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Structure–activity relationship-guided development of retinoic acid receptor-related orphan receptor gamma (RORγ)-selective inverse agonists with a phenanthridin-6(5*H*)-one skeleton from a liver X receptor ligand

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ABSTRACT

Retinoic acid receptor-related orphan receptors (RORs), which belong to the nuclear receptor superfamily, regulate many physiological processes, including hepatic gluconeogenesis, lipid metabolism, immune function and circadian rhythm. Since RORs resemble liver X receptors (LXRs) in the fold structure of their ligand-binding domains, we speculated that ROR-mediated transcription might be modulated by LXR ligands, in line with the multi-template hypothesis. Therefore, we screened our LXR ligand library for compounds with ROR ligand activity and identified a novel ROR ligand with a phenanthridin-6(5*H*)-one skeleton. Structure–activity relationship studies aimed at separating ROR inverse agonistic activity from LXR-agonistic activity enabled us to develop a series of ROR inverse agonists based on the phenanthridin-6(5*H*)-one skeleton, including a RORγ-selective inverse agonist.

1. Introduction

Nuclear receptors have highly conserved DNA-binding and ligand-binding domains, and regulate DNA transcription by binding transcription factors such as hormones. However, many of these receptors are still classified as orphan receptors, since their natural ligands have not been identified. Retinoic acid receptor-related orphan receptor (ROR) is the one of these orphan receptors, and it is thought to be involved in immunity, cellular metabolism and circadian rhythm.[1] There are three subtypes, RORα, RORβ and RORγ, which are generated by alternative splicing or alternative transcriptional initiation.[1] These subtypes have different tissue expression patterns and different functions. RORα is highly expressed in brain, particularly cerebellum and thalamus, and it has been reported that RORα-deficient mice exhibit ataxia and severe cerebellar atrophy.[2] RORβ is highly expressed in the central nervous system, especially brain and retina,[3] and is thought to be involved in the processing of sensory information,[1] although its precise function is not yet fully established. RORγ has two different isoforms, RORγ1 and RORγt (RORγ2). RORγ1 is expressed in liver, adipose tissue, kidney, small intestines, and skeletal muscle.[4] Recent studies indicate that RORγ1 is related to insulin resistance, and the loss or potentially the inhibition of RORγ1 might protect against insulin resistance and type 2 diabetes.[4] On the other hand, RORγt is exclusively expressed in cells of the immune system, and is considered to play critical roles in differentiation of Th17 cells and secretion of inflammatory cytokines such as IL-17.[5–7] Th17 cells have been identified as key mediators of immune responses in autoimmune diseases such as multiple sclerosis (MS), Crohn's disease and rheumatoid arthritis (RA). Therefore, novel inverse agonists targeting RORγ are considered to be candidates for treatment of autoimmune diseases and diabetes.

Several studies have been directed at development of ROR inverse agonists. The synthetic liver X receptor (LXR) agonist T0901317 was the first reported inverse agonist for both RORα and RORγ,[8] but T0901317 also binds to several other nuclear receptors.[9] Development of this compound led to a variety of selective ROR ligands, such as RORα/γ-selective inverse agonist SR1001,[10] RORα-selective inverse agonist SR3335,[11] and RORγ-selective modulator SR2211.[12] Other compounds such as SR9805,[13] and digoxin[14] were also identified as ROR-specific inverse agonists

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(Fig. 1). Nevertheless, structure–activity relationships (SAR) of ROR ligands are generally not well established. However, a recent SAR study of compounds derived from high-throughput screening of the GSK in-house compound library led to the discovery of potent indole-based RORγt inverse agonists.[15]

Here, to obtain highly subtype-specific ROR ligands, we focused on screening LXR ligands for ROR activity, and then set out to separate ROR inverse agonistic activity from LXR agonistic activity. We describe here the identification and SAR study of ROR inverse agonists based on the phenanthridin-6(5*H*)-one skeleton, and the development of ROR subtype-specific ligands.

2. Results and discussion

2.1. Identification of lead compound

The multi-template approach to drug discovery is based on the fact that the number of three-dimensional spatial structures (fold structures) of human proteins is at least 50 times smaller than the number of human proteins (50,000–70,000).[16] Therefore, in principle, a template/scaffold structure that is spatially complementary to onefold structure might serve as a multi-template for structural development of ligands that would interact specifically with more than 50 different human proteins, neglecting physical/chemical interactions. In other words, the structures of ligands that bind to one member of a class of fold structures may be used for the development of novel lead compounds for other members of the same fold structure class. However, lead compounds obtained on the basis of this approach are likely to possess polypharmacological character. Therefore, structural modification of polypharmacological lead compounds, aimed at optimizing selectivity for the particular target, is required. Based on the fact that the fold structures of nuclear receptor ligand-binding domains (LBDs) are similar, we have used the multi-template approach to create ligands for several nuclear receptors, including farnesoid X receptor,[17] LXR,[18] vitamin D receptor,[19,20] androgen receptor,[21] and estrogen receptor.[22] We have also reported improvement of selectivity versus other target proteins.[18,21,22] Based on those results, we hypothesized that ROR ligands would be found among ligands of other nuclear receptors possessing similar fold structure to ROR.

Our group has developed LXR ligands based on the phenanthridin-6(5*H*)-one scaffold, which is a cyclized carba-analog of T0901317 (Fig. 2).[23] As shown in Figure 3, RORs are similar to LXR in the fold structure of the LBD. In addition, LXR agonist T0901317 binds to RORα and RORγ. Therefore, we hypothesized that our LXR ligands might bind to the T0901317-binding pocket of ROR, and would be candidate ligands for RORs. To test this idea, we used a full-length receptor linked to an ROR response element (RORE) driving luciferase gene expression. Screening of our compound library[23–25] identified compound **1** (Fig. 2) as a weak inverse agonist of ROR (IC_{50} values: RORα; 9.9 μM, RORβ; 10.1 μM, RORγ; 9.0 μM). For comparison, the IC_{50} values of T0901317 were RORα; >20 μM, RORγ; 6.5 μM. Next, we examined the effects of *N*-substituents. The ROR transcriptional activity of a series of *N*-alkylated derivatives was measured by luciferase reporter gene assay.

As shown in Table 1, introduction of a longer-chain alkyl group on the nitrogen atom resulted in enhancement of ROR inverse agonistic activity. However, the longest alkyl analog (**2j**) showed decreased activity, suggesting that the binding pocket hosting the N-alkylated derivatives might not be able to accommodate a group larger than a hexyl group. In this series, compound **2h** showed the most potent effect on RORγ activity, and therefore we selected the *N*-butylphenanthridinone skeleton (**2h**) as a lead structure for further structural development studies.

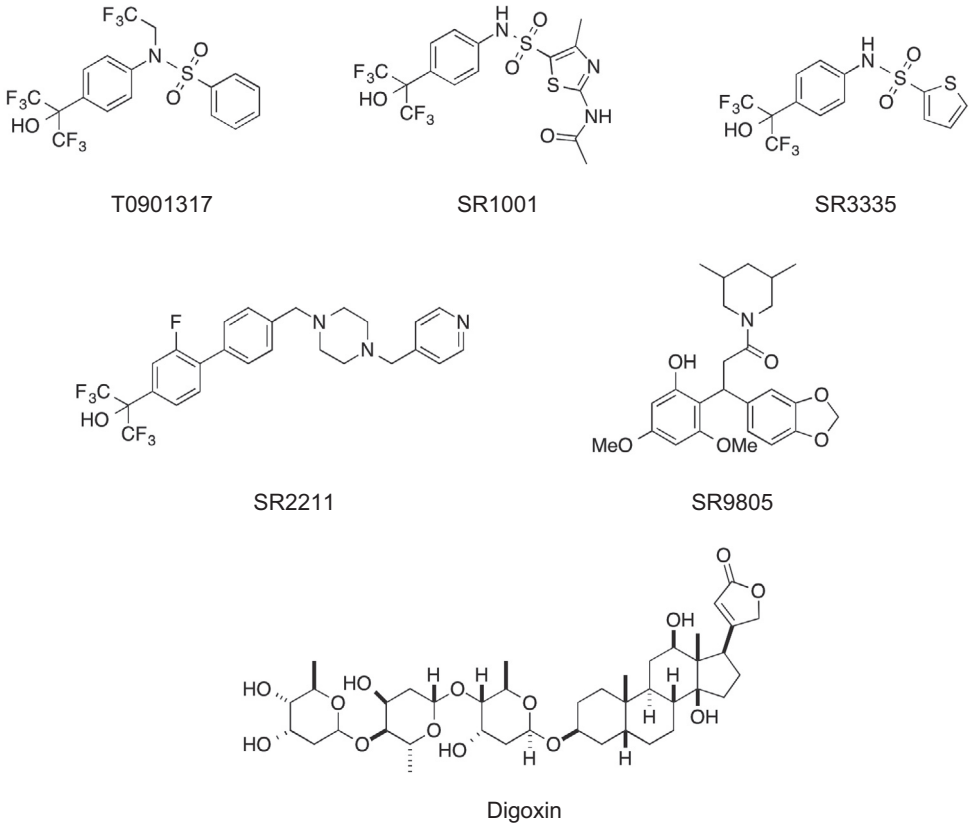


Figure 1. Chemical structure of reported ROR inverse agonists.

