Discovery Of Novel Tankyrase Inhibitor Chemotypes: An Insightful Test Case For Virtual Screening And Molecular Modeling Approaches

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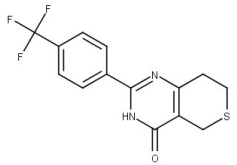
Targets of interest: tankyrase and PI3Kα

Tankyrase (TNKS) – PARP family enzyme, activates Wnt pathway

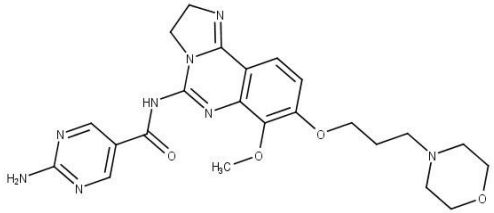
- Canonical Wnt signaling pathway is responsible for cell growth and development
- Selective tankyrase inhibitors have shown promising results in the therapy of colorectal cancer



XAV939

PI3K – phosphoinositide 3-kinase, part of the PI3K/AKT/mTOR signaling pathway

- PI3K signaling pathway is responsible for cell growth, proliferation and development
- Genes encoding the PI3K pathway enzymes are often mutated in various forms of cancer



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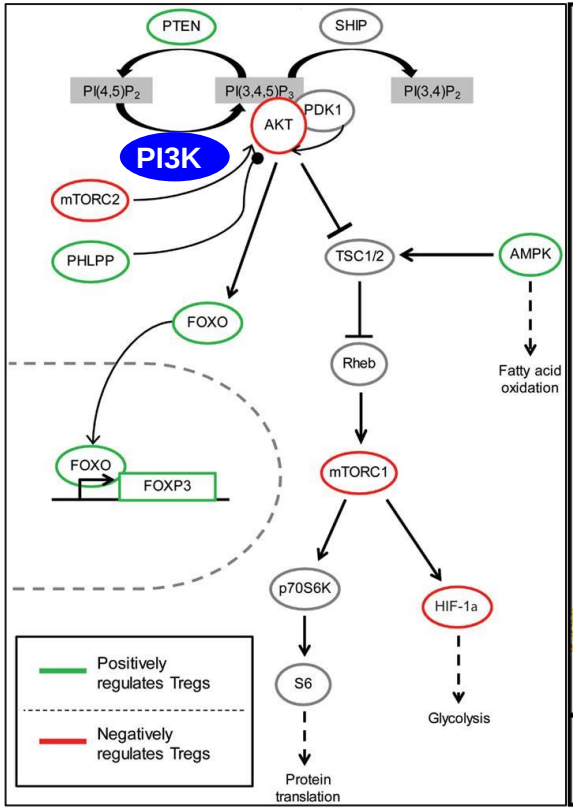
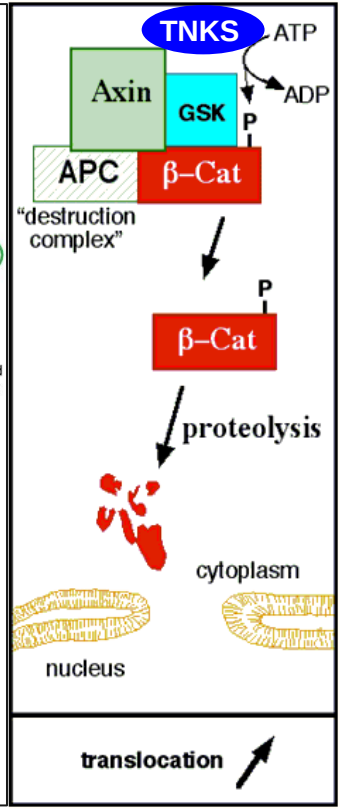




Synergistic effects of simultaneous inhibition for cancer treatment

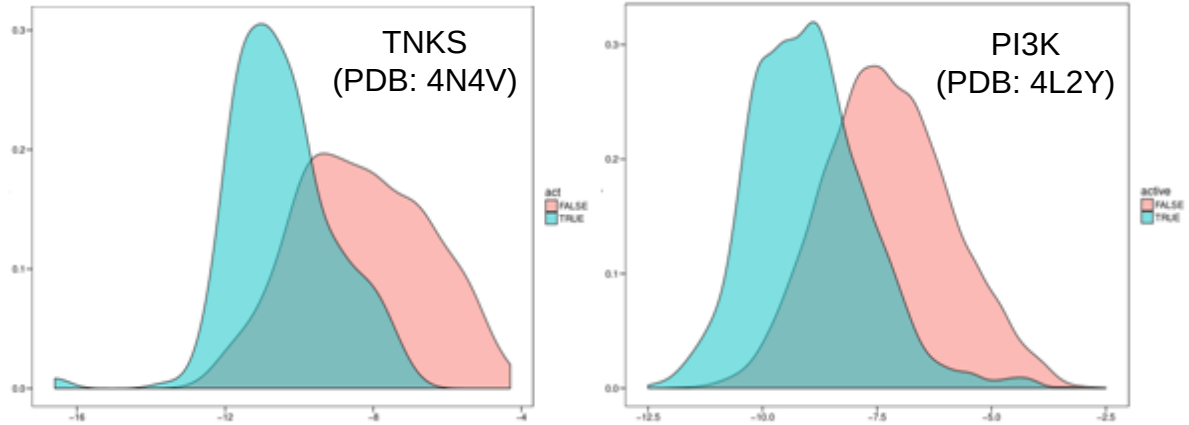
Target-optimized scoring functions for molecular docking

