Approved Drug Mimics of Short Peptide Ligands from Protein Interaction Motifs

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Received May 19, 2008

Most biological functions are regulated through complex networks of transient protein interactions, and, thus, finding effective ways to modulate them would represent an important step towards defining the next generation of drugs. In this study, we set out to determine if existing approved drugs may represent a good source of compounds from which initial lead inhibitors of protein−protein interactions mediated by short peptide regions may be drawn. Peptide structures were defined in terms of pharmacophores and searched against U.S. Food and Drug Administration (FDA)-approved drugs to identify similar compounds. The top ranking matches (using a score that corrects root-mean-square deviation (rmsd) for the number of matched pharmacophores and for the number of drug rotatable bonds) included a number of nuclear receptor ligands that matched allosterically to the corepressor binding site of peroxisome proliferator-activated receptor alpha (PPARα). The top ranking drug matches were docked to the peptide-binding site using AUTODOCK. The majority of the top-ranking matches showed a negative estimated free energy change upon binding that is comparable to, or greater than, that of the original peptide. We conclude that the usage of certain approved drugs may represent a useful strategy in inhibiting specific protein−protein interactions. Such a strategy may benefit from the increased likelihood that developed compounds might have favorable bioactivity and safety profiles in clinical use.

INTRODUCTION

Many proteins exert their biological roles as components of complexes, and the functions of proteins are often determined by their specific interactions with other proteins. Because of the central importance of protein−protein interactions (PPI) for cellular processes, the ability to interfere with specific PPIs provides a powerful means of influencing the functioning of pathways within the cell. PPIs are central to most biological processes—from intracellular communication to programmed cell death—and therefore represent a large and important class of targets for human therapeutics. While the size of typical PPI surfaces may have initially discouraged targeting such surfaces, the realization that the majority of the binding energy is in some cases quite localized has encouraged the development of small molecule inhibitors.[1] This is most evident for PPIs that are primarily mediated by domain-oligopeptide interactions.[2] Such interactions may be typically more druggable than domain-domain interactions, since the interaction surface is not only typically smaller[3] but also more easily defined. Examples of such drugs include the nutlins, *cis*-imidazoline analogs that inhibit the interaction of murine double minute protein 2 (MDM2) and p53, by mimicking the p53 α helix.[4] Other examples are the small-molecule anti-integrin mimetics of the Arg-Gly-Asp (RGD) tripeptide.[5] Small organic modulators of PPIs are therefore highly desirable tools for the study of physiological cellular processes and for the treatment of disease.[6] The Eukaryotic Linear Motif (ELM) database[7] describes a number of such linear motifs, typically of 3−10 residues in length, that form the basis of protein−protein interactions. The structural conformation of such motifs is often disordered in the free protein but takes on a particular structure when bound to its partner. For a number of these motifs, the structure of the motif in complex with its bound partner is available either from X-ray crystallography or nuclear magnetic resonance spectroscopic (NMR) studies.

Challenges to overcome in identifying clinically useful small molecule modulators of PPIs include the structural characterization of binding pockets involved in PPIs and identifying templates and early druglike hits. Here, we take a set of peptide-domain interactions which have been structurally characterized by X-ray crystallography or NMR and investigate whether one group of druglike molecules, the set of approved drugs, represents a useful library of scaffolds that may target such interactions.

METHODOLOGY

The approach has five main steps. First, identification of all cases of peptide-mediated PPIs of known three-dimensional (3D) structure; second, creation of pharmacophore queries based on the 3D structures of known eukaryotic linear motifs (ELMs) in complexes; third, creation of a conformer library of FDA approved drugs; fourth, searching the pharmacophore queries of the motifs against the FDA conformer library to define and rank potential drug mimics

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10.1021/ci800174c CCC: $40.75
Published on Web 10/01/2008

