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Synthesis and Biological Evaluation of Cyclopentaquinoline Derivatives as Nonsteroidal Glucocorticoid Receptor Antagonists

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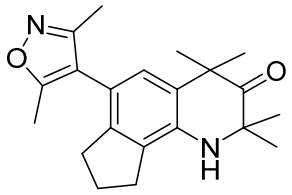
ABSTRACT: The steroidal glucocorticoid antagonist mifepristone has been reported to improve the symptoms of depression. We report the discovery of 6-(3,5-dimethylisoxazol-4-yl)-2,2,4,4-tetramethyl-2,3,4,7,8,9-hexahydro-1H-cyclopenta[h]

quinolin-3-one **3d** (QCA-1093) as a novel nonsteroidal glucocorticoid receptor antagonist. The compound displayed potent *in vitro* activity, high selectivity over other steroid hormone receptors, and significant antidepressant-like activity *in vivo*.



3d
hGR (Ki): 15 nM
hPR (Ki): 1479 nM
hAR, hERα, hERβ (Ki): >2300 nM
hMR >3000 nM

KEYWORDS: *glucocorticoid receptor antagonist, QCA-1093, antidepressant, tetrahydroquinoline, mifepristone*.

Introduction

Physiological and psychological stress promotes the activation of the hypothalamic–pituitary–adrenal axis causing the secretion of glucocorticoid hormones from the adrenal cortex. Glucocorticoid hormones bind to glucocorticoid receptors (GRs) in the brain and induce various anxiety/depression-related symptoms such as increased sadness,[1] suppression of food ingestion,[2] and neuronal loss in the hippocampus.[3] The steroidal GR antagonist mifepristone (**1**, Figure 1) has been reported to improve the symptoms of depression.[4] However, an abortifacient effect due to the potent progesterone receptor (PR) antagonist activity,[5] rapid clearance from the body, and cross-reactivity with other steroid hormone receptors hampered its use as an antidepressant agent. Multiple groups attempted to address these limitations through the synthesis of selective nonsteroidal GR antagonists, namely chromene[6] or arylindazole derivatives.[7] We focused on developing a novel, potent, and selective nonsteroidal GR antagonist for the treatment of depression.

In a high-throughput screening of compounds using luciferase reporter assays, we identified tetrahydroquinoline derivative **3a** as a human GR (hGR) antagonist (IC_{50} = 37 nM). The binding affinities (Ki) of **3a** to hGR and human PR (hPR) were 16 nM and 22 nM, respectively, which were similar to those of mifepristone (hGR, Ki = 6 nM; hPR, Ki = 15

nM). Tetrahydroquinoline **2**, a nonsteroidal PR antagonist, with a high binding affinity to PR (Ki = 3.9 nM), exhibits a pan-steroid receptor inhibitory activity for hPR, hGR, the human androgen receptor (hAR), and the human mineralocorticoid receptor (hMR) in competitive binding assays.[8] We modified the structures of suitable tetrahyrdroquinolines, such as compound **2** and **3a,** as lead compounds to obtain a GR selective antagonist with selectivity over other steroid hormone receptors.

In this paper, we demonstrated that the optimization of **3a** led to the identification of 6-(3,5-dimethylisoxazol-4-yl)-2,2,4,4-tetramethyl-2,3,4,7,8,9-hexahydro-1H-cyclopenta[h] quinolin-3-one **3d** (QCA-1093) as a novel nonsteroidal GR antagonist. The compound displayed potent *in vitro* activity, high selectivity over other steroid hormone receptors, and significant antidepressant-like activity *in vivo*.

Chemistry

Schemes 1 and 2 present an outline of the synthesis of tetrahydroquinoline-3-ones **3a-f** and **12a-k** following the published procedures with a slight modification.[8,9] Dihydroquinoline **4** was prepared by the Skraup reaction of acetone with commercially available substituted aniline in the presence of iodine[10] or catalytic scandium trifluoromethanesulfonate.[11] The hydroboration of olefin and conversion of the hydroxyl

group followed by bromination of **4** afforded alcohol **6**. After protection of the alcohol with a tert-butyldimethylsilyl (TBDMS) group and protection of the amine with a Boc group, the TBDMS group was removed by tetrabutylammonium fluoride to yield alcohol **7**. Alcohol **7** was oxidized by pyridinium chlorochromate and methylated by methyl iodide to give ketone **9**. Ketone **9** was esterified with bis(pinacolato)diboron in the presence of a catalytic amount of [1,1′-bis(diphenylphosphino)ferrocene] palladium to synthesize boronic acid ester **11**. A series of compounds (**3a-f** and **12a-k**) were obtained by the Suzuki coupling reaction between the boronic acid ester and appropriate halides (aryl halide or compound **9**) followed by acidic deprotection.

Biological results and discussion

The synthesized compounds were evaluated for their hGR antagonistic activity (IC_{50}) in a whole-cell luciferase assay and binding affinities (Ki) to hGR, hPR, and hMR. The initial modification of the aromatic and heteroaromatic rings at the isoxazole moiety of **3a** did not produce a potent and selective hGR antagonist (see supporting information, Table 6). The structures and *in vitro* data of the compounds with substituents on the tetrahydroquinoline ring are summarized in Table 1. The introduction of a chloro (**3b**) or methoxy (**3c**) group at the 7 position (R^1) on the tetrahydroquinoline ring of compound **3a** led to a significant

decrease in the binding potency to hGR. Cyclopentaquinoline **3d**, in which R^1 and R^2 were joined by a three methylene linker, displayed hGR binding potency and antagonistic activity comparable to those of **3a** with a remarkable improvement in its selectivity for hPR and hMR. Dexamethasone increased the luciferase signal by approximately 200% at 10 nM. In the same luciferase assay without dexamethasone, **3d** itself was not an agonist because **3d** increased the luciferase activity by only 4% from baseline, even at a concentration of 1 μM. Increasing the length of the methylene linker as cyclohexaquinoline **3e** significantly decreased the binding potency to hGR. The insertion of oxygen to the linker as [1, 3]dioxoloquinoline **3f** led to a 3-fold decrease in the hGR binding potency with a significant selectivity for hPR and hMR.

We examined the introduction of a variety of aromatic and heteroaromatic rings at the isoxazole moiety (R^3) while maintaining the cyclopentaquinoline template. Exchanging R^3 with a benzene or mono substituted benzene ring system resulted in a reduction of the binding potency (*ortho*-, *meta*-, or *para*-F, Cl, OMe, OH, CN, see supporting information, Table 7). The results of substitution with the five-membered heterocycles in R^3 are presented in Table 2. The 1,4-dimethylimidazol-5-yl derivative **12b** displayed a 4-fold decrease in hGR binding potency and the 1,4-dimethylpyrazol-5-yl derivative **12a** exhibited

similar hGR binding potency and selectivity as those of **3d**. It was noted that the hGR binding potencies of 1,3-dimethylpyrazol-5-yl **12c** and 1,4-dimethylimidazoyl-2-yl **12d** were significantly low. These results indicate that the high affinity hGR binding required the electronegativity of oxygen and/or nitrogen and two lipophilic methyl substituents at suitable positions on the five-membered heterocycle structure.

In an attempt to expand structure–activity relationship (SAR), we modified the isoxazole moiety (R^3) to a variety of 5,6- or 6,6- condensed ring systems. Because the imidazopyridine derivative **12e** displayed similar GR binding potency and selectivity as those of **3d**, it is likely that a desirable combination of the electronic and lipophilic effects in the R^3 unit was represented with this compound. Replacement of the imidazopyridine structure in **12e** with a pyrazolopyridine group in **12f** led to a 3-fold decrease in the GR binding activity. A systematic investigation of the effect of the addition of nitrogen atoms on **12e** revealed that a reduction of lipophilicity resulted in 4-fold decline in the hGR binding activity in **12g** and **12h** and a considerable loss of potency in **12i**. Substitution with a 6,6- condensed ring system reduced the hGR binding activity (1,8-naphthyridine **12k**) 3-fold. Based on the observed low binding potency for hPR and hMR, the scaffold of cyclopentaquinoline in R^3 was essential for the hGR selectivity.

