Multicomponent Macrocyclic IL-17a Modifier

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ABSTRACT: IL-17a is a major inflammation target, with several approved antibodies in clinical use. Small-molecule IL-17a antagonists are an emerging hot topic, with the recent advancement of three compounds into clinical trials. Here, we describe the design, discovery, synthesis, and screening of macrocyclic compounds that bind to IL-17a. We found that all currently described IL-17a modifiers belong to the same pharmacophore model, likely resulting in a similar receptor binding mode on IL-17a. A pipeline of pharmacophore analysis, virtual screening, resynthesis, and protein biophysics resulted in a potent IL-17a macrocyclic modifier.

KEYWORDS: interleukin 17a, IL-17a, multicomponent reaction, Ugi, isocyanide, MST, pharmacophore

The cytokine interleukin 17a (IL-17a) is involved in pathogenesis of several immunoinflammatory diseases, including psoriasis, psoriatic arthritis, and rheumatoid arthritis. After binding to the receptor on the surface of T helper cells, IL-17 activates several signaling cascades that, in turn, lead to the induction of chemokines. The chemokines act as chemoattractants and recruit immune cells, such as monocytes and neutrophils, to the site of inflammation to help eliminate invading pathogens. Activation of IL-17 signaling is also observed in the pathogenesis of various autoimmune disorders, such as psoriasis. Antagonizing the IL-17−receptor interaction can abrogate the inflammatory overreaction of this cytokine in a pathogenic setting. The potential applications of IL17-directed drugs could go well beyond the above-mentioned indications, e.g., multiple sclerosis (MS), Alzheimer’s disease, or ischemic brain injury; however, they are limited by the mAb nature of the currently available drugs.

With an extensive buried surface area between the receptor and the IL-17 dimer of ~2220 Å², not surprisingly, all current anti-IL-17a therapies are based on antibodies. Given the success of the marketed antibody drugs secukinumab (Cosentyx) and ixekizumab (Talz) in psoriasis, psoriatic arthritis, and anklyosing spondylitis, the race to a commercial small-molecule IL-17a antagonist has started (Figure 1). While the early attempts to discover IL-17a modifiers were focused on macrocycles, with the idea to cover a large surface area (1−4), more recent attempts successfully discovered non-macrocyclic small molecules (5−7). It started as early as 2016, when screening a macrocyclic DEL-derived library

Figure 1. Timeline of macrocyclic and small-molecule IL-17a modifier discovery. The smallest common denominator α-aminoacyl amide substructure is marked in blue.

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yielded the 18-membered compound 1. Several years later, the co-crystal structures of related macrocycles 2 (20-membered) and 3 (21-membered), with the IL-17a homodimer, were published, revealing that these compounds bind at the interface of the monomers of the IL-17a dimer, thereby allosterically reducing its ability to engage the IL-17 receptor. Another interesting IL-17a-modifying macrocycle is the 18-membered 4, which is built on a macrolide scaffold. Recently, three small non-macrocyclic molecules, 5, 6, and 7, entered early clinical trials. In parallel, several peptides potently binding IL-17a were disclosed. Here we describe our efforts to discover yet another macrocyclic IL-17a modifier, based on our recently developed efficient multi-component reaction-based macrocycle chemistry.

Analysis of the co-crystal structure of the two macrocycles, 2 and 3, bound to the interface of the IL-17a dimer revealed in both structures four key hydrogen bonds of the bis-amide substructure with the backbone of Leu97A and Leu97B (Figure 2B). We figured that these hydrogen bonds could be used to anchor moieties into the IL-17a dimer interface. Interestingly, all currently described IL-17a modifiers (Figure 1) contain the same bis-amide substructure or a bioisostere thereof. Our bis-hydrogen bond donor/hydrogen bond acceptor pharmacophore model (Figure 2C) serves to screen a compound library of ~1000 macrocycles by virtual screening (VS). To test our VS hypothesis in a timely manner, we focused on a recently described (by us) short and convergent two-step macrocycle syntheses of 6-SS, 7-SS, and 8-SS. To further characterize the most active compound, we separated the stereoisomers using semi-preparative supercritical fluid chromatography (SFC) on a Chiralpak IC chiral column, 10.0 × 250 mm (Figure 3). We were able to separate two isomers, 6C and 6D. Rescreening of the two isolated stereoisomers and the remaining inseparable mix revealed 6AB and showed that the isomer 6C gave the best affinity, at 170 nM.

Among the four possible stereoisomers of 6, the SS one shows the best cooperativity score (14.6), followed by RS (12.8), SR (11.6), and RR (7.2). Figure 4A shows a dense network of van der Waals interactions between 6-SS and hydrophobic residues like Ile-96, Leu-97, Val-98, Leu-99, Leu-112, and Leu-117. Similarly, these types of interactions are also prevalent among the other three stereoisomers (Figure SI-7). To a lesser but still present extent, there are π–π contacts with Tyr-62 and Leu-99 (Figure 4B). Interestingly, the four hydrogen bonds with Leu-97A/B are shared only by the three compounds to the IL-17a dimer using microscale thermophoresis (MST). The mixture of stereoisomers of 6, 7, and 8 showed promising binding affinities of 507 nM, 94 μM, and 51.1 μM, respectively. To further characterize the most active compound, we separated the stereoisomers using semi-preparative supercritical fluid chromatography (SFC) on a Chiralpak IC chiral column, 10.0 × 250 mm (Figure 3). We were able to separate two isomers, 6C and 6D. Rescreening of the two isolated stereoisomers and the remaining inseparable mix revealed 6AB and showed that the isomer 6C gave the best affinity, at 170 nM.

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A 17a MST assay was performed. Purified IL-17a protein was considered crucial for macrocycle binding to IL-17a.

**Figure 3.** SFC-MS chromatograms of the separation of the stereoisomers of 6 on a Chiralpak IC chiral column and their respective binding affinities, \( K_d \): (A) (rac)-6, (B) 6AB, (C) 6C, and (D) 6D.

The compounds described in this application were tested for their ability to bind IL-17. The biophysical data obtained from testing the above representative examples revealed binding affinities of 507 nM, 94 \( \mu \)M, and 51.1 \( \mu \)M for the diastereomeric meso mixture of compounds 6, 7, and 8, respectively (Figure 3). Subsequent separation of compound 6 into 6AB, 6C, and 6D revealed \( K_d \) values of 328 nM, 170 nM, and 309 nM, respectively.

Using a rational drug design approach, enabled by computational macrocycle screening and a very short, two-step macrocycle synthesis, we were able to discover low nM binders to the important anti-inflammatory target IL-17a. Moreover, pharmacophore analysis of currently described small-molecule IL-17a antagonists revealed a common multifurcated hydrogen-bonding pattern. Our findings are significant and will be of help for future design of small-molecule IL-17a antagonists to test medical indications which are out of reach of the current mAb-based therapeutics.

**ASSOCIATED CONTENT**

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.2c00257.

Experimental procedures and full characterization for compounds, including Figures SI-1–SI-8 and Tables SI-1 and SI-2 (PDF).

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**Notes**
The authors declare no competing financial interest.

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