CHAPTER II: Design of Nevirapine Derivatives Insensitive to the K103N and Y181C HIV-1 Reverse Transcriptase Mutants

363 nevirapine derivatives were designed using a combinatorial library design approach and docked into the binding pocket of K103N and Y181C HIV-1 RT using the GOLD program. Compounds having a GoldScore higher than that of nevirapine (55.00 and 52.00 for K103N and Y181C mutants, respectively) were first retrieved and submitted to a topological analysis with the SILVER program. Compounds presenting a significant percentage of their surface buried upon binding (>80%) and exhibiting H-bonds to either N103 or C181 residues of the HIV-RT were selected. Finally, the hits were performed quantum chemical calculations for calculating the interaction energy with N103 or C181 residues.

1. Docking of nevirapine derivatives and post docking analysis

After removing the duplicated compounds in all libraries, 339 nevirapine derivatives were docked into the K103N and the Y181C protein mutants. The GoldScore of each docked conformation are shown in Table 14. The compounds having GoldScore higher than that of nevirapine (55.00 and 52.00 for K103N and Y181C mutants, respectively) were selected for further study. 124 compounds were retrieved to perform post-docking with SILVER program. In this study, docked compounds which have H-bonding with N103 or C181 for K103N and Y181C mutants, respectively and percentage of their surface buried upon binding higher than 80% were selected. There are 25 compounds having H-bonding with N103 and percentage of their surface buried upon binding higher than 80%, as shown in Table 15. 3 compounds have H-bonding with backbone atom of N103 and the others have H-bonding with sidechain atom of N103. In Y181C mutant, 6 compounds have H-bonding with backbone atom of C181, as shown in Table 15. The orientation of all 31 compounds are the same as nevirapine in K103N and Y181C binding pockets (like butterfly-like shape), as shown in Figure 18 and 19, respectively.

Table 14 GoldScore of docked nevirapine derivatives into the both protein mutants.

Cpd	GoldScore		Cpd	GoldScore	
	K103N	Y181C		K103N	Y181C
Nevirapine	57.16	54.23	Str40	61.30	36.10
Str1	49.54	51.78	Str41	42.87	47.95
Str2	52.17	28.44	Str42	48.92	39.95
Str3	55.18	49.61	Str43	51.18	50.22
Str4	49.03	47.86	Str44	47.91	45.03
Str5	57.96	59.28	Str45	58.07	39.20
Str6	55.37	58.28	Str46	55.47	51.64
Str7	57.73	56.09	Str47	50.66	50.57
Str8	59.16	54.20	Str48	55.75	50.24
Str9	56.52	49.09	Str49	52.06	52.58
Str10	59.60	36.86	Str50	36.12	37.36
Str11	56.43	44.97	Str51	44.05	46.81
Str12	49.05	40.47	Str52	31.61	39.10
Str13	57.59	38.47	Str53	42.54	44.43
Str14	59.41	58.95	Str54	49.67	41.25
Str15	57.67	44.53	Str55	60.39	37.38
Str16	56.99	55.26	Str56	50.15	43.12
Str17	54.34	52.80	Str57	48.49	42.09
Str18	58.35	54.54	Str58	38.18	51.82
Str20	50.61	38.60	Str60	45.20	36.75
Str21	55.80	45.33	Str61	41.16	37.32
Str22	46.35	37.80	Str62	37.09	10.93
Str23	57.97	48.61	Str63	41.01	44.02
Str24	54.76	37.91	Str64	39.21	35.44
Str25	60.05	39.86	Str65	41.47	32.90
Str26	54.64	51.42	Str66	40.22	39.42
Str27	54.28	54.24	Str67	43.53	44.85
Str28	60.64	58.37	Str68	43.58	38.18
Str30	62.56	38.60	Str70	23.92	6.40
Str31	46.04	46.80	Str71	34.73	20.25
Str32	39.20	29.55	Str72	11.66	21.05
Str33	52.61	49.32	Str73	29.06	39.27
Str34	45.04	47.77	Str74	32.23	29.79
Str35	63.12	47.45	Str75	33.47	32.49
Str36	55.06	48.65	Str76	43.07	32.83
Str37	55.10	53.18	Str77	40.64	31.96
Str38	55.40	56.28	Str78	49.66	41.75
Str39	55.38	52.99	Str80	38.92	-0.51

Table 14 (cont'd)

