Catalytic Asymmetric Synthesis of CPZEN-45

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ABSTRACT:

A catalytic asymmetric total synthesis of CPZEN-45 (**1**), anti-XDR-TB agent, was accomplished using a direct aldol reaction of a glycine Schiff base (**3**) and uridine-derived aldehyde (**4**) catalyzed by Zn(II)-(*R,R*)-Linked-BINOL complex to give the desired stereoisomer selectively. The diazepinone ring system, another key structural element, was successfully constructed by vinyl halide-amide coupling promoted by Cu(I) salt.

(Graphical Abstract)

Keywords: CPZEN-45; Catalytic asymmetric aldol reaction; Anti-XDR-TB agent

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(Figure, no legend)

Tuberculosis (TB) has long been a serious threat to public health. In 2014, the World Health Organization reported 9.6 million new cases of TB worldwide and 1.5 million TB-related human deaths.¹ Most alarming is that 3.3% of new cases and 20% of treated cases are multidrug-resistant TB (MDR-TB), 9.7% of which are categorized as extensively drug-resistant TB (XDR-TB). XDR-TB is resistant to many clinically available anti-TB drugs, and has been reported in 105 counties to date.¹ A promising tactic to combat the rapid spread of drug-resistance is the development of novel anti-TB agents with a mode of action distinct from that of the anti-TB drug precedents.

In 2003, caprazamycin B (2) was reported as a secondary metabolite of the actinomycete *Streptomyces* sp. MK730-62F2 that exhibits antibacterial activity against MDR-TB (minimum inhibitory concentration [MIC]: 3.13 $\mu\text{g/mL}$ for *Mycobacterium tuberculosis* (*M. tuberculosis* H37Rv) as well as against drug-sensitive strains (DS-TB, MIC ranging from 6.25 - 12.5 $\mu\text{g/mL}$).²

The low water-solubility of **2**, however, prevented the use of caprazamycin (a mixture of caprazamycin B and related compounds with varying side-chain substructures) for further preclinical studies, and led to structure-activity relationship (SAR) studies guided by the anti-TB activity and water miscibility. In 2013, Takahashi and co-workers disclosed the preparation and anti-TB activity of CPZENs,³ which were semi-synthetically derived from natural samples of caprazamycins. Among them, CPZEN-45 (**1**) displayed better anti-TB activity toward both MDR-TB (H37Rv; MIC: 1.56 µg/mL) and clinically isolated DS-TB strains (MIC: 0.78 to 12.5 µg/mL) compared with caprazamycin B, with improved water-solubility and negligible acute toxicity. Notably, CPZEN-45 exhibited outstanding therapeutic efficacy in a murine TB model infected with an XDR-TB. Extensive biological studies revealed that the anti-TB activity stems from the inhibition of WecA,⁴ a key enzyme in the biosynthesis of mycolyl arabinogalactan, which is essential for the viability of *Mycobacterium tuberculosis* and yet has never been a molecular target of clinical anti-TB drugs. Due to its unique anti-TB profile and favorable physicochemical properties, CPZEN-45 is currently in the pipeline for anti-TB drug development at the stage of preclinical study (as of April 2016).⁵

The success of CPZEN-45 validated WecA as a convincing molecular target for exploratory studies of anti-TB medicines; thus, additional SAR studies using CPZEN-45 should be a reasonable strategy for obtaining anti-XDR-TB agents with more potent biological activity allowing for per os administration. Semi-synthetic SAR studies are effective when ample starting material of natural origin is available, but a major drawback of this strategy is the limited diversity of accessible

compounds. For CPZEN-45, removal of the aminoribose moiety without damaging the rest of the structure, and skeletal alteration of the diazepinone core have not been achieved, and selective deletion or transformation of one particular functionality is nontrivial. Therefore, we planned a total synthesis of CPZEN-45 to expand the repertoire of available derivatives. Very recently, Takemoto and co-workers reported the first total synthesis of CPZEN-45.⁶

Figure 1. Synthetic strategy of CPZEN-45 (**1**).

