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Structural Modification of the Natural Product Valerenic Acid Tunes RXR Homodimer Agonism

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Retinoid X receptors (RXR) are ligand-sensing transcription factors with a unique role in nuclear receptor signaling as universal heterodimer partners. RXR modulation holds potential in cancer, neurodegeneration and metabolic diseases but adverse effects of RXR activation and lack of selective modulators prevent further exploration as therapeutic target. The natural product valerenic acid has been discovered as RXR agonist with unprecedented preference for RXR subtype and homodimer activation. To capture structural determinants of this activity profile and identify potential for optimization, we have studied effects of structural modification of the natural product on RXR modulation and identified an analogue with enhanced RXR homodimer agonism.

Introduction

Ligand-activated transcription factors, termed nuclear receptors (NRs), act as sensors for multiple endogenous metabolites and signaling molecules and regulate gene expression in response to these ligand stimuli.[1] NRs hence enable pharmacological control of gene expression rendering them as attractive drug targets.[1] Among the 48 human NRs, the three highly conserved retinoid X receptors (RXRs, NR2B1-3)[2,3] have particular importance as they represent the universal heterodimer partners for other NRs.[4,5] Therefore, RXRs participate in multiple NR dependent regulatory systems and are involved in a vast number of physiological processes. This may suggest attractive therapeutic potential of RXR modulation in various pathologies[6–8] but also gives rise to adverse effects observed with RXR agonists like bexarotene (**1**, Figure 1a).[9–11] The natural product valerenic acid (**2**) has emerged from a virtual screening campaign as new type of RXR modulator with pronounced preference for RXR homodimer (EC_{50} = 7 μM) and RXRβ activation (RXRα: EC_{50} = 27 μM, 9-fold activation; RXRβ: EC_{50} = 5.2 μM, 69-fold activation; RXRγ: EC_{50} = 43 μM, 4-fold activation) demonstrating that functionally selective and subtype-preferential RXR ligands can be obtained[12] as a potential avenue to pharmacological RXR modulation with reduced adverse effects.[13] Here we evaluated the effects of structural modifications on the hexahydroindene motif of **2** on RXR agonism. We observed a steep SAR in terms of RXRβ-preference but identified a valerenic acid derivative (**7**) with enhanced agonism on the RXR homodimer.

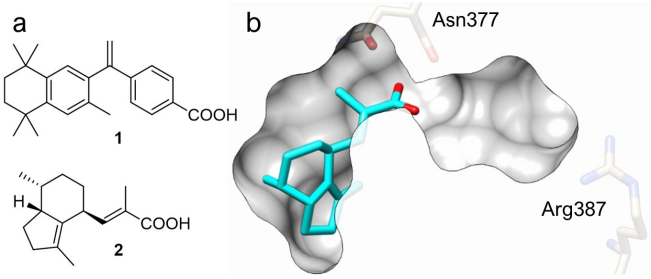

Figure 1. (a) Chemical structures of RXR agonists bexarotene (**1**) and valerenic acid (**2**). (b) Binding of **2** to RXR. **2** (blue) was predicted to bind to the hydrophobic region of the RXR ligand binding site close to the activation function in helix 12 with no contact to Arg387 but forming a polar interaction to Asn377. RXRβ (PDB ID 7a78)[3] is shown as example.

Results and Discussion

The RXR ligand binding site constitutes an L-shaped hydrophobic tunnel that narrows towards its polar end which is defined by an arginine residue (Arg387 in RXRβ) forming a strong ionic contact with most RXR agonists as typically exclusive polar interaction.[3,6] Docking of valerenic acid (**2**) interestingly suggested binding close to the activation function at the hydrophobic end of the RXR ligand binding sites in all RXR subtypes (Figure 1). This result was obtained with AutoDock Vina,[14] which we recently found well-suitable for RXR ligand docking,[13] and reproduced by the Molecular Operating Environment (MOE)[15] docking algorithm with rigid receptor and induced fit. The predicted binding mode indicated that mainly the hydrophobic hexahydroindene motif mediated binding of **2** to RXR and RXR activation. Therefore, we evaluated the impact of modifications in this two-ring scaffold on RXR

