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Integrating DNA-encoded chemical libraries with virtual combinatorial library screening: Optimizing a PARP10 inhibitor

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Abstract

Two critical steps in drug development are 1) the discovery of molecules that have the desired effects on a target, and 2) the optimization of such molecules into lead compounds with the required potency and pharmacokinetic properties for translation. DNA-encoded chemical libraries (DECLs) can nowadays yield hits with unprecedented ease, and lead-optimization is becoming the limiting step. Here we integrate DECL screening with structure-based computational methods to streamline the development of lead compounds. The presented workflow consists of enumerating a virtual combinatorial library (VCL) derived from a DECL screening hit and using computational binding prediction to identify molecules with enhanced properties relative to the original DECL hit. As proof-of-concept demonstration, we applied this approach to identify an inhibitor of PARP10 that is more potent and druglike than the original DECL screening hit.

Graphical Abstract

Keywords

DNA-encoded chemical libraries; Computer-guided drug discovery; Poly-(ADP-ribose) polymerase; Hit-to-lead development; Virtual combinatorial libraries

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Conflict of Interest

The authors declare no conflict of interest.

Appendix A. Supplementary Material

Supplementary material to this article can be found online at TBD.

ADP-ribosylation is a ubiquitous post-translational protein modification that regulates important cellular processes.¹ Poly ADP-ribose polymerases (PARPs) constitute a family of enzymes that form ADP-ribose protein modifications from nicotinamide adenine dinucleotide (NAD⁺) substrates.² PARP-modulating pharmaceuticals are of high medical importance, and four inhibitors of PARP1 are in clinical use.^{3, 4} The majority of PARPs are mono (ADP-ribose) transferases,⁵ and both their cellular roles and medicinal chemistry are less well understood than that of the *bona fide* PARP polymerases.^{3, 4} PARP10 is an example of a mono-ADP ribose transferase that regulates important cellular processes.⁶ PARP10 is involved in immune signaling,⁷ regulation of metabolism,⁸ response to DNA-damage,⁹ and transcriptional regulation.¹⁰ The development of chemical probes to study the biological functions of PARP10 is therefore an active research area,^{11–14} and there is a need to further elucidate its medicinal chemistry and to develop chemical probes with more favorable properties.

As part of our research to establish focused DNA-encoded chemical libraries (DECLs) for drug development, we discovered micromolar inhibitors of PARP10.¹⁵ The most prominent PARP10 hit series that emerged from this study contained a 6-carboxy tetralone fragment connected to aromatic heterocycles or phenyl groups via a 2,3-diaminopropionyl methylcarboxamide (MeNHDAP) linker. The MeNH-DAP linker is a remnant of the DECL designed to target NAD⁺-binding proteins (NADEL; NAD⁺-mimicking DNA-encoded library), which consists of two carboxylic acid groups assembled on the conserved DAP scaffold (the structure of NADEL is shown in Fig. 2).¹⁵ While the diverse carboxylic acids contained in NADEL efficiently map out parts of the NAD⁺-binding sites, the conserved MeNH-DAP linker samples the protein surface between these fragment-binding pockets suboptimally. Replacing the MeNH-DAP moiety may provide additional binding energy. Furthermore, the MeNH-DAP linker negatively affects the compounds physicochemical properties by contributing a large topological polar surface area (TPSA = 87.3 Å²), as well as several hydrogen bond donors (n = 3) and acceptors (n = 6). The MeNH-DAP group also has four rotatable bonds. Replacing the MeNH-DAP moiety emerges therefore as a priority towards developing NADEL hits for PARP10 and other targets into lead molecules.

The encountered situation is representative for DECL-screening hits in general. DECLs allow screening vast compound libraries rapidly and economically^{16–20} and have provided clinical lead compounds.^{21–23} The combinatorial nature of DECLs and the need to attach molecules to DNA codes leads to libraries of compounds for which most of the structural diversity is concentrated in some parts of the molecules (often capping fragments such as carboxylic acids or amines) whereas other parts of the molecule (e.g. scaffolds) have lower structural diversity.²⁴ Extensive research efforts have been dedicated to alleviate this problem by developing new DNA-compatible chemistry,²⁵ constructing DECLs with variable scaffolds,^{26–28} natural product derived DECLs,^{29–31} and building libraries in which different reactions are performed during a synthesis cycle.^{32, 33} We aim to address the challenge of low-diversity substructures at the level of hit-to-lead development instead of during DECL construction. To date, only few studies have explored how to take advantage of the unique combinatorial characteristics of DECLs²⁴ to facilitate lead development. In one report, McCloskey *et al.* combined DECL-screening data with machine learning to

discover compounds with improved potency and properties.³⁴ In another approach, Wellaway *et al.* merged DECL hits with fragment-based screening.³⁵ Here we present a method that integrates DECL screening with structure-based computational drug development methods. In the outlined strategy, virtual combinatorial libraries (VCLs) are designed based on the structure of a DECL screening hit; moieties that are highly diverse in the DECL are kept constant in these VCLs, and parts that have low structural diversity in the underlying DECL are varied (Fig. 1). To evaluate this concept, we used one of the PARP10 screening hits discovered from NADEL¹⁵ and replaced the unfavorable MeNH-DAP moiety by alternative linkers.

