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Melanin-concentrating hormone receptor 1 antagonists: Synthesis, structure–activity relationship, docking studies, and biological evaluation of 2,3,4,5-tetrahydro-*1H*-3-benzazepine derivatives

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ABSTRACT

Melanin-concentrating hormone receptor 1 (MCHR1) antagonists have been studied as potential agents for the treatment of obesity. Initial structure–activity relationship studies of in-house hit compound **1a** and subsequent optimization studies resulted in the identification of tetrahydroisoquinoline derivative **23**, 1-(2-acetyl-1,2,3,4-tetrahydroisoquinolin-7-yl)-4-[4-(4-chlorophenyl)piperidin-1-yl]butan-1-one, as a potent hMCHR1 antagonist. A homology model of hMCHR1 suggests that these compounds interact with Asn294 and Asp123 in the binding site of hMCHR1 to enhance binding affinity. Oral administration of compound **23** dose-dependently reduced food intake in diet-induced obesity (DIO)-F344 rats.

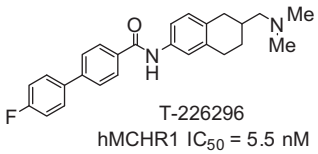
1. Introduction

Melanin-concentrating hormone (MCH) is a cyclic peptide hormone first isolated from the chum salmon pituitary and was shown to regulate melanin pigment aggregation in melanocytes.[1] MCH was subsequently found to be present in mammals,[2] and its amino acid sequence is highly conserved in fish, rats, and humans.[3] In the mammalian brain, MCH is predominantly expressed in the perikarya of the lateral hypothalamus and zona incerta, with projections toward various regions of the brain.[4] Recent studies on MCH have focused on its role in the regulation of feeding behavior and energy balance, which was confirmed by the following biological evidences. (i) Intracerebroventricular (icv) injection of MCH acutely stimulates feeding behavior in rats.[5] (ii) Fasting in genetically obese *ob*/*ob* mice[6] and A^y/a (agouti) mice results in the upregulation of MCH mRNA levels.[7] (iii) MCH reverses the action of alpha-melanocyte-stimulating hormone (α-MSH), an anorexigenic melanocortin peptide.[8a–c] (iv) MCH-knockout mice exhibit reduced body weight due to hypophagia and an increase in metabolic rate.[9] (v) Overexpression of MCH mRNA in mice results in hyperphagia, mild obesity, and insulin resistance.[10]

MCH was identified as the endogeneous ligand for the orphan G-protein-coupled receptor (GPCR), MCHR1 (aka SLC-1, GPR24),[11a–e] and MCH receptor 2 (MCHR2, aka SLT).[12a–f] Interestingly, MCHR2 is not present in rodents.[13]

MCHR1 is widely expressed in the CNS,[14] and its expression, as well as that of its ligand MCH, was found to be upregulated in *ob*/*ob* mice and by fasting.[15] MCHR1 deficient (*Mch1r*−/−) mice are hyperphagic; however, *Mch1r*−/− mice are less susceptible to diet-induced obesity due to their hyperactivity and increased energy expenditure.[16a,b]

An MCHR1 peptide antagonist suppressed MCH-induced food intake in satiated Sprague–Dawley rats. Furthermore, chronic infusion of the peptide antagonist in diet-induced obese (DIO)-mice reduced food intake, prevented body weight gain, and reduced body weight in obese mice.[17a,b] These findings have made MCHR1 an attractive pharmaceutical target; small-molecule MCHR1 antagonists have been heavily investigated by many pharmaceutical and biotech companies in an attempt to identify an effective anti-obesity agent.[18a–c]



T-226296
hMCHR1 IC_{50} = 5.5 nM

Figure 1. Structure of T-226296.

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We previously reported the tetrahydronaphthalene-based compound T-226296 (Fig. 1) as the first small molecule MCHR1 antagonist.[19] Further effort has been devoted toward identifying a novel class of non-peptidic MCHR1 antagonists. High-throughput screening of our in-house compound library against hMCHR1 resulted in the identification of benzazepine derivative **1a** (Fig. 2) as a new lead compound with moderate affinity (IC_{50} = 20 nM) in a receptor-binding assay. To explore the initial SAR of **1a**, we designed 4 types of molecules (Types I–IV, Fig. 2), especially to identify the basic nitrogen that is essential for in vitro activity. Next, the replacement of the phenylpiperidine and 3-benzazepine moieties was investigated. Herein, we describe the SAR investigation of this series of compounds, which led us to identify the potent, orally active hMCHR1 antagonist **23** with favorable pharmacokinetic properties.

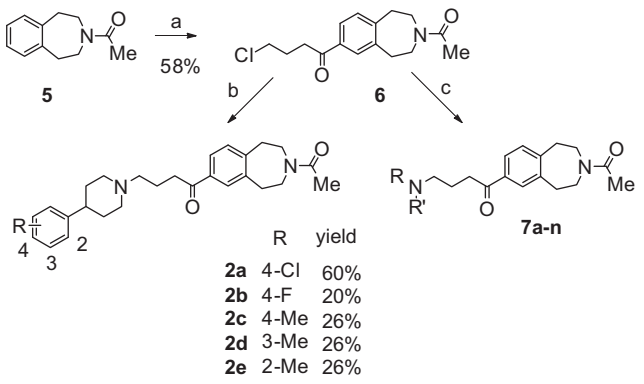
2. Chemistry

Benzazepine derivatives **2a–e** and **7a–n** were synthesized as shown in Scheme 1. *N*-Acetylbenzazepine **5** was subjected to the Friedel–Crafts reaction with 4-chlorobutyryl chloride to generate a key intermediate, 4-chloro-1-(2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)butan-1-one **6**; subsequent displacement of the chlorine with phenylpiperidines afforded **2a–e**. Compounds **7a–n** were synthesized from **6** through parallel synthesis reaction with a variety of amines under similar conditions to those used for **2a–e**. Replacement of the nitrogen substituent on the benzazepine ring is shown in Scheme 2. The acetyl group of compound **2a** was hydrolyzed using concentrated HCl to yield the amine compound **1b**. Reductive amination of **1b** with formaldehyde afforded the *N*-methyl derivative **1c**. Coupling of **1b** with acid chloride, sulfonyl chloride, methyl chlorocarbamte, and ethyl isocyanate provided amides **2f–j**, sulfonamides **2k** and **2l**, carbamate **2m**, and urea **2n**, respectively.

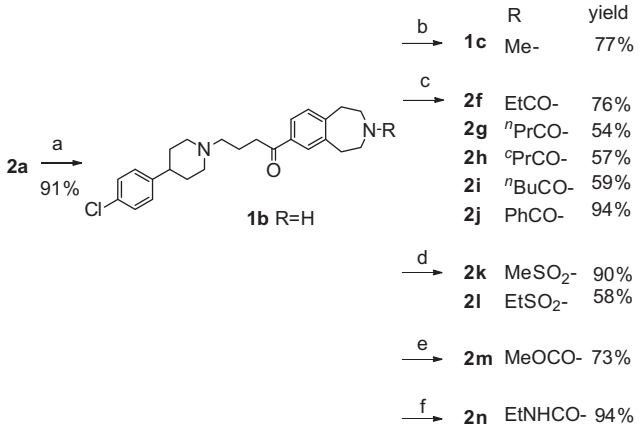
Replacements of the 3-benzazepine moiety of **2a** consisting of other bicyclic (**22–27**) or monocyclic (**28**) templates are depicted in Scheme 3. The syntheses of these compounds were conducted according to the reactions shown in Scheme 1. For bicyclic analogues **8**, **9**, **11**, and **12**, the Friedel–Crafts reaction was used to regioselectively furnish desired intermediates **15**, **16**, **18**, and **19**, respectively.

The syntheses of benzazepine derivatives **3a**, **3b**, and **4** are illustrated in Scheme 4. *N*-Trifluoroacetylbenzazepine **29** was subjected to the Friedel–Crafts reaction with succinic anhydride to afford carboxylic acid **30**. Compound **30** was coupled with 4-chlorophenylpiperidine to yield amide **31**, which was treated with aqueous K_2CO_3 to generate amine **3a**. Compound **3a** was subjected to reductive amination with formaldehyde or a reaction with acetyl chloride to afford *N*-methyl analogue **3b** or *N*-acetyl analogue **4**, respectively.

The alkoxy derivative **37** was synthesized as shown in Scheme 5. Removal of the methyl group from 7-methoxy-2,3,4,5-tetrahydro-1*H*-3-benzazepine **32** using aqueous HBr followed by Boc-protection of the nitrogen atom provided the 7-hydroxybenzazepine derivative **33**. Alkylation of the hydroxy group using 1-bromo-3-chloropropane in the presence of K_2CO_3 generated the ether **34**, which was sequentially reacted with 4-chlorophenylpiperidine to afford **35**. Deprotection of the Boc group followed by a reaction with acetyl chloride yielded the desired ether analogue **37**.



Scheme 1. Synthesis of benzazepine derivatives **2a–e** and **7a–n**. Reagents and conditions: (a) 4-chlorobutyryl chloride, $AlCl_3$, nitroethane; (b) phenylpiperidines, *N*,*N*′-diisopropylethylamine, K_2CO_3, KI, DMF, 60 °C; (c) HNRR′, *N*,*N*′-diisopropylethylamine, DMF, 60 °C.



Scheme 2. Synthesis of benzazepine derivatives **1c** and **2f–n**. Reagents and conditions: (a) concd. HCl, reflux; (b) formaldehyde, formic acid, 100 °C; (c) acid chloride, Et_3N, THF; (d) sulfonyl chloride, Et_3N, THF; (e) methyl chlorocarbamate, Et_3N, THF; (f) ethyl isocyanate, Et_3N, THF.

