



Research paper

Demethylgorgosterol-derived steroids as a new class of ROR γ -preferential inverse agonistsSavumiga Shanmugamani^{a,1}, Till Kasch^{b,1}, Nicolai Rosenbaum^a, Christoph W. Grathwol^c, Annkatrin Kuhn^a, Lisa Schmidt^a, Julian A. Marschner^{b,1}, Stefan Bräse^{a,c,1,*}^a Institute of Organic Chemistry (IOC), Karlsruhe Institute of Technology (KIT), Kaiserstraße 12, 76131, Karlsruhe, Germany^b Department of Pharmacy, Ludwig-Maximilians-University (LMU) Munich, Munich, Germany^c Institute of Biological and Chemical Systems, Functional Molecular Systems (IBCS-FMS), Karlsruhe Institute of Technology (KIT), Kaiserstraße 12, 76131, Karlsruhe, Germany

A B S T R A C T

23-Demethylgorgosterol is an unusual marine sterol scaffold characterized by an atypical 22,23-cyclopropyl-containing side chain and an uncommon sterol substitution pattern. Its recent access through an efficient semi-synthetic route enables systematic structural diversification. In this study, we completed the semi-synthesis and prepared the unnatural diastereomer together with a series of structurally diverse intermediates and derivatives. Based on their structural similarity to sterols and oxysterols, these compounds were evaluated for modulation of the ligand-activated nuclear receptors farnesoid X receptor (FXR), liver X receptor α (LXR α), and the retinoic acid receptor-related orphan receptors ROR α , ROR β , and ROR γ . While little or no activity was observed at FXR and LXR α , several derivatives displayed low-micromolar inverse agonism at ROR γ . Alcohol- and bromide-bearing derivatives showed a pronounced preference for ROR γ , whereas oxidation of the terminal alcohol to the corresponding carboxylic acid broadened activity across all three ROR isoforms, yielding a low-micromolar pan-ROR inverse agonist with a modest preference for ROR γ . These findings identify the terminal region of the cyclopropyl side chain as a key structural feature associated with ROR activity, efficacy, and isoform preference and establish demethylgorgosterol-derived structures as a natural product-inspired scaffold for the exploration of ROR γ -preferential and pan-ROR inverse agonists.

1. Introduction

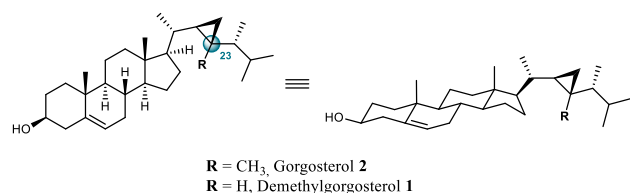
23-Demethylgorgosterol (1, Scheme 1) belongs to the class of gorgostanes, a group of marine sterols that are known for the presence of a cyclopropyl ring within the side chain at C-22 and C-23 [1], which was first isolated from the soft coral *Gorgonia flabellum* by SCHMITZ et al. [2] in 1970. Soft corals themselves are unable to synthesize sterols de novo [3]. Instead, they rely on endosymbiotic photosynthetically active algae belonging primarily to the Symbiodiniaceae family. These algae produce and transfer sterols, together with numerous other metabolites, including glycerol, glucose, various amino acids, fatty acids, and glycoconjugates, to the coral host. This metabolic exchange substantially contributes to the survival and growth of the host coral under nutrient-poor conditions [4]. It has been proposed that the exotic cyclopropyl moiety of gorgosterane sterols confers an evolutionary defensive advantage. Two hypotheses have been put forward: Firstly, the unusual cyclopropane structure makes the sterol resistant to the metabolic degradation by predators and thereby may offer passive

protection. Secondly, a more active defense mechanism has been debated including the formation of reactive cyclopropylcarbinyl cations, which lead to the inactivation of metabolic enzymes in predators [4a].

Beyond their ecological role, gorgostane sterols earned increasing attention due to their diverse biological activities, including anti-inflammatory [5] or antiproliferative [5] properties. In particular, gorgosterol (2), was reported by PUTRA et al. to act as an antagonist of farnesoid X receptor (FXR, NR1H4), a nuclear receptor (NR), which is highly expressed in liver, lungs and intestine [6]. FXR binds to and is activated by specific endogenous bile acid metabolites, particularly 3,7- and 3,12-substituted bile acids. It then induces the expression of genes that suppress cholesterol-7 α -hydroxylase (CYP7A1) expression, the rate-limiting enzyme in bile acid biosynthesis [7]. Accordingly, FXR agonists can prevent the accumulation of bile acids and have emerged as promising agents for the treatment of cholestasis [6,8]. Their clinical relevance is represented by obeticholic acid, which was the first FXR agonist approved for primary biliary cholangitis, although its US marketing authorization was recently withdrawn. [9]. While FXR responds