GR is a part of a large multiprotein complex in the cytoplasm that includes a heat shock protein. Upon ligand binding, a conformational change of the GR-ligand complex leads to the dissociation of these chaperones and the translocation of GR to the nucleus.[12] Once in the nucleus, GR interacts with the DNA sequences and transcription factors to initiate or repress gene transcription. Ligands that compete only with agonists for GR binding are called "passive" antagonists, whereas those that compete also for the binding of the agonist receptor complex and DNA sequences are called "active" antagonists.[13] As Schoch reported that the "active" and "passive" antagonist conformations from the X-ray crystal structure of the hGR ligand-binding domain (LBD) bind to mifepristone (PDB code: 3H52),[14] we analyzed the conformation of the two types of X-ray cocrystal structures with the docking simulation of **3d** and GR using the Glide program, as shown in Figure 2. In both models, the negative charge in the izoxazole moiety of **3d** accepted a hydrogen bond from Arg611 in the same manner as the C_3 ketone in mifepristone. We noted that the cyclopentyl moiety of **3d** interacted with the lipophilic cavity of GR formed by Met601, Ala605, Leu608, Met646, and Leu732. However, the corresponding amino acid Ala605 of PR was Val760. The lipophilic cavity in PR was presumably unable to adjust to the cyclopentyl moiety because of a steric clash. Therefore, **3d** may be unfavorable as a PR ligand, although it is

unclear as to which simulation models better explains why **3d** has high affinity and selectivity to GR.

The cross-reactivity of **3d** with other steroid receptors was assessed by binding assays using the human estrogen receptors (hERα and hERβ) and hAR (Table 3). Compound **3d** displayed high selectivity for hGR over these steroidal receptors.

The pharmacokinetic analysis of compound **3d** (10 mg/kg, p.o. and 1 mg/kg, i.v. in male Sprague–Dawley rats) revealed a moderate half-life and excellent oral bioavailability of the compound in the rats (Table 4). The unbound brain to plasma ratio (Kp, uu, brain), which is a parameter of the brain penetration potential,[15] was determined to estimate the potential of **3d** as an antidepressant drug candidate. The ratio of the unbound brain concentration (Cb,u: 25.7 nmol/kg) to the unbound plasma concentration (Cp,u: 14.1 nmol/L) was 1.8 at 1 h after the oral dosing (30 mg/kg) of compound **3d** in the rats. From Kp, uu, brain, and Cb,u we estimated that a sufficient brain penetration of **3d** would be achieved after 30 mg/kg oral dosing for *in vivo* evaluation.

We tested the antidepressant-like activity of **3d** in the rat forced swimming test, which is used as a model of depression.[16] In this test, rats are forced to swim in a cylinder with water and they struggle to escape, after which they become resigned to float on the surface of

water with minimum movements. It was reported that tricyclic antidepressants and mifepristone decrease the immobility time.[17] In our result, the immobility time was significantly decreased by mifepristone (30 mg/kg, 3 times, p.o.) and compound **3d** (30 and 100 mg/kg 3 times, p.o.) (Table 5). The effect of **3d** (30 mg/kg, 3 times) was comparable with that of mifepristone (30 mg/kg, 3 times). The significant effects of both mifepristone and **3d** on the immobility time in the forced swimming test suggest that the antagonism of GR contributed to the antidepressant-like effects of these compounds.

Currently, pharmacological, pharmacokinetic, and toxicological studies on this series of GR antagonist are underway. We are preparing another detailed report on the pharmacological evaluation of a representative GR antagonist. Therefore, a further discussion on this compound series will be present in this later report.

Conclusion

A series of novel cyclopentaquinolines were developed and examined for their activity in the luciferase reporter assay and binding affinity to hGR. The new compounds demonstrated improved selectivity for hGR over other steroid hormone receptors. Among them, compound **3d** (QCA-1093) exhibited excellent penetration into the brain and *in vivo* activity in the forced swimming test in rats. These results indicate that QCA-1093 has

potential as a new antidepressant and warrants further pharmacological evaluation as a GR antagonist.

Experimental Section

Chemistry

General. Reagents, starting materials, and solvents were purchased from commercial suppliers and were used as received. Reactions were run under a nitrogen atmosphere at an ambient temperature unless otherwise specified. Chromatography refers to the flash chromatography conducted on Kieselgel 60 230–400 mesh (E. Merck, Darmstadt) using the indicated solvents. Medium pressure liquid chromatography was performed on Purif-α (Shoko Scientific) with Purifi pack normal phase Silica gel (30–60 μm) columns and UV detection at 254 nm. The melting points were determined with a Yanaco melting point apparatus and were uncorrected. ^{1}H and ^{13}C NMR spectra were measured on Bruker DPX-300 or AVANCE III 400. The NMR signals were expressed in ppm downfield from tetramethylsilane as the internal standard ($\delta = 0$). Splitting patterns are designed as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad. Mass spectra (MS) were recorded on a Waters ACQUITY SQD instrument operating with electrospray ionization and Thermo Scientific MSQ Plus instrument operating with atmospheric pressure chemical ionization. Elemental analyses for carbon, hydrogen, and nitrogen were conducted with Yanaco MT-6 and were within ±0.4% of the theoretical values for the

formulae given. The purity of the final compounds was determined by HPLC with UV detection at 220 nm and 254 nm. The conditions of the isocratic elution method (isocratic HPLC) were as follows: column, SUMIPAX ODS D-210SLP (Sumika Chemical Analysis Service, Ltd.); isocratic elution, mixture of CH_3CN and H_2O with 0.05% TFA for 12 min; and flow rate, 1 mL/min. The conditions of the linear gradient method (gradient HPLC) were as follows: column, Chromolish Speed ROD (Merck Millipore); gradient elution, 10–100% CH_3CN in water with 0.05% TFA for 4.5 min and maintaining 100% CH_3CN with 0.05% TFA for 2.5 min; and flow rate, 2 mL/min. All final compounds were >95% pure. The high-resolution mass spectrometry (HRMS) experiments were performed with Thermo Fisher Scientific LTQ Orbitrap Velos Pro using electrospray positive ionization.

Synthesis of 6-(3,5-Dimethylisoxazol-4-yl)-2,2,4,4-tetramethyl-2,3,4,7,8,9-hexahydro-*1H*-cyclopenta[h]quinolin-3-one (3d). *Step 1: 2,2,4-Trimethyl-2,7,8,9-tetrahydro-1H-cyclopenta[h]quinoline (**4d**).*

Scandium (III) trifluoromethanesulfonate (4.99 g, 0.010 mol) was added to a solution of 4-aminoindane (22.5 g, 0.169 mol) in acetone (500 mL), and the mixture was stirred at room temperature for 7 days. The reaction mixture was filtered through celite. The solvent

was evaporated under reduced pressure, and the residue was purified by flash chromatography using hexane/AcOEt (20:1) as an eluent. After evaporation, the product was obtained as a slightly orange solid (21.8 g, 60%). MS *m*/*z* $[M+H]^+$ 214. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 (d, J = 7.6 Hz, 1H), 6.55 (d, J = 7.6 Hz, 1H), 5.24 (s, 1H), 2.86 (t, J = 7.4 Hz, 2H), 2.63 (t, J = 7.4 Hz, 2H), 2.06 - 2.13 (m, 2H), 1.98 (s, 3H), 1.29 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 144.9, 139.2, 128.9, 126.7, 126.0, 122.2, 119.0, 112.6, 51.8, 33.3, 31.5, 28.8, 25.0, 19.0.

*Step 2: 2,2,4-Trimethyl-2,3,4,7,8,9-hexahydro-1H-cyclopenta[h]quinolin-3-ol (**5d**)*. One molar borane/THF solution (200 mL) under ice cooling for over 1 h was added dropwise to a solution of **4d** (23.7 g, 0.111 mol) in THF (200 mL), and the mixture was stirred at 15 °C for 6 h. THF/water (1:1, 100 mL) was added dropwise to the reaction solution over 15 min. Aqueous sodium hydroxide solution (3 N, 67 mL) was added over 15 min and 30% aqueous hydrogen peroxide (22.5 mL) was added dropwise over 5 min. The mixture was stirred at room temperature for 1.5 h. The reaction solution was poured into water, and the mixture was extracted with AcOEt. The organic layer was washed with saturated aqueous sodium hydrogen carbonate and saturated brine and was dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the residue was purified by flash

chromatography and was eluted with a gradient of hexane/AcOEt (20/1) to hexane/AcOEt (3/1). After the evaporation, the product was obtained as a brown oil (23.9 g, 90%). MS *m/z* $[M+H]^+$ 232. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.18 (d, J = 7.7Hz, 1H), 7.08 (br, 1H), 3.30 (d, J = 9.4 Hz, 1H), 3.04 (m, 1H), 2.83 (t, J = 6.9 Hz, 2H), 2.72 (m, 1H), 2.63 (m, 1H), 2.03 (m, 2H), 1.46 (s, 3H), 1.33 (d, J = 6.6 Hz, 3H), 1.11 (s, 3H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 143.7, 134.9, 129.8, 126.9, 121.7, 74.3, 57.3, 35.0, 32.2, 30.1, 24.7, 24.4, 18.1, 17.3.