We previously reported the catalytic asymmetric total synthesis of caprazol,^{7,8} a caprazamycin-related natural product lacking its side-chain moiety, and the synthesis of caprazamycin B (**2**).^{9,10} In the syntheses, catalytic asymmetric reactions developed in this laboratory¹¹ were successfully applied to furnish key stereochemical elements, such as a β -hydroxy- α -amino acid structure within the diazepanone ring system, a β -hydroxyester of the side-chain portion, and an asymmetrically esterified 3-glutaric acid. As part of our ongoing synthetic studies of caprazamycin-related compounds, our synthetic strategy of CPZEN-45 is summarized in Figure 1. The β -hydroxy- α -amino acid structure at the juncture between the uridine and diazepinone parts can be constructed by an aldol reaction of a glycine Schiff base with uridine-derived aldehyde,

taking advantage of a catalyst system developed by our laboratory, a 2:1 complex of Zn(II) metal and (*R,R*)-linked-BINOL (**5**).¹² These conditions considerably improved the diastereoselectivity of the *syn*-amino alcohol (**7**) from the isocyanoacetate-aldol reaction adopted in the total synthesis of caprazol (dr = ca. 4/1). The diazepinone ring system, another characteristic substructure of CPZEN-45, should be constructed by Cu(I)-mediated vinyl halide-amide coupling,¹³ followed by glycosylation and amide formation.

Table 1 Optimization of aldol reaction catalyzed by Zn(II)-(*R,R*)-Linked-BINOL.

entry	solvent	temp.	time (d)	yield of 6 (%)	<i>syn/anti</i> of 7	dr of <i>syn</i> - 7
1	toluene	rt	3	37	1.28/1	6.4/1
2	THF	rt	14	85	9.3/1	79/1
3	THF	40 °C	3	60	4.4/1	30/1
4 ^a	THF	40 °C	3	57	3.35/1	11.4/1
5	1,4-dioxane	40 °C	3	69	4.6/1	13.8/1

6	DME	40 °C	3	60	3.0/1	6.67/1
7	Et ₂ O	rt	14	51	2.55/1	3.4/1
8	CH ₂ Cl ₂	rt	3	27	1/1	1/1

^a 0.4 equiv of Hunig's base was added.

At the outset of the synthesis, we screened the aldol reaction conditions using glycine Schiff **3** and uridine-derived aldehyde **4** as substrates to give the oxazolidine product **6**. The product was hydrolyzed to result in the corresponding amino alcohol **7** with which stereoselectivity could be determined by NMR. After the initial disappointing results when using (*R*)-LLB (37%, *syn/anti* = 1.4/1, dr of *syn* = 3.9/1)¹⁴ and the Zn(II)-(*R*)-Schiff base catalyst (42%, *syn/anti* = 2.5/1, dr of *syn* = 3.6/1),¹⁵ we found that the diastereoselectivity (6.4/1) of the *syn*-product was promising when using a catalyst system comprising Et₂Zn (20 mol%) and (*R,R*)-linked-BINOL¹² (**5**, 10 mol%) in toluene at room temperature for 3 days (Table 1, entry 1). Even in this case, however, the chemical yield (37%) and the *syn/anti* selectivity (1.28/1) were unsatisfactory. Changing the solvent to THF increased the *syn/anti* ratio to 9.3/1, and the diastereoselectivity of the *syn*-product was increased up to 79/1 (entry 2). Notably, a reaction time as long as 14 days was required for isomerization of *cis*-oxazolidine to the *trans*-isomer to attain excellent *syn*-selectivity of the amino alcohol **7**. To shorten the reaction time, we tried to facilitate the isomerization with gentle warming up to 40 °C (entry 3) and the addition of an external base (0.4 equiv of Hünig's base, entry 4), but failed to improve the reaction

time. Other ethereal solvents, such as 1,4-dioxane, 1,2-dimethoxyethane (DME), and diethyl ether (entries 5-7), failed to give better results, and no selectivity was observed when using CH_2Cl_2 (entry 8), which rendered entry 2 as the best condition. This is the first example in which an iminoester was successfully used as a substrate for Zn-linked-BINOL-catalyzed asymmetric aldol reaction conditions.

Scheme 1. Catalytic asymmetric synthesis of CPZEN-45 (**1**).

