

## **Title: Molecular docking of aptamer sequence and Comparative study of original vs annotated sequences:**

DATE: March, 2025

### **OVERVIEW:**

The original aptamer sequences targeting five opioids, Fentanyl, Tramadol, Oxycodone, Codeine and Methadone, were systematically retrieved from reported literature and used as the primary dataset in this study. These unmodified sequences, previously identified to exhibit binding potential, served as the baseline for comparative evaluation. To establish their performance, each aptamer was subjected to molecular docking against its respective opioid ligand, allowing assessment of binding affinity, molecular interactions, and conformational stability. The docking results revealed variation in binding strengths across the five aptamers, enabling the identification of the sequence with the most favourable interaction profile. This lead aptamer was subsequently selected for further investigation through modification approaches, including mutation and truncation, with the aim of enhancing affinity, specificity, and structural efficiency.

### **OBSERVATIONS:**

The primary sequences obtained from the literature showed notable variability in nucleotide composition and length across the five opioid-specific aptamers. These differences were reflected in the GC content and sequence motifs, both of which play critical roles in structural stability and ligand recognition. The primary sequences provided the fundamental framework upon which subsequent structural predictions were built.

Secondary structure analysis revealed the presence of characteristic stem-loop and hairpin motifs, which are crucial for target binding. Variations in the number and stability of these structural elements were observed across different aptamers. Aptamers with well-defined loop regions and stable stems demonstrated stronger predicted binding tendencies, consistent with their docking scores.

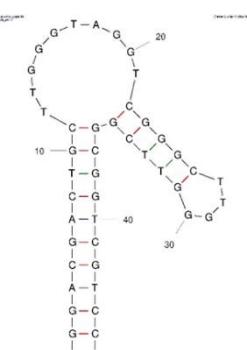
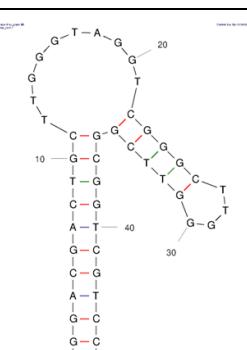
The tertiary structures generated through computational modelling highlighted the three-dimensional folding patterns responsible for ligand recognition. Compact and stable folding conformations were observed in sequences with higher GC content and stronger secondary structural motifs. Binding pockets formed predominantly within loop and bulged regions, enabling favourable ligand accommodation. Comparative docking confirmed that aptamers with clearly defined tertiary binding cavities exhibited enhanced affinity and specificity toward their respective opioids.

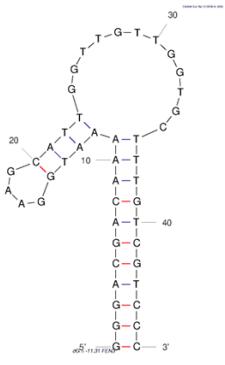
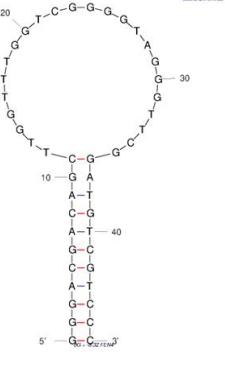
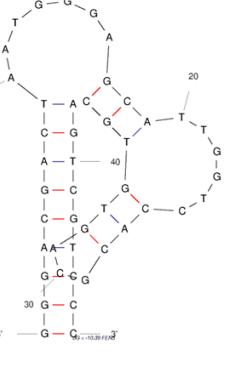
## Docking Analysis: Fentanyl

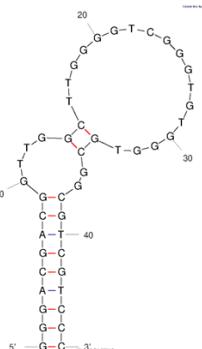
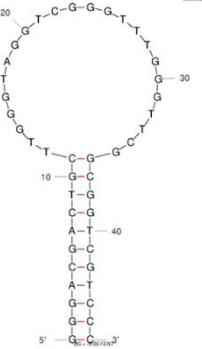
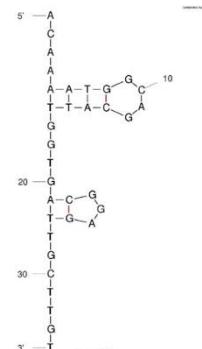
March 15, 2025

### RESULTS:

#### APTAMER: FENTANYL

Aptamer	Sequence	Structure	Delta G	Reference
FEN 1	GGGACGACTGCTTGGGTAGGTCGGGCTTGGGTTC GGCGGT CGTCCC		<b>-9.70</b>	
FEN 2	GGGACGACTGCTTGGGTAGGTCGGGCTTGGGTTC GGCGGT CGTCCC		<b>-11.07</b>	

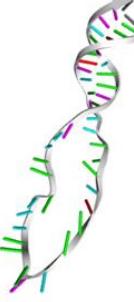
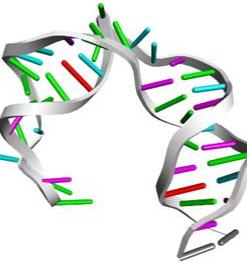
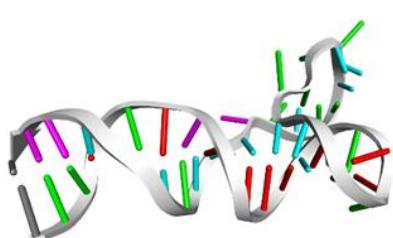
FEN 3	GGGACGACAAAAATGGAAGCATTGGTGTGGTG CTTGTGTCGTCCC		<b>-11.31</b>	
FEN 4	GGGACGACAGCTTGGTTGGTCGGGGTAGGGTTC GGATGTCGTCCC		<b>-8.32</b>	
FEN 5	GGGACGACTAATGGGAGCATTGGTCCACGCAGTG TGCAGTCGTCCC		<b>-10.39</b>	

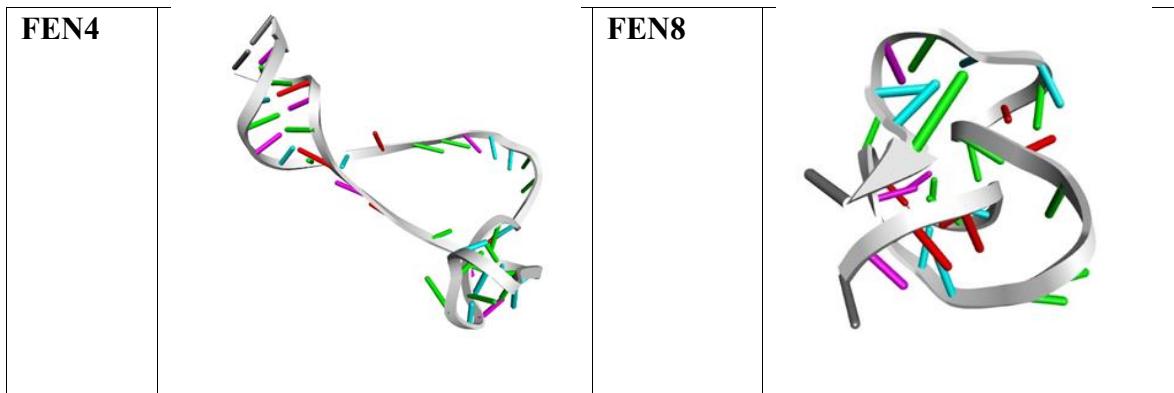
FEN 6	GGGACGACGGTGGCTTGGGTAGGTGGTTGGTGC CGCGTCGTCCC		<b>-8.54</b>	
FEN 7	GGGACGACTGCTTGGGTAGGTGGTTGGTTC GGCGGTTCGTCCC		<b>-9.66</b>	
FEN 8			<b>-2.59</b>	

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Tertiary structure prediction using 3dRNA/DNA.

*Tertiary structure were predicted using 3dRNA/DNA web server and were obtained as follows.*

Aptamer	Structure	Aptamer	Structure
FEN1		FEN5	
FEN2		FEN6	
FEN3		FEN7	

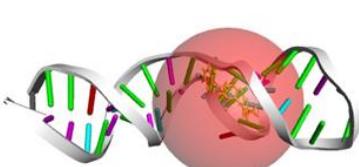
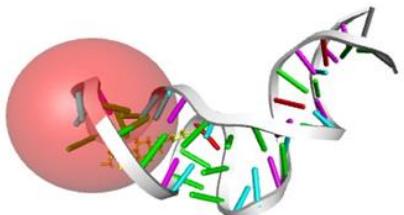


March 20, 2025

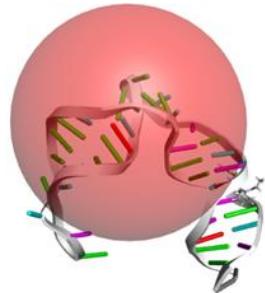
### Molecular Docking using Biovia Discovery Studio

LibDock protocol was used and optimized for the docking process. The loop regions were defined as the binding sphere for docking the aptamers against fentanyl. Hence 2 different results for each loop were generated.

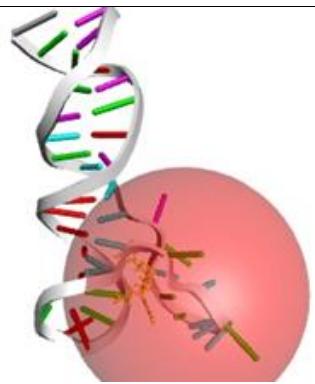
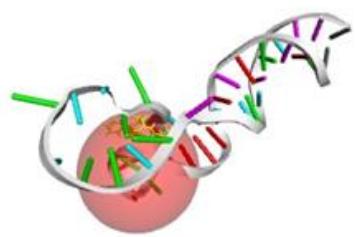
#### *Loop-specific docking spheres for eight aptamer sequences*

	Loop 1	Loop 2
FEN1		

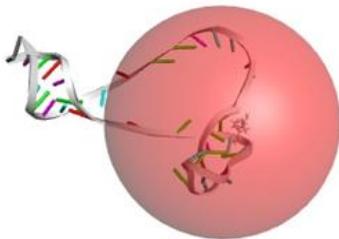
FEN2

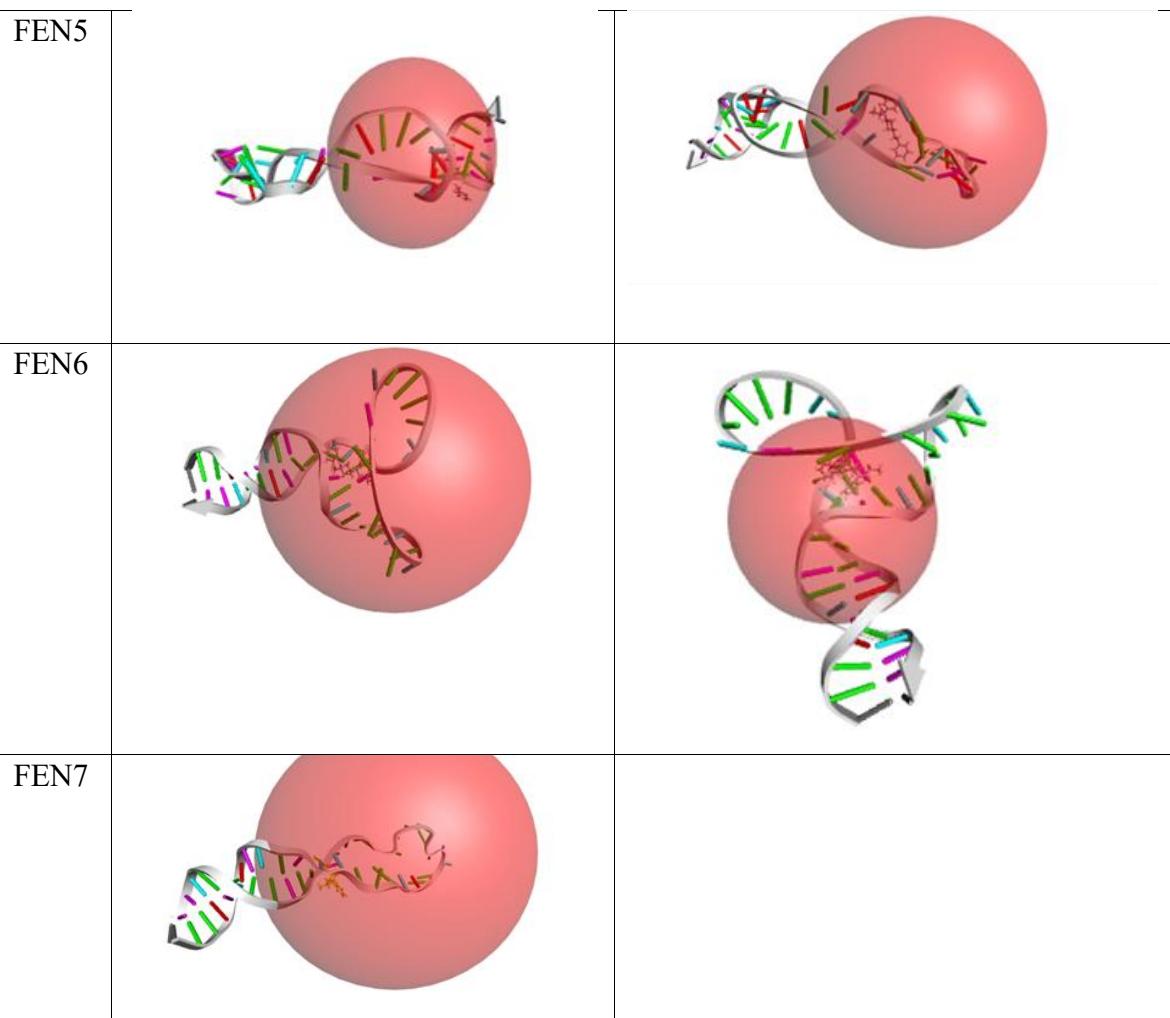


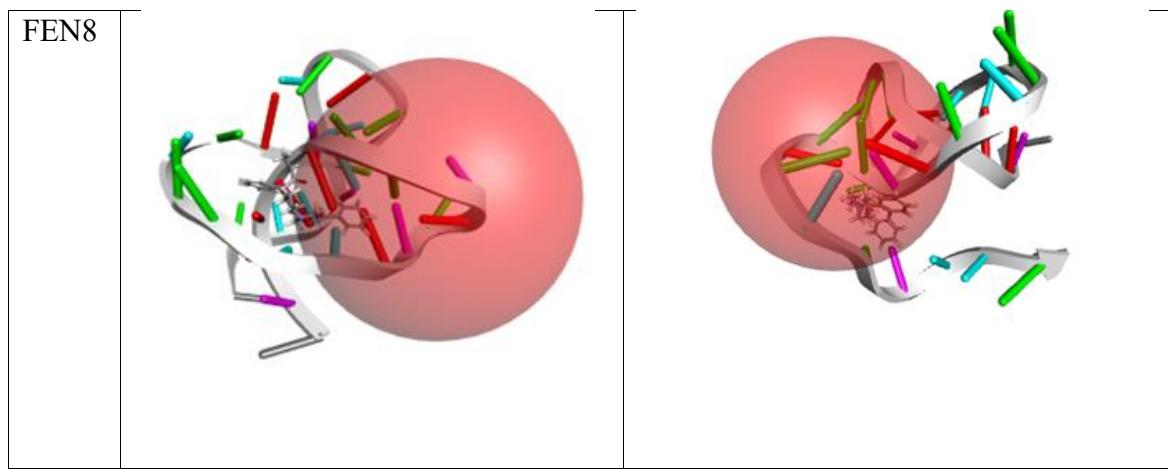
FEN3



FEN4

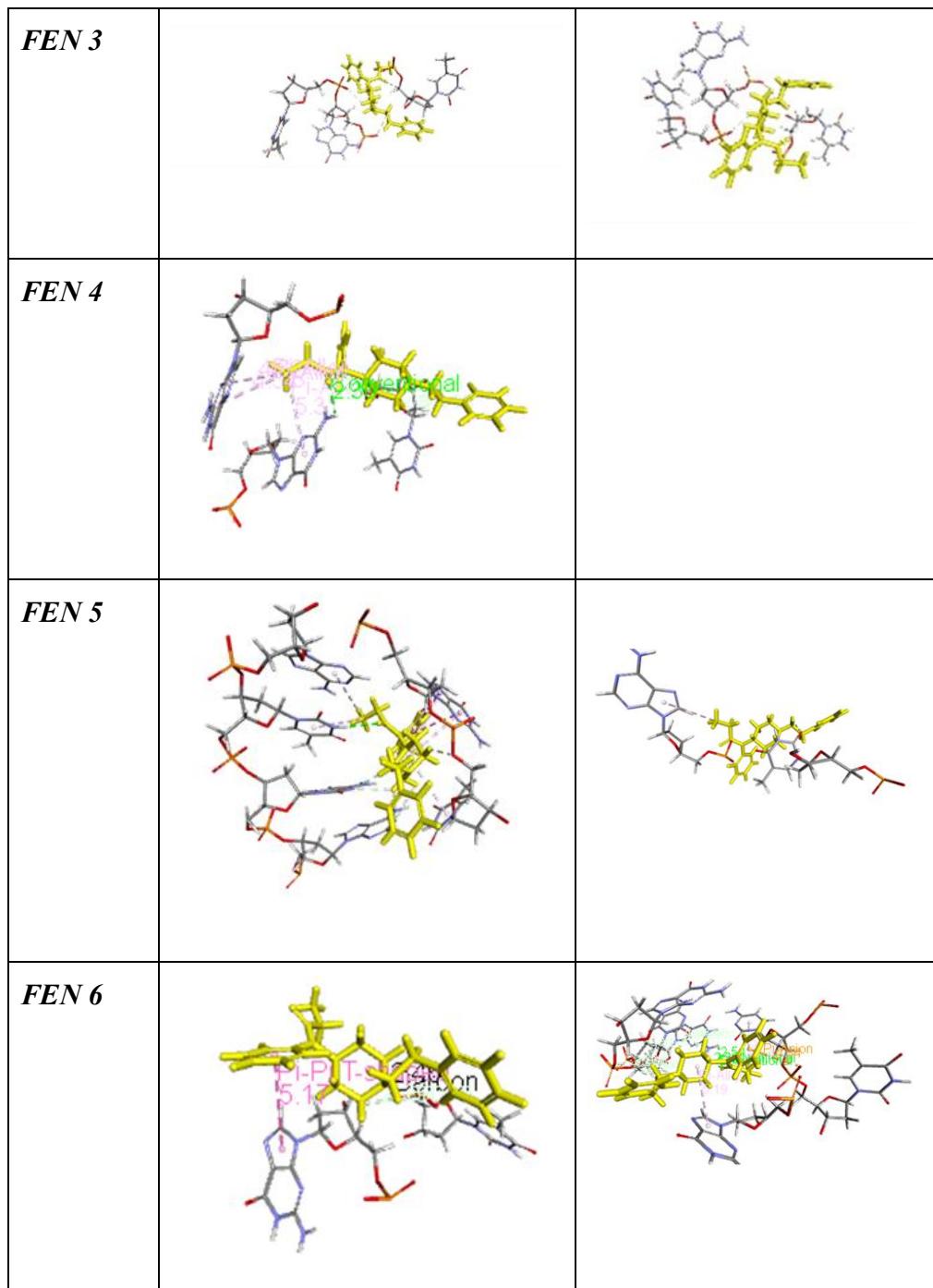


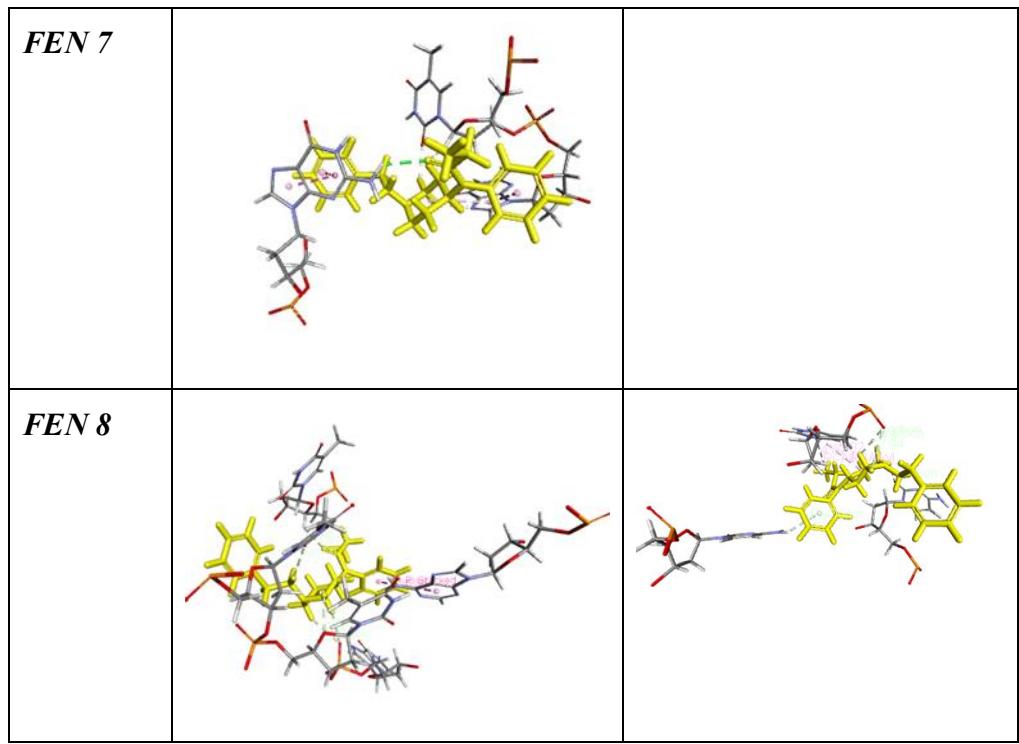




*Ligand interaction diagrams of the eight aptamer sequences*

Sequence	Loop 1	Loop 2
<i>FEN 1</i>		
<i>FEN 2</i>		





***Thermodynamic details of the eight aptamer sequences***

	Loop	LibDock score	Poses	Potential Energy (kcal/mol)	Van der Waals Energy (kcal/mol)	Electrostatic Energy (kcal/mol)
FEN1	Loop 1	115.556	65	99.19146	-596.18458	-1493.03567
	Loop 2	93.768	78	99.19146	-596.18458	-1493.03567

FEN2	Loop1	134.521	63	562.7039	-571.463	-1257.57
FEN3	Loop 1	122.414	60	356.97107	-568.22767	-1313.24612
	Loop 2	122.262	62	356.97107	-568.22767	-1313.24612
FEN4	Loop 1	91.2529	55	930.422	-506.789	-1552.07
FEN5	Loop 1	114.618	65	6853.89072	248.01299	-1250.7119
	Loop 2	101.681	86	6813.47707	246.63195	-1292.19898
FEN6	Loop 1	94.639	99	647.7185	-359.549	-1641.45
	Loop 2	109.259	79	422.1538	-559.353	-1661.99
FEN7	-	-	-	-	-	-
FEN8	Loop 1	105.855	90	649.8279	-398.417	-659.075
	Loop 2	119.446	80	676.4631	-392.879	-654.635

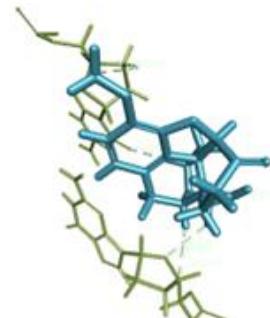
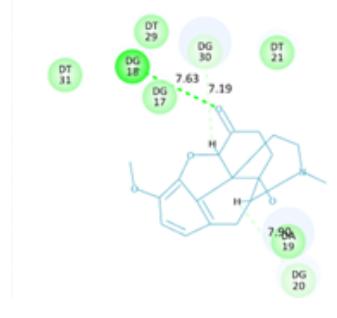
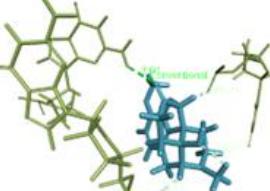
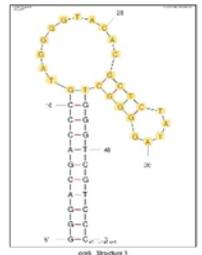
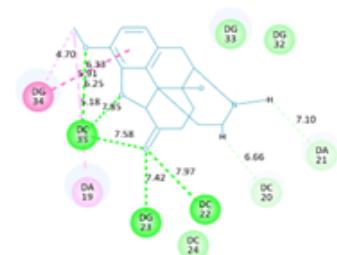
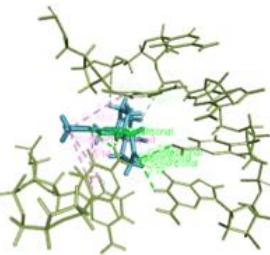
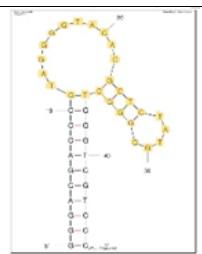
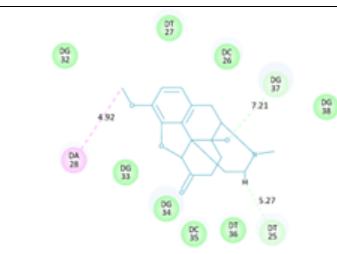
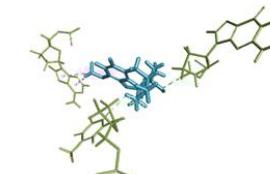
***Summary of ligand- aptamer interaction types and Bond lengths***

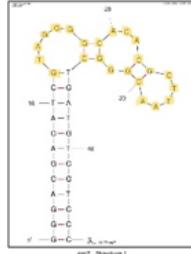
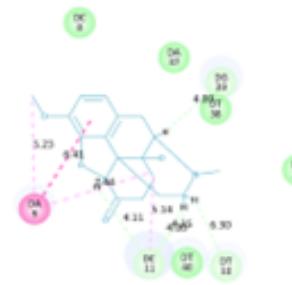
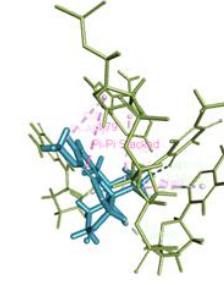
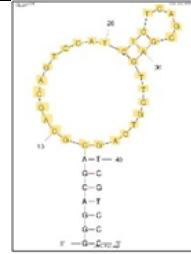
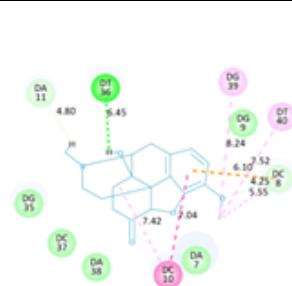
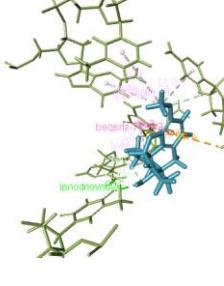
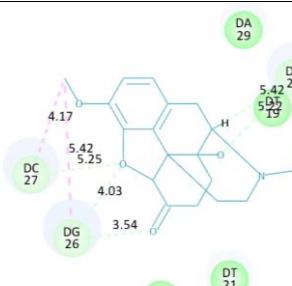
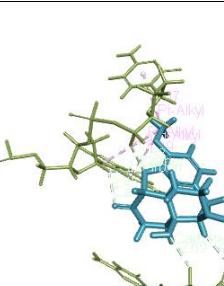


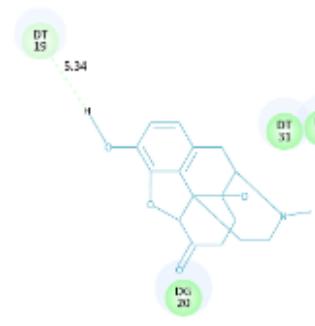
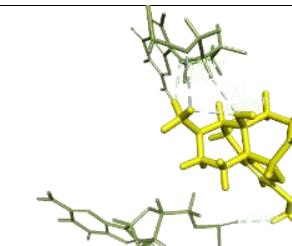
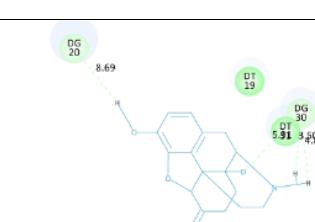
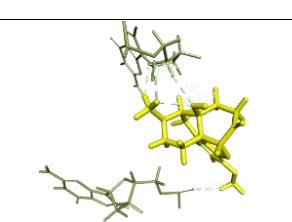
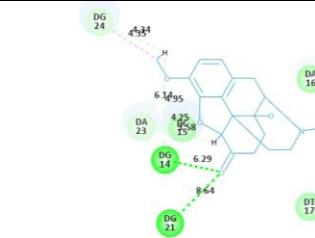
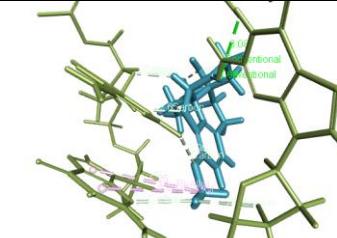
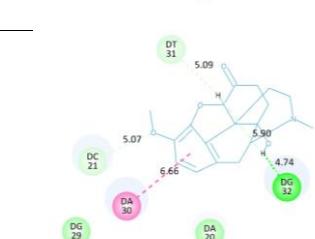
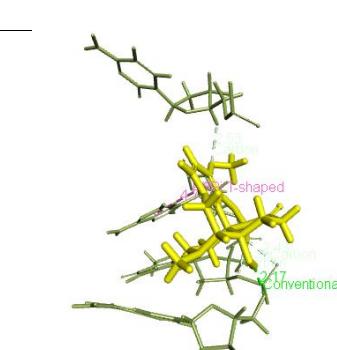
FEN8	-	-	5	2.65	-	-	Pi – Pi stacked	5.36
	-	-	4	2.86	-	-	2 Pi - alkyl	4.85

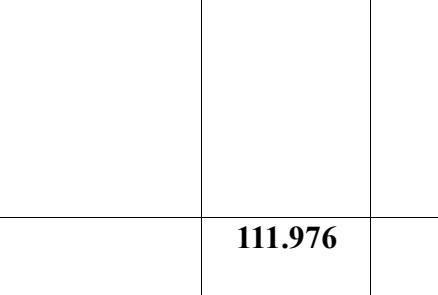
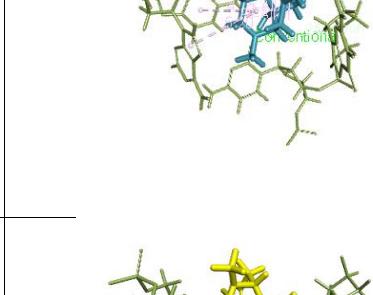
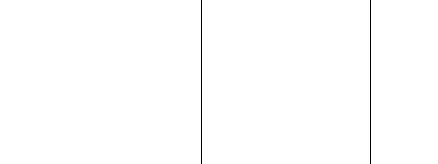
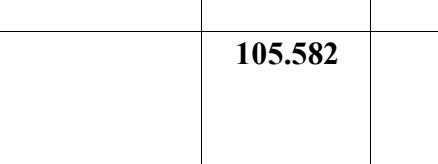
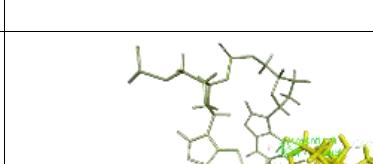
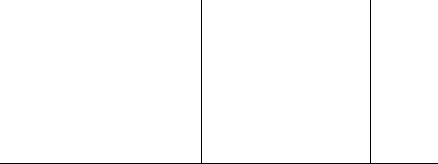
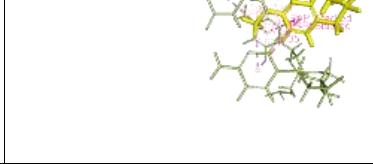
**APTAMER: OXYCODONE: June, 2025**

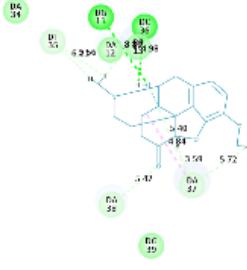
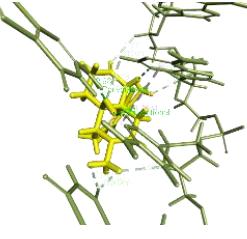
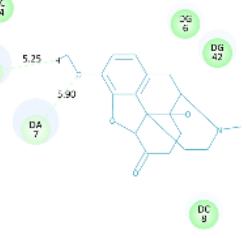
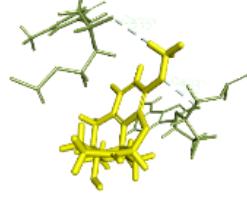
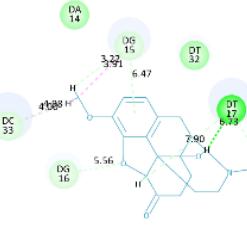
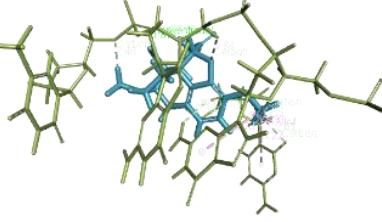
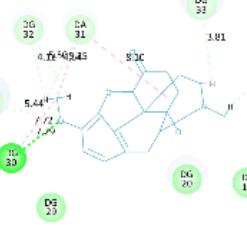
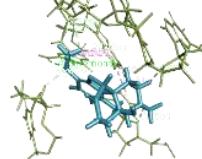
Sequence	Secondary strcuture	Libdock score	2d diagram	Ligand interactions	Mean length
Om1		<b>96.0374</b>			<b>Carbon- hydrogen = 2.61</b>
Om2		<b>85.2247</b>			<b>Carbon- hydrogen = 2.71</b>

Om3		100.829			Carbon-hydrogen = 2.57
Om4		55.8756			Conventional hydrogen = 3.01 Carbon hydrogen = 2.44
Om5		103.179			Conventional hydrogen = 2.556 Carbon hydrogen = 2.66
Om6		97.2575			Carbon hydrogen = 2.44

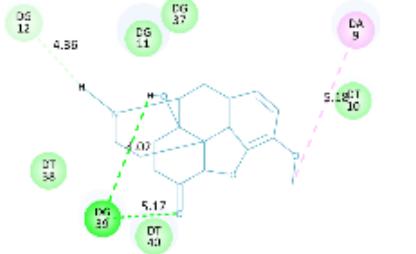
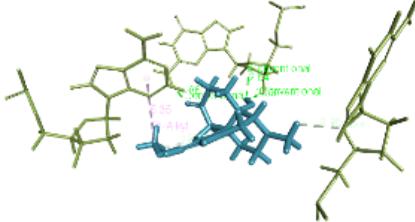
Om7		112.009			Carbon hydrogen=2.47
Om8		104.886			Conventional hydrogen =2.09407  Carbon hydrogen= <b>2.59</b>
Om9		90.9723			Carbon hydrogen= <b>2.56</b>

<b>Om10</b> <b>(Loop 1)</b>					Carbon hydrogen= 2.46
<b>Om10</b> <b>(Loop 2)</b>					Carbon hydrogen= 2.578
<b>Om11</b>		<b>104.823</b>			Conventional hydrogen = 2.52 Carbon hydrogen=2.64
<b>Om12</b>		<b>102.648</b>			Conventional hydrogen = 2.17484 Carbon hydrogen=2.43

Om13		<b>88.2669</b>			Conventional hydrogen = 1.87143 Carbon hydrogen=2.81
Om14		<b>111.976</b>			Conventional hydrogen = 2.17694 Carbon hydrogen=2.65
Om15		<b>105.582</b>			Conventional hydrogen = 2.17 Carbon hydrogen= 2.37895
Om16		<b>96.7257</b>			Conventional hydrogen = 2.49 Carbon hydrogen= 2.66

Om17		<b>88.3048</b>			Conventional hydrogen = 2.56 Carbon hydrogen= 2.62
Om19		<b>94.4124</b>			Carbon hydrogen= 2.66
Om20		<b>95.3504</b>			Conventional hydrogen = 1.87366 Carbon hydrogen= 2.61
Om22		<b>111.647</b>			Conventional hydrogen = 2.35173 Carbon hydrogen= 2.64



<b>Om36</b>		<b>101.165</b>	 	Conventional hydrogen = 2.36 Carbon hydrogen= 2.56	

*Thermodynamic details of the original aptamer sequences:*

Aptamer	Loop	LibDock Score	Poses	Potential Energy (Kcal/mol)	Van der Waals Energy (Kcal/mol)	Electrostatic Energy (Kcal/mol)
<b>Om1</b>	Loop 1	96.0374	61	-200.73488	-580.47973	-1881.85719
<b>Om2</b>	Loop 1	85.2247	3	363.76322	-510.24311	-1791.58823
<b>Om3</b>	Loop 1	100.829	34	203.59272	-558.30750	-1537.87002
<b>Om4</b>	Loop 1	55.8756	3	263.33386	-591.30110	-1416.59646
<b>Om5</b>	Loop 1	103.179	54	527.19474	-534.53132	-1454.27076
<b>Om6</b>	Loop 1	97.2575	34	737.07330	-545.39207	-1194.34724
<b>Om7</b>	Loop 1	112.009	33	3720.54413	339.82160	-1235.72556
<b>Om8</b>	Loop 1	104.886	43	688.18495	-561.87882	-1121.97511
<b>Om9</b>	Loop 1	90.9723	43	-33.93414	-541.31739	-1719.75534
<b>Om10</b>	Loop 1	99.1992	48	-325.22818	-587.95594	-2011.09443
<b>Om10</b>	Loop 2	99.6803	58	-316.56818	-586.06136	-2005.36331
<b>Om11</b>	Loop 1	104.823	20	563.62782	-505.15916	-1634.75519
<b>Om12</b>	Loop 1	102.648	14	3413.47779	177.68932	-1227.66480

<b>Om13</b>	Loop 1	88.2669	21	-82.72360	-574.90705	-1818.74369
<b>Om14</b>	Loop 1	111.976	25	-116.83076	-593.01786	-1785.20808
<b>Om15</b>	Loop 1	105.582	7	-521.69179	-568.47614	-2143.05190
<b>Om16</b>	Loop 1	96.7257	52	185.03648	-512.74172	-1579.63574
<b>Om17</b>	Loop 1	88.3048	12	62.22770	-576.87280	-1669.16842
<b>Om19</b>	Loop 1	94.4124	9	481.42475	-565.70551	-1191.92489
<b>Om20</b>	Loop 1	95.3504	49	26.93459	-546.74518	-1711.29622
<b>Om22</b>	Loop 1	111.647	16	3478.39814	166.10728	-1338.16969
<b>Om25</b>	Loop 1	96.4731	12	147.67846	-591.91642	-1558.35047
<b>Om28</b>	Loop 1	91.7221	47	-274.95603	-611.68420	-1862.39485
<b>Om29</b>	Loop 1					
<b>Om31</b>	Loop 1	98.6253	22	54.09763	-548.55827	-1613.96784
<b>Om36</b>	Loop 1	101.165	25	526.23299	-596.99538	-1340.70276

