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Ergoline-Derived Inverse Agonists of the Human H3 Receptor for the Treatment of Narcolepsy

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Ergoline derivative (6aR,9R)-4-(2-(dimethylamino)ethyl)-N-phenyl-9-(pyrrolidine-1-carbonyl)-6,6a,8,9-tetrahydroindolo[4,3-fg]quinoline-7(4H)-carboxamide (**1**), a CXCR3 antagonist, also inhibits human histamine H3 receptors (H3R) and represents a structurally novel H3R inverse agonist chemotype. It displays favorable pharmacokinetic and in vitro safety profiles, and served as a lead compound in a program to explore ergoline derivatives as potential drug candidates for the treatment of narcolepsy. A key objective of this work was to enhance the safety and efficacy profiles of **1**, while minimizing its duration of action to mitigate the episodes of insomnia documented with previously reported clinical candidates during the night following administration. Modifications to the ergoline core at positions 1, 6 and 8 were systematically investigated, and derivative **23** (1-((4a*R*,8*R*,9a*R*)-8-(hydroxymethyl)-1-(2-((*R*)-2-methylpyrrolidin-1-yl)ethyl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-6(1*H*)-yl)ethanone) was identified as a promising lead compound. Derivative **23** has a desirable pharmacokinetic profile and demonstrated efficacy by enhancing brain concentrations of tele-methylhistamine, a major histamine metabolite. This validates the potential of the ergoline scaffold to serve as a template for the development of H3R inverse agonists.

Introduction

The human H3 receptor (H3R) has been the subject of extensive research as a potential drug target since its discovery in 1983[1] and subsequent cloning in 1999.[2] H3R inverse agonists have been proposed as agents for the treatment of numerous maladies,[3] ranging from cardiovascular, inflammatory, respiratory and pain disorders to migraine, attention deficit hyperactivity disorder (ADHD), as well as multiple sclerosis[4] and Tourette's syndrome.[5] A particular focus of research has been on the development of H3R inverse agonists for the management of sleep disorders such as narcolepsy.

The underlying cause of human narcolepsy is the degeneration of orexinergic neurons,[6] which leads to decreased orexin and histamine levels in the cerebrospinal fluid (CSF). Narcoleptic patients suffer from sleep disturbances manifested as excessive daytime sleepiness, frequent sleep-onset rapid eye movement (SOREM) periods and sleep paralysis.[7] They also experience cataplectic events, expressed as sudden episodes of muscle weakness, which may be precipitated by strong emotional responses or rapidly initiated physical activity.

Orexinergic neurons promote arousal via activation of the histaminergic system in the tubero-mamillary nucleus, where the presynaptic H3R limit histamine release and regulate wakefulness.[8,9] A deficient orexin system results in narcolepsy,[10] and inverse agonists capable of dampening the histamine negative feedback at the H3R have the potential to be of therapeutic benefit by elevating histamine to promote wakefulness and thus compensate for the loss of orexinergic tone.

Orexin-deficient (orexin−/−) mice exhibit behavioral arrests similar to cataplectic episodes in humans, and therefore, these mice are considered to be a suitable animal model for this condition.[11] H3R inverse agonists that decrease narcoleptic episodes in orexin−/− mice[12] can reasonably be expected to reverse narcolepsy and possibly even cataplexy in humans.

Pitolisant (Figure 1), the most advanced H3R inverse agonist clinical candidate, was shown to be efficacious in the treatment of narcolepsy.[13] After one week treatment with pitolisant, narcoleptic patients showed a significant decrease in the sleepiness score, as well as sleep suppression. This suggests that by elevating brain histamine transmission, an H3R inverse agonist can be used for treating narcolepsy. The prolonged duration of action of pitolisant ($T_{1/2}$ = 11 h), however, leads to the

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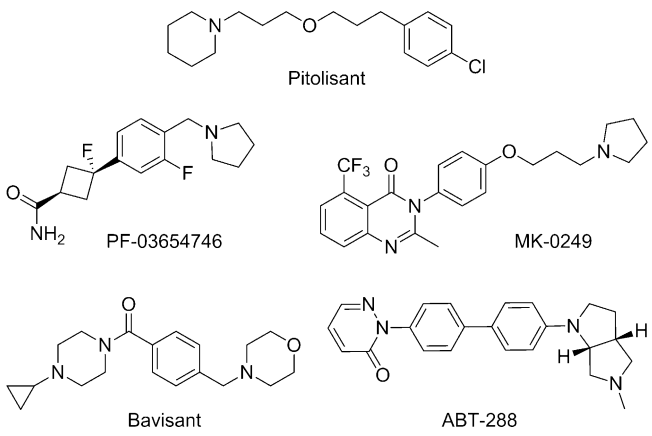


Figure 1. Clinical candidate H3R inverse agonists.

undesirable effect of insomnia.[14,15] Other drug candidates, such as PF-03654746 ($T_{1/2}$ = 9–18 h),[16] MK-0249 ($T_{1/2}$ = 14 h),[17] JNJ-31001074 (bavisant; $T_{1/2}$ = 13 to 20 h),[18] and ABT-288 ($T_{1/2}$=40–61 h)[19] also have prolonged duration of action. This severely limits their administration at doses that are efficacious for treating narcolepsy while avoiding insomnia. The recently described clinical candidate AZD5213 displays a half-life of approximately 5 h.[20] Clinical positron emission tomography (PET) imaging confirmed its fast receptor binding and rapid disengagement kinetics in the human brain, resulting in a pharmacokinetic profile predicted to be compatible with daytime efficacy and a limited risk of insomnia during the following night.

The objective of the present work was to identify a compound with the potential to demonstrate efficacy in narcolepsy patients, without concomitant insomnia during the following night. Our working hypothesis was predicated on the notion that achieving a rapid, high H3R occupancy, combined with rapid clearance, would lead to the selection of a candidate fulfilling the desired profile.

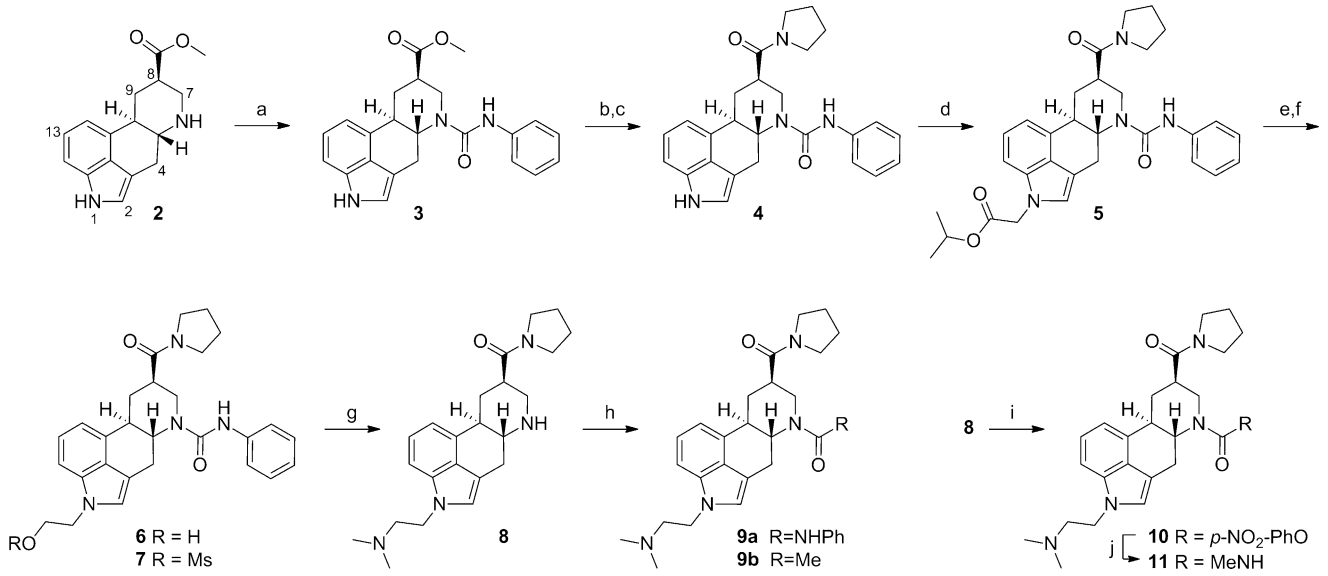
Results and Discussion

Chemistry

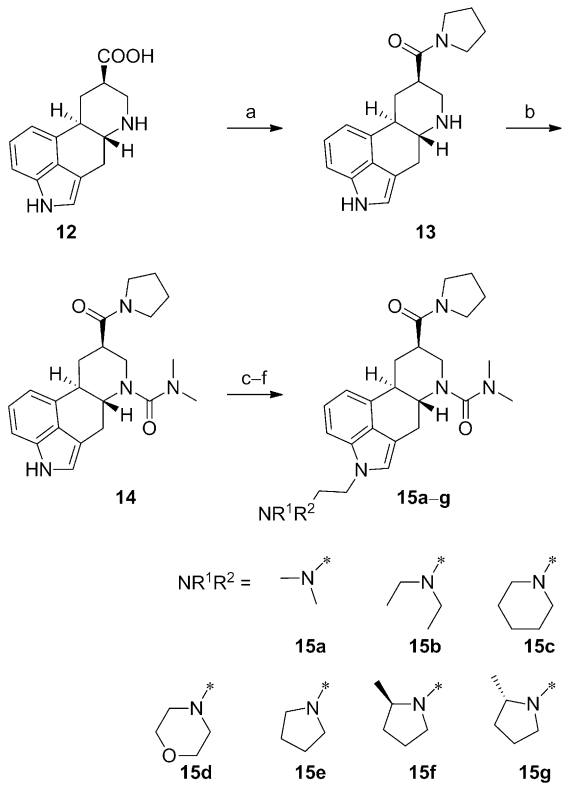
The modifications of the ergoline skeleton in lead compound **1** were focused on positions 1, 6 and 8. As depicted in Scheme 1, to access position-6 analogues, the penultimate compound (**8**) was prepared by treating ergoline-8-carboxylic acid methyl ester **2**[21] with isocyanatobenzene to afford urea **3**. Hydrolysis of the methyl ester, followed by coupling with pyrrolidine in the presence of 2-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexa- fluorophosphate (HATU) gave carboxamide **4**. Alkylating **4** with isopropyl 2-bromoacetate yielded the isopropyl acetate (**5**), which was then reduced and mesylated to afford **7**. Displacement of the mesylate with dimethylamine also simultaneously unmasked the 7-amino group to provide amine **8**. The latter compound was reacted with isocyanatobenzene and acetyl chloride to afford **9a** and **9b**, respectively. Compound **11** was obtained from **8** via the activated 4-nitrophenyl carbamate intermediate **10**.

The syntheses of **15a–g** with variations in position 1 are depicted in Scheme 2. Ergoline-8-carboxylic acid **12** was sequentially coupled with pyrrolidine and dimethylcarbamic chloride to afford **14**. Target molecules **15a–g** were readily obtained from compound **14**, introducing the side chain in a manner similar to the procedure described for compounds **9a** and **9b**. The low isolated yields for some analogues were primarily attributable to their poor solubility in purification solvents.

Compounds **18a–d**, with variations on position 8, were accessed as illustrated in Scheme 3. Starting from ergoline-8-car-



Scheme 1. Variation of the substituent at position 6. *Reagents and conditions*: a) isocyanatobenzene, CH_2Cl_2, RT, 12 h, 90%; b) 1 M NaOH, THF/H_2O (4:1), RT, 2 h, 89%; c) pyrrolidine, HATU, Et_3N, CH_2Cl_2, RT, o/n, 73%; d) isopropyl 2-bromoacetate, TBAI, NaOH, CH_2Cl_2, 0 °C→RT, o/n, 90%; e) $LiBH_4$, THF, 0 °C→RT, o/n, 63.5%; f) MsCl, Et_3N, CH_2Cl_2, 0 °C→RT, 4 h; g) Me_2NH, MeCN, 50 °C, sealed tube, o/n, 68% (two steps); h) For **9a**: isocyanatobenzene, CH_2Cl_2, RT, 12 h, 16%; For **9b**: AcCl, DIPEA, CH_2Cl_2, 0 °C→RT, 4 h, 37%; i) 4-nitrophenyl chloroformate, DIPEA, CH_2Cl_2, 0 °C→RT, o/n, 74%; j) $MeNH_2$, DIPEA, DMAP, CH_2Cl_2, RT, o/n, 22%.