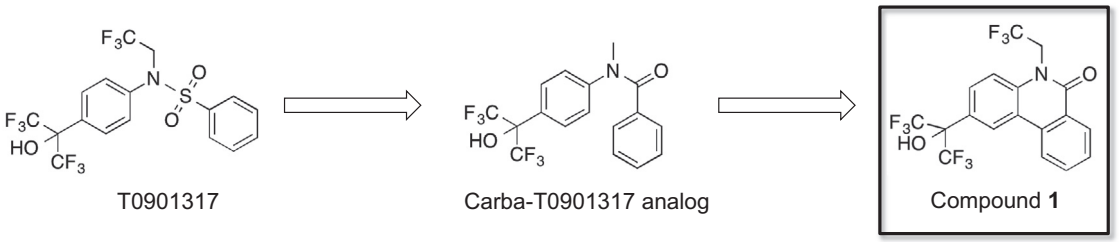


Figure 2. Structural development of candidate ROR ligands.

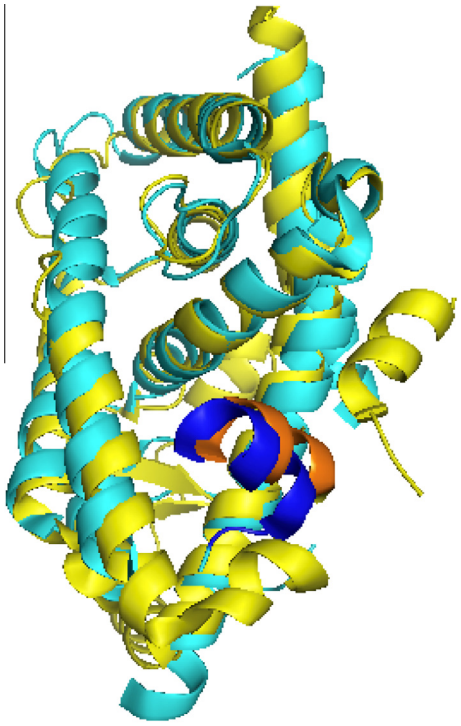


Figure 3. Superposition of X-ray crystal structures of the LBDs of RORγ (yellow, PDB ID: 3KYT) and LXRβ (cyan, PDB ID: 1PQC). Orange represents H12 of RORγ and blue represents H12 of LXRβ.

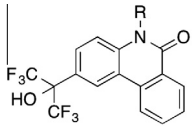
2.2. Synthesis and evaluation of 2-substituted phenanthridin-6(5*H*)-one analogues

Next, we examined whether a 1,1,1,3,3,3-hexafluoro-2-hydroxypropyl group at the 2-position is essential for inverse agonistic activity towards ROR. We prepared various compounds in which the hexafluoroisopropanol moiety was replaced with an alkyl group, as shown in Scheme 1. Briefly, *p*-alkyl anilines **6** were condensed with *o*-Br/I benzoic chloride. After introduction of an *n*-butyl group on the nitrogen atom, intramolecular cyclization of **8a**–**h** proceeded smoothly with $Pd(OAc)_2$–$PCy_3{\cdot}HBF_4$–Cs_2CO_3/K_2CO_3 as the coupling reagent[24] to afford **3a**,**c**–**i**. Compound **3b** was obtained by reduction of **3a**.

The synthesized compound **3b** showed weak inhibitory activity, suggesting that the ditrifluoromethyl group might be associated with more potent repressive activity and the hydroxyl group might not form a strong hydrogen bond with histidine in RORγ, in contrast to the case of LXRα and LXRβ (Table 2). This idea is consistent with the reported co-crystal structure of T0901317 complexed with RORγ.[26] The analogues **3c**–**g** showed moderate ROR inverse agonistic activities, whereas an analog with a large group at the 2-position, that is, 2-*n*-dodecyl analog **3i**, was inactive.

Although compound **3e** exhibited strong inverse agonistic activity towards ROR in this series of compounds (IC_{50} values: RORα; 9.0 μM, RORβ; 7.9 μM, RORγ; 9.2 μM), the activity toward RORγ was reduced by half as compared to that of the lead compound **2h**. These results indicated that the bulky hexafluoroisopropanol moiety is important for ROR ligand recognition.

Table 1
Inverse agonistic activities of phenanthridin-6(5*H*)-one analogues **1** and **2a**–**j**

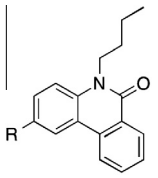


Compound	R	IC_{50} (μM)		
		RORα	RORβ	RORγ
T0901317	—	>20	NA	6.5
1	CH_2CF_3	9.9	10.1	9.0
2a	CH_2-*c*-Pr	13.6	11.8	9.3
2b	CH_2-*c*-Hex	10.3	8.8	5.8
2c	H	>20	>20	>20
2d	Me	>20	>20	>20
2e	Et	>20	>20	>20
2f	*i*-Pr	12.3	9.7	6.0
2g	*n*-Pr	10.8	8.2	4.2
2h	*n*-Bu	9.8	6.5	3.9
2i	*n*-C_6H_{13}	7.6	5.4	4.7
2j	*n*-C_9H_{19}	>20	>20	>20

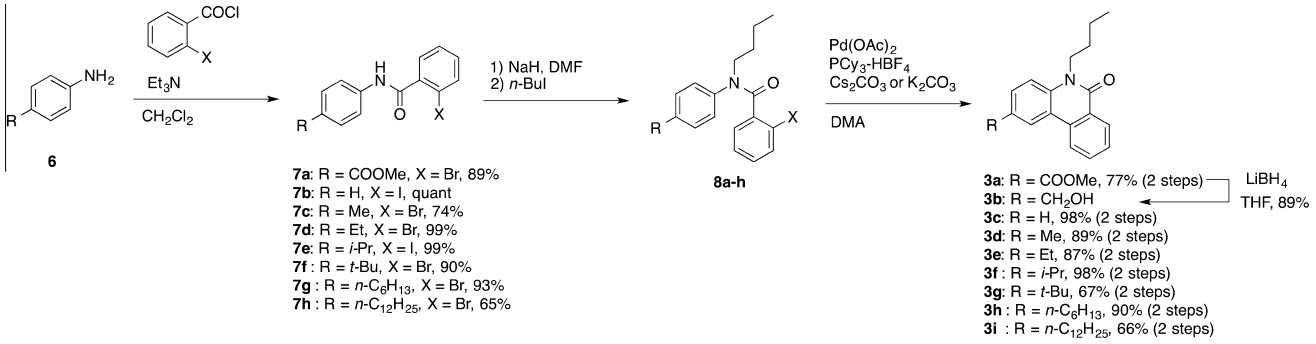
2.3. Synthesis and evaluation of monomethoxy-substituted phenanthridin-6(5*H*)-one analogues

Based on the evaluation of the lead compound, we next investigated the effect of methoxy substitution and synthesized regioisomers of methoxy-substituted phenanthridin-6(5*H*)-one analogues

Table 2
Inverse agonistic activities of phenathridin-6(5*H*)-one analogues **2h** and **3b**–**i**



Compound	R	IC_{50} (μM)		
		RORα	RORβ	RORγ
2h	$C(CF_3)_2(OH)$	9.8	6.5	3.9
3b	CH_2OH	>20	>20	>20
3c	H	10.2	13.4	14.5
3d	Me	17.6	17.1	16.8
3e	Et	9.0	7.9	9.2
3f	*i*-Pr	16.7	17.0	11.9
3g	*t*-Bu	11.8	12.8	10.4
3h	*n*-C_6H_{13}	10.3	11.3	13.0
3i	*n*-$C_{12}H_{25}$	NA	NA	NA

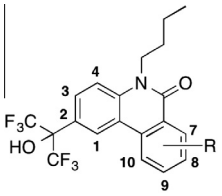


Scheme 1.

4a–**f** as shown in Scheme 2. Briefly, *N*-butylamine derivatives **9a**–**c**, which are commercially available anilines, were treated with hexafluoroacetone sesquihydrate to afford hexafluoropropanol derivatives **10a**–**c**. They were condensed with 2-iodobenzoyl chloride or regioisomers of methoxyl-substituted 2-bromobenzoic acid and the resulting anilides **11a**,**b** and **12a**–**d** were cyclized in the presence of Pd catalyst to give compounds **4a**–**f**.

The effect of methoxylation on the ROR inverse agonistic activity seemed to depend on the position at which the methoxy group is introduced. Introduction of a methoxy group at the 3-, 9- or 10-position repressed ROR transcriptional activities (**4a**, **4e**, and **4f**, respectively) (Table 3). Compounds **4c** and **4d**, with a monomethoxy group at the 7- and 8-position, respectively, were cytotoxic at 20 μM. Interestingly, **4e** showed 5-fold higher activity towards RORγ than RORα (IC_{50} values: RORα; 5.3 μM, RORγ; 1.1 μM).

