Design of sp³-Enriched α-Helix-Mimetic Library

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Although PPIs are among the most screened target class in high-throughput screening (HTS), success rate of finding hit compounds in many HTS campaigns using small molecule compounds remains generally very low [1,2]. This suggests that the most of the available chemical libraries are not suitable for screening PPI targets, and special design for PPI-focused libraries is required to achieve suitable chemical space [3]. Indeed, discovered to date PPI inhibitors are remarkably different if compared with the majority of known drugs: usually they are larger, more hydrophobic, contain more (hetero)aromatic rings, and therefore belong to "beyond Ro5" chemical space.

Numerous attempts have been undertaken to define PPI-related chemical space in order to reduce attrition rate in PPI-targeted HTS campaigns. The most general approach is to define some simple molecular descriptors that would be applied for preliminary filtering of commercially available HTS libraries. Thus, based on general analysis of the molecular descriptors of selected known PPI-inhibitors, Morelli et al. [4] have proposed 'Rule-of-Four' (Ro4) to describe the chemical space covered by these compounds. By its definition, a molecule belongs to this space if it obeys three of four following properties: MW ≥ 400 Da, cLogP ≥ 4, number of H-bond acceptors (HBA) ≥ 4, number of rings ≥ 4. In addition, filtering protocol $2P2I_{HUNTER}$ has been proposed by this research group as a learning machine tool for filtering of general screening libraries to design PPI-focused libraries [1,5,6].

However, such molecular-descriptors-based approaches are not indisputable, because, in fact, they equate non-drug-like and a PPI-related chemical spaces. This means that even if some potent PPI-modulators are found in this space, their future as potential drugs will be uncertain (solubility, permeability etc. issues), and significant efforts should be required at hit-to-lead optimization stage. In our view, this contradiction can be waived if PPI-focused libraries are designed on natural-like sp³-enriched scaffolds. Indeed, the most recently developed PPI inhibitors tend to be non-planar and non-linear, contain more saturated (hetero)cycles, more sp³ carbons and stereo-centers and therefore to be more natural-like [7,8]. In addition, many natural products or natural-product-based compounds have been found as potent PPI-modulators [9,10]. It is obvious that the nature is three-dimensional and therefore recognizes small molecules in a complementary 3D-fashion, and so drugs are likely to be more selective for their targets (especially in PPIs) if they are three-dimensional too [11,12]. Not coincidentally, compounds with diverse and well-developed 3D-shapes have gained most attractiveness ones on the market of screening compounds for HTS over last several years. Furthermore, Fsp³ parameter has become one of the most important criterion of HTS libraries value since it was introduced in 2009 by Frank Lovering et.al [13] as a measure of three-dimensionality and therefore complexity for libraries members. According to their findings and some our further observations, scaffold/molecule saturation may benefit:

- ► Better diversity;
- ► Complexity;
- ► Access to greater chemical space;
- ► Improved phys-chem parameters (logP; PSA; water solubility etc.);
- ► Better opportunity to reduce scaffold MW;
- ► Better opportunity for further scaffold modification;
- ► Natural product-likeness;
- ► Better affinity to target proteins
- ► Greater selectivity;
- ► Easy access to IP-clean field.

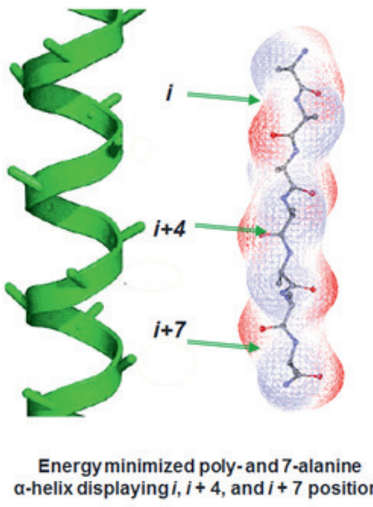
This inspired us to create new subset of our PPI-focused library, namely α-Helix 3D-mimetics Library. The library has been designed on selected sp³-enriched scaffolds from our DOS chemistry and populated with members that are able to mimic α-helices as key recognition elements of protein secondary structure.

