Design of Small-Sized Libraries by Combinatorial Assembly of Linkers and Functional Groups to a Given Scaffold: Application to the Structure-Based Optimization of a Phosphodiesterase 4 Inhibitor

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Received January 21, 2005

Combinatorial chemistry and library design have been reconciled by applying simple medicinal chemistry concepts to virtual library design. The herein reported "Scaffold-Linker-Functional Group" (SLF) approach has the aim to maximize diversity while minimizing the size of a scaffold-focused library with the aid of simple molecular variations in order to identify critical pharmacophoric elements. Straightforward rules define the way of assembling three building blocks: a conserved scaffold, a variable linker, and a variable functional group. By carefully selecting a limited number of functional groups not overlapping in pharmacophoric space, the size of the library is kept to a few hundred. As an application of the SLF approach, a small-sized combinatorial library (320 compounds) was derived from the scaffold of the known phosphodiesterase 4 inhibitor zardaverine. The most interesting analogues were further prioritized for synthesis and enzyme inhibition by FlexX docking to the X-ray structure of the enzyme, leading to a 900-fold increased affinity within nine synthesized compounds and a single screening round.

Introduction

With combinatorial chemistry as a tool, infinite variations on a core template are theoretically possible.[1] However, in the drug discovery process, it is desirable to gain the maximum of information[2] out of a minimum of experiments. For the medicinal chemist, this means the optimization of a screening library, i.e., a minimal size with a maximal chemical diversity. Hence, focused libraries designed around a selected scaffold can only span a wide range of physicochemical and structural properties, when the decorations are diverse enough. To date, a lot of combinatorial structure generation tools have been developed and most of them are generating rather large virtual libraries.[3,4] However, awareness that the highest possible number of compounds do not automatically increase the hit rate and the fact that most of the generated molecules are synthetically not easily available make computational chemists apply a second algorithm to select a representative subset. Indeed, to stay in the order of magnitude of a hundred compounds,[5] virtual combinatorial libraries are assessed by different techniques such as Monte Carlo calculations,[6] genetic algorithms,[7] artificial neutral network, or simply statistical sampling with user-defined property ranges.[8]

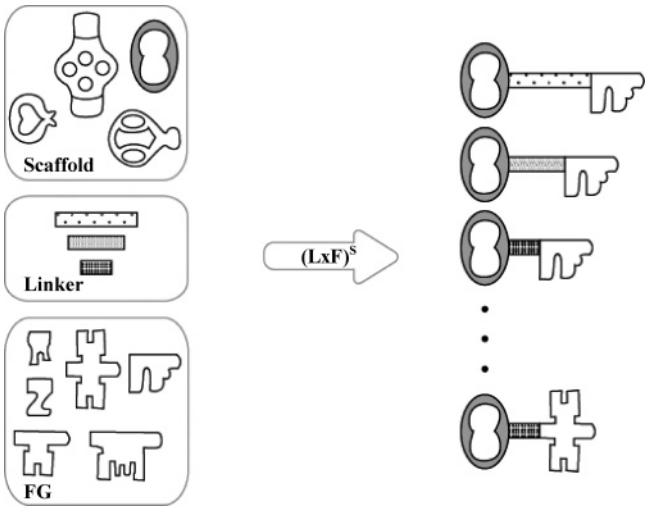

Figure 1. Schematic representation of the three types of molecular fragments and the assembly rule for the complete library enumeration.

In contrast to the latest, we propose an alternative approach to optimize size versus diversity that relies on the combinatorial assembly of user-selected building blocks: a scaffold, a linker and a functional group (Figure 1). Thus, each enumerated molecule can be considered as a chemical tool to probe the protein active site. Similar approaches published recently are implemented in COREGEN[9] and SMILIB.[10] On the basis of homology and molecular diversity concepts, combining a limited number of linkers and functional groups (cations, anions, hydrogen bond acceptor−donor systems, aromatics/lipophilics) easily affords small-sized polyfunctionalized compounds.[11,12]

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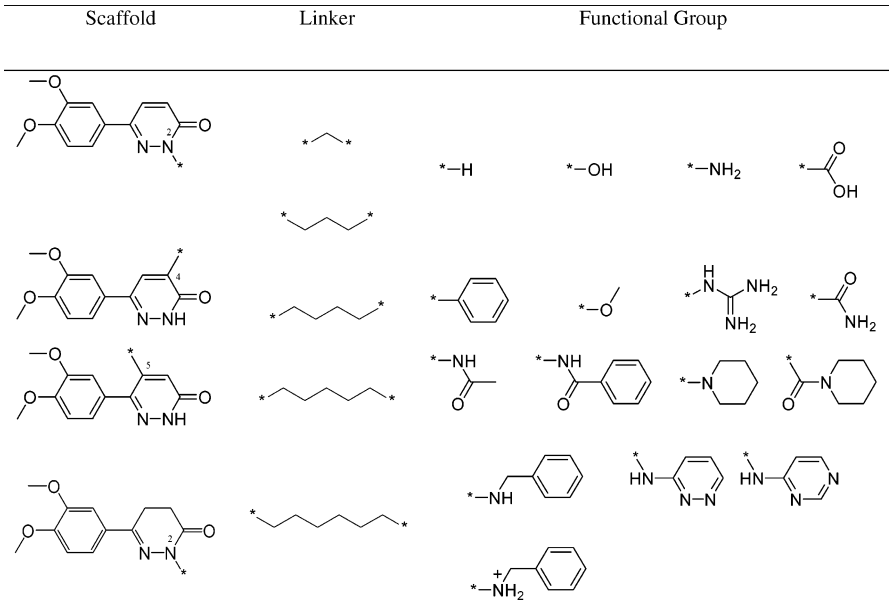
10.1021/jm050063y CCC: $30.25
Published on Web 05/07/2005

