IDENTIFICATION OF SPECIFIC ANTAGONISTS FOR THE MEMBRANE RECEPTOR OF ANDROGENS, OXER1 FROM THE ZINC NATURAL PRODUCT DATABASE

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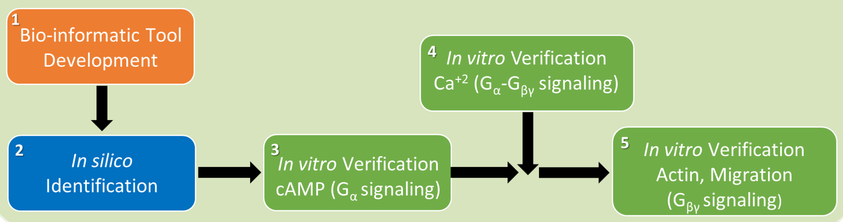
Introduction and Aim

Prostate cancer is known as hormone-sensitive, androgen dependent tumor and the second leading cause of cancer death in men. It is clear that androgens and androgen receptor signaling are crucial for prostate cancer growth and have been exploited therapeutically. However, hormone resistant prostate cancer is an unsolved problem with limited therapeutic choices. The action of androgens is mediated mainly through intracellular androgen receptors, which belong to the nuclear family of receptors. These receptors are transcription factors that determine key cell processes. A recent study by our team identified an alternative androgen receptor on the membrane of prostate cancer cells, OXER1 (5-oxo-6E, 8Z, 11Z, 14Z-eicosatetraenoic acid receptor). Interestingly, androgens via OXER1 inhibit cancer cell growth and migration.

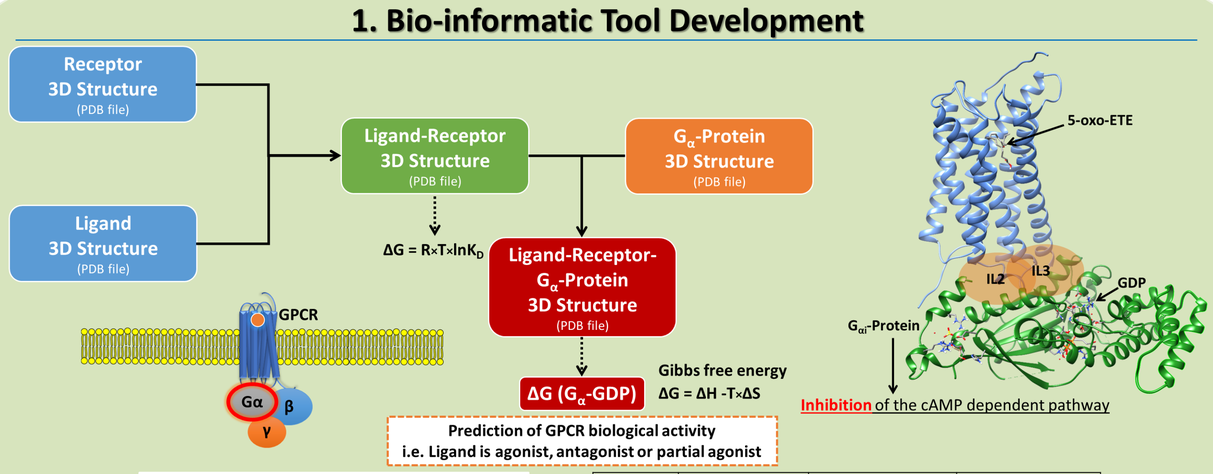
Aim of this research is to identify new molecules that will bind to the membrane receptor of androgens, OXER1 and will have antagonistic effects such as testosterone. To achieve this, we focus on natural products which there are data that may have a pharmacological effect and a therapeutic benefit in prostate cancer.

