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Discovery of Tertiary Amine and Indole Derivatives as Potent RORγt Inverse Agonists

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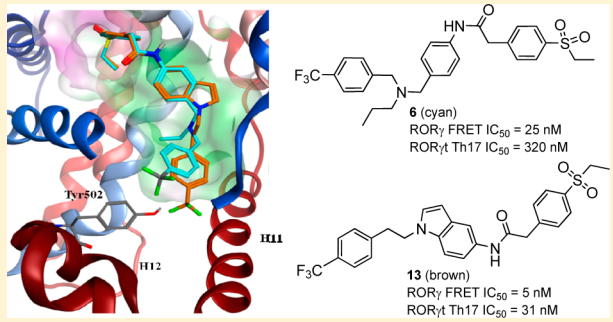
Supporting Information

ABSTRACT: A novel series of tertiary amines as retinoid-related orphan receptor gamma-t (RORγt) inverse agonists was discovered through agonist/inverse agonist conversion. The level of RORγt inhibition can be enhanced by modulating the conformational disruption of H12 in RORγt LBD. Linker exploration and rational design led to the discovery of more potent indole-based RORγt inverse agonists.

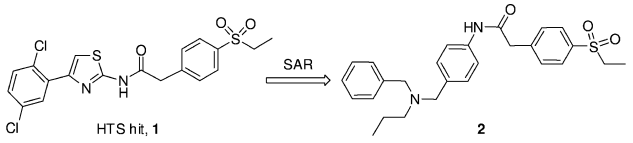


KEYWORDS: *RORγt, agonists, inverse agonists, Th17 cell differentiation, cocrystal structure, structure-based design*

Retinoid-related orphan receptor gamma-t (RORγt) is a member of the nuclear receptor superfamily. RORγt is a key regulator of the development and functions of T-helper 17 (Th17) cells which are implicated in the pathology of a variety of human inflammatory and autoimmune disorders.[1,2] The RORγt inhibitors have potential utility in controlling the activity of Th17 cells and can be developed as therapeutic agents for treatment of Th17-related autoimmune diseases. A few small molecule inhibitors of RORγt have been reported in the literature.[3−10] In this paper, we report the discovery of tertiary amines and indoles as potent RORγt inverse agonists using structure- and knowledge-based compound design.

A high-throughput screen (HTS) of the GSK in-house compound collection using a RORγ fluorescence resonance energy transfer (FRET) assay[11] resulted in identification of thiazole amide **1** as a RORγt inverse agonist with IC_{50} of 1.0 μM. The binding of **1** to the RORγt ligand binding domain (LBD) was confirmed with a thermal shift of 7.1 °C in a thermal shift assay.[11] SAR exploration on the left-hand side (LHS) of **1** led to the identification of tertiary amine **2** as a potent RORγt agonist with a EC_{50} of 0.02 μM in dual FRET assay (Scheme 1).[12] Dual FRET assay, using the same technology as the FRET assay but without adding a surrogate agonist, only relies on the basal level of RORγt activity and is able to measure both agonists and inverse agonists. Peptide profiling study using dual FRET assay showed that coactivator peptide (e.g., steroid receptor coactivator 1 (SRC1)) was recruited upon binding of **2** to RORγt LBD whereas

Scheme 1. From HTS Hit (1) to Tertiary Amine RORγt Agonist (2)



corepressor peptide (e.g., nuclear receptor corepressor 2 (NCOR2)) was not.[12] Given the structure similarity of **1** and **2**, we assume that they adopt a similar binding mode within RORγt LBD despite their difference as agonist and inverse agonist. To understand the binding mode of the chemical series, an in-silico docking study for **2** based on a reported RORγt crystal structure[13] was conducted.

A RORγt LBD crystal structure (PDB accession code: 3KYT) was selected and processed for the docking study. A total of 40 poses with the best scores were obtained and visually inspected after docking with Surflex-Dock v2.3[14−16] in Sybyl 8.1,[17] among which the top 10 poses were found to be representative and thus further ranked using MM/GBSA[18−20] affinity scores based on the VSGB2.0 solvent model.[21,22] As a

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result, the binding mode with the most favored MM/GBSA binding energy was selected and illustrated in Figure 1.[12] In this