Cpd	GoldScore	
	K103N	Y181C
Str81	48.64	43.67
Str82	28.97	16.71
Str83	41.36	43.85
Str84	52.07	42.34
Str85	33.95	30.35
Str86	44.26	31.23
Str87	51.89	45.73
Str88	43.65	39.09
Str90	47.10	26.27
Str91	49.12	49.99
Str92	49.34	28.64
Str93	53.35	51.81
Str94	60.10	49.02
Str95	37.89	39.99
Str96	55.59	54.72
Str97	54.49	48.15
Str98	55.83	49.82
Str100	44.76	46.20
Str101	48.45	50.29
Str102	49.73	45.98
Str103	49.25	43.65
Str104	54.62	48.29
Str105	53.33	52.31
Str106	52.24	50.97
Str107	46.97	46.48
Str108	51.72	52.61
Str109	47.82	45.15
Str110	59.78	58.21
Str111	48.66	49.45
Str112	42.96	35.46
Str113	43.47	46.32
Str114	47.22	46.10
Str115	57.77	45.50
Str116	45.65	46.92
Str117	50.14	48.31
Str118	55.02	48.28
Str120	47.14	36.11
Str122	40.59	41.22

Cpd	GoldScore	
	K103N	Y181C
Str123	50.99	42.91
Str124	48.21	46.17
Str125	53.81	42.60
Str126	46.79	52.69
Str127	49.79	44.34
Str128	52.83	53.75
Str130	51.15	52.25
Str131	55.55	40.17
Str132	51.76	38.18
Str133	57.82	39.04
Str134	51.44	22.89
Str135	57.68	49.34
Str136	58.18	39.54
Str137	48.46	41.20
Str138	56.19	48.30
Str139	48.60	44.18
str140	59.00	43.85
str141	43.36	41.37
str142	40.53	24.70
str143	48.64	39.21
str144	57.53	45.80
str145	49.61	41.09
str146	53.72	48.42
str147	50.36	43.85
str148	55.58	44.11
Str150	33.17	39.48
Str151	42.99	39.48
Str152	36.20	20.35
Str153	51.02	36.90
Str154	50.15	45.68
Str155	45.85	38.37
Str156	49.00	45.88
Str157	49.97	41.52
Str158	50.38	41.68
Str160	54.89	36.97
Str161	45.39	28.56
Str162	25.70	12.59
Str163	55.51	33.66

Table 14 (cont'd)

Cpd	GoldScore		Cpd	GoldScore	
	K103N	Y181C		K103N	Y181C
Str164	48.95	34.09	Str204	36.17	41.01
Str165	55.88	43.91	Str205	23.09	33.91
Str166	54.17	43.13	Str206	35.96	46.82
Str167	56.74	52.22	Str207	30.62	38.82
Str168	53.05	50.73	Str208	42.69	35.41
Str169	57.44	51.07	Str210	15.24	31.55
Str170	52.82	28.34	Str211	46.81	46.25
Str171	49.15	45.35	Str212	18.49	16.93
Str172	41.94	31.46	Str213	45.34	39.23
Str173	50.86	50.72	Str214	49.75	46.50
Str174	47.41	48.79	Str215	29.26	25.73
Str175	61.34	47.50	Str216	43.72	35.30
Str176	54.59	53.25	Str217	53.70	50.07
Str177	49.61	52.82	Str218	50.94	44.02
Str178	51.84	53.57	Str220	28.58	18.10
Str179	54.19	51.47	Str221	52.07	49.96
Str180	51.52	21.26	Str222	35.11	36.22
Str181	53.23	52.42	Str223	50.83	43.22
Str182	49.51	38.38	Str224	56.70	46.39
Str183	48.21	43.59	Str225	53.26	45.33
Str184	46.53	41.49	Str226	56.76	34.83
Str185	44.24	44.72	Str227	54.15	54.02
Str186	52.26	49.86	Str228	47.66	44.32
str187	51.14	47.67	Str230	45.75	43.75
str188	50.94	59.01	Str232	43.04	39.41
Str190	48.89	40.49	Str233	51.39	39.49
Str191	50.01	43.25	Str234	51.36	41.35
Str192	20.73	15.10	Str235	49.34	51.40
Str193	33.38	48.14	Str236	54.19	54.36
Str194	45.36	27.13	Str237	51.67	50.14
Str195	50.13	26.72	Str238	52.99	53.67
Str196	41.59	46.70	Str240	53.58	56.44
Str197	50.56	49.04	Str241	59.04	53.02
Str198	45.03	38.13	Str242	23.52	23.94
Str200	8.97	11.92	Str243	49.96	43.78
Str201	34.97	30.99	Str244	58.86	46.62
Str202	20.61	28.85	Str245	35.79	44.20
Str203	41.47	33.09	Str246	42.52	39.28

