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Investigating Enzyme Selectivity and Hit Enrichment by Automatically Interfacing Ligand- and Structure-Based Molecular Design

Orazio Nicolotti,* Ilenia Giangreco, Teresa Fabiola Miscioscia, Angelo Carotti

Dipartimento Farmaco-Chimico, Università di Bari, Via Orabona 4, 70125, Bari, Italy
*e-mail: nicolotti@farmchim.uniba.it

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Abstract

A new method was implemented in the in-house program *@lic&* to rationally combine structure- and ligand-based design through nondeterministic rescoring of docking poses for the automated generation of structural alignments of a series of benzamidine compounds acting as protease inhibitors. Such alignments enable 3D-QSAR models to explain enzyme selectivity and to retrieve with high sensitivity active hits from a pool of 260 structurally-related benzamidine derivatives.

Introduction

Docking has become the most popular method for structure-based design assuming an important role in the various stages of drug research. It is normally applied for three basic challenges. The first deals with reproduction of the binding mode of known active ligands; the second with identification of new ligands through virtual screening and the third concerns prediction of binding affinities of a congeneric series of ligands. As well known, major problems arose when differences between active and inactive compounds of a single chemotype were quantified in terms of affinity values. The cause of this failure is widely agreed upon as being strictly related to the fact that scoring functions are not tailored to correctly measure the desolvation and the small differences of ligand-protein interaction energy [1]. However, with the availability of X-ray structure of the target proteins and affinity data of a series of their ligands, the effort of joining structure- to ligand-based design may represent an effective tool for predicting binding affinity [2] and, ultimately, for obtaining interpretable results to design new compounds. In this perspective, the importance of selecting suitable docking poses to build quantitative models is clear. As described in a recent work [2], molecular alignments herein used were constructed through *@lic&* which enabled the automated selection of docking poses that were optimal to regress docking scores with biological responses and yielded the smallest RMSD values. Shedding new light on the potential of our approach, the present analysis has a twofold aim. The first is the elucidation of molecular selectivity on a more solid basis by integrating structure- and ligand-based design. The second is the use of QSAR as a diagnostic tool for ligand enrichment experiments with the ultimate purpose of designing small focused combinatorial library of new active molecules.

Methods

Database

Based on a recent innovative method, 3D-QSAR models were derived from a collection of 88 3-amidinophenylalanine protease inhibitors divided into a training and test set (1 – 72 and 73 – 88 of Table 1 in Supplementary Materials) [3, 4]. Each ligand was attributed a pair of affinity values referring to the inhibition of thrombin and trypsin whose X-ray entries into the PDB were 1ETS and 1PPH. Apart from the 72 inhibitors of training set, a pool of 260 additional ligands having the benzamidine core structure was used as benchmarking set for assessing ligand enrichment.

Supporting information for this article is available on the WWW under www.qcs.wiley-vch.de

Docking Rescoring Through the In-house Program @lic&

Although top-scored solutions supposedly engage in stronger binding interactions, their energies are not always capable of explaining the variance in biological data and/or of reproducing expected bound conformations. A genetic algorithm [5] was implemented to sample the combinatorial space whose boundaries are represented by correlation between docking scores and experimental biological affinity and by the RMSDs computed in respect to a well-defined cocrystallized inhibitor (Fig.1 in Supplementary Materials). The interested reader is referred to our original paper [2] for more details.

Results and Discussion

A relevant objective of the present investigation was to obtain valid explanations of enzyme selectivity [6] in order to identify molecular determinants distinguishing the thrombin and trypsin inhibitors. In this framework, selective agents should interact with nonconserved protein residues of the two proteases, whereas nonselective inhibitors should basically contact conserved amino acid side-chains or backbone. Unlike other approaches which utilized only one biased and subjective structural alignment, two diverse superimpositions were constructed for the two serine proteases by applying *@lic&* to the training set compounds having 20 diverse docking solutions per ligand. After obtaining two diverse structural overlays, GRID-GOLPE analyses were performed to derive 3D-QSAR models (see Table 2 in Supplementary Materials), which were able to explain molecular selectivity by recalculating the exact pK_i values for both targets. This result became even clearer by plotting experimental vs. recalculated pK_i differences. As shown in Figure 1, thrombin and trypsin selective inhibitors were located in quadrants I and III. The bisector intercepts compounds whose selectivity was fully explained. Conversely, the over- or under-estimation of selectivity was related to the vertical distance and position above or below the bisector. In the worst of cases, if inversion of selectivity should occur after recalculating pK_i values, reversed thrombin and trypsin selective compounds will move down to quadrant IV and up to quadrant II, respectively.