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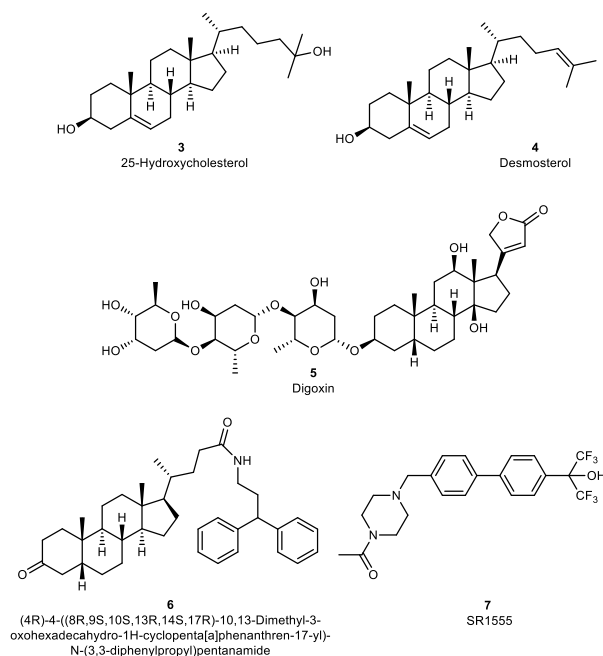
Scheme 1. Absolute configuration of gorgosterol (**2**) and demethylgorgosterol (**1**).

downstream to bile-acid products, the closely related liver X receptors (LXR α/β , NR1H3/2) respond upstream to cholesterol/oxysterol abundance and can promote cholesterol catabolism [7b]. Concurrently, it was reported that the structurally related 24(*S*)-saringosterol from *Sargassum fusiforme* acts as a selective LXR β agonist, inducing target gene expression in colon and liver epithelial, adrenal, and immune cell lines [10]. Although there are multiple tool compounds published [11], clinical application of LXR modulation is still pending. While FXR and LXR isoforms are phylogenetically within the same subfamily of nuclear receptors (NR1H) and govern target gene expression via dimerization with the retinoid X receptors (RXRs, NR2B), retinoic acid receptor-related orphan receptors (RORs, NR1F) act as monomers but are adjacent in terms of ligand chemistry. ROR α binds cholesterol and cholesterol sulfate structurally [12], and ROR α /ROR γ are modulated by oxysterols and cholesterol-biosynthesis intermediates, with ROR γ t linking sterol metabolism to immune cell differentiation [13], which all together makes ROR isoforms interesting targets for pharmaceutical intervention. Their natural function strongly depends on the specific isoform and localization. For example, the ROR β isoform is found in cells of the brain, retina and pineal gland where it is involved in the differentiation of specific neuronal cells and impacts circadian behavior and vision [14]. The other two isoforms, ROR α and ROR γ , however, are less exclusively distributed. ROR α is expressed in tissues like liver [15], skeletal muscle [15], kidney [15] and the brain [13], whereas ROR γ is predominantly found in the thymus (as ROR γ t isoform), but also in liver and kidney [16], skeletal muscles [17] and lymphoid organs [18]. Both ROR α and ROR γ play a key role in regulating the development of T helper 17 (Th17) cells. These cells produce pro-inflammatory cytokines, such as interleukin-17 (IL-17), which recruit neutrophils to the site of infection to fight bacteria and fungi. However, increased Th17 cell activation is also closely linked to the emergence of autoimmune diseases like Multiple Sclerosis and rheumatoid arthritis, while dysregulation of ROR- γ has been linked to metabolic disorders and certain cancers [19]. Although numerous natural and synthetic ligands of ROR γ have been identified, the development of selective and pharmacologically viable inverse agonists remains challenging. Early investigations focused primarily on endogenous sterols such as 25-hydroxycholesterol [20] (**3**) and desmosterol [21] (**4**), which act as agonists (see Scheme 2). However, these ligands showed only moderate potency and limited selectivity. Naturally occurring antagonists are rare. One of the few reported examples is the cardiac glycoside digoxin [22] (**5**), which inhibits ROR γ activity in the low micromolar range ($\text{IC}_{50} = 4.1 \mu\text{M}$). Due to its known cardiotoxicity, however, digoxin (**5**) is unsuitable for therapeutic use [23].

Subsequent research efforts have resulted in the development of inverse agonists that are both semi-synthetic and fully synthetic, with enhanced potency. These include 3-oxo-lithocholic acid derivatives [24] (**6**) and small-molecule scaffolds, such as SR1555 [25] (**7**).

Recent advancements have yielded highly potent nanomolar inhibitors with diverse chemical backbones [26], several of which have entered clinical development. However, the therapeutic translation of ROR γ modulation has remained challenging, considering both compound-specific properties and broader target related limitations [27].

Recently, we reported a convenient and high-yielding formal semi-



Scheme 2. Selected natural and (semi-)artificial ROR γ ligands.