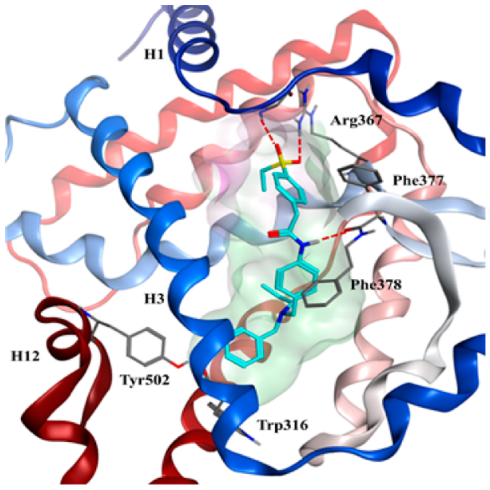


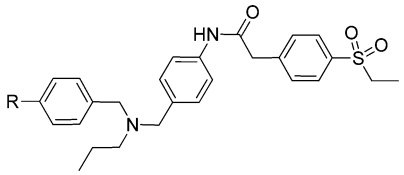
Figure 1. Predicted binding mode of **2** in RORγt LBD is illustrated, where **2** is in cyan stick and RORγt LBD in ribbon expression. Residues involved in key molecular interactions with **2** are highlighted and labeled.

binding mode, two H-bondings were observed: One from the sulfone moiety with Arg367 and a backbone amide, and the other between the linker amide and a backbone carbonyl. In addition, two $\pi-\pi$ stacking interactions are formed: one between the sulfone-substituted phenyl ring and Phe377 and the other between the middle phenyl ring and Phe378. The LHS benzyl group of the ligand occupies the hydrophobic site near Tyr502 and Trp316, which is believed to be important for activating RORγt by stabilizing the activation function 2 (AF2) domain (H12) essential for the downstream peptide recruitment.[23]

On the basis of literature evidence of agonist/inverse agonist conversion reported for estrogen receptor ligands,[24] we decided to design RORγt inverse agonists based on **2** and its predicted binding mode by introducing substituents to the para-position of the LHS phenyl ring near the AF2 domain to interfere H12's packing and thus affect the downstream peptide recruitment.

A series of compounds with different size and/or shape of 4-substituents on the LHS phenyl ring were designed and synthesized.[12] Potency (IC_{50} or XC_{50}) and maximum response (max %) were measured in FRET and dual FRET assays (Table 1). As expected, the compounds showed a range of maximum responses depending on the properties (size, shape, and electrostatics) of the 4-substituents. The maximum inhibition in the FRET assay improved in an order of Me < Et <*t*-Bu < CF_3 < O-*c*-Pen ~ Ph. In the dual FRET assay, maximum % activation of compounds **2**–**4** decreased from 125% to 65% with the increase in size of the substituents. With a larger substituent than **4**, compound **5** showed no effect in modulating RORγt basal activity and is considered as a neutral antagonist. Enlarging 4-substituents further from that of **5** resulted in inverse agonists with an increased level of inhibition. Shape and electrostatic effect also play a role for the substituents with similar size (**5** vs **6**; **7** vs **8**). The results of dual FRET assay together with the structural insight from the docking study revealed the molecular mechanism of action for the compounds. The strong correlation between bulk of the substituent and level of inhibition can be interpreted as severity

Table 1. SAR of 4-Substituents on LHS Phenyl of Amines



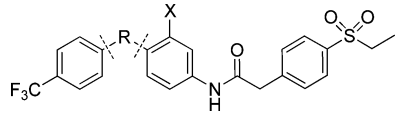
		FRET		dual FRET	
compd	R	IC_{50} (nM)	max %	XC_{50} (nM)[a]	max %
2	H	>25 000		20[b]	125
3	Me	>25 000		25[b]	89
4	Et	13	38	25[b]	65
5	*t*-Bu	40	84	>10 000	
6	CF_3	25	92	6	25
7	O-*c*-Pen	20	115	32	85
8	Ph	50	115	20	94

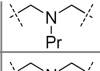
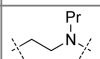
[a]IC_{50} (nM) unless indicated. [b]EC_{50} (nM).

of the structural interference of the substituent to H12 based on the predicted binding mode. The severity of the structural interference is translated to the magnitude of conformational change of H12 and thus the ability of coactivator and corepressor recruitment. In contrast to the notable change in the level of inhibition, potency of the compounds was merely affected by bulk of the substituents. Compound **7** was further characterized by peptide recruitment profiling based on the dual FRET assay.[12] As a result, neither coactivator peptide nor corepressor peptide tested was recruited. This was the first time to observe that a corepressor peptide was not recruited by a type of inverse agonists. Further biological investigation is needed to interpret the results.

