Three-dimensional quantitative structure-activity relationship analysis and ADME predictions of guanylhydrazone coactivator binding inhibitors of estrogen receptors

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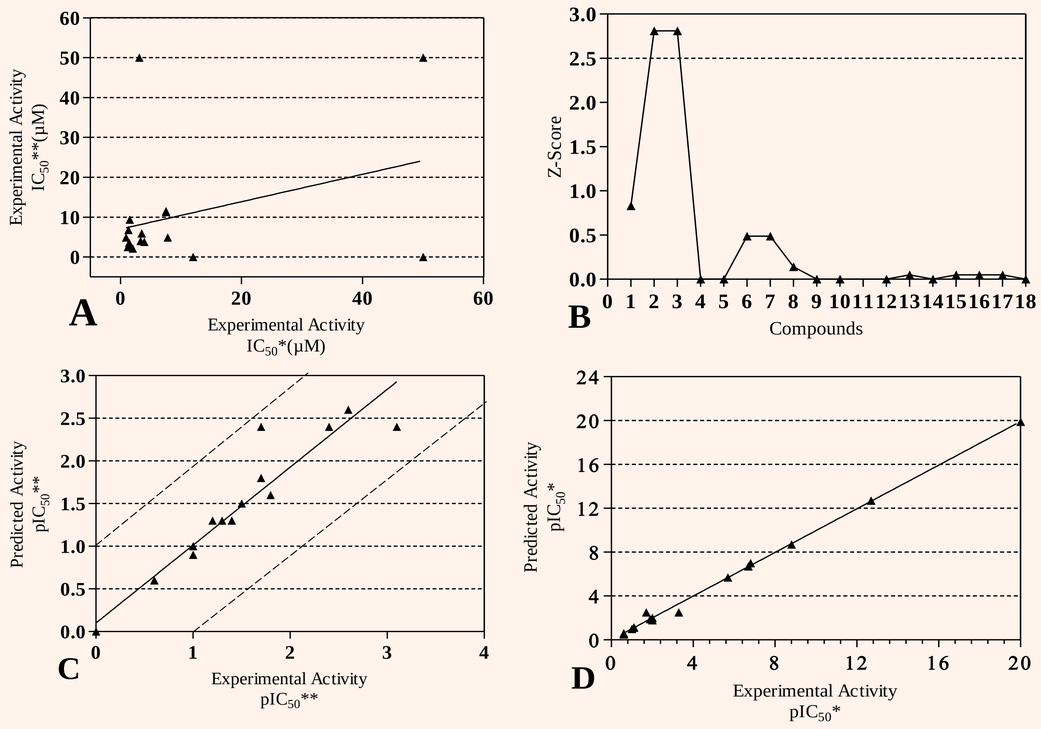
Introduction

The estrogen receptors (ER) refer to a group of the nuclear hormone receptor superfamily of ligand-mediated transcriptional factors. They bind to a DNA and regulates gene expression. Over expression of this type of receptors leads to a breast cancer progression. Hormone-responsive breast cancer develops resistance to conventional anti-cancer therapy, and this becomes a major problem in a breast cancer therapy. ER inhibitors (Tamoxifen) can effectively block ER to treat the tumor, but no more effective due to ER resistance to them [**1**]. Here, we report the exploration of the series of guanylhydrazone molecules, which block ER transcription through different mechanisms than traditional antagonists.

Computational methods

The three-dimensional database was created on a basis of the MOE molecular modeling package. The molecules contain different IC_{50}: cell-based assay of reporter gene activity and mammalian two-hybrid assay (M2H). The IC_{50} was converted to pIC_{50} scale (-log IC_{50}), in which higher values represent higher exponential potency. The QSAR models were built for both sets of p IC_{50} values separately to distinguish the best model. The predicted pIC_{50} parameters of entire training set (best-fit model) were cross evaluated and validated with the descriptors of the test set of molecules. The dataset included the same 17 molecules with the different IC_{50} values (-log IC_{50}) (M2H assay). The molecules are aromatic, polar and properties such as molar refractivity and the logarithm of the (octanol/water) partition coefficients are important in describing such systems.

Results



*- cell-based assay of reporter gene activity

**- mammalian two-hybrid system assay (M2H)

Figure 1: Measured activities (-log IC_{50}) versus predicted activities **(A, C, D)**. Correlation plot for: **-log IC_{50}***: R = 0.9984, R^2= 0.9969, -log IC_{50} predicted: 0.996895 (-log (IC_{50}*)) + 0.0140789; **–log IC_{50}**:** R = 0.9529, R^2 = 0.9080, -log IC_{50} predicted = 0.908014(-log (IC_{50}**)) + 0.12391. The 'residual' deviation is not exceeding 1.0 or more units of ΔpIC_{50}, which represents a good fit to the experimental data. 'Residual' deviation (**$SD_{\Delta G}$= +/-1.0)** is equal to experimental activity (-log IC_{50}) except to the predicted activity (-log IC_{50}). The dashed lines mark deviations of 1.0 (0.5 χ factor) logarithmic unit from the ideal prediction. There are only two Z-score outliers, the choice of descriptors can be considered as adequate **(B)**.