Table 14 (cont'd)

Cpd	GoldScore	
	K103N	Y181C
Str247	54.95	51.91
Str248	54.64	40.99
Str250	30.27	28.64
Str251	58.56	52.65
Str252	56.71	52.56
Str253	47.85	50.22
Str254	50.99	-
Str255	41.44	-
Str256	52.81	-
Str257	55.67	-
Str258	56.35	-
Str259	56.32	-
Str260	55.99	-
Str261	52.59	-
Str262	52.54	-
Str263	53.01	-
Str264	49.91	-
Str265	59.74	-
Str266	50.39	-
Str267	45.19	-
Str268	46.92	-
Str269	47.88	-
Str270	56.81	-
Str271	54.31	-
Str273	44.43	-
Str274	32.72	-
Str275	39.47	-
Str276	41.55	-
Str277	48.40	-
Str278	43.45	-
Str279	47.30	-
Str280	54.83	-
Str281	51.02	-
Str283	51.53	-
Str284	41.48	-
Str285	29.25	-
Str286	56.11	-
Str287	49.25	-

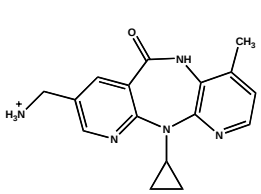
Cpd	GoldScore	
	K103N	Y181C
Str288	56.34	-
Str289	57.01	-
Str290	60.52	-
Str291	57.05	-
Str292	61.71	-
Str293	58.09	-
Str294	-	46.39
Str295	-	40.63
Str296	-	49.99
Str297	-	51.92
Str298	-	52.57
Str299	-	49.98
Str300	-	46.05
Str301	-	54.31
Str302	-	47.01
Str303	-	39.75
Str304	-	46.06
Str305	-	23.89
Str306	-	45.47
Str307	-	42.90
Str308	-	43.18
Str309	-	45.24
Str310	-	47.13
Str311	-	46.25
Str313	-	20.50
Str314	-	23.10
Str315	-	57.96
Str316	-	39.73
Str317	-	43.68
Str318	-	35.21
Str319	-	38.01
Str320	-	34.91
Str321	-	51.18
Str323	-	20.32
Str324	-	42.84
Str325	-	21.90
Str326	-	51.22
Str327	-	49.60

Table 14 (cont'd)

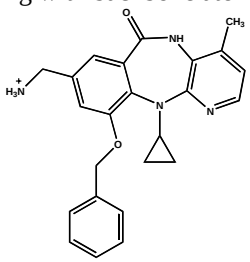
Cpd	GoldScore	
	K103N	Y181C
Str328	-	45.12
str329	-	45.11
Str330	-	53.72
Str331	-	49.21
Str332	-	54.77
Str333	-	39.12
Str334	53.66	41.89
Str335	54.96	35.97
Str336	30.79	32.86
Str337	50.86	37.10
Str338	41.47	31.65
Str339	56.53	54.19
Str340	61.83	57.29
Str341	58.09	58.73
Str342	48.26	61.77
Str343	60.74	54.55
Str344	59.41	43.84
Str345	48.17	40.18
Str346	43.00	33.22
Str347	49.14	24.59
Str348	48.57	36.99
Str349	54.43	58.01
Str350	55.31	51.41
Str351	45.17	51.32
Str352	59.23	48.03
Str353	45.39	52.01
Str354	67.49	11.15
Str355	59.95	31.15
Str356	64.68	36.32
Str357	65.81	53.70
Str358	61.91	25.49
Str359	65.59	37.73
Str360	44.95	36.06
Str361	48.19	22.11
Str362	54.83	26.68
Str363	50.77	26.63

Table 15 Selected which have H-bonding with N103 or C181 for K103N and Y181C mutants, respectively and percentage of their surface buried upon binding higher than 80%.

