Bioorganic & Medicinal Chemistry Letters 25 (2015) 2345–2352

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

De novo design of caseinolytic protein proteases inhibitors based on pharmacophore and 2D molecular fingerprints

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ARTICLE INFO

Article history:
Received 24 December 2014
Revised 25 March 2015
Accepted 7 April 2015
Available online 17 April 2015

Keywords:
Caseinolytic protein proteases inhibitors
De novo drug design
Pharmacophore
Molecular fingerprint
Molecular docking

ABSTRACT

Caseinolytic protein proteases (ClpP) are large oligomeric protein complexes that contribute to cell homeostasis as well as virulence regulation in bacteria. Inhibitors of ClpP can significantly attenuate the capability to produce virulence factors of the bacteria. In this work, we developed a workflow to expand the chemical space of potential ClpP inhibitors based on a set of β-lactones. In our workflow, an artificial pharmacophore model was generated based on HipHop and HYPOGEN method. A de novo compound library based on molecular fingerprints was constructed and virtually screened by the pharmacophore model. The results were further investigated by molecular docking study. The workflow successfully achieved potential ClpP inhibitors. It could be applied to design more novel potential ClpP inhibitors and provide theoretical basis for the further optimization of the hit compounds.

The caseinolytic protein protease (ClpP) is a highly conserved serine protease present in bacteria and higher organisms. ClpP is responsible for cell homeostasis and among other duties for regulating bacterial virulence in several pathogens including *Staphylococcus aureus* and *Listeria monocytogenes*.[1,2] Significant interests in ClpP inactivation started with the discovery of its crucial role in the virulence of these pathogens that cause severe infections in the clinics and are difficult to treat through the occurrence of multidrug resistance.[3–10]

There have been efforts to discover and develop small molecules that perturb the activity of ClpP[11–13] Sieber and co-workers demonstrated that selective inhibition of ClpP in *Staphylococcus aureus* resulted in a drastically decreased expression of major virulence factors.[14–16] To date, β-lactone is the main inhibitor scaffold that exhibits specificity for ClpP. It is important to systematically analyze the structural features and expand the chemical space of putative inhibitors.

Based on the biological assays provided by Prof. Stephan A. Sieber, we developed a workflow (shown in Fig. 1) based on the structural information of these β-lactones and ClpP protease. Two pharmacophore models were generated based on HYPOGEN and HipHop method, respectively. Because of the structural oneness, we combined two pharmacophore models artificially to expand the features and validated by the training set and decoy set. Meanwhile, we extracted the bioactive molecular fingerprints to generate de novo compound library to expand the scaffold. The workflow consisting of various methods provided a feasible solution for discovery of novel potential ClpP protease inhibitors and theoretical basis for further optimization of hit compounds.

In the Discovery Studio (DS), two of the most powerful methods applied to generate pharmacophore model are the Common Feature Pharmacophore Generation protocol (HipHop method) and 3D QSAR Pharmacophore Generation protocol (HYPOGEN method). The Common Feature Pharmacophore Generation protocol identifies configurations or three-dimensional spatial arrangements of chemical features that are common to molecules in a training set. The configurations are identified by a pruned exhaustive search, starting with small sets of features and extending them until no larger common configuration is found. 3D QSAR Pharmacophore Generation protocol derives Structure Activity Relationship (SAR) pharmacophore models from a set of molecules with known activity values on a given biological target.

A set of 37 β-lactone analogues with well-defined ClpP inhibitory activity (Table 1) obtained from Stephan A. Sieber laboratory was used to generate HYPOGEN hypotheses featuring quantitative predictive character. The HipHop hypotheses were generated using a set of 27 active analogues from the 37 inhibitors. For each compound, possible diverse sets of conformations were generated within a relative energy threshold of 20 kcal/mol using the BEST flexible conformation generation option. Selecting the chemical feature is one of the most important steps in generating pharmacophore. Feature Mapping module from DS was used to select the chemical features for hypothesis generation. While generating

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http://dx.doi.org/10.1016/j.bmcl.2015.04.035
0960-894X/