3. Results and discussion

Compounds synthesized in this study were tested for their binding affinities to the human MCH receptor 1 (hMCHR1) and rat MCH receptor 1 (rMCHR1) expressed in Chinese hamster ovary (CHO) cells using [^{125}I]-MCH(4–19) as a ligand.[19] Compounds with high hMCHR1 affinities were evaluated further for antagonistic activity in an in vitro functional assay to examine the inhibition of MCH-stimulated arachidonic acid release from CHO cells expressing hMCHR1.[19]

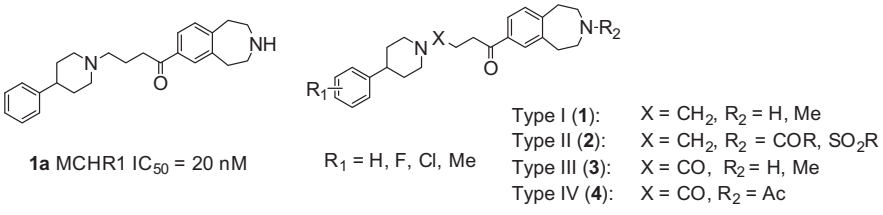
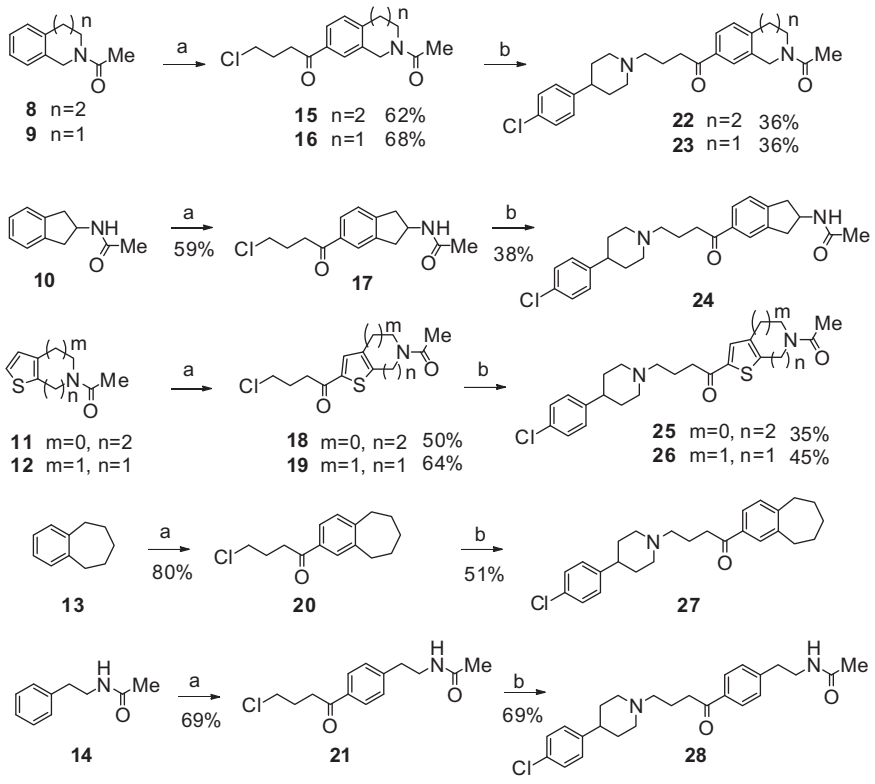
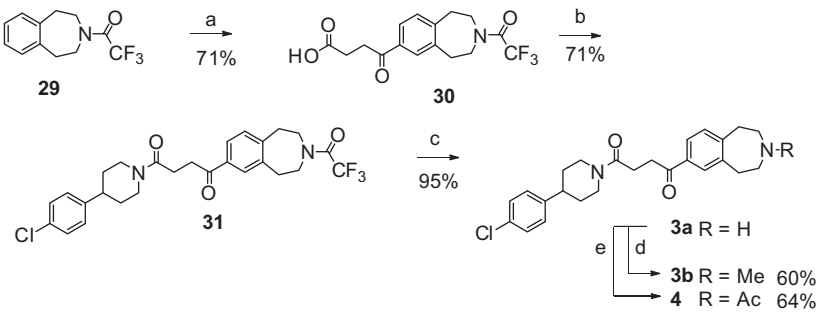


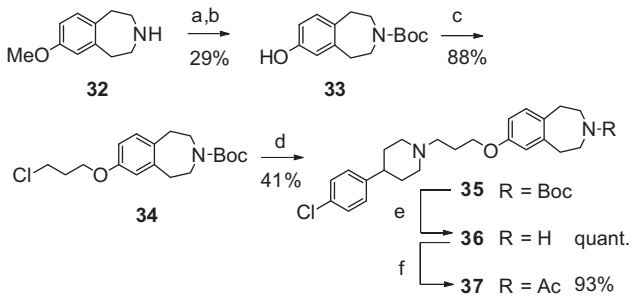
Figure 2. Structure of HTS hit compound **1a** and analogues designed; Types I–IV.



Scheme 3. Synthesis of compound **22**–**28**. Reagents and conditions: (a) 4-chlorobutyryl chloride, $AlCl_3$, 1,2-dichloroethane; (b) 4-chlorophenylpiperidine, Et_3N, K_2CO_3, KI, DMF, 60 °C.



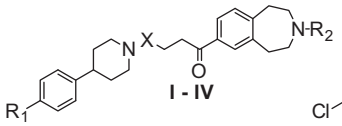
Scheme 4. Syhtnesis of compound **3a**, **3b** and **4**. Reagents and conditions: (a) succinic anhydride, $AlCl_3$, 1,2-dichloroethane; (b) 4-chlorophenylpiperidine hydrochloride, diethyl phosphorocyanidate, Et_3N, DMF; (c) K_2CO_3, MeOH-H_2O; (d) HCHO, HCOOH, 100 °C; (e) acetyl chloride, K_2CO_3, MeCN.

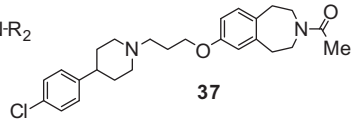


Scheme 5. Synthesis of compound **37**. Reagents and conditions: (a) 48% HBr, 80 °C, 16 h; (b) Boc_2O, *N*,*N*′-diisopropylethylamine, THF–DMF (2:1), rt to 50 °C; (c) 1-bromo-3-chloropropane, K_2CO_3, DMF, 80 °C; (d) 4-chlorophenylpiperidine hydrochloride, K_2CO_3, KI, DMF, 80 °C, 3 h; (e) TFA, rt, 16 h; (f) acetyl chloride, Et_3N, THF, rt.

The results of the initial SAR study of lead compound **1a** (IC_{50} = 20 nM) are shown in Tables 1 and 2. Table 1 depicts the in vitro activities of the compounds, grouped as Types I–IV and the ether analogue **37**. Addition of a chlorine atom at the 4-position of the terminal benzene ring in compound **1a** resulted in a 20-fold increase in potency (**1b**, IC_{50} = 1.2 nM). Introduction of a methyl group onto the nitrogen in the benzazepine ring afforded **1c** (IC_{50} = 0.96 nM), which showed comparable in vitro activity to **1b**. Acetylation of the nitrogen atom in the benzazepine ring of **1b** resulted in a modest loss of binding affinity (**2a**, IC_{50} = 6.6 nM). Interestingly, **2a** showed the most potent antagonistic activity against hMCHR1 (arachidonic acid release, IC_{50} = 11 nM). However, **1b** and **1c** exhibited substantial loss in antagonistic activity (IC_{50} = 22 and 34 nM, respectively), although these compounds displayed potent binding affinities. Type III compounds **3a** and **3b** showed moderate binding affinities (IC_{50} = 20 and 30 nM, respectively), and a neutral compound **4** (Type IV) resulted in a 10-fold decrease in potency (IC_{50} = 190 nM). These results suggest that it is essential to have at least one basic nitrogen atom to obtain potent binding affinity, and that compounds with a basic nitrogen

Table 1
In vitro activities of 3-benzazepine derivatives

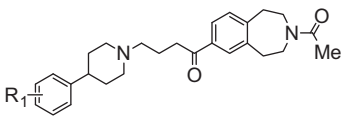




Compd	Type	R_1	R_2	X	IC_{50} (nM)	
					hMCHR1[a]	AA release[b]
1a	I	H	H	CH_2	20	NT[c]
1b	I	Cl	H	CH_2	1.2	22
1c	I	Cl	Me	CH_2	0.96	34
2a	II	Cl	Ac	CH_2	6.6	11
3a	III	Cl	H	CO	20	NT[c]
3b	III	Cl	Me	CO	30	NT[c]
4	IV	Cl	Ac	CO	190	NT[c]
37	–	–	–	–	100	NT[c]

[a] Binding affinity for hMCHR1.
[b] Antagonistic activity against hMCHR1, inhibition of MCH-stimulated arachidonic acid release from CHO cells expressing hMCHR1.
[c] Not tested. IC_{50} values are calculated with one experiment performed in duplicate.

Table 2
Binding affinities of 3-benzazepine derivatives **2b–e**



Compd	R_1	IC_{50} (nM)
		hMCHR1[a]
2b	4-F	86
2c	4-Me	19
2d	3-Me	1200
2e	2-Me	900

[a] Refers to Table 1.

atom in the center of the molecule (**1b**,**c** = Type I, **2a** = Type II) are favored over those with terminal amines (**3a**,**b** = Type III). In addition, the ether analogue **37** showed a 15-fold loss in binding affinity (IC_{50} = 100 nM) compared with **2a**. These results suggest that the carbonyl group of **2a** may interact with the binding site of the receptor. On the basis of these results, we selected the acetylbenzazepine analogue **2a** (Type II) for further optimization studies.

Table 2 shows the effects of varying substituents on the terminal benzene ring of **2a**. Replacement of chlorine at the 4-position with fluorine (**2b**, IC_{50} = 86 nM) or a methyl group (**2c**, IC_{50} = 19 nM) resulted in 13- and 3-fold decreases in binding affinity, respectively. Furthermore, binding affinities dramatically decreased when the methyl group position in **2c** was changed from the benzene 4-position to the 3- or 2-position (**2d**, IC_{50} = 1200 nM; **2e**, IC_{50} = 900 nM).

Replacement of the 4-chlorophenylpiperidine group of **2a** was investigated (Table 3). Compounds possessing various amino groups **7a–n** showed lower affinity than the original compound **2a**. Only one compound, 4-(4-chlorophenyl)piperidine-4-ol derivative **7l**, showed moderate binding affinity (IC_{50} = 62 nM), whereas the other amine analogues exhibited a significant loss in activity.

After establishing 4-chlorophenylpiperidine as a suitable substituent, we replaced the nitrogen substituent on the benzazepine ring. Table 4 depicts the in vitro activities of amides **2f–j**, sulfonamides **2k** and **2l**, carbamate **2m**, and urea **2n**. All compounds displayed high hMCHR1 and rMCHR1 binding affinities, except for the benzoyl-substituted **2j** (IC_{50} = 70 nM). Among these, the small alkyl amides **2f–h** and the small alkyl sulfonamides **2k** and **2l** exhibited considerably potent antagonistic activities (IC_{50} = 2.8–9.2 nM), and their IC_{50} values were comparable to those of hMCHR1 binding.

