Supporting Information

Binding Free Energies for Nicotine Analogs Inhibiting Cytochrome P450 2A6 by a Combined Use of Molecular Dynamics Simulations and QM/MM-PBSA Calculations

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Running title: CYP2A6-inhibitor Binding Free Energies

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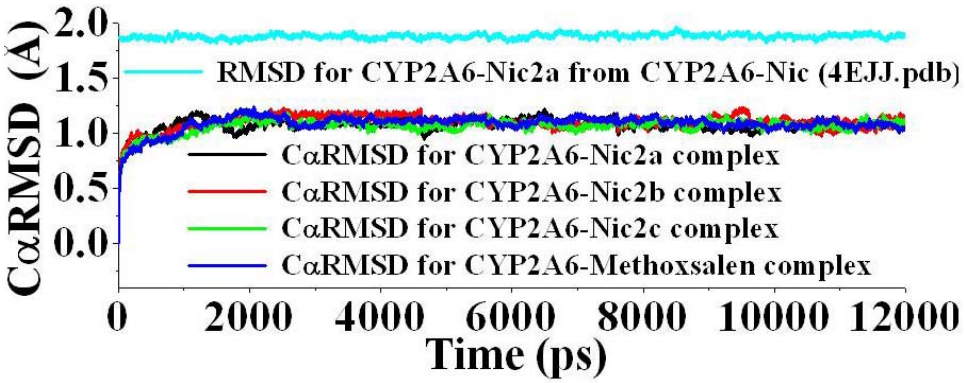

Figure S1. Tracked positional root-mean square deviation (RMSD) for Cα atoms of the binding structure of CYP2A6 binding with each of nicotine analogs along MD trajectories. The Cα RMSD was calculated based on the starting X-ray crystal structure (PDB entry code: 2FDW for CYP2A6-Nic2a complex, 2FDV for CYP2A6-Nic2b complex, 2FDU for CYP2A6-Nic2c complex). The positional RMSD for non-hydrogen atoms of CYP2A6-Nic2a complex was calculated based on the X-ray crystal structure of CYP2A6-Niconine (PDB entry code: 4EJJ).

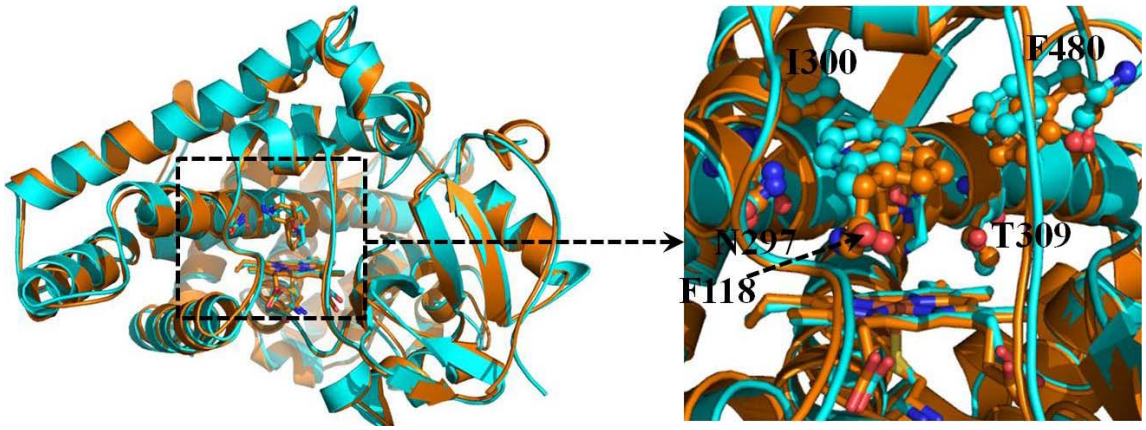

Figure S2. Superimposition of CYP2A6-Nic2a binding structure with the X-ray crystal structure of CYP2A6-Nicotine complex (PDB entry code: 4EJJ). The CYP2A6-Nic2a complex (colored in cyan) is the same as that in Figure 1 in the text, *i.e*. derived from MD trajectory and minimized using QM/MM approach. The proteins are represented as ribbons, with CYP2A6-Nicotine complex colored in gold. The heme group of CYP2A6 is shown in stick-style. Residues F118, N297, I300, T309 and F480 of CYP2A6, which are within the 5 Å of the ligand, are shown in ball-and-stick style. Both ligands (Nic2a and Nicotine) are shown in stick style and colored by atom-types.

