LINXS Event - Membrane Protein workshop: Structural Resolution of Membrane Proteins: From Expression to Sample Preparation

Study of anti-cancer effects of TTA-A2 and paclitaxel due to antagonistic interactions with T-type calcium channels

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Antagonistic interaction between TTA-A2 and paclitaxel for anti-cancer effects by complex formation with T-type calcium channel

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ABSTRACT

Studies have shown that in cancer cells, there is an increased T-type calcium channel (TTCC) expression compared to healthy cells. Therefore, the studies targeting TTCC for cancer therapy have shown many positive outcomes. Here, we have used TTA-A2- a potent TTCC inhibitor as a test drug, and paclitaxel (PTX)- a tubule-binding anti-cancer agent as a positive control. Blocking TTCC has shown to overcome resistance in cancer cells towards anti-cancer drugs by reducing calcium influx, and some studies have shown that PTX treatment also reduces the intracellular calcium signaling in cells. So, there is a possibility that PTX might be interacting with calcium channels. Since, drug-drug interaction can cause severe side-effects, or alter the actions of each other; we aim to study the interactions among TTA-A2, PTX, and TTCC. In this study, we have used computational analysis to test the binding of TTA-A2 and PTX with TTCC. To confirm the *in-silico* result, we further tested these drugs in a 3D spheroid model of A549, a lung adenocarcinoma cell line. The *in-silico* result showed that both the drugs, TTA-A2 and PTX, could interact at the same site of TTCC to form a higher stable complex as compared to the TTCC-native. The *in vitro* result showed the antagonistic interaction between the drugs when they are used at the same time. By using the sequential treatment, the spheroids were sensitized by TTA-A2, before treating with PTX. The result indicated that sequential treatment could help to overcome the antagonistic interaction between the two drugs.

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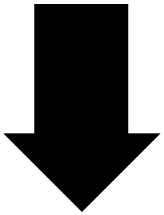
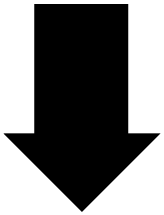
KEYWORDS
A549; Lung cancer; paclitaxel; TTA-A2; T-type calcium channel; synergistic/antagonistic effect

