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Identification of inhibitors for vascular endothelial growth factor receptor by using dynamic combinatorial chemistry

Zhao Yang [a], Zheng Fang [b], Wei He [c], Zhixiang Wang [a], Haifeng Gan [c], Qitao Tian [c], Kai Guo [c,*]

[a] School of Pharmaceutical Engineering, China Pharmaceutical University, No. 639 Longmian Avenue, Nanjing 211816, China
[b] School of Pharmaceutical Science, Nanjing Technology University, No. 30 Puzhu South Road, Nanjing 211816, China
[c] College of Biotechnology and Pharmaceutical Engineering, Nanjing Technology University, No. 30 Puzhu South Road, Nanjing 211816, China

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ABSTRACT

The novel analysis method consisting of size-exclusion chromatography (SEC) and HRMS analysis was firstly applied in the discovery of potential inhibitors towards cancer drug targets. With vascular endothelial growth factor receptor (VEGFR-2) as a target, dynamic combinatorial libraries (DCLs) were prepared by reacting aldehydes with amines. Four sensitive binders targeted VEGFR-2 were directly isolated from the library. Antitumor activity test in vitro and inhibition experiments toward angiogenesis were also carried out.

Over the past decade, a great deal of efforts have been made to generate efficient lead compounds. Generally, the preferred methods used for this strategy are high-throughput screening,[1] rational design,[2] and fragment-based approaches.[3] Due to its higher efficiency, the latter has been increasingly a powerful strategy to identify ligands for biological targets.[4,5] Meanwhile, a novel fragment-based methodology, dynamic combinatorial chemistry (DCC) was developed. Typically, analog compounds in traditional approaches need to be synthesized individually and subsequently evaluated in a parallel fashion. However, DCC allows the chemical synthesis with biological tests in one single pot. Prior to addition of external templates, the concentration of library members is dictated by its relative stability.[6–8] Upon addition of a target, the equilibrating species interact with the template via non-covalent interactions, thereby leading to selection and amplification of the strongest binders in the DCLs at the expense of other species.[9] Recent advances include the discovery of aspartic protease endothiapepsin inhibitors,[10] diselenide exchange reaction,[11] and the study of dynamic boronic acid/boronate ester systems.[12] Consequently, DCC has considerable potential as a tool for the discovery of new ligands targeted bioactive molecules.

Larger DCLs are necessary for screening high bioactive compounds. However, a particular important challenge in DCC is the associated analytical methods. Although high-resolution techniques, such as NMR spectroscopy and X-ray crystallography are applied in the ligand identification, library size is limited to tens of compounds at most due to restricted analytic ability.[10,13,14] Our research group has used HRMS analysis coupled with size-exclusion chromatography (SEC) to identify the inhibitors of BSA and lysozyme.[15,16] New highly sensitive binders were directly isolated from the related DCLs, which demonstrated that SEC–HRMS method has emerged as a powerful tool to complement the lead identification in the drug discovery field. However, this method has not been used in the screening of inhibitors aimed at drug targets.

Protein kinase plays a vital role in the regulation of different cellular processes mainly through catalyzing endocellular phosphorylation reaction of specific amino acids, which are closely related to the growth of tumor. Recently, inhibitors of receptor protein tyrosine kinases (RPTKs) have drawn much attention due to its importance in the treatment of cancer.[17] Therefore, it is very significant to explore effective and targeted inhibitors of protein kinase. VEGFR-2 has emerged as an important receptor protein tyrosine kinase in the past several years, thus considerable efforts have been put into developing efficient inhibitors targeted at VEGFR-2.[18] In the previous research, studies concerning 6-urea-3-substituted vinyl indazole compounds have been intensively conducted. Moreover, Schiff base group in compound **1** was replaced by ethylene groups, resulting in compound **2**. Interestingly, it was profitable to exert an anti-neoplastic effect when the R^1 and R^2 (Fig. 1) groups were occupied by aryl groups.[19] However, tedious

* Corresponding author.

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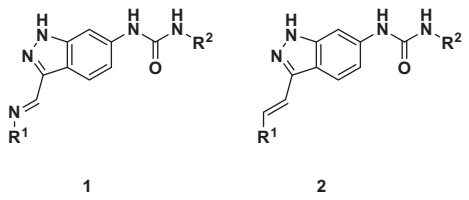


Figure 1. General structural formula of 6-urea-3-substituted vinyl indazole.

and immense work is necessary if a traditional drug screening process is employed. Besides, the synthesis and indeed biological screening of bioactive ligands are very expensive, in term of time, money and resources.

In this study, we reported our recent progress in identifying the most potential compounds through the establishment of DCLs, combing with the SEC–HRMS analysis method.