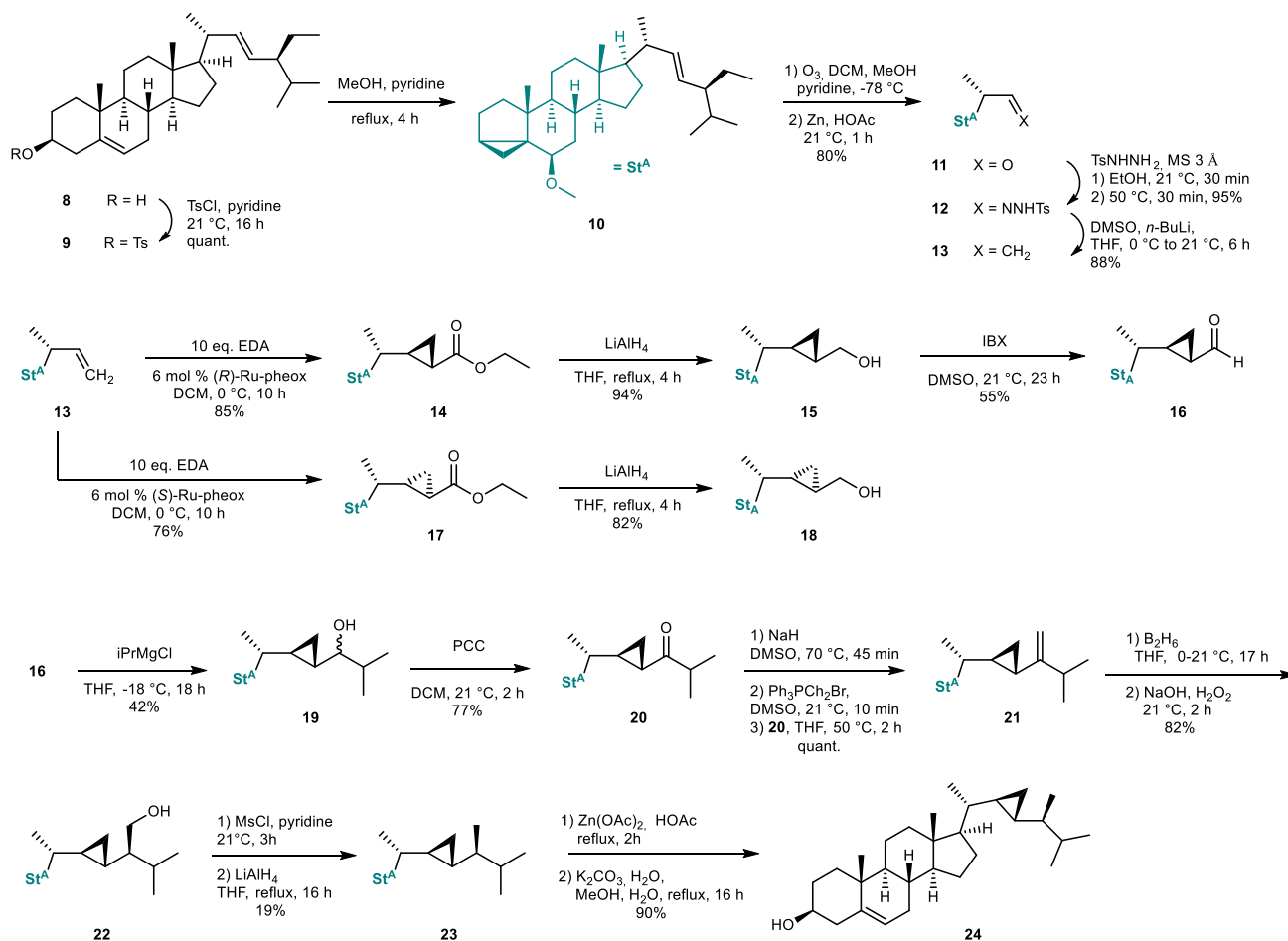
synthesis of 23-demethylgorgosterol (**1**) [28] providing access not only to the natural-product scaffold but also to structurally diverse intermediates and derivatives. Here, we exploit this synthetic route to investigate the nuclear-receptor pharmacology of the demethylgorgosterol chemotype. A focused series of compounds was profiled in cell-based reporter gene assays for the sterol-responsive receptors FXR, LXR α , ROR α , ROR β , and ROR γ . By linking systematic side-chain modifications to receptor activity, this study defines the nuclear-receptor selectivity and structure–activity relationships of the demethylgorgosterol scaffold and identifies chemically accessible, ROR γ -preferential inverse agonists as starting points for further ligand development.

2. Experimental

2.1. Semi-synthesis of 22*R*,23*R*,24*S*-Demethylgorgosterol

Following the route described by ROSENBAUM et al. [28], we initiated the semi-synthesis of demethylgorgosterol (**1**) with the commercially available compound stigmasterol (**8**). Ketone **20** was synthesized in ten steps with a yield of 23%, applying a ruthenium-catalyzed stereoselective intermolecular cyclopropanation reaction developed by IWASA et al. [29] for assembling the characteristic side chain cyclopropane motif. Through the use of the (*R*)-Ru-pheox catalyst, the 22*R*,23*S*-configuration was selectively obtained, providing ester **14** and alcohol **15** in optical purity (see Scheme 3).