The following requirements were used for preferable scaffolds selection:

- ► sp3 – enriched (Fsp3 ≥ 0.4) to ensure their complexity and therefore 3D-diversity;
- ► Contain at least 2 points of diversification;
- ► Contain moieties of "privileged structures" such as piperazines, piperidines (including 3- or 4-amines, carboxamides etc.), pyrrolidines (including 3- amines, carboxamides etc.), (benz)-1.4-diazepines, prolines (including unusual) and others;
- ► Contain moieties of naturally occurring compounds;
- ► Lipophilitciy / hydrophilicity balanced;
- ► Conformationally constrained (e.g. spiro- and bridged heterocyclic systems).

Strategy for α-Helix 3D-Mimetics Library Design

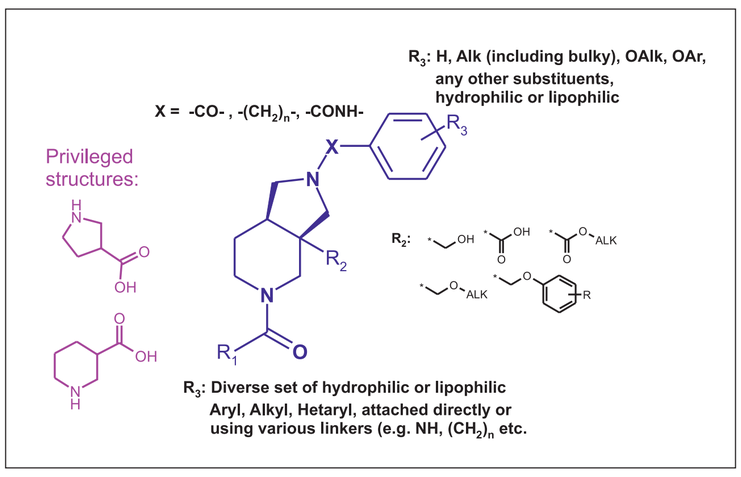
Our strategy for the design of helix-mimetics involves:



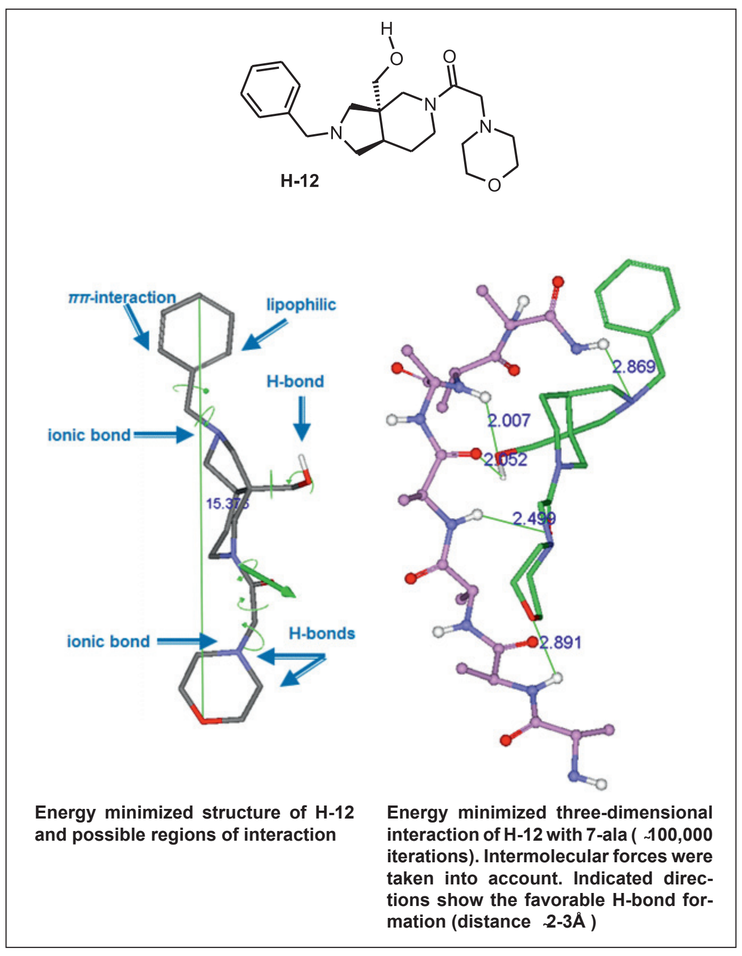
Energy minimized poly- and 7-alanine α-helix displaying *i*, *i* + 4, and *i* + 7 positions