Table 1. Zardaverine-Derived Scaffolds, Linkers, and Functional Groups Used for the Combinatorial Enumeration of a PDE4-Focused Library



[a] * indicates a connecting pseudo-atom used for the combinatorial assembly of scaffolds, linkers and fragments.

Indeed, chemists succeeded over the years to apply combinatorial synthesis strategies to simple rings and chains to form small organic molecules[13,14] and not stay limited to peptide and oligonucleotide polymers. Thus, the virtual combinatorial library has to be designed in order to have its physical counterpart and to guarantee that all compounds are synthesizable. We herewith present the combinatorial assembly method encoded in the SLF_Libmaker program and its coupling to the structure-based prioritization of the most interesting compounds applied to the optimization of a known phosphodiesterase 4 (PDE4) inhibitor.

Results

Selection of the Most Appropriate Docking Tool. Predicting the best possible docking/scoring strategy from the simple knowledge of a protein binding site is still very difficult.[15] Therefore, three accurate docking engines[16] (FlexX, Gold, Surflex) in combination with four scoring functions (FlexXscore, Goldscore, Chemscore, Surflex) were selected for a preliminary study aimed at determining which X-ray structure (1q9m, 1mkd) is the most suitable and which docking strategy recovers the X-ray pose of zardaverine. Out of the three docking tools tested herein, FlexX was the only program able to predict with a reliable accuracy (below 2.0 Å rmsd) the X-ray pose of zardaverine, whatever the scoring function used and the protein coordinates (Table 2). FlexX was then selected for further docking the zardaverine-focused library, using the original FlexX score for primary sorting the virtual hits and the 1q9m coordinates of the holoprotein.

Setup and Docking of a PDE4 Focused Library. The molecular fragments (Table 1) were assembled according to the rules described above. Four scaffolds with a single substitution point were derived from zardaverine, replacing the difluoromethoxy by a methoxy group. Moreover, the unsaturated pyridazinone moiety was topologically explored at position N2, C4, and C5, whereas the dihydropyridazinone was substituted only at position N2 (Table 1). The linkers were chosen to be three odd-numbered (C_1, C_3, C_5) and two even-numbered polymethylene chains (C_4, C_6). The functional groups were finally selected for their pharmacophoric properties and for their synthetic feasibility. Three hundred twenty structures were altogether generated to be part of the virtual library that was docked against the PDE4 target. FlexX scores range from −42.9 to −13.5 kJ/mol, zardaverine being scored at −22.3 kJ/mol. For most of the structures, a single binding mode of the dimethoxyphenyl substructure, very close to that observed for zardaverine, was found (Figure 2). Other energetically favored binding modes were not discovered through visual inspection of all poses. Browsing the top-ranked pose of all compounds suggest that two additional pockets (named A and B in Figure 2) could be targeted by numerous compounds. Hitlist prioritization was then achieved by selecting any compound whose Flex score was lower than −15 kJ/mol and for which the rmsd of the dimethoxyphenyl substructure from that of zardaverine in its X-ray pose was lower than 1.0 Å. The latter filter was simply used in order to avoid the selection of misdocked false positives. Nine compounds exploring additional pockets A and B unoccupied by zardaverine were finally selected, synthesized, and tested (Tables 3 and 4). Seven out of these nine compounds were N2-substituted dihydropyridazinones exploring two additional pockets of PDE4 not investi-

Table 2. Docking of Zardaverine to Two 1mkd and 1q9m Coordinates of Human PDE4

docking/scoring method	1mkd		1q9m	
	best score[a]	best rmsd[b]	best score	best rmsd
FlexX/FlexScore	0.66	0.66 (1)	1.02	0.51 (7)
Gold/GoldScore	8.14	6.44 (29)	10.04	7.46 (10)
Gold/ChemScore	6.73	1.40 (7)	1.39	1.27 (3)
Surflex	7.05	1.49 (3)	5.84	5.56 (4)

[a] Best-scored solution. [b] Solution with the lowest rmsd from the X-ray pose. The ranking of the corresponding pose is indicated in commas.

