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Combining structure-based drug design and pharmacophores

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Abstract

Development towards integrated computer-aided drug design methodologies is presented by utilising crystal structure complexes to produce structure-based pharmacophores. These novel pharmacophores represent the ligand features that are involved in interactions with the target protein, as well as the space around the ligand occupied by the protein. The protein–ligand complexes can also yield information about all interactions that ligands could potentially form with the binding site, as well as about the size of the binding cavity. Together, these describe a 'superligand', which can also be viewed as a pharmacophore. Various types of novel pharmacophores are discussed and compared, using HIV-1 reverse transcriptase (RT) as the target protein, and their application in database searching is presented.

Keywords: Drug design; Ligand-based pharmacophore; Structure-based pharmacophore; Novel pharmacophores; Database searching

1. Introduction

Traditionally, the first consideration before embarking on a computer-aided drug design (CADD) project is whether the detailed three-dimensional structure of the drug target is known. This determines whether a ligand-based (QSAR, CoMFA, pharmacophore) or a structure-based approach (docking, de novo ligand design) is undertaken to generate new lead compounds, which are then evaluated in an iterative process. This methodology proceeds to the selection of a small number of the best candidates, which are synthesised or purchased and tested for activity at the target. The results are then fed back into the CADD process.

The strict separation of ligand- and structure-based CADD methods has numerous drawbacks. Most ligand-based strategies propose and evaluate potential lead compounds so as to conserve the three-dimensional arrangement of functional groups on a scaffold believed to be most important in the activity of existing ligands. This precludes the discovery of novel ligands, which undertake different interactions with the target protein. However, docking methods, where a potential new ligand is placed into the binding site of the target and its 'fit' evaluated are computationally expensive, especially if induced fit of both ligand and protein are evaluated. Conformational changes, especially large scale changes, in the protein upon ligand binding are often ignored in these studies. Structure-based methods are also limited by the availability of detailed structures of the target, ideally in different conformations, with and without ligands complexed to it.

We propose that integration between ligand- and structure-based CADD methodologies which model separate facets of the natural system will allow us to use all available information in a particular drug design project in a quantitative and objective way.

Other such combinations of computational tools have been utilised by different groups to augment the capabilities of the individual tools: 3D QSAR and receptor modelling [1], pharmacophores and molecular docking [2,3], pharmacophores and receptor modelling [2,4], pharmacophores and pseudoreceptor modelling [5,6] and pharmacophores and 3D QSAR with excluded volumes from crystallographic protein structures [7,8], as well as structure-based pharmacophores from crystal structures [9,10]. Some groups have developed in house software [11,12], e.g. 'Relibase', a database system

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designed to analyse protein–ligand complexes from the Protein Data Bank (PDB, [13]). Relibase incorporates ligand similarity and substructure searches of data from the PDB.

2. Methodology

We have developed a method that utilises a three-step process to generate a 'superligand' pharmacophore that accounts for protein flexibility. It involves the creation of ligand binding pockets that describe the movements of pocket residues upon ligand binding, which are then enhanced with pharmacophoric features. The applicability of our method was tested using HIV-1 reverse transcriptase. Strategies for database searching with this feature rich pharmacophore are also presented.

2.1. Defining the residues of the binding pocket

A rational and impartial definition of the residues comprising the binding pocket was generated for structures obtained from crystallography or NMR. The residues of the binding pocket were defined by studying the solvent accessibility of all residues within a defined radius of the ligand. The ligand was removed and the binding pocket solvated. Solvent accessibility and thereby the likelihood of being part of the binding pocket was determined by summation of the number of residues within a certain radius of each solvent molecule.

2.2. Consideration of protein flexibility

Protein flexibility was considered via the creation of different binding pockets with residue coordinates representing the 'average', 'largest' and 'smallest' movements residues displayed upon inhibitor binding. These pockets were not meant to represent actual conformations that the pocket might adopt, but rather to estimate the range of movements that each pocket residue realised when different inhibitors were bound to the pocket. An activity-weighted pocket could also be calculated by taking into consideration the activity of the inhibitor bound to a particular crystal structure.

2.3. 'Superligand' generation and database searching

Pharmacophoric features and excluded volumes were added to the pockets. Manual selection processes were used to edit and reduce the number of features. Database searching could then be performed using a sequential query process where the number of structures to be searched was successively reduced and the complexity of the queries increased.

