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Original article

Discovery of flavonoid derivatives as anti-HCV agents via pharmacophore search combining molecular docking strategy

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ABSTRACT

Common feature based pharmacophore and structure-based docking approaches have been employed in the identification of novel anti-HCV candidates from our in-house database. A total of 31 hits identified *in silico* were screened *in vitro* assay. 20 Compounds demonstrated anti-HCV activities ($EC_{50} < 50$ μM), including two naturally occurring flavones apigenin (**21**) and luteolin (**22**) with low micromole EC_{50} values and three compounds (**23**, **24** and **25**) of novel scaffolds with moderate potencies. In addition, pharmacophore refinement was also conducted based on the current knowledge of flavone-derived anti-HCV candidates and the results of combined *in silico* and *in vitro* assays.

1. Introduction

Hepatitis C virus (HCV) infection remains to be an important health-care problem and has been identified as a leading cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma [1]. It is estimated that a minimum of 3% of the world's population (about 180 million people) are chronically infected, with additional approximate 3–4 million new cases of HCV infection each year [2]. The current care standard for HCV, composed of Pegylated Interferon-α and Ribavirin, can only achieve a sustained viral response in less than 50% of patients infected with the predominant genotype 1 [3]. Furthermore, patients are often intolerant to the serious adverse reactions of flu-like symptoms, depression, anemia, which might lead to poor treatment compliance [4]. It is urgent to develop novel anti-HCV agents with improved efficiency and minimal side effects.

The RNA-dependent RNA polymerase (RdRp) of HCV, also known as protein NS5B, is a key enzyme for the synthesis of a complementary minus-strand RNA, using the genome as template, and the subsequent synthesis of genomic plus-strand RNA from this minus-strand RNA template [5]. Since NS5B is crucial for viral infectivity, it has been recognized as a promising and validated target for HCV therapies [6].

α,γ-Diketoacids (DKAs) were initially revealed to be selective and reversible inhibitors against NS5B through high throughput screening (HTS) approaches [7]. Follow-up works demonstrated these compounds may serve as natural substrate UTP mimics which bind to the active site of NS5B [8]. However, due to poor physicochemical properties of these compounds, many DKA analogs or mimics with NS5B inhibitory activities have been designed and prepared [8–13]. Moreover, the replacement of DKA scaffold with naturally occurring flavonoid by scaffold hopping strategies, which led to the discovery of galangin derivatives, were among the most promising candidates (Fig. 1) [14–16].

In this study, considering the high interest of developing novel anti-HCV chemical candidates, a HipHop pharmacophore model, established from 8 reference NS5B inhibitors, was used as a filtrating tool to screening our in-house database. Then 246 hits were further screened by NS5B structural-based docking, among which 31 hits were identified. Finally, 20 compounds out of these hits were found be novel anti-HCV candidates through *in vitro* assays (Fig. 2). More, the pharmacophore for flavonoid-like analogs with

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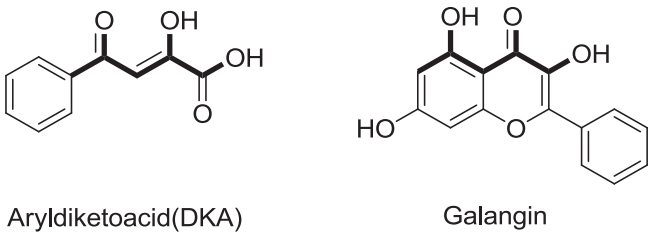

Fig. 1. The structures of Aryldiketoacid (aryl-DKA) and its bioisostere galangin (mimic substructure was shown in thick lines).

anti-HCV activities has been refined based on ligand-based and structure-based approaches.