- ► Mimicry of helix by the structure of polycyclic small molecule scaffolds
- ► Mimicry of side-chain residues on one face of the α-helix
- ► At least three points of interaction with 7-ala helix at *i*, *i+4* (or *i+3*) and *i+7* positions
- ► Possible H-bond, hydrophobic, electrostatic or π–π-interaction (not electrophilic or redox!!!)
- ► Include hydrophilic and lipophilic regions in the scaffolds
- ► Avoidance of polycyclic aromatics (such as terphenyls and their hetero-analogs, oligo-benzamides and their hetero-analogs etc.)
- ► High Fsp³ for core scaffolds
- ► High solubility of mimetics

Example of selected scaffold:



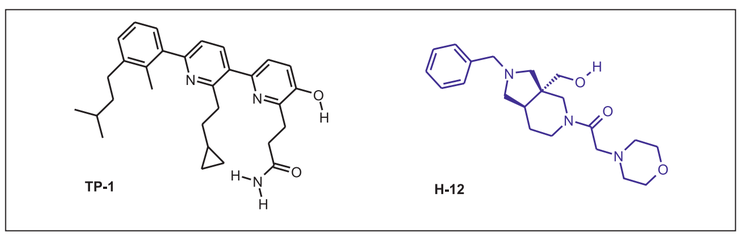
Example of modeling – interaction of H12 with 7-Ala:



Energy minimized structure of H-12 and possible regions of interaction

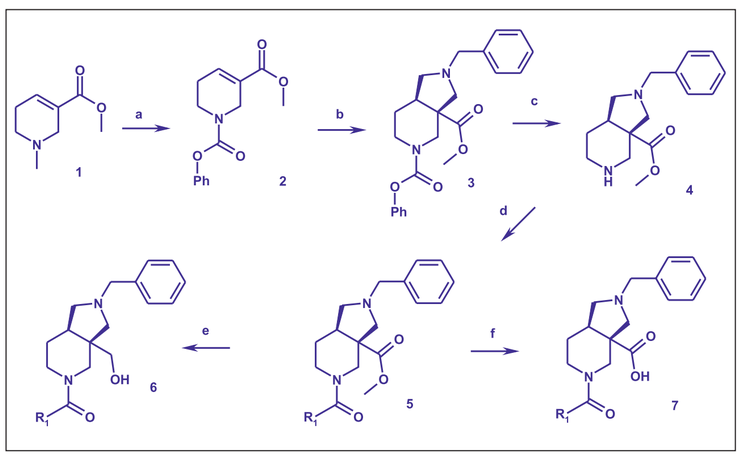
Energy minimized three-dimensional interaction of H-12 with 7-ala (~100,000 iterations). Intermolecular forces were taken into account. Indicated directions show the favorable H-bond formation (distance ~2-3Å)

Phys-Chem Properties for TP-1 (reported [14] design) and H-12 (ChemDiv's design):

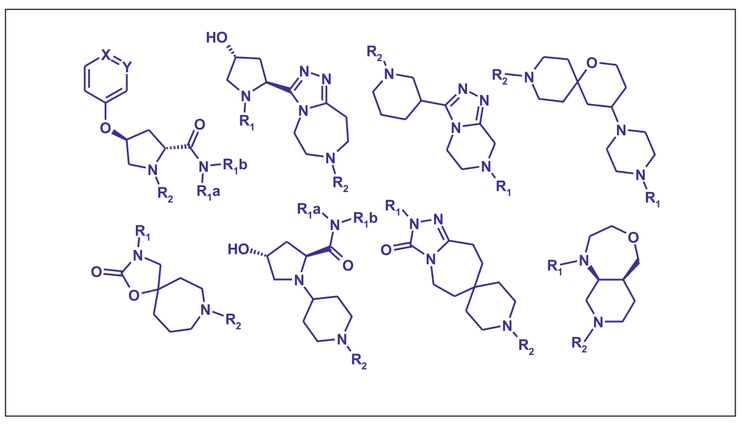


Phys-Chem parameter	TP-1 (Reported design)		H-12 (ChemDiv's design)
MW	471.6		373.5
clogP	6.43		2.23
LogSW (pH 7.5)	-5.81	vs	-0.3
PSA	55.32		56.25
HBD	3		1
HBA	5		6
NRB	12		7
Fsp3,%	43.3		66.7

