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Autoreferát dizertačnej práce

Modeling of Drug-Receptor Molecular Interactions by Means of Combination of Quantum Mechanical Methods and Empirical Potential Functions and Computer-Aided Design of New Anticancer Drugs

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Abstract

Considering the multitude of diseases lacking accessible and efficient therapy, the necessity to discover and develop new therapeutics persists. This dissertation is devoted to computational design of new inhibitors of histone deacetylase 4 (HDAC4), an enzyme responsible for epigenetic regulation of gene expression by means of nucleosomal histones and non-histone proteins deacetylation. Inhibition of class IIa histone deacetylases, that comprises HDAC4, was associated with treatment of various types of cancer. Nowadays, HDAC4 is being studied as a perspective pharmacological target for treatment of neurodegenerative diseases such as Alzheimer's or Huntington's disease. Molecular modeling plays significant role in drug discovery since it is able to decipher the structural characteristics of a drug responsible for its activity and also for its ADME properties. Therefore, molecular modeling and computational studies accelerate research and decrease costs of new drug development. Molecular mechanics (MM) and docking are fast and relatively precise methods traditionally used in structure-based drug design (SBDD). However, if charge or electron transfer, metal chelation, or strong polarization upon ligand binding occurs, as well as in projects requiring higher precision of results, it is wise to use quantum mechanics (QM) methods. Nevertheless, QM methods are computationally more expensive and their usage for macromolecular systems can become prohibitive. This is why hybrid QM/MM methods that combine QM description of the active center of the protein receptor with MM description of more distant parts of the macromolecular system, were applied.

We have devoted the first part of the dissertation to systematically and quantitatively assess the hybrid QM/MM DFT-B3LYP/6-31G*//OPLS-2005 method in terms of accuracy of computed intermolecular interaction energies. We have selected B3LYP functional and 6-31G* basis set thanks to its balanced tradeoff between accuracy and speed of calculation and its widespread usage for description of macromolecules (considered in the second part of the dissertation). We have performed the assessment on a series of small-molecule model systems of variable polarity and chemical properties, representing different non-bonding interactions. Molecules were arranged in clusters composed of the same type of small molecules. We have studied dependence of description of individual interactions and precision of QM/MM calculation on type and number of cluster components included into the QM region of the model system. Subsequently, we utilized the results of the QM/MM method and QSite program (Schrödinger, Inc., New York, NY, USA) tests in the second part of the dissertation, where we optimized and designed HDAC4 inhibitors from the class of diarylsubstituted cyclopropane hydroxamic acids (DCHA) taken from literature. Here, we suggested new and

potentially more potent HDAC4 inhibitors using three-tier approach. In the first phase, we created combinatorial library of DCHA analogs and screened them by docking into the HDAC4 active site. The scoring functions of Glide program (Schrödinger, Inc., New York, NY, USA) was used to identify new molecules with stronger binding affinity towards the HDAC4 target. For the most promising complexes obtained by docking, we used MM methods to predict the inhibitory activities of new DCHA analogs towards HDAC4. In the third phase, we calculated Gibbs free energy of interaction (ΔG_{bin}) between the molecule and HDAC4 enzyme using the QM/MM approach. This was performed for molecules predicted to bind strongest in the MM phase. For the resulting best DCHA analogs from the QM/MM phase, ADME properties were also predicted by means of QikProp program (Schrödinger, Inc., New York, NY, USA). In both MM and QM/MM phase of the inhibitor design, we created QSAR models to correlate calculated Gibbs free energies of interaction between DCHA inhibitor and HDAC4 receptor and experimental IC_{50}^{exp} values of known DCHAs to calibrate our computational approach.

KEYWORDS

histone deacetylases; computer-aided drug design; hybrid QM/MM approach; QSAR models; non-covalent drug – receptor interactions

Introduction

Histone deacetylases (HDACs) catalyze the removal of acetyl groups from acetylated ε-amino groups of lysine residues of both nucleosomal histones and non-histone proteins and participate in the epigenetic control of the gene expression. The ε-amino group of lysine is protonated and positively charged at the cellular pH. Its acetylation, which occurs at the N-terminal part of the histone proteins neutralizes this positive charge. In the global extent, the acetylation of numerous lysines in histone proteins neutralizes the net positive charge of these DNA-binding proteins. As a result, negatively charged DNA is bound to histone proteins more loosely and becomes thus more easily accessible to the cellular transcription machinery [1]. As part of complexes with other proteins, HDACs partake in the regulation of diverse cellular pathways including cell differentiation and growth arrest, DNA repair, and apoptosis [2–4]. Thanks to their involvement in diverse biochemical pathways, HDACs are implied in various pathologies, such as cancer, neurodegenerative disorders and inflammatory diseases. Thus, HDACs have emerged as promising pharmacological targets [5–7]. Eleven HDAC isoforms use Zn-dependent catalytic mechanism to deacetylate the lysines, and they comprise classes I, IIa, IIb, and IV [8,9].

Histone deacetylase class IIa comprises HDAC 4, 5, 7 and 9, isoforms that shuttle between nucleus and cytoplasm [10]. They share well-conserved N-terminus containing transcription factor binding sites connected with regulatory functions [11]. HDAC4 is an isoform containing 1084 residues. 3D structure of the whole protein is unknown, however, the crystal structure of its active site has been published in *Protein Data Bank* database [12]. The link between this HDAC isoform and several diseases has been observed. Initially, HDAC4 has been primarily linked with cancers such as leukemia or renal carcinoma [13–15]. In the course of time HDAC4 was proven to be linked to neurodegenerative diseases, such as Huntington's disease and Alzheimer's disease. Later on, evidence surfaced about HDAC4 being potential therapeutic targets for a number of other indications including muscular atrophy, metabolic disorders such as type II diabetes, acute ischemic injury and inflammation [14,16–20].

In this dissertation, we have focused on quest for HDAC4 inhibitors due to several reasons. First, this isoform seems to be promising drug target in development of therapeutics for diseases mentioned above. Second, HDAC4 together with its homological partners from the class IIa were not as much explored, therefore only a few inhibitors of this HDAC class are known. Third, crystal structure of HDAC4 is available and experimental inhibitory potencies of a homologic series of hydroxamic acid inhibitors were published [21,22]. For a more detailed treatise on HDACs and the role of different isoforms, we refer the reader to our published review article [4].

Most of HDAC inhibitors currently known bind non-selectively to all HDAC isoforms. Many inhibitors are partially selective, however, only to the whole HDAC class, not a single HDAC isoform. Molecular mechanisms of HDAC action are not yet well understood. It is possible that in each type of cancer (or disease), it is different HDAC isoform that is overexpressed and therefore desirable to be inhibited [23]. Consequently, regarding all these issues, the pursuit for isoform selective HDAC inhibitors is thus of great importance.

The majority of drug design studies involving large drug-receptor complexes are traditionally carried out by computationally efficient force field-based methods, such as molecular mechanics (MM) and dynamics (MD). Lately, quantum mechanics (QM) has been increasingly used for more rigorous description of the drug-receptor interactions in biological systems. The QM treatment of ligand-protein interactions is required when massive rearrangement of electron density, such as formation of covalent bonds, proton transfer, charge transfer, chelation of metals, cation - π interactions or strong polarization of ligand occur during the process of drug binding [24–31]. Such phenomena cannot be fully described by force fields that rely on predetermined atomic parameters, molecular topologies and fixed net atomic charges [24,25,32]. Full QM calculations including the entire macromolecular system composed of thousands of atoms are still computationally expensive. However, hybrid quantum mechanical/molecular mechanical (QM/MM) methods can be applied also to relatively large and complex biological systems [24,25,32–35]. Mixed QM/MM methods describe the drug and the binding site of a receptor by QM methods (QM region), consequently leading to improved accuracy of the calculated binding affinities. Protein domains more distant to the binding site as well as the surrounding solvent are included into QM/MM calculations by means of computationally less expensive MM methods (MM region) [24,25,32,33,36]. DFT calculations with the B3LYP functional [37] were used quite successfully in studies on macromolecular systems of pharmacological interest [24,25,30,34], even if the frequently used DFT-B3LYP combination is known to under-represent stacking and London dispersion interactions (similarly to the Hartree-Fock method) [24,38]. Even the HDAC deacetylation reaction mechanism itself has been studied using QM/MM methods employing B3LYP functional with 6-31G* basis set by several groups [39–42].