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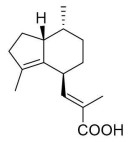
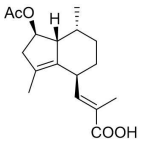
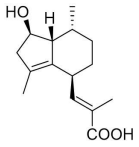
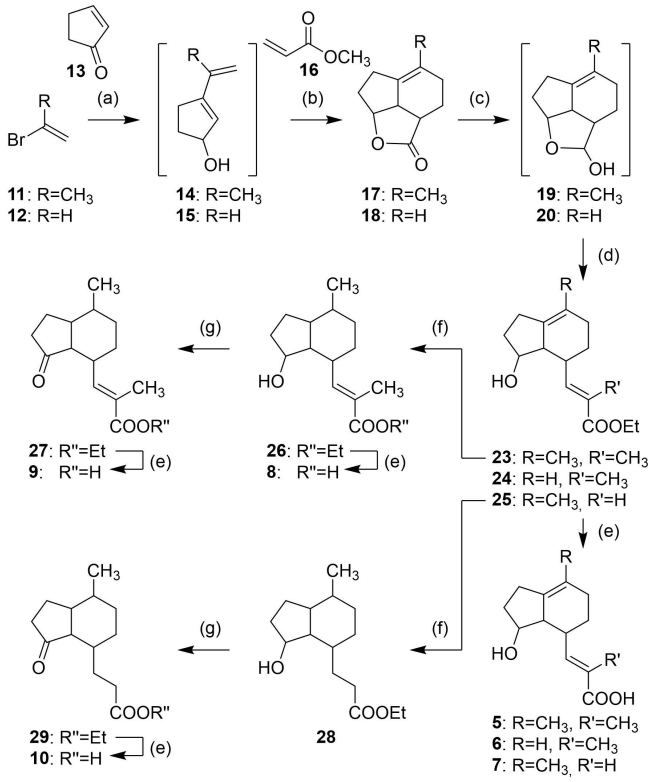
Table 1. Activities of the natural products **3** and **4** on RXR. **2** for comparison.

		EC_{50} [μM] (max. fold activation)[a]			
ID	structure	RXRα	RXRβ	RXRγ	RXR:RXR
2		27 ± 3 (9 ± 1)[b]	5.2 ± 0.4 (69 ± 1)[b]	43 ± 1 (4 ± 1)[b]	6 ± 2 (1.7 ± 0.1)
3		inact. (200 μM)	inact. (200 μM)	inact. (200 μM)	inact. (200 μM)
4		inact. (200 μM)	inact. (200 μM)	inact. (200 μM)	inact. (200 μM)

[a] Activities on RXR subtypes were determined in Gal4-hybrid reporter gene assays; effects on the RXR homodimer were determined in a reporter gene assay based on DR1 and the full-length human RXR; data are the mean ± SD, n ≥ 3. Fold activation refers to the fold induction of reporter activity vs. control (0.1% DMSO treated cells). Inact. – no significant effect at the highest tested concentration as indicated. [b] Hybrid reporter gene assay data for **2** from Ref. [12].



Scheme 1. Synthesis of **5**–**10**. Reagents and Conditions: (a) *t*-BuLi, TFA, Et_2O, −78 °C, 4 h; (b) $MgBr_2 \cdot Et_2O$, DIPEA, DCM, RT, 16 h, 12–40% over two steps; (c) DIBAL-H, DCM, −78 °C, 2 h; (d) $Ph_3P{=}CMeCO_2Et$ (**21**) or $Ph_3P{=}CHCO_2Et$ (**22**), benzene, reflux, 16 h, 20–47% over two steps; (e) LiOH, H_2O, reflux, 16–48 h, 20–97%; (f) Crabtree, H_2, DCM, 0 °C-RT, 2.5 −3.5 h, 61–90%; (g) IBX, DMSO, RT, 3–4 h, 45–70%.

agonism to obtain preliminary insights into the structure-activity relationship of valerenic acid (**2**) as RXR agonist.