General Scheme for the Scaffold Synthesis:



More examples of α-Helix-mimetic scaffolds:



Phys-Chem Parameters for α-Helix 3D-Mimetic Library

Phys-Chem Parameter	Range	Average
MW	283…593	399
cLogP	-3.4...7.8	2.5
NRB	0…4	2.64
HBA	2…13	6.6
HBD	0…3	0.6
PSA	7…172	74.9
Fsp³	0.11…1.0	0.47
Chiral centers	0…3	0.7
cLogD (pH 7.4)	-4.2…7.8	2.4
cLogSW (pH 7.4)	-10.8…1.8	-4.0

Distribution of the library members by Fsp3 is represented in Fig. 1 (blue plots). We have compared it with distribution for two other ChemDiv's PPI-focused libraries, namely:

- ► **Shape mimetics library** – library of analogs of reported helix, turn, beta-sheet, strand, loop mimetics, created in 2012, consists of 9.5K compounds (brown plots);
- ► **Natural-product-derived compounds (NPB)** - library of derivatives of natural products, created in 2013, consists of 4K compounds (grey plots).

The graph shows clearly that new 3D-Mimetics library is more nature-like. As a result, the library members have improved LogP parameter, so their distribution by this property is similar to the distribution for NPB library (Fig. 2).

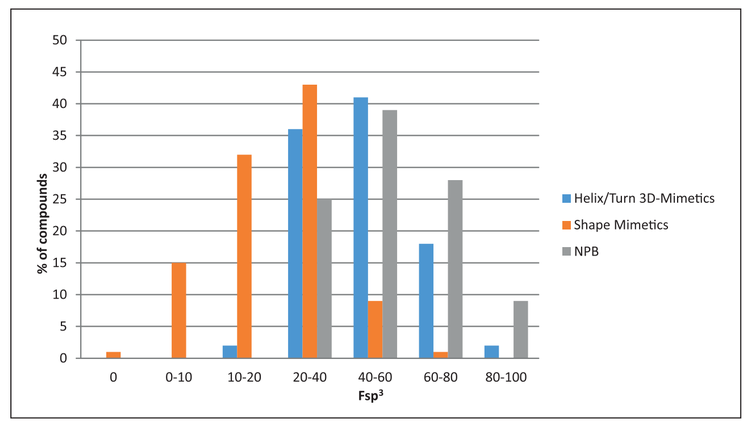


Fig. 1.

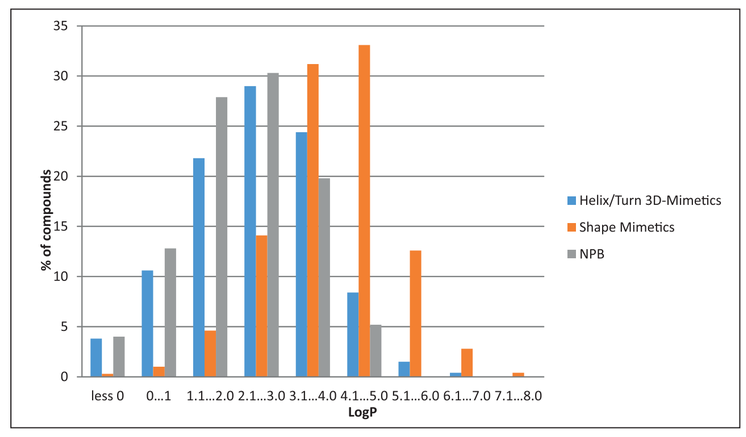


Fig. 2.

α-Helix 3D-Mimetics Library Summary:

- ► The library consists of more than 42.7K α-helix mimetics;
- ► Assured chemical diversity (diversity coefficient 0.9);
- ► 19.2K (45%) compounds meet Rule of Four criteria ("PPI-related chemical space", see above);
- ► 41.1K (96%) compounds meet Rule of Five criteria (belong to "Drug-like chemical space);
- ► 18.3K (43%) compounds belong to both, "PPI-related" and "Drug-like" chemical spaces;
- ► 25.3K library members contain at least one chiral center in the structure;
- ► ***Confirmed relative and/or absolute stereochemistry for all library members.***

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