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Binding characterization, synthesis and biological evaluation of RXRα antagonists targeting the coactivator binding site

Dingyu Xu [a,†], Shangjie Guo [a,†], Ziwen Chen [a], Yuzhou Bao [a], Fengyu Huang [a], Dan Xu [a], Xindao Zhang [a], Zhiping Zeng [a], Hu Zhou [a], Xiaokun Zhang [a,b], Ying Su [a,b,*]

[a] School of Pharmaceutical Sciences, Xiamen University, Xiamen 361005, China
[b] Sanford Burnham Prebys Medical Discovery Institute, 10901 N. Torrey Pines Road, La Jolla, CA 92037, USA

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ABSTRACT

Previously we identified the first retinoid X receptor-alpha (RXRα) modulators that regulate the RXRα biological function via binding to the coregulator-binding site. Here we report the characterization of the interactions between the hit molecule and RXRα through computational modeling, mutagenesis, SAR and biological evaluation. In addition, we reported studies of additional new compounds and identified a molecule that mediated the NF-κB pathway by inhibiting the TNFα-induced IκBα degradation and p65 nuclear translocation.

Nuclear receptors (NRs) are a superfamily of transcription factors of which many function via a ligand-mediated mechanism.[1,2] As nuclear receptors are essential players in various biological processes such as differentiation, apoptosis, metabolism, and inflammation and NRs are implicated in many diseases including cancer, diabetes and obesity, NRs have become important drug targets.[3,4] Members of the nuclear receptor superfamily share conserved domains, including a N-terminal domain, a DNA-binding domain (DBD) and a ligand-binding domain (LBD).[5–7] The LBD plays a crucial role in ligand-regulated nuclear receptor activities. The LBD consists of a canonical ligand-binding pocket (LBP) for the binding of small molecule ligands, a transactivation function domain termed AF-2 composed of helix 12 of the LBD, a coregulator-binding surface groove, and a dimerization surface. A well-accepted mechanism for ligand-mediated nuclear receptor activities is that ligand binds to the LBP to induce a major conformational change, converting the corepressor-binding site into a coactivator-binding site and triggering a cascade of events that lead to biological activities. Therefore, many nuclear receptor drugs are developed to target the LBP.[8,9] However, drugs acting by binding to the LBP are associated with undesirable side effects. Protein crystallographic studies have revealed various alternate sites on NRs,[10–12] suggesting that targeting alternate binding areas on the nuclear receptor surface may offer opportunities to mitigate side effects and to discover new therapeutic stratregies.[13–15] Among these alternate sites, the coregulator-binding site has attracted increasing attention. Compounds that bind to the coregulator-binding site of some nuclear receptors,[15–17] including estrogen receptor, androgen receptor, vitamin D receptor and thyroid hormone receptor, have been reported. Recently we reported the first example of an RXRα modulator that acts via the coregulator-binding site.[18] The reported binder, **23** (Fig. 1A) was identified through employing a docking-based virtual screening approach. Various RXRα mutants were studied to demonstrate that the identified binder, **23** does not bind to the LBP. Modeling and mutagenesis studies further show that **23** binds directly to the coregulator-binding surface. **23** could regulate the biological functions of tRXRα, an N-terminally truncated form of RXRα that is overexpressed in many cancer cells and is implicated in diseases.[11,19,20] **23** inhibits tumor necrosis factor-alpha (TNFα)-induced interaction of tRXRα with the p85α subunit of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), resulting in the inhibition of AKT activation in vitro and the induction of apoptosis. These results demonstrate the feasibility of targeting the alternate binding sites on the surface of RXRα for therapeutic intervention.[21]

Here we describe the further characterization of the binding nature of **23** and some important features of the structure–activity relationships (SAR) resulting from molecular modeling, biological

* Corresponding author.
E-mail address: ysu@sbpdiscovery.org (Y. Su).
† These authors contribute equally to this work.

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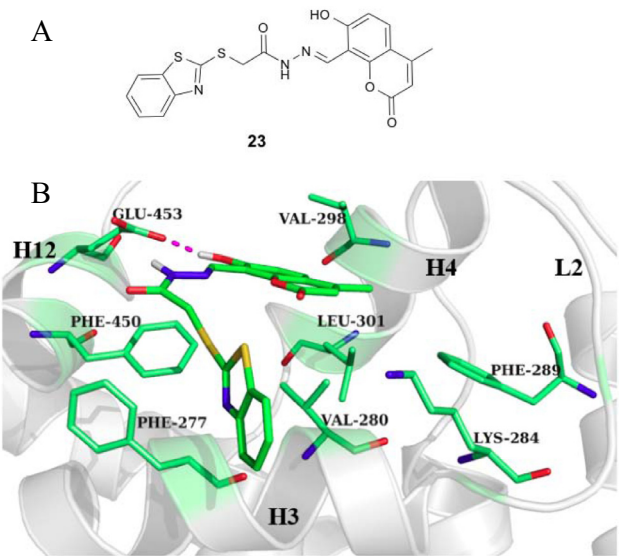

Figure 1. (A) Structure **23**. (B) Binding model of **23** in the coactivator-binding site of RXRα. Protein is shown in ribbon diagram and **23** and the interacting side chains are in shown in stick representation.

testing and chemical synthesis. Based on the obtained structural insights, we designed and synthesized a series of new molecules. Biological testing of the new molecules led to the identification of a novel compound with new biological function.

