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Novel acetylcholinesterase inhibitor as increasing agent on rhythmic bladder contractions: SAR of 8-{3-[1-(3-fluorobenzyl)piperidin-4-yl]propanoyl}-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (TAK-802) and related compounds

Yuji Ishichi,[a] Mitsuru Sasaki,[a] Masaki Setoh,[a] Tetsuya Tsukamoto,[a] Seiji Miwatashi,[a] Hiroshi Nagabukuro,[b] Satoshi Okanishi,[b] Shigemitsu Imai,[b] Reiko Saikawa,[b] Takayuki Doi[b] and Yuji Ishihara[a,*]

[a]*Medicinal Chemistry Research Laboratories, Pharmaceutical Research Division, Takeda Pharmaceutical Company Ltd, 2-17-85, Jusohonmachi, Yodogawa-ku, Osaka 532-8686, Japan*
[b]*Pharmaceutical Research Laboratories I, Pharmaceutical Research Division, Takeda Pharmaceutical Company Ltd, 2-17-85, Jusohonmachi, Yodogawa-ku, Osaka 532-8686, Japan*

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Abstract—As part of an on-going investigation to develop an increasing agent on rhythmic bladder contractions, 1-aryl-3-(1-benzylpiperidin-4-yl)propanones were synthesized and examined as noncarbamate acetylcholinesterase (AChE) inhibitors. Among compounds with various aryl groups, 1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one derivative **9c** was found to possess a potent AChE inhibition activity with an IC_{50} value of 1.3 nM. The compound **9c** increased rhythmic bladder contractions in Guinea pigs and rats without affecting the basal intravesical pressure, which suggests that **9c** may be useful for the treatment of voiding dysfunction caused by detrusor underactivity.
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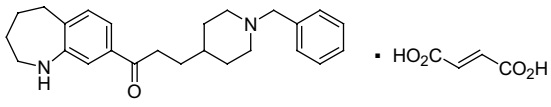
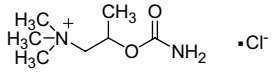
1. Introduction

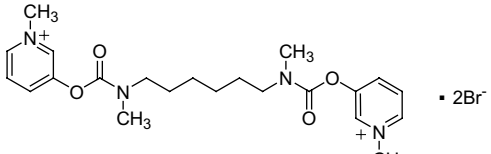
Demographic changes resulting in a progressively aging society have seen a growing incidence of lower urinary tract symptoms (LUTS), and increased attention is being paid to quality of life (QOL) maintenance of LUTS patients. LUTS are classified into two categories: storage dysfunction (including urinary frequency, nocturia, and urinary incontinence) and voiding dysfunction (characterized by difficulty in urination or urinary retention).[1] Difficulty in urination frequently results in loss in QOL, making voiding dysfunction a significant medical target. One of the causes of the voiding dysfunction is



TAK-147



Bethanechol Chloride



Distigmine Bromide

Figure 1. Structures of TAK-147, bethanechol chloride, and distigmine bromide.

Keywords: Acetylcholinesterase inhibitor; Pyrrolos[3,2,1-*ij*]quinolin-4-one; Rhythmic bladder contraction; TAK-802.
* Corresponding author. Tel.: +81 6 6300 6413; fax: +81 6 6300 6306; e-mail: Ishihara_Yuji@takeda.co.jp

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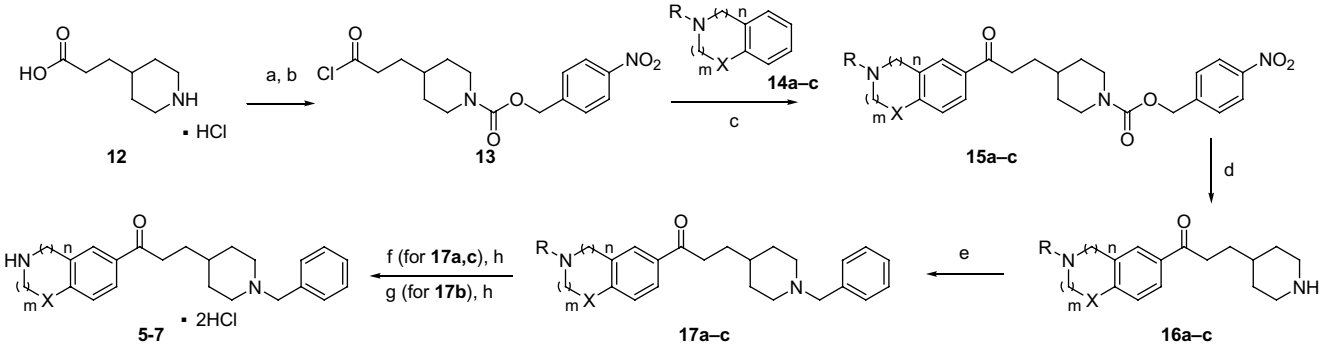
impaired detrusor contractility known as detrusor underactivity. The detrusor underactivity is associated with aging, prostatic hypertrophy, diabetes, and multiple sclerosis. The detrusor muscle is known to be under the control of the parasympathetic nerve, for which one of the major transmitters is acetylcholine (ACh). At present, some cholinergic agents such as bethanechol[2] or distigmine[3] are clinically used to treat patients with detrusor underactivity in order to potentiate bladder contractions. Bethanecol, an ACh analogue that undergoes only slight hydrolysis by AChE, is considered to induce bladder contractions (Fig. 1) by acting on muscarinic ACh receptors of the detrusor muscle. However, continuous cholinergic stimulation of the detrusor muscle is considered to cause reduction in bladder compliance. Distigmine, a long acting carbamate AChE inhibitor, does not have sufficient therapeutic effect[4] presumably owing to increasing urethral resistance. This undesirable action is supposed to be a result of the direct agonist effect of carbamate agents on nicotinic ACh receptors in the urethra.[5] We therefore hypothesized that an AChE inhibitor, which does not possess a carbamate structure could avoid direct activation of nicotinic ACh receptors and improve bladder voiding function more effectively than carbamates. As a novel class of AChE inhibitor bearing 1-aryl-3-(1-benzylpiperidin-4-yl)propanone structure, one of us (Y.I.) and his coworkers have previously reported a SAR of TAK-147 (Fig. 1) and its preclinical potential for the treatment of Alzheimer's disease.[6] In this study, preliminary examination revealed that TAK-147 increased rhythmic bladder contractions in Guinea pigs. Thus, taking TAK-147 as a lead compound, we planned to synthesize and evaluate 1-aryl-3-(1-benzylpiperidin-4-yl)propanones as increasing agents on rhythmic bladder contractions. On the optimization, we focused a modification of 1-aryl group, which is supposed to locate at the entrance of the enzyme's active site and is thought to be tolerable for modification.[6c] Among various aryl groups, we found 1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one is preferable for the potent AChE inhibition. In this report, we describe the synthesis and SAR of 8-{3-[1-(3-fluorobenzyl)piperidin-4-yl]propanoyl}-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one **9c** and related compounds **1**–**11**.