Table 1. List of Peptide Classes Used for Building 3D Pharmacophore Queries

peptide (ELM database[7] name)	regular expression	PDB ID
LIG_14−3−3_1	R[SFYW].S.P	1QJB
LIG_14−3−3_2	R.[SYFWTQAD].[ST].[PLM]	1QJA
LIG_AP2alpha_1	F.D.F	1KY7
LIG_AP2alpha_2	DP[FW]	1KYD
LIG_Clathr_ClatBox_2	PWDLW	1UTC
LIG_CORNRBOX	L[^P]{2}[HI]I[^P]{2}[IAV][IL]	1KKQ
LIG_CYCLIN_1	[RK].L.{0,1}[FYLIVMP]	1H27
LIG_Dynein_DLC8_1	[KR].TQT	1F95
LIG_EVH1_II	PP..F	1DDV
LIG_FHA_1	T..[ILA]	1K3N
LIG_GYF	[QHR].{0,1}P[PL]PP[GS]H[RH]	1L2Z
LIG_HOMEOBOX	[FY][DEP]WM	1B8I
LIG_HP1_1	P[MVLIRWY]V[MVLIAS][LM]	1S4Z
LIG_IQ	...[SACLIVTM]..[ILVMFCT]Q.{3}[RK].{4,5}[RKQ]..	1M45
LIG_MDM2	F...W..[LIV]	1YCQ
LIG_NRBOX	[^P](L)[^P][^P](L)(L)[^P]	1M2Z
LIG_PCNA	(^.{0,3}|Q).[^FHWY][ILM][^P][^FHILVWYP][DHFM][FMY]..	1AXC
LIG_PDZ_1	.[ST].[VIL]$	1BE9, 1L6O, 1Q3P, 1RGR, 1VJ6, 2FNE, 2PDZ
LIG_PDZ_2	.[VYF].[VIL]$	1U38, 1V1T
LIG_PDZ_3	.[DE].[IVL]	1ZUB, 2PDZ
LIG_PP1	..[RK].{0,1}[VI][^P][FW].	1S70
LIG_RB	[LI].C.[DE]	1GH6
LIG_SH3_1	[RKY]..P..P	1AZG, 1GCQ, 1K4U, 1W70
LIG_SH3_2	P..P.[KR]	2BZ8
LIG_SH3_3	...[PV]..P	1GCQ
LIG_Sin3_1	[LIV]..[LM]L.AA.[FY][LI]	1E91
LIG_TPR	EEVD$	2C2L
LIG_TRAF2_1	[PSAT].[QE]E	1D0J
LIG_TRAF2_2	P.Q..D	1CZY
LIG_WW_1	PP.Y	1EG4
LIG_WW_4	...[ST]P.	1I8G

of the ELMs; and fifth, evaluation of matching pharmacophores by docking.

Identification of Peptide-Mediated PPIs of Known 3D Structure. To detect all cases of peptide-mediated protein interactions of known 3D structure, we first parsed the PDB and identified all those entries containing two or more interacting proteins. We extracted all the information regarding the 66 different types of peptide ligands involved in peptide-mediated interactions from the ELM database and assigned Pfam families[8] to all the globular domains involved in the interactions via literature curation. We then used BLAST (E-value ≤ 0.01) to assign Pfam families to all interactions of known 3D structure. Whenever we identified a protein chain containing an ELM-binding domain, we searched all contacting chains for occurrences of the linear consensus motif. If we found a motif match in close vicinity of the globular domain (≤ 10 Å), we considered it as a potential domain-peptide interaction. Finally, we went manually through the 2200 potential hits, comparing the interacting structures to those described in the literature and removing false positives where the interaction was not mediated by the consensus peptide.

To avoid composition biases, we initially created a nonredundant set of interactions by clustering those pairs sharing a 100% sequence identity on, both, the domain and the peptide. Second, we selected a subset of 41 interactions which spanned 30 ELMs. The criteria used in selecting these were to ensure each ELM class had at least one representative structure available and was represented at least once, to avoid having too many representative structures from one ELM class and to favor X-ray crystal structures over NMR.