*Interaction and Bonding details of the sequences:*

Types of Bonds								
	Conventional hydrogen bond	Mean Length	C-H bond	Mean Length	Electrostatic interaction	Mean Length	Hydrophobic interaction	Mean Length
<b>Om1</b>			2	2.61				
<b>Om2</b>			5	2.71				
<b>Om3</b>			5	2.57				
<b>Om4</b>	1	3.01	3	2.44				
<b>Om5</b>	5	2.556	3	2.66			1(Pi-Pi T-shaped) 3(Pi-Alkyl)	5.13537 5.14
<b>Om6</b>			2	2.44			2(Pi-Alkyl)	4.25
<b>Om7</b>			5	2.47			1(Pi-Pi T-shaped) 3(Pi-Alkyl)	4.79165 5.37
<b>Om8</b>	1	2.09407	3	2.59	Pi-Anion	4.41921	1(Pi-Pi T-shaped) 4(Pi-Alkyl)	5.45643 5.035
<b>Om9</b>			6	2.56			3(Pi-Alkyl)	4.45

<b>Om10</b>			2	2.46				
<b>Om10</b>			5	2.578				
<b>Om11</b>	2	2.52	6	2.64			2(Pi-Alkyl)	5.08
<b>Om12</b>	1	2.17484	3	2.43			Pi-Pi T-shaped	4.7921
<b>Om13</b>	1	1.87143	2	2.81			2(Pi-Alkyl)	5.02
<b>Om14</b>	1	2.17694	6	2.65			2(Pi-Alkyl)	5.15
<b>Om15</b>	2	2.17	1	2.37895			2(Pi-Pi Stacked) 4(Pi-Alkyl)	5.61 4.347
<b>Om16</b>	8	2.49	4	2.66			1(Pi-Pi Stacked) 4(Pi-Alkyl)	4.6563 4.45
<b>Om17</b>	2	2.56	7	2.62			1(Pi-Alkyl)	5.17104
<b>Om19</b>			2	2.66				
<b>Om20</b>	1	1.87366	5 1(Pi-Donor Hydrogen Bond)	2.61 (2.68577)			2(Pi-Alkyl)	5.22
<b>Om22</b>	1	2.35173	6	2.64			3(Pi-Alkyl)	5.02
<b>Om25</b>	2	2.11	1	2.76507				
<b>Om28</b>		2.21585	5	2.64				
<b>Om29</b>								
<b>Om31</b>			3	2.37			5 (Pi-Alkyl) 1(Pi-Pi Stacked)	5.088 5.48349
<b>Om36</b>	3	2.36	2	2.56			1 (Pi-Alkyl)	5.3462

### APTAMER: TRAMADOL : July 1, 2025

Aptamer	Loop no.	Poses	Libdock score	2d diagram	Ligand interactions	Mean length
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TR_OG	<b>1</b>	<b>61</b>	<b>113.947</b>		<p>Conventional H = 2.03, (1) CH = 2.62, (4) Pi- alkyl = 4.88 (4)</p>
	<b>2</b>	<b>60</b>	<b>90.3457</b>		<p>Conventional H= 1.94, (1) CH = 2.57 (5) Pi- pi T shaped = 5.73 (2) Pi- alkyl = 3.81</p>
	<b>3</b>	<b>62</b>	<b>86.0369</b>		<p>Conventional H= 1.80 (1) CH = 2.61 (3) Pi- pi T shaped = 4.27 (2) Pi- alkyl = 3.55</p>

*Thermodynamic details of the aptamer sequence:*

Aptamer Sequence	Loop	Libdock score	Poses	Potential Energy (kcal/ mol)	Vander Waals Energy (Kcal/mol)	Electrostatic Energy (Kcal/mol)
TR_OG	1	113.947	61	585.80865	-488.87541	-1122.62781
	2	90.3457	60	599.63969	-505.46356	-1095.13311
	3	86.0369	62	579.21985	-496.55865	-1121.39748

*Interaction and Bonding details of the sequence:*

Type of Bonds									
Aptamer	Loop	Conventional hydrogen bond	Mean Length	C- H bond	Mean length	Electrostatic Interaction	Mean length	Hydrophobic Interaction	Mean length
TR_OG	1	1	2.03	4	2.62	NA	NA	4 (Pi- alkyl)	4.88
	2	1	1.94	5	2.57	NA	NA	3 (2- Pi- pi T shaped, 1- Pi- alkyl)	Pi- pi T shaped = 5.73, Pi- alkyl = 3.81
	3	1	1.80	3	2.61	NA	NA	3 (2- Pi- pi T shaped, 1- Pi- alkyl)	Pi- pi T shaped = 4.27, Pi- alkyl = 3.55

## APTAMER: CODEINE: AUGUST, 2025

Aptamers binding to **Codeine** are **FC5** and **FC45**. Sequences for these aptamers-

FC5=> 5'-GGGACAGGGCUAGCAGUAGGAUUGGGUGAGGGGAUGUGCUG-3'

FC45=> 5'-GGGACAGGGCUAGCUUAGUGCUAUGUGAGAAAAGGGUGUGGGGG-3'

Aptamer	Libdock score	Poses	2d diagram	Ligand interactions	Mean length
FC5	89.6173	60			CH = 2.62 (11) Pi- anion = 4.15 (1) Pi- alkyl = 4.66 (4)
FC45	70.938	1			Conventional H = 1.84 (1) CH = 2.69 (5) Pi- alkyl = 4.28 (4)

*Thermodynamic details of the aptamer sequences:*

Aptamer	Loop	Libdock score	Poses	Potential energy (kcal/mol)	Vander Waals Energy (Kcal/mol)	Electrostatic energy (Kcal/mol)
FC5	1	89.6173	60	-91.11823	327.38661	-2336.55048
FC45	1	70.938	1	155.87331	538.38718	-2437.30236

***Interaction and Bonding details of the sequences:***

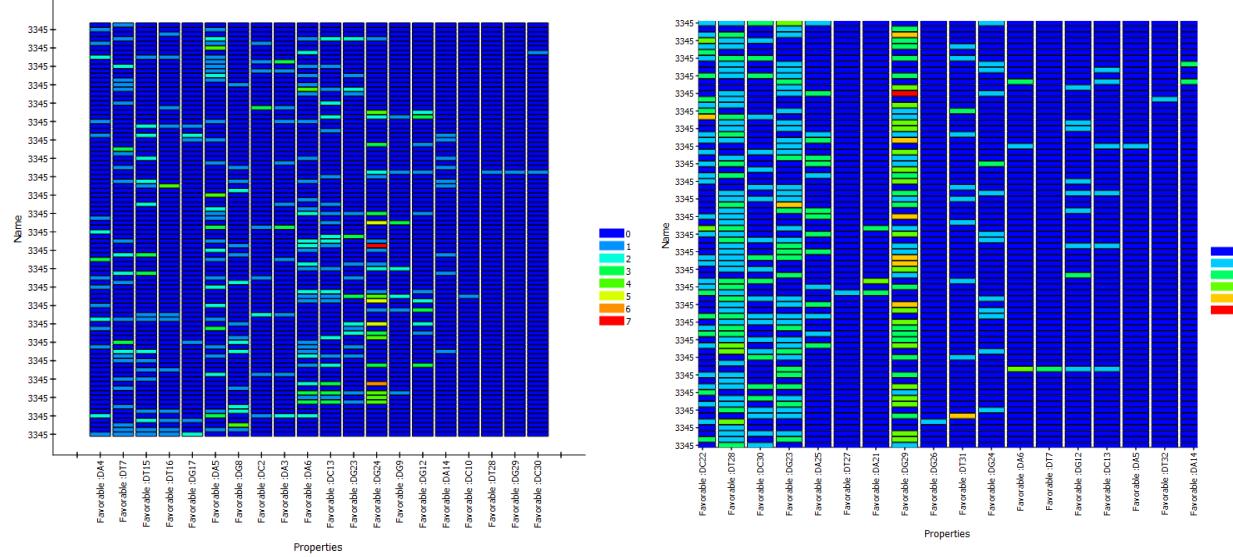
Type of Bonds									
Aptamer	Loop	Conventional hydrogen bond	Mean Length	C- H bond	Mean length	Electrostatic Interaction	Mean length	Hydrophobic Interaction	Mean length
FC5	1	-	-	11	2.62	Pi- anion (1)	4.15	4 (Pi- alkyl)	4.66
FC45	1	1	1.84	5	2.69	NA	NA	Pi- alkyl (4)	4.28

*March 15, 2025*

***POINT MUTATION APPROACH FOR THE BEST ORIGINAL SEQUENCES OF APTAMERS:***

***Fentanyl: Aptamer FEN 8:***

As **FEN8** was the best identified aptamer, we carried out the mutations based on the data of heatmap obtained for the two poses.



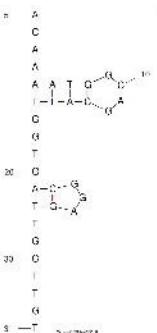
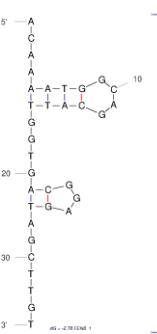
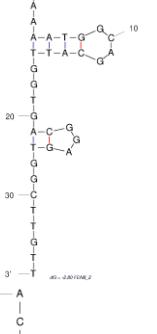
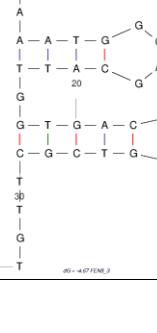
**Fig. Heatmap plot of residue stability for mutation**

According to the heat map, position 29 of the nucleotide sequence showed the most stable binding as most of the poses generated through LibDock for fentanyl showed a high-level binding affinity towards the aptamers 29<sup>th</sup> residue.

Hence, we mutated on 28<sup>th</sup> and 29<sup>th</sup> residue and carry out its permutations and combinations based on this data to check the effect of the 2D constructs and overall molecular docking parameters to generate a novel sequence. Similarly based on this data, mutation on position 5 was carried out.

**Point mutation at the 28<sup>th</sup> and 29<sup>th</sup> position of the aptamer sequence**

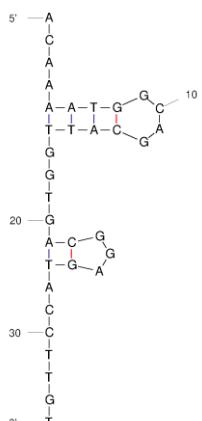
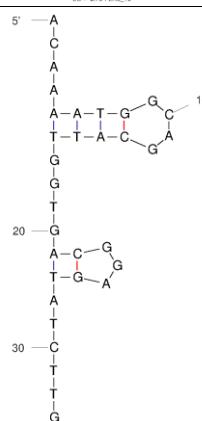
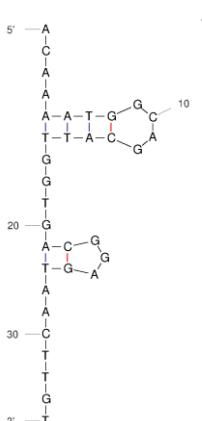
Sr. no.	SEQUENCE	2D Diagram	$\Delta G/\text{kcal mol}^{-1}$	GC %
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OG	ACAAAATGGCAGCATTGGTGACGGAGT <b>TG</b> CTTGT	 <p>5' — A C A A A — T — G — C — 10   —   —   —   T — T — A — C — G G G T G 39 —   — T G — 3' — T</p>	-2.59	47.1
1	ACAAAATGGCAGCATTGGTGACGGAGT <b>AG</b> CTTGT	 <p>5' — A C A A A — T — G — C — 10   —   —   —   T — T — A — C — G G G T G 39 —   — T G — 3' — T</p>	-2.78	47.1
2	ACAAAATGGCAGCATTGGTGACGGAGT <b>GG</b> CTTGT	 <p>5' — A C A A A — T — G — C — 10   —   —   —   T — T — A — C — G G G T G 39 —   — T G — 3' — T</p>	-2.80	50
3	ACAAAATGGCAGCATTGGTGACGGAGT <b>CG</b> CTTGT	 <p>5' — A C A A A — T — G — C — 10   —   —   —   T — T — A — C — G G G — T — G — A — C — G — 20   —   —   —   C — G — C — T — G — A T 39 —   — T G — 3' — T</p>	-4.67	50

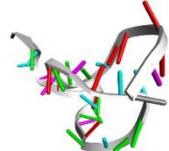
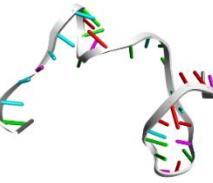
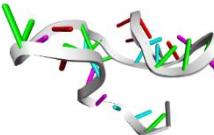
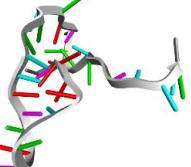
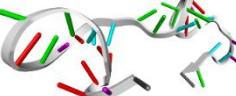
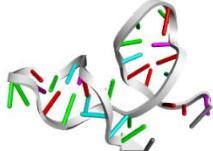
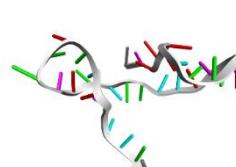
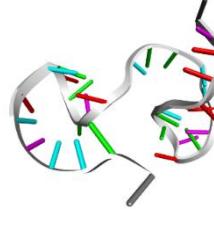
4	ACAAAATGGCAGCATTGGTGACGGAGT <b>TA</b> CTTGT		-2.98	44.1
5	ACAAAATGGCAGCATTGGTGACGGAGT <b>TC</b> CTTGT		-2.59	47.1
6	ACAAAATGGCAGCATTGGTGACGGAGT <b>TT</b> CTTGT		-2.59	44.1

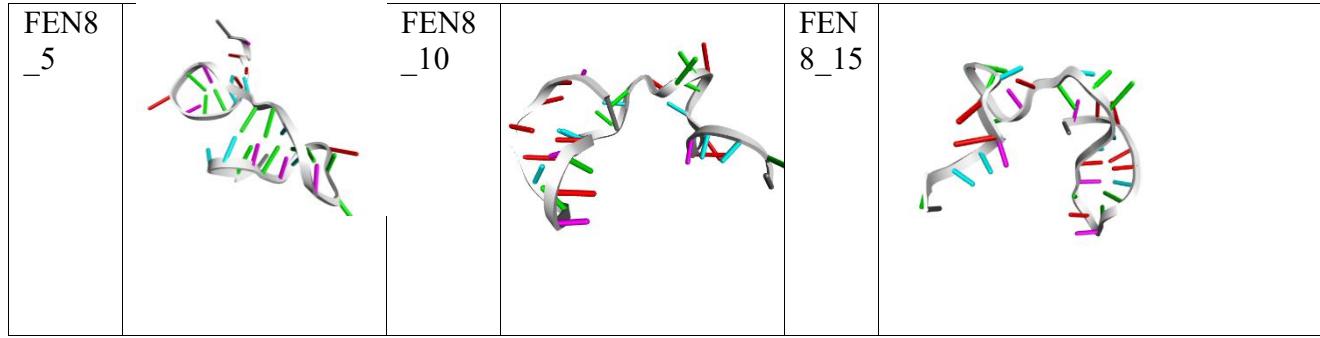
7	ACAAAATGGCAGCATTGGTGACGGAGT <b>CC</b> CTTGT	<p>5' — A   C   A   A   A — A — T — G — C — G — C — 10   T — T — A — C — G — A   G   G   T   G — C — G — G — A   G — C — T — G — T — G   T   G   T   G 3' — T</p> <p>ds = -2.89 FENN 5</p>	-2.89	50
8	ACAAAATGGCAGCATTGGTGACGGAGT <b>GT</b> CTTGT	<p>5' — A   C   A   A   A — A — T — G — C — G — C — 10   T — T — A — C — G — A   G   G   T   G — A — C — G — G — A   C — T — G — T — G   T   T   G   T 3' — T</p> <p>ds = -3.36 FENN 5</p>	-3.36	50
9	ACAAAATGGCAGCATTGGTGACGGAGT <b>GA</b> CTTGT	<p>5' — A   C   A   A   A — A — T — G — C — G — C — 10   T — T — A — C — G — A   G   G   G — T — G — A — C — G — G — A   C — A — G — T — G — A   T   T   G   T   T   T 3' — T</p> <p>ds = -2.87 FENN 5</p>	-2.87	47.06

10	ACAAAATGGCAGCATTGGTGACGGAGT <b>CC</b> CTTGT	<p style="text-align: center;">dG = -3.95 FEB 8</p>	-3.95	50
11	ACAAAATGGCAGCATTGGTGACGGAGT <b>CT</b> CTTGT	<p style="text-align: center;">dG = -4.16 FEB 11</p>	-4.16	47.06
12	ACAAAATGGCAGCATTGGTGACGGAGT <b>CA</b> CTTGT	<p style="text-align: center;">dG = -6.50 FEB 12</p>	-6.50	47.06

13	ACAAAATGGCAGCATTGGTGACGGAGT <b>AC</b> CTTGT	 <small>dG = -2.78 FENB_13</small>	-2.78	47.06
14	ACAAAATGGCAGCATTGGTGACGGAGT <b>AT</b> CTTGT	 <small>dG = -2.78 FENB_14</small>	-2.78	44.12
15	ACAAAATGGCAGCATTGGTGACGGAGT <b>AA</b> CTTGT	 <small>dG = -2.78 FENB_15</small>	-2.78	44.12

*3D structures of the mutated aptamers:*

Apt	3D structure	Apt	3D structure	Apt	3D structure
FEN8_1		FEN8_6		FEN8_11	
FEN8_2		FEN8_7		FEN8_12	
FEN8_3		FEN8_8		FEN8_13	
FEN8_4		FEN8_9		FEN8_14	

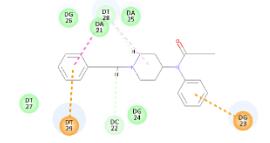
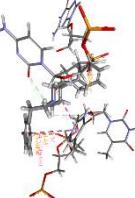
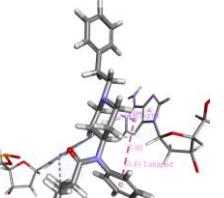
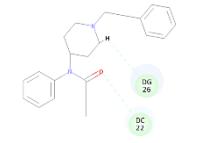
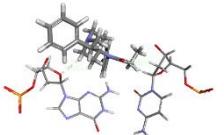
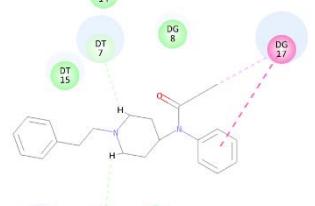
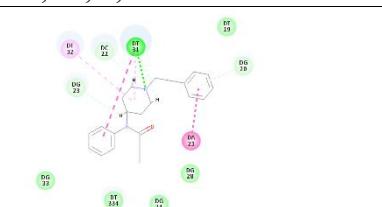
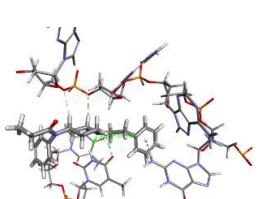


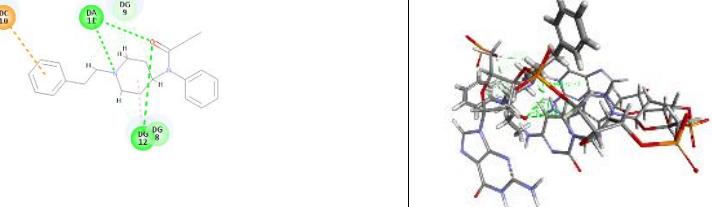
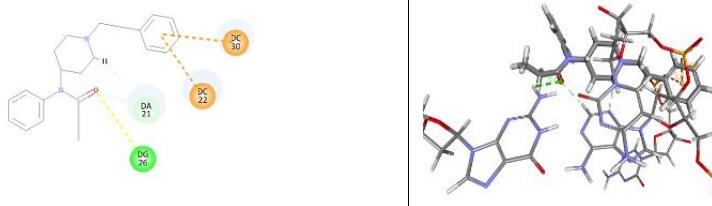
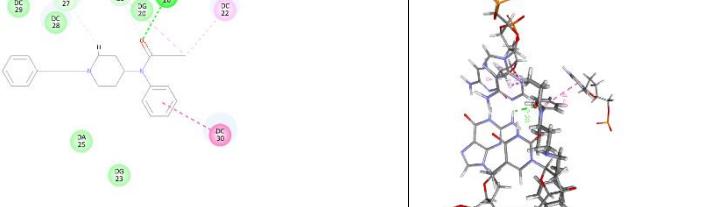
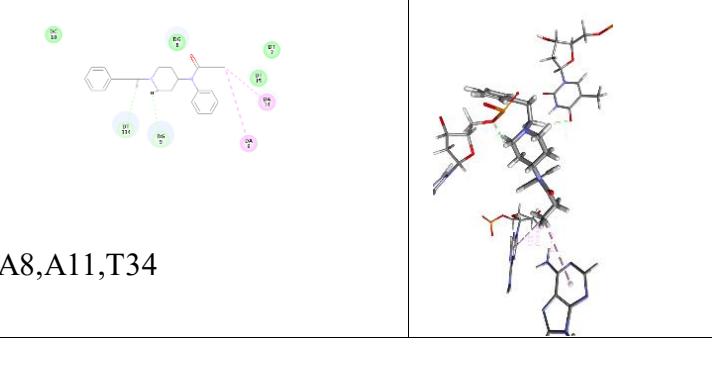
**Docking analysis of the mutated aptamers: JUNE 16, 2025**

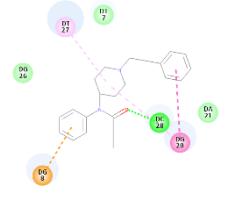
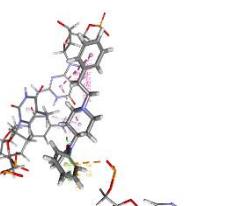
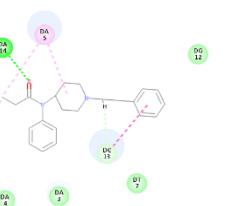
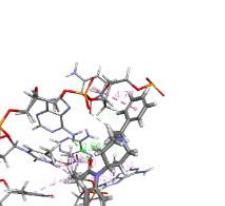
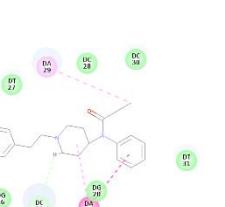
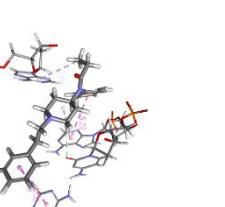
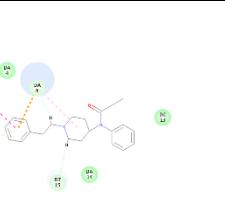
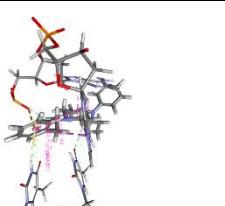
Aptamer	Loop	2D diagram	Interactions	LibDOCK score	Poses
FEN8_1	L1	 C:10 G:33		106.471	79
	L2	 C:22, A:25, T:27, A:28		109.797	58

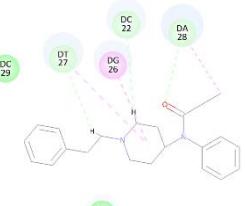
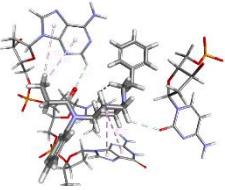
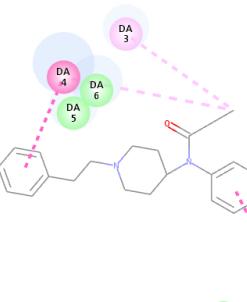
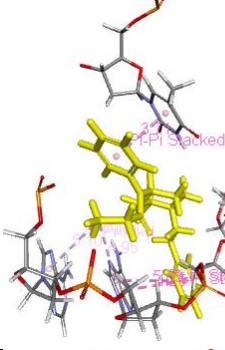
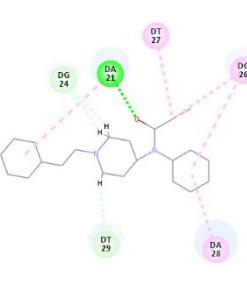
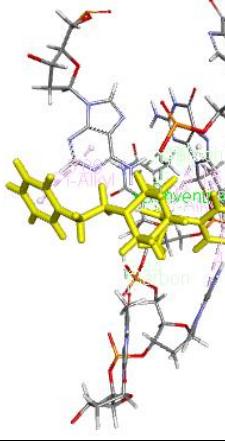
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	L2	<p>A:3,A:5,G:26,G:28</p>		86.0762	33
FEN8_3	L1	<p>G:8</p>		102.148	63
	L2	<p>G:8</p>		113.235	74

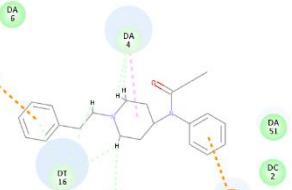
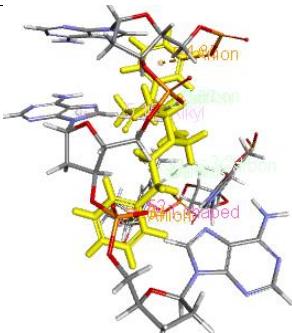
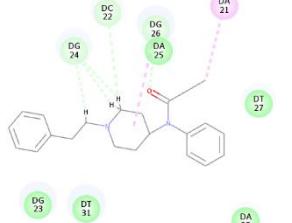
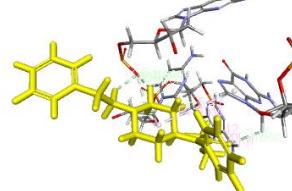
FEN8_4	L1	<p>A:3,A:4,A:5</p>		107.107	73
	L2	<p>A:21,T:28,A:29</p>		114.251	72
FEN8_5	L1	<p>A:3,A:4,A:5,T:16</p>		107.085	77
	L2	<p>T:27,C:29,C:30,T:31</p>		77.2522	18
FEN8_6	L1	<p>A6, G8, T1</p>		65.637	3

FEN8_6	L2	 C22,G23,T28,T29		106.72	61
FEN8_7	L1	 A4, A14		98.51	43
FEN8_7	L2			102.97	75
FEN8_8	L1	 A6,T7,G,17		105.28	75
FEN8_8	L2	 G20,A21,C22,G23,T31,T32		94.33	81

FEN8_9	L1	 G8,G9,C10,A11,G12	80.26	8
FEN8_9	L2	 A21,C22,G26,C30	111.57	72
FEN8_10	L2		86.84	23
FEN8_11	L1	 G6,A8,A11,T34	67.94	7

FEN8_11	L2	 G8,G20,T27,C28		85.1629	77
FEN8_12	L1	 A5,A6,C13,A14,T15,T16		88.9658	31
FEN8_12	L2	 A21,C22,A25,A29		108.249	80
FEN8_13	L1	 A5,A6,T15,T16		67.701	1

FEN8_13	L2	 C22,G26,T27,A28		98.8606	69	
FEN_14	L1		A6 T7 G8 G9 C10 A11 G12 C13 A14 T15		114.208	75
	L2		C22 G23 G24 A25 G26		102.006	66

FEN_15	L1		A6, T7, G8, G9, C10, A11, G12, C13, A14, T15		108.029	69
	L2		C22,G23,G24,A25,G 26		84.0115	68

***Summary of the thermodynamics, Interaction types and Bond lengths of the mutated aptamers***

		Potential Energy (kcal/mol)	Van der Waals Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Favourable interactions	Conventional Hydrogen bonds	Mean length	Non – conventional)	Mean length
FEN8	L1	649.8279	-398.417	-659.075		-	-	5	2.65
	L2	676.4631	-392.879	-654.635		-	-	4	2.86
FEN8_1	L1	926.16360	-367.73344	-545.27817	7	0	N/A	4	2.65
	L2	957.15658	-371.77999	-500.76781	5	0	N/A	4	2.71

FEN8_2	L1	738.03736	-393.61092	-720.52470	7	2	2.73	2	2.77
	L2	714.20594	-395.92531	-737.77166	7	0	N/A	3	2.63
FEN8_3	L1	3233.40923	569.24893	-831.89973	7	0	NA	6	2.60
	L2	3237.30276	543.85668	-790.65194	2	2	2.72	0	0
FEN8_4	L1	2215.50117	-67.91270	-1095.64800	7	0	0	2	2.45
	L2	2223.07248	-76.93442	-1077.96599	8	2	2.695	2	2.95
FEN8_5	L1	442.45160	-335.38257	-977.87224	9	1	2.78	1	2.56
	L2	383.00582	-361.24298	-988.81565	5	1	2.96	1	2.58
FEN8_6	L1	67.92325	-379.257	-1157.13	7	N/A	N/A	3	2.55
	L2	87.10741	-390.696	-1128.39	6	N/A	N/A	2	2.9
FEN8_7	L1	307.6314	-382.699	-988.592	5	N/A	N/A	1	2.72
	L2	314.812	-389.083	-977.491	2	N/A	N/A	2	2.4
FEN8_8	L1	452.36139	-386.42246	-834.18073	4	N/A	N/A	2	2.59
	L2	474.7887	-388.129	-812.682	8	1	1.95	4	2.55
FEN8_9	L1	927.6301	-144.967	-1034.79	11	3	2.37	6	2.50
	L2	902.6833	-168.295	-1030.67	5	1	2.94	2	2.39
FEN8_10	L1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	L2	-34.8585	-386.061	-1293.65	6	1	2.28	2	2.68
FEN8_11	L1	412.8482	-402.14	-855.845	4	N/A	N/A	2	2.74
	L2	457.7802	-372.308	-817.924	4	1	1.90	N/A	N/A
FEN8_12	L1	809.782	-403.751	-683.742	7	1	2.66	2	2.46
	L2	797.9246	-406.455	-690.53	5	N/A	N/A	1	2.73
FEN8_13	L1	328.7943	-357.872	-977.644	6	N/A	N/A	3	2.55
	L2	292.0211	-372.195	-1003.14	6	N/A	N/A	3	2.85
FEN8_14	L1	351.19447	-379.04085	-917.42673	7	N/A	N/A	3	2.64
	L2	375.81768	-343.82951	-905.42269	7	1	1.87	4	2.53
FEN8_15	L1	500.1722	-353.63802	-788.43528	10	N/A	N/A	5	2.45

	L2	489.69709	-360.75214	-785.97364	7	N/A	N/A	5	2.71
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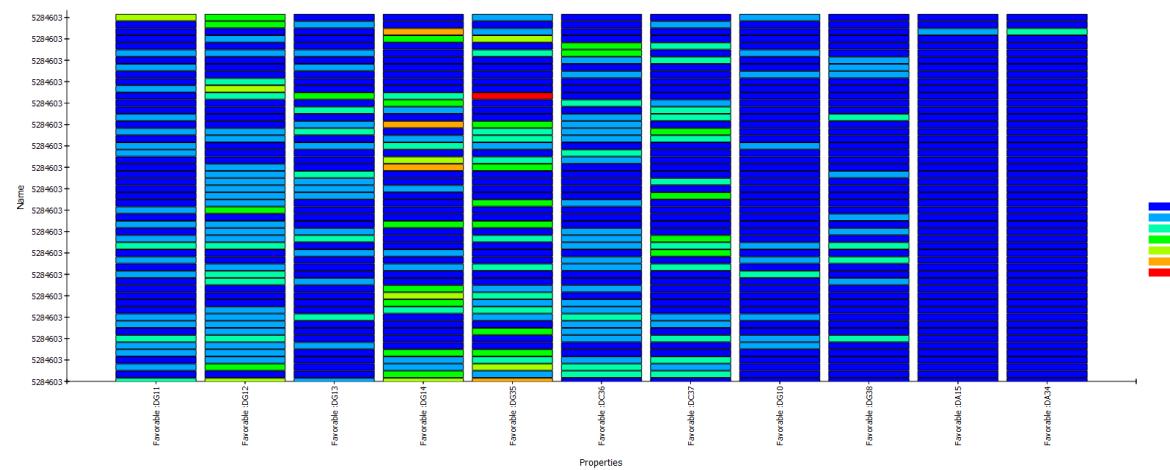
**Oxycodone: Aptamer: OM16**

**JUNE 20, 2025**

**Mutations at the 34<sup>th</sup> and 35<sup>th</sup> residue:**

Aptamer binding to Oxycodone is OM16. Sequence for Om16-

5'-GGGACGACGGGGGGAGTAATGTTGTGGGGGAGCCGGTGTCCC -3'



	MUT	SEQUENCE	2D diagram	$\Delta G/\text{kcal mol}^{-1}$	GC %	T <sub>m</sub>
	NM	GGGACGACGGGGGGAGTAATGTTGTGTGGGGG <b>A</b> GCGGTCGTCCC				
1	A:34 A:35	GGGACGACGGGGGGAGTAATGTTGTGTGGGGG <b>A</b> ACC CGGT CGTCCC				
2	A:34 T:35	GGGACGACGGGGGGAGTAATGTTGTGTGGGGG <b>A</b> TCC CGGT CGTCCC				
3	A:34 C:35	GGGACGACGGGGGGAGTAATGTTGTGTGGGGG <b>A</b> CC CGGT CGTCCC				
4	T:34 A:35	GGGACGACGGGGGGAGTAATGTTGTGTGGGGG <b>T</b> ACCG GT CGTCCC				
5	T:34 T:35	GGGACGACGGGGGGAGTAATGTTGTGTGGGGG <b>T</b> TCC CGGT CGTCCC				
6	T:34 G:35	GGGACGACGGGGGGAGTAATGTTGTGTGGGGG <b>T</b> GCC CGGT CGTCCC				
7	T:34 C:35	GGGACGACGGGGGGAGTAATGTTGTGTGGGGG <b>T</b> CCC CGGT CGTCCC				
8	G:34 A:35	GGGACGACGGGGGGAGTAATGTTGTGTGGGGG <b>G</b> ACCG GT CGTCCC				
9	G:34 T:35	GGGACGACGGGGGGAGTAATGTTGTGTGGGGG <b>G</b> TCC CGGT CGTCCC				
10	G:34 G:35	GGGACGACGGGGGGAGTAATGTTGTGTGGGGG <b>G</b> GCC CGGT CGTCCC				
11	G:34 C:35	GGGACGACGGGGGGAGTAATGTTGTGTGGGGG <b>G</b> CCC CGGT CGTCCC				
12	C:34 A:35	GGGACGACGGGGGGAGTAATGTTGTGTGGGGG <b>C</b> ACCG GT CGTCCC				
13	C:34 T:35	GGGACGACGGGGGGAGTAATGTTGTGTGGGGG <b>C</b> TCC CGGT CGTCCC				
14	C:34 G:35	GGGACGACGGGGGGAGTAATGTTGTGTGGGGG <b>C</b> GCC CGGT CGTCCC				
15	C:34 C:35	GGGACGACGGGGGGAGTAATGTTGTGTGGGGG <b>C</b> CCC CGGT CGTCCC				

## Docking analysis: JUNE 20, 2025

Aptamer	Loop	Loop co-ordinates	2D diagram	Interactions	Mean length	LibDOCK score	Poses
OM16_1	1	11- 36	<p>Interactions</p> <ul style="list-style-type: none"> <li>van der Waals</li> <li>Conventional Hydrogen Bond</li> <li>Carbon Hydrogen Bond</li> <li>Pi-alkyl</li> </ul>		CH = 2.66 Conventional H = 1.97 Pi- alkyl = 4.6	87.9748	54
OM16_2	1	11- 36	<p>Interactions</p> <ul style="list-style-type: none"> <li>van der Waals</li> <li>Conventional Hydrogen Bond</li> <li>Carbon Hydrogen Bond</li> <li>Pi-alkyl</li> </ul>		CH = 2.67 Conventional H = 2.24 Pi- alkyl = 5.14	107.014	27
OM16_3	1	12- 35	<p>Interactions</p> <ul style="list-style-type: none"> <li>van der Waals</li> <li>Conventional Hydrogen Bond</li> <li>Carbon Hydrogen Bond</li> <li>Pi-Sigma</li> <li>Pi-H T-shaped</li> <li>Pi-alkyl</li> </ul>		CH = 2.77 Conventional H = 2.46 Pi- alkyl = 4.80 Pi-pi T shaped= 5.41 Pi- sigma = 2.43	98.8269	11

OM16_4	1	11- 36	<p><b>Interactions:</b></p> <ul style="list-style-type: none"> <li>van der Waals (green)</li> <li>Conventional Hydrogen Bond (green dashed)</li> <li>Carbon Hydrogen Bond (light green)</li> <li>Pi-Sigma (purple)</li> <li>Pi-Pi Stacked (pink)</li> <li>Pi-Alkyl (light blue)</li> </ul>		CH = 2.48 Conventional H = 2.45	82.7639	31
OM16_5	1	11-36	<p><b>Interactions:</b></p> <ul style="list-style-type: none"> <li>van der Waals (green)</li> <li>Carbon Hydrogen Bond (light green)</li> <li>Pi-Anion (orange)</li> </ul>		CH= 2.70	109.799	47
OM16_6	1	11-36	<p><b>Interactions:</b></p> <ul style="list-style-type: none"> <li>van der Waals (green)</li> <li>Conventional Hydrogen Bond (green dashed)</li> </ul>		CH= 2.56 Conventional H= 2.70	108.906	10

OM16_7	1	12- 35	<p><b>Interactions</b></p> <ul style="list-style-type: none"> <li>van der Waals</li> <li>Carbon Hydrogen Bond</li> </ul>		CH = 2.68	107.966	29
OM16_8	1	11-36	<p><b>Interactions</b></p> <ul style="list-style-type: none"> <li>van der Waals</li> <li>Conventional Hydrogen Bond</li> <li>Carbon Hydrogen Bond</li> <li>Pi-Pi Stacked</li> <li>Pi-Alkyl</li> </ul>		CH = 2.52 Conventional H = 1.85	80.4167	55
OM 16_9	1	11-36	<p><b>Interactions</b></p> <ul style="list-style-type: none"> <li>van der Waals</li> <li>Conventional Hydrogen Bond</li> <li>Carbon Hydrogen Bond</li> <li>Pi-Pi Stacked</li> <li>Pi-Alkyl</li> </ul>		CH= 2.88 Conventional H= 2.30	108.578	48

OM 16_10	1	11-36	<p>Interactions</p> <ul style="list-style-type: none"> <li>van der Waals</li> <li>Conventional Hydrogen Bond</li> <li>Carbon Hydrogen Bond</li> <li>Pi-Alkyl</li> </ul>		CH = 2.47 Conventional H = 1.87	73.1858	66
OM 16_11	1	12- 35	<p>Interactions</p> <ul style="list-style-type: none"> <li>van der Waals</li> <li>Conventional Hydrogen Bond</li> <li>Carbon Hydrogen Bond</li> <li>Pi-Alkyl</li> </ul>		CH = 2.68 Conventional H = 2.51	101.511	12
OM 16_12	1	11- 36	<p>Interactions</p> <ul style="list-style-type: none"> <li>van der Waals</li> <li>Conventional Hydrogen Bond</li> <li>Carbon Hydrogen Bond</li> <li>Pi-Alkyl</li> </ul>		CH = 2.82	92.8741	6
Om16_13	1	12-35			CH = 2.54 Conventional H = 2.41	89.428	9

