Influenza Virus Neuraminidase Inhibitors: Generation and Comparison of Structure-Based and Common Feature Pharmacophore Hypotheses and Their Application in Virtual Screening

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X-ray crystallographic data of the influenza virus neuraminidase in complex with different inhibitors were used to generate chemical feature-based pharmacophore models of the binding site of this enzyme. The models were built using the software package Catalyst. Pharmacophore hypotheses derived from the 3-D structure of ligands cocrystallized with the enzyme were then compared with automatically generated common feature pharmacophore hypotheses for neuraminidase inhibitors. The latter models were found to contain fewer features and exhibited lower selectivity in virtual screening experiments. Some functions of the inhibitors obviously participate in more than one mode of interaction with the enzyme (charge−charge interaction and hydrogen bond) or form hydrogen bonds to several amino acids. Since such multiple interactions of one chemical function cannot be included into the Catalyst data format, strategies are presented to overcome these limitations. Finally, the results of 3-D database searching experiments using these hypotheses are described.

INTRODUCTION

Influenza is an acute respiratory disease, which afflicts large parts of the population in annual epidemic outbreaks. It often causes severe, sometimes even lethal, effects. Since the virus is highly contagious and transmitted by aerosol, it spreads rapidly. There is always the threat of particularly virulent strains, which lead to many more cases of disease and death than usual.[1] Aside from the considerable morbidity and mortality, there are several other reasons why there is still a need to develop novel, selective drugs for the treatment of influenza: because of the fast mutations in viral antigens, vaccines against influenza often become ineffective. Resistance problems exist for all drugs used in the treatment of this viral infection.[2] More severe outbreaks of influenza can even result in pandemics, as has happened four times in the last century. Apart from the affliction for the people concerned, the immense economic damage that results from lost productivity and medical expenses must not be disregarded.

Adamantine and its analogue rimantadine were the first antiinfluenza drugs and block the ion channel of virus protein M2.[3] However, they are only active against type A influenza viruses and cause many side effects. Two drugs that have been launched in the last years (zanamivir, which has to be administered via inhalation, and the orally active prodrug oseltamivir) present the latest class of antiinfluenza drugs, the neuraminidase (NA)[1] inhibitors (see Chart 1).

Aside from hemaglutinine, NA is one of the two major surface proteins in both type A and type B influenza viruses. It is essential for the release of the virus of the infected cell and for its transport through the mucus in the respiratory tract. Although the amino acid sequence homology between NA of influenza A and B only comes up to about 25%, the active site of the enzyme is conserved, and therefore, presents an attractive target for structure-based drug design.[1,4] Designed according to information obtained from the crystallographic data of the viral NA complexed with its natural substrate sialic acid, zanamivir and oseltamivir present a big success in the history of rational, structure-based drug development.

The aim of this study was to generate pharmacophore models for NA inhibitors in a structure-based approach, as well as to compare these models with those obtained in an automatically performed common feature-based pharmacophore generation process.

Built with the software package Catalyst, these pharmacophore models are suitable for 3-D database searching, which may serve to validate them and to detect potential new lead compounds. Known NA inhibitors should selectively be retrieved as hits from the databases, which makes the models valuable tools for checking substances for their possible NA inhibitory activity.

Furthermore, we show how limitations within the Catalyst software can be overcome. So far, within the Catalyst environment, it is not possible to superimpose two features (e.g., negative ionizable with hydrogen-bond acceptor) onto one chemical function in a molecule, to retrieve only hits that fit both feature definitions at this special position. Besides, it is not supported that two or more hydrogen-bond acceptors or donors radiate from one heteroatom. The solution applied was to create a set of multiple hypotheses together with defined connection strategies.

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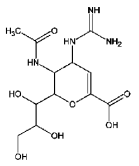
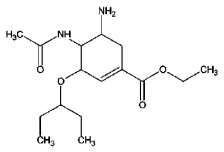
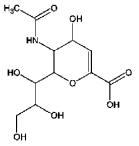
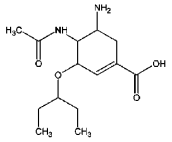
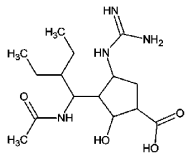
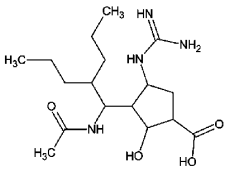
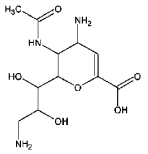
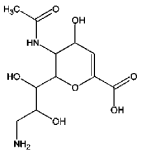
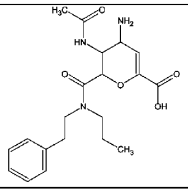
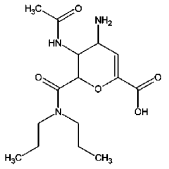
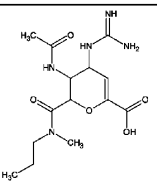
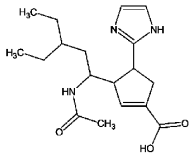
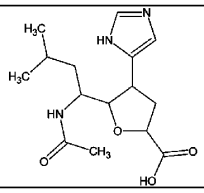
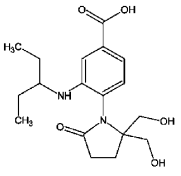
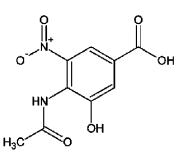
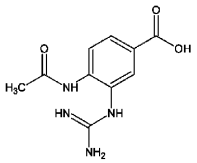
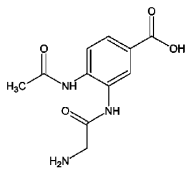
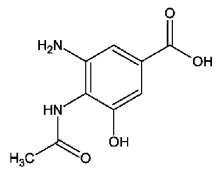
Chart 1. Structures of NA Inhibitors Used for Both Structure-Based and Automatic Pharmacophore Hypothesis Generation, as Well as for Validation of the Models by Forming an Internal Inhibitor Database

