Design, Synthesis & Screening of Natural Product DEL

WuXi Biology

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Introduction

DNA-encoded library (DEL) technology has been recognized as one of the major screening methods with its unique advantages in vast chemical diversity and multiplexed affinity-based screening. However, current DEL library mostly focuses on small molecular and peptide, with only limited cases reported on natural products. Natural product has its unique advantages, including diverse skeletons and structural complexity. Thus, we constructed two natural products libraries with a diversity of around 100,000 and further utilized these natural product libraries to Acetylcholinesterase (AChE), a classic neuro-related target. Various Chemotypes were observed in the post-selection data analysis and a total of 12 on-DNA compounds were synthesized for primary validation. 9 of them were validated as potential AChE binders. In Summary, our results demonstrate the feasibility of constructing natural products-based DEL Library and the application of them on certain targets to further expand drug discovery chemical space.

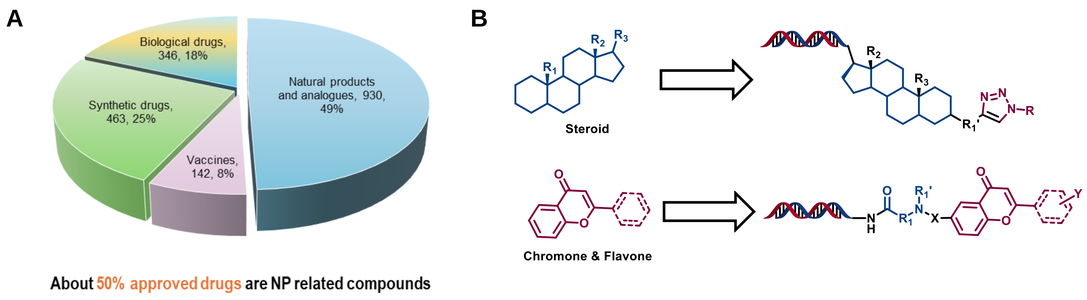


Figure 1. (A) In approved drugs, molecules related to natural products account for more than half of the total. (B) Design and of DEL libraries based on natural product scaffolds.

Design and Synthesis of Natural Product DEL

Steroidal compounds are classes of natural products widely found in plants, animals, and microorganisms. They possess various biological activities and medicinal values, including antitumor activity, metabolic regulation, antibacterial, and antifungal properties. Modifications at different sites on the steroidal skeleton can significantly impact their biological activities.

Chromone and flavone are classes of polyphenolic secondary metabolites commonly found in plants, exhibiting a range of biological activities such as antioxidant, anti-inflammatory, antitumor, and immune-regulatory effects. The flavonoid skeleton has also been extensively studied due to its diverse biological activities and broad medicinal value.

Based on steroid, chromone and flavonoid natural product scaffolds, we designed two DEL libraries, aiming to effectively explore the chemical space around them and facilitate the discovery and optimization of natural product drugs.

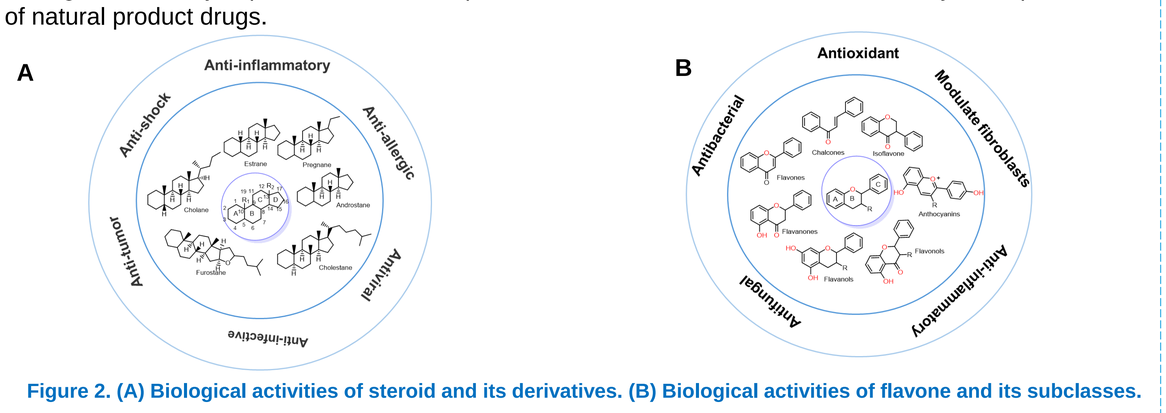


Figure 2. (A) Biological activities of steroid and its derivatives. (B) Biological activities of flavone and its subclasses.