Molecular docking – the most widely used virtual screening method

- Determine possible binding mode [pose]
- Score/rank poses and ligands by expected affinity
- Should be fast and reasonably reliable

- Existing scoring functions often not accurate enough

Distribution density of Vinardo scoring function values



- AutoDock Vina, Smina
- Actives: ChEMBL23
- Decoys: DUD-E from ZINC

AUC ROC ~ 0.81

Machine learning scoring functions

Datasets: 3618 PI3Kα and 247 tankyrase inhibitors

(Refined model: 6445 and 682 inhibitors from ChEMBL 24)

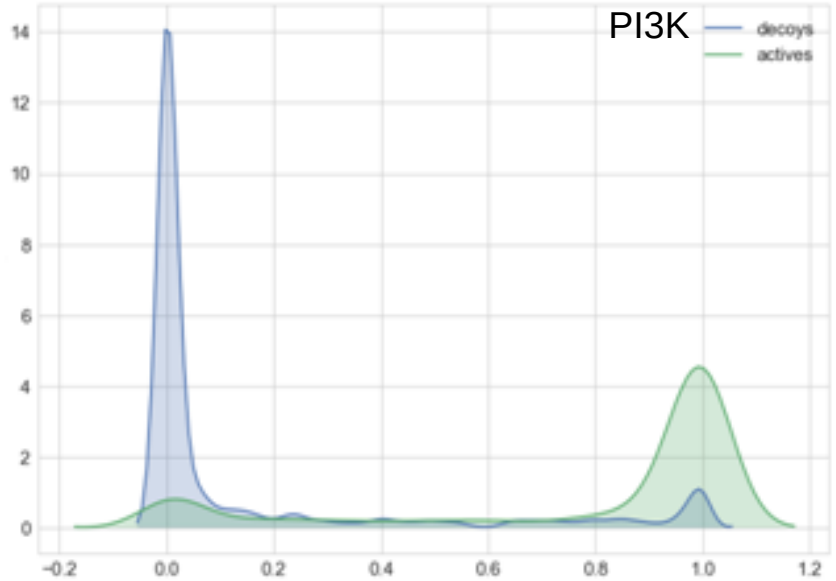
Training/test set split in approximately 70:30 ratio

Classification Deep Neural Network model

Descriptors are Smina scoring function terms

Method	AUC ROC			
	Random split		Time split	
	PI3K	TNKS	PI3K	TNKS
DNN	**0.96**	**0.93**	**0.90**	**0.84**

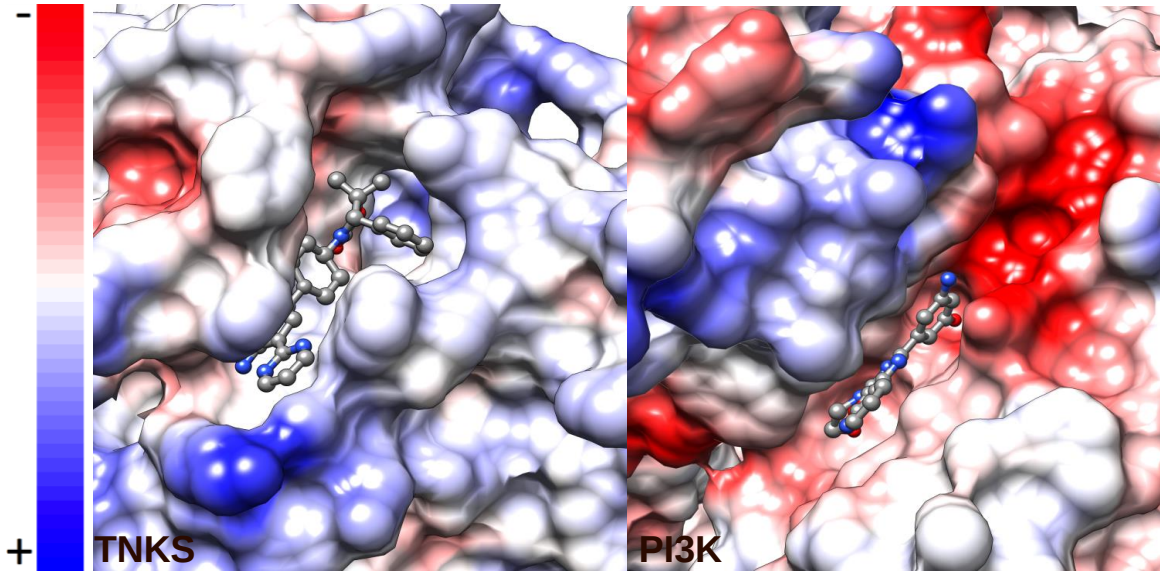
Distribution density of scoring function values