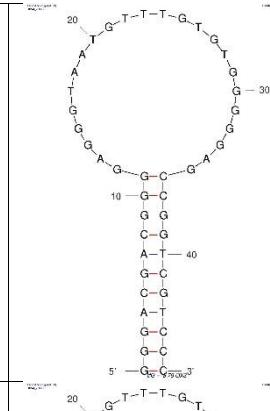
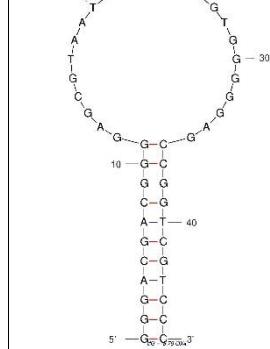
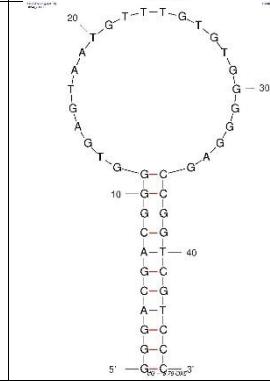
			<p><b>Interactions</b></p> <ul style="list-style-type: none"> <li>van der Waals</li> <li>Conventional Hydrogen Bond</li> <li>Pi-Anion</li> <li>Pi-Alkyl</li> </ul>			
OM 16_14	1	11- 36	<p><b>Interactions</b></p> <ul style="list-style-type: none"> <li>van der Waals</li> <li>Carbon Hydrogen Bond</li> <li>Pi-Alkyl</li> </ul>		CH = 2.42	113.754
OM 16_15	1	13- 34	<p><b>Interactions</b></p> <ul style="list-style-type: none"> <li>van der Waals</li> <li>Carbon Hydrogen Bond</li> </ul>		CH = 2.21	107.636

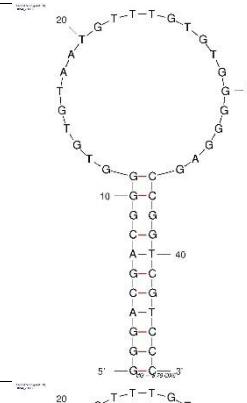
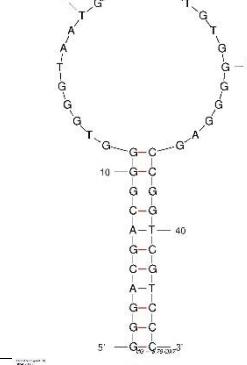
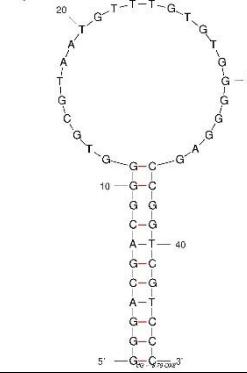
***Summary of Thermodynamics, Interaction types and Bond lengths of mutated aptamers:***

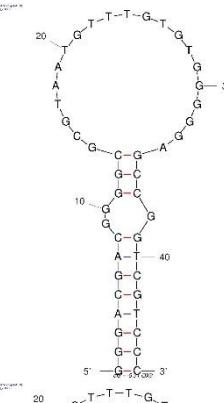
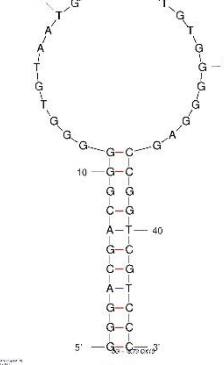
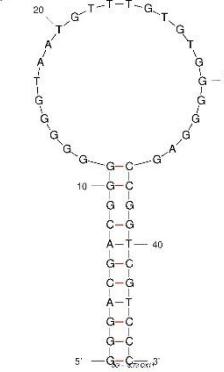
Mutated Aptamer	Loop	Potential Energy	Vander Waals Energy	Electrostatic Energy	Favourable Interactions	Conventional Hydrogen Bonds	Mean length	Non-conventional	Mean length
OM 16	L1	185.036	-512.742	-1579.64	5	8	2.49	1	2.37
OM 16_1	L1	1.7739e+12	1.7739e+12	-1639.6	4	1	1.97	1	2.66
OM16_2	L1	401.253	-556.017	-1642.73	6	2	2.24	2	2.67
OM16_3	L1	-50.0504	-591.75	-1890.39	6	3	2.46	2	2.77
OM16_4	L1	19.4459	-512.665	-1737.11	5	2	2.45	2	2.48
OM16_5	L1	368.195	-610.567	-1415.28	5	-	-	7	2.70
OM16_6	L1	476.806	-603.307	-1294.05	3	1	2.70	2	2.56
OM16_7	L1	122.563	-596.938	-1743.08	2	-	-	2	2.68
OM16_8	L1	-83.8139	-502.611	-1885.46	4	1	1.85	2	2.52
OM16_9	L1	21305.6	19176	-1581.83	4	4	2.30	3	2.88
OM16_10	L1	259.614	-489.695	-1795.99	3	1	1.87	1	2.47
OM16_11	L1	-238.747	-604.895	-1898.13	6	4	2.51	3	2.68
OM16_12	L1	617.656	-511.377	-1723.15	2	-	-	4	2.82
OM16_13	L1	1411.61	-557.228	-605.474	5	2	2.41	2	2.54
OM16_14	L1	425.362	-582.116	-1358.5	3	-	-	3	2.42
OM16_15	L1	445.786	-599.469	-1322.26	3	-	-	6	2.21

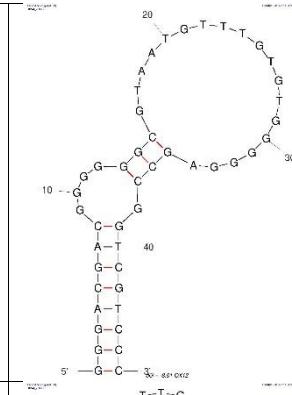
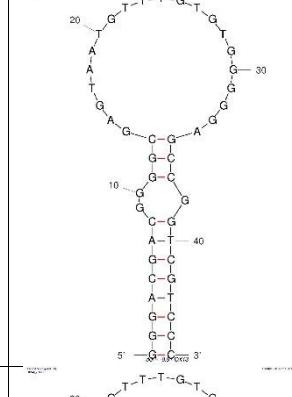
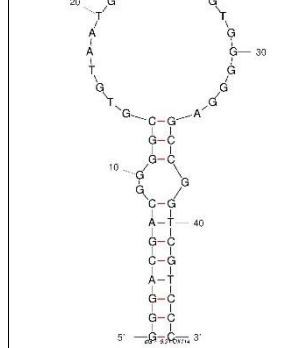
***Mutations at the 13<sup>th</sup> and 15<sup>th</sup> residue: JULY, 2025***

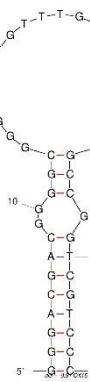
	MUT	SEQUENCE	2D diagram	$\Delta G/$ kcal $mol^{-1}$
	NM	GGGACGACGGGG <b>GG</b> <b>A</b> GTAATGTTGTGGGGAGCCGGTCG TCCC		-8.79
OX1	A:13 A:15	GGGACGACGGGG <b>AG</b> <b>A</b> GTAATGTTGTGGGGAGCCGGTCG TCCC		-8.79
OX 2	A:13 T:15	GGGACGACGGGG <b>AG</b> <b>T</b> GTAATGTTGTGGGGAGCCGGTCG TCCC		-8.79

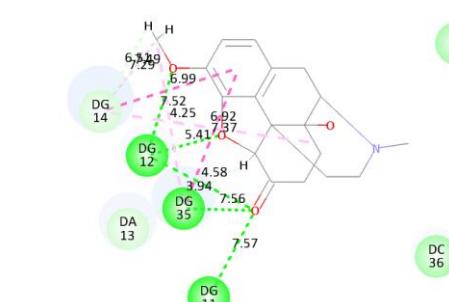
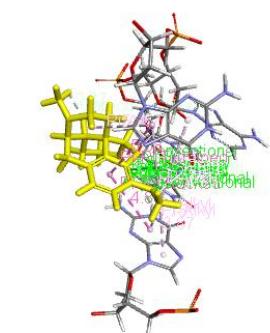
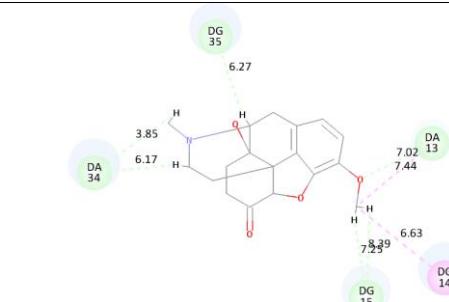
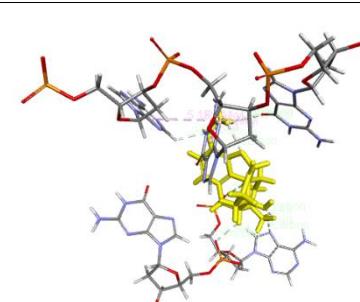
OX 3	A:13 G:15	GGGACGACGGGG <b>AGG</b> <b>G</b> TAAATGTTGTGTGGGGAGCCGGTCG TCCC		-8.79
OX 4	A:13 C:15	GGGACGACGGGG <b>AGC</b> <b>G</b> TAAATGTTGTGTGGGGAGCCGGTCG TCCC		-8.79
OX 5	T:13 A:15	GGGACGACGGGG <b>TGAG</b> TAAATGTTGTGTGGGGAGCCGGTCG TCCC		-8.79

OX 6	T:13 T:15	GGGACGACGGGGTGTGTAATGTTGTGTGGGGAGCCGGTCG TCCC		- 8.79
OX 7	T:13 G:15	GGGACGACGGGGTGGGTAATGTTGTGTGGGGAGCCGGTCG TCCC		-8.79
OX 8	T:13 C:15	GGGACGACGGGGTGCCTAATGTTGTGTGGGGAGCCGGTCG TCCC		-8.79

OX 9	C:13 C:15	GGGACGACGGGGCG <b>G</b> <b>C</b> GTAATGTTGTGTGGGGAGCCGGTCG TCCC		-9.91
OX 10	G:13 T:15	GGGACGACGGGG <b>G</b> <b>T</b> GTAATGTTGTGTGGGGAGCCGGTCG TCCC		-8.79
OX 11	G:13 G:15	GGGACGACGGGG <b>G</b> <b>G</b> GTAATGTTGTGTGGGGAGCCGGTCG TCCC		-8.79

OX 12	G:13 C:15	GGGACGACGGGG <b>GGC</b> GTAATGTTGTGTGGGGAGCCGGTCG TCCC		-8.79
OX 13	C:13 A:15	GGGACGACGGGG <b>CGA</b> GTAATGTTGTGTGGGGAGCCGGTCG TCCC		-9.91
OX 14	C:13 T:15	GGGACGACGGGG <b>CGT</b> GTAATGTTGTGTGGGGAGCCGGTCG TCCC		-9.91

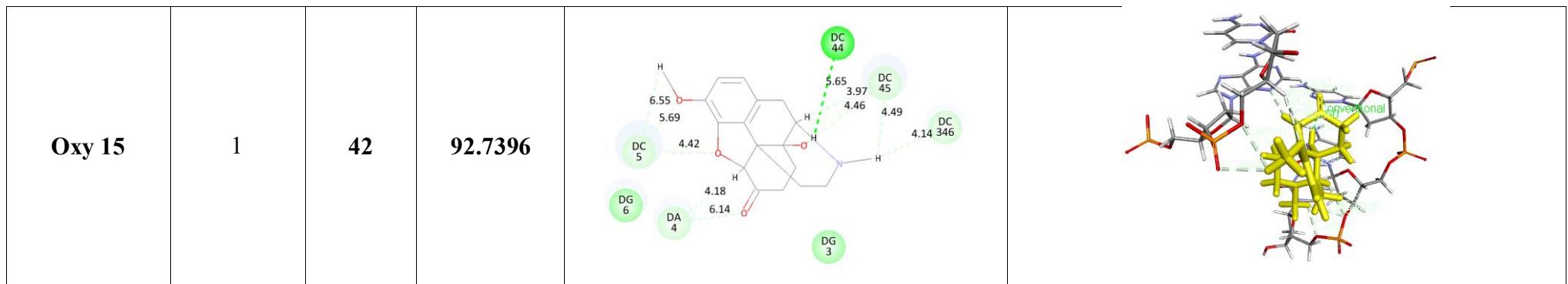
OX 15	C:13 G:15	GGGACGACGGGG <b>CGG</b> TAATGTTGTGGGGAGCCGGTCG TCCC		- 9.91
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Aptamer	Loop no.	Poses	Libdock score	2d diagram	Ligand interactions
Oxy 1	1	49	100.636		
Oxy 2	1	0			
Oxy 3	1	18	61.6697		

Oxy 4	1	1	32.7432		
Oxy 5	1	52	101.284		
Oxy 6	1	59	91.8696		

Oxy 7	1	64	98.9549		
Oxy 8	1	0			
Oxy 9	1	30	92.535		
Oxy 10	1	0			
Oxy 11	1	5	64.6889		

Oxy 12	1	6	<b>68.4958</b>		
Oxy 13	1	33	<b>89.7462</b>		
Oxy 14	1	39	<b>90.4808</b>		

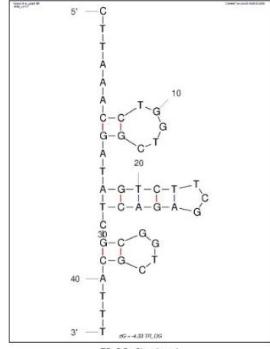
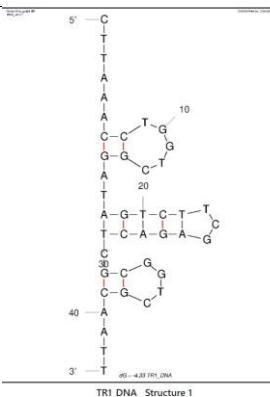
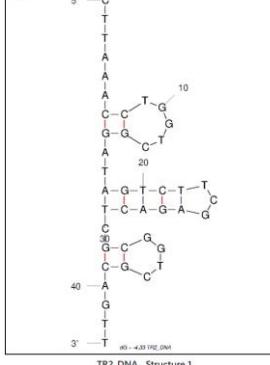


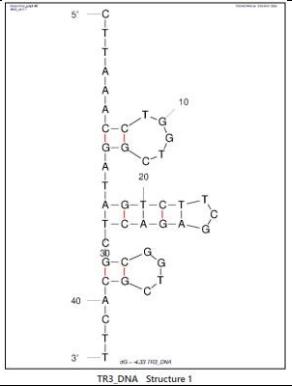
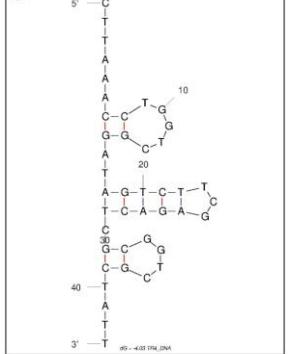
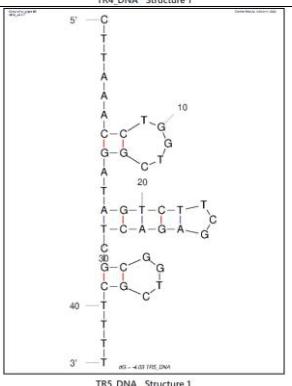
**Summary of Thermodynamics, Interaction Types and Bond lengths of mutated aptamers:**

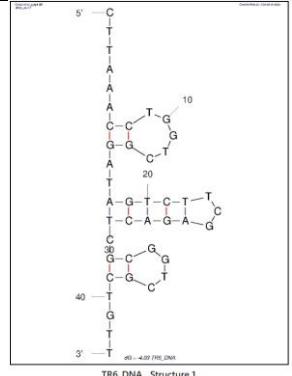
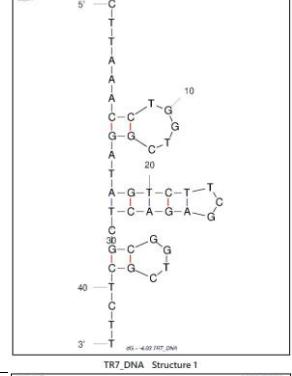
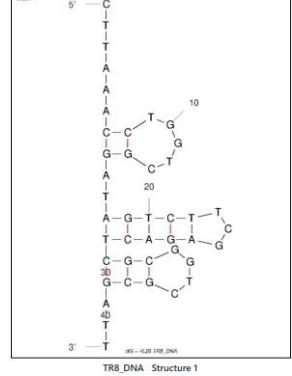
Aptamer Sequence	Loop	Potential Energy (kcal/ mol)	Vander Waals Energy (Kcal/ mol)	Electrostatic Energy (Kcal/ mol)	Favourable Interactions	Conventional Hydrogen bond	Mean length	Non-conventional Bond	Mean length
Oxy 1	1	197.421	-513.371	-1558.16	19	8	2.55	4	2.5
Oxy 2	1				NA	NA			
Oxy 3	1	757.795	-493.817	-1396.96	10	-	-	8	2.63
Oxy 4	1	532.858	-493.27	-1404.17	8	1	2.63	6	2.53
Oxy 5	1	217.959	-517.387	-1558.45	18	7	2.48	5	2.6
Oxy 6	1	317.88	-525.228	-1460.56	7			5	2.68
Oxy 7	1	227.53	-517.937	-1562.94	15	5	2.38	4	2.55
Oxy 8	1				NA	NA			
Oxy 9	1	406.528	-477.432	-1769.23	11	1	2.59918	10	2.61
Oxy 10	1				NA	NA			
Oxy 11	1	478.913	-478.743	-1467.86	7	1	1.82706	2	2.55
Oxy 12	1	444.216	-465.05	-1804.79	6	2	2	2	2.7
Oxy 13	1	368.107	-470.003	-1743.75	9	1	1.90882	6	2.53
Oxy 14	1	395.195	-476.55	-1735.8	8	1	1.8682	5	2.76
Oxy 15	1	461.97	-477.29	-1727.79	1	1	2.59932	10	2.61

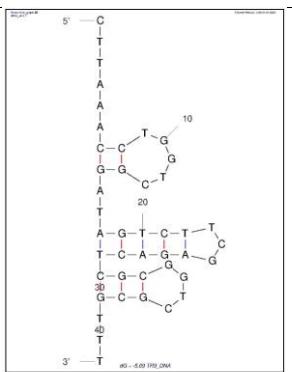
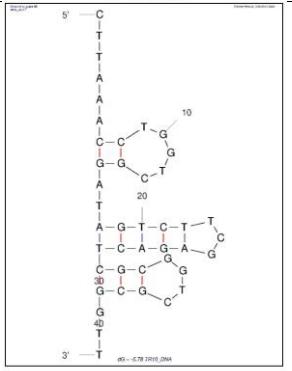
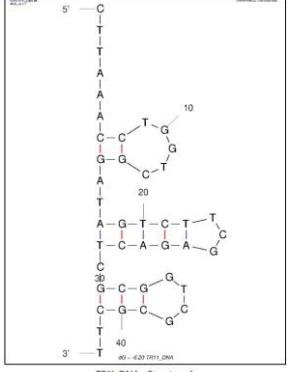
*Tramadol: Aptamer: APT39*

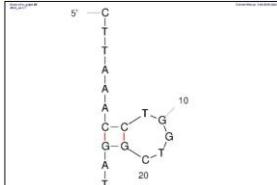
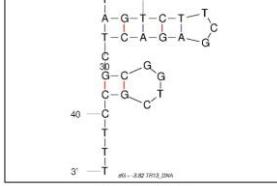
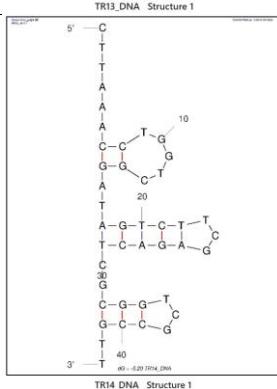
*Mutations at the 40<sup>th</sup> and 41<sup>st</sup> residue: JULY 15, 2025*

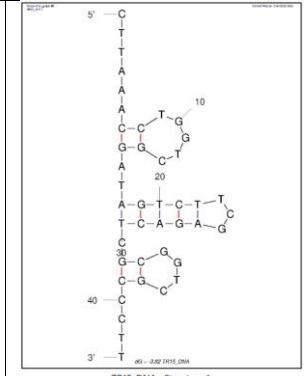
	MU T	SEQUENCE	2D diagram	ΔG/ kcal mol <sup>-1</sup>
	NM	CTTAAACCTGGTCGGATAGTCTCGAGACTCGCGGTCGC <b>A</b> TT		-4.33
TR 1	A:4 0 A:4 1	CTTAAACCTGGTCGGATAGTCTCGAGACTCGCGGTCGC <b>A</b> TT		-4.33
TR 2	A:4 0 G:4 1	CTTAAACCTGGTCGGATAGTCTCGAGACTCGCGGTCGC <b>A</b> G <b>T</b>		-4.33

TR 3	A:4 0 C:4 1	CTTAAACCTGGTCGGATAGTCTTCGAGACTCGCGGTCGC <b>ACTT</b>	 <p>rs5 - 4.33 TR3 DNA</p> <p>TR3 DNA Structure 1</p>	-4.33
TR 4	T:4 0 A:4 1	CTTAAACCTGGTCGGATAGTCTTCGAGACTCGCGGTCGC <b>TATT</b>	 <p>rs5 - 4.03 TR4 DNA</p> <p>TR4 DNA Structure 1</p>	-4.03
TR 5	T:4 0 T:4 1	CTTAAACCTGGTCGGATAGTCTTCGAGACTCGCGGTCGC <b>TTTT</b>	 <p>rs5 - 4.03 TR5 DNA</p> <p>TR5 DNA Structure 1</p>	-4.03

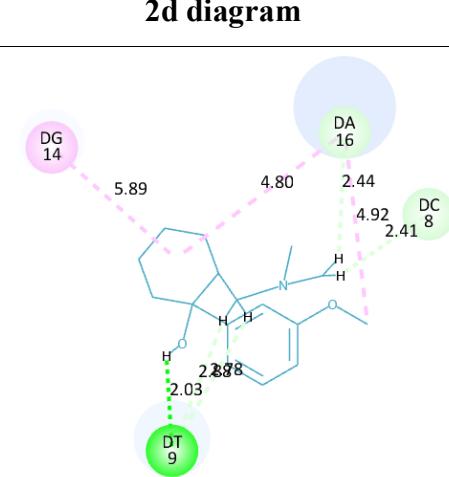
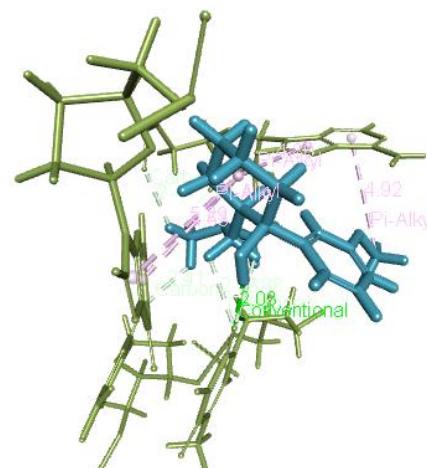
TR 6	T:4 0 G:4 1	CTTAAACCTGGTCGGATAGTCTCGAGACTCGCGGTCGCTGTT		-4.03
TR 7	T:4 0 C:4 1	CTTAAACCTGGTCGGATAGTCTCGAGACTCGCGGTCGCTCTT		-4.03
TR 8	G:4 0 A:4 1	CTTAAACCTGGTCGGATAGTCTCGAGACTCGCGGTCGCGATT		-6.26

TR 9	G:4 0 T:4 1	CTTAAACCTGGTCGGATAGTCTCGAGACTCGCGGTCGCCGT TT	 <p>5' — C T T A A — C C G — T G G — A T G T C T C G A G A C T C G C G G T C G C G C T T — 3'      TR9_DNA_Structure 1      rG = -5.69 TR9_DNA</p>	-5.69
TR 10	G:4 0 G:4 1	CTTAAACCTGGTCGGATAGTCTCGAGACTCGCGGTCGCCGGT T	 <p>5' — C T T A A — C C G — T G G — A T G T C T C G A G A C T C G C G G T C G C G C T T — 3'      TR10_DNA_Structure 1      rG = -5.78 TR10_DNA</p>	-5.78
TR 11	G:4 0 C:4 1	CTTAAACCTGGTCGGATAGTCTCGAGACTCGCGGTCGCCGCTT	 <p>5' — C T T A A — C C G — T G G — A T G T C T C G A G A C T C G C G G T C G C G C T T — 3'      TR11_DNA_Structure 1      rG = -6.20 TR11_DNA</p>	-6.20

TR 12	C:4 0 A:4 1	CTTAAACCTGGTCGGATAGTCTTCGAGACTCGCGGTCGCC <b>CATT</b>		-3.82
TR 13	C:4 0 T:4 1	CTTAAACCTGGTCGGATAGTCTTCGAGACTCGCGGTCGCC <b>CTTT</b>		-3.82
TR 14	C:4 0 G:4 1	CTTAAACCTGGTCGGATAGTCTTCGAGACTCGCGGTCGCC <b>CGTT</b>		-5.20

TR 15	C:4 0 C:4 1	CTTAAACCTGGTCGGATAGTCTTCGAGACTCGCGGTCGCC <b>CCTT</b>		-3.82
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### Docking analysis:

Aptamer	Loop no.	Poses	Libdock score	2d diagram	Ligand interactions	Mean length
TR_OG	<b>1</b>	<b>61</b>	<b>113.947</b>			Conventional H = 2.03, (1) CH = 2.62, (4) Pi- alkyl = 4.88 (4)

	2	60	90.3457			Conventional H = 1.94, (1) CH = 2.57 (5) Pi- pi T shaped = 5.73 (2) Pi- alkyl = 3.81
	3	62	86.0369			Conventional H = 1.80 (1) CH = 2.61 (3) Pi- pi T shaped = 4.27 (2) Pi- alkyl = 3.55
TR_1	1	65	99.8259			Conventional H = 1.88 (1) CH = 2.65 (6) Pi- alkyl = 4.92 (2)

2	61	95.388	<p>Chemical structure diagram of compound 61 showing hydrogen bonding interactions with residues DA28, DG27, DG25, DA26, DT22, DT23, and DC24. Distances are labeled: 5.29, 7.22, 6.02, 4.46, 5.85, 4.52, and 5.58.</p>	<p>3D surface plot of the binding site for compound 61, showing Pi-Pi Stacked, Pi-alkyl, and CH interactions.</p>	$\text{CH} = 2.81 (5)$ $\text{Pi-pi T shaped} = 5.47$ $\text{Pi- alkyl} = 4.90$	
3	59	113.536	<p>Chemical structure diagram of compound 59 showing hydrogen bonding interactions with residues DC33, DG32, DG39, DG34, DG35, DG38, DG37, DT36, and DA40. Distances are labeled: 4.63, 4.86, 4.08, 5.66, 5.70, 3.78, 5.47, 6.15, and 4.71.</p>	<p>3D surface plot of the binding site for compound 59, showing Conventional, Chelatable, and Pi-alkyl interactions.</p>	$\text{Conventional H} = 2.38 (3)$ $\text{CH} = 2.63 (4)$ $\text{Pi- alkyl} = 4.70$	

TR_2	1	69	92.3606		Conventional H= 2.86 (2) CH= 2.77 (5) Pi- pi T shaped = 5.59 Pi- alkyl = 4.99 (4)
	2	72	95.1408		Conventional H= 2.34, CH= 2.80 (6) Pi- pi T shaped = 4.15 Pi- alkyl = 5.27
	3	68	87.7886		Conventional H = 2.65, CH= 2.52 (5)

TR_3	1	60	<b>98.5637</b>			Conventional H = 2.18, (1) CH = 2.51 (6) Pi- pi t shaped = 5.36, Pi- Alkyl = 5.09 (6)
	2	64	<b>94.4791</b>			Conventional H = 2.88 (1) CH= 2.84 (8) Pi- pi T shaped = 5.30, Pi- alkyl = 4.95 (2)
	3	67	<b>100.48</b>			Conventional H = 2.03, CH= 2.70 Pi- sigma = 2.70 Pi- alkyl = 4.59

TR_4	1	69	<b>81.8945</b>			Conventional H = 2.37 (2)  CH= 2.8 (2)  Pi- pi T shaped = 5.44  Pi- Alkyl = 4.45
	2	59	<b>91.546</b>			Conventional H= 2.67 (2)  CH = 2.67(10)  Pi- pi T shaped = 4.48
	3	56	<b>116.637</b>			Conventional H = 2.56 (1)  CH= 2.42 (8)  Pi- alkyl = 4.61 (3)

TR_5	1	8	70.6988			<b>CH = 2.53 (4)</b> <b>Pi-anion = 3.98 (1)</b> <b>Pi-alkyl = 5.16 (1)</b>
	2	78	82.4699			<b>Conventional H = 2.61 (2)</b> <b>CH = 2.67 (7)</b> <b>Pi-alkyl = 4.97 (5)</b>
	3	59	108.862			<b>Conventional = 2.47 (2)</b> <b>CH = 2.62 (2)</b> <b>Pi-alkyl = 4.66 (5)</b>
TR_6	1	0	NA	NA	NA	NA

	2	64	<b>100.651</b>			<b>Conventional</b> $H = 2.17 (2)$ $CH = 2.54 (2)$ <b>Pi- anion =</b> $3.29 (1)$ <b>Pi- alkyl =</b> $4.72 (3)$
TR_7	1	67	<b>86.6841</b>			<b>Conventional</b> $H = 1.83 (1)$ $CH = 2.46 (3)$ <b>Pi- sigma =</b> $2.37 (1)$ <b>Pi- Alkyl =</b> $4.43 (3)$
	2	51	<b>91.9318</b>			<b>Conventional</b> $H = 2.09 (1)$ $CH = 2.59 (7)$

	<b>3</b>	<b>50</b>	<b>111.466</b>	<p>Detailed description: A chemical structure of compound 3 is shown with several substituents labeled DG 35, DG 38, DG 34, DC 37, DC 39, DC 33, DT 36, and DC 36. Bond lengths are indicated: 4.00, 5.47, 6.50, 6.12, 4.99, 3.90, 3.48, and 4.43.</p>	<p>Detailed description: A 3D space-filling model of compound 3 is shown in green, interacting with a protein binding site. Key interactions are labeled: Pi-sigma = 2.59 (1), Pi-pi t shaped = 5.90 (1), and Pi-alkyl = 4.54.</p>	<b>Conventional H = 2.50 (2)</b> <b>CH= 2.49 (3)</b> <b>Pi- sigma = 2.59 (1)</b> <b>Pi- pi t shaped = 5.90 (1)</b> <b>Pi- alkyl = 4.54</b>
TR_8	<b>1</b>	<b>70</b>	<b>105.501</b>	<p>Detailed description: A chemical structure of compound 1 is shown with several substituents labeled DG 15, DG 14, DT 9, DG 10, DG 16, DG 11, DC 8, and DC 13. Bond lengths are indicated: 4.77, 5.56, 6.57, 4.45, and 5.16.</p>	<p>Detailed description: A 3D space-filling model of compound 1 is shown in green, interacting with a protein binding site. Key interactions are labeled: Conventional alkyl = 2.88, Conventional pi = 2.88, and Conventional sigma = 2.03.</p>	<b>Conventional H = 2.34 (2)</b> <b>CH= 2.81 (1)</b> <b>Pi- alkyl = 10.44 (2)</b>
	<b>2</b>	<b>0</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>	<b>NA</b>

	<b>3</b>	<b>53</b>	<b>112.08</b>		
TR_9	<b>1</b>	<b>56</b>	<b>126.15</b>		

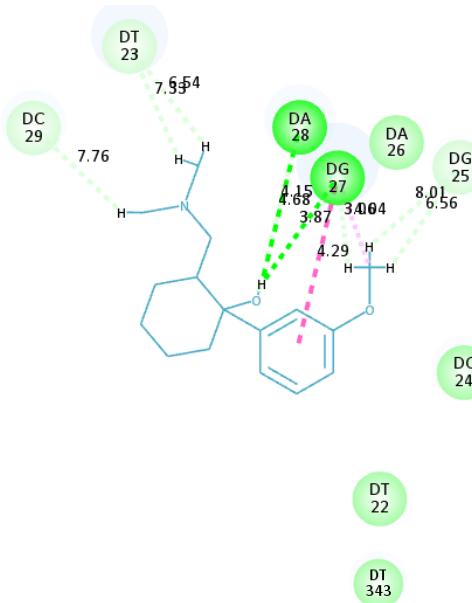
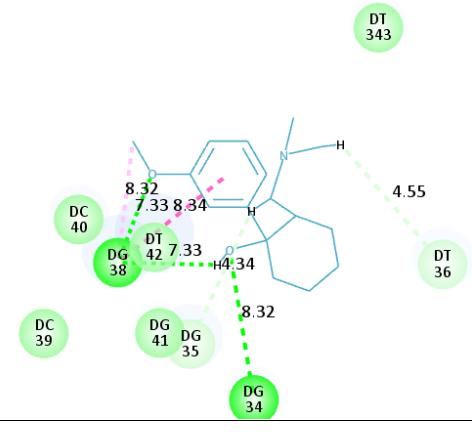
	2	69	<b>89.0144</b>			CH= 2.57 (6) Pi- alkyl = 4.55 (1)
	3	67	<b>93.1002</b>			Conventional H = 2.91 (3) CH= 2.65 (4)

TR_10	1	74	102.7			Conventional H = 2.17 (2)  CH = 2.68 (2)  Pi- pi T shaped = 4.19 (1)  Pi- alkyl = 4.71 (4)
	2	59	94.1082			Conventional H = 1.99 (1)  CH = 2.76 (3)  Pi- sigma = 2.88 (1)
	3	0	NA	NA	NA	NA
TR_11	1	65	120.404			Conventional H = 1.96 (1)  CH = 2.75 (5)  Pi- pi T shaped = 4.1 (2)  Pi- alkyl = 5.17 (2)

	2	0	NA	NA	NA	NA
	3	82	87.5798			Conventional H = 1.97 (2) CH= 2.59 (5) Pi- lone pair = 2.90(1) Pi- pi t shaped = 5.43 (1) Pi- alkyl = 5.35 (1)
TR_12	1	64	107.016			Conventional H = 2.00 (1) CH = 2.42 (3) Pi- sigma = 2.84 (1) Pi- pi t shaped = 4.13 (2) Pi- alkyl = 5.20 (2)

	2	58	97.0725		<b>Convnetional H = 2.23 (1)</b> <b>CH = 2.76 (2)</b> <b>Pi- pi stacked = 4.18 (1)</b> <b>Pi- alkyl = 5.21 (1)</b>
	3	77	94.5929		<b>Conventional H = 1.89 (1)</b> <b>CH= 2.55 (3)</b> <b>Pi- anion = 3.62 (1)</b> <b>Pi- alkyl = 4.97 (4)</b>
TR_13	1	61	102.175		<b>CH= 2.53 (5)</b> <b>Pi- pi stacked = 4.65 (3)</b> <b>Pi- alkyl = 5.05 (3)</b>

	2	58	93.6295	<p>Chemical structure diagram of compound 2 showing various interactions with residues labeled DC, DA, DG, DT, and DG. Bond lengths are indicated in pink dashed lines.</p> <ul style="list-style-type: none"> <li>DC 24: 5.72</li> <li>DA 28: 5.38</li> <li>DG 25: 4.58</li> <li>DA 26: 4.07</li> <li>DG 27: 3.94</li> <li>DG 27: 3.92</li> <li>DT 22: 6.57</li> <li>DT 23: 5.96</li> </ul>	<b>Conventional</b> <b>H = 2.05 (1)</b> <b>CH = 2.65 (5)</b> <b>Pi-pi stacked = 5.95 (1)</b> <b>Pi-alkyl = 4.52 (1)</b>
	3	50	109.323	<p>Chemical structure diagram of compound 3 showing various interactions with residues labeled DT, DG, DC, and DG. Bond lengths are indicated in pink dashed lines.</p> <ul style="list-style-type: none"> <li>DT 343: 4.96</li> <li>DG 34: 4.81</li> <li>DG 32: 5.27</li> <li>DG 33: 5.56</li> <li>DG 32: 5.21</li> <li>DG 38: 5.29</li> <li>DC 40: 7.66</li> <li>DC 35: 6.03</li> <li>DC 37: 6.55</li> <li>DT 36: 6.02</li> </ul>	<b>Conventional</b> <b>H = 2.08 (1)</b> <b>CH = 2.53 (5)</b> <b>Pi-pi stacked = 5.13 (2)</b> <b>Pi-alkyl = 4.47 (7)</b>
TR_14	1	80	96.1587	<p>Chemical structure diagram of compound TR_14 showing various interactions with residues labeled DG, DC, DT, DG, and DA. Bond lengths are indicated in pink dashed lines.</p> <ul style="list-style-type: none"> <li>DG 11: 4.37</li> <li>DC 13: 4.14</li> <li>DG 10: 5.19</li> <li>DT 9: 6.32</li> <li>DG 15: 6.32</li> <li>DA 16: 6.32</li> </ul>	<b>Conventional</b> <b>H = 1.88 (1)</b> <b>CH = 2.45 (2)</b> <b>Pi-alkyl = 4.96 (1)</b>

	<b>2</b>	<b>11</b>		
			<b>76.7456</b>	
	<b>3</b>	<b>45</b>	<b>81.2622</b>	

**Conventional**  
**H = 2.53 (2)**

**CH = 2.51 (7)**

**Pi- pi stacked**  
**= 4.01 (2)**

**Pi- alkyl =**  
**4.26 (1)**

**Conventional**  
**H = 2.13 (3)**

**CH= 2.64 (2)**

**Pi- pi stacked**  
**= 4.78 (1)**

**Pi- alkyl =**  
**5.45 (1)**

TR_15	1	69	80.4712			Conventional H = 2.18 (1)  CH = 2.56 (3)  Pi- alkyl = 4.93 (2)
	2	0	NA	NA	NA	NA
	3	71	113.273			CH= 2.54 (7)  Pi- anion = 4.69 (1)  Pi- alkyl = 5.14 (4)

**Summary of Thermodynamics:**

Aptamer Sequence	Loop	Libdock score	Poses	Potential Energy (kcal/ mol)	Vander Waals Energy (Kcal/mol)	Electrostatic Energy (Kcal/mol)
<b>TR_OG</b>	1	113.947	61	585.80865	-488.87541	-1122.62781
	2	90.3457	60	599.63969	-505.46356	-1095.13311
	3	86.0369	62	579.21985	-496.55865	-1121.39748
<b>TR_1</b>	1	99.8259	65	489.31977	-486.62720	-1162.81638
	2	95.388	61	517.01489	-484.60583	-1130.62520
	3	113.536	59	518.65331	-490.57575	-1129.01188
<b>TR_2</b>	1	92.3606	69	525.29237	-499.51201	-1067.28599
	2	95.1408	72	524.23253	-501.92841	-1059.92722
	3	87.7886	68	577.69924	-488.69858	-1029.82761
<b>TR_3</b>	1	98.5637	60	577.41927	-466.76050	-1171.22853
	2	94.4791	64	557.27411	-482.27050	-1163.23470
	3	100.48	67	571.81076	-478.82028	-1153.57586
<b>TR_4</b>	1	81.8945	69	708.46020	-480.12301	-988.48807
	2	91.546	59	688.69331	-481.40432	-996.04283
	3	116.637	56	696.23872	-477.35782	-1000.29333
<b>TR_5</b>	1	70.6988	8	193.30956	-492.84927	-1481.99858
	2	82.4699	78	186.90682	-492.57954	-1486.66017