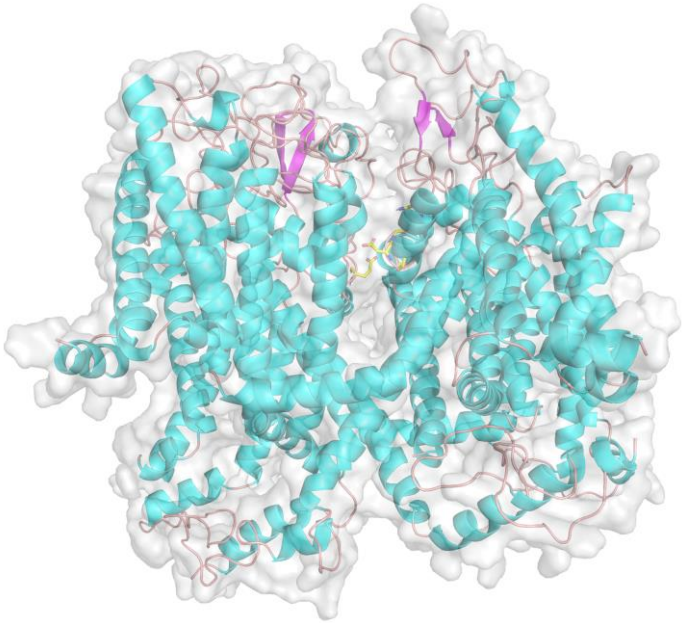


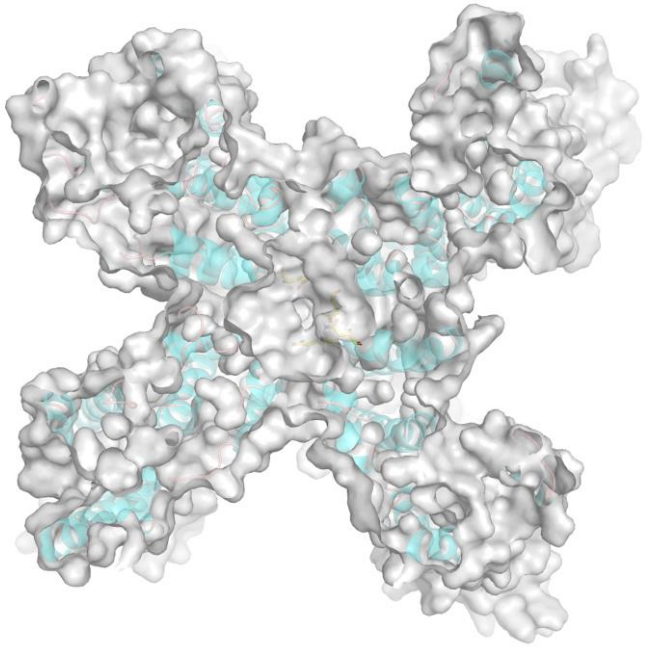
Introduction

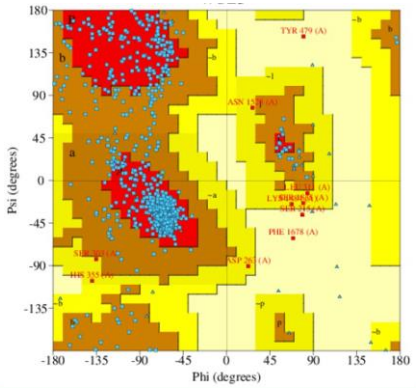
- T-type calcium channel (TTCC)-a low voltage-gated calcium channel (VGCC)
- Maintains a continuous low influx of calcium in the cells.
- Calcium plays a vital role in the cells, such as cell division, cell signaling, hormonal release, and gene expression
- Blocking TTCCs has shown a decrease in the proliferation of various cancer cells
- TTCC consist of two blocking sites- pore blocker (PB) and peptide gating (PG) site