Compound name (PDB entry code)	Structure	Compound name (PDB entry code)	Structure
zanamivir (1A4G)		oseltamivir	
DANA (1F8B)		GS4071 (2QWK)	
BCX1812 (1L7F)		BCX1898	
4,9-diamino-DANA (1F8E)		9-amino-DANA (1F8D)	
GR-210729 (1BJI)		CAS: 182251-67-8	
CAS: 182251-63-4 (2QWI)		CAS: 247159-37-1	
CAS: 495397-50-7		BANA 206	
BANA 105 (1IVD)		BANA 113	
CAS : 170447-95-7 (1INH)		BANA 106 (1IVC)	

MATERIALS AND METHODS

Training Set Selection and Conformational Analysis. The selection of a suitable training set is essential for the quality of automatically generated pharmacophore models.[5] Of equal importance is the definition of an appropriate set of active molecules that are not used for model generation but serve for validation purposes. For this study, a set of highly active NA inhibitors in late phases of development was derived either from the Protein Databank (PDB),[6] the Ensemble database,[7] or several other literature sources.[2−4,8,9] The compounds are listed in Chart 1. All structure models were built and minimized within the Catalyst software package version 4.7,[10] installed on a SGI Octane R10000 dual processor workstation. Before starting the pharmacophore generation process, conformational models for the molecules were calculated using the best conformer generation method with a maximum conformational energy of 20 kcal/mol above the lowest energy conformation found. The poling algorithm was used, which seeks to provide a broad coverage of conformational space.[11−13] The number of conformers generated for each compound was limited to a maximum number of 250.

Generation of Pharmacophore Models. (a) Direct Approach. Direct, structure-based pharmacophore generation uses the spatial information of the target protein for topological description of ligand−receptor interactions and for the subsequent discovery of new structural leads. In this study, two different models were built upon the X-ray structures of inhibitors from the PDB entries 1F8E and 1BJI. Using the coordinates of the ligand and the surrounding amino acids, the features and their projected points were positioned and linked according to the information on interactions present in the literature.

(b) Indirect Approach. The HipHop algorithm provides a common feature-based alignment of highly potent ligands,[14] thus yielding a pharmacophore model giving the possibility to distinguish between active and inactive compounds. Before starting the generation process, a selection is done among the features defined in Catalyst for those that should be present in the resulting model. In this study, positive ionizable (PI), negative ionizable (NI), hydrogen-bond donor (HBD), hydrogen-bond acceptor (HBA), and hydrophobic (H) were selected. Catalyst also allows the user the customization of predefined features as well as the creation of new ones.

Database Searching. Chemical feature-based 3-D pharmacophore models built within the Catalyst software may be used as queries for 3-D database searching. Virtual screening of such databases can serve two main purposes: first, validating the quality of the generated pharmacophore models by selective detection of compounds with known NA inhibitory activity, and second, finding novel, potential leads suitable for further development. Compared to de novo design methods,[15] database searching offers the advantage that the retrieved compounds usually are easily available for testing. In a database searching approach, to be retained as a hit, a molecule must be able to map all features of the pharmacophore hypothesis used for the search process.

In Catalyst, two algorithms for database searching can be chosen: the *Fast Flexible Search* command retrieves hits more quickly by only taking the already existing precalculated conformers of the database into account. Better results

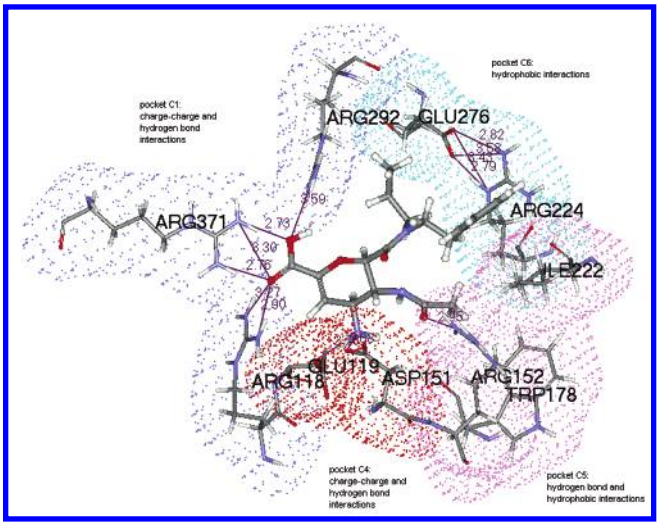


Figure 1. Display of important amino acids forming four pockets of the NA binding site. Surfaces of the pockets are color-coded (blue: pocket C1; red: pocket C4; pink: pocket C5; and light blue: pocket C6).