Various laboratories have incorporated the QM/MM approach into their drug discovery projects using different methodology and different QM region sizes. They applied these methods to study systems and design inhibitors for HIV-1 protease inhibitors, matrix-metalloproteinase inhibitors, Cytochrome P450 and others [30,42–50]. The importance of using QM charges in ligand docking has been raised in several works [32,51–53]. For more detailed treatise on QM/MM calculation in medicinal chemistry and drug discovery, we direct the reader to [32,33,54] or to recent review on QM docking and scoring algorithms [51].

3. AIMS OF THE DISSERTATION

This dissertation aims at computational design of new inhibitors of histone deacetylase 4 (HDAC4), an enzyme responsible for epigenetic regulation of gene expression associated with treatment of various types of cancer and neurodegenerative diseases such as Alzheimer's or Huntington's disease.

The primary goal of the dissertation was to optimize and design new more potent HDAC4 inhibitors based on diarylsubstituted cyclopropane hydroxamic acids (DCHA) by means of computer-aided drug design methods including combinatorial library design and rational structure-based drug design (SBDD) using hybrid quantum mechanical/molecular mechanical (QM/MM) method.

The secondary goal was to assess the performance and precision of hybrid QM/MM method implemented in QSite program of Schrödinger [55–57] at the popular DFT-B3LYP/6-31G*//OPLS-2005 level in terms of the QM region definition, in order to obtain the best description of nonbonding drug - receptor interactions when performing SBDD of DCHAs.

Results

Tests of the DFT B3LYP/6-31G*//OPLS-2005 method

Before applying the QM/MM method itself to ligand-protein systems of interest, we have carried out testing of the method and dependence of the results accuracy on the size and nature of the quantum region. Our study aimed at systematic and quantitative examination of the accuracy of description of non-covalent interactions by the advanced, however, approximate QM/MM approach compared to the full QM description of the system for the DFT B3LYP/6-31G*//OPLS-2005 method depending on the composition and size of the QM region, using the QSite and Jaguar software modules of Schrödinger [55–57]. To this end, we compared calculated non-covalent equilibrium ligand–protein model interaction energies and interaction energy curves for a series of molecular clusters with well-defined configurations stabilized by non-covalent interactions. Small-molecule model systems used for this purpose were composed of water, methane, methanol, formamide and benzene. These clusters composed of the molecules of the same type. Moreover, we had one model system consisting of acetic acid anion and methylamine cation to model ionic interactions.

In this part, we did not attempt to compute the precise interaction energies of the model systems by employing high level QM calculations, large basis sets or counterpoise correction. Instead, we intended to assess the performance of the QM/MM *vs.* QM approach at the same level of theory that is computationally accessible and applicable to macromolecular systems.

Comparison of the computed equilibrium interaction energies obtained by the full QM and hybrid QM/MM approaches showed us the details of QM/MM method performance at the selected level of theory. For the purposes of this dissertation and other drug design studies, we suggested some priorities for incorporation of receptor residues or function groups into the QM region of studies carried out at the cost-effective DFT-B3LYP/6-31G*//OPLS-2005 level leading to more realistic description of the ligand - protein interactions. One needs to be careful when selecting the protein residues to include into QM region. The size of the QM region is basically tradeoff between accuracy and speed. Generally, it holds true, that bigger QM region leads to better and more precise results. However, certain residues are better to be excluded from QM region in order for the calculation to produce best results. In the following we briefly summarize the results on each and every cluster tested:

Before we start talking about individual clusters, let us explain a bit on terminology and namely on a system of linearly arranged methane molecules. The middle molecule is called A, or layer A. This is subsequently surrounded by molecules of layer B and afterwards by layer C and D. Layer A of our methane model system resembles ligand in real protein-ligand

system. Layer B represents the residues immediately adjacent to ligand while layer C is the one further away from ligand immediately adjacent to layer B residues and so on.

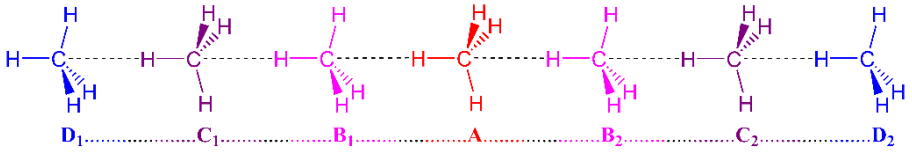


Figure 1. Model system composed of 7 linearly arranged methane molecules (middle molecule A plus three surrounding layers B, C and D)

In clusters of polar water molecules stabilized predominantly by hydrogen bonding and electrostatic interactions, the inclusion of the first coordination layer B into the QM region of DFT-B3LYP hybrid QM/M calculation increases the accuracy of the computed interaction energy of the ligand in the tetrahedral clusters with denser packing from about 54% to more than 93% of the full QM treatment. Thus, the extension of the QM region from ligand (A) to ligand + first coordination layer of polar molecules (A + B) may lead to nearly 40% higher accuracy of description of the ligand - protein-model interactions. Similar to the water clusters, the model systems containing polar formamides stabilized by HBs and electrostatic interactions, inclusion of the first coordination layer B into the QM region of DFT-B3LYP hybrid QM/MM calculation increased the accuracy of the computed QM/MM interaction energy of the ligand A from about 80% to 96% of the interaction energy obtained by full QM treatment. Therefore, in systems containing small polar molecules capable of HB formation, incorporation of polar first coordination layer into the QM region containing a polar ligand of the QM/MM DFT-B3LYP/6-31G*//OPLS-2005 calculation increases the precision of ligand - protein-model description, and is strongly recommended. However, addition of the second coordination layer C into the QM region adds only up to 5% to the interaction energy accuracy, but increases the CPU time considerably, therefore it is not advisable.

For the methylamine - acetate ion-pair, and other similar charged function groups and molecular ions, the difference of ~20% between the QM/MM and full QM calculated interaction energies is smaller than in waters. However, taking into account large absolute values of interaction energies (ca. -135 kcal·mol^{-1}), this type of interaction represents significant contribution to the precision of description of ligand - protein-model interactions. Therefore, placement of ligand as well as the first coordination layer residue, which forms an ion-pair with the ligand into the QM region of a QM/MM calculation, is highly recommended.

In clusters containing molecules of medium polarity, such as aliphatic alcohols, amines and others, the accuracy parameter improved by nearly 20% when the first coordination layer B was added to the QM region. Therefore, addition of semi-polar molecules and function groups

to the QM region containing semi-polar or polar ligands of the hybrid QM/MM calculation is encouraged.

As known, and reproduced by us in the dissertation, DFT-B3LYP/6-31G* fails in describing dispersion interactions in model systems composed of non-polar molecules. Thus, for small non-polar molecules such as aliphatic hydrocarbons and similar, inclusion of one or more coordination layers (B, C *etc.*) of a non-polar ligand into the QM region of DFT-B3LYP calculation leads to incorrect ligand - protein-model interaction energies, and is therefore discouraged. However, QM/MM calculation predicts the interaction between the ligand and adjacent protein residues correctly when it comes to non-polar interactions. Therefore, small non-polar molecules are not suitable for incorporation into the QM region of QM/MM DFT-B3LYP calculations and should be left for MM treatment for the sake of both accuracy and speed of the calculation. The very same conclusion holds true for π-π stacking interactions of the London dispersion nature as exemplified in our study by benzenes.

Having briefly summarized our results, we conclude that in studies involving mixed QM/MM DFT-B3LYP/6-31G*//OPLS-2005 methodology applied on protein-ligand systems, it is worthwhile to incorporate into the QM region of QM/MM calculation the first layer of residues directly interacting with ligand by proton transfer, charge transfer, metal ion chelation, hydrogen bonding, electrostatic interactions, as well as moderately polar interactions. This will lead to more realistic description of the ligand - protein non-bonding interactions, as indicated on similar systems studied by other laboratories [58–60]. On the other hand, it is advisable to leave out non-polar and aromatic molecules that bind with the ligand predominantly via van der Waals dispersion and π-π stacking interactions.