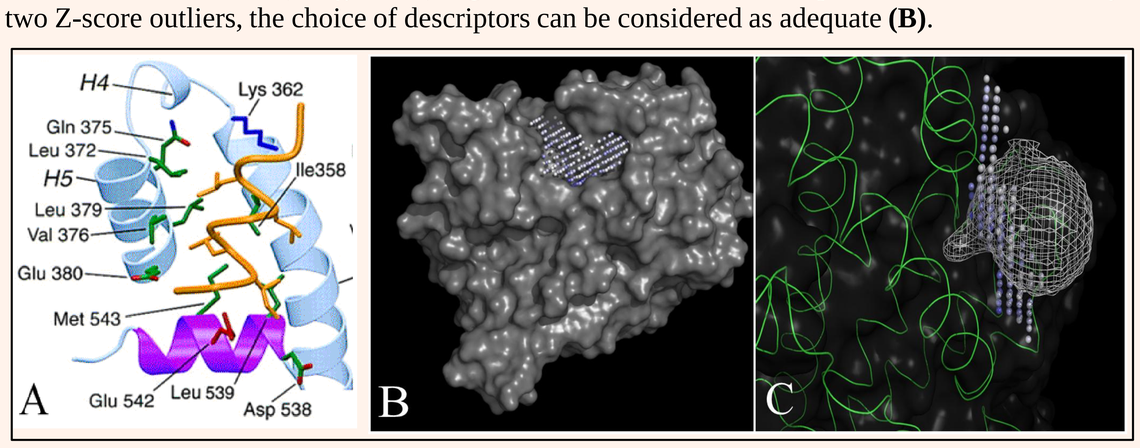


Figure 2: Center of the grid box. The affinity grid coordinates are crucial for successful and correct ligand - protein simulation. The crystallographic structure of the ERα-GRIP1 complex was inspected for protein - ligand interactions manually. As was previously shown **[2,3],** the GRIP1 LxxLL domain (**A**) is important for binding, hence the center of the grid was placed upon LxxLL relative position in the ERα (**B**). The GRIP1 peptide was removed prior to docking. Established grid coordinates (Å) and other parameters were set to -7.98 (x), -17.09 (y), -0.67 (z) as a grid box center, 64000 of current grid points per map and 0.375 Å of spacing (**C**).

Compound	ΔG_{exp}	$\Delta G_{exp\ pred}$	$\Delta G_{dock\ actual}$	$\Delta\Delta G_{dock\ actual}$	$\Delta G_{dock\ refined}$
1	8.38	8.43	-7.38	0.05	9.04
2	8.98	8.78	-7.15	-0.18	8.81
3	8.59	8.78	-7.08	-0.25	8.74
4	8.28	8.28	-6.12	-1.21	7.78
5	8.42	8.42	-7.6	0.27	9.26
6	8.34	8.31	-7.53	0.20	9.19
7	8.89	8.86	-8.11	0.78	9.77
8	9.39	9.39	-7.41	0.08	9.07
9	8.85	8.85	-7.16	-0.17	8.82
10	8.92	8.92	-7.13	-0.20	8.79
11	9.65	9.65	-6.99	-0.34	8.65
12	8.11	8.11	-7.41	0.08	9.07
13	8.38	8.38	-7.30	-0.03	8.96
14	10.49	10.49	-8.11	0.78	9.77
15	9.37	9.37	-6.82	-0.51	8.48
16	9.37	9.37	-7.58	0.25	9.24
17	10.49	10.49	-7.77	0.44	9.43