Advantages of target-specific scoring functions

- Take into account significant differences in electrostatics and lipophilic interactions inside the binding sites
- Significant differences in the importance of the descriptors (Smina scoring function terms)

Protein surface and electrostatic potential



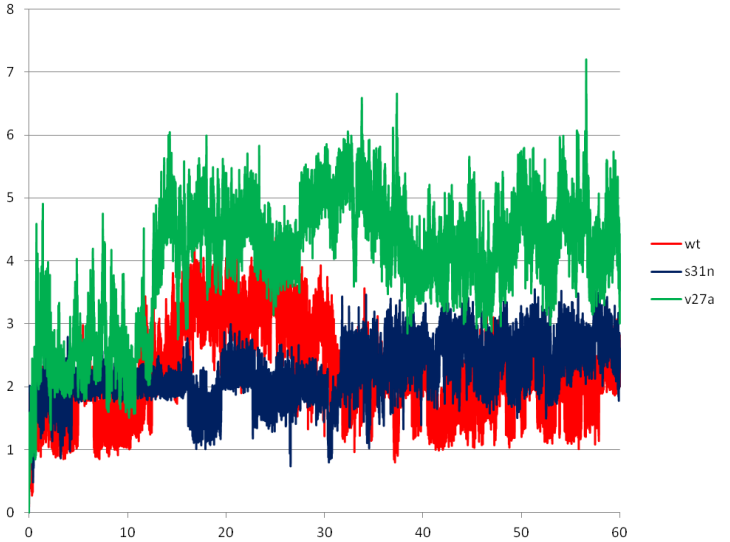
Target-specific scoring functions are more efficient than general-purpose scoring functions

Berishvili V.P., Voronkov A.E., Radchenko E.V., Palyulin V.A., *Mol. Inf.*, 2018, 37(11), 1800030

Direct time-domain analysis of molecular dynamics trajectories

Hypothesis: the nature of changes in the ligand-protein interaction descriptors over the molecular dynamics trajectory can be **directly analyzed** to build more accurate predictive models compared to the ones based on the static complex structures.

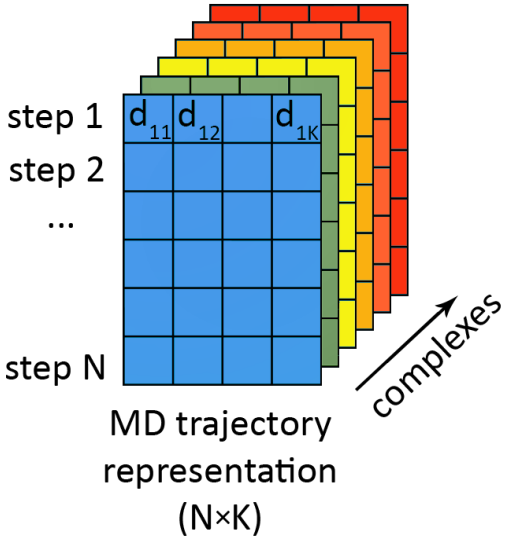
Different behavior of an influenza virus M2 channel inhibitor active and inactive to specific channel variants



Klimochkin Yu.N., Shiryaev V.A., Petrov P.V., Radchenko E.V., Palyulin V.A., Zefirov N.S. *Curr. Comp.-Aided Drug Des.*, 2016, 12(2), 154–164.