Scheme 2. Variation of the side chain at position 1. *Reagents and conditions*: a) HATU, pyrrolidine, DMF, RT, o/n, 76%; b) dimethylcarbamic chloride, DIPEA, DMAP, MeCN, 65 °C, 4 h, 71%; c) isopropyl 2-bromoacetate, TBAI, NaOH, CH_2Cl_2, 0 °C→RT, o/n, 67%; d) $LiBH_4$, THF, 0 °C→RT, o/n, 81%; e) MsCl, Et_3N, CH_2Cl_2, 0 °C→RT, o/n, 55%; f) amines, MeCN, 50 °C, o/n, 10–68%.

boxylic acid **12**, acylation of position 6 with acetic anhydride afforded **16**. Esterification of position 8 with benzyl chloride, and alkylation of position 1 with isopropyl 2-bromoacetate, followed by debenzylation under hydrogenation conditions, gave acid **17**. The latter was coupled with various amines, and the isopropyl ester group was reduced, mesylated and reacted with (*R*)-2-methylpyrrolidine to yield products **18a–d**.

Cyclization of **16** with (*E*)-*N*′-hydroxyacetimidamide gave the 1,2,4-oxadiazole derivative **19**. The latter compound was subjected to a similar sequence of reactions as described above for **18a–d** to yield compound **20**. Beginning with compound **17**, selective reduction of the isopropyl ester group with lithium borohydride, followed by esterification with methanol and thionyl chloride gave methyl ester **21**. Mesylation and displacement of the mesylate with (*R*)-2-methylpyrrolidine afforded **22**. Straightforward reduction of the methyl ester led to alcohol **23**, and thereafter to the methyl ether **24**.

Pharmacological evaluation

The present work had its genesis with the observation that ergoline derivative **1** (Figure 2), previously reported as a CXC chemokine receptor 3 (CXCR3) inhibitor,[22] also has potent affinity for human H3 receptors (K_i = 8.3 nM). Its selectivity against a panel of more than 100 receptors, proteases, and kinases (data not shown), its favorable pharmacokinetic properties in rats,[23a] and its unique structure relative to previously reported H3R inverse agonists provided the impetus to further explore the optimization of its properties. Inverse agonism of **1** was proven in GTPγS assays (data not shown), based on the well-described activation of histamine release by H3R inverse agonists.[1] Additional incentive to pursue the investigation of **1** as an H3R inverse agonist was provided by preliminary in vivo experiments, which confirmed that it shows histaminergic activities in rats after oral administration[23b] and strongly decreased the narcoleptic episodes in orexin-deficient mice (Figure 3).

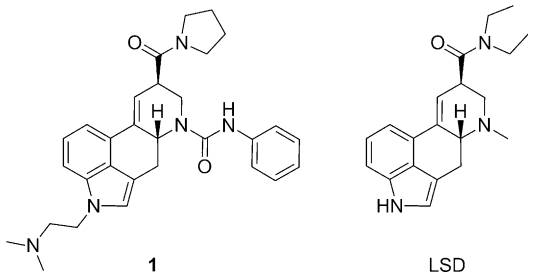


Figure 2. Comparison of the core structures of ergoline (**1**) and lysergic acid diethylamide (LSD).

At the outset of the derivatization effort, we identified and sought to address several potential deficiencies associated with the ergoline derivative **1**. As is evident in Figure 2, essentially the entire structure of lysergic acid diethylamide (LSD) is embedded within the lead compound **1**. Therefore, one concern was that the demonstrated selectivity of **1** versus serotonin, adrenergic and dopamine receptors (Table 1) could be eroded subsequent to derivatization, and that these analogues or their corresponding metabolites might display unwanted LSD-like mind-altering effects.[24] Another concern was the

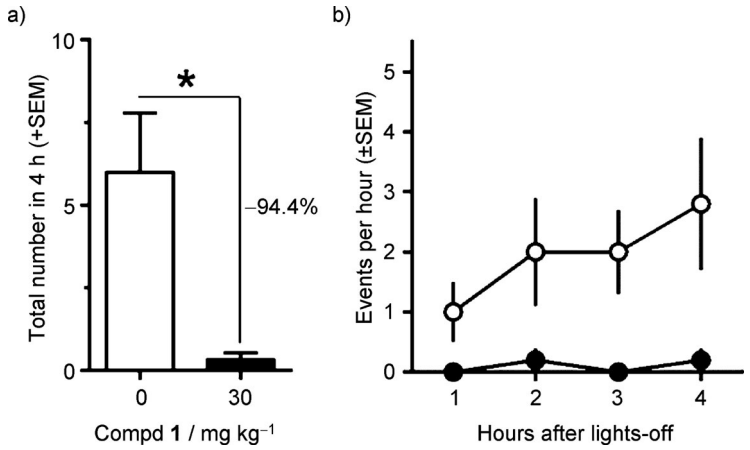
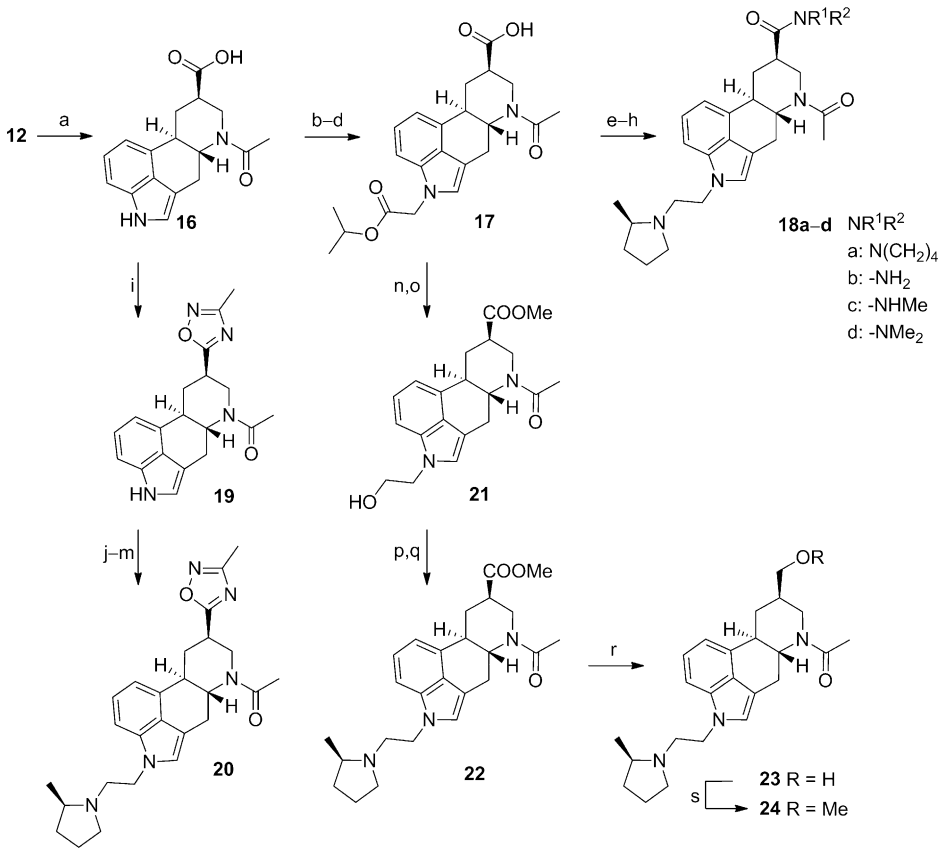


Figure 3. Effect of a single 30 mg kg^{-1} dose of orally administered **1** on behavioral arrests in orexin−/− mice. a) The mean total number and b) the time course of behavior arrests in orexin-deficient mice after oral administration of vehicle (○) or compound **1** at 30 mg kg^{-1} (●). Compound **1** significantly decreased the number of behavioral arrests (Wilcoxon matched-pairs signed rank test: W = −21.00, p = 0.03).



Scheme 3. Variation of the substituent at position 8. *Reagents and conditions*: a) Et_3N, Ac_2O, THF, 0 °C→RT, o/n, 86%; b) CbzCl, Et_3N, DMAP, 0 °C→RT, 1 h, 71%; c) isopropyl 2-bromoacetate, TBAI, NaOH, CH_2Cl_2, 0 °C→RT, o/n, 79%; d) H_2, Pd/C THF, RT, o/n, 74%; e) R_1R_2NH, HATU, DIPEA, DMF, RT, 4 h, 78–88%; f) $LiBH_4$, THF, 0 °C→RT, o/n; g) MsCl, Et_3N, CH_2Cl_2, 0 °C→RT, o/n, 82–88%; h) (*R*)-2-methylpyrrolidine, MeCN, Et_3N, 50 °C, o/n, 31–35%; i) (*E*)-*N*′-hydroxyacetimidamide, HATU, DIPEA, RT→100 °C, 4 h, 60%; j) isopropyl 2-bromoacetate, NaOH, TBAI, 0 °C→RT, o/n, 80%; k) $LiAlH_4$, THF, −78 °C, 10 min, 70%; l) Et_3N, CH_2Cl_2, MsCl, 0 °C→RT, 4 h, 75%; m) (*R*)-2-methylpyrrolidine, MeCN, 60 °C, o/n, 85%; n) 1. $LiBH_4$, THF, 0 °C→RT, o/n; 2. AcOH, 0 °C, 30 min, 44%; o) $SOCl_2$, CH_3OH, 0 °C→RT, 4 h, 83%; p) MsCl, Et_3N, CH_2Cl_2, 0 °C→RT, 4 h, 88%; q) (*R*)-2-methylpyrrolidine, MeCN, Et_3N, 50 °C, o/n, 33%; r) $LiBH_4$, THF, 0 °C→RT, o/n, 40%; s) CH_3I, NaH, THF, RT, o/n, 23%.

chemical reactivity of the D-ring double bond common to both **1** and LSD, which renders them photosensitive and susceptible to Michael addition.[25] To circumvent these issues, we probed the hydrogenation of this double bond, which led to chemically more stable derivatives in line with published data for dihydro-LSD (lumi-LSD).[25] Thus the transformation of **1** to **9a** yielded a compound that retains full H3R activity but displays dramatically diminished affinity for serotonin, adrenergic and dopamine receptors, as well as for CXCR3. A final shortcoming associated with ergoline derivative **1** was the potential to induce phospholipidosis, a problem common to many cationic amphiphilic compounds acting at H3R.[26] Both **1** and **9a** induced strong signals in an in vitro phospholipidosis assay,[27] (204% and 218%, respectively, versus the positive control amiodarone at 50 μM; Table 2). This activity was eliminated by transforming the phenyl urea in **1** and **9a** to the less lipophilic acetamide **9b** and dimethyl urea **15a**.

The aim of the ensuing optimization effort was to identify derivatives that would maintain an H3R affinity profile similar to **9b** and **15a**, while displaying pharmacokinetic properties predictive of a short duration of action in man. Along these lines, exploration of the side chain substituents at position 1 (**15a–g**) indicated that the dimethyl (**15a**) to diethyl (**15b**) transformation increased affinity to both H3R and cardiac hERG channels (Table 2). Affinity for the latter was decreased by cyclization to a piperidine ring (**15c**); this change had minimal impact on the interaction with H3R, and it decreased hERG liability. Decreasing the pK_a of the piperidine amine by changing the ring to morpholine (**15d**) was detrimental to affinity, whereas contracting the cycle to the pyrrolidine ring (**15e**) enhanced potency. The best side chain substituent proved to be 2-methylpyrrolidine, with the *R* isomer (**15f**) being slightly more potent than the *S* isomer (**15g**), and parallels observations made with other H3R inverse agonists, such as ABT-239.[28]

Profiling of **15f** confirmed that it had no significant affinity for hERG channels and gave no measureable signal in the

Table 1. Affinity profile of lead compound **1**, lysergic acid diethylamide (LSD) and **9a** as inverse agonist for H3R and agonist at serotonin, adrenergic and dopamine receptors.

Compd	K_i [nM]	IC_{50} [nM]									
	H3R	5HT1A	5HT2A	5HT2B	5HT2C	α1A	β1	β2	D1	D2	D3
1	8.3	18200	7700	4900	2800	>10000	>20000	>20000	8700	10000	>20000
9a	23	>30000	>30000	>30000	>30000	>30000	>30000	>30000	>30000	>30000	>30000
LSD[a]	n.t.	1.1	2.7	30	5.5	220	140	740	180	120	27

[a] Data for LSD from the literature.[24] Note that the hallucinogenic effects of LSD are attributed mostly to its strong partial agonist effects at 5-HT2A receptors. Specific 5-HT2A agonist drugs are hallucinogenic, and 5-HT2A-specific antagonists largely block the stimulatory activity of LSD.[32]

Table 2. In vitro and in vivo properties of H3R inverse agonists.