2. Chemistry

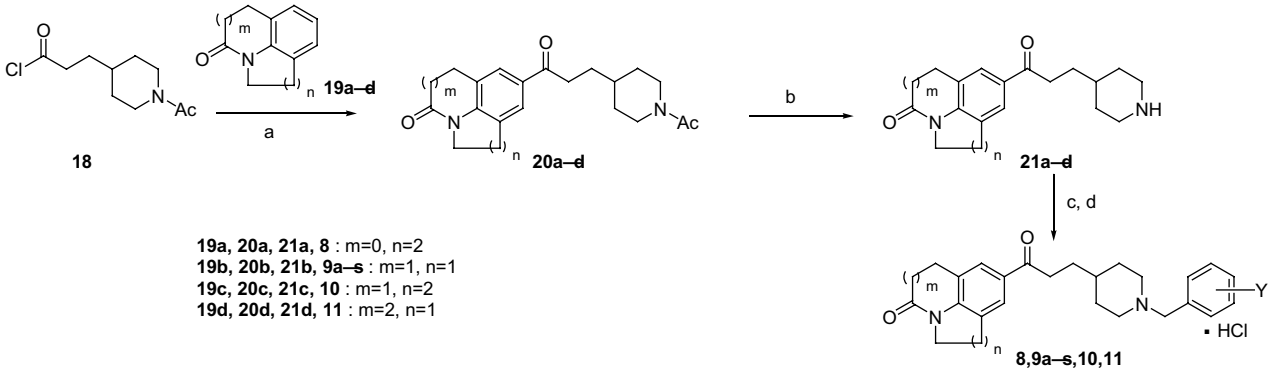
Compounds **1**–**4** were synthesized according to our previous report.[6b] The syntheses of bicyclic compounds **5**–**7** is outlined in Scheme 1. We have reported that Friedel–Crafts acylation of NH-protected 2-benzazepine **14a** and 1,4-benzoxazepine **14c** proceeded in a highly regioselective manner to give 8-acyl-2-benzazepine and 7-acyl-1,4-benzoxazepine, respectively.[7a] For the NH-protected 3-benzazepine, only 7-acylation products have been reported.[7b] We applied these findings to the synthesis of 2-benzazepine **5**, 3-benzazepine **6**, and 1,4-benzoxazepine derivative **7**. In the previous procedure of the synthesis of 2-benzazepine derivative **5**, methoxycarbonyl group was used as a NH-protecting group of piperidine nitrogen.[7a] For the ease of deprotection, we have chosen a *para*-nitrobenzyloxycarbonyl as the protecting group. 4-Piperidinepropanoic acid **12** was protected with a *para*-nitrobenzyloxycarbonyl group,[8] which is stable to Friedel–Crafts acylation conditions, and the resulting carboxylic acid was converted to acid chloride **13** by treating with thionyl chloride. Friedel–Crafts acylation of appropriately NH-protected bicyclic compounds **14a**–**c**[9] with acid chloride **13** were carried out in the presence of 3 equiv of aluminum chloride to give **15a**–**c**. After selective deprotection of the *para*-nitrobenzyloxycarbonyl group of **15a**–**c**, **16a**–**c** were alkylated with benzyl bromide to afford **17a**–**c**, which were then deprotected under acidic or basic hydrolysis conditions to give **5**–**7**.

The syntheses of tricyclic compounds **8**, **9a**–**s**, **10**, and **11** is shown in Scheme 2. The tricyclic heterocycles **19a**–**d** were synthesized according to literature methods.[10] Friedel–Crafts acylation of 3-(1-acetylpiperidin-4-yl)propanoyl chloride (**18**)[11] with **19a**–**d** afforded **20a**–**d** as single regioisomers. After deprotection of the acetyl group, **21a**–**d** were alkylated with substituted benzyl halides to give **8**, **9a**–**s**, **10**, and **11**.



2-Benzazepines: **5, 14a, 15a, 16a, 17a**; X=-(CH_2)-, m=2, n=1, R=Ac
3-Benzazepines: **6, 14b, 15b, 16b, 17b**; X=-(CH_2)-, m=1, n=2, R=$COCF_3$
1,4-Benzoxazepines: **7, 14c, 15c, 16c, 17c**; X=O, m=2, n=1, R=CHO

Scheme 1. Reagents and conditions: (a) PNB-OCOCl, THF, H_2O, KOH; (b) $SOCl_2$; (c) **14a**–**c**, $AlCl_3$, CH_2Cl_2; (d) H_2, Pd–C, EtOH–THF; (e) $PhCH_2Br$, K_2CO_3, CH_3CN; (f) concd HCl,120 °C; (g) K_2CO_3, H_2O, MeOH; (h) HCl.



Scheme 2. Reagents and conditions: (a) **19a–d**, $AlCl_3$, CH_2Cl_2; (b) concd HCl, 140 °C; (c) substituted benzyl halides, K_2CO_3, CH_3CN; (d) HCl.

3. Results and discussion

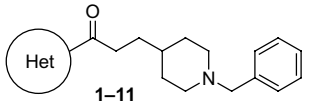
3.1. AChE inhibition activity

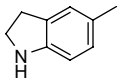
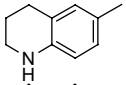
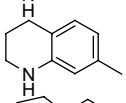
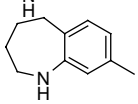
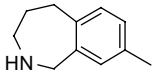
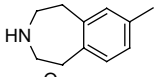
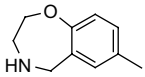
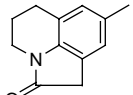
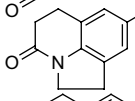
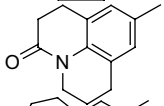
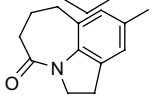
The AChE inhibition activity was measured by Ellman's method[12] using human-erythrocyte-derived AChE. Distigmine was tested as a reference compound and the IC_{50} value was measured as 380 nM. The effect of 1-aryl groups was evaluated and the results are shown in Table 1. All compounds exhibited potent activities with IC_{50}'s in the 1–200 nM range. In general, compounds bearing tricyclic groups showed 10 to 100 times more potent than those with bicyclic groups. Among compounds **1**–**4** bearing bicyclic aromatic amines, the activity decreased slightly with increasing size of the heterocyclic ring. With regard to the attachment position of 3-(1-benzylpiperidin-4-yl)propanoyl group, 6-position was found to be better than the 7-position (cf. **2** vs **3**). These results correspond well with our previous data using rat cerebral cortex as the enzyme source.[6b] The AChE inhibition activities of 2-benzazepine **5**, 3-benzazepine **6**, and oxazepine **7** were slightly potent as compared to those of compounds **1**–**4**. Compounds bearing tricyclic heterocycles (**8**, **9a**, **10**, and **11)** exhibited potent activity with IC_{50} values in the range of 1.3–16 nM, and the activity again tended to decrease slightly with increasing size of the heterocyclic ring. Among various heterocyclic frameworks, 1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one derivative **9a** possessed the most potent activity. Thus, the effect of substituents (Y) on benzylamino moiety of pyrrolo[3,2,1-*ij*]quinoline derivatives **9** was evaluated and the results are shown in Table 2. Among substituents, fluoro, chloro, and hydroxy groups retained the activity. The order of IC_{50} potency on substitution position was 3-, 2- > 4-, except for hydroxy series **9k**–**m**. Compound **9m** (Y = 4-OH) exhibited remarkably potent enzyme inhibition with an IC_{50} value of 0.49 nM.

3.2. Effect on rhythmic bladder contractions in Guinea pigs and rats

Selected compounds with potent AChE inhibition activity were examined for their effect on rhythmic bladder contractions. In vivo efficacy was evaluated by measuring distention-induced rhythmic bladder contractions in Guinea pigs and rats. Figure 2 shows the intravesical

Table 1. AChE inhibitory activity of compounds with various heterocyclic frameworks **1**–**11**



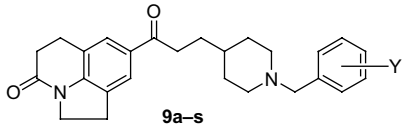
Compd	Het	Salt	*h*AChE inhibition	
			IC_{50} (nM)[a]	95% CI[b]
1		Fumarate	53	(50–57)
2		2HCl	64	(60–70)
3		Fumarate	166	(153–180)
4 (TAK-147)		Fumarate	193	(178–210)
5		2HCl	23	(21–24)
6		2HCl	25	(23–27)
7		2HCl	40	(38–42)
8		HCl	3.6	(3.3–3.9)
9a		HCl	1.3	(1.0–1.7)
10		HCl	9.8	(8.7–11)
11		HCl	16	(14–19)
Distigmine[c]			380	(366–394)

[a] The IC_{50} value is the concentration required to inhibit control enzyme activity by 50%. These values were calculated from the results of two experiments conduced in duplicate. All compounds were dissolved in distilled water.

[b] 95% confidence interval for each IC_{50} value.

[c] Distigmine bromide.

