Natural Product Mimetics

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From Complex Natural Products to Simple Synthetic Mimetics by Computational De Novo Design

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Abstract: *We present the computational de novo design of synthetically accessible chemical entities that mimic the complex sesquiterpene natural product (−)-Englerin A. We synthesized lead-like probes from commercially available building blocks and profiled them for activity against a computationally predicted panel of macromolecular targets. Both the design template (−)-Englerin A and its low-molecular weight mimetics presented nanomolar binding affinities and antagonized the transient receptor potential calcium channel TRPM8 in a cell-based assay, without showing target promiscuity or frequent-hitter properties. This proof-of-concept study outlines an expeditious solution to obtaining natural-product-inspired chemical matter with desirable properties.*

Natural products are of fundamental interest as chemical matter for interrogating biological systems.[1,2] They contain biologically pre-validated architectures that allow the exploration of drug-relevant chemical space.[3] However, drug discovery still awaits the full exploitation of pharmacologically active natural products because, among other reasons, their supply is limited and they often possess complex chemical structures rendering total syntheses difficult.[4] Herein, we present the computational de novo design of synthetically accessible, isofunctional mimetics of the structurally intricate natural product (−)-Englerin A (**1**), a sesquiterpene from *Phyllanthus engleri*. Nanomolar binding affinities and high ligand efficiencies to the transient receptor potential melastatin 8 (TRPM8) ion channel designate these designer compounds.

The molecular basis for the potent antiproliferative activity of (−)-Englerin A was discovered by Waldmann and co-workers.[5] The natural product selectively kills renal cancer cells ($GI_{50} = 1$–87 nM) through activation of TRP canonical 4/5 (TRPC4/5) calcium channels, but its development into a clinical candidate may be precluded by acute toxicity.[6] Its total synthesis was first achieved by Willot et al.[7] and has recently been simplified to 14 steps.[8] Recently, the rational design of terpene-based (−)-Englerin A mimetics has failed to deliver chemical entities with potent anticancer activity.[9]

Bioactivity-guided scaffold trees have proven useful for the stepwise exploration of natural-product-derived molecular frameworks.[10] We therefore envisaged that ligand-based, computer-assisted de novo design might be able to translate the complex pharmacophore patterns of natural product templates into simpler and synthetically more accessible entities.

We started off by using the ligand-based software tool DOGS (Design of Genuine Structures)[11] to computationally generate mimetics of (−)-Englerin A (Figure 1). This algorithm performs a reaction-based molecular design process using 83 organic synthesis schemes and 25214 molecular building blocks, and has been successfully used for the de novo design of lead- and drug-like new chemical entities (NCEs) before.[12] The automated design process resulted in 903 in silico structures (Figure 2), which were then compared to (−)-Englerin A in terms of their topological pharmacophore feature similarity (CATS method).[13] This re-scoring step was done to increase the chance of finding isofunctional NCEs as a consensus of two similarity metrics, namely the DOGS scoring function[14] and the CATS similarity (Supporting Information).[15] The resulting top-scoring compounds were further analyzed for their potential macromolecular targets. For this purpose, we used software that has been shown to be able to predict the targets of drug- and fragment-like compounds and complex natural products (SPiDER method).[16] SPiDER infers potential drug targets from pharmacophore and property similarities between the query compound and known pharmacologically active ligands with known targets.

Based on the similarity analysis and the target predictions, we selected designs 1 and 2 on ranks 4 and 18 of the result list for further consideration, taking into account the availability of starting materials, absence of reactive moieties, and predicted solubility (Figure 1; Supporting Information). For ease and reduced cost of synthesis, we converted the aliphatic rings to arene systems. Compound **2** was obtained from the reaction of the required oxazolidinone and acyl chloride, followed by installation of the furanyl moiety. Suzuki coupling of *N*- and *C*-protected 3-bromophenylalanine afforded compound *rac*-**3** (Scheme 1).

With both compounds in hand, we tested them in functional cell-based assays for TRPM8 and TRPV1 modulation (Table 1). Compounds **2** and **3** potently blocked the TRPM8 subtype ($K_B = 1.4$ and 0.2 μM, respectively), but did not inhibit the V-type TRP (VR1) calcium channel at a concentration of 10 μM. (−)-Englerin A equipotently blocked TRPM8 ($K_B = 0.4$ μM), thereby revealing an apparently broader activity