	3	108.862	59	187.87109	-491.08514	-1488.48661
<b>TR_6</b>	1	NA	0	NA	NA	NA
	2	100.651	64	2168.73483	819.84049	-1441.11791
	3			2166.62224	821.52810	-1447.43964
<b>TR_7</b>	1	86.6841	67	450.42865	-495.78140	-1241.33310
	2	91.9318	51	398.69430	-501.93606	-1285.59393
	3	111.466	50	387.38103	-505.15991	-1293.44143
<b>TR_8</b>	1	105.501	70	723.70949	-475.78308	-975.56767
	2	NA	0	NA	NA	NA
	3	112.08	53	697.15890	-472.94880	-1011.57611
<b>TR_9</b>	1	126.15	56	429.51398	-521.51803	-1135.92156
	2	89.0144	69	445.79399	-524.26261	-1122.30469
	3	93.1002	67	0.44368E+15	-0.11700E+04	0.8748E+15
<b>TR_10</b>	1	102.7	74	0.32183	-487.36914	-1627.20717
	2	94.1082	59	-34.72904	-496.24758	-1647.76924
	3	NA	0	NA	NA	NA
<b>TR_11</b>	1	120.404	65	929.04027	-505.79776	-724.23297
	2	NA	0	NA	NA	NA
	3	87.5798	82	932.58630	-505.53604	-718.02939

<b>TR_12</b>	1	107.016	64	0.17578E + 17	0.17578E + 17	-0.31203E+ 03
	2	97.0725	58	741.47369	-513.20088	-928.32997
	3	94.5929	77	710.51656	-512.48483	-966.42413
<b>TR_13</b>	1	102.175	61	478.31215	-505.11564	-1101.88352
	2	93.6295	58	484.05428	-503.44375	-1098.59398
	3	109.323	50	492.83605	-505.81367	-1089.94196
<b>TR_14</b>	1	96.1587	80	423.47242	-489.53441	-1240.37718
	2	76.7456	11	418.63384	-478.89040	-1261.84927
	3	81.2622	45	386.72572	-487.48199	-1281.07220
<b>TR_15</b>	1	80.4712	69	638.19940	-447.74122	-1130.04618
	2	NA	0	NA	NA	NA
	3	113.273	71	629.62174	-462.08904	-1124.41620

**Interaction and Bonding details:**

Aptamer	Loop	Type of Bonds							
		Conventional hydrogen bond	Mean Length	C- H bond	Mean length	Electrostatic Interaction	Mean length	Hydrophobic Interaction	Mean length
<b>TR_OG</b>	1	1	2.03	4	2.62	NA	NA	4 (Pi- alkyl)	4.88
	2	1	1.94	5	2.57	NA	NA	3 (2- Pi- pi T shaped, 1- Pi-alkyl)	Pi- pi T shaped = 5.73, Pi-alkyl = 3.81
	3	1	1.80	3	2.61	NA	NA	3 (2- Pi- pi T shaped, 1- Pi-alkyl)	Pi- pi T shaped = 4.27, Pi-alkyl = 3.55
<b>TR_1</b>	1	1	1.88	6	2.65	NA	NA	2 (Pi- alkyl)	4.92
	2	NA	NA	5	2.81	NA	NA	2 (Pi- pi T shaped, Pi-alkyl )	Pi- pi T shaped = 5.47, Pi-alkyl = 4.90
	3	3	2.38	4	2.63	NA	NA	Pi- alkyl	4.70
<b>TR_2</b>	1	2	2.86	5	2.77	NA	NA	5 (4- Pi-alkyl, 1- Pi-pi T shaped)	Pi- alkyl = 4.99, Pi- pi T shaped= 5.59
	2	1	2.34	6	2.80	NA	NA	2 ( Pi- pi T shaped, Pi-alkyl)	Pi- pi T shaped= 4.15, Pi-alkyl = 5.27
	3	1	2.65	5	2.52	NA	NA	NA	NA

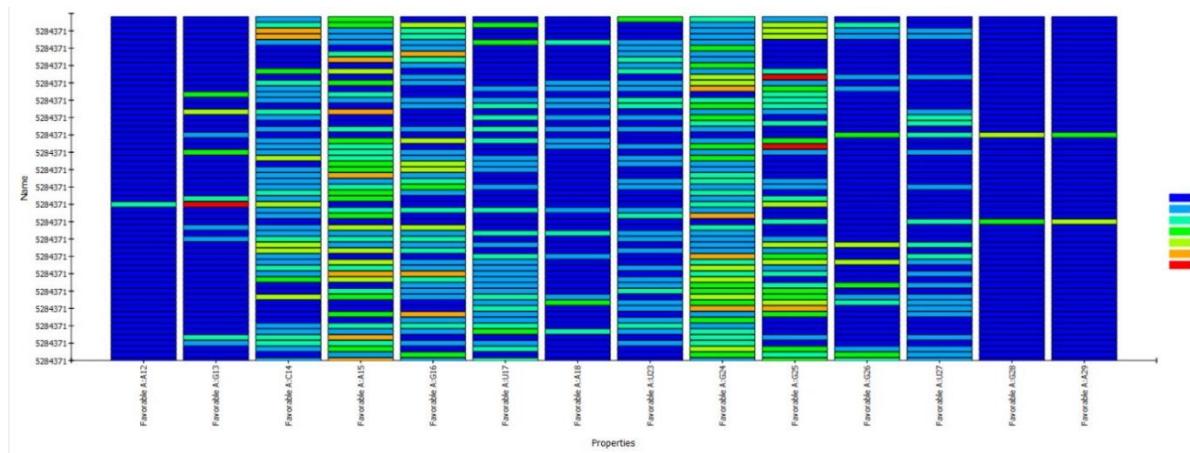
<b>TR_3</b>	1	1	2.18	6	2.51	NA	NA	7 (6- Pi-alkyl, 1- Pi-pi T shaped)	Pi- pi T shaped = 5.36, Pi-alkyl = 5.09
	2	1	2.88	8	2.84	NA	NA	3 (2- Pi-alkyl, 1- Pi-pi T shaped)	Pi- alkyl = 4.95, Pi- pi T shaped = 5.30
	3	1	2.03	1	2.70	NA	NA	2 (1- Pi-sigma, 1- Pi-alkyl)	Pi- sigma = 2.70, Pi-alkyl = 4.59
<b>TR_4</b>	1	2	2.37	2	2.8	NA	NA	2 ( Pi- pi T shaped, Pi-alkyl)	Pi- pi T shaped = 5.44, Pi alkyl = 4.45
	2	2	2.67	10	2.67	NA	NA	1 ( Pi- pi T shaped)	4.48
	3	1	2.56	8	2.42	NA	NA	1 (Pi- alkyl)	4.61
<b>TR_5</b>	1	4	2.53	NA	NA	1	3.98	1 (Pi- alkyl)	5.16
	2	2	2.61	7	2.67	NA	NA	5 (Pi- alkyl)	4.97
	3	2	2.47	2	2.62	NA	NA	5 (Pi- alkyl)	4.66
<b>TR_6</b>	1	NA	NA	NA	NA	NA	NA	NA	
	2	2	2.17	2	2.54	1	3.29	3 (Pi- alkyl)	4.72
	3								
<b>TR_7</b>	1	1	1.83	3	2.46	NA	NA	4 (1- Pi-sigma, 3- Pi-alkyl)	Pi- sigma = 2.37, Pi-alkyl

									alkyl = 4.43
	2	1	2.09	7	2.59	NA	NA	NA	NA
	3	2	2.50	3	2.49	NA	NA	3 (Pi- sigma, Pi- pi T shaped, Pi- alkyl )	2.59, 5.90, 4.54
<b>TR_8</b>	1	2	2.34	1	2.81	NA	NA	2 (Pi- alkyl)	5.22
	2	NA	NA	NA	NA	NA	NA	NA	NA
	3	3	2.13	5	2.46	NA	NA	3 (2- Pi-sigma, 1- Pi- alkyl)	5.58, 4.24
<b>TR_9</b>	1	2	2.45	6	2.54	NA	NA	2 (Pi- pi T shaped, Pi- alkyl)	5.85, 5.31
	2	NA	NA	6	2.57	NA	NA	Pi- alkyl	4.55
	3	3	2.91	4	2.65	NA	NA	NA	NA
<b>TR_10</b>	1	2	2.17	2	2.68	NA	NA	5 (4- Pi- alkyl, 1- Pi- pi T shaped)	4.71, 4.19
	2	1	1.99	3	2.76	NA	NA	1 (Pi- sigma)	2.88
	3	NA	NA	NA	NA	NA	NA	NA	NA
<b>TR_11</b>	1	1	1.96	5	2.75	NA	NA	4 ( 2- Pi- pi T shaped, 2- Pi- alkyl)	4.1, 5.17
	2	NA	NA	NA	NA	NA	NA	NA	
	3	2	1.97	5	2.59	1 (pi-lone pair)	2.90	2 (Pi- pi T shaped, Pi- alkyl)	5.43, 5.35

<b>TR_12</b>	1	1	2.00	3	2.42	NA	NA	5 (2- Pi- pi T shaped, 2- Pi- alkyl, 1- Pi- sigma)	4.13, 5.20, 2.84
	2	1	2.23	2	2.76	NA	NA	2 (Pi- pi stacked, Pi- alkyl)	4.18, 5.21
	3	1	1.89	3	2.55	1	3.62	4 (Pi- alkyl)	4.97
<b>TR_13</b>	1	NA	NA	5	2.53	NA	NA	6 (3- pi- pi stacked, 3- Pi- alkyl)	4.65, 5.05
	2	1	2.05	5	2.65	NA	NA	2 (Pi- pi stacked, Pi- alkyl)	5.95, 4.52
	3	1	2.08	5	2.53	NA	NA	9 (7- Pi- alkyl, 2- Pi- pi stacked)	4.47, 5.13
<b>TR_14</b>	1	1	1.88	2	2.45	NA	NA	1 (Pi- alkyl)	4.96
	2	2	2.35	7	2.51	NA	NA	3 (Pi- pi stacked, Pi- alkyl)	4.01, 4.26
	3	3	2.13	2	2.64	NA	NA	2 (Pi- pi stacked, Pi- alkyl)	4.78, 5.45
<b>TR_15</b>	1	1	2.18	3	2.56	NA	NA	2 (Pi- alkyl )	4.93
	2	NA	NA	NA	NA	NA	NA	NA	NA
	3	NA		7	2.54	1	4.69	4 (Pi- alkyl)	5.14

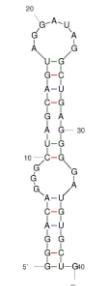
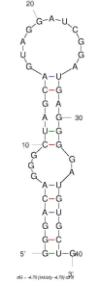
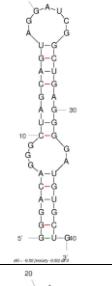
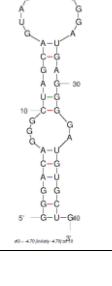
# Codeine: Aptamer FC5: AUGUST, 2025

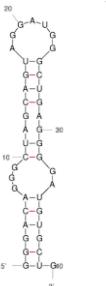
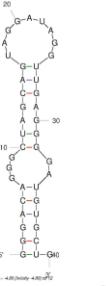
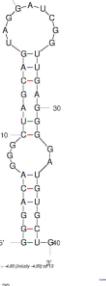
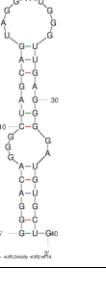
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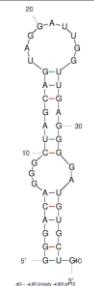


Aptamer	MUT	SEQUENCE	2D diagram	$\Delta G/ \text{ kcal mol}^{-1}$
CD OG	NM	GGGACAGGGCUAGCAGUAGGAU <b>UGG</b> <b>G</b> UGAGGGGAUGUGCUG		-3.80
CD 1	<b>A:23 G:26</b>	GGGACAGGGCUAGCAGUAGGAU <b>AGG</b> <b>G</b> UGAGGGGAUGUGCUG		-3.80

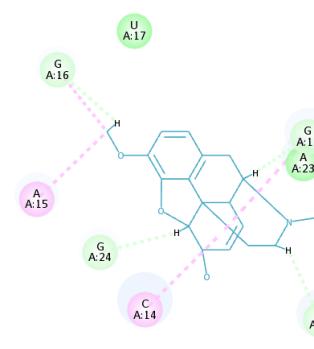
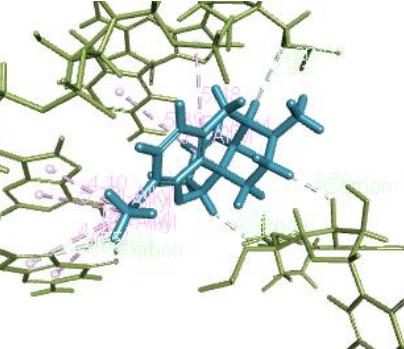
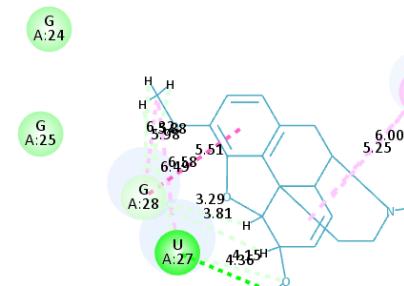
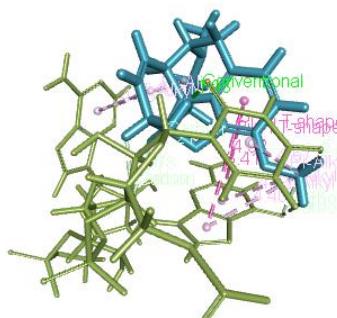
<b>CD 2</b>	<b>C:23 G:26</b>	GGGACAGGGCUAGCAGUAGGAU <b>CGG</b> <b>G</b> UGAGGGGAUGUGCUG		<b>-3.90</b>
<b>CD 3</b>	<b>G:23 G:26</b>	GGGACAGGGCUAGCAGUAGGAU <b>GGG</b> <b>G</b> UGAGGGGAUGUGCUG		<b>-3.80</b>
<b>CD 4</b>	<b>U:23 A:26</b>	GGGACAGGGCUAGCAGUAGGAU <b>UGG</b> <b>A</b> UGAGGGGAUGUGCUG		<b>-4.70</b>
<b>CD 5</b>	<b>U:23 C:26</b>	GGGACAGGGCUAGCAGUAGGAU <b>UGG</b> <b>C</b> UGAGGGGAUGUGCUG		<b>-9.50</b>
<b>CD 6</b>	<b>A:23 A:26</b>	GGGACAGGGCUAGCAGUAGGAU <b>AGG</b> <b>A</b> UGAGGGGAUGUGCUG		<b>-4.70</b>

<b>CD 7</b>	<b>A:23 C:26</b>	GGGACAGGGCUAGCAGUAGGAU <b>AGG</b> <b>C</b> UGAGGGGAUGUGCUG		<b>-9.50</b>
<b>CD 8</b>	<b>C:23 A:26</b>	GGGACAGGGCUAGCAGUAGGAU <b>CGG</b> <b>A</b> UGAGGGGAUGUGCUG		<b>-4.70</b>
<b>CD 9</b>	<b>C:23 C:26</b>	GGGACAGGGCUAGCAGUAGGAU <b>CGG</b> <b>C</b> UGAGGGGAUGUGCUG		<b>-9.50</b>
<b>CD 10</b>	<b>G:23 A:26</b>	GGGACAGGGCUAGCAGUAGGAU <b>CGG</b> <b>A</b> UGAGGGGAUGUGCUG		<b>-4.70</b>

CD 11	G:23 C:26	GGGACAGGGCUAGCAGUAGGAU <b>GGG</b> <b>C</b> UGAGGGGAUGUGCUG	 <p>20 G-A-U A-U-C A-G-A A-U-C G-A-G A-U-C G-A-G A-U-C G-A-G S' -G-U-G60 <i>dT - 4.00 (molar dGDP)</i></p>	-9.50
CD 12	A:23 U:26	GGGACAGGGCUAGCAGUAGGAU <b>AGG</b> <b>U</b> UGAGGGGAUGUGCUG	 <p>20 G-A-U A-U-C A-G-A A-U-C G-A-G A-U-C G-A-G A-U-C G-A-G S' -G-U-G60 <i>dT - 4.00 (molar dGDP)</i></p>	-4.80
CD 13	C:23 U:26	GGGACAGGGCUAGCAGUAGGAU <b>CGG</b> <b>U</b> UGAGGGGAUGUGCUG	 <p>20 G-A-U A-U-C A-G-A A-U-C G-A-G A-U-C G-A-G A-U-C G-A-G S' -G-U-G60 <i>dT - 4.00 (molar dGDP)</i></p>	-4.80
CD 14	G:23 U:26	GGGACAGGGCUAGCAGUAGGAU <b>GGG</b> <b>U</b> UGAGGGGAUGUGCUG	 <p>20 G-A-U A-U-C A-G-A A-U-C G-A-G A-U-C G-A-G A-U-C G-A-G S' -G-U-G60 <i>dT - 4.00 (molar dGDP)</i></p>	-4.80

CD 15	U:23 U:26	GGGACAGGGCUAGCAGUAGGAU <b>UGGU</b> UGAGGGGAUGUGCUG		<b>-4.80</b>
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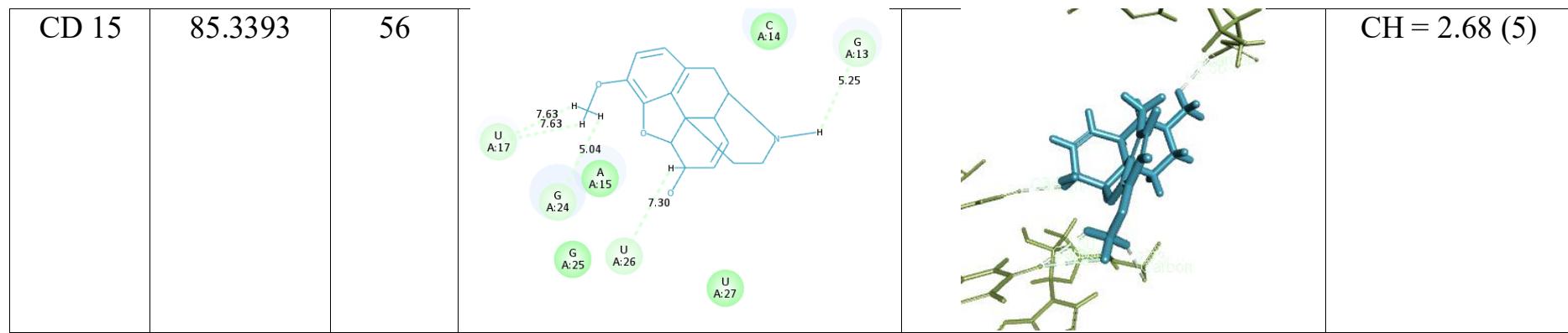
**Docking analysis: AUGUST, 2025**

Aptamer	Libdock score	Poses	2D Interactions	Ligand Interactions	Mean length
CD 1	103.246	43			CH = 2.44 (5) Pi- alkyl = 4.89 (6)
CD 2	90.4085	59			Conventional H = 1.93 (1) CH = 2.52 (6) Pi- Pi T shaped = 5.15 (2) Pi- alkyl = 4.49 (5)

CD 3	101.481	39	<p>Detailed description: This diagram shows the RNA structure of CD 3 with atoms labeled A, G, U, C, and H. Bond lengths are indicated by pink and green dashed lines, with values such as 5.19, 4.59, 4.89, 9.92, 7.33, and 4.88.</p>	<p>Detailed description: A 3D ribbon representation of the CD 3 RNA structure. A blue stick model represents a bound ligand. Various interactions are labeled: 'Depar-Donor' (red), 'Depar-Acceptor' (green), 'Pi-Sigma' (purple), 'Pi-Pi T shaped' (orange), and 'Pi-Alkyl' (blue).</p>	Conventional H = 2.61 (1) CH = 2.40 (4) Pi- alkyl = 4.84 (5)
CD 4	92.3469	47	<p>Detailed description: This diagram shows the RNA structure of CD 4 with atoms labeled A, G, U, C, and H. Bond lengths are indicated by pink and green dashed lines, with values such as 5.19, 4.59, 4.89, 9.92, 7.33, and 4.88.</p>	<p>Detailed description: A 3D ribbon representation of the CD 4 RNA structure. A blue stick model represents a bound ligand. Various interactions are labeled: 'Depar-Donor', 'Depar-Acceptor', 'Pi-Sigma', 'Pi-Pi T shaped', and 'Pi-Alkyl'.</p>	Conventional H = 2.20 (3) CH = 2.28 (7) Pi- anion = 4.33 (1) Pi- sigma = 2.92 (1) Pi- alkyl = 5.10 (2)
CD 5	89.6163	47	<p>Detailed description: This diagram shows the RNA structure of CD 5 with atoms labeled A, G, U, C, and H. Bond lengths are indicated by pink and green dashed lines, with values such as 5.19, 4.59, 4.89, 9.92, 7.33, and 4.88.</p>	<p>Detailed description: A 3D ribbon representation of the CD 5 RNA structure. A blue stick model represents a bound ligand. Various interactions are labeled: 'Depar-Donor', 'Depar-Acceptor', 'Pi-Sigma', 'Pi-Pi T shaped', and 'Pi-Alkyl'.</p>	Conventional H = 2.44 (2) CH = 2.65 (7) Pi- anion = 4.91 (1) Pi- Pi T shaped = 5.69 (1) Pi- alkyl = 4.33 (5)

CD 6	77.9743	1			CH = 2.66 (3) Pi- pi T shaped = 4.41 (1) Pi- alkyl = 4.53 (8)
CD 7		0	-	-	-
CD 8	92.1921	51			Conventional H = 2.05 (2) CH = 2.76 (4) Pi- anion = 4.04 (1) Pi- alkyl = 5.23 (5)
CD 9	84.453	51			Conventional H = 1.94 (1) CH = 2.73 (5) Pi - anion = 3.90 (1) Pi- pi T shaped = 5.92 (1) Pi- alkyl = 4.79 (3)

CD 10	-	0	-	-	-
CD 11	-	0	-	-	-
CD 12	-	0	-	-	-
CD 13	77.0739	1	 	<p>Conventional H = 1.92 (2) CH = 2.85 (4) Pi- alkyl = 4.83 (3)</p>	
CD 14	100.835	52	 	<p>Conventional H = 2.19 (3) CH = 2.61 (6) Pi- anion = 4.23 (1) Pi- alkyl = 4.54 (4)</p>	



**Summary of Thermodynamics, Interaction profile and Bond types:**

Aptamer	Loop	Potential energy (kcal/ mol)	Vanderwals energy (kcal/mol)	Electrostatic energy (kcal/mol)	Total favourable interactions	Conventional Hydrogen bond	Mean length	Non-conventional bond	Mean length
CD 1	1	111.370	230.668	-2049.85	13	-	-	5 (Carbon hydrogen), 6 (Pi- alkyl)	2.44, 4.89
CD 2	1	101.592	336.573	-2150.996	12	1	1.93	6 (CH), 2 (Pi- pi T shaped), 5 (Pi- alkyl)	2.52, 5.15, 4.49
CD 3	1	258.176	356.862	-2024.274	10	1	2.61	4 (CH), 5 (Pi- alkyl)	2.40, 4.84
CD 4	1	-302.483	139.480	-2360.956	14	3	2.20	7 (CH), 1 (Pi-anion), 1( Pi- sigma), 2 (Pi-alkyl)	2.28, 4.33, 2.92, 5.10
CD 5	1	-150.331	250.769	-2322.187	16	2	2.44	7 (CH), 1 (Pi- anion), 1 (Pi- pi T	2.65, 4.91, 5.69, 4.33

								shaped), 5( Pi- alkyl)	
CD 6	1	-285.891	233.057	-2446.331	12	-	-	3 (CH), 1 (Pi- pi T shaped), 8 (Pi- alkyl)	2.66, 4.41, 4.53
CD 7	1	-	-	-	-	NA	NA	NA	NA
CD 8	1	-376.974	109.694	-2401.059	12	2	2.05	4 (CH), 1 (Pi- anion), 5 (Pi- alkyl)	2.76, 4.04, 5.23
CD 9	1	-303.548	238.107	-2457.643	11	1	1.94	5 (CH), 1 (Pi- anion), 1 (Pi- pi T shaped), 3 (Pi- alkyl)	2.73, 3.90, 5.92, 4.79
CD 10	1	-	-	-	-	NA	NA	NA	NA
CD 11	1	-	-	-	-	NA	NA	NA	NA
CD 12	1	-	-	-	-	NA	NA	NA	NA
CD 13	1	-465.626	223.950	-2608.208	9	2	1.92	4 (CH), 3 (Pi- alkyl)	2.85, 4.83
CD 14	1	-196.838	242.452	-2354.566	14	3	2.19	6 (CH), 1 (Pi- anion), 4 (Pi- alkyl)	2.61, 4.23, 4.54
CD 15	1	-80.448	228.559	-2223.030	5	-	-	5 (CH)	2.68

## ***In-silico Modification of aptamers: JUNE 12, 2025***

Aptamer modifications are a method of chemically modifying the aptamer structure to improve its functionality, structural stability, and binding affinity. Chemical modifications enhance the structural integrity of aptamers by conferring protection from nuclease degradation, thermal stability, and inhibition of secondary structure collapse. Terminal modifications such as thiol, enable them to be immobilised on sensor surfaces such as gold electrodes, making them more applicable in the development of biosensors.

Recent in vitro analysis have shown that chemical modification of aptamers is a key factor for augmenting their biochemical stability, biological activity, and therapeutic efficacy. One such highlighted case is the modification of RNA aptamers to chemically modified DNA aptamers with the use of thiophosphate, dithiophosphate, and 5' PEGylation. These modifications significantly reduced nuclease degradation, and improved retention in circulation, leading to effective inhibition of tumor growth in ovarian cancer models. (Jeong et al., 2020). Similarly, Spiegelmers – mirror image aptamers composed of L- nucleotides – have shown resistance to enzymatic digestion, enabling their stable function in complex biological environments (Vater & Klussman, 2015). Sulphur based modifications, such as phosphorothioate and phosphorodithioate linkages have also been explored to increase structural durability and prolong aptamer performance in vitro (Ghosh et al., 2020). PEGylation that is attaching polyethylene glycol chains- has proven effective in extending aptamer half- life and reducing non- specific interactions without compromising functional binding capabilities (Liang et al., 2017)

The mutated aptamers showing the best thermodynamic and interaction profile were then modified in silico, that is the addition of functional groups like thiol, amine, and Biotin at the 5' or 3' end of the aptamer. This was facilitated by the software Avogadro, which is a principal software package for 3D structural visualization, manual modification of molecules, and forcefield geometry optimization of aptamers following terminal chemical modification. In this work.

### **Thiol modification:**

Thiol functionalization was achieved by forming a stable phosphorothioate (P-S) bond through nucleophilic substitution. Here, the sulphur atom from the thiol group replaced a hydroxyl group on the phosphorus atom, forming a stable bond.

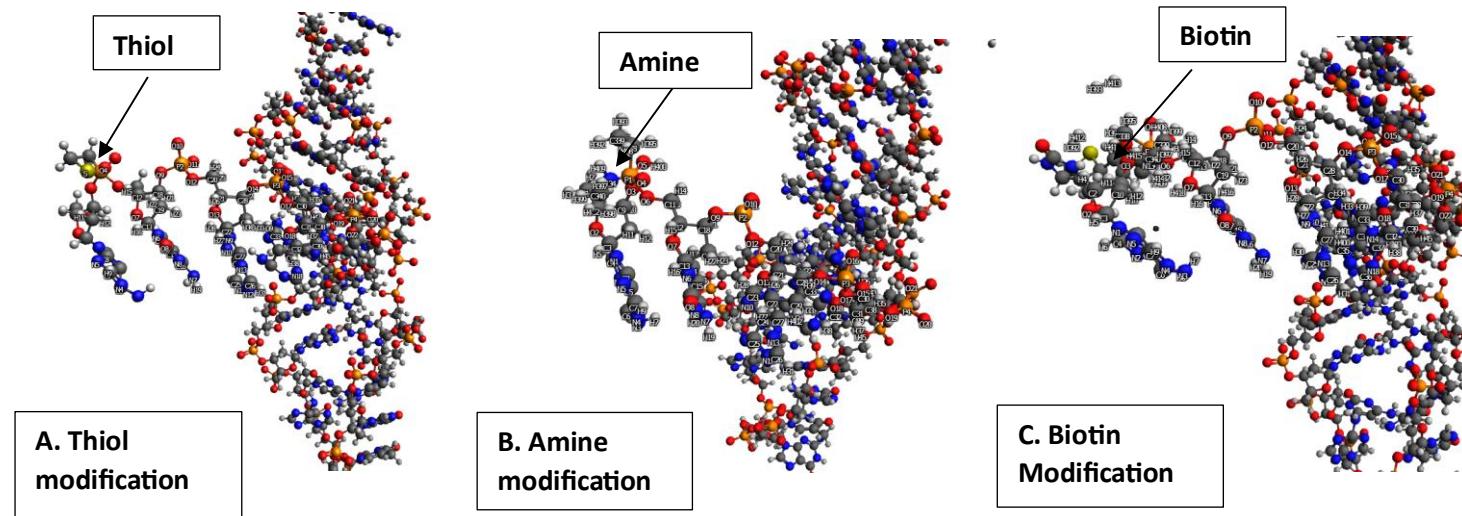
### **Amine modification:**

5' end amine modification was accomplished by adding a primary amine group to the terminal phosphate in a phosphoramidate linkage. The reaction follows a nucleophilic substitution pathway, in which the free electron pair on the nitrogen of the amine attacks the electrophilic phosphorus, resulting in the removal of a hydroxyl group and the formation of a stable P-N bond.

### **Biotin modification:**

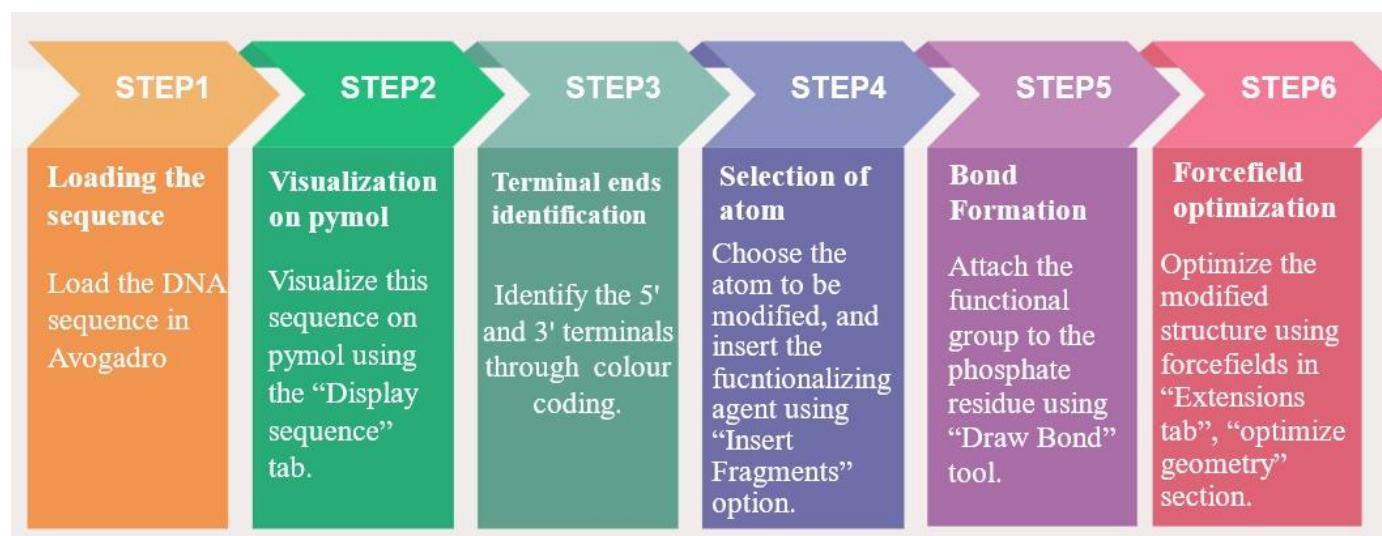
Biotin was conjugated to the aptamer using a two-step approach. First, a primary amine group was attached to the 5' phosphate of the aptamer via a covalent, stable phosphoramidate bond formed by nucleophilic substitution reaction. This site serves as a coupling point. In the second

step, the carboxyl group of biotin was attached covalently to the terminal amine through an amide bond, where the amine spacer group reduced steric hindrance.



*Fig.: Representation of In silico modified aptamers*

#### *Methodology:*



Oxygen	
Phosphorous	
Hydrogen	
Carbon	
Nitrogen	

*Colour coding scheme in Avogadro*

In this work, the FEN\_8 aptamer was modified, and forcefield optimisation with different forcefields was done for the same.

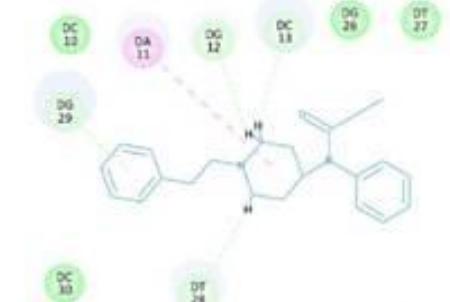
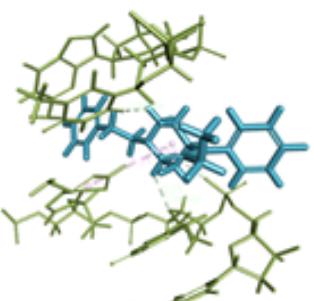
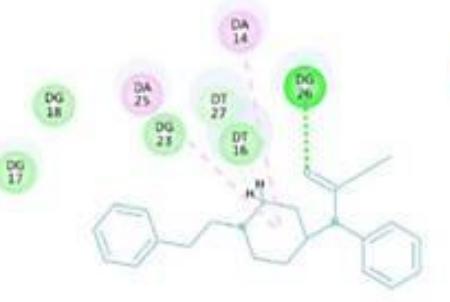
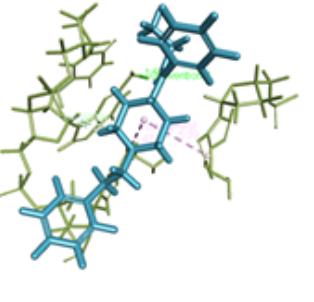
*Table: Forcefield optimization of functionalised aptamer (FEN\_8):*

Forcefield	Energy (kJ/mol)
UFF	24,072 kJ/mol
GAFF	66,045 kJ/mol
Ghemical	5615.62 kJ/mol

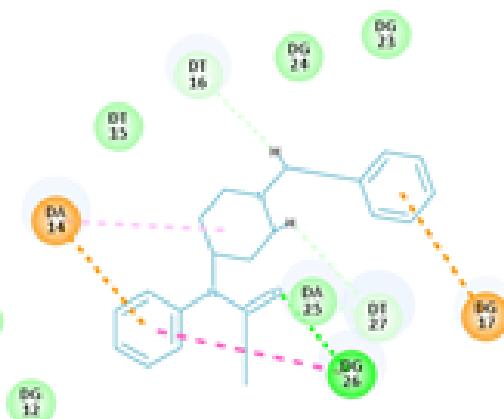
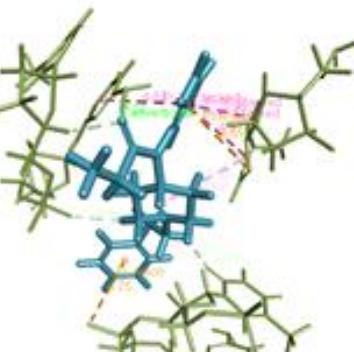
The ideal range for the optimized forcefields lies 5000 to 6000 kJ/mol, so the structure optimized with Ghemical forcefield was preferred.