Creation of Pharmacophore Queries. The pharmacophore queries were built using MOE's pharmacophore tool based on the 3D structures of the peptide motifs in complexes (NMR or X-ray crystal structures reported in the Protein Data bank (PDB)). We built 41 pharmacophore queries encompassing the 30 ELM classes selected (Table 1). We have built one pharmacophore query for each PDB entry. A pharmacophore scheme defines the set of attributes used to construct labeled ligand annotation points that can be matched to the query. The pharmacophore points were defined based on the interaction points of the ELMs with the parent protein. Each query was built based on a single ELM instance, rather than constructing a consensus pharmacophore across multiple instances. Certain ELMs are represented by more than one pharmacophore representation, which typically share some features but differ in other features (see Supporting Information Table S2).The features in the pharmacophore query were built using the PCH_All Scheme (Polar-Charged-Hydrophobic scheme for all atoms: hydrophobic features coincide with centroids of hydrophobic rings, chains and groups; donors and acceptors do not include tautomeric cases; and cations and anions include resonance cases). The PCH_All scheme (Supporting Information Table S2) has the following ligand annotation points, Don: H-bond donor, Acc: H-bond acceptor, Don & Acc: H-bond donor as well as H-bond acceptor, Cat: cation (excess positive charge), Ani: anion (excess negative charge), Aro: aromatic

Table 2. List of the Best Matching Hits[a]

PDB ID	peptide sequence	matched ph4 rmsd (Å)[b]	ratio matched to total ph4s	RBC[c]	drug name	Pubchem ID	score	AUTODOCK binding energy (rmsd, Å)[d]	AUTODOCK peptide binding energy[e]
1KKQ	LEAIIRKAL	0.28	1	7	testosterone enanthate	9416	−0.42	−7.1 (1.96)	−4.49
1KKQ	LEAIIRKAL	0.37	1	5	drisdol	5280793	−0.33	−6.8 (1.71)	
1KKQ	LEAIIRKAL	0.40	1	5	depovirin (testosterone cipionate)	6012	−0.30	−7.5 (1.32)	
1KKQ	LEAIIRKAL	0.40	1	5	doxercalciferol	5281107	−0.30	−6.7 (1.67)	
1KKQ	LEAIIRKAL	0.41	1	5	clocortolone pivalate	36673	−0.29	−6.0 (1.81)	
1KKQ	LEAIIRKAL	0.41	1	6	calcifediol	5280447	−0.29	−6.7 (1.76)	
1KKQ	LEAIIRKAL	0.43	1	5	calciferol	5284358	−0.27	−7.3 (1.69)	
1CZY	PQQATDD	0.33	0.83	10	capreomycin	3000502	−0.27	−1.8 (2.38)	−4.24
1KKQ	LEAIIRKAL	0.43	1	5	calcipotriol	5282134	−0.26	−6.7 (2.01)	
1KKQ	LEAIIRKAL	0.43	1	5	paricalcitol	5281104	−0.26	−7.0 (1.78)	
1KKQ	LEAIIRKAL	0.43	1	10	retabolil	9677	−0.25	−7.0 (2.24)	
1KKQ	LEAIIRKAL	0.44	1	5	cortexone M	13126	−0.25	−6.8 (1.54)	
1KKQ	LEAIIRKAL	0.44	1	7	digitoxin	3061	−0.25	−7.3 (4.45)	
1KKQ	LEAIIRKAL	0.47	1	6	calcitriol	5280453	−0.22	−6.4 (1.97)	
1CZY	PQQATDD	0.18	0.67	10	capreomycin	3000502	−0.21	−1.8 (2.38)	
1KKQ	LEAIIRKAL	0.49	1	6	pipecuronium	50192	−0.20	−6.1 (2.23)	
2FNE	ETSV	0.34	0.62	5	gadoteridol	60714	−0.20	−2.3 (4.47)	−4.13
1KKQ	LEAIIRKAL	0.49	1	9	raplon	177969	−0.19	−5.7 (3.04)	
1KKQ	LEAIIRKAL	0.50	1	7	hydroxyprogesterone caproate	3653	−0.19	−5.8 (1.91)	
1VJ6	VTSV	0.35	1	5	gadoteridol	60714	−0.19	−5.4 (2.99)	−6.13

[a] Those with the lowest score between FDA drugs with ≤10 rotatable bonds, where at least 60% of the searched pharmacophore points searched are matched, and drugs match ≤10 of the searched peptides. [b] Matched pharmacophore (ph4) rmsd: This represents the rmsd between the pharmacophore spheres of the compound and peptide. [c] RBC: rotatable bond count. [d] AUTODOCK rmsd: results are based on local docking to binding region. rmsd represents the shift in rmsd of the compound from the initial pharmacophore placement for all heavy atoms of the compound (the model presented is the model among the top ten most energetically favored with the lowest rmsd). In parentheses is the estimated free energy of binding for the drug in kcal/mol. [e] AUTODOCK peptide binding energy: estimated free energy of binding for the native peptide in kcal/mol.