Table 2. The effect of substituents in 8-[3-(1-benzylpiperidin-4-yl)propanoyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3, 2,1-*ij*]quinolin-4-ones **9a–s**



9a–s

Compd[a]	Y	*h*AChE inhibition	
		IC_{50} (nM)[b]	95% CI[c]
9a	H	1.3	(1.0–1.7)
9b	2-F	2.5	(2.3–2.8)
9c	3-F	1.3	(1.2–1.5)
9d	4-F	4.6	(4.1–5.3)
9e	2-Cl	5.1	(4.6–5.6)
9f	3-Cl	4.9	(4.2–5.6)
9g	4-Cl	153	(143–163)
9h	2-OMe	64	(61–68)
9i	3-OMe	125	(116–135)
9j	4-OMe	345	(321–371)
9k	2-OH	1.1	(0.97–1.2)
91	3-OH	8.7	(7.9–9.6)
9m	4-OH	0.49	(0.45–0.54)
9n	2-NO_2	90	(83–98)
9o	3-NO_2	2.9	(2.7–3.2)
9p	4-NO_2	43	(40–47)
9q	2-CN	55	(51–58)
9r	3-CN	6.8	(6.4–7.2)
9s	4-CN	32	(30–34)
Distigmine[c]		380	(366–394)

[a] All compounds were tested as their HCl salts except distigmine.
[b,c] See corresponding footnotes of Table 1.

Table 3. AUC200 value and bioavailability of selected compounds

Compd	*h*AChE inhibition	AUC200[a]		BA[d,f]
	IC_{50} (nM)	Guinea pigs (μg/kg, iv)[b]	Rats (μg/kg, id)[d,e]	Rats (%)
4 (TAK-147)	193	660		
5	23	195		
6	25	560		
8	3.6	5.4		
10	9.8	87		
11	16	11.6		
9a	1.3	3.0	55	16
9b	2.5	15		
9c	1.3	0.769 (0.075–1.877)[c]	22	33
9f	4.9	5.0		
9k	1.1	1.0	122	7.3
91	8.7	27		
9m	0.49	76		
Distigmine[g]	380	21.1 (6.5–39.4)[c]		

[a] Dose necessary to double the area under the curve (AUC) of the vesical contraction. All compounds were dissolved in distilled water.
[b] Values are an average of 5–8 independent experiments.
[c] 95% Confidence interval for AUC200 value (iv). In the blanks, 95% confidence levels could not be calculated.
[d] Compounds that exhibited potent AUC200 value in Guinea pigs were examined.
[e] An average of 7–10 independent experiments.
[f] Preliminary pharmacokinetic data.
[g] Distigmine bromide.

pressure (cystometrogram) before and after administration of **9c**. Intravenous (iv) administration of **9c** increased bladder contractions mainly by prolonging the duration of the contractions, and was evaluated using the AUC200 value, which is the dose of compound necessary to double the area under the curve (AUC) of the vesical contraction.[13] The AUC200 value of selected compounds in Guinea pigs are shown in Table 3. AUC200 efficacies in Guinea pigs (iv) were approximately parallel to the corresponding AChE inhibition activity. Although the reason is not clear, 4-hydroxy derivative **9m** exhibited significantly low AUC200 efficacy compared with its AChE IC_{50} value. Compounds (**9a**, **9c**, and **9k**), which showed excellent iv AUC200 values, were evaluated for their efficacies in rats after intraduodenal (id) administration.[14] The values were comparable to those of the iv examination except for **9k**. AUC200 efficacy (id) of **9k** was weak compared to that of the iv administration, suggesting that **9k** had some pharmacokinetic deficiencies such as permeability or metabolic stability. Preliminary pharmacokinetic studies were conducted for compounds **9a**, **9c**, and **9k**. The bioavailability (BA) of **9k** in rats was lower than those of **9a** and **9c** (7.3% for **9k** vs 16% for **9a**, 33% for **9c**). In this intraduodenal study, **9c** exhibited the most potent increasing effect with an AUC200 value of 22 μg/kg, id. Based on these results, **9c** was selected as a candidate for further evaluation. Distigmine was also tested in the rhythmic bladder contraction study, and showed a modest AUC200 value of 21.1 μg/kg, iv, which is better than was expected from its weak AChE inhibition activity (IC_{50} = 380 nM).[15] However, it was reported that distigmine heightened the risk of urinary retention. Distigmine is presumed to increase intraure-

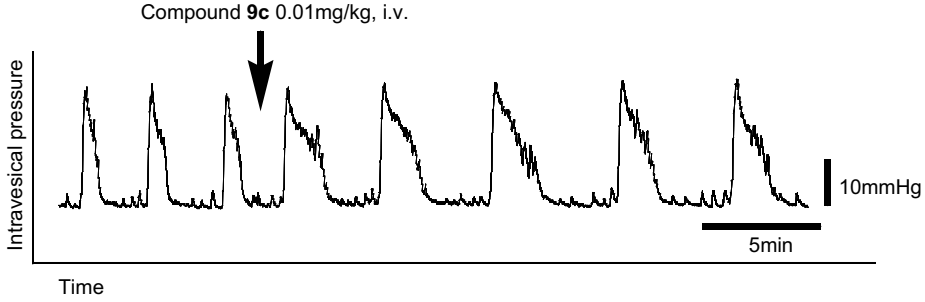


Figure 2. Cystometrogram of anesthetized Guinea pig and the effect of **9c**.

thral pressure owing to its excitation effect on nicotinic receptors. We have previously studied the muscarinic and nicotinic effects of **9c** and distigmine,[16] and the nicotinic effect of **9c** was found to be about 1/3 of distigmine by relative efficacy. Furthermore, distigmine inhibited butyrylcholinesterase (BChE) activity with the IC_{50} value of 537 nM while that of **9c** was >10,000 nM, suggesting that **9c** is more selective for parasympathetic nervous.[16] Thus, **9c** is more effective than distigmine for its more potent AUC200 value, lower nicotinic effect, and high selectivity against BChE. On the other hand, bethanechol, another agent for increasing bladder contractions, was concerned about its continuous stimulation of parasympathetic nervous system. And in our rhythmic bladder contraction study, bethanechol had increased basal intravesical pressure and diminished maximum intravesical pressure, indicating lowering bladder compliance.[16] Compound **9c** increased bladder contractions only while the micturition reflex was evoked, and did not affect the basal intravesical pressure. Thus, **9c** is considered to be more effective in the treatment of voiding dysfunction caused by detrusor underactivity.

4. Conclusion

We have synthesized a series of novel noncarbamate acetylcholinesterase inhibitors to identify improved agents for the treatment of voiding dysfunction. Among these compounds, 1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one derivative **9c** exhibited potent AChE inhibition activity with an IC_{50} value of 1.3 nM. Compound **9c** displayed an increasing effect on bladder contractions with excellent efficacy, showing AUC200 values of 0.769 μg/kg, iv in Guinea pigs and 22 μg/kg, id in rats. Furthermore, the nicotinic effect of **9c** has been shown to be about a third of that of carbamate acetylcholinesterase inhibitor distigmine. Therefore, compound **9c** is expected to be an effective agent for the treatment of voiding dysfunction caused by detrusor underactivity, and clinical development of **9c** as the free base (TAK-802) is currently underway.

5. Experimental section

5.1. Chemistry

Melting points were determined on a Yanagimoto micro-melting point apparatus and were uncorrected. Infrared (IR) spectra were obtained on Jasco IR-810. ^{1}H NMR spectra were taken on Varian Gemini 200 (200 MHz) or Mercury 300 (300 MHz) spectrometers. Chemical shifts were given in ppm with tetramethylsilane as the internal standard. The following abbreviations were used: s = singlet, d = doublet, t = triplet, m = multiplet, dd = double doublet, dt = double triplet, br = broad. Elemental analyses (C, H, N) were measured on Vario EL (EL-04) instrument. Column chromatography was carried out using Merck silica gel 60 (63–200 μm). TLC was performed on silica gel 60 F_{254} (Merck), and the spots were detected by UV light (wavelength 254 nm). Abbreviations for solvents are the following: Et_2O, diethyl ether; EtOH, ethanol; EtOAc, ethyl acetate; Hex, hexane; IPE, diisopropyl ether; MeOH, methanol; THF, tetrahydrofuran.