can be achieved by applying the *Best Flexible Search* operation, which changes the conformations of the molecules during the virtual screening process in torsion angle space to check whether other than the presaved conformers map the pharmacophore hypothesis. In our study, all screening experiments were performed by using the *Best Flexible Search* algorithm. For validation purposes, an internal database was built within Catalyst, consisting exclusively of compounds with known NA inhibitory activity. Furthermore, the Derwent World Drug Index (WDI),[16] containing approximately 50 000 drugs and other bioactive molecules, the Interbioscreen database,[17] and the Maybridge database[18] were used for screening.

RESULTS AND DISCUSSION

Part One: Structure-Based Pharmacophore Design. All NA inhibitors on the market or in clinical phases of development possess strong structural resemblance in those parts corresponding to the four pockets critical for interaction in the active site of NA.[2,4,9,19] The interacting residues of the ligands are carried by a central ring system, often represented by a dihydropyran moiety. The four pockets defining the binding site are displayed in Figure 1.

Pocket C1 is created by a triarginyl cluster (Arg 118, Arg 292, and Arg 371) and anchors the carboxylate group of the inhibitors. Not only charge−charge interaction is decisive here but also a pattern of hydrogen bonds formed from the carboxylate oxygen atoms to the arginine guanidine residues. For the acetamide moiety positioned in the C5 pocket, several interactions can be observed: its oxygen atom functions as an HBA to Arg 152, whereas the nitrogen atom acting as donor forms a hydrogen bond to a water molecule buried in the cleft. The methyl group undergoes a favorable hydrophobic contact with Trp 178 and Ile 222. In pocket C4, the basic substituent, usually a guanidine or an amine group, participates in charge−charge interactions and hydrogen bonds to Glu 119, Asp 151, and/or Glu227. Comparison with the 4-hydroxy analogues developed earlier proves the significance of these basic substituents for increased inhibitory activity.[4,8] Structural differences of the inhibitors can

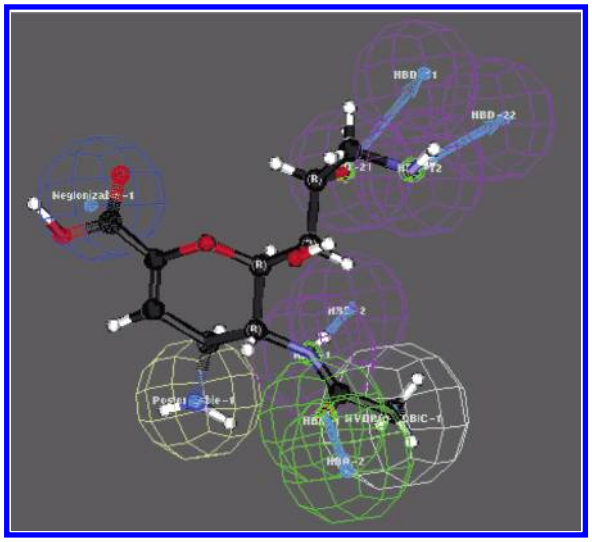


Figure 2. NA inhibitor 4,9-diamino-DANA (PDB identification 1F8E) aligned with a structure-based pharmacophore hypothesis (seven features, 11 points), derived using the Catalyst program. Pharmacophore features are color-coded (blue: NI; violet: HBD; light yellow: PI (customized function); white: H (customized function); and green: HBA).

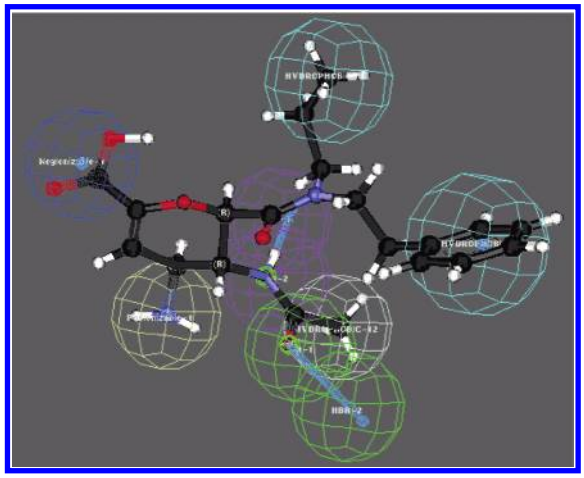


Figure 3. NA inhibitor GR-210729 (PDB 1BJI) aligned with a structure-based pharmacophore hypothesis (seven features, nine points), derived using the Catalyst program. Pharmacophore features are color-coded (blue: NI; violet: HBD; light yellow: PI (customized function); white: H (customized function); green: HBA; and light blue: H).