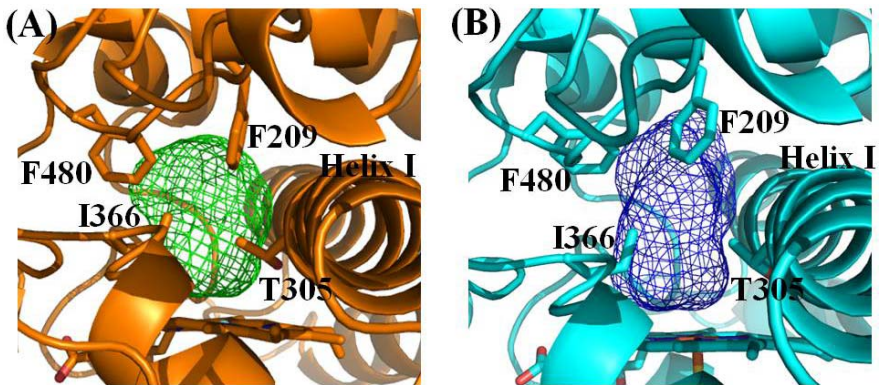

Figure S3. (A) The cavity around the Nicotine-binding site in CYP2A6-Nicotine (PDB entry code: 4EJJ). (B) The cavity around Nic2a-binding site in CYP2A6-Nic2a complex. CYP2A6 is represented as colored ribbon, and the cavity is represented as mesh surface calculated by using program HOLLOW.[1] The ligands (Nicotine and Nic2a) are not shown for the reason of clarity. The orientation of CYP2A6 is along the Helix I, ~180° turn for residue F480 when compared with the position of F480 in Figure S2. Residues F209, T305, I366 and F480 are shown in stick-style and colored by atom types. As indicated in the X-ray crystal structures of CYP2A6 and CYP2A13,[2] the conformational change of residues F209, T305, I366 and F480 is recognized as the gating of the channel for ligands accessing to the binding site.

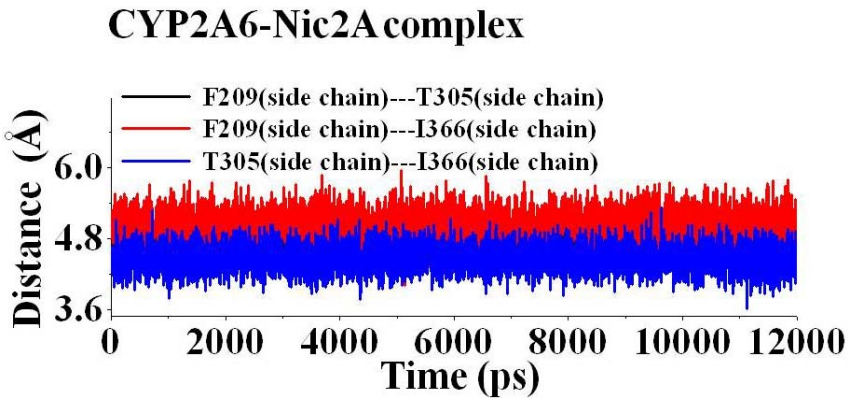

Figure S4. The tracked shortest distances for CYP2A6-Nic2A binding structure along the MD trajectories. F209(side chain0---T305(side chain) represents the shortest distance between non-hydrogen atoms at F209 side chain and the non-hydrogen atoms at T305 side chain; F209(side chain0---I366(side chain) represents the shortest distance between non-hydrogen atoms at F209 side chain and the non-hydrogen atoms at I366 side chain; and T305(side chain0---I366(side chain) represents the shortest distance between non-hydrogen atoms at T305 side chain and the non-hydrogen atoms at I366 side chain.

Table S1. Detailed energetic results (kcal/mol) obtained from the QM/MM-PBSA calculations at T = 298.15 K and P = 1 atm for the ten snapshots (No.1 to 10) of CYP2A6 complexed with Nic2a.