5.1.1. Compounds 1–4 were prepared according to our previous report.[6b]

5.1.2. 4-Nitrobenzyl 4-(3-chloro-3-oxopropyl)piperidine-1-carboxylate (13). A solution of 4-nitrobenzyl chloroformate (16.7 g, 77.5 mmol) in THF (50 mL) was added dropwise to a mixture of 3-piperidin-4-yl-propanoic acid hydrochloride (**12**)[11] (15.0 g, 44.3 mmol), aqueous KOH solution (8.70 g, 155 mmol in H_2O 100 mL), and THF (50 mL) at 0 °C. The mixture was stirred at room temperature for 12 h. The mixture was concentrated in vacuo, and acidified using 1 N HCl. The mixture was extracted with EtOAc (2 times), dried over $MgSO_4$, and concentrated to give a colorless oil. The oil was crystallized from Et_2O–IPE to afford 3-(1-{[(4-nitrobenzyl)oxy]carbonyl}piperidin-4-yl)propanoic acid as colorless crystals (17.9 g, 69%). Mp 99–100 °C (Et_2O–IPE). IR (KBr) 2932, 1695, 1520, 1348, 852 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05–1.25 (m, 2H), 1.40–1.60 (m, 1H), 1.62 (q, J = 7.2 Hz, 2H), 1.65–1.80 (m, 2H), 2.40 (t, J = 7.2 Hz, 2H), 2.65–2.95 (m, 2H), 4.05–4.30 (m, 2H), 5.22 (s, 2H), 7.51 (d, J = 6.9 Hz, 2H), 8.00–10.00 (br, 1H), 8.22 (d, J = 6.9 Hz, 2H). Anal. Calcd for $C_{16}H_{20}N_2O_6$: C, 57.14; H, 5.99; N, 8.33. Found: C, 57.08; H, 5.89; N, 8.39.

The 3-(1-[(4-nitrobenzyl)oxy]carbonylpiperidin-4-yl)propanoic acid (8.00 g, 23.8 mmol) was added portionwise to thionyl chloride (20 mL) at 0 °C with stirring. The mixture was stirred at room temperature for 1 h. The thionyl chloride was removed in vacuo, and the residue was crystallized from Et_2O to afford **13** as colorless crystals (7.00 g, 83%). Mp 64–65 °C (Et_2O). IR (KBr) 2936, 1799, 1699, 1607, 1522, 1346, 853 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05–1.25 (m, 2H), 1.40–1.90 (m, 5H), 2.65–2.90 (m, 2H), 2.94 (t, J = 7.5 Hz, 2H), 4.10–4.30 (m, 2H), 5.22 (s, 2H), 7.51 (d, J = 8.1 Hz, 2H), 8.22 (d, J = 8.1 Hz, 2H).

5.1.3. 4-Nitrobenzyl 4-{3-oxo-3-[3-(trifluoroacetyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl]propyl}piperidine-1-carboxylate (15b). Aluminum chloride (10.0 g, 75.0 mmol) was added portionwise to a mixture of **13** (8.87 g, 25.0 mmol) and 3-(trifluoroacetyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine **14b**[8] (6.08 g, 25.0 mmol) in CH_2Cl_2 (30 mL) at room temperature. The mixture was stirred at room temperature for 12 h. The reaction mixture was poured into ice, extracted with EtOAc, dried over $MgSO_4$, and concentrated to give a pale yellow oil. The oil was subjected to chromatography on SiO_2 (100 g, Hex–EtOAc = 2:1–1:1) and crystallized from EtOH–Et_2O to afford **15b** as colorless crystals (5.02 g, 36%). Mp 132–133 °C. IR (KBr) 2938, 1682, 1520, 1346, 770 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.10–1.30 (m, 2H), 1.45–1.65 (m, 1H), 1.65–1.90 (m, 4H), 2.70–2.95 (m, 2H), 2.95–3.20 (m, 6H), 3.65–3.85 (m, 4H), 4.10–4.30 (m, 2H), 5.22 (s, 2H), 7.20–7.30 (m, 1H), 7.51 (d, J = 8.4 Hz, 2H), 7.70–7.80 (m, 2H), 8.22

(d, $J = 8.4$ Hz, 2H). Anal. Calcd for $C_{28}H_{30}F_3N_3O_6$: C, 59.89; H, 5.38; N, 7.48. Found: C, 59.84; H, 5.31; N, 7.47.

The following compounds **15a**,**c** were prepared in a similar manner to that described for **15b**. The compounds were obtained as a mixture of amide bond isomers and were used for the next reaction after appropriate identification (e.g. ^{1}H NMR).

5.1.4. 4-Nitrobenzyl 4-[3-(2-acetyl-2,3,4,5-tetrahydro-1*H*-2-benzazepin-8-yl)-3-oxopropyl]piperidine-1-carboxylate (15a). 24%, Amorphous. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.05–1.30 (m, 2H), 1.40–1.90 (m, 7H), 2.05 (s, 3H × 1/2), 2.11 (s, 3H × 1/2), 2.65–3.10 (m, 6H), 3.70–3.80 (m, 2H × 1/2), 3.80–3.90 (m, 2H × 1/2), 4.10–4.30 (m, 2H), 4.55 (s, 2H × 1/2), 4.60 (s, 2H × 1/2), 5.22 (s, 2H), 7.20–7.30 (m, 1H), 7.51 (d, $J = 8.4$ Hz, 2H), 7.75–7.80 (m, 1H+1H × 1/2), 7.93 (s, 1H × 1/2), 8.22 (d, $J = 8.4$ Hz, 2H).

5.1.5. 4-Nitrobenzyl 4-[3-(4-formyl-2,3,4,5-tetrahydro-1,4-benzoxazepin-7-yl)-3-oxopropyl]piperidine-1-carboxylate (15c). 83%, Amorphous. IR (KBr) 2926, 2680, 1678, 1605, 1522, 1346, 1236, 735 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.10–1.30 (m, 2H), 1.45–1.85 (m, 5H), 2.70–2.95 (m, 2H), 2.98 (t, $J = 7.5$ Hz, 2H), 3.75–3.80 (m, 2H × 1/2), 3.90–3.95 (m, 2H × 1/2), 4.10–4.30 (m, 4H), 4.56 (s, 2H × 1/2), 4.67 (s, 2H × 1/2), 5.22 (s, 2H), 7.08 (d, $J = 3.6$ Hz, 1H × 1/2), 7.11 (d, $J = 3.9$ Hz, 1H × 1/2), 7.45–7.55 (m, 2H), 7.80–7.85 (m, 2H), 7.94 (s, 1H × 1/2), 8.09 (s, 1H × 1/2), 8.20–8.25 (m, 2H).

5.1.6. 3-Piperidin-4-yl-1-[3-(trifluoroacetyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl]propan-1-one (16b). Compound **15b** (4.60 g, 8.19 mmol) was dissolved in EtOH (150 mL) and THF (50 mL), and was hydrogenated at room temperature with 10% Pd–C (wet, 1.0 g) as catalyst at atmospheric pressure. The mixture was stirred for 90 min under hydrogen atmosphere. The catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residue was subjected to chromatography on SiO_2 (60 g, EtOAc–MeOH = 9:1) and crystallized from EtOH–Et_2O to afford **16b** as colorless crystals (1.86 g, 59%). Mp 124–125 °C (EtOH–Et_2O). IR (KBr) 2924, 1682, 1464, 1167, 756 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.10–1.30 (m, 2H), 1.35–1.55 (m, 1H), 1.60–1.80 (m, 4H), 2.00–2.20 (br, 1H), 2.60 (dt, $J = 12.0$, 2.4 Hz, 2H), 2.97 (t, $J = 7.5$ Hz, 2H), 3.00–3.20 (m, 6H), 3.65–3.85 (m, 4H), 7.20–7.30 (m, 1H), 7.75–7.80 (m, 2H). Anal. Calcd for $C_{20}H_{25}F_3N_2O_2{\cdot}0.25H_2O$: C, 62.08; H, 6.64; N, 7.24. Found: C, 61.79; H, 6.50; N, 6.95.

The following compounds **16a**,**c** were prepared in a similar manner to that described for **16b**. The compounds were obtained as a mixture of amide bond isomers and were used for next reaction after appropriate identification (e.g., ^{1}H NMR).