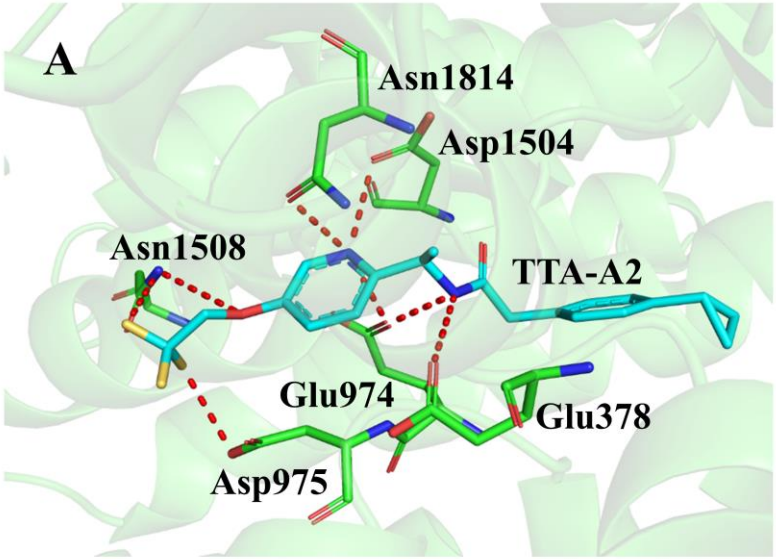
Homology Modeling

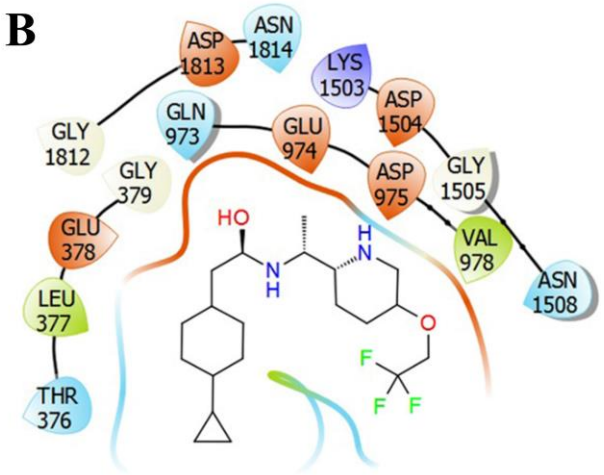


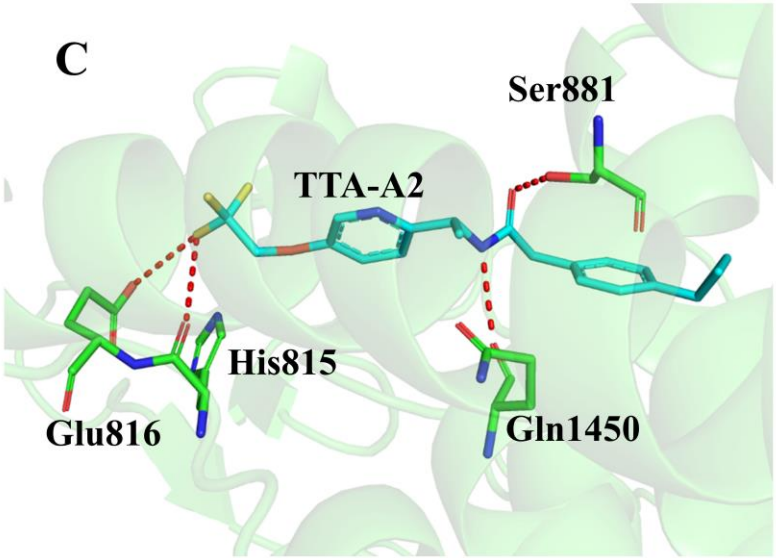


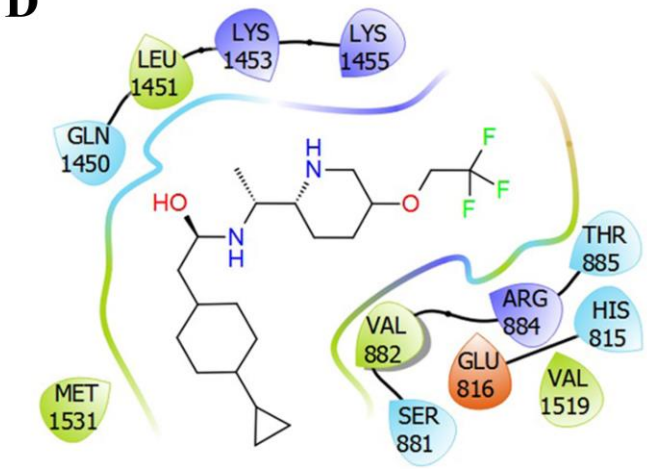


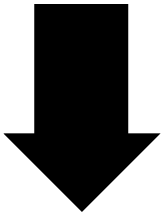
Molecular Docking

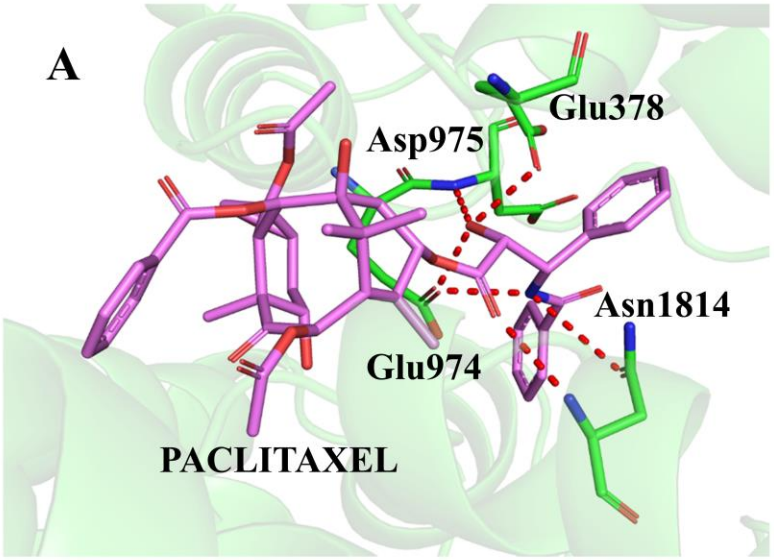


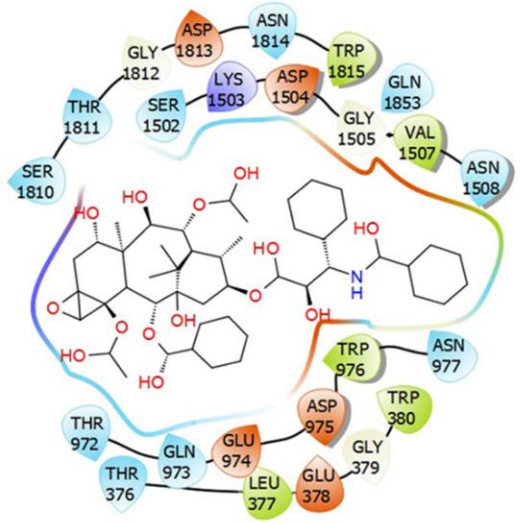


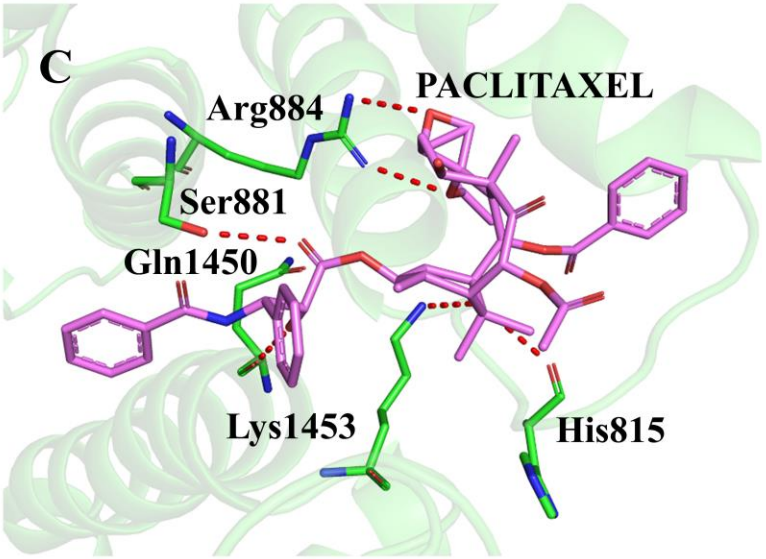