The data set analyzed contains mostly selective thrombin inhibitors. Thus, quadrant I of Figure 1 was more populated compared to quadrant III which comprised only a small number of selective trypsin inhibitors. In addition, a closer look at Figure 1 revealed that limited inversion of selectivity was calculated for only 6 out of 72 compounds in the training set. Their position close to the origin of the axes indicated that they were nonselective inhibitors and, thus, not very promising references for such analyses. Interestingly, very good performances were observed also when analyzing selectivity of test set compounds except

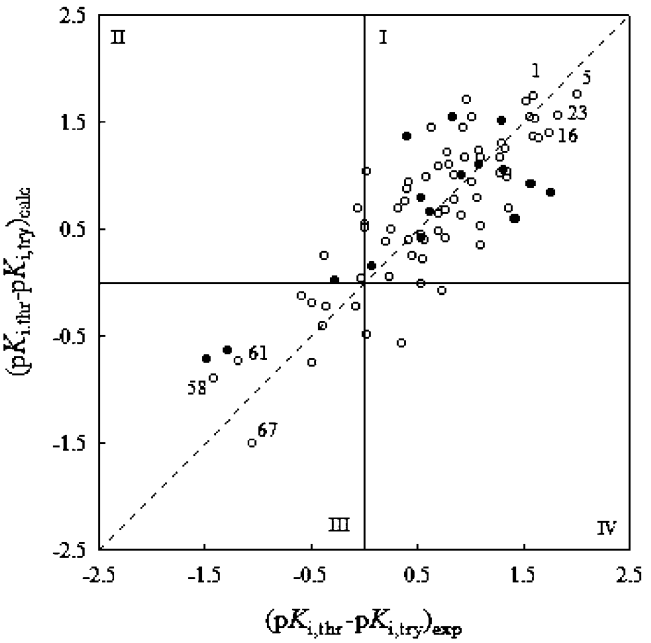

Figure 1. Selectivity plot representing for each compound projections of experimental vs. recalculated thrombin and trypsin pK_i differences. Empty and solid circles indicate training and test set compounds. Bisector is drawn as a dashed line. Labeled compounds are discussed in the text.

for one nonselective inhibitor that was misplaced in quadrant II (solid circle in Fig. 1).

For ease of presentation, pK_i difference and amino acid residue mutations always referred to thrombin in comparison to trypsin; a number of examples were reported herein to illustrate some benefits obtained by applying our strategy. Two different orientations were observed for R1 cyclic substituents approaching thrombin and trypsin binding sites when considering selective thrombin inhibitor **16** (Fig. 2a). The Q221R mutation is likely to better stabilize the methoxy group of the R1 substituent of the thrombin conformer through polar interaction. In addition, a more suitable orientation was observed for the sulfonyl group in R2 facing the K60 residue which belongs to the P subsite that is a specific region of thrombin. The thrombin selectivity of inhibitor **1** (Fig. 2b) was probably due to the interaction between its dimethylamino group in R1 and T149 which is favorably oriented in the case of thrombin and not optimally positioned for trypsin; reinforcing binding interactions were those occurring between the specific K60 residue and the sulfonyl group in R2. Our investigation subsequently analyzed selectivity vs. trypsin bearing in mind that the data set had been basically designed to obtain selective thrombin inhibitors. Despite this intrinsic limitation, a few examples of trypsin selective inhibitors were chosen and discussed herein. With respect to the selective trypsin inhibitor **67**, the R1 and R2 substituents exchanged their relative positions (Fig. 2c); a putative contact between the piperidine protonated nitrogen and the