Our methodology was applied to HIV-1 RT. Numerous high resolution crystal structures are available of the HIV-1 RT enzyme complexed with various non-nucleoside inhibitors, with DNA fragments, as well as of the uncomplexed enzyme (see [14] for a listing and also see [13], which allows a search of all published crystal structures of the enzyme, including references). This enzyme is thus highly suitable for structure-based approaches and we [15] and others (see [14] for a listing) have already performed such studies in the traditional way. There are also a large number of studies investigating non-nucleoside inhibitors of HIV-1 RT with classical ligand-based methods (see, for example [16], and references therein).

The HIV-1 RT enzyme presents a challenging drug design target, because it is a particularly flexible protein. We want to account for this flexibility by looking at complexes of the protein with various ligands and compare these to the unliganded protein. A composite binding site, represented by the surface area encompassed by the inhibitors, has previously been created for 9 RT/inhibitor complexes [17]. This was compared qualitatively to small molecules docked and minimised in a single structure of RT.

The other challenge presented by the HIV-1 RT protein as a drug target is its high mutability, which confers high drug resistance. One approach to the design of 'mutation resistant' inhibitors has consisted of the design of ligands making extensive main chain hydrogen bonding contacts with the enzyme [18]. We intend to work towards 'mutation resistant' inhibitors by analysing the whole binding pocket and concentrating on new interactions which have not been utilised by known inhibitors, particularly interactions involving mutation resistant side chains in the binding pocket which have never been mutated in any of the structures of resistant enzymes.

3. Experimental

The methodology described above was applied to HIV-1 RT.

3.1. Defining the residues of the binding pocket

Identification of the residues that form the binding pocket in each of the RT/inhibitor complexes was performed with 19 crystal structures (Table 1). Residues within a 25 Å radius of the inhibitor were defined for each crystal structure and all other protein residues were deleted. Using the biopolymer module of Sybyl (Tripos) [19], hydrogens and charges were added (Kollman_All charge set), and the hydrogens were minimised (Powell method, Tripos forcefield, termination after 500 iterations or RMS force <0.05 kcal mol^{-1} Å^{-1}).

The inhibitor was removed from its binding pocket and the SiteID module of Sybyl was then used to solvate the truncated protein with a single layer of water molecules. The number of non-hydrogen atoms lying within an 8 Å radius (default) of each solvent molecule was counted. If this exceeded 75 (default) the solvent molecule was retained, otherwise it was removed. Two series of parameters were

Table 1
The RMSD of RT crystal structures after superimposition onto unliganded RT (1dlo) using the DDM derived superimposition subset (1528 atoms)

PDB file	RMSD (Å)	Research group
1hmv	0.878	Harvard I
1rtd	0.733	Harvard II
2hmi	0.997	CABM
1bqm	1.770	CABM
1bqn	1.809	CABM
1hni	1.807	CABM
1hnv	1.823	CABM
1klm	0.967	OCMS
1rev	1.204	OCMS
1rt1	1.035	OCMS
1rt2	0.975	OCMS
1rt3	1.173	OCMS
1rt4	1.081	OCMS
1rt5	1.082	OCMS
1rt6	1.052	OCMS
1rt7	1.073	OCMS
1rth	1.142	OCMS
1rti	1.033	OCMS
1tvr	1.829	CABM
1uwb	1.855	CABM
1vrt	1.304	OCMS
1vru	1.385	OCMS
1dlo		CABM

The different research groups and their associates that resolved the crystal structures have also been noted; Center for Advanced Biotechnology and Medicine (CABM), Rutgers University, New Jersey; Oxford Centre for Molecular Sciences (OCMS), Oxford; Harrison research group, Department of Molecular and Cellular Biology, Harvard University, Cambridge (Harvard I); Verdine research group, Department of Chemistry and Chemical Biology, Harvard University, Cambridge (Harvard II).

used to characterise the binding pocket, by varying the type of surface depressions considered as pockets. A small pocket (series 1) was defined using default parameters, including the minimum pocket concavity (MPC) (default of 2, flat pocket) and the van der Waals bump scaling factor (default of 0.8 Å). A large pocket (series 2) was defined using a more lenient set of parameters. Default parameters were used except MPC set to 10 (narrow well) and bump scaling factor set to 0.7 Å. Finally, all protein atoms lying within a 3 Å radius of each retained solvent atom in the pockets identified as above, were characterised as solvent accessible and considered as belonging to the binding pocket. In series 1, the known non-nucleoside inhibitor binding pocket was, in several cases, identified as being composed of two pockets separated by a non-solvent volume—these were combined. The number of times a particular residue was listed as part of a binding pocket was tallied (the maximum tally being 19 for a residue always identified as being part of the binding pocket) (Table 2).