When considering the presented recommendations, it is wise to keep in mind that they were obtained for six model systems and 44 different clusters, and have their limitations for several reasons: (1) the number of the considered model systems and cluster topologies was low compared to the variability of ligand coordination in real systems; (2) all the calculations were performed *in vacuo*; (3) mixed clusters of polar and non-polar molecules were not considered; (4) the imposed constraints needed to conserve the model system symmetry and conformation prevented the conformational energy hypersurface crossing to configurations with lower total energy values, which in reality may occur frequently and thus modify the values of computed ΔE_{int}^{QM}, $\Delta E_{int}^{QM/MM}$ energies; and (5) the results and recommendations are valid only for the DFT-B3LYP/6-31G*//OPLS-2005 approach, although cautious extrapolations may be possible. In spite of these limitations, we believe that the presented recommendations will be useful in molecular or material design research by providing an estimate of the accuracy that can be expected from the QM/MM ligand binding studies.

Keeping these results in mind and using the same QM/MM level of theory in design of HDAC4 inhibitors, we proceeded to the second part of the dissertation in the next chapter.

Computational Design of Inhibitors of Histone Deacetylase 4

Motivation

Researchers headed by Celia Dominguez [21,22] have recently synthesized and tested trans-(2R,3R)-arylsubstituted-cyclopropane hydroxamic acids (DCHA) (Figure 2), which were found to be potent and selective inhibitors of the class IIa HDACs. Docking studies showed that the DCHAs may form effective HDAC inhibitors since their cyclopropane scaffold properly positioned the inhibitors with its aryl substituents into the cavity of the HDAC4 catalytic site and into the hydrophobic channel of the enzyme. At the same time, the hydroxamic group coordinates the catalytic Zn^{2+}, Fig. 2. [21]. Among the studied DCHAs several HDAC4 inhibitors displayed the half-maximal inhibitory concentrations (IC_{50}^{exp}) in the low nanomolar range as well as favorable pharmacokinetic profiles with distribution to the brain and muscles [61,62]. In this lead optimization study, we decided to perform *in-silico* screening and test more diverse modifications and analogs of the HDAC4 inhibitors than those experimentally tested by Dominguez *et al.* Moreover, we tried exploit lower specificity pocket (LSP) found at the bottom of the HDAC4 catalytic cavity by means of using different substituents filling this pocket. Our goal was to suggest new potential inhibitors selective to HDAC4 with higher binding affinities towards this biological target.

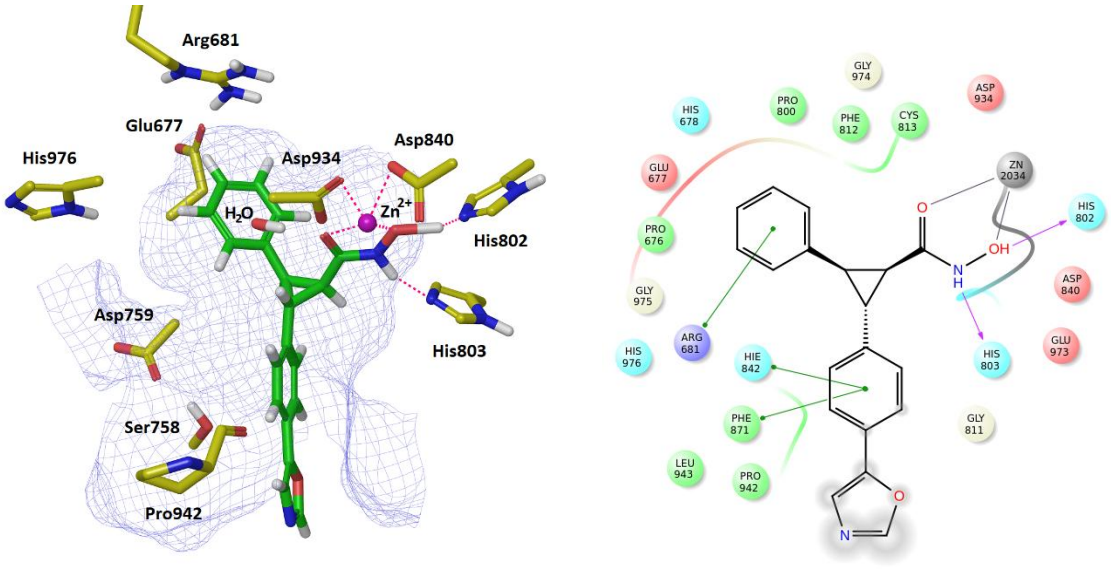


Figure 2. **A.** Crystal structure of active site of the HDAC4 with bound diarylcyclopropane hydroxamic acid inhibitor DCHA14 (PDB entry 4CBY) [21] with observed inhibitory potency IC_{50}^{exp} of 30 nM. [21] Carbon atoms of the inhibitor are colored green. The contour surface delineates the catalytic site of the HDAC4. **B.** Schematic depiction of the inhibitor interactions at the active site of HDAC4.

In our quest for new, more potent inhibitors, we have used combinatorial library design, computer-aided drug design, docking and *in silico* screening, MM and hybrid QM/MM approach to establish quantitative structure activity relationships (QSAR) and optimize the series of known DCHA inhibitors of HDAC4. We have decided to incorporate QM/MM approach to this drug design study since the process of HDACI binding to the catalytic zinc and polar amino acid residues of the deacetylase active site induces considerable rearrangement of electron density of the inhibitor.

Computational Details

First, virtual combinatorial library was generated and *in silico* screened with help of the CombiGlide module of the SMDD of Schrödinger [63]. The scaffold of trans-(2R,3R)-arylsubstituted-cyclopropane hydroxamic acids (DCHA) was taken from the crystal structure of inhibitor bound to the active site of HDAC4 [21]. The initial diversity library of DCHA analogs consisted of 12 180 compounds composed of 210 aromatic substituents (R_1-groups), 29 aromatic rings (R_2-groups) and 2 R_3-groups (---H and ---F). Each analog was built as a neutral molecule containing the cyclopropane hydroxamic acid scaffold and substituents. The virtual library of analogs was enumerated by attaching the R-groups (fragments) onto the DCHA scaffold, which was kept in its refined bound conformation.

Standard precision docking of the enumerated virtual library into the refined crystal structure of the active site of HDAC4 [21] was performed. *In silico* screening of the virtual library was performed using GlideScore [64,65], an empirical scoring function which comprises contributions from protein-ligand Coulombic and van der Waals interactions, hydrogen bonds, lipophilic-lipophilic term, rotatable bond penalty and a hydrophobic enclosure term. Ten best scoring ligand poses were retained. To reduce the size of the initial large diversity library, the best scoring DCHA analogs with predicted GlideScore binding affinities lower than -12.5 kcal·mol^{-1} were selected and analyzed in terms of the frequency of distinct R-group occurrence. Among the 701 analyzed compounds, 427 molecules contained fluorine atom in the R_3 position, 9 R_2 fragments occurred more than 25 times and 42 R_1 fragments occurred more than 5 times in the chemical structures of the selected analogs, Fig. 3. These DCHA fragments (frequently found in best scoring compounds) were then used for preparation of a focused virtual combinatorial subset enriched in potentially interesting inhibitor candidates.

Subsequently, QM-polarized ligand docking (QPLD) into the active site of the HDAC4 was carried out for a combinatorial subset of the focused virtual library consisting of $42 \times 9 = 378$ analogs of DCHA inhibitors to account for the polarization effects induced by the protein environment upon the bound ligand. The QPLD procedure started with the extra precision (XP) docking that generated 20 geometrically unique ligand poses at the binding site of the

protein receptor. Then single point QM/MM calculations were performed on the protein-ligand complex with ligand included in the quantum region surrounded by the electric field created from the protein. Partial atomic charges of the bound ligand were derived by electrostatic potential fitting. Glide then repeated the ligand docking using each of the calculated ligand charge sets and the QPLD algorithm returned 10 ligand poses with the highest interaction energies to the HDAC4 receptor [63].