2. Results and discussion

2.1. Establishment and validation of the ligand-based pharmacophore

Known as a powerful tool to identify novel compounds with similar biological activities, the pharmacophores could be developed by ligand-based method [17]. As shown in Fig. 3, the training set which was used to establish the pharmacophore was composed of eight NS5B inhibitors selected from literature [9,10,12–16] according to the following criteria: 1. they should share certain structural diversity; 2. they should be the most active compounds identified in each series; 3. they should be visually examined to contain similar pharmacophore components in order to ensure their similar binding models against NS5B. Due to limited activity scale (<2 log units) and set size of the training set, the HipHop module available in Discovery Studio (DS) [18] was adaptively used. Considering the more druggable properties of the three flavonoids (compounds **5**, **7** and **8**) in the training set, 'principal' values of 2 and 'Max-Omit-Feat' values of 0 were assigned to these compounds, while 'principal' and 'Max-Omit-Feat' values were set 1 for the other 5 compounds. Ten hypotheses (Hypos) were generated and scored as shown in Table 1. Considering it could both attain the highest score of 70.367 and accept all compounds in the training set statistically well enough, the Hypo 1 was selected and was used for further validation. On the other hand, other established hypotheses mapped poorly to at least one component of training set (fit value <1). The Hypo 1 has four features, namely one hydrophobic feature (H), one H-bond donor (D), and two H-bond accepters (A1 and A2) (Fig. 4A) and Compound **5** was mapped onto Hypo 1 with highest fit values of 3.99 (Fig. 4B).

Hypo 1 was further validated by the goodness of hit (GH) scoring method [19,20]. An external database of decoy set, which was used for pharmacophore validation, was made up of other 40 independent active and 1000 inactive compounds. 38 Positive compounds were successfully identified among total 55 hits and a set of statistical parameters, such as yield of actives, ratio of actives, enrichment factor (EF) and GH scores, were presented in Table 2. Thus the validated Hypo 1 was qualified to conduct virtual screening [21].

2.2. Virtual screening and molecular docking

Our in-house database, in which 15,568 commercially available natural products with searchable 3D structure were collected, was screened by Hypo1 to discover potential anti-HCV candidates. 246 Compounds were initially identified and most of which were flavonoids and flavonoids glycosides. Moreover, in order to minimize the number of hits and to maximize the probability of positive

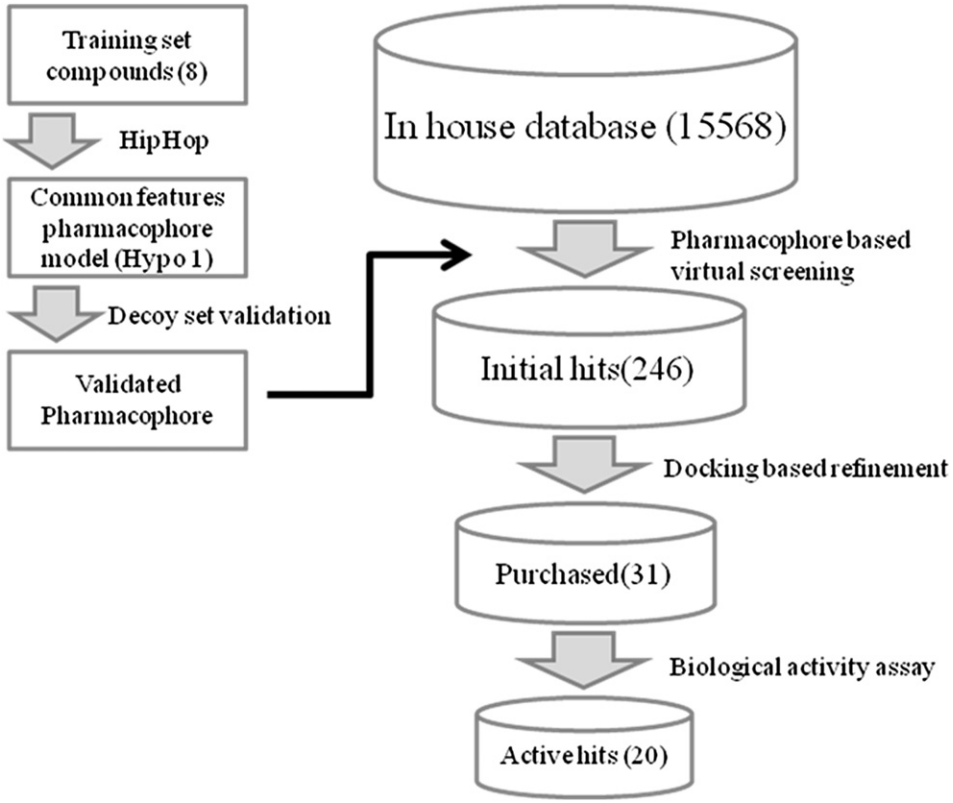

Fig. 2. Virtual screening flow chart.