New antagonists of the membrane androgen receptor OXER1 from the ZINC natural product database

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Supplemental Material

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Supplemental Information: In silico structure-activity relationships of OXER1 antagonists in the binding pocket of OXER1

In a previous work, we have simulated the binding site for 5-oxo-ETE (the natural ligand for OXER1) and testosterone. Here, in view of the above finding of the \( \text{G}_a \text{ii} \) antagonism of ZINC15959779 on OXER1, we have used the online resource GalaxyWeb (http://galaxy.seoklab.org), in order to refine the binding site prediction. Our results (Figure S5) identified a binding pocket for 5-oxo-ETE and testosterone, proximal to the extracellular part of the receptor, similar to the one we have reported before. Simulation binding of these two molecules, after hydration of the receptor, reveals a number of common hydrogen or Van der Waals bond formation with Leu\textsubscript{172}, Ser\textsubscript{173}, Arg\textsubscript{176}, Leu\textsubscript{227}, Ser\textsubscript{239}, Tyr\textsubscript{240}, His\textsubscript{253}, Tyr\textsubscript{257}, Ser\textsubscript{311} and Thr\textsubscript{341}, without or with the participation of water molecules. In addition, 5-oxo-ETE forms specific bonds with Val\textsubscript{180} and Ile\textsubscript{312}, while testosterone (and B2-OPC) forms specific bonds with Thr\textsubscript{231}, Phe\textsubscript{337}, Ser\textsubscript{340} and Leu\textsubscript{341}, suggesting that these latter amino acids might confer the antagonistic activity of testosterone (Supplemental Table 1).

The interaction of ZINC15959779 with OXER1, with an estimated \( \Delta G = -19.655 \text{ kcal/mol} \) is shown in Figure S6, together with the remaining 8 retained compounds. As shown, all of them share the same binding pocket, in which also bind 5-oxo-ETE, Testosterone and B2-OPC. The specific best poses of ZINC, testosterone, B2-OPC and 5-oxo-ETE in the OXER1 binding pocket, calculated with GemDock is shown in Figure 2. As shown, the natural compound ZINC15959779 final pose differs significantly from the agonist 5-oxo-ETE, both in the steric interactions that can be formed and in their functional groups. Interestingly, a similar conformation of the compounds was equally detected, using the Discovery Studio 2021 program (https://www.3ds.com/products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/), validating our results. ZINC15959779, has an aromatic ring with two hydroxyl groups that play an important role in its antagonistic action, as they interact strongly with the amino acid Leu\textsubscript{227} in loop 4 and the amino acid His\textsubscript{253} in loop 5 with which they form a hydrogen bond. Comparing ZINC15959779 with testosterone and B2-OPC, we found that all three molecules form similar bonds with the amino acids Arg\textsubscript{176}, Leu\textsubscript{227}, Thr\textsubscript{231}, Tyr\textsubscript{240}, His\textsubscript{253}, Ser\textsubscript{311}, Ser\textsubscript{340}, Leu\textsubscript{341} and Thr\textsubscript{344}. After the three antagonists binding to the binding groove of OXER1, we observed that amino acids Leu\textsubscript{227} and His\textsubscript{253} attain a similar conformation; in contrast these residues attain an opposite change after 5-oxo-ETE binding. This, as well as other changes, detailed in Figures S7 and S8 and Supplemental Table 1, results in a significant modification of transmembrane helices 3 and 4 (resulting in a significant reorientation of aminoacid residues in IL2) and helices 5 and 6, with a significant change in IL4 (Figure S8). These changes of IL2 and 3 are responsible for the decreased binding interaction of G\textsubscript{ai}-GDP, as previously reported.
Table S 1
Predicted hydrogen bonds in the binding pocket of OXER1 formed by agonist and antagonists.