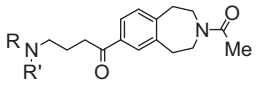
We replaced the 3-benzazepine core of **2a** with other templates; the corresponding in vitro data are summarized in Table 5. The 2-benzazepine derivative **22** showed a 7-fold decrease in binding affinity (IC_{50} = 46 nM) compared with **2a**. On the other hand, the tetrahydroisoquinoline derivative **23** displayed equipotent hMCHR1 affinity (IC_{50} = 5.6 nM) and antagonistic activity (IC_{50} = 6.2 nM) to **2a**. Replacement of the 3-benzazepine scaffold with other 6,5- or 5,6-membered rings (**24**–**26**) markedly decreased the hMCHR1 affinity (IC_{50} = 19–280 nM). Replacement of the N-Ac group with a CH_2 group in the benzazepine ring of **2a** yielded **27**, which exhibited in decreased binding affinity (IC_{50} = 61 nM). Ring-opening of the azepane part in the benzazepine core, phenethylamide **28**, also showed attenuated binding affinity (IC_{50} = 450 nM). These results suggest that the benzene-fused 6- or 7-membered ring systems possessing a nitrogen atom confer high binding affinities. In addition, arranging the amide moiety on the ring system in an optimal position is required to obtain an effective interaction with the receptor.

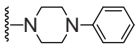
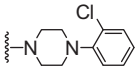
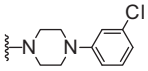
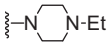
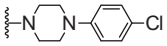
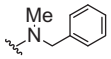
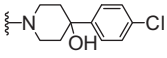
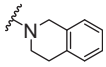
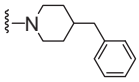
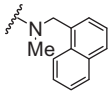
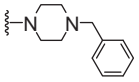
4. Homology model of hMCHR1 and automated docking

Further analysis of the SAR results will be discussed in terms of a homology docking model we constructed. A homology model of hMCHR1 was constructed based on the crystal structure of bovine rhodopsin (PDB code 1F88) as a structural template using MOE 2005.06,[20] after truncating a portion of the extracellular region (residues 179–200). A primary sequence alignment of hMCHR1 and bovine rhodopsin was performed using MOE. Several models were prepared by probing the possible rotamer states of residues in the TM ligand binding region, which included Asp123, Tyr272, Tyr273, and Asn294. hMCHR1 antagonists were docked into hMCHR1 models using the automated docking program GOLD.[21–23] Reasonable binding modes were selected considering both the GoldScore and the consistency of the observed SAR. To account for side chain flexibility, the resulting binding modes were refined using energy minimization in MOE. Automatic docking with distance constraints between the ligand atom and the oxygen atom of the Asp123 carboxyl group was also performed. For this case, however, reasonable binding modes could not be obtained.

Figure 3 depicts an overall model of the receptor transmembrane helical region docked with compound **2a**, which is embedded in the region between TM3, 5, 6, and 7. In this mode, compound **2a** sits in a binding pocket located in the upper half of the helices, inserting its terminal chlorophenyl moiety into the bottom of the cavity formed by TM5 and 6. Figure 4 shows a close-up view of the interactions between the compound and hMCHR1. Asp123 on TM3, which plays a critical role in the binding of MCH, is located near the center of compound **2a** and interacts with the protonated nitrogen atom. This orientation varies from that observed in the binding mode of T-226296 analogue: the amide carbonyl group of T-226296 analogue interacts with Gln127 instead of Asp123.[24] Figure 5 shows the binding interactions of the 3-benzazepine moiety of **2a**. This mode shows a detailed view of where the amide carbonyl group makes a hydrogen bond interaction with the side chain carbamoyl nitrogen of Asn294 on TM7. The SAR study of replacements for the

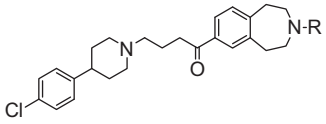
Table 3
Binding affinities of 3-benzazepine derivatives **7a–n**



Compd	–NRR′	IC_{50} (nM) hMCHR1[a]	Compd	–NRR′	IC_{50} (nM) hMCHR1[a]
7a		>1000	**7h**		>1000
7b		>1000	**7i**		480
7c		>1000	**7j**		>1000
7d		>1000	**7k**		>1000
7e		>1000	**7l**		62
7f		>1000	**7m**		>1000
7g		>1000	**7n**		>1000

[a] Refers to Table 1.

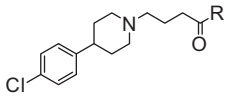
Table 4
In vitro activities of 3-benzazepine derivatives **2f–n**

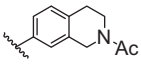
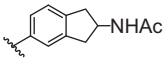
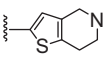
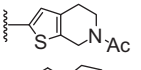
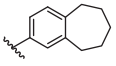
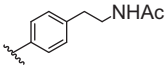


Compd	R	IC_{50} (nM)		
		hMCHR1[a]	rMCHR1[b]	AA release[c]
2f	EtCO	5.2	8.4	2.8
2g	^{n}PrCO	4.7	7.5	7.4
2h	^{c}PrCO	3.9	7.3	5.4
2i	^{n}BuCO	7.2	11	11
2j	PhCO	70	79	NT[d]
2k	$MeSO_2$	6.7	8.5	9.2
2l	$EtSO_2$	4.5	6.4	5.3
2m	MeOCO	12	17	NT[d]
2n	EtNHCO	8.1	11	NT[d]

[a] Binding affinity for hMCHR1.
[b] Binding affinity for rMCHR1.
[c] Antagonistic activity against hMCHR1, inhibition of MCH-stimulated arachidonic acid release from CHO cells expressing hMCHR1.
[d] Not tested. IC_{50} values are calculated with one experiment performed in duplicate.

Table 5
In vitro activities of compounds **22–28**



Compd	R	IC_{50} (nM)		
		hMCHR1[a]	rMCHR1[b]	AA release[c]
22		46	86	NT[d]
23		5.6	11	6.2
24		77	NT[d]	NT[d]
25		19	NT[d]	NT[d]
26		280	NT[d]	NT[d]
27		61	80	NT[d]
28		450	NT[d]	NT[d]

[a,b,c,d] Refers to Table 4.

benzazepine moiety (Table 5) supports this model. Replacement of the 3-benzazepine ring with other bicyclic rings (**22, 24–26**), deletion of the amide group (**27**), and ring-opening (**28**) decreased the potency because these modifications cannot place the amide carbonyl group in the appropriate position to interact with Asn294. Furthermore, Asn294 can form a hydrogen bond with the protonated basic nitrogen atom in compounds **1b**, **1c**, **3a**, and **3b** (Table 1) that possess moderate to potent binding affinities for hMCHR1. This model also suggests that there is a sufficient space for a substituent around the nitrogen atom of the 3-benzazepine ring of compound **2a**. This space accommodates relatively large, polar groups, such as *n*-butylcarbonyl (**2i**), methoxycarbonyl (**2m**), and ethylaminocarbonyl (**2n**) groups, but not a bulky benzoyl group (**2j**), as shown in Table 4. The 3-benzazepine core interacts with

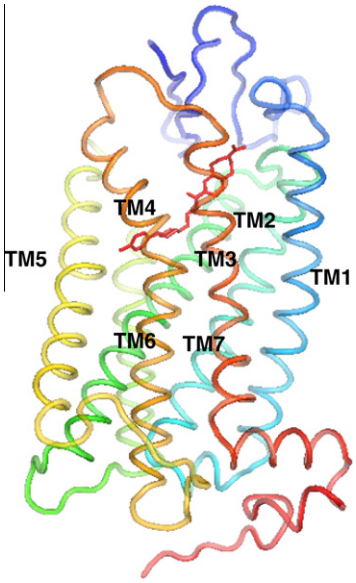


Figure 3. Binding mode of compound **2a** (red). The overall view shows that **2a** is positioned between TM3 (green), TM5 (yellow), TM6 (orange), and TM7 (red).

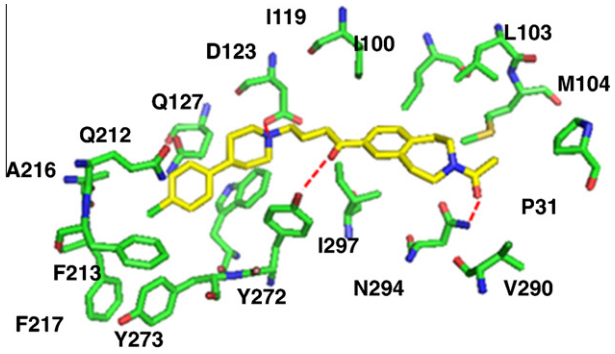


Figure 4. Interactions between compound **2a** (carbon atoms in yellow, nitrogen atoms in blue, oxygen atoms in red, and chlorine atoms in light green) and MCHR1 (carbon atoms in green).

lipophilic amino acid residues, i.e., Ile100, Leu103, Met104, and Ile119, in the binding pocket. The carbonyl group at the 7-position of the benzazepine ring makes a hydrogen bond with the side chain oxygen atom of Tyr272 on TM6, which can explain the decreased potency of the ether analogue **37** (Table 1). Figure 6 shows a detailed view of the binding mode for the 4-chlorophenylpiperidine moiety; the chlorophenyl group is situated in a lipophilic binding pocket comprised of Phe213, Ala216, and Phe217 on TM5 and Tyr273 on TM6. This model provides a small lipophilic space around the 4-position of the terminal benzene ring, while the 2- and 3-positions appear too crowded to allow substituents. The narrow SAR observed for compounds **2b–e** (Table 2) and **7a–n** (Table 3) exemplifies the situation described in this model. The protonated nitrogen atom of the piperidine group in **2a** forms an ionic interaction and a hydrogen bond with the carboxy group of Asp123 on TM3; this is consistent with the decreased binding affinities of the carbonylpiperidine analogue **4** (Table 1) that cannot interact with Asp123.