The binding pocket was refined by comparing residue conservation across all the crystal structures. The final pocket was formed by truncating the residue list to only those residues with a tally greater than seven for the larger pocket (series 2), or if the tally was greater than seven in series 1, which only occurred with Ile142. The remaining pocket comprised 63 residues ensuring complete identification of potentially important residues. Further, six additional residues (identified by an * in Table 2), cited in the literature as having contact with inhibitors, but not identified by SiteID, were also included. The residues of the defined pocket were divided into two sets to be applied to database searching. The set called 'primary features' contained residues with a tally of greater than 10 in the smaller pocket (series 1), as well as those identified in the literature as having hydrogen bonding interactions with inhibitors. The remaining residues were designated 'secondary features'.

3.2. Consideration of protein flexibility

From the data generated above, several different binding pockets were constructed to account for the flexibility of the enzyme. To generate the coordinates for these modified pockets, the original crystal coordinates of each protein were superimposed upon the unliganded crystal structure (1dlo), utilising a superimposition subset of residues, which do not move upon binding of inhibitors and/or substrates to the RT enzyme. The superimposition subset was previously derived using our difference distance matrix approach [14]. The superimpositions were performed using Insight II (Accelrys) [20]. All residues except those identified as part of the binding pocket (Table 2) were then deleted. The coordinates of the remaining structures were then saved in the orientation obtained after superimposition.

The average location for the backbone and side chain of each residue of each crystal structure was calculated and its distance from a central inhibitor reference point was determined. The crystal structure with the minimum and maximum distance values for the backbone and side chain of each residue was noted. 'Average', as well as 'largest' and 'smallest' pocket structures with respect to the backbone or side chain displacements were created by using the complete coordinates for each residue from the appropriate crystal structure, without resorting to expensive dynamics calculations.

An activity 'weighted' pocket was created by taking into consideration the activity of the inhibitor bound to a particular crystal structure by multiplying the coordinates of each residue in a crystal structure by a weighting factor.[1] The pocket structures were saved in pdb format suitable for the generation of structure-based queries. Only studies with the 'weighted' pocket are presented below, as it allows an indirect comparison with the techniques and results of our ligand-based pharmacophore [16].

[1] The coordinates for each residue were summed up over all crystal structures and divided by the sum of the weighting factors. The weighting factor was calculated from the inverse of the logarithm of IC_{50} values in nM, normalised in such a way that the least active compound had a weight of one.

Table 2
List of all the residues defined as forming the binding pocket in one or more of the 19 RT-inhibitor crystal structures, by being in contact distance (3 Å) of a solvent sphere; A refers to the p66 subunit and B refers to the p51 subunit of RT