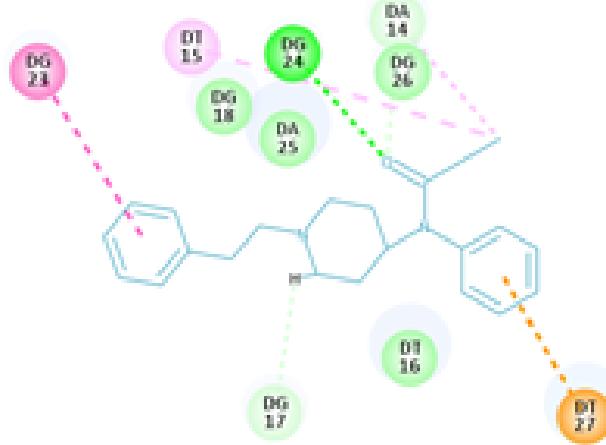
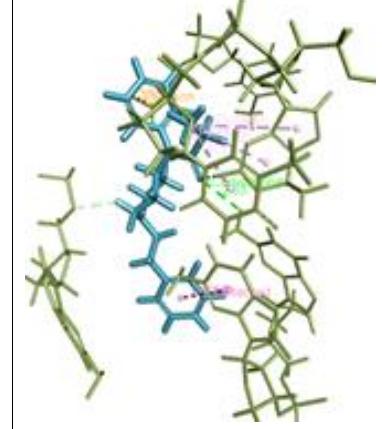
**Docking results and analysis: JUNE 16, 2025**

**Thiol modification:**

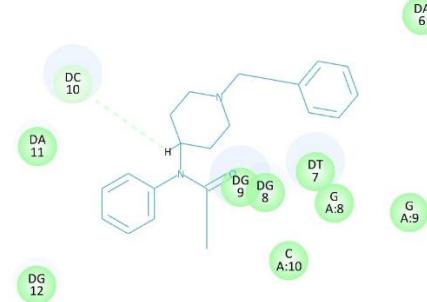
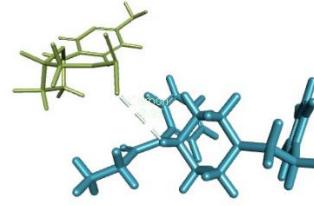
Modifying agent	Loop	Favorable residue count	2d diagram	Ligand interactions	Mean length	Libdock score
Thiol	L1	5	 A:11, G:12, C:13, G:29, T:28		Carbon hydrogen = 2.65, Pi-donor = 2.34, Pi- alkyl = 5.34	93.372
	L2	6	 T:27, T:16, G:26, A:25, A:14, G:23		Conventional hydrogen= 1.94, Carbon hydrogen= 2.55, Pi- alkyl = 5.43	113.96

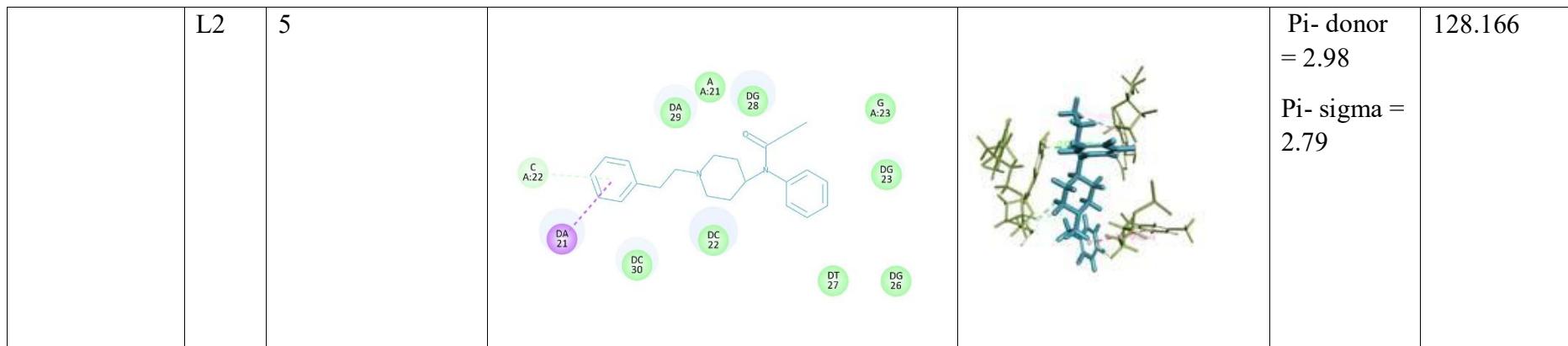
### *Amine modification:*

Modifying agent	Loop	Favorable residue count	2d diagram	Ligand interactions	Mean length	Libdock score
Amine	L1	7	 <p>T:16, T:27, G:26, A:14, G:27</p>		Conventional hydrogen = 2.27, Carbon hydrogen = 2.45, Pi-pi stacked = 4.59, Pi- alkyl = 5.36, Pi-anion = 4.72	117.858

	L2	7	 <p>G:24, A:14, T:27, G:23, T:15, G:17</p> 	Conventional hydrogen= 2.83, Carbon hydrogen= 2.66, Pi-pi stacked= 5.60, Pi- alkyl= 4.89, Pi- anion = 3.98	122.639
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### Biotin Modification:

Modifying agent	Loop	Favorable residue count	2d diagram	Ligand interactions	Mean length	Libdock score
Biotin	L1	5			CH = 2.95	93.4567



**Summary of Thermodynamics, interaction profile and bond types:**

Modifying agent	Loop	Potential energy	Vanderwals Energy	Electrostatic energy	Total Favourable interactions	Conventional Hydrogen bond	Mean length	Non-conventional bond	Mean length
<b>Thiol</b>	L1	843510.194	825452.540	-1047.333	6	0	-	4 (CH), 1(Pi-alkyl), 1 (Pi-donor)	2.65, 5.34, 2.34
	L2	843491.305	825445.172	-1059.573	6	1	1.94	2 (CH), 3 (Pi- alkyl)	2.55, 5.43
<b>Amine</b>	L1	0.19415E+14	0.19415E+14	-0.1168E+04	8	2	2.27	2 (CH), 2 (Pi- pi stacked), 1 (Pi- alkyl), 1 (pi- anion)	2.45, 4.59, 5.36, 4.72
	L2	0.19415E+14	0.19415E+14	-0.11273E+04	8	2	2.83	2 (CH), 2 (Pi- alkyl), 1 (Pi- pi stacked), 1(pi- anion)	2.66, 4.89, 5.60, 3.98
<b>Biotin</b>	L1	3540215.020	3530809.346	-1086.866	5	0	-	2 (CH)	2.95
	L2	3540212.842	3530808.639	-1112.456	5			1 (CH), 1(Pi-donor), 1 (Pi- sigma)	2.84, 2.98, 2.79

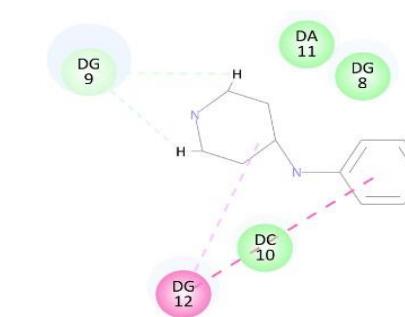
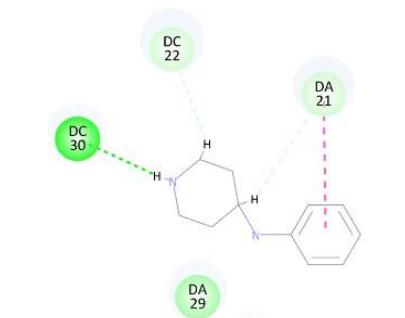
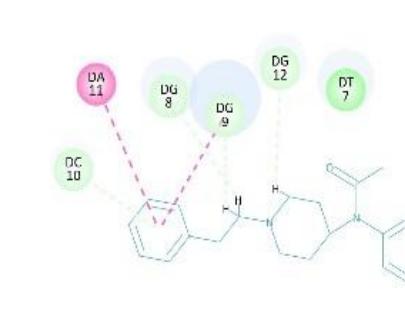
**Analogs data: JULY, 2025**

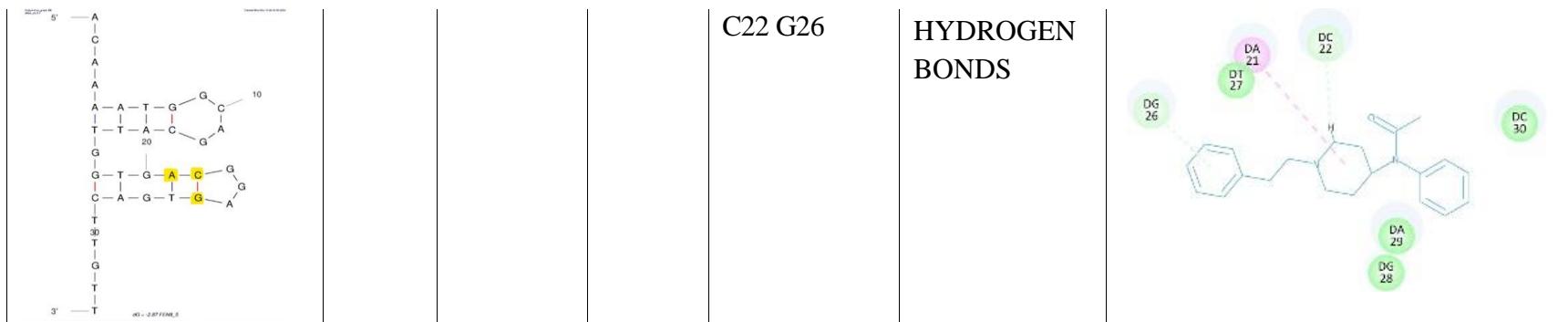
The selected opioids were docked to with their corresponding analogs, to check the affinity and interaction profile and Binding of the analogs to the opioids.

**Analogs for Fentanyl:**

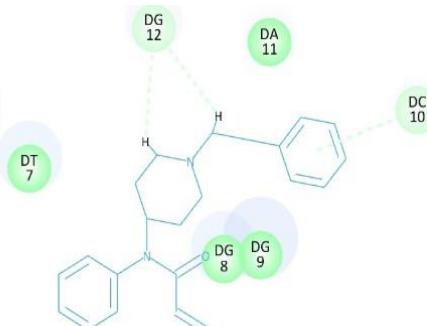
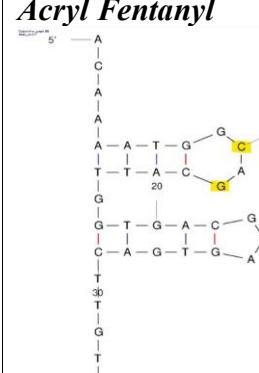
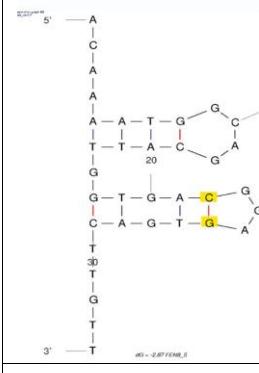
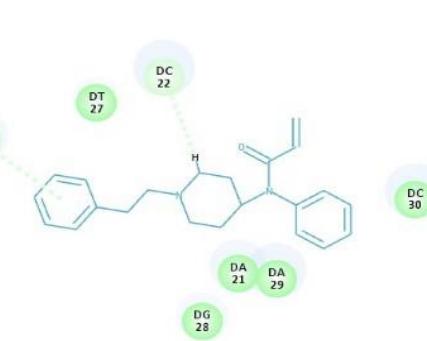
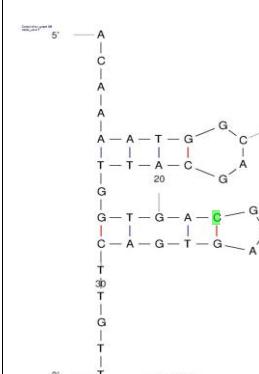
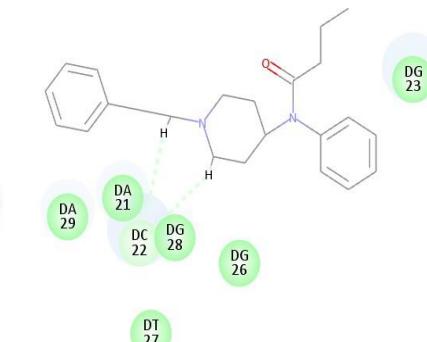
<b>Sr no.</b>	<b>Analogs</b>
1	4- Anilinopiperidine
2	Acetyl Fentanyl
3	Furanyl Fentanyl
4	Acryl Fentanyl
5	Butyryl Fentanyl
6	Valeryl Fentanyl
7	Cyclopropyl Fentanyl
8	Methoxyacetyl fentanyl
9	P- fluorofentanyl
10	Ortho- methyl furanyl fentanyl
11	P- Methoxy furanyl Fentanyl Hydrochloride
12	P- Methoxybutyryl Fentanyl- d7 hydrochloride
13	cis-(-) -3- Methylfentanyl
14	Alphamethyl Thiofentanyl

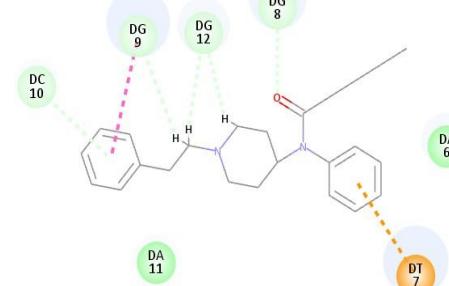
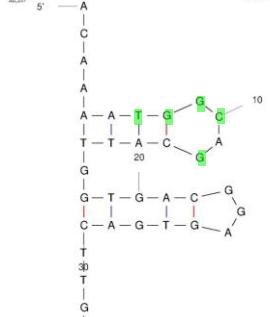
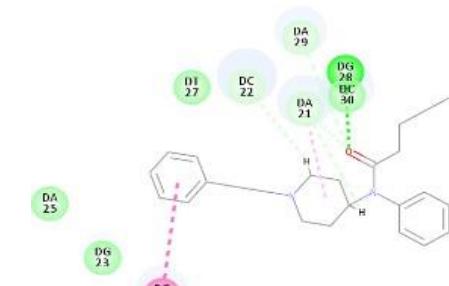
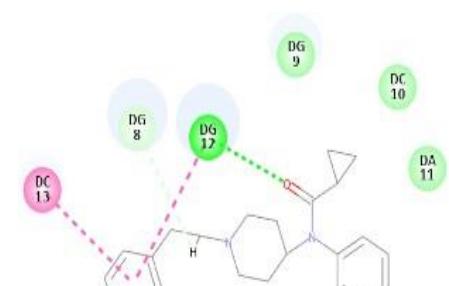
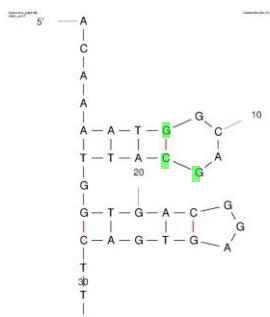
**Docking results and analysis:**

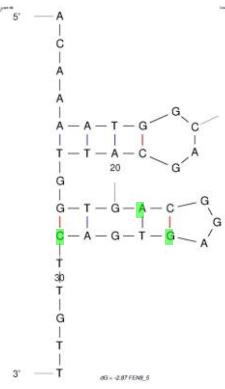
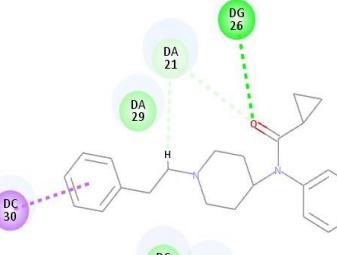
NAME	LOOP	LIBDOCK SCORE	POSES	INTERACTING RESIDUES	TYPES	2D DIAGRAM
4-Anilinopiperidine	1	67.5927	3	G9 G12	CARBON HYDROGEN BONDS	
	2	80.2055	46	A21 C22 C30	CARBON HYDROGEN BONDS  CONV H BONDS	
Acetyl Fentanyl	1	76.2085	5	G8 G9 C10 A11 G12	CARBON HYDROGEN BONDS	
	2	109.204	63	A21	CARBON	

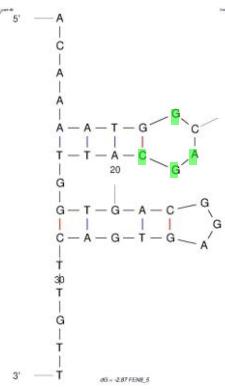
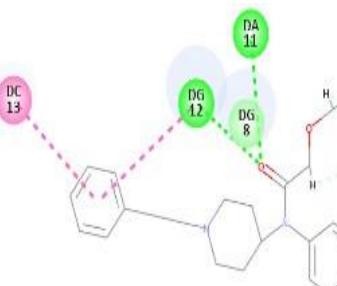


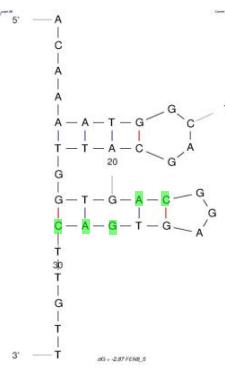
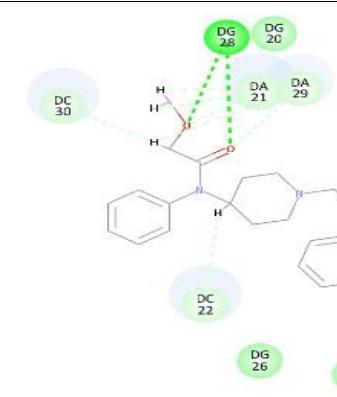
<b>Furanyl Fentanyl</b> <p><math>\Delta G = -2.87 \text{ kcal/mol}</math></p>	<b>1</b> <p><math>\Delta G = -2.87 \text{ kcal/mol}</math></p>	<b>102.392</b>	<b>8</b>	<b>G9, C10, A11, G12</b>	Carbon Hydrogen Bonds, Conventional Hydrogen Bonds	
<p><math>\Delta G = -2.87 \text{ kcal/mol}</math></p>	<b>2</b> <p><math>\Delta G = -2.87 \text{ kcal/mol}</math></p>	<b>108.423</b>	<b>75</b>	<b>NA</b>	<b>NA</b>	

<b>Acryl Fentanyl</b>	1	86.0688	12	C10, G12	Carbon Hydrogen Bonds	
						
<b>Butyryl Fentanyl</b>	1	NA	NA	NA	NA	NA
	2	113.014	73	C22, G26	Carbon Hydrogen Bonds	
	2	74.3449	3	C22	Carbon Hydrogen Bonds	

<b>Valeryl Fentanyl</b>	1	97.6193	33	T7 G8 G9 C10 G12	CARBON HYDROGEN BONDS	
	2	111.182	77	A21 C22 G26 G28 A29	CARBON HYDROGEN BONDS  CONV H BONDS	
<b>H - Cyclopropyl fentanyl</b>	1	84.4494	5	G8 G12 C13	CARBON HYDROGEN BONDS  CONV H BONDS	
						

 <p><math>\Delta G = -2.87 \text{ kJ/mol}_S</math></p>	2	115.749	67	A21 C30	CARBON HYDROGEN BONDS  CONV H BONDS	
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<p>Methoxyacetylentanyl</p>  <p><math>\Delta G = -2.87 \text{ kJ/mol}_S</math></p>	1	98.0739	36	G9 A11 G12 C13	CARBON HYDROGEN BONDS  CONV H BONDS	
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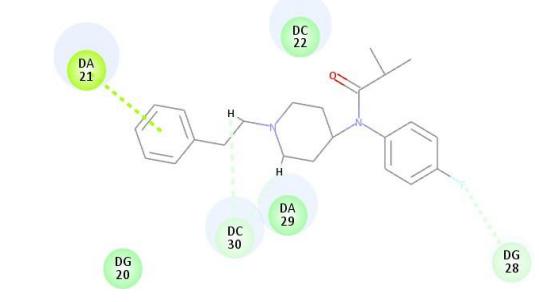
 <p><math>\Delta G = -2.87 \text{ kJ/mol}_S</math></p>	2	113.183	82	A21 C22 G28 A29 C30	CARBON HYDROGEN BONDS  CONV H BONDS	
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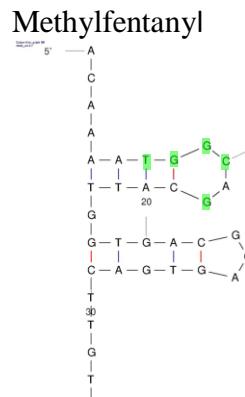
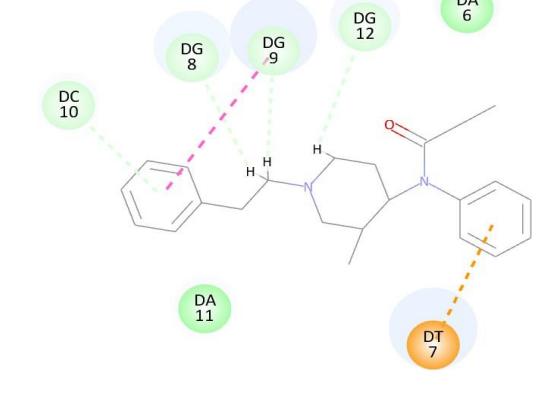
<p><b>p-fluorofentanyl</b></p> <p>5' — A C A A — A — A — T — G — C — 10 T — T — A — C — G — 20 G — T — G — A — C — G C — A — G — T — G — A / T — 30 T G T — T 3' — T</p> <p>MS = 287.11685</p>	1	109.315	21	G9 C10 A11 G12	CONV H BONDS	
<p>5' — A C A A — A — A — T — G — C — 10 T — T — A — C — G — 20 G — T — G — A — C — G C — A — G — T — G — A / T — 30 T G T — T 3' — T</p> <p>MS = 287.11685</p>	2	117.719	67	A21 G26 A29 C30	CARBON HYDROGEN BONDS  CONV H BONDS	

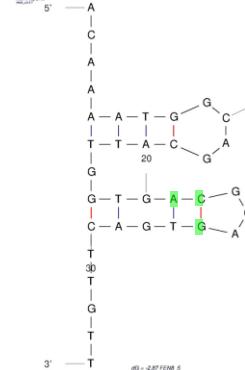
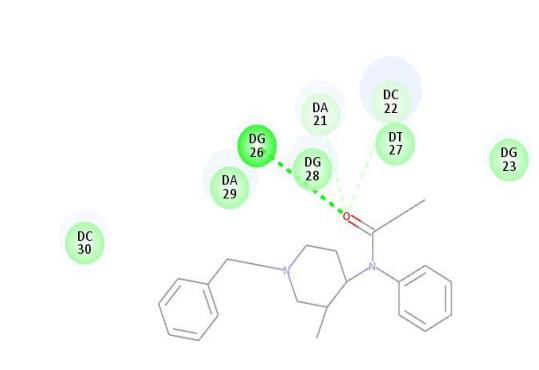
<p><b>Ortho-methylfuranyl fentanyl</b></p> <p>5' — A C A A — A — A — T — G — C — 10 T — T — A — C — G — 20 G — T — G — A — C — G C — A — G — T — G — A / T — 30 T G T — T 3' — T</p> <p>MS = 287.11685</p>	1	97.8321	10	T7 G12	CARBON HYDROGEN BONDS	
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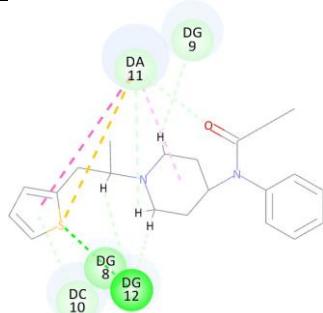
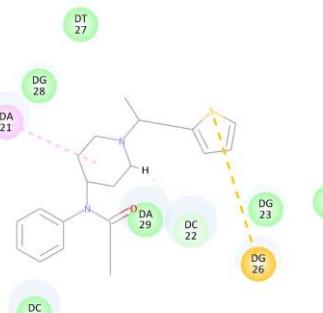


p-Methoxy butyryl fentanyl-d7 hydrochloride	1	107.986	50	T7 G8 G9 C10 A11 G12	CARBON HYDROGEN BONDS  CONV H BONDS	
	2	125.014	77	A21 G26 T27 A29 C30	CARBON HYDROGEN BONDS  CONV H BONDS	
p-Fluoroisobutyryl Fentanyl	1	88.966	3	A6 G9 C10 G12	CARBON HYDROGEN BONDS	

	2	105.313	8	A21 G28 C30	CARBON HYDROGEN BONDS	
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<b>cis-(−)-3-Methylfentanyl</b> 	1	83.9103	17	T7 G8 G9 C10 G12	CARBON HYDROGEN BONDS CONV H BONDS	
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	2	106.051	69	A21 C22 G26	CARBON HYDROGEN BONDS CONV H BONDS	
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alpha- Methyl ThioFentanyl	1	75.121	9	G9 C10 A11 G12	CARBON HYDROGEN BONDS CONV H BONDS	
	2	105.347	22	A21 C22 G26	CARBON HYDROGEN BONDS	

**Summary of Thermodynamics, Interaction profiling and Bond types:**

Analog name	Loop	Potential Energy	Vanderwals Energy	Electrostatic energy	Favourable interactions	Conventional Hydrogen bond	Mean length	Non-conventional bond	Mean length
<b>4-anilinopiperidine</b>	1	932.86795	-143.72286	-1025.26517	2	0	NA	2	2.415
	2	897.39442	-149.6968	-1047.99723	3	1	1.93	2	2.72
<b>Acetyl Fentanyl</b>	1	913.40149	-156.75841	-1040.41069	5	0	NA	5	2.56
	2	885.32846	-161.82973	-1054.60484	3	0	NA	2	2.41
<b>Furanyl Fentanyl</b>	1	913.89225	-164.29955	-1028.85627	4	1	1.95	2	2.90
	2	919.99437	-169.03469	-1018.31281	NA	NA	NA	NA	NA

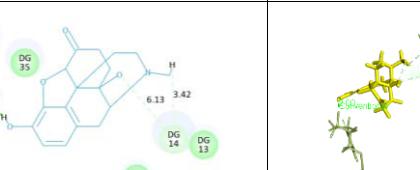
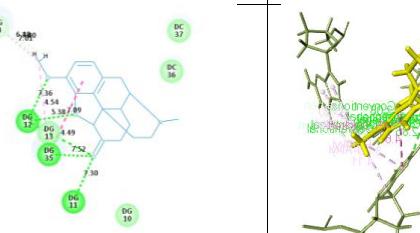
<b>Acryl Fentanyl</b>	1	912.76121	-164.62426	-1029.79751	2	0	NA	3	2.48
	2	886.03672	-161.3869	-1056.32227	2	0	NA	2	2.45
<b>Butyryl Fentanyl</b>	1	NA	NA	NA	NA	NA	NA	NA	NA
	2	912.58648	-164.76596	-1026.37519	2	0	NA	2	2.52
<b>Valeryl Fentanyl</b>	1	889.35421	-170.81407	-1047.75034	5	3	2.32	4	2.39
	2	901.66322	-165.02228	-1042.05787	5	1	1.95	4	2.52
<b>Cyclopropyl Fentanyl</b>	1	890.76865	-163.2397	-1056.75404	3	2	2.05	2	2.65
	2	923.94049	-166.94836	-1024.3639	3	2	2.77	2	2.69
<b>Methoxyacetyl Fentanyl</b>	1	879.87219	-163.75007	-1058.71107	4	2	2.61	3	2.32
	2	939.72583	-161.24538	-1009.32534	5	2	2.67	8	2.68
<b>p- Fluorofentanyl</b>	1	917.14472	-156.9778	-1033.23938	4	1	2.13	0	NA
	2	885.43272	-169.62132	-1051.49309	4	0	NA	5	2.63
<b>Ortho- methyl Furanyl Fentanyl</b>	1	878.44289	-170.61825	-1070.9922	2	0	NA	3	2.71
	2	910.70167	-170.76417	-1033.58147	4	2	2.52	7	2.51
<b>p- Methoxy Furanyl Fentanyl</b>	1	925.88726	-161.10309	-1026.68743	5	0	NA	9	2.58
	2	906.00937	-172.35438	-1031.35991	5	1	2.22	10	2.64
<b>p- Methoxy Butyryl Fentanyl</b>	1	869.34396	-172.20503	-1066.88289	6	1	1.91	2	2.53
	2	880.12522	-172.10991	-1054.62716	5	1	1.95	8	2.63
<b>p- fluoroisobutyryl Fentanyl</b>	1	893.23784	-160.83774	-1056.15379	4	0	NA	2	2.32
	2	914.01612	-165.07949	-1034.17842	3	0	NA	3	2.63
<b>Cis-(-)3-methylfentanyl</b>	1	915.03379	-166.85219	-1031.0527	5	0	NA	3	2.47
	2	929.7104	-171.65312	-1011.68255	3	1	2.67	3	2.55

<b>Alpha- methyl thiofentanyl</b>	1	899.28017	-159.51612	-1050.79455	4	1	2.27	6	2.48
	2	879.9781	-166.38538	-1060.55386	3	0	0	1	2.44

### ANALOGS: OXYCODONE:

Sr. no.	Analogs
1	Oxymorphone
2	Hydrocodone
3	Hydromorphone
4	Noroxycodeone
5	Thebacon

### Docking results and analysis: Original (OM16) aptamer:

Analogue	Loop	2D diagram	Ligand interaction	LibDock Score	No. Poses
<b>Oxymorphone</b>	1			89.438	52
<b>Hydrocodone</b>	1			93.1532	61

<b>Hydromorphone</b>	1			89.5736	60
<b>Noroxycodone</b>	1			91.58	33
<b>Thebacon</b>	1			84.7699	70

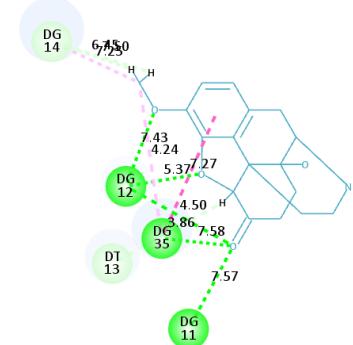
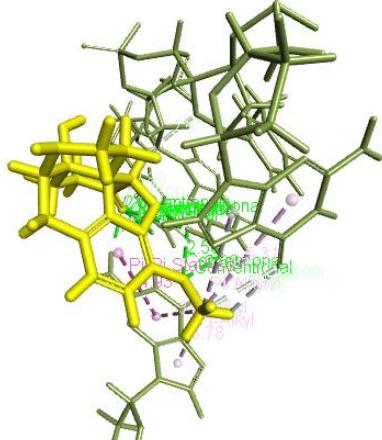
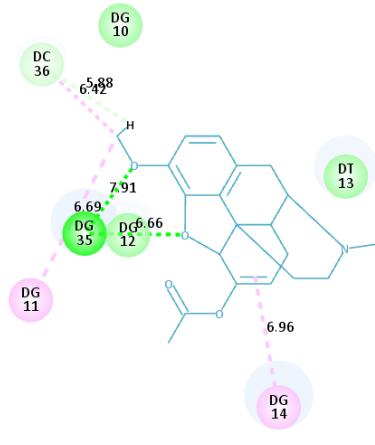
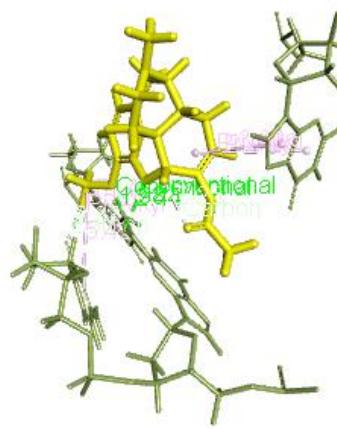
*Summary of Thermodynamics, Interaction profile, and Bond types:*

Analogue	Potential Energy (kcal/mol)	Vander Waals Energy (Kcal/mol)	Electrostatic Energy (Kcal/mol)	Conventional hydrogen bond	Mean Length	C - H bond	Mean length	Hydrophobic Interaction	Mean length	Others
Oxymorphone	236.216	-515.464	-1522.89	1	1.99849	3	2.5			
Hydrocodone	195.547	-511.956	-1563.13	7	2.37	2	2.6	5	4.44	

Hydromorphone	217.428	-518.137	-1531.46	1	2			1	4.93646	
Noroxycodone	183.756	-512.865	-1575.08	7	2.4	2	2.6	5	4.48	
Thebacon	207.728	-515.748	-1564.18	3	2.23	4	2.6	7	4.51	Pi-Lone Pair (2.92011)

**Docking analysis with the mutated OX5 aptamer:**

Analogue	Loop	Binding Site	2D diagram	Ligand interaction	LibDock Score	No. Poses
Oxymorphone	1				95.5027	51
Hydrocodone	1					
Hydromorphone	1				94.0159	74

Noroxycodone	1			94.7009	31
Thebacon	1			86.079	68

*Summary of Thermodynamics, Interaction profile, and Bond types:*

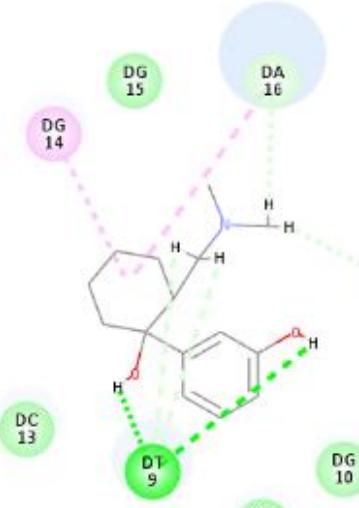
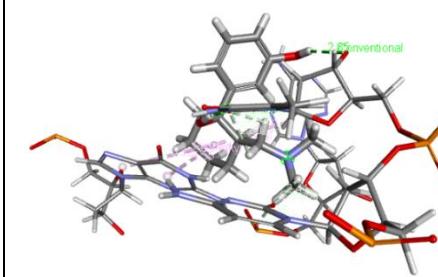
Analogue	Potential Energy (kcal/mol)	Vander Waals Energy (Kcal/mol)	Electrostatic Energy (Kcal/mol)	Conventional hydrogen bond	Mean Length	C - H bond	Mean length	Hydrophobic Interaction	Mean length	Others(Electrostatic)
Oxymorphone	228.693	-514.01	-1551.66	5	2.08	3	2.4	3	5	0
Hydrocodone										0
Hydromorphone	235.232	-512.929	-1540.09	6	2.24	2	2.6	3	4.66	0

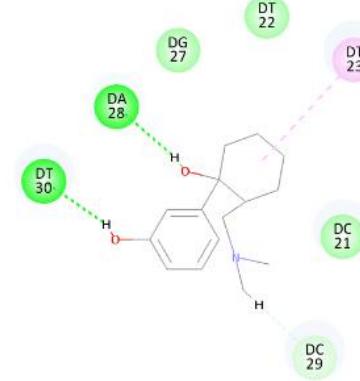
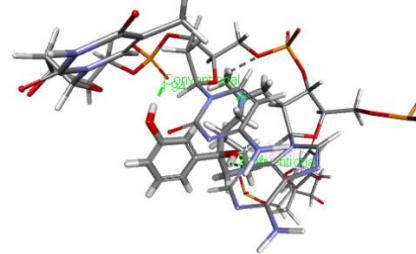
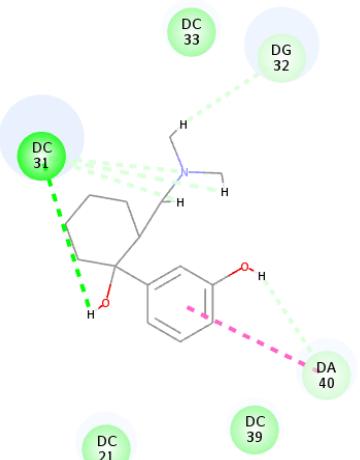
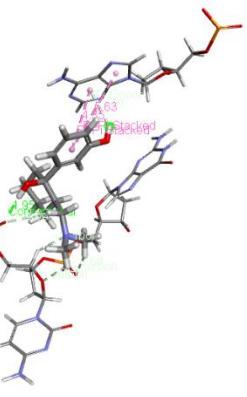
Noroxycodone	222.501	-494.153	-1521.21	7	2.45	4	2.67	5	4.52	0
Thebacon	222.501	-494.153	-1521.21	2	2.15	2	2.3	4	4.87	0

***ANALOGS: TRAMADOL:***

Sr. no.	Analogs
1	O- desmethyltramadol
2	N- Desmethyltramadol
3	N,O- Desmethyltramadol
4	Tramadol N- oxide
5	Faxeladol
6	Ciramadol
7	Axomadol
8	1,6- Dehydro Tramadol
9	O- Desmethyl- N,N bidesmethyl Tramadol
10	O- methyl Tramadol

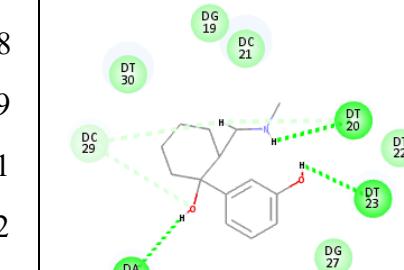
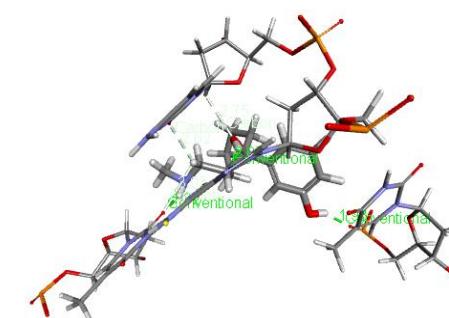
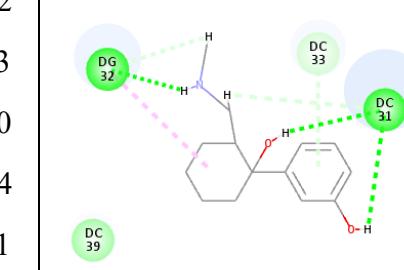
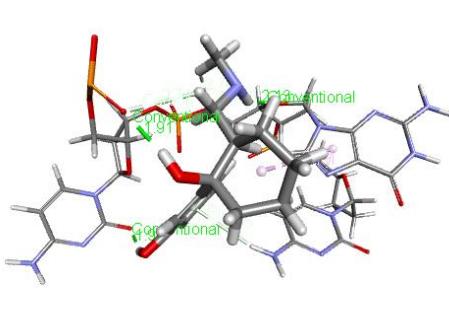
*Docking analysis and results: Original aptamer: APT39:*

ANALOGUE	Loo p	Bindin g Site	2D diagram	Ligand Interaction	LibDoc k Score	No. Pose s
<i>1. O-Desmethyltramadol</i>	1	:DC8 :DT9 :DG14 :DA16 :DC13 :DT12 :DG10 :DG15 :DT17 :DA18 :DT3 :DA5 :DA6 :DC7			109.784	60

	2	:DT23 :DA28 :DC29 :DT30 :DC21			87.6036	65
	3	:DC31 :DG32 :DA40 :DC21 :DC33 :DC39 :DG38 :DG34 :DT30			77.6949	60

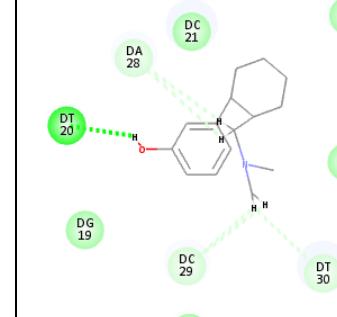
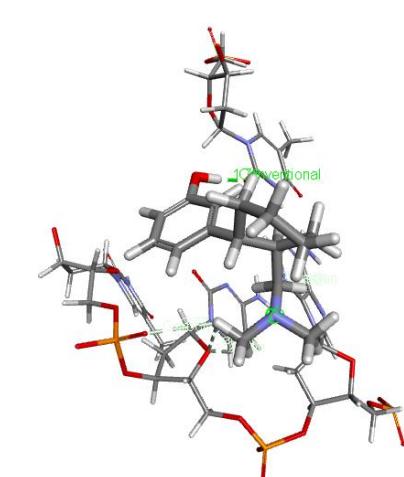
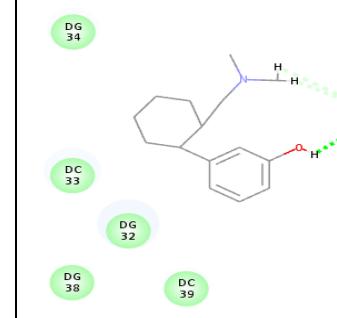
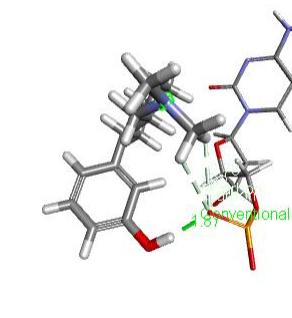
2. <i>N</i> -DesmethylTramadol	1	:DT9 :DG10 :DC13 :DG14 :DA16 :DT12 :DC8 :DT17 :DG15 :DA18			99.578	64
	2	:DC21 :DT23 :DT30 :DT20 :DT22 :DC31 :DG27 :DA28 :DC29 :DG19			92.3541	59