5. Pharmacokinetic analysis and pharmacology

The metabolic stabilities of compounds **2a**, **2f**, **2h**, **2l**, and **23** were evaluated in hepatic microsomes (Table 6). Tetrahydroisoquinoline **23** showed good metabolic stability in human and rat hepatic microsomes (29 and 18 μL/min/kg at 10 μM, respectively). In contrast, the benzazepines **2a**, **2f**, and **2l** displayed relatively poor metabolic stability, and the cyclopropyl amide compound **2h** was considerably unstable metabolically in vitro. Compounds **2a**, **2l**, and **23** were orally administered to F344 rats at a dose of 10 mg/kg, and the concentration of these compounds in the plasma and brain were measured. The concentrations of **23** in both plasma and brain were found to be higher than those of **2a**. The C_{max} value for **23** in the rat brain was 0.90 ± 0.15 μg/g, 3-fold higher than that for **2a**. Furthermore, the AUC_{0-24h} value for **23** in rat brain was 5.07 μg h/g, which was 5-fold higher than that for **2a**. The favorable pharmacokinetic profile may be ascribed to the excellent metabolic stability and BBB permeability of compound **23**. On the other hand, sulfonamide **2l** showed poor pharmacokinetic properties and, in particular, the C_{max} and AUC_{0-24h} values for brain were extremely low (0.06 ± 0.02 μg/g and 0.06 μg h/g, respectively). On the basis of these results, the tetrahydroisoquinoline derivative **23** was se-

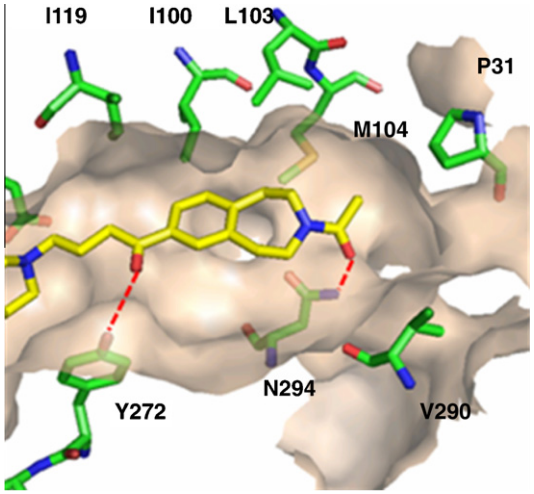


Figure 5. Binding mode of 3-benzazepine of **2a**. The acetyl carbonyl group makes a hydrogen bond with Asn294 on TM7. Asn294 can also interact with the benzazepine nitrogen atom even when the nitrogen is protonated as a basic amine. The carbonyl group at the 7-position of the benzazepine ring makes a hydrogen bond with Try272 on TM6.

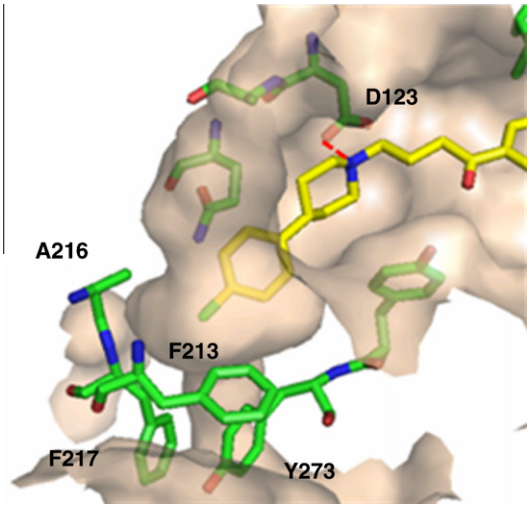
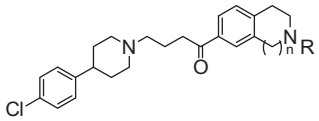


Figure 6. Binding interaction of 4-chlorophenylpiperidine of **2a**. The protonated nitrogen atom of the piperidine makes an ionic interaction and a hydrogen bond with Asp123 on TM3. The chlorophenyl moiety is packed tightly within the lipophilic pocket composed of Phe213 (TM5), Ala216 (TM5), Phe217 (TM5), and Tyr273 (TM6).

Table 6
Profiles of **2a**, **2f**, **2h**, **2l**, and **23**



Compd	R	*n*	CL(int.)[a] (μL/min/kg)		Plasma[b]			Brain[b]		
			Human	Rat	C_{max} (μg/mL)	T_{max} (h)	AUC_{0-24h} (μg h/mL)	C_{max} (μg/g)	T_{max} (h)	AUC_{0-24h} (μg h/g)
2a	Ac	2	66	78	0.26 ± 0.01	2	1.35	0.32 ± 0.08	1	0.97
2f	EtCO	2	121	109	NT[c]	NT[c]	NT[c]	NT[c]	NT[c]	NT[c]
2h	[c]PrCO	2	209	238	NT[c]	NT[c]	NT[c]	NT[c]	NT[c]	NT[c]
2l	$EtSO_2$	2	105	107	0.11 ± 0.03	1	0.18	0.06 ± 0.02	1	0.06
23	Ac	1	29	18	0.48 ± 0.06	2	3.40	0.90 ± 0.15	2	5.07

[a] Internal Clearance: In vitro metabolic stability in hepatic microsome at a concentration of 10 μM.
[b] Concentrations were measured using HPLC 1, 2, 4, 8, and 24 h after oral administration of compounds (10 mg/kg, po) in F344 rats. $n = 3$.
[c] Not tested.

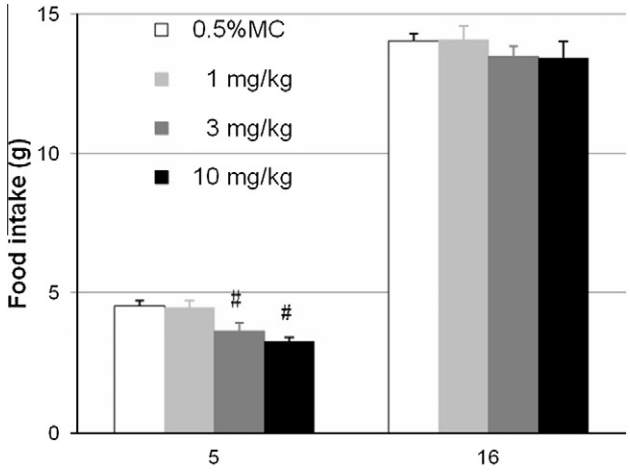


Figure 7. Effects of **23** (1, 3, and 10 mg/kg, po) on food intake in DIO-F344 rats. Cumulative food intake after 5 and 16 h was measured. #: $P < 0.025$ versus 0.5% MC (Williams test). $n = 6$.

lected for further evaluation. Oral administration of **23** (1, 3, and 10 mg/kg) dose-dependently and significantly suppressed food intake (1.5%, 19.9% and 28.1%, respectively) in DIO-F344 rats (Fig. 7).

Although **23** showed promising anti-obesity profiles, further development was suspended because **23** (and the related compounds) displayed potent hERG channel inhibitory activity (64.2% at 1 μM, 100.0% at 10 μM) in a patch clamp study. Thus, **23** may substantially inhibit the hERG channel at pharmacologically effective doses in DIO-F344 rats (10 mg/kg, $C_{max} = 0.48 \pm 0.06$ μg/mL; 1.08 μM). Further optimization study addressing hERG activity is necessary for development of this analogue.

6. Conclusion

We developed a benzazepine derivative **1a** as a hit compound, representing a new class of hMCHR1 antagonists. SAR studies and subsequent optimization studies led to the identification of a potent, orally active, non-peptidic hMCHR1 antagonist, tetrahydroisoquinoline derivative **23**. Homology modeling of these analogues suggested that some key interactions, particularly those with Asn294 and Asp123, enhance the binding affinity. Compound **23** exhibited nanomolar hMCHR1 affinity ($IC_{50} = 5.6$ nM) and potent antagonistic activity ($IC_{50} = 6.2$ nM). Pharmacokinetic studies revealed that **23** exhibited good pharmacokinetic properties with high BBB permeability in rats. This compound effectively and dose-dependently attenuated food intake in DIO-F344 rats after oral administration (1, 3, 10 mg/kg). These results suggest that **23** represents a new class of orally active non-peptidic hMCHR1 antagonists; however, further optimization is necessary to avoid hERG-associated liability.

7. Experimental section

Melting points (mp) were determined with a Yanagimoto micro melting point apparatus or BÜCHI Melting Point B545 and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Varian Gemini 200 or Varian Mercury 300 NMR spectrometer. Chemical shifts were reported in δ value (ppm) with tetramethylsilane as an internal standard. Splitting patterns are designed as follows: s, singlet; d, doublet; t, triplet; dd, double doublet; q, qualtet; quint, quintet; sext, sextet; m, multiplet; br, broad. Coupling constants (J) are reported in hertz (Hz). LC/MS (ESI positive) spectra were recorded on a Waters Micromass ZQ 2000. We carried out Elemental analysis (C, H, N) to determine purity of test compounds by the Analytical Department of Takeda Pharmaceutical Co., and the results were within 0.4% of theoretical values. Purity of compounds (>95%) was established by elemental analysis. The data of Elemental analysis was attached Supplementary data. Thin-layer chromatography (TLC) analyses were performed with silica gel 60_{F254} plate (Merck Art. 5715) or alumina 60_{F254} plate (TypeE). Chromatographic separations were performed with Merck Silica gel 60 (Merck Art.7734), ICN Alumina B, Akt. I (Activity grade III) or basic silica gel (ChromatorexNH, 100–200 mesh, Fuji Sylysia Chemical Ltd) using the indicated eluents. Yields are unoptimized. Chemical intermediates were characterized by ^{1}H NMR.

7.1. 1-(3-Acetyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)-4-chlorobutan-1-one (6)

To a mixture of **5** (20.0 g, 105.7 mmol) and 4-chlorobutyryl chloride (16.4 g, 116.2 mmol) in nitroethane (200 mL) was added $AlCl_3$ (31.0 g, 232.5 mmol) and the mixture was stirred at room temperature for 2 h. The reaction mixture was poured onto crashed ice and the mixture was partitioned between AcOEt and water. The AcOEt layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was triturated with diisopropylether to give **6** (17.9 g, 58 %) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.19 (3H, s), 2.20–2.28 (2H, m), 2.93–3.05 (4H, m), 3.16 (2H, t, $J = 7.0$ Hz), 3.57–3.64 (2H, m), 3.68 (2H, t, $J = 6.2$ Hz), 3.71–3.79 (2H, m), 7.19–7.25 (1H, m), 7.69–7.81 (2H, m).