5.1.7. 1-(2-Acetyl-2,3,4,5-tetrahydro-1*H*-2-benzazepin-8-yl)-3-piperidin-4-yl-propan-1-one (16a). 72%, Amorphous. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05–1.30 (m, 2H), 1.35–1.50 (m, 1H), 1.60–1.90 (m, 7H), 2.03 (s, 3H × 1/2), 2.10 (s, 3H × 1/2), 2.58 (t, $J = 12.0$ Hz, 2H), 2.90–3.10 (m, 6H), 3.70–3.80 (m, 2H × 1/2), 3.80–3.90 (m, 2H × 1/2), 4.54 (s, 2H × 1/2), 4.60 (s, 2H × 1/2), 7.20–7.30 (m, 1H), 7.75–7.80 (m, 1H+1H × 1/2), 7.93 (s, 1H × 1/2).

5.1.8. 7-(3-Piperidin-4-yl-propanoyl)-2,3-dihydro-1,4-benzoxazepine-4(5*H*)-carbaldehyde (16c). 89%, Amorphous. IR (KBr) 2921, 1674, 1603, 1427, 733 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05–1.55 (m, 2H), 1.60–1.90 (m, 5H), 2.50–2.70 (m, 3H), 2.96 (t, $J = 7.5$ Hz, 2H), 3.00–3.15 (m, 2H), 3.79 (t, $J = 4.5$ Hz, 1H), 3.94 (t, $J = 4.5$ Hz, 1H), 4.10–4.20 (m, 2H), 4.55 (s, 2H × 1/2), 4.67 (s, 2H × 1/2), 7.07 (d, $J = 4.5$ Hz, 1H), 7.10 (d, $J = 4.5$ Hz, 1H), 7.80–8.00 (m, 2H), 8.09 (s, 1H × 1/2), 8.22 (s, 1H × 1/2).

5.1.9. 3-(1-Benzylpiperidin-4-yl)-1-[3-(trifluoroacetyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl]propan-1-one (17b). Benzyl bromide (600 mg, 3.51 mmol) was added dropwise to a suspension of **16b** (1.34 g, 3.50 mmol) and $NaHCO_3$ (0.6 g, 7 mmol) in CH_3CN (10 mL) at room temperature. The mixture was stirred at room temperature for 5 h, then concentrated in vacuo, and the residue was diluted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and concentrated to give a pale yellow oil. The oil was subjected to chromatography on SiO_2 (10 g, Hex–EtOAc = 1:1) to afford **17b** as a colorless oil (1.01 g, 61%). ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.20–1.45 (m, 3H), 1.60–1.80 (m, 4H), 1.85–2.05 (m, 2H), 2.80–3.10 (m, 8H), 3.49 (s, 2H), 3.65–3.85 (m, 4H), 7.20–7.40 (m, 6H), 7.70–7.80 (m, 2H).

The following compounds **17a,c** were prepared in a similar manner to that described for **17b**. The compounds were obtained as a mixture of amide bond isomers and were used for next reaction after appropriate identification (e.g. ^{1}H NMR).

5.1.10. 1-(2-Acetyl-2,3,4,5-tetrahydro-1*H*-2-benzazepin-8-yl)-3-(1-benzylpiperidin-4-yl)propan-1-one (17a). 66%, Amorphous. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20–1.40 (m, 3H), 1.60–1.75 (m, 4H), 1.80–1.90 (m, 2H), 1.90–2.00 (m, 2H), 2.04 (s, 3H × 1/2), 2.11 (s, 3H × 1/2), 2.80–3.05 (m, 6H), 3.49 (s, 2H), 3.70–3.75 (m, 2H × 1/2), 3.80–3.90 (m, 2H × 1/2), 4.54 (s, 2H × 1/2), 4.59 (s, 2H × 1/2), 7.20–7.40 (m, 6H), 7.75–7.80 (m, 1H+1H × 1/2), 7.93 (s, 1H × 1/2).

5.1.11. 7-[3-(1-Benzylpiperidin-4-yl)propanoyl]-2,3-dihydro-1,4-benzoxazepine-4(5*H*)-carbaldehyde (17c). 58%, Amorphous. IR (KBr) 2922, 1682, 1603, 1451, 734 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20–1.40 (m, 3H), 1.60–1.80 (m, 4H), 2.80–3.00 (m, 4H), 3.49 (s, 2H), 3.77 (t, $J = 4.8$ Hz, 1H), 3.93 (t, $J = 4.8$ Hz, 1H), 4.10–4.20 (m, 2H), 4.53 (s, 2H × 1/2), 4.65 (s, 2H × 1/2), 7.05 (d, $J = 4.2$ Hz, 1H × 1/2), 7.08 (d, $J = 4.2$ Hz, 1H × 1/2), 7.20–7.35 (m, 5H), 7.75–7.80 (m, 1H+1H × 1/2), 7.90–7.95 (m, 1H × 1/2), 8.06 (s, 1H × 1/2), 8.20 (s, 1H × 1/2).

5.1.12. 3-(1-Benzylpiperidin-4-yl)-1-(2,3,4,5-tetrahydro-1*H*-2-benzazepin-8-yl)propan-1-one dihydrochloride (5).[7a] A solution of **17a** (1.27 g, 3.03 mmol) in concentrated HCl (20 mL) was stirred at 120 °C for 12 h. The mixture was concentrated to give **5** as a pale yellow amorphous (1.20 g, 88%), which was crystallized from ethanol to afford colorless crystals. Mp 147–150 °C. IR (KBr) 2921, 1682, 1604, 1452, 740 cm^{-1}. ^1H NMR (free base; 300 MHz, $CDCl_3$) δ 1.20–1.40 (m, 3H), 1.55–1.80 (m, 7H), 1.85–2.00 (m, 2H), 2.80–3.10 (m, 6H), 3.21 (t, J = 5.4 Hz, 2H), 3.47 (s, 2H), 3.98 (s, 2H), 7.20–7.35 (m, 6H), 7.65–7.75 (m, 2H). Anal. Calcd for $C_{25}H_{32}N_2O{\cdot}2HCl{\cdot}0.5H_2O$: C, 65.49; H, 7.69; N, 6.11. Found: C, 65.60; H, 7.66; N, 6.08.

5.1.13. 3-(1-Benzylpiperidin-4-yl)-1-(2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)propan-1-one dihydrochloride (6). A mixture of **17b** (1.00 g, 2.12 mmol), saturated aqueous K_2CO_3 (10 mL), H_2O (10 mL), and MeOH (30 mL) was stirred at room temperature for 12 h. The mixture was concentrated in vacuo, extracted with EtOAc (3 times), and the extract was washed with brine. The organic phase was dried over $MgSO_4$ and concentrated to give a colorless oil. The oil was subjected to chromatography on basic SiO_2 (20 g, EtOAc–MeOH = 9:1) to afford a free base of **6** as a colorless oil (602 mg, 75%). The free base (602 mg) was treated with HCl–EtOH and crystallized from EtOH–Et_2O to give **6** as colorless crystals. Mp 232–234 °C. IR (KBr) 2948, 2728, 1680, 1456, 752 cm^{-1}. ^1H NMR (free base; 300 MHz, $CDCl_3$) δ 1.20–1.40 (m, 3H), 1.65–1.75 (m, 4H), 1.90–2.00 (m, 3H), 2.80–3.05 (m, 12H), 3.48 (s, 2H), 7.10–7.40 (m, 6H), 7.65–7.75 (m, 2H, 6-H, and 8-H of 3-benzazepine). Anal. Calcd for $C_{25}H_{32}N_2O{\cdot}2HCl{\cdot}0.25H_2O$: C, 66.14; H, 7.66; N, 6.17. Found: C, 66.44; H, 7.82; N, 6.19.

