Applications of biophysical methods in small-molecule modulators targeting protein function

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Chapter 2

Artificial Macrocycles as IL17A/IL17RA Antagonists

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Interleukin 17(A) is a pro-inflammatory cytokine involved in several auto-immune and inflammatory diseases. Current antagonists against IL17(A) or its receptor (IL17RA) that show efficacy in clinical trials are monoclonal antibodies (mAbs). However, recently designed artificial macrocycles are potent IL17A/IL17RA antagonists. Based on co-crystal structures, a better understanding of the biological activity and SAR of the macrocycles has been gained, demonstrating that they can compete with mAbs for difficult targets such as PPIs.

Inflammation is a complex biological protective response of body tissue against harmful stimuli (either acute or chronic) in which monocytes and lymphocytes play a role in the later stages. In recent decades, antagonists of inflammatory factors have been developed for the treatment of various inflammatory diseases, such as Crohn's disease, rheumatoid arthritis, ankylosing spondylitis and psoriasis, among which antagonists against tumor necrosis factor (TNF) are the most promising. Currently, while TNF inhibitors are widely used in different diseases, they behave differently in patients, and some side effects appear after long-term treatment. Thus, a major priority is the investigation of new approaches to treat TNF related inflammatory diseases. As interleukin 17 (IL17A) and TNF share similar effector functions, IL17A could be targeted in patients with inflammation who are not responsive to TNF inhibitors. Therefore, much attention is being devoted to drugs targeting IL17A.

Interleukin 17, synonymous with IL17A (the archetype protein in the IL17 family consisting of IL17A/F), was first discovered in 1993 as a pro-inflammatory cytokine predominantly produced by a subset of $CD4^+$ cells (T helper cells (Th17)). However, it has been found that other cell types, such as mast cells and neutrophils, produce IL17A as well [1]. For several decades, IL17A has been well known to participate in various acute inflammation reactions, e.g. the release of pro-inflammatory cytokines IL6 and IL8 from mesenchymal cells leading to fever, and the accumulation of neutrophils in blood and tissue [2]. IL17A also contributes to chronic inflammation associated with matrix destruction [3], resulting in joint damage and defective tissue repair. Additionally, IL17A increases the expression of the receptor activator of NF-κB ligand (RANKL) on osteoblasts, increasing the RANK signal in osteoclasts, as shown in the bone destruction of rheumatoid arthritis [4]. When acting on endothelial cells, IL17A stimulates inflamma-

tion and pro-coagulant activity [5]. IL17A also promotes endothelial cells and dendritic cells to release cytokines and enzymes [6-7]. In monocytes and dendritic cells, IL17A is involved in inflammation by modulating the production of pro-inflammatory cytokines.

The first receptor identified for IL17A was IL17A receptor A (IL17RA), and soon after that, other components required for the IL17 pathway were found [8-9]. Since IL17F shares 50% sequence homology with IL17A, a heterodimer consisting of IL17A and IL17F can also interact with the IL17 receptor complex. The receptor complex contains IL17RA and IL17RC ligands [9]. After the release of IL17A, two IL17A and one 17RA complex related intracellular signal pathways are activated. In the first pathway, IL17RA can recruit adapter NF-κB activator 1 (Act-1) to form a complex with its conserved cytoplasmic domain SEFIR, which is common to all IL17R family members [10-11]. Subsequently, Act-1 binds to TNF receptor-associated factor 6 (TRAF6) and acts as an E3 ubiquitin ligase on TRAF6, which recruits transforming growth factor activated kinases (TAK) 1 to mediate nuclear transportation of transcription factors such as nuclear factor-κB (NF-κB), activator protein 1 (AP1) and CCAAT/enhancer-binding proteins (C/EBP) [12]. It is well established that mitogen-activated protein (MAP) kinase located downstream of TRAF6 is also required for AP1 activation [13].

The second pathway is activated by IKKi-dependent phosphorylation of Act-1 at three Serine sites, which in turn suppresses the recruitment of TRAF6, thereby blocking the NF-κB pathway. In addition, Act-1 can modulate the mRNA stability of alternative splicing factor 1 (ASF) and ELAV-like protein 1 (ELAV1) through the ubiquitination of TRAF5 and its binding efficiency to TRAF2. At the same time, Act-1 can interact with and ubiquitinate ELAV1 through TRAF2 and TRAF5 [14].

Based on these observations, several agents have been designed to block the IL17 pathway. These can be broadly divided into antibody inhibitors and small molecule inhibitors. In the first approach, several mAbs have been generated and are now undergoing clinical trials (Table 1). Rheumatoid arthritis and multiple sclerosis are diseases that have been widely studied using mouse models, and preclinical studies in humans related to IL17A and trials of IL17A mAb inhibitors to treat rheumatoid arthritis and multiple sclerosis have been reported [15].