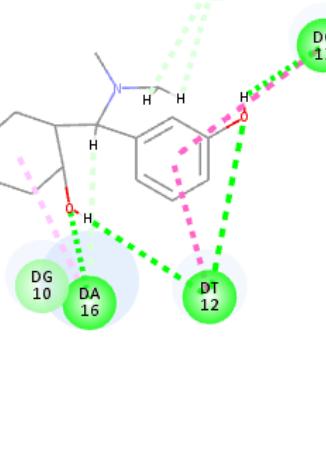
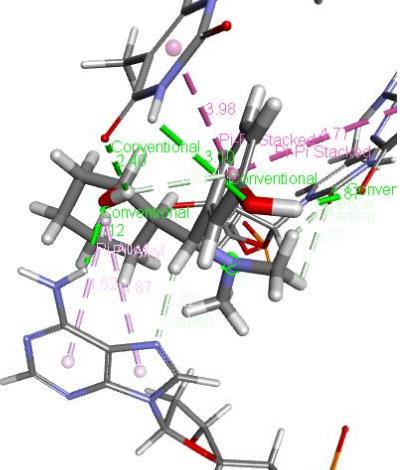
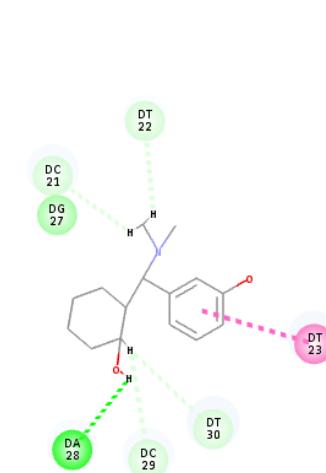
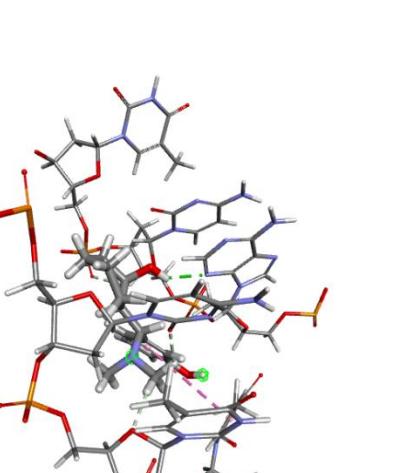
	3	:DC31 :DG32 :DC33 :DA40 :DC39 :DG34 :DC21 :DG38 :DC37 :DT20 :DC29			80.0316	60
	1	:DC8 :DT9 :DG14 :DA16 :DG15 :DT12 :DC13 :DG10 :DT17 :DA18			106.397	65

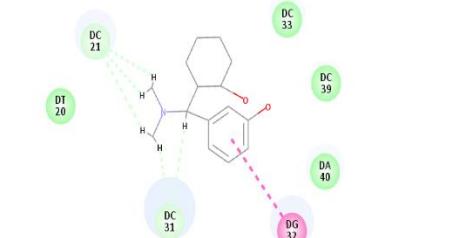
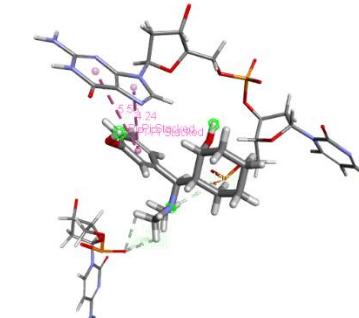
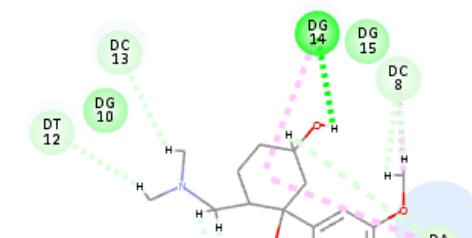
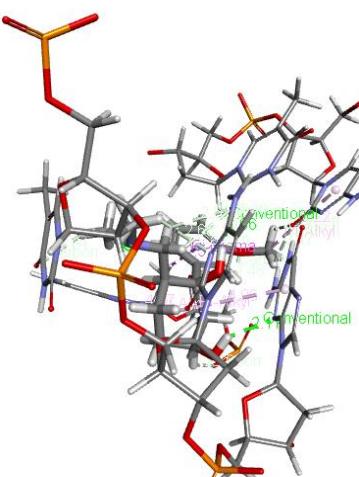
	2	:DT20 :DT23 :DA28 :DC29 :DC21 :DT22 :DT30 :DG27 :DG19 :DC31			88.484	63
	3	:DC31 :DG32 :DC33 :DA40 :DG34 :DC21 :DC39 :DG38			79.6842	65
<i>Tramadol N-oxide</i>	1	:DT9 :DT12 :DC13			98.0697	57

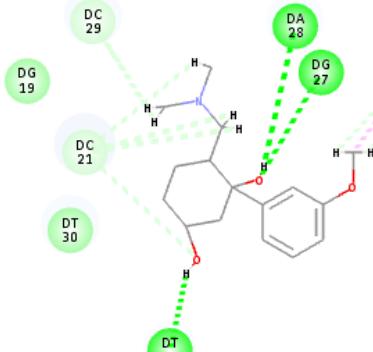
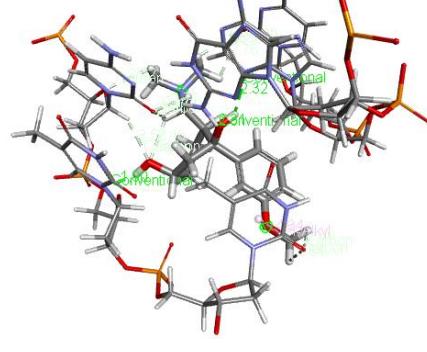
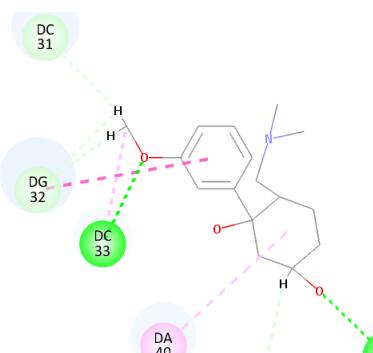
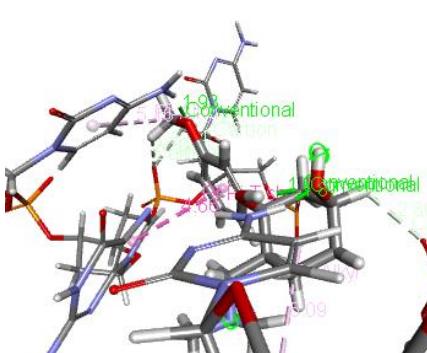
		: <dg14>  :<dg15>  :<da16>  :<dg10>  :<dc8>  :<dt17>  :<da18> </da18></dt17></dc8></dg10></da16></dg15></dg14>				
	2	: <dt20>  :<dc21>  :<dt23>  :<da28>  :<dc29>  :<dt22>  :<dg27>  :<dc31>  :<dt30>  :<dg19> </dg19></dt30></dc31></dg27></dt22></dc29></da28></dt23></dc21></dt20>				

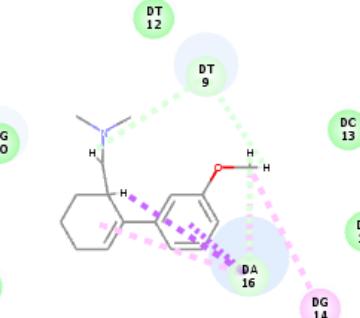
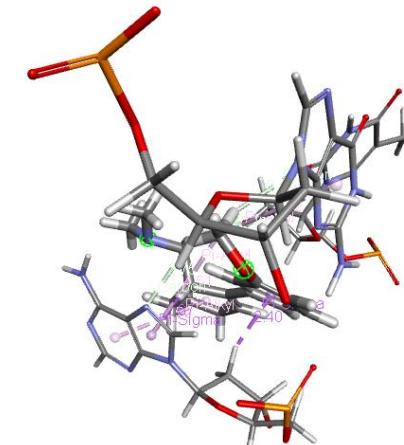
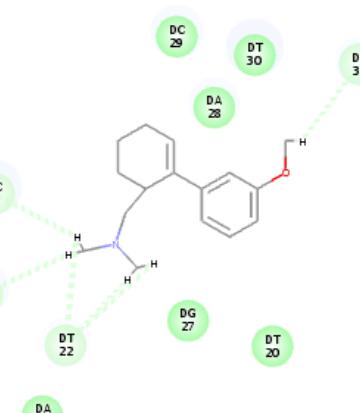
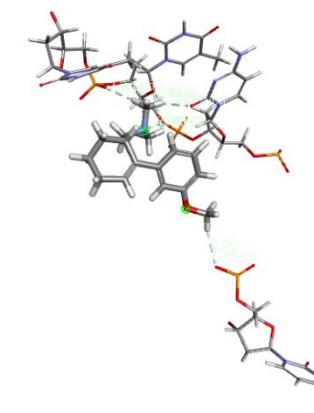
	3	:DC21 :DC31 :DG32 :DC33 :DA40 :DC39 :DG38 :DT30 :DG34 :DC37			84.9829	58
<i>Faxeladol</i>	1	:DT9 :DG10 :DG14 :DG15 :DA16 :DT17 :DC13 :DT12 :DC8 :DA18 :DG11			107.518	63

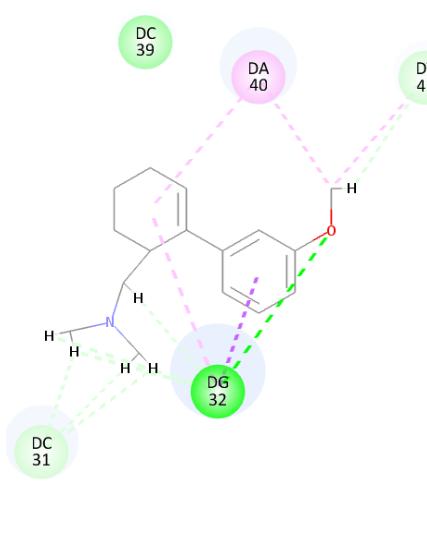
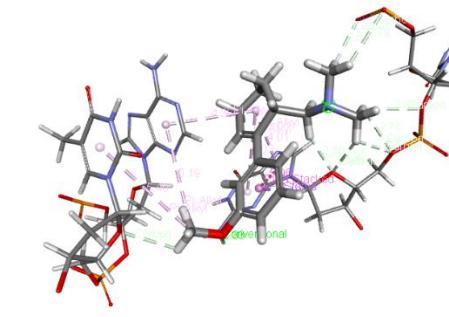
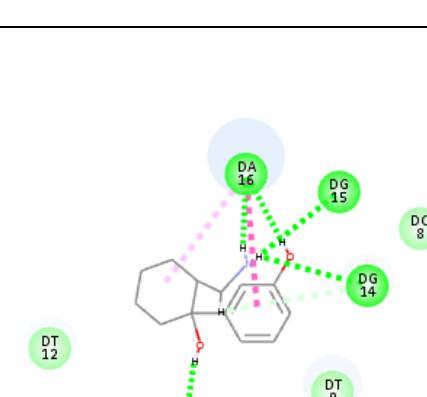
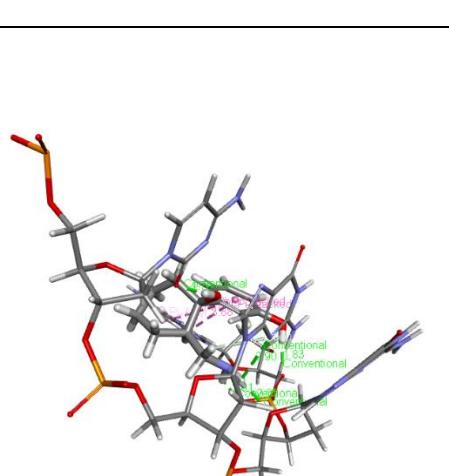
	2	:DT20 :DA28 :DC29 :DT30 :DC21 :DT22 :DT23 :DG27 :DG19 :DC31			85.5464	65
	3	:DC31 :DG32 :DA40 :DC21 :DC39 :DG38 :DC33 :DG34			79.0907	56

<i>Ciramadol</i>	1	:DT9 :DT12 :DC13 :DA16 :DG10 :DA18 :DT17 :DG14 :DC8 :DG15 :DG19 :DG11			98.0628	79
	2	:DC21 :DT22 :DT23 :DA28 :DC29 :DT30 :DT20 :DG19 :DG27 :DC31			90.5142	60

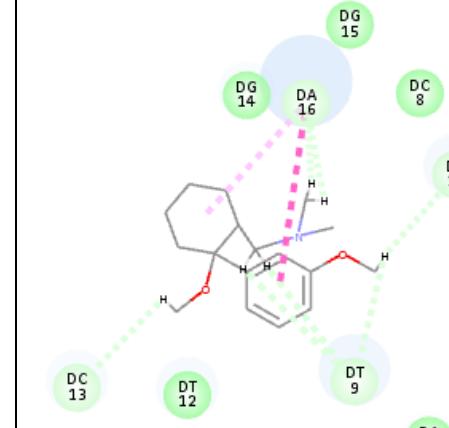
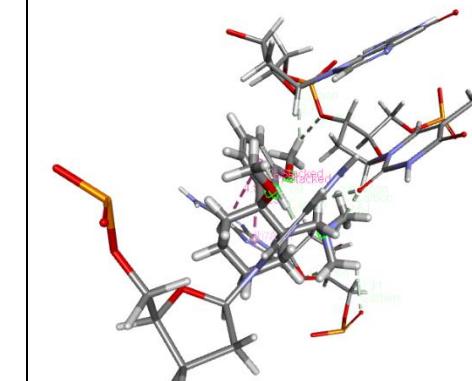
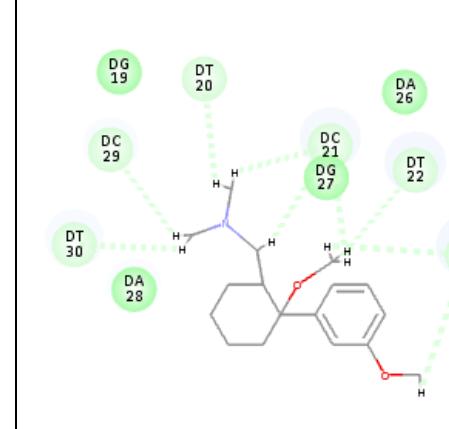
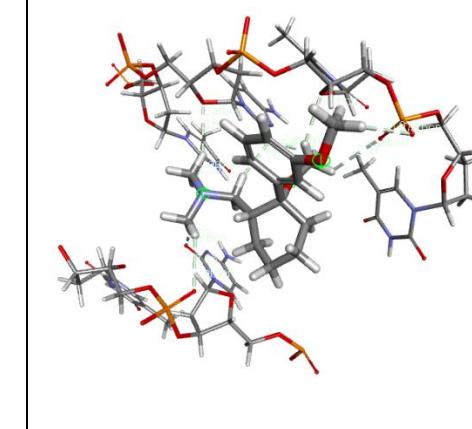
	3	:DC21 :DC31 :DG32 :DA40 :DG38 :DC39 :DC33 :DG34			76.1322	72
<i>Axomadol</i>	1	:DC8 :DT9 :DT12 :DC13 :DG14 :DA16 :DG10 :DG15 :DT17 :DA18 :DG11			118.22	69

	2	:DC21 :DT22 :DT23 :DG27 :DA28 :DC29 :DT20 :DT30 :DG19 :DA40 :DC31			97.473	61
	3	:DC21 :DC31 :DG32 :DC33 :DC39 :DA40 :DG34 :DG38 :DG19 :DT20 :DC37			84.5274	75

<i>1,6-DehydroTramadol</i>	1	:DT9 :DG14 :DA16 :DG10 :DT12 :DC13 :DC8 :DT17 :DA18 :DG15			109.238	562
	2	:DC21 :DT22 :DT23 :DC31 :DT20 :DA28 :DT30 :DC29 :DG27 :DG19			91.0053	60

	3	:DC31 :DG32 :DA40 :DT41 :DC21 :DC33 :DC39 :DG38 :DG34 :DT30 :DG35 :DC37			79.1473	65
<i>O</i> -Desmethyl- <i>N,N</i> - <i>bidesmethyl Tramadol</i>	1	:DC13 :DG14 :DG15 :DA16 :DT9 :DT12 :DG10 :DT17 :DA18			99.8576	56

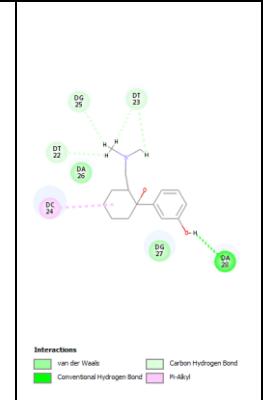
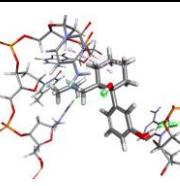
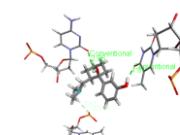
	2	:DC21 :DT22 :DT23 :DG27 :DA28 :DT20 :DC29 :DT30 :DC31			89.4453	68
	3	:DC31 :DG32 :DA40 :DC33 :DG34 :DG38 :DC21 :DC39			74.7039	61

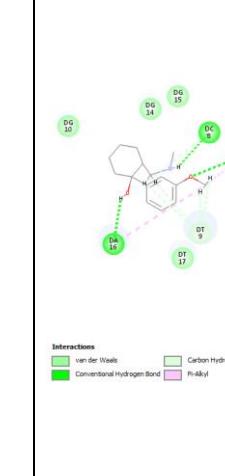
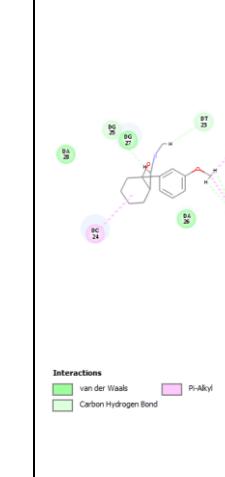
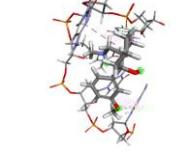
<i>O</i> -methyl Tramadol	1	:DT9 :DG10 :DC13 :DA16 :DG14 :DG15 :DA18 :DT12 :DT17 :DC8 :DG19			104.158	60
	2	:DT20 :DC21 :DT22 :DT23 :DC29 :DT30 :DA28 :DG27 :DC31 :DA40			85.7251	59

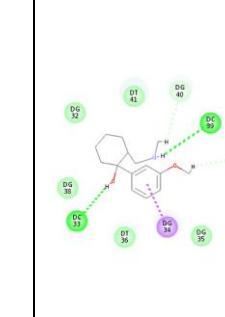
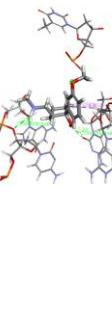
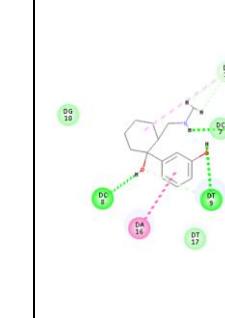
	3	:DC21 :DC31 :DA40 :DC33 :DG34 :DG32 :DG38 :DC39 :DT30 :DG35			<b>84.7369</b>	<b>64</b>
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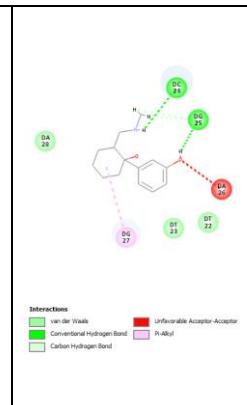
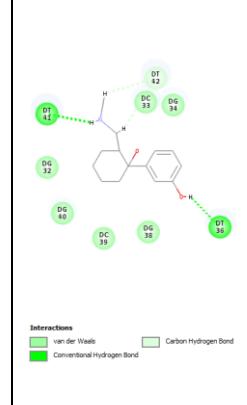
*Analog docking analysis with mutated sequence TR\_9:*

ANALOGUE	Loop	Binding Site	2D diagram	Ligand Interaction	LibDock Score	No. Poses
O-Desmethyltramadol	1	:DC8 :DT9 :DG14 :DG15 :DA16 :DG10 :DT17 :DC7			120.425	61

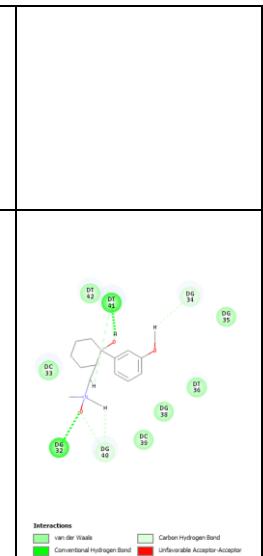
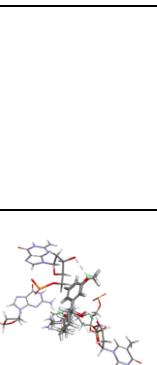
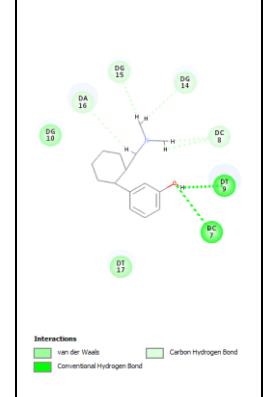
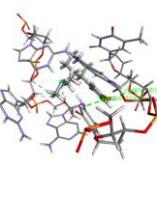
	2	:DT22 :DT23 :DC24 :DG25 :DA28 :DG27 :DA26 :DC21 :DA18 :DT17 :DG19 :DT20			93.2218	77
	3	:DC33 :DT36 :DT42 :DG34 :DG38 :DT41 :DG32 :DG40 :DC39 :DG35			94.2209	54

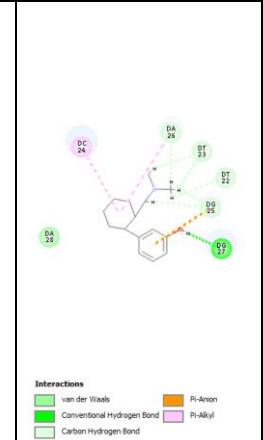
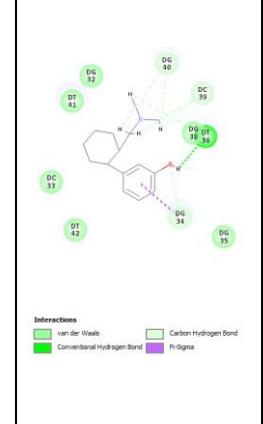
N-Desmethyl Itramadol	1	:DC7 :DC8 :DT9 :DA16 :DG14 :DG15 :DG10 :DT17 :DA18 :DT30			115.238	60
	2	:DC21 :DT22 :DT23 :DC24 :DG25 :DA26 :DG27 :DA28 :DT17 :DG19 :DA16 :DT20 :DG15			87.6698	72

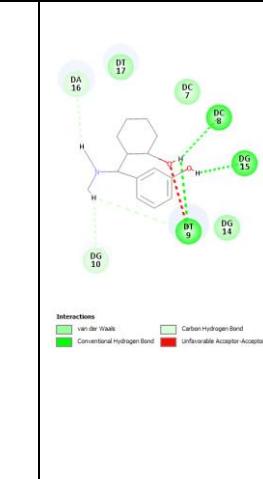
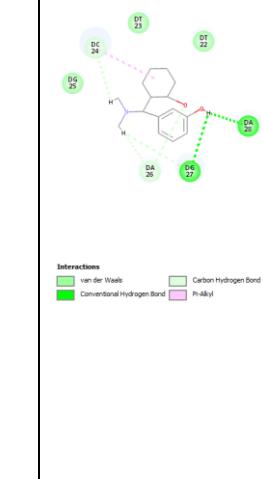
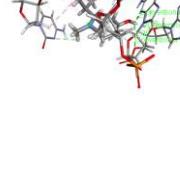
		:DA18 :DC7				
	3	:DC33 :DG34 :DC39 :DG40 :DT42 :DG35 :DT36 :DG38 :DG32 :DT41			94.6119	59
N,O-Desmet hyltramadol	1	:DC8 :DT9 :DG14 :DG15 :DA16 :DT17 :DC7 :DG10			120.888	45

	2	:DC24 :DG25 :DG27 :DA26 :DT22 :DT23 :DA28 :DA16 :DT17 :DG19 :DT20 :DC21	 Interactions: - van der Waals (green circles) - Conventional Hydrogen Bond (green dashed lines) - P-Absl (pink dashed lines) - Carbon Hydrogen Bond (yellow dashed lines)		87.5971	72
	3	:DT36 :DT41 :DT42 :DG32 :DG38 :DC33 :DC39 :DG40 :DG34 :DG35	 Interactions: - van der Waals (green circles) - Conventional Hydrogen Bond (green dashed lines)		92.1077	59

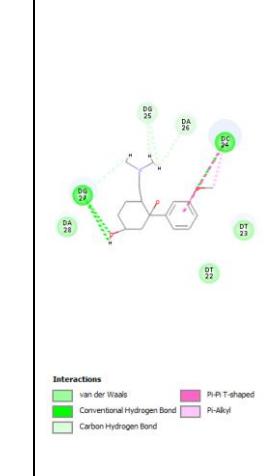
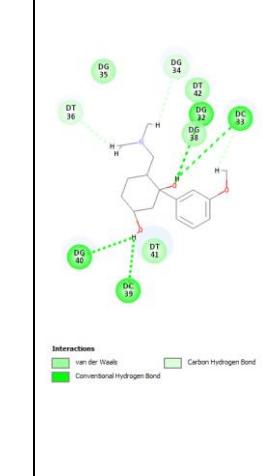
Tramadol N-oxide	1	:DT9 :DG14 :DG15 :DA16 :DT17 :DC7 :DC8 :DG10 :DA18	 Interactions: van der Waals Carbon-Hydrogen Bond Pi-Pi T-shaped Pi-Alkyl Unfavorable Negative-Negative		125.325	63
	2	:DC24 :DA26 :DG27 :DA28 :DT22 :DT23 :DG25 :DC21 :DA16 :DT17 :DA18 :DG19	 Interactions: van der Waals Conventional Hydrogen Bond Pi-Pi T-shaped Pi-Alkyl Unfavorable Negative-Negative		97.1377	70

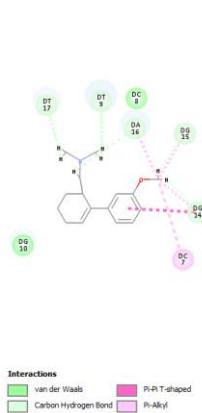
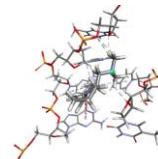
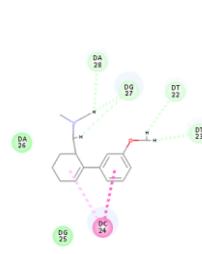
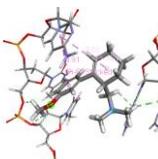
		:DT20 :DC29 :DG15				
	3	:DG32 :DG34 :DG40 :DT41 :DC39 :DC33 :DG35 :DT36 :DG38 :DT42 :DT343	 <p>Interactions</p> <ul style="list-style-type: none"><li>van der Waals (green)</li><li>Carbon Hydrogen Bond (light green)</li><li>Conventional Hydrogen Bond (dark green)</li><li>Unfavorable Acceptor-Acceptor (red)</li></ul>		96.1479	74
Faxeladol	1	:DC7 :DC8 :DT9 :DG14 :DG15 :DA16 :DT17 :DG10	 <p>Interactions</p> <ul style="list-style-type: none"><li>van der Waals (green)</li><li>Carbon Hydrogen Bond (light green)</li><li>Conventional Hydrogen Bond (dark green)</li><li>Unfavorable Acceptor-Acceptor (red)</li></ul>		116.65	44

	2	:DT22 :DT23 :DC24 :DG25 :DA26 :DG27 :DC21 :DA28 :DT17 :DG19 :DT20 :DA16	 <b>Interactions:</b> - van der Waals (green circles) - Conventional Hydrogen Bond (green dashed lines) - Pi-Aro (orange dashed lines) - Pi-Allyl (pink dashed lines) - Carbon Hydrogen Bond (light green dashed lines)	88.3191	65
	3	:DG34 :DT36 :DC39 :DG40 :DT41 :DT42 :DC33 :DG38 :DG32	 <b>Interactions:</b> - van der Waals (green circles) - Conventional Hydrogen Bond (green dashed lines) - Pi-Sigma (purple dashed lines)	88.572	54

Ciramadol	1	:DC8 :DT9 :DG10 :DG15 :DA16 :DT17 :DC7 :DG14 :DA18 :DT30			118.393	67
	2	:DC24 :DA26 :DG27 :DA28 :DT22 :DT23 :DG25 :DC21 :DA16 :DT17 :DG19 :DT20 :DG15			92.1628	73

		:DA18				
	3	:DG32 :DT36 :DC39 :DG40 :DT41 :DT42 :DC33 :DG34 :DG38			92.8239	69
Axomadol	1	:DC7 :DT9 :DG10 :DG14 :DG15 :DA16 :DT17 :DC8			131.421	55

	2	:DC24 :DG25 :DA26 :DG27 :DT22 :DT23 :DA28 :DG15 :DA16 :DT17 :DC21 :DG19 :DT20 :DA18	 <p>Interactions</p> <ul style="list-style-type: none"><li>van der Waals</li><li>Conventional Hydrogen Bond</li><li>π-π T-shaped</li><li>π-Allyl</li><li>Carbon Hydrogen Bond</li></ul>	98.2846	69
	3	:DG32 :DC33 :DG34 :DT36 :DC39 :DG40 :DG38 :DT41 :DT42	 <p>Interactions</p> <ul style="list-style-type: none"><li>van der Waals</li><li>Conventional Hydrogen Bond</li><li>Carbon Hydrogen Bond</li></ul>	104.789	39

		:DG35				
1,6-Dehydro Tramadol	1	:DC7 :DT9 :DG14 :DG15 :DA16 :DT17 :DC8 :DG10				54
	2	:DT22 :DT23 :DC24 :DG27 :DA28 :DC21 :DG25 :DA26 :DT17 :DG19 :DT20			88.3828	64

	3	:DG32 :DC33 :DG40 :DT41 :DT42 :DG34 :DC39 :DG35 :DT36 :DG38	<p>Interactions</p> <ul style="list-style-type: none"> <li>van der Waals</li> <li>Carbon Hydrogen Bond</li> </ul>		84.6634	57
-O-Desmethyl-N,N-bisdesmethyl Tramadol	1	:DC7 :DC8 :DT9 :DG14 :DG15 :DA16 :DT17 :DG10	<p>Interactions</p> <ul style="list-style-type: none"> <li>van der Waals</li> <li>Conventional Hydrogen Bond</li> <li>pi-Sigma</li> <li>pi-pi T-shaped</li> <li>Carbon Hydrogen bond</li> </ul>		109.669	47

	2					66
	3	:DT36 :DT41 :DT42 :DC39 :DG40 :DG32 :DC33 :DG34 :DG38 :DG35			87.6372	60

O-methyl tramadol	1	:DT9 :DG14 :DG15 :DA16 :DT17 :DC7 :DC8 :DG10 :DA18 :DT30 :DC31 :DG32			115.288	61
	2	:DA16 :DT17 :DA26 :DG27 :DT23 :DC24 :DG25 :DG19 :DT20 :DG15 :DT22			83.6895	52

		:DA28 :DC21				
	3	:DG34 :DG35 :DT36 :DG38 :DG40 :DT41 :DG32 :DC33 :DT42 :DC39			90.054	50

*Summary of Thermodynamics, Interaction profile and Bond types:*

ANALOGUE	Lo op	POTENTIAL ENERGY	VANDER WAALS ENERGY	ELECTROSTATIC ENERGY	CONVENTIONAL HYDROGEN BOND	MEAN LENGTH	NON CONVENTIONAL	MEAN LENGTH	Hydrophobic	
1.O-Desmethyltramadol	1	574.05011	-487.92223	-1133.54258	2.02962 2.84976	2.79528 2.8772 2.44693 2.40989		5.31728 4.54697 4.7689		

	2	587.34865	-501.14894	-1105.65775	2.4391 1.94129	2.49922 2.63567		4.6479 2	0
	3	577.931	-500.579	-1111.97	2.55922 2.64231 2.53871 2.70202 3.02121	1.94862	1.94862	5.6310 5 4.3519 1	0
N-Desmethyl tramadol	1	565.52901	-493.79764	-1142.30780	2.20878 1.89813	2.64649 2.70035		5.2405 2 5.3632 4.4836 4 4.5243 7 5.0823 7 4.8521 2	0
	2	564.21530	-499.30780	-1125.50512	1.80495	2.61125		0	0

					1.8743		2.51967 2.46541			
	3	552.962	-496.557	-1144.53	1.99753	1.9975 3	2.74779 2.56963 3.04666		4.8433 9 5.0381 2 5.1470 8	0
N,O-Desmeth yltramadol	1	555.47986	-497.31847	-1142.94225	1.89939 2.77072 1.96744		2.57413 2.68203		5.2357 6 4.47111 4.8391	0
	2	537.48176	-502.57491	-1155.47948	2.07211 1.93044 1.8242		2.74606 3.03397 2.61621		0	0
	3	528.87233	-494.57676	-1168.60959	1.90649 1.96958 2.13326		2.71435 2.76445 3.05477		4.7874 1	0

4.Tramadol N-oxide	1	281.46460	-500.27821	-1416.74532	2.00932	2.00932	2.83109 2.63607 2.79022 2.58669 2.94227 2.70322 2.60455		4.05089 5.77699 4.86543 4.82106 3.95 4.2259	4.02537
	2	285.22382	-506.50207	-1400.93236	2.41952	2.41952	2.3908 2.52974 2.86238		5.78067 5.46062	3.98833 3.1874
	3	311.29574	-498.62917	-1390.98947	1.98437 2.00625		2.63347 2.52283 2.57178 2.52085 2.61271		5.16524	4.8499
5.Faxeladol	1	572.81276	-487.14582	-1137.18659	2.24119		2.76302		5.51459	

				2.71583		2.53172 2.60569		4.2222 1 4.6795 8 5.4315 4	
	2	588.85566	-505.74174	- 1096.68706	1.76072		2.9163 2.9227 2.537 3.09667 2.7739	0	0
	3	537.14730	-494.26411	- 1150.36422	1.86785		2.78122 2.82477	0	0
Ciramadol	1	586.37207	-496.19169	- 1116.04223	3.09882 2.11877 2.40071 1.86581		2.22189 2.41857 2.77212 2.81323	3.9845 1 4.7677 6 4.8700 3 4.5205 5	0
	2	573.93170	-505.51168	- 1115.90386	2.09455	2.0945	2.65994	5.5996	0

					5	2.63624 2.57931 2.50345		5	
	3	587.15352	-494.19626	-1107.81336	0	0	2.42868 2.73681 2.4977 2.70036 2.78595	4.2402 9 5.5544 8	
Axomadol	1	597.94077	-494.00288	-1116.71734	1.95662 2.10747		2.48299 2.78731 2.78347 2.82001 2.67592 2.60511 2.46689 2.26538	2.5132 3 5.2209 9 4.9586 9 4.3712 6	
	2	565.88956	-504.03257	-1130.61762	2.3073 2.32016 1.89757		2.57956 2.90909 2.45908 2.76955	4.3365 2	

							2.62129 2.76271 2.6601 2.6616 2.64274			
	3	575.59989	-497.25312	-1120.77213	1.92819 1.87068 1.85022		2.8005 2.70486 2.56392 3.04411	4.6599 8 5.1583 4 5.0853 1		
1,6-Dehydro Tramadol	1	587.16664	-495.26917	-1120.50620	0	0	2.57415 2.74549 2.67206	2.40119 2.6744 3 4.8153 4 4.5100 9 4.5864 8		
	2	625.76272	-502.97433	-1069.65741	0	0	2.50075 2.33236	0	0	

						2.40527 3.03631 2.62873 2.97058 2.56127			
	3	582.73126	-496.44126	-1123.40473	2.8289	2.8289	2.35722 2.88076 2.7807 2.71708 2.64661 2.56585 2.78887 2.79045	2.7760 7 5.0553 4 4.0088 7 5.0203 8 4.3594 3 5.1925 8 5.3964 9	0
-O- Desmethyl- N,N- bisdesmethyl Tramadol	1	-1120.50620	-486.97997	-1162.35827	1.75881 1.82953 2.00506 2.9014 2.09257		2.67228	2.67228	3.8761 3 5.6071 8 4.9037 3

	2	540.42409	-498.01314	-1152.90432	2.78688 1.82917 3.02506 1.85267		2.80292 2.82973 3.09024 2.68173		4.3086	0
	3	540.22321	-491.10454	-1162.10993	1.79044 2.91825 1.90316 2.73636 2.01151		2.90936	2.90936	4.5991 7 5.4837	0
O-methyl tramadol	1	600.92549	-502.03304	-1096.43925	0	0	2.51171 2.66736 2.88543 2.30678 2.71468 2.77304 2.73602		4.2874 3 4.5037 5 4.7026 3	

	2	575.19761	-506.44861	-1116.95506	0	0	2.65571 2.90768 2.42468 2.57867 2.55039 2.56426 2.64934 2.52426 3.06866	0	0
	3	577.17579	-501.97393	-1120.92459	0	0	2.359 2.69485 2.68281	4.6555 8	0

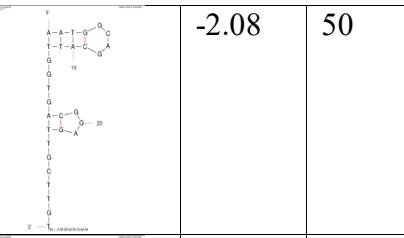
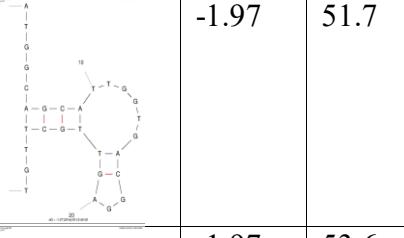
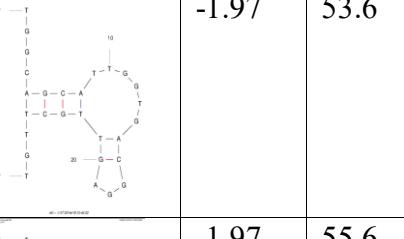
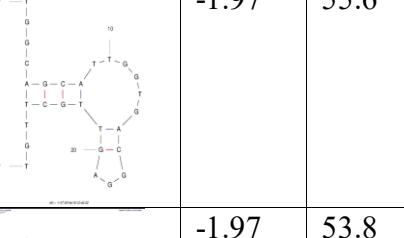
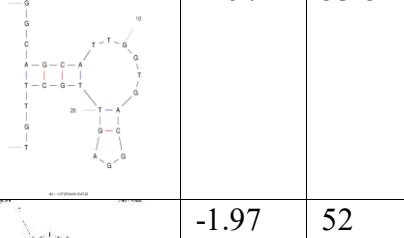
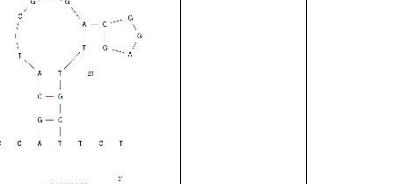
**The Truncation approach:**

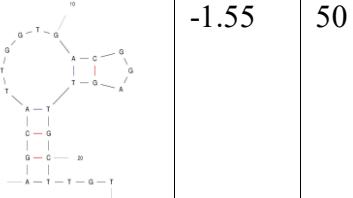
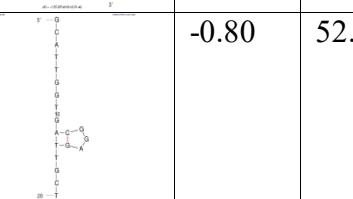
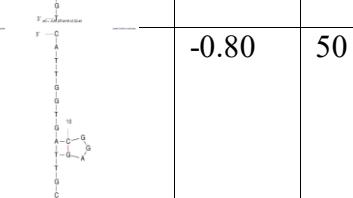
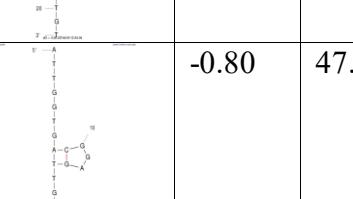
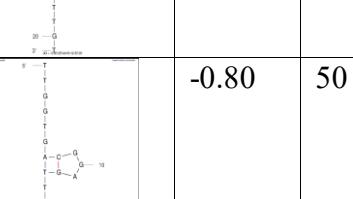
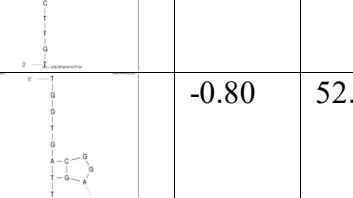
Truncation was another mutation approach where the sequences were truncated from 5' and 3' ends, and the respective thermodynamics and interaction profile for each of the sequence was checked after docking with the five opioids Fentanyl, Tramadol, Oxycodone, Codeine and Methadone.