Time series representation of MD trajectories

- **Each complex is characterized by a 2D N×K tensor**
- N is the number of trajectory frames
- K is the number of descriptors



GROMACS descriptors

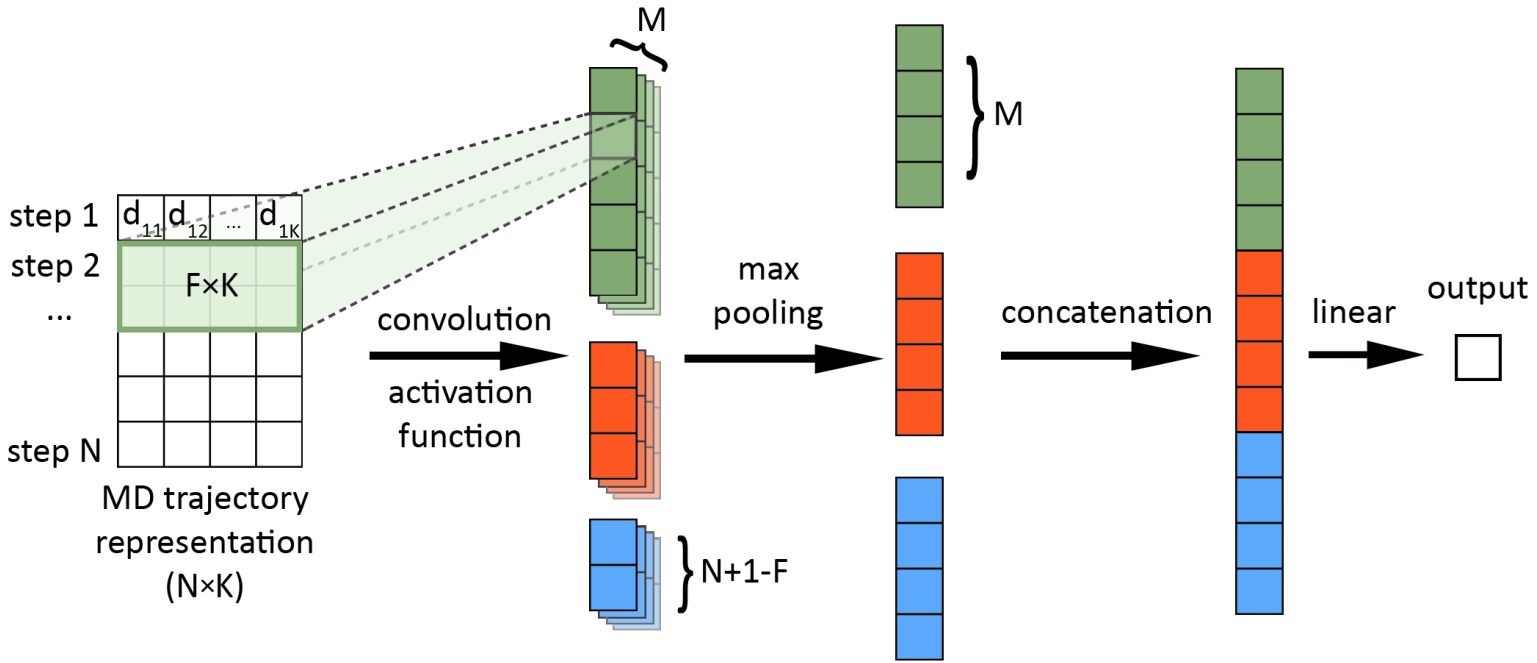
- RMSD for the ligand and binding site atoms
- Electrostatic and van der Waals ligand interaction energies
- Ligand radius of gyration
- Squared relative distance between the ligand and binding site centers of mass
- Solvation energy

Smina descriptors (scoring function components)

- Steric potential
- Hydrophobic potential
- Coulomb interactions
- Solvation potential
- Hydrogen bonds