ring center, and Hyd: hydrophobic region. The pharmacophore queries for the ELM 3D structures were then searched against the pharmacophore preprocessed FDA conformer database using MOE's pharmacophore matching algorithm. Only one pharmacophore query was constructed for each peptide structure, taking the conformation derived from the X-ray crystallographic model or from the consensus NMR model. Details of the actual matches are given in Supporting Information Table 2.

Creation of FDA Conformer Library and Pharmacophore Preprocessing. The conformer library of FDA drugs was created in-house using molecular operating environment (MOE),[9] by linking the FDA "Orange" list (Approved Drug Products with Therapeutic Equivalence Evaluations)[10] to PUBCHEM. We used MOE's conformation import option, which constructed conformers using a parallelized fragment-based approach: molecules are subdivided into overlapping fragments each of which is subjected to a rigorous stochastic search. The fragment conformations were rapidly assembled by superposing the overlapping atoms. In order to reduce the computational requirements, we limited the number of conformations to 50 per compound. Conformations were modeled in vacuum. The failure limit was increased to 100, i.e., the algorithm will search for conformers until it reaches the 100th attempt. Thus, the algorithm checks whether the atom positions of the current conformer are within the rms tolerance of the atom positions of a saved conformer 100 times. With greater failure limit, the chance of getting the minimum energy conformer is higher. The default value is 20, but we increased it to 100 to make it more stringent. Merck molecular force field MMFF94[11] was chosen for molecular mechanics calculations.The pharmacophore finger prints for the FDA conformers were calculated using the PCH_All scheme. The PCH_All scheme has the following ligand annotation points, Don: H-bond donor, Acc: H-bond acceptor, Don & Acc: H-bond donor as well as H-bond acceptor, Cat: cation (excess positive charge), Ani: anion (excess negative charge), Aro: aromatic ring center, and Hyd: hydrophobic region. The pharmacophore queries were then searched against the pharmacophore preprocessed FDA conformer library to look for possible hits. The searches were done such that initially we searched for drugs that perfectly match the queries, and if no such drugs were returned, we also searched for partial matching.

Statistical Treatment of the Results To Identify Top Hits. We found that, within each ELM class, rmsd showed a significant linear relationship with the number of pharmacophore points matched (Figure S1) and the number of rotatable bonds in the drug. We wanted to correct the rmsd values for these sources of variation, and therefore we defined a score for results returned for a given peptide search as the residuals of a linear regression of rmsd against the number of points matched and the number of rotatable bonds. A large negative score implies a match with a much lower rmsd than would be expected for the same values of the predictor variables. The final ranking of matches is based on this score.

Docking of Drugs to Peptide Binding Proteins. We took the compound-protein pairs in the shortlisted Table 2 and performed docking experiments using AutoDock4.[12] For each compound we used an initial spatial configuration corresponding to the matched pharmacophore points and ran the docking experiment with default settings as generated by AutoDockTools utility scripts, which assigned hydrogens and partial charges to ligand and receptor. The protein portion was immobile, but the ligand was permitted to sample conformations other than those predicted by the pharmacophore constraints. AutoDock's potential energy grid maps were centered on the protein's peptide binding site and

extended 11.5 Angstroms along each of the coordinate axes which confined the ligand movements to that region. The numerical docking resulted in 10 final poses for each compound, ranked according to free energy changes. The rmsd to the initial (reference) state was recorded to verify whether the docked ligands remained in the vicinity of the binding site. The native peptides for each domain in Table 2 were also docked, starting from 3D structure coordinates, to establish a comparison for favorable free energy of binding.