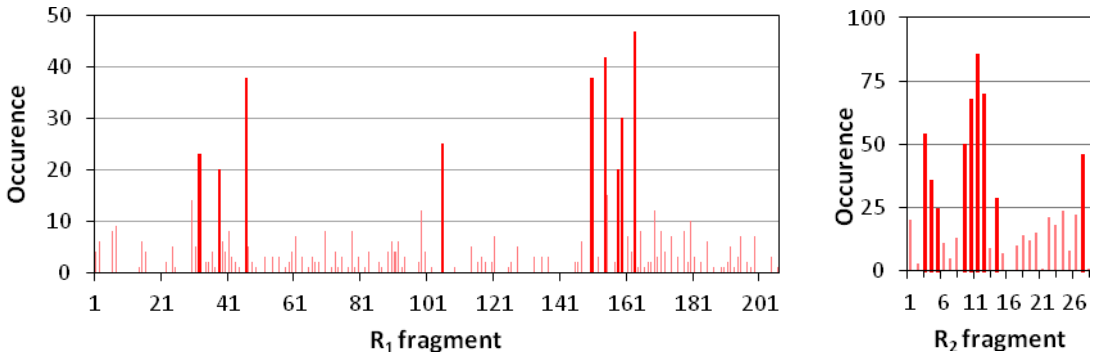


Figure 3. Graphs of the frequency of occurrence of individual R-groups (fragments) among the 701 best predicted DCHA analogs of the initial virtual library.

The QPLD algorithm returned 85 analogs with GlideScore of their best poses lower than -12.5 kcal·mol^{-1}. These 85 best analogs were then forwarded to more precise force field-based (OPLS-2005) molecular mechanics (MM) calculation of relative Gibbs free energies $\Delta\Delta G_{com}^{MM}$ of the HDAC4-DCHA complex formation (predicted binding affinities), which involved also consideration of the receptor flexibility.

The structures of the studied tri and tetrasubstituted DCHAs of the training set [21,22], and their complexes with human HDAC4, were prepared by *in situ* modifications of the bound native ligand DCHA14 in the crystal structure of the complex (Figure 2) [21]. For inhibitors containing rotatable bonds, restricted conformational sampling was carried out. Then the complexes were energy-minimized *in vacuo* using OPLS-2005 force field [66,67], with the distance cutoff extended to 20 Å and effective permittivity set to 2 to account for dielectric shielding in proteins using the MacroModel [68]. Minimization of the enzyme-inhibitor complexes and of the free enzyme was carried out by relaxing the structures gradually, starting with the residue side chains and concluding with the relaxation of all atoms including the protein backbone. The structures of HDAC4-DCHA complexes with the lowest total MM energies were chosen for the calculation of enzyme-inhibitor interaction Gibbs free energies. The electrostatic effect of hydration on the molecular structures optimized *in vacuo* was added by employing the Poisson-Boltzmann Solver of Impact of the Schrödinger SMDD suite [69].

Prior to the $\Delta\Delta G_{com}^{MM}$ calculation on the new DCHA analogs, we have examined whether the MM calculation procedure and 3D models of HDAC4-DCHA complexes lead to realistic predictions of ligand binding affinities. Hence, we have modeled the enzyme-inhibitor complexes and computed the $\Delta\Delta G_{com}^{MM}$ for a training set of known DCHA inhibitors studied by Dominguez *et al.* [21,22] with published inhibitory potencies IC_{50}^{exp} towards the HDAC4.

The computed Gibbs free energies $\Delta\Delta G_{com}^{MM}$ were correlated with the observed potencies IC_{50}^{exp} of the training set of DCHA inhibitors (representing experimental measure of the enzyme-inhibitor binding strength), in a linear regression model. The resulting quantitative structure-activity relationship (MM-QSAR) was able to explain 88 % of the variance in the inhibitory potencies of the training set. It documented significant correlation between the predicted binding affinities and observed inhibitory potencies towards the HDAC4 and validated the computational approach used.

Calculation of Gibbs free energies of the HDAC4-DCHA complex formation $\Delta\Delta G_{com}^{MM}$ for the 85 analogs identified by the QPLD and prediction of their inhibitory potencies (IC_{50}^{pre}) from the QSAR model (Fig. 4.19): $IC_{50}^{pre} = 10^{[0.1192 \cdot \Delta\Delta G_{com}^{MM} + 1.0235]}$ led to identification of 21 new potent DCHA analogs with $IC_{50}^{pre} \leq 35$ nM. For these 21 analogs we have used the advanced and computationally most expensive QM/MM calculation of the relative Gibbs free energies $\Delta\Delta G_{com}^{QM/MM}$ of HDAC4-DCHA complex formation.

The hybrid QM/MM approach applied to the HDAC4-DCHA complexes have used the density functional theory (DFT) [70,71] with hybrid exchange-correlation energy functional B3LYP [72–74] and split-valence 6-31G* basis set with polarization functions on all heavy atoms [75]. Based on results from model systems and QM/MM systems tests on non-covalent interactions description using DFT-B3LYP/6-31G*//OPLS-2005 QM/MM approach [36], ten HDAC4 active site residues (side chains: Glu677, Arg681, Ser758, Asp759, His802, His803, Asp840, Asp934, His976 and complete residue: Pro942), which displayed the strongest attractive or repulsive interactions with the ligand, the Zn^{2+} ion and the structural water, were included into the quantum region of the HDAC4-DCHA complexes. The quantum region contained approximately 160 atoms (binding site + inhibitor) and its total charge was equal to -1 è. The QM and MM regions interact in QSite via Coulombic interactions between the MM charges and the QM wave function (external electrostatic potential entering the system Hamiltonian). In addition, van der Waals interactions were considered between QM and MM atoms (all atoms employ the OPLS-2005 van der Waals parameters) [55,56,76,77]. The HDAC4-DCHA complexes, receptor and free inhibitors underwent QM/MM minimization *in vacuo* using the QSite and Jaguar modules of the Schrödinger SMDD suite [56,77]. Single point

calculation was performed on the minimized structures employing the Poisson-Boltzmann Solver of Jaguar to account for the effects of hydration [56,69,77,78].

The QM/MM total energy landscapes of complex biochemical systems typically display a rugged topography. Therefore, multiple starting configurations of the enzyme-inhibitor complexes have to be considered to obtain reasonable interaction free energies. Out of several optimized configurations of the enzyme-inhibitor complex with low mutual RMSD value, the structure with the lowest total energy was selected and processed further. Even small changes in the configuration of the minimized complex may lead to considerable changes in the computed total energy because of the strong electrostatic interactions involving the Zn^{2+}, hydroxamic acid functional group, charged residues included in the quantum region (Arg681, Asp840 and Asp934) and charged residues in the classical region located within 4 Å distance from the bound inhibitor (Glu677 and Glu973).

The QM/MM approach was also validated for the precision of calculated binding affinities $\Delta\Delta G_{com}^{QM/MM}$ of the new analogs on the training set of DCHA inhibitors studied by Dominguez *et al.* [21,22,61]. The computed quantities were correlated with the observed potencies IC_{50}^{exp} of the training set of DCHA inhibitors. The resulting quantitative structure-activity relationship (QM/MM-QSAR) was able to explain 91 % of the variance in the inhibitory potencies of the training set, documented superior correlation between the predicted and observed binding affinities and validated the QM/MM calculation procedure, Fig. 4. This QSAR model also allows to predict inhibitory potencies of new analogs which bind to the HDAC4 in the same mode as the training set of DCHA inhibitors by the QM/MM approach used here.

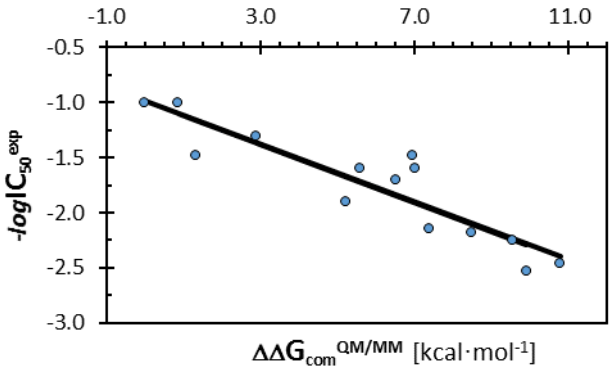
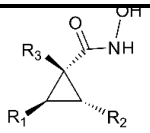
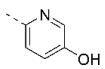
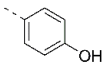
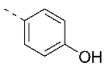
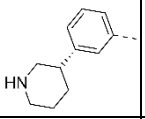
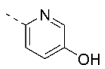
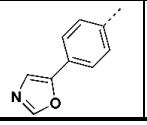
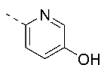


Figure 4. Plot of linear regression of the QM/MM-QSAR model of HDAC4 inhibition by DCHAs: $pIC_{50}^{exp} = -log_{10}IC_{50}^{exp} = -0.1310 \cdot \Delta\Delta G_{com}^{QM/MM} - 0.9875$ (number of compounds n = 14, squared regression coefficient $r^2 = 0.83$, leave-one-out cross-validated squared regression coefficient $r_{xv}^2 = 0.77$, standard error of regression $\sigma = 0.22$, Fischer F-test F = 55.24, level of statistical significance $\alpha > 95\%$)

Prediction of inhibitory potencies of DCHA analogs

The most promising 21 new DCHA analogs identified by the MM-QSAR model advanced to the QM/MM-QSAR model for a more precise inhibitory potencies IC_{50}^{pre} prediction. Our computer-aided drug design approach yielded 6 new likely inhibitor candidates with promising predicted activities. For some of them (dDCHA1, dDCHA4 and dDCHA5) the calculated IC_{50}^{pre} were lower than those of the most potent HDAC4 inhibitor studied by Dominguez *et al.* [21,22] (Table 1).