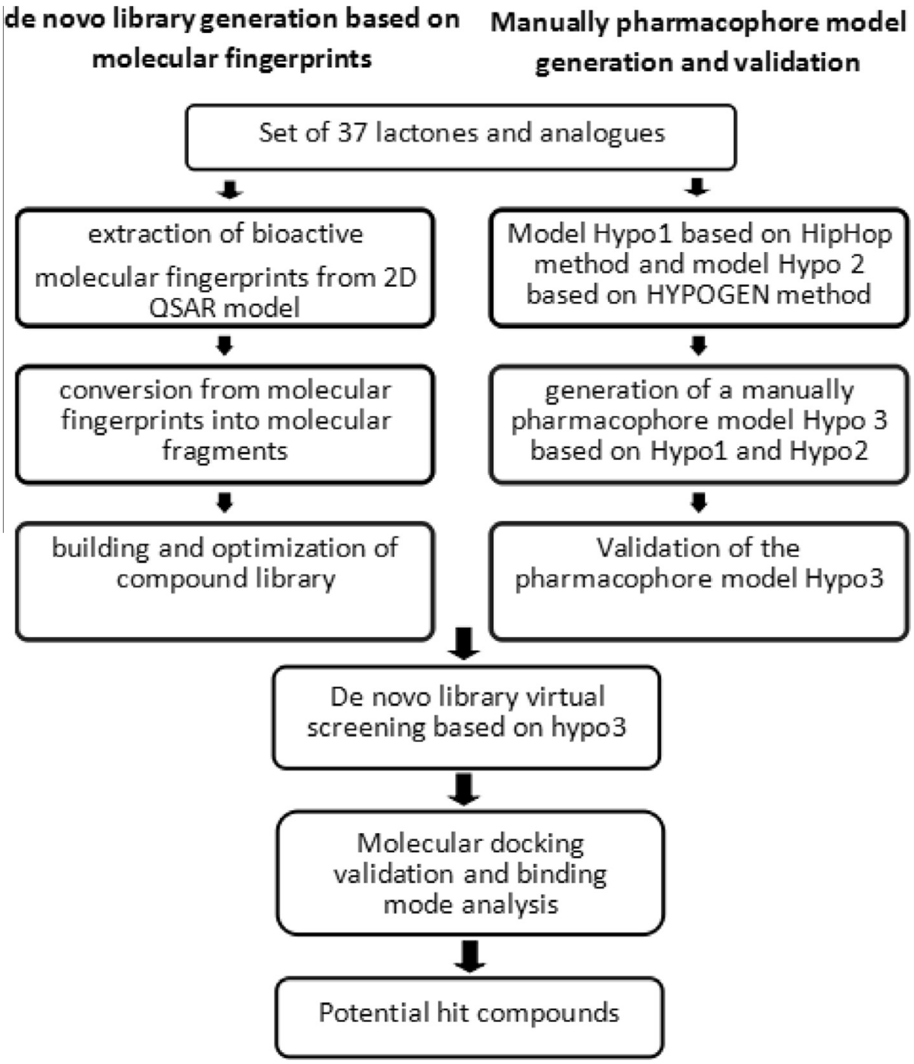
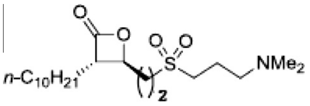
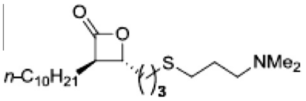
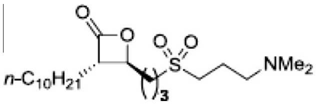
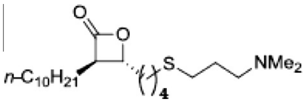
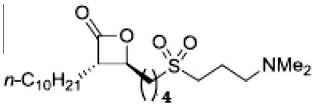
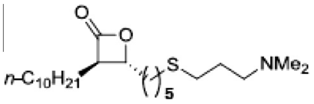
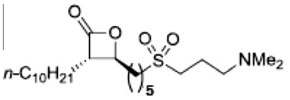
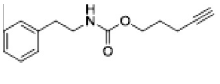
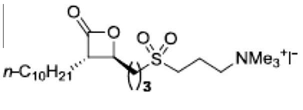
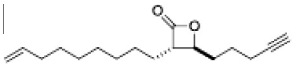


Figure 1. Workflow for the generation of ClpP inhibitors.

Table 1
The chemical structure, experimental active data of compounds

No.	Structure	A[a] %	No.	Structure	A%
1		1	20		26
2		0	21		6
3		0	22		15
4		5	23		92
5		0	24		5