be seen in the side chain in the C6 pocket (see Figures 2 and 3), which leads to a division into two groups:

In the first group, the side chain consists of a glycerol moiety (or a closely related residue) and forms hydrogen bonds to Glu 276. Zanamivir shares this binding mechanism. For the components of the second group, which have a bulky hydrophobic group in common, a rearrangement of the active site residue Glu 276 takes place, which folds away forming a new hydrogen bond with Arg 224.[2,8] This results in the formation of a new binding pocket for the hydrophobic side chain.

For both groups, pharmacophore hypotheses including all those key interactions were created within the program Catalyst using information on the active conformations of the inhibitors in complex with the target protein originating from the PDB. Inhibitors crystallized in the NA of influenza virus type A and B show little differences regarding their

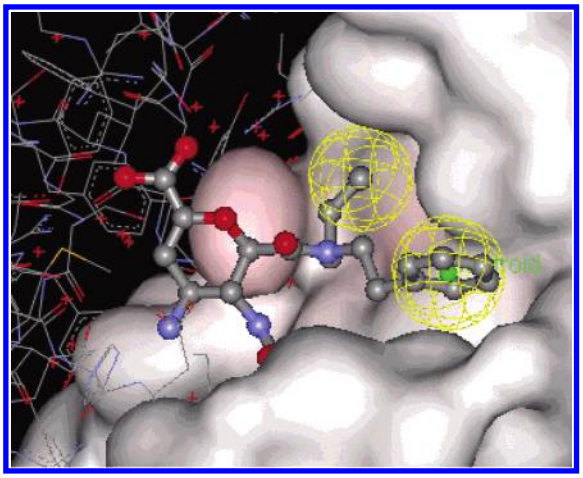


Figure 4. GR-210729 in the NA active site, solid surface partly added to the enzyme, hydrophobic side-chain residues supplied with location constraints (yellow spheres) for measurement of maximally available space.

interactions with the enzyme or their active conformations, so that the creation of separate models for the two NA types was not necessary.

Hypotheses for the first group were designed starting from the PDB entry 1F8E[19] detected with a solution of 1.4 Å. 1F8E contains the zanamivir analogue 4,9-diamino-DANA, where an amine function replaces both the guanidine and the terminal hydroxyl groups of the glycerol moiety. The locations of features in the pharmacophore model were defined by the crystallographic coordinates of the atoms of 1F8E and the essential amino acids in the active site of NA. Finally, the features were merged into one hypothesis consisting of one small hydrophobic region, one HBD, and one HBA, representing the acetamide moiety, two HBDs standing for the glycerol oxygen atoms, and finally one PI and one NI feature, which encode for the C4 amine and the carboxylic acid, respectively. Since the definition for an H feature in Catalyst does not include the acetamide methyl group, it had to be customized accordingly. An extension of the PI function was also necessary because otherwise ligands containing an imidazole as a basic substituent cannot be recognized. The ligand extracted from the 1F8E complex in its bioactive conformation was able to successfully map all features of the described pharmacophore without conformational change, when a compare/fit operation was performed (see Figure 2).

For pharmacophore model generation based on molecules of the second group, the complex 1BJI from the PDB was chosen.[9] Prominent examples for this group that also carry bulky hydrophobic residues causing a rearrangement of Glu 276 are oseltamivir and BCX1812, which already reached clinical phase three of development. Following the observed binding mode, in this case, the C6 side chain is now represented by two hydrophobic features (see Figure 3).

According to the dimension of the C6 pocket, these hydrophobic features were given location constraints of 1.85 Å for the phenyl ring and 1.70 Å for the terminal propyl CH_3 group (see Figure 4).

For further restriction and enhanced steric selectivity, excluded volume spheres that the ligand is not allowed to penetrate were added to the model. They were centered onto the amino acids Trp 178 and Ile 222, surrounding the