Table 1. Designed analogs of diarylcyclopropane hydroxamic acid inhibitors DCHA, predicted relative Gibbs free energies of enzyme-inhibitor complex formation $\Delta\Delta G_{com}^{QM/MM}$ and their components calculated by hybrid QM/MM method (DFT-B3LYP/6-31G*//OPLS-2005), and predicted inhibitory potencies IC_{50}^{pre} estimated from the QM/MM-QSAR model, Fig. 4.

Designed analog				**Relative Gibbs free energy of complex formation** [a]			**Predicted inhibitory potency** [b]
Inhibitor	**R-groups**			$\Delta\Delta G_{int}^{QM/MM}$ [kcal·mol^{-1}]	$\Delta\Delta G_{sol}^{QM/MM}$ [kcal·mol^{-1}]	$\Delta\Delta G_{com}^{QM/MM}$ [kcal·mol^{-1}]	IC_{50}^{pre} [nM]
	R_1	**R_2**	**R_3**				
dDCHA1			---F	-2.4	-1.2	-3.6	3
dDCHA2			---F	-0.1	2.0	1.9	17
dDCHA3			---F	0.6	3.5	4.1	33
dDCHA4			---F	-2.7	3.3	0.6	12
dDCHA5			---F	2.0	-5.9	-3.9	3

[a] calculated relative values with respect to reference training set inhibitor DCHA11, $\Delta\Delta G_{com}^{QM/MM} = \Delta\Delta G_{int}^{QM/MM} + \Delta\Delta G_{sol}^{QM/MM}$ (all energy values were rounded off to one decimal),

[b] predicted inhibitory activities towards HDAC4 were calculated from the QM/MM-QSAR model (Fig. 8) as: $IC_{50}^{pre} = 10^{[0.1310 \cdot \Delta\Delta G_{com}^{QM/MM} + 0.9875]}$

The best designed DCHA analogs contain indazole, phenylpiperidine and phenyloxazole in the R_1 position and hydroxypyridine moiety in the R_2 position. In the dDCHA1, the nitrogen of the R_2 hydroxypyridine stabilizes the catalytic water molecule at the active site by a HB and the hydroxyl forms another HB with the backbone carbonyl group of Gly975. In addition,

the heterocyclic –NH– group of indazole in the R_1 position forms a HB with the carbonyl group of Pro942 (Fig. 1). In dDCHA4, the –NH– group of the phenylpiperidine forms a HB with the side chain of Asp757. These specific attractive interactions (negative values of the $\Delta\Delta G_{com}^{QM/MM}$ contributions, Table 1) are expected to enhance not only the binding affinity but also the isoform selectivity of the proposed analogs. The attractive interactions of the hydroxypyridine, indazole and phenylpiperidine moieties are reinforced by the solvent effect (negative values of the $\Delta\Delta G_{solv}^{QM/MM}$ contribution, Table 1). In the analog dDCHA5 that contains R_1 phenyloxazole, which remains exposed to solvent in the HDCA4-dDCHA5 complex, the contribution of the solvent effect to the inhibitor binding is dominant. In this case however, the driving force of the complex formation, originating primarily from the solvent effect, is less specific.

The computed relative enzyme-inhibitor binding affinities $\Delta\Delta G_{com}^{QM/MM}$ of the designed analogs and predicted inhibitory potencies IC_{50}^{pre} calculated from the regression equation of the superior QM/MM-QSAR model (Figure 4) suggest that the analogs dDCHA1 and dDCHA5 with the IC_{50}^{pre} = 3 nM may form HDAC4 inhibitors up to 3-times more potent than the most active compounds of the training set of DCHA inhibitors (IC_{50}^{exp} = 10 ± 1 nM, Table 4.9) [21,22,61]. The results indicate that aromatic rings bearing heteroatoms and/or polar function groups, which fill the lower specificity pocket of the binding site of the HDAC4, may increase the potencies of DCHA inhibitors towards this enzyme.

ADME properties prediction of DCHA analogs

In the course of drug development many drug candidates fail due to unsatisfactory pharmacokinetic properties. Therefore, it is crucial to incorporate consideration of ADME properties into the compound selection process. Thus, we have computed 36 physicochemical molecular properties (ADME-related descriptors) by the QikProp SMDD module of Schrödinger [79]. The drug likeness parameter (#stars - number of descriptors that do not fall into the optimum intervals of descriptor values satisfied by 95% of known drugs) was used to assess the fitness of the predicted pharmacokinetic profile of the designed DCHA inhibitors. All of the selected DCHA analogs were forecast to comply with the pharmacokinetic profiles of drug-like compounds, equally to the reference inhibitor DCHA14. Therefore, we believe that the most potent analogs dDCHA1 and dDCHA5 display the potential to be further developed into orally bioavailable drug candidates.

CONCLUSIONS

This dissertation consists of two parts. Though separate and different from each other, the second one follows upon the first one. At the outset of my doctoral studies we aimed at applying advanced hybrid quantum mechanical / molecular mechanical (QM/MM) methods in computational drug design. QM/MM methods are advanced and can produce description of intermolecular interactions superior to traditional approaches based on force fields and molecular mechanics only. However, using these methods presents significant computational burden in terms of both CPU and run time. Therefore, deliberate user needs to select proper tradeoff between computational expenses and precision of the calculation and, as always, verify that the selected QM/MM method represents the system of interest adequately.

Thus, we have devoted the first part of the dissertation to systematically and quantitatively assess the hybrid QM/MM DFT-B3LYP/6-31G*//OPLS-2005 method in terms of accuracy of computed intermolecular interaction energies. We selected B3LYP/6-31G* method thanks to its balanced tradeoff between accuracy and speed. We have performed the assessment on a series of small-molecule model systems of variable polarity and chemical properties. Molecules were arranged in clusters composed of the same type of small molecules. Based on the results from first part we proposed guidelines for the definition of the QM region to be used in studies involving complex structures such as proteins or eventually also condensed matter. These recommendations should lead to improved accuracy of the computed ligand–receptor interaction energies $\Delta E_{int}^{QM/MM}$ using the DFT method. Our results suggest that it is desirable to include into the QM region of a QM/MM calculation molecules (residues) of the first layer directly surrounding the ligand that interact with the ligand by electrostatic, hydrogen bonding, charge/proton transfer, and ion chelation as well as by moderately polar interactions. Conversely, it is advisable to leave out of the quantum region of hybrid QM/MM DFT-B3LYP/6-31G*//OPLS-2005 calculation all non-polar and aromatic molecules/residues that bind to the ligand predominantly via van der Waals dispersion and π−π stacking non-covalent interactions. Excluding these residues from the QM region and leaving the force field to describe the interactions at the QM–MM interface will contribute to both precision and speed of the calculations. Furthermore, it is recommended to select the QM region as large as available computer resources allow, since the accuracy of the computed $\Delta E_{int}^{QM/MM}$ increases further with the growing size of the QM region.