Convolutional Neural Network architecture

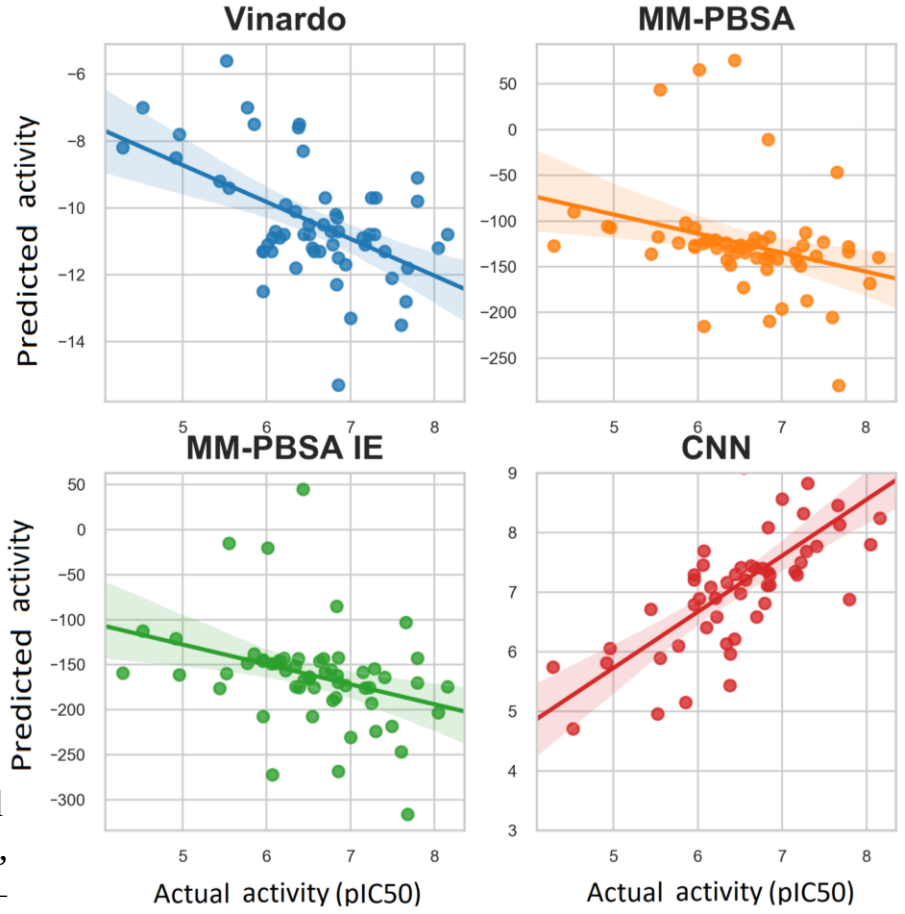
M filters with different convolution kernel lengths F



Applicability in virtual screening

Tankyrase test dataset (57 complexes)

Method	Docking scoring function (Vinardo)	MM-PBSA, 30 ns	MM-PBSA with interaction entropy, 30 ns	Best NN model (CNN)
Spearman correlation	**–0.42**	**–0.46**	**–0.41**	**0.73**
Pearson correlation	**–0.52**	**–0.29**	**–0.32**	**0.70**



Berishvili V.P., Perkin V.O., Voronkov A.E., Radchenko E.V., Syed R., Venkata Ramana Reddy C., Pillay V., Kumar P., Choonara Y.E., Kamal A., Palyulin V.A., *J. Chem. Inf. Model.*, 2019, 59 (8), 3519–3532

Search for novel tankyrase inhibitors

ZINC subset: 1.7 mln compounds (Russian vendors)

Tankyrase docking & Machine-learning rescoring: 174

Preliminary ADMET filtering (LogP, solubility, HIA, hERG): 17

Visual analysis and selection

Selected for purchase and activity testing: 10

Purchased and tested: 7

Virtual screening of tankyrase/PI3Kα inhibitors

Preliminary ADMET filtering

MW < 600

LogP < 6 (MSU in-house model, OCHEM ALogPS)

Solubility > 10^{-5} M (OCHEM ALogPS)

HIA > 75% (MSU in-house model)

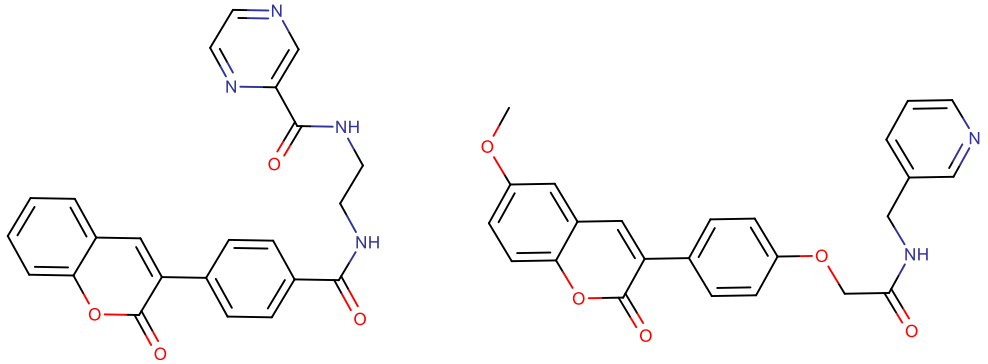
hERG: pK_i < 6, pIC_{50} < 6 (MSU in-house model)

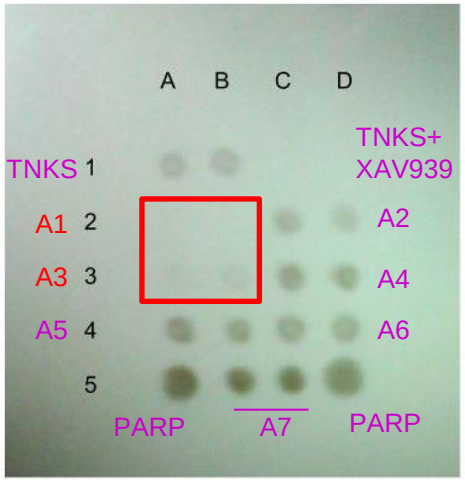
Biochemical *in vitro* studies of tankyrase inhibitors