Table 3
Inverse agonistic activities of phenanthridin-6(5*H*)-one analogues **2h** and **4a**–**f**

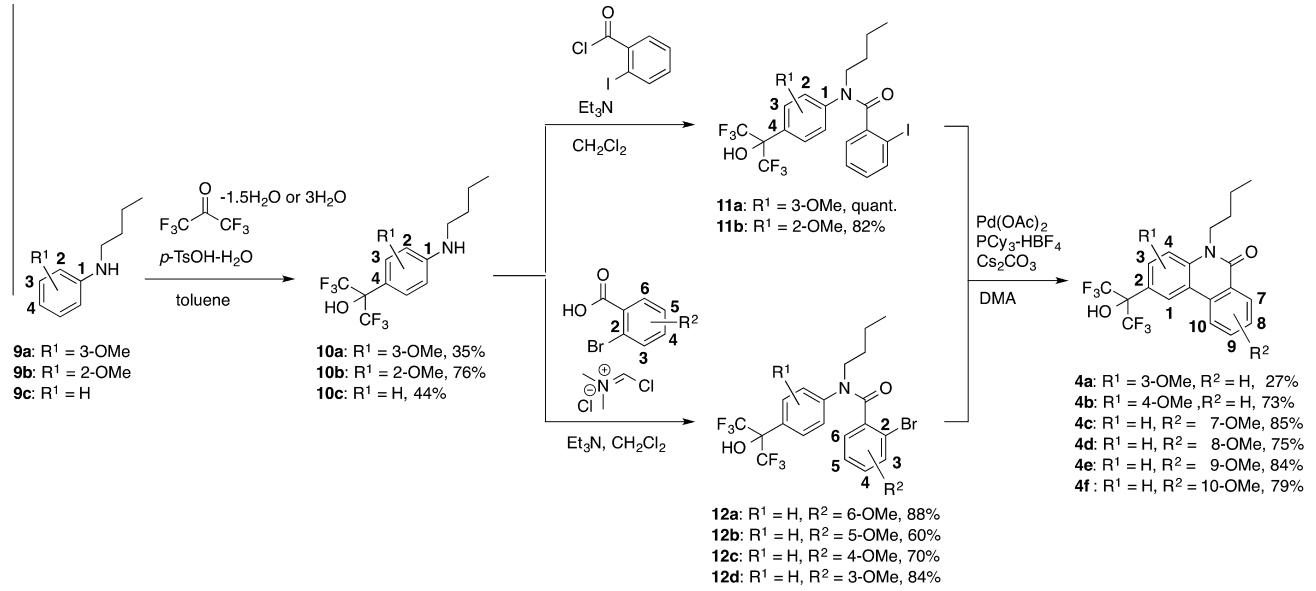


Compound	R	IC_{50} (μM)		
		RORα	RORβ	RORγ
2h	–	9.8	6.5	3.9
4a	3-OMe	7.8	10.9	9.2
4b	4-OMe	>10	>10	>10
4c	7-OMe	Toxic	Toxic	Toxic
4d	8-OMe	Toxic	Toxic	Toxic
4e	9-OMe	5.3	7.7	1.1
4f	10-OMe	9.1	7.5	5.7

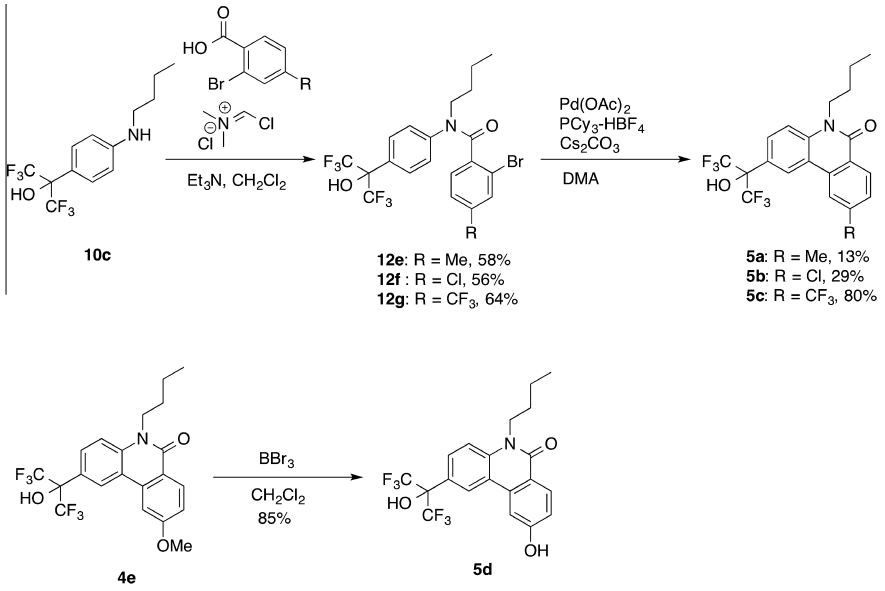
2.4. Synthesis and evaluation of 9-substituted phenanthridin-6(5*H*)-one analogues

Since a methoxy group at the 9-position provided selectivity for RORγ, we next synthesized 9-substituted phenathridin-6(5*H*)-one analogues **5a**–**d** to investigate the effects of substituent bulkiness and electronic factors (Scheme 3). Briefly, **10c** was condensed with 2-bromo-4-substituted benzoic acids and the products were cyclized by using the same methods as in Schemes 1 and 2. As 2-bromo-4-(trifluoromethyl)benzoic acid is not commercially available, we prepared it from 2-amino-4-(trifluoromethyl)benzoic acid by means of the Sandmeyer reaction, as reported.[27]

As shown in Table 4, introduction of a hydroxyl group at the 9-position (**5d**) reduced the inverse agonistic effect on RORγ, whereas introduction of a chloro group (**5b**) enhanced it. In

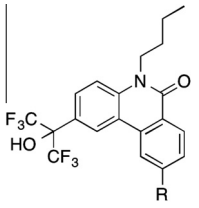


Scheme 2.



Scheme 3.

Table 4
Inverse agonistic activities of phenanthridin-6(5*H*)-one analogues **4e** and **5a–d**



Compound	R	IC_{50} (μM)		
		RORα	RORβ	RORγ
4e	OMe	5.3	7.7	1.1
5a	Me	7.6	7.1	1.0
5b	Cl	5.6	4.9	0.69
5c	CF_3	5.5	5.6	2.6
5d	OH	6.4	5.5	4.1

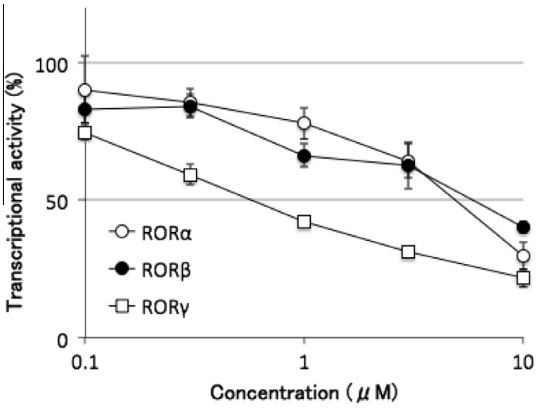


Figure 4. Compound **5b** inhibited ROR transcriptional activities in a dose-dependent manner.

addition, **5b** was highly selective for RORγ over RORα (**4e**: 5-fold, **5b**: 8-fold). Introduction of a methyl group (**5a**) or a trifluoromethyl group (**5c**) did not enhance the inverse agonistic effect. These results suggest that RORγ might contain a binding pocket for the 9-chloro group on the phenanthridin-6(5*H*)-one scaffold, resulting in high selectivity for RORγ (see Fig. 4).

2.5. Selectivity for LXRs

Since our compounds were originally developed from LXR ligands, compound **5b** might possess LXR agonistic activity. Therefore, we investigated the LXR-agonistic activity using a reporter gene assay as previously reported.[28] As shown in Figure 5, we confirmed that T0901317 showed agonistic activity toward both LXRα and LXRβ. However, **5b** exhibited no agonistic effect towards either of these receptors, like RORα/γ inverse agonist SR1001. On the other hand, compound **5b** also exhibited potent antagonistic activity, with IC_{50} value for LXRβ of 0.68 μM (Table 5). For these reasons, although compound **5b** has LXR antagonistic activity, we obtained an RORγ-selective inverse agonist that lacks LXR agonistic activity.

3. Conclusion

Based on the similarity of the fold structures of LXR and ROR, we speculated that our LXR ligand library might contain ROR ligands. Screening assay identified compound **1** as an ROR pan inverse agonist. Then, SAR study enabled us to develop a series of nuclear receptor ROR inverse agonists based on the phenanthridin-6(5*H*)-one skeleton. Among them, compound **5b** was the most potent ROR inverse agonist. Further, it showed RORγ selectivity and lacked LXR-agonistic activity, as determined by luciferase reporter gene assay. Further optimization of the structure of this compound should provide useful tools for elucidation of RORγ function and candidate agents for treatment of insulin resistance and type 2 diabetes.