These results from the first part were subsequently utilized in the second part concerning optimization of lead compounds to inhibit histone deacetylase 4 (HDAC4) [21,22]. HDAC4 is an enzyme involved in epigenetic regulation, cancer and neurodegenerative diseases. Its

inhibition has been found useful in the latter. Our aim was to show that consideration of a wider range of heteroaromatic substituents in the R_1 and R_2 positions of the molecular scaffold of the DCHA, may enhance the inhibitory potencies of these new analogs. Especially the role of the R_2 group that samples the lower specificity pocket of the HDAC4, which was not explored in the papers of Dominguez *et al.* [21,22], was elaborated. We have used four-tier approach. In the first step we performed regular docking of virtual combinatorial diversity library into HDAC4 receptor, downloaded under the entry 4CBY and prepared in Schrödinger *Small-Molecule Drug Discovery* suite. The second step involved docking of smaller focused subset of the combinatorial library into the same receptor, however, using quantum polarized ligand docking. In the third step we performed molecular mechanical calculations on the best candidates from the previous step to predict their binding affinities to HDAC4 using MM methods. Last but not least, best hits from the third step were further processed using QM/MM methods. In the end, we predicted ADME properties for the best scoring candidates.
For the molecular mechanical and hybrid QM/MM step, we have created quantitative structure-activity relationship between experimentally measured inhibitory activities of known compounds synthesized by Dominguez *et al.* [21,22] and our computationally predicted binding energy. Using these models, we predicted binding affinity of the suggested compounds. We believe that utilization of more precise description of the enzyme-inhibitor interactions by the first principle QM/MM approach enhances the quality of the computational prediction of inhibitor binding affinities and results in more accurate prediction of the inhibitory potencies of the designed DCHA analogs. We have designed several more potent analogs of diarylcyclopropane hydroxamic acids, such as dDCHA1 and dDCHA5 with predicted HDAC4 inhibitory potencies in the low nanomolar concentration range (IC_{50}^{pre} = 3 nM), *i.e.* 3-times more potent than the published DCHAs. The designed analogs displayed favorable ADME profiles and are recommended for synthesis and experimental verification of the inhibitory potencies towards the HDAC4 isoform in medicinal chemistry laboratories.

References

[1] Kim H-J, Bae S-C. Histone Deacetylase Inhibitors: Molecular Mechanisms of Action and Clinical Trials as Anti-cancer Drugs. *Am J Transl Res* 2011; 3: 166–79.

[2] Marks P, Rifkind R, Richon VM, Breslow R, Miller T, Kelly WK. Histone Deacetylases and Cancer: Causes and Therapies. *Nat Rev Cancer* 2001; 1: 194–202.

[3] Marks P. Histone Deacetylase Inhibitors: a Chemical Genetics Approach to Understanding Cellular Functions. *Biochim Biophys Acta* 2010; 1799: 717–25.

[4] Kollar J, Frecer V. Selective Inhibitors of Zinc-Dependent Histone Deacetylases. Therapeutic Targets Relevant to Cancer. *Curr Pharm Des* 2015; 21: 1472–502.

[5] Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in Human Disease and Prospects for Epigenetic Therapy. *Nature* 2004; 429: 457–63.

[6] Chakrabarti A, Melesina J, Kolbinger FR, *et al*. Targeting Histone Deacetylase 8 as a Therapeutic Approach to Cancer and Neurodegenerative Diseases. *Future Med Chem* 2016; 8: 1609–34.

[7] Hull EE, Montgomery MR, Leyva KJ. HDAC Inhibitors as Epigenetic Regulators of the Immune System: Impacts on Cancer Therapy and Inflammatory Diseases. *Biomed Res Int* 2016: 1–15.

[8] Dokmanovic M, Clarke C, Marks PA. Histone Deacetylase Inhibitors: Overview and Perspectives. *Mol Cancer Res* 2007; 5: 981–9.

[9] de Ruijter AJM, van Gennip AH, Caron HN, Kemp S, van Kuilenburg ABP. Histone Deacetylases (HDACs): Characterization of the Classical HDAC Family. *Biochem J* 2003; 370: 737–49.

[10] Gryder BE, Sodji QH, Oyelere AK. Targeted Cancer Therapy: Giving Histone Deacetylase Inhibitors all they Need to Succeed. *Future Med Chem* 2012; 4: 505–24.

[11] Verdin E, Dequiedt F, Kasler HG. Class II Histone Deacetylases: Versatile Regulators. *Trends Genet* 2003; 19: 286–93.

[12] Berman HM, Westbrook J, Feng Z, *et al*. The Protein Data Bank. *Nucleic Acids Res* 2000; 28: 235–42.

[13] Chauchereau A, Mathieu M, de Saintignon J, et al. HDAC4 Mediates Transcriptional Repression by the Acute Promyelocytic Leukaemia-associated Protein PLZF. *Oncogene* 2004; 23: 8777–84.

[14] Qian DZ, Kachhap SK, Collis SJ, *et al.* Class II Histone Deacetylases are Associated with VHL-independent Regulation of Hypoxia-inducible Factor 1 alpha. *Cancer Res* 2006; 66: 8814–21.

[15] Ozdağ H, Teschendorff AE, Ahmed AA, *et al.* Differential Expression of Selected Histone Modifier Genes in Human Solid Cancers. *BMC Genomics* 2006; 7: 90.

[16] Clocchiatti A, Florean C, Brancolini C. Class IIa HDACs: from Important Roles in Differentiation to Possible Implications in Tumourigenesis. *J Cell Mol Med* 2011; 15: 1833–46.

[17] Bertos NR, Wang AH, Yang XJ. Class II Histone Deacetylases: Structure, Function, and Regulation. *Biochem Cell Biol* 2001; 79: 243–52.

[18] Mihaylova MM, Vasquez DS, Ravnskjaer K, et al. Class IIa Histone Deacetylases are Hormone-activated Regulators of FOXO and Mammalian Glucose Homeostasis. *Cell* 2011; 145: 607–21.

[19] Granger A, Abdullah I, Huebner F, *et al.* Histone Deacetylase Inhibition Reduces Myocardial Ischemia-reperfusion Injury in Mice. *FASEB J* 2008; 22: 3549–60.

[20] Hancock WW, Akimova T, Beier UH, Liu Y, Wang L. HDAC Inhibitor Therapy in Autoimmunity and Transplantation. *Ann Rheum Dis* 2012; 71: i46–54.

[21] Bürli RW, Luckhurst CA, Aziz O, *et al.* Design, Synthesis, and Biological Evaluation of Potent and Selective Class IIa Histone Deacetylase (HDAC) Inhibitors as a Potential Therapy for Huntington's Disease. *J Med Chem* 2013; 56: 9934–54.

[22] Luckhurst CA, Breccia P, Stott AJ, *et al.* Potent, Selective, and CNS-Penetrant Tetrasubstituted Cyclopropane Class IIa Histone Deacetylase (HDAC) Inhibitors. *ACS*