The construction of the natural product DELs is depicted in Figure 3. The DELs are accomplished within two chemical cycles.

Regarding the steroid-derived DEL, in Cycle 1, it was connected to 37 steroid scaffolds (BB1) by amidation. Subsequently, 1,645 amino compounds were transformed into azides and linked to the steroid scaffold through a click reaction in Cycle 2. The diversity of the resulting library amounted to 60,865.

The synthesis of the Chromone/Flavone DEL is shown in Figure 3B. In Cycle 1, 883 protected amino acids were linked to DNA and subsequently deprotected. Cycle 2 involved reactions around BB1's amino group through four routes: acylation with 2 chromones and 14 flavone scaffolds, reductive alkylation with 4 chromones, Sn2 reaction with 1 flavone scaffold, and Sonogashira reaction with 4 chromones. The final library size was 28,600.

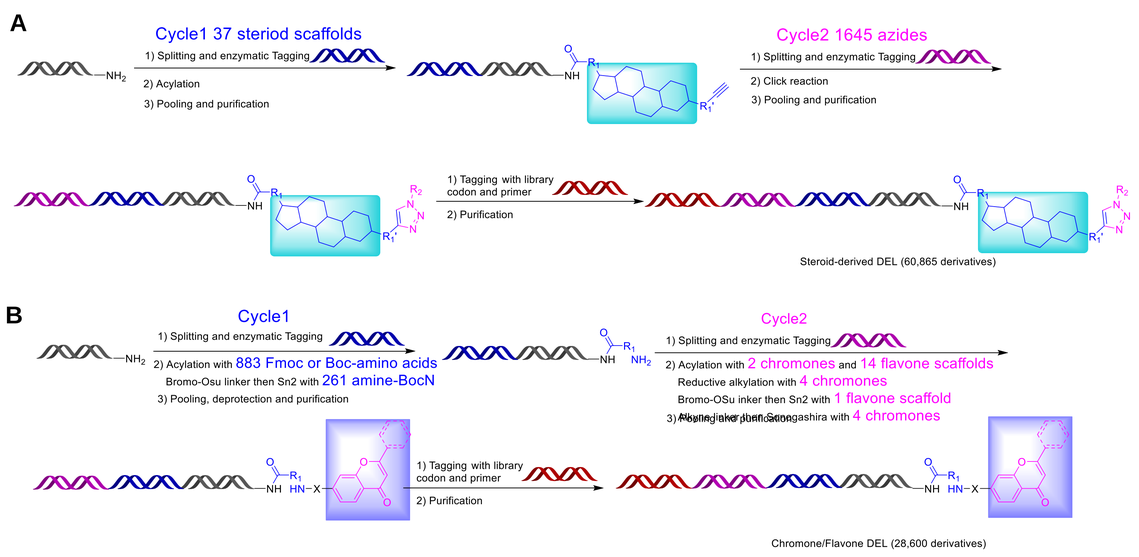


Figure 3. (A) Synthesis route of steroid-derived DEL. (B) Synthesis route of Chromone/Flavone DEL

Screening and Post-Selection Chemistry

AChE is a key enzyme involved in biological neurotransmission and serves as a critical target for various neurological disorders. Several AChE inhibitors (AChEI) have been developed and are currently in clinical use. Among them, natural products are an important source of AChEI, with various natural products and their analogs, such as alkaloids, demonstrating significant AChE inhibitory activity. Due to the good biocompatibility and safety of natural products, the development of new natural product-based AChE inhibitors could provide patients with potentially safer and more effective drugs.

To discover novel natural product derivatives targeting AChE, we conducted an affinity screening against AChE with the natural product DELs. We used AChE protein and NTC as two parallel channels for screening, employing Ni-NTA magnetic beads for protein capture. After elution, qPCR, and PCR, High-throughput Decoding was performed. Data analysis of the screened results indicated that the natural product library with a steroidal scaffold showed greater data enrichment during screening and displayed significant SAR information. In contrast, the libraries based on isoketones and flavonoids did not exhibit any obvious structural patterns.