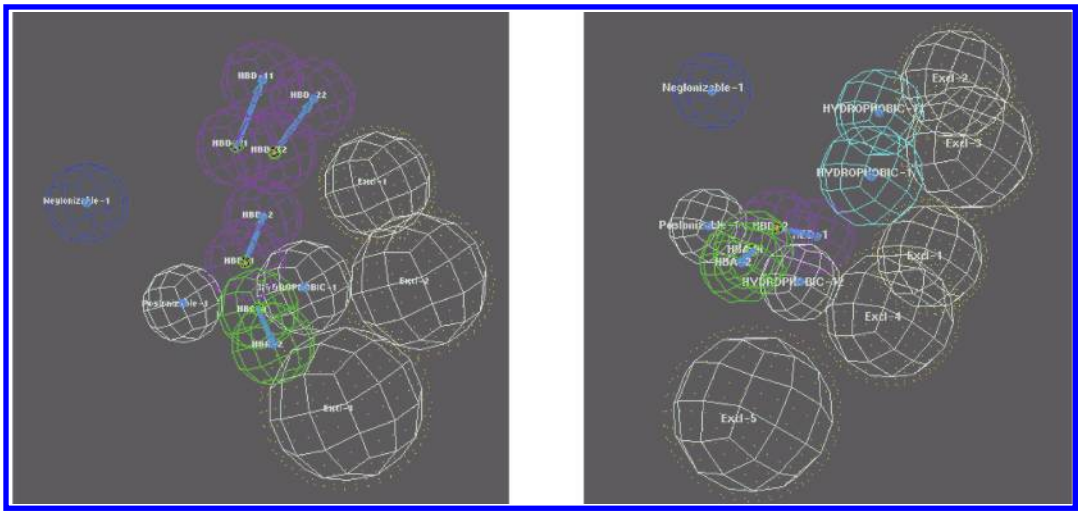


Figure 5. Addition of excluded volume spheres for further hypothesis refinement resulting in 1F8EhypExVol (left) and 1BJIhypExVol (right). Color codes are explained in Figures 2 and 3.

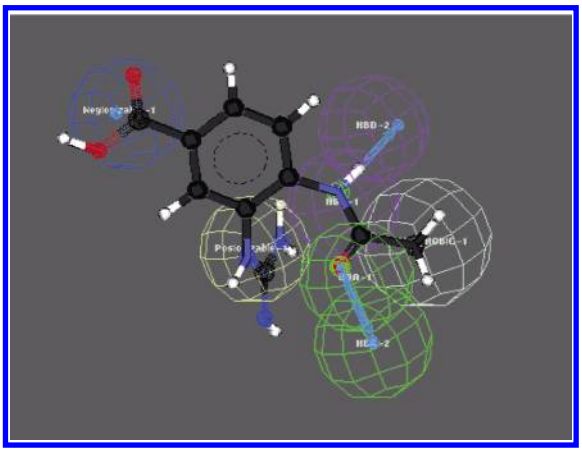


Figure 6. Aromatic NA inhibitor BANA113 aligned with a structure-based pharmacophore hypothesis (five features, seven points), derived using the Catalyst program. Pharmacophore features are color-coded (blue: NI; violet: HBD; light yellow: PI (customized function); white: H (customized function); and green: HBA).

acetamide methyl group, and onto Glu 276, Arg 224, and Ala 246, which form the larger C6 pocket. The resulting hypotheses are displayed in Figure 5.

Those two models, obtained as mentioned, were the basis for further improvement (see description in part three) to take into account the net of hydrogen bonds from the acid and the basic group to the surrounding amino acids. Moreover, they were used for 3-D database searches (part four).

From the 1F8E hypothesis, a general model was created by deleting the C6 side chain features (see Figure 6). This basis model contains only features common to the components of both groups, as well as other NA inhibitors, like the intensively investigated class of benzoic acid derivatives designed after the lead compound BANA 113[1,20] (Chart 1).

Part Two: Ligand-Based Pharmacophore Generation. The software-assisted automatic pharmacophore generation was carried by using the Catalyst module HipHop. In this procedure, the molecules with their associated conformers were divided into the two groups, according to their structural features as mentioned before. Hypothesis generation was performed, which resulted in each set of input molecules in 10 pharmacophore models with up to a total of four features. Many of these alternative models were found to be in fact closely related. The structural requirements detected by Catalyst for NA inhibitors consist of a PI and more often, however, a HBD, for the basic substituent, a NI representing the carboxylate group, and an HBA for the acetamide oxygen, or alternatively a HBD for its nitrogen atom. The C6 side chain was represented by only one feature, a HBD in case of the first group or an H for the bulky hydrophobic residue of the second group. The proposed mapping modes of the inhibitors in these hypotheses are homogeneous and match the information from the crystallized enzyme−ligand complexes. Automatic generation processes with a total feature minimum higher than four, however, could not be carried out. Obviously, the important regions for interaction with the enzyme could only partly be detected or not entirely be included into a single hypothesis by the automatic model generation procedure within Catalyst. Even though the number of features was extended by manual addition and hypotheses merging operations, the common feature models still proved to be inferior to the structure-based ones. When searching our self-compiled NA inhibitors database containing 18 compounds (Chart 1), we obtained fewer hits, and the pharmacophore models were less likely to identify the bioactive conformations. In conclusion, it can be stressed that the detailed information about the key interactions with NA, the necessary features, their location constraints, and the directions of the vectorized functions can be transferred into a better pharmacophore hypothesis by applying a structure-based approach than a ligand-based one. All further attempts for improvement were therefore focused on the structure-based pharmacophores.

Part Three: AND/OR Pharmacophore Connections. The carboxylic acid and the basic substituent both contribute to the high binding affinity of the ligands not only because of their charge but also their ability to form hydrogen bonds (see Figure 7). We describe methods that permit us to take into account multiple contributions of a single chemical structure with a complementary group on the target side.