RESULTS AND DISCUSSION

We compiled 41 pharmacophore representations of 30 distinct classes of peptides based on the determined structure of their complexes with peptide-binding domains[13] (Table 1). A pharmacophore[14] is the ensemble of features necessary to ensure the optimal supramolecular interactions with a specific biological target structure and to trigger (or to block) its biological response. The pharmacophore comprises chemical features such as hydrogen bonding and electrostatic and hydrophobic interactions among others and can be used as a query for database searching.[15,16] The 41 derived pharmacophores were then searched against a data set of 1253 FDA approved drug compounds, to check for similar features. Pharmacophores defined in both peptides and approved drugs were H-bond donor, H-bond acceptor, H-bond donor/acceptor, cation, anion, aromatic ring center, and hydrophobic region. Drugs with an rmsd lower than 1.0 were retained for further analysis. A statistical score was chosen that ranks compounds highly if they match many of the defined pharmacophores, with a low root mean squared deviation (rmsd) and with a low number of rotatable bonds (calculated as the residual from the regression of rmsd versus number of pharmacophores matched and number of rotatable bonds; see Methods; Supporting Information Figure S1(a). The linear model accounting for both number of rotatable bonds and number of pharmacophores matched predicts much of the variation in the observed data (Figure S1(b)), with a correlation of 0.82 between the observed and predicted rmsd.

Figure 1 visually summarizes the findings of the searches. Given the clear tendency of some compounds to match many peptide models, we sought to focus on compounds that did not, in particular compounds that had fewer rotatable bonds and with a better coverage of the identified pharmacophore points. Table 2 indicates the top 20 matches for drugs with $\leq$10 rotatable bonds, where at least 60% of the searched pharmacophore points searched are matched and where the drug matches $\leq$10 of the searched peptides. These top 20 hits were identified for 4 matched peptide models. To evaluate further these matches, we carried out molecular docking using AUTODOCK4,[12] as an independent test to check whether the identified drugs would indeed fit in the peptide-recognition regions. These docking models indicated that, in general, the energy of binding was of approximately the same level as that seen for the original peptides docked in the original pose defined by their identified X-ray structure (Table 2). This indicates that that the ligands do indeed have some potential to form a stable complex with the peptide-binding sites. The compounds identified in Table 2, and further compounds listed in Table S1, provide potential scaffold compounds from which novel drugs targeting PPIs may be developed.

A noticeable feature of the matched compounds is the similarity between a number of drugs, which are themselves ligands of nuclear receptors (NRs) and the corepressor peptide that binds the PPARα nuclear receptor. These NR binding drugs fall into three classes: (a) androgen receptor agonists[17]—testosterone enanthate, depovirin (testosterone cipionate), and retabolil; (b) vitamin D receptor ligands—drisdol, doxercalciferol, calcifediol, calcitriol, calciferol, and paricalcitol; and (c) corticosteroids—clocortolone pivalate, and cortexone M. We note that the mean of the best scores for the 18 NR ligands in the data set was significantly lower than the mean of the best scores for the 294 other compounds with matches to the peptide classes (nonparametric mean difference test (Mann−Whitney) $p = 0.0002$). The result is a little surprising, since the corepressor is binding in these searches not to the well studied ligand-binding pocket but to a nearby site that is normally thought to bind peptides. While this finding raises the possibility that NR ligands could bind additionally to the corepressor site of the NR, to date no structural studies have identified this mode of action. Figure 2 shows the binding of the corepressor peptide (CORNRBOX motif) to the PPAR receptor[18] and illustrates the suggested binding of the drug hit testosterone enanthate to PPAR α at the corepressor binding site, based on the initial pharmacophore matching. We note that testosterone enanthate did not provide such a good match to the coactivator (NRBOX) peptides (Table S1(a)).

We found that although the nuclear receptor targeting drugs appear to have affinity for the nuclear corepressor site, docking them to the more hydrophobic internal pocket of the ligand binding domain reveals that each compound has a more favorable binding energy for the ligand-binding pocket (ranging from −8.6 to −10.5) compared to the corepressor site (Table 2). This is expected as the internal binding pocket is homologous to the receptor sites in which those ligands naturally bind, and being more fully surrounded by hydrophobic residues, it is expected that the hydrophobic contribution to the binding energy is significantly higher. If there is any biological relevance of nuclear receptor targeting drugs binding to the corepressor site, it is likely to involve much weaker binding compared to the ligand-binding site.