	No. of selected compounds
- compounds have H-bonding with backbone atom of N103	3



Cpd1



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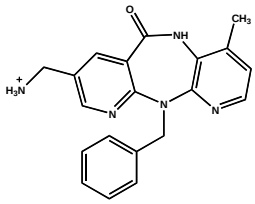
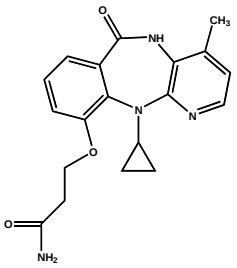


Cpd3

- compounds have H-bonding with sidechain atom of N103 22



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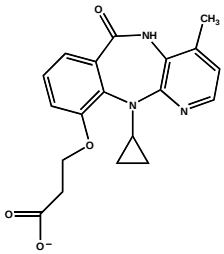

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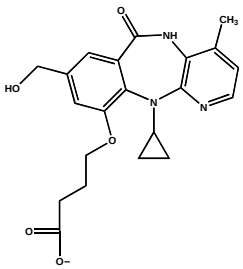

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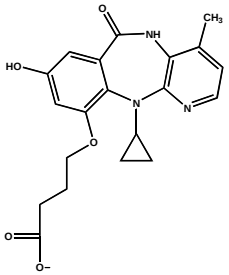

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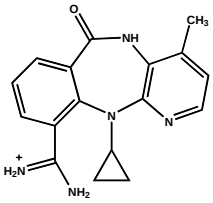

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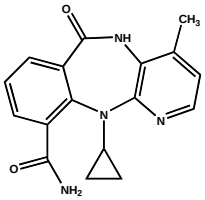

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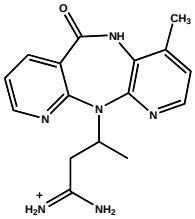

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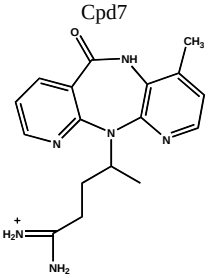

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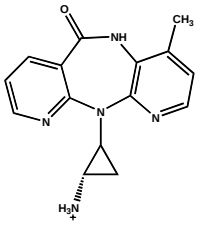

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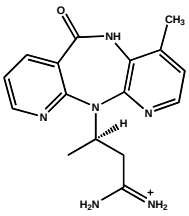

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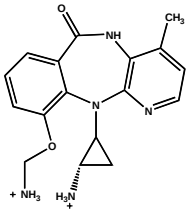

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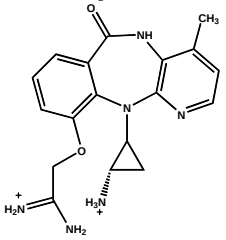

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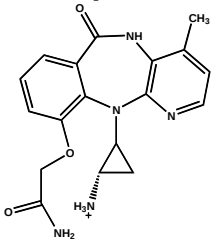

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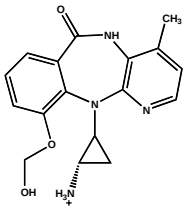

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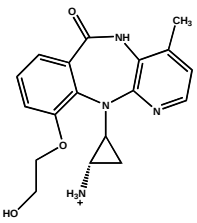

