Discovery, SAR, and X-ray Binding Mode Study of BCATm Inhibitors from a Novel DNA-Encoded Library

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S *Supporting Information*

ABSTRACT: As a potential target for obesity, human BCATm was screened against more than 14 billion DNA encoded compounds of distinct scaffolds followed by off-DNA synthesis and activity confirmation. As a consequence, several series of BCATm inhibitors were discovered. One representative compound (R)-3-((1-(5-bromothiophene-2-carbonyl)-pyrrolidin-3-yl)oxy)-N-methyl-2′-(methylsulfonamido)-[1,1′-biphenyl]-4-carboxamide (**15e**) from a novel compound library synthesized via on-DNA Suzuki−Miyaura cross-coupling

showed BCATm inhibitory activity with IC$_{50}$ = 2.0 μM. A protein crystal structure of **15e** revealed that it binds to BCATm within the catalytic site adjacent to the PLP cofactor. The identification of this novel inhibitor series plus the establishment of a BCATm protein structure provided a good starting point for future structure-based discovery of BCATm inhibitors.

KEYWORDS: *BCATm, obesity, DNA Encoded Library, ELT, on-DNA Suzuki−Miyaura cross-coupling reaction*
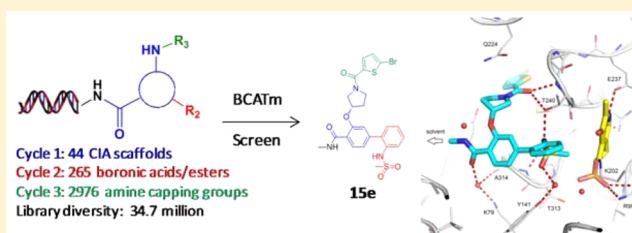
Branched chain aminotransferases (BCAT) catalyze trans-amination of the branched chain amino acids (BCAAs) leucine, isoleucine, and valine to their respective α-keto acids, which are further converted to succinyl-CoA and acetyl-CoA, and participate in tricarboxylic acid cycle and glycolysis.[1] There are two isozymes, mitochondrial (BCATm) and cytosolic (BCATc), and the human BCAT isozymes are 58% identical in amino acid sequence.[2] While BCATm is in most tissues, BCATc is largely restricted to the central nervous system.[1] BCAAs are essential amino acids that must be consumed in the diet of mammals since they do not possess the necessary enzymes to synthesize these molecules *de novo*. An increasing body of evidence supports the notion that BCAAs stimulate protein synthesis in skeletal muscle, with leucine being primarily responsible for the stimulation of protein synthesis after intake of a mixed meal.[3] Conversely, it is also known that the product of leucine transamination, α-keto isocaproic acid, inhibits protein degradation resulting in an overall accumu-lation of protein.[4] A recent mouse BCATm knockout study demonstrated that lack of this enzyme increased the mice's energy expenditure, associated with a futile protein turnover

cycle, and protected them from obesity when subjected to a high fat diet.[5] Furthermore, metabolomic profiling of obese versus lean humans reveals a BCAA-related metabolite signature that is suggestive of increased catabolism of BCAAs and correlated with insulin resistance.[6] To further validate BCATm as a therapeutic target for the intervention of metabolic diseases, a suitable small molecular inhibitor is highly desirable. To date, no human BCATm inhibitors have ever been reported. Herein we report our effort in identifying small molecule inhibitors of human BCATm via DNA encoded library technology. More than 14 billion compounds from a superpool of various DNA encoded libraries of distinct scaffolds were screened against BCATm followed by off-DNA hit synthesis and activity confirmation. As a consequence, several potent BCATm chemotypes were discovered. In this report we describe the design and synthesis of a 34.7-million-member

DNA-encoded library and BCATm hits identified from this library.