Residue details	Parameters		Literature cited	Features	
	Series 1	Series 2		Primary	Secondary
Trp 88 A	4	11			x
Glu 89 A	8	9			x
Val 90 A	7	12			x
Gln 91 A	13	17		x	
Leu 92 A	9	13			x
Gly 93 A	12	18		x	
Ile 94 A	9	18			x
Pro 95 A	12	19		x	
His 96 A	4	17			x
Pro 97 A	1	13			x
Gly 99 A	5	15			x
Leu 100 A	13	19		x	
Lys 101 A	14	18		x	
Lys 102 A	1	1	*	x	
Lys 103 A	9	18			x
Val 106 A	8	18			x
Gln 161 A	14	19		x	
Ser 162 A	5	8			x
Met 164 A	1	8			x
Thr 165 A	9	17			x
Leu 168 A	1	7			x
Arg 172 A	8	12			x
Val 179 A	17	19		x	
Ile 180 A	14	19		x	
Tyr 181 A	16	18		x	
Gln 182 A	13	19		x	
Tyr 183 A	7	19			x
Met 184 A	6	16			x
Asp 186 A	1	10			x
Leu 187 A	0	8			x
Tyr 188 A	13	18		x	
Val 189 A	1	6	*	x	
Gly 190 A	3	9			x
Glu 224 A	–	–	*	x	
Pro 225 A	–	2	*	x	
Pro 226 A	–	–	*	x	
Phe 227 A	7	19			x
Trp 229 A	13	19		x	
Met 230 A	6	13			x
Tyr 232 A	2	9			x
Leu 234 A	1	18			x
His 235 A	2	9			x
Pro 236 A	1	6	*	x	
Tyr 318 A	7	14			x
Ile 380 A	3	7			x
Val 381 A	6	16			x
Ile 382 A	2	16			x
Pro 25 B	1	9			x
Leu 26 B	2	10			x
Ile 31 B	5	10			x
Thr 131 B	1	8			x
Ile 132 B	3	7			x
Pro 133 B	6	15			x
Ser 134 B	13	18			
Ile 135 B	9	17			x
Asn 136 B	4	17			x
Asn 137 B	16	19		x	
Glu 138 B	19	19		x	
Thr 139 B	19	18		x	
Pro 140 B	15	19		x	
Gly 141 B	13	16		x	
Ile 142 B	8	3			x
Arg 143 B	5	8			x

The number of times that a residue is listed as part of the binding pocket for the first or second series of parameters (see text) is tallied out of 19. The residues included in the pocket definition due to references in the literature are noted by (*). All of the residues of the pocket are segregated into a primary or secondary features set (see text).

3.3. 'Superligand' generation and database searching

The Unity 3D module of Sybyl was used for the generation of structure-based queries. The residues of the 'weighted' pocket were searched for all possible hydrogen bond donor and acceptor features. The software then places and displays graphically a pharmacophoric feature at an optimal position for a putative ligand functional group to interact with the hydrogen bond donor or acceptor group on the protein. A manual selection process was then required to choose those pharmacophoric features suitable for inclusion in the structural query. Features projecting into the binding pocket were selected, while those that projected out of the pocket, and likely into the space of the protein that had been cut away during the initial preparation and definition of the pocket, were deleted. Hydrophobic residues could not be selected automatically, and so all of the aromatic residues listed in the pocket definition were selected to generate the corresponding hydrophobic feature of the ligand in the binding pocket. Aliphatic amino acid residues were not selected, as this would have increased the already large number of features in the binding pocket even further. Fig. 1 illustrates the query features used. Another significant element of the structural query was the inclusion of excluded volumes, which can be placed to represent residues of the binding pocket. The excluded volume features in Unity 3D permits the user to specify the radius of the excluded volume sphere around each atom. By reducing this value, one can allow for protein flexibility to some degree.

Three queries were created to sequentially screen the National Cancer Institute (NCI) database as implemented in Unity 3D (Flex Query) for the 'weighted' binding pocket. The first query generated used the two hydrophobic features interacting with Tyr181 and Tyr188, and the excluded volumes of all residues in the secondary feature set with the default van der Waals scaling factor of 1.0. The results were saved as a separate database, which was then searched with the second query. The second query generated was the same as the first query with the addition of all residues of the primary feature set, except Tyr181 and Tyr188, defined as excluded volumes with a van der Waals scaling factor of 0.5, resulting in a smaller database for the third query. The third query, illustrated in Fig. 2, was composed of all features