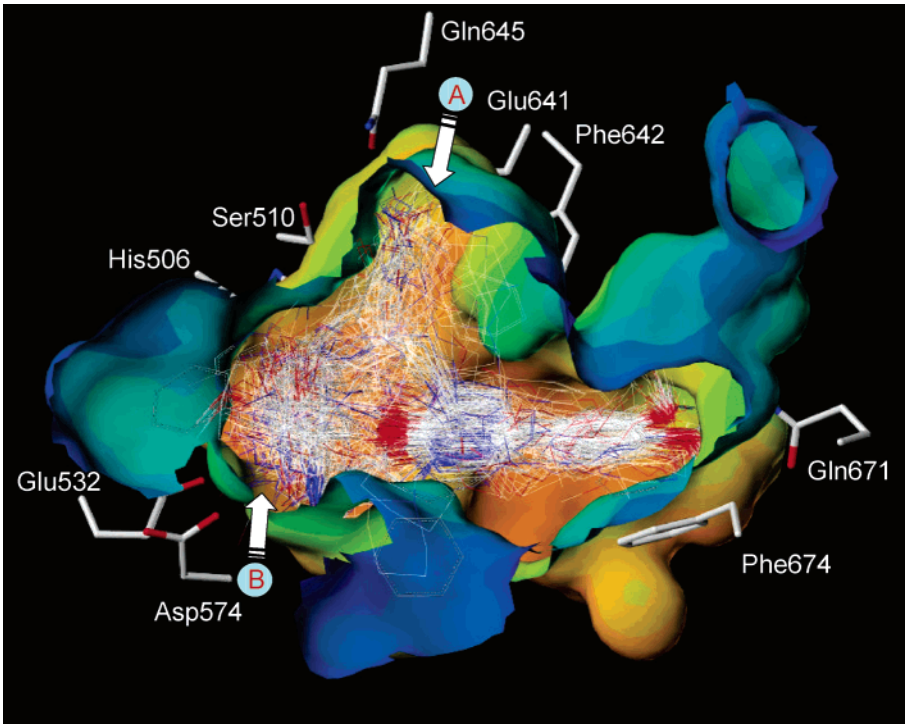
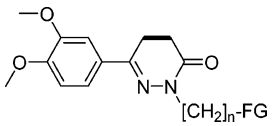


Figure 2. Docking of a scaffold-based library of 320 compounds into the X-ray structure of the human PDE4 catalytic domain. The best-ranked pose of each compound is displayed as a color-coded wireframe in the active site of PDE4D represented as a MOLCAD[37] solid surface color-coded by cavity depth (blue → yellow: accessible → buried surfaces). Important side chains are displayed as capped sticks and labeled at the Cα atom. Subsites A and B are indicated by white arrows.

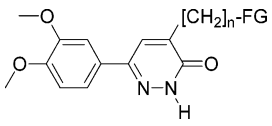
Table 3. PDE4 Inhibition of Compounds **1**−**8**



compound	functional group (FG)	*n*	FlexX score[a]	IC_{50}, nM[b]
1 (zardaverine)			−22.31	800
2	H	0	−20.84	2000
3	Ph	1	−19.45	60
4	Ph	3	−17.17	20
5	Ph	5	−15.63	0.9
6	Ph	6	−16.85	80
7	NH_2	6	−26.84	20
8	NHC(=NH)NH_2	6	−20.74	60000

[a] FlexX score, in kJ/mol. [b] The IC_{50} was calculated by nonlinear regression and represents the mean value of three independent determinations. The experimental error is about 15%.

Table 4. PDE4 Inhibition of Compounds **9** and **10**



compound	functional group (FG)	n	FlexX score[a]	IC_{50}, nM[b]
9	Ph	1	−22.08	8000
10	Ph	3	−20.40	>10000

[a] FlexX score, in kJ/mol. [b] The IC_{50} was calculated by nonlinear regression and represents the mean value of three independent determinations. The experimental error is about 15%.

gated by either zardaverine or rolipram. A first hydrophobic channel (His462, His506, Phe642; site A) topped by polar side chains (Glu641, Gln645) favors a phenyl ring one to six carbon atoms from the N2-pyridazine ring (Figure 3B). A second negatively charged subsite around Glu532 and Asp574 (site B) favors basic amines (primary amine or amidine) six carbon atoms from the N2 pyridazine ring (Figure 3C). The last two selected compounds (Table 4) belong to the series of 4-substituted pyridazinones, with again a phenyl ring connected via one or three carbon atoms to the C4 pyridazine atom and predicted to interact with the above-mentioned hydrophobic channel after 180° rotation of the dihedral angle linking the dimethoxyphenyl moiety to the pyridazine ring.