4. Experimental section

4.1. Biology

4.1.1. Cell culture condition

Human embryonic kidney (HEK) 293 cells were cultured in DMEM supplemented with 5% FBS at 37 °C in a humidified atmosphere containing 5% CO_2.

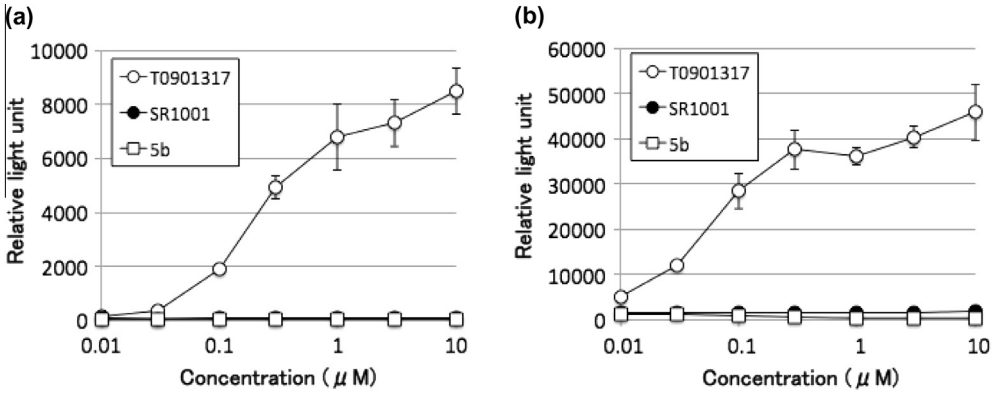


Figure 5. Compound **5b** showed no LXR-agonistic activity. (a) LXRα agonist assay. (b) LXRβ agonist assay.

Table 5
Agonistic activity (EC_{50}) and antagonistic activity (IC_{50}) of T0901317, SR1001 and compound **5b** on LXRs

Compound	LXRα		LXRβ	
	EC_{50} (μM)	IC_{50} (μM)	EC_{50} (μM)	IC_{50} (μM)
T0901317	0.29	NA	0.12	NA
SR1001	NA	NA	NA	NA
5b	NA	>3.0	NA	0.68

4.1.2. ROR inverse agonistic activity assay

HEK293 cells were seeded in 96-well culture plates (1.0×10^4 cells per well) the day before transfection. Each cell was co-transfected with 30 ng of a nuclear receptor expression plasmid (pcDNA3.1(−)-hRORα1; accession no. NM_134261, pcDNA3.1(−)-hRORβ; accession no. NM_006914, or pcDNA3.1(−)-hRORγ1; accession no. NM_005060), 50 ng of a luciferase reporter (RORE-TK-Luc) and 10 ng of CMX-β-galactosidase expression vector. Transfections were performed according to the calcium phosphate precipitation method. At 8 h post-transfection, the cells were treated with dimethyl sulfoxide (DMSO) or compounds for 16 h. Treated cells were assayed for luciferase activity in a luminometer. The luciferase activity of each well was normalized by the level of β-galactosidase activity. Each sample was carried out in triplicate and the experiments were repeated at least three times.

4.1.3. LXR agonistic/antagonistic activity assay

HEK293 cells were seeded in 96-well culture plates (1.0×10^4 cells per well) the day before transfection. Each cell was co-transfected with 30 ng of a nuclear receptor expression plasmid (CMX-GAL4N hLXRα, or CMX-Gal4N hLXRβ), 50 ng of a luciferase reporter (TK-MH100x4-Luc) and 10 ng of CMX-β-galactosidase expression vector. Transfections were performed according to the calcium phosphate precipitation method. At 24 h post-transfection, the cells were treated with dimethyl sulfoxide (DMSO) or compounds for 16 h. For antagonistic activity assay, cells were treated test compound in the presence of T0901317 (for LXRα; 0.30 μM, for LXRβ; 0.10 μM). Treated cells were assayed for luciferase activity in a luminometer. The luciferase activity of each well was normalized by the level of β-galactosidase activity. Each sample was carried out in triplicate.

4.2. Chemistry

4.2.1. General comments

Melting points were determined on a Yanagimoto hot-stage melting point apparatus, without correction. ^{1}H NMR spectra were recorded on a JEOL JNM-GX500 or JNM-ECA500 (500 MHz) spectrometer. Chemical shifts are expressed in δ (ppm) values, with tetramethylsilane (TMS) as an internal reference. Fast atom bombardment mass spectra (FAB-MS) and high-resolution mass spectra (HRMS) were recorded on a JEOL JMS-HX110 spectrometer with *m*-nitrobenzyl alcohol as the matrix.

4.2.2. General procedure A: synthesis of phenylhexafluoropropanol analogues

4.2.2.1. 2-(4-Butylamino-2-methoxyphenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol (10a). To a solution of hexafluoroacetone trihydrate (707 mg, 3.21 mmol) in toluene (5.00 mL) were added *N*-butyl-3-methoxyaniline (**9a**) (384 mg, 2.14 mmol) and *p*-TsOH-H_2O (81.5 mg, 428 μmol), and the mixture was stirred at 120 °C for 10 h. After cooling to room temperature, the mixture was diluted with ethyl acetate, washed with satd $NaHCO_3$ aqueous solution and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane/AcOEt = 1:0–2:1) to give **10a** (258 mg, 0.749 mmol, 35%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38 (s, 1H), 7.27 (d, 1H, *J* = 8.5 Hz), 6.26 (dd, 1H, *J* = 8.5, 2.4 Hz), 6.18 (d, 1H, *J* = 2.4 Hz), 4.15 (br s, 1H), 3.91 (s, 3H), 3.12 (m, 2H), 1.61 (tt, 2H, *J* = 7.3, 7.3 Hz), 1.43 (tq, 2H, *J* = 7.3, 7.3 Hz), 0.97 (t, 3H, *J* = 7.3 Hz); MS (FAB) *m*/*z* 346 $(M+H)^+$.

4.2.2.2. 2-(4-Butylamino-3-methoxyphenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol (10b). Prepared from *N*-butyl-2-methoxyaniline (**9b**) in accordance with general procedure A. Colorless oil (76%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (d,1H, *J* = 8.5 Hz), 7.05 (s, 1H), 6.58 (d, 1H, *J* = 8.5 Hz), 4.36 (br s, 1H), 3.85 (s, 3H), 3.14 (t, 2H, *J* = 7.3 Hz), 1.64 (tt, 2H, *J* = 7.3, 7.3 Hz), 1.44 (tq, 2H, *J* = 7.3, 7.3 Hz), 0.98 (t, 3H, *J* = 7.3 Hz); MS (FAB) *m*/*z* 376 $(M+H)^+$.

4.2.2.3. 2-(4-(Butylamino)phenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol (10c). Prepared from *N*-butylaniline (**9c**) using a slight modification of general procedure A. To a solution of hexafluoroacetone sesquihydrate (1.16 g, 3.00 mmol) and *N*-butylaniline (**8c**) (448 mg, 3.00 mmol) in toluene (20.0 mL) was added *p*-TsOH-H_2O (62.1 mg, 0.360 mmol), and the mixture was stirred at 100 °C for 3 h. After cooling to room temperature, the mixture was diluted with ethyl acetate, washed with satd $NaHCO_3$ aqueous solution and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane/AcOEt = 10:1) to give **10c** (414 mg, 1.31 mmol, 44%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (d, 2H, *J* = 8.0 Hz), 6.54 (d, 2H, *J* = 9.2 Hz), 3.05 (t, 2H, *J* = *J* = 7.2 Hz), 1.57–1.51 (m, 2H), 1.37–1.34 (m, 2H), 0.89 (t, 3H, *J* = 7.4 Hz).

4.2.3. General procedure B: synthesis of benzamide analogues 7 and 11

4.2.3.1. *N*-(4-Acetylphenyl)-2-bromobenzamide (7a). To a solution of 4-aminoacetophenone (41.0 mg, 0.300 mmol) in CH_2Cl_2 (5.00 mL) were added dropwise 2-bromobenzoyl chloride (72.4 mg, 0.333 mmol) and Et_3N (82.5 μl, 750 μmol) at 0 °C, and the mixture was stirred for 3 h, then allowed to warm to room temperature. After cooling to 0 °C, the mixture was diluted with ethyl acetate and water was added. The organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane/AcOEt = 1:0–1:1) to give **7a** (85.0 mg, 0.270 mmol, 89%) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.07 (d, 1H, J = 8.5 Hz), 7.83 (br s, 1H), 7.73 (d, 2H, J = 8.5 Hz), 7.68 (d, 1H, J = 7.5 Hz), 7.66 (d, 1H, J = 7.5 Hz), 7.44 (t, 1H, J = 7.5 Hz), 7.36 (td, 1H, J = 7.5, 1.5 Hz), 3.92 (s, 3H).

4.2.3.2. 2-Iodo-*N*-phenylbenzamide (7b). Prepared from aniline and 2-iodobenzoyl chloride in accordance with general procedure B. White solid (99%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.90 (d, 1H, J = 7.9 Hz), 7.62 (d, 2H, J = 7.3 Hz), 7.52 (dd, 1H, J = 8.0, 1.9 Hz), 7.40 (m, 4H), 7.15 (m, 2H).