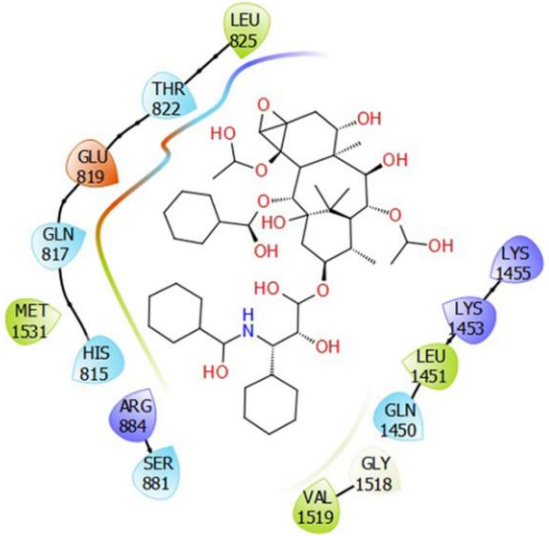


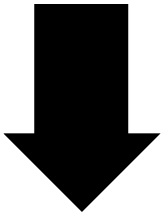



A
Glu378
Asp975
Asn1814
Glu974
PACLITAXEL

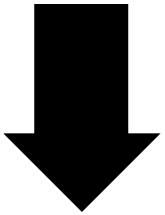

B
ASP 1813
ASN 1814
GLY 1812
TRP 1815
LYS 1503
ASP 1504
GLN 1853
THR 1811
SER 1502
GLY 1505
VAL 1507
SER 1810
ASN 1508
TRP 976
ASN 977
ASP 975
TRP 380
THR 972
GLU 974
GLY 379
GLN 973
THR 376
LEU 377
GLU 378