Reagents and conditions: (a) 1. TFA/ Et_3SiH (9:1), rt, 2.5 h, then aqueous MeOH, rt, 1 h; 2. MeNH₂, EDCI, HOBt, CH_2Cl_2 rt, 14 h, 58% (2 steps); (b) *p*NsCl, Et_3N , CH_2Cl_2 , 1 h, rt, 72%; (c) **10**, $\text{BF}_3 \cdot \text{OEt}_2$, MS4A, CH_2Cl_2 , 40 °C, 15 h, 70%; (d) HSCH₂CO₂H, K₂CO₃, MeOH, rt, 24 h, 74%; (e) **13**, AcOH, NaBH₃CN, AcOEt, rt, 15 min; then HCHO aq, rt, 1 h, 92% (2 steps); (f) CuI, DMEDA,

Cs₂CO₃, 1,4-dioxane, 120 °C, 8 h, 72%; (g) 3HF·Et₃N, Et₃N, CH₃CN, rt, 8 h, 86%; (h) 1. DMP, CH₂Cl₂, rt, 25 min; 2. NaClO₂, 2-methyl-2-butene, THF, then NaH₂PO₄ aq, rt, 20 min; 3. 4-*n*-butylaniline, COMU, Et₃N, DMF, rt, 5.5 h, 81% (3 steps); (i) 3HF·Et₃N, Et₃N, CH₃CN, 60 °C, 12 h, 97%; (j) 1. Zn, *n*-BuOH, NH₄Cl aq, rt, 2 h; 2. 80% TFA, rt, 1 h, then purification by HPLC and Dowex® 66 free base, 64% (2 steps).

For total synthesis, the oxazolidine intermediate **6** was treated with a mixture of TFA and Et₃SiH (9:1) followed by the addition of aqueous MeOH to give the β-hydroxy-α-amino acid, which was transformed into amide **8** by coupling with methylamine in 61% yield over 2 steps. After protection of the primary amine by a *p*Ns group (**9**, 63% yield),¹⁶ BF₃·OEt₂-promoted glycosylation^{8b,17} with the fluororibose derivative **10** according to the protocol reported by Matsuda and Ichikawa effectively furnished the corresponding glycoside **11** in 70% yield. Next, removal of the *p*Ns group gave rise to **12** in 74% yield, which was followed by introduction of the iodoolefin substructure in sequential one-pot reductive amination reactions using NaBH₃CN as a reductant. The first step with **12** and crotyl aldehyde derivative **13**,¹⁸ and the second step with formalin resulted in the uneventful production of diazepinone precursor **14** (92% yield). Next, we examined the second key transformation, Cu(I)-mediated vinyl halide-amide coupling^{13a}; cyclization proceeded smoothly by heating a mixture of **14**, CuI (1 equiv), DMEDA (2 equiv), and Cs₂CO₃ (2 equiv) in 1,4-dioxane under reflux at 120 °C to construct the enamide structure (**15**) with a reasonable yield of 72%. An

alternative approach in which the anilide portion was installed prior to the cyclization was not successful due to the unavoidable β -elimination at the iodoolefin functionality under the basic conditions.¹⁹

The endgame of the total synthesis was initiated with deprotection to unveil the primary hydroxyl group (**16**, 86%). Carboxylic acid **17** was obtained by the standard two-step oxidation procedure, and subsequent condensation with 4-*n*-butylaniline using COMU as the coupling reagent afforded **18** with the whole carbon framework of CPZEN-45 in its structure (81% over 3 steps).²⁰ After removal of the TIPS groups by 3HF·Et₃N (97% yield), the azide group was reduced under Zn-mediated conditions. Eventually, hydrolysis of the acetal with wet TFA gave CPZEN-45 in a form of TFA salt, which was purified by HPLC to give CPZEN-45 2TFA salt. The resulting CPZEN-45 2TFA salt was treated with ion-exchange resin, Dowex® 66 free base, to complete total synthesis of CPZEN-45 TFA salt. All of the physicochemical data, including ¹H and ¹³C NMR of the synthetic material, were fully identical to that obtained from the natural sample.³

In conclusion, a catalytic asymmetric total synthesis of CPZEN-45 (**1**), an anti-XDR-TB agent, was accomplished using an aldol reaction of a glycine Schiff base and a uridine-derived aldehyde catalyzed by the Zn(II)-(*R,R*)-linked-BINOL complex to give the desired stereoisomer selectively. The diazepinone part, another key structural element, was successfully constructed by vinyl halide-amide coupling promoted by Cu(I) salt. Further SAR studies of CPZEN-45-related compounds to search for anti-TB agents with more potent activity through this synthetic route are

currently underway.

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For example, the reaction using **19** bearing an anilide moiety suffered from facile β -elimination to give **20** exclusively.

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Legends of Artworks

Figure 1. Synthetic strategy of CPZEN-45 (**1**).

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