7.2. 1-(3-Acetyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)-4-[4-(4-chlorophenyl)piperidin-1-yl]-butan-1-one (2a)

A mixture of **6** (1.18 g, 4.00 mmol), 4-chlorophenylpiperidine hydrochloride (1.11 g, 4.80 mmol), diisopropylethylamine (0.62 g, 4.80 mmol), K_2CO_3 (0.66 g, 4.80 mmol) and KI (0.80 g, 4.80 mmol) in DMF (15 mL) was stirred at 60 °C for 16 h. The reaction mixture was partitioned between AcOEt and water. The AcOEt layer was washed with water, brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (AcOEt, AcOEt/MeOH = 20/1) to give **2a** (1.08 g, 60%) as a white solid: mp 131–132 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.54–2.25 (11H, m), 2.37–2.56 (3H, m), 2.90–3.12 (8H, m), 3.53–3.64 (2H, m), 3.66–3.79 (2H, m), 7.07–7.31 (5H, m), 7.72–7.82 (2H, m). Anal. ($C_{27}H_{33}ClN_2O_2\cdot 0.2H_2O$) C, H, N.

7.3. 1-(3-Acetyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)-4-[4-(4-fluorophenyl)piperidin-1-yl]-butan-1-one (2b)

The title compound was prepared by a procedure similar to the one described for **2a** to provide **2b** (20%) as a white solid: mp 111–112 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.65–1.82 (4H, m), 1.95–2.15 (4H, m), 2.19 (3H, s), 2.48 (3H, t, J = 7.0 Hz), 2.95–3.09 (8H, m), 3.60 (2H, m), 3.73 (2H, m), 6.92–7.01 (2H, m), 7.13–7.28 (3H, m), 7.77 (2H, m). Anal. ($C_{27}H_{33}FN_2O_2$) C, H, N.

7.4. 1-(3-Acetyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)-4-[4-(4-methylphenyl)piperidin-1-yl]-butan-1-one (2c)

The title compound was prepared by a procedure similar to the one described for **2a** to provide **2c** (26%) as a white solid: mp 103–104 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.77–1.82 (4H, m), 2.01 (2H, m), 2.06 (2H, m), 2.19 (3H, s), 2.32 (3H, s), 2.51 (3H, t, J = 6.9 Hz), 2.95–3.13 (8H, m), 3.59 (2H, m), 3.73 (2H, m), 7.10–7.27 (5H, m), 7.78 (2H, m). Anal. ($C_{28}H_{36}N_2O_2$) C, H, N.

7.5. 1-(3-Acetyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)-4-[4-(3-methylphenyl)piperidin-1-yl]-butan-1-one (2d)

The title compound was prepared by a procedure similar to the one described for **2a** to provide **2d** (26%) as a solid: mp 82–83 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.67–1.81 (4H, m), 1.96–2.09 (3H, m), 2.19 (3H, s), 2.32 (3H, s), 2.45 (3H, t, J = 7.2 Hz), 2.88–3.00 (9H, m), 3.58–3.74 (4H, m), 7.00 (3H, m), 7.16–7.27 (2H, m), 7.78 (2H, m). Anal. ($C_{28}H_{36}N_2O_2$) C, H, N.

7.6. 1-(3-Acetyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)-4-[4-(2-methylphenyl)piperidin-1-yl]-butan-1-one (2e)

The title compound was prepared by a procedure similar to the one described for **2a** to provide **2e** (26%) as a solid: mp 82–83 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.73–1.75 (4H, m), 1.97–2.15 (3H, m), 2.20 (3H, s), 2.33 (3H, s), 2.51 (3H, t, J = 7.2 Hz), 2.70–2.73 (2H, m), 2.95–3.10 (7H, m), 3.61 (2H, m), 3.74 (2H, m), 7.09–7.27 (5H, m), 7.79 (2H, m). Anal. ($C_{28}H_{36}N_2O_2$) C, H, N.

7.7. Combinatorial chemistry

Parallel reactions were carried out in disposal polypropylene test tube with cap (SARSTEDT AG&Co., 101 × 16.5 mm) using a shaker. Liquid handling platform (Genesis, TECAN AG) was used for sampling of the reaction mixture. Liquid Analytical HPLC analysis (CAPCELL PAK UG120 C18 Column; 2.0 mm id × 50 mm, 3 μm particle size, 0.5 mL/min; solvent A: 0.1% TFA, solvent B: 0.1% MeCN; gradient cycle: 0.00 min (A/B = 90/10), 4.00 min (A/B = 5/95), 5.50 min (A/B = 5/95), 8.00 min (A/B = 90/10); UV 220 nm) was performed on a Shimadzu LC-10Av System. The mass spectrometer used was Micromass Platform II (Atmospheric Pressure Chemical ionization: APCI or Electronspray ionization: ESI). Preparative HPLC purification (YMC Combiprep ODS-A, S-5 μm, 50 mm id × 20 mm, 25 mL/min; solvent A: 0.1% TFA, solvent B: 0.1% MeCN; gradient cycle: 0.00 min (A/B = 90/10), 1.00 min (A/B = 90/10), 4.20 min (A/B = 10/90), 5.40 min (A/B = 10/90), 5.50 min (A/B = 90/10), 5.60 min (A/B = 90/10); UV 220 nm) was performed on a Gilson high-throughput purification System.

7.8. General procedure for the amine derivatives 7a–n

A mixture of **6** (0.05 g, 0.148 mmol), amine (0.150 mmol), *N*,*N*-diisopropylethylamine (30.4 mg, 0.30 mmol) and DMF (1.0 mL) charged in a test tube was shaken at 60 °C for 12 h. After this time, the reaction mixture was transferred to a 96-well plate and the solvent was removed. The resulting residue was partitioned between AcOEt (1.5 mL) and water (1.5 mL). The AcOEt layer was separated and concentrated. The resulting residue was dissolved in DMSO (0.5 mL) and the mixture was purified by preparative HPLC to give the desired compound. Yield (mg) and LC–MS data of **7a**–**n** were described in Supplementary data.

7.9. 4-[4-(4-Chlorophenyl)piperidin-1-yl]-1-(2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)-butan-1-one dihydrochloride (1b)

A solution of **2a** (9.80 g, 21.6 mmol) in concentrated hydrochloric acid (150 mL) was refluxed at 120 °C for 24 h. The reaction mixture was basified with 8 M NaOH solution under ice-cooling and the resulting mixture was partitioned between AcOEt and water. The extract was washed with water, brine, dried over anhydrous $MgSO_4$, and filtered. To this filtrate was added 4 M HCl/AcOEt (13 mL) under ice-cooling. After removal of the solvent under reduced pressure, the resulting solid was triturated with isopropanol–diisopropyl ether to give **1b** (9.49 g, 91%) as a white powder: mp 243–247 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.99–2.20 (6H, m), 2.80–3.39 (15H, m), 3.56 (2H, br d), 7.26–7.43 (5H, m), 7.81–7.85 (2H, m), 9.48 (1H, br s). Anal. ($C_{25}H_{31}ClN_2O\cdot 2HCl$) C, H, N.

7.10. 4-[4-(4-Chlorophenyl)piperidin-1-yl]-1-(3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)-butan-1-one (1c)

A mixture of **1b** (0.30 g, 0.73 mmol), formaldehyde (0.09 mL, 1.09 mmol) and formic acid (0.9 mL) was stirred at 100 °C for 4 h. The reaction mixture was poured into water (20 mL) and the mixture was basified with 8 M NaOH solution. The mixture was extracted with AcOEt (20 mL × 2) and the extract was washed with water and brine, dried over $MgSO_4$. After removal of the solvent under reduced pressure, the resulting residue was chromatographed on NH-silica gel (hexane/AcOEt = 1/1) to give **1c** (free amine, 0.24 g, 77%) as a colorless solid. This was treated with 4 M HCl (AcOEt solution) to give a white solid: mp 248–252 °C (decomp.). ^{1}H NMR (200 MHz, $CDCl_3$, free amine) δ: 1.62–2.20 (8H, m), 2.36–2.67 (10H, m), 2.93–3.15 (8H, m), 7.07–7.30 (5H, m), 7.70–7.80 (2H, m). Anal. ($C_{26}H_{33}ClN_2O\cdot 2HCl\cdot 0.5H_2O$) C, H, N.

7.11. 4-[4-(4-Chlorophenyl)piperidin-1-yl]-1-(3-propionyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)-butan-1-one (2f)

To a mixture of **1b** (0.30 g, 0.62 mmol) and triethylamine (0.28 mL, 2.05 mmol) in THF (8 mL) was added a solution of propionyl chloride (0.060 g, 0.68 mmol) in THF (2.0 mL) under ice-cooling. After being stirred at room temperature for 1 h, the reaction mixture was poured into 5% $NaHCO_3$ (40 mL) and the mixture

was extracted with AcOEt (20 mL × 2). The extract was washed with water (20 mL) and brine (20 mL), dried over $MgSO_4$. After removal of the solvent under reduced pressure, the resulting pale yellow solid was triturated with Et_2O/diisopropyl ether solution to yield **2f** (0.22 g, 76%) as a colorless powder: mp 110–112 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.19 (3H, t, J = 7.5 Hz), 1.62–1.80 (4H, m), 1.95–2.08 (4H, m), 2.41–2.48 (5H, m), 2.97–3.04 (8H, m), 3.59–3.61 (2H, m), 3.73–3.76 (2H, m), 7.12 (2H, d, J = 8.1 Hz), 7.20–7.26 (3H, m), 7.56–7.78 (2H, m). Anal. ($C_{28}H_{35}Cl\ N_2O_2$) C, H, N.

7.12. 1-(3-Butyryl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)-4-[4-(4-chlorophenyl)piperidin-1-yl]-butan-1-one (2g)

The title compound was prepared by a procedure similar to the one described for **2f** to provide **2g** (54%) as a colorless powder: mp 93–94 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.99 (3H, t, J = 7.5 Hz), 1.62–1.80 (6H, m), 1.95–2.07 (4H, m), 2.37–2.46 (5H, m), 2.95–3.04 (8H, m), 3.60–3.61 (2H, m), 3.73–3.75 (2H, m), 7.12 (2H, d, J = 8.1 Hz), 7.20–7.26 (3H, m), 7.76–7.78 (2H, m). Anal. ($C_{29}H_{37}Cl\ N_2O_2$) C, H, N.