PDE4 Inhibitory Potency. Out of the nine synthesized compounds, five exhibit a stronger in vitro inhibition of bovine smooth muscle PDE4 than zardaverine **1**. Considering the very high sequence conservation among PDE4s in mammals, we can expect very similar results with the human PDE4 that has been modeled in the current study. A significant enhancement of enzymatic inhibition is observed by adding a phenyl ring at various distance (one to six carbon atoms) from the N2-pyridazine atom, the best inhibitor being compound **5**, bearing a phenylpentyl substituent and exhibiting a subnanomolar IC_{50} (Table 3). A potent inhibitor (compound **7**, IC_{50} = 20 nM) combining a hexyl linker and a primary amine functional group was also discovered (Table 3). Surprisingly, the corresponding amidine **8** was found much less potent. Last, the two 4-substituted pyridazinones **9** and **10** (Table 4) were much less active than the corresponding 2-substituted dihydropyridazinones **3** and **4** and show only micromolar affinities for the PDE4.

Discussion

We herewith present a simple and straightforward method to design small combinatorial libraries while

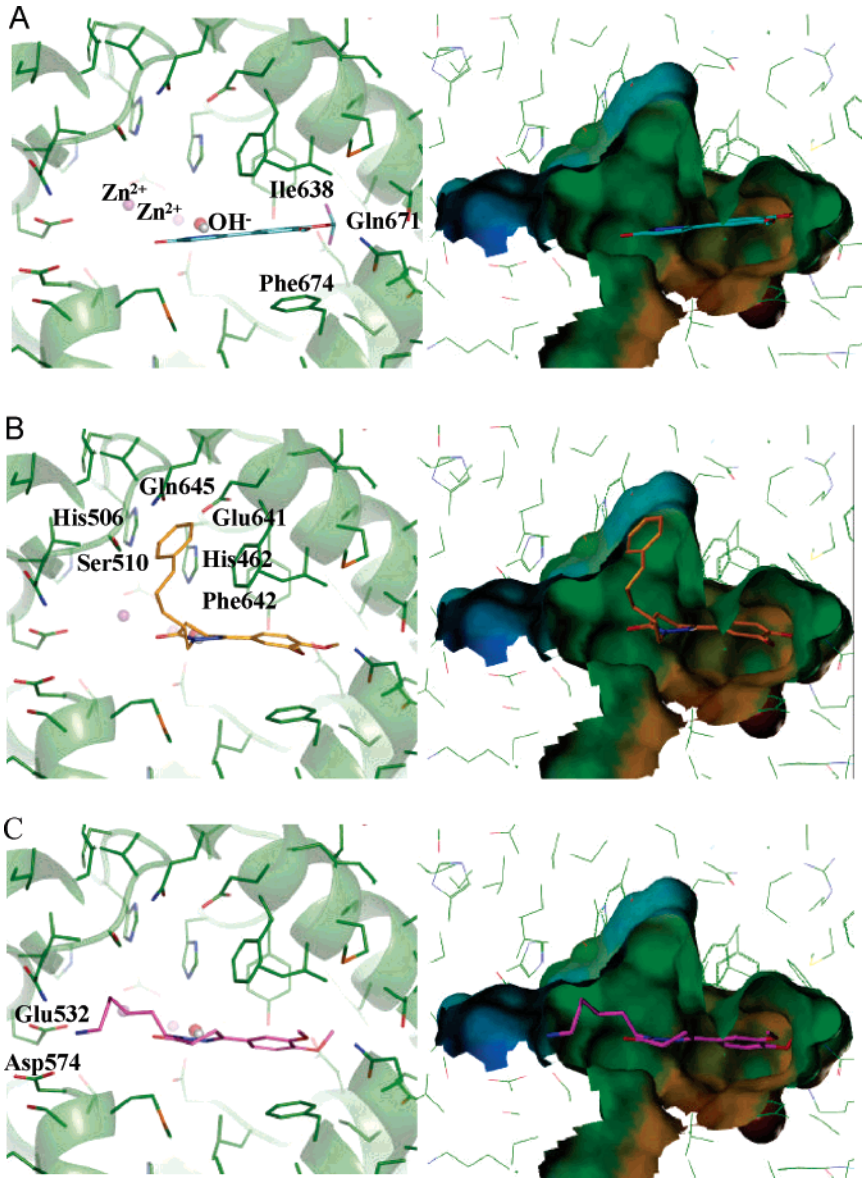


Figure 3. Close up to the human PDE4 inhibitor binding site filled with zardaverine **1** (A, X-ray pose), compound **5** (B, FlexX best-scored pose), and compound **7** (C, FlexX best-scored pose). Left panels represent the inhibitor (zardaverine, cyan; compound **5**, orange; compound **7**, magenta) bound to PDE4 (green). Zn^{2+} and OH^{-} ions are displayed by balls. The molecular surface of the binding site was rendered using the SYBYL implementation of MOLCAD[37] and color-coded by hydrophobicity (brown → blue: hydrophobic → hydrophilic). The view was prepared with PyMol version 0.95 (http://www.pymol.org).

optimizing size versus diversity. A key advantage of the SLF approach is that diversity is encoded in a simple pharmacophoric space. The method relies on three building blocks that are all under the control of the user: an invariable scaffold, a variable linker, a variable functional group. The linker has the simple role of varying the distance between the core of the molecule (the scaffold) and a few functional groups carefully selected to cover all possible intermolecular interactions. Therefore, additional interactions may be gained either locally or at a remote site within a single round of library design.

