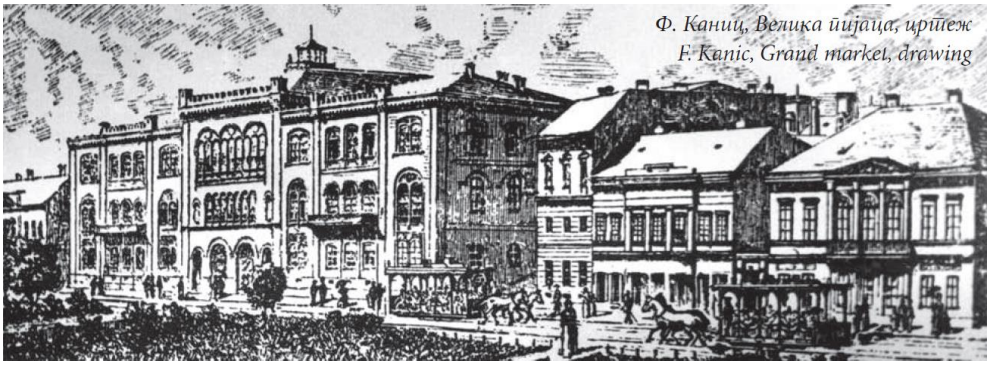
PHYSICAL CHEMISTRY 2018

14th International Conference on Fundamental and Applied Aspects of Physical Chemistry



The Conference is dedicated to the 210th Anniversary of the University of Belgrade

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September 24-28, 2018

PHYSICAL CHEMISTRY 2018

14th International Conference
on Fundamental and Applied Aspects of
Physical Chemistry

Organized by
The Society of Physical Chemists of Serbia

FINAL PROGRAM

September 24-28, 2018
Belgrade, Serbia

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RATIONAL DESIGN OF SELECTIVE HISTONE DEACETYLASE INHIBITORS

D. Ruzić[1], N. Djoković[1], M. Petković[2], D. Agbaba[1], M. Lahtela-Kakkonen[3], A. Ganesan[4] and K. Nikolić[1]

[1] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia (knikolic@pharmacy.bg.ac.rs)
[2] Department of Organic Chemistry, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11000 Belgrade, Serbia
[3] School of Pharmacy (Pharmaceutical Chemistry) University of Eastern Finland, Kuopio, Finland
[4] School of Pharmacy, University of East Anglia, Norwich Research Park, NR4 7TJ Norwich, United Kingdom

ABSTRACT

The concept of gene expression is continuously explained with epigenetic modifications. One of the most studied enzymes which have an influence on histone posttranslational modifications are histone deacetylases (HDACs). The overexpression and alterations in the structure of HDACs isoforms are described in the pathogenesis of cancer, inflammation and neurodegeneration. With different cellular function and tissue distribution of HDACs, scientists introduced them as attractive targets used in novel drug discovery protocols. Rational drug design of novel small molecules is usually guided by computational approaches. In our laboratory, we use ligand-based (pharmacophore modeling and virtual screening) and structure-based (molecular docking and molecular dynamics) drug design methodologies. The main focus in our drug design project is identification of selective HDAC6 and SIRT2 inhibitors. With respect to all published data, very small number of selective HDAC modulators has been reported so far. Here, we present rational design of novel selective HDAC6 and SIRT2 inhibitors as promising drug candidates for further development and structure optimization.

INTRODUCTION

Histone modifications such as histone acetylation, deacetylation, methylation, demethylation, phosphorylation, and deamination, are defined as epigenetic hallmarks with crucial influence on regulation of gene expression and chromatin organization [1, 2]. The chromatin condensation

and gene expression are mainly regulated by level of activities of Histone Acetyl Transferases (HATs) and Histone Deacetylases (HDACs), which catalyzes acetylation of ε-amino group of particular lysine residues and hydrolysis of acetyl-lysine residues of the histone tails, respectively [3].
Histone deacetylases are clinically confirmed drug targets for cancer, neurological diseases and immune disorders, with four FDA approved HDAC inhibitors (HDACi) for clinical use as antineoplastic drugs [4, 5]. One of the main goals in current epigenetic research is to rationally design selective HDACi for specific isoform as drug candidates with increased efficacy and safety.

The HDAC enzyme family is divided into four classes, where classes I, II and IV are zinc-dependent hydrolases and class III (sirtuins) are NAD+-dependent enzymes [6].

Histone deacetylase 6 (HDAC6) is mainly cytoplasmatic enzyme with two catalytic domains. In the study was used crystal structure of the trichostatin A in complex with second catalytic domain of human HDAC6 (PDB code - 5EDU). HDAC6 is involved in regulation of activity of cytoplasmatic proteins and therefore play a crucial role in cytoskeleton remodelling, dynamics of microtubule and triggering of apoptosis [5]. Inhibition of HDAC6 is confirmed to strongly influence on migration and viability of the cancer cells. Recent discovery of hydroxamic acid derivatives as potent and selective HDAC6 inhibitors [7], indicated on severe cardiotoxicity, mutagenicity, and poor solubility, as crucial problems for clinical use of the agents.