5.1.14. 3-(1-Benzylpiperidin-4-yl)-1-(2,3,4,5-tetrahydro-1,4-benzoxazepin-7-yl)propan-1-one dihydrochloride (7). A solution of **17c** (2.41 g, 5.93 mmol) in concentrated HCl (10 mL) and MeOH (10 mL) was stirred at reflux for 1 h. The mixture was concentrated and crystallized from EtOH to give **7** as colorless crystals (1.17 g, 44%). Mp 234–236 °C; IR (KBr) 2919, 1678, 1601, 1494, 1236, 740 cm^{-1}. ^1H NMR (free base; 300 MHz, $CDCl_3$) δ 1.20–1.45 (m, 3H), 1.60–2.10 (m, 7H), 2.80–3.00 (m, 4H), 3.24 (t, J = 4.5 Hz, 2H), 3.50 (s, 2H), 4.01 (s, 2H), 4.10 (t, J = 4.5 Hz, 2H), 7.06 (d, J = 8.1 Hz, 1H), 7.20–7.35 (m, 5H), 7.75–7.80 (m, 2H). Anal. Calcd for $C_{24}H_{30}N_2O_2{\cdot}2HCl{\cdot}0.5H_2O$: C, 62.61; H, 7.22; N, 6.08. Found: C, 62.55; H, 7.05; N, 6.10.

5.1.15. 8-[3-(1-Acetylpiperidin-4-yl)propanoyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (20b). 3-(1-Acetyl-4-piperidinyl)propanoic acid (**18**)[11] (8.82 g, 44.3 mmol) was added portionwise to thionyl chloride (20 mL) at 0 °C with stirring. The mixture was stirred at room temperature for 20 min. The reaction mixture was concentrated in vacuo, and the residue was collected by filtration, washed with Et_2O to give the corresponding acid chloride of **18** as a colorless solid. Aluminum chloride (16.2 g, 121 mmol) was added portionwise to a mixture of the acid chloride and 1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one**19b**[10] (6.40 g, 36.9 mmol) in 1,2-dichloroethane (10 mL) at room temperature. The mixture was stirred at room temperature for 12 h. The reaction mixture was poured into ice, extracted with EtOAc (4 times), dried over $MgSO_4$, and concentrated to give a pale yellow oil. The oil was subjected to chromatography on SiO_2 (50 g, EtOAc: MeOH = 10:1) to afford **20b** as colorless crystals (12.3 g, 94%). Recrystallization from MeOH–EtOAc gave 11.8 g of **20b**. Mp 157–159 °C. IR (KBr) 2928, 1672, 1640, 1597, 1493, 1446, 1381, 1152, 972, 750 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.00–1.30 (m, 2H), 1.50–1.95 (m, 5H), 2.09 (s, 3H), 2.53 (dt, J = 12.9, 2.4 Hz, 1H), 2.72 (t, J = 7.6 Hz, 2H), 2.90–3.15 (m, 5H), 3.24 (t, J = 8.6 Hz, 2H), 3.75–3.90 (m, 1H), 4.14 (t, J = 8.6 Hz, 2H), 4.55–4.70 (m, 1H), 7.68 (s, 1H), 7.73 (s, 1H). Anal. Calcd for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.39; N, 7.90. Found: C, 71.11; H, 7.58; N, 7.82.

The following compounds, **20a**, **20c**, and **20d**, were prepared in a similar manner to that described for **20b**.

5.1.16. 8-[3-(1-Acetylpiperidin-4-yl)propanoyl]-5,6-dihydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-2(1*H*)-one (20a). 80%, Mp 80–82 °C (EtOH–Et_2O). IR (KBr) 2930, 1715, 1622, 1495, 1343, 1152, 731 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 1.05–1.30 (m, 2H), 1.50–2.10 (m, 7H), 2.09 (s, 3H), 2.53 (dt, J = 8.7, 2.0 Hz, 1H), 2.83 (t, J = 6.0 Hz, 2H), 2.90–3.10 (m, 3H), 3.56 (s, 2H), 3.70–3.85 (m, 3H), 4.55–4.65 (m, 1H), 7.73 (s, 2H). Anal. Calcd for $C_{21}H_{26}N_2O_3{\cdot}0.5H_2O$: C, 69.40; H, 7.49; N, 7.71. Found: C, 69.50; H, 7.79; N, 7.44.

5.1.17. 9-[3-(1-Acetylpiperidin-4-yl)propanoyl]-2,3,6,7-tetrahydro-1*H*,5*H*-pyrido[3,2,1-*ij*]quinolin-5-one (20c). 85%, Mp 135–136 °C (EtOH–Et_2O). IR (KBr) 2930, 1674, 1435, 1159, 752 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.00–1.30 (m, 2H), 1.45–1.85 (m, 5H), 1.90–2.05 (m, 2H), 2.09 (s, 3H), 2.53 (dt, J = 12.8, 2.6 Hz, 1H), 2.65–2.70 (m, 2H), 2.85 (t, J = 6.2 Hz, 2H), 2.90–3.10 (m, 5H), 3.70–3.90 (m, 3H), 4.55–4.70 (m, 1H), 7.61 (s, 2H). Anal. Calcd for $C_{22}H_{28}N_2O_3$: C, 71.71; H, 7.66; N, 7.60. Found: C, 71.63; H, 7.78; N, 7.61.

5.1.18. 9-[3-(1-Acetylpiperidin-4-yl)propanoyl]-1,2,6,7-tetrahydroazepino[3,2,1-*hi*]indol-4(5*H*)-one (20d). 63%, Mp 93–95 °C (EtOH–Et_2O). IR (KBr) 2928, 2859, 1713, 1605, 1146, 754 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.00–1.30 (m, 2H), 1.45–1.85 (m, 7H), 1.90–2.25 (m, 2H), 2.09 (s, 3H), 2.53 (dt, J = 12.8, 2.6 Hz, 1H), 2.90–3.10 (m, 6H), 3.56 (s, 2H), 3.75–3.90 (m, 1H), 3.95–4.05 (m, 2H), 4.55–4.70 (m, 1H), 7.66 (s, 1H), 7.68 (s, 1H). Anal. Calcd for $C_{22}H_{28}N_2O_3{\cdot}0.1H_2O$: C, 71.36; H, 7.68; N, 7.57. Found: C, 71.29; H, 7.81; N, 7.63.

5.1.19. 8-(3-Piperidin-4-yl-propanoyl)-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one (21b). A solution of **20b** (11.9 g, 33.5 mmol) in concentrated HCl (60 mL) was stirred at 140 °C for 4 h. The reaction mixture was concentrated in vacuo, and the residue was adjusted to pH 12 using aqueous 8 N NaOH solution, and extracted with EtOAc. The organic phase was washed with brine,

dried over $MgSO_4$, and concentrated. The residue was crystallized from EtOAc–Et_2O to afford **21b** (10.4 g, 99%). mp 114–115 °C (EtOH–Et_2O). IR (KBr) 1914, 1672, 1597, 1493, 1381, 1157, 754 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.00–1.30 (m, 2H), 1.30–1.90 (m, 7H), 2.59 (dt, J = 12.0, 2.4 Hz, 2H), 2.72 (t, J = 7.6 Hz, 2H), 2.85–3.15 (m, 5H), 3.23 (t, J = 8.6 Hz, 2H), 4.14 (t, J = 8.6 Hz, 2H), 7.68 (s, 1H), 7.73 (s, 1H). Anal. Calcd for $C_{19}H_{24}N_2O_2$: C, 73.05; H, 7.74; N, 8.97. Found: C, 72.62; H, 7.76; N, 8.87.

The following compounds, **21a**, **21c**, and **21d**, were prepared in a similar manner to that described for **21b**.

5.1.20. 8-(3-Piperidin-4-yl-propanoyl)-5,6-dihydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-2(1*H*)-one (21a). 31%, Mp 134–136 °C (EtOH–Et_2O). IR (KBr) 2926, 1715, 1671, 1603, 1497, 1343, 1155, 752 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.00–1.25 (m, 2H), 1.30–1.80 (m, 7H), 1.95–2.10 (m, 2H), 2.58 (dt, J = 12.0, 2.4 Hz, 2H), 2.83 (t, J = 6.2 Hz, 2H), 2.94 (t, J = 7.6 Hz, 2H), 3.00–3.15 (m, 2H), 3.55 (s, 2H), 3.74 (t, J = 6.2 Hz, 2H), 7.73 (s, 2H). Anal. Calcd for $C_{19}H_{24}N_2O_2 \cdot 0.25H_2O$: C, 72.01; H, 7.79; N, 8.84. Found: C, 72.46; H, 7.87; N, 8.88.

