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Towards the automatic design of synthetically accessible protein ligands: Peptides, amides and peptidomimetics

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Summary

The computer program LUDI for the de novo design of protein ligands was extended so that it is now able to take into account the synthetic accessibility of the constructed molecules. As an example, the design of peptides, amides and peptidomimetics using amino acids as building blocks is described. Two new libraries containing natural and non-natural amino acids were constructed for this purpose. Conformational flexibility is taken into account by using multiple conformers for each amino acid. The program was applied to the design of ligands for the enzymes elastase, renin and thermolysin.

Introduction

Recently, a novel computer program for the de novo design of protein ligands [1,2] was described. This program LUDI builds protein ligands by fragment joining. The fragments are simple small organic molecules with typically up to 50 atoms. The program can construct a large diversity of possible structures if a sufficiently large fragment library is available. Initially, a standard library consisting of roughly 1000 fragments was used. Recently, also larger fragment libraries have been employed, such as for example a 30 000-compound subset of the fine-chemicals directory (FCD) [3].

In principle, LUDI can be used to construct specific classes of ligands by using the appropriate fragment libraries. For example, peptides can be constructed by using amino acids as fragments. However, when linking fragments together, the first version of LUDI did not constrain the dihedral angle of the newly formed bond between the fragments to a certain value. Therefore, the possibility existed that the actual value of the dihedral angle did not correspond to an energetically favorable conformation. This turned out to be particularly troublesome for amide bonds which are found experimentally to be planar with a strong preference for the *trans* conformation. The first version of LUDI therefore required the inspection of the generated structures. The current experience is that the rate of structures that had to be rejected due to nonplanar bonds was unsatisfactorily high, making the approach impracticable.

In the present communication, some modifications in the LUDI algorithm are reported that now enable a better control of the dihedral angle of the newly formed bond between the fragments. Used together with fragment libraries specifically designed for fragment joining with a specified chemical bond as link, LUDI is now able to construct synthetically accessible protein ligands. As an example, the automatic construction of peptides, amides and peptidomimetics is described. For this purpose, two new fragment libraries compiled from natural and non-natural amino acids have been constructed. Some details on these libraries are given.

A computer program for the automatic construction of peptides has previously been described by Moon and Howe [4]. In their pioneering work, implemented in the computer program GROW, they use a multiconformer library of amino acids to construct peptides in a build-up procedure. The conformers are generated by conformational analysis using a molecular mechanics force field. The present approach shows some similarity to the work of Moon and Howe, mainly concerning two aspects. Firstly, similar to GROW, a ligand is formed by superim-

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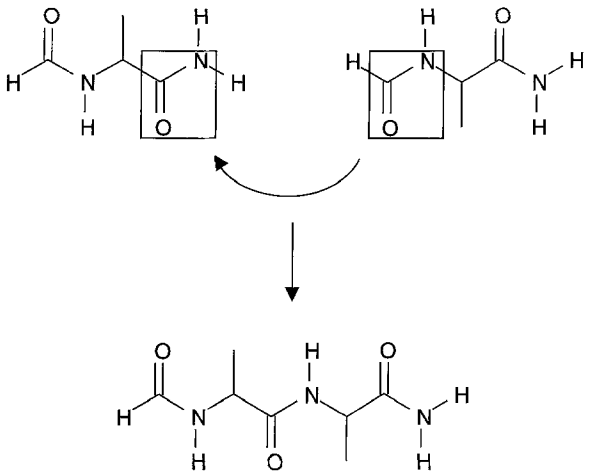


Fig. 1. Formation of a peptide bond with LUDI. The three atoms C, O and N (encircled by the square) of the fragment are superimposed onto the corresponding atoms of the already-positioned peptide.

posing atoms forming the amide group. Secondly, as for GROW, the present approach requires a seed group. However, as will be discussed below, the LUDI build mode also differs from GROW in a number of aspects. The most important differences between LUDI and GROW relate to the placement of the seed group, the algorithm used for the docking of the individual amino acids, the scoring of the resulting protein–ligand complex and the construction of the fragment libraries.