Step 3: 6-Bromo-2,2,4-trimethyl-2,3,4,7,8,9-hexahydro-1H-cyclopenta[h]quinolin-3-ol ***(6d)***. A solution of bromine (16.0 g, 0.100 mol) in $CHCl_3$ (50 mL) under ice cooling for over 1 h was added to a solution of **5d** (23.9 g, 0.100 mol) in $CHCl_3$ (240 mL) dropwise, and the mixture was stirred at room temperature for 3 h. The reaction solution was adjusted to pH 12 with 10% aqueous sodium hydroxide solution (240 mL) and was poured into water. The mixture was then extracted with $CHCl_3$. The organic layer was washed with water and saturated brine and was dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography and was eluted with a gradient of hexane/AcOEt (10/1) to hexane/AcOEt (3/1). After the evaporation, the product was obtained as a white solid (21.5 g, 71%). MS *m/z* $[M+H]^+$ 310.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.13 (s, 1H) 3.36 (s, 1H), 3.31 (dd, J = 9.4, 6.1 Hz, 1H), 2.90 (t, J = 7.5 Hz, 2H), 2.72 (t, J = 7.5 Hz, 2H), 2.68 - 2.74 (m, 1H), 2.07 - 2.15 (m, 2H), 1.71 (d, J = 6.1 Hz, 1H), 1.41 (d, J = 6.5 Hz, 3H), 1.31 (s, 3H), 1.08 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.6, 138.0, 128.9, 128.7, 124.5, 106.9, 78.4, 52.9, 35.9, 34.5, 30.5, 27.8, 23.8, 21.2, 18.2.

Step 4:

6-Bromo-3-(tert-butyldimethylsilyloxy)-2,2,4-trimethyl-2,3,4,7,8,9-hexahydro-1H-cyclopent a[h]quinoline. Imidazole (9.80 g, 144 mmol) and *tert*-butyldimethylchlorosilane (10.9 g, 72.0 mmol) were added to a solution of **6d** (11.2 g, 36.0 mmol) in DMF (47 mL), and the mixture was stirred at 100 °C for 8.5 h. The reaction solution was diluted with ice water and was adjusted to pH 10 with saturated aqueous sodium hydrogen carbonate. The mixture was then extracted with ethyl acetate. The organic layer was washed with water and saturated brine and was dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography and was eluted with a gradient of hexane to hexane/AcOEt (20/1). After the evaporation, the product was obtained as a slightly orange solid (14.3 g, 96%). MS m/z $[M+H]^+$ 424. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 (s, 1H), 3.37 (t, J = 6.9Hz, 1H), 2.90 (t, J = 5.8 Hz, 2H), 2.73 (brt, 2H), 2.11

(m, 2H), 1.33 (d, J = 5.2 Hz, 3H), 1.25 (s, 3H), 1.06 (s, 3H), 0.96 (s, 9H), 0.14 (s, 3H), 0.12 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 142.4, 138.1, 128.6, 128.6, 125.5, 106.8, 79.6, 53.6, 36.5, 34.5, 30.5, 29.4, 26.2, 23.8, 21.4, 18.8, 18.5, -3.2, -3.2.

Step 5: tert-Butyl 6-Bromo-3-(tert-butyldimethylsilyloxy)-2,2,4-trimethyl-2,3,4,7,8,9-hexahydro-1H-cyclopenta[h]quinoline-1-carboxylate. One point six molar *n*-butyllithium-hexane solution (19.1 mL, 30.5 mmol) was added dropwise over 20 min to a solution of the compound of the previous step (12.3 g, 29.0 mmol) in THF (70 mL) cooled to −45 °C, and the mixture was stirred at −45 °C for 30 min and then at 0 °C for 2 h. Di-tert-butyl dicarbonate (9.80 g, 58.0 mmol) in THF (32 mL) was added to this solution at 0 °C, and the mixture was stirred at room temperature for 6 h. The reaction solution was poured into ice water (150 mL), and the mixture was extracted with ethyl acetate (150 mL × 2). The organic layer was washed with saturated brine and was dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography and was eluted with a gradient of hexane to hexane/AcOEt (9/1). After the evaporation, the product was obtained as a yellow oil (16.2 g, quant.). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (s, 1H) 3.22 (d, J = 5.8 Hz, 1H), 2.91 (m, 2H), 2.74 (m, 2H), 1.93 - 2.20 (m, 2H), 1.50 (s, 3H), 1.46 (s, 9H),

1.30 (s, 3H), 1.27 (d, J = 5.3Hz, 3H), 0.92 (s, 9H), 0.13 (s, 3H), 0.11 (s, 3H).

Step 6: tert-Butyl

*6-Bromo-3-hydroxy-2,2,4-trimethyl-2,3,4,7,8,9-hexahydro-1H-cyclopenta[h]quinoline-1-carboxylate (**7d**).* One molar tetrabutylammonium fluoride-THF solution (170 mL, 170 mmol) was added to a solution of the compound of the previous step (47.0 g, 89.6 mmol) in THF (180 mL), and the mixture was stirred at 60 °C for 3 h. The reaction solution was diluted with ice water, and the mixture was extracted with ethyl acetate. The organic layer was washed with saturated brine and was dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography and was eluted with a gradient of hexane/AcOEt (20/1) to hexane/AcOEt (5/1). After the evaporation, the product was obtained as a colorless powder (31.0 g, 83%). MS m/z $[M+H]^+$ 410. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10 (s, 1H), 3.16 (t, J = 4.7Hz, 1H), 2.90 (m, 2H), 2.74 (m, 2H), 1.93 - 2.20 (m, 2H), 1.91 (d, J = 4.7 Hz, 1H), 1.50 (s, 3H), 1.47 (s, 9H), 1.38 (s, 3H), 1.37 (d, J = 4.7 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 154.1, 143.3, 141.6, 136.1, 134.6, 126.0, 115.6, 81.0, 80.7, 60.5, 36.9, 34.7, 33.0, 28.3, 27.2, 24.6, 20.2, 16.0.

Step 7: tert-Butyl

6-Bromo-2,2,4-trimethyl-2,3,4,7,8,9-hexahydro-1H-cyclopenta[h]quinolin-3-one-1-carboxy

*late (**8d**)*. Pyridinium chlorochromate (23.5 g, 109 mmol) and 4A molecular sieves (29.8 g) awere added to a solution of **7d** (29.8 g, 72.5 mmol) in CH_2Cl_2 (90 mL), and the mixture was stirred at room temperature for 7 h. The reaction mixture was filtered through celite, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography and was eluted with a gradient of hexane to hexane/AcOEt (10/1). After the evaporation, the product was obtained as a colorless powder (25.5 g, 86%). MS *m*/*z* $[M+H]^+$ 408. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.08 (s, 1H), 3.88 (br, 1H), 3.00 - 3.06 (m, 1H), 2.93-2.96 (m, 2H), 2.63 - 2.68 (m, 1H), 2.16 (br, 1H), 1.93 - 2.03 (m, 1H), 1.71 (br, 3H), 1.52 (s, 9H), 1.44 (d, J = 6.7 Hz, 3H), 1.31 (br, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.5, 144.7, 141.2, 134.7, 130.6, 125.3, 117.2, 81.4, 65.5, 34.7, 33.0, 28.4, 25.6, 24.7.

*Step 8: tert-Butyl 6-Bromo-2,2,4,4-tetramethyl-2,3,4,7,8,9-hexahydro-1H-cyclopenta[h]quinolin-3-one-1-carboxylate (**9d**).* One point six molar lithium bis(trimethylsilyl)amide-THF solution (15.7 mL, 16.8 mmol) was added drop wise over 10 min to a solution of **8d** (1.71 g, 4.19 mmol) in THF (29 mL)cooled to −50 °C, and the mixture was heated to −20 °C. Iodomethane (1.05 mL, 16.8 mmol) was the added, and the mixture was stirred at room temperature for 3 h. The reaction mixture was poured into ice water, and the mixture was extracted with ethyl

acetate. The organic layer was washed with saturated brine and was dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography and was eluted with a gradient of hexane to hexane/AcOEt (20/1). After the evaporation, the product was obtained as a white powder (1.60 g, 90%). MS *m*/*z* $[M+H]^+$ 422. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (s, 1H), 2.94 (t, J = 7.5 Hz, 2H), 2.84 (br, 2H), 2.07 (br, 2H), 1.50 (s, 9H), 1.47 (s, 6H), 1.41 (br, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 212.7, 152.4, 144.8, 142.2, 135.2, 134.4, 125.9, 117.0, 81.3, 65.3, 47.7, 34.7, 32.8, 28.4, 24.6.