The concept of enumerating combinatorial libraries by assembling building elements (scaffolds, ring systems, linkers, building blocks) has been recently described in several methods.[9,10] COREGEN[9] is a fragment-based design method for assembling linkers and rings frequently occurring in known kinase inhibitors. By decomposing a molecule into R ring-building blocks and L linkers, a combinatorial library of R * L * P compounds (P being the number of positions that can be derivatized) is generated. SMILIB[10] assembles scaffolds, linkers, and functional groups in product-space. However, the latter do not necessarily describe the pharmacophoric space well. Thus, the combinatorial assembly is unrestricted and generates very large libraries unless subset selection according to user-defined queries (e.g. drug-likeness) is performed to reduce the size of the library. A basic difference with the above-mentioned methods is that our approach does not fully optimize the starting lead in a single round, but ensures at each design step a significant affinity gain by an incremental optimization of both the linker and the functional group. Once a linker-functional group combination has been identified in the first design round, both building blocks may be optimized in a second round to fine-tune the best possible combination by exploring the local chemical space around the selected building blocks.

The SLF approach has been applied to the structure-based optimization of a known micromolar PDE4 inhibitor, zardaverine. By carefully selecting, in agreement with medicinal chemists, a limited number of linkers and functional groups, a zardaverine-focused library of 320 compounds has been enumerated and docked to the X-ray structure of PDE4 according to settings previously known to reproduce the X-ray pose of zardaverine in the enzyme. The catechol substructure in both zardaverine and rolipram, two known PDE4 inhibitors, are positioned in the most hydrophobic subpocket of the active site between Ile638 and Phe-674.[17,18] Both ether oxygen atoms are involved in bifurcated hydrogen bonds to the side-chain of Gln671 (Figure 3A). The same binding mode is observed for the very large majority of compounds in the virtual library (Figure 2), indicating that unrestrained FlexX docking is able to properly locate most of these ligands in the protein active site. The advantage of the herein proposed library design is exemplified by a series of zardaverine-based compounds for which additional interactions with remote pockets have been disclosed (Figures 2, 3). N2-substituted pyridazines **3**−**6** (Table 3) interact with hydrophobic pocket A (His462, His506, Phe642) through a phenyl functional group that can be linked to the pyridazine core by polymethylene spacers of various lengths (one to six carbon atoms; Figure 3B). Compound **7** discloses another remote polar subsite B (Glu532, Asp574) through a primary amine six carbon atoms from the N2 pyridazine atom. The folded conformation of the spacer however suggests that shorter polymethylene spacers (e.g. butyl) may be appropriate as well.

In the current study, the design effort has only been focused toward potency for a given PDE. However, the lack of selectivity of most PDE inhibitors toward other PDE isoforms[19] and genes probably account for observed side effects such as emesis and arrhythmia, which dramatically restricts the clinical development of PDE4 inhibitors as antiinflammatory compounds.[20] Thus, it may be valuable to identify specific 3-D features in the selected PDE target to direct the design of potent and selective inhibitors. A systematic survey of the amino acid sequence of 21 human PDEs in the UniProt database[21] and a subsequent multiple alignment indicates that the acidic subsite B is fully conserved in all PDEs (data not shown). We therefore anticipate that the additional interactions gained by compound **7** will not affect its selectivity profile with respect to zardaverine. Conversely, hydrophobic subsite A targeted by compounds **3**−**6** shows some degree of variation among PDEs, especially at Ser510 and Cys660, as exemplified by the recently described crystal structure of phosphodiesterase 4B in complex with (*S*)- and (*R*)-rolipram.[22] The present data can thus be used to try and design potent and selective PDE inhibitors by simultaneously targeting the two remote subsites and directing the interaction with variable residues of the hydrophobic pocket A.

Conclusion

The SLF method allows medicinal chemists to use their knowledge in an iterative "design-synthesize-screen-analyze" process. There are still certain shortcomings to the current implementation of the method, and many further refinements are possible. For example, a user-defined 3-D conformation (e.g. X-ray conformation) of a scaffold could be selected as a rigid body to avoid incorrect conformer generation from a simple 1D representation (i.e. complex ring systems). Furthermore, the automatic detection of symmetry centers and/or axes would avoid the enumeration of duplicates and spare an additional postprocessing step. Last, a scaffold library designed from commercially available screening collections[23] will soon enable the choice of multiple scaffolds fulfilling similarity/diversity-based queries. The SLF method can be used for a fast lead optimization consisting of the systematic search of remote subpockets in the neighborhood of a given scaffold by optimizing both the length of the necessary linker and the nature of the terminal functional groups.