Methods

Formation of the amide bond

In order to facilitate the construction of peptides (and also of other molecules with fixed dihedral angles) we have implemented a new link mode in the program which we call 'triple-atom link mode', which is shown schematically in Fig. 1. In contrast to the normal link mode which uses two atoms for the link, the new link mode now uses three atoms for the superposition. The standard building block is $HCONHCH(R)CONH_2$. Two amino acids are linked together by superimposing the C-terminal three atoms C, O and N of the amide group of building block i with the corresponding N-terminal atoms of building block (i + 1). In parallel, the functional groups of the amino acid to be appended are superimposed with the interaction sites that represent favorable binding positions in the protein binding site. The program builds a peptide chain in the active site by appending one amino acid at a time. The building can be done both in the N-terminal and C-terminal direction.

In a further mode of operation, called the peptide-bridge mode, the program can replace an amino acid in the middle of a peptide chain by a peptidomimetic. This mode can also be used to search for spacers to link two separate peptide chains together. In the peptide-bridge mode, both the N-terminal and the C-terminal CON groups are used for the superposition. Again, simultaneous with the formation of two amide bonds, the fitting of the amino acids is done in such a way that the direct interactions with the protein through side-chain interactions are optimized.

Construction of the amino acid libraries

An important aspect of peptides is their backbone flexibility. Ramachandran plots of high-resolution protein structures show several regions in the ϕ,ψ space that are occupied. On the other hand, high-level quantum-mechanical calculations and also force-field calculations show that there are only few local minima for Ac-Gly-$NHCH_3$ and Ac-Ala-$NHCH_3$ [5]. Thus, it appeared insufficient to use the local minima for the backbone conformation only. We have therefore decided to extract amino acids from high-resolution protein structures (resolution better than 2 Å) deposited in the Brookhaven protein databank (PDB) [6] and use them as fragment libraries. In order to remove duplicate entries from the fragment library, all amino acid conformers were removed that exhibit a root-mean-square (rms) deviation of less than 1 Å with all heavy atoms superimposed. In doing so, we generated a fragment library consisting of roughly 100 conformers per amino acid. The PDB entries used in the construction of the fragment library were taken from a subset of the PDB used by others to investigate folding patterns [7]. All hydrogens were added using the graphics program INSIGHT [8]. The side chains of the amino acids aspartate, glutamate, lysine and arginine were assumed to be charged. The library contains L-amino acids only. However, D-amino acids are also accounted for by simply taking the mirror image for every individual L-amino acid conformer.

Furthermore, we have constructed a library of currently 50 non-natural amino acids. Examples from this library are shown in Fig. 2. The library contains several amino acids that are closely related to naturally occurring amino acids, such as homo-phenylalanine, cyclohexyl-alanine, phenyl-glycine or norleucine. However, several entries could better be described as peptidomimetics. An example is the pyridone shown in Fig. 2. For the construction of the second library, no experimental information on possible conformers was available. We were therefore forced to construct the conformers by a computational procedure. We have used the program MIMUMBA [9] to generate multiple conformers. About 100 conformers per amino acid were generated on average and stored in the library. Most non-natural amino acids selected in this library are commercially available [10]. A few entries were taken from the recent literature on peptidomimetics. Therefore, all structures generated from the library should be synthetically easily accessible by standard peptide synthesis.

The design of a peptide or a peptidomimetic can be done as follows. The first step is the selection of a seed group. The seed group can be placed using interactive computer graphics following a molecular mechanics optimization of the position in the binding site. Alternatively, one may start from a known protein–ligand complex and just keep a small part of the known ligand and use this as seed in the design of novel ligands. This approach was taken in the example presented below. A third option is to use LUDI for the initial placement of the seed group. In the bridge mode, the user has to specify either the amino acid to be replaced or the terminus to be linked.

The basic computational methodology is the same as described previously [1,2]. Firstly, a new fragment is appended. Then, the position of this new fragment is checked for steric overlap with the protein and with other parts of the ligand, for electrostatic repulsion and for the presence of buried unpaired polar groups. Optionally, one can also specify some further requirements. For example, it can be requested that a minimum percentage of the fragment surface has to be in contact with the protein or that an interaction with a specified atom of the protein is made (e.g., interaction with the zinc atom of a metalloprotease).

All hits satisfying the criteria mentioned above are scored using an empirical scoring function described previously [11]. The full fragment library is searched and only the top-scoring hits are kept. The construction of a peptide proceeds similar to the program GROW described by Moon and Howe [4]. The result of a LUDI run using the new 'triple-atom' build mode is a list of peptides or peptidomimetics with a predicted 3D structure that are prioritized according to the calculated score.