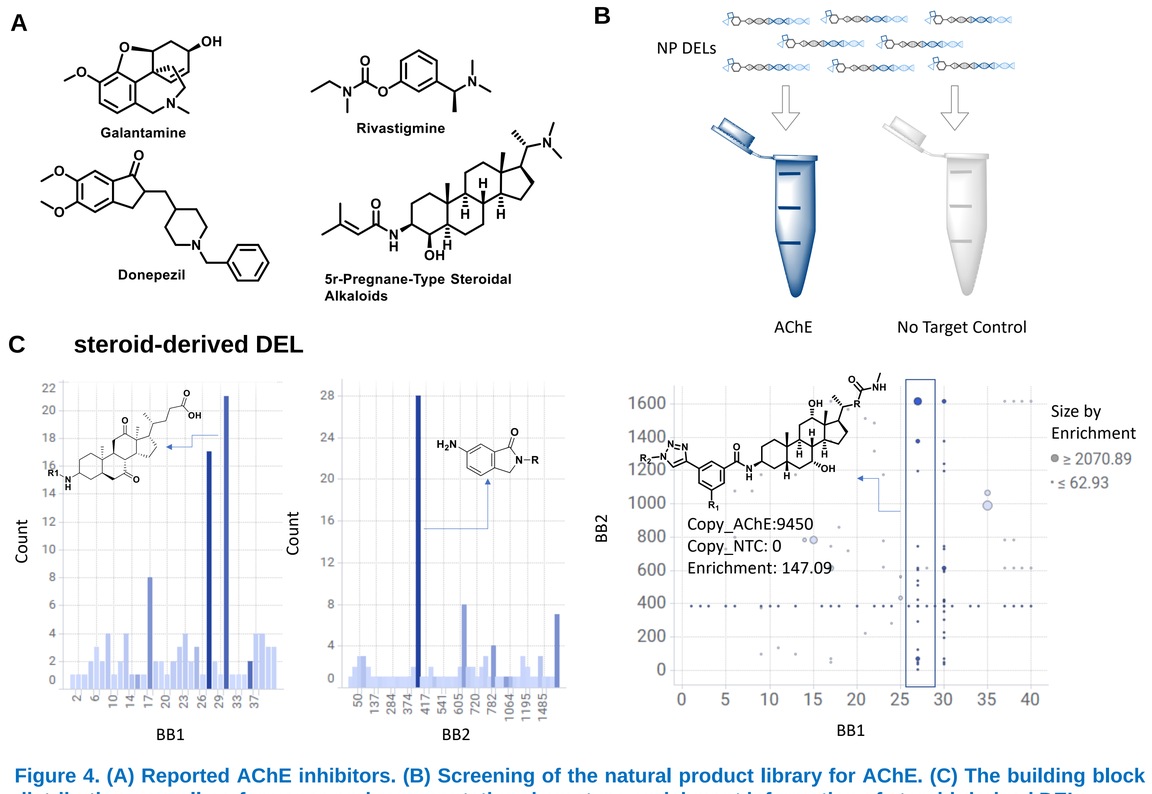
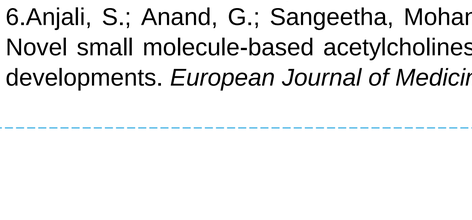


Figure 4. (A) Reported AChE inhibitors. (B) Screening of the natural product library for AChE. (C) The building block distribution, overall performance and representative chemotype enrichment information of steroid-derived DEL.
Each of the x, y axes represent the single cycle of reaction in a two-cycle DEL. The size of each dot is proportional to the enrichment values of each compound.

On-DNA Validation

Subsequently, we selected 12 compounds from the screened chemotypes for on-DNA resynthesis. These compounds were then validated using affinity selection mass spectrometry (ASMS). The results showed that 9 out of the 12 synthesized compounds exhibited potential binding to AChE. Regarding the structure of these compounds, aside from the triazole that is generated as a fixed product, the modifications to the steroidal scaffold in these molecules primarily involve electron-deficient aromatic rings. The structural uniqueness of these natural product analogs demonstrates their potential to become novel AChE ligands.



Item	Retention time (min)	Area	Recovery rate
Reference	3.34	6.52E+04	8.16%
AChE-Elution	3.35	5.32E+03	
NTC-Elution	NA	NA	

Structure Information

Figure 5. On-DNA ASMS result of representative compound NP-1. The desired product and a partial product is determined as binder to AChE. The exacted peak was determined according to the molecular mass of corresponding compound. The area was analyzed by UNIFI and recovery rate was calculated.

Reference

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