Materials and Methods

Virtual Library Construction. The three-fragment assembly rule is "scaffold, linker, functional group"[24] instead of a more common use of binary combinations of building blocks.[25] A more detailed look at the different building blocks is given as follows. Two hypotheses about the scaffold[26] are usually cited: (i) a suitable scaffold is believed to optimally orient the attached substituents for binding and (ii) the scaffold itself interacts with the protein as an anchor. The maximum number of possible connection points equals the number of removable hydrogen atoms, but the most encountered examples of scaffolds have from one to four substituted positions.

The linker has two substitutions points. Its main role is frequency variation, i.e., to modulate the distance between the molecular scaffold and the protein active site. For a first screening round, the linkers are chosen in the acyclic polymethylenic series (Scaffold-$[CH_2]_n$-FG; FG: Functional Group). Thus, by expanding an alkyl chain, the hydrophobicity of the molecule is increased.[27]

Functional groups represent basic pharmacophoric features resulting from steric, electronic, lipophilic, and H-bonding properties. "H" is always the reference substituent. The other substituents are smallest possible representative fragments and will mostly mix property information, e.g. carboxylate shows anionic or H-bond acceptor behavior depending on its interaction partner.

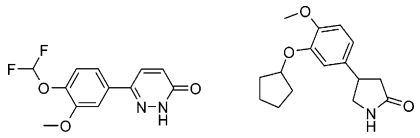
The complete number N of enumerated molecules can be expressed as:

$$N = (L \times F)^S$$

where L is the number of linker fragments, F is the number of functional groups, and S is the number of substitution points marked on the scaffold. The complete enumeration with a core structure showing a local symmetry in substitution points gives a lower number of unique structures according to the Pólya counting theory.[28] The complete enumeration method is implemented in SLF_LibMaker, a C++ program based on OpenEye's OEChem 1.3 library.[29] The molecular fragments (scaffolds, linkers, functional groups) are encoded as SMILES[30] or SDF[31,32] file formats with connecting pseudo-atoms represented by "*" in both formats. Depending on the user-defined selection of scaffolds, linkers, and functional groups in separate data files, the desired combinatorial library is assembled in SMILES or SD file format and converted into 3-D structures using Concord.[33] In the current example, a library of 320 compounds was built from a selection of 4 scaffolds, 5 linkers, and 16 functional groups, chosen in agreement with medicinal chemists. OpenEye's Filter[29] program was finally used to ionize compounds at physiological pH. For the specific case of the benzylamino linker, it should be noticed that both neutral and ionized states were explicitly considered.

Automated Docking. The crystal structures of the human phosphodiesterase 4D (PDE4D) catalytic domain in complex with zardaverine[18] and rolipram[17] (Chart 1) were retrieved from the Protein Data Bank (pdb entries 1mkd and 1q9m,

Chart 1. Structure of Two PDE4 Inhibitors



zardaverine (**1**) rolipram

respectively).[34] The numbering of the UniProt[21] entry CN4D_HUMAN (Q08499) was selected as a reference. These structures were used to generate two series of input coordinates including the holo-protein, the corresponding active site, and its native ligand. The protein active site was defined as the set of amino acids for which at least one atom is included in a 6.5 Å-radius sphere surrounding any non-hydrogen atom of the bound ligand. All metal ions were assigned as Zn^{2+} ions, although their real nature is still a matter of debate[17,35,36] and included in the binding site. All water molecules were removed, except the one supposed to be a hydroxide ion[4] that is thought to be a bridging element between the metal ions and the pyridazinone moiety of zardaverine. Atomic types and protonation states of protein atoms were manually checked. Hydrogen atoms were finally added by using the BIOPOLYMER module of SYBYL package.[37]

To determine which docking tool was the most appropriate in the current context, FlexX1.12,[38] Gold2.1,[39] and Surflex1.1[40] programs were used as previously described,[16] to reproduce the enzyme-bound pose of zardaverine. Docking was considered successful, when the best-scored pose was found within 2.0 Å root-mean-square deviation (rmsd) from the X-ray pose. Cross-docking of zardaverine to the 1q9m entry and of rolipram to the 1mkd structure was then achieved in order to select the best set of holoprotein coordinates for both inhibitors.

Full database docking was realized using the 1q9m coordinates and FlexX as described above. The final hitlist was prioritized (i) by FlexX-score; (ii) by analysis of binding modes achieved by a nearest-neighbor clustering of FlexX poses based on the Cartesian coordinates of the common dimethoxyphenyl substructure; and (iii) visual inspection of all compounds.

Synthesis. The synthesis of compounds **2**−**10** and structurally related molecules will be described elsewhere (M.S and J.−J.B., manuscript in preparation).