5.1.21. 9-(3-Piperidin-4-yl-propanoyl)-2,3,6,7-tetrahydro-1*H*,5*H*-pyrido[3,2,1-*ij*]quinolin-5-one (21c). 72%, Mp 87–88 °C (EtOH–Et_2O). IR (KBr) 2922, 1674, 1603, 1483, 1163, 752 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.00–1.30 (m, 2H), 1.35–1.80 (m, 5H), 1.90–2.05 (m, 2H), 2.59 (dt, J = 12.0, 2.4 Hz, 2H), 2.65–2.75 (m, 2H), 2.90–3.15 (m, 9H), 3.89 (t, J = 5.8 Hz, 2H), 7.62 (s, 2H). Anal. Calcd for $C_{20}H_{26}N_2O_2$: C, 73.59; H, 8.03; N, 8.58. Found: C, 73.39; H, 8.12; N, 8.43.

5.1.22. 9-(3-Piperidin-4-yl-propanoyl)-1,2,6,7-tetrahydroazepino[3,2,1-*hi*]indol-4(5*H*)-one (21d). 61%, Amorphous. IR (KBr) 2926, 1717, 1672, 1603, 1337, 1151, 733 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$) δ 1.00–1.30 (m, 2H), 1.35–1.90 (m, 6H), 1.95–2.20 (m, 4H), 2.58 (dt, J = 8.1, 1.6 Hz, 2H), 2.85–3.15 (m, 6H), 3.56 (s, 2H), 3.98 (t, J = 5.8 Hz, 2H), 7.66 (s, 1H), 7.68 (s, 1H).

5.1.23. 8-{3-[1-(3-Fluorobenzyl)piperidin-4-yl]propanoyl}-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one hydrochloride (9c). 3-Fluorobenzyl bromide (660 mg, 3.49 mmol) was added dropwise to a suspension of **21b** (1.04 g, 3.32 mmol) and K_2CO_3 (0.8 g, 5.8 mmol) in CH_3CN (10 mL) at room temperature. The mixture was stirred at room temperature for 12 h, then concentrated in vacuo, and the residue was diluted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$, and concentrated to give a pale yellow oil. Crystallization from EtOH–Et_2O afforded the free base of **9c** (1.31 g, 94%). Mp 111–112 °C. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.20–1.50 (4H, m), 1.55–1.80 (4H, m), 1.85–2.05 (2H, m), 2.71 (2H, t, J = 7.6 Hz), 2.80–3.15 (5H, m), 3.22 (2H, t, J = 8.6 Hz), 3.47 (2H, s), 4.13 (2H, t, J = 8.6 Hz), 6.85–7.15 (3H, m), 7.20–7.35 (1H, m), 7.67 (1H, s), 7.72 (1H, s). Anal. Calcd for $C_{26}H_{29}FN_2O_2$: C, 74.26; H, 6.95; N, 6.66. Found: C, 74.28; H, 7.02; N, 6.58. The free base (1.00 g, 2.38 mmol) was treated with an excess amount of 10 N HCl in EtOH, and crystallized from EtOH–Et_2O to give **9c** as colorless crystals (1.03 g, 95%). Mp 201–203 °C. IR (KBr) 1652, 1594, 1259, 1149 cm^{-1}. Anal. Calcd for $C_{26}H_{29}FN_2O_2 \cdot HCl$: C, 68.34; H, 6.62; N, 6.13. Found: C, 68.15; H, 6.66; N, 6.04.

The following compounds, **8**, **10**, **11**, **9a**,**b**, and **9d**–**s**, were prepared in a similar manner to that described for **9c**.

5.1.24. 8-[3-(1-Benzylpiperidin-4-yl)propanoyl]-5,6-dihydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-2(1*H*)-one hydrochloride (8). 85%, Mp 244–246 °C (EtOH–Et_2O). IR (KBr) 1718, 1670, 1604, 1344, 1147 cm^{-1}. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.20–1.42 (m, 3H), 1.61–1.77 (m, 4H), 1.85–2.08 (m, 4H), 2.78–2.96 (m, 6H), 3.48 (s, 2H), 3.54 (s, 2H), 3.71–3.77 (m, 2H), 7.22–7.33 (m, 5H), 7.72 (s, 2H). Anal. Calcd for $C_{26}H_{30}N_2O_2 \cdot HCl \cdot 0.25H_2O$: C, 70.41; H, 7.15; N, 6.31. Found: C, 70.26; H, 7.26; N, 6.20.

5.1.25. 9-[3-(1-Benzylpiperidin-4-yl)propanoyl]-2,3,6,7-tetrahydro-1*H*,5*H*-pyrido[3,2,1-*ij*]quinolin-5-one hydrochloride (10). 86%, Mp 236–238 °C (EtOH–Et_2O). IR (KBr) 2938, 1669, 1603, 1364, 1167, 750 cm^{-1}. ^1H NMR (free base; 300 MHz, $CDCl_3$) δ 1.20–1.40 (m, 3H), 1.60–1.75 (m, 4H), 1.80–2.00 (m, 4H), 2.65–2.79 (m, 2H), 2.80–3.00 (m, 8H), 3.48 (s, 2H), 3.89 (t, J = 6.0 Hz, 2H), 7.20–7.35 (m, 5H), 7.60 (s, 1H), 7.61 (s, 1H). Anal. Calcd for $C_{27}H_{32}N_2O_2 \cdot HCl \cdot 0.25H_2O$: C, 70.88; H, 7.38; N, 6.12. Found: C, 70.79; H, 7.49; N, 6.13.

5.1.26. 9-[3-(1-Benzylpiperidin-4-yl)propanoyl]-1,2,6,7-tetrahydroazepino[3,2,1-*hi*]indol-4(5*H*)-one hydrochloride (11). 80%, Mp 209–211 °C (EtOH–Et_2O). IR (KBr) 2928, 1738, 1603, 1143, 737 cm^{-1}. ^1H NMR (free base; 300 MHz, $CDCl_3$) δ 1.20–1.40 (m, 2H), 1.45–2.20 (m, 11H), 2.60–3.10 (m, 5H), 3.16 (d, J = 12.6 Hz, 1H), 3.30 (d, J = 12.6 Hz, 1H), 3.49 (s, 2H), 3.49–4.10 (m, 3H), 6.80–7.40 (m, 5H), 7.62 (s, 1H), 7.99 (s, 1H). Anal. Calcd for $C_{27}H_{32}N_2O_2 \cdot HCl \cdot 0.5H_2O$: C, 70.19; H, 7.42; N, 6.06. Found: C, 69.78; H, 7.37; N, 6.03.

5.1.27. 8-[3-(1-Benzylpiperidin-4-yl)propanoyl]-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one hydrochloride (9a). 80%, Mp 245–248 °C (EtOH–Et_2O). IR (KBr) 3026, 1672, 1595 cm^{-1}. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.19–1.43 (m, 3H), 1.60–2.03 (m, 6H), 2.71 (t, J = 7.6 Hz, 2H), 2.81–3.09 (m, 6H), 3.22 (t, J = 8.6 Hz, 2H), 3.48 (s, 2H), 4.13 (t, J = 8.6 Hz, 2H), 7.20–7.32 (m, 5H), 7.66 (s, 1H), 7.70 (s, 1H). Anal. Calcd for $C_{26}H_{30}N_2O_2 \cdot HCl$: C, 71.14; H, 7.12; N, 6.38. Found: C, 70.97; H, 7.14; N, 6.18.

5.1.28. 8-3-[1-(2-Fluorobenzyl)piperidin-4-yl]propanoyl-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one hydrochloride (9b). 81%, Mp 110–112 °C (EtOH–Et_2O). IR (KBr) 1644, 1590, 1234, 1148 cm^{-1}. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.18–1.43 (m, 3H), 1.60–1.80 (m, 4H), 1.91–2.13 (m, 2H), 2.71 (t, J = 7.8 Hz, 2H), 2.83–3.10 (m, 6H), 3.22 (t, J = 8.6 Hz, 2H), 3.56

(s, 2H), 4.13 (t, J = 8.6 Hz, 2H), 6.94–7.41 (m, 4H), 7.66 (s, 1H), 7.70 (s, 1H). Anal. Calcd for $C_{26}H_{29}FN_2O_2$·HCl·2.5H_2O: C, 62.21; H, 7.03; N, 5.58. Found: C, 62.17; H, 6.73; N, 5.40.