7.13. 4-[4-(4-Chlorophenyl)piperidin-1-yl]-1-[3-(cyclopropylcarbonyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl]butan-1-one (2h)

The title compound was prepared by a procedure similar to the one described for **2f** to provide **2h** (57%) as a white solid: mp 122–124 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.77–0.84 (2H, m), 1.00–1.04 (2H, m), 1.62–1.86 (4H, m), 1.93–2.07 (6H, m), 2.42–2.47 (1H, m), 2.44 (2H, t, J = 7.2 Hz), 2.97–3.04 (7H, m), 3.75–3.81 (4H, m), 7.12 (2H, d, J = 8.4 Hz), 7.23–7.26 (3H, m), 7.76–7.78 (2H, m). Anal. ($C_{29}H_{35}Cl\ N_2O_2$) C, H, N.

7.14. 4-[4-(4-Chlorophenyl)piperidin-1-yl]-1-(3-pentanoyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)-butan-1-one (2i)

The title compound was prepared by a procedure similar to the one described for **2f** to provide **2i** (59%) as a pale red solid: mp 88 °C (decomp.). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.94 (3H, t, J = 7.5 Hz), 1.39 (2H, sext, J = 7.5 Hz), 1.60–1.80 (6H, m), 1.95–2.08 (4H, m), 2.39–2.46 (5H, m), 2.95–3.04 (8H, m), 3.60–3.61 (2H, m), 3.72–3.75 (2H, m), 7.12 (2H, d, J = 8.1 Hz), 7.20–7.26 (3H, m), 7.76–7.79 (2H, m). Anal. ($C_{30}H_{39}Cl\ N_2O_2$) C, H, N.

7.15. 1-(3-Benzoyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)-4-[4-(4-chlorophenyl)piperidin-1-yl]-butan-1-one (2j)

The title compound was prepared by a procedure similar to the one described for **2f** to provide **2j** (94%) as a colorless powder: mp 103–105 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.65–1.80 (6H, m), 1.93–2.08 (6H, m), 2.44 (2H, t like), 2.95–3.11 (5H, m), 3.52 (2H, br m), 3.89 (2H, br s), 7.09–7.46 (10H, m), 7.77–7.80 (2H, m). Anal. ($C_{30}H_{35}Cl\ N_2O_2$) C, H, N.

7.16. 4-[4-(4-Chlorophenyl)piperidin-1-yl]-1-[3-(methylsulfonyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl]butan-1-one (2k)

A mixture of **1b** (0.15 g, 0.34 mmol), methanesulfonyl chloride (0.05 g, 0.41 mmol) and triethylamine (0.16 mL, 1.12 mmol) in THF (10 mL) was stirred at room temperature for 2 h. The reaction mixture was poured into 5% $NaHCO_3$ (50 mL) and the mixture was extracted with AcOEt (30 mL × 2). The extract was washed with water and brine, dried over $MgSO_4$. After removal of the solvent under reduced pressure, **2k** (0.15 g, 90%) was obtained as a pale yellow solid: mp 156–158 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.64–1.75 (4H, m), 1.95–2.14 (4H, m), 2.47 (2H, t like), 2.79 (3H, s), 2.97–3.13 (9H, m), 3.43-–3.47 (4H, m), 7.12 (2H, d, J = 8.4 Hz), 7.22–7.26 (3H, m), 7.77–7.81 (2H, m). Anal. ($C_{26}H_{33}ClN_2O_3S{\cdot}0.5H_2O$) C, H, N.

7.17. 4-[4-(4-Chlorophenyl)piperidin-1-yl]-1-[3-(ethylsulfonyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl]butan-1-one (2l)

The title compound was prepared by a procedure similar to the one described for **2k** to provide **2l** (58%) as a colorless solid: mp 138–139 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.33 (3H, t, J = 7.4 Hz), 1.59–1.74 (4H, m), 1.94–2.09 (4H, m), 2.44 (3H, t like), 2.92–3.11 (10H, m), 3.46–3.50 (4H, m), 7.11 (2H, d, J = 8.8 Hz), 7.21–7.27 (3H, m), 7.76–7.80 (2H, m). Anal. ($C_{27}H_{35}ClN_2O_3S$) C, H, N.

7.18. Methyl-7-{4-[4-(4-chlorophenyl)piperidin-1-yl]butanoyl}-1,2,4,5-tetrahydro-3*H*-3-benzazepine-3-carboxylate (2m)

A mixture of **1b** (0.20 g, 0.41 mmol) in AcOEt (20 mL) was washed with 2 M NaOH (20 mL) and brine (20 mL), dried over anhydrous $MgSO_4$. After removal of the solvent under reduced pressure, the resulting residue was dissolved in THF (10 mL). To this solution was added triethylamine (0.070 mL, 0.50 mmol) and methyl chlorocarbonate (0.050 g, 0.50 mmol) successively. After being stirred at room temperature for 16 h, the reaction mixture was poured into water (40 mL) and the mixture was extracted with AcOEt (20 mL × 2). The extract was washed with brine and dried over anhydrous $MgSO_4$. After removal of the solvent under reduced pressure, the resulting residue was purified by alumina column chromatography (hexane/AcOEt = 1/1) to give **2m** (0.14 g, 73%) as a colorless oil. This oil was treated with 4 M HCl (AcOEt solution) to give a colorless solid: mp 61 °C. ^{1}H NMR (300 MHz, $CDCl_3$, free amine) δ: 1.66–1.80 (4H, m), 1.95–2.07 (6H, m), 2.34 (2H, t, J = 7.2 Hz), 2.96–3.04 (7H, m), 3.61 (4H, br s), 3.75 (3H, s), 7.11 (2H, d, J = 8.7 Hz), 7.20–7.27 (3H, m), 7.75–7.77 (2H, m). Anal. ($C_{27}H_{33}Cl\ N_2O_3{\cdot}HCl$) C, H, N.

7.19. 7-{4-[4-(4-Chlorophenyl)piperidin-1-yl]butanoyl}-*N*-ethyl-1,2,4,5-tetrahydro-3*H*-3-benzazepine-3-carboxamide (2n)

The title compound was prepared by a procedure similar to the one described for **2f** to provide **2n** (94%) as a colorless solid. This solid was triturated with diisopropyl ether to give a colorless powder: mp 146–148 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.17 (3H, t, J = 7.4 Hz), 1.76 (4H, br m), 1.99–2.02 (4H, br m), 2.44–2.47 (2H, m), 3.00–3.03 (9H, m), 3.32 (2H, m), 3.55 (4H, m), 4.47 (1H, m), 7.12 (2H, d, J = 8.4 Hz), 7.19–7.26 (3H, m), 7.74–7.77 (2H, m). Anal. ($C_{28}H_{36}\ Cl\ N_3O_2{\cdot}0.3H_2O$) C, H, N.

7.20. 1-(2-Acetyl-2,3,4,5-tetrahydro-1*H*-2-benzazepin-8-yl)-4-chlorobutan-1-one (15)

The title compound was prepared by a procedure similar to the one described for **6** to provide **15** (62%) as a colorless solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.76–1.90 (2H, m), 2.05 and 2.12 (3H, s), 2.14–2.27 (2H, m), 2.94–3.06 (2H, m), 3.00–3.17 (2H, m), 3.63–3.77 (2H, m), 3.70–3.86 (2H, br m), 4.56 and 4.61 (2H, s), 7.13–7.39 (2H, m), 7.77–7.97 (1H, m).

7.21. 1-(2-Acetyl-1,2,3,4-tetrahydroisoquinolin-7-yl)-4-chlorobutan-1-one (16)

The title compound was prepared by a procedure similar to the one described for **6** to provide **16** (68%) as colorless crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.19 (3H, s), 2.25 (2H, m), 2.95 (2H, m), 3.17 (2H, quint, J = 6.9 Hz), 3.68 (3H, m), 3.85 (1H, t, J = 6.3 Hz), 4.71 (2H, s), 7.25 (1H, m), 7.78 (2H, m).

7.22. 1-(2-Acetyl-2,3,4,5-tetrahydro-1*H*-2-benzazepin-8-yl)-4-[4-(4-chlorophenyl)piperidin-1-yl]butan-1-one hydrochloride (22)

The title compound was prepared by a procedure similar to the one described for **2a** to provide **22** (free amine, 36%) as a light brown oil. This oil was treated with 4 M HCl (AcOEt solution) and the resulting precipitate was triturated with Et_2O/AcOEt solution to give **22** as a colorless powder: mp 153–155 °C (decomp.). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.50–1.83 (2H, m), 1.87–2.23 (9H, m), 2.78–3.26 (9H, m), 3.58 (2H, d, J = 12.1 Hz), 3.66–3.86 (2H, m), 4.47–4.74 (2H, m), 7.17–7.51 (5H, m), 7.67–8.03 (2H, m), 10.6–10.7 (1H, br m). Anal. ($C_{27}H_{33}Cl\ N_2O_2 \cdot HCl \cdot 1.0H_2O$) C, H, N.

7.23. 1-(2-Acetyl-1,2,3,4-tetrahydroisoquinolin-7-yl)-4-[4-(4-chlorophenyl)piperidin-1-yl]butan-1-one hydrochloride (23)

The title compound was prepared by a procedure similar to the one described for **2a** to provide **23** (free amine, 36%) as a colorless crystalline solid. This solid was treated with hydrochloric acid (EtOH solution) to afford **23** as a colorless powder: mp 204–206 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 1.78–2.28 (9H, m), 2.71–3.28 (9H, m), 3.49–3.62 (2H, m), 3.62–3.75 (2H, m), 4.53–4.84 (2H, m), 7.13–7.51 (5H, m), 7.63–7.96 (2H, m), 10.7 (1H, br s). Anal. ($C_{26}H_{31}ClN_2O_2 \cdot HCl \cdot 0.3H_2O$) C, H, N.

7.24. *N*-[5-(4-Chlorobutanoyl)-2,3-dihydro-1*H*-inden-2-yl]acetamide (17)

The title compound was prepared by a procedure similar to the one described for **6** to provide **17** (59%) as colorless crystals. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.97 (3H, s), 2.22 (2H, quint., J = 6.0 Hz), 2.86 (2H, dd, J = 16.2 Hz, 4.2 Hz), 3.16 (2H, t, J = 6.9 Hz), 3.34 (2H, dd, J = 16.5 Hz, 7.2 Hz), 3.68 (2H, t, J = 6.0 Hz), 4.78 (1H, m), 5.81 (1H, m), 7.32 (1H, d, J = 7.8 Hz), 7.84 (2H, m).