Results

Elastase

The first example presented is the serine protease elastase. We used the 3D structure of human leukocyte elastase complexed with the peptidic chloromethylketone inhibitor Suc-Ala-Ala-Pro-Ala-CH_2Cl (**1**, see Scheme 1; PDB refcode 1HNE) [12] in our calculations. The C-terminal amino acid alanine with the covalent link to the protein (**2**) was kept without modifications of the heavy atom positions. LUDI was then used to search the library of natural amino acids for suitable groups for P_2-P_4. In the first step the position P_2 was considered. LUDI finds the amino acids proline, alanine and glycine. The highest

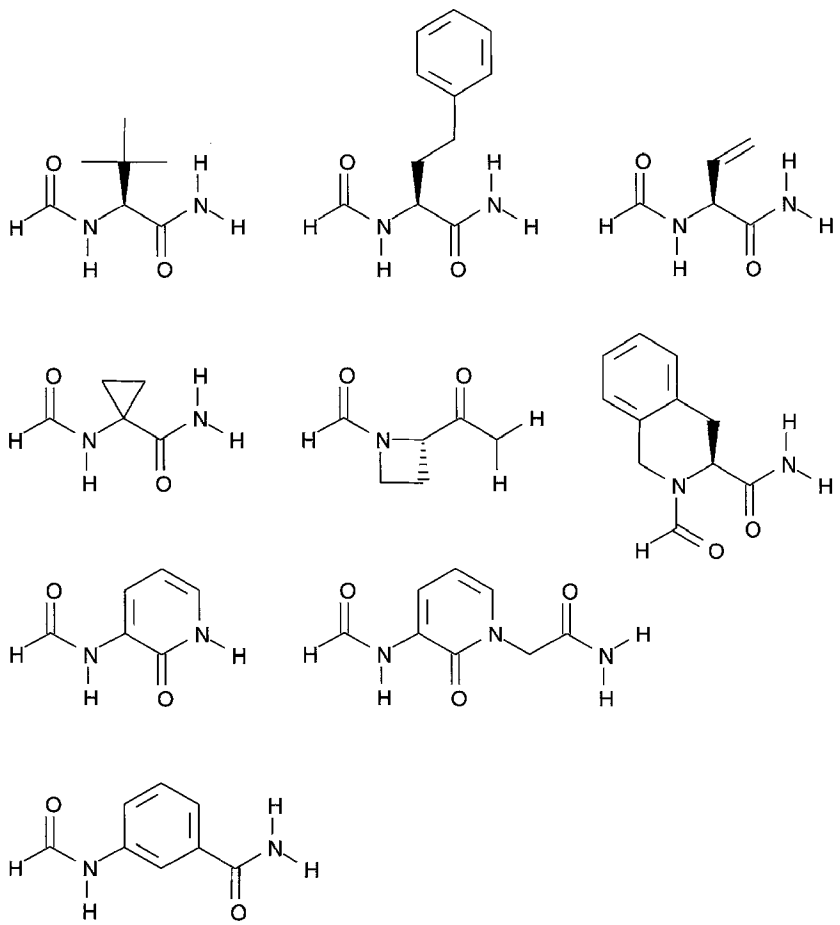
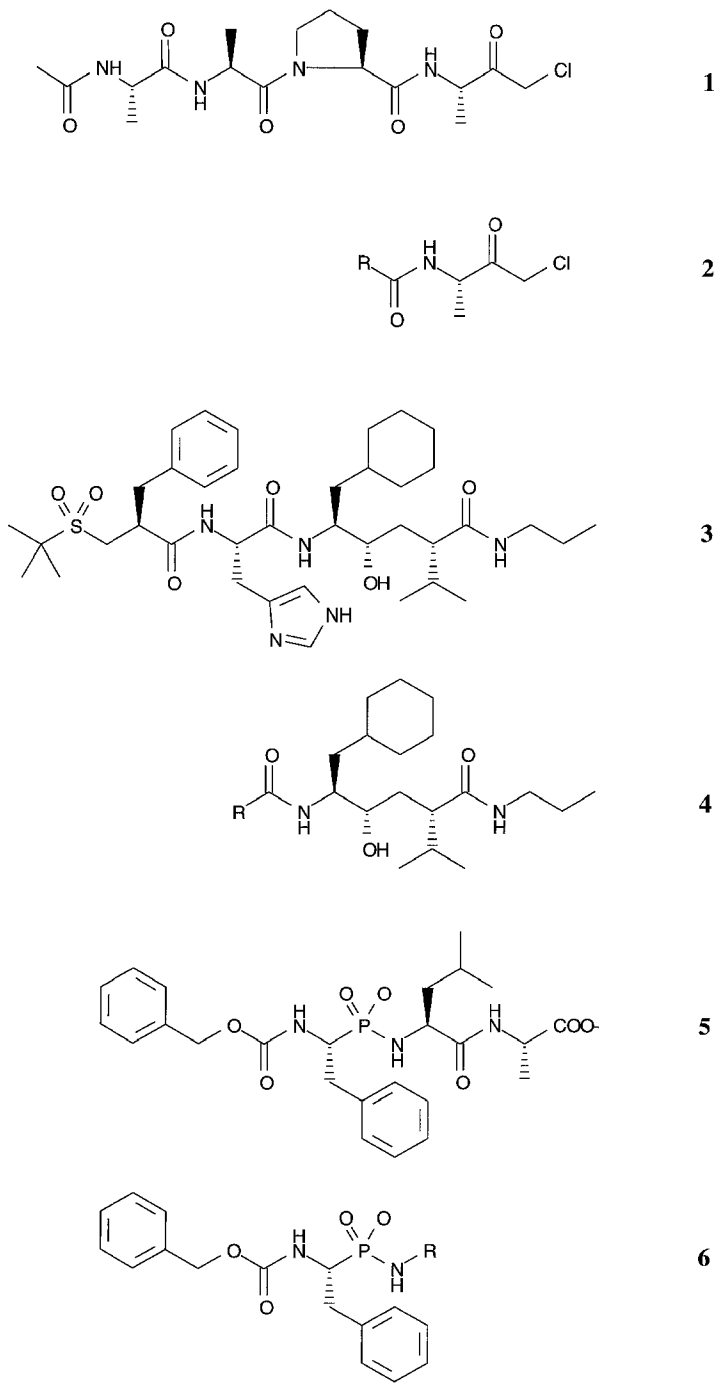