C
PACLITAXEL
Arg884
Ser881
Gln1450
Lys1453
His815

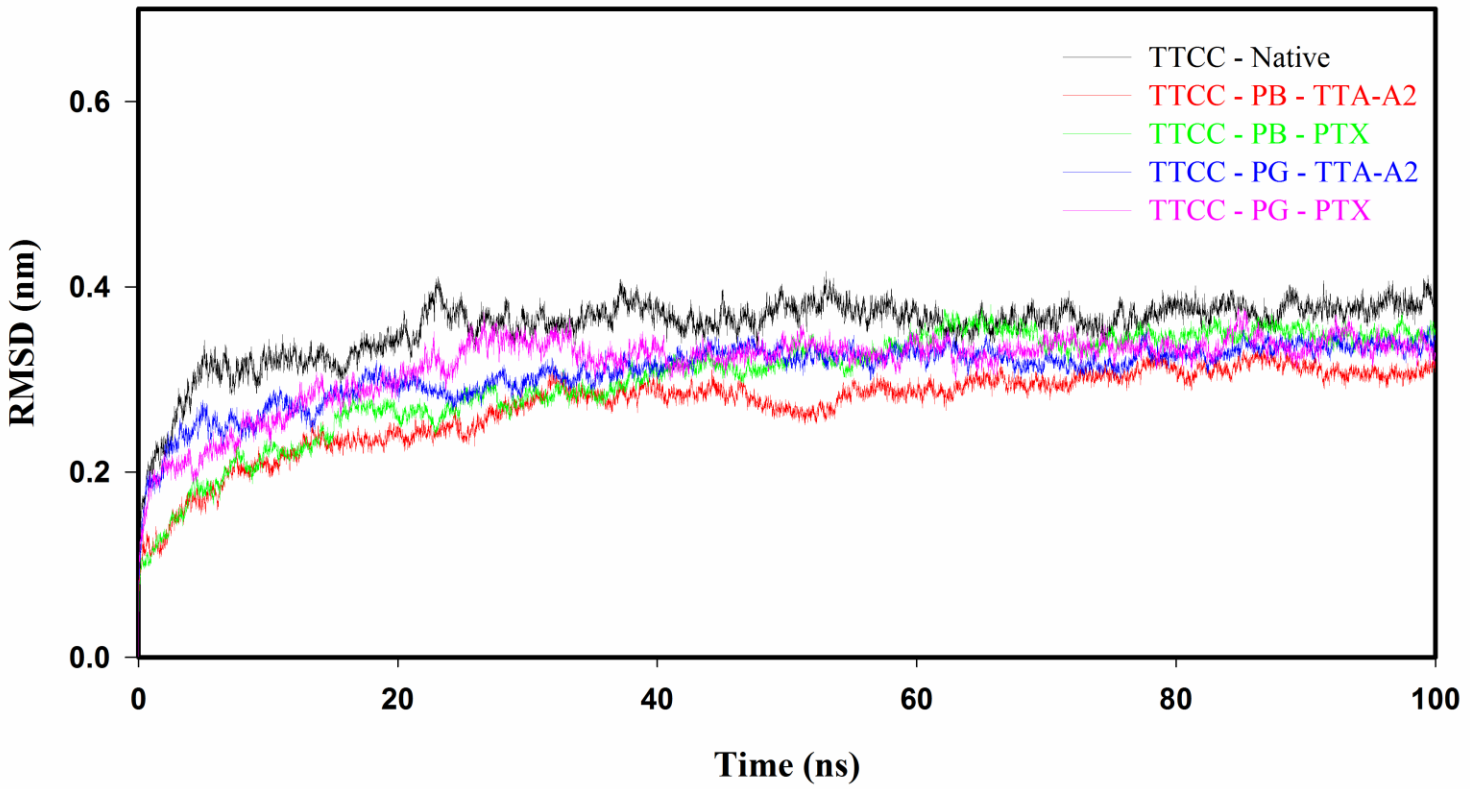

D
LEU 825
THR 822
GLU 819
GLN 817
LYS 1455
LYS 1453
MET 1531
LEU 1451
HIS 815
GLN 1450
ARG 884
GLY 1518
SER 881
VAL 1519

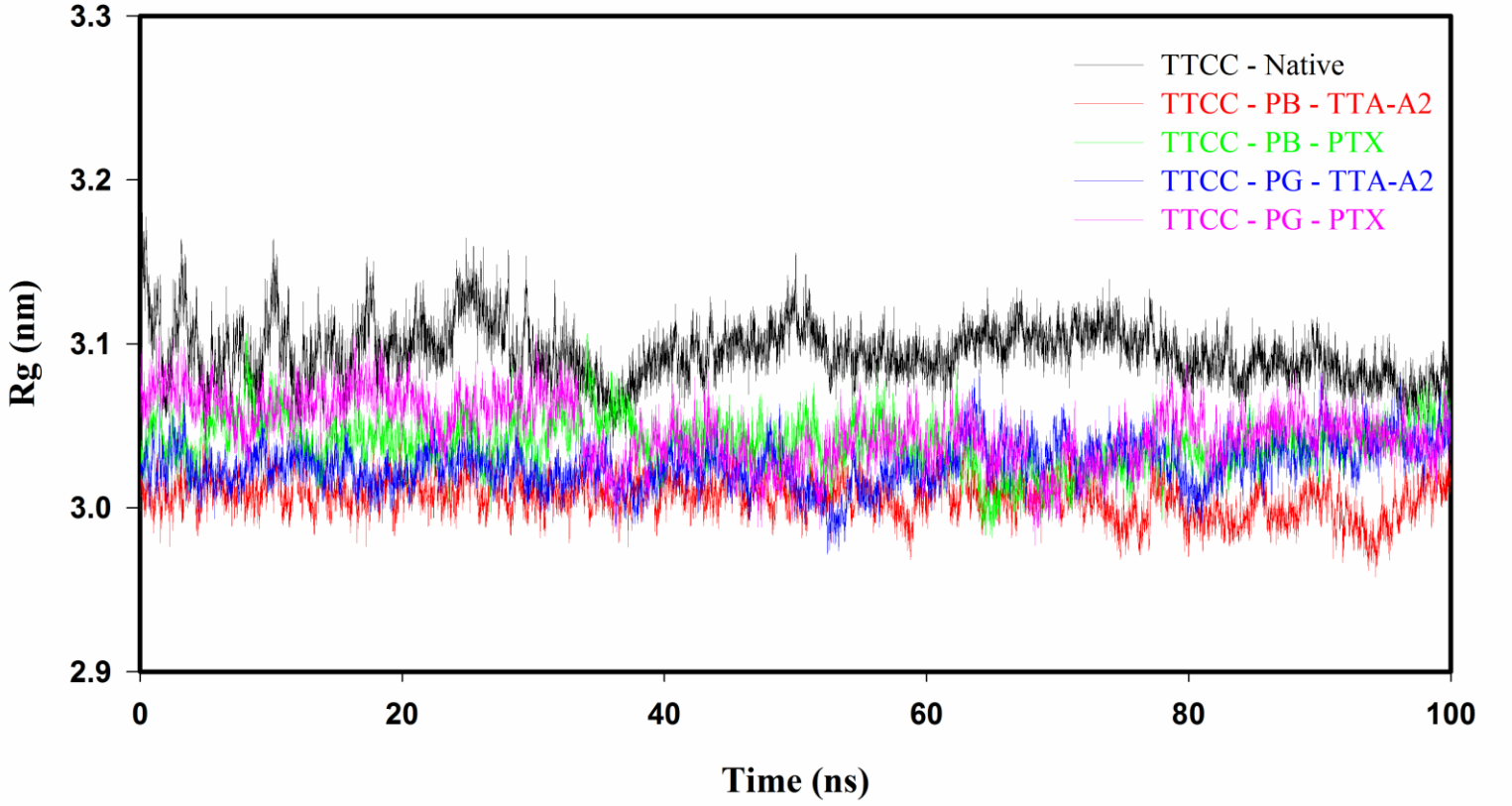


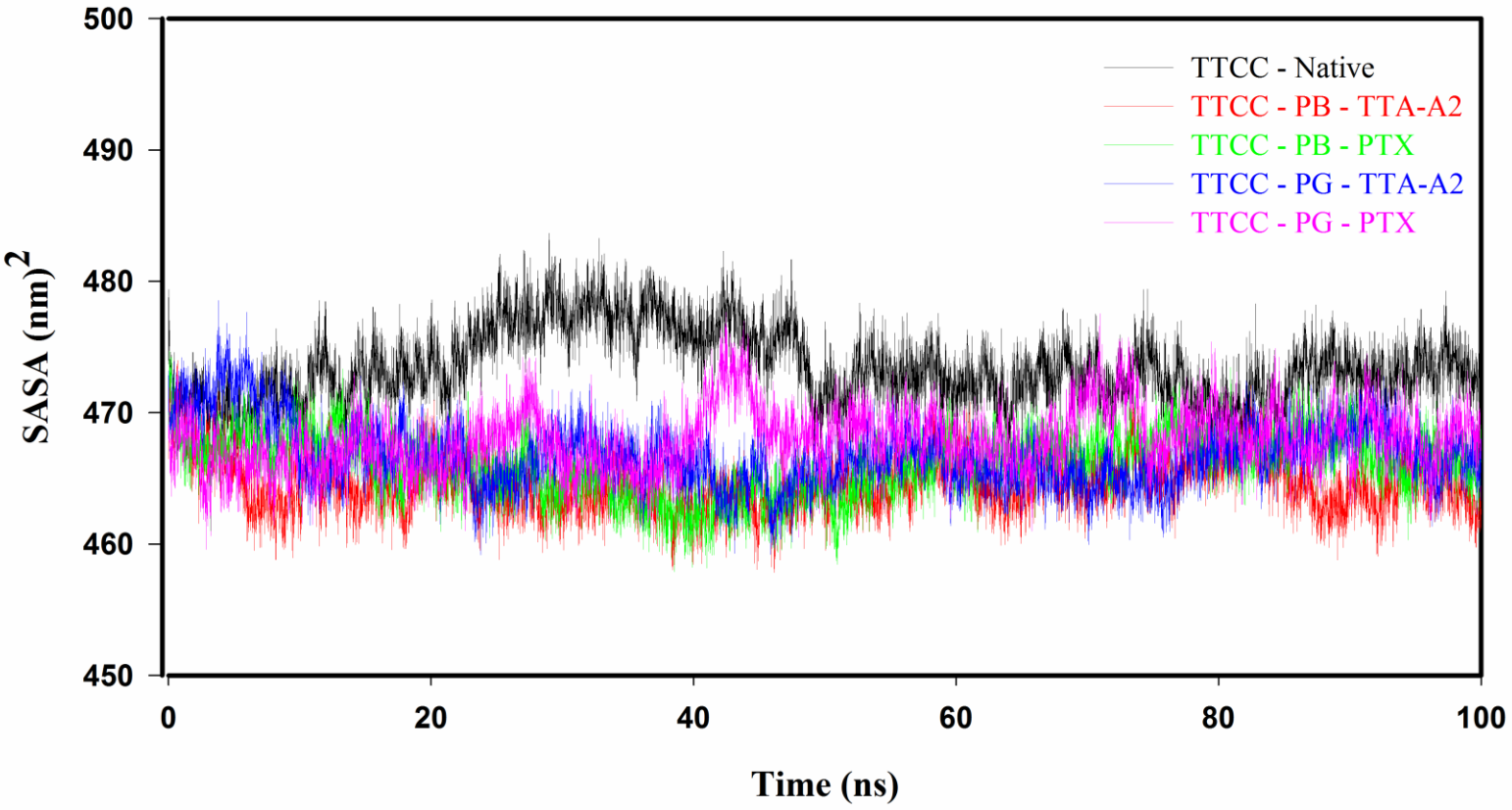
	Compound	Binding affinity	Hydrogen bonding Interaction
PB position	TTA-A2	-7.1	Glu378, Glu974, Asp975, Asp1504, Asn1508, and Asn1814
	PTX	-5.1	Glu378, Glu974, Asp975 and Asn1814
PG modifier	TTA-A2	-6.4	His815, Glu816, Ser881 and Gln1450
	PTX	-6.0	His815, Ser881, Arg884, Gln1450 and Lys1453