| Compound    | Leu141 | Lys166 | Leu169 | Leu172 | Ser173 | Asn175 | Arg176 | Val180 | Leu227 | Leu228 | Thr231 | Ser233 | Ser239 | Tyr240 | Arg241 | His253 |
|-------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 5-oxo-ETE   | 3.4    | 3.3    | 2.5    | 3.3    | 3.3    | 3.2    | 2.9    | 3.4    | 2.5    | 2.7    | 2.8    | 3.2    | 2.8    | 3.5    | 3.5    |
| Testosterone| 2.9    | 3.1    | 3.0    | 3.3    | 3.6    | 2.0    | 2.9    | 4.3    | 3.4    | 2.9    | 3.5    | 2.9    | 3.5    | 4.3    |
| B2          | 3.6    | 1.8    | 3.6    | 2.8    | 2.6    | 2.8    | 1.9    | 3.7    | 2.7    | 2.6    | 2.4    | 2.3    | 2.3    | 3.1    | 3.0    |
| ZINC15959779| 2.6    | 2.1    | 2.4    | 2.8    | 2.3    | 2.7    | 3.2    | 2.7    | 2.6    | 3.2    | 2.7    | 2.6    | 3.3    | 2.3    |
| Gue1564     | 2.7    | 3.4    | 2.2    | 2.6    | 2.5    | 3.6    | 4.4    | 2.8    | 2.6    | 3.4    | 2.8    | 2.9    | 1.8    | 2.1    | 2.5    |

| Compound    | Tyr257 | Glu260 | Phe308 | Ser311 | Ile312 | Gly315 | Phe337 | Ser340 | Leu341 | Tyr345 | Thr344 | Thr347 |
|-------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 5-oxo-ETE   | 3.1    | 2.9    | 2.9    | 3.3    | 3.2    | 2.8    | 3.3    | 2.7    | 2.8    |
| Testosterone| 3.2    | 3.0    | 2.7    | 4.5    | 2.9    | 3.4    | 2.7    | 3.3    | 2.5    |
| B2          | 3.4    | 2.6    | 3.7    | 2.2    | 2.4    | 3.4    | 2.4    | 3.4    | 3.5    |
| ZINC15959779| 2.2    | 2.7    | 2.9    | 3.1    | 3.1    | 2.6    | 2.8    |
| Gue1564     | 2.9    | 3.6    | 4.4    | 4.2    | 3.0    | 3.0    | 3.0    | 3.3    | 3.4    | 4.0    |

Table presents the hydrogen bonds in the binding pocket of OXER1, formed by the agonist (5-oxo-ETE), the antagonists (testosterone and B2-OPC) and the retained compound ZINC15959779. Gue 1564, the reported Gβγ OXER1 antagonist is also presented for comparison.
Table S2
Absorption, Distribution, Metabolism and Excretion (ADME) values, calculated with the online resource www.swissadme.ch.

See the resource’s help and the main text for further details and corresponding references.
**Table S3**

Similarity Ensemble Approach of the retained molecules (SEA).

| Compound | Interaction 1 | P-Value 1 | Interaction 2 | P-Value 2 | Interaction 3 | P-Value 3 | Interaction 4 | P-Value 4 | Interaction 5 | P-Value 5 | Interaction 6 | P-Value 6 | Interaction 7 | P-Value 7 | Interaction 8 | P-Value 8 |
|----------|--------------|-----------|--------------|-----------|--------------|-----------|--------------|-----------|--------------|-----------|--------------|-----------|--------------|-----------|--------------|-----------|

The interaction of each compound with a number of targets is presented, together with the corresponding probability. A cut-off of \( p=1\times10^{-10} \) was arbitrary chosen. The estimation was made with the online resource [https://sea.bkslab.org/](https://sea.bkslab.org/).
Supplemental Figures

Figure S1

QSAR Analysis. **A.** Molecular descriptors of the retained QSAR model. Table presents the loading coefficients of the descriptors and their statistical significance. **B.** Linear correlation between the experimental (abscissa) and the calculated endpoint (ordinate) of the training and predicted set of compounds. As shown and described in the text, a significant linear correlation ($R^2=0.9898$) was obtained. The retained model also predicts very well a small set of compounds, presented in Table 1. **C.** Figure presents the applicability domain of the retained model. Abscissa shows the HAT (leverage value, diagonal elements of the HAT matrix “HAT i/i") of each compound, while the ordinate shows the predicted value by the model equation. Vertical line shows the leverage values the $h^*$ value for our training set, defined as $3p'/n$, where $p'$ is the number of the model variables + 1 and $n$ is the number of training compounds. Horizontal values shows the applicability domain of the model. **D and E.** Principal component analysis of compounds used for the building of the QSAR model. The two first components account for 43% and 27% of the estimated variability. In D the identification of the three main categories of compounds (lipids, steroids and polyphenols) used for its construction is presented, while in E the variable loading on each of the two first components is presented.