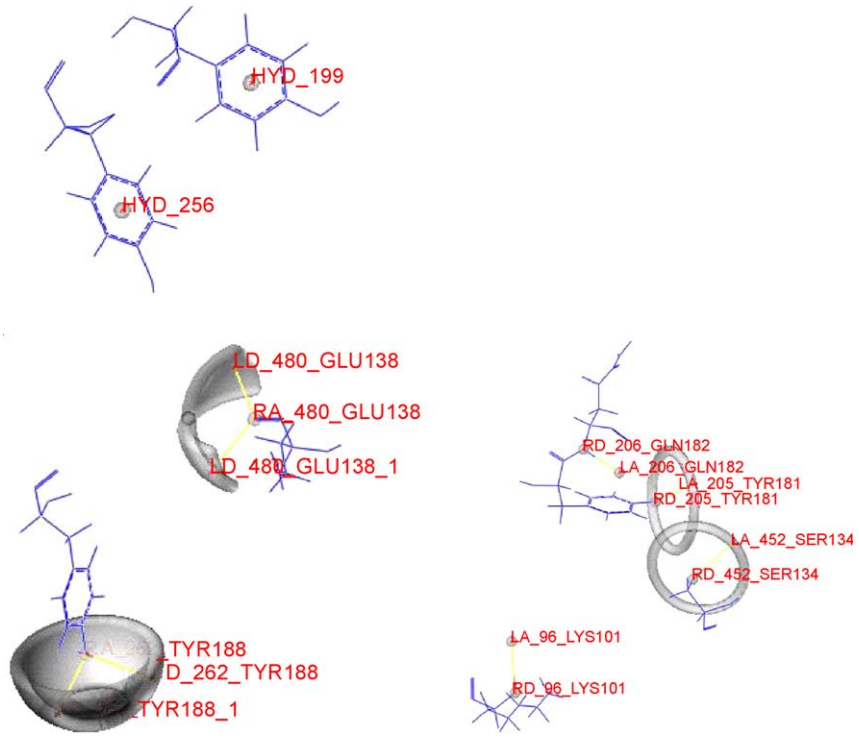

Fig. 1. (Left, top) Hydrophobic features for Tyr181 and Tyr188; the residues of the protein are shown in blue and the features of the query are shown in grey. (Left, bottom) Hydrogen bond acceptor functions on the residues of the protein project into the possible spatial volume (bifurcated cap for Glu138 and cap for Tyr188) in which a hydrogen bond donor function on the ligand could lie to make an intermolecular contact with the protein. (Right) Hydrogen bond donor functions on the residues of the protein project into the possible spatial volume (torus for Ser134 and Tyr181, and small spheres for Lys101 and Gln182) in which a hydrogen bond acceptor function on the ligand could lie to make an intermolecular contact with the protein.

generated, including 52 donor, 46 acceptor and 10 hydrophobic features. This search used the Partial Match Constraint Dialog, using default parameters with the exception of the minimum and maximum features required for acceptance of a hit.

This query can be described as a 'superligand', because it defines all potential binding interactions the residues comprising the pocket are capable of. Structural queries were saved in Sybyl mol2 file format, providing Cartesian coordinates and spatial information of the features of the query that could be used in other programs.

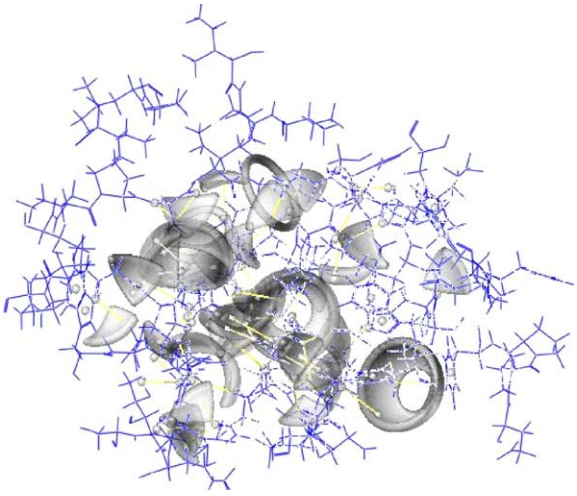

Fig. 2. Structural query 3, the superligand of the 'weighted' binding pocket. The residues of the defined binding pocket are seen in blue, while the features of the structural query are seen as grey volumes.

4. Results

The first stage of our integrated drug design process involved the generation of classical ligand-based pharmacophores using the standard tools within catalyst (Accelrys) [16]. The resultant pharmacophores were used to elucidate at least one of the mechanisms of action for the known anti-HIV agent gossypol.

Here, we present the next stage, the creation of structure-based pharmacophores, or 'superligands', which take into account all possible interactions between a ligand and a binding pocket, as well as the size of the binding pocket.

A common non-nucleoside inhibitor binding pocket (NNIBP) was defined from the data of 19 different RT-inhibitor complexes. An activity-weighted pocket was created to attempt to consider the flexibility of the enzyme, and to parallel the ligand-based pharmacophore development. Other binding pockets were also created, such as the 'largest' pocket, and could be converted into queries in exactly the same way as described for the activity-weighted pocket.