Med Chem Lett 2016; 7: 34–9.
[23] Khan N, Jeffers M, Kumar S, *et al.* Determination of the Class and Isoform Selectivity of Small-molecule Histone Deacetylase Inhibitors. *Biochem J* 2008; 409: 581–9.
[24] Cavalli A, Carloni P, Recanatini M. Target-related Applications of First Principles Quantum Chemical Methods in Drug Design. *Chem Rev* 2006; 106: 3497–519.
[25] Sgrignani J, Magistrato A. First-Principles Modeling of Biological Systems and Structure-based Drug-design. *Curr Comput - Aided Drug Des* 2013; 9: 15–34.
[26] Ma JC, Dougherty DA. The Cation−π Interaction. *Chem Rev* 1997; 97: 1303–24.
[27] Dal Peraro M, Raugei S, Carloni P, Klein ML. Solute-Solvent Charge Transfer in Aqueous Solution. *ChemPhysChem* 2005; 6: 1715–8.
[28] Raha K, Merz KM. Large-scale Validation of a Quantum Mechanics Based Scoring Function: Predicting the Binding Affinity and the Binding Mode of a Diverse Set of Protein−ligand Complexes. *J Med Chem* 2005; 48: 4558–75.
[29] Komeiji Y, Ishida T, Fedorov DG, Kitaura K. Change in a Protein's Electronic Structure Induced by an Explicit Solvent: An ab initio Fragment Molecular Orbital Study of Ubiquitin. *J Comput Chem* 2007; 28: 1750–62.
[30] Ryde U, Söderhjelm P. Ligand-binding Affinity Estimates Supported by Quantum-mechanical Methods. *Chem Rev* 2016; 116: 5520–66.
[31] Sapse A-M, Schweitzer BS, Dicker AP, Bertino JR, Frecer V. Ab initio Studies of Aromatic-aromatic and Aromatic-Polar Interactions in the Binding of Substrate and Inhibitor to Dihydrofolate Reductase. *Int J Pept Protein Res* 1992; 39: 18–23.
[32] Barbault F, Maurel F. Simulation with Quantum Mechanics/Molecular Mechanics for Drug Discovery. *Expert Opin Drug Discov* 2015; 10: 1047–57.
[33] Menikarachchi L, Gascon J. QM/MM Approaches in Medicinal Chemistry Research. *Curr Top Med Chem* 2010; 10: 46–54.
[34] Friesner RA, Guallar V. Ab initio Quantum Chemical and Mixed Quantum Mechanics/Molecular Mechanics (QM/MM) Methods for Studying Enzymatic Catalysis. *Ann Rev Phys Chem* 2005; 56: 389–427.
[35] Senn HM, Thiel W. QM/MM Methods for Biomolecular Systems. *Angew Chem Int Ed Engl* 2009; 48: 1198–229.
[36] Kollar J, Frecer V. How Accurate is the Description of Ligand–protein Interactions by a Hybrid QM/MM Approach? *J Mol Model* 2018; 24: Article Number 11.
[37] Becke AD. A New Mixing of Hartree–Fock and Local Density- Functional Theories. *J Chem Phys* 1993; 98: 1372–7.
[38] Grimme S, Antony J, Ehrlich S, Krieg H. A Consistent and Accurate ab initio Parametrization of Density Functional Dispersion Correction (DFT-D) for the 94 Elements H-Pu. *J Chem Phys* 2010; 132: 154104.
[39] Wu R, Hu P, Wang S, Cao Z, Zhang Y. Flexibility of Catalytic Zinc Coordination in Thermolysin and HDAC8: A Born−Oppenheimer ab Initio QM/MM Molecular Dynamics Study. *J Chem Theory Comput* 2010; 6: 337–43.
[40] Wu R, Wang S, Zhou N, Cao Z, Zhang Y. A Proton-Shuttle Reaction Mechanism for Histone Deacetylase 8 and the Catalytic Role of Metal Ions. *J Am Chem Soc* 2010; 132: 9471–9.
[41] Chen K, Zhang X, Wu Y-D, Wiest O. Inhibition and Mechanism of HDAC8 Revisited. *J Am Chem Soc* 2014; 136: 11636–43.
[42] Gleeson D, Gleeson MP. Application of QM/MM and QM Methods to Investigate Histone Deacetylase 8. *Med Chem Commun* 2015; 6: 477–85.
[43] Hensen C, Hermann JC, Nam K, Ma S, Gao J, Höltje H-D. A Combined QM/MM Approach to Protein−ligand Interactions: Polarization Effects of the HIV-1 Protease on Selected High Affinity Inhibitors. *J Med Chem* 2004; 47: 6673–80.
[44] Gräter F, Schwarzl SM, Dejaegere A, Fischer S, Smith JC. Protein/Ligand Binding Free Energies Calculated with Quantum Mechanics/Molecular Mechanics. *J Phys Chem B* 2005; 109: 10474–83.
[45] Khandelwal A, Lukacova V, Comez D, Kroll DM, Raha S, Balaz S. A Combination of Docking, QM/MM Methods, and MD Simulation for Binding Affinity Estimation of

Metalloprotein Ligands. *J Med Chem* 2005; 48: 5437–47.
[46] Gleeson MP, Gleeson D. QM/MM Calculations in Drug Discovery: A Useful Method for Studying Binding Phenomena? *J Chem Inf Model* 2009; 49: 670–7.
[47] Rathore RS, Sumakanth M, Reddy MS, *et al.* Advances in Binding Free Energies Calculations: QM/MM-Based Free Energy Perturbation Method for Drug Design. *Curr Pharm Des* 2013; 19: 4674–86.
[48] Moraca F, Rinaldo D, Smith AB, Abrams CF. Specific Noncovalent Interactions Determine Optimal Structure of a Buried Ligand Moiety: QM/MM and Pure QM Modeling of Complexes of the Small-Molecule CD4 Mimetics and HIV-1 gp120. *ChemMedChem* 2018; 13: 627–33.
[49] Shaik S, Cohen S, Wang Y, Chen H, Kumar D, Thiel W. P450 Enzymes: Their Structure, Reactivity, and Selectivity—Modeled by QM/MM Calculations. *Chem Rev* 2010; 110: 949–1017.
[50] Denisov IG, Makris TM, Sligar SG, Schlichting I. Structure and Chemistry of Cytochrome P450. *Chem Rev* 2005; 105: 2253–78.
[51] Crespo A, Rodriguez-Granillo A, Lim VT. Quantum-Mechanics Methodologies in Drug Discovery: Applications of Docking and Scoring in Lead Optimization. *Curr Top Med Chem* 2017; 17: 2663–80.
[52] Cho AE, Guallar V, Berne BJ, Friesner R. Importance of Accurate Charges in Molecular Docking: Quantum Mechanical/Molecular Mechanical (QM/MM) Approach. *J Comput Chem* 2005; 26: 915–31.
[53] Zhong H, Kirschner KN, Lee M, Bowen JP. Binding Free Energy Calculation for Duocarmycin/DNA Complex Based on the QPLD-Derived Partial Charge Model. *Bioorg Med Chem Lett* 2008; 18: 542–5.
[54] Lang EJM, Mulholland AJ. Molecular Dynamics, Quantum Mechanics, and Combined Quantum Mechanics/Molecular Mechanics Methods for Drug Discovery and Development. In: Chackalamannil S, Rotella D, Ward S, editors. Compr Med Chem III, Bristol, United Kingdom: Elsevier; 2017, p. 51–66.
[55] Philipp DM, Friesner RA. Mixed ab initio QM/MM Modeling Using Frozen Orbitals and Tests with Alanine Dipeptide and Tetrapeptide. *J Comput Chem* 1999; 20: 1468–94.
[56] QSite, version 6.3, Small-Molecule Drug Discovery Suite 14-2, Schrödinger, LLC, New York, NY, 2014.
[57] Murphy RB, Philipp DM, Friesner RA. A Mixed Quantum Mechanics/Molecular Mechanics (QM/MM) Method for Large-Scale Modeling of Chemistry in Protein Environments. *J Comput Chem* 2000; 21: 1442–57.
[58] Ryde U. QM / MM Calculations on Proteins. *Methods Enzymol* 2016; 577: 119–58.
[59] Hu L, Söderhjelm P, Ryde U. On the Convergence of QM/MM Energies. *J Chem Theory Comput* 2011; 7: 761–77.
[60] Hu L, Eliasson J, Heimdal J, Ryde U. Do Quantum Mechanical Energies Calculated for Small Models of Protein-Active Sites Converge. *J Phys Chem A* 2009; 113: 11793–800.
[61] Beconi M, Aziz O, Matthews K, *et al.* Oral Administration of the Pimelic Diphenylamide HDAC Inhibitor HDACi 4b is Unsuitable for Chronic Inhibition of HDAC Activity in the CNS in vivo. *PLoS One* 2012; 7: e44498.
[62] Bradner J. Fluorinated HDAC Inhibitors and Uses Thereof. WO2011084991, 2011.
[63] CombiGlide, version 3.3, Small-Molecule Drug Discovery Suite 14-2, Schrödinger, LLC, New York, NY, 2014.
[64] Halgren TA, Murphy RB, Friesner RA, *et al.* Glide: A New Approach for Rapid, Accurate Docking and Scoring. 2. Enrichment Factors in Database Screening. *J Med Chem* 2004; 47: 1750–9.
[65] Friesner RA, Banks JL, Murphy RB, *et al.* Glide: A New Approach for Rapid, Accurate Docking and Scoring. 1. Method and Assessment of Docking Accuracy. *J Med Chem* 2004; 47: 1739–49.
[66] Banks JL, Beard HS, Cao Y, *et al.* Integrated Modeling Program, Applied Chemical Theory (IMPACT). *J Comput Chem* 2005; 26: 1752–80.