No	$E_{QM/MM}$(com) [a] (Hartree)	E_{QM}(lig) [b] (Hartree)	E_{MM}(rec) [c] (kcal/mol)	$\Delta E_{QM/MM}$ [d] (kcal/mol)	ΔG_{solv} (kcal/mol)	ΔE_{bind} or ΔH [e] (kcal/mol)	-TΔS (0.93) [f] (kcal/mol)	ΔG_{bind} [g] (kcal/mol)
1	-565.2844124	-571.7842825	4134.60	-55.87	22.94	-32.93	22.33	-10.60
2	-565.4536488	-571.7842825	4026.90	-54.36	23.20	-31.16	22.53	-8.63
3	-565.3879875	-571.7842825	4068.00	-54.26	23.24	-31.02	24.90	-6.12
4	-565.25158	-571.7842825	4155.00	-55.66	22.24	-33.43	24.08	-9.35
5	-565.3443749	-571.7842825	4095.40	-54.29	22.87	-31.42	22.14	-9.29
6	-565.4685668	-571.7842825	4017.90	-54.73	22.79	-31.93	23.62	-8.31
7	-565.5077392	-571.7842825	3993.00	-54.41	23.63	-30.78	23.09	-7.68
8	-565.4607552	-571.7842825	4023.30	-55.22	23.77	-31.46	21.58	-9.88
9	-565.6032297	-571.7842825	3934.00	-55.33	22.97	-32.36	23.11	-9.25
10	-565.2777678	-571.7842825	4137.70	-54.80	22.92	-31.88	24.42	-7.46
Ave. [h]				**-54.89**	**23.06**	**-31.84**	**23.18**	**-8.66**

[a] $E_{QM/MM}$(com) represents the energy of complex which is calculated at the B3LYP/6-31G(d):Amber level using the combined program of Gaussian03 and Amber8 software developed in our lab.[1,2]

[b] E_{QM}(lig) is the energy of ligand which is the most stable structure calculated at the B3LYP/6-31G(d) level in gas phase.

[c] E_{MM}(rec) represents the energy of MM subsystem calculated by Amber8 programm.

[d] The QM/MM gas-phase binding energy. $\Delta E_{QM/MM} = E_{QM/MM}(com) - E_{MM}(rec) - E_{QM}(lig)$.

[e] The binding energy. $\Delta E_{bind} = \Delta E_{QM/MM} + \Delta G_{solv}$.

[f] The entropy contribution. The scaling factor w was 0.93 for the entropy calculations.

[g] The binding free energy. $\Delta G_{bind} = \Delta E_{QM/MM} + \Delta G_{solv} - T\Delta S$.

[h] The average values calculated for 10 snapshots.

Table S2. Detailed energetic results (kcal/mol) obtained from the QM/MM-PBSA calculations at T = 298.15 K and P = 1 atm for the ten snapshots (No.1 to 10) of CYP2A6 complexed with Nic2b.

No	$E_{QM/MM}$(com) [a] (Hartree)	E_{QM}(lig) [b] (Hartree)	E_{MM}(rec) [c] (kcal/mol)	$\Delta E_{QM/MM}$ [d] (kcal/mol)	ΔG_{solv} (kcal/mol)	ΔE_{bind} or ΔH [e] (kcal/mol)	-TΔS (0.93) [f] (kcal/mol)	ΔG_{bind} [g] (kcal/mol)
1	-604.5891176	-611.0913628	4135.70	-55.48	25.84	-29.63	21.28	-8.35
2	-604.6484445	-611.0913628	4098.30	-55.30	25.35	-29.95	22.33	-7.63
3	-604.5578043	-611.0913628	4154.50	-54.63	26.65	-27.98	22.51	-5.46
4	-604.5788341	-611.0913628	4139.70	-53.02	24.71	-28.32	22.07	-6.24
5	-604.3259586	-611.0913628	4297.30	-51.94	25.33	-26.61	22.31	-4.30
6	-604.4627184	-611.0913628	4214.10	-54.56	26.28	-28.28	23.07	-5.20
7	-604.5913711	-611.0913628	4131.00	-52.19	24.77	-27.42	21.45	-5.98
8	-604.5449415	-611.0913628	4159.80	-51.86	24.80	-27.05	22.32	-4.73
9	-604.4303878	-611.0913628	4233.70	-53.87	25.49	-28.39	22.29	-6.10
10	-604.5900034	-611.0913628	4135.70	-56.03	25.74	-30.29	21.45	-8.84
Ave. [h]				**-53.89**	**25.50**	**-28.39**	**22.11**	**-6.28**