In our study were developed 3D-QSAR (Quantitative Structure Activity Relationship) models for HDAC1 and HDAC6 inhibitors in order to define specific molecular determinants for selective HDAC6 inhibition. The HDAC6 and HDAC1 enzymes significantly differ in structure, function, and localization and therefore present optimal pair for the rational design of selective HDAC6 inhibitors.

Sirtuin 2 (SIRT2), another cytoplasmatic NAD-dependent histon deacetylase, is recently confirmed as promising drug target for the treatment cancers, depression and Parkinson's disease. Until today none of SIRT2 inhibitors has been approved for the clinical use. Some of the main problems with current SIRT2 inhibitors are poor potency, selectivity and pharmacokinetic.

Therefore in our study was examined conformational space of sirtuin2-inhibitor complexes, further refinement of crystallographic structures, and development of more efficient virtual screening (VS) protocol.

EXPERIMENTAL

The 3D-QSAR data sets of 36 HDAC1 and 32 HDAC6 inhibitors (hydroxamic acid derivatives) were selected from ChEMBL database (https://www.ebi.ac.uk/chembl/). Negative logarithm of the inhibitory constant pKi = -log (Ki) was further used as dependent variable in 3D-QSAR modeling. The wide range of pKi values (pKi = -log (Ki)), as dependent variable in the HDAC1 (pKi: 6.486-9.699) and HDAC6 (pKi: 6.548-9.602) data sets, provide wide applicability domain of the formed 3D-QSAR models.

The HDAC1 and HDAC6 data sets contain more than 80% of common compounds.

Dominant forms of the HDAC1 and HDAC6 inhibitors at the physiological pH=7.4 were defined by use of Marvin Sketch 6.1.0 program (Chem Axon 2013). Conformations of the ligands for 3D-QSAR modelling were generated by virtual docking of data set ligands into the crystal structures of HDAC1 (PDB: 5ICN) and HDAC6 (PDB: 5EDU). Molecular docking was performed by use of GOLD Software 5.6.0 [8].

Calculation of GRIND molecular descriptors and development of 3D-QSAR models was performed by Pentacle program 1.07 [http://www.moldiscovery.com/software/pentacle/].

The data set compounds used for 3D-QSAR modelling were divided into training and test set.

The General Fragment Library (40142 fragments) and Epigenetic Targeted Library (7019 fragments), from Life Chemicals Inc. (http://www.lifechemicals.com/), were used for fragment-based design of novel HDAC6 inhibitors.

The ADMET Predictor Software v.8.5.0 [http://www.simulations-plus.com/] was used to predict physico-chemical parameters, pharmacokinetic properties and toxicity of the examined and designed HDAC6 inhibitors.

The SIRT2 study has started from five different crystallographic structures of SIRT2 with inhibitors. Molecular dynamics (MD) simulations in explicit solvent has been done for 1.5 μs. Structure based virtual screening was performed by use of GRID-based 3D descriptors and linear discriminant analysis.

RESULTS AND DISCUSSION

The developed and validated 3D-QSAR models for HDAC1 and HDAC6 inhibitors were successfully used to define specific GRIND variables as molecular determinants for inhibiting activity on HDAC6. For the comparative GRIND analysis were used HDAC6 selective inhibitors such as scriptaid and tubacin.

Based on the comparative study and novel drug design protocols is hypothesized that hydrophobic and steric interactions of the CAP group should be potentiated, distance between the CAP and zinc binding groups should be optimized, and diversity in linker chemistry should be applied in order to improve the selectivity for the HDAC6 isoform.

The employed design strategies were based on the replacement of the 1.8-naphthalimide CAP group of scriptaid, while retaining the aliphatic linkers of six or eight carbon atoms.

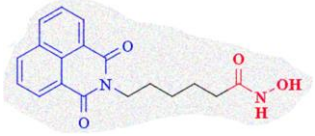
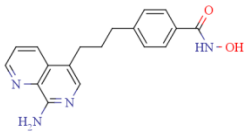
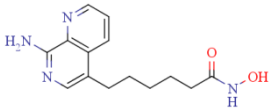
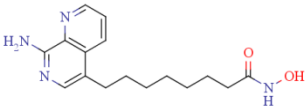
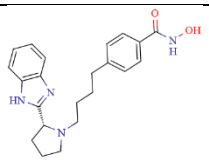
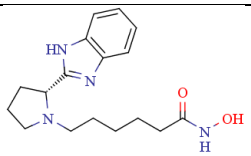
The Epigenetic Targeted Library and General Fragment Library were used for fragments selection based on similar McGowan Volume (Vx) and total surface area (SAtot) values to 1.8-naphthalimide (**Table 1**).