PDE4 Inhibition. PDE4 was isolated from the media layer of bovine aorta by anion exchange chromatography as previously described,[41,42] and its activity was measured at a concentration of 1 μM cAMP by radioenzymatic assay.[43] To prevent the interaction of contaminating PDE3 in the assay of isolated PDE4, studies were always carried out in the presence of 100 μM cGMP. New compounds were dissolved in DMSO or ethanol with a final concentration (1%) which did not significantly affect PDE activity. The inhibition study on PDE4 activity included six concentrations of the drug. The IC_{50} values were calculated by nonlinear regression using the Prism Software (GraphPad Software, Inc., San Diego, CA 92130).

Acknowledgment. Financial support (CIFRE grant no. 329/2002) of IDEALP Pharma (Villeurbanne, France) to M.K. and of CNPq/Brasil to J.X.A.-Jr. is acknowledged.

References

(1) Alper, J. Drug discovery on the assembly line. *Science* **1994**, *264*, 1399−1401.
(2) Bradley, E. K.; Miller, J. L.; Saiah, E.; Grootenhuis, P. D. J. Informative library design as an efficient strategy to identify and optimize leads: Application to cyclin-dependent kinase 2 antagonists. *J. Med. Chem.* **2003**, 4360−4364.
(3) Beavers, M. P.; Chen, X. Structure-based combinatorial library design: methodologies and applications. *J. Mol. Graphics Modell.* **2002**, *20*, 463−468.
(4) De Julian-Ortiz, J. V. Virtual Darwinian drug design: QSAR inverse problem, virtual combinatorial chemistry, and computational screening. *Comb. Chem. High Throughput Screening* **2001**, 295−310.
(5) Nilakantan, R.; Nunn, D. S. A fresh look at pharmaceutical screening library design. *Drug Discovery Today* **2003**, *8*, 668−672.
(6) Langer, T.; Wolber, G. Virtual combinatorial chemistry and in silico screening: Efficient tools for lead structure discovery? *Pure Appl. Chem.* **2004**, *76*, 991−996.
(7) Wright, T.; Gillet, V. J.; Green, D. V. S.; Pickett, S. D. Optimizing the size and configuration of combinatorial libraries. *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 381−390.
(8) Weaver, D. C. Applying data mining techniques to library design, lead generation and lead optimization. *Curr. Opin. Chem. Biol.* **2004**, 264−270.
(9) Aronov, A. M.; Bemis, G. W. A minimalist approach to fragment-based ligand design using common rings and linkers: application to kinase inhibitors. *Proteins* **2004**, *57*, 36−50.
(10) Schuller, A.; Schneider, G.; Byvatov, E. SMILIB: Rapid assembly of combinatorial libraries in SMILES notation. *QSAR Comb. Sci.* **2003**, *22*, 719−721.
(11) De Laet, A.; Hehenkamp, J. J. J.; Wife, R. L. Finding drug candidates in virtual and lost/emerging chemistry. *J. Heterocycl. Chem.* **2000**, 669−674.
(12) Bemis, G. W.; Murcko, M. A. The properties of known drugs. 1. Molecular frameworks. *J. Med. Chem.* **1996**, *39*, 2887−2893.
(13) Janvier, P.; Sun, X.; Bienayme, H.; Zhu, J. Ammonium chloride-promoted four-component synthesis of pyrrolo[3,4-b] pyridin-5-one. *J. Am. Chem. Soc.* **2002**, 2560−2567.
(14) Kuznetsov, V.; Gorohovsky, S.; Levy, A.; Meir, S.; Shkoulev, V. et al. Approaches for introducing high molecular diversity in scaffolds: Fast parallel synthesis of highly substituted 1H-quinolin-4-one libraries. *Mol. Diversity* **2004**, 437−448.
(15) Bissantz, C.; Folkers, G.; Rognan, D. Protein-based virtual screening of chemical databases. 1. Evaluation of different docking/scoring combinations. *J. Med. Chem.* **2000**, *43*, 4759−4767.
(16) Kellenberger, E.; Rodrigo, J.; Muller, P.; Rognan, D. Comparative evaluation of eight docking tools for docking and virtual screening accuracy. *Proteins* **2004**, *57*, 225−242.
(17) Huai, Q.; Wang, H.; Sun, Y.; Kim, H. Y.; Liu, Y. et al. Three-dimensional structures of PDE4D in complex with roliprams and implication on inhibitor selectivity. *Structure (Camb)* **2003**, *11*, 865−873.
(18) Lee, M. E.; Markowitz, J.; Lee, J. O.; Lee, H. Crystal structure of phosphodiesterase 4D and inhibitor complex. *FEBS Lett.* **2002**, *530*, 53−58.
(19) Bischoff, E. Potency, selectivity, and consequences of nonselectivity of PDE inhibition. *Int. J. Impot. Res.* **2004**, *16 Suppl 1*, S11−14.
(20) Lipworth, B. J. Phosphodiesterase-4 inhibitors for asthma and chronic obstructive pulmonary disease. *Lancet.* **2005**, *365*, 167−175.
(21) Apweiler, R.; Bairoch, A.; Wu, C. H.; Barker, W. C.; Boeckmann, B. et al. UniProt: the Universal Protein knowledgebase. *Nucleic Acids Res.* **2004**, *32 Database issue*, D115−119.
(22) Xu, R. X.; Rocque, W. J.; Lambert, M. H.; Vanderwall, D. E.; Luther, M. A. et al. Crystal structures of the catalytic domain of phosphodiesterase 4B complexed with AMP, 8-Br-AMP, and rolipram. *J. Mol. Biol.* **2004**, *337*, 355−365.
(23) Baurin, N.; Baker, R.; Richardson, C.; Chen, I.; Foloppe, N. et al. Drug-like annotation and duplicate analysis of a 23-supplier chemical database totalling 2.7 million compounds. *J. Chem. Inf. Comput. Sci.* **2004**, *44*, 643−651.
(24) Lewell, X. Q.; Jones, A. C.; Bruce, C. L.; Harper, G.; Jones, M. M. et al. Drug rings database with Web interface. A tool for identifying alternative chemical rings in lead discovery programs. *J. Med. Chem.* **2003**, 3257−3274.
(25) De Julian-Ortiz, J. V.; Galvez, J.; Munoz-Collado, C.; Garcia-Domenech, R.; Gimeno-Cardona, C. Virtual combinatorial syntheses and computational screening of new potential anti-herpes compounds. *J. Med. Chem.* **1999**, 3308−3314.
(26) Lipkus, A. H. Exploring chemical rings in a simple topological-descriptor space. *J. Chem. Inf. Comput. Sci.* **2001**, 430−438.
(27) Wermuth, C. G. *The Practice of Medicinal Chemistry*, 2nd ed.; Academic Press: New York, 2003.
(28) van Almsick, M.; Dolhaine, H.; Honig, H. Efficient algorithms to enumerate isomers and diamutamers with more than one type of substituent. *J. Chem. Inf. Comput. Sci.* **2000**, *40*, 956−966.
(29) *OEChem*; 1.3 ed.; OpenEye Scientific Software, Inc.
(30) Weininger, D. SMILES, a chemical language and information system. 1. Introduction to methodology and encoding rules. *J. Chem. Inf. Comput. Sci.* **1988**, *28*, 31−36.
(31) MDL, E.: San Leandro, CA 94577.
(32) Dixon, J. S. Evaluation of the CASP2 docking section. *Proteins* **1997**, *Suppl 1*, 198−204.
(33) Pearlman, R. S. *Concord User's Manual*, v4.08 ed.; Tripos, Inc.: 1699 South Hanley Road, St. Louis, MO 63144-2319.

