

# Nutraceutical Evaluation of Trigonelline's Therapeutic Potential By Targeting Bladder Cancer Stem Cells And Cancer-Associated Fibroblasts Via Downregulation Of TGFβ3/GLI2/YAP1 Signaling Hub

## SUPPLEMENTARY FIGURES

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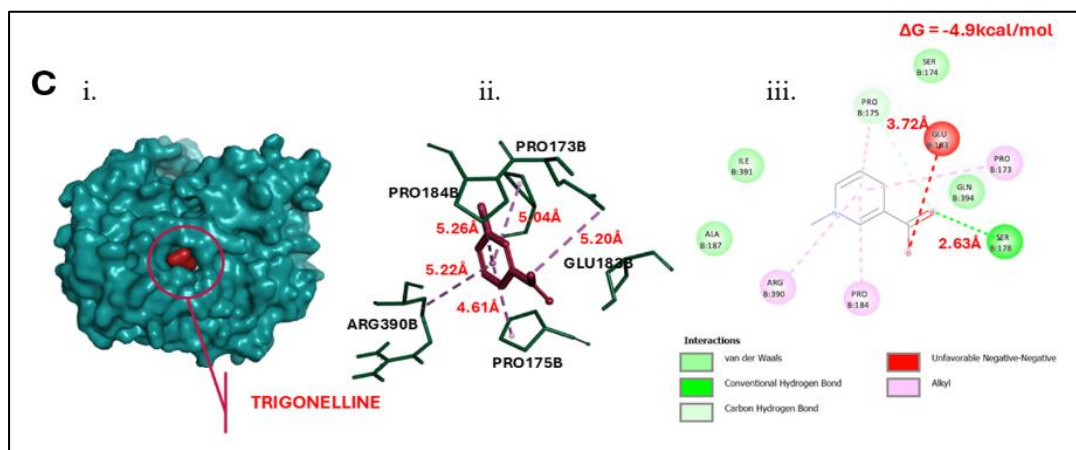
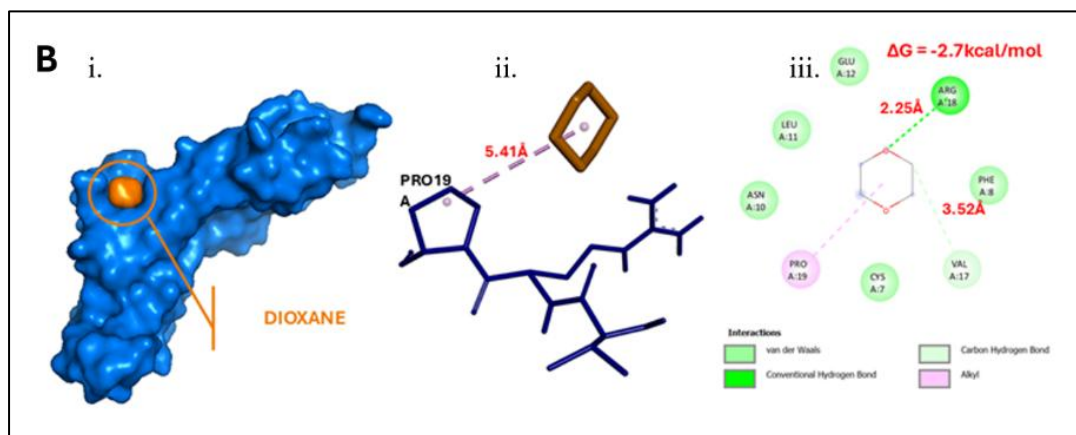
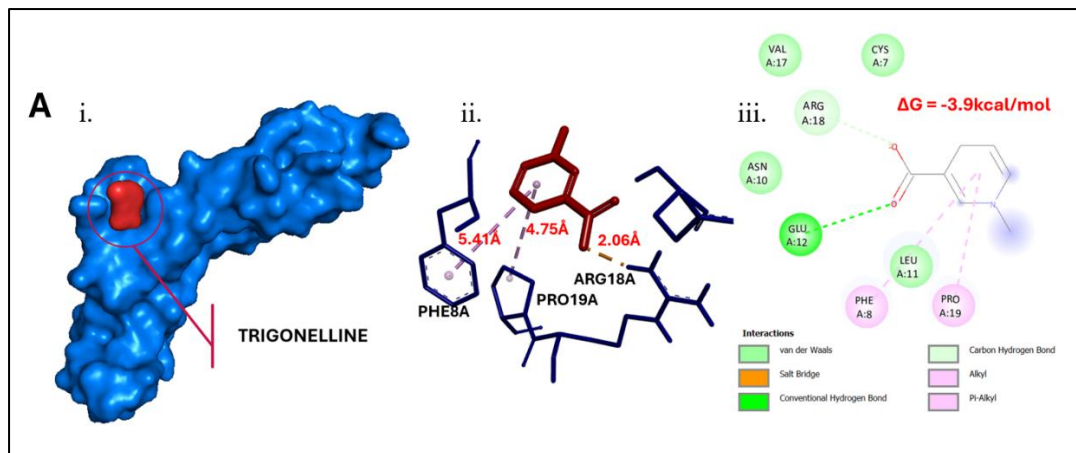
**Table S1.** List of primer sequences used for q-PCR experiments in this study.