The relative and absolute configuration of the cyclopropane-containing side chain is set exclusively in this cyclopropanation step and was unambiguously confirmed by single-crystal X-ray diffraction for the key intermediates **14–20** in our previous work [28]. As this configuration is not affected by any of the subsequent transformations, all downstream intermediates and derivatives possess the same cyclopropane configuration. The corresponding 22*S*,23*R*-configured diastereomers **17** and **18** were additionally prepared by employing the opposite enantiomer of the Ru-pheox catalyst, which provides defined and inverted configurations at both cyclopropane stereocenters [29]. Their diastereomeric relationship to **14** and **15** was confirmed by characteristic differences in their ^{13}C NMR chemical shifts and by their distinct optical rotations: **14** ($[\alpha] = +68.4^\circ$, $c = 0.56$, CHCl_3) and **17** ($[\alpha] = -3.9^\circ$, $c = 0.49$, CHCl_3); **15** ($[\alpha] = +44.0^\circ$, $c = 0.50$, CHCl_3) and



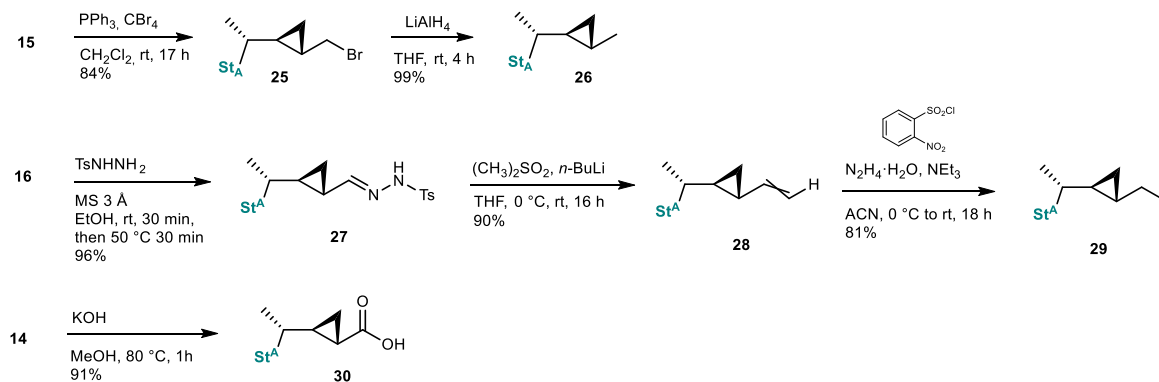
Scheme 3. Synthesis of 22R,23R,24S-demethylgorgosterol (**24**) starting from stigmasterol (**8**) in 3% overall yield, using a 14-step protocol, combining the methodologies described by ROSENBAUM et al.^[28] and DJERASSI et al.^[30] Compound **19** was obtained as a mixture of diastereomers; all other compounds were isolated as single diastereomers.

18 ([α] = +17.1°, c = 0.45, CHCl₃).

With ketone **20** in hand, we proceeded with the methodology reported by DJERASSI et al. [30] to access 22R,23R,24S-demethylgorgosterol (**24**) in a 14% yield over four additional steps. Starting from stigmasterol, the overall sequence comprised 14 steps and provided 22R,23R,24S-demethylgorgosterol in 3% total yield (see Scheme 3).

Unfortunately, the hydroboration-oxidation reaction afforded only the unnatural 22R,23S,24S-diastereomer **22** in optical purity. Although the desired 22R,23S,24R diastereomer was also formed, it could not be separated from **22**. The configuration at the newly formed C-24

stereocenter was assigned by analogy to the chromatographic behaviour described by DJERASSI et al., [30] where the isomer leading to the natural configuration shows the higher R_f value. As this less polar isomer could not be isolated in sufficient purity, biological evaluation was performed with the isolated 22R,23S,24S-diastereomer **22**; a direct assignment of the C-24 configuration by NOESY was not possible owing to extensive signal overlap.



Scheme 4. Synthesis of demethylgorgosterol side-chain derivatives **25–30** via bromination–reduction, JULIA-KOCIENSKI olefination, diimide reduction and ester saponification.

2.2. Synthesis of further derivatives for SAR exploration

To study structure-activity relationships (SAR) around the demethylgorgosterol scaffold, various analogues were synthesized based on the intermediates of the semi-synthesis. These retained the characteristic cyclopropane motif but varied in the adjacent alkyl region (see Scheme 4).