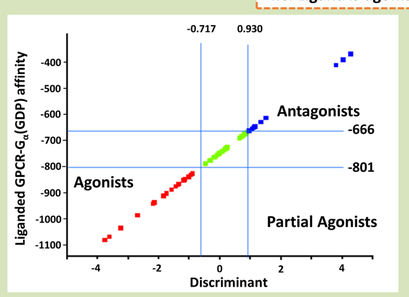
Method and Results

Flowchart of study



1. Bio-informatic Tool Development

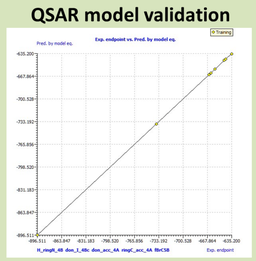




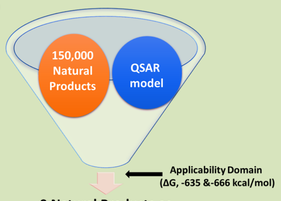
OXER1-Ligand	ΔG GPCR-G_αGDP (kcal/mol)	% cAMP inhibition (Experimental data)	Action Bibliography/ (Experimental)/ Model
5-oxo-ETE	-876.5	100	Ago/(Ago)/Ago
Testosterone	-667.0	51	Antago/Antago/Antago
TC150	-677.3	48	NA/Antago/Antago
TC151	-695.2	66	NA/Antago/Antago
TC153	-685.2	54	NA/Antago/Antago
B2	-665.2	48	NA/Antago/Antago
B5	-736.2	25	NA/PA/PA
Epicatechin	-642.8	67	NA/Antago/Antago
5-HETE	-710.8	NA	PA/PA
12-HpETE	-713.8	NA	PA/PA
15-HpETE	-758.3	NA	PA/PA
12-HETE	-717.7	NA	PA/PA
15-HETE	-723.8	NA	PA/PA

2. *In Silico* Identification

QSAR model validation



Training Name	Training G_α-Interaction (kcal/mol)	Prediction Name	Prediction G_α-Interaction (kcal/mol)
5-oxo-ETE	-896.5	5-HETE	-710.8
Testosterone	-663	12-HpETE	-713.8
TC150	-657.3	15HpETE	-758.3
TC151	-645.2	12-HETE	-717.7
TC153	-635.2	15-HETE	-723.8
B2	-665.2	Progesterone	-773.6
B5	-736.2	DHEA	-660.8
Epicatechin	-642.8	TC404	-680.8
		TC405	-692
		TC406	-662.8
		TC407	-732.4
		B1	-676.6
		B3	-663.8
		B4	-682.9
		Catechin	-669.2
		Catechin_Galate	-718.5
		Epicatechin-3-O-gallate	-680.2
		Epigallocatechin_gallate	-721.7



9 Natural Products as Antagonists of OXER1

Generation of molecular descriptors → Selection of most relevant molecular descriptors → Mapping of descriptors with activity → Model validation

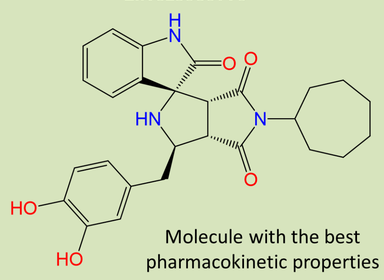
QSAR model application

Name	Chemical Structure	kcal/mol Predicted interaction with $G_{\alpha i}$	kcal/mol Pred. by QSAR
1. ZINC15959779		-662,44	-661,7837
2. ZINC02274955		-661,12	-662,2376
3. ZINC01805555		-663,94	-639,4079
4. ZINC05397075		-656,03	-669,4895
Testosterone			

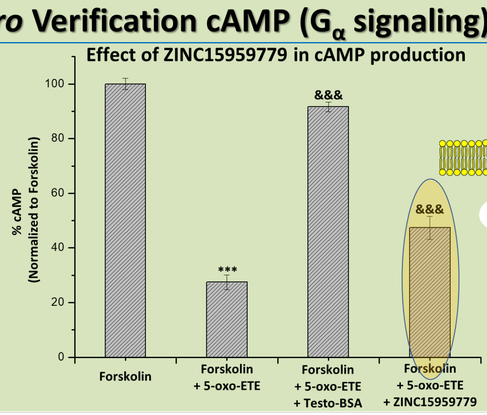
Name	Chemical Structure	kcal/mol Predicted interaction with $G_{\alpha i}$	kcal/mol Pred. by QSAR
5. ZINC08790433		-664,7	-670,9441
6. ZINC12864636		-659,28	-660,6397
7. ZINC02121631		-664,77	-666,8884
8. ZINC04081886		-660,97	-645,0612
9. ZINC12881427		-665,69	-665,897

3. *In vitro* Verification cAMP (G_α signaling)