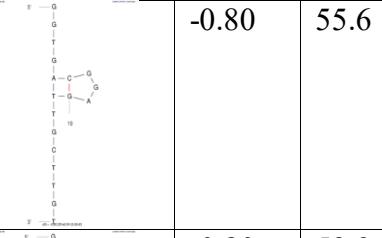
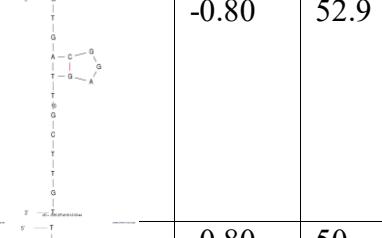
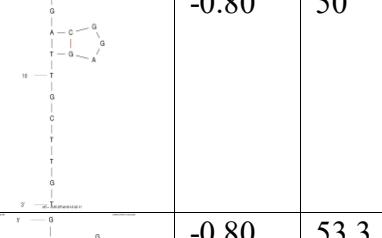
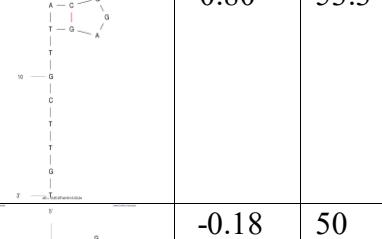
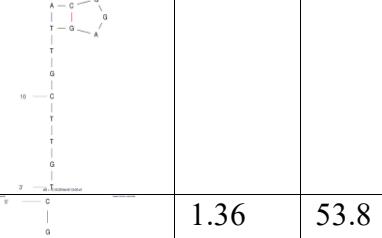
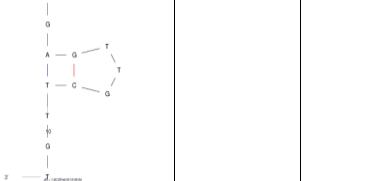
**Truncation for Fentanyl: May, 2025**

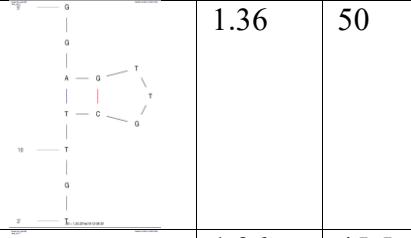
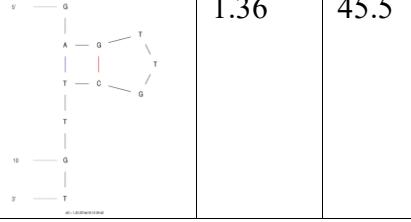
**5' Truncation of nucleotides of the aptamer sequence**

Sr.No.	SEQ3UENCE (5' reduction)	2D STRUCTURE	ΔG/kcal mol <sup>-1</sup>	GC %
1	ACAAAATGGCAGCATTGGTGAC GGAGTTGCTTGT		-2.59	47.1
2	CAAAATGGCAGCATTGGTGACG GAGTTGCTTGT		-2.59	48.5
3	AAAATGGCAGCATTGGTGACGG AGTTGCTTGT		2.59	46.9
4	AAATGGCAGCATTGGTGACGGA GTTGCTTGT		2.59	48.4

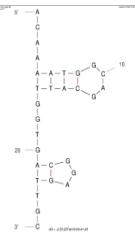
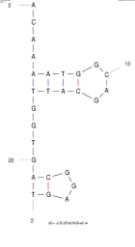
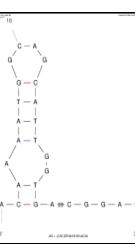
5	AATGGCAGCATTGGTGACGGAG TTGCTTGT		-2.08	50
6	ATGGCAGCATTGGTGACGGAGT TGCTTGT		-1.97	51.7
7	TGGCAGCATTGGTGACGGAGTT GCTTGT		-1.97	53.6
8	GGCAGCATTGGTGACGGAGTTG CTTGT		-1.97	55.6
9	GCAGCATTGGTGACGGAGTTGC TTGT		-1.97	53.8
10	CAGCATTGGTGACGGAGTTGCT TGT		-1.97	52

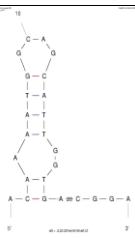
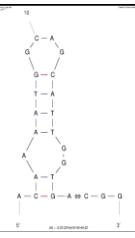
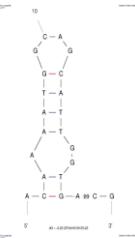
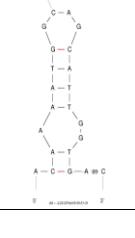
11	AGCATTGGTGACGGAGTTGCTT GT		-1.55	50
12	GCATTGGTGACGGAGTTGCTTG T		-0.80	52.2
13	CATTGGTGACGGAGTTGCTTGT		-0.80	50
14	ATTGGTGACGGAGTTGCTTGT		-0.80	47.6
15	TTGGTGACGGAGTTGCTTGT		-0.80	50
16	TGGTGACGGAGTTGCTTGT		-0.80	52.6

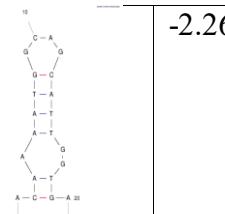
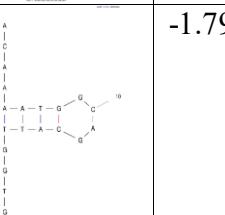
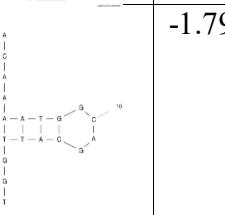
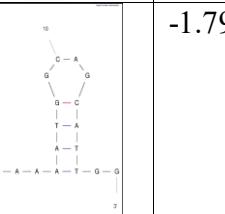
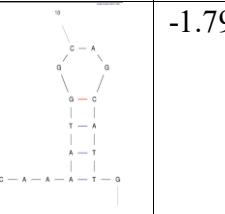
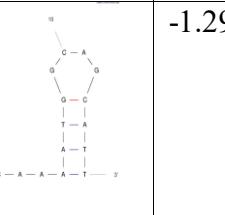
17	GGTGACGGAGTTGCTTGT		-0.80	55.6
18	GTGACGGAGTTGCTTGT		-0.80	52.9
19	TGACGGAGTTGCTTGT		-0.80	50
20	GACGGAGTTGCTTGT		-0.80	53.3
21	ACGGAGTTGCTTGT		-0.18	50
22	CGGAGTTGCTTGT		1.36	53.8

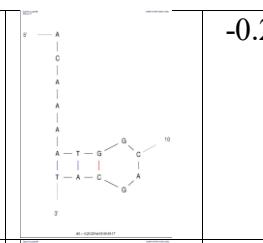
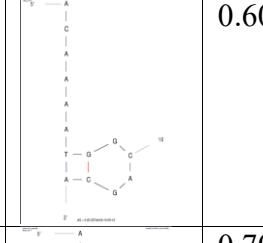
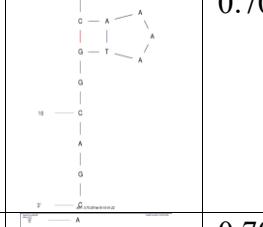
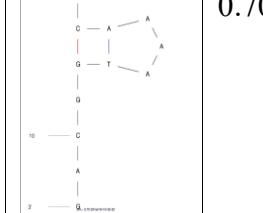
23	GGAGTTGCTTGT		1.36	50
24	GAGTTGCTTGT		1.36	45.5

Sr.No	SEQUENCE (3' reduction)	2D structure	$\Delta G/\text{kcal mol}^{-1}$	GC%
1	ACAAAATGGCAGCATTGGTGA CGGAGTTGCTTGT		-2.59	47.1
2	ACAAAATGGCAGCATTGGTGA CGGAGTTGCTTG		-2.59	48.5
3	ACAAAATGGCAGCATTGGTGA CGGAGTTGCTT		-2.59	46.9
4	ACAAAATGGCAGCATTGGTGA CGGAGTTGCT		-2.59	48.4

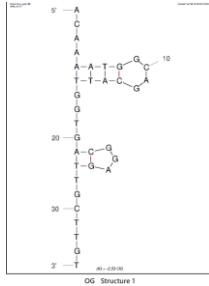
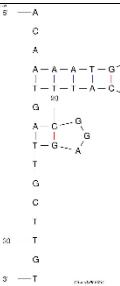
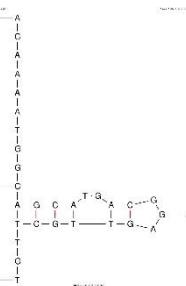
5	ACAAAATGGCAGCATTGGTGA CGGAGTTGC		-2.59	50
6	ACAAAATGGCAGCATTGGTGA CGGAGTTG		-2.59	48.3
7	ACAAAATGGCAGCATTGGTGA CGGAGTT		-2.59	46.4
8	ACAAAATGGCAGCATTGGTGA CGGAGT		-2.30	48.1
9	ACAAAATGGCAGCATTGGTGA CGGAG		-2.26	50

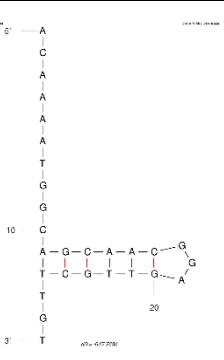
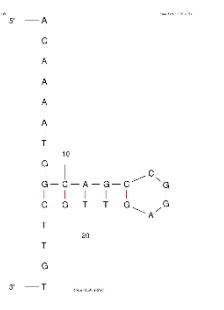
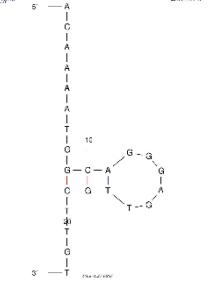
10	ACAAAATGGCAGCATTGGTGA CGGA		-2.26	48
11	ACAAAATGGCAGCATTGGTGA CGG		-2.26	50
12	ACAAAATGGCAGCATTGGTGA CG		-2.26	47.8
13	ACAAAATGGCAGCATTGGTGA C		-2.26	45.5

14	ACAAAATGGCAGCATTGGTGA		-2.26	42.9
15	ACAAAATGGCAGCATTGGTG		-1.79	45
16	ACAAAATGGCAGCATTGGT		-1.79	42.1
17	ACAAAATGGCAGCATTGG		-1.79	44.4
18	ACAAAATGGCAGCATTG		-1.79	41.2
19	ACAAAATGGCAGCATT		-1.29	37.5

20	ACAAAATGGCAGCAT		-0.29	40
21	ACAAAATGGCAGCA		0.60	42.9
22	ACAAAATGGCAGC		0.70	46.2
23	ACAAAATGGCAG		0.70	41.7

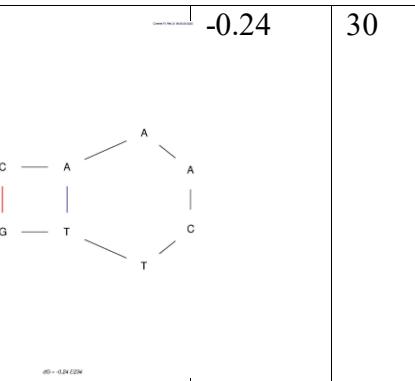
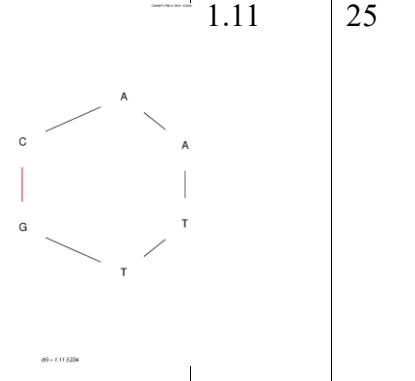
*Mid truncation of nucleotides of the aptamer sequence*

Sr no.	Sequence ( Splitting approach)	Secondary structure	$\Delta G/\text{kcal mol}^{-1}$	GC%
1.	ACAAAATGGCAGCATTGGTGA CGGAGTTGCTTGT		-2.59	
2.	ACAAAATGGCAGCATTGACG GAGTTGCTTGT		-3.09	43.8
3.	ACAAAATGGCAGCATGACGG AGTTGCTTGT		-2.57	46.7

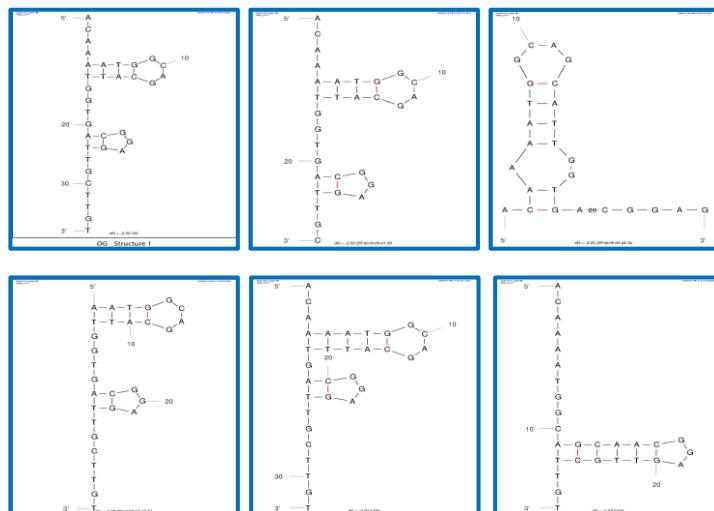
4.	ACAAAATGGCAGCAACGGAG TTGCTTGT		-6.57	46.4
5.	ACAAAATGGCAGCCGGAGTT GCTTGT		-1.95	50
6.	ACAAAATGGCAGGGAGT TGCTTGT		-1.27	48.53

7.	ACAAAATGGCAGAGTTGCTT		-1.77	40.9
8.	ACAAAATGGCAGTTGCTTGT		-0.82	40
9.	ACAAAATGGGTTGCTTGT		-0.14	38.9

10.	ACAAAATGTTGCTTGT	<p>5' — A   C — A — A   G — T — T   C   T   T   G   T</p> <p style="text-align: right;">-0.64</p> <p>31.3</p>
11.	ACAAAATTGCTTGT	<p>T — T   A — G   A — A — T   A — A — T   C — G   A — T — 3'</p> <p style="text-align: right;">-0.14</p> <p>28.6</p>
12.	ACAAAAGCTTGT	<p>A — G   C   A — T   A — A — T — 10   C — G   A — T — 3'</p> <p style="text-align: right;">-0.64</p> <p>33.3</p>

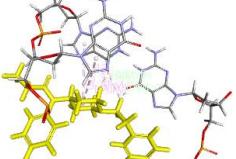
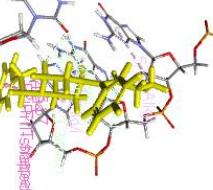
13.	ACAAACTTGT		-0.24	30
14.	ACAATTGT		1.11	25

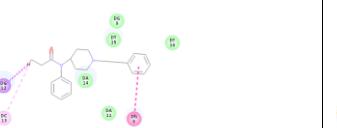
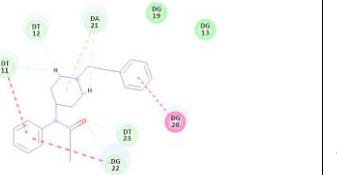
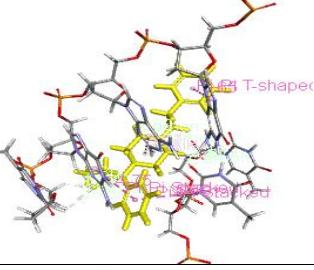
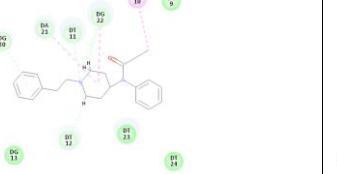
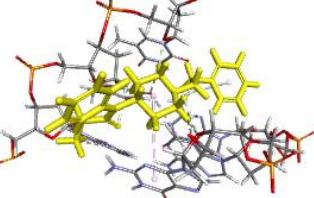
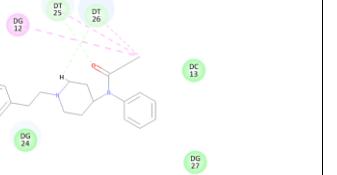
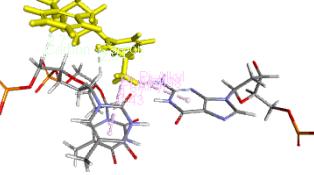
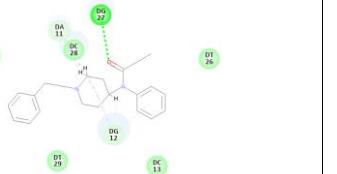
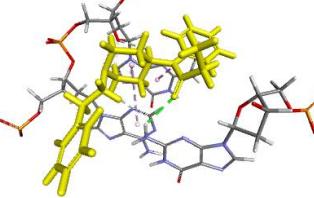
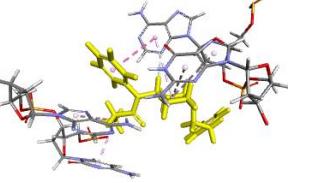
*Top 5 most favourable sequences from Truncation approach:*



Sr No.	Sequence	length	$\Delta G/\text{kcalmol}^{-1}$	GC%
1	ACAAAATGGCAGCATTGGTGACGGAGTTGCTTGT	34	-2.59	47
2	ACAAAATGGCAGCATTGGTGACGGAGTTGC	30	-2.59	50
3	ACAAAATGGCAGCATTGGTGACGGAG	26	-2.26	50
4	AATGGCAGCATTGGTGACGGAGTTGCTTGT	30	-2.08	50
5	ACAAAATGGCAGCATTGACGGAGTTGCTTGT	32	-3.09	43.8
6	ACAAAATGGCAGCAACGGAGTTGCTTGT	28	-6.57	46.4

*Summary of ligand- aptamer interaction of truncated sequences*

Aptamer	Loop	2D diagram	Interactions	LibDOCK score	Poses
T2	L1			90.5702	83
	L2			106.221	100

T3	L1			85.4313	7
T4	L1			117.435	100
	L2			116.353	100
T5	L1			44.775	4
	L2			98.4182	100
T6	L1			94.9582	98

***Summary of the thermodynamics, Interaction types and Bond lengths of the mutated aptamers***

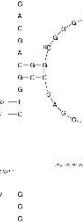
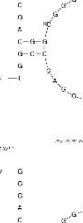
	L	Potential Energy (kcal/mol)	Van der Waals Energy (kcal/mol)	Electrostatic Energy (kcal/mol)	Favourable interactions	Conventional Hydrogen bonds	Mean length	Non – conventional	Mean length
T2	L1	1635.42561	989.44182	-800.80773	5	NA	NA	3	2.179
	L2	606.06152	-10.84226	-830.87382	10	NA	NA	6	2.584
T3	L1	487.27610	-7.97423	-794.06718	4	NA	NA	NA	NA
T4	L1	-85.8419	-175.49299	-1343.48516	11	NA	NA	7	2.579
	L2	20759.39211	20700.33107	-1375.22518	11	NA	NA	7	2.786
T5	L1	1435.52711	1006.35550	-1065.92452	5	NA	NA	2	2.856
	L2	87.84553	-354.36073	-1059.51429	8	1	2.397	4	2.476
T6	L1	283.29990	-27.49391	-1023.74018	7	NA	NA	1	2.556

*Truncation for Oxycodone: Original aptamer: OM16:*

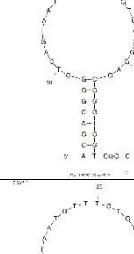
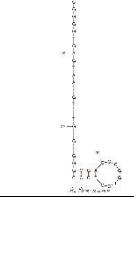
Sr no	Sequence	2D diagram	Dg
OG	5'GGGACGACGGGGGGAGTAATGTTGTGGGGGAGCCGGTCGTCCC3'		-8.79
O1(5')	GGACGACGGGGGGAGTAATGTTGTGGGGGAGCCGGTCGTCCC		-7.26 10-35
O2	GACGACGGGGGGAGTAATGTTGTGGGGGAGCCGGTCGTCCC		-5.42 9-34
O3	ACGACGGGGGGAGTAATGTTGTGGGGGAGCCGGTCGTCCC		-3.95 8-33
O4	CGACGGGGGGAGTAATGTTGTGGGGGAGCCGGTCGTCCC		-2.72

05	GACGGGGGGAGTAATGTTGTGTGGGGGAGCCGGTCGTCCC		-1.64
06	ACGGGGGGAGTAATGTTGTGTGGGGGAGCCGGTCGTCCC		-1.64
07	CGGGGGGAGTAATGTTGTGTGGGGGAGCCGGTCGTCCC		-1.64

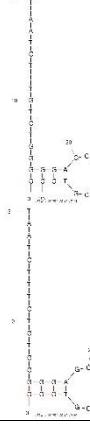
Sr No	Sequence	2D diagram	Dg
OG	GGGACGACGGGGGGAGTAATGTTGTGTGGGGGAGCCGGTCGTCCC		
08(3')	GGGACGACGGGGGGAGTAATGTTGTGTGGGGGAGCCGGTCGTCC		-7.51 11-36
09	GGGACGACGGGGGGAGTAATGTTGTGTGGGGGAGCCGGTCGTC		-5.67 11-36

O10	GGGACGACGGGGGGAGTAATGTTGTGGGGGAGCCGGTCGT		-4.38 11-36
O11	GGGACGACGGGGGGAGTAATGTTGTGGGGGAGCCGGTCG		-3.33
O12	GGGACGACGGGGGGAGTAATGTTGTGGGGGAGCCGGTC		-0.71
O13	GGGACGACGGGGGGAGTAATGTTGTGGGGGAGCCGGT		-0.71
O14	GGGACGACGGGGGGAGTAATGTTGTGGGGGAGCCGG		-0.71

*Truncation for Mutated Oxycodone aptamer: OX5:*

Sr no	OX 5 Sequence	2D Diagram	Dg value
1	5'GGGACGACGGGTGAGTAATGTTGTGGGGAGCCGGTCGTCCC3'		- 8.79
X1	GGACGACGGGTGAGTAATGTTGTGGGGAGCCGGTCGTCCC		- 7.26 9-35
X2	GACGACGGGTGAGTAATGTTGTGGGGAGCCGGTCGTCCC		- 5.42 9-34
X3	ACGACGGGTGAGTAATGTTGTGGGGAGCCGGTCGTCCC		- 3.95 8-33
X4	CGACGGGTGAGTAATGTTGTGGGGAGCCGGTCGTCCC		- 2.72
X5	GACGGGTGAGTAATGTTGTGGGGAGCCGGTCGTCCC		- 1.64

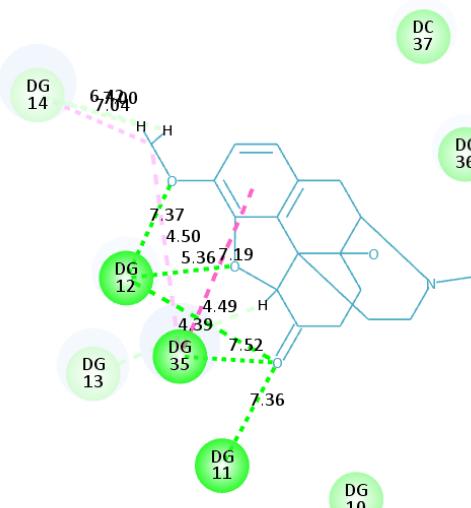
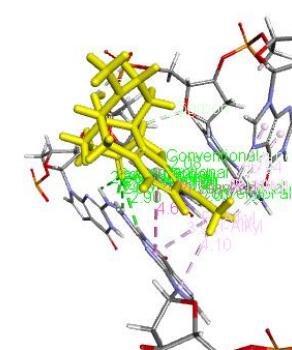
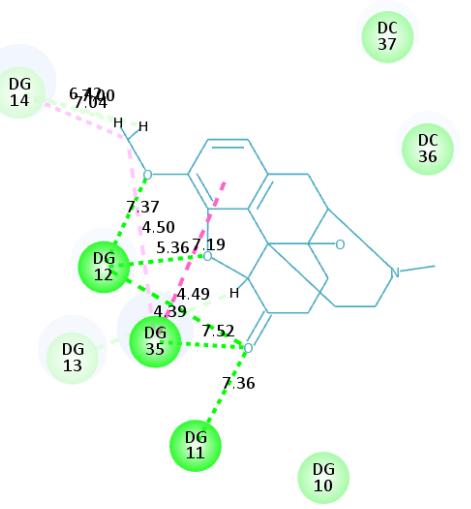
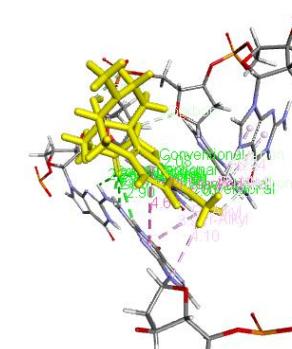
X6	ACGGGGTGAGTAATGTTGTGTGGGGGAGCCGGTCGTCCC		- 1.64
X7	CGGGGTGAGTAATGTTGTGTGGGGGAGCCGGTCGTCCC		- 1.64
OX 5 SEQ	GGGGTGAGTAATGTTGTGTGGGGGAGCCGGTCGTCCC		-1.64
X8	GGTGAGTAATGTTGTGTGGGGGAGCCGGTCGTCCC		- 1.64
X9	GTGAGTAATGTTGTGTGGGGGAGCCGGTCGTCCC		-1.64
X10	TGAGTAATGTTGTGTGGGGGAGCCGGTCGTCCC		- 1.64

X11	GAGTAATGTTGTGGGGGAGCCGGTCGTCCC		-1.64
X12	AGTAATGTTGTGGGGGAGCCGGTCGTCCC		-1.64
X13	GTAATGTTGTGGGGGAGCCGGTCGTCCC		-1.64
X14	TAATGTTGTGGGGGAGCCGGTCGTCCC		-1.64

*Docking analysis for truncated aptamers:*

Analogue	Loop	Binding Site	2D diagram	Ligand interaction	LibDock Score	No. Poses
<b>5' Truncation</b>						
O1	1				97.1788	62
O2	1				96.2037	58

O3	1	<p><b>3' Truncation</b></p>		85.0261	58
O8	1			96.7257	52

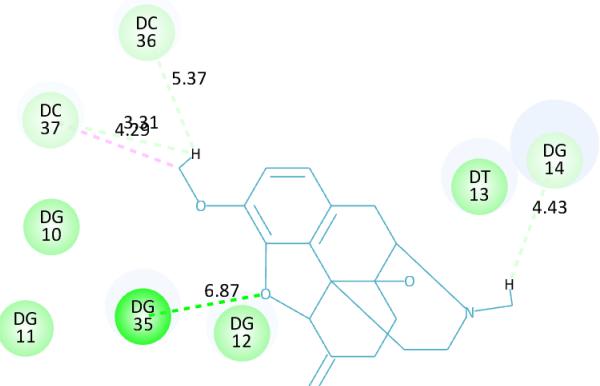
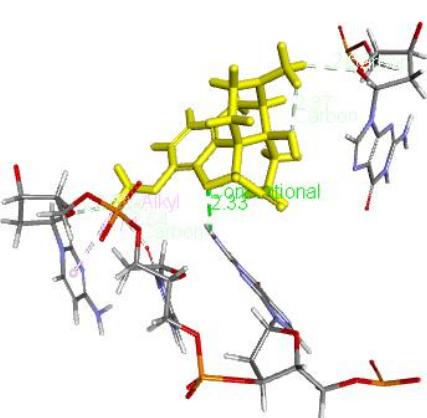
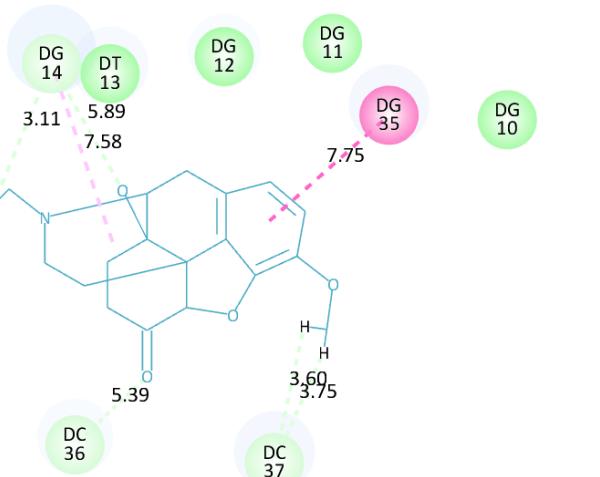
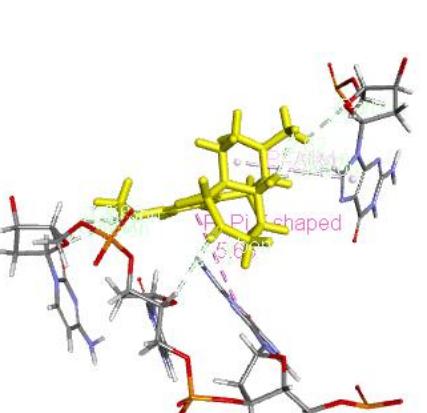
O9	1			96.7257	52
O10	1			96.7257	52

*Summary of thermodynamics, Interaction profile and Bond types:*

Sequence	Potential Energy (kcal/mol)	Vander Waals Energy (Kcal/mol)	Electrostatic Energy (Kcal/mol)	Conventional hydrogen bond	Mean Length	C-H bond	Mean length	Hydrophobic Interaction	Mean length	Others(Electrostatic)
O1	216.885	-505.359	-1514.62	5	2.22	4	2.7	6	4.6	NA
O2	228.641	-492.024	-1476.44	7	2.5	2	2.5	4	4.3	NA
O3	294.857	-477.353	-1373.69	2	2.55	1	2.8	1	5.1	NA
O8	197.885	-496.742	-1542.83	8	2.45	4	2.6	5	4.4	NA
O9	233.827	-484.904	-1474.15	8	2.45	4	2.6	5	4.4	NA
O10	317.541	-472.08	-1358.25	8	2.45	4	2.6	5	4.4	NA

*Truncation for mutated Oxycodone sequence: Aptamer: OX5:*

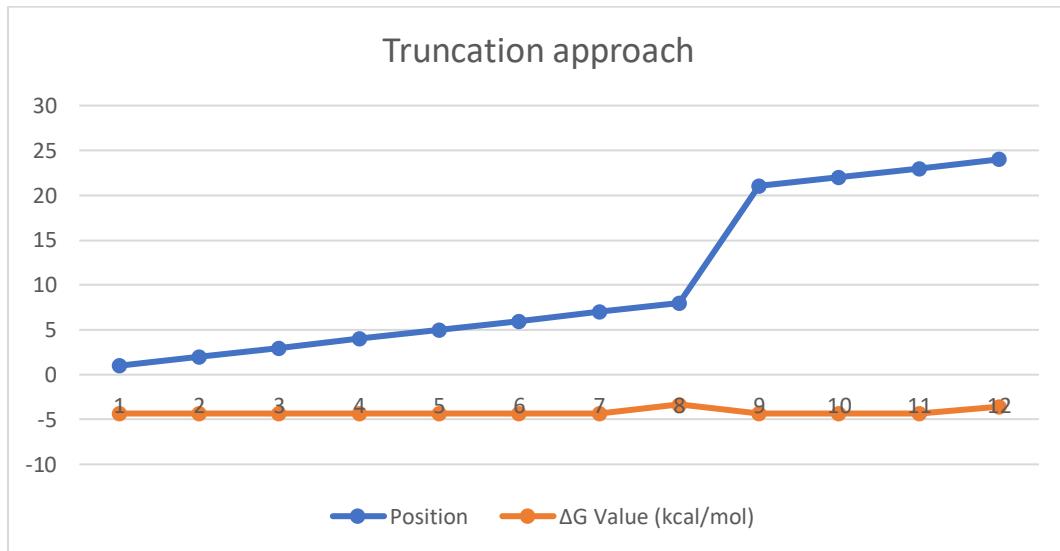
Analogue	Loop	Binding Site	2D diagram	Ligand interaction	LibDock Score	No. Poses
Ox1	1				98.0076	61

Ox2	1			88.3364	65
Ox3	1			88.1794	52

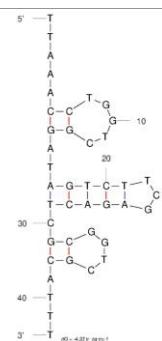
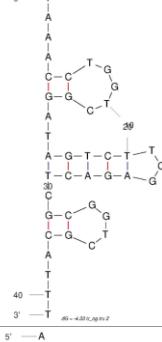
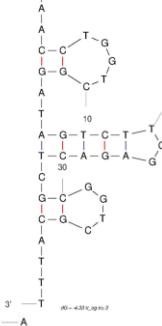
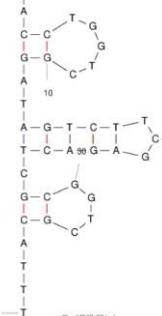
*Summary of Thermodynamics, Interaction profile and Bond types:*

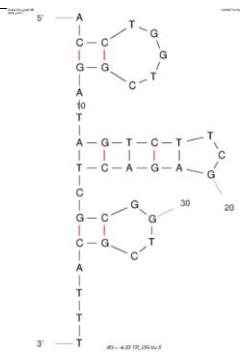
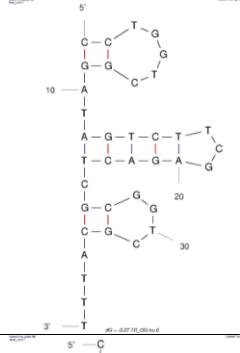
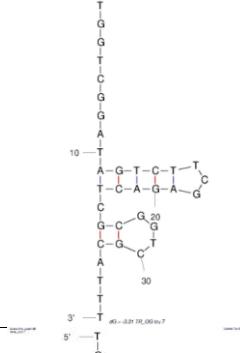
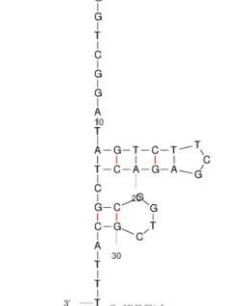
Analogue	Potential Energy (kcal/mol)	Vander Waals Energy (Kcal/mol)	Electrostatic Energy (Kcal/mol)	Conventional hydrogen bond	Mean Length	C - H bond	Mean length	Hydrophobic Interaction	Mean length	Others( Electrostatic)
Ox1	246.08	-511.562	-1499.46	6	2.45	3	2.45	5	4.36	NA
Ox2	277.695	-496.226	-1439.05	1	2.33	4	2.52	1	4.74	NA
Ox3	359.93	-487.144	-1321.5	NA	NA	7	2.6	2	5.45	NA

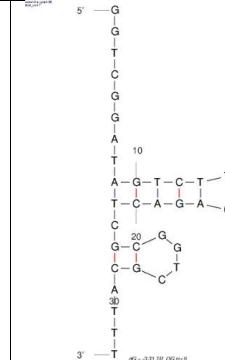
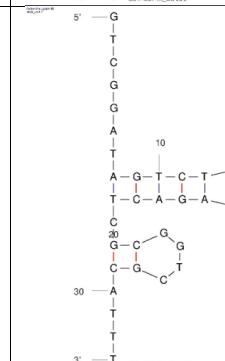
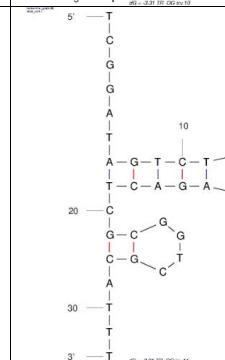
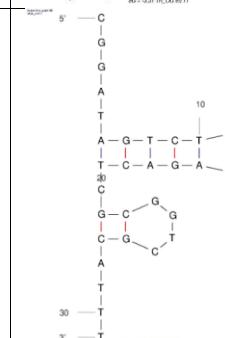
**Truncation for Tramadol: Original Aptamer: TR\_OG:**

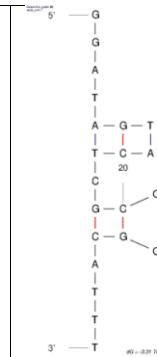
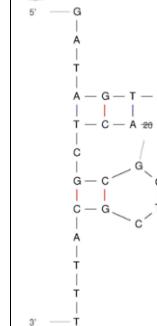
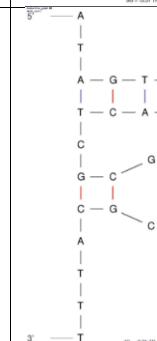
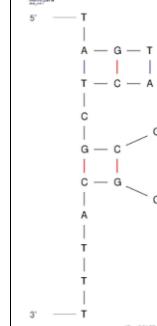


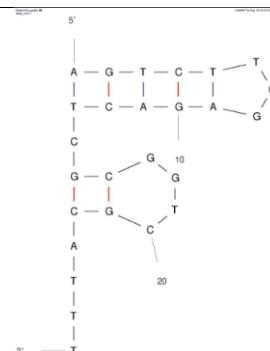
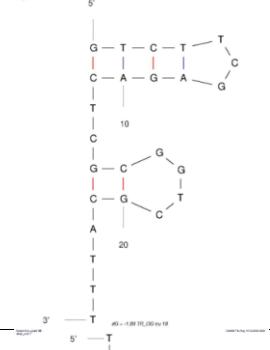
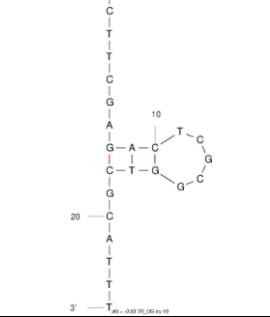
Sr no.	Sequence	Dot bracket structure	2D Diagram	Dg value
<b>5' truncation</b>				
1	CTTAAACCTGGTCGGATAGTCTTCGAGACTCGCGGTC GCATT	.....((.....)).((((.....))).((.....))....		-4.33