Encoded Library Technology (ELT) has proven to be an important lead discovery platform for various therapeutic targets.[7−16] To increase library diversity and expand drug-like chemical space, we recently developed a trifunctional scaffold based novel DNA encoded library dubbed the "CIA" (carboxylic acid, iodide, and amine) library. Each of the trifunctional scaffolds contains a carboxylic acid, an aryl iodide group, and an Fmoc-protected amine group. Similar to our previously reported libraries, this library was synthesized using the split-and-pool strategy, beginning from a short sequence of duplex DNA stabilized by a synthetic hairpin (the "head-piece").[10,11] The design of the headpiece allows synthesis of the small molecule library starting from the free amine group, while DNA tags encoding the building blocks (BBs) are enzymatically ligated at the nonlinked end of the DNA duplex. In this instance, 44 CIA scaffolds (referred to as cycle 1 building blocks or BB1) were first installed onto the headpiece DNA starting material (1, Scheme 1) through amide bond formation

Scheme 1. Synthesis of the Three Cycle DNA Encoded CIA Library
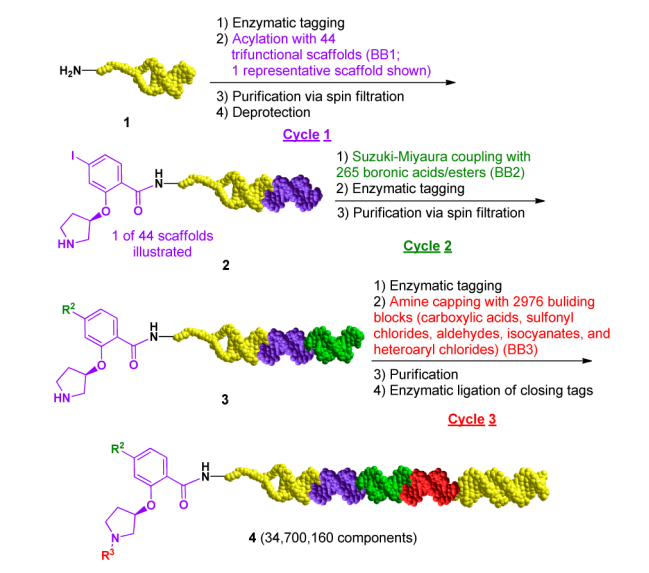
followed by pooling and Fmoc-deprotection.[11] After splitting into separate reaction wells, 265 cycle 2 boronic acids/ester building blocks (BB2) were introduced through on-DNA Suzuki−Miyaura cross-coupling reactions.[17] This is our first reported application of on-DNA cross-coupling reactions in a library synthesis setting. After standard pooling, purification via spin filtration, and splitting into different reaction wells, 2976 unique cycle 3 building blocks (BB3) including carboxylic acids, sulfonyl chlorides, aldehydes, isocyanates, and heteroaryl chlorides were reacted with the amine group thus providing the final library with a size of 34.7 million. After pooling and purification, the DNA sequence was then "closed" with a final tag that encodes a library identifier, a priming region for PCR amplification, and a degenerate sequence to control for PCR amplification biases.

The affinity selections were performed on a chemically biotinylated human BCATm protein construct. The target protein was immobilized on a streptavidin agarose resin packed column. After immobilization, the column was washed with

buffer containing 1 mM biotin to ensure occupation of all biotin binding sites. The immobilized protein was then exposed to the on-DNA library prior to extensive washing to remove nonbinders. Bound library molecules were eluted by heat denaturation of the protein at 80 °C. The eluant was then incubated with fresh immobilized protein to start a new round of affinity selection. A parallel selection against a column lacking protein was done to allow for the identification of molecules enriched by nontarget affinity. After three rounds of selection, the DNA tags of the eluted population were PCR amplified and sequenced, and the sequences were translated to identify structures of putative BCATm binders.