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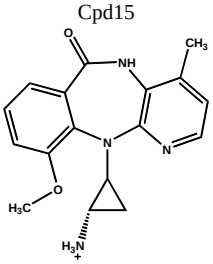
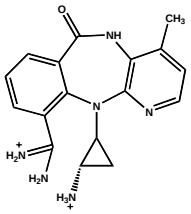
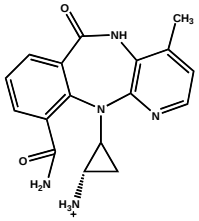
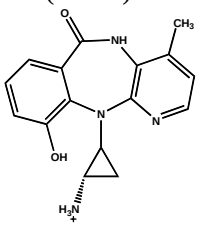
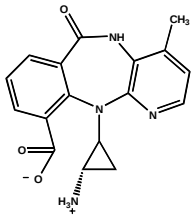
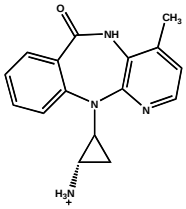
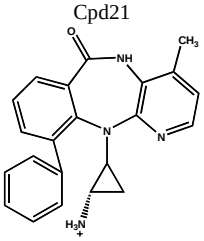
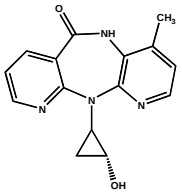
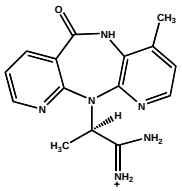
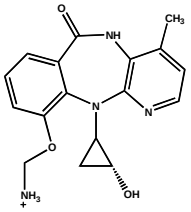
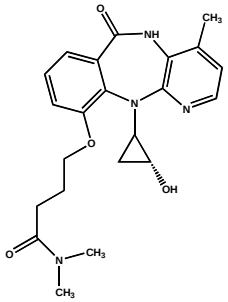
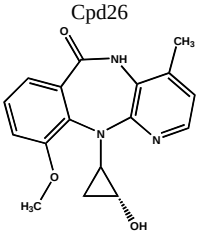
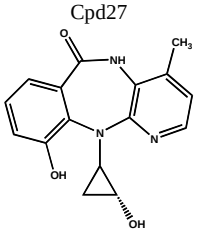
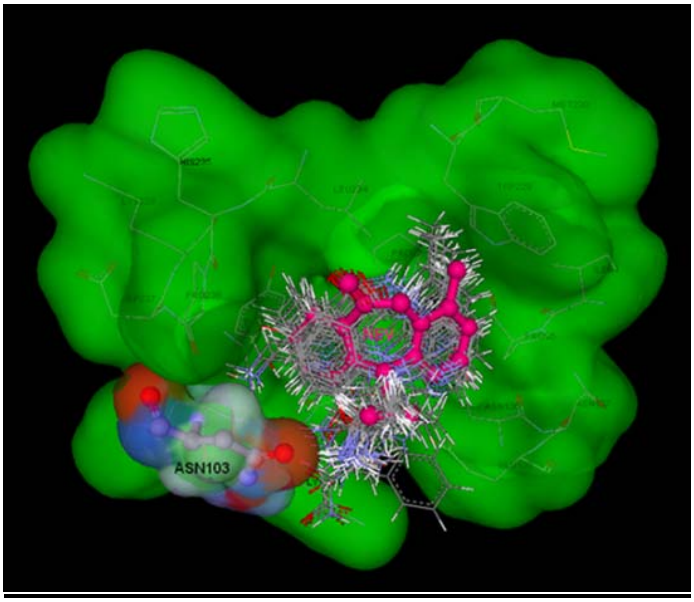


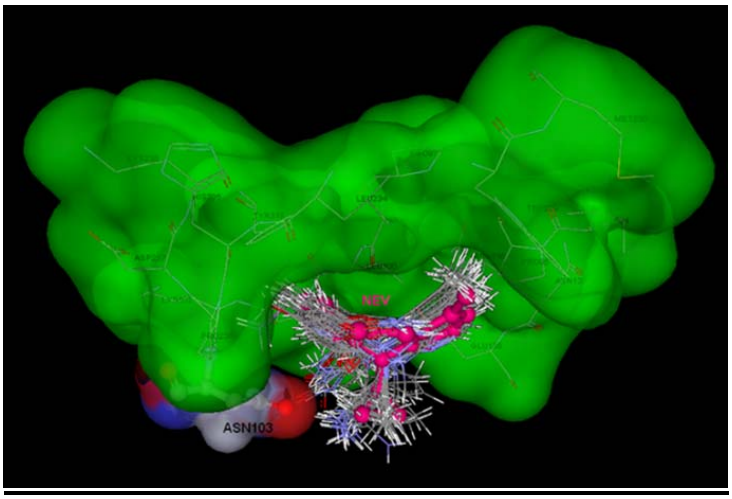
Cpd19

Table 15 (cont'd)

	No. of selected compounds
- compounds have H-bonding with sidechain atom of N103 (cont'd)	
 Cpd20  Cpd21  Cpd22  Cpd23  Cpd24  Cpd25	
- compounds have H-bonding with backbone atom of C181	6
 Cpd26  Cpd27  Cpd28  Cpd29  Cpd30  Cpd31	

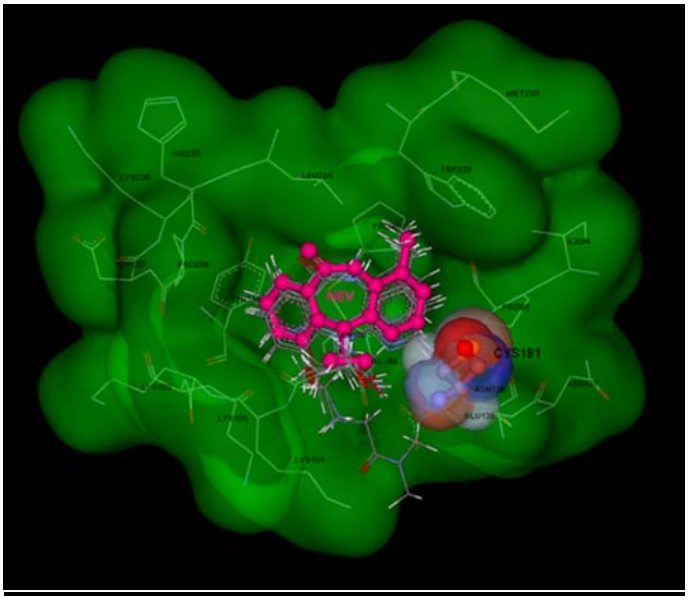


a.