In a first step, the PI feature from the hypotheses derived in the structure-based approach from 1F8E and 1BJI was displaced by a HBD to Glu 119. Then, instead of the NI, a HBA was introduced with one of the carboxylic acid oxygen atoms functioning as HBA. Five hypotheses were built

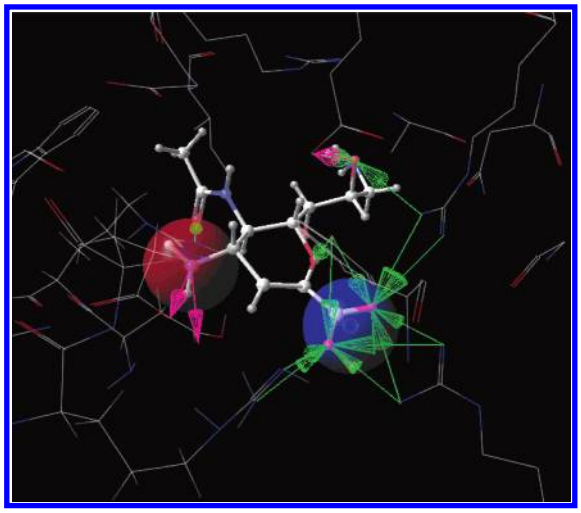


Figure 7. Pharmacophore model displaying a set of hydrogen bonds from the basic and acid residues of NA inhibitors to the surrounding amino acids in the enzyme active site. Illustration computed with our recently developed program LigandScout.[21] Pharmacophore features are color-coded (blue: NI; pink: HBD; red: PI; and green: HBA).

according to the five possible hydrogen bonds from the carboxylate to the three surrounding arginines (Arg 118, Arg 292, and Arg 371). They were linked via the Boolean operator OR connection, a process supported by the Catalyst program (see Figure 8). Database searching with such a combined model provides a hit list, whose components have to match at least one of the connected hypotheses.

In the active site of the NA, there are several possibilities to form hydrogen-bond networks to the surrounding amino acids starting from a single atom of a ligand. Since in Catalyst, by default only one hydrogen bond can be formed from one atom, combined hypotheses help to overcome this limitation. Nevertheless, such an OR connection does not serve the purpose to take into account the ionizability of the carboxylate and the C4 residue, characteristic for highly active inhibitors. The requirement of such an AND connection between the original models, containing the PI and NI features, and the combined hydrogen bonds hypotheses can only indirectly be met in Catalyst. A two-step database searching is necessary to obtain the intersection of hits fulfilling both criteria. For the initial searching, the models containing ionizable features are used. The resulting hit list is then checked for its ability to match the combined hypothesis. The hits found possess ionizable and hydrogen-bond donating qualities. AND/OR connections are valuable tools to include as much knowledge as possible about the ligand−enzyme interactions into the pharmacophore models. They provide higher selectivity of the hypotheses as can be seen from the restricted hit lists in database searches.

Part Four: Database Searching. Database searches with structure-based models built upon the zanamivir analogue 4,9-diamino-DANA, PDB entry 1F8E, were carried out first. They proved to be valuable for the selective detection of this zanamivir-like group of NA inhibitors. When searching our internal NA inhibitor database and the WDI, all components belonging to this group were present in the hit lists, moreover exhibiting high fit values. The resulting number of 34 hits from the WDI could be reduced to 19 using the hypothesis refined by excluded volume spheres and finally to 17 (less than 0.04% of the entire database) screening these hits with the combined model, where HBDs and HBAs replace the PI and NI feature. The NA inhibitors among them were not eliminated, demonstrating the quality and selectivity of the hypotheses. The majority of the other molecules was found to be classified as antibiotic substances, according to their activity keyword in the 1-D data.