Fig. 2. Examples of non-natural amino acids and peptidomimetics contained in the fragment library.



Scheme 1. Structures of compounds **1**–**6**.

score is found for proline, in very good agreement with published binding data on peptides [13]. The docked proline was then used to append a further amino acid at position P_3. The LUDI hits for P_3 are leucine, glycine and alanine, with the highest score obtained for leucine, which was then again used to search for P_4 substituents. However, no hit was found in this calculation. It should be noted that the one requirement for LUDI to append another amino acid is that at least one hydrogen bond is formed by the peptide backbone. LUDI therefore failed to find an amino-acid-backbone conformation that could both be attached to the P_3-Leu and hydrogen bonds with elastase. Therefore, the best peptide for P_3-P_2 obtained from LUDI is Leu-Pro. The docked conformation of this dipeptide is compared with the experimentally observed conformation of Suc-Ala-Ala-Pro-Ala-CH_2Cl in Fig. 3. The rms deviation of the backbone atoms for the P_3-P_2 section is 0.82 Å.

In a further calculation, the library of non-natural amino acids and peptidomimetics was searched for suit-

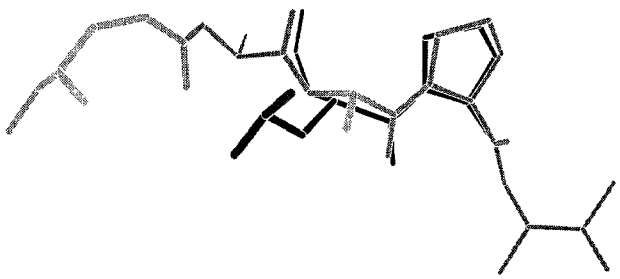


Fig. 3. Comparison of the fitted P_2-P_3 dipeptide Leu-Pro (dark bonds) with the protein-bound conformation of Suc-Ala-Ala-Pro-Ala-CH_2Cl.

able groups at position P_3. In particular, we were interested in the ability of LUDI to dock the 3-amino-pyridone into the binding site of elastase. This group was recently disclosed as a useful peptidomimetic [14], leading to potent orally active inhibitors of human leukocyte elastase [15]. Using proline or alanine at position P_2, the pyridone is rejected by LUDI due to intramolecular steric overlap between the pyridone and the side chain at position P_2. Using glycine at position P_2, 3-amino-pyridone is docked successfully into the binding site of elastase. The docked conformation is compared with Suc-Ala-Ala-Pro-Ala-CH_2Cl in Fig. 4.