Table 1. IL17A directed mAbs in clinical trials or on the market

mAb[a]	Target	Indication	Development Phase
Secukinumab (Novartis)	IL-17A	Plaque Psoriasis,	On the market
		Ankylosing spondylitis,	On the market
		Rheumatoid arthritis,	Phase III
		Uveitis.	Phase III
Ixekizumab (Lilly)	IL-17A	Plaque Psoriasis,	On the market
		Rheumatoid arthritis.	Phase III
Brodalumab (Amgen)	IL-17RA	Plaque Psoriasis, Rheumatoid arthritis.	Phase III
ABT-122 (AbbVie)	IL-17A TNF	Plaque Psoriasis, Rheumatoid arthritis	Phase II
KHK4827 (Kyowa Hakko Kirin)	IL-17RA	Plaque Psoriasis	Phase III
Perakizumab (Roche)	IL-17A	Psoriatic arthritis	Discontinued
RG 7624 (Roche)	IL-17A IL-17F	Autoimmune diseases	Discontinued
ANB004 (AnaptysBio)	IL-17	Autoimmune, inflammatory diseases	No information available
COVA322 (Covagen AG)	IL-17A TNF	Inflammatory diseases	No information available

[a] clinical trial information: https://clinicaltrials.gov/

IL17A signals through the formation of a heterodimeric receptor complex involving IL17RA and IL17RC and the compounds described here act by disrupting the interaction of IL17A with IL17RA, which is believed to be the first step in receptor activation. There are several ways to block IL17A signalling by targeting IL17A proteins or receptors. The priority option is a direct action against IL17A or IL17RA. Two monoclonal antibodies directed against IL17A are FDA approved: Ssecukinumab (AIN457), a fully human IL17A specific monoclonal antibody derived from human lgG1 kappa isotype and ixekizumab (LY2439821), a humanized lgG4 antibody. Other IL17A targeted antibodies that have completed phase II are shown in Table 1. Secukinumab and ixekizumab are

both specific for IL17A homodimer and IL17A but not effective on IL17F homodimer [15-16]. CJM112 is another IgG1 against IL17A that is being tested for the potential treatment of hidradenitis suppurativa and psoriasis [17]. CNT06785 is a fully human IgM IL17A antibody in phase II trials for rheumatoid arthritis and moderate-to-severe chronic obstructive pulmonary disease (COPD). However, there are no published data or further plans for development [18-19]. Unlike secukinumab and ixekizumab, bimekizumab (previously UCB 4940) is a mAb targeting both IL17A and IL17F. A phase II trial for the add-on use of bimekizumab to certolizumab pegol in patients with rheumatoid arthritis has been completed with failure [20]. Clinical trials of ABT-122, a dual-variable-domain immunoglobulin targeting both TNF and IL17A for amplifying efficiency, which was designed to treat moderate-severe psoriatic arthritis and rheumatoid arthritis, have currently completed phase II [21-22]. The other targets for potential intervention are members of the IL17RA complex, of which IL17A/17RA inhibition is the broadest way to regulate the IL17A signal pathway. Brodalumab (AMG827) is a human mAb neutralizing IL17A/17RA with the high affinity that can block the biological activity of IL17, IL17F, a heterodimer composed of 17A/17F, or 17E. During phase II trials, brodalumab showed efficiency but with a greater risk of adverse events compared to the placebo group [23].

In addition to biological therapy, small-molecule inhibitors that target IL17A signalling by binding to the soluble ligand have been developed and are being tested in various clinical trials. Recently, some studies have suggested that some macrocycles might interfere with the interaction between IL17A and IL17RA with efficiency comparable to that of mAbs.

The crystal structure of the human IL17A/IL17RA interaction reveals an IL17A homodimer which forms two symmetrical interactions with IL17RA [24] (Fig 1). Both chains (A and B) of the IL17A interact with the IL17RA D1 or D2 domain. Compared with the apo structure, the entire N-terminus of chain A bends away from IL17RA. Thus the N-terminus of the IL17A homodimer is slightly separated in a buried cavity of the complex. The buried surface area of IL17A/IL17RA is around 2000Å^2. The overall interaction comprises a large, flat and featureless binding interface, resulting in numerous additive but weak polar and hydrophobic interactions.