Compd	hH3R K_i [nM][a]		CXCR3	pK_a	hERG	PLD	log P	MDR1-MDCK[d]			Brain	tMeHA[f]
	cAMP	binding	IC_{50} [μM]		[%][b]	[%][c]		A–B	B–A	ratio	level[e]	% induction
1	0.2	8.3	0.062	7.8	17	204	3.7	–	–	–	–	–
9a	0.7	23	11	7.9	13	218	4.6	–	–	–	–	–
9b	24	275	>100	8.0	4	0	1.7	–	–	–	–	–
11	23	97	>100	-	8	–	–	–	–	–	–	–
15a	178	252	>100	8.0	14	0	2.6	–	–	–	–	–
15b	5.1	73	-	8.6	76	0	3.5	–	–	–	–	–
15c	7.6	129	-	8.4	10	–	3.0	–	–	–	–	–
15d	535	4307	-	5.9	16	0	2.6	–	–	–	–	–
15e	5.3	214	-	8.6	14	–	3.3	–	–	–	–	–
15f	0.2	6.8	>100	8.3	18	0	3.9	3.2	72.2	22.9	95[f]	86[f]
15g	1.7	80	–	8.7	15	–	2.3	–	–	–	–	–
18a	0.9	13	>100	–	2	0		2.6	43.1	16.7	–	–
18b	2.0	51	–	8.3	11	0	2.15	0.7	19.4	28.9	–	–
18c	2.5	39	–	–	19	–		0.9	24.2	26.1	–	–
18d	0.7	9.1	–	8.3	20	1	2.3	1.5	49.9	32.7	30	69
20	1.0	13	–	8.2	18	–	2.8	18.0	28.5	1.58	–	–
22	1.4	21	–	8.5	20	1	2.6	23.8	32.8	1.38	56	103
23	0.5	19	–	8.6	16	0	2.8	7.8	48.7	6.3	59	64
24	1.2	22	–	8.6	−7	–	3.0	9.9	37.7	3.81	18	129
Bavisant	6.8	102	–	6.3	8	0	1.1	–	–	–	4087	82

[a] cAMP and K_i values were determined from 2–3 samples; [b] Patch-clamp assay, % inhibition at 10 μM; [c] PLD: phospholipidosis, % of control (amiodarone) at 50 μM; [d] Permeability values expressed as $P_{app} \times 10^{-6}$ cm s^{-1} in a Madin Darby Canine Kidney (MDCK) cell line transfected with the human multidrug resistance (MDR1) gene; [d] Brain concentration in ng g^{-1}, 1 h after oral administration of 10 mg kg^{-1}; [e] % increase of tele-methylhistamine brain concentration 1 h after oral administration of 10 mg kg^{-1}, compared to vehicle group ($n = 5$ rats per dose group); [f] Measured 0.5 h after oral administration of 10 mg kg^{-1}.

phospholipidosis assay. Testing **15f** in Madin Darby Canine Kidney (MDCK) cells transfected with the human multidrug resistance (MDR1) gene showed that it had limited membrane permeability due to strong Pgp transporter-mediated efflux. Accordingly, after oral administration of **15f** to rats (10 mg kg^{-1}), brain levels were low, despite rapid absorption and high plasma levels (2895 ng mL^{-1} after 30 min). Nonetheless, **15f** elicited a very pronounced pharmacodynamic effect, manifested in an increased brain concentration of the major histamine metabolite tele-methylhistamine (tMeHA), by more than two-fold, two hours post-dosing. This encouraging result hinted at the possibility that an even more robust interaction on histaminergic neurons might be achievable with better brain penetrable ergoline derivatives.

Toward this end, the optimized side chain in **15f** was combined with acetamide **9b** to give **18a**. The latter retained a profile similar to **15f**, but displayed poor transcellular transport properties. To address this issue, we turned our focus toward exploring variations of the pyrrolidinyl amide. Replacement of the pyrrolidine with primary, mono- or di-methylated amides yielded analogues **18b–d**. Although they maintained high affinity for H3R, these molecules have low permeability and are rapidly effluxed. Measurement of the rat brain concentration of the most potent compound, **18d**, one hour after oral administration, confirmed its low brain exposure. These amides nevertheless showed a clear pharmacological effect by increasing tMeHA concentrations in the frontal cortex.

We reasoned that modulation of Pgp efflux, leading to even modest improvements in brain exposure, could lead to correspondingly higher efficacies. Since carbonyl amides are a recognition element for Pgp transporter-mediated efflux, their replacement is a frequently used strategy to decrease efflux and enhance brain exposure.[29] As a first step, the amide was replaced with a 1,2,4-oxadiazole ring (**20**), a classical amide bioisostere.[30] This modification dramatically improved the permeation ratio in the MDCK assay, indicating decreased affinity for the Pgp transporter. The simple replacement of the pyrrolidinylamide with a methyl ester (**22**) confirmed that the amide plays a limited role in terms of binding as well as cAMP inhibition to H3R and can be removed without loss of affinity. Surprisingly, **22** did not display markedly enhanced brain penetration relative to **18d** (56 vs 30 ng g^{-1}, 1 h after 10 mg kg^{-1} p.o.). However, **22** was equally effective in increasing tMeHA levels in vivo (103% increase vs vehicle group, 1 h after 10 mg kg^{-1} p.o.) as the H3R antagonist clinical candidate, bavisant (82% increase). This is a striking result, since **22** reaches a brain concentration in rats that is 73-fold lower than bavisant (56 vs 4087 ng g^{-1}). This difference can't be explained by a fivefold ratio in affinity for human H3R, and might indicate that bavisant is functionally weaker at rat as compared with human receptors. Although ester **22** displays a desirable pharmacokinetic profile ($F = 33\%$; $T_{1/2} = 0.6$ h), it comes at the cost of decreased chemical stability—it was completely degraded in solution at pH 7.4 at 80 °C within two days, thereby precluding its further development.

Compared with **22**, methyl ether **24** displays very low brain levels after one hour, contrasting with its high tMeHA induction. This suggests a strong initial efficacy, followed by a very rapid clearance of **24**, likely resulting from its demethylation to the active metabolite **23**. Interestingly, **23** has a profile similar

to **22** and was proven to be stable in solution. It has no significant cross-reactivity against a panel of 80 ion channels, GPCRs (including 5HT2A and 5HT2B up to 30 μM, and H4R, 25% inhibition at 10 μM), or kinases and proteases (data not shown). It inhibits the constitutive activity of the human H3R with a K_i value of 1.2 nM in the GTPγS assay, demonstrating its inverse agonism. A good permeability (Papp = 26×10^{-6} cm s^{-1}), with a moderate efflux ratio of 1.8 was observed in Caco2 cells. In MDCK-MDR1 cells, its passive permeability was similar (Papp = 28×10^{-6} cm s^{-1}), but its efflux ratio of 6.3 is indicative of a Pgp substrate. Compound **23** does not significantly bind to hERG channels (16% at 10 μM) or inhibit common CYP450 enzymes (< 25% at 10 μM, 3A4, 2C9, 2D6, 1A2, 2C19). It displays rapid absorption (brain T_{max} = 0.5 h after 1 mg kg^{-1} p.o.) and a 54% oral bioavailability in rats, and desirable pharmacodynamics properties, including a fast elimination half-life (Table 3). The in vitro clearance values are almost identical in rat and human liver microsomes (CLint = 59 and 58 μL min^{-1} mg), suggesting that **23** might also have a fast clearance in human.

Table 3. Pharmacokinetic properties of **23** after i.v. administration (0.5 mg kg^{-1}).[a]

Parameter	Mouse	Rat	Dog
CL [L h^{-1} kg^{-1}]	10.2	2.57	2.29
Vd_{SS} [L kg^{-1}]	2.27	2.78	3.67
$T_{1/2,z}$ [h]	0.21	1.20	1.44
AUC_∞ [h ng mL^{-1}]	49.2	196	219

[a] CL: total blood clearance; Vd_{SS}: volume of distribution at steady state; $T_{1/2,z}$: apparent terminal half-life; AUC_∞ : area under the curve (t = 0–∞).

Evaluation of the pharmacokinetic/pharmacodynamic properties in rats confirmed the efficacy of **23** in increasing brain tMeHA levels (Figure 4a), with a brain-to-plasma concentration ratio of 0.15 (Figure 4b). In the key experiment, an orally administered single oral dose of **23** (10 mg kg^{-1}) to rats achieved a maximal H3R occupancy in the brain of only 54%, indicative of properties unlikely to provide robust wake-promoting activity.[31]

The optimization of these novel ergoline structures to achieve higher receptor occupancy, while retaining a short duration of action, is ongoing in order to identify a candidate with a suitable profile for clinical development.

Conclusions

The serendipitous discovery that ergoline derivative **1** displayed H3R inverse agonist properties uncovered a novel chemotype interacting with this target. However, the lead compound (**1**) is encumbered with a number of limitations (e.g., chemical reactivity, phospholipidosis, modest selectivity, and extended terminal half-life), which preclude its development. Accordingly, its physicochemical and pharmacological profiles were systematically improved by iterative structural modifications. The most advanced molecule to emerge from this effort was ergoline **23**, whose overall profile confirms the possibility of combining desirable selectivity, efficacy and pharmacokinetic properties in a single molecule. Further optimization of ergoline derivatives is anticipated to yield analogues with enhanced brain penetration and correspondingly higher pharmacological efficacy.

Experimental Section

Chemistry

General methods: All reagents and anhydrous solvents were obtained from commercial sources and used as received. ^{1}H NMR spectra were obtained on a Bruker 400 MHz instrument in the solvent indicated, with tetramethylsilane (TMS) as an internal standard. Coupling constants (*J*) are reported in Hertz (Hz). Liquid chromatography/mass spectrometry (LC/MS) analyses were run on an Agilent 1200 HPLC/6110 SQ system; mobile phase: 10 mM aq NH_4HCO_3 (A)/MeCN (B); gradient: 5% B for 0.2 min, increase to 95% B within 1.2 min, 95% B for 1.5 min, back to 5% B within 0.01 min; flow rate = 1.8 mL min^{-1}; XBridge C18 column (4.6× 50 mm, 3.5 μm), oven temperature: 50 °C. Compounds were purified by silica gel chromatography, and reactions were monitored using thin-layer chromatography (TLC) at 254 nm. Preparative HPLC conditions: XBridge Prep C18 column (10 μm OBD, 19× 250 mm); mobile phase: 0.1% aq NH_3 (A); MeCN (B); gradient: 25 to 55% B in 10 min; flow rate = 30 mL min^{-1}.

(4a*R*,8*R*,9a*R*)-Methyl 6-(phenylcarbamoyl)-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxylate (3): Isocyanatobenzene (0.44 g, 3.7 mmol) was added to a solution of **2** (1 g, 3.7 mmol) in CH_2Cl_2 (10 mL) at RT, then stirred for 2 h. The mixture was washed with saturated aq $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was dried (anhyd $MgSO_4$), filtered, and concentrated in vacuo to give the title compound as a yellow solid (1.3 g, 90%): LC/MS: t_R = 1.83 min, m/z = 390.2 $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-*N*-Phenyl-8-(pyrrolidine-1-carbonyl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinoline-6(1*H*)-carboxamide (4): To a solution of **3** (1.3 g, 3.3 mmol) in THF/water (25 mL, v/v = 4:1) was added 1 M aq NaOH (10 mL). The reaction mixture was stirred at RT for 2 h. The mixture was adjusted to pH 5 with 1 N aq HCl and extracted with EtOAc (3×30 mL). The combined organic layers were dried (anhyd $MgSO_4$), filtered and concentrated in vacuo to give the crude intermediate as a yellow solid (1.1 g, 89%): LC/MS: t_R = 1.42 min, m/z = 376.2 $[M+1]^+$. This intermediate (750 mg, 2 mmol) was dissolved in CH_2Cl_2 (20 mL), and the solution was treated with Et_3N (606 mg, 6 mmol), 2-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) (838 mg, 2.2 mmol) and pyrrolidine (296 mg, 4 mmol). The reaction mixture was then stirred at RT overnight. After quenching with water (20 mL), the resulting mixture was extracted with EtOAc (3× 50 mL). The organic layer was dried (anhyd $MgSO_4$), filtered, and concentrated in vacuo. The resultant residue was purified by silica gel chromatography (CH_2Cl_2/MeOH, 20:1) to yield **4** as a white solid (0.71 g, 83%): LC/MS: t_R = 1.85 min, m/z = 429.3 $[M+H]^+$.