Each run took about 2 CPU min on a Silicon Graphics workstation with an R4000 CPU.

Renin

The second example that we present is the design of inhibitors for the aspartic protease renin. The 3D structure of renin complexed with the inhibitor CGP38560 (**3**) solved by Rahuel, Grütter and Priestle was used as starting point (PDB refcode 1RNE) [16]. CGP38560 is an inhibitor largely peptidic in nature with histidine at position P_2 and a close analog of phenylalanine at position P_3. The purpose of the calculation was to assess the ability of LUDI to search for possible replacements of the Phe-His part of CGP38560. The coordinates of the protein and of the C-terminal part of the inhibitor (**4**) were taken from the PDB without any modification.

The search for the P_2 position using the natural amino acids library retrieved three hits, comprising valine, isoleucine and glycine. Using valine as seed for a further search for the P_3 position, the amino acids isoleucine, phenylalanine, leucine, proline, methionine and serine are retrieved. If the calculation is started from the experimentally determined conformation of P_2-His, then the search in the library of natural amino acids retrieves tryptophan, phenylalanine, leucine, proline, methionine, valine and alanine as possible candidates for position P_3. Therefore, the present calculation suggests Ile-Val, Phe-Val and Trp-His as candidates for P_3-P_2.

Valine at position P_2 of renin inhibitors has been shown to yield potent inhibitors with nanomolar binding affinity [17]. Likewise, phenylalanine and tryptophan have been shown to yield excellent binding affinities at position P_3 [17]. LUDI fails to place histidine into the P_2 pocket, which is known to yield very potent inhibitors of renin and is one of the best-suited amino acids at position P_2. However, a visual inspection of the X-ray structure of the complex renin–CGP38560 reveals, that no hydrogen bonds are formed between the protein and the P_2 side chain of the inhibitor. However, the present algorithm will only place histidine into the binding site if it forms at least one hydrogen bond with the side chain. Such an interaction is not found for any of the stored conformers of histidine.

Thermolysin

The last example reported in this paper is the design of peptidic inhibitors of thermolysin. The 3D structure of thermolysin complexed with ZF^PLA (**5**) was used in the calculations (PDB refcode 4TMN) [18]. The N-terminal Leu-Ala dipeptide fragment was clipped off and the remaining inhibitor substructure (**6**) was used as seed in the LUDI calculation. One technical problem arises because the seed fragment does not contain a terminal amide group. In order to facilitate the peptide link, an amide group was fitted onto the PO_2NH group with the carbonyl oxygen between the two oxygens of the phosphonamide group. This amide group was then employed in the calculations. LUDI was used to search for suitable amino acids at positions P_1' and P_2'. For P_1', LUDI retrieved valine, alanine, glycine and serine, with the highest score for valine. Leucine, which is present at position P_1' in the protein structure, is not retrieved. Using the docked valine at postion P_1', the hits for position P_2' are valine, alanine, leucine and glycine. Inhibitors containing leucine and alanine at position P_2' are indeed known to be very potent inhibitors of thermolysin [19]. The suggested inhibitor containing the Val-Val moiety is compared with ZF^PLA in Fig. 5. The rms deviation of the backbone atoms between the two structures is 0.27 Å.

Finally, the library of non-natural amino acids and peptidomimetics was searched for entries suitable for the P_1' position. The calculation retrieved five hits which are shown in Fig. 6. The highest score is obtained for thia-

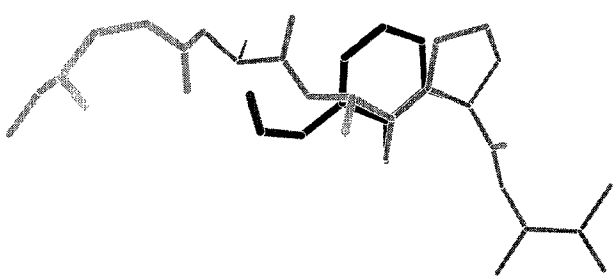


Fig. 4. Comparison of the fitted 3-amino-pyridin-2-one derivative (dark bonds) with the protein-bound conformation of Suc-Ala-Ala-Pro-Ala-CH_2Cl.