7.25. *N*-(5-{4-[4-(4-Chlorophenyl)piperidin-1-yl]butanoyl}-2,3-dihydro-1*H*-inden-2-yl)-acetamide (24)

The title compound was prepared by a procedure similar to the one described for **2a** to provide **24** (38%) as colorless crystals: mp 133–134 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.61–1.82 (4H, m), 1.94 (3H, s), 1.98–2.14 (3H, m), 2.51 (4H, m), 2.90 (2H, dd, J = 16.4, 4.8 Hz), 2.99 (4H, m), 3.34 (2H, dd, J = 16.8, 7.4 Hz), 4.78 (1H, m), 5.87 (1H, m), 7.10–7.32 (5H, m), 7.84 (2H, m). Anal. ($C_{26}H_{31}ClN_2O_2$) C, H, N.

7.26. 1-(5-Acetyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)-4-chlorobutan-1-one (18)

The title compound was prepared by a procedure similar to the one described for **6** to provide **18** (50%) as a pale brown powder. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.14–2.28 (5H, m), 2.88–2.99 (2H, m), 3.06 (2H, t, J = 7.0 Hz), 3.67 (2H, t, J = 6.2 Hz), 3.76 (1H, t, J = 5.7 Hz), 3.92 (1H, t, J = 5.7 Hz), 4.56 and 4.68 (2H, s), 7.46 (1H, s).

7.27. 1-(6-Acetyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)-4-chlorobutan-1-one (19)

The title compound was prepared by a procedure similar to the one described for **6** to provide **19** (64%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.15–2.31 (2H, m), 2.19 and 2.21 (3H, s), 2.75–2.83 (2H, m), 3.02–3.11 (2H, m), 3.65 (2H, t, J = 6.2 Hz), 3.72 and 3.88 (2H, t, J = 6.2 Hz), 4.70 and 4.84 (2H, s), 7.46 (1H, s).

7.28. 1-(5-Acetyl-4,5,6,7-tetrahydrothieno[3,2-c]pyridin-2-yl)-4-[4-(4-chlorophenyl)piperidin-1-yl]-butan-1-one (25)

The title compound was prepared by a procedure similar to the one described for **2a** to provide **25** (35%) as a pale brown solid: mp 116–118 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.61–1.73 (4H, m), 1.92–2.09 (4H, m), 2.17 and 2.20 (3H, s), 2.39–2.46 (1H, m), 2.42 (2H, t, J = 7.0 Hz), 2.88 (2H, t, J = 7.0 Hz), 2.88–3.03 (4H, m), 3.75 and 3.91 (2H, t, J = 5.8 Hz), 4.54 and 4.67 (2H, s), 7.12 (2H, d, J = 8.4 Hz), 7.25 (2H, d, J = 8.4 Hz), 7.43 and 7.44 (1H, s). Anal. ($C_{24}H_{29}ClN_2O_2S$) C, H, N.

7.29. 1-(6-Acetyl-4,5,6,7-tetrahydrothieno[2,3-c]pyridin-2-yl)-4-[4-(4-chlorophenyl)piperidin-1-yl]-butan-1-one (26)

The title compound was prepared by a procedure similar to the one described for **2a** to provide **26** (45%) as a pale yellow solid: mp 137–138 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.62–1.80 (4H, m), 1.89–2.09 (4H, m), 2.17 and 2.20 (3H, s), 2.38–2.45 (3H, m), 2.74–3.02 (6H, m), 3.71 and 3.87 (2H, t, J = 5.4 Hz), 4.68 and 4.83 (2H, s), 7.12 (2H, d, J = 8.6 Hz), 7.25 (2H, d, J = 8.6 Hz), 7.45 (1H, s). Anal. ($C_{24}H_{29}ClN_2O_2S$) C, H, N.

7.30. 4-Chloro-1-(6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-2-yl)butan-1-one (20)

The title compound was prepared by a procedure similar to the one described for **6** to provide **20** (80%) as a brown oil. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.58–1.70 (4H, m), 1.80–1.86 (2H, m), 2.22 (2H, m), 2.82–2.89 (4H, m), 3.15 (2H, t, J = 7.0 Hz), 3.67 (2H, t, J = 6.2 Hz), 7.18 (1H, d like), 7.68–7.72 (2H, m).

7.31. 4-[4-(4-Chlorophenyl)piperidin-1-yl]-1-(6,7,8,9-tetrahydro-5*H*-benzo[7]annulen-2-yl)-butan-1-one hydrochloride (27)

The title compound was prepared by a procedure similar to the one described for **2a** to provide **27**(free amine, 51%) as a pale yellow oil. This was treated with 4 M hydrochloric acid (AcOEt solution) and the resulting precipitate was triturated with diisopropyl ether to give **27** as a colorless powder: mp 256–257 °C. ^{1}H NMR (200 MHz, $CDCl_3$, free amine) δ: 1.61–2.10 (10H, m), 2.39–2.47 (4H, m), 2.81–3.11 (11H, m), 7.09–7.28 (5H, m), 7.69–7.73 (2H, m). Anal. ($C_{26}H_{32}ClNO \cdot HCl$) C, H, N.

7.32. *N*-{2-[4-(4-Chlorobutanoyl)phenyl]ethyl}acetamide (21)

The title compound was prepared by a procedure similar to the one described for **6** to provide **21** (69%) as a pale yellow powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.95 (3H, s), 2.23 (2H, quint. J = 6.5 Hz), 2.89 (2H, t, J = 6.5 Hz), 3.16 (2H, t, J = 6.9 Hz), 3.53 (2H, q, J = 6.9 Hz), 3.68 (2H, t, J = 6.5 Hz), 5.44 (1H, br s), 7.29 (2H, d, J = 8.1 Hz), 7.92 (2H, d, J = 8.1 Hz).

7.33. *N*-[2-(4-{4-[4-(4-Chlorophenyl)piperidin-1-yl]butanoyl}phenyl)ethyl]acetamide (28)

The title compound was prepared by a procedure similar to the one described for **2a** to provide **28** (69%) as a colorless powder: mp 158 –160 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.60–1.75 (4H, m), 1.94 (3H, s), 1.94–2.04 (4H, m), 2.40–2.48 (3H, m), 2.85–3.03 (6H, m), 3.54 (2H, q like), 5.43 (1H, br s), 7.11 (2H, d, J = 13.2 Hz), 7.23–7.31 (4H, m), 7.94 (2H, d, J = 12.0 Hz). Anal. ($C_{25}H_{31}ClN_2O_2 \cdot 1.5\ H_2O$) C, H, N.

7.34. 4-Oxo-4-[3-(trifluoroacetyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl]butanoic acid (30)

To a mixture of **29** (10.0 g, 41.0 mmol) and succinic anhydride (4.1 g, 41.0 mmol) in 1,2-dichloroethane (40 mL) was added $AlCl_3$ (27.0 g, 205 mmol) at room temperature. After being stirred at 45 °C for 1 h, the reaction mixture was poured onto ice and the resulting mixture was partitioned between AcOEt and water. The AcOEt layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane/AcOEt = 1/1) to give **30** (10.0 g, 71%) as a yellow solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 2.81 (2H, t, J = 6.4 Hz), 2.90–3.15 (4H, m), 3.29 (2H, t, J = 6.4 Hz), 3.65–3.85 (4H, m), 7.20–7.33 (1H, m), 7.75–7.85 (2H, m), 10.0 (1H, br s).

7.35. 4-[4-(4-Chlorophenyl)piperidin-1-yl]-4-oxo-1-[3-(trifluoroacetyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl]butan-1-one (31)

A mixture of **30** (7.50 g, 21.8 mmol), 4-chlorophenylpiperidine hydrochloride (5.00 g, 21.8 mmol), and triethylamine (4.41 g, 43.6 mmol) in DMF (30 mL) was stirred at room temperature for 30 min, then cooled to 0 °C. To this mixture was added diethyl phosphorocyanidate (3.56 g, 21.8 mmol) and the mixture was stirred at 0 °C for 1 h. The reaction mixture was partitioned between AcOEt and water, and the AcOEt layer was washed with water, brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane/AcOEt = 1/1) to give **31** (8.10 g, 71%) as a colorless solid. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.45–2.00 (4H, m), 2.55–2.90 (4H, m), 2.97–3.28 (5H, m), 3.34 (2H, t, J = 6.4 Hz), 3.64–3.84 (4H, m), 4.04–4.21 (1H, m), 4.68–4.84 (1H, m), 7.14 (2H, d, J = 8.4 Hz), 7.20–7.34 (3H, m), 7.80–7.90 (2H, m).

7.36. 4-[4-(4-Chlorophenyl)piperidin-1-yl]-4-oxo-1-(2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)butan-1-one (3a)

A mixture of **31** (8.00 g, 15.4 mmol) and K_2CO_3 (6.40 g, 46.0 mmol) in MeOH/H_2O (4/1, 250 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and the resulting residue was partitioned between AcOEt and water. The AcOEt layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure to afford **3a** (6.20 g, 95%) as a white solid: mp 148–149 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.47–2.00 (4H, m), 2.57–2.88 (5H, m), 2.98 (8H, br m), 3.07–3.27 (1H, m), 3.50 (2H, t, J = 6.6 Hz), 4.05–4.21 (1H, m), 4.72–4.84 (1H, m), 7.10–7.34 (5H, m), 7.74–7.83 (2H, m). Anal. ($C_{25}H_{29}ClN_2O_2$) C, H, N.

7.37. 4-[4-(4-Chlorophenyl)piperidin-1-yl]-1-(3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)-4-oxobutan-1-one (3b)

The title compound was prepared by a procedure similar to the one described for **1c** to provide **3b** (60%) as a colorless solid: mp 143–145 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.47–2.00 (4H, m), 2.38 (3H, s), 2.47–2.88 (8H, m), 2.95–3.06 (4H, m), 3.08–3.28 (1H, m), 3.35 (2H, t, J = 6.8 Hz), 4.07–4.21 (1H, m), 4.71–4.86 (1H, m), 7.08–7.34 (5H, m), 7.75–7.85 (2H, m). Anal. ($C_{26}H_{31}ClN_2O_2$) C, H, N.