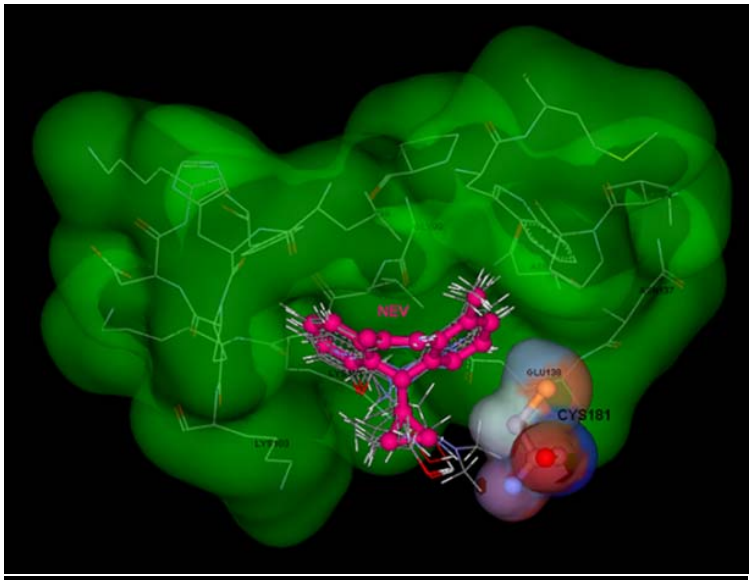


b.

Figure 18 Orientation of selected nevirapine derivatives in K103N binding pocket (side view (a) and top view (b))



a.



b.

Figure 19 Orientation of selected nevirapine derivatives in Y181C binding pocket (side view (a) and top view (b))

2. Calculation of interaction energy

For confirming the selected compounds having H-bonding interaction with N103 or C181, the calculated interaction energies were performed by using quantum chemical calculations. For K103N mutant, nevirapine (pdb code 1fk9) and each of 25 selected compounds were used to calculate interaction energies with N103 at B3LYP/6-31G(d) and MP2/6-31G(d) levels of theory and the results are shown in Table 16. Interaction energies showed that nevirapine slightly interacted with N103 in both methods. The interaction energies are about 0.3 and -0.7 kcal/mol at B3LYP/6-31G(d) and MP2/6-31G(d) levels of theory, respectively.

The orientation of the selected compounds having H-bonding with backbone N103 are shown with N103 and compared with nevirapine's orientation in Figure 20. Two of them (Cpd1 and Cpd3) have repulsive interactions with N103 in both methods. These repulsive interactions were caused by steric interaction of R_1 substituent (methyl ammonium group), as shown in Figure 20a and 20b. Instead of the formation of attractive interaction with N103, the methyl ammonium group of Cpd1 and Cpd3 has formed H-bonding interaction with P236. Attractive interaction energies of Cpd2 (-3.1 and -5.6 kcal/mol) are contributed by H-bonding interaction between nitrogen atom of backbone N103 and hydrogen atom of ammonium group in R_1 substituent with the length of 2.49 Å (Figure 20c).

In the case of other selected compounds having H-bonding with the sidechain of N103, except Cpd9, these compounds show tighter interaction energies than that of nevirapine (0.3 kcal/mol by B3LYP/6-31G(d) calculations and -0.7 kcal/mol by MP2/6-31G(d) calculations). The repulsive interaction of Cpd9 are due to steric hindrance between amide group at R_2 substituent and oxygen atom of N103 sidechain, as shown in Figure 21a. In addition, the oxygen atom of amide group at R_2 substituent has attractive interaction with L100 and K101, therefore, its GoldScore is found to be higher than GoldScore of nevirapine. For the compounds having the lowest interaction energy with sidechain N103, Cpd20, the interaction energies are -33.7 and