A crystal structure of RORγt LBD in a complex with **2** was later resolved at a resolution of 2.01 Å (PDB accession code: 4NIE).[12] To our delight, the overall binding mode of **2** in RORγt LBD is essentially the same as the predicted one. With the established binding mode, we explored SAR of the amine containing linker with the LHS fixed as 4-CF_3–Ph (Table 2). Removal of the propyl group from the tertiary amine lowered the potency by 50-fold (**9**), indicating that the hydrophobic interaction between the propyl group and RORγt LBD has significant impact on compound potency. Interestingly, shifting the NH by one position (**10**) regained RORγt potency by more

Table 2. SAR of Amine Linkers



Compd	R	X	FRET		Th17
			IC_{50} (nM)	max%	IC_{50} (nM)
6		H	25	92	320
9		H	1,259	115	>10,000
10		H	100	117	-
11		H	79	113	733
12		Cl	16	109	1,023

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than 12-fold from that of **9**. Attaching a propyl moiety to the amine did not improve much of the potency (**11** vs **10**). While introducing a Cl group on the ortho-position of the middle phenyl ring led to the RORγt potency increased by more than 6-fold (**12** vs **10**). The potency improvement of **12** can be attributed to both molecular interaction and conformational effect. Compounds, **6**, **9**, **11**, and **12**, were also tested in mouse Th17 cell differentiation assay,[11] and their IC_{50} values were more than 100 nM (Table 2). Therefore, further optimization was needed for achieving better Th17 potency.

To mimic the aniline geometry of **11** and **12**, we designed a more rigid indole compound **13** as a potential RORγt inverse agonist. Figure 2 shows the predicted binding mode of **13**,

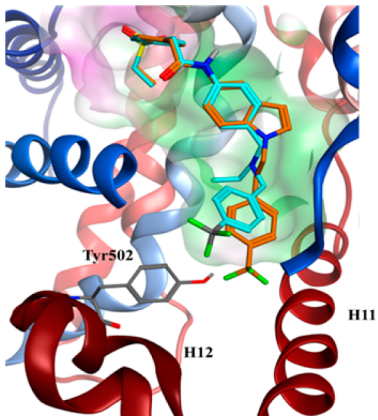
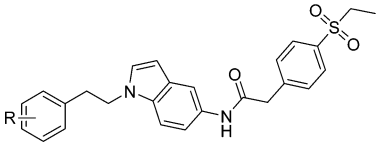


Figure 2. Structural overlay of **13** (in brown stick) and **6** (in cyan stick) in RORγt LBD.

compared with that of **6** in RORγt crystal structure. The 4-CF_3−Ph moiety of **13** extends naturally toward H12 in a low energy conformation, where CF_3 is in close contact with the side chain of Tyr502 on H12. Comparing to phenyl, indole seems to be more efficient for the design of inverse agonists with AF2 domain disrupting mechanism. With this assessment, **13** was synthesized and confirmed to be a potent RORγt inverse agonist with FRET IC_{50} of 5 nM (max % = 104) (Table 3).

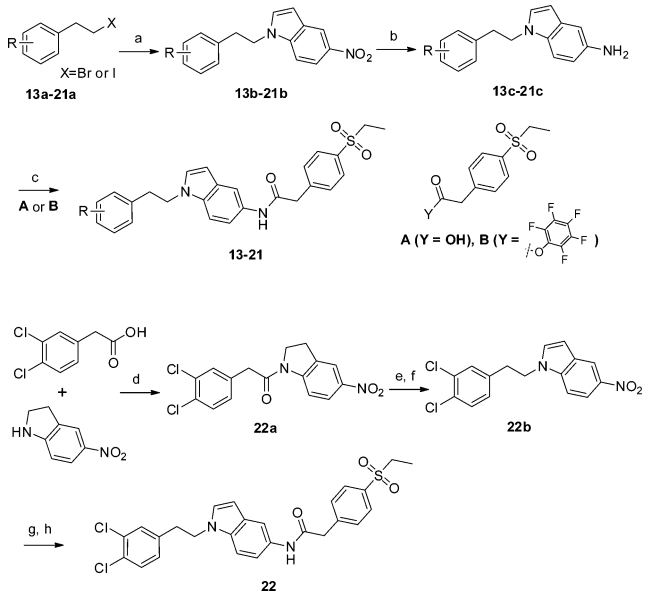
Table 3. SAR of Substituents on LHS Phenyl of Indoles



