

IN SILICO SCREENING IN LEAD DISCOVERY APPLIED TO POLY(ADP-RIBOSE) POLYMERASE

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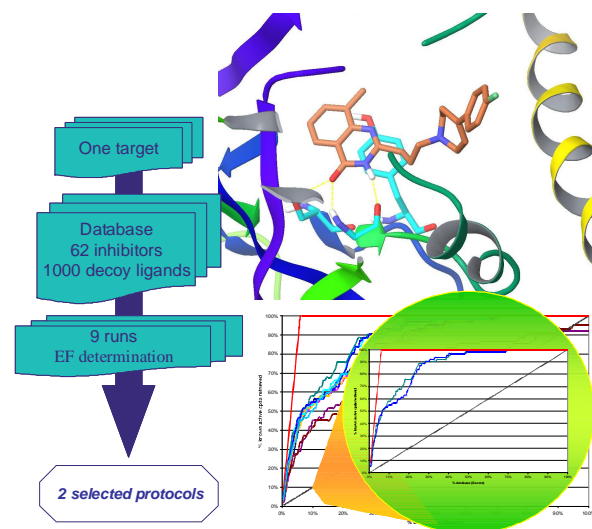
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Introduction

Poly (ADP-ribose) polymerase (PARP-1: EC 2.4.2.30) activation plays a role in the pathogenesis of various cardiovascular and inflammatory diseases. PARP-1 activation is also relevant for the ability of cells to repair injured DNA. Thus, pharmacological inhibitors of PARP-1 may attenuate organ injury caused by ischemic and inflammatory cell or enhance the cytotoxicity of antitumor agents¹. As an initial step of our lead discovery program, we developed a virtual screen protocol to discriminate known PARP-1 inhibitors and inactive compounds using GLIDE². The maximal enrichment factor (EF = 5.81) suggests that our protocol effectively identifies potential PARP-1 inhibitors. This protocol was employed to select molecules from a compounds library to be tested and 7 new micromolar inhibitors were discovered.

Binding Site: 1UK0-FR257517



| | 10, 0.9 | 10, 0.8 | 0.9, 0.9 | 0.9, 0.8 | 0.8, 0.9 | 0.8, 0.8 | 0.8, 0.7 | 0.7, 0.9 | 0.7, 0.8 |
|---------------|---------|---------|----------|----------|----------|----------|----------|----------|----------|
| n actives 2% | 9 | 9 | 11 | 13 | 10 | 10 | 13 | 15 | 14 |
| n actives 5% | 23 | 26 | 25 | 28 | 17 | 17 | 26 | 28 | 24 |
| n actives 10% | 33 | 34 | 33 | 33 | 30 | 30 | 36 | 34 | 31 |
| EF (2%) | 7.26 | 7.26 | 8.87 | 10.48 | 8.06 | 8.06 | 10.48 | 12.10 | 11.29 |
| EF (5%) | 7.42 | 8.39 | 8.06 | 9.03 | 5.48 | 5.48 | 8.39 | 9.03 | 7.74 |
| EF (10%) | 5.32 | 5.48 | 5.32 | 5.32 | 4.52 | 4.19 | 5.81 | 5.48 | 5.00 |

Table 1
10, 0.9 and 0.8 indicates respectively no-scaling, 0.9 and 0.8 scaling of Van der Waals radii of non-polar atoms in the ligand and in the receptor. First number refers to protein, second one to ligand.

Figure 1

Experimental determination of IC50

Universal Colorimetric PARP assay Kit with Histone-coated Plate was purchased from Trevigen, Inc. (USA). Reference inhibitors NU1025 and DPO were from Calbiochem. Results are shown in table 2.

Computational methodology

A preliminary analysis of available X-ray crystal structures of PARP-1 detected no significant conformational differences among them, so a single crystal complex structure was employed (PDB ID: 1UK0, co-crystallized ligand: FR257517).

Crystallographic studies provided evidences that the amino group of the inhibitor forms hydrogen bonds with Gly202 e Ser243 and the aryl group occupies hydrophobic space in the region of the active site, predominantly interacting with the side chain of Tyr246.