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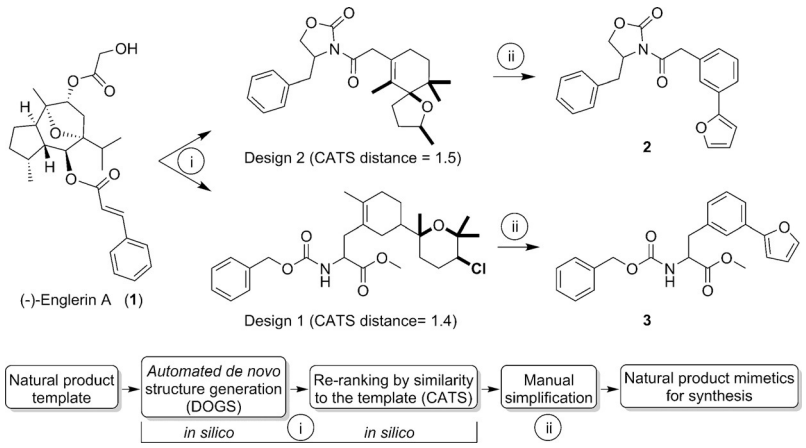


Figure 1. The molecular design strategy. In the first step (i), the design template 1 was computationally converted into 903 de novo designs by reaction-based building block fusion. Re-scoring of the computer-generated structures with the topological pharmacophore metric CATS[13] (lower values suggest greater similarity to (−)-Englerin A) and the assessment of synthetic feasibility led to the selection of the original designs 1 and 2. Simplification of their chemical structure for ease of synthesis resulted in compounds **2** and **3**. The substructures of designs 1 and 2 that are not contained in the final compounds **2** and **3** are highlighted in bold. The chiral centers of the designed compounds are not assigned, because the software did not consider stereochemistry.

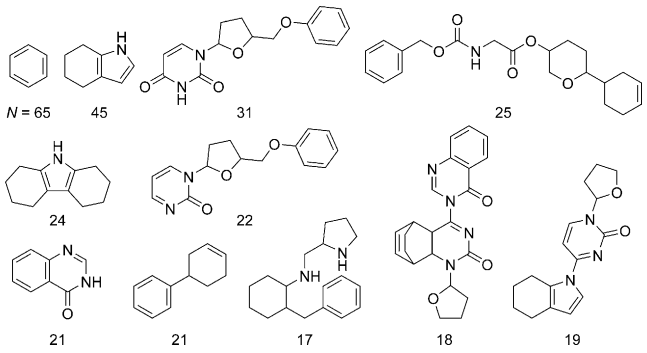
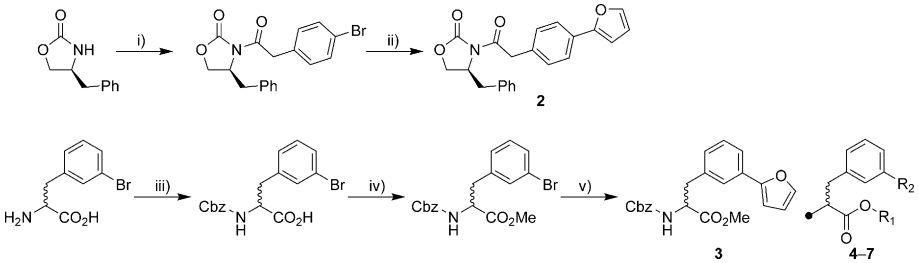


Figure 2. The most frequent molecular scaffolds of 903 de novo designed (−)-Englerin A mimetics. The numbers are absolute frequencies.



Scheme 1. Synthesis of the (−)-Englerin A mimetics **2** and **3**. Reagents and conditions: i) *n*-BuLi, 2-(4-bromophenyl)acetyl chloride, THF, −78 °C→RT, 4 h; ii) furan-2-ylboronic acid, $Pd(PPh_3)_2Cl_2$, Na_2CO_3, DMF/H_2O, 100 °C, 5 h; iii) Cbz-Cl, Na_2CO_3, dioxane, 0 °C→RT, 24 h; iv) H_2SO_4, $MgSO_4$, MeOH, CH_2Cl_2, RT 24 h; v) furan-2-ylboronic acid, $Pd(PPh_3)_4$, Cs_2CO_3, DMF/H_2O, 100 °C, 5 h.

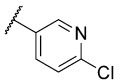
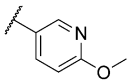
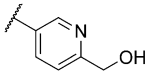
spectrum than originally assumed by Akbulut et al.[5] Significantly, the screening results showed that the de novo designs inherited the binding potential to the TRP target family from their mother natural product. Both (−)-Englerin A and the designer compounds are isofunctional antagonists of the TRPM8 calcium channel. While (−)-Englerin A shows acute toxicity,[6] the two de novo designed compounds **2** and **3** were non-cytotoxic in a preliminary study with human primary glioblastoma cells (U-87 MG cells), which do not express TRPM8 (Supporting Information). Furthermore, these compounds display high ligand efficiencies ($LE > 0.30$), rendering them suitable for further exploration as TRPM8 antagonists.[17]