Inhibitory activity measurements

7 compounds were purchased out of 10 selected
Immunochemical measurement of PAR buildup
Preliminary screening followed by more detailed studies for active inhibitors

Two inhibitors found
A1: **IC_{50} = 3.1 ± 0.5 nM, non-competitive, reversible**
A3: IC_{50} = 4 ± 2 μM





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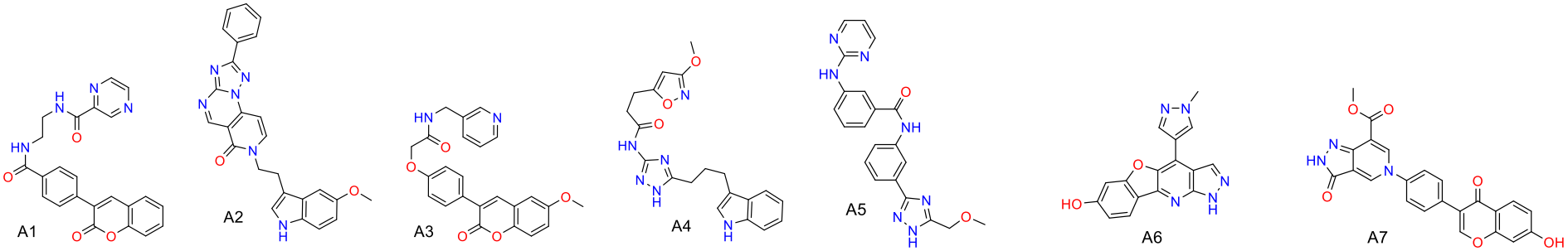
Retrospective analysis of the virtual screening results

Could the methods used for the hit-oriented virtual screening be employed during further lead optimization?

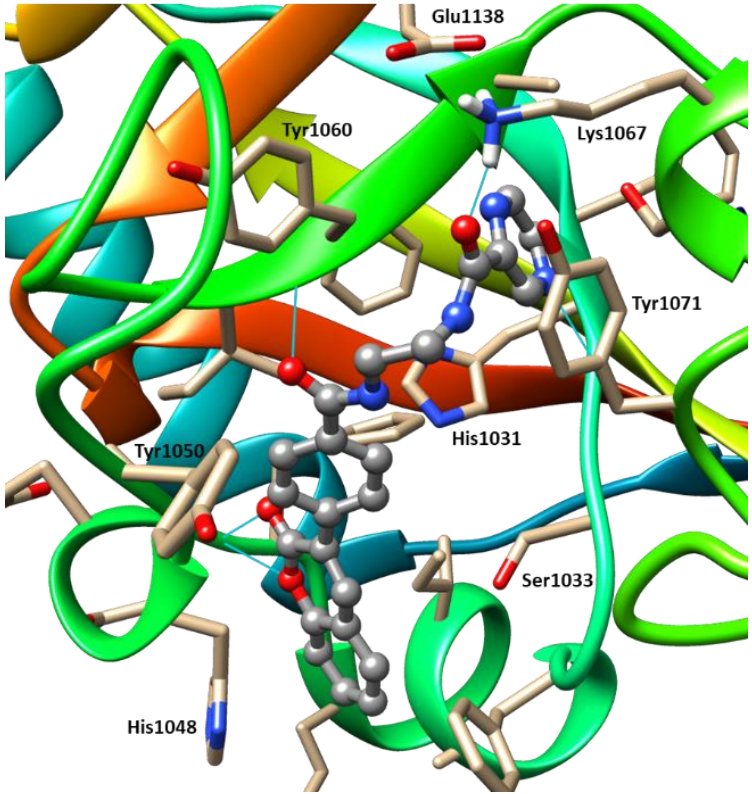
No differentiation or correlation: apparently significant likelihood of binding for all compounds