Further analysis of the affinity selection output could be conveniently performed in a Spotfire cube view. In Figure 1, encoded small molecule compounds enriched at the level of two independent DNA sequences or greater are shown with the cube axes representing building blocks used in cycle 1, cycle 2, and cycle 3 chemistry, respectively. As discussed in earlier reports, families of related compounds are of particular interest because they contain one or more building blocks in common and are easily recognized in these views as lines or planes.[10,11] Here, several such features were evident. First of all, there is a strong selection for BB1 building block (R)-2-((1-(((9H-fluoren-9-yl)methoxy)carbonyl)pyrrolidin-3-yl)oxy)-4-iodobenzoic acid (5) as most of the data points shown in the cube are on the plane defined by this particular building block (i.e., all of the corresponding compounds to each of the data points have the same building block at cycle 1). Second, on this plane there are several lines: two major horizontal ones and three major perpendicular ones. The horizontal lines are defined by BB2 borates N-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-phenyl)methanesulfonamide (6) and 3-boronobenzoic acid (7), correspondingly, and the three perpendicular lines are defined by BB3 carboxylates 5-bromo-2-((tert-butoxycarbonyl)-amino)benzoic acid (8), 5-bromothiophene-2-carboxylic acid (9), and 2-amino-5-bromobenzoic acid (10). The structures corresponding to these lines are enumerated as general structures I, II, III, IV, and V.

The above analysis suggested that affinity selection of the 34.7 million member CIA library over BCATm provides several series of molecules with a common cycle 1 trifunctional moiety. To confirm these hits, we synthesized representative off-DNA compounds from each of these series by standard synthetic methods as outlined in Scheme 2. Starting from the trifunctional scaffold 12, the methyl carboxylate was first converted to methylamide (13) followed by Suzuki−Miyaura cross-coupling to install the second substituted phenyl ring (14a−d). Then Boc-deprotection of the pyrrolidine nitrogen followed by acylation affords the desired compounds 15a−j. Meanwhile palladium mediated reduction of 13 removed the iodo group, which was followed by pyrrolidine nitrogen Boc-deprotection and subsequent acylation to afford compound 16.

Representative compounds 15a−c (Figure 2) from combination of lines 1−3 and 4 were first synthesized and tested in a BCATm enzymatic assay. As indicated in Figure 2, while both compounds 15a and 15b showed IC_{50} greater than 100 μM, compound 15c seems better than 15a and 15b with a measurable IC_{50} = 74.1 μM. We then switched the 3-carboxylphenyl group in 15c to the 2-methylsulfonamidophenyl group featured in line 5 thus leading to compound 15d, which displayed significant BCATm enzyme inhibition with IC_{50} = 3.9 μM.
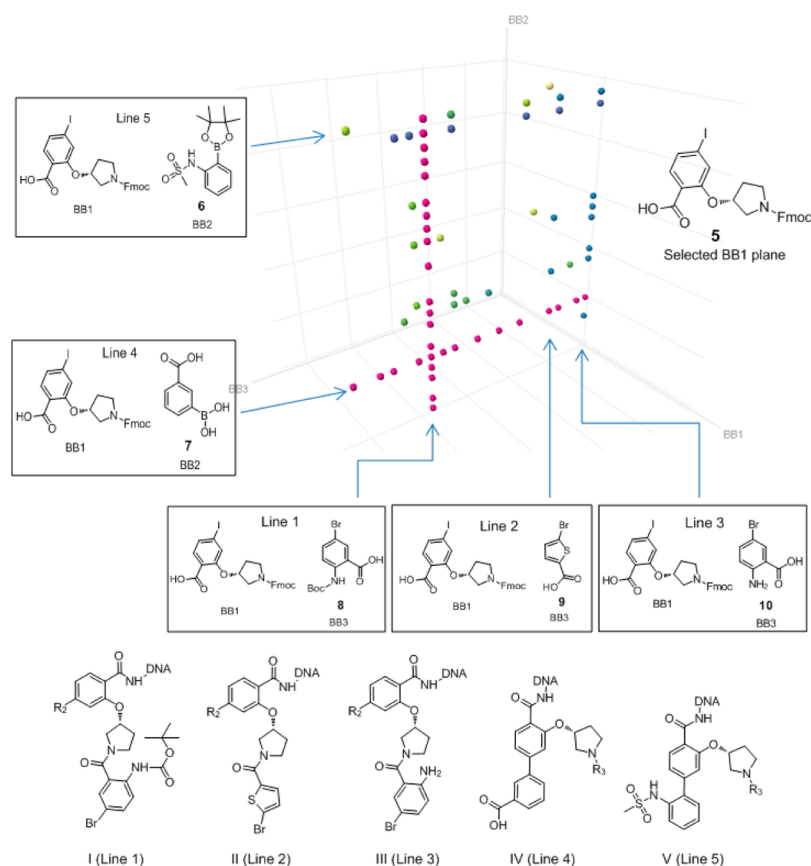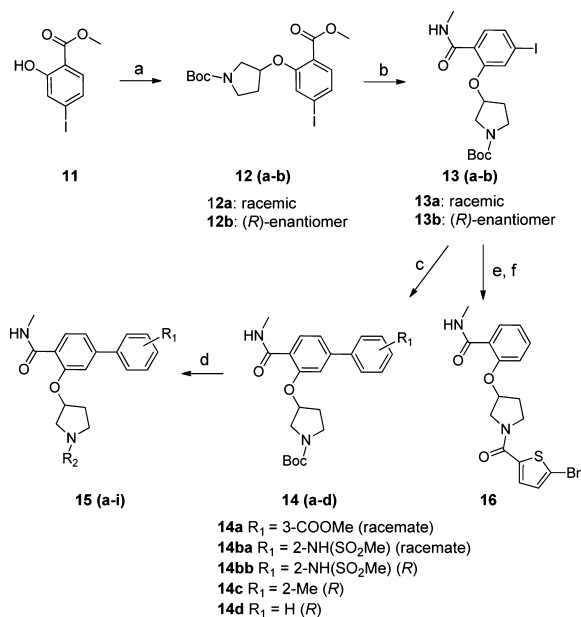
Figure 1. Spotfire cube data view of BCATm selection features. BB1, cycle 1 building blocks; BB2, cycle 2 building blocks; and BB3, cycle 3 building blocks. Color of the dots represents different levels of enrichment (pink is 50-fold, blue is 20-fold, green is 10-fold, and yellow is 5-fold). To simplify the visualization, only selection features of building block 5 with copy counts ≥2 were shown in the cube.