In addition to valerenic acid (**2**), *Valeriana officinalis* contains the close analogues acetoxy- (**3**) and hydroxyvalerenic acid (**4**). In vitro profiling of these natural products in reporter gene assays (Table 1) revealed no effect on RXR activity up to 200 μM concentration suggesting that modifications in 1-position of the indane skeleton of **2** were detrimental. This observation, however, aligned with the predicted binding mode of **2** in which the 1-position of the indane is buried in a hydrophobic cavity with no space available to accommodate additional substituents.

As **3** and **4** failed to modulate RXR, we next centered our attention on modifications on the opposite side of the indane scaffold. As no previous SAR knowledge was available for **2** as RXR ligand, we took (economic) synthetic accessibility into consideration for analogue design and focused in this study on hydroxylated derivatives of **2** which were accessible via the synthesis strategy developed by Ramharter and Mulzer[16,17] as intermediates or by using alternative starting materials.

The valerenic acid derivatives **5**–**10** were prepared according to Scheme 1 following the published route.[16,17] As first step, 2-bromoprop-1-ene (**11**) or bromoethene (**12**) was treated with *n*-BuLi and subsequently reacted with cyclopent-2-en-1-one (**13**) to obtain the cyclopentenols **14** and **15** after workup with TFA. In the interest of an economic synthesis for rapid SAR exploration we skipped the enantiomeric resolution of **14** and **15** but directly treated the dienes with the dienophile **16** in presence of $MgBr_2 \cdot Et_2O$ to obtain the key lactones **17** and **18** in

a metal-coordinated Diels-Alder reaction in up to 40% isolated yield over two steps without intermediate isolation. The lactones **17** and **18** were subsequently reduced to the hemiacetals **19** and **20** using DIBAL-H from which Wittig reaction with the ylides **21** and **22** afforded **23**–**25** and ester hydrolysis of **23**–**25** yielded **5**–**7**. **23** was further derivatized by reduction with the Crabtree catalyst to obtain the saturated analogue **26** whose ester hydrolysis gave **8**, and oxidation of **26** with IBX gave the ketone **27** which was hydrolyzed to **9**. Additionally, reduction of **25** with the Crabtree catalyst produced the fully saturated analogue **28** which was oxidized to ketone **29** and hydrolyzed to **10**. **5**–**10** were obtained as diastereomerically pure enantiomeric pairs.

In vitro profiling in Gal4-hybrid reporter gene assays (Table 2) revealed reduced RXR agonism of **5**–**10** on all RXR subtypes compared to **2**. Compound **5** with a hydroxy substituent replacing the 3-methyl group of **2** and a 7,8-double bond retained weak RXR agonism while **6** additionally lacking the 7-methyl group was inactive thus indicating importance of the 7-methyl motif for interaction with RXR. Interestingly, enhanced RXR agonism was detected for **7** comprising the 3-hydroxy and 7-methyl groups but lacking the side chain methyl substituent. The saturated analogue **8** of **5** exhibited similarly weak RXR agonism as **5** and the 3-oxo derivatives with α-methyl acrylic acid (**9**) or propanoic acid (**10**) side chain revealed no detectable activity on RXR.

RXRs can act as various dimeric forms with other nuclear receptors mediating their widespread roles in health and disease. While the Gal4-RXR hybrid assays are very useful to reveal activity on the different RXR subtypes, this system cannot capture the potentially different effects on RXR dimers. Hence, we determined the activity of the natural product **2** and the descendants **5**–**10** on the human full length RXR homodimer and heterodimers with retinoic acid receptor (RAR), liver X receptor (LXR) and farnesoid X receptor (FXR). Valerenic acid (**2**) activated the RXR homodimer with low activation efficacy but with preference over all studied heterodimers (Table 2, Figure 2a). The analogues **6**, **9** and **10** showing no activity on the Gal4-RXR subtypes were also inactive on the homodimer, while the active derivatives **5**, **7** and **8** exhibited consistently higher potency on the homodimer than on the hybrid receptors. Among them, **7** emerged with similar low micromolar potency as **2** but significantly increased RXR homodimer activation efficacy.