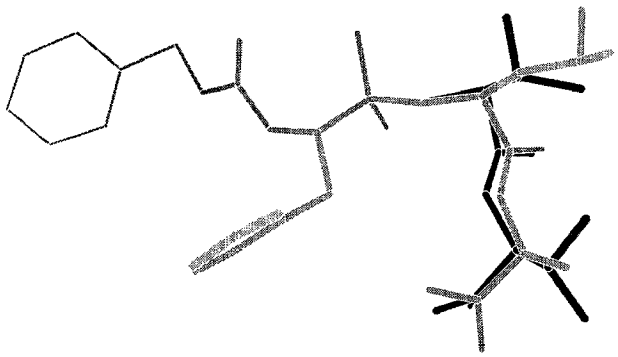


Fig. 5. Comparison of the fitted P_1'-P_2' dipeptide Val-Val (dark bonds) with the protein-bound conformation of the thermolysin inhibitor Cbz-PheΨ[PO_2NH]Leu-Ala.

zolyl-alanine followed by norleucine. No experimental binding data could be found in the literature for these compounds.

Discussion and Conclusions

In the present paper, we report further developments of the computer program LUDI with respect to the building of synthetically accessible protein ligands. LUDI is now able to construct peptides by joining amino acids. Two new libraries were constructed for this purpose. Firstly, amino acid fragments HCONHCH(R)$CONH_2$ were extracted from high-resolution protein structures taken from the PDB. The conformational flexibility of the amino acids is taken into account by using several conformers for each amino acid. On the average, 100 conformers are stored per amino acid. Secondly, a library of commercially available non-natural amino acids and peptidomimetics was constructed. In this case, the conformers were generated using the computer program MIMUMBA [9]. As examples, we have used LUDI to construct inhibitors for the proteins elastase, renin and thermolysin. In all cases, LUDI retrieves amino acids which are known from experiment to bind in the subsites of the corresponding enzymes. For elastase, LUDI is able to dock 3-amino-pyridone into the binding site which is a known peptidomimetic. Therefore, the current experience indicates that LUDI is indeed able to suggest successfully peptides, amides and peptidomimetic building blocks as new substituents for known protein ligands.

The present algorithm can be easily extended to other classes of molecules, such as N-substituted polyglycines ('peptoides'), polycarbamates or polyesters. For example, ethers can be formed by joining fragments from a library containing hydroxy compounds with fragments from a library containing R-CH_2Cl or R-CH_2Br. As the current method does not use any force field, it can also easily be extended to fragments involving no standard functional groups, such as sulfonamides or phophonamides. It is also possible to combine the new build mode with the standard link mode of LUDI [2]. A possible approach to de novo ligand design would then be to firstly construct a peptide and then to replace parts of the peptide (for example the N- and the C-terminus or some side chains) by novel groups, thereby reducing the peptidic character. Alternatively, one may search for bridging groups to form cyclic structures, e.g. lactams.

The present approach should also prove to be useful to guide combinatorial chemistry approaches. As pointed out recently by Martin et al. [20], one major problem in the construction of combinatorial libraries is the abundance of potential building blocks which calls for computational schemes for the selection of building blocks. If the 3D structure of the target protein is known, the present approach can be used for this purpose. Due to the build-up procedure, the present algorithm can easily be extended to a very large number of diverse monomers and is therefore useful for monomer selection. If a compound from a combinatorial library shows detectable

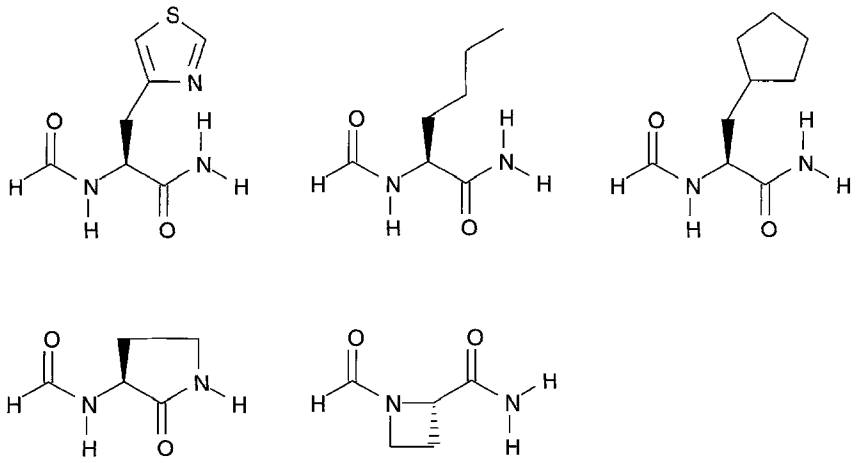


Fig. 6. Hits obtained from a search through the library of non-natural amino acids and peptidomimetics for suitable groups at position P_1' in inhibitors of thermolysin.

biological activity, its docked conformation suggested by LUDI immediately offers a starting point for further optimizations. On the other hand, some classes of enzyme inhibitors may not be easily amenable to combinatorial chemistry approaches. In such a situation, the present approach can be used to guide the design and synthesis of protein ligands using classical organic chemistry.