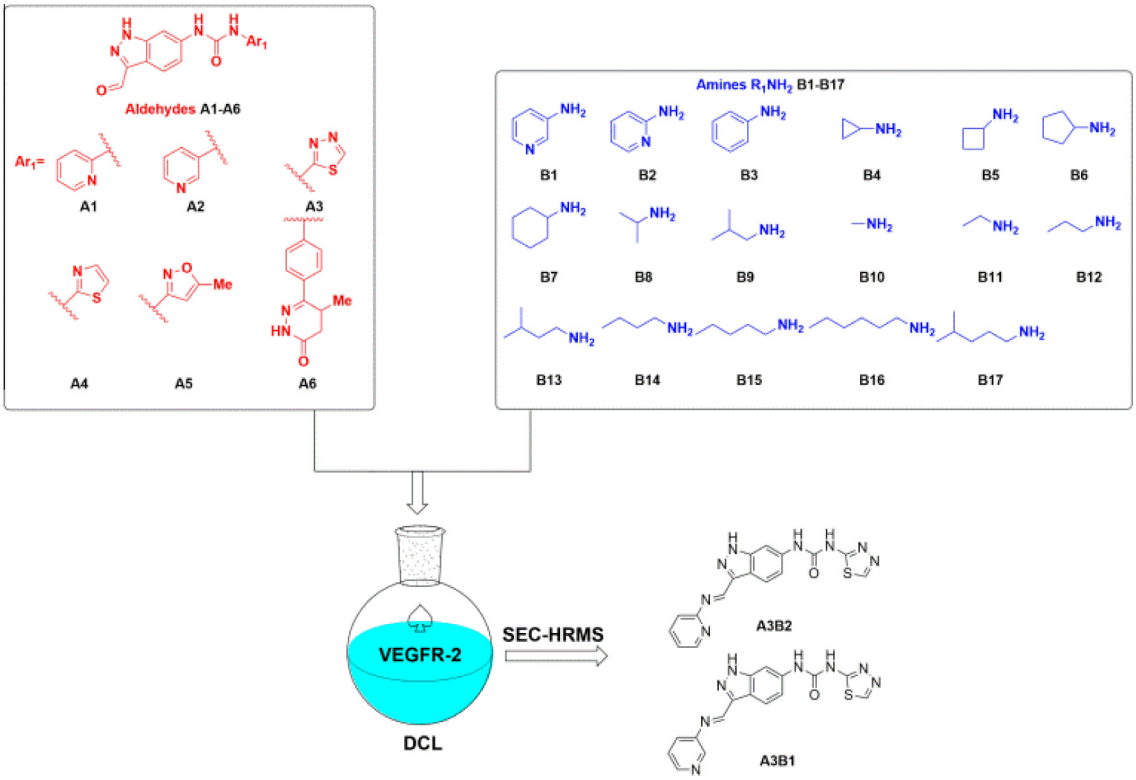
Initially, DCL 1 (Scheme 1) was designed based on analogous derivatives by reacting aldehydes A1–A6 with amine B1–B17. Some aliphatic amines and cyclamines were selected. Besides, isomers of these aliphatic amines were also added in the library. Occasionally, two heterocyclic compounds were included in the library. Interestingly, 365.0921 was observed in the HRMS results (Fig. 2), while, no ligand was detected in the control test. These results demonstrated that A3B1 or A3B2 might be the potential inhibitors. On the contrary, imines formed between aliphatic amine and aldehydes were not identified from the library.

In theory, the larger the library designed, the higher the probability of discovering potential binders towards targets. Thus, a larger DCL was prepared by adding more aldehydes and amines into the library. Five aromatic aldehydes were supplied. However, the formed imines showed worse binding ability towards VEGFR-2 when R^1 was occupied by aryl groups. In addition, more heterocyclic compounds were also added in the DCL 2 (Scheme 2) allowing for the interesting results revealed in the previous section. A blank experiment proceeded with the same protocol without VEGFR-2 as a target. Interestingly, no molecular ion peaks were detected in the HRMS results. On the contrary, the same molecular ion peak (365.092) was detected again in the HRMS analysis of DCL 2 with VEGFR-2 as a target (Fig. 3). Moreover, another molecular ion peak (473.1501) was observed. Through the analysis of library components, four compounds with the corresponding molecular weight detected in the HRMS were discovered. Two other potential binders (A6B27 and A4B21) were observed apart from A3B1 and A3B2. Therefore, further investigation was necessary for identifying the real binders in the library.

To further validate the bioactivity of these selected compounds, inhibiting effect of four potential binders in vitro was determined according to classic MTT method.[20] T lymphocyte leukemia cells (Hun 78), human lung cancer cells (A546), human prostate cells (PC3), human breast cancer cells (MDA-MB-435), human colon cancer cells (HT-29), human gastric cancer cells (BGC-823) were applied in the biological activity assay. IC50 values were calculated according to BLLIS method.[21]

As revealed by the data in Table 1, A3B2 showed better inhibitory activity towards MDA-MB-435 and HT-29, while, A3B1 showed better inhibitory activity towards A546 and PC3. Similarly, a discriminating inhibitory activity was observed when A6B27 and A4B21 were added into the culture medium. Generally, different growth and metabolic mechanism in various cells resulted in diverse therapeutic effect when one anti-cancer drug was applied in the treatment of various cancers. Despite all this, the suppression tests still displayed that the screened compounds showed a moderate to good inhibitory activity towards common cancer cells compared with Sunitinib.

Another important feature of inhibitors targeted VEGFR-2 was inhibition rate toward neoangiogenesis. Thus, inhibitory experiments towards chick chorioallantoic membrane angiogenesis model were also conducted in vitro. When the chicken embryo was incubated for 1 week, chick chorioallantoic membrane was



Scheme 1. Synthesis of DCL 1 targeted VEGFR-2.