7.38. 1-(3-Acetyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)-4-[4-(4-chlorophenyl)piperidin-1-yl]-4-oxobutan-1-one (4)

To a mixture of **3a** (0.20 g, 0.47 mmol) and K_2CO_3 (0.14 g, 1.00 mmol) in acetonitrie (5 mL) was added acetyl chloride (37 mg, 0.47 mmol) and the mixture was stirred at room temperature for 16 h. The reaction mixture was partitioned between AcOEt and water, and the AcOEt layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to afford **4** (0.14 g, 64%) as a colorless solid: mp 109–112 °C. ^{1}H NMR (200 MHz, $CDCl_3$) δ: 1.64 (2H, m), 1.89 (2H, m), 2.58 (4H, m), 2.66 (3H, s), 2.87 (2H, t, J = 6.6 Hz), 3.11 (4H, m), 3.17 (1H, m), 3.34 (2H, t, J = 6.6 Hz), 3.58–3.76 (4H, m), 4.13 (1H, d, J = 15.6 Hz), 4.73 (1H, d, J = 12 Hz), 7.11–7.31 (5H, m), 7.81 (2H, m). Anal. ($C_{27}H_{31}ClN_2O_3$) C, H, N.

7.39. *tert*-Butyl 7-hydroxy-1,2,4,5-tetrahydro-3*H*-3-benzazepine-3-carboxylate (33)

A mixture of **32** (1.76 g, 9.92 mmol) and HBr (48% solution, 20 mL) was stirred at 80 °C for 16 h. After this time, the reaction mixture was cooled to room temperature and concentrated under reduced pressure. The resulting residue was dissolved in THF/DMF (2/1, 300 mL). To this solution was added *N*,*N*-diisopropylethylamine (2.82 g, 21.82 mmol) and di-*tert*-butyl dicarbonate (2.17 g, 9.92 mmol). The mixture was stirred at room temperature for 16 h, and then heated at 50 °C for 2 h. The reaction mixture was concentrated under reduced pressure and the resulting residue was purified by alumina column chromatography (hexane/AcOEt = 1/1) to give **33** (0.75 g, 29%) as a light brown amorphous powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.48 (9H, s), 2.80–2.84 (4H, m), 3.53 (4H, dd like), 4.77 (1H, s), 6.56–6.61 (2H, m), 6.97 (1H, d, J = 7.8 Hz).

7.40. *tert*-Butyl 7-(3-chloropropoxy)-1,2,4,5-tetrahydro-3*H*-3-benzazepine-3-carboxylate (34)

A mixture of **33** (0.70 g, 2.66 mmol), 1-bromo-3-chloropropane (0.50 g, 3.19 mmol), and K_2CO_3 (1.10 g, 7.98 mmol) in DMF (20 mL) was stirred at 80 °C for 3 h. After this time, the reaction mixture was cooled to room temperature and partitioned between AcOEt and water. The AcOEt layer was washed with water, brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The resulting residue was purified by alumina column chromatography (hexane/AcOEt = 3/2) to give **34** (0.80 g, 88%) as a light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.49 (9H, s), 2.16–2.33 (2H, m), 2.83–2.85 (4H, m), 3.52–3.71 (4H, m), 4.05–4.18 (4H, m), 6.63–6.69 (2H, m), 7.00–7.26 (1H, m).

7.41. *tert*-Butyl 7-{3-[4-(4-chlorophenyl)piperidin-1-yl]propoxy}-1,2,4,5-tetrahydro-3*H*-3-benzazepine-3-carboxylate (35)

The title compound was prepared by a procedure similar to the one described for **2a** to provide **35** (41%) as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.49 (9H, s), 1.69–1.84 (4H, m), 1.95–2.11 (4H, m), 2.44–2.57 (3H, m), 2.84–2.85 (4H, m), 3.04–3.08 (2H, m), 3.53 (4H, br m), 4.00 (2H, t, J = 6.3 Hz), 6.64–6.69 (2H, m), 7.01 (1H, d, J = 8.4 Hz), 7.14––7.18 (2H, m), 7.24–7.28 (2H, m).

7.42. 7-{3-[4-(4-Chlorophenyl)piperidin-1-yl]propoxy}-2,3,4,5-tetrahydro-1*H*-3-benzazepine (36)

A mixture of **35** (0.48 g, 0.96 mmol) and TFA (5 mL) was stirred at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure and the resulting residue was partitioned between AcOEt and 1 M NaOH solution. The AcOEt layer was washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure to give **36** (0.38 g, quant.) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.79–1.81 (4H, m), 1.99–2.06 (4H, m), 2.48–2.59 (6H, m), 2.88–3.10 (7H, m), 4.00 (2H, t, J = 6.2 Hz), 6.63–6.68 (2H, m), 6.98–7.02 (1H, m),

7.15 (2H, d, J = 8.6 Hz), 7.27 (2H, d, J = 8.6 Hz). This oil was treated with 4 M HCl/AcOEt solution to give the dihydrochloride of **36**.

7.43. 3-Acetyl-7-{3-[4-(4-chlorophenyl)piperidin-1-yl]propoxy}-2,3,4,5-tetrahydro-1*H*-3-benzazepine (37)

To a solution of **36** (0.10 g, 0.21 mmol) and triethylamine (0.070 g, 0.69 mmol) in THF (10 mL) was added a solution of acetyl chloride (0.018 g, 0.23 mmol). After being stirred at room temperature for 2 h, the reaction mixture was partitioned between AcOEt and water. The AcOEt layer was separated and washed with 5% $NaHCO_3$, brine, dried over anhydrous $MgSO_4$. After removal of the solvent under reduced pressure, the resulting residue was purified by alumina column chromatography (hexane/AcOEt = 3/2 to AcOEt) to give **37** (0.080 g, 93%) as a white solid: mp 109 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.72–1.83 (4H, m), 1.96–2.11 (4H, m), 2.18 (3H, s), 2.50 (1H, m), 2.55 (2H, t like), 2.87–2.89 (4H, m), 3.04–3.09 (2H, m), 3.53–3.58 (2H, m), 3.67–3.74 (2H, m), 4.01 (2H, t, J = 6.3 Hz), 6.66–6.70 (2H, m), 7.00–7.07 (1H, m), 7.15 (2H, d, J = 8.4 Hz), 7.27 (2H, d, J = 8.4 Hz). Anal. ($C_{26}H_{33}ClN_2O_2$) C, H, N.

7.44. Homology modeling and ligand docking

The homology model of hMCHR1 was constructed using the crystal structure of bovine rhodopsin (PDB code 1F88), which obtained from the RCSB Protein Data Bank, as a structural template.[25] The primary alignment of the amino acid sequences between hMCHR1 and rhodopsin using MOE 2005.06. Construction of hMCHR1 homology models and side chain rotamer search were performed with MOE. Antagonists were docked into the obtained receptor models using the program GOLD. Resulting docking modes with receptor models were subjected energy minimization with MOE. In the energy minimization process, the MMFF94s forcefield was used.

7.45. Metabolic stability in hepatic microsomes

Human hepatic microsomes were purchased from Xenotech, LLC (Lenexa, KS). An incubation mixture with a final volume of 0.1 mL consisted of microsomal protein in 50 mmol/L KH_2PO_4–K_2HPO_4 phosphate buffer (pH 7.4) and 1 μmol/L test compound. The concentration of hepatic microsomal protein was 0.2 mg/mL. An NADPH-generating system containing 50 mmol/L $MgCl_2$, 50 mmol/L glucose-6-phosphate, 5 mmol/L beta-$NADP^+$ and 15 unit/mL glucose-6-phosphate dehydrogenase was prepared and added to the incubation mixture with a 10% volume of the reaction mixture. After the addition of the NADPH-generating system, the mixture was incubated at 37 °C for 0 and 20 min. The reaction was terminated by the addition of acetonitrile equivalent to the volume of the reaction mixture. All incubations were made in duplicate. Test compound in the reaction mixture was measured by LC system equipped with a UV detector. For metabolic stability determinations, chromatograms were analyzed for parent compound disappearance from the reaction mixtures.

7.46. In vivo pharmacological study

All animal experiments were performed in compliance with the Guidelines for the Care and Use of Laboratory Animals of Takeda Pharmaceutical Co., Ltd.

7.46.1. Rat pharmacokinetic study (concentration in plasma and brain)

Test compounds were administered to fed F344 rats (male, 9 weeks, n = 3) orally (10 mg/kg, 0.5% methylcellulose suspension). At 1, 2, 4, 8 and 24 h after oral administration, blood samples were collected from tail vein. The rats were then sacrificed, and the brain tissues were collected and immediately stored at −80 °C. The blood samples were centrifuged to obtain the plasma fraction. The brain samples were homogenized in saline to obtain 20% (w/v) homogenates. The 100 μL of plasma or brain homogenate was deproteinized with 100 μL acetonitrile. After centrifugation, the supernatant obtained was diluted 2-fold with 0.01 mol/L HCO_2NH_4 (adjusted to pH 3.0 with HCO_2H) and centrifuged again. The compound concentration in the supernatant was measured by LC system with a UV detector or LC/MS/MS system with an API3000 triple quadrupole mass spectrometer (Perkin–Elmer Sciex). The mass spectrometer was equipped with a turbo ionspray source and operated in positive ion mode. The LC conditions were as follows: column, an L-column ODS (4.6 × 250 mm, Chemicals evaluation and research institute, Japan); mobile phase, mixture solution of 0.01 mol/L $HCOONH_4$ (adjusted to pH 3.0 with HCO_2H) and acetonitrile; flow rate (isocratic) 1.0 mL/min; column temperature, 25 °C.

7.46.2. Rat food intake inhibition study

Male F344 rats (10-week-old: CLEA Japan) were housed individually and given chow diet (CE-2: CLEA Japan). Two weeks later, the rats were grouped based on the food intake and the body weight as indices. At 3:00PM, the rats were weighed and their food bins were removed from their cages. Simultaneously, 0.5% methylcellulose solution was administered orally to the control group, and 0.5% methylcellulose suspension (1, 3 and 10 mg/kg) of the compound was administered orally to the compound administration group at 2 mL/kg (6 per group). Food bin for each rat was inserted 1 h after administration. Five-hour and 16-hour after presentation of food bin), food intake of each rat was measured.

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Supplementary data

Supplementary data (synthesis of starting materials (**5**, **9**, **19**, **11**, **12**, **13**, and **29**), parallel synthesis of compound **7a–n**, and elemental analysis data) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.09.007.

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