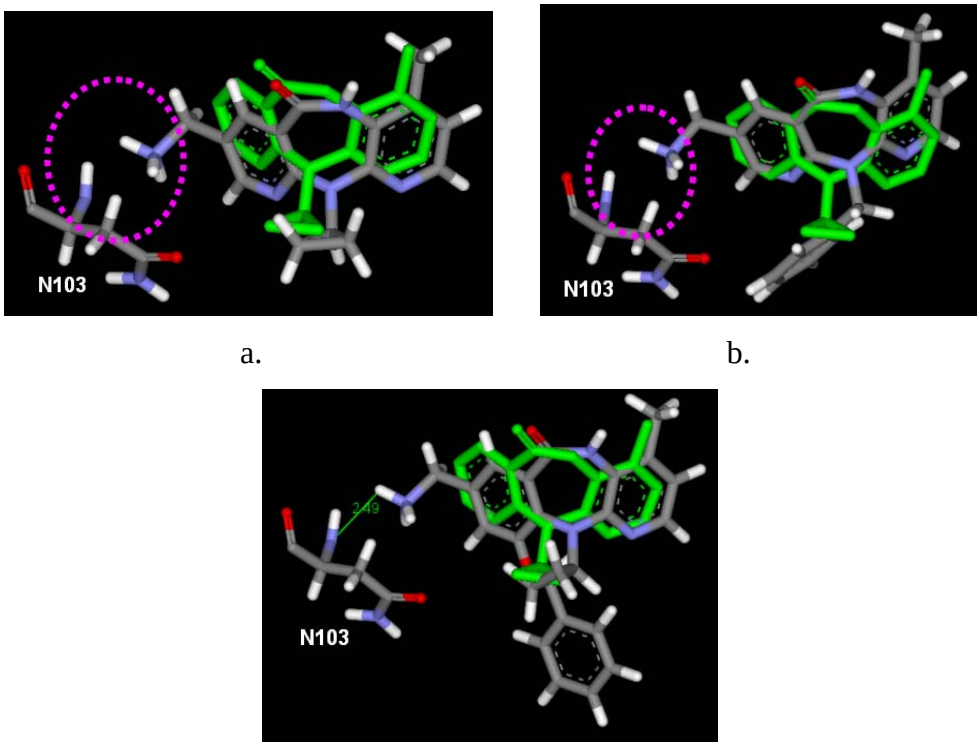
-35.0 kcal/mol by B3LYP/6-31G(d) and MP2/6-31G(d) calculations, respectively. The attractive interaction is caused by forming double hydrogen bonding with carbonyl group of sidechain of N103, as shown in Figure 21b.

In the case of Y181C mutant, the interaction energy between nevirapine and C181 was calculated by both methods (Table 17). The interaction energies are 1.7 and -2.3 kcal/mol for B3LYP/6-31G(d) and MP2/6-31G(d) calculations, respectively. There are 6 selected nevirapine derivatives that interact with C181 and the results are shown in Table 4. All 6 compounds showed stronger interaction energies than that of nevirapine and it was found that H-bonding with nitrogen backbone atom of C181 was formed to these nevirapine derivatives. H-bonding interactions were occurred from substituted hydroxyl group of cyclopropyl (Cpd 26, Cpds 28-30) and from amino group of R_3 substituent (Cpd27). The orientation of Cpds 26 and 27 are shown in Figure 22.

By using B3LYP/6-31G(d) and MP2/6-31G(d) levels of theory, the interaction energies between all hits and N103 or C181 are agreed well with the results from M. Kuno *et al.* (2003) as density functional method can not handle the H-¶ interaction but MP2 method has taken into account this interaction. Therefore, in this study, more attractive interaction of nevirapine derivatives were generally found by using MP2/6-31G(d) level of theory.

Table 16 Interaction energies between N103 and 25 selected compounds of K103N mutant.