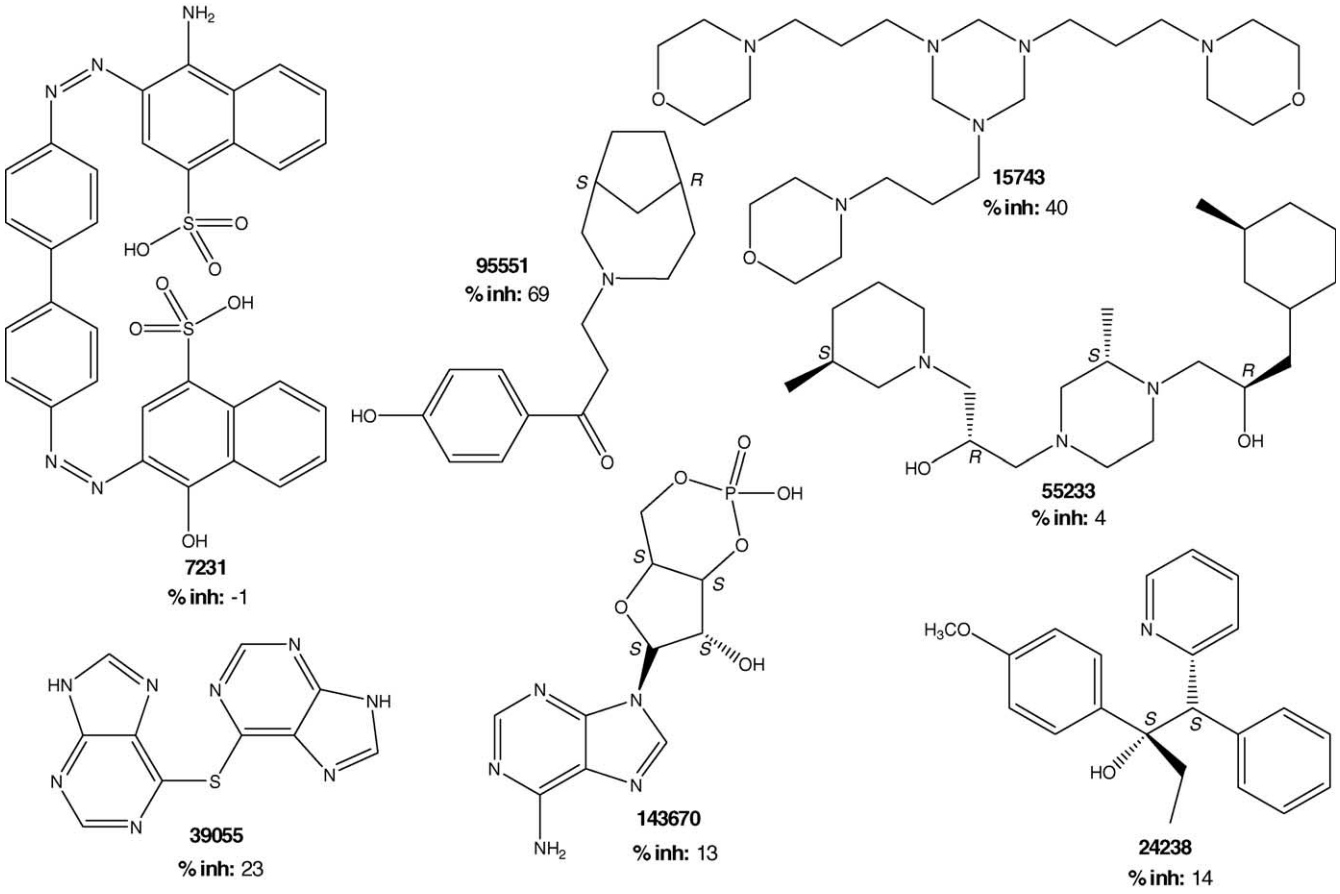

Fig. 3. Structures of NCI donated compounds selected from database search with NSC code number. Compounds were tested against HIV-1 RT [23]. Percent inhibition at 66 μg/mL is shown.