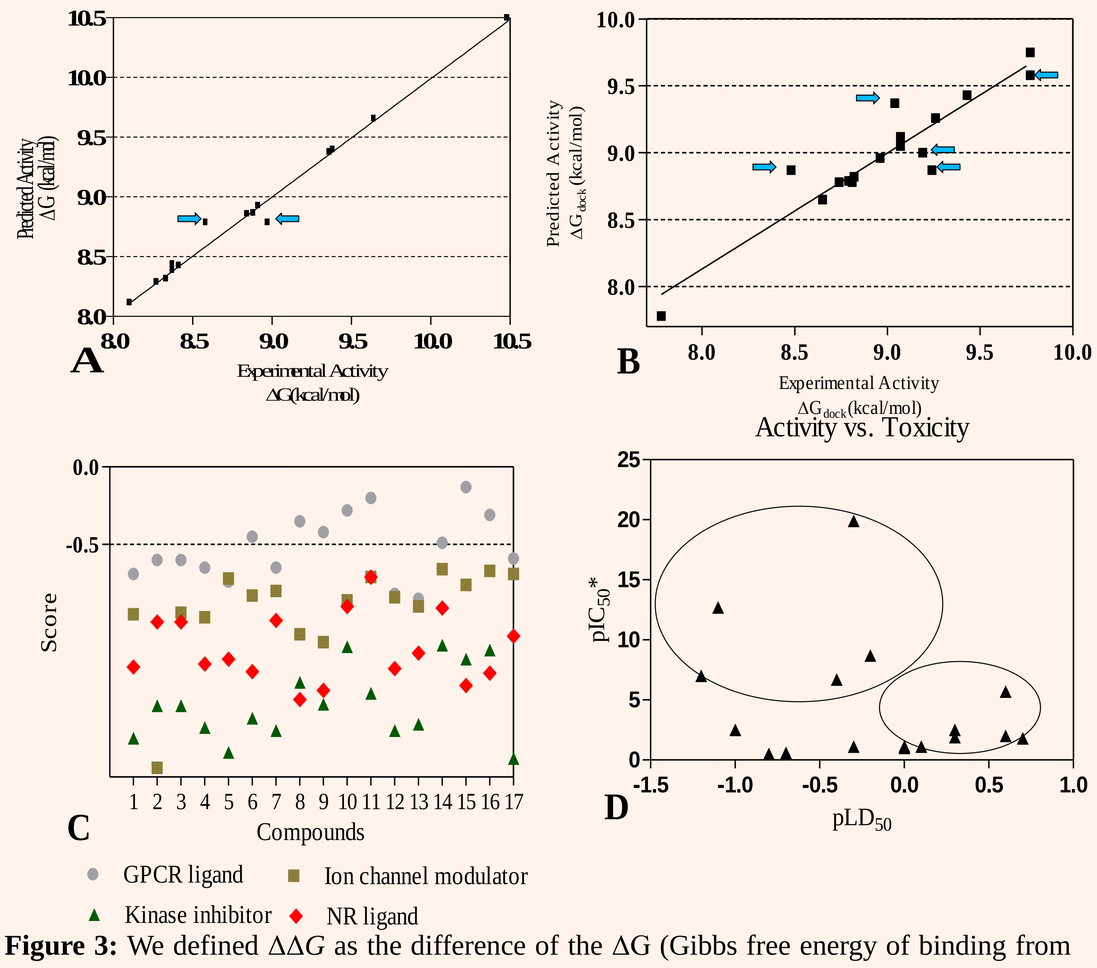


Figure 3: We defined $\Delta\Delta G$ as the difference of the ΔG (Gibbs free energy of binding from Autodock output files) for experimental and docking values, (**$\Delta\Delta G_{dock\ actual}$= 1 / ($\Delta G_{dock\ actual}$ - mean $\Delta G_{dock\ actual}$); $\Delta G_{dock\ refined}$= mean ΔG_{exp} + $\Delta\Delta G_{dock\ actual}$); $SD_{\Delta G}$= +/-0.04.** ΔG was calculated as: ΔG = RTln(IC_{50}) **(Table, A, B)**. ADME properties show predicted possibility of these compounds to inhibit some of the key cellular proteins such as G-protein coupled receptors and nuclear receptors **(C).** The therapeutic window is the activity vs. toxicity ratio of the compounds. Hence, there might be a different probability to hit a highly toxic compound at randomly given pIC_{50} range. The motivation for the toxicity is that the strongest inhibitors are most probably less toxic **(D).**

Conclusion

In the present study, we have analyzed a series of 17 guanylhydrazone coactivator binding inhibitors for the ERα. We prepared and characterized the dataset of potential inhibitors of estrogen receptors; build a QSAR model, which is based on the experimental data (IC_{50}). The compounds represent binding affinity modes in cell-based assays and docking studies, which have strong correlations upon the 3D QSAR model. IC_{50} values were converted to Gibbs free energy of binding parameters to evaluate deviations in experimentally obtained and *in silico* calculated data. Additional work related to the activity and receptor specificity of these and other coactivator binding inhibitors will be the subject of the further analyses.

Acknowledgment

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References

[1] A. L. LaFrate *et al*., *Bioorg. Med. Chem*., 16(23): 10075-10084 (2008)

[2] A. L. Rodriguez *et al., J. Med. Chem.*, 47(3):600-611 (2004)

[3] A. K. Galande *et al., J. Pept. Res.*, 63(3):297-302 (2004)