[a] $E_{QM/MM}$(com) represents the energy of complex which is calculated at the B3LYP/6-31G(d):Amber level.
[b] E_{QM}(lig) is the energy of ligand which is the most stable structure calculated at the B3LYP/6-31G(d) level in the gas phase.
[c] E_{MM}(rec) represents the energy of the MM subsystem calculated .
[d] The QM/MM gas-phase binding energy. $\Delta E_{QM/MM} = E_{QM/MM}(com) - E_{MM}(rec) - E_{QM}(lig)$.
[e] The binding energy. $\Delta E_{bind} = \Delta E_{QM/MM} + \Delta G_{solv}$.
[f] The entropy contribution. The scaling factor w was 0.93 for the entropy calculations.
[g] The binding free energy. $\Delta G_{bind} = \Delta E_{QM/MM} + \Delta G_{solv} - T\Delta S$.
[h] The average values calculated for 10 snapshots.

Table S3. Detailed energetic results (kcal/mol) obtained from the QM/MM-PBSA calculations at T = 298.15 K and P = 1 atm for the ten snapshots (No.1 to 10) of CYP2A6 complexed with Nic2c.

No	$E_{QM/MM}$(com) [a] (Hartree)	E_{QM}(lig) [b] (Hartree)	E_{MM}(rec) [c] (kcal/mol)	$\Delta E_{QM/MM}$ [d] (kcal/mol)	ΔG_{solv} (kcal/mol)	ΔE_{bind} or ΔH [e] (kcal/mol)	-TΔS (0.93) [f] (kcal/mol)	ΔG_{bind} [g] (kcal/mol)
1	-644.017397	-650.4014525	4055.70	-49.64	23.00	-26.64	20.63	-6.01
2	-643.8258067	-650.4014525	4177.70	-51.42	24.83	-26.59	20.46	-6.12
3	-644.1779389	-650.4014525	3957.00	-51.68	24.16	-27.52	20.50	-7.02
4	-644.0601322	-650.4014525	4026.40	-47.16	22.50	-24.65	20.02	-4.63
5	-643.8355732	-650.4014525	4169.50	-49.35	23.30	-26.05	20.68	-5.37
6	-643.8687773	-650.4014525	4147.70	-48.38	24.55	-23.83	22.20	-1.63
7	-644.0144532	-650.4014525	4059.80	-51.89	25.00	-26.90	20.63	-6.26
8	-644.2459105	-650.4014525	3915.00	-52.34	24.66	-27.67	21.49	-6.18
9	-644.182057	-650.4014525	3952.70	-49.97	24.30	-25.67	18.79	-6.88
10	-643.9320033	-650.4014525	4108.00	-48.36	23.46	-24.90	18.86	-6.03
Ave. [h]				**-50.02**	**23.98**	**-26.04**	**20.43**	**-5.61**

[a] $E_{QM/MM}$(com) represents the energy of complex which is calculated at the B3LYP/6-31G(d):Amber level.
[b] E_{QM}(lig) is the energy of ligand which is the most stable structure calculated at the B3LYP/6-31G(d) level in the gas phase.
[c] E_{MM}(rec) represents the energy of MM subsystem calculated .
[d] The QM/MM gas-phase binding energy. $\Delta E_{QM/MM} = E_{QM/MM}(com) - E_{MM}(rec) - E_{QM}(lig)$.
[e] The binding energy. $\Delta E_{bind} = \Delta E_{QM/MM} + \Delta G_{solv}$.
[f] The entropy contribution. The scaling factor w was 0.93 for the entropy calculations.
[g] The binding free energy. $\Delta G_{bind} = \Delta E_{QM/MM} + \Delta G_{solv} - T\Delta S$.
[h] The average values calculated for 10 snapshots.

Table S4. Detailed energetic results (kcal/mol) obtained from the QM/MM-PBSA calculations at T = 298.15 K and P = 1 atm for the ten snapshots (No.1 to 10) of CYP2A6 complexed with Methoxsalen.