Isothermal titration calorimetry (ITC) orthogonally confirmed binding of **7** to all RXR subtypes with low micromolar affinity (Table 3, Supporting Information Figure 1). This observation of consistent affinity and potency for homodimer activation but lower potency on the Gal4-hybrid receptors may suggest different molecular determinants for activation of RXR as homodimer or other mono-/oligomeric forms,[18] but this hypothesis requires further structural evaluation.

Overall, our preliminary observations on the SAR of **2** as RXR ligand indicated that larger modifications on the hexahydroindene scaffold or introduction of more polar hydroxy substituents were not favored for activity on the RXR subtypes. Additionally, comparison of **5**–**8** suggested importance of the 7-methyl group for RXR activation by this scaffold as it is contained in all active derivatives **5**, **7** and **8** but lacking in the inactive analogue **6**.

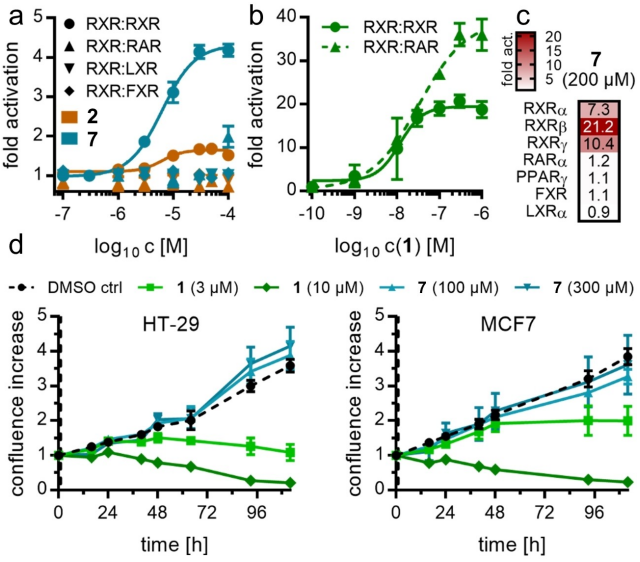
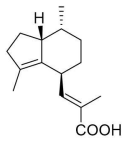
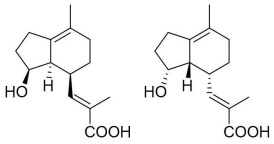
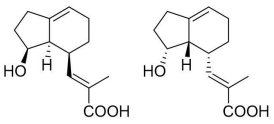
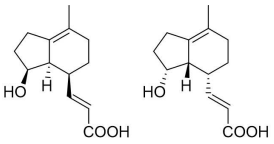
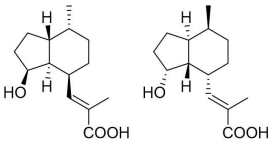
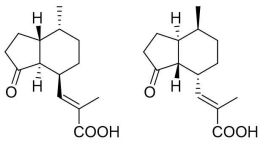
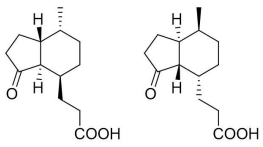


Figure 2. (a) The natural product **2** (orange) and derivative **7** (blue) exhibit preference for the RXR homodimer over RXR heterodimers. **7** has improved efficacy in RXR homodimer activation. Data are the mean ± S.E.M. fold activation vs. DMSO control; $n \geq 3$. (b) The reference RXR agonist **1** is a potent activator of the RXR homodimer ($EC_{50} = 0.012 \pm 0.003$ µM) and the RXR:RAR heterodimer ($EC_{50} = 0.033 \pm 0.006$ µM). Data are the mean ± S.E.M. fold activation vs. DMSO control; $n \geq 3$. (c) **7** was selective for RXRs over other lipid-sensing nuclear receptors. Heatmap shows mean fold activation vs. DMSO control; $n \geq 3$. (d) In contrast to **1**, **7** did not inhibit proliferation of HT-29 colon cancer and MCF7 breast cancer cells. Data are the mean ± S.E.M., $n \geq 4$.