Table 1. Selected fragments for design of novel HDAC6 inhibitors.

Fragment Epigenetic Targeted Library	**Total Surface Area**	**McGowan Volume**
1.8-naphthalimide	191.659	205.864
F1957-0054	191.012	190.864
F1956-0006	207.766	206.03
G3000-0080	191.38	196.113

The selected CAP groups were further used in fragment based design of novel selective HDAC6 inhibitors. Based on the 3D-QSAR predicted pKi values against HDAC1 and HDAC6 enzymes (**Table 2**) and *in silico* ADMET parameters of the designed ligands were selected group of the most promising candidates for further study.

Table 2. Designed HDAC inhibitors with improved HDAC6 selectivity.

Designed compound	Pred. pKi_{HDAC1}	Pred. pKi_{HDAC6}	ΔpKi
 Scriptaid CHEMBL96051	8.824 (exp.)	9.602 (exp.)	**-0.778**
 D1-3	7.796	8.879	-1.083
 D1-5	7.380	9.175	-1.795
 D1-6	7.895	8.974	-1.079
 D3-4	7.834	8.896	-1.062
 D3-5	7.911	9.004	-1.093

Specific binding modes and ChemScore docking scoring functions of selected designed HDAC6 inhibitors and scriptaid (CHEMBL96051) were further examined by virtual docking in crystal structures of human HDAC1 (5ICN) and HDAC6 (5EDU) (**Figure 1, Table 3**).

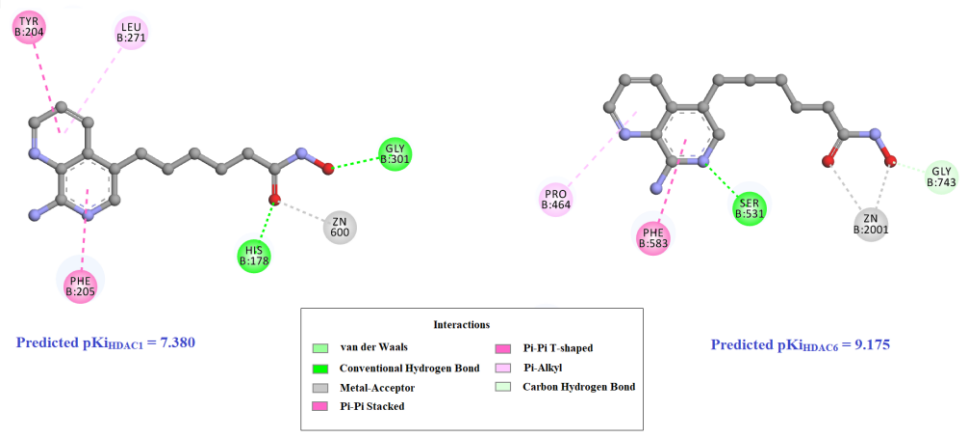


Figure 1. Binding mode of designed **D1-5** obtained by virtual docking on HDAC1 (left) and HDAC6 (right).

Table 3. The ChemScore docking of designed HDAC inhibitors.

	ChemScore	
Compound	HDAC1	HDAC6
Scriptaid	**25.432**	**30.6137**
D1-3	32.9064	35.1829
D1-5	27.1201	31.1112
D1-6	28.3858	30.9245
D3-4	34.0629	37.5489
D3-5	29.1502	31.1169

The binding modes and ChemScore docking scoring functions of the selected designed compounds have confirmed potent and selective binding with HDAC6 enzyme.

Molecular dynamic simulation of SIRT2-inhibitor complexes has indicated on significant conformational flexibility of the system, flexibility of binding site and multiple binding modes of inhibitors. Structure-based VS models, generated from three extracted molecular dynamic SIRT2-inhibitor complexes, were significantly improved related to VS models created from crystallographic structures and to published virtual screening studies. These VS results clearly indicate on significance of considering flexibility of binding site in rational design of SIRT2 inhibitors. The

developed VS models were successfully applied for screening of commercial databases of compounds and several types of novel SIRT2 inhibitors have been identified.

CONCLUSION

The 3D-QSAR models for HDAC1 and HDAC6 inhibition were developed with the conformers generated by virtual docking study.
Based on the 3D-QSAR study and GRIND analysis were defined molecular determinants for design of selective HDAC6 inhibitors.
Based on the 3D-QSAR predicted activities against HDAC1 and HDAC6 enzymes, *in silico* ADMET parameters, and virtual docking results of the designed ligands were selected group of the most promising candidates for further study.

Refined atomistic models of SIRT2-inhibitor complexes have been created. Significant progress in the virtual screening performance will be applied in rationalize design of SIRT2 inhibitors with improved selectivity and potency.

Acknowledgement
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