		FRET		Th17
compd	R	IC_{50} (nM)	max %	IC_{50} (nM)
13	4-CF_3	5	104	31
14	4-H	3	65	
15	4-F	8	92	
16	4-Cl	4	99	164
17	4-CN	6	108	457
18	4-O*i*Pr	6	119	70
19	2-CF_3	6	83	
20	3-CF_3	4	57	
21	2-Cl, 4-Cl	6	100	9
22	3-Cl, 4-Cl	6	98	40

A series of indole analogues were designed and prepared subsequently to explore SAR of the LHS phenyl substitution. Synthetic procedures for preparation of indole analogues **13**−**21** and **22** were shown in Scheme 2. Reaction of bromides

Scheme 2. Synthetic Procedures for Indole Analogues 13−21 and 22[a]



[a]Reagents and conditions: (a) for X = Br: 5-nitro-*1H*-indole, Cs_2CO_3, KI, DMF, 90 °C. For X = I: 5-nitro-*1H*-indole, K_2CO_3 (or Cs_2CO_3), DMF, RT; or 5-nitro-*1H*-indole, NaH, DMF, 0−90 °C. (b) $SnCl_2 \cdot 2H_2O$, ethanol, reflux. (c) For acid **A**, HATU, DIPEA, DCM, RT; for perfluorophenyl ester **B**, DIPEA, DCM, RT; (d) HATU, DIPEA, DCM; (e) $NaBH_4$, $BF_3 \cdot Et_2O$, THF, RT; (f) toluene, 4,5-dichloro-3,6-dioxocyclohexa-1,4-diene-1,2-dicarbonitrile, 80 °C; (g) $SnCl_2 \cdot 2H_2O$, ethanol, reflux; (h) perfluorophenyl ester **B**, DIPEA, DCM, RT.

13a−**21a** and 5-nitro-1*H*-indole gave 1-substituted-5-nitro-indoles **13b**−**21b**. Reduction of the nitro-indoles to amino-indoles **13c**−**21c**, followed by amide formation with acid **A** or activated ester **B** produced the targeted compounds **13**−**21**. Synthesis of compound **22** started with reaction of 2-(3,4-dichlorophenyl)acetic acid and 5-nitro-indoline, which led to formation of amide **22a**. Reduction of the amide, followed by oxidation of indoline gave the nitro-indole **22b**, which upon nitro reduction and amide formation with **B** afforded the targeted compound **22**.

All indole-containing compounds tested showed strong RORγt inhibition with FRET IC_{50} values below 10 nM. Similar to the tertiary amine, the bulk of the para-substitution clearly impacts the level of RORγt inhibition. The nonsubstituted analogue **14** showed only 65% of inhibition, and the level of inhibition generally improved with the increase in size of the substituted moieties (**14**−**18**), provided that shape and electrostatic effect of the substituents are taken into consideration when comparing compounds with similar size of the substituents. Substitution on other positions of the LHS phenyl was also explored. The reduced maximum response of **19** and **20** suggests that 4-substitution is more effective on H12 disruption than 2- or 3-substitution. The RORγt potencies of the disubstituted compounds **21** and **22** are similar to that of **16** but the Th17 potencies are higher. This suggests that lipophilicity might have played a role in cellular potency. With

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proper substituents on LHS phenyl of the indoles, we were able to achieve high Th17 potency with IC_{50}s equal or below 100 nM (**13**, **18**, **21**, and **22**).

In summary, we identified a novel series of tertiary amines as RORγt inverse agonists using structure- and knowledge-based design. Relationship between ligand/H12 structural disruption and the level of RORγt inhibition was established for the first time. Linker exploration and rational design led to a series of indole-based analogues as more potent RORγt inverse agonists. Compound **21** was discovered as a potent RORγt lead with both FRET and Th17 IC_{50}s lower than 10 nM. Further optimization of the PK profile of the aryl amide series is ongoing and will be reported in due course.

■ ASSOCIATED CONTENT

Supporting Information

RORγt FRET, dual FRET and Th17 assays description; peptide profiling studies; modeling studies; cocrystal structure data; synthetic procedures; and compound characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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