Preference for the RXR homodimer and selectivity over related lipid-activated nuclear receptors (Figure 2) distinguish **7** from the widely used RXR reference agonist bexarotene (**1**). **1** is a potent activator of the RXR homodimer and the RXR:RAR heterodimer with even higher efficacy on the heterodimer (Figure 2). The clinical anticancer effect of bexarotene (**1**) has been ascribed to RXR-mediated apoptosis induction but whether the molecular mechanism of this activity involves RXR homodimer or RXR:RAR heterodimer activation is debated.[19,20] When we compared the effects of **7** and **1** on cancer cell proliferation (Figure 2d), we detected no effect of **7** on proliferation of colorectal (HT-29) and breast (MCF7) cancer cells even at high concentrations suggesting that RXR homodimer activation is insufficient for the antiproliferative effects of rexinoids like **1** which thus rather require heterodimer activation. Selective RXR homodimer activation may have unprecedented biological effects and open new therapeutic opportunities of RXR modulation. The homodimer preference of **7** further highlights the potential of the valerenic acid scaffold for the development of a novel type of RXR modulators.

Table 2. Activities of **5**–**10** on RXR.[a]

ID	structure	EC_{50} [μM] (max. fold activation)[b] RXRα	RXRβ	RXRγ	RXR:RXR
2		27 ± 3 (9 ± 1)	5.2 ± 0.4 (69 ± 1)	43 ± 1 (4 ± 1)	6 ± 2 (1.7 ± 0.1)
5		> 200	> 200	> 200	106 ± 31 (1.5 ± 0.1)
6		inact. (200 μM)	inact. (200 μM)	inact. (200 μM)	inact. (200 μM)
7		89 ± 9 (7 ± 1)	125 ± 13 (21 ± 4)	127 ± 13 (10 ± 2)	6.3 ± 0.7 (4.3 ± 0.1)
8		> 200	> 200	> 200	52 ± 25 (1.4 ± 0.1)
9		inact. (200 μM)	inact. (200 μM)	inact. (200 μM)	inact. (200 μM)
10		inact. (200 μM)	inact. (200 μM)	inact. (200 μM)	inact. (200 μM)

[a] Activities on RXR subtypes were determined in Gal4-hybrid reporter gene assays; effects on the RXR homodimer were determined in a reporter gene assay based on DR1 and the full-length human RXR; data are the mean ± SD, n ≥ 3. Fold activation refers to the fold induction of reporter activity vs. control (0.1% DMSO treated cells). Inact. – no significant effect at the highest tested concentration as indicated. [b] Hybrid reporter gene assay data for **2** from Ref. [12].

Table 3. Binding affinities of **7** to the RXR LBDs determined by ITC.

	K_d(RXRα)	K_d(RXRβ)	K_d(RXRγ)
7	3.3 μM	3.5 μM	5.3 μM

Conclusions

The natural product valerenic acid (**2**) exhibits an appealing selective RXR modulator profile but has limited potency and weak homodimer activation efficacy disqualifying as a tool. Structural modification of **2** revealed a steep structure-activity relationship and differences for Gal4-RXR and RXR homodimer

activation. The valerenic acid derivative **7** emerged as improved selective RXR homodimer agonist and may serve as an early tool for *in vitro* studies. Our results highlight the potential of valerenic acid (**2**) for further optimization towards selective RXR modulators to open new therapeutic opportunities via fine-tuned RXR activation.

Supporting Information

The Supporting Information contains analytical data, synthetic procedures, *in vitro* assay methods, and computational procedures. Additional references are cited in the Supporting Information.[21–31]

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords: retinoid X receptor · transcription factor · natural product synthesis · neurodegeneration · cancer

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