Isopropyl 2-((4a*R*,8*R*,9a*R*)-6-(phenylcarbamoyl)-8-(pyrrolidine-1-carbonyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)acetate (5): A mixture of **4** (428 mg, 1 mmol), NaOH (400 mg, 10 mmol) and tetrabutylammonium iodide (TBAI, 37 mg, 0.1 mmol) in CH_2Cl_2 (20 mL) was cooled to 0 °C and treated slowly with isopropyl 2-bromoacetate (362 mg, 2 mmol). The reaction mixture was then allowed to warm to RT while stirring overnight. The resulting mixture was diluted with CH_2Cl_2 (100 mL), washed with

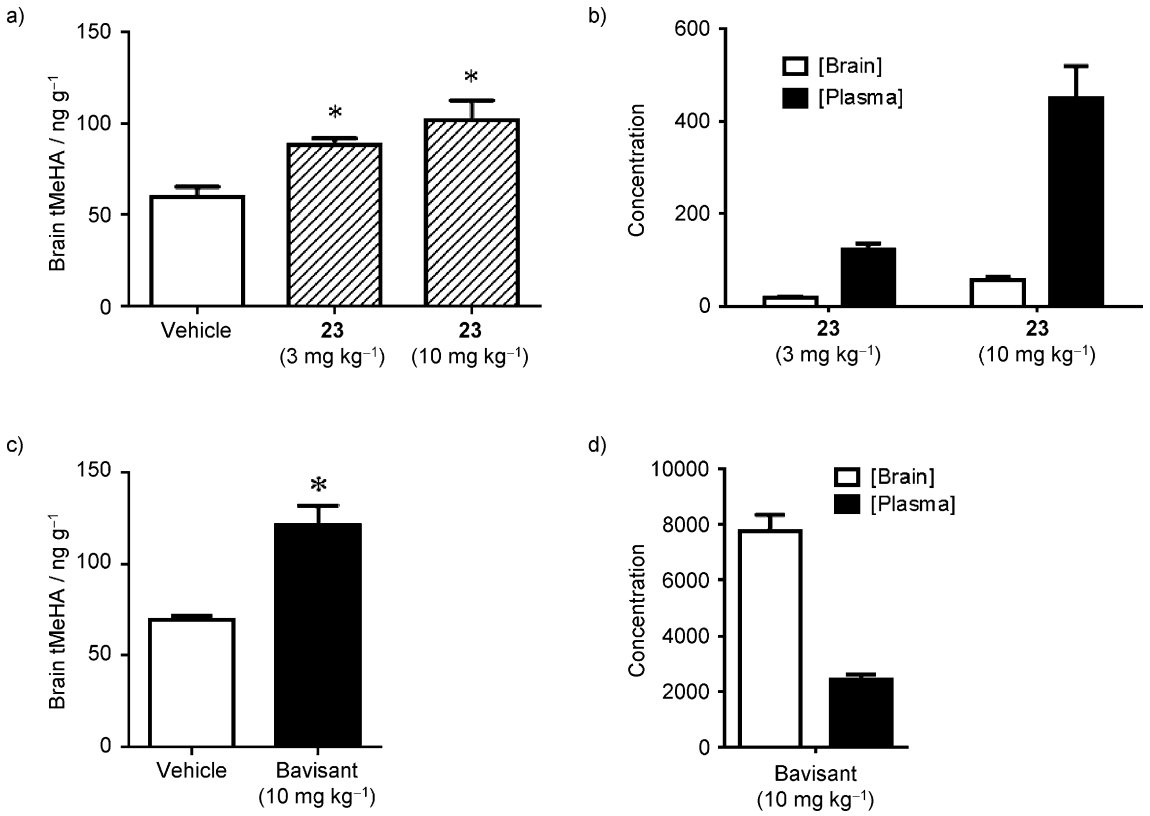


Figure 4. Effect of **23** compared with bavisant on tMeHA (panels a,c) and corresponding plasma and brain concentration (panels b,d), 1 h after oral administration. a) Dose-dependent increase of brain (frontal cortex) tele-methyl histamine (tMeHA), a major metabolite of histamine, caused by **23**; vehicle is 50 mM citrate buffer. b) Brain (frontal cortex) (ng g^{-1}) and plasma (ng mL^{-1}) exposure of **23**, 1 h after oral administration; a dose-dependent change can also be observed, with plasma exposure many fold greater than brain exposure. c,d) Bavisant also elevated brain tMeHA, with about a fourfold higher exposure in the brain than in plasma.

water (50 mL), saturated aq $NaHCO_3$ (50 mL) and brine (80 mL). The organic layer was dried (anhyd $MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by silica gel chromatography (petroleum ether/EtOAc, 2:1) to give the title compound as a yellow oil (475 mg, 90%): LC/MS: $t_R = 1.95$ min, $m/z = 529.3$ $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-1-(3-Hydroxyethyl)-*N*-phenyl-8-(pyrrolidine-1-carbonyl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinoline-6(1*H*)-carboxamide (6): $LiBH_4$ (2 M in THF, 3 mL, 6 mmol) was slowly added to a mixture of **5** (800 mg, 1.5 mmol) in THF (15 mL) at 0 °C under argon. The reaction mixture was stirred at 0 °C for 3 h, and then at RT overnight. The resulting mixture was quenched by careful addition of water (5 mL), stirred at RT for 30 min, and then extracted with EtOAc (3×30 mL). The combined organic layers were dried (anhyd $MgSO_4$), filtered, and concentrated in vacuo to give the title compound as a yellow solid (450 mg, 63.5%), which was used without further purification: LC/MS: $t_R = 1.82$ min, $m/z = 473.3$ $[M+H]^+$.

2-((4a*R*,8*R*,9a*R*)-6-(Phenylcarbamoyl)-8-(pyrrolidine-1-carbonyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)ethyl methanesulfonate (7): A mixture of **6** (472 mg, 1 mmol) and Et_3N (303 mg, 3 mmol) in CH_2Cl_2 (15 mL) was cooled to 0 °C. MsCl (170 mg, 1.5 mmol) in CH_2Cl_2 (5 mL) was added slowly, and the resulting mixture was stirred at RT for 4 h. The organic solvent was then removed in vacuo. The residue was diluted with CH_2Cl_2 (20 mL) and washed with saturated aq $NaHCO_3$ (10 mL). The organic layer was dried (anhyd $MgSO_4$), filtered, and concentrated in vacuo to give crude **7** as a yellow solid (500 mg, 91%), which was used directly in the next step without further purification: LC/MS: $t_R = 1.91$ min, $m/z = 551.2$ $[M+H]^+$.

((4a*R*,8*R*,9a*R*)-1-(2-(Dimethylamino)ethyl)-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinolin-8-yl)(pyrrolidin-1-yl)methanone (8): To a solution of **7** (500 mg, 0.91 mmol) in MeCN (8 mL) was added Me_2NH (648 mg, 8 mmol) under argon. The reaction mixture was stirred at 50 °C overnight in a sealed tube. After cooling to RT, the resulting mixture was diluted with EtOAc (20 mL) and washed with water (15 mL). The organic layer was dried (anhyd $MgSO_4$), filtered, and concentrated in vacuo to give the title compound as a white solid (260 mg, 68%): LC/MS: $t_R = 1.54$ min, $m/z = 381.3$ $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-1-(2-(Dimethylamino)ethyl)-*N*-phenyl-8-(pyrrolidine-1-carbonyl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinoline-6(1*H*)-carboxamide (9a): To a solution of **8** (380 mg, 1 mmol) in CH_2Cl_2 (10 mL) was added isocyanatobenzene (119 mg, 1 mmol) at RT. The reaction mixture was stirred at RT for 12 h. The mixture was diluted with CH_2Cl_2 (100 mL) and washed with saturated aq $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was dried (anhyd $MgSO_4$), filtered and concentrated in vacuo to give a crude product, which was purified by preparative HPLC to afford the title compound as a white solid (80 mg, 16%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.87$ (s, 1H), 7.45 (dd, $J = 8.6$, 1.0 Hz, 2H), 7.27 (d, $J = 2.0$ Hz, 1H), 7.23 (s, 1H), 7.18–7.07 (m, 2H), 6.93 (t, $J = 7.3$ Hz, 1H), 6.86–6.70 (m, 2H), 4.19 (dd, $J = 9.9$, 5.8 Hz, 3H), 3.98 (td, $J = 11.1$,

3.6 Hz, 1H), 3.70–3.44 (m, 6H), 3.21 (t, J=11.8 Hz, 1H), 3.08–2.88 (m, 3H), 2.71 (dt, J=10.5, 5.4 Hz, 2H), 2.31 (d, J=2.2 Hz, 6H), 2.13–1.92 (m, 4H), 1.58–1.48 ppm (m, 1H); LC/MS: t_R=1.88 min, m/z=500.4 $[M+H]^+$.

1-((4a*R*,8*R*,9a*R*)-1-(2-(Dimethylamino)ethyl)-8-(pyrrolidine-1-carbonyl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-6(1*H*)-yl)ethanone (9b): A mixture of **8** (75 mg, 0.2 mmol) and *N*,*N*-diisopropylethylamine (DIPEA, 52 mg, 0.4 mmol) in CH_2Cl_2 (20 mL) was cooled to 0 °C. The mixture was slowly treated with AcCl (19 mg, 0.24 mmol). The reaction mixture was stirred at 0 °C to RT for 4 h. The solvent was removed in vacuo, and the residue was purified by preparative HPLC to give the title compound as a yellow oil (31 mg, 37%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.16–7.10 (m, 2H), 6.81–6.83 (m, 2H), 4.17 (t, J=7.2 Hz, 3H), 4.08–4.01 (m, 1H), 3.59–3.55 (m, 2H), 3.51–3.48 (m, 3H), 3.39–3.35 (m, 1H), 3.23 (t, J=11.2 Hz, 1H), 3.03–2.91 (m, 2H), 2.79–2.67 (m, 3H), 2.30 (s, 6H), 2.27 (s, 3H), 2.05–1.89 ppm (m, 5H); LC/MS: t_R=1.75 min, m/z=423.3 $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-4-Nitrophenyl 1-(2-(Dimethylamino)ethyl)-8-(pyrrolidine-1-carbonyl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinoline-6(1*H*)-carboxylate (10): To a mixture of **8** (150 mg, 0.39 mmol) in CH_2Cl_2 (20 mL) was slowly added DIPEA (100 mg, 0.78 mmol) and 4-nitrophenyl chloroformate (96 mg, 0.48 mmol) at 0 °C. The resulting reaction mixture was stirred at RT overnight. The solvent was removed in vacuo, and the crude product was purified by prep-TLC (EtOAc/MeOH, 10:3, R_f=0.4) to give the title compound as a yellow oil (160 mg, 74%).

(4a*R*,8*R*,9a*R*)-1-(2-(Dimethylamino)ethyl)-*N*-methyl-8-(pyrrolidine-1-carbonyl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinoline-6(1*H*)-carboxamide (11): To a mixture of **10** (150 mg, 0.28 mmol) and DIPEA (72 mg, 0.56 mmol)) in CH_2Cl_2 (20 mL) was added $MeNH_2$ (0.28 mL, 0.56 mmol) and 4-dimethylaminopyridine (DMAP, 7 mg, 0.056 mmol). The reaction mixture was stirred at RT overnight. The solvent was removed in vacuo, and the residue purified by preparative HPLC to give the title compound as yellow oil (26 mg, 22%): ^{1}H NMR (400 MHz, CD_3OD): δ=7.18 (d, J=8.4 Hz, 1H), 7.12–7.08 (m, 1H), 6.91 (s, 1H), 6.85 (d, J=6.8 Hz, 1H), 4.59 (s, 1H), 4.25 (t, J=7.2 Hz, 2H), 3.89–3.84 (m, 1H), 3.68–3.61 (m, 3H), 3.56–3.41 (m, 3H), 3.29–3.24 (m, 2H), 3.17–3.04 (m, 2H), 2.83–2.72 (m, 6H), 2.30 (s, 6H), 2.08–2.01 (m, 2H), 1.96–1.90 (m, 2H), 1.70–1.61 ppm (m, 1H); LC/MS: t_R=1.53 min, m/z=438.2 $[M+H]^+$.

((4a*R*,8*R*,9a*R*)-1,4,4a,6,7,8,9,9a-Octahydroindolo[1,14-fg]quinolin-8-yl)(pyrrolidin-1-yl)methanone (13): To a solution of **12** (2 g, 7.8 mmol) in DMF (10 mL) was added pyrrolidine (3.2 mL, 39 mmol), HATU (4.4 g, 11.7 mmol) and Et_3N (3.2 mL, 23.4 mmol). The reaction mixture was stirred at RT overnight, then diluted with water (15 mL) and extracted with EtOAc (3×50 mL). The combined organic layers were washed with brine (3×50 mL), dried (anhyd $MgSO_4$), filtered and concentrated to give the title compound as a brown solid (1.8 g, 76%), which was used without further purification: LC/MS: t_R=1.67 min, m/z=310.2 $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-*N*,*N*-dimethyl-8-(pyrrolidine-1-carbonyl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinoline-6(1*H*)-carboxamide (14): To a solution of **13** (1.7 g, 5.5 mmol) in MeCN (120 mL) was added dimethylcarbamic chloride (0.65 g, 6.1 mmol), DIPEA (2.1 g, 16.5 mmol) and DMAP (0.13 g, 1.1 mmol). The reaction mixture was stirred at 65 °C for 4 h, then cooled to RT, diluted with CH_2Cl_2 (50 mL) and washed with water (50 mL) and brine (50 mL). The organic layer was dried (anhyd $MgSO_4$), filtered and concentrated to yield the title compound as a yellow solid (1.5 g, 71%): LC/MS: t_R=1.80 min, m/z=381.1 $[M+H]^+$.