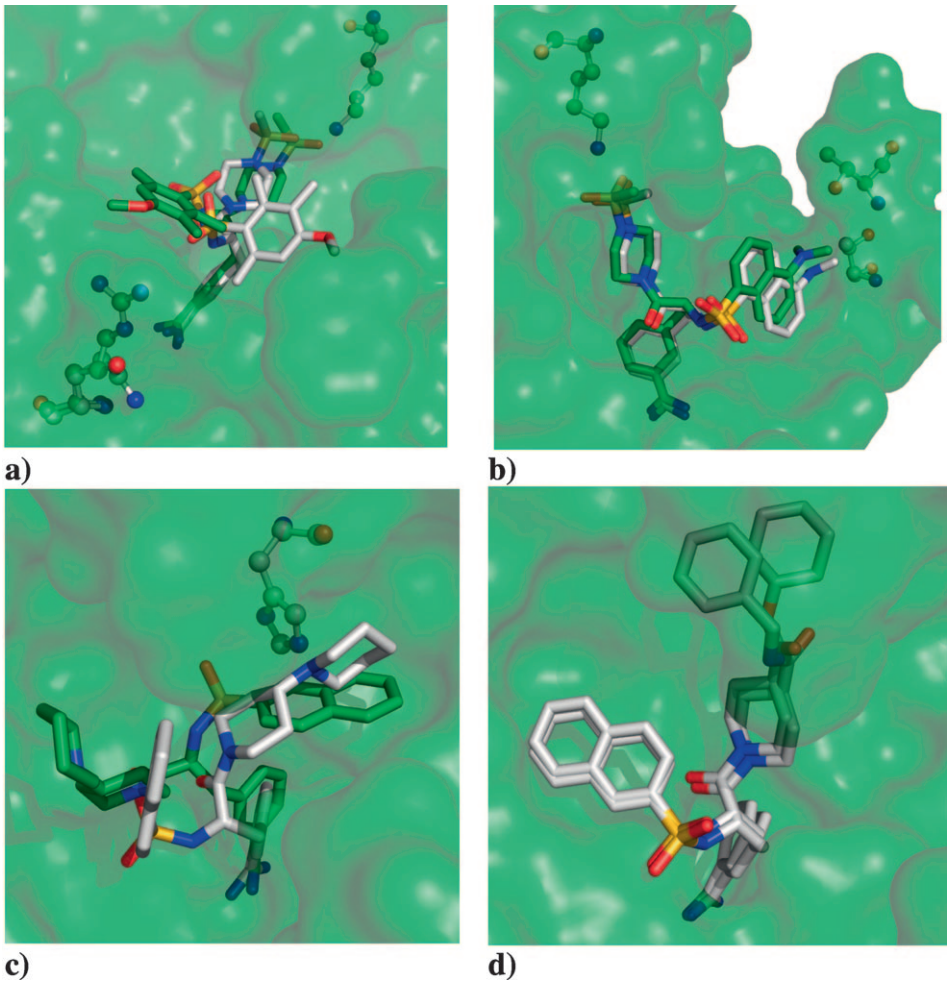

Figure 2. Thrombin and trypsin relevant amino acid residues along with the corresponding docking solutions selected by *@lic&* are colored in green and white, respectively. Green background surface was rendered from 1ETS thrombin crystal structure. Thrombin selective inhibitors: a) **16** ($\Delta pK_i = 1.750$) with thrombin specific K60 and mutated Q221R residues; b) **1** ($\Delta pK_i = 1.607$) with thrombin specific K60 and conserved T149 residues. Trypsin selective inhibitors: e) **67** ($\Delta pK_i = -1.176$) with catalytic H57 residue; f) **58** ($\Delta pK_i = -1.419$) and **61** ($\Delta pK_i = -1.060$).

Nε atom of the catalytic H57 was likely to occur. Inhibitors **58** and **61** disclosed selectivity towards trypsin. This finding was probably related to the steric hindrance and length of the R2 substituent that would badly fit the space available in the specific P subsite of thrombin (Fig. 2d). Interestingly, this trend was also observed in the case of other trypsin inhibitors (i.e., **37**, **42**, **54** and **57**).

As a second main objective, the 3D-QSAR thrombin model obtained from *@lic&* was challenged for its ability to fish active hits from an external benchmarking set of 260 freely available thrombin ligands. Among these, 165 were annotated with experimental pK_i values while 95 came from the DUD database [7]. With the aim of minimizing random retrieving and of performing an even stringent test, benchmarking structures shared the same identical benzamidine core being both physically and topologically similar to 3D-QSAR training set compounds. Hit-inhibitors (81 out of 260) were designated only those molecules having known pK_i values higher than 6.50 ($K_i <$ 316 nM) while all the remaining were considered as decoys (179 out of 260). As normally done in standard docking screen, ligand enrichment among top-ranking hits was assumed as a key metric for assessing 3D-QSAR performances. Maximum predicted pK_i solutions from 3D-QSAR and top-scored poses from docking were herein used in the enrichment experiments. To this end, Figure 3a profiles the percentage of hit-inhibitors as a function of the percentage of the ranked benzamidine pool. The line 2 and 3 indicated enrichments obtained through 3D-QSAR and docking (i.e., GOLD) while the line 4 represented the baseline random selection. Interestingly, it was observed that the line 2 (i.e., 3D-QSAR) was pretty dominant even when screening small percentages (i.e., less than 30%) of ranked database. This concept became even clearer by profiling the receiver operator characteristic (ROC) curve which related the term [1 – specificity] (i.e., % of selected