Step 9:

*6-(3,5-Dimethylisoxazol-4-yl)-2,2,4,4-tetramethyl-2,3,4,7,8,9-hexahydro-1H-cyclopenta[h] quinolin-3-one (**3d**).* (Method A). Two molar aqueous potassium carbonate solution (60 mL, 120 mmol), 3,5-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoxazole (13.4 g, 60.0 mmol), and tetrakis(triphenylphosphine)palladium (0) (3.70 g, 3.20 mmol) were added to a solution of **9d** (16.9 g, 40.0 mmol) in isopropanol (320 mL), and the mixture was stirred at 80 °C for 16 h. The reaction mixture was poured into water, and the mixture was extracted with ethyl acetate. The organic layer was washed with saturated brine and was dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the

residue was purified by flash chromatography and was eluted with a gradient of hexane/AcOEt (20/1) to hexane/AcOEt (3/1). After the evaporation, the *t*-Boc-protected intermediate **10d** was obtained as a white powder (9.15 g). Trifluoroacetic acid (51 mL) was added to the powder (11.3 g) dissolved in $CHCl_3$ (26 mL), and the mixture was stirred at room temperature for 4 h. The reaction mixture was poured into ice water, neutralized with 3N aqueous NaOH solution (220 mL), and extracted with $CHCl_3$. The organic layer was washed with saturated brine and was dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography and was eluted with a gradient of hexane to hexane/AcOEt (4/1). After the evaporation, the product (7.77 g, 46%, two step yield) was obtained as a white powder, mp 192.5-193.1 °C. HRMS-ESI *m*/*z* $[M+H]^+$ calcd for $C_{21}H_{26}N_2O_2$, 339.20670; found 339.20519. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.79 (s, 1H) 2.80 (t, J = 7.5 Hz, 2H), 2.68 (t, J = 7.5 Hz, 2H), 2.27 (s, 3H), 2.16 (s, 3H), 2.11 - 2.18 (m, 2H), 1.44 (s, 6H), 1.36 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 214.8, 164.9, 159.5, 143.4, 138.4, 129.2, 127.4, 125.4, 117.5, 116.3, 59.9, 46.7, 32.4, 29.3, 27.8, 24.7, 24.7, 11.7, 10.7. Found C, 74.67; H, 7.75; N, 8.26. $C_{21}H_{26}N_2O_2$ requires C, 74.53; H, 7.74; N, 8.28%. 99.8% purity at UV220 and 254 nm based on isocratic HPLC (t_R = 4.05 min, CH_3CN/water 60/40).

Synthesis of

2,2,4,4-Tetramethyl-6-([1,2,4]triazolo[4,3-a]pyridin-5-yl)-2,3,4,7,8,9-hexahydro-*1H*-cyclopenta[h]quinolin-3-one (12h). *Step 1: tert-Butyl 2,2,4,4-Tetramethyl-6-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-2,3,4,7,8,9-hexahydro-1H-cyclopenta[h]quinolin-3-one-1-carboxylate (**11**).* Bis(pinacolato)diboron (3.61 g, 14.2 mmol), potassium acetate (2.09 g, 21.3 mmol), and [1,1'-bis(diphenylphosphino)ferrocene]palladium (II) dichloride (260 mg, 0.31 mmol) were added to a solution of the compound of **9d** (3.00 g, 7.10 mmol) in isopropanol (42 mL) , and the mixture was stirred at 80 °C for 6 h. The reaction solution was diluted with ethyl acetate, and the precipitate was filtered off. The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography and was eluted with hexane/AcOEt (20/1). After the evaporation, the product (3.30 g, 100%) was obtained as a brown powder. MS *m*/*z* $[M+H]^+$ 470. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.45 (s, 1H), 3.12 (br, 2H), 2.73 (br, 2H), 2.01 (br, 2H), 1.55 (s, 6H), 1.49 (s, 9H), 1.49 (br, 6H), 1.29 (s, 12H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 213.4, 152.5, 152.3, 139.8, 137.7, 131.7, 129.8, 83.4, 80.9, 65.4, 47.7, 34.2, 31.2, 28.4, 26.6, 24.9.

Step 2:

*2,2,4,4-Tetramethyl-6-([1,2,4]triazolo[4,3-a]pyridin-5-yl)-2,3,4,7,8,9-hexahydro-1H-cyclopenta[h]quinolin-3-one (**12h**).* (Method B). Two molar aqueous potassium carbonate solution (6.4 mL, 12.8 mmol), 5-chloro-1,2,4-triazolo[4,3-a]pyridine (0.654g, 4.26 mmol), and tetrakis(triphenylphosphine)palladium (0) (0.148 g, 0.13 mmol) were added to a solution of **11** (2.00 g, 4.26 mmol) in DMF (30 mL) , and the mixture was stirred at 110 °C for 4 h. The reaction mixture was poured into water, and the mixture was extracted with ethyl acetate. The organic layer was washed with saturated brine and was dried over sodium sulfate. The solvent was evaporated under reduced pressure. Trifluoroacetic acid (20 mL) was added to the residue dissolved in CH_2Cl_2 (20 mL), and the mixture was stirred at room temperature for 1 h. The reaction mixture was poured into ice water, neutralized with 1N aqueous NaOH solution, and extracted with $CHCl_3$. The organic layer was washed with saturated brine and was dried over sodium sulfate. The solvent was evaporated under reduced pressure, and the residue was purified by flash chromatography and was eluted with a gradient of $CHCl_3$ to $CHCl_3$/MeOH (9/1). After evaporation, the product (0.624 g, 41%, two step yield) was obtained as a slightly yellowish powder. HRMS-ESI *m*/*z* $[M+H]^+$ calcd for $C_{22}H_{24}N_4O$, 361.20229; found 361.20100. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (s, 1H), 7.74 (d, J = 9.2 Hz, 1H), 7.44 (dd, J = 9.2, 2.4Hz, 1H), 7.25 (s, 1H), 6.89 (d, J = 6.6

Hz, 1H), 5.61 (s, 1H), 2.87 (t, J = 7.3 Hz, 2H), 2.70 (t, J = 7.3 Hz, 2H), 2.01 - 2.08 (m, 2H), 1.38 (s, 6H), 1.31 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 214.4, 149.3, 142.0, 140.6, 136.5, 135.1, 129.8, 128.2, 126.6, 124.0, 118.9, 113.5, 113.0, 59.1, 45.9, 31.9, 29.6, 26.7, 24.5. 98.3% purity at UV220 nm and 99.4% purity at UV254 nm based on isocratic HPLC (t_R = 2.55 min, CH_3CN/water 40/60).

Synthesis of

6-(2,4-Dimethyl-2H-pyrazol-3-yl)-2,2,4,4-tetramethyl-2,3,4,7,8,9-hexahydro-*1H*-cyclopenta[h]quinolin-3-one (12a). With a method using 5-bromo-1,4-dimethyl-*1H*-pyrazole instead of 5-chloro-1,2,4-triazolo[4,3-a]pyridine in the method B, compound 12a was obtained as a white powder (19 mg, 32%). HRMS-ESI m/z $[M+H]^+$ calcd for $C_{21}H_{27}N_3O$, 338.22269; found 338.22125. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.30 (s, 1H), 6.84 (s, 1H), 3.53 (s, 3H), 2.83 (t, J=7.41 Hz, 2H), 2.57 (t, J=7.61 Hz, 2H), 1.97 - 2.10 (m, 2H), 1.83 (s, 3H), 1.35 (s, 3H), 1.34 (s, 3H), 1.25 - 1.30 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 214.7, 142.9, 140.4, 139.4, 137.7, 129.0, 126.3, 125.0, 116.1, 113.2, 59.1, 45.8, 36.6, 31.9, 29.5, 26.6, 24.7, 24.6, 24.4, 9.0. 97.4% purity at UV 220 nm and 97.0% purity at UV 254 nm based on isocratic HPLC (t_R = 2.12 min, CH_3CN/water 55/45).

Synthesis of

6-(3,5-Dimethyl-3H-imidazol-4-yl)-2,2,4,4-tetramethyl-2,3,4,7,8,9-hexahydro-*1H*-cyclopenta[h]quinolin-3-one (12b). With a method using 5-bromo-1,4-dimethyl-*1H*-imidazole instead of 5-chloro-1,2,4-triazolo[4,3-a]pyridine in the method B, compound 12a was obtained as a white powder (21 mg, 94%). HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{21}H_{27}N_3O$, 338.22269; found 338.22157. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (s, 1H). 6.68 (s, 1H), 3.59 (br, 1H), 3.39 (s, 3H), 2.80 (t, J = 7.5 Hz, 2H), 2.57 - 2.77 (m, 2H), 2.11 - 2.18 (m, 2H), 2.11 (s, 3H), 1.44 (s, 6H), 1.38 (s, 3H), 1.36 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 214.8, 144.5, 138.8, 136.1, 134.9, 129.0, 128.3, 127.2, 126.2, 117.3, 59.9, 46.7, 32.5, 31.9, 29.3, 27.8, 24.8, 24.7, 13.4. 97.9% purity at UV220 nm and 97.5% purity at UV254 nm based on gradient HPLC (t_R = 2.15 min).