Recently, several groups have discovered artificial macrocycles that efficiently antago-

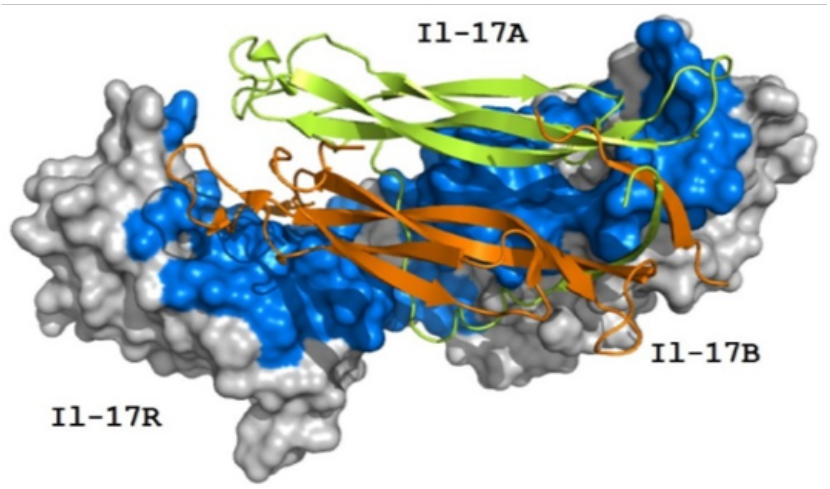
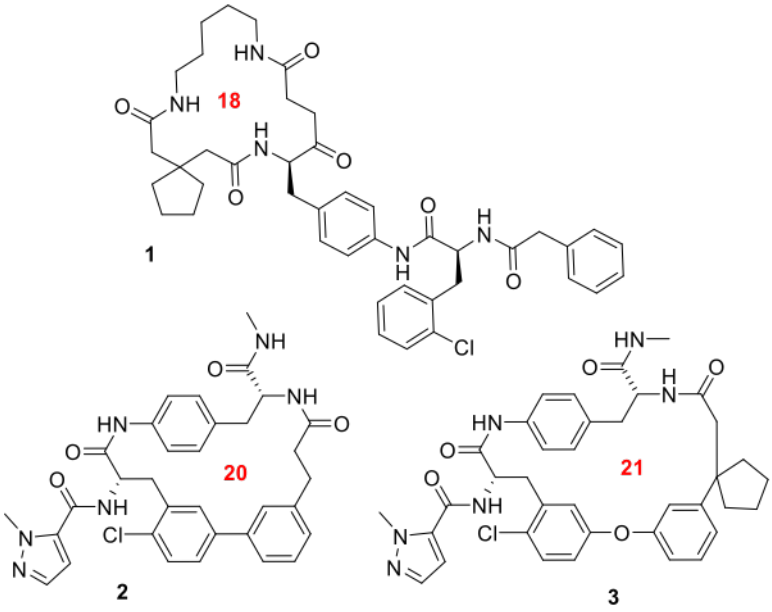


Fig 1. Complex between IL17A dimer and its receptor (PDB ID 4HSA). The IL17A dimer is shown in orange and green cartoon and IL17R as a surface representation. The footprint of IL17A on IL17RA is shown in blue.

nize the IL17A/IL17RA interactions (Scheme 1). The biotech company Ensemble Therapeutics disclosed macrocycles binding to IL17A [25]. The discovery of compound 1 involved synthesis of large libraries of macrocycles using DNA-encoded library (DEL) synthesis technology. Recently, H/D exchange MS was used to determine the binding region of macrocycle 1 on IL17A, which is predicted to bind to the β-hairpin pocket [26]. Another group from Pfizer designed potent derivative macrocycles 2 and 3 and elucidated their high-resolution co-crystal structure with IL17A [27-28]. Both compounds bind into the homodimeric interface. Compound 3 shows multiple interactions with its receptor including hydrogen bonds, hydrophobic interactions and stacking interactions of the aromatic components of the macrocycle (Fig 2).

Co-crystallisation of the macrocycles with IL17A was achieved using antibody antigen-binding fragment (Fab) co-crystallization chaperones – a technique known to facilitate the crystallization of difficult targets (Fig 2). Macrocycle 3 shows multiple interactions with its dimeric receptor, including hydrogen bonds, hydrophobic interactions and stacking interactions of the aromatic components of the macrocycle (Fig 2C–E). Notably, the atoms of the spiro-cyclopentyl moiety are important activity elements in all active macrocycle series, which can be rationalized by the shape and electrostatic complementarity with the Lys114, Leu97 pocket (Fig 2E).

Recently, design guidelines for macrocycles were formulated based on the analysis



Scheme 1. Structures of macocycles interacting with IL17A. Ring size is indicated in red.

of orally available cycles to obtain drug-like compounds. Thus, macrocycle 3 with a ring size of 21, a MW of 682, clogP of 5, tPSA of 140, HBD = 4, HBA = 6, four substituents, an overall polar/nonpolar atom balance of 0.3 and an N/O ratio of 6/5 fits well into these guidelines. Macrocycle 3 exhibits five hot-spot interacting areas (Fig 2G). Besides, it shows measurable activity in the keratinocyte-based bioassay for IL17A inhibition, indicating reasonable membrane permeation.