4.2.3.3. 2-Bromo-*N*-*p*-tolylbenzamide (7c). Prepared from *p*-toluidinein accordance with general procedure B. White solid (74%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.66 (dd, 1H, J = 7.6, 1.8 Hz), 7.64 (d, 1H, J = 7.6 Hz), 7.58 (br s, 1H), 7.53 (d, 1H, J = 8.0 Hz), 7.42 (t, 1H, J = 7.6 Hz), 7.33 (td, 1H, J = 7.6, 1.8 Hz), 7.19 (d, 2H, J = 8.0 Hz), 2.35 (s, 3H); MS (FAB) m/z 290, 292 $(M+H)^+$.

4.2.3.4. 2-Bromo-*N*-(4-ethylphenyl)benzamide (7d). Prepared from *p*-ethylaniline in accordance with general procedure B. White solid (99%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (ddd, 2H, J = 10.0, 8.0, 1.5 Hz), 7.59 (br s, 1H), 7.55 (d, 2H, J = 8.0 Hz), 7.42 (td, 1H, J = 8.0, 1.5 Hz), 7.32 (td, 1H, J = 8.0, 1.5 Hz), 7.22 (d, 2H, J = 8.0 Hz), 2.65 (q, 2H, J = 7.5 Hz), 1.24 (t, 3H, J = 7.5 Hz); MS (FAB) m/z 304, 306 $(M+H)^+$.

4.2.3.5. 2-Iodo-*N*-(4-isopropylphenyl)benzamide (7e). Prepared from 4-isopropylaniline and 2-iodobenzoyl chloride in accordance with general procedure B. White solid (99%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 (d, 1H, J = 8.0 Hz), 7.54 (d, 2H, J = 8.6 Hz), 7.51 (dd, 1H, J = 5.0, 1.9 Hz), 7.41 (td, 1H, J = 7.3, 1.3 Hz), 7.23 (d, 2H, J = 8.5 Hz), 7.13 (td, 1H, J = 7.9, 1.9 Hz), 2.89 (sep, 1H, J = 7.3 Hz), 1.23 (d, 6H, J = 6.7 Hz).

4.2.3.6. 2-Bromo-*N*-(4-*tert*-butylphenyl)benzamide (7f) Prepared from 4-*tert*-butylaniline in accordance with general procedure B. Colorless oil (90%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (t, 2H, J = 8.0 Hz), 7.59 (br s, 1H), 7.57 (d, 2H, J = 8.0 Hz), 7.42 (t 1H, J = 8.0 Hz), 7.41 (d, 2H, J = 8.0 Hz), 7.33 (td, 1H, J = 8.0, 1.8 Hz), 1.33 (s, 9H); MS (FAB) m/z 332, 334 $(M+H)^+$.

4.2.3.7. 2-Bromo-*N*-(4-hexylphenyl)benzamide (7g). Prepared from *p*-hexylaniline in accordance with general procedure B. White solid (93%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (td, 2H, J = 8.0, 1.5 Hz), 7.60 (br s, 1H), 7.54 (d, 2H, J = 8.5 Hz), 7.41 (td, 1H, J = 8.0, 1.5 Hz), 7.32 (td, 1H, J = 8.0, 1.5 Hz), 7.19 (d, 2H, J = 8.5 Hz), 2.60 (t, 2H, J = 7.6 Hz), 1.62–1.53 (m, 2H), 1.37–1.26 (m, 6H), 0.89 (t, 3H, J = 7.0 Hz); MS (FAB) m/z 360, 362 $(M+H)^+$.

4.2.3.8. 2-Bromo-(4-dodecylphenyl)benzamide (7h). Prepared from *p*-dodecylaniline and 2-bromobenzoyl chloride in accordance with general procedure B. White solid (65%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.65 (td, 2H, J = 8.5, 1.5 Hz), 7.59 (br s, 1H), 7.54 (d, 2H, J = 7.5 Hz), 7.41 (td, 1H, J = 7.5, 1.5 Hz), 7.32 (td, 1H, J = 7.5, 1.5 Hz), 7.19 (d, 2H, J = 8.5 Hz), 2.59 (t, 2H, J = 8.0 Hz), 1.64–1.52 (m, 2H), 1.35–1.22 (m, 18H), 0.88 (t, 3H, J = 7.0 Hz); MS (FAB) m/z 444,446 $(M+H)^+$.

4.2.3.9. *N*-Butyl-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-3-methoxyphenyl]-2-iodo-benzamide (11a). To a solution of **10a** (104 mg, 0.300 mmol) in CH_2Cl_2 (5.00 mL) were added dropwise Et_3N (82.5 μl, 750 μmol) and 2-iodobenzoyl chloride (88.0 mg, 0.330 mmol) at 0 °C, and the mixture was stirred for 3 h, then allowed to warm to room temperature. After cooling again to 0 °C, the mixture was diluted with ethyl acetate, and water was added. The organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane/AcOEt = 1:0–1:1) to give **11a** (quant.) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 (d, 1H, J = 7.3 Hz), 7.41 (d, 1H, J = 7.9 Hz), 7.11 (t, 1H, J = 7.3 Hz), 6.93–6.90 (m, 3H), 6.80 (s, 1H), 4.01–3.98 (br m, 2H), 3.78 (s, 3H), 1.69–1.67 (m, 2H), 1.46–1.43 (m, 2H), 0.96 (t, 3H, J = 7.0 Hz).

4.2.3.10. *N*-Butyl-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-2-methoxyphenyl]-2-iodobenzamide (11b). Prepared from **10b** in accordance with general procedure B. White solid (82%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, 1H, J = 7.9 Hz), 7.30 (d, 1H, J = 8.5 Hz), 7.14 (s, 1H), 7.06 (d, 1H, J = 8.5 Hz), 6.99–6.92 (m, 2H), 6.80–6.77 (m, 1H), 3.85 (s, 3H), 3.60–3.28 (m, 2H), 1.72–1.51 (m, 2H), 1.51–1.32 (m, 2H), 0.94 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 576 $(M+H)^+$.

4.2.4. General procedure C: synthesis of benzamide analogues 12

4.2.4.1. 2-Bromo-*N*-butyl-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl]-6-methoxybenzamide (12a). To a solution of 2-bromo-6-methoxybenzoic acid (104 mg, 397 μmol) in CH_2Cl_2 (3.00 mL) was added chloromethylenedimethyliminium chloride (50.8 mg, 397 μmol) at 0 °C under an Ar atmosphere, and the mixture was stirred at room temperature for 1 h. Then, 2-(4-butylaminophenyl)-1,1,1,3,3,3-hexafluoropropan-2-ol (**10c**) (50.0 mg, 159 μmol) and Et_3N (166 μl, 1.19 mmol) were added at 0 °C. Stirring was continued for 3 h, then the mixture was allowed to warm to room temperature, and diluted with ethyl acetate. Water was added, and the organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane/AcOEt = 1:0–1:1) to give **12a** (73.5 mg, 139 μmol, 88%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.50 (d, 2H, J = 8.5 Hz), 7.30 (d, 2H, J = 8.5 Hz), 6.96–6.93 (m, 2H), 6.51 (d, 1H, J = 7.3 Hz), 4.20–4.08 (m, 1H), 4.06 (br s, 1H), 3.75–3.67 (m, 2H), 3.65 (s, 3H), 1.81–1.54 (m, 2H), 1.52–1.34 (m, 2H), 0.94 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 528 $(M+H)^+$.

4.2.4.2. 2-Bromo-*N*-butyl-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl]-5-methoxybezamide (12b). Prepared from **10c** and 2-bromo-5-methoxybenzoic acid in accordance with general procedure C. White solid (60%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (d, 2H, J = 7.9 Hz), 7.25 (s, 1H), 7.22 (d, 2H), 6.57 (dd, 1H, J = 8.9, 2.7 Hz), 6.51 (d, 1H, J = 2.4 Hz), 3.95 (br s, 2H), 3.59 (s, 3H), 1.67–1.59 (m, 2H), 1.42 (tq, 2H, J = 7.3, 7.3 Hz), 0.94 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 528 $(M+H)^+$.

4.2.4.3. 2-Bromo-*N*-butyl-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl]-4-methoxybenzamide (12c). Prepared from **10c** and 2-bromo-4-methoxybenzoic acid in accordance with general procedure C. White solid (70%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.70–7.47 (m, 2H), 7.32–7.10 (m, 3H), 7.10–6.85 (m, 1H), 6.70–6.48 (m, 1H), 4.05–3.78 (m, 3H), 3.70 (s, 3H),

1.70–1.50 (m, 2H), 1.50–1.33 (m, 2H), 0.99–0.78 (m, 3H); MS (FAB) *m*/*z* 528 $(M+H)^+$.