First, simpler hydrocarbon derivatives were synthesized, closely resembling the natural side chain. Alcohol **15** or aldehyde **16** served as starting materials. Defunctionalization of **15** to the methyl derivative **26** as the simplest hydrocarbon analogue was achieved in very good yield by conversion of **15** to the bromide **25** via Appel reaction and successive reductive debromination using lithium aluminium hydride. Further hydrocarbon derivative was obtained from aldehyde **16** via Julia-Kocienski-type olefination and diimide-based reduction of the C,C-double bond. Aldehyde **16** was first converted to the tosylhydrazone **27** and then reacted with dimethyl sulfone to yield alkenyl compound **28** (see Scheme 4).

For the successive reduction of the olefin **28**, transition metal-catalyzed hydrogenation was not applied, due to potential cleavage of the adjacent cyclopropane ring. Instead, diimide-based protocols were used, that yielded the alkyl compound **29** in very good yields.

In addition, the saponification of the ester derivative **14** with KOH provided the corresponding carboxylic acid **30** in excellent yield. This offers direct access to a polar reference compound within this series.

3. Results

3.1. Demethylgorgosterol derivatives preferentially modulate ROR γ but are inactive at FXR and LXR α

Marine sterols, including methylcholestanes and gorgostanes, have previously been reported to modulate FXR activity [6]. Based on these observations, the synthesized gorgostane analogues were screened for activity at the sterol- and bile-acid-responsive nuclear receptors FXR, LXR α , and ROR γ using Gal4-hybrid luciferase reporter gene assays. Unexpectedly, most derivatives showed no significant activity at FXR or LXR α , with reporter signals remaining close to those of the vehicle control (Supplementary Fig. S1). In contrast, several compounds reduced ROR γ -dependent reporter activity to approximately 0.4-fold of control, identifying ROR γ as the principal responsive receptor within the initial screening panel (Fig. 1).

To substantiate the ROR γ activity observed in the initial Gal4-hybrid screen, the compounds were evaluated in two complementary control experiments. First, all derivatives were reassessed in an orthogonal assay employing full-length human ROR γ and an RORE-driven luciferase reporter. Compounds showing inhibitory activity in the Gal4-hybrid assay reproduced this profile in the orthogonal reporter format (Fig. S2). Since compound-induced cytotoxicity could likewise reduce reporter output, compounds showing reduced ROR γ -dependent reporter activity were additionally assessed in a multiplex cytotoxicity assay at 10 μ M. No relevant cytotoxic effects were observed at this concentration (Supplementary Fig. S3).

The chain-shortened methylcyclopropyl analogue **26** was inactive at ROR γ , and extension of the hydrophobic alkyl chain, as in **31a**, did not restore activity. By contrast, introduction of a terminal hydroxymethyl group in compound **15** resulted in pronounced inverse agonism. The corresponding cyclopropyl stereoisomer **18** retained activity, indicating that this stereochemical alteration was tolerated. Replacement of the hydroxymethyl group with a bromomethyl substituent, as in **25**, also preserved inverse agonist activity, suggesting that an obligatory hydrogen-bond donor interaction at this position is unlikely.

Moderate enlargement and branching of the polar side chain were likewise tolerated. The branched alcohol **19** retained pronounced inverse agonist activity, while the corresponding ketone **20** remained active but displayed a less preferential profile in subsequent

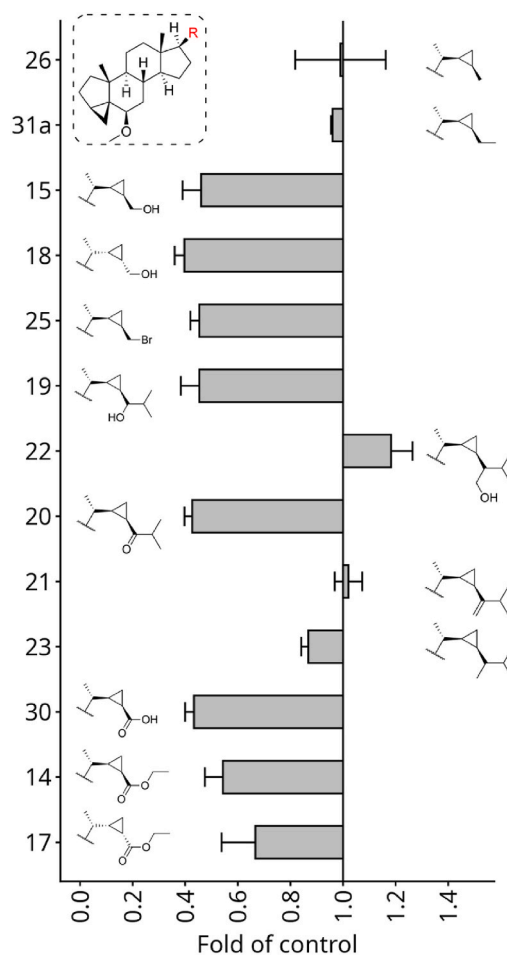


Fig. 1. Gorgosterol analogues carrying a polar head group displayed activity on ROR γ in a Gal4-hybrid reporter gene assay. Compounds were tested at 10 μ M in three independent experiments. Data are the mean $\pm$ S.E.M.

concentration–response experiments. Further extension to the 3-methylbutan-1-ol side chain of compound **22** produced a slight increase in ROR γ -dependent reporter activity in the initial screen. However, this apparent weak agonistic response was neither reproduced in the orthogonal RORE-based reporter assay nor in subsequent concentration–response experiments, in which compound **22** was inactive at all three ROR isoforms. The initial screening signal was therefore not considered evidence of agonistic receptor modulation.