ZINC15959779

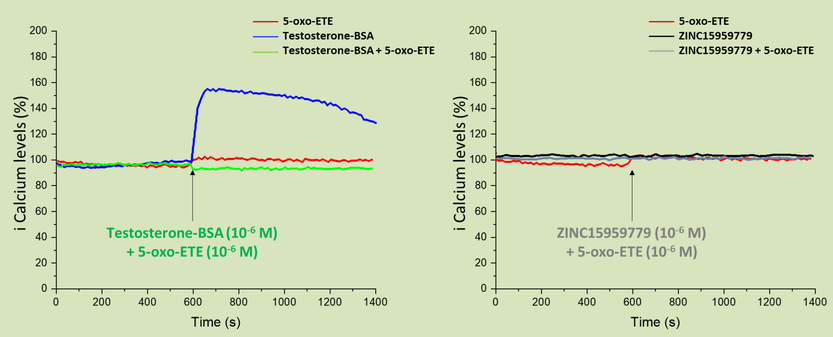


Molecule with the best pharmacokinetic properties



4. *In vitro* Verification Ca^{+2} (G_α-$G_{\beta\gamma}$ signaling)

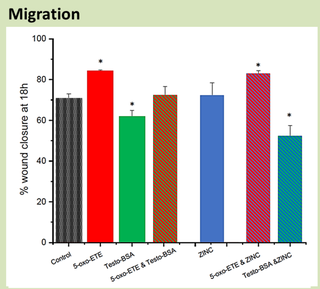
No effect of ZINC15959779 in iCa^{+2} levels



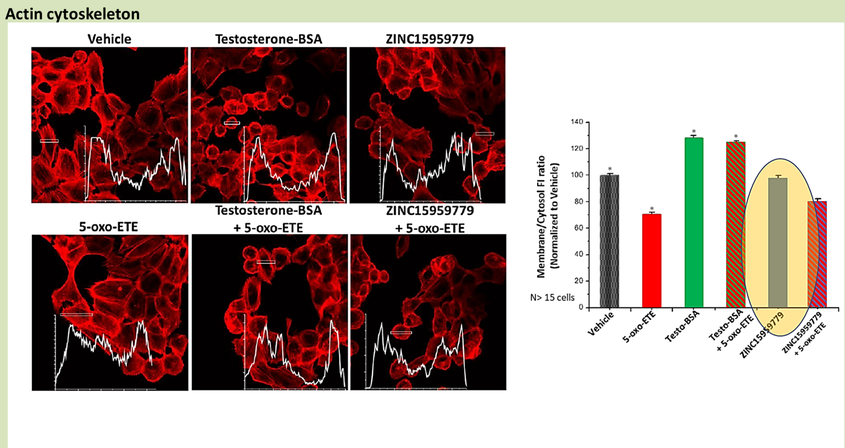
- OXER1 is the receptor that mediates testosterone-induced calcium changes from endoplasmic reticulum.
- The major signaling molecules involved in OXER1-mediated calcium increase are c-Src, FAK, PI3K, RACK-1, PLC.

5. *In vitro* Verification Actin and Migration ($G_{\beta\gamma}$ signaling)

Migration



Actin cytoskeleton



- No effect of ZINC15959779 in $G_{\beta\gamma}$ signaling
- **ZINC15959779 is selective OXER1 $G_{\alpha i}$-Antagonist**

Conclusions

We discovered new antagonists of the OXER1 receptor, using a pioneering bioinformatics method that we developed in the context of this study. This method has a general character and combines molecular simulation methods as well as experimental data, turning it into an excel-lent tool for the study of biochemical systems. We came up with 9 natural products and finally one, ZINC15959779, based on their pharmacokinetic properties, as OXER1 antagonists. We then successfully confirmed the *in vitro* antagonistic activity of this compound, as well as of the polyphenol B2-OPC, similar to the antagonistic profile of testosterone on OXER1 receptor-$G_{\alpha i}$-Protein signaling. In addition, we explored the signaling molecules triggered by OXER1 receptor, as they are poorly studied, in order to elucidate the total G_α-$G_{\beta\gamma}$ signaling pathway of testosterone via this receptor. The later further supports testosterone actions at the membrane level, via OXER1, and reveals the significant role of such actions in the interplay between androgens and lipids in controlling cancer cell fate.

References

1. *Panagiotopoulos, A. et al. OXER1 mediates testosterone-induced calcium responses in prostate cancer cells. Molecular and Cellular Endocrinology (**2022**) 539: 111487.*
2. *Kalyvianaki, K. et al. Antagonizing effects of membrane-acting androgens on the eicosanoid receptor OXER1 in prostate cancer. Sci Rep (**2017**) 7: 44418.*
3. *Panagiotopoulos, A. et al. A simple open source bio-informatic methodology for initial exploration of GPCR ligands' agonistic/ antagonistic properties. Pharmacology Research & Perspectives (**2020**) 8: 1-12.*

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