Since compound **15d** was initially prepared as a racemic mixture, its (*R*)-enantiomer (**15e**, Figure 3), which incorporates the actually selected BB1 (**5**), was subsequently synthesized. Compound **15e** showed about 2-fold increase in BCATm enzyme inhibition potency (IC_{50} = 2.0 μM). Further structure–activity relationship exploration around compound **15e** indicated that the 5-bromo group on the thiophene ring can be replaced by a chloro group (**15f**) with mostly maintained potency (IC_{50} = 2.8 μM). However, cyano substitution at the same position (**15g**) led to more than 5-fold decrease of activity (IC_{50} = 15.1 μM) suggesting either size or electron negativity of the cyano group is not tolerated at this position. It was further discovered that the 2-methylsulfonamidophenyl group is also critical for the observed activity. As shown earlier, the corresponding 3-carboxylphenyl analogue **15c** was much less active than **15d**. Replacement of the 2-methylsufonamidophenyl group with either 2-methylphenyl (**15h**, IC_{50} = 64.6 μM) or a simple unsubstituted phenyl group (**15i**, IC_{50} > 100 μM) caused more than 10-fold decrease in enzymatic activity, and removal of the entire phenyl group (**16**) rendered the compound almost inactive.

To better understand its binding mode and mechanism of action, a high resolution (1.6 Å) X-ray crystal structure of BCATm in complex with **15e** was obtained through cocrystallization in the presence of aldimine cofactor pyridoxal 5′-phosphate (PLP). As shown in Figure 4a, compound **15e** sits deep in the BCATm active site with the methylamide moiety pointing into a solvent exposed area accounting for the tolerance of DNA linkage at this position. Compound **15e** adopts a T-shaped conformation within the BCATm active site (Figure 4a). The phenyl 2-methylsulfonamido is directed toward the covalently bound PLP cofactor, such that one of the sulfone oxygens makes a direct H-bond with the backbone amide of A314, while the other oxygen engages in a water bridged interaction with the hydroxyl of Y141 (Figure 4b). The bromothiophene of **15e** sits snugly in a hydrophobic pocket surrounded by residues including C318, V182, M241, V238, and V320 (Figure 4c). This results in a decrease in activity when the bromine is replaced by the longer cyano group of **15g** but tolerates its substitution by chlorine (**15f**). We also briefly considered the importance of the chirality of the ether linkage. The T-shape binding mode of **15e** is helped by a quasi-planar pyrrolidine with an axially branching methoxy moiety. Modeling suggests that the alternative enantiomer could adopt a similar binding mode with only small changes to the solvent-exposed pyrrolidinyl ring pucker and equatorial branching of the methoxy moiety. The key interactions offered by the phenyl 2-methylsulfonamido and the bromothiophene would still be in place, potentially explaining the relatively small difference between the enzyme inhibition for **15e** and the racemate **15d**.