The starting point to explore the proposed approach of integrating DECL screens and computational drug discovery was the PARP10 inhibitor A82-(CONHMe)-B354 (Fig. 2). This compound inhibited PARP10 in a histone ADP-ribosylation assay with $IC_{50} = 6.0 \mu M$ and showed selectivity over other PARPs tested.¹⁵ A82-(CONHMe)-B354 has liabilities with regard to chemical features that are used to predict favorable ADME properties and druglikeness such as a problematically high topological polar surface area (TPSA) of 145.8 Å². The objective of the study was to replace the MeNH-DAP group by a linker to reduce the polar surface area and to improve the overall properties of the compound while maintaining or enhancing the potency of PARP10 inhibition.

The first step of the study involved the assembly of a VCL based on the structure of A82-(CONHMe)-B354 (PARP10-VCL; Fig. 2). The tetralone and 2-quinoxalinol fragments of A82-(CONHMe)-B354 were kept constant in PARP10-VCL because they are the result of sampling during the DECL screen, whereas the connectivity of the two fragments was varied. 350 different diamine scaffolds were selected based on commercial accessibility and favorable structural features. As the linkers changed, it was possible that the ideal site of attachment of the fragments to the linkers differs from the carboxylic acids in NADEL. We therefore included ten tetralone derivatives with different positions of the amine-reactive group and three different 2-quinoxalinol moieties. In addition to carboxylic acids, the tetralone fragments used for VCL construction also contained isocyanates to form ureas, sulfonyl chlorides to form sulfonamides, and halogenated derivatives for nucleophilic aromatic substitution. The Pipeline Pilot software (3dsbiovia.com) was used to enumerate the 10500 PARP10-VCL compounds.

PARP1-VCL was screened *in silico* by means of the Glide docking algorithm³⁶ to identify compounds that fit into the PARP10 binding pocket. A crystal structure of PARP10 in complex with a small molecule inhibitor (PDB 5LX6)³⁷ was used as the target. Top 1% of Glide hits were rescored using Molecular Mechanics Generalized Born Surface Area (MM-GBSA) method.³⁸ The rescored hit list was visually inspected from the top based on qualitative criteria, such as exposure to solvent or entropic penalty upon binding, to select hits for synthesis. We also paid attention to structural diversity of the selected hits, including fragments with different attachment chemistry and connection geometry and a broad range of diamine linkers, as well as their synthetic feasibility. Table 1 shows the structures of the ten selected compounds and the docking pose of hit compound **5a** is shown in Figure 3.

The synthesis of the ten selected compounds is shown in Scheme 1. Compounds **5a-f** were synthesized as outlined in Scheme 1a. The tetralone carboxylic acids **1a-c**, were coupled with the Boc-protected diamines **2a-e** using standard HATU or EDC coupling conditions in good yields. The deprotection of the resulting amides **3a-e** was accomplished using TFA followed by coupling with the quinoxaline propanoic acid **4** to afford the final compounds **5a-e**. In a similar manner compound **5f** was synthesized by switching the order of the two coupling reactions.

Compounds **9a-c** were synthesized according to Scheme 1b. The tetralone carboxylic acids **7a,b** were coupled with the Boc-protected diamines **2e,g-h** to afford the intermediate amides **8a-c**. Deprotection of the Boc group followed by coupling with carboxylic acid **4** afforded the final compounds **9a-c**.

The urea derivative **14** was synthesized as outlined in Scheme 1c. The amino tetralone **10** reacted with the Boc-piperazine carbamoyl chloride **11** to afford compound **12** in 40% yield. Deprotection followed by coupling with carboxylic acid **13** afforded the final compound **14** in 34% yield.

The inhibitory potency of the synthesized compounds towards PARP10 was evaluated in an ADP-ribosyltransferase assay (BPS Bioscience) at concentrations of 1 μ M and 10 μ M (Table 1). Inhibition of PARP14 was measured as control because PARP10 and PARP14 are structurally similar. Compound **5a** with the lowest Glide score (-12.20) distinctly inhibited PARP10 (25 % at 1 μ M and 90 % at 10 μ M; Table 1). Compound **5a** also inhibited PARP14 with similar potency (27% at 1 μ M). Inhibition of PARP10 was $<50\%$ for the other tested compounds at 10 μ M, indicating that their inhibitory potency is at least 2-fold lower than that of the original starting compound A82-(CONHMe)-B354 ($IC_{50} = 6.0 \mu$ M). Of these compounds, **14** (46% inhibition), **9c** (37% inhibition), and **5f** (35% inhibition) showed the most promise and selectivity over PARP14 was observed (Table 1). Even though the VCL-protocol did not enhance the potency of these compounds, they still may serve as starting point for future hit-to-lead efforts given that the MeNH-DAP moiety was replaced by more favorable structures.

