing 5% DMSO. Dissociation constant was calculated by fitting the fluorescence intensity values to a quadratic equation. The mean values of three independent measurements are presented

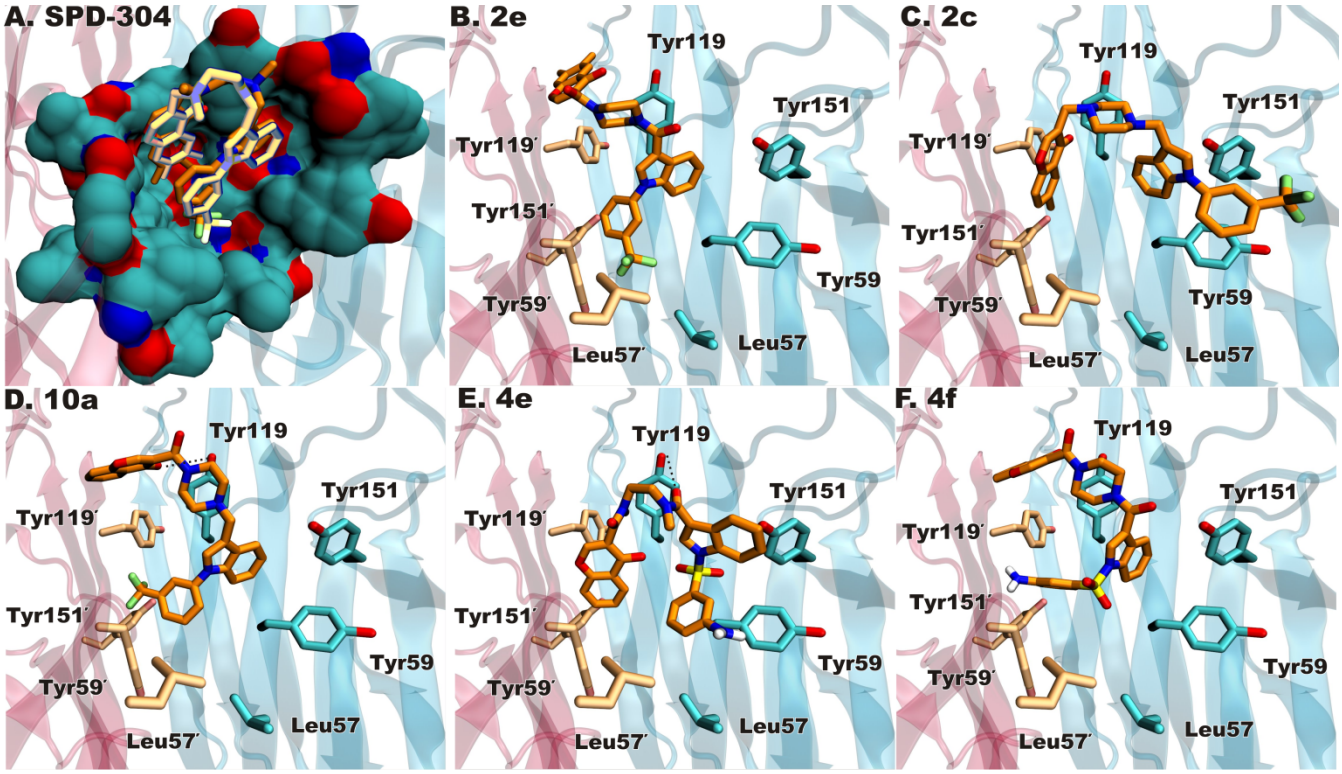

Figure S2. Predicted conformations of the most active compounds in comparison with SPD-304 shown at exactly the same orientation. (A) Surface representation of the SPD-304 binding pocket with the x-ray (yellow carbon atoms) and the best-matched (orange carbon atoms) conformations of the inhibitor. Predicted conformations of the most active compounds **2e** (B), **2c** (C), **10a** (D), **4e** (E), and **4f** (F) showing the major interacting residues of the SPD-304 binding site that are colored with bright orange or cyan carbons for each TNF monomer, respectively. All other atoms are colored with blue for nitrogen, red for oxygen and green for fluorine.

Table S1. Results of the docking calculations for the most active analogs in comparison with SPD-304. The most populated conformational cluster with the lowest estimated free energy of binding is designated as top-ranked, whereas the best-matched cluster is the one that resembles more closely the crystallographic pose of SPD-304. The estimated free energy of binding ΔG (Kcal/mol) is the mean value of each conformational ensemble that was clustered with a RMSD cutoff of 2.0 Å and its population is given as percent. The experimental dissociation constant is designated as K_d^{exp}, whereas K_d^{est} was estimated using the equation: $\Delta G = R \cdot T \cdot \ln K_d$ where R=1.9872 cal·K^{-1}·mol^{-1} and T=298.15 K.

Compound	Top-ranked cluster				Best-matched cluster				K_d^{exp} (µM)
	Rank	Pop. (%)	ΔG (Kcal/mol)	K_d^{est} (µM)	Rank	Pop. (%)	ΔG (Kcal/mol)	K_d^{est} (µM)	
SPD-304^{xray}	7	36	-7.39	3.8	23	1	-6.85	9.5	5.4
SPD-304	2	21	-7.31	4.4	12	3	-7.13	5.9	5.4
2c	1	21	-8.52	0.57	15	1	-7.58	2.8	4.8
10e	2	38	-8.42	0.67	5	2	-7.83	1.8	2.1
10a	1	45	-9.28	0.16	5	1	-8.04	1.3	1.6
11a	1	20	-8.94	0.28	14	2	-7.72	2.2	5.2
2d	3	9	-8.32	0.80	16	1	-8.15	1.1	2.5
2e	4	52	-9.70	0.08	4	3	-8.54	0.55	5.1
8c	1	54	-9.63	0.09	5	2	-8.86	0.32	0.74
4c	1	16	-9.87	0.06	22	2	-8.24	0.91	3.2
4e	1	22	-8.85	0.33	30	1	-7.84	1.8	0.96
4f	2	43	-9.33	0.14	7	4	-8.51	0.57	5.4
8c	1	54	-9.63	0.09	5	2	-8.86	0.32	0.74

SPD-304^{xray} refers to the results reported in ref[17] by using the crystallographic coordinates of the inhibitor and a larger search space with respect to this study (SPD-304).