In summary, a novel 34.7 million member trifunctional scaffold-based DNA encoded library was synthesized utilizing the recently reported on-DNA Suzuki–Miyaura reaction. Affinity based screening of this library against BCATm led to the discovery of a novel and attractive biphenyl pyrrolidine ether hit series that showed potent BCATm inhibition. SAR exploration indicated that both the methylsulfonamido and the

Scheme 2. Off-DNA Synthesis of Representative Compounds Selected by BCATm[a]

11 → **12 (a-b)** → **13 (a-b)**

12a: racemic
12b: (R)-enantiomer

13a: racemic
13b: (R)-enantiomer

15 (a-i) ← **14 (a-d)** → **16**

14a R₁ = 3-COOMe (racemate)
14ba R₁ = 2-NH(SO₂Me) (racemate)
14bb R₁ = 2-NH(SO₂Me) (R)
14c R₁ = 2-Me (R)
14d R₁ = H (R)

[a]Reagents and conditions: (a) diisopropyl azodicarboxylate, PPh₃, *tert*-butyl 3-hydroxypyrrolidine-1-carboxylate; (b) (i) NaOH, THF-MeOH, H₂O; (ii) HATU, DIEA, MeNH₂·HCl, AcCN; (c) boronic acids, Pd(PPh₃)₄, Cs₂CO₃, THF/H₂O, N₂, 80 °C, 2 h; (d) (i) 10% TFA/DCM; (ii) HATU, DIEA, R₂COOH, AcCN; (e) H₂ balloon, 10% Pd/C, MeOH; (f) (i) 10% TFA/DCM; (ii) HATU, DIEA, 5-bromothiophene-2-carboxylic acid, AcCN.
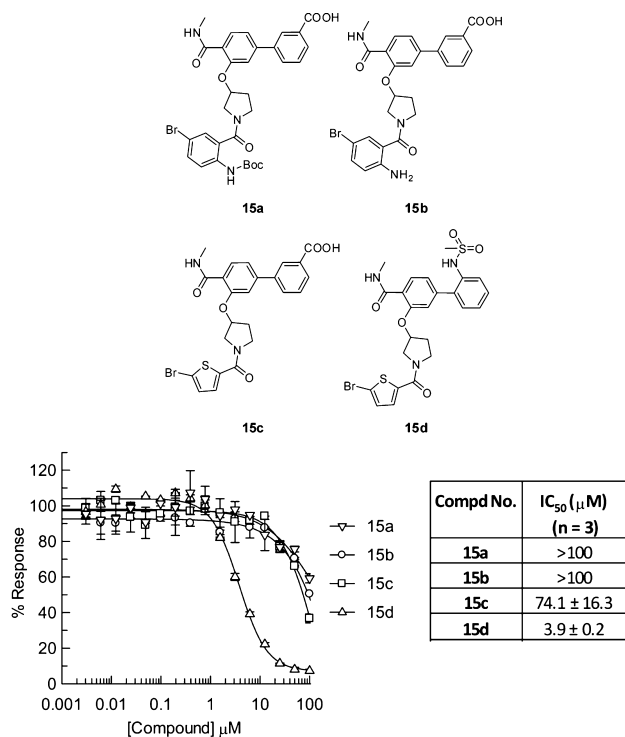

Figure 2. First group of off-DNA compounds and activities against BCATm.