Queries were generated by including all hydrophobic interactions with aromatic amino acid side chains, and all side chains capable of hydrogen bonding in the pocket, to develop a 'superligand', a query, or structure-based pharmacophore, depicting all potential interactions a ligand could form with the residues of the NNIBP. For database searching, the NCI database was first filtered by size, so that only molecules which could potentially fit into the pocket and which contained at least two hydrophobic features in the correct relative orientation were retrieved. This was necessary, because it proved too computationally expensive to use a single query encompassing all features and excluded volumes to search the database while allowing conformational flexibility to all database ligands, as well as the possibility of only matching a specified number of features (partial-match searching). The two tyrosines (181 and 188) used as anchor or reference points in the first query have been shown to provide the most significant intermolecular binding interactions with the known ligands in the pocket [21,22]. Searching the NCI database with this query resulted in 27,329 hits from 117,649 compounds. The second query refined this subset by size to 11,110 hits. For the first two searches, around 700 of the searched compounds 'timed out' each time. This meant that in the preset time of 90 s, no fit was found for these compounds, and the search algorithm moved on to the next structure, despite not having exhaustively searched all conformers of the timed out compound. The most likely reason for time outs at this stage was a large number of rotatable bonds in a compound and it was felt that these compounds would probably be too flexible to be of interest and thus they were discarded. The third query search of the refined database subset allowed for partial matches, where a minimum of 8 and a maximum of 16 features were required to fit for a compound to be considered a hit. This search returned 172 hits. Because of the considerable computational effort required for this search, a large number of compounds (7537) timed out.

From the 172 hits thus obtained, a number of compounds were selected for biological testing, using the following criteria. The activities of these compounds were estimated with our ligand-based pharmacophores, but this was only a small point of consideration in the selection. Additional criteria included structural diversity, flexibility and rigidity, size and molecular weight. Familiar structural motifs encountered in antiviral natural products and current anti-HIV drugs, such as several aromatic rings, a possible 'butterfly' conformation, and dimeric structures, were also taken into account. Ten compounds were thus selected and

requested from the NCI, seven of which were donated. These seven compounds, shown in Fig. 3, were tested against HIV-1 RT [23]. Most compounds exhibited nominal inhibition at 66 μg/mL, with NSC 95551 having the highest activity of 69% (Fig. 3). NSC 7231 was not active in this assay despite anti-HIV data being given in the NCI Development Therapeutics Program database [24]. Testing of further compounds is currently under investigation.

5. Discussion and conclusions

This work has taken a significant step towards the full integration of ligand- and structure-based computer-aided drug design methodologies. This has been achieved by creating a 'superligand', a structure-based pharmacophore incorporating all possible interactions ligands can form within a binding pocket, as well as information about the size of the pocket. By creating different 'superligand' pharmacophores, we believe we can also take the flexibility of the binding pocket into account, not just in terms of its size, but also in terms of the different orientations observed for the amino acid side chains that are potential binding partners for ligands. We have shown how such a 'superligand' pharmacophore can be used to search databases of compounds in a way similar to the use of classical ligand-based pharmacophores in database mining.

Problems remain in the integration of the results of our ligand-based (pharmacophore) [16] and structure-based (superligand) studies to create our target 'combiphores'. We have successfully converted the structure-based queries of this study into a format suitable for importation into the catalyst pharmacophore development software [25]. Further elaboration will use these 'superligand' pharmacophores within catalyst, and compare them to the ligand-based pharmacophores or to fit compounds onto them. Preliminary attempts with catalyst find no possible fits, presumably because the algorithm does not allow a compound to miss out on the majority of the features of the pharmacophore. Furthermore, database searching was not possible with these structure-based pharmacophores with the version of catalyst available to us, as it did not allow the necessary partial match searching. Even the partial match-searching algorithm in the later catalyst versions (4.7 and 4.9) appears to allow the ligand to miss out only one feature.

To improve integration between the ligand- and structure-based pharmacophores we are investigating the possibility of 'importing' our ligand-based pharmacophores from catalyst to unity. Cross searching could then be attempted. That is, searching a selection of compounds considered hits on the ligand-based pharmacophore with the structure-based pharmacophore and vice versa. The complete exploration of the binding interactions of a pocket should provide us with the ability to target the residues of a protein that have not previously been utilised in interactions with ligands. In the case of the NNIBP of HIV-1 RT this should enable us to design novel agents effective against mutant strains of the enzyme resistant to present drugs.

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