To validate the method used, we have carried out a pharmacophore search based on the query for a modified version of the RGD (Arg-Gly-Asp) peptide [PDB ID: 1l5g chain C]. The interaction points in this peptide were analyzed by the ligand interaction tool in MOE and LIGPLOT.[19] The drug, tirofiban, a known RGD mimetic is included in the FDA database. The RGD Ph4 query was searched against the entire FDA conformer database. Tirofiban matches with 5 out of the 7 Ph4 points included in the query for RGD with an rmsd of 0.56. While tirofiban is ranked relatively high (23rd out of the 273 FDA compounds with 5 out of 7 matches that came out as hits for RGD Ph4 query), it is clear that based on this single example, the method we have applied is likely to only enrich for true positives but not to unequivocally identify them. A larger validation data set would be desirable in the future; however, currently such a data set of drugs or compounds mimicking known ELMs, along with the relevant structures, is not available.

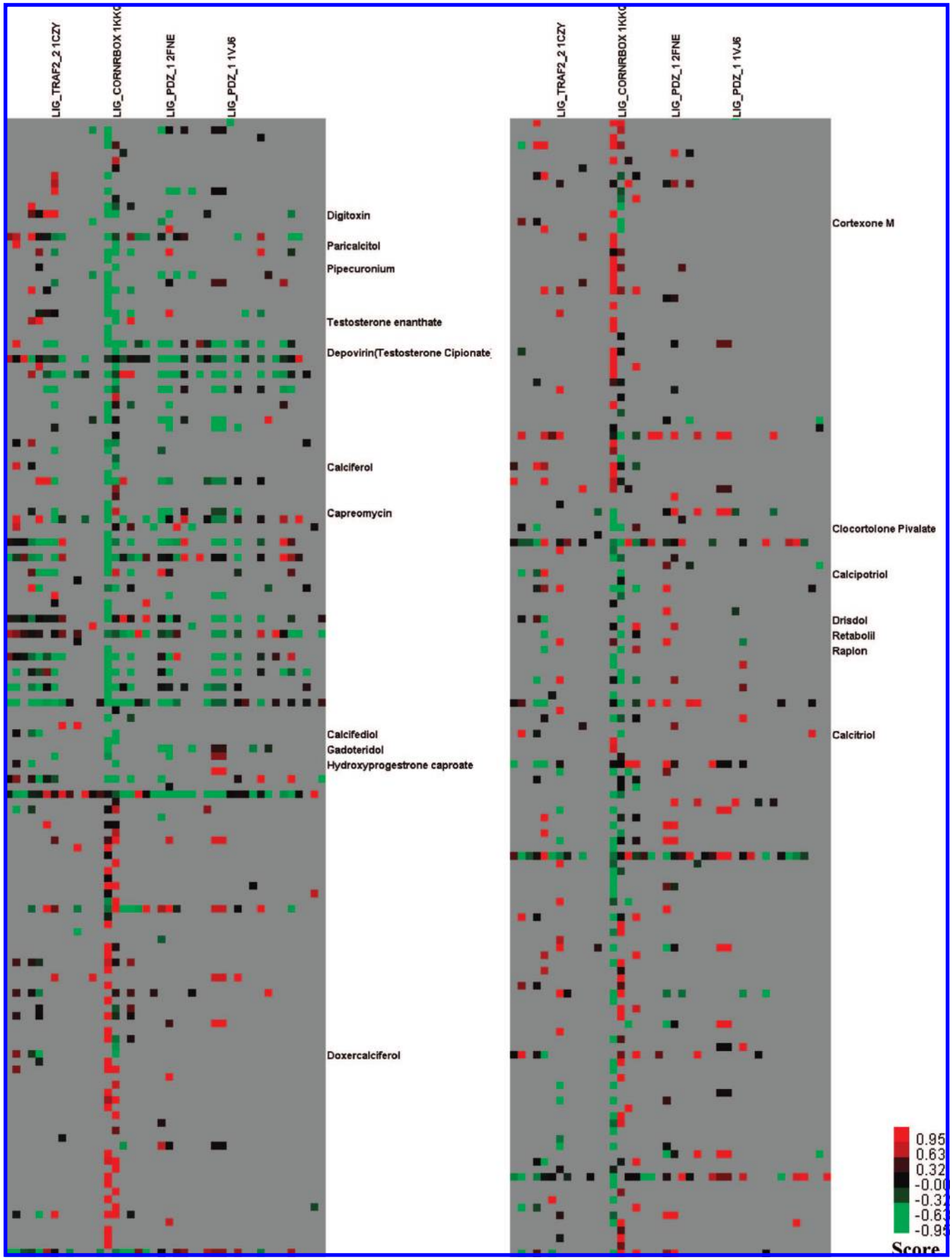

Figure 1. Heat map of peptide versus FDA searches. Column names: ELM name for peptide plus PDB ID for structure. Row names: drug name. Most significant matches are in bright green. Labels for rows and columns are given for data points indicated in Table 2.

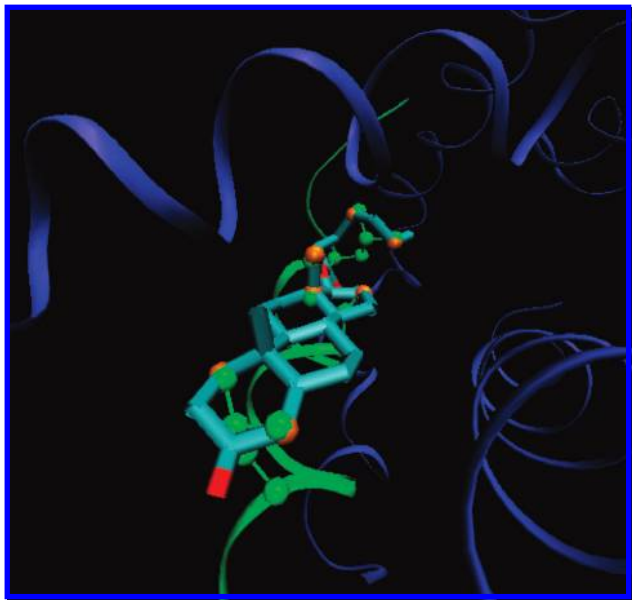

Figure 2. Superimposition of pharmacophore-matched compound on peptide binding to domain. The top ranking testosterone match (tubular) is shown superimposed on the PPARα ligand-binding domain (1KKQ) structure (blue ribbon) and the CORNRBOX motif containing peptide (green ribbon). All seven pharmacophore points in testosterone, colored orange, are matched to corresponding atoms in CORNRBOX peptide.

This survey illustrates the potential for comparison among known PPIs and approved drugs to identify templates for future drug development targeting PPIs. If drugs based on these ligands are to be developed that mimic or antagonize the action of peptide binding, they are likely to require modification of side chains not only to stabilize the potential interactions suggested in the models but also to eliminate potential activity through the drug's usual mechanism of action.