Protein–protein interactions (PPIs) on membranes are promising drug targets, and many mAb-based drugs are currently marketed. Traditionally, PPI inhibitors are designed as antibodies rather than small molecules, such as herceptin (anti-HER2), secukinumab (anti-IL17A) or atezolizumab (anti-PDL1). mAbs efficiently target large featureless protein surfaces and can be developed in a straightforward process to the market. Moreover, the attrition rate of mAbs during clinical development appears to be much lower than that of small molecules. Hence, some traditional small molecule-oriented pharma companies have recently announced a strong focus on biologics or their complete exit from the field of small molecule drug research and development. However, mAbs have a number of disadvantages as well, such as high cost-of-good, non-oral applications, poor tissue penetration, often long half-life times and most importantly being applicable only to extracellular targets. Recent findings from several groups demonstrate that several potent macrocycles show strong affinity to IL17A and may lead to the discovery of lower

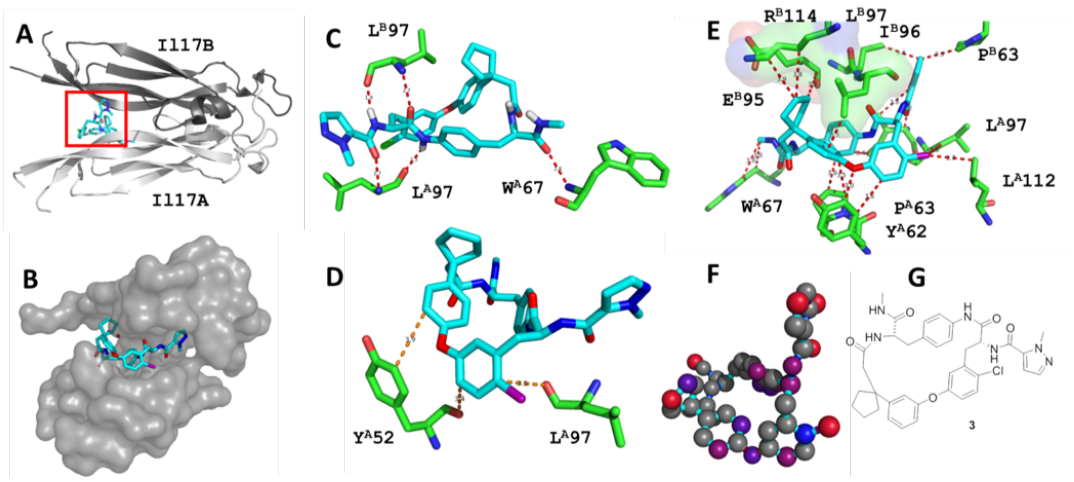


Fig 2. Macrocycle 3 (63P) bound to IL17A dimer (PDB ID 5HI4).A: Secondary structure elements of IL17A chain A (light grey) and B (dark grey) and boxed macrocycle 3 (cyan sticks); B: Surface representation of IL17A with 3; C: The hydrogen bonding network involves L97 from the IL17A and B monomers and W67 of IL17A A monomer; D: pi-stacking interactions with Y52A and L97A; E: van der Waals interactions involving P63B, L97A, L112A, Y62A, P63A, W67A; the subpocket (surface representation) formed by R114B, E95B and L97B harbours the cyclopentenyl moiety which is of great importance for the SAR; F: the relative contribution of the heavy atoms of 3 to the binding to IL17A form low to high (grey to red) calculated using SCORPION [33]; G: 2D structure of 3.

cost and more effective treatments for IL17A related inflammatory diseases. In nature, macrocycles are not uncommon and frequently exhibit useful biological activities [29] and have major advantages over open chain analogues: including higher affinity and selectivity [30], preferable entropic signature, better oral bioavailability or higher stability [31-32].

In summary, IL17A/IL17RA antagonists derived from medicinal chemistry might offer better options by re-investigating beyond r-o-5 small compound classes such as artificial macrocycles, natural products, or peptidomimetics to treat inflammatory diseases. These compounds will also potentially enable research into novel targets such as large receptor ligand interactions, DNA and RNA. The application of synthetic macrocycles to challenging PPI targets represents an important emerging area, with potential implications for the future balance of effort between biological and small molecule drug discovery.

Contributions

Writing-Original Draft Preparation, all authors; Writing-Review and Editing, all authors.

Conflicts of interest

There are no conflicts to declare.

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