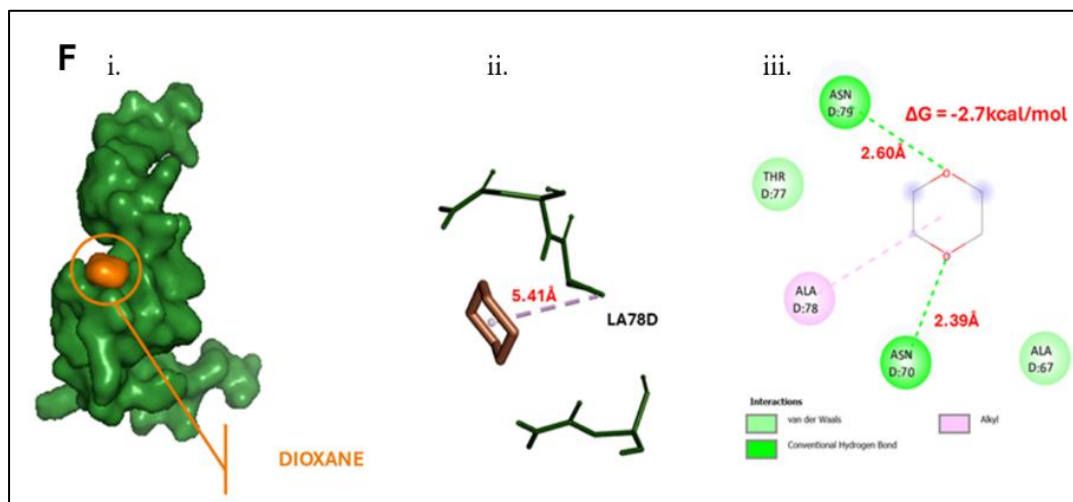
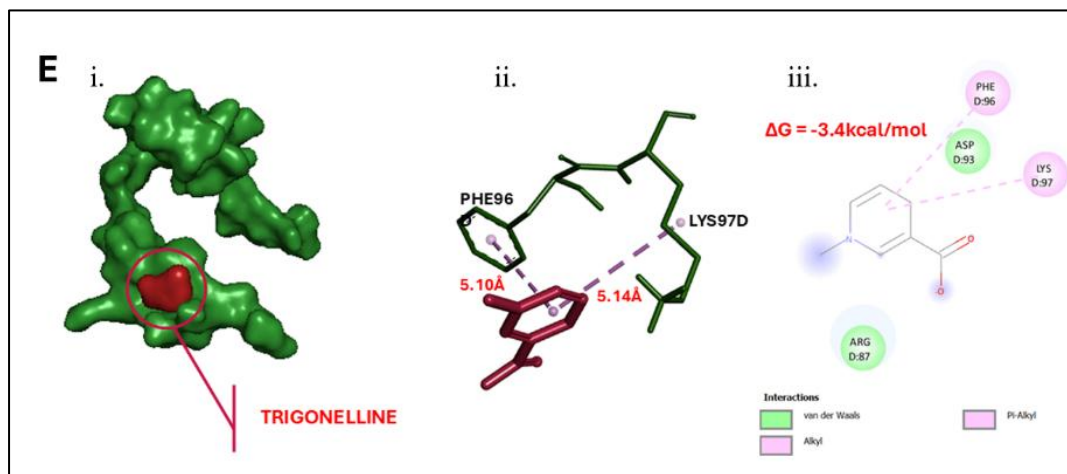
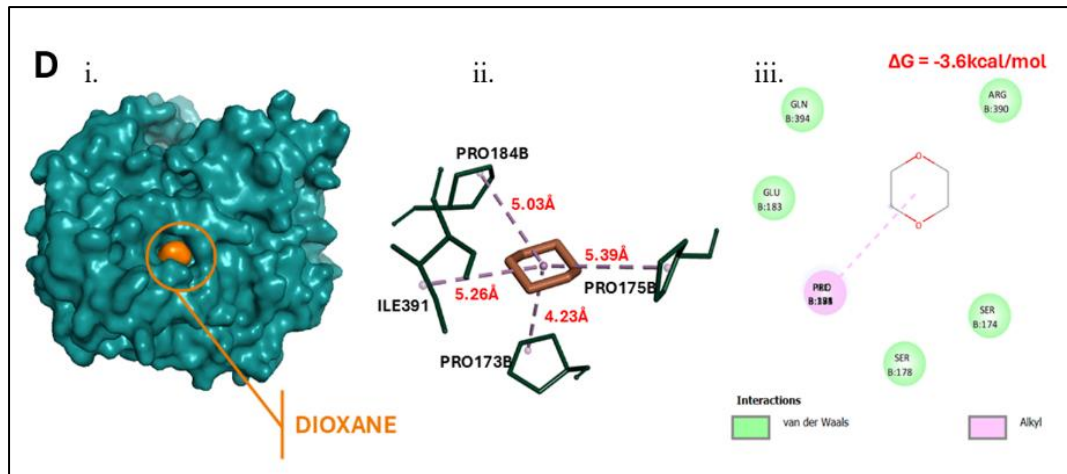
<b>Gene target</b>	<b>Forward</b>	<b>Reverse</b>
Actin	TTGTTACAGGAAGTCCCTTGCC	ATGCTATCACCTCCCCTGTGTG
YAP1	TAGCCCTGCGTAGCCAGTTA	TCATGCTTAGTCCACTGTCTGT
GLI2	GGTACCATTACGAGCCTCATTC	AAAGCCGGATCAAGGAGATG
TGFb3	CTAAGCGGAATGAGCAGAGGATC	TCTCAACAGCCACTCACGCACA
Vimentin	TGGCACGTCTTGACCTTGAA	GGTCATCGTGATGCTGAGAA
FAP	GGAAGTGCCTGTTCCAGCAATG	TGTCTGCCAGTCTTCCCTGAAG
$\alpha$ -SMA	CTATGCCTCTGGACGCACAACCT	CAGATCCAGACGCATGATGGCA