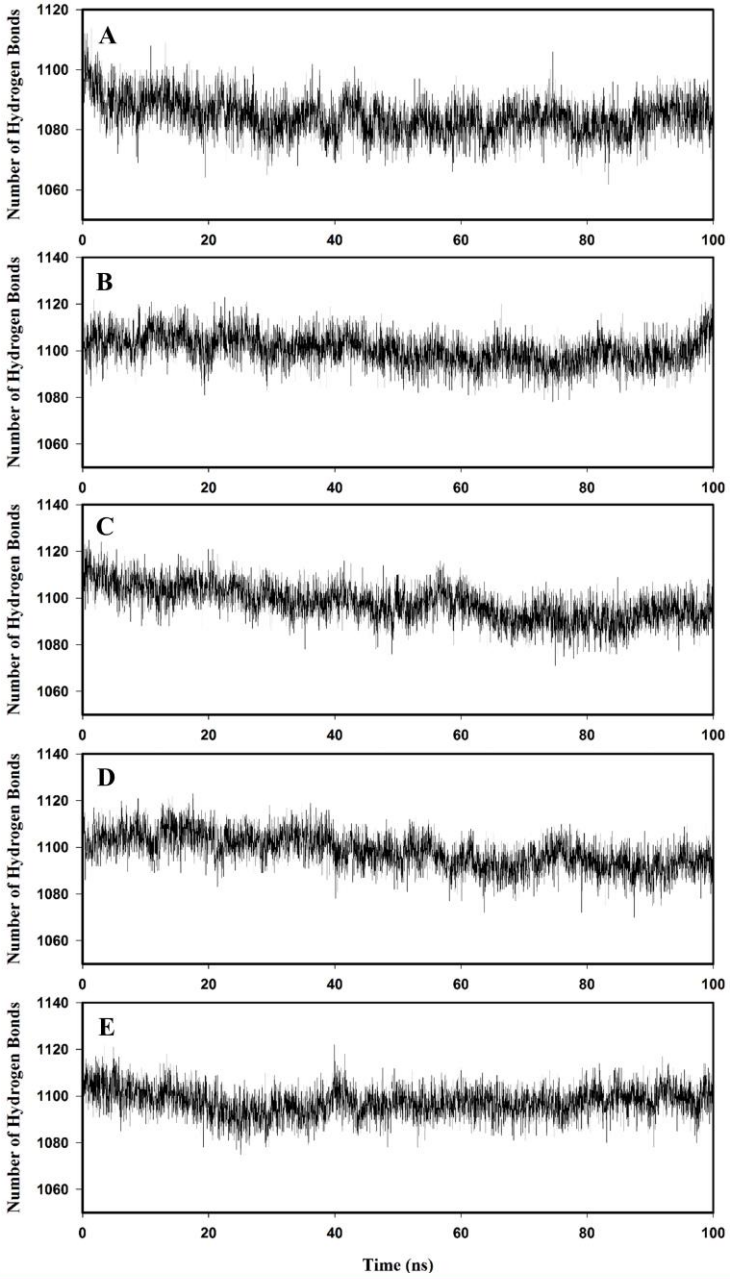


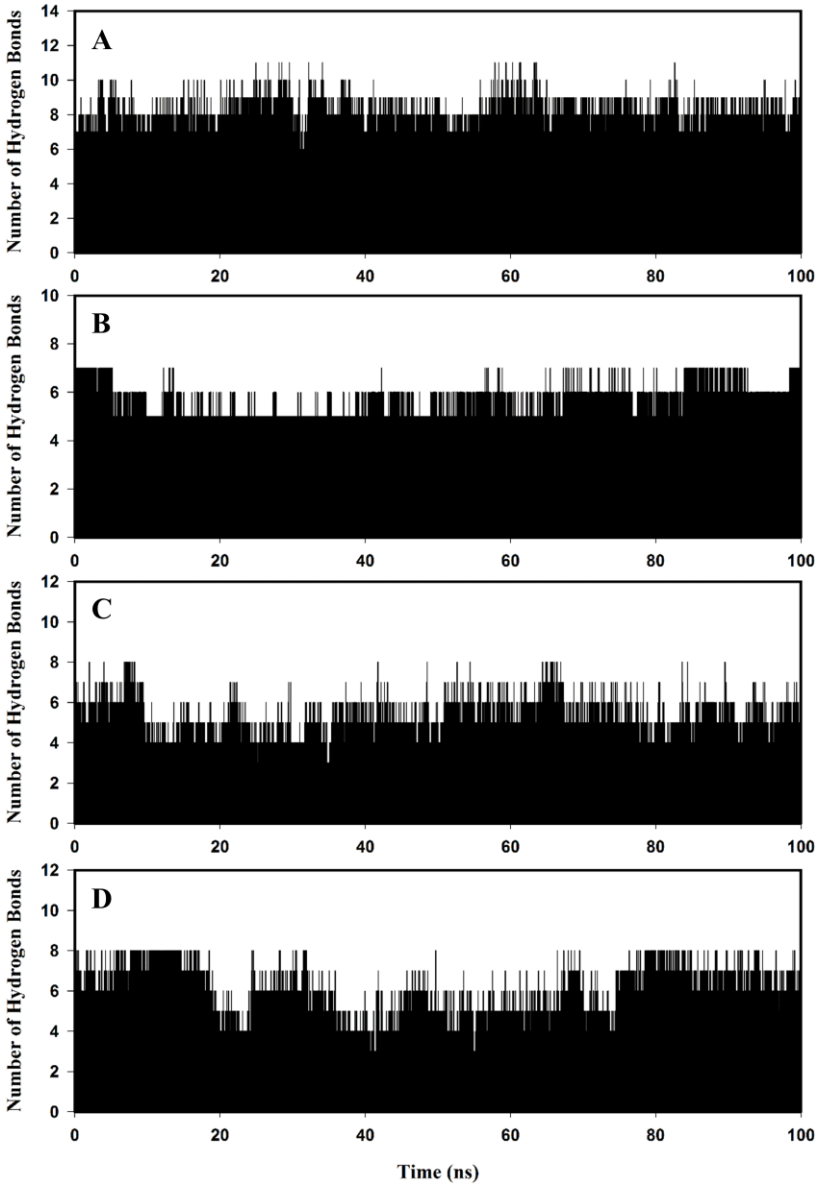
Molecular Dynamics Simulation



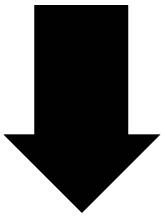

TTCC - Native
TTCC - PB - TTA-A2
TTCC - PB - PTX
TTCC - PG - TTA-A2
TTCC - PG - PTX
Rg (nm)
3.3
3.2
3.1
3.0
2.9
0
20
40
60
80
100
Time (ns)


500
490
480
470
460
450
SASA (nm)2
0
20
40
60
80
100
Time (ns)
TTCC - Native
TTCC - PB - TTA-A2
TTCC - PB - PTX
TTCC - PG - TTA-A2
TTCC - PG - PTX


A
B
C
D
E
Number of Hydrogen Bonds
Time (ns)

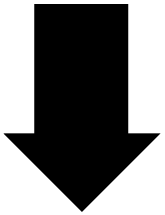

A
B
C
D
Number of Hydrogen Bonds
Time (ns)

S. no.	Compound	RMSD (nm)	Rg (nm)	SASA $(nm)^2$	H-bond numbers
1	TTCC-Native	0.36 ± 0.03	3.09 ± 0.02	474.5 ± 2.9	1084.6 ± 6.4
	PB Site				
1	TTCC-TTA-A2	0.27 ± 0.04	3.00 ± 0.01	464.7 ± 2.2	1100.7 ± 6.8
2	TTCC-PTX	0.30 ± 0.04	3.04 ± 0.01	466.2 ± 2.4	1098.1 ± 7.8
	PG modifier Site				
1	TTCC-TTA-A2	0.31 ± 0.03	3.03 ± 0.02	466.8 ± 2.5	1098.4 ± 7.6
2	TTCC-PTX	0.31 ± 0.03	3.04 ± 0.02	468.0 ± 2.4	1097.7 ± 6.2



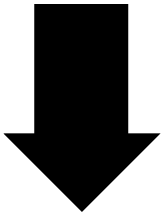
MMPBSA