4.2.4.4. 2-Bromo-*N*-butyl-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl]-3-methoxybenzamide (12d). Prepared from **10c** and 2-bromo-3-methoxybenzoic acid in accordance with general procedure C. White solid (84%); ^1H NMR (500 MHz, $CDCl_3$) δ 7.51 (d, 2H, *J* = 8.5 Hz), 7.22 (m, 2H), 7.00 (dd, 1H, *J* = 7.9, 7.9 Hz), 6.66 (d, 1H, *J* = 7.9 Hz), 6.59 (d, 1H, *J* = 7.9 Hz), 4.05–3.90 (m, 2H), 3.80 (s, 3H), 1.73–1.63 (m, 2H), 1.50–1.35 (m, 2H), 0.94 (t, 3H, *J* = 7.3 Hz); MS (FAB) *m*/*z* 528 $(M+H)^+$.

4.2.4.5. 2-Bromo-*N*-Butyl-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl]-4-methylbenzamide (12e). Prepared from **10c** and 2-bromo-4-methylbenzoic acid in accordance with general procedure C. Colorless oil (58%); MS (FAB) *m*/*z* 512, 514 $(M+H)^+$.

4.2.4.6. 2-Bromo-*N*-butyl-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl]-4-chlorobenzamide (12f). Prepared from **10c** and 2-bromo-4-chlorobenzoic acid in accordance with general procedure C. Colorless oil (56%); ^1H NMR (500 MHz, $CDCl_3$) δ 7.49 (d, 2H, *J* = 8.6 Hz), 7.35 (s, 1H), 7.14 (d, 2H, *J* = 8.6 Hz), 6.97 (d, 1H, *J* = 5.2 Hz), 6.86 (d, 1H, *J* = 8.0 Hz), 3.93–3.81 (m, 2H), 1.60–1.52 (m, 2H), 1.39–1.30 (m, 2H), 0.88 (t, 3H, *J* = 7.5 Hz).

4.2.4.7. 2-Bromo-*N*-butyl-*N*-[4-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenyl]-4-(trifluoromethyl)benzamide (12g) Prepared from **10c** and 2-bromo-4-(trifluoromethyl)benzoic acid in accordance with general procedure C. 2-Bromo-4-(trifluoromethyl)benzoic acid was prepared by the reported method. Colorless oil (64%); ^1H NMR (500 MHz, $CDCl_3$) δ 7.63 (s, 1H), 7.53 (d, 2H, *J* = 8.6 Hz), 7.29 (d, 1H, *J* = 7.4 Hz), 7.21 (d, 2H, *J* = 8.6 Hz), 7.08 (d, 1H, *J* = 8.0 Hz), 3.94 (br s, 2H), 1.67–1.60 (m, 2H), 1.41 (tq, 2H, *J* = 7.4, 7.4 Hz), 0.94 (t, 3H, *J* = 7.4 Hz).

4.2.5. General procedure D: introduction of butyl group

4.2.5.1. *N*-(4-Acetylphenyl)-2-bromo-*N*-butylbenzamide (8a) To a solution of **7a** (104 mg, 1.00 mmol) in DMF (2.00 mL) was added sodium hydride (40.0 mg, 1.67 mmol) at 0 °C under an Ar atmosphere, and the mixture was stirred at room temperature for 30 min. Then, 1-iodobutane (276 mg, 1.50 mmol) was added at 0 °C. The mixture was stirred overnight, and allowed to warm to room temperature. Then, it was diluted with ethyl acetate, and water was added. The organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane/AcOEt = 4:1) to give **8a** (348 mg, 0.930 mmol, 93%) as a colorless oil.

4.2.5.2. *N*-Butyl-2-iodo-*N*-phenylbenzamide (8b). Prepared from **7b** in accordance with general procedure D. Colorless oil (98%); ^1H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, 1H, *J* = 7.3 Hz), 7.16 (d, 4H, *J* = 4.3 Hz), 7.09 (m, 1H), 7.04 (dd, 1H, *J* = 7.5, 1.2 Hz), 6.95 (dd, 1H, *J* = 7.4, 1.3 Hz), 6.79 (td, 1H, *J* = 7.4, 1.3 Hz), 3.91 (br s, 2H), 1.60 (m, 2H), 1.40 (m, 2H), 0.91 (t, 3H, *J* = 7.4 Hz).

4.2.5.3. 2-Bromo-*N*-butyl-*N*-*p*-tolylbenzamide (8c). Prepared from **7c** in accordance with general procedure D. Oily solid (quant.); MS (FAB) *m*/*z* 346, 348 $(M+H)^+$.

4.2.5.4. 2-Bromo-*N*-butyl-*N*-(4-ethylphenyl)benzamide (8d) Prepared from **7d** in accordance with general procedure D. Colorless oil (quant.); MS (FAB) *m*/*z* 360, 362 $(M+H)^+$.

4.2.5.5. *N*-Butyl-2-iodo-*N*-(4-isopropylphenyl)benzamide (8e) Prepared from **7e** in accordance with general procedure D. Colorless oil (98%); ^1H NMR (500 MHz, $CDCl_3$) δ 7.63 (d, 1H, *J* = 8.0 Hz), 7.29 (s, 1H), 7.04 (t, 1H, *J* = 8.5 Hz), 7.00 (d, 1H, *J* = 8.5 Hz), 6.92 (dd, 1H, *J* = 7.3, 1.8 Hz), 6.78 (td, 1H, *J* = 7.9, 1.8 Hz), 3.88 (br s, 2H), 2.75 (sep, 1H, *J* = 6.7 Hz), 1.62 (m, 2H), 1.40 (m, 2H), 1.12 (d, 6H, *J* = 6.7 Hz), 0.91 (t, 3H, *J* = 7.3 Hz); MS (FAB) *m*/*z* 422 $(M+H)^+$.

4.2.5.6. 2-Bromo-*N*-butyl-*N*-(4-*tert*-butylphenyl)benzamide (8f) Prepared from **7f** in accordance with general procedure D. Colorless oil (quant.); MS (FAB) *m*/*z* 388, 390 $(M+H)^+$.

4.2.5.7. 2-Bromo-*N*-butyl-*N*-(4-hexylphenyl)benzamide (8g) Prepared from **7g** in accordance with general procedure D. Colorless oil (quant.); MS (FAB) *m*/*z* 416, 418 $(M+H)^+$.

4.2.5.8. 2-Bromo-*N*-butyl-*N*-(4-dodecylphenyl)benzamide (8h) Prepared from **8h** in accordance with general procedure D. Colorless oil (quant.); MS (FAB) *m*/*z* 500, 502 $(M+H)^+$.

4.2.6. General procedure E: synthesis of phenanthridine analogues by Pd-catalyzed cyclization

4.2.6.1. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-3-methoxyphenanthridin-6(5*H*)-one (4a). To a solution of **11a** (41.4 mg, 71.9 μmol) in DMA (3.00 mL) were added PCy_3-HBF_4 (13.2 mg, 35.9 μmol), Cs_2CO_3 (141 mg, 431 μmol) and $Pd(OAc)_2$ (4.04 mg, 18.0 μmol) under an Ar atmosphere, and the mixture was stirred for 3 h at 120 °C. Then, the mixture was cooled to 0 °C, and diluted with ethyl acetate. Water was added, and the organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (*n*-hexane/AcOEt = 1:0–2:1) to give **4a** (8.7 mg, 19.4 μmol, 27%) as a white solid; mp 101.0–104.0 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.52 (d, 1H, *J* = 7.9 Hz), 8.47 (s, 1H), 8.12 (d, 1H, *J* = 8.6 Hz), 7.79 (dd, 1H, *J* = 8.6, 6.7 Hz), 7.59 (dd, 1H, *J* = 7.9, 6.7 Hz), 6.98 (s, 1H), 4.38 (t, 2H, *J* = 7.3 Hz), 4.12 (s, 3H), 1.82 (tt, 2H, *J* = 7.3, 7.3 Hz), 1.54 (tq, 2H, *J* = 7.3, 7.3 Hz), 1.05 (t, 3H, *J* = 7.3 Hz); HRMS (FAB) calcd for $C_{21}H_{20}F_6NO_3$ 448.1347; found: 448.1350 $(M+H)^+$.

4.2.6.2. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-4-methoxyphenanthridin-6(5*H*)-one (4b). Prepared from **11b** in accordance with general procedure E. White solid (73%); mp 91.0–95.0 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.50 (d, 1H, *J* = 7.9 Hz), 8.26 (s, 1H), 8.22 (d, 1H, *J* = 7.9 Hz), 7.75 (dd, 1H, *J* = 7.9, 7.3 Hz), 7.59 (dd, 1H, *J* = 7.9, 7.3 Hz), 7.36 (s, 1H), 4.54 (t, 2H, *J* = 7.3 Hz), 4.03 (s, 1H), 3.97 (s, 3H), 1.84 (tt, 2H, *J* = 7.3, 7.3 Hz), 1.44 (tq, 2H, *J* = 7.3, 7.3 Hz), 0.99 (t, 3H, *J* = 7.3 Hz); MS (FAB) *m*/*z* 448 $(M+H)^+$; Anal. calcd for $C_{21}H_{19}F_6NO_3$-1/2H_2O: C, 55.30; H, 4.42; N, 3.07. Found: C, 55.27; H, 4.46; N, 3.09.