The new link mode of LUDI is not restricted to pure peptides. For example, one can start from a nonpeptidic structure and search for an amino acid as a substituent to improve the binding affinity of the lead structure. Furthermore, the library of non-standard amino acids can easily be extended to contain more structures that should be metabolically stable. As shown in the last example (thermolysin), the 'triple-atom' link mode can also be used to join fragments without actually forming an amide bond.

The new build mode of LUDI is fully implemented in the program. Therefore, it is possible to combine the construction of a peptide with a LUDI run using the 'default' link mode. This will for example allow to make C- or N-terminal modifications using nonpeptide building blocks, or to search for novel side chains that make interactions with the protein which are not feasible with available amino acids. Furthermore, the seed group can be positioned in the binding site using LUDI running in the standard mode [1]. The seed can be selected from a large number of fragments.

The present approach is similar to the program GROW developed by Moon and Howe [4] and also to the very recent work by Frenkel et al. [21]. In comparison with this work, the present approach is believed to have a number of advantages. Firstly, the present algorithm uses a new scoring function described recently [11], which was calibrated to experimentally determined binding constants of 45 protein–ligand complexes. The calibration data set contained both very small ligands, like benzene, and large ligands up to nonapeptides. Therefore, we expect that this scoring function should allow for a good prioritization even between ligands of different size. Secondly, we refrain from using computationally expensive force-field calculations. Therefore, the present approach is faster than GROW. Another difference relates to the amide bond formation. LUDI superimposes three atoms of the amide group to ensure a reasonable geometry close to planarity. At the same time, atoms suitable to form favorable interactions with the protein are superimposed onto precalculated interaction sites. Therefore, the present algorithm will tolerate small deviations from planarity of the amide bond if this enables the formation of favorable protein–ligand interactions. We note that small deviations from planarity ($\omega = 180°$) of the amide bond are also observed in high-resolution crystal structures of small peptides [22,23]. By contrast, GROW and the approach by Frenkel et al. [21] do not permit deviations of the amide bond from planarity.

Another difference between GROW and LUDI relates to the conformer selection and prioritization. In GROW, the amino acid conformers are generated by a force-field-based computational procedure. The conformational energy is used to prioritize the conformers. Moon and Howe note that typically the 300 conformers with the lowest energy are used in the design. The selection scheme of GROW therefore relies on the accuracy of the molecular mechanics force field. In LUDI, we use experimentally determined conformers for the natural amino acids with roughly 100 conformers on average. The use of experimental structures from high-resolution structures ensures that only low-energy conformers are stored in the fragment library. Our approach relies on the assumption that amino acids in a protein and in a ligand adopt similar conformations. For the non-standard amino acid library, the conformer generation is done using the computer program MIMUMBA [9]. However, the results obtained with this program for the conformational analysis of a large set of diverse protein ligands suggest that MIMUMBA is indeed able to generate biologically relevant conformers [9].

The present work faces a number of limitations. Firstly, similar to other previously published algorithms, the protein is kept rigid. Therefore, if ligand binding is accompanied by a conformational change being significantly different from that of known ligands, the present approach may run into problems. Furthermore, we cannot rule out the possiblity that we miss important conformations of the amino acids in our fragment library. Another limitation relates to the fragment-placement algorithm. Amino acids with polar side chains will only be accepted if the side chain is involved in a hydrogen bond. As shown for the P_2 binding site of renin, LUDI does not retrieve histidine as a suitable amino acid for this position, despite its good overall complementarity with the protein.

In summary, a new method for the automatical design of synthetically accessible protein ligands described. In the present communication, the design of peptides, amides and peptidomimetics is described. Currently, work is in progress to extend the appraoch to incorporate other simple chemical reactions. The ultimate goal is to develop a method that can automatically design protein ligands which are synthetically easily accessible.

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