To characterize the binding nature of **23** to the protein, we first investigated the potential binding mode of **23** in the coactivator-binding region using the Glide docking program from Schrodinger.[22] The 10 top-scored docking modes were visually evaluated and one docking mode was intuitively selected as the binding mode shown in Figure 1B. In this mode, **23** sits in the co-activator binding groove consisting of Phe277 and Val280 of H3, Phe289 of L2, Val298 and Leu301 of H4 and Phe450 of H12 (Fig. 1B). **23** interacts with RXRα through both hydrophobic interactions and H-bond. The 7-OH-4-Me-2-oxo-2*H*-Chromen-8-yl portion of the compound is located near H4 with the ring system making hydrophobic interactions with the side chains of Phe289, Val298 and Leu301, and the *para* —OH group forming a H-bond with Glu453. The contribution of Val298 to the ligand–protein interaction has proved to be critical.[18] Here, to evaluate the involvement of the *para* —OH group, compound **24** is synthesized where the —OH group in **23** was methylated (Fig. 3 and scheme in the Supplementary data) and became incapable of acting as an H-bond donor. As anticipated, **24** showed a weaker inhibitory effect on the transactivation of RXRα (Fig. 2), demonstrating a role of the —OH group in the ligand–protein interaction. Binding of **24** to the RXRα-LBD was also evaluated by the surface plasma resonance (SPR) method. In consistency with the transactivation result, **24** binds weaker to RXRα (Fig. 3). In the proposed binding mode, this —OH group forms an H-bond with side chain of Glu453. Thus, mutant E543A could have an impact on **23** binding and its activity. However mutating this residue can preclude our evaluation of **23** binding from using the reporter gene assay that depends on the binding of coactivator. This is because Glu453 plays a key role in the recruitment of the coactivator, an essential step leading to transactivation after the binding of an agonist.[5] Indeed, mutant E453A is inactive (Fig. 2). Therefore, in order to confirm the involvement of E453 in the binding of **23**, we performed an SPR experiment to directly measure the binding of compound **23** to E453A mutant. Our SPR result showed that **23** bound to the E453A mutant protein 10 fold weaker than to the wild type RXRα (Fig. S1 in the Supplementary data), suggesting that Glu453 is

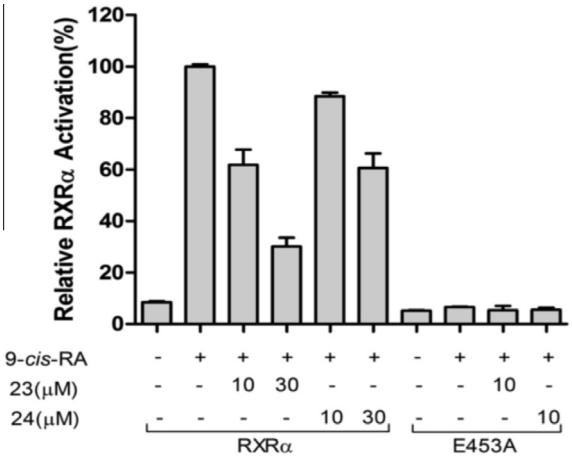

Figure 2. The antagonist effect of **23** and **24** on the transactivation activity of RXRα or RXRα-E453A. HEK-293T cells cotransfected with pG5-Luc, RXRα or mutant E453A expression vector were treated with 9-*cis*-RA (10^{-7} M), and the indicated concentration of **23** or **24** for 12 h.

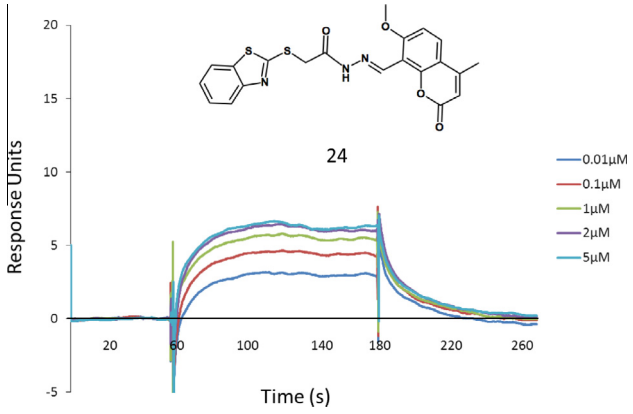

Figure 3. Structure of **24** and the binding of **24** to RXRα-LBD by SPR assay. The sensorgrams were obtained from injection of series of concentration of **24** over the immobilized RXRα-LBD chip.

involved in the protein/ligand interaction. This data, together with data from **24**, supports the binding mode proposed by the docking study (Fig. 1B).

We then asked if the binding of molecule **23** prefers the coactivator-binding site to the corepressor-binding site as the coactivator-binding region and the corepressor binding region overlap.[2] The role played by E453 in the binding of **23** supports that **23** binds to the coactivator-binding region. E453 is located in H12 which is part of the coactivator-binding site, whereas H12 does not contribute to the formation of the corepressor-binding region and E453 is likely not available for interacting with ligand. Furthermore, classical ligand like 9-*cis*-retinoic acid (9-*cis*-RA) binds to the LBD of RXRα, which stabilizes the coactivator-binding region and can augment the binding of **23** if **23** binds to the coactivator-binding site. Indeed we observed that in the presence of the 9-*cis*-RA, **23** binds tighter to RXRα in the SPR experiment (Fig. S2 in the Supplementary data). Therefore, **23** binds to the coactivator-binding site of RXRα.

We then examined the binding nature of compound **23** in the coactivator-binding site to identify a strategy to optimize its binding property. First we were interested in the region where the ring system of 2-oxo-2*H*-Chromen-8-yl binds. The binding mode shows that there is limited space around 2-oxo-2*H*-Chromen-8-yl to accommodate substituents on 2-oxo-2*H*-Chromen-8-yl. In