(34) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N. et al. The Protein Data Bank. *Nucleic Acids Res.* **2000**, *28*, 235−242.
(35) Huai, Q.; Colicelli, J.; Ke, H. The crystal structure of AMP-bound PDE4 suggests a mechanism for phosphodiesterase catalysis. *Biochemistry* **2003**, *42*, 13220−13226.
(36) Xu, R. X.; Hassell, A. M.; Vanderwall, D.; Lambert, M. H.; Holmes, W. D. et al. Atomic structure of PDE4: insights into phosphodiesterase mechanism and specificity. *Science* **2000**, *288*, 1822−1825.
(37) *SYBYL*; 6.91 ed.; TRIPOS, Assoc., Inc.: St-Louis, MO.
(38) Rarey, M.; Kramer, B.; Lengauer, T.; Klebe, G. A fast flexible docking method using an incremental construction algorithm. *J. Mol. Biol.* **1996**, *261*, 470−489.
(39) Verdonk, M. L.; Cole, J. C.; Hartshorn, M. J.; Murray, C. W.; Taylor, R. D. Improved protein−ligand docking using GOLD. *Proteins* **2003**, *52*, 609−623.
(40) Jain, A. N. Surflex: fully automatic flexible molecular docking using a molecular similarity-based search engine. *J. Med. Chem.* **2003**, *46*, 499−511.
(41) Lugnier, C.; Schoeffter, P.; Le Bec, A.; Strouthou, E.; Stoclet, J. C. Selective inhibition of cyclic nucleotide phosphodiesterases of human, bovine and rat aorta. *Biochem. Pharmacol.* **1986**, *35*, 1743−1751.
(42) Lugnier, C.; Komas, N. Modulation of vascular cyclic nucleotide phosphodiesterases by cyclic GMP: role in vasodilatation. *Eur. Heart J.* **1993**, *14 Suppl I*, 141−148.
(43) Keravis, T. M.; Wells, J. N.; Hardman, J. G. Cyclic nucleotide phosphodiesterase activities from pig coronary arteries. Lack of interconvertibility of major forms. *Biochim. Biophys. Acta* **1980**, *613*, 116−129.

JM050063Y