Isopropyl 2-((4a*R*,8*R*,9a*R*)-6-(dimethylcarbamoyl)-8-(pyrrolidine-1-carbonyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)acetate: To a solution of **14** (1.9 g, 4.9 mmol) in CH_2Cl_2 (160 mL) was slowly added NaOH (1.96 g, 49 mmol), TBAI (0.36 g, 0.98 mmol) and isopropyl 2-bromoacetate (3.55 g, 19.6 mmol) at 0 °C. The reaction mixture was then stirred at RT overnight. The precipitate was filtered out, and the filtrate was sequentially washed with water (50 mL), saturated aq $NaHCO_3$ (50 mL) and brine (80 mL). The organic layer was dried (anhyd $MgSO_4$), filtered and concentrated in vacuo to give the title compound as a yellow oil (1.6 g, 67%): LC/MS: t_R=1.91 min, m/z=481.2 $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-1-(2-Hydroxyethyl)-*N*,*N*-dimethyl-8-(pyrrolidine-1-carbonyl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinoline-6(1*H*)-carboxamide: To a solution of 2-((4a*R*,8*R*,9a*R*)-6-(dimethylcarbamoyl)-8-(pyrrolidine-1-carbonyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]-quinolin-1(4*H*)-yl)acetate (2.1 g, 4.36 mmol) in THF (150 mL) was added $LiBH_4$ (2M in THF, 6.5 mL, 13 mmol) at 0 °C under argon. The reaction mixture was stirred at 0 °C for 3 h, followed by RT overnight. After cooling to 0 °C, AcOH (6 mL) was added slowly. The resulting mixture was stirred at 0 °C for 30 min, then adjusted to pH 8 with saturated aq $NaHCO_3$ (40 mL) and extracted with EtOAc (2×100 mL). The combined organic layers were washed with brine (30 mL), dried (anhyd $MgSO_4$), filtered and concentrated in vacuo to give the title compound as a brown oil (1.5 g, 81%): LC/MS: t_R=1.71 min, m/z=425.1 $[M+H]^+$.

2-((4a*R*,8*R*,9a*R*)-6-(Dimethylcarbamoyl)-8-(pyrrolidine-1-carbonyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)ethyl methanesulfonate: A mixture of (4a*R*,8*R*,9a*R*)-1-(2-hydroxyethyl)-*N*,*N*-dimethyl-8-(pyrrolidine-1-carbonyl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinoline-6(1*H*)-carboxamide (0.9 g, 2.1 mmol) and Et_3N (636 mg, 6.3 mmol) in CH_2Cl_2 (50 mL) was cooled to 0 °C. MsCl (460 mg, 3.2 mmol) in CH_2Cl_2 (5 mL) was slowly added to the cooled solution. The reaction mixture was stirred at 0 °C for 3 h, followed by RT overnight. The solvent was removed, and the residue was purified by silica gel chromatography (EtOAc/petroleum ether, 2:1) to give the title compound as a white solid (580 mg, 55%): LC/MS: t_R=1.79 min, m/z=503.2 $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-1-(2-(Dimethylamino)ethyl)-*N*,*N*-dimethyl-8-(pyrrolidine-1-carbonyl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinoline-6(1*H*)-carboxamide (15a): To a solution of 2-((4a*R*,8*R*,9a*R*)-6-(dimethylcarbamoyl)-8-(pyrrolidine-1-carbonyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)ethyl methanesulfonate (75 mg, 0.15 mmol) in MeCN (10 mL) was added Me_2NH (1.36 g, 16 mmol) under argon. The reaction mixture was stirred at 50 °C overnight. After cooling to RT, the organic solvent was removed in vacuo to yield the crude product. The residue was purified by preparative HPLC to give **15a** as a white solid (16 mg, 24%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.17–7.11 (m, 2H), 6.88–6.87 (m, 1H), 6.75 (s, 1H), 4.19 (t, J=7.2 Hz, 2H), 3.64–3.40 (m, 6H), 3.18–3.12 (m, 4H), 2.98 (s, 6H), 2.88–2.69 (m, 4H), 2.31 (s, 6H), 2.05–1.86 ppm (m, 5H); LC/MS: t_R=1.50 min, m/z=452.3 $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-1-(2-(Diethylamino)ethyl)-*N*,*N*-dimethyl-8-(pyrrolidine-1-carbonyl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinoline-6(1*H*)-carboxamide(15b): Reaction of 2-((4a*R*,8*R*,9a*R*)-6-(dimethylcarbamoyl)-8-(pyrrolidine-1-carbonyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)ethyl methanesulfonate and Et_2NH in a manner similar to **15a** but using 200 mg starting material (0.4 mmol) gave **15b** as a white solid (95 mg, 50%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.18–7.16 (d, 2H), 6.88–6.87 (m, 1H), 6.76 (s, 1H), 4.17 (t, J=7.2 Hz, 2H), 3.62–3.41 (m, 5H), 3.20–3.14 (m, 4H), 3.00 (s, 6H), 2.87–2.81 (m, 4H), 2.62 (q, J=7.2 Hz, 4H), 2.05–2.02

(m, 2H), 1.93–1.92 (m, 2H), 1.85–1.82 (m, 2H), 1.07–1.04 ppm (t, J= 7.2 Hz, 6H); LC/MS: t_R=1.97 min, m/z=480.3 $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-*N*,*N*-dimethyl-1-(2-(piperidin-1-yl)ethyl)-8-(pyrrolidine-1-carbonyl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinoline-6(1*H*)-carboxamide (15c): Reaction of 2-((4a*R*,8*R*,9a*R*)-6-(dimethylcarbamoyl)-8-(pyrrolidine-1-carbonyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)ethyl methanesulfonate and piperidine in a manner similar to **15a** gave **15c** as a white solid (31 mg, 42%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.18–7.16 (m, 2H), 6.88–6.87 (m, 1H), 6.77 (s, 1H), 4.21 (t, J=7.6 Hz, 2H), 3.60–3.41 (m, 5H), 3.20–3.14 (m, 4H), 3.00 (s, 6H), 2.87–2.70 (m, 4H), 2.48 (br s, 4H), 2.01–1.80 (m, 6H), 1.78–1.65 ppm (m, 6H); LC/MS: t_R=1.99 min, m/z=492.3 $[M+1]^+$.

(4a*R*,8*R*,9a*R*)-*N*,*N*-dimethyl-1-(2-morpholinoethyl)-8-(pyrrolidine-1-carbonyl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinoline-6(1*H*)-carboxamide (15d): Reaction of 2-((4a*R*,8*R*,9a*R*)-6-(dimethylcarbamoyl)-8-(pyrrolidine-1-carbonyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)ethyl methanesulfonate and morpholine in a manner similar to **15a** gave **15d** as a white solid (46 mg, 62%): ^{1}H NMR (400 MHz, CD_3OD): δ=7.19–7.09 (m, 2H), 6.87–6.86 (m, 2H), 4.24 (t, J=6.8 Hz, 2H), 3.70–3.64 (m, 6H), 3.47–3.42 (m, 3H), 3.22–3.18 (m, 2H), 3.04–2.96 (m, 10H), 2.74–2.71 (m, 3H), 2.50–2.48 (m, 4H), 2.06–2.03 (m, 2H), 1.95–1.92 (m, 2H), 1.67–1.70 ppm (m, 1H); LC/MS: t_R=1.19 min, m/z=494.1 $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-*N*,*N*-Dimethyl-1-(2-(pyrrolidin-1-yl)ethyl)-8-(pyrrolidine-1-carbonyl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinoline-6(1*H*)-carboxamide (15e): Reaction of 2-((4a*R*,8*R*,9a*R*)-6-(dimethylcarbamoyl)-8-(pyrrolidine-1-carbonyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)ethyl methanesulfonate and pyrrolidine in a manner similar to **15a** gave **15e** as a white solid (23 mg, 32%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.22–7.06 (m, 2H), 6.87(dd, J=4.7, 1.9 Hz, 1H), 6.75 (s, 1H), 4.23 (t, J=7.2 Hz, 2H), 3.61–3.41 (m, 5H), 3.25–3.08 (m, 4H), 2.99 (s, 6H), 2.91–2.76 (m, 4H), 2.57 (m, 4H), 2.12–1.78 ppm (m, 10H); LC/MS: t_R=1.88 min, m/z=478.3 $[M+H]^+$.

***(4a*R*,8*R*,9a*R*)-*N*,*N*-dimethyl-1-(2-((*R*)-2-methylpyrrolidin-1-yl)ethyl)-8-(pyrrolidine-1-carbonyl)-4,4a,7,8,9,9a-hexahydroindolo-[1,14-fg]quinoline-6(1*H*)-carboxamide (15f)**: Reaction of 2-((4a*R*,8*R*,9a*R*)-6-(dimethylcarbamoyl)-8-(pyrrolidine-1-carbonyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)ethyl methanesulfonate and (*R*)-2-methylpyrrolidine in a manner similar to **15a** gave **15f** as a white solid (43 mg, 58%): ^{1}H NMR (400 MHz, CD_3OD): δ=7.10–7.18 (m, 2H), 6.88–6.86 (m, 2H), 4.25–4.22 (m, 2H), 3.70–3.60 (m, 3H), 3.45–3.32 (m, 3H), 3.32–3.29 (m, 5H), 3.22–3.14 (m, 4H), 2.97–2.93 (m, 4H), 2.45–2.52 (m, 2H), 2.22–2.19 (m, 1H), 2.06–2.03 (m, 5H), 1.86–1.76 (m, 3H), 1.19–1.40 ppm (m, 5H); LC/MS: t_R=1.98 min, m/z=492.3 $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-*N*,*N*-Dimethyl-1-(2-((*S*)-2-methylpyrrolidin-1-yl)ethyl)-8-(pyrrolidine-1-carbonyl)-4,4a,7,8,9,9a-hexahydroindolo-[1,14-fg]quinoline-6(1*H*)-carboxamide (15g): Reaction of 2-((4a*R*,8*R*,9a*R*)-6-(dimethylcarbamoyl)-8-(pyrrolidine-1-carbonyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)ethyl methanesulfonate and (*S*)-2-methylpyrrolidine in a manner similar to **15a** but using 150 mg starting material (0.3 mmol) gave **15g** as a white solid (85 mg, 58%): ^{1}H NMR(400 MHz, CD_3OD): δ=7.20–7.10 (m, 2H), 6.88–6.86 (m, 2H), 4.26–4.22 (m, 2H), 3.75–3.62 (m, 3H), 3.51–3.40 (m, 3H), 3.30–3.28 (m, 5H), 3.20–3.08 (m, 4H), 3.02–2.78 (m, 4H), 2.50–2.35 (m, 2H), 2.22–2.19 (m, 1H), 2.06–1.85 (m, 5H), 1.72–1.60 (m, 3H), 1.38–1.22 (m, 1H), 1.08 (t, J=7.0 Hz, 1H), 1.05 ppm (d, J=6.0 Hz, 3H); LC/MS: t_R=1.98 min, m/z=492.3 $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-6-Acetyl-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]-quinoline-8-carboxylic acid (16): To a solution of **12** (5 g, 19.5 mmol) in THF (200 mL) at 0 °C was slowly added Et_3N (43 mL, 156 mmol) and Ac_2O (18.5 mL, 97.6 mmol). The reaction mixture was stirred at RT overnight. The precipitate was collected by filtration to yield crude **16** as a white solid (5 g, 86%), which was used without further purification: LC/MS: t_R=1.46 min, m/z=299.1 $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-Benzyl-6-acetyl-1,4,4a,6,7,8,9,9a-octahydroindolo-[1,14-fg]quinoline-8-carboxylate: To a mixture of **16** (5 g, 16.8 mmol) and Et_3N (7.5 mL, 50.3 mmol) in CH_2Cl_2 (150 mL) was added CbzCl (3.8 mL, 26.8 mmol) slowly at 0 °C. After stirring for 5 min, DMAP (0.2 g, 1.68 mmol) was added. The reaction mixture was stirred at RT for 1 h, and then the solvent was removed in vacuo. The residue was diluted with CH_2Cl_2 (100 mL) and washed with saturated aq $NaHCO_3$ (30 mL) and brine (30 mL). The organic layer was dried (anhyd $MgSO_4$), filtered and concentrated to give the crude product, which was used directly in the next step without further purification (4.6 g, 71%): LC/MS: t_R=1.92 min, m/z= 389.2 $[M+1]^+$.

(4a*R*,8*R*,9a*R*)-Benzyl 6-acetyl-1-(2-isopropoxy-2-oxoethyl)-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxylate: To a solution of (4a*R*,8*R*,9a*R*)-benzyl-6-acetyl-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxylate (1.9 g, 4.9 mmol) in CH_2Cl_2 (160 mL) was slowly added NaOH (1.96 g, 49 mmol) and TBAI (0.36 g, 0.98 mmol), followed by isopropyl 2-bromoacetate (3.55 g, 19.6 mmol) at 0 °C. The resulting reaction mixture was stirred at RT overnight. After addition of ice water (50 mL), the organic layer was separated, dried (anhyd $MgSO_4$), filtered and concentrated in vacuo to yield the crude product. The residue was purified by silica gel chromatography (EtOAc/petroleum ether, 1:5) to give the title compound as a yellow solid (1.9 g, 79%): LC/MS: t_R= 2.05 min, m/z=489.2 $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-6-Acetyl-1-(2-isopropoxy-2-oxoethyl)-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxylic acid (17): To a solution of (4a*R*,8*R*,9a*R*)-benzyl-6-acetyl-1-(2-isopropoxy-2-oxoethyl)-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxylate (0.93 g, 1.9 mmol) in THF (300 mL) was added 10% Pd/C (100 mg, 0.19 mmol). The reaction mixture was stirred under an H_2 atmosphere at RT overnight. The resulting mixture was filtered through Celite, and the filter cake was washed with THF (50 mL). The filtrate was concentrated in vacuo to give the crude product, which was washed with hexane/EtOAc (50 mL, v/v=30:1) to give the title compound as a white solid (0.56 g, 74%): LC/MS: t_R=1.56 min, m/z=399.1 $[M+H]^+$.