No	$E_{QM/MM}$(com) [a] (Hartree)	E_{QM}(lig) [b] (Hartree)	E_{MM}(rec) [c] (kcal/mol)	$\Delta E_{QM/MM}$ [d] (kcal/mol)	ΔG_{solv} (kcal/mol)	ΔE_{bind} or ΔH [e] (kcal/mol)	-TΔS (0.93) [f] (kcal/mol)	ΔG_{bind} [g] (kcal/mol)
1	-756.3811177	-762.9582147	4179.70	-52.51	26.51	-26.00	19.17	-6.83
2	-756.3065483	-762.9582147	4228.40	-54.41	26.81	-27.60	20.27	-7.33
3	-756.3631751	-762.9582147	4193.70	-55.25	27.74	-27.51	20.05	-7.46
4	-756.4086644	-762.9582147	4164.80	-54.89	27.08	-27.81	18.14	-9.67
5	-756.5171634	-762.9582147	4096.80	-54.98	28.04	-26.94	18.26	-8.68
6	-756.4214671	-762.9582147	4157.00	-55.13	27.13	-28.00	19.97	-8.02
7	-756.4366903	-762.9582147	4147.40	-55.08	27.60	-27.48	18.09	-9.40
8	-756.3556229	-762.9582147	4199.00	-55.81	27.78	-28.03	19.23	-8.80
9	-756.4381811	-762.9582147	4145.70	-54.31	26.72	-27.59	21.02	-6.57
10	-756.5856228	-762.9582147	4051.70	-52.83	26.81	-26.02	20.17	-5.85
Ave. [h]				**-54.52**	**27.22**	**-27.30**	**19.44**	**-7.86**

[a] $E_{QM/MM}$(com) represents the energy of complex which is calculated at the B3LYP/6-31G(d):Amber level.
[b] E_{QM}(lig) is the energy of ligand which is the most stable structure calculated at the B3LYP/6-31G(d) level in the gas phase.
[c] E_{MM}(rec) represents the energy of MM subsystem calculated.
[d] The QM/MM gas-phase binding energy. $\Delta E_{QM/MM} = E_{QM/MM}(com) - E_{MM}(rec) - E_{QM}(lig)$.
[e] The binding energy. $\Delta E_{bind} = \Delta E_{QM/MM} + \Delta G_{solv}$.
[f] The entropy contribution. The scaling factor w was 0.93 for the entropy calculations.
[g] The binding free energy. $\Delta G_{bind} = \Delta E_{QM/MM} + \Delta G_{solv} - T\Delta S$.
[h] The average values calculated for 10 snapshots

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Table S5. Calculated binding free energies (kcal/mol, with standard deviations) for CYP2A6 binding with inhibitors by using the MM-PBSA method in comparison with the experimentally-derived binding free energies.

	ΔE_{MM}	ΔG_{solv}	ΔE_{bind}	$-T\Delta S$	$\Delta G_{\mathrm{bind}}^{\mathrm{calc}}$	$\Delta G_{\mathrm{bind}}^{\mathrm{expt}}$ [a]
Nic2a	-47.3±2.0	26.8±1.2	-20.5±2.0	10.8±0.7 (12.7)[b]	-9.7±1.3 (-7.8)[b]	-8.5
Nic2b	-48.0±2.3	30.9±1.4	-17.1±2.3	9.6±1.1 (11.3)[b]	-7.5±2.1 (-5.8)[b]	-7.2
Nic2c	-45.3±2.2	27.7±1.4	-17.6±2.1	8.6±0.7 (10.2)[b]	-9.0±1.4 (-7.5)[b]	-5.5
Methoxsalen	-49.4±2.0	31.3±1.0	-18.1±1.9	8.2±1.1 (9.7)[b]	-9.9±0.7 (-8.4)[b]	-7.8

[a] The experimental binding free energies were calculated from the corresponding experimental K_{i} values *via* $\Delta G_{\mathrm{bind}}^{\mathrm{expt}} = RT \ln K_{\mathrm{d}} = RT \ln K_{\mathrm{i}}$. The binding free energies as listed in Table S5 were calculated by using the molecular mechanics-Poisson-Boltzmann surface area (MM-PBSA) method.[3] The procedure for the MM-PBSA calculations is similar to that used in our previous studies,[4,5] and is also similar to the QM/MM-PBSA methods described in the text of the present paper (with w=0.93). The final binding free energy ΔG_{bind} for CYP2A6 inhibitor each inhibitor was taken as the average of the ΔG_{bind} values calculated for the equally-distributed 100 snapshots of the last 9.0 ns MD trajectory.

[b] The values in parentheses were obtained by re-calibrating the w based on the MM-PBSA data.

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