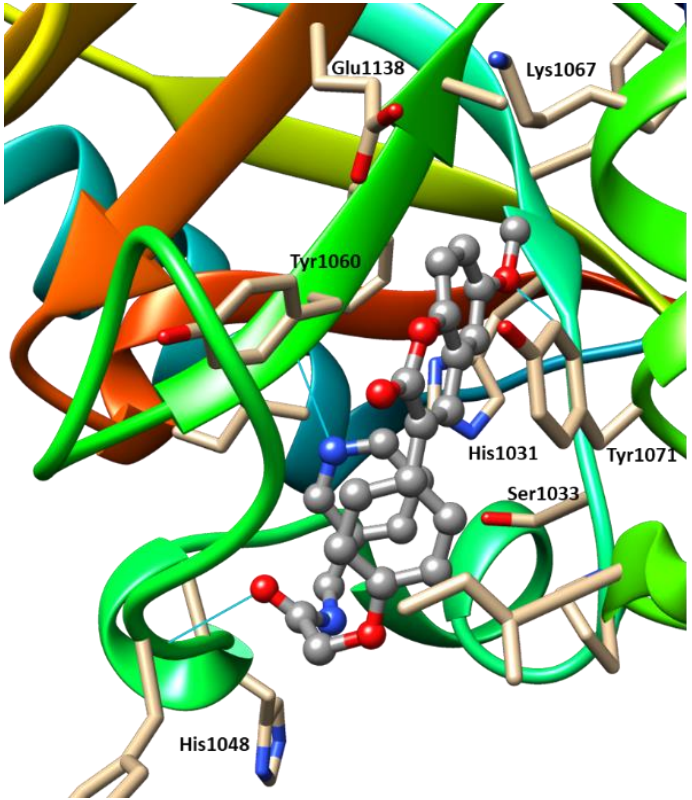


Compound	Binding affinity predicted by docking scoring function, kcal/mol	Binding probability predicted by ML scoring function	Binding energy calculated by MM-PBSA, kcal/mol
A1	–12.8 ± 0.1	0.61 ± 0.1	–32.5 ± 10.3
A2	–12.4 ± 0.2	0.70 ± 0.1	–36.3 ± 9.8
A3	–12.4 ± 0.1	0.62 ± 0.1	–30.8 ± 9.2
A4	–11.7 ± 0.1	0.24 ± 0.1	–28.1 ± 9.6
A5	–12.6 ± 0.2	0.15 ± 0.1	–29.1 ± 9.7
A6	–12.5 ± 0.1	0.46 ± 0.1	–31.2 ± 8.0
A7	–12.6 ± 0.1	0.56 ± 0.1	–32.0 ± 8.8

Molecular dynamics studies: Binding modes

Stable binding for A1 and A3 but different binding modes





Molecular dynamics studies: Free energy perturbation (FEP)

Alchemical (non-physical) thermodynamic cycle
Ligand uncoupling and conformational restraints
Computationally intensive

A1 likely has more favorable interactions in the binding site compared to A3 which can be attributed to the differences in the binding modes and the presence of additional polar groups

FEP predictions are not quite perfect but more reliable compared to the docking and MM-PBSA methods

Binding free energy (kcal/mol)		A1	A2	A3	A7
Total free energy of binding	ΔG^0_{bind}	–10.8 ± 0.2	–8.2 ± 0.2 (+2.6)	–4.0 ± 0.2 (+6.8)	7.6 ± 0.2 (+18.4)
Predicted	$\mathbf{pK_d}$	**7.9**		**2.9**	
Experimental	$\mathbf{pIC_{50}}$	**8.0**	**n/a**	**5.0**	**n/a**

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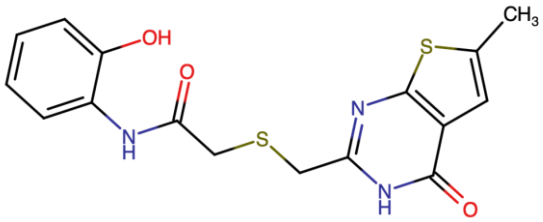
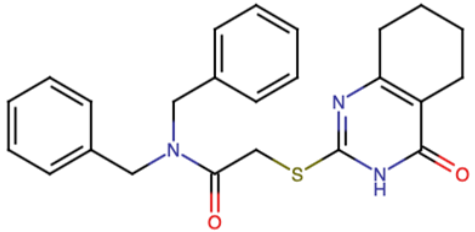
Second round of *in silico* and *in vitro* screening

20 diverse compounds selected by virtual screening based on refined models

Two tankyrase inhibitors were found with micromolar activity



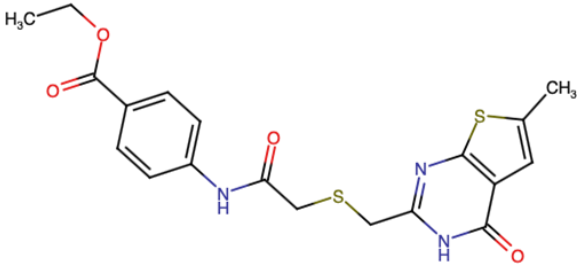
Y042-4555



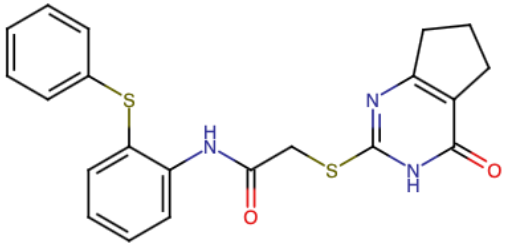
D365-0076

Additional analogs: 9 (5+4) compounds selected based on structural similarity

All 9 compounds have inhibitory activity (at different levels)