Isopropyl 2-((4a*R*,8*R*,9a*R*)-6-acetyl-8-carbamoyl-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)acetate: To a solution of **17** (239 mg, 0.6 mmol) in DMF (10 mL) was added NH_4Cl (255 mg, 4.8 mmol), DIPEA (744 mg, 6 mmol) and HATU (456 mg, 1.2 mmol). The reaction mixture was stirred at RT for 4 h. After diluting with water (10 mL), the resulting mixture was extracted with CH_2Cl_2 (3×10 mL). The combined layers were dried (anhyd $MgSO_4$), filtered and concentrated in vacuo to yield the crude product as a yellow oil (200 mg, 84%), which was used directly in the next step without further purification: LC/MS: t_R=1.73 min, m/z=398.2 $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-6-Acetyl-1-(2-hydroxyethyl)-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxamide: To a solution of isopropyl 2-((4a*R*,8*R*,9a*R*)-6-acetyl-8-carbamoyl-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)acetate (1.65 g, 4.15 mmol) in THF (150 mL) was slowly added $LiBH_4$ (2 M in THF, 6.5 mL,

13 mmol) at 0 °C. The reaction mixture was stirred at RT overnight. The reaction was then re-cooled to 0 °C, AcOH (60 mL) was slowly added, and the resulting mixture was stirred for an additional 30 min. After dilution with EtOAc (150 mL), the pH was adjusted to 8 with saturated aq $NaHCO_3$ (40 mL), followed by washing with brine (30 mL). The organic layer was dried (anhyd $MgSO_4$), filtered, and concentrated in vacuo to give the title compound as a brown oil (0.9 g, 64%), which was used without further purification: LC/MS: $t_R = 1.63$ min, $m/z = 342.3$ $[M+H]^+$.

2-((4a*R*,8*R*,9a*R*)-6-Acetyl-8-carbamoyl-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)ethyl methanesulfonate: (4a*R*,8*R*,9a*R*)-6-acetyl-1-(2-hydroxyethyl)-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxamide (716 mg, 2.1 mmol) and Et_3N (636 mg, 6.3 mmol) were combined in CH_2Cl_2 (50 mL) and cooled to 0 °C. MsCl (460 mg, 3.2 mmol) in CH_2Cl_2 (5 mL) was slowly added to the mixture, which was stirred at 0 °C for 3 h, and then overnight at RT. The organic solvent was removed in vacuo to give the title compound as a yellow oil (0.7 g, 82%), which was used directly in the next step without further purification. LC/MS: $t_R = 1.03$ min, $m/z = 420.2$ $[M+H]^+$.

1-((4a*R*,8*R*,9a*R*)-1-(2-((*R*)-2-Methylpyrrolidin-1-yl)ethyl)-8-(pyrrolidine-1-carbonyl)-,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-6(1*H*)-yl)ethanone (18a): The title compound was prepared as a white solid (100 mg, 35%) in a manner similar to **18d**: ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.20$–7.04 (m, 2H), 6.83 (s, 2H), 4.32–3.92 (m, 4H), 3.69–3.44 (m, 5H), 3.38 (d, $J = 11.9$ Hz, 1H), 3.30–3.11 (m, 3H), 3.08–2.89 (m, 2H), 2.81–2.70 (m, 1H), 2.58–2.45 (m, 2H), 2.33–2.26 (m, 4H), 2.08–1.74 (m, 8H), 1.52–1.40 (m, 1H), 1.12 ppm (d, $J = 5.9$ Hz, 3H); LC/MS: $t_R = 1.74$ min, $m/z = 463.1$ $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-6-Acetyl-1-(2-((*R*)-2-methylpyrrolidin-1-yl)ethyl)-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxamide (18b): To a solution of 2-((4a*R*,8*R*,9a*R*)-6-acetyl-8-carbamoyl-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)ethyl methanesulfonate (461 mg, 1.1 mmol) in MeCN (10 mL) was added (*R*)-2-methylpyrrolidine (468 mg, 5.5 mmol). The reaction mixture was stirred at 50 °C overnight. The reaction mixture was cooled, and the solvent was removed in vacuo to yield the crude product. Purification by preparative HPLC yielded **18b** as a white solid (150 mg, 33%): ^1H NMR (400 MHz, CD_3OD): $\delta = 7.21$ (d, $J = 8.4$ Hz, 1H), 7.12 (t, $J = 7.2$ Hz, 1H), 6.94 (s, 1H), 6.86 (d, $J = 7.8$ Hz, 1H), 4.59 (m, 1H), 4.32–4.26 (m, 2H), 3.99–3.98 (m, 1H), 3.60 (m, 1H), 3.19–3.15 (m, 2H), 2.97–2.84 (m, 3H), 2.57–2.46 (m, 2H), 2.30–2.22 (m, 4H), 1.97–1.94 (m, 1H), 1.81–1.77 (m, 2H), 1.64–1.61 (m, 1H), 1.60–1.46 (m, 2H), 1.14–1.12 (d, $J = 6.4$ Hz, 3H), 0.96–0.92 ppm (m, 1H); LC/MS: $t_R = 1.71$ min, $m/z = 409.3$ $[M+H]^+$.

Isopropyl 2-((4a*R*,8*R*,9a*R*)-6-acetyl-8-(methylcarbamoyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)acetate: To a solution of **17** (0.24 g, 0.6 mmol) in DMF (10 mL) was added $MeNH_2$ (74 mg, 2.4 mmol), DIPEA (0.3 g, 2.4 mmol) and HATU (0.46 g, 1.2 mmol). The reaction mixture was stirred at RT for 4 h. After diluting with water (10 mL), the resulting mixture was extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were dried (anhyd $MgSO_4$), filtered and concentrated in vacuo to give the crude product (217 mg, 88%), which was used directly in the next step without further purification: LC/MS: $t_R = 1.78$ min, $m/z = 412.3$ $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-6-Acetyl-1-(2-hydroxyethyl)-*N*-methyl-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxamide: To a solution of isopropyl 2-((4a*R*,8*R*,9a*R*)-6-acetyl-8-(methylcarbamoyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)acetate (1.65 g, 4.36 mmol) in THF (150 mL) was added $LiBH_4$ (2 M in THF, 6.5 mL, 13 mmol). The reaction mixture was stirred at RT overnight. After cooling to 0 °C, AcOH (6 mL) was slowly added to the mixture, which was stirred at 0 °C for an additional 30 min. The pH of the resulting mixture was adjusted to 8 with saturated aq $NaHCO_3$ (40 mL), and extracted with EtOAc (2×100 mL). The combined organic layers were washed with brine (30 mL), dried (anhyd $MgSO_4$), filtered and concentrated in vacuo to give the title compound as a brown oil (481 mg, 31%): LC/MS: $t_R = 1.22$ min, $m/z = 356.2$ $[M+H]^+$.

2-((4a*R*,8*R*,9a*R*)-6-Acetyl-8-(methylcarbamoyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)ethyl methanesulfonate: A solution of (4a*R*,8*R*,9a*R*)-6-acetyl-1-(2-hydroxyethyl)-*N*-methyl-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxamide (0.20 g, 0.56 mmol) and Et_3N (0.22 mL, 1.56 mmol) in CH_2Cl_2 (25 mL) was cooled to 0 °C. MsCl (89 mg, 0.78 mmol) in CH_2Cl_2 (5 mL) was slowly added, and the resulting reaction mixture was warmed to RT and stirred for 4 h. The solvent was removed in vacuo to yield the crude product, which was diluted with CH_2Cl_2 (50 mL). The organic layer was washed with saturated aq $NaHCO_3$ (30 mL), dried (anhyd $MgSO_4$), filtered and concentrated in vacuo to give the title compound as a yellow solid (0.21 g, 88%): LC/MS: $t_R = 1.64$ min, $m/z = 433.9$ $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-6-Acetyl-*N*-methyl-1-(2-((*R*)-2-methylpyrrolidin-1-yl)ethyl)-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxamide (18c): To a mixture of 2-((4a*R*,8*R*,9a*R*)-6-acetyl-8-(methylcarbamoyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)ethyl methanesulfonate (147 mg, 0.34 mmol) and Et_3N (0.22 mL, 1.6 mmol) in MeCN (15 mL) was added (*R*)-2-methylpyrrolidine (275 mg, 3.2 mmol) under argon. The reaction mixture was stirred at 50 °C overnight. The solvent was removed in vacuo to yield the crude product, which was purified by preparative HPLC to give **18c** as a white solid (47 mg, 33%): ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.20$–7.14 (m, 2H), 6.88–6.86 (m, 2H), 4.62–4.59 (m,1H), 4.25 (t, $J = 7.6$ Hz, 2H), 3.88 (m, 1H), 3.29–3.17 (m, 4H), 3.08–2.92 (m, 3H), 2.88–2.84 (d,3H), 2.55–2.37 (m, 2H), 2.26–2.19 (m, 4H), 1.78–1.70 (m, 5H), 1.50–1.40 (m, 2H), 1.11–1.10 ppm (d, $J = 6.0$ Hz, 3H); LC/MS $t_R = 1.77$ min, $m/z = 423.3$ $[M+H]^+$.

Isopropyl 2-((4a*R*,8*R*,9a*R*)-6-acetyl-8-(dimethylcarbamoyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)acetate: To a solution of **17** (239 mg, 0.6 mmol) in DMF (10 mL) was added $Me_2NH{\cdot}HCl$ (154 mg, 1.9 mmol), DIPEA (300 mg, 2.4 mmol) and HATU (456 mg, 1.2 mmol). The reaction mixture was stirred at RT for 4 h, then diluted with water (10 mL) and extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were dried (anhyd $MgSO_4$), filtered, and concentrated in vacuo to yield the crude product as a yellow oil (200 mg, 78%), which was used directly in the next step without further purification: LC/MS: $t_R = 1.87$ min, $m/z = 426.2$ $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-6-Acetyl-1-(2-hydroxyethyl)-*N*,*N*-dimethyl-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxamide: To a mixture of isopropyl 2-((4a*R*,8*R*,9a*R*)-6-acetyl-8-(dimethylcarbamoyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl) acetate (0.89 g, 2.1 mmol) in THF (80 mL) was slowly added $LiBH_4$ (2 M in THF, 3.1 mL, 6.2 mmol) at 0 °C under argon. The reaction mixture was stirred at RT overnight, then cooled to 0 °C before slowly adding AcOH (30 mL). The mixture was then stirred for an additional 30 min, and then adjusted to pH 8 with saturated aq $NaHCO_3$ (40 mL). The mixture was extracted with EtOAc (2×80 mL), and the combined organic layers were dried (anhyd $MgSO_4$), filtered and concentrated in vacuo to give the title product as a brown oil (319 mg, 41 %), which was used in the next step without further purification: LC/MS: $t_R = 1.62$ min, $m/z = 370.1$ $[M+H]^+$.

2-((4a*R*,8*R*,9a*R*)-6-Acetyl-8-(dimethylcarbamoyl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)ethyl methanesulfonate: A solution of MsCl (460 mg, 3.2 mmol) in CH_2Cl_2 (5 mL) was slowly added to a mixture of (4a*R*,8*R*,9a*R*)-6-acetyl-1-(2-hydroxyethyl)-*N*,*N*-dimethyl-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxamide (775 mg, 2.1 mmol) and Et_3N (636 mg, 6.3 mmol) in CH_2Cl_2 (50 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 3 h, then warmed to RT and stirred overnight. The organic solvent was removed in vacuo to give the crude product, which was purified by silica gel chromatography (EtOAc/petroleum ether, 2:1) to afford the title compound as a white solid (790 mg, 84%): LC/MS: $t_R = 1.69$ min, $m/z = 448.1$ $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-6-Acetyl-*N*,*N*-dimethyl-1-(2-((*R*)-2-methylpyrrolidin-1-yl)ethyl)-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxamide (18 d): (*R*)-2-Methylpyrrolidine (468 mg, 5.5 mmol) was added to a solution of 2-((4a*R*,8*R*,9a*R*)-6-acetyl-8-(dimethylcarbamoyl)-4a,6,7,8,9,9a-hexahydro indolo[1,14-fg]quinolin-1(4*H*)-yl)ethyl methanesulfonate (492 mg, 1.1 mmol) in MeCN (10 mL). The reaction mixture was stirred at 50 °C overnight. The solvent was removed in vacuo to give the crude product, which was purified by preparative HPLC to yield **18 d** as a white solid (150 mg, 31%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.17$–7.14 (m, 2H), 6.84–6.83 (m, 2H), 4.23 (t, $J = 7.6$ Hz, 2H), 4.15–4.04 (m, 2H), 3.48–3.36 (m, 2H), 3.26–3.15 (m, 7H), 2.99–2.97 (m, 4H), 2.78–2.72 (m, 1H), 2.52–2.48 (m, 1H), 2.42–2.23 (m, 5H), 2.02–1.73 (m, 4H), 1.43–1.40 (m, 1H), 1.12–1.10 ppm (d, $J = 6.0$ Hz, 3H); LC/MS: $t_R = 1.85$ min, $m/z = 437.2$ $[M+H]^+$.