Synthesis of 6-(1,3-Dimethyl-1H-pyrazol-5-yl)-2,2,4,4-tetramethyl-2,3,4,7,8,9-hexahydro-*1H*-cyclopenta[h]quinolin-3-one (12c). With a method using 5-bromo-1,3-dimethyl-*1H*-pyrazole instead of 5-chloro-1,2,4-triazolo[4,3-a]pyridine in the method B, compound 12c was obtained as a white powder (17 mg, 31%). HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{21}H_{27}N_3O$, 338.22269; found 338.22150. ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.92 (s, 1H), 5.99 (s, 1H), 5.33 (brs., 1H), 3.59 (s, 3H), 2.81 (t, J = 7.29 Hz, 2H), 2.73 (t, J = 7.43 Hz, 2H), 2.15 (s,

3H), 2.02 (m, 2H), 1.35 (s, 6H), 1.22 - 1.29 (m, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 214.7, 145.5, 143.0, 142.2, 139.2, 129.1, 126.2, 124.3, 116.9, 105.0, 59.1, 45.8, 36.4, 32.3, 29.6, 26.6, 24.6, 24.3, 13.2. 98.4% purity at UV220 nm and 97.7% purity at UV254 nm based on isocratic HPLC (t_R = 2.12 min, CH_3CN/water 55/45).

Synthesis of

6-(1,4-Dimethyl-1H-imidazol-2-yl)-2,2,4,4-tetramethyl-2,3,4,7,8,9-hexahydro-*1H*-cyclo penta[h]quinolin-3-one (12d). With a method using 2-bromo-1,4-dimethyl-*1H*-imidazole instead of 5-chloro-1,2,4-triazolo[4,3-a]pyridine in the method B, compound 12d was obtained as a white powder (19 mg, 32%). HRMS-ESI *m*/*z* $[M+H]^+$ calcd for $C_{21}H_{27}N_3O$, 338.22269; found 338.22153. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.47 (d, J = 0.83 Hz, 1H), 7.26 (s, 1H), 5.86 (s, 1H), 3.66 (s, 3H), 2.84 (m, 4H), 2.29 (d, J = 0.83 Hz, 3H), 2.08 (m, 2H), 1.37 (s, 6H), 1.30 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 213.9, 143.8, 143.7, 142.1, 129.7, 128.2, 126.5, 125.7, 120.2, 108.1, 59.1, 45.8, 34.7, 31.6, 29.6, 26.6, 24.7, 24.3, 9.6. 98.8% at UV220 nm and 97.8% purity at UV254 nm based on isocratic HPLC (t_R = 2.68 min, CH_3CN/water 35/65).

Synthesis of

6-(Imidazo[1,2-a]pyridin-5-yl)-2,2,4,4-tetramethyl-2,3,4,7,8,9-hexahydro-*1H*-cyclopent

a[h]quinolin-3-one (12e). With a method using 5-bromo-imidazo[1,2-a]pyridine instead of 5-chloro-1,2,4-triazolo[4,3-a]pyridine in the method B, compound 12e was obtained as a white powder (41 mg, 77%). HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{23}H_{25}N_3O$, 360.20704; found 360.20555. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.54 (d, J = 8.8 Hz, 1H), 7.43 (s, 1H), 7.29 (dd, J = 8.8, 6.6 Hz, 1H), 7.16 (s, 1H), 6.81 (d, J = 6.6 Hz, 1H), 5.52 (s, 1H), 2.86 (t, J = 7.1 Hz, 2H), 2.63 (t, J = 7.1 Hz, 2H), 1.99 - 2.05 (m, 2H), 1.36 (s, 6H), 1.31 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 214.5, 145.2, 141.9, 140.2, 138.1, 133.1, 129.6, 126.5, 124.5, 123.8, 120.4, 115.2, 112.3, 111.3, 59.1, 45.9, 31.8, 29.6, 26.7, 24.5, 24.4. Found C, 76.07; H, 6.82; N, 11.68. $C_{23}H_{25}N_3O\cdot 0.2H_2O$ requires C, 76.09; H, 7.05; N, 11.57%. 96.9% at UV220 nm and 96.7% purity at UV254 nm based on isocratic HPLC (t_R = 2.31 min, CH_3CN/water 40/60).

Synthesis of 2,2,4,4-Tetramethyl-6-(pyrazolo[1,5-a]pyridin-4-yl)-2,3,4,7,8,9-hexahydro-*1H*-cyclopenta[h]quinolin-3-one (12f). With a method using pyrazolo[1,5-a]pyridyn-4-yl trifluoromethanesulfonate instead of 5-chloro-1,2,4-triazolo[4,3-a]pyridine in the method B, compound 12f was obtained as a gray powder (14 mg, 24%). HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{23}H_{25}N_3O$, 360.20704; found 360.20584.^{1}H NMR (300 MHz, DMSO-d_6) δ 8.61 (d, J =

6.8 Hz, 1H), 7.98 (d, *J* = 2.3Hz, 1H), 7.17 (s, 1H), 7.12 (d, *J* = 6.8 Hz, 1H), 6.93 (dd, *J* = 6.8, 6.8 Hz, 1H), 6.42 (d, *J* = 2.3 Hz, 1H), 5.38 (s, 1H), 2.79 - 2.87 (m, 4H), 1.99 - 2.08 (m, 2H), 1.37 (s, 6H), 1.30 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 214.8, 141.5, 141.3, 139.6, 139.0, 131.4, 129.6, 126.8, 126.3, 124.1, 123.6, 122.4, 112.0, 97.0, 59.2, 45.9, 32.3, 29.6, 26.7, 24.7, 24.5. Found C, 72.67; H, 6.75; N, 15.27. $C_{22}H_{24}N_4O \cdot 0.2H_2O$ requires C, 72.58; H, 6.76; N, 15.39%. 99.5% purity at UV220 and 98.2% purity at UV254 nm based on isocratic HPLC (t_R = 3.33 min, CH_3CN/water 65/35).

Synthesis of

6-(Imidazo[1,2-a]pyrazin-5-yl)-2,2,4,4-tetramethyl-2,3,4,7,8,9-hexahydro-*1H*-cyclopenta[h]quinolin-3-one (12g). With a method using 5-chloro-imidazo[1,2-a]pyrazine instead of 5-chloro-1,2,4-triazolo[4,3-a]pyridine in the method B, compound 12g was obtained as a white powder (22 mg, 37%). HRMS-ESI *m*/*z* $[M+H]^+$ calcd for $C_{22}H_{24}N_4O$, 361.20229; found 361.20083. ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.14 (s, 1H), 7.99 (s, 1H), 7.97 (d, *J* = 1.1 Hz, 1H), 7.87 (s, 1H), 7.27 (s, 1H), 5.68 (s, 1H), 2.87 (t, *J* = 7.5 Hz, 2H), 2.71 (t, *J* = 7.5 Hz, 2H), 2.00 - 2.10 (m, 2H), 1.37 (s, 6H), 1.32 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 214.4, 142.5, 140.7, 140.7, 140.6, 135.4, 131.6, 129.8, 128.4, 126.6, 124.3, 117.0, 112.8, 59.1, 45.9, 31.8, 29.6, 26.7, 24.5, 24.5. 98.7% purity at UV220 nm and 99.0% purity at

UV254 nm based on isocratic HPLC (t_R = 2.76 min, CH_3CN/water 40/60).

Synthesis of

6-(1,2,4-Triazolo[4,3-a]pyridin-8-yl)-2,2,4,4-tetramethyl-2,3,4,7,8,9-hexahydro-*1H*-cyclopenta[h]quinolin-3-one (12i). With a method using 8-bromo-1,2,4-triazolo[4,3-a]pyridine instead of 5-chloro-1,2,4-triazolo[4,3-a]pyridine in the method B, compound 12i was obtained as a white powder (17 mg, 31%). HRMS-ESI *m/z* $[M+H]^+$ calcd for $C_{22}H_{24}N_4O$, 361.20229; found 361.20083. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.32 (s, 1H) 8.53 (dd, J = 6.74, 0.96 Hz, 1H), 7.39 (s, 1H), 7.31 (d, J = 6.33 Hz, 1H), 7.05 (t, J = 6.74 Hz, 1H), 5.38 (brs, 1H), 2.74 - 2.97 (m, 4H), 2.02 (m, 2H), 1.32 - 1.47 (m, 6H), 1.30 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 214.8, 170.2, 148.0, 142.0, 139.3, 136.8, 129.2, 128.7, 125.9, 125.8, 125.0, 122.9, 122.5, 113.7, 79.1, 59.7, 59.2, 45.9, 32.8, 29.5, 26.7, 24.7, 24.4, 20.7, 14.0. 98.3% purity at UV220 nm and 98.7% purity at UV254 nm based on isocratic HPLC (t_R = 2.55 min, CH_3CN/water 40/60).