S. No.	Compound	Van der Waals energy	Electrostatic energy	Polar solvation energy	SASA energy	Binding energy
	PB site					
1.	TTA-A2	-278.43 ± 2.12	-21.51 ± 1.83	153.78 ± 3.29	-34.12 ± 0.41	-180.28 ± 3.78
2.	PTX	-254.53 ± 1.83	-23.39 ± 1.31	167.23 ± 2.32	-29.14 ± 0.27	-139.83 ± 1.92
	PG modifier Site					
1.	TTA-A2	-248.27 ± 2.85	-35.92 ± 3.62	142.73 ± 2.89	-28.21 ± 0.57	-169.67 ± 4.78
2.	PTX	-231.92 ± 2.77	-29.69 ± 2.36	132.25 ± 2.52	-28.77 ± 0.38	-158.13 ± 3.72



Conclusion

- TTA-A2 showed good binding affinity at PB and PB site of TTCC.
- PTX exhibit form stable interactions with TTCC at both sites.
- Study indicated that drugs can also interact with off target with the off target proteins in cells.




Спасибо
Gracias
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Спасибо
Dank U
Grazie
Ευχαριστώ
Danke
Merci
Dziękuję
Diolch
Ngiyabonga
Dank U
Thank You
Obrigado
Tack
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Grazie
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Terima Kasih
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