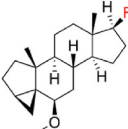
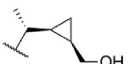
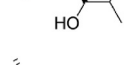
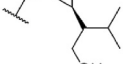
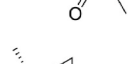
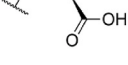
Oxidation of the terminal alcohol in **15** to the corresponding carboxylic acid **30** retained ROR γ inverse agonism. The related ethyl esters **14** and **17** were also active, although their fold changes at 10 μ M differed from that of the free acid. Taken together, the screening results demonstrate substantial tolerance toward chemically distinct terminal functionalities, including alcohol, ketone, bromide, carboxylic acid, and ester groups. Thus, ROR γ modulation by this series appears to depend less on a single defined hydrogen-bonding motif than on the overall geometry, polarity, and steric dimensions of the side chain. However, alternative explanations, including solvent-exposed or water-mediated interactions and differences in cellular exposure, cannot be distinguished using the reporter assay alone.

3.2. Hit validation and activity across the ROR isoforms

Activity in the single-concentration screening assays was evaluated using defined combined criteria incorporating both the magnitude of the reporter response and the strictly standardized mean difference (SSMD) relative to vehicle control. Hit classification additionally required

concordant inhibition in the orthogonal full-length ROR γ /ROR ϵ assay. Thus, SSMD was used as a measure of standardized effect size and reproducibility rather than as an isolated hit criterion (Supplementary Table S2; see Supporting Information for detailed classification criteria). Compounds qualifying as inhibitory or inconclusive hits in the initial screening were characterized in concentration–response experiments at ROR α , ROR β , and ROR γ using analogous Gal4-hybrid reporter assays (Table 1 and Supplementary Fig. S4). The validated active derivatives reduced ROR γ -dependent reporter activity in a concentration-dependent manner, with IC₅₀ values predominantly in the single-digit micromolar range and residual activities of approximately 20–50%, depending on the compound. Compound 22, which had produced a

Table 1
Potency and efficacy of selected gorgosterol analogues on ROR isoforms.

ID		IC ₅₀ [ROR α] μ M (min. act.) ^a	IC ₅₀ [ROR β] μ M (min. act.) ^a	IC ₅₀ [ROR γ] μ M (min. act.) ^a
15		inactive ^b	inactive ^b	6.0 $\pm$ 1.7 (0.2 $\pm$ 0.04)
18		inactive ^b	inactive ^b	8.8 $\pm$ 3.2 (0.37 $\pm$ 0.04)
25		inactive ^b	inactive ^b	8.0 $\pm$ 6.3 (0.38 $\pm$ 0.01)
19		inactive ^b	inactive ^b	4.52 $\pm$ 3.41 (0.32 $\pm$ 0.04)
22		inactive ^b	inactive ^b	inactive ^b
20		9.5 $\pm$ 0.7 (0.54 $\pm$ 0.05)	10.3 $\pm$ 0.6 (0.52 $\pm$ 0.04)	9.6 $\pm$ 0.8 (0.36 $\pm$ 0.06)
30		9.79 $\pm$ 0.65 (0.14 $\pm$ 0.03)	10.79 $\pm$ 0.79 (0.2 $\pm$ 0.04)	3.7 $\pm$ 0.6 (0.22 $\pm$ 0.02)
14		8.5 $\pm$ 3.0 (0.52 $\pm$ 0.1)	11.6 $\pm$ 1.9 (0.54 $\pm$ 0.13)	6.1 $\pm$ 2.6 (0.42 $\pm$ 0.11)
17		n.d. ^c (0.59 $\pm$ 0.06)	n.d. ^c (0.67 $\pm$ 0.07)	n.d. ^c (0.5 $\pm$ 0.06)

^a IC₅₀ values were determined in Gal4-hybrid reporter gene assays using four-parameter logistic regression. Minimal activity is reported as the fraction of reporter activity remaining relative to the vehicle-treated control. Data are mean $\pm$ SD; corresponding 95% confidence intervals and fitting parameters are provided in Supplementary Table S1. n $\geq$ 3 independent biological experiments, each performed in technical duplicates.

^b >50% remaining receptor activity at the highest concentration tested with no detectable dose-response-profile within the tested concentration range.^c The concentration–response relationship could not be fitted reliably within the tested concentration range. Higher concentrations could not be evaluated because of limited compound solubility.

slight increase in ROR γ -dependent reporter activity in the initial screen, showed no concentration-dependent activity at ROR α , ROR β , or ROR γ .