| Variable | Coeff. | Stds Coeff. | Co.Int. % | pc |
|----------|--------|-------------|-----------|----|
| Intercept | -316.5664 | 41.9398 | 0.00000 |
| h_ring6_4B | -1.5181 | -0.5425 | 0.00000 |
| dna_la_4F | 1.2765 | 0.0946 | 0.00000 |
| dna_ac_4A | 1.1816 | 0.6993 | 0.00000 |
| ring_ac_4A | -0.5772 | -0.1697 | 0.00000 |
| FRCSB | 13.0913 | 0.7727 | 0.00000 |

![Figure S1 A](image1)
![Figure S1 B](image2)
![Figure S1 C](image3)
![Figure S1 D](image4)
![Figure S1 E](image5)
Figure S2
Effect of ZINC15959779 on intracellular Ca\(^{2+}\) release. Cells were incubated in an acidic pH (5.6) in a Ca\(^{2+}\)-free medium (Panagiotopoulos et al, 2021, submitted). Testosterone-BSA (A.) and B2-OPC (B.) at 10\(^{-6}\)M increase intracellular Ca\(^{2+}\). This effect was reverted by 5-oxo-ETE, which had no effect over the basal intracellular Ca\(^{2+}\) levels. In contrast, ZINC15959779 (C.) had no effect. Arrow shows the time point (in seconds) at which substances were introduced (all at 10\(^{-6}\)M). A typical experiment is presented, repeated three times with identical results.
Figure S3
High magnification of DU-145 cells, treated with $10^{-6}$M of the different compounds, for 30 min. Actin cytoskeleton was visualized in a confocal microscope, after staining with rhodamine-phalloidin, at 20 min. A typical experiment is presented.
Figure S4
Microphotographs of an experiment of wound healing. A scratch was induced in a DU-145 cell monolayer and its closure was monitored for 18h. Agents were introduced at 10-6M, and cells were cultured in the presence of DMSO+ethanol, as detailed in the Material and Methods section. A typical experiment is presented, which was repeated 3 times (in triplicate) with similar results.
Figure S5

In A, the 3D conformation of OXER1 (in blue) and the aminoacids implicated in the binding pocket formation (in red sticks) is shown. The ligand pocket is shown in B, as extracted with PyMol (see Material and Methods). The ligand-accessible area of the binding pocket, also calculated with the PyMol program, is 3888.5 Å². In C, a 90° rotation counterclockwise of the receptor molecule, intracellular loops 2 and 3 (IL2 and IL3) are presented, which, as reported previously³, is the site of Gα protein binding.
Figure S6
A. Binding of retained ZINC molecules (in red) to OXER1 (blue ribbons). ZINC15959779 is presented in a higher magnification, with the receptor in yellow.
Figure S7

Figure presents the comparison of the amino acid residues and 3D conformation in the binding groove of OXER1 receptor after binding of the agonist 5-oxo-ETE and the antagonists Testosterone (green), B2-OPC (blue) or ZINC15959779 (yellow).
Figure S8
Comparison of OXER1 transmembrane helices and intracellular loops 2 and 3 change. A. After the agonist 5-oxo-ETE (white) or antagonists (Testosterone, green; B2-OPC, blue; ZINC15959779, yellow) binding. The $G_{\alpha_i}$-interacting intracellular loops are shown as blue ribbons. B. A zoom of IL2 and 3 after 5-oxo-ETE (white) or Testosterone (green) residue changes are shown.
Figure S9
Alignment of ZINC15959779 with testosterone and B2-OPC in the binding pocket of OXER1.

In the left panel the alignment of ZINC15959779 with testosterone is shown and in the right panel the alignment of ZINC15959779 with B2-OPC. Calculations were performed with the iGemDock v2.1 program, analyzing the best retained solution. 4,5
Supplemental References

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