Readily Accessible Host Library and Screening Approach for High Binding Affinity Host-Guest Complex Formation: Case Study for Cyclodextrin-Bilirubin Complex

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ABSTRACT

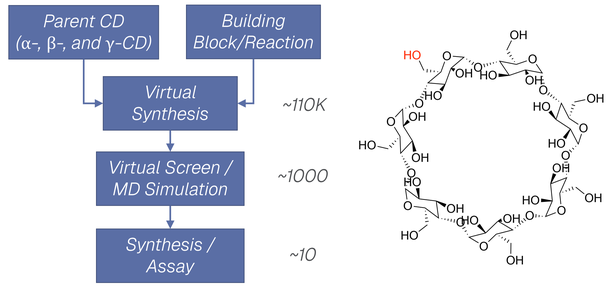
We report an efficient and cost-effective approach to rapidly identify novel, high-affinity, selective, derivatized-cyclodextrin–based chemical entities. We demonstrated this approach through a case study with bilirubin, a particularly challenging target due to its amphiphilic character, conformational flexibility, and poor aqueous solubility. Cyclodextrins (CDs) are versatile scaffolds for ligand sequestration, but their challenging derivatization chemistry has limited broader applications in drug delivery and toxin removal. Here, we generated a readily synthesizable library of approximately 100,000 β- and γ-CD derivatives by modifying monomeric units using diverse, scalable reactions. This extensive library was initially screened in silico to eliminate low-scoring compounds, followed by molecular dynamics simulations to refine hit selection.

w yielded 12 promising candidates, 10 of which were selected and synthesized. Binding interactions were confirmed through circular dichroism, UV, and NMR spectroscopy, and our top compound showed ~30-fold higher binding affinity than native β-CD. NMR revealed a unique binding mode where bilirubin engages the CD host with one portion inside the hydrophobic cavity and the other interacting with the polar exterior, suggesting that targeted derivatizations could further improve performance.

Preliminary in vivo validation underscores the translational potential of this platform. By successfully addressing the complex challenge of bilirubin binding, this campaign provides direct evidence that derivatized CDs can be rationally designed to target a broad spectrum of therapeutically relevant molecules.

MONO SUBSTITUTED LIBRARY SCREENING AND STRUCTURE DETERMINATION

110K Virtual CD Library Screened, and 10 Compounds were Synthesized



- **6-mono-substituted virtual library (110K β- and γ-CDs) assembled from commercially available reagents.**
- *The three distinct bilirubin poses were identified from MD simulation. Virtual screen docked CDs to each of these bilirubin poses as rigid receptors.*
- *MD simulations are performed for the top 1000 docked compounds, and the unstable poses during the MD simulation were discarded.*
- *10 compounds representing a range of predicted binding were synthesized.*

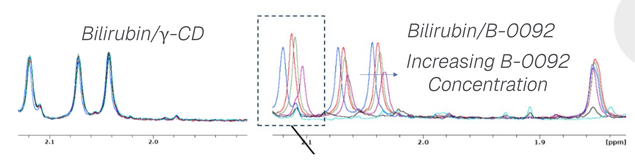
B-0092 is Identified as a Hit Compound, and Its Complex structure with bilirubin is determined

ID	Scaffold	CiDi K_a (M^{-1})	UV K_a (M^{-1})	NMR K_a (M^{-1})
β-CD	β	32	312	-
B-0081	β	526	526	
B-0088	β	250	1369	< 100
B-0085	β	Min. change	208	
γ-CD	γ	23	66	-
B-0092	γ	909	588	833
B-0089	γ	714	Min. change	
B-0094	γ	126	Min. change	

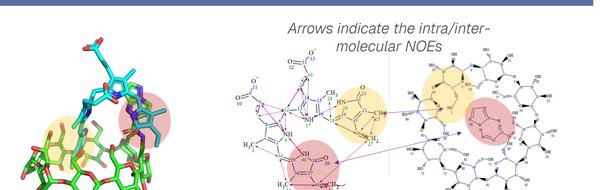
**4 of 10 synthesized CDs culled due to low solubility*

- *Association constants (K_a) determined by circular dichroism (CiDi) and UV spectra, calculated using 1:1 binding mode.*
- *An order of magnitude increases in binding relative to unmodified CD's were observed*
- **1D ^{1}H NMR chemical shifts in bilirubin resonances confirm binding for B-0092 and B-0088**

1D ^{1}H NMR Spectra



Bilirubin/B-0092 Complex Structure



- *2D ROESY NMR experiment was used to find inter/intra-molecular NOEs.*
- *MD simulations determined conformers satisfying NOE distances.*
- **Cavity-bound structure places one pyrrolone partly embedded in cavity and the other pi-stacked against the pyrazolopyrimidine sidechain.**

POC TARGET: BILIRUBIN

A POC Target with Medium Predicted Binding Affinity was Chosen

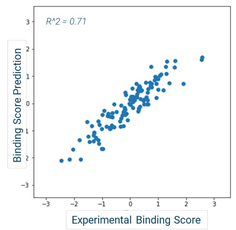
We first trained an ML model using +125 molecular descriptors of hundreds of guest molecules amongst >1500 measurements found in literature. The total number of unique host/guest measurements are 328, 471, and 40 for α-, β-, and γ-CD, respectively.

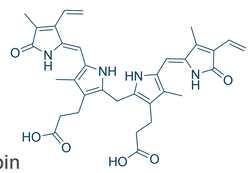
Using the ML model, we evaluated the compounds found in Therapeutic Target Database (TTD). As a proof of concept (POC) target, we chose bilirubin, because of its predicted middling CD binding and complex structure provides a challenging target to demonstrate the versatility of our platform to rapidly derive CDs with superior binding to unmodified parent CDs.