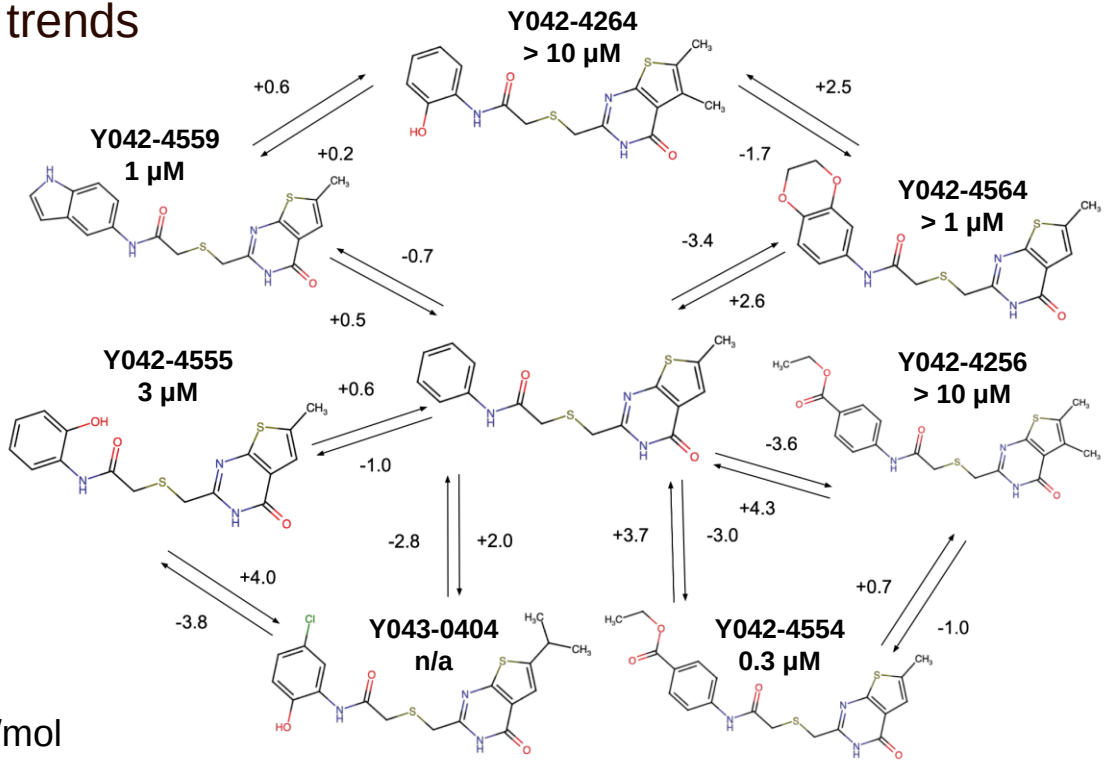
Y042-4554 (IC_{50} = 300 nM)



D467-0063 (IC_{50} = 30 nM)

RFEP analysis of structure-activity relationships

Relative Free Energy Perturbation (RFEP) analysis for matching molecular pairs
Good accuracy and lower computational cost compared to FEP
Correctly predicts SAR trends



RFEP ΔΔG in kcal/mol

Conclusions

Combination of virtual screening and molecular modeling methods significantly improves hit discovery success rate: out of 1.7 million compounds, 36 were selected for *in vitro* testing and 13 compounds were found to be active (including several inhibitors with nanomolar activity)

Three promising new chemotypes of tankyrase inhibitors were discovered

General-purpose and even target-specific scoring techniques are useful for virtual screening but still not suitable for activity ranking and optimization of similar structures

Free Energy Perturbation and Relative Free Energy Perturbation (RFEP) can predict the effects of structural modifications on binding energies

Acknowledgements

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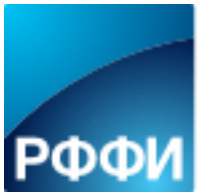
Indian and South Africa partner teams

ChemDiv company for samples of compounds

STI FRAMEWORK PROGRAMME



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ФУНДАМЕНТАЛЬНЫХ
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Thank you for your attention!