[67] Jorgensen WL, Maxwell DS, Tirado-Rives J. Development and Testing of the OPLS All-Atom Force Field on Conformational Energetics and Properties of Organic Liquids. *J Am Chem Soc* 1996; 118: 11225–36.
[68] MacroModel, version 10.4, Small-Molecule Drug Discovery Suite 14-2, Schrödinger, LLC, New York, NY, 2014.
[69] Impact, version 6.2, Small-Molecule Drug Discovery Suite 14-2, Schrödinger, LLC, New York, NY 2014.
[70] Kohn W, Sham LJ. Self-Consistent Equations Including Exchange and Correlation Effects. *Phys Rev* 1965; 140: A1133–8.
[71] Hohenberg P, Kohn W. Inhomogeneous Electron Gas. *Phys Rev* 1964; 136: B864–71.
[72] Becke AD. Density-Functional Exchange-Energy Approximation with Correct Asymptotic Behavior. *Phys Rev A* 1988; 38: 3098–100.
[73] Becke AD. Density-Functional Thermochemistry. III. The Role of Exact Exchange. *J Chem Phys* 1993; 98: 5648–52.
[74] Stephens PJ, Devlin FJ, Chabalowski CF, Frisch MJ. Ab initio Calculation of Vibrational Absorption and Circular Dichroism Spectra Using Density Functional Force Fields. *J Phys Chem* 1994; 98: 11623–7.
[75] Ditchfield R, Hehre WJ, Pople JA. Self-Consistent Molecular- Orbital Methods. IX. An Extended Gaussian- Type Basis for Molecular-Orbital Studies of Organic Molecules. *J Chem Phys* 1971; 54: 724–8.
[76] Murphy RB, Philipp DM, Friesner R. Frozen Orbital QM/MM Methods for Density Functional Theory. *Chem Phys Lett* 2000; 321: 113–20.
[77] Bochevarov AD, Harder E, Hughes TF, *et al.* Jaguar: A High-Performance Quantum Chemistry Software Program with Strengths in Life and Materials Sciences. *Int J Quantum Chem* 2013; 113: 2110–42.
[78] Baker NA, Sept D, Joseph S, Holst MJ, McCammon JA. Electrostatics of Nanosystems: Application to Microtubules and the Ribosome. *Proc Natl Acad Sci* 2001; 98: 10037–41.
[79] QikProp, version 3.9, Small-Molecule Drug Discovery Suite 14-2, Schrödinger, LLC, New York, NY, 2014.

Publications of the author related to the dissertation

ADC Vedecké práce v zahraničných karentovaných časopisoch

ADC02 Kollár, Jakub [UKOMFKJFBd] (80%) - Frecer, Vladimír [KAUT] [UKOFAFYZ] (20%): Selective inhibitors of zinc-dependent histone deacetylases. Therapeutic targets relevant to cancer
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[o1] 2015 Wang, J. - Schwartz, R.J.: Post-translational modification. In: Congenital Heart Diseases: The Broken Heart: Clinical Features, Human Genetics and Molecular Pathways. Wien : Springer-Verlag, 2015, S. 202 - SCOPUS
[o1] 2017 Chistiakov, D.A. - Myasoedova, V.A. - Orekhov, A.N. - Bobryshev, Y.V.: Current Pharmaceutical Design, Vol. 23, No. 8, 2017, s. 1174 - SCOPUS
[o1] 2017 Zagni, C. - Floresta, G. - Monciino, G. - Rescifina, A.: Medicinal Research Reviews, Vol. 37, No. 6, 2017, s. 1428 - SCOPUS

ADC04 Kollár, Jakub [UKOMFKJFBd] (60%) - Frecer, Vladimír [KAUT] [UKOFAFYZ] (40%): How accurate is the description of ligand-protein interactions by a hybrid QM/MM approach?
Lit. 79 zázn.
In: Journal of Molecular Modeling. - Vol. 24, No. 1 (2018), s. 11-11 [20 s.]. - ISSN 1610-2940
Registrované v: wos
Indikátor časopisu:
IF (JCR) [2016-1,425]

ADC05 Kollár Jakub, Frecer Vladimír: Diarylcyclopropane Hydroxamic Acid Inhibitors of Histone Deacetylase 4 Designed by Combinatorial Approach and QM/MM Calculations.
J Mol Graph Model; submitted May 2018

Presentation of the results related to the dissertation at the conferences

AFG Abstrakty príspevkov zo zahraničných vedeckých konferencií

AFG02 Kollár, Jakub [UKOMFKJFBd] (40%) - Polonec, P. (10%) - Tučeková, Zuzana (10%) - Miertuš, Stanislav (10%) - Frecer, Vladimír [UKOFAFYZ] (30%): Computational Design of Histone Deacetylase Inhibitors as Antitumor Agents
Lit. 3 zázn.
In: Regional Biophysics Conference 2016. Book of abstracts. - Trieste : Universitá di Trieste, 2016. - S. 27. - ISBN 978-88-8303-757-3
[Regional Biophysics Conference 2016. Trieste, 25.-28.8.2016]

AFG03 Kollár, Jakub [KAUT] [UKOMFKJFBd] (70%) - Frecer, Vladimír [UKOFAFYZ] (30%): Computational design of histone deacetylase inhibitors as epigenetic agents targeting cancer
Lit. 3 zázn.
In: ABC of Physics of Life. Book of abstracts. - Zagreb : Croatian Biophysical Society & Institute of Physics, 2016. - S. 110-111. - ISBN USBN 978-953-7666-14-9

[International "Greta Pifat Mrzljak" School of Biophysics. 13th, Split, 1.-10.9.2016]

AFH Abstrakty príspevkov z domácich vedeckých konferencií

AFH03 Kollár, Jakub [UKOMFKJFBd] (70%) - Frecer, Vladimír [UKOFAFYZ] (30%): How precise are hybrid QM/MM methods for description of drug-receptor interactions?
Lit. 2 zázn.
In: Applied Natural Sciences 2015 - Book of Abstracts. - Trnava : Univerzita sv. Cyrila a Metoda, 2015. - S. 45. - ISBN 978-80-8105-723-6
[ANS 2015 : Applied Natural Sciences : International Scientific Conference. 5th, Jasná, 30.9.-2.10.2015]

AFH05 Kollár, Jakub [UKOMFKJFBd] (70%) - Frecer, Vladimír [UKOFAFYZ] (30%): QSAR of anticancer drugs inhibitors of histone deacetylase 4
In: 46th EuroCongress on Drug Synthesis and Analysis. Book of Abstracts [elektronický zdroj]. - Bratislava : FaF UK, 2017. - S. 35-36 [online]. - ISBN 978-80-223-4388-6
[EuroCongress on Drug Synthesis and Analysis. 46th, Bratislava, 5.-8.9.2017]
URL: https://drive.google.com/file/d/0B0x72_8GZy7mMk5LejBvQVJRVDQ/view

AFH07 Kollár, Jakub [UKOMFKJFBd] (70%) - Frecer, Vladimír [UKOFAFYZ] (30%): Molecular modelling and design of histone deacetylase inhibitors as anticancer agents
In: 46th EuroCongress on Drug Synthesis and Analysis. Book of Abstracts [elektronický zdroj]. - Bratislava : FaF UK, 2017. - S. 28-29 [online]. - ISBN 978-80-223-4388-6
[EuroCongress on Drug Synthesis and Analysis. 46th, Bratislava, 5.-8.9.2017]
URL: https://drive.google.com/file/d/0B0x72_8GZy7mMk5LejBvQVJRVDQ/view

BFA Abstrakty odborných prác zo zahraničných podujatí (konferencie, ...)

BFA01 Kollár, Jakub [KAUT] [UKOMFKJFBd] (60%) - Polonec, Peter (5%) - Frecer, Vladimír [UKOFAFYZ] (35%): QSAR and proposition of new anticancer drugs - histone deacetylase 4 inhibitors
Lit. 3 zázn.
In: 45. konference Syntéza a analýza léčiv [elektronický zdroj]. - Hradec Králové : ČFS ČLS J. E. Purkyně, 2016. - S. 87-88 [online]
[Syntéza a analýza léčiv. 45., Hradec Králové, 22.-24.6.2016]
URL: http://www.faf.cuni.cz/getattachment/Special/REG-SAL2016/Sbornik-konference/Sbornik_SAL_2016.pdf.aspx