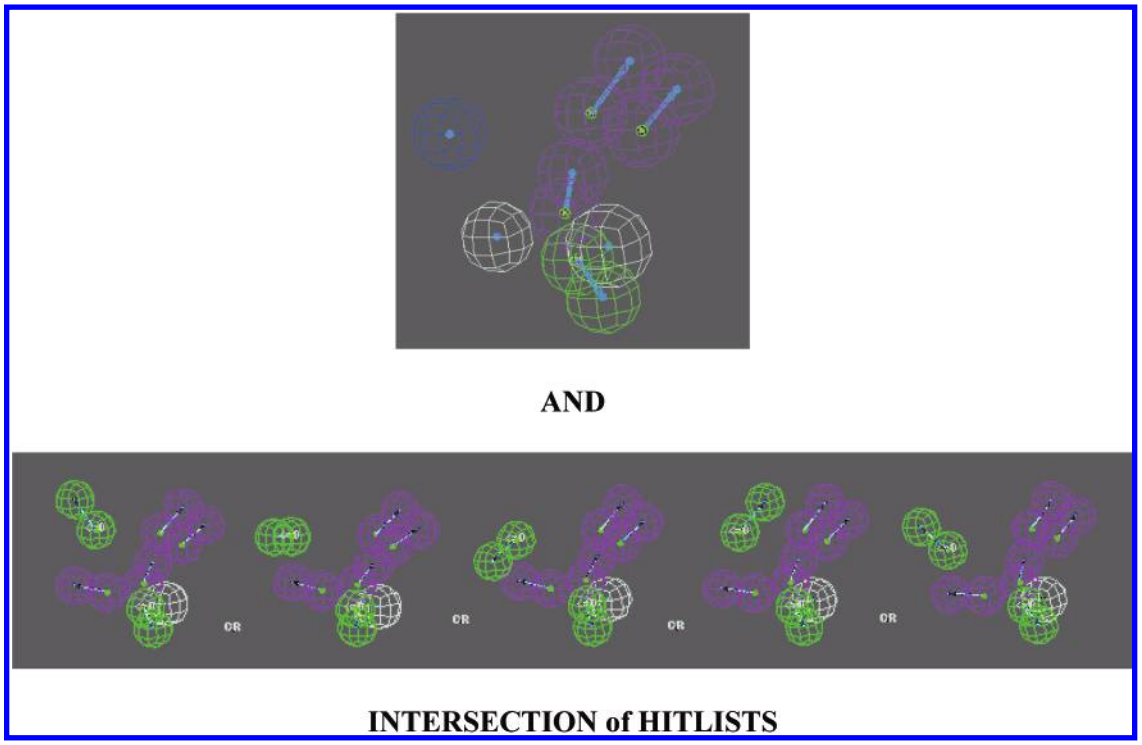


Figure 8. Five structure-based pharmacophore hypotheses designed based upon crystallographic data file 1F8E. Upper figure: hypothesis containing one PI and one NI feature. Lower figure: five hypotheses, OR connection, displaying a set of hydrogen bonds formed by the basic and the acid residue of NA inhibitors to the surrounding amino acids.

Figure 9. Examples of hits derived from the Maybridge (left) and the Interbioscreen (right) databases searched with the basis hypothesis.

The screening experiment of our internal NA inhibitor database and the WDI with the models obtained from the ligand GR-210729, representing NA inhibitors with a hydrophobic residue in the C6 side chain, confirmed just as high selectivity for these hypotheses. The prodrug oseltamivir, belonging to this class, could not be detected since the otherwise positive ionizable carboxylate is substituted with an ethyl moiety.[8] Its active form, compound GS4071, containing the free acid, however, was found as a hit.

The basis hypothesis, where no features had been placed onto the C6 side chain, retrieved 80% of the compounds, when searching the internal NA inhibitor database. Among the hits, we retrieved ligands from both classes, as well as other molecules lacking C6 substitution. On the other hand, the prodrug form of oseltamivir and earlier inhibitors, substituted with a hydroxy group instead of the basic residue, like DANA,[8] were not detected. Furthermore, molecules where an attempt had been made to incorporate the buried water from the active site into the inhibitor structure by adding a hydroxy group[4] were also not present in the hit lists. Because of a decreased selectivity of this hypothesis containing two features less than the original models, WDI searching yielded a hit list of 752 compounds, of which 13 display an antiviral mode of action. Among them, all NA inhibitors were present, now also including BANA 113. Because of the similar substitution pattern, this benzoic acid derivative maps the basis hypothesis, although it displays a somewhat different binding mode in the NA active site.

Other databases were screened to find compounds with novel structural characteristics and scaffolds that might present new classes of influenza NA inhibitors. Substances that were retrieved from the Maybridge database often contained a thioxazolidine as a central ring system carrying the residues for interaction, whereas hits from the Interbioscreen database were usually open chained systems, Figure 9.

CONCLUSIONS

In this study, we describe the development of highly selective pharmacophore models for inhibitors of viral NA within the Catalyst software package. They reflect the binding mode and the important interactions of the ligands with certain amino acids in the active site.

Models were generated in both an automated ligand-based approach and a structure-based approach, the latter providing more detailed information and accuracy in its description of ligand binding. In our study, efforts were made to take multiple contributions of ligand functions and their importance for high binding affinity into account. We concentrated on including all these influences into the models, which created the need for special hypotheses connections. Subsequently, our hypotheses underwent validation in virtual screening processes, returning very selective hit lists from 3-D databases containing NA inhibitors. Such an application of pharmacophore models in database screening can therefore be useful in the detection of new leads.

The hypotheses provide illustrations of the important interactions between the viral NA and its inhibitors and may serve as a preselection tool in drug development to check substances for their potential NA inhibitory activity, even before synthesis and further investigations are implemented.

Abbreviations: NA, neuraminidase; PDB, Protein Databank; PI, positive ionizable; NI, negative ionizable; HBD, hydrogen-bond donor; HBA, hydrogen-bond acceptor; H, hydrophobic; WDI, World Drug Index.

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REFERENCES AND NOTES

(1) Chand, P.; Babu, Y. S.; Bantia, S.; Chu, N.; Cole, B. L.; et al. Design and Synthesis of Benzoic Acid Derivatives as Influenza NA Inhibitors Using Structure-Based Drug Design, *J. Med. Chem.* **1997**, *40*, 4030−4052.

