α-HELIX 3D-MIMETIC LIBRARY

Sergey Tkachenko, Volodymyr Kysil, Fred Lakner, Yan Ivanenko

Protein–protein interactions (PPIs) play a central role in many cellular processes and are involved in numerous pathways, including diseases, different stages of cancer development and host–pathogen interactions. With a recently estimated number of 300,000 in humans, the possibilities for therapeutic intervention are significantly larger compared to 'classical' drug targets. Despite the long-held assumption that PPIs were poorly druggable, they have shown increasing potential as new therapeutic targets over the last 10 years. Modulators of these original interactions are likely to lead to the next generation of highly innovative drugs that should reach the market in the next decade [1]. As a result, PPIs are today the most screened target class in high-throughput screening (HTS).

However, success rate of finding hit compounds in many HTS campaigns using small molecule compounds remains generally very low [2]. This suggests that 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 by the date PPI inhibitors are remarkably different if compared with the majority of known drugs. PPI inhibitors tend to be larger, more hydrophobic, more rigid, non-planar and non-linear, contain more (hetero)cycles, more sp^3 carbons and stereo-centers [4, 5]. It's obvious that most of them 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. [ ] 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 [1,7].

Importantly, this tool has been applied for analysis of numerous commercially available HTS libraries from various suppliers including ChemDiv. Compared with all other chemical libraries analyzed, the percentage of selected compounds was higher for the PPI library from ChemDiv. Thus, selected compounds from the Eccentric subset of ChemDiv (83.1%) cover almost the entire PPI chemical space described by SVM model. The compounds from the Cyclic Ugi subset of ChemDiv overlap with 50% of the PPI inhibitors in 2P2I database. This confirms ChemDiv's expertise in PPI-focused library design.

However, known PPI-modulators are predominantly flat hydrophobic (terphenyl- and oligobenzamide-like) structures with high molecular weight and obviously low solubility, and their future as potential drugs therefore is questionable. On the other hand, 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 PPI) if they are three-dimensional too [8, 9]. Not coincidentally, compounds with diverse and well-developed 3D-shapes have become the most attractive ones on the market of screening compounds for HTS for last several years. Furthermore, Fsp^3 parameter has become one of the most important criterion of HTS libraries value since it was introduced in 2009 by Frank Lovering et.al [ ] as a measure of three-dimensionality and therefore complexity for libraries members. According to their findings and our further observations, scaffold/molecule saturation may benefit:

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

This inspired us to create new subset of our PPI-focused library, namely Helix/Turn 3D-mimetics Library. The library has been designed on selected sp^3-enriched scaffolds arisen from our DOS chemistry and populated with members that are able to mimic α-helices and β-turns as a key protein recognition element.

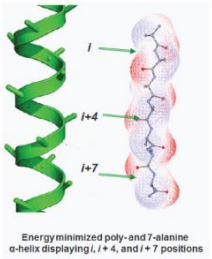
The following requirements were used for preferable scaffolds selection:

- **sp^3 – enriched ($Fsp^3 \geq 0.4$) 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).**

A-HELIX 3D-MIMETICS SUB-LIBRARY DESIGN

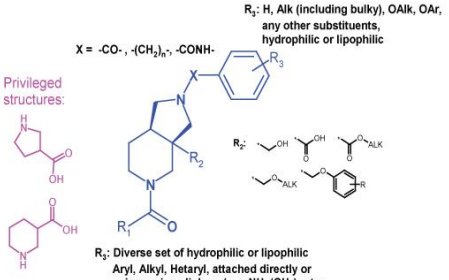
OUR STRATEGY FOR THE DESIGN OF HELIX-MIMETICS INVOLVES:

- **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 mimetics 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**