bromothiophene moieties are crucial for the activity. A binding mode study revealed that representative compound **15e** binds to BCATm at its catalytic site through multiple H-bond and
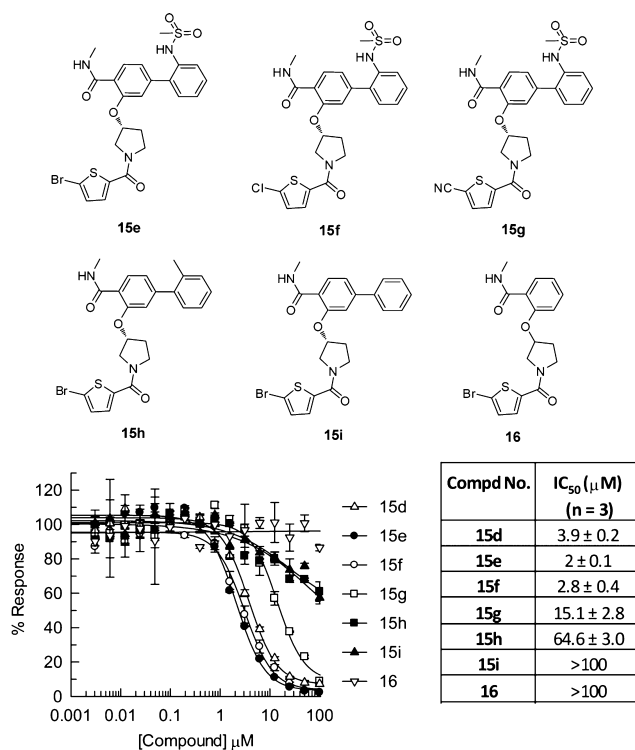

Figure 3. Structures and activities for analogues of compound **15d**.

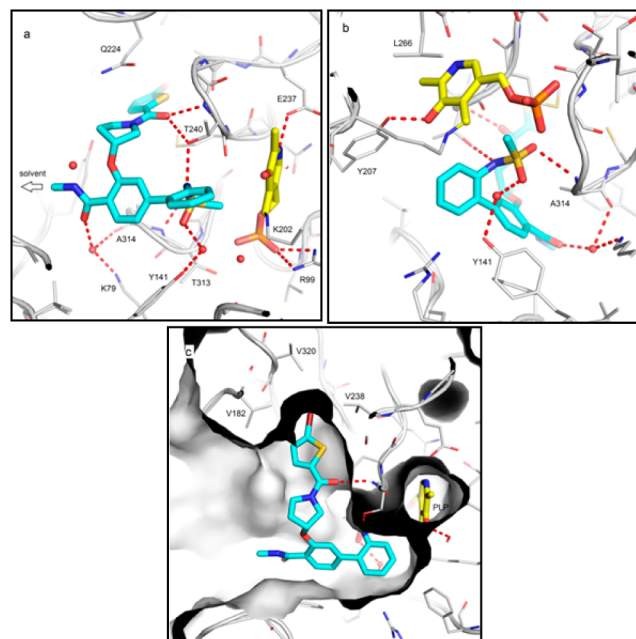| Compd No.	IC₅₀ (µM) (n = 3)
15d	3.9 ± 0.2
15e	2 ± 0.1
15f	2.8 ± 0.4
15g	15.1 ± 2.8
15h	64.6 ± 3.0
15i	>100
16	>100

Figure 4. Views of X-ray crystal structure of BCATm in complex with **15e** (in cyan) and cofactor PLP (in yellow).

van der Waals interactions. While this work exemplifies another successful application of DNA encoded libraries to hit/lead identification for novel therapeutic targets, the identification of the novel biphenyl pyrrolidine ether BCATm inhibitor series and the establishment of the liganded BCATm crystal structure also lays a good foundation for future structure-based BCATm inhibitor design.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

BCAAs, branched chain amino acids; BCATm, mitochondrial branched-chain aminotransferase; BCATc, cytosolic branched-chain aminotransferase; ELT, encoded library technology; CIA, carboxylic acid, iodide, and amine; BB, building block; NTC, no-target control; PLP, pyridoxal 5′-phosphate

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