Synthesis of

2,2,4,4-Tetramethyl-6-(quinolin-4-yl)-2,3,4,7,8,9-hexahydro-*1H*-cyclopenta[h]quinolin-3-one (12j). With a method using 4-chloroquinoline instead of 5-chloro-1,2,4-triazolo[4,3-a]pyridine in the method B, compound 12j was obtained (12 mg,

20%). HRMS-ESI *m*/*z* $[M+H]^+$ calcd for $C_{25}H_{26}N_2O$, 371.21179; found 371.21040. ^1H NMR (300 MHz, DMSO-d_6) δ 9.09 (d, *J* = 5.3 Hz, 1H), 8.19 (d, *J* = 8.3 Hz, 1H), 7.96 (dd, *J* = 8.3, 6.8 Hz, 1H), 7.87 (d, *J* = 8.3 Hz, 1H), 1.33 (s, 6H), 7.72 - 7.77 (m, 2H), 7.07 (s, 1H), 5.63 (br, 1H), 2.88 (br, 2H), 2.73 (br, 1H), 2.59 (br, 1H), 2.00 - 2.05 (m, 2H), 1.37 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 214.5, 141.8, 139.9, 129.5, 127.6, 126.6, 126.3, 125.0, 123.0, 121.7, 59.2, 45.9, 40.4, 40.3, 32.2, 29.6, 26.7, 24.5. 97.4% purity at UV220 nm and 96.4% purity at UV254 nm based on isocratic HPLC (t_R = 2.20 min, CH_3CN/water 45/55).

Synthesis of

2,2,4,4-Tetramethyl-6-([1,8]naphthyridin-4-yl)-2,3,4,7,8,9-hexahydro-*1H*-cyclopenta[h]quinolin-3-one (12k). With a method using 4-chloro-1,8-naphthyridine instead of 5-chloro-1,2,4-triazolo[4,3-a]pyridine in the method B, compound 12k was obtained as a white powder (19 mg, 32%). HRMS-ESI *m*/*z* $[M+H]^+$ calcd for $C_{24}H_{25}N_3O$, 372.20704; found 372.20574. ^1H NMR (400 MHz, DMSO-d_6) δ 9.18 (dd, *J* = 4.5, 1.9 Hz, 1H), 9.16 (d, *J* = 4.9 Hz, 1H), 8.32 (dd, *J* = 8.7, 1.9 Hz, 1H), 7.77 (dd, *J* = 8.7, 4.5 Hz, 1H), 7.74 (d, *J* = 4.9 Hz, 1H), 7.06 (s, 1H), 5.63 (s, 1H), 2.89 (t, *J* = 6.8 Hz, 2H), 2.67 - 2.73 (m, 2H), 2.00 - 2.05 (m, 2H), 1.37 (s, 6H), 1.32 (s, 6H). ^{13}C NMR (101 MHz, DMSO-d_6) δ 214.7, 156.0,

153.1, 152.8, 149.4, 141.7, 139.6, 135.7, 129.4, 126.4, 125.0, 122.8, 122.3, 122.2, 121.3, 59.2, 45.9, 32.2, 29.6, 26.7, 24.5. Found C, 76.59; H, 6.90; N, 10.99. $C_{24}H_{25}N_3O \cdot 0.3H_2O$ requires C, 76.49; H, 6.85; N, 11.15%. 99.9% purity at UV220 and 254 nm based on isocratic HPLC (t_R = 2.84 min, CH_3CN/water 40/60).

Docking simulation. The docking experiment was conducted using the GLIDE program.[18] The protein structures were built based on the "active" and "passive" antagonist conformation from the X-ray crystal structure of hGR LBD with mifepristone at 2.80 Å resolution (PDB ID: 3H52).[14] The original protein structure was prepared following the standard "Protein Preparation Wizard" in Maestro.[19] The initial 3D coordinates of **3d** were generated using LigPrep.[20]

Pharmacology

Steroid receptor binding assays. *GR.* The cytosol fraction including the human recombinant GR (P2812, Life Technologies) was incubated at 4 °C for 30 min with 5 nM (or 22 nM in some experiments) [^{3}H]-dexamethasone (85–91 C_i/mmol; TRK645, GE Healthcare) in the absence or presence of a 200-fold excess of dexamethasone (Sigma), all prepared in an assay buffer consisting of 25 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 mM DTT, and 0.1% bovine serum albumin. The incubation volume was 50 µL. The incubation

was terminated with the addition of a volume of dextran-coated charcoal (0.75% Norit® "SX-II" and 0.75% 70-kD dextran in the assay buffer). This mixture was shaken and incubated at 4 °C for 10 min and was then centrifuged at 2000 rpm for 5 min (or at 3000 rpm for 10 min). The aliquots of the supernatants were then transfused into a glass filter (Printed filtermat A, PerkinElmer) and were then dried. The solid scintillator (MeltiLex A, PerkinElmer or MicroScinti40, Packard) was mounted on the filter and melted by heat. After cooling, the radioactivity was counted using 1450 MicroBeta Trilux (PerkinElmer) or TopCount (PerkinElmer). Specific binding was defined as the difference between bindings measured in the absence (total) and presence (nonspecific) of dexamethasone.

PR. The cytosol fraction including the human recombinant PR-B isoform (P2835, Life Technologies) was incubated at 4 °C for 30 min with 5 nM [^{3}H]-progesterone (90–103 C_i/mmol; NET-381, PerkinElmer) in the absence or presence of a 200-fold excess of progesterone (Sigma), all prepared in an assay buffer consisting of 10 mM Tris-HCl (pH 7.4), 10 % glycerol, 1 mM DTT and 0.1 % bovine serum albumin. The incubation volume was 50 μL. The remaining procedures were identical to those of the GR binding assay. Specific binding was defined as the difference between the bindings measured in the absence (total) and presence (nonspecific) of progesterone.

MR. The cytosol fraction including the human recombinant MR (Mitsubishi Tanabe Pharma) was incubated at room temperature for 30 min with 4 nM [^{3}H]-aldosterone (73–88 C_i/mmol; NET-419, PerkinElmer) in the absence or presence of a 250-fold excess of aldosterone (Steraloids), all prepared in an assay buffer consisting of 20 mM Tris-HCl (pH 7.4), 1 mM EDTA, 10% glycerol, and 20 mM sodium tungstate. The incubation volume was 50 μL. The remaining procedures were identical to those of the GR binding assay. Specific binding was defined as the difference between the bindings measured in the absence (total) and presence (nonspecific) of aldosterone.

AR and ER. Binding assays for human AR, human ERα, and human ERβ were performed at Cerep (France, www.cerep.fr/). Briefly, affinity to AR was determined using the binding of [^{3}H]methyltrienolone to cytosol AR of LNCaP cells. Affinities to ERs were examined using human recombinant ERα expressed in Sf9 cells and ERβ in Hi5 cells with the fluorescence polarization method using fluormone^{TM}ES2.

Determination of K_i values for GR, PR and MR. The values of dissociation constants (K_d) were determined beforehand by Scatchard analysis as follows: 3.1 nM (or 5.6 nM in some experiments) for GR, 3.5 nM for PR, and 2.0 nM for MR. The IC_{50} values to inhibit the specific binding by 50% were estimated from the nonlinear regression (Prism 3.0). K_i

values were obtained from the equation $Ki = IC_{50}/(1 + C/K_d)$, where C is the concentration of each radioligand used in the experiment, and K_d is the dissociation constant of the radioligand (Microsoft Excel 2000).

Luciferase reporter assay. CHO-K1 cells were stably transfected with the two copies of a consensus glucocorticoid responsive element site positioned upstream of a thymidine kinase promoter driving a luciferase gene and separate neomycin gene to confer the resistance to geneticin.[21] These cells were grown in Dulbecco's modified Eagle's medium/nutrient mixture F-12 containing 10% fetal bovine serum, 2.5 nM L-glutamine, 50 U/mL penicillin, 25 μg/mL streptomycin, and 500 μg/mL geneticin sulfate (G418) at 37 °C under 5% CO_2/95% air. The cells were detached using trypsin-EDTA, centrifuged, and suspended in Opti-MEM reduced serum medium (Invitrogen). The cells were then seeded into white 96-well plates (Corning) at a density of 2×10^4 cells per well. After overnight incubation, the cells were treated with the compound for the evaluation and 10 nM dexamethasone. After a 6-h incubation period, the luciferase activity assay was conducted by adding the luciferase substrate Bright-Glo™ (Promega) into each well. The agonist activity of the compounds was evaluated at 1 μM concentration in the same assay system in the absence of stimulation with dexamethasone.