This study has been restricted to short linear motif interactions. The logic for this is that short motif interactions are often sufficient to induce weak binding, without a role for additional regions involved in protein−protein interactions. However, the findings are likely to be relevant for a wider range of protein interaction regions, since short bioactive peptides that mediate protein−protein interactions are not restricted only to those that have been identified as recurrent motifs.[20]

Developing drug compounds based on scaffolds of existing drugs has the benefit that the safety and bioavailability profiles of any such developed compounds may be closer to those of approved FDA compounds, compared to compounds obtained from other libraries. Many approved compounds are larger than typical lead compound libraries, and, therefore, they may provide a more reasonably sized scaffold for PPI inhibition.

ACKNOWLEDGMENT

This work was supported by Science Foundation Ireland and by an Irish Research Council for Science, Engineering and Technology fellowship (to F.C.). A.S. and P.A. were supported by the Spanish Science Ministry (BIO2007-62426).

Supporting Information Available: Results for peptides versus FDA drug compound pharmacophore searches (a comma-delimited file, compatible with Microsoft Excel) (Table S1), pharmacophore features defined on the basis of interactions (includes motif and context) (Table S2), and (a) relationship between the rmsd and the number of matched pharmacophore points between peptides and drugs and (b) observed RMSDs for each pharmacophore hit versus the regression model rmsd prediction based on the number pharmacophore points and rotatable bonds of the match (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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CI800174C