PARP-1 crystal structure was prepared for docking with the default procedure implemented in GLIDE [Protein Preparation]. Nine virtual screening runs were carried out in order to determine the best Wan der Waals scaling parameters combinations to be used. A database formed by 1000 drug-like decoy ligands (Rognan database³) and 127 known active inhibitors⁴, prepared with LigPrep⁵, was employed. Multiple protonation and tautomerization states were employed when appropriate. EF were obtained with two different combinations (Table 1) and an ensemble docking protocol was planned employing two docking runs. It is shown in figure 1.

Further tests showed that EF can be improved re-docking the 10% of best SP ranked molecules with XP scoring function and with a final visual inspection to eliminate evident false positives verifying the existence of the key interactions in the active site. Molecular descriptors were calculated with DRAGON, a software package developed by Milano Chemometrics and QSAR Research Group. It allows calculation of more than 1600 molecular descriptors for thousands of molecules.

Post docking analysis

A post-docking analysis by means of Linear Interaction Approximation (LIA), a way of combining molecular mechanics calculations with experimental data, was used to develop a more predictive scoring method able to discriminate between active and inactive compounds. Unfortunately, we were not able to find a stable correlation model also using other methods as MM-GBSA and Embrace because we did not have an homogenous dataset for QSAR studies.

Results

A compounds library of about 5,000 molecules, both vendors and corporate compounds, was submitted to virtual screening employing the described protocols.

The resulting 500 hits were analysed by means of Multivariate Data Analysis (MVDA) to select the most diverse subset of structures.

A Principal Component Analysis was performed on the DRAGON descriptors matrix using the software SIMCA-P⁶. Extracted principal components were directly read into MODDE⁶ and employed to perform an Onion/D-optimal Design (DOE) with the aim to select diversity-enriched subsets from the original hits. Workflow is shown in figure 2.

52 compounds were selected and experimentally screened for their ability to inhibit PARP-1 activity. The results obtained by the experimental screen are shown in table 2. We obtained 7 active compounds with a pIC50 range from 6.5 to 5.3.

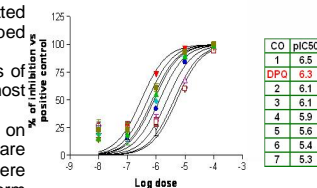


Table 2

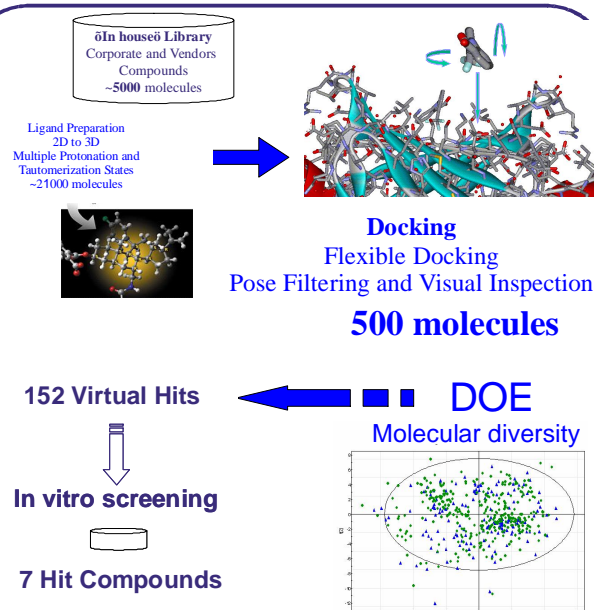


Figure 2

Conclusion

This study demonstrates that structure-based virtual screening represents a viable approach to the development of new PARP-1 inhibitors. Although this is a relatively small-scale study 7 new micromolar inhibitors were discovered. These compounds appear to be suitable as leads for further studies.

References

- 1) *J. Med. Chem.*, 2004, 47, 5467-5481.
- 2) *J. Med. Chem.*, 2004, 47, 1739-1749.
- 3) *J. Med. Chem.*, 2000, 43, 4759-4767.
- 4) *J. Med. Chem.*, 2004, 47, 4151; *Expert. Opin. Ther. Patent* 2002, 12, 7; *J. Med. Chem.*, 2001, 44, 3786; *FEBS Letters* 579/2005 1389-1393
- 5) Schrödinger, L.L.C., New York, www.schrodinger.com
- 6) Umetrics AB, www.umetrics.com.

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