Forced swimming test. The experiments were performed on male Wistar rats (8–9 weeks old). The animals were kept at a room temperature of 21–23°C in a normal day-night cycle (light from 7:00 to 19:00). They had free access to food and tap water before the experiment. The forced swimming test was performed using the method of Porsolt.[16] Briefly, the rats were placed for 15 min individually in transparent cylinders (40 cm in height, 19 cm in diameter) containing 15 cm of water (25 °C). The water was changed at the beginning of each session. The rats were wiped with dry towels and were then returned to their home cages. After 24 h, they were placed again in the cylinder for 5 min for test sessions. The test sessions were recorded by a video camera. The test compounds were administered 23.5, 5, and 1 h before the test sessions. All drugs were freshly prepared. Mifepristone (Sigma-Aldrich) and **3d** were suspended in saline with 2–3% Tween 80. All drugs and vehicles were orally administered at a volume of 1 or 3 mL/kg.

An observer blind to the treatments measured the immobility time during the 5-min test sessions. The rats were judged to be immobile only if they took the minimum movement necessary to keep their noses just above the water. The immobility time was analyzed by Student's *t*-test or Dunnett's two-tailed test. All the procedures were conducted in accordance with the guidelines for animal experimentation set by the Ethics Committee for

Animal Use at Mitsubishi Pharma Corporation.

In Vivo Pharmacokinetic Experiments

Intravenous and oral administration to rats. Compound **3d** was intravenously administrated at 1 mg/kg (n = 4) or orally at 10 mg/kg (n = 4) to male Sprague–Dawley rats. Blood samples were collected from the jugular vein at 0.05, 0.25, 0.5, 1, 2, 4, 8, and 24 h and at 0.25, 0.5, 1, 2, 4, 6, and 24 h after the administration in i.v.- and p.o.-treated rats, respectively. Heparin sodium salt was used as anticoagulant and plasma was obtained from blood samples by centrifugation.

Estimation of tissue-to-plasma concentration ratio. Compound **3d** was orally administered at 30 mg/kg to male Sprague–Dawley rats (n = 3 at each time point). The animals were sacrificed at 1 and 3 h after the administration, and blood was collected with heparin sodium salt. The brain was removed and rinsed with saline, weighed, and was homogenized in five volumes (w/v) of saline.

Procedure for pharmacokinetic analysis. The concentration of compound **3d** in the plasma and brain homogenates was determined with liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS). Pharmacokinetic parameters were calculated with non-compartmental analysis.

Supporting Information Available

Additional SAR information and experimental details, and ^{1}H and ^{13}C NMR spectrum. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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Abbreviations used

GR, glucocorticoid receptor; PR, progesterone receptor; MR, mineralocorticoid receptor; AR, androgen receptor; ER, estrogen receptor; LBD, ligand-binding domain

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Figure legend

Figure 1. Chemical structures of mifepristone and tetrahydroquinolines.

Figure 2. Docking study of compound **3d** (magenta) in the active and passive antagonist conformations of GR. (A) Active antagonist conformation. (B) Passive antagonist conformation.

Scheme legend

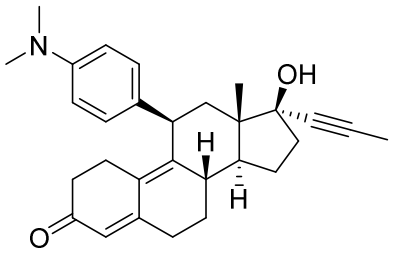
Scheme 1. Synthesis of tetrahydroquinoline analogue [a]

[a] Reagents and conditions: (i) $Sc(OTf)_3$, acetone, room temperature, or I_2, catechol, reflux; (ii) BH_3 in THF; (iii) 3 N aq NaOH, 30% aq H_2O_2; (iv) Br_2, $CHCl_3$; (v) TBDMS, imidazole, DMF; (vi) n-BuLi in hexane, Boc_2O, THF; (vii) 1 M n-Bu_4NF in THF; (viii) PCC, CH_2Cl_2; (ix) LiHMDS in THF, MeI, THF; (x) 3,5-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoxazole, 2 M K_2CO_3, $Pd(PPh_3)_4$, DMF or iPrOH; (xi) TFA, CH_2Cl_2 or $CHCl_3$.

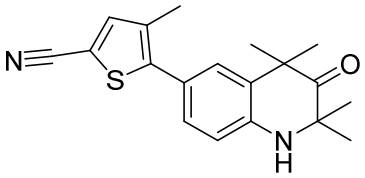
Scheme 2. Synthesis of hexahydrocyclopentaquinoline analogue [a]

[a] Reagents and conditions: (xii) bis(pinacolato)diboron, $PdCl_2$(dppf), KOAc, isopropanol; (xiii) Aryl halide, 2 M K_2CO_3, $Pd(PPh_3)_4$, DMF.

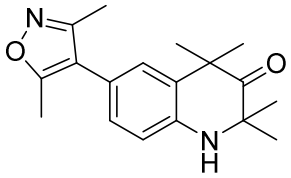
Figure 1.



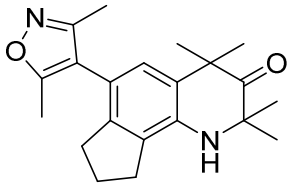
mifepristone **1**
hGR (Ki): 6 nM
hPR (Ki): 15 nM