4.2.6.3. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-7-methoxyphenanthridin-6(5*H*)-one (4c). Prepared from **12a** in accordance with general procedure E. White solid (85%); mp 226.0–230.0 °C; ^1H NMR (500 MHz, $CDCl_3$) δ 8.64 (s, 1H), 8.37 (d, 1H, *J* = 9.1 Hz), 7.82 (d, 1H, *J* = 8.5 Hz), 7.48 (d, 1H, *J* = 2.4 Hz), 7.37 (d, 1H, *J* = 8.5 Hz), 7.10 (dd, 1H, *J* = 9.1, 2.4 Hz), 5.09 (br s, 1H), 4.26 (t, 2H, *J* = 7.3 Hz), 3.94 (s, 3H), 1.72 (tt, 2H, *J* = 7.3, 7.3 Hz), 1.44 (tq, 2H, *J* = 7.3, 7.3 Hz), 0.95 (t, 3H, *J* = 7.3 Hz); MS (FAB) *m*/*z* 448 $(M+H)^+$; Anal. calcd for $C_{21}H_{19}F_6NO_3$-3/4H_2O: C, 54.73; H, 4.48; N, 3.04. Found: C, 54.71; H, 4.51; N, 2.91.

4.2.6.4. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-8-methoxyphenanthridin-6(5*H*)-one (4d). Prepared from **12b** in accordance with general procedure E. White solid

(75%); mp 195.0–197.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.59 (s, 1H), 8.18 (d, 1H, J = 9.2 Hz), 7.94 (d, 1H, J = 3.0 Hz), 7.79 (d, 1H, J = 9.2 Hz), 7.44 (d, 1H, J = 9.2 Hz), 7.34 (dd, 1H, J = 9.2, 3.0 Hz), 4.59 (s, 1H), 4.38 (t, 2H, J = 7.3 Hz), 3.95 (s, 3H), 1.77 (tt, 2H, J = 7.3, 7.3 Hz), 1.52 (tq, 2H, J = 7.3, 7.3 Hz), 1.02 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 448 (M+H)$^+$; Anal. calcd for $C_{21}H_{19}NO_3$–H_2O: C, 54.20; H, 4.55; N, 3.01. Found: C, 53.89; H, 4.12; N, 2.96.

4.2.6.5. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-9-methoxyphenanthridin-6(5*H*)-one (4e). Prepared from **12c** in accordance with general procedure E. White solid (84%); mp 152.0–154.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.51 (s, 1H), 7.83 (d, 1H, J = 9.1 Hz), 7.81 (d, 1H, J = 7.9 Hz), 7.60 (dd, 1H, J = 7.9, 7.9 Hz), 7.33 (d, 1H, J = 9.1 Hz), 7.06 (d, 1H, J = 7.9 Hz), 5.22 (br s, 1H), 4.20 (t, 2H, J = 7.3 Hz), 4.04 (s, 3H), 1.68 (tt, 2H, J = 7.3, 7.3 Hz), 1.48 (tq, 2H, J = 7.3, 7.3 Hz), 0.99 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 448 (M+H)$^+$; Anal. calcd for $C_{21}H_{19}F_6NO_3$–H_2O: C, 54.20; H, 4.55; N, 3.01. Found: C, 54.32; H, 4.54; N, 3.13.

4.2.6.6. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-10-methoxyphenanthridin-6(5*H*)-one (4f). Prepared from **12d** in accordance with general procedure E. White solid (79%); mp 175.5–177.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.75 (s, 1H), 8.16 (d, 1H, J = 7.9 Hz), 7.84 (d, 1H, J = 9.2 Hz), 7.50 (dd, 1H, J = 7.9, 7.9 Hz), 7.38 (d, 1H, J = 9.2 Hz), 7.22 (d, 1H, J = 7.9 Hz), 4.68 (br s, 1H), 4.30 (t, 2H, J = 7.3 Hz), 4.01 (s, 3H), 1.74 (tt, 2H, J = 7.3, 7.3 Hz), 1.49 (tq, 2H, J = 7.3, 7.3 Hz), 1.00 (t, 3H, J = 7.3 Hz); MS (FAB) m/z 448 (M+H)$^+$; Anal. calcd for $C_{21}H_{19}F_6NO_3$: C, 56.38; H, 4.28; N, 3.13. Found: C, 56.15; H, 4.41; N, 3.14.

4.2.6.7. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-9-methylphenanthridin-6(5*H*)-one (5a). Prepared from **12e** in accordance with general procedure E. White solid (13%); mp 194.5–196.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.58 (d, 1H, J = 2.3 Hz), 8.35 (d, 1H, J = 8.0 Hz), 7.98 (s, 1H), 7.75 (d, 1H, J = 9.2 Hz), 7.39–7.36 (m, 2H), 4.30 (dd, 2H, J = 7.7, 3.9 Hz), 2.51 (s, 3H), 1.75–1.69 (m, 2H), 1.48–1.42 (m, 2H), 0.95 (t, 3H, J = 7.4 Hz); HRMS (FAB) calcd for $C_{21}H_{19}F_6NO_2$ 431.1320. Found: 431.1320 (M)$^+$.

4.2.6.8. 5-Butyl-9-chloro-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)phenanthridin-6(5*H*)-one (5b). Prepared from **12f** in accordance with general procedure E. White solid (29%); mp 194.0–198.5 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56 (d, 1H, J = 2.3 Hz), 8.46 (d, 1H, J = 8.6 Hz), 8.22 (d, 1H, J = 1.7 Hz), 7.84 (d, 1H, J = 9.2 Hz), 7.55 (dd, 1H, J = 8.6, 2.3 Hz), 7.45 (d, 1H, J = 8.6 Hz), 4.35 (t, 2H, J = 8.0 Hz), 1.80–1.74 (m, 2H), 1.53–1.47 (m, 2H), 1.01 (t, 3H, J = 7.4 Hz); HRMS (FAB) calcd for $C_{20}H_{17}ClF_6NO_2$ 452.0852. Found: 452.0868 (M+H)$^+$.

4.2.6.9. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-9-(trifluoromethyl)phenathridin-6(5*H*)-one (5c). Prepared from **12g** in accordance with general procedure E. White solid (80%); mp 182.5–185.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.61 (dd, 2H, J = 10.0, 5.4 Hz), 8.46 (s, 1H), 7.83 (d, 1H, J = 9.2 Hz), 7.76 (d, 1H, J = 8.6 Hz), 7.44 (d, 1H, J = 9.2 Hz), 4.33 (t, 2H, J = 8.0 Hz), 1.77–1.71 (m, 2H), 1.47 (td, 2H, J = 14.9, 7.4 Hz), 0.96 (t, 3H, J = 7.2 Hz); HRMS (FAB) calcd for $C_{21}H_{17}F_9NO_2$ 486.1116. Found: 486.1113 (M+H)$^+$.

4.2.6.10. Methyl 5-butyl-6-oxo-5,6-dihydrophenanthridine-2-carboxylate (3a). Prepared from **8a** in accordance with general procedure E. White solid (77%, two steps); mp 114.0–118.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.92 (d, 1H, J = 1.8 Hz), 8.53 (d, 1H, J = 7.3 Hz), 8.37 (d, 1H, J = 7.9 Hz), 8.10 (dd, 1H, J = 9.2, 1.8 Hz), 7.79 (t, 1H, J = 7.3 Hz), 7.61 (dd, 1H, J = 7.9, 7.3 Hz), 7.44 (d, 1H, J = 9.2 Hz), 4.39 (t, 2H, J = 7.9 Hz), 2.69 (s, 3H), 1.77 (tt, 2H, J = 7.9, 7.9 Hz), 1.52 (tq, 2H, J = 7.3, 7.3 Hz), 1.01 (t, 3H, J = 7.3 Hz); HRMS (FAB) calcd for $C_{19}H_{19}NO_2$ 294.1494. Found: 294.1501 (M+H)$^+$.

4.2.6.11. 5-Butylphenanthridin-6(5*H*)-one (3c). Prepared from **8b** in accordance with general procedure E. Colorless oil (98%, two steps); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54 (dd, 1H, J = 7.9, 1.3 Hz), 8.29 (dd, 1H, J = 8.6, 1.3 Hz), 8.27 (d, 1H, J = 8.6 Hz), 7.74 (ddd, 1H, J = 8.0, 7.4, 1.2 Hz), 7.57 (t, 1H, J = 7.4 Hz), 7.53 (ddd, 1H, J = 8.5, 7.4, 1.2 Hz), 7.40 (d, 1H, J = 8.5 Hz), 7.30 (dd, 1H, J = 8.0, 7.4 Hz), 4.38 (t, 2H, J = 7.9 Hz), 1.78 (tt, 2H, J = 7.9, 7.3 Hz), 1.52 (tq, 2H, J = 7.3, 7.3 Hz), 1.00 (t, 3H, J = 7.3 Hz); HRMS (FAB) calcd for $C_{17}H_{17}NO$ 252.1388. Found: 252.1349 (M+H)$^+$.