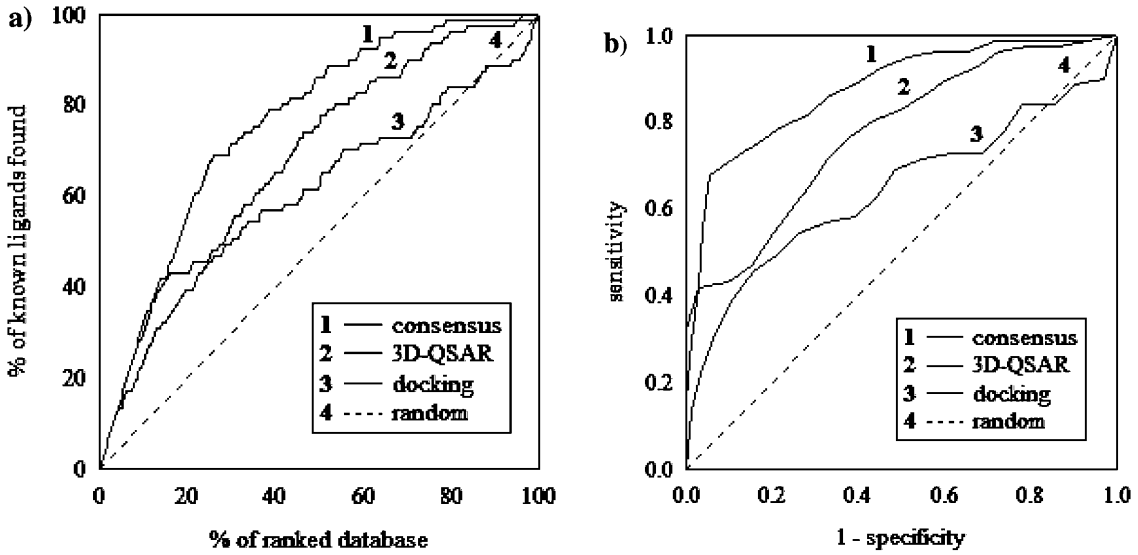

Figure 3. Enrichment plot (a) and ROC curve (b) profiling consensus (line 1), 3D-QSAR (line 2), docking (line 3) and random (line 4) retrievings.

decoys) vs. sensitivity (i.e., % of selected hit-inhibitors). As illustrated in Figure 3b, our 3D-QSAR model denoted a higher sensitivity in comparison to docking. As a further confirmation, a larger area under curve (*AUC*) was obtained for 3D-QSAR ($AUC = 0.775$) than for docking ($AUC = 0.642$). Interestingly, visual inspection of hits revealed that 3D-QSAR was able to correctly rank true active molecules with remarkable structural similarity to training set. In this perspective, our 3D-QSAR model would serve as a useful support for designing focused combinatorial libraries and as a diagnostic protocol for their successful screening. For completeness, consensus scoring analysis [8] was also performed. Among the fusion rules, the consensus by minimum yielded better results and, thus, an overall improved hit retrieving ($AUC = 0.877$) was obtained.

Conclusions

The development of *@lic&* represented a successful effort to overcome some intrinsic limitations of docking and 3D-QSAR methods. The program proved to be particularly suited to generate robust statistical models capable of explaining both binding affinity and, more importantly, binding selectivity. The present investigation resulted two key results. The first is the ability to determine the putative reasons for enzyme selectivity on the basis of occurrence of nonequivalent interactions of diversely oriented inhibitors into the active sites of the two distinct serine proteases. The selectivity plot revealed that inversion of selectivity was never observed in the case of strong selective inhibitors and that good performances were observed even for all compounds belonging to the test set. The second result is the increased sensitivity of 3D-QSAR in retrieving active hits. This would hopefully have the greatest pragmatic impact in the designing of focused chemical libraries of enzyme inhibitors and, more importantly, in their successful screening. In our ongoing research, *@lic&* is being further improved by implementing a multiobjective [9] fitness function.

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