2

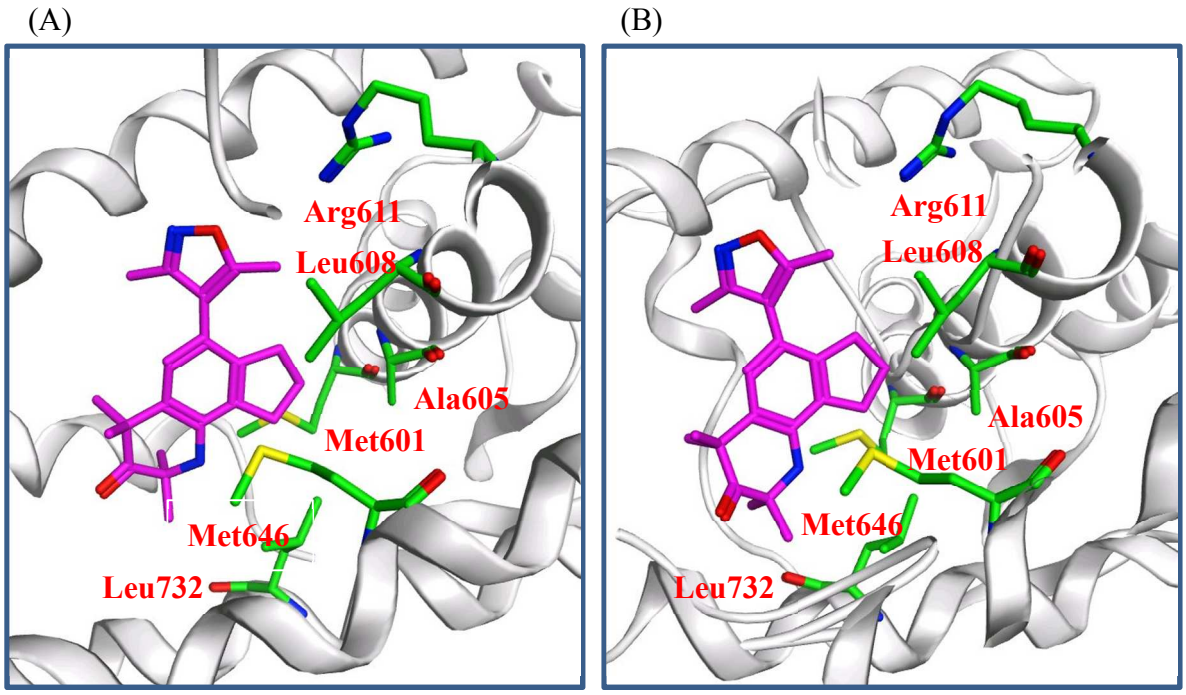


3a
hGR (Ki): 16 nM
hPR (Ki): 22 nM

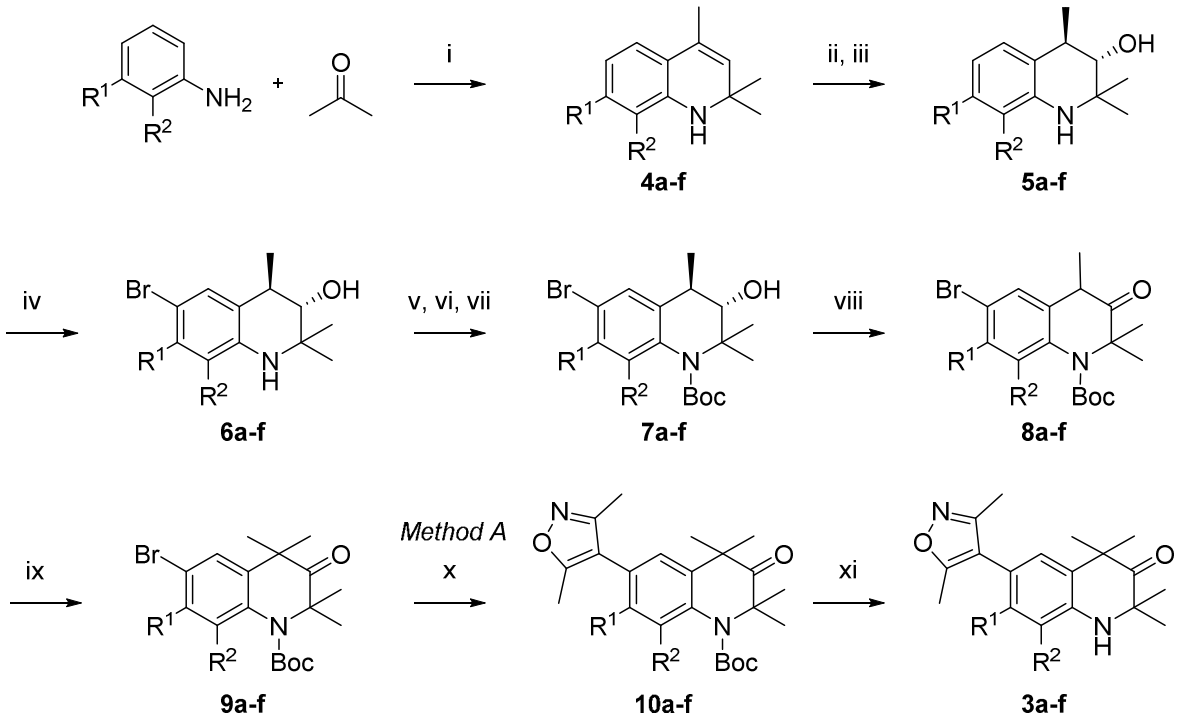


3d
hGR (Ki): 15 nM
hPR (Ki): 1479 nM

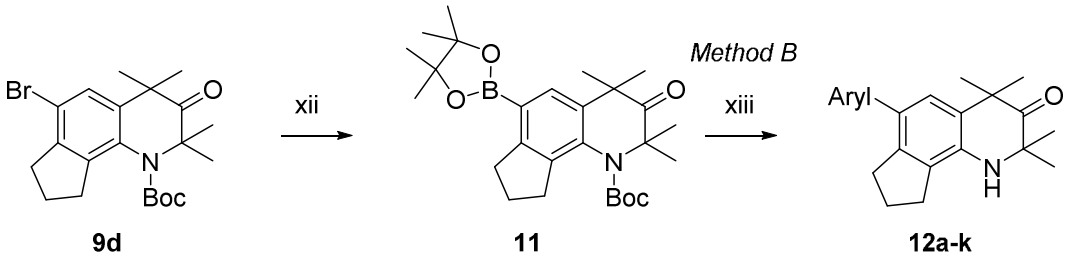
Figure 2.


(A)
Arg611
Leu608
Ala605
Met601
Met646
Leu732
(B)
Arg611
Leu608
Ala605
Met601
Met646
Leu732

Scheme 1.


i
ii, iii
iv
v, vi, vii
viii
ix
Method A
x
xi
4a-f
5a-f
6a-f
7a-f
8a-f
9a-f
10a-f
3a-f

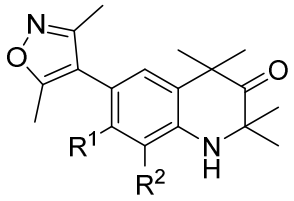
Scheme 2.



9d **11** **12a-k**

TABLES

Table 1. SAR for substituent on tetrahydroquinoline



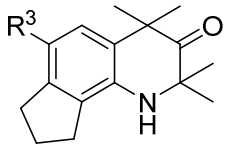
Compound	R^1	R^2	hGR				hPR	hMR
				Reporter gene assay				
			Ki (nM)	IC_{50} (nM) [a]	Inhibition (%) [b]	Agonist activity [c]	Ki (nM)	Ki (nM)
3a	H	H	16	37	96	NT	22	337
3b	Cl	H	151	<30	98	+8%	403	>3000
3c	OMe	H	146	NT	NT	NT	NT	NT
3d	$-(CH_2)_3-$		15	30	96	+4%	1479	>3000
3e	$-(CH_2)_4-$		106	167	70	+1%	NT	NT
3f	$-OCH_2O-$		55	77	88	+1%	2269	>3000

[a] inhibition of transcription activity of hGR (reporter gene assay). NT = not tested.

[b] inhibition at 300 nM.

[c] percent change from baseline at 1 μM without dexamethasone (cf. typical change by 10 nM dexamethasone: +200%).

Table 2. SAR for isoxazole moiety (R^3) at the 6 position on tetrahydroquinoline



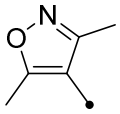
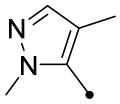
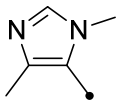
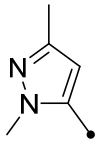
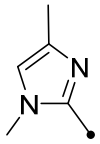
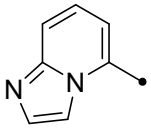
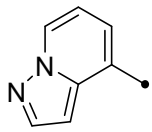
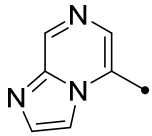
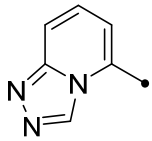
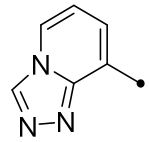
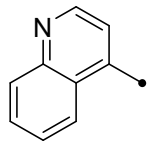
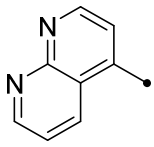
Compound	R^3	hGR				hPR	hMR
			Reporter gene assay				
		Ki (nM)	IC_{50} (nM) [a]	Inhibition (%) [b]	Agonist activity [c]	Ki (nM)	Ki (nM)
3d		15	30	96	+4%	1479	>3000
12a		28	128	79	+7%	>1250	NT
12b		69	150	63	+9%	NT	NT
12c		>384	NT	NT	NT	NT	NT
12d		>384	NT	NT	NT	NT	NT
12e		11	21	92	+4%	>1250	>1000

Table 2 (Continued)

Compound	R^3	hGR				hPR	hMR
		Ki (nM)	Reporter gene assay			Ki (nM)	Ki (nM)
			IC_{50} (nM) [a]	Inhibition (%) [b]	Agonist activity [c]		
12f		35	84	103	-24%	1194	NT
12g		46	93	89	-3%	>1250	>3333
12h		53	91	87	-5%	>1250	>1000
12i		>384	NT	NT	NT	NT	NT
12j		151	NT	NT	NT	NT	NT
12k		35	53	92	-6%	>1250	>1000

[a] inhibition of transcription activity of hGR (reporter gene assay). NT = not tested.

[b] inhibition at 300 nM.

[c] percent change from baseline at 1 μM without dexamethasone.

Table 3. Cross-reactivity of **3d** to steroid receptors

Compound	Ki (nM)					
	hGR	hPR	hMR	hERα	hERβ	hAR
3d	15	1479	>3000	>2300	>2300	>2300

Table 4. Pharmacokinetic profiles of compound **3d** in SD rats [a]

Route	Dose (mg/kg)	$C_{o/max}$ (ng/mL)	t_{max} (h)	AUC_{0-t} (ng·h/mL)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng·h/mL)	CL_t (mL/h/kg)	Vd_{ss} (mL/kg)	$BA_{0-\infty}$ (%)
iv	1	633 ± 52		410 ± 53	3.2 ± 0.8	460 ± 80	2227 ± 399	6131 ± 946	
po	10	273 ± 45	3.5 ± 1.0	2420 ± 870	4.6 ± 1.8	2715 ± 528			59 ± 12

[a] mean ± standard deviation (n = 3)

Table 5. The effect of **3d** and mifepristone in the forced swimming test in rats

Compd	dose [a] (mg/kg)	immobility time [b] (s)	% of vehicle (%)
vehicle		232.2 ± 10.3	100.0
3d	10 × 3	206.8 ± 4.1	89.1
3d	30 × 3	176.2 ± 13.6 **	75.9
3d	100 × 3	187.3 ± 9.7 *	80.7
vehicle		211.3 ± 10.2	100.0
3d	30 × 3	170.7 ± 9.3 #	80.8
mifepristone	10 × 3	187.0 ± 12.3	88.5
mifepristone	30 × 3	169.3 ± 10.5 *	80.1

[a] Mifepristone and **3d** were orally administered 23.5, 5 and 1 h before the test. [b] Values are the mean ± S.E.M. (n = 6 per group). **; $p < 0.01$, *; $p < 0.05$ vs. vehicle (Dunnett's two-tailed test). #; $p < 0.05$ vs. vehicle (t-test).