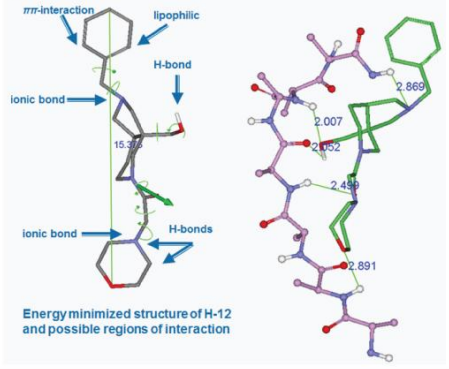


Energy minimized poly-and 7-alanine α-helix displaying *i*, *i* + 4, and *i* + 7 positions

EXAMPLE OF SELECTED SCAFFOLD:



EXAMPLE OF MODELING – INTERACTION OF H12 WITH 7-ALA:

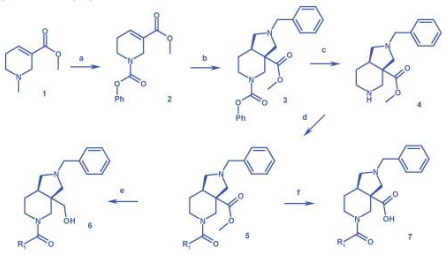


Energy minimized structure of H-12 and possible regions of interaction

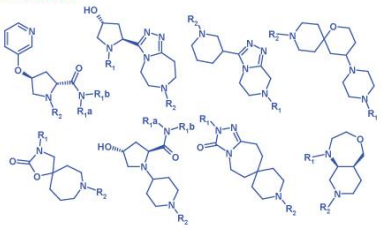
PHYS-CHEM PROPERTIES FOR TP-1 (REPORTED DESIGN) AND H-12 (CHEMDIV'S DESIGN)

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		-0.3
PSA	55.32	vs	56.25
HBD	3		1
HBA	5		6
NRB	12		7
Fsp3,%	43.3		66.7

GENERAL SCHEME FOR THE SCAFFOLD SYNTHESIS:

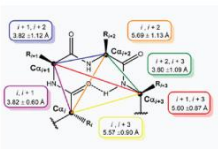


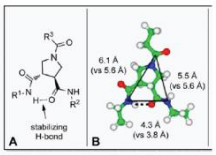
MORE EXAMPLES OF AHELIX-MIMETIC SCAFFOLDS:



β-TURN MIMETICS SUB-LIBRARY DESIGN

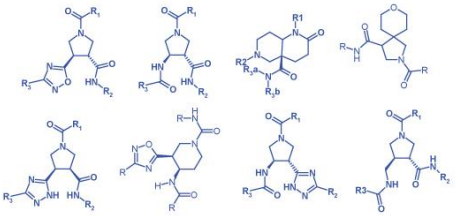
Proven approach proposed by L.R.Witby and D.L.Boger [12] for β-turn mimetic scaffolds selection was used for the sub-library design.


Mean and standard deviations of the Cα distances taken from a set of 10,245 β-turns in PDB [1]



Trans-pyrrolidine-3,4-dicarboxamide scaffold (A) and measured distances between substituted centers in low-energy conformation of its representative (R_1=R_2=R_3=Et) (B) [1]

SELECTED EXAMPLES OF β-TURN MIMETIC SCAFFOLDS:



HELIX/TURN 3D-MIMETICS LIBRARY SUMMARY:

- **The library consists of more than 42.7 α-helix- and β-turn 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);**
- **??K (??%) 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 related and/or absolute stereochemistry for all library members.**

PHYS-CHEM PARAMETERS FOR HELIX/TURN 3D-MIMETICS 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

Ziwei Huang, Gui-In Lee and Andrew D. Hamilton. Alpha-Helix Mimetics in Drug Discovery. In: Drug Discovery Research: New Frontiers in the Post-Genomic Era. P. 280-298. DOI: 10.1002/9780470131862.ch11

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ChemDiv, Inc Introduction, 6605 Nancy Ridge Drive, San Diego, CA 9212, USA. Tel: +1 858-794-4860, Fax: +1 858-794-4931, E-mail: sb@chemdiv.com
WWW.CHEMDIV.COM