4.2.6.12. 5-Butyl-2-methylphenanthridin-6(5*H*)-one (3d) Prepared from **8c** in accordance with general procedure E. Colorless oil (89%, two steps); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (dd, 1H, J = 8.0, 1.2 Hz), 8.28 (d, 1H, J = 8.0 Hz), 8.10 (s, 1H), 7.74 (td, 1H, J = 8.0, 1.2 Hz), 7.57 (m, 1H), 7.37–7.30 (d, 1H, J = 7.6 Hz), 4.38 (t, 2H, J = 7.6 Hz), 2.49 (s, 3H), 1.82–1.75 (m, 3H), 1.56–1.48 (m, 2H), 1.01 (t, 3H, J = 7.3 Hz); HRMS (FAB) calcd for $C_{18}H_{19}NO$ 266.1545. Found: 266.1584 (M+H)$^+$.

4.2.6.13. 5-Butyl-2-ethylphenathridin-6(5*H*)-one (3e). Prepared from **8d** in accordance with general procedure E. Pale brown oil (87%, two steps); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (dd, 1H, J = 8.0, 1.5 Hz), 8.30 (d, 1H, J = 8.0 Hz), 8.12 (d, 1H, J = 1.5 Hz), 7.75 (td, 1H, J = 8.0, 1.5 Hz), 7.58 (t, 1H, J = 8.0 Hz), 7.39 (dd, 1H, J = 8.0, 1.5 Hz), 7.34 (d, 1H, J = 8.0 Hz), 4.39 (t, 2H, J = 8.0 Hz), 2.79 (q, 2H, J = 8.0 Hz), 1.83–1.76 (m, 2H), 1.57–1.49 (m, 2H), 1.34 (t, 3H, J = 7.6 Hz), 1.02 (t, 3H, J = 7.3 Hz); HRMS (FAB) calcd for $C_{19}H_{21}NO$ 280.1701. Found: 280.1696 (M+H)$^+$.

4.2.6.14. 5-Butyl-2-isopropylphenanthridin-6(5*H*)-one (3f) Prepared from **8e** in accordance with general procedure E. Colorless oil (98%, two steps); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.53 (dd, 1H, J = 7.9, 1.3 Hz), 8.30 (d, 1H, J = 8.0 Hz), 8.12 (d, 1H, J = 1.9 Hz), 7.73 (t, 1H, J = 7.8 Hz), 7.55 (t, 1H, J = 7.8 Hz), 7.41 (dd, 1H, J = 8.7, 1.9 Hz), 7.34 (d, 1H, J = 8.6 Hz), 4.36 (t, 2H, J = 7.9 Hz), 3.04 (sep, 1H, J = 7.0 Hz), 1.77 (m, 2H), 1.51 (m, 2H), 1.33 (d, 3H, J = 7.3 Hz), 1.00 (t, 3H, J = 7.3 Hz); HRMS (FAB) calcd for $C_{20}H_{23}NO$ 294.1858. Found: 294.1851 (M+H)$^+$.

4.2.6.15. 2-*tert*-Butyl-5-butylphenanthridin-6(5*H*)-one (3g) Prepared from **8f** in accordance with general procedure E. Pale brown oil (67%, two steps); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.56 (dd, 1H, J = 8.0, 1.5 Hz), 8.32 (d, 1H, J = 8.0 Hz), 8.31 (d, 1H, J = 1.5 Hz), 7.78–7.74 (m, 1H), 7.60 (dd, 1H, J = 8.0, 1.5 Hz), 7.57 (d, 1H, J = 8.0 Hz), 7.37 (d, 1H, J = 8.0 Hz), 4.39 (t, 2H, J = 7.6 Hz), 1.83–1.76 (m, 2H), 1.561.50 (m, 2H), 1.44 (s, 9H), 1.02 (t, 3H, J = 7.3 Hz); HRMS (FAB) calcd for $C_{21}H_{25}NO$ 308.2014. Found: 308.2008 (M+H)$^+$.

4.2.6.16. 5-Butyl-2-hexylphenanthridin-6(5*H*)-one (3h) Prepared from **8g** in accordance with general procedure E. Colorless oil (90%, two steps); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (d, 1H, J = 8.0 Hz), 8.30 (d, 1H, J = 8.0 Hz), 8.09 (s, 1H), 7.75 (t, 1H, J = 8.0 Hz), 7.57 (t, 1H, J = 8.0 Hz), 7.37 (dd, 1H, J = 8.0, 1.5 Hz), 7.33 (dd, 1H, J = 8.0, 1.5 Hz), 4.39 (t, 2H, J = 7.6 Hz), 2.74 (t, 2H, J = 7.9 Hz), 1.83–1.76 (m, 2H), 1.73–1.66 (m, 2H), 1.58–1.49 (m, 2H), 1.41–1.32 (m, 6H), 1.02 (t, 3H, J = 7.3 Hz), 0.90 (t, 3H, J = 7.0 Hz); HRMS (FAB) calcd for $C_{23}H_{29}NO$ 336.2327. Found: 336.2310 (M+H)$^+$.

4.2.6.17. 5-Butyl-2-dodecylphenanthridin-6(5*H*)-one (3i)

Prepared from **8h** in accordance with general procedure E. Colorless oil (66%, two steps); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.55 (dd, 1H, J = 8.0, 1.5 Hz), 8.30 (d, 1H, J = 8.0 Hz), 8.09 (d, 1H, J = 1.5 Hz), 7.75 (ddd, 1H, J = 8.0, 6.5, 1.5 Hz), 7.57 (td, 1H, J = 8.0, 1.5 Hz), 7.36 (dd, 1H, J = 8.0, 1.5 Hz), 7.33 (d, 1H, J = 8.0 Hz), 4.38 (t, 2H, J = 7.6 Hz), 2.74 (t, 2H, J = 7.6 Hz), 1.83–1.76 (m, 2H), 1.73–1.66 (m, 2H), 1.57–1.49 (m, 2H), 1.45–1.20 (m, 18H), 1.02 (t, 3H, J = 7.3 Hz), 0.88 (t, 3H, J = 7.0 Hz); HRMS (FAB) calcd for $C_{29}H_{41}NO$ 420.3266. Found: 420.3297 $(M+H)^+$.

4.2.7. 5-Butyl-2-hydroxymethylphenanthridin-6(5*H*)-one (3b)

To a solution of **3a** (288 mg, 0.770 mmol) in THF (5.00 mL) was added lithium borohydride (86.8 mg, 4.00 mmol) at 0 °C. The mixture was stirred overnight, and then allowed to warm to room temperature and diluted with ethyl acetate. Water was added, and the organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (n-hexane/AcOEt = 2:1) to give **3b** (193 mg, 0.680 mmol, 89%) as a white solid; mp 150.0–153.0 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54 (d, 1H, J = 8.0 Hz), 8.28–8.26 (m, 2H), 7.75 (t, 1H, J = 8.0 Hz), 7.59 (t, 1H, J = 8.0, 1.8 Hz), 7.52 (dd, 1H, J = 8.0, 1.8 Hz), 7.36 (d, 1H, J = 8.0 Hz), 4.83 (d, 2H, J = 6.0 Hz), 4.37 (t, 2H, J = 7.5 Hz), 1.99–1.94 (m, 1H), 1.81–1.74 (m, 2H), 1.55–1.48 (m, 2H), 1.01 (t, 3H, J = 7.5 Hz); Anal. calcd for $C_{18}H_{19}NO_2$: C, 76.84; H, 6.81; N, 4.98. Found: C, 76.94; H, 6.71; N, 5.01.

4.2.8. 5-Butyl-2-(1,1,1,3,3,3-hexafluoro-2-hydroxypropan-2-yl)-9-hydroxyphenathridin-6(5*H*)-one (5d)

To a solution of **4e** (55.9 mg, 0.125 mmol) in CH_2Cl_2 (1.20 mL) was added boron tribromide (17% in CH_2Cl_2, 0.400 mL) under an Ar atmosphere. The mixture was stirred overnight at room temperature, then $NaHCO_3$ was added to it. Water was added, and the organic layer was separated, washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by column chromatography (n-hexane/AcOEt = 2:1) to give **5d** (45.9 mg, 0.106 mmol, 85%) as a white solid; mp 250.0–252.5 °C; ^{1}H NMR (500 MHz, DMSO) δ 8.50 (d, 1H, J = 1.7 Hz), 8.22 (d, 1H, J = 9.2 Hz), 7.81 (d, 1H, J = 8.6 Hz), 7.69 (d, 1H, 9.2 Hz), 7.64 (d, 1H, J = 2.3 Hz), 7.10 (dd, 1H, J = 8.6, 2.3 Hz), 4.30 (t, 2H, J = 7.7 Hz), 1.66–1.60 (m, 2H), 1.41 (tq, 2H, J = 7.4, 7.4 Hz), 0.94 (t, 3H, J = 7.4 Hz); HRMS (FAB) calcd for $C_{20}H_{17}F_6NO_3$ 433.1113. Found: 433.1118 $(M)^+$.

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