	# unique host:guest measurements
α-CD	328
β-CD	471
γ-CD	40

**Katrizky et al; Suzuki, et al; Rekharsky, et al.*

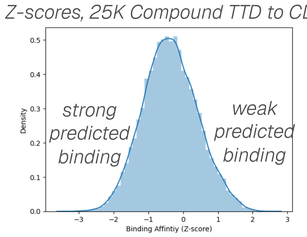
model training





Bilirubin
- Z-score = 0.07
- middling predicted CD affinity
- Less hydrophobic than other common CD binders

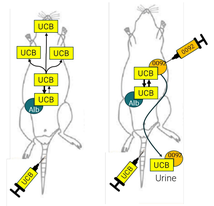
Z-scores, 25K Compound TTD to CD



In Vivo Pharmacodynamics POC

B-0092 Showed Increased Retention of Bilirubin in Plasma and Increased Excretion via Urine

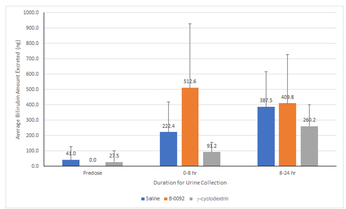
- *Sprague-Dawley rats dose subcutaneously with either B-0092 or unmodified γ-CD*
- *At B-0092 vs unmodified γ-CD C_{max} (1 h), dose UCB bolus iv in excess of calculated available albumin capacity (ca 15 mpk to saturate)*
- *Measure retention of UCB in plasma and increased UCB elimination in urine*



Treatment	Analyte	Dose (mg/kg)	C_{max} (ng/mL)	AUC_{0-inf} (hr*ng/mL)
Saline + bilirubin	bilirubin	30 iv	71600	36600
B-0092 + bilirubin	B-0092	2000 sc	881000	2220000
	bilirubin	30 iv, 1 h post TA	189000	91300
γ-CD + bilirubin	γ-CD	2000 sc	867000	1410000
	bilirubin	30 iv, 1 h post TA	73700	34600

3X

- *5-6-week Sprague-Dawley male rats were dosed with saline or test article (B-0092 or γ-CD, 2000 mpk sc) followed 1 h later (timed for test article C_{max}) by bilirubin dosing, 30 mpk, iv*
- *Urine was collected for 0-8 h (test article PK) and for 8-24 h period (post-clearance)*

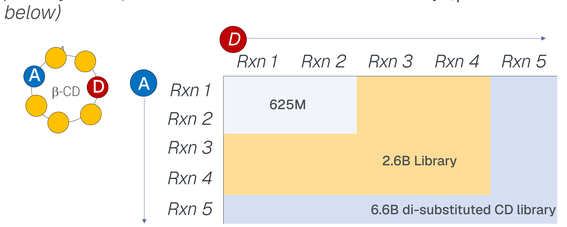


- *Relative to γ-CD, an enhanced trend of urinary excretion of bilirubin was observed in the timeframe correlating test article AUC (0-8 h)*

Library Expansion and Di-Substituted Cyclodextrin Library

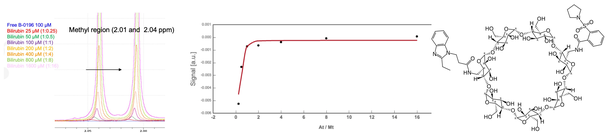
Broadening Chemical Diversity by Discovering New Reaction

- *Beren has identified five diverse derivatization reactions with consistent yield with various CD scaffolds*
- *Beren has made significant advancements in synthesis and conceptual knowledge enabling the practical application of primary rim A, D-heterofunctionalization chemistry (β-CD shown below)*



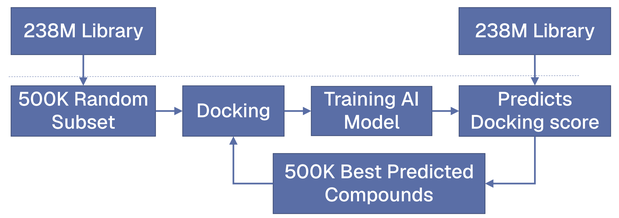
First of seven synthesized compounds, B-0196 has a 1500X increase in binding affinity compared to B-0092 as determined by NMR

- *For dissolution of B-0196 to non-aggregated form (low water solubility), DMSO-d_6 was used for ^{1}H NMR K_a measurement*
- *B-0196 displayed an average K_a = 1.2 x 10^6 M^{-1} compared to B-0092's K_a of 833 M^{-1} (PBS) and 1000 M^{-1} (DMSO-d_6)*
- *B-0196's solubility may be improved in hit to lead*



AI-Assisted Docking Accelerated Screening of Large Chemical Library

- *We developed an AI-assisted docking approach which uses active learning to build AI model that predicts docking scores:*
  - *Selecting a 500K random subset of compounds for docking → Training the AI model to predict their docking scores*
  - *Using the model to identify the highest-scoring compounds from the full library*
  - *Employing these top-predicted compounds in subsequent docking and training rounds*
  - *Repeating this process a minimum of five times to ensure the model's quality*
- **The AI-assisted docking reduces the total cost to 16 days and $8K for AWS compute cost (vs ~ 40 days and $240K for AWS compute cost)**



Competing Interest

The authors are listed as inventors on a patent application covering this work and filed by Beren Therapeutics P.B.C. (international patent application no. PCT/IB2025/057291).

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