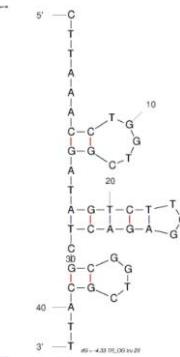
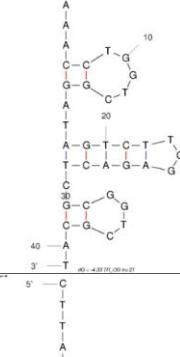
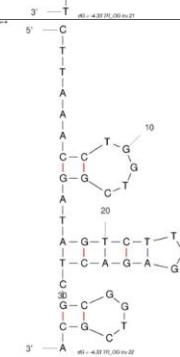
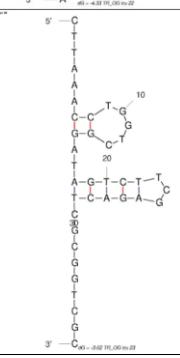
2	TTAACCTGGTCGGATAGTCTTCGAGACTCGCGGTGCG CATT	.....((....)).((((....))))((....))....		-4.33
3	TAAACCTGGTCGGATAGTCTTCGAGACTCGCGGTGCGC ATT	.....((....)).((((....))))((....))....		-4.33
4	AAACCTGGTCGGATAGTCTTCGAGACTCGCGGTGCGC ATT	...((....)).((((....))))((....))....		-4.33
5	AACCTGGTCGGATAGTCTTCGAGACTCGCGGTGCGCAT TT	..((....)).((((....))))((....))....		-4.33

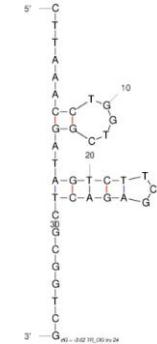
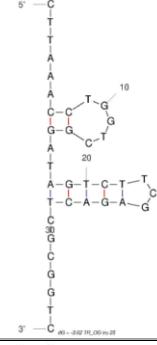
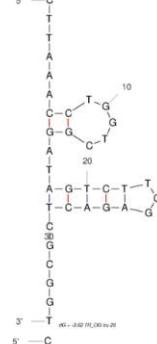
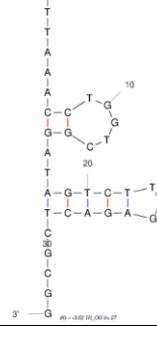
6	ACCTGGTCGGATAGTCTTCGAGACTCGCGGTGCATT T  TR_OG tru 5	.((.....)).((((....))))..((....))....		-4.33
7	CCTGGTCGGATAGTCTTCGAGACTCGCGGTGCATT  TR_OG tru 6	((.....)).((((....))))..((....))....		-3.37
8	CTGGTCGGATAGTCTTCGAGACTCGCGGTGCATT  TR_OG tru 7	.....((((....))))..((....))....		-3.31
9	TGGTCGGATAGTCTTCGAGACTCGCGGTGCATT  TR_OG tru 8	.....((((....))))..((....))....		-3.31

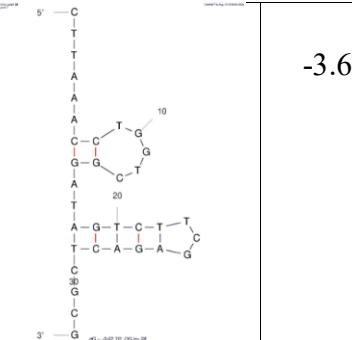
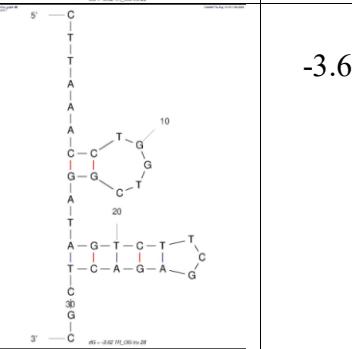
10	GGTCGGATAGTCTCGAGACTCGCGGTCGCATT  TR_OG tru 9	.....((((....))))((....))....		-3.31
11	GTCGGATAGTCTCGAGACTCGCGGTCGCATT  TR_OG tru 10	.....((((....))))((....))....		-3.31
12	TCGGATAGTCTCGAGACTCGCGGTCGCATT  TR_OG tru 11	.....((((....))))((....))....		-3.31
13	CGGATAGTCTCGAGACTCGCGGTCGCATT  TR_OG tru 12	.....((((....))))((....))....		-3.31

14	GGATAGTCTTCGAGACTCGCGGTCGCATT  TR_OG tru 13	....((((...))))((....))....		-3.31
15	GATAGTCTTCGAGACTCGCGGTCGCATT  TR_OG tru 14	...((((...))))((....))....		-3.31
16	ATAGTCTTCGAGACTCGCGGTCGCATT  TR_OG tru 15	..((((...))))((....))....		-3.31
17	TAGTCTTCGAGACTCGCGGTCGCATT  TR_OG tru 16	.((((...))))((....))....		-3.31

18	AGTCTTCGAGACTCGCGGTGCGATT  TR_OG tru 17	(((((...))))..((....))....		-2.60
19	GTCTTCGAGACTCGCGGTGCGATT  TR_OG tru 18	(((((...))))..((....))....		-1.89
20	TCTTCGAGACTCGCGGTGCGATT  TR_OG tru 19	.....(((....)))......		-0.93
<b>3' Truncation</b>				

21	CTTAAACCTGGTCGGATAGTCTTCGAGACTCGCGGTC GCATT  TR-OG tru 20	.....((....)).((((...))))((....))...		-4.33
22	CTTAAACCTGGTCGGATAGTCTTCGAGACTCGCGGTC GCAT  TR_OG tru 21	.....((....)).((((...))))((....))..		-4.33
23	CTTAAACCTGGTCGGATAGTCTTCGAGACTCGCGGTC GCA  TR_OG tru 22	.....((....)).((((...))))((....)).		-4.33
24	CTTAAACCTGGTCGGATAGTCTTCGAGACTCGCGGTC GC  TR_OG tru 23	.....((....)).((((...)))).....		-3.62

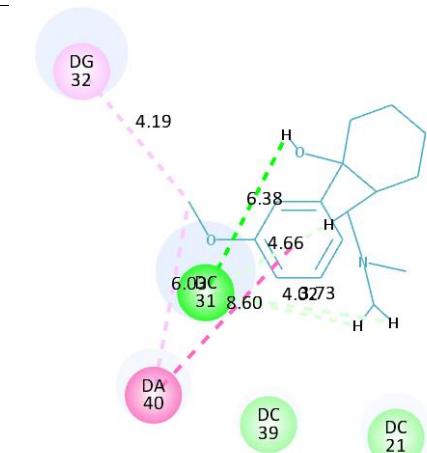
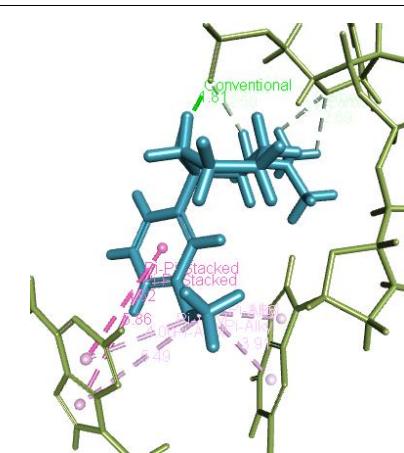
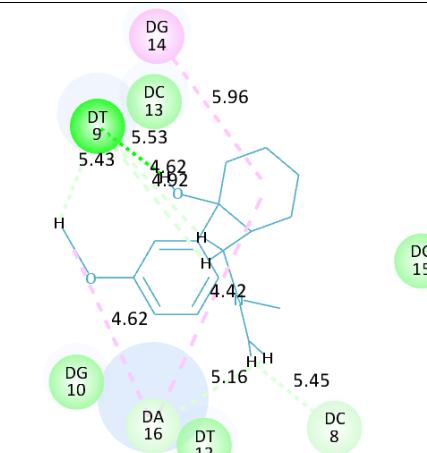
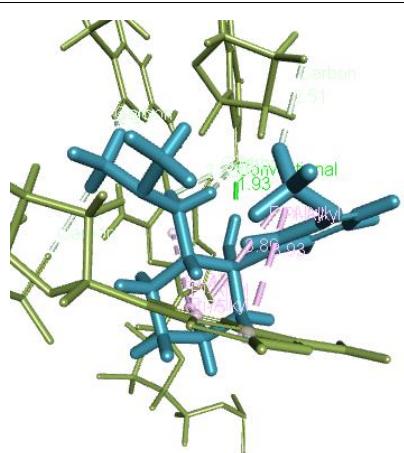
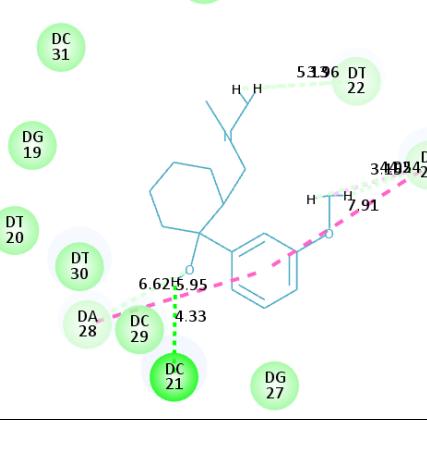
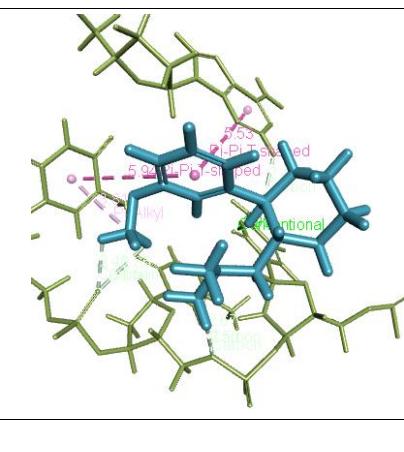
25	CTTAAACCTGGTCGGATAGTCTTCGAGACTCGCGGT G  TR_OG tru 24	.....((....))..((((...)))).....		-3.62
26	CTTAAACCTGGTCGGATAGTCTTCGAGACTCGCGGT C  TR_OG tru 25	.....((....))..((((...)))).....		-3.62
27	CTTAAACCTGGTCGGATAGTCTTCGAGACTCGCGGT T  TR_OG tru 26	.....((....))..((((...)))).....		-3.62
28	CTTAAACCTGGTCGGATAGTCTTCGAGACTCGCGG A  TR_OG tru 27	.....((....))..((((...)))).....		-3.62

29	<p>CTTAAACCTGGTCGGATAGTCTTCGAGACTCGCG TR_OG tru 28</p>	<p>.....((.....))..((((((...))))))....</p>		<p>-3.62</p>
30	<p>CTTAAACCTGGTCGGATAGTCTTCGAGACTCGC TR_OG tru 29</p>	<p>.....((.....))..((((((...))))))....</p>		<p>-3.62</p>

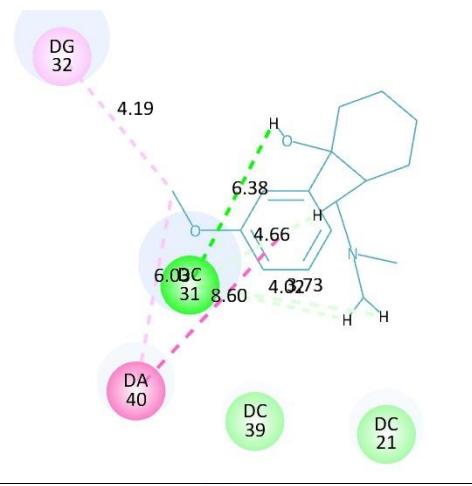
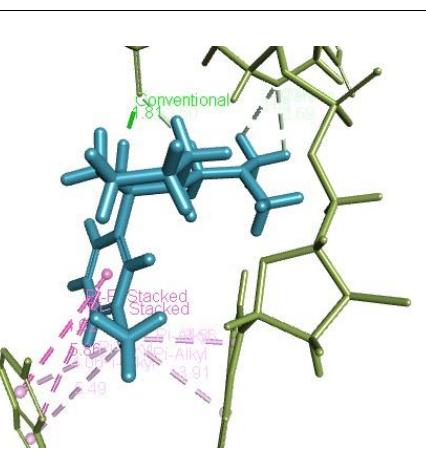
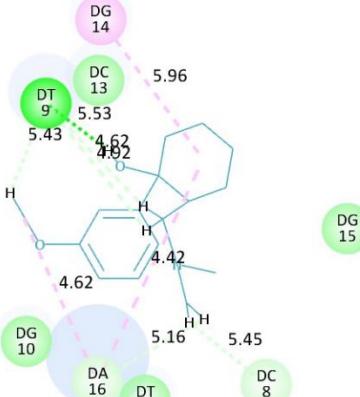
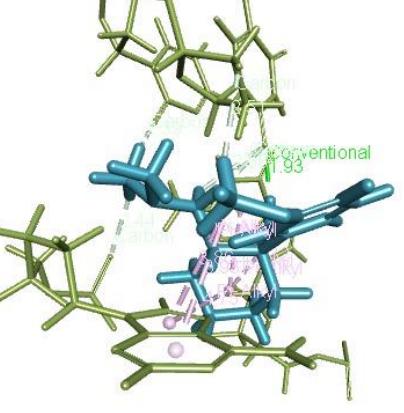
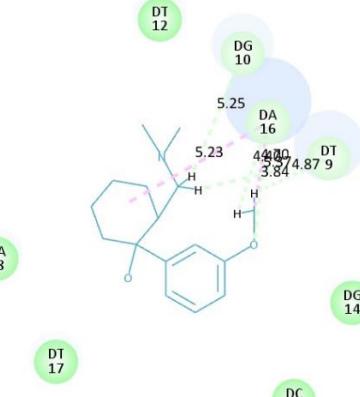
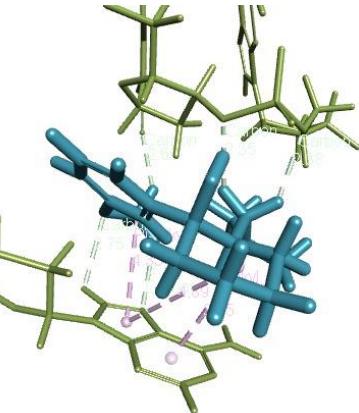
**Docking analysis:**

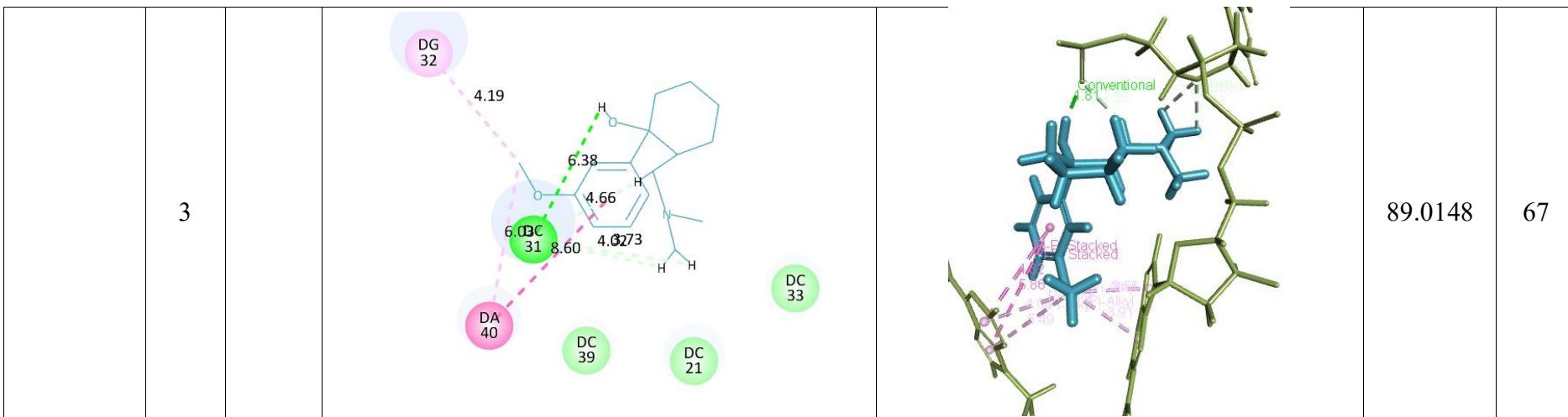
Sequence	Loo p	Binding Site	2D diagram	Ligand interaction	LibDock Score	No. Poses
<b>5' truncation</b>						
Tru 1	1				113.947	61
	2				90.3457	60

		<p>3</p> <p>DG 32 DA 40 DC 31 DC 39 DC 21 DC 33</p> <p>4.19 6.38 4.66 8.60 4.0273 6.03C</p>	<p>Conventional Anti-Stacked Anti-Alkyl</p>	86.0369	62
Tru 2	1	<p>DG 14 DA 16 DC 13 DT 9 DG 10 DG 15 DC 8</p> <p>5.89 4.46 5.11 6.23 5.48 5.64 4.66 9.5</p>	<p>Conventional Anti-Stacked Anti-Alkyl</p>	113.289	61
	2	<p>DG 19 DT 20 DT 30 DA 28 DC 29 DC 21 DG 27</p> <p>6.62 5.95 4.33 3.40 3.44 2.23 7.91</p>	<p>Conventional Anti-Stacked Anti-Alkyl</p>	91.3457	60

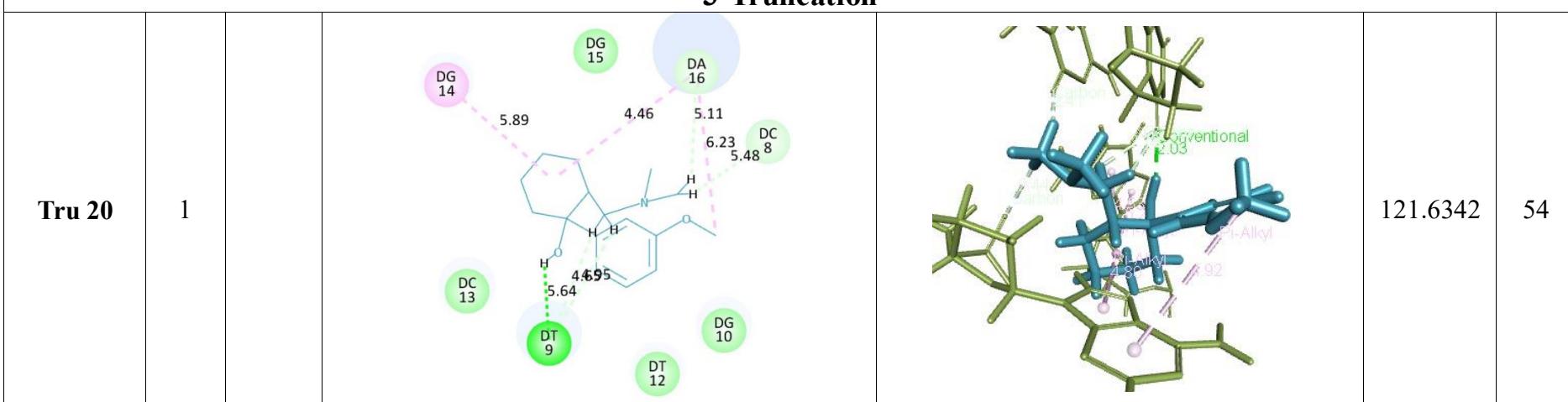
					87.8349	62
Tru 3	1				116.606	61
	2				89.8628	71

	3		<p>Detailed description: This diagram shows a chemical structure of a ligand interacting with a protein. Key interactions include:  - DT 20 (4.10) and DG 19 (4.44) with a nitrogen atom.  - DC 21 (7.84) and DT 30 (5.34) with a carbonyl oxygen.  - DG 29 (6.48) and DA 28 (4.12) with another carbonyl oxygen.  - DG 27 (4.358) and DG 28 (5.454) with a hydrogen atom.  - DC 31 (4.12) with a nitrogen atom.  - DG 14 (5.96) with a carbonyl oxygen.  - DG 13 (5.53) and DT 9 (5.43) with a nitrogen atom.  - DG 15 (5.45) and DC 8 (5.16) with a carbonyl oxygen.  - DG 10 (4.62) and DA 16 (4.62) with a nitrogen atom.  - DT 12 (4.42) with a carbonyl oxygen.</p>	<p>Detailed description: A 3D ribbon model of the protein-ligand complex. The ligand is shown as a blue stick model, and the protein backbone is shown as green sticks. Several magenta dashed lines represent interactions between the ligand and the protein backbone or side chains.</p>	90.2898	67
Tru 4	1		<p>Detailed description: This diagram shows a chemical structure of a ligand interacting with a protein. Key interactions include:  - DG 14 (5.96) with a carbonyl oxygen.  - DG 13 (5.53) and DT 9 (5.43) with a nitrogen atom.  - DG 15 (5.45) and DC 8 (5.16) with a carbonyl oxygen.  - DG 10 (4.62) and DA 16 (4.62) with a nitrogen atom.  - DT 12 (4.42) with a carbonyl oxygen.  - DG 16 (5.45) and DC 8 (5.16) with a carbonyl oxygen.</p>	<p>Detailed description: A 3D ribbon model of the protein-ligand complex. The ligand is shown as a blue stick model, and the protein backbone is shown as green sticks. Several magenta dashed lines represent interactions between the ligand and the protein backbone or side chains.</p>		
	2		<p>Detailed description: This diagram shows a chemical structure of a ligand interacting with a protein. Key interactions include:  - DC 31 (4.10) and DG 19 (4.44) with a nitrogen atom.  - DT 20 (7.84) and DT 30 (5.34) with a carbonyl oxygen.  - DA 28 (6.48) and DC 29 (5.16) with a nitrogen atom.  - DC 21 (4.358) and DG 27 (5.454) with a hydrogen atom.  - DT 22 (4.12) and DT 23 (4.12) with a carbonyl oxygen.</p>	<p>Detailed description: A 3D ribbon model of the protein-ligand complex. The ligand is shown as a blue stick model, and the protein backbone is shown as green sticks. Several magenta dashed lines represent interactions between the ligand and the protein backbone or side chains.</p>		

						
Tru 5	1				87.8634	57
	2				91.2436	61

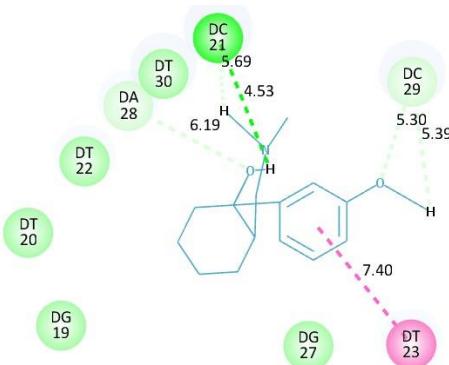
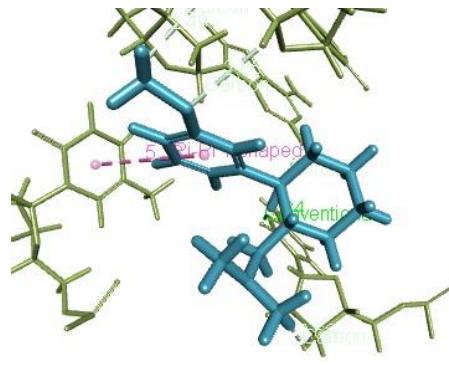
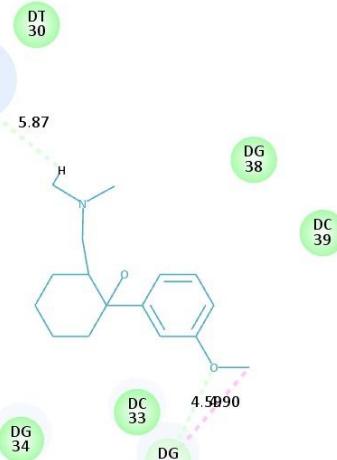
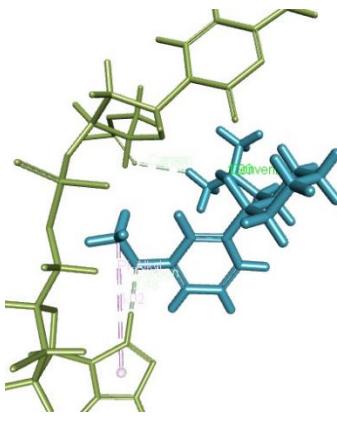


### 3' Truncation



	2			119.2438	61
	3			87.9234	61
Tru 21	1			113.4347	57

	2	<p>Chemical structure diagram of compound 2 showing interactions with residues DT 30, DG 27, DC 29, DA 28, DT 21, DT 22, H 4.05, H 5.22, H 5.20, H 5.10, and DT 23.</p>	<p>3D surface plot of binding site for compound 2.</p>	112.3454	62
	3	<p>Chemical structure diagram of compound 3 showing interactions with residues DG 32, DA 40, C 6.08, C 8.60, C 31, H 4.19, H 6.38, H 4.66, H 4.03, H 272, DC 33, DC 39, DC 21.</p>	<p>3D surface plot of binding site for compound 3.</p>	91.6723	61
Tru 22	1	<p>Chemical structure diagram of Tru 22 showing interactions with residues DG 14, DC 13, DT 9, DG 15, H 5.89, H 5.64, H 4.46, H 5.11, H 6.23, H 5.48, DA 16, DC 8, DG 10, and DT 12.</p>	<p>3D surface plot of binding site for Tru 22.</p>	97.8234	60

	2			93.3423	54
	3			74.562	67

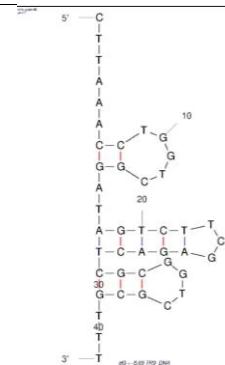
*Summary of Thermodynamics, Interaction Profile and Bond types:*

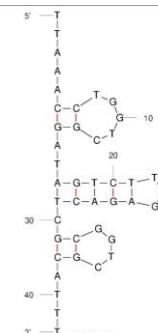
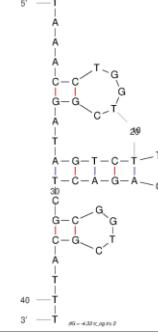
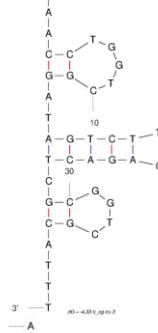
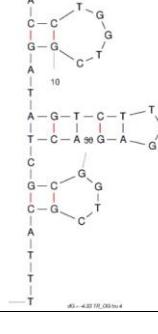
Sequence	Loop	Potential Energy (kcal/ mol)	Vander Waals Energy (Kcal/ mol)	Electrostatic Energy (Kcal/ mol)	Conventional hydrogen bond	Mean Length	C- H bond	Mean length	Hydrophobic Interaction	Mean length	Others( Electrostatic)
TRU 1	1	595.86905	-476.75621	-1088.88412	1	2.025	4	2.62	4 (Pi- alkyl)	4.92	NA
	2	609.70009	-493.34436	-1061.38943	1	1.94	5	2.57	2 (Pi- pi T shaped), 1 (Pi- alkyl)	5.73, 3.81	NA

	3	589.28025	-484.43945	-1087.65379	1	1.80	3	2.61	2 (Pi- pi T shaped), 4 (Pi- alkyl)	5.23	NA
TRU 2	1	599.65593	-461.73011	-1057.89896	1	1.94	5	2.57	1 (Pi- pi T shaped, 1 (Pi- alkyl)	3.94, 3.82	NA
	2	613.68672	-478.31729	-1030.20617	1	1.84	4	2.63	3 (Pi- alkyl)	4.75	NA
	3	593.26688	-469.41237	-1056.47054	2	2.37	5	2.70	2 (Pi- pi T shaped, 2 (Pi- alkyl)	5.55, 5.17	NA
TRU 3	1	564.71806	-452.86416	-1060.36874	1	1.93	5	2.61	5 (Pi- alkyl)	3.41	NA
	2	586.18799	-468.90650	-1024.51866	2	1.53	3	5.12	1 (Pi- alkyl), 2 (Pi- pi T shaped)	4.45, 6.93	NA
	3	518.67796	-470.56786	-1085.03667	1	2.13	4	4.45	2 (Pi- pi T shaped)	5.14	NA
TRU 4	1	548.82007	-441.57773	-1039.95416	1	1.93	5	2.60	5 (Pi- alkyl)	4.47	NA
	2	488.10786	-462.13200	-1072.99805	1	1.94	5	2.57	2 (Pi- pi T shaped), 1 (Pi- alkyl)	5.73, 3.81	NA
	3	522.93579	-460.79382	-1037.93661	1	1.80	3	2.61	2 (Pi- pi T shaped), 4 (Pi- alkyl)	5.24, 4.25	NA
TRU 5	1	497.75458	-435.60901	-1045.26518	1	1.95	3	2.74	3 (Pi- pi T shaped)	5.16	NA
	2	483.78387	-443.77758	-1048.06915	0	NA	3	2.82	0	NA	NA
	3	498.36149	-442.74815	-1036.12770	1	1.86	2	2.36	2 (Pi- alkyl), 1 (Pi- pi T shaped)	5.12, 3.86	NA
TRU 20	1	546.7124	-445.8234	-1047.0916	1	2.02	4	2.62	4 (Pi- alkyl)	4.88	NA
	2	468.9342	-440.5681	-1078.923	1	1.94	5	2.57	2 (Pi- pi T shaped), 1 (Pi- alkyl)	5.73, 3.81	NA
	3	542.5649	-432.7182	-1045.6902	1	1.82	3	2.64	2 (Pi- pi T shaped), 1 (Pi- alkyl)	5.27, 4.28	NA

<b>TRU 21</b>	1	567.8912	-454.6782	-1023.7032	1	2.12	4	2.88	4 (Pi- alkyl)	5.71	NA
	2	494.8561	-442.3846	-1084.365	1	1.82	5	2.65	1 (Pi- alkyl)	3.97	NA
	3	485.6120	-538.9240	-1046.8923	1	1.90	3	2.71	2 (Pi- pi T shaped), 4 (Pi- alkyl)	5.17, 4.56	NA
<b>TRU 22</b>	1	456.8729	-589.9123	-1061.2884	1	2.05	4	2.63	4 (Pi- alkyl)	4.88	NA
	2	490.3418	-512.8734	-1056.7184	1	1.84	4	2.59	1 (Pi- pi T shaped)	5.38	NA
	3	437.1214	-562.8134	-1089.2134	1	1.97	3	2.67	2 (Pi- pi T shaped)	5.14	NA

**Docking analysis for mutated sequences:**

Sr no.	Sequence	Dot bracket structure	2D Diagram	Dg value
<b>5' truncation</b>				
1	CTTAAACCTGGTCGGATAGTCTTCGAGACTCGCGGTG GC GTTT  TR_9	.....((.....)).((((....))))((.....))....		-5.69

2	TTAACCTGGTCGGATAGTCTTCGAGACTCGCGGTGCG CGTT  <b>R1</b>	.....((....)).((((....))))((....))....		-5.69
3	TAAACCTGGTCGGATAGTCTTCGAGACTCGCGGTGCGC GTTT  <b>R2</b>	.....((....)).((((....))))((....))....		-5.69
4	AAACCTGGTCGGATAGTCTTCGAGACTCGCGGTGCGC GTTT  <b>R3</b>	...((....)).((((....))))((....))....		-4.33
5	AACCTGGTCGGATAGTCTTCGAGACTCGCGGTGCGCT TT  <b>R4</b>	..((....)).((((....))))((....))....		-4.33

**Docking analysis:**

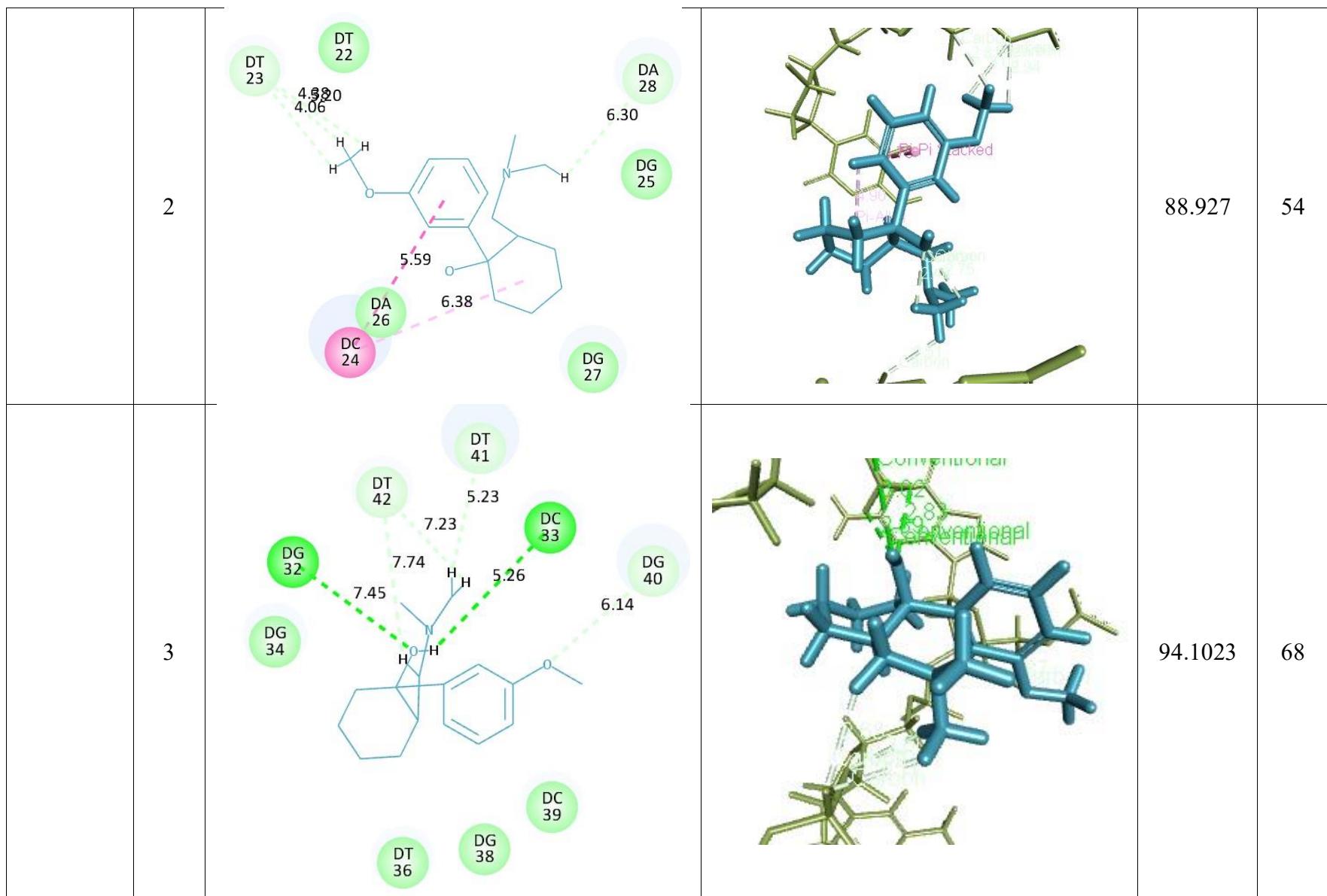
Sequence	Loo p	2D diagram	Ligand interaction	LibDock Score	No. Poses
R1	1			126.15	62
	2			89.0144	61

				93.1002	60
R2	1			126.15	71

2			88.0162	62
3			94.1063	60

R3	1			121.165	54
	2			94.0722	62

R4	1	<p>Chemical structure diagram of compound R4-1 showing interactions with residues DT 9, DC 7, DG 10, DA 16, DT 17, DG 14, DG 15, DG 16, and DG 17. Distances are labeled: 7.25, 6.85, 6.94, 6.80, 5.91, 5.25, 6.69, 3.74, 2.89.</p>	<p>3D ribbon diagram of the R4-1 binding site showing the ligand in blue and orange sticks, surrounded by green sticks representing the protein backbone. Labels include Conventional, T-shaped, and Alkyl.</p>	123.152	60
	3	<p>Chemical structure diagram of compound R4-3 showing interactions with residues DT 36, DG 38, DG 34, DG 35, DG 39, DC 33, DC 34, DG 40, DT 41, and DG 32. Distances are labeled: 4.91, 4.40, 5.33, 4.49, 4.48, 4.01, 6.92.</p>	<p>3D ribbon diagram of the R4-3 binding site showing the ligand in blue and orange sticks, surrounded by green sticks representing the protein backbone. Labels include Conventional, T-shaped, and Alkyl.</p>	87.3624	61



*Summary of Thermodynamics, Interaction Profile and Bond types:*

Sequence	Loop	Potential Energy (kcal/ mol)	Vander Waals Energy (Kcal/ mol)	Electrostatic Energy (Kcal/ mol)	Conventional hydrogen bond	Mean Length	C- H bond	Mean length	Hydrophobic Interaction	Mean length	Others( Electrostatic)
R1	1	421.68921	-519.45281	-1111.81738	2	2.45	6	2.54	1 (Pi- pi T shaped), 2(Pi- alkyl)	5.85, 5.31	NA
	2	402.54160	-515.54160	-1129.37780	0	NA	6	2.57	1 (Pi- alkyl)	4.55	NA
	3	418.82161	-518.35521	-1115.76092	3	2.91	4	2.65	0	NA	NA
R2	1	407.43339	-503.17547	-1095.72062	2	2.44	6	2.64	3 (Pi- alkyl)	5.49	NA
	2	388.42060	-499.33147	-1113.14379	0	NA	6	2.59	1 (Pi- alkyl)	4.53	NA
	3	404.56579	-502.07787	-1099.66417	3	2.53	4	2.65	0	NA	NA
R3	1	346.80710	-490.68252	-1127.29054	2	2.39	6	2.44	1 (Pi- pi T shaped), 2 (Pi- alkyl)	5.85, 5.31	NA
	2	328.09220	-486.83978	-114.42190	0	NA	6	2.34	1 (Pi- alkyl)	4.56	NA
	3	343.93950	-489.58492	-1131.23409	3	2.92	4	2.45	0	NA	NA
R4	1	292.29434	-482.79139	-1136.13524	2	2.54	6	2.45	1 (Pi- pi T shaped), 2 (Pi- alkyl)	5.85, 5.17	NA
	2	302.02093	-475.79311	-1133.79288	0	NA	6	2.44	1 (Pi- alkyl), 1 (Pi- Pi T shaped)	4.73	NA
	3	289.42674	-481.69379	-1140.07879	3	2.94	6	2.66	0	NA	NA