The concentration-dependent inhibition of PARP10 was measured to determine the IC_{50} values of **5a** and **14** (Fig. 4a). Compound **5a** inhibited PARP10 with an IC_{50} value of 3.9 μ M. Replacing the MeNH-DAP linker by a 4-aminomethyl-4-methylpiperidine linker therefore improved the inhibitory potency by 1.5-fold. Importantly, the topological polar surface area decreased to 117.1 $\text{\AA}^2$ from 145.8 $\text{\AA}^2$, and the druglikeness score (calculated by the algorithm integrated in DataWarrior) increased to 3.82 from 2.22. Therefore, **5a** offers a superior starting point for lead development relative to A82-(CONHMe)-B354. Compound **14** inhibited PARP10 with an IC_{50} value of $\sim 28 \mu$ M. Testing the inhibition of 12 other PARPs provided a selectivity profile of **5a** (Fig. 4b). While **5a** exhibited high selectivity towards some PARPs (Inhibition at 10 μ M: PARP3 = 22%; PARP6 = 0 %; PARP8 = 5%; PARP12 = 16%; PARP15 = 10%), this compound inhibited the activity of the other PARPs by $>50\%$ at 10 μ M and completely blocked the activity of PARP2. In contrast, the initial DECL-screening hit A82-(CONHMe)-B354 exhibited satisfactory target selectivity, and inhibition of other PARPs at 10 μ M was below 25%.¹⁵

In summary, this report introduces an innovative approach to develop early lead compounds from DECL hits by *in silico* screening of a VCL. The study validated the concept by identifying a PARP10 inhibitor that differs from the DECL screening hit by the structure of the scaffold and the linkage geometry on the tetralone. Overall, the modification yielded a favorable decrease in polar surface area, increased druglikeness, and modestly enhanced potency. Having established the conceptual framework of the approach, the next step will be to gain further understanding on how to design VCLs and enhance the predictability of the *in silico* screen. It is noteworthy that the original DECL screening hit A82-(CONHMe)-B354 had higher potency than most of the tested virtual screening hits. While this result suggests that further improvements of the workflow are needed, it also bears testimony to the effectiveness of DECLs to intimately sample protein binding sites. The MeNH-DAP moiety evidently provides critical interactions to the affinity of A82-(CONHMe)-B354 to PARP10. This outcome is in line with previous studies that demonstrated that even subtle linker modifications of DECL hits often have a profound effect on compound potency.²⁶ Likewise, the higher target selectivity of A82-(CONHMe)-B354 compared to **5a** emphasize the unique ability of DECLs to address target selectivity in early design stages.³⁹ In conclusion, integrating DECL-screens with computer-guided drug discovery emerges as an attractive strategy to rapidly access lead compounds with high potency and favorable characteristics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Pursued approach of integrating DNA-encoded chemical library (DECL) screens with structure-based computational drug discovery methods. A DECL screen provides candidate hit molecules that consist of structural elements that have been effectively selected with regard to the target, but also moieties that had low diversity in the DECL. A virtual combinatorial library (VCL) is constructed from a screening hit keeping some substructures constant and varying others. *In silico* screening identifies molecules in the VCL that are likely to bind the protein with high affinity and have more favorable physicochemical properties. Selected hits are synthesized and tested to identify compounds with enhanced properties for further lead development.

Figure 2.

Design of the virtual combinatorial library (PARP10-VCL) used to replace the MeNH-DAP substructure (highlighted in red) of the PARP10 DECL screening hit A82-(CONHMe)-B354. The PARP10-VCL compounds consist of 350 diamines onto which ten tetralones and three 2-quinoxalinols were assembled, providing a virtual library with 10500 distinct structures for docking-based screening.

Figure 3.
Docking pose of the predicted PARP10 inhibitor **5a** binding to PARP10 (crystal structure PDX 5LX6).

Figure 4.

Inhibitory activity of VCL-derived molecules. a) Dose-response curve of PARP10 inhibition by compounds **5a** and **14**. Inset: Structural properties of **5a** (MW: molecular weight; TPSA: topological polar surface area; RB: rotatable bonds; structural properties of **14** are provided in Table S1 in the Supporting Information). b) Selectivity profile of **5a** to PARP enzymes.

Scheme 1.

Synthesis of PARP10 inhibitor candidates. Reagents and conditions: (a) EDC or HATU, DMF, DIEA, 40–48%; (b) 1. TFA, DCM, quant. 2. EDC or HATU, DIEA, DMF, 13–33%. (c) DCM, DIEA, 40%.

Table 1.

Predicted PARP10 inhibitors identified from screening PARP10-VCL.

Compound ^a	Structure	Glide Score	PARP10 1 μ M	10 μ M	PARP14 1 μ M
5a		-12.20	25	90	21
5b		-12.07	15	9	12
5c		-11.86	13	10	2
9b		-11.70	13	4	0
14		-11.65	17	46	0
5f		-11.63	1	15	17
5d		-11.57	11	16	27
9a		-11.57	13	12	7
9c		-11.51	0	37	0 (5 ^b)
5e		-11.49	0	35	6 (10 ^b)

^aCompounds were sorted by Glide Scores and numbered according to synthetic method (see Scheme 1). Predicted physicochemical properties of compounds are summarized in Table S1 in the Supporting Information.

^bInhibition at a compound concentration of c = 10 μ M.