The alcohol derivatives 15, 18, and 19 and the bromide 25 showed a pronounced preference for ROR γ . These compounds did not reduce ROR α - or ROR β -dependent activity by more than 50% within the tested concentration range, whereas they inhibited ROR γ with IC₅₀ values of 4.5–8.8 μ M. As no reliable IC₅₀ values could be determined for ROR α or ROR β within the tested concentration range, numerical selectivity ratios were not calculated; ROR γ preference is therefore defined operationally by measurable concentration-dependent activity at ROR γ in the absence of corresponding activity at ROR α and ROR β . Compound 15 displayed the greatest efficacy within this subgroup, reducing residual ROR γ activity to 0.20 $\pm$ 0.04-fold of control. Its cyclopropyl stereoisomer 18 was slightly less potent and less efficacious, with an IC₅₀ of 8.8 $\pm$ 3.2 μ M and a residual activity of 0.37 $\pm$ 0.04. A similar preference for ROR γ was observed for compounds 19 and 25.

In contrast, ketone 20 displayed comparable micromolar potency at all three ROR isoforms. Although it reduced ROR γ activity more strongly than ROR α or ROR β activity, the similar IC₅₀ values indicate limited isoform selectivity. The carboxylic acid 30 was likewise active at all three receptors but retained a modest preference for ROR γ , with IC₅₀ values of 9.79 $\pm$ 0.65 μ M at ROR α , 10.79 $\pm$ 0.79 μ M at ROR β , and 3.7 $\pm$ 0.6 μ M at ROR γ . Compound 30 also produced substantial suppression of reporter activity across all three isoforms, with residual activities of 0.14–0.22-fold of control.

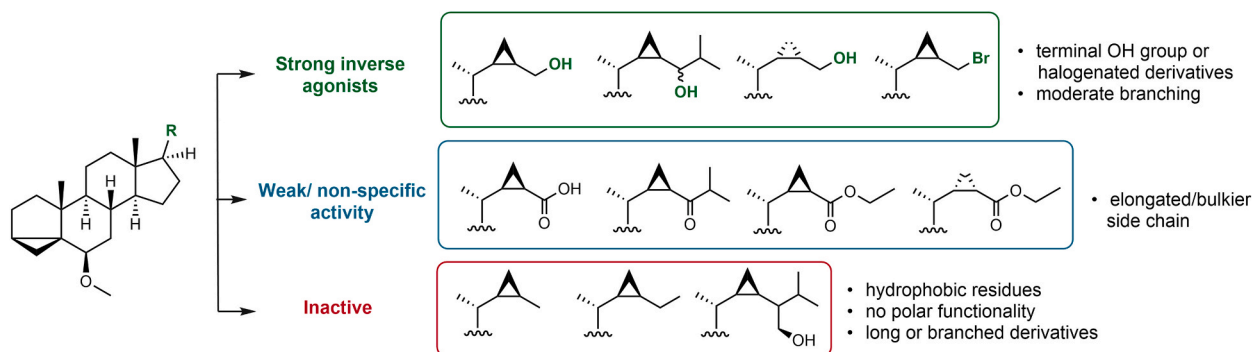
The corresponding ethyl ester 14 showed broadly similar IC₅₀ values but lower maximal efficacy, leaving 0.42–0.54-fold residual activity. This difference suggests that the ionization state, steric properties, or cellular disposition of the terminal group can affect efficacy without strongly altering apparent potency. However, the present cellular data do not establish whether the greater efficacy of 30 arises from a direct contribution of the carboxylate to receptor binding, altered intracellular exposure, or possible metabolism of the ester.

The stereoisomeric ester 17 was less efficacious than compound 14, with residual activities of 0.50–0.67-fold of control. Its concentration–response curves could not be fitted reliably within the experimentally accessible concentration range of up to 30 μ M. Higher concentrations could not be evaluated because of limited compound solubility, precluding reliable IC₅₀ determination. The modest differences between the stereoisomeric pairs 15/18 and 14/17 suggest that the stereochemical configuration of the cyclopropyl side chain influences activity but is not an absolute requirement for receptor modulation. These data are consistent with a stereochemically preferred presentation of the terminal substituent, although they do not by themselves demonstrate conformational hindrance.

To further assess whether the reporter-based activity translated into modulation of endogenous ROR signaling, compound 18 was selected as a representative ROR γ -active derivative and evaluated for its effect on interleukin-17 receptor A (IL17RA) [31], a reported ROR α / γ target gene, in MCF-7 cells. Compound 18 significantly reduced IL17RA expression, consistent with the response to the reference ROR inverse agonist SR1001. In contrast, the structurally related but reporter-inactive analogue 31a showed only a nonsignificant trend toward reduced IL17RA expression (Supplementary Fig. S5). Together, these data support translation of the reporter-based activity of 18 to an endogenous transcriptional readout.