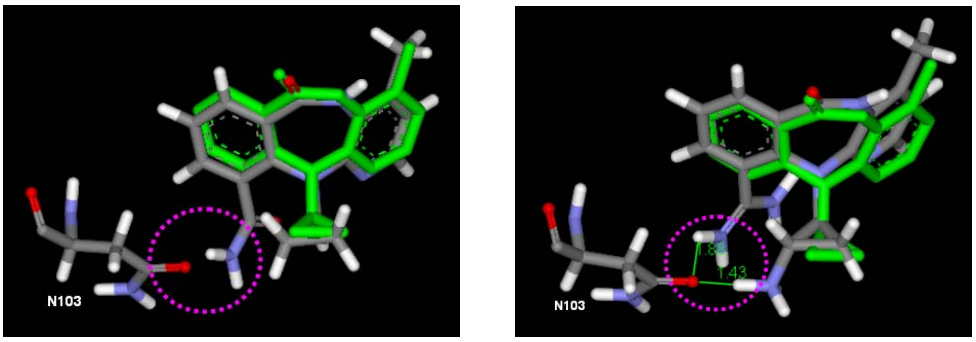
Compound	Interaction energy (kcal/mol)	
	B3LYP/6-31G(d)	MP2/6-31G(d)
Nevirapine (pdb code 1fk9)	0.313	-0.746
- H-bonding with backbone N103		
Cpd 1 (Str 251)	7.311	4.510
Cpd 2 (Str 357)	-3.064	-5.629
Cpd 3 (Str 352)	6.110	2.623
- H-bonding with sidechain N103		
Cpd 4 (Str 16)	0.165	-2.364
Cpd 5 (Str 18)	-4.584	-7.649
Cpd 6 (Str 343)	-16.608	-20.563
Cpd 7 (Str 340)	-9.906	-13.501
Cpd 8 (Str 35)	-17.074	-18.845
Cpd 9 (Str 36)	7.958	6.580
Cpd 10 (Str 45)	-18.086	-19.005
Cpd 11 (Str 55)	-12.297	-13.779
Cpd 12 (Str 94)	-25.484	-25.782
Cpd 13 (Str 175)	-1.619	-3.197
Cpd 14 (Str 257)	-30.320	-31.654
Cpd 15 (Str 258)	-23.239	-25.296
Cpd 16 (Str 259)	-19.143	-21.622
Cpd 17 (Str 260)	-24.628	-26.082
Cpd 18 (Str 270)	-17.768	-19.959
Cpd 19 (Str 286)	-24.659	-26.272
Cpd 20 (Str 288)	-33.725	-35.056
Cpd 21 (Str 289)	-14.161	-14.910
Cpd 22 (Str 290)	-24.568	-25.542
Cpd 23 (Str 291)	-14.969	-16.698
Cpd 24 (Str 292)	-26.726	-27.573
Cpd 25 (Str 293)	-19.121	-21.362



a. b.

c.

Figure 20 Orientation of selected nevirapine derivatives (Cpd1 (a), Cpd3 (b), and Cpd2 (c) in atom-type color) having H-bonding with backbone N103 compared with N103 and nevirapine (green color).

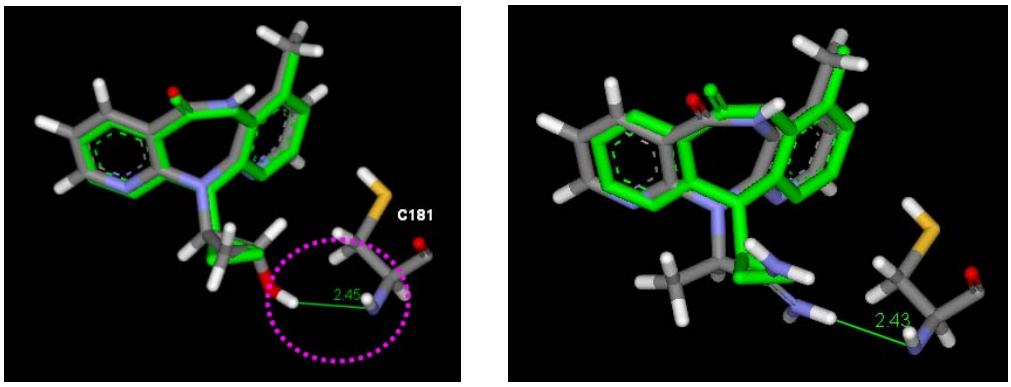


a. b.

Figure 21 Orientation of selected nevirapine derivatives (Cpd9 (a) and Cpd20 (b) in atom-type color) having H-bonding with sidechain N103 compared with N103 and nevirapine (green color).

Table 17 Interaction energy between C181 and selected compounds of Y181C mutant.

Compound	Interaction energy (kcal/mol)	
	B3LYP/6-31G*	MP2/6-31G*
Nevirapine (pdb code 1jlb) - H-bonding with backbone Cys181	1.691	-2.326
Cpd 26 (Str 227)	-1.825	-5.744
Cpd 27 (Str 235)	-3.294	-6.948
Cpd 28 (Str 297)	-0.807	-5.550
Cpd 29 (Str 322)	1.663	-4.242
Cpd 30 (Str 326)	-1.259	-5.723
Cpd 31 (Str 330)	-1.663	-4.614



a. b.

Figure 22 Orientation of selected nevirapine derivatives (Cpd26 (a) and Cpd27 (b) in atom-type color) having H-bonding with C181 compared with C181 and nevirapine (green color).