1-((4a*R*,8*R*,9a*R*)-8-(3-Methyl-1,2,1-oxadiazol-2-yl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-6(1*H*)-yl)ethanone (19): DIPEA (8.8 g, 68 mmol), HATU (12.9 g, 34 mmol) and (*E*)-*N*′-hydroxyacetimidamide (3.8 g, 51 mmol) were added to a solution of **16** (5 g, 17 mmol) in DMF (50 mL). The reaction mixture was stirred at RT for 1 h, and then at 100 °C for 3 h. After cooling to RT, the reaction mixture was quenched with water (30 mL) and extracted with EtOAc (3×60 mL). The combined organic layers were dried (anhyd $MgSO_4$), filtered, and concentrated in vacuo to give the crude product, which was purified by silica gel chromatography (EtOAc/petroleum ether, 1:1) to afford **19** as a yellow solid (3.4 g, 60%): LC/MS: $t_R = 1.63$ min, $m/z = 337.2$ $[M+H]^+$.

Isopropyl 2-((4a*R*,8*R*,9a*R*)-6-acetyl-8-(3-methyl-1,2,1-oxadiazol-2-yl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)acetate: A mixture of **19** (2.5 g, 7.4 mmol), NaOH (2.7 g, 35.5 mmol) and TBAI (0.54 g, 1.48 mmol) in CH_2Cl_2 (50 mL) was cooled to 0 °C. Isopropyl 2-bromoacetate (2.7 g, 14.8 mmol) was slowly added and the reaction mixture, which was then stirred at RT overnight. The precipitate was removed by filtration, and the filtrate was washed with water (100 mL). The organic layer was dried (anhyd $MgSO_4$), filtered, and concentrated in vacuo. Purification by silica gel chromatography (petroleum ether/EtOAc, 2:1) gave the title compound as a yellow oil (2.6 g, 80%), which was used in the next step without further purification: LC/MS: $t_R = 1.83$ min, $m/z = 437.3$ $[M+H]^+$.

1-((4a*R*,8*R*,9a*R*)-1-(2-Hydroxyethyl)-8-(3-methyl-1,2,1-oxadiazol-2-yl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg] quinolin-6(1*H*)-yl)-ethanone: A suspension of $LiAlH_4$ (0.24 g, 6.3 mmol) in THF (10 mL) was cooled to −78 °C. Isopropyl 2-((4a*R*,8*R*,9a*R*)-6-acetyl-8-(3-methyl-1,2,1-oxadiazol-2-yl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]-quinolin-1(4*H*)-yl)acetate (1 g, 2.1 mmol) was slowly added at −78 °C under argon. After stirring at −78 °C for 10 min, the mixture was quenched with the careful addition of water (5 mL), then filtered through Celite, and the filter cake washed with THF (20 mL). The filtrate was extracted with EtOAc (3×100 mL). The combined organic extracts were dried (anhyd $MgSO_4$), filtered, and concentrated in vacuo to give the title compound as a yellow solid (560 mg, 70%): LC/MS: $t_R = 1.59$ min, $m/z = 381.1$ $[M+H]^+$.

2-((4a*R*,8*R*,9a*R*)-6-Acetyl-8-(3-methyl-1,2,1-oxadiazol-2-yl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)ethyl methanesulfonate: A mixture of 1-((4a*R*,8*R*,9a*R*)-1-(2-hydroxyethyl)-8-(3-methyl-1,2,1-oxadiazol-2-yl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]-quinolin-6(1*H*)-yl)ethanone (0.18 g, 0.52 mmol) and Et_3N (0.22 mL, 1.56 mmol) in CH_2Cl_2 (25 mL) was cooled to 0 °C, and MsCl (89 mg, 0.78 mmol) in CH_2Cl_2 (5 mL) was slowly added to the mixture. After stirring at RT for 4 h, the reaction was quenched with saturated aq $NaHCO_3$ (30 mL). The aqueous and organic phases were separated, and the organic layer was dried (anhyd $MgSO_4$), filtered, and concentrated in vacuo to give the title compound as a yellow solid (0.24 g, 75%): LC/MS: $t_R = 1.68$ min, $m/z = 459.1$ $[M+H]^+$.

1-((4a*R*,8*R*,9a*R*)-8-(3-Methyl-1,2,1-oxadiazol-2-yl)-1-(2-((*R*)-2-methylpyrrolidin-1-yl)ethyl)-4,4a,7,8,9,9a-hexahydroindolo [1,14-fg]quinolin-6(1*H*)-yl)ethanone (20): To a mixture of 2-((4a*R*,8*R*,9a*R*)-6-acetyl-8-(3-methyl-1,2,1-oxadiazol-2-yl)-4a,6,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-1(4*H*)-yl)ethyl methanesulfonate (230 mg, 0.5 mmol) in MeCN (10 mL) was added (*R*)-2-methylpyrrolidine (212 mg, 2.5 mmol) under argon. The reaction mixture was stirred at 60 °C overnight. The solvent was removed in vacuo, and the crude product was purified by preparative HPLC to give the title compound as a yellow solid (190 mg, 85%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.20$–7.17 (m, 2H), 6.88–6.87 (m, 2H), 4.27–4.13 (m, 4H), 3.90–3.68 (m, 1H), 3.62–3.58 (m, 1H), 3.40–2.90 (m, 6H), 2.60–2.50 (m, 1H), 2.43–2.30 (m, 4H), 2.22–2.20 (m, 1H), 2.12 (s, 3H), 2.02–1.73 (m, 4H), 1.52–1.37 (m, 1H), 1.13–1.09 ppm (m, 3H); LC/MS: $t_R = 1.86$ min, $m/z = 448.2$ $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-6-Acetyl-1-(2-hydroxyethyl)-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxylic acid: $LiBH_4$ (2 M in THF, 6.5 mL, 13 mmol) was slowly added to a solution of **17** (1.65 g, 4.36 mmol) in THF (150 mL) at 0 °C under argon. The resulting reaction mixture was then stirred at RT overnight. After re-cooling to 0 °C, AcOH (6 mL) was slowly added to the reaction mixture, which was stirred for an additional 30 min before adjusting the pH to 8 with saturated aq $NaHCO_3$ (40 mL). The reaction mixture was extracted with EtOAc (2×100 mL), and the combined organic layers were washed with brine (30 mL), dried (anhyd $MgSO_4$), filtered, and concentrated to give the crude product as a brown oil (620 mg, 44%), which was used directly in the next step without further purification: LC/MS: $t_R = 1.23$ min, $m/z = 343.2$ $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-Methyl 6-acetyl-1-(2-hydroxyethyl)-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxylate (21): $SOCl_2$ (173 mg, 1.46 mmol) was slowly added to a solution of (4a*R*,8*R*,9a*R*)-6-acetyl-1-(2-hydroxyethyl)-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxylic acid (100 mg, 0.29 mmol) in CH_3OH (15 mL) at 0 °C. The reaction mixture was stirred at RT for 4 h. The solvent was removed in vacuo to yield the crude product, which was diluted with EtOAc (20 mL). The organic layer was washed with saturated aq $NaHCO_3$ (15 mL), dried (anhyd $MgSO_4$), filtered and concentrated in vacuo to give the title compound as a yellow solid (86 mg, 83%), which was used in the next step without further purification: LC/MS: $t_R = 1.69$ min, $m/z = 357.2$ $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-Methyl 6-acetyl-1-(2-(methylsulfonyloxy)ethyl)-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxylate: A mixture of **21** (0.19 g, 0.52 mmol) and Et_3N (0.22 mL, 1.56 mmol) in CH_2Cl_2 (25 mL) was cooled to 0 °C. MsCl (170 mg, 1.5 mmol) in CH_2Cl_2 (5 mL) was slowly added to the mixture, which was then stirred at RT for 4 h. The organic solvent was removed in

vacuo to yield the crude product. The residue was diluted with CH_2Cl_2 (50 mL), washed with saturated aq $NaHCO_3$ (30 mL) and concentrated in vacuo to give the title compound as a yellow solid (0.22 g, 88%), which was used in the next step without further purification: LC/MS: $t_R = 1.68$ min, $m/z = 435.2$ $[M+H]^+$.

(4a*R*,8*R*,9a*R*)-Methyl 6-acetyl-1-(2-((*R*)-2-methylpyrrolidin-1-yl)ethyl)-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]quinoline-8-carboxylate (22): To a mixture of (4a*R*,8*R*,9a*R*)-methyl-6-acetyl-1-(2-(methylsulfonyloxy)ethyl)-1,4,4a,6,7,8,9,9a-octahydroindolo[1,14-fg]-quinoline-8-carboxylate (139 mg, 0.32 mmol) and Et_3N (0.22 mL, 1.6 mmol) in MeCN (15 mL) was added (*R*)-2-methylpyrrolidine (275 mg, 3.2 mmol) under argon. The reaction mixture was then stirred at 50 °C overnight. The solvent was removed to yield the crude product, which was purified by preparative HPLC to give **22** as a white solid (50 mg, 33%): ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.20$ (d, $J = 8.4$ Hz, 1H), 7.12 (t, $J = 7.2$ Hz, 1H), 6.92 (s, 1H), 6.83 (d, $J = 6.8$ Hz, 1H), 4.33–4.22 (m, 2H), 4.08–4.03 (m, 1H), 3.76 (s, 3H), 3.54–3.49 (m, 1H), 3.40–3.37 (s, 2H), 3.26–3.16 (m, 3H), 3.05–2.96 (m, 1H), 2.90–2.81 (m, 2H), 2.51–2.48 (m, 1H), 2.38–2.34 (m, 1H), 2.26–2.22 (m, 4H), 1.98–1.94 (m, 1H), 1.79–1.73 (m, 2H), 1.62–1.58 (m, 1H), 1.47–1.42 (m, 1H), 1.09–1.08 ppm (d, $J = 6.0$ Hz, 3H); LC/MS: $t_R = 1.76$ min, $m/z = 424.1$ $[M+H]^+$.

1-((4a*R*,8*R*,9a*R*)-8-(Hydroxymethyl)-1-(2-((*R*)-2-methylpyrrolidin-1-yl)ethyl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-6(1*H*)-yl)ethanone (23): To a solution of **22** (0.2 g, 0.48 mmol) in THF (30 mL) was slowly added $LiBH_4$ (2 M in THF, 0.72 mL, 1.42 mmol) at 0 °C under argon. The reaction mixture was stirred at 0 °C for 3 h, then at RT overnight. The reaction was quenched at 0 °C with the careful addition of water (5 mL). After stirring for 30 min, the mixture was extracted with EtOAc (3×50 mL). The combined organic layers were dried (anhyd $MgSO_4$), filtered, and concentrated in vacuo to give the crude product. Purification by preparative HPLC yielded the title compound as a white solid (76 mg, 40%): ^{1}H NMR (400 MHz, CD_3OD): $\delta = 7.33$ (d, $J = 8.4$ Hz, 1H), 7.20 (t, $J = 7.6$ Hz, 1H), 7.04 (s, 1H), 6.91 (d, $J = 7.6$ Hz, 1H), 4.60 (t, $J = 6.4$ Hz, 2H), 3.92–3.84 (m, 3H), 3.63–3.34 (m, 8H), 3.10–2.70 (m, 3H), 2.25–2.18 (m, 5H), 2.10–1.94 (m, 2H), 1.75–1.65 (m, 1H), 1.46 (d, $J = 6.8$ Hz, 3H), 0.86–0.84 ppm (m, 1H); LC/MS: $t_R = 1.69$ min, $m/z = 396.3$ $[M+1]^+$.