3.3. Structure–activity relationships

The structure–activity relationships of the demethylgorgosterol series identify the cyclopropyl side chain, particularly its terminal region, as an important structural feature associated with cellular ROR γ modulation. The steroid core and cyclopropane motif alone were insufficient for activity, as demonstrated by the inactive, weakly functionalized analogues 26 and 31a. Instead, receptor modulation required an appropriately sized and positioned terminal substituent (Scheme 5).



Scheme 5. SAR summary figure.

Terminally functionalized derivatives generally displayed the strongest inverse agonist activity. The primary alcohol **15**, its stereoisomer **18**, and the branched alcohol **19** were preferentially active at ROR γ , indicating that moderate branching and variation in cyclopropyl stereochemistry are compatible with activity. However, retention of activity after replacement of the hydroxyl group by bromide, carboxylic acid, ester, or ketone shows that hydrogen-bond donation is not an essential requirement. The terminal functionality is therefore more appropriately interpreted as contributing to the physicochemical and steric properties associated with activity rather than as evidence for one obligatory receptor interaction.

The bromomethyl derivative **25** retained ROR γ -preferential inverse agonism, although with somewhat reduced efficacy compared with alcohol **15**. Oxidation to the carboxylic acid **30** broadened activity to ROR α and ROR β while maintaining a modest potency preference for ROR γ . Esterification, as in compound **14**, largely preserved potency but reduced efficacy, whereas the stereoisomeric ester **17** was weaker and did not reach a sufficiently complete response for reliable IC₅₀ determination. Together, these findings indicate that both the terminal functional group and its stereochemical presentation influence efficacy and isoform selectivity.

Side-chain dimensions also influenced receptor activity. Moderate branching, as in compound **19**, was compatible with ROR γ inverse agonism, whereas further elongation in compound **22** resulted in loss of activity. Although compound **22** produced a slight increase in ROR γ -dependent reporter activity in the initial screen, this observation was not confirmed in concentration–response experiments and therefore does not support a switch toward agonism. These findings suggest that increasing side-chain dimensions beyond a certain range is not compatible with activity in the present cellular assay system.

Overall, the data define a permissive but spatially constrained side-chain pharmacophore. A terminal polar or polarizable substituent is favourable for activity, whereas a specific hydrogen-bond donor is not required. Moderate side-chain branching and both cyclopropyl configurations are tolerated, although stereochemistry can modulate potency and efficacy. Excessive elongation, by contrast, can abolish activity. Within the present series, compounds **15**, **18**, **19**, and **25** combine low-micromolar potency with a pronounced preference for ROR γ , whereas compounds **20**, **30**, and **14** display broader activity across the ROR isoforms.

4. Conclusion

In this study, the nuclear-receptor pharmacology of the unusual demethylgorgosterol scaffold was systematically explored across FXR, LXR α , and the three ROR isoforms. Despite previous reports of FXR and LXR modulation by structurally related marine sterols, the investigated derivatives were largely inactive at these receptors and instead preferentially modulated ROR γ . Structure–activity analysis identified the terminal region of the cyclopropyl side chain as a major structural

feature associated with cellular ROR activity: appropriately positioned polar or polarizable substituents supported low-micromolar inverse agonism, whereas the steroid core and cyclopropane motif alone were insufficient. However, as the present conclusions are based on cell-based reporter assays, the data do not distinguish direct differences in receptor binding from effects on cellular exposure, intracellular disposition, or metabolism. These findings establish demethylgorgosterols as a distinct class of ROR modulators and provide initial structural principles linking the characteristic gorgostane side chain to receptor activity, isoform preference, and efficacy.

CRediT authorship contribution statement

Savumiga Shanmugamani: Writing – review & editing, Writing – original draft, Investigation. **Till Kasch:** Writing – review & editing, Validation, Investigation, Data curation. **Nicolai Rosenbaum:** Investigation. **Christoph W. Grathwol:** Writing – original draft, Supervision, Conceptualization. **Annkatriin Kuhn:** Investigation. **Lisa Schmidt:** Investigation. **Julian A. Marschner:** Writing – review & editing, Supervision. **Stefan Bräse:** Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejmech.2026.119391>.

Data availability

The Supporting Information covers detailed material on the conducted experiments and their results, including the characterization of the obtained compounds. Data that refer to the experiments described herein were submitted to the repository Chemotion (<https://www.chemotion-repository.net/>). All DOIs minted for the data are linked in the SI. New data obtained in this study are assigned to the collection

embargo numbers SAS_2024-08-26 (https://dx.doi.org/10.14272/collection/SAS_2024-08-26) [32] and NR_2021-08-17 (https://dx.doi.org/10.14272/collection/NR_2021-08-17) [33]. The material obtained in this study was submitted to the Molecule Archive at KIT and can be requested from there (<https://compound-platform.eu/home>).

SAS_2024-08-26, NR_2021-08-17 (Original data)

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