To exclude artifact measurements, we measured the solubility of compound **3** and performed in silico frequent hitter assessment. We did not observe colloidal aggregation in water up to a concentration of 60 μM. Its molecular structure is free from apparent reactive and otherwise undesired substructure flags, and possesses a predicted low target promiscuity score (<2%).[18] We therefore concluded that compound **3** blocks the TRPM8-mediated Ca^{2+} flux in a specific concentration-dependent fashion and has a low off-target potential. This conclusion was further substantiated by activity testing against a panel of 12 potential macromolecular targets predicted by the SPiDER software (Supporting Information). Of these targets, only PXR was mildly antagonized by compound **3** in a concentration of 10 μM (52% replacement of radiolabeled agonist T0901317, $K_i = 100$ nM). This outcome indicates natural products as suitable templates for the de novo design of synthetic mimetics. It equally corroborates the target promiscuity prediction model (inSili.com GmbH, Zurich, Switzerland), which correctly designated compound **3** as a target-family selective ligand.[18]

To obtain preliminary structure–activity relationship data, we synthesized derivatives of compound **3**. We chose the site of structural variation by flexibly aligning compound **3** in the *R* and *S* configurations, respectively, on a low-energy conformation of (−)-Englerin A and analyzing their superimposed pharmacophores (Figure 3). The *S* enantiomer led to a better overall match and we decided to explore the least well aligned region in more detail. The alignment suggested (*S*)-**3** as the bioactive enantiomer. Derivatives **4**–**7** were obtained in modular fashion to interrogate the effect of substituting the furanyl moiety on activity against TRPM8 (Table 1). With the exception of compound **7**, all derivatives potently blocked the ion channel with sub-micromolar activity.

The results of this study demonstrate that computational de novo design can be productively applied to finding synthetically accessible NCEs from structurally complex natural products. Although we used a two-dimensional

Table 1: Inhibitory activities of (−)-Englerin A, **1**, and the designed compounds **2**–**7** (see Scheme 1) against the target ion channel TRPM8.

No.	R_1	R_2	IC_{50}/ μM	K_B [μM]
1[a]			3.0 ± 0.08 log units	0.4
2[b]			10 ± 0.08 log units	1.4
3	Me		1.7 ± 0.12 log units	0.2
4	t-Bu		2.0 ± 0.13 log units	0.3
5	Me		4.5 ± 0.07 log units	0.6
6	Me		3.7 ± 0.50 log units	0.5
7	Me		>10	

[a] (−)-Englerin A was purchased from Axon Lab AG (Baden, Switzerland). [b] $K_B = IC_{50}[1 + (C/EC_{50.C})]^{-1}$, where C is the concentration of reference agonist in the assay and $EC_{50.C}$ its EC_{50} value.

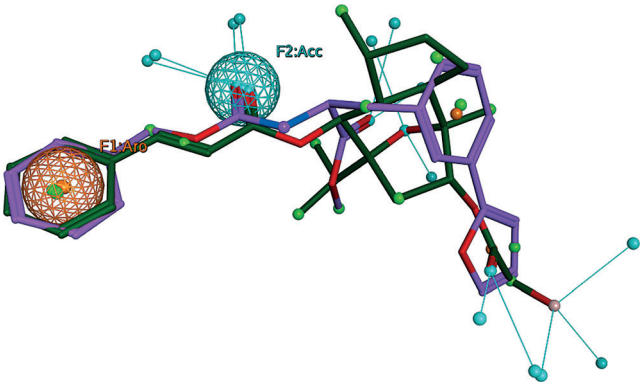


Figure 3. Alignment of low-energy conformations of (−)-Englerin A (green) and compound (*S*)-**3** (magenta). Two perfectly superimposed pharmacophore features are highlighted (F1: aromatic, F2: H-bridge acceptor).

ligand-based approach, one can speculate that the intricate stereochemistry of the template (−)-Englerin A was implicitly captured by the similarity metrics that guided the in silico design process. Evidently, the simplified structural and pharmacophore features of the designed compounds were sufficient for blocking the TRPM8 channel. This observation may not be transferable to structurally more demanding natural product templates (for example, flexible macrocycles) and other targets. For such cases, the design approach could be extended by conformation-sensitive compound re-scoring, as a first next step. By substituting some of the "three-dimensionality" of the natural product with planar arene systems in the molecule simplification step (Figure 1), we eliminated a perceived advantage of natural products.[19] It remains to be shown if this idea is generally applicable. Comparing our design strategy to alternative approaches that aimed at finding TRPM8 ligands, the main advantage lies in the discovery of new chemotypes without the need for high-throughput screening.[20] It represents an automated and largely unbiased design approach. However, de novo design does not eliminate the need for subsequent hit-to-lead optimization.

Despite providing an attractive solution to drive the design of biologically-relevant chemical matter, a single proof-of-concept application should not be over-interpreted and oversold. A thorough assessment of the potential and the limitations of this de novo design approach with regard to diverse natural product templates will be mandatory. Keeping a healthy skepticism, the molecular design concept outlined here might open a new path for natural-product-inspired chemical biology and medicinal chemistry.[2,21]

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