1-((4a*R*,8*R*,9a*R*)-8-(Methoxymethyl)-1-(2-((*R*)-2-methylpyrrolidin-1-yl)ethyl)-4,4a,7,8,9,9a-hexahydroindolo[1,14-fg]quinolin-6(1*H*)-yl)ethanone (24): To a solution of **23** (126 mg, 0.32 mmol) in MeCN (15 mL) was added CH_3I (70 mg, 0.48 mmol) and K_2CO_3 (0.22 g, 1.6 mmol) under argon. The reaction mixture was stirred at RT overnight. The solvent was removed to yield the crude product, which was purified by preparative HPLC to give the title compound as a white solid (30 mg, 23%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta = 7.23$ (d, $J = 8.4$ Hz, 1H), 7.10–7.04 (m, 2H), 6.80 (d, $J = 8.4$ Hz, 1H), 4.85–4.75 (m, 1H), 4.62–4.50 (m, 2H), 3.75–3.68 (m, 2H), 3.42–3.39 (m, 2H), 3.26–3.10 (m, 5H), 2.92–2.90 (m, 1H), 2.78–2.70 (m, 3H), 2.59–2.50 (m, 1H), 2.18–2.10 (m, 1H), 2.05 (s, 3H), 1.93–1.72 (m, 4H), 1.32–1.28 (m, 2H), 1.18–1.16 (d, $J = 6.0$ Hz, 3H), 0.68–0.66 ppm (m, 1H); LC/MS: $t_R = 1.68$ min, $m/z = 410.2$ $[M+H]^+$.

Biology

General: LC/MS–MS analyses were performed on an Agilent 6410, triple quadrupole mass spectrometer.

Radioligand binding assay: Human H3R membrane preparations in CHO-K1 cells (PerkinElmer) were incubated with 1 nM [^{3}H]-*N*-α-methylhistamine ([^{3}H]-NAMH, PerkinElmer) in the presence or absence of increasing concentrations (10-point dose titrations with 3-fold serial dilutions) of test compound to determine H3R competition binding. The binding incubations (120 min at 28 °C) were performed in a final volume of 0.1 mL buffer (50 mM Tris pH 7.5, 5 mM $MgCl_2$). (*R*)-(−)-α-Methylhistamine thioperamide (10 μM) was used to define nonspecific binding. All binding reactions were terminated by transferring 70 μL binding reaction from reaction plate into gel filtration plates (Zeba 96-well spin desalting plates, Thermo Scientific), followed by centrifugation at 1000 *g* for 2 min to collect the protein with bound radioligand. Microscint-40 (200 μL) was added to determine the bound radiolabel by using a Wallac Microbeta Trilux 2450 microplate liquid scintillation counter (PerkinElmer). IC_{50} values and Hill slopes were determined by using GraphPad Prism: log(inhibitor) vs response−variable slope. The corresponding K_i values were calculated using the Chang–Prusoff equation: $K_i = IC_{50}/\{1+([\text{radioligand}]/K_d)\}$.

Adenylate cyclase (cAMP) assay: In the presence of 10 mM 3-isobutyl-1-methylxanthine, CHO-A8 cells stably expressing the full-length human, mouse or rat H3R were incubated at 28 °C for 50 min with increasing concentrations of test compound in the presence of 5 μM (human and mouse) or 1.5 μM (rat) (*R*)-α-methylhistamine and 3 mM forskolin. cAMP formation was determined using the Ultra LANCE cAMP assay kit (PerkinElmer). Plates were read using an EnVision reader (PerkinElmer). Data were normalized to the amount of cAMP produced in control wells and are expressed as a percentage of inhibition. Experiments were run in duplicate, and data were analyzed using GraphPad Prism to obtain IC_{50} values and Hill slopes. pK_b values were determined by the generalized Cheng–Prusoff equation.

In vivo PD/PK: Male Sprague–Dawley rats (Shanghai Laboratory Animal Center, China) at the age of 8 weeks were used. They had access to food and water ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee of Chempartner Co., LTD (IACUC Protocol NO: A998HL0076). On the study day, the body weights of each animal were recorded before dosing. Compounds were dissolved in 50 mM citrate acid buffer and sonicated briefly until there was little or no suspension in the solution. Different groups of animals ($n = 5$ rats/group) were dosed orally 1 h or at other time points (1 mL kg^{-1}) before they were sacrificed using CO_2. Oral bavisant was used as a reference in all studies.

Blood sample collection and bioanalyses: A cardiac puncture was performed to collect blood sample from the cardiac cavity. The collected blood was immediately mixed with EDTA-K2 (20 μL mL^{-1}) to avoid clotting. The blood samples in tubes were then centrifuged (15 min, 6000 rpm), and the plasma transferred to new tubes and cooled in dry ice then stored in a −70 °C freezer until bioanalysis (usually within 2–3 days). A 30 μL aliquot of sample was added with 30 μL of the internal standard (dexamethasone, 300 ng mL^{-1}) and then followed by 150 μL MeCN for protein precipitation. The mixture was vortexed for 2 min and centrifuged at 12000 rpm for 5 min. The 5 μL supernatant was analyzed by using LC/MS–MS.

Frontal cortex brain tissue collection and bioanalyses: The rat brains were taken out of the skull and rinsed with ice-cold saline. Resting on top of a petri dish of ice, the frontal cortices were then removed from the rest of the brain with a sharp blade. The wet weight of the frontal cortex was immediately weighed and recorded. The frontal cortex samples were then kept in dry ice until being transfer to a −70 °C freezer for storage. The brain sample was homogenized for 2 min with 3 volumes (*v/w*) of homogenizing solution (EtOH/PBS, 85:15), and then centrifuged at 12000 rpm for

5 min. Finally, 60 μL supernatant was added into a 96-well plate and 3 μL supernatant was analyzed by using LC/MS–MS.

Ex vivo receptor occupancy assay: At specific times after oral treatment, the animals were sacrificed and the frontal cortices were dissected out on ice. The crude homogenate samples of rat frontal cortex were used to measure H3R binding as described above with [^{3}H]-*N*-α-methylhistamine as the radioligand. The protein concentration of each sample was determined by Pierce BCA protein assay kit (Thermo, #23227). The cortical homogenate was added to a 96-well plate containing [^{3}H]-*N*-α-methylhistamine (0.1 nM), in a volume of 0.2 mL (final protein concentration 4 μg μL^{-1}), incubated at 28 °C for 15 min, then the reaction was stopped by rapid filtration using a filtration plate (Millipore multiscreen GF/B plate, #F6HN58185). The filters were washed three times with ice-cold buffer (50 mM Tris, pH 7.5), then mixed with 250 μL of Microscint-40 to determine the bound radiolabel by using a Wallac Microbeta Trilux 2450 microplate liquid scintillation counter. Nonspecific binding was determined in the presence of 10 μM thioperamide. Each data point was obtained from a total of at least four animals. The inhibition of specific [^{3}H]NAMH binding, calculated relative to vehicle-treated samples, was determined to provide an indication of receptor occupancy by the compound. The dose-dependent % receptor occupancy and time course were analyzed using GraphPad Prism: Spikes, Points & collecting line. The dose–response was fit using GraphPad Prism: log(inhibitor) vs response < −variable slope.

Aqueous stability: Test compounds were prepared as solutions in potassium phosphate buffer (pH 7.4) or aqueous H_2SO_4 (pH 1) at 14 μM, and stirred at RT and 80 °C, respectively for 7 days, with LC/MS monitoring every 24 h. The samples for LC/MS were prepared through fivefold dilution with MeOH/H_2O (5:1; *v*/*v*).

CYP450 time-dependent inhibition assay: Time-dependent CYP450 inhibition was assessed in parallel with reference inhibitors by a preincubation and secondary incubation protocol. During preincubation, an aliquot of 20 μL of 5 mM NADPH (+NADPH) or buffer (−NADPH) was added into 80 μL of a mixture of positive control, test compound, or solvent, and P450 enzymes (1.25 mg mL^{-1} of human liver microsomes or 62.5 pmol mL^{-1} of recombinant CYP2C19). After 30 min, an aliquot of 20 μL from the preincubation mixture was transferred into 180 μL of a secondary incubation mixture containing substrates and 1 mM NADPH. The substrate concentrations for CYP1A2 (phenacetin at 75 μM), CYP2C9 (diclofenac at 10 μM), and CYP3A4 (midazolam at 10 μM) were at reported K_m values, and those for CYP2D6 (bufurolol at 40 μM) and CYP2C19 (S-mephenytoin at 100 μM) were at threefold the reported K_m values. The secondary incubation was allowed for 15 min. The reactions were quenched by transferring 60 μL of the aliquot from the secondary incubation system into 120 μL MeCN containing the stable isotopes of the metabolites for the corresponding CYP isoforms as internal standards. After 5 min vortexing, proteins were precipitated by 15 min centrifugation at 3220 *g*. The resulting supernatants were transferred into 96-well sample plates for LC/MS–MS analysis. Changes in P450 enzyme activities were evaluated by comparing the production of the metabolites based on the peak area ratios (metabolite/internal standard) between the preincubation systems with and without inhibitor after normalization for the activity loss in the absence of NADPH.

Liver microsomal stability: Stock solutions of test compounds at 10 mM in DMSO were prepared and stored at −80 °C. Before the assay, spiking solutions for the test compounds were prepared in MeCN at 500 μM and then further diluted to 1.5 μM in microsomes (0.75 mg mL^{-1} prepared in 0.1 M potassium phosphate buffer containing 1.0 mM EDTA, pH 7.4). The compound and microsomes mixtures were loaded (30 μL) into 96-well assay plates and preincubated at 37 °C for 10 min. Metabolism was initiated at designated time points (0 and 30 min) by adding 15 μL of prewarmed NADPH stock solution (6 mM prepared in 0.1 M potassium phosphate buffer containing 1.0 mM EDTA, pH 7.4). At the end of the incubation, 135 μL of MeCN containing 200 ng mL^{-1} osalmide was added into each well to stop the reaction. For the 0 min time point, the NADPH solution was added after the system was quenched with MeCN. The reaction mixtures were centrifuged at 3220 *g* for 10 min. The supernatants were mixed with Milli-Q water (1:1, *v*/*v*) and analyzed by LC/MS–MS.

MDCK-MDR1 assay: Seeded and washed MDCK-MDR1 cells were put in a 24-well plate. The transepithelial electrical resistance (TEER) across the monolayer was measured at room temperature for each well using a Millicell ERSohm meter. The cell monolayers with TEER higher than 250 ohm cm^2 were used for permeability assay. Donor solution containing test compound (in duplicate) (10 μM) were loaded either to the apical chamber (600 μL) for A-to-B (apical to basolateral) or to the basolateral chamber (900 μL) for B-to-A (basolateral to apical) permeation. The receiver chambers were loaded with buffered Hank's balanced salt solution (HBSS) (800 μL in the basolateral chamber for A-to-B permeation and 500 μL in the apical chamber for B-to-A permeation). All solutions loaded to apical chambers also contained 5 μM Lucifer Yellow as a quality control for monolayer integrity. An aliquot of 100 μL was taken from all the donor chambers as time zero (T_0) samples before transport was initiated. A second aliquot of 100 μL was also taken at T_0 from all apical chambers containing Lucifer Yellow. Transport was initiated by placing the apical plate seeded with MDCK-MDR1 monolayers onto the basolateral plate. The assembled plates were kept in a culture incubator (5% CO_2 and 95% relative humidity) at 37 °C for 90 min.

Caco-2 permeability: Caco-2 cells were seeded in a 24-well plate at a density of 4.8×10^4/well 21–27 days prior to the assay and maintained in culture (37 °C, 5% CO_2 with 95% relative humidity) with change of growth medium (minimum essential medium + 10% fetal bovine serum + 1% non-essential amino acids) every other day. On the assay day, Caco-2 monolayers were washed once with HBSS containing 25 mM HEPES (pH 7.4), and the TEER across the monolayer was measured at room temperature for each well using a Millicell ERSohm meter. The cell monolayers with TEER higher than 250 ohm cm^2 were used for permeability assay. The procedures of permeability assay conducted in Caco-2 cells were the same as those described in MDCK-MDR1 assay.

Test in orexin-deficient mice: Homozygous orexin-deficient mice ($n = 11$) were used for this study. For testing compound effects on narcoleptic episodes, mice were treated immediately before lights-off and then put into large Plexiglas boxes equipped with saw dust bedding, a nest box, and enrichments such as wooden chew block, transparent running wheel, transparent mouse tunnel, plastic disc, and five glass marbles. The behavior of the mice was videotaped from above using infrared cameras. Videotapes were scored offline by two independent experienced observers. Narcoleptic episodes were defined as periods of total inactivity outside the nest box, lasting longer than 10 s and preceded by activity lasting longer than 40 s.[11,33] After a one-week recovery period in the home cage, the animals were retested. Each animal was treated with either saline or compound **1** (30 mg kg^{-1}) in a pseudo-randomized order. An experimental session started 2 h before lights-off when the animals were put into the exposure boxes. Immediately before lights-off, the animals were treated and the video

recording was started (recording duration: 4 h). The animals were left in the exposure box until the next morning. Then, they were put back into the home cage. The first experimental session was without any treatment to determine a pre-test baseline (pre-test). Then, every animal was repeatedly tested with the different treatment in a pseudo-randomized order. At the end, a further session without any treatment was added to determine the post-test baseline (post-test).

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