(2) Smith, B. J.; McKimm-Breshkin, J. L.; McDonald, M.; Fernley, R. T.; Varghese, J. N.; et al. Structural Studies of the Resistance of Influenza Virus NA to Inhibitors, *J. Med. Chem.* **2002**, *45*, 2207−2212.

(3) Smith, P. W.; Sollis, S. L.; Howes, P. T.; Cherry, P. C.; Starkey, I. D.; et al. Dihydropyrancarboxamides Related to Zanamivir: A New Series of Inhibitors of Influenza Virus Sialidases. 1. Discovery, Synthesis, Biological Activity, and Structure−Activity Relationships of 4-Guanidino- and 4-Amino-4H-pyran-6-carboxamides, *J. Med. Chem.* **1998**, *41*, 787−797.

(4) Wang, T.; Wade, R. C. Comparative Binding Energy (COMBINE) Analysis of Influenza NA−Inhibitor Complexes, *J. Med. Chem.* **2001**, *44*, 961−971.

(5) Sutter, J.; Gruener, O.; Hoffmann, R.; Li, H.; Waldman, M. *Pharmacophore Perception, Development, and Use in Drug Design: Effect of Variable Weights and Tolerances on Predictive Model Generation*; International University Line: La Jolla, CA, 2000; pp 501−511.

(6) Brookhaven National Laboratory: Protein Data Bank http://www.rcsb.org/pdb/.

(7) Prous Science Ensemble: A new Database affording a unique, integrated view of drug information, User's Guide; http://www.prous.com, 2001.

(8) Babu, Y. S.; Chand, P.; Bantia, S.; Kotian, P.; Dehghani, A.; et al. BCX-1812 (RWJ-270201): Discovery of a Novel, Highly Potent, Orally Active, and Selective Influenza NA Inhibitor through Structure-Based Drug Design, *J. Med. Chem.* **2000**, *43*, 3482−3486.

(9) Taylor, N. R.; Cleasby, A.; Singh, O.; Skarzynski, T.; Wonacott, A. J.; et al. Dihydropyrancarboxamides Related to Zanamivir: A New Series of Inhibitors of Influenza Virus Sialidases. 2. Crystallographic and Molecular Modeling Study of Complexes of 4-Amino-4H-pyran-6-carboxamides and Sialidase from Influenza Virus Types A and B, *J. Med. Chem.* **1998**, *41*, 798−807.

(10) Accelrys Inc. *Catalyst, version 4.7*; San Diego, 2002; http://www.accelrys.com.

(11) Smellie, A.; Kahn, S. D.; Teig, S. L. Analysis of Conformational Coverage. 1. Validation and Estimation of Coverage, *J. Chem. Inf. Comput. Sci.* **1995**, *35*, 285−294.
(12) Smellie, A.; Kahn, S. D.; Teig, S. L. Analysis of Conformational Space. 2. Applications of Conformational Models, *J. Chem. Inf. Comput. Sci.* **1995**, *35*, 295−304.
(13) Smellie, A.; Teig, S. L.; Towbin, P. Promoting Conformational Variation, *J. Comput. Chem.* **1995**, *16*, 171−187.
(14) Clement, O. O.; Mehl, A. T. *Pharmacophore Perception, Development, and Use in Drug Design: HipHop: Pharmacophores Based on Multiple Common-Feature Alignments*; International University Line: La Jolla, CA, 2000; pp 71−84.
(15) Böhm, H. J.; Klebe, G.; Kubinyi, H. *Wirkstoffdesign*; Spektrum Akademischer Verlag GmbH: Heidelberg, Berlin, Oxford, 1996; pp 451−466.
(16) The Derwent World Drug Index is available in Catalyst multi-conformational data format from Accelrys Inc., San Diego, http://www.accelrys.com.
(17) Interbioscreen, Bioscreen NP Database, Natural Products Catalog of Screening Compounds, 16 000 structures, Daylight Databases; http://www.daylight.com.
(18) Maybridge Organic Chemical Catalog, 62 000 compounds, Daylight Databases; http://www.daylight.com.
(19) Smith, B. J.; Colman, P. M.; von Itzstein, M.; Danylec, B.; Varghese, J. N. Analysis of inhibitor binding in influenza virus NA, *Protein Sci.* **2001**, *10*, 689−696.
(20) Atigadda, V. R.; Brouilette, W. J.; Duarte, F.; Ali, S. M.; Babu, Y. S.; et al. Potent Inhibition of Influenza Sialidase by a Benzoic Acid Containing a 2-Pyrrolidinone Substituent, *J. Med. Chem.* **1999**, *42*, 2332−2343.
(21) Wolber, G.; Langer, T. LigandScout: 3-D Pharmacophores Derived from Protein-Bound Ligands and their Use as Virtual Screening Filters, *J. Chem. Inf. Comput. Sci.*, accepted for publication.

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