**Table S2.** List of antibodies used for Western blot analysis in this study.

<b>Target</b>	<b>Dilution</b>	<b>Company and Catalog No</b>	<b>Predicted MW (kDA)</b>
$\beta$ -catenin	1:1000	Cell Signaling, $\beta$ -catenin (6B3) Rabbit mAb, #9582	92
YAP1	1:1000	Proteintech, YAP1 Antibody, Rabbit Polyclonal, 13584-1-AP	70
GLI2	1:1000	Affinity Biosciences, GLI2 Antibody, Rabbit Polyclonal, DF7541	167
TGFb3	1:1000	Proteintech, TGF Beta 3 Antibody, Rabbit Polyclonal, 18942-1-AP	45-50
GAPDH	1:1000	Proteintech, GAPDH Antibody, Rabbit pAb, 10494-1-AP	36

Supplementary Figure S1.





**Figure S2. Molecular docking of Trigonelline, Dioxane (standard drug) bound to TGFβ3, GLI2, YAP1.**

**A, B: Trigonelline and dioxane docked to TGFβ3.** Trigonelline - TGFβ3 complex was stabilized with two hydrogen bonds, two alkyl bonds, one salt bridge, and Van der Waals. Their  $\Delta G$  energy was higher than dioxane-TGFβ3 complex. **C, D: Trigonelline and dioxane bound to GLI2.** Trigonelline docked to GLI2 with two hydrogen bonds and five alkyl bonds with the affinity binding was  $-4.9 \text{ kcal/mol}$  while the stability of

dioxane\_GLI2 complex formed solely alkyl bonds and lower binding energy. **E, F:** *Trigonelline and dioxane docked to YAP1*. Although dioxane targeted YAP1 with two hydrogen bonds, the affinity binding of this complex was lower than the  $\Delta G$  energy of the trigonelline-YAP1 complex, which stabilized by alkyl interaction and Van der Waals.

**\*i:** 3D conformation presenting the binding pocket between the target gene and drug, **ii:** stick illustration showing the salt bridge (orange dash line), hydrophobic interactions between atoms and drug (pink dash line) and corresponding distance, **iii:** 2D diagram illustrating binding free energy, the Hydrogen interaction and corresponding distance (dash green line).