5.1.29. 8-{3-[1-(4-Fluorobenzyl)piperidin-4-yl]propanoyl}-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one hydrochloride (9d). 84%, Mp 245 °C (Decomposed), (EtOH–Et_2O). IR (KBr) 1671, 1594, 1295, 1149 cm^{-1}. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.18–1.42 (m, 3H), 1.60–1.81 (m, 4H), 1.83–2.06 (m, 2H), 2.71 (t, J = 7.8 Hz, 2H), 2.80–3.08 (m, 6H), 3.22 (t, J = 8.4 Hz, 2H), 3.44 (s, 2H), 4.13 (t, J = 8.4 Hz, 2H), 6.91–7.03 (m, 2H), 7.19–7.33 (m, 2H), 7.66 (s, 1H), 7.70 (s, 1H). Anal. Calcd for $C_{26}H_{29}FN_2O_2$·HCl: C, 68.34; H, 6.62; N, 6.13. Found: C, 68.01; H, 6.56; N, 5.99.

5.1.30. 8-{3-[1-(2-Chlorobenzyl)piperidin-4-yl]propanoyl}-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one hydrochloride (9e). 79%, Mp 191–193 °C (EtOH–Et_2O). IR (KBr) 1659, 1594, 1162, 1147 cm^{-1}. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.20–1.48 (m, 3H), 1.58–1.82 (m, 4H), 1.93–2.15 (m, 2H), 2.71 (t, J = 7.6 Hz, 2H), 2.84–3.09 (m, 6H), 3.22 (t, J = 8.6 Hz, 2H), 3.59 (s, 2H), 4.13 (t, J = 8.6 Hz, 2H), 7.15–7.36 (m, 3H), 7.42–7.52 (m, 1H), 7.67 (s, 1H), 7.71 (s, 1H). Anal. Calcd for $C_{26}H_{29}ClN_2O_2$·HCl·0.5H_2O: C, 64.73; H, 6.48; N, 5.81. Found: C, 65.42; H, 6.49; N, 5.79.

5.1.31. 8-{3-[1-(3-Chlorobenzyl)piperidin-4-yl]propanoyl}-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one hydrochloride (9f). 86%, Mp 208–210 °C (EtOH–Et_2O). IR (KBr) 1654, 1596, 1162, 1149 cm^{-1}. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.19–1.42 (m, 3H), 1.58–1.79 (m, 4H), 1.86–2.02 (m, 2H), 2.71 (t, J = 7.7 Hz, 2H), 2.81–3.08 (m, 6H), 3.22 (t, J = 8.6 Hz, 2H), 3.45 (s, 2H), 4.13 (t, J = 8.6 Hz, 2H), 7.16–7.28 (m, 3H), 7.32 (s, 1H), 7.66 (s, 1H), 7.71 (s, 1H). Anal. Calcd for $C_{26}H_{29}ClN_2O_2$·HCl·0.5H_2O: C, 64.73; H, 6.48; N, 5.81. Found: C, 64.97; H, 6.39; N, 5.65.

5.1.32. 8-{3-[1-(4-Chlorobenzyl)piperidin-4-yl]propanoyl}-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one hydrochloride (9g). 80%, Mp 235–237 °C (EtOH–Et_2O). IR (KBr) 1670, 1594, 1162, 1145 cm^{-1}. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.18–1.41 (m, 3H), 1.58–1.78 (m, 4H), 1.84–2.02 (m, 2H), 2.72 (t, J = 7.7 Hz, 2H), 2.80–3.08 (m, 6H), 3.22 (t, J = 8.6 Hz, 2H), 3.44 (s, 2H), 4.13 (t, J = 8.6 Hz, 2H), 7.20–7.33 (m, 4H), 7.67 (s, 1H), 7.71 (s, 1H). Anal. Calcd for $C_{26}H_{29}ClN_2O_2$·HCl·H_2O: C, 63.54; H, 6.56; N, 5.70. Found: C, 63.26; H, 6.53; N, 5.54.

5.1.33. 8-{3-[1-(2-Methoxybenzyl)piperidin-4-yl]propanoyl}-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one hydrochloride (9h). 76%, Amorphous. IR (KBr) 2922, 1671, 1596, 1491, 1151, 755, 729 cm^{-1}. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.23–1.49 (m, 3H), 1.61–1.79 (m, 4H), 1.96–2.17 (m, 2H), 2.72 (t, J = 7.7 Hz, 2H), 2.87–3.06 (m, 6H), 3.22 (t, J = 8.6 Hz, 2H), 3.58 (s, 2H), 3.82 (s, 3H), 4.14 (t, J = 8.6 Hz, 2H), 6.83–6.96 (m, 2H), 7.18–7.41 (m, 2H), 7.67 (s, 1H), 7.72 (s, 1H). Anal. Calcd for $C_{27}H_{32}N_2O_3$·HCl·2.5H_2O: C, 63.09; H, 7.45; N, 5.45. Found: C, 63.10; H, 7.21; N, 5.30.

5.1.34. 8-{3-[1-(3-Methoxybenzyl)piperidin-4-yl]propanoyl}-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one hydrochloride (9i). 84%, Mp 157–159 °C (EtOH–Et_2O). IR (KBr) 2915, 1665, 1597, 1490 cm^{-1}. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.22–1.43 (m, 3H), 1.59–1.76 (m, 4H), 1.83–2.05 (m, 2H), 2.71 (t, J = 7.9 Hz, 2H), 2.86–3.06 (m, 6H), 3.22 (t, J = 8.5 Hz, 2H), 3.26 (s, 2H), 3.81 (s, 3H), 4.13 (t, J = 8.5 Hz, 2H), 6.76–6.82 (m, 1H), 6.87–6.92 (m, 2H), 7.22 (t, J = 8.4 Hz, 1H), 7.66 (s, 1H), 7.71 (s, 1H). Anal. Calcd for $C_{27}H_{32}N_2O_3$·HCl·0.5H_2O: C, 67.84; H, 7.17; N, 5.86. Found: C, 67.96; H, 7.25; N, 5.70.

5.1.35. 8-3-[1-(4-Methoxybenzyl)piperidin-4-yl]propanoyl-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one hydrochloride (9j). 61%, Amorphous. IR (KBr) 1654, 1596, 1492, 1382, 1255 cm^{-1}. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.23–1.49 (m, 3H), 1.61–1.79 (m, 4H), 1.96–2.17 (m, 2H), 2.72 (t, J = 7.7 Hz, 2H), 2.87–3.06 (m, 6H), 3.22 (t, J = 8.6 Hz, 2H), 3.43 (s, 2H), 3.77 (s, 3H), 4.12 (t, J = 8.5 Hz, 2H), 6.83 (d, J = 8.5 Hz, 2H), 7.20 (d, J = 8.5 Hz, 2H), 7.67 (s, 1H), 7.72 (s, 1H). Anal. Calcd for $C_{27}H_{32}N_2O_3$·HCl·0.75H_2O: C, 67.21; H, 7.21; N, 5.81. Found: C, 67.31; H, 7.05; N, 5.75.

5.1.36. 8-{3-[1-(2-Hydroxybenzyl)piperidin-4-yl]propanoyl}-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one hydrochloride (9k). 47%, Mp 223–225 °C (EtOH–Et_2O). IR (KBr) 3543, 1661, 1592, 1270, 1148 cm^{-1}. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.20–1.53 (m, 3H), 1.60–1.84 (m, 4H), 1.98–2.16 (m, 3H), 2.72 (t, J = 7.8 Hz, 2H), 2.84–3.10 (m, 6H), 3.23 (t, J = 8.6 Hz, 2H), 3.68 (s, 2H), 4.13 (t, J = 8.6 Hz, 2H), 6.70–6.85 (m, 2H), 6.88–7.00 (m, 1H), 7.12–7.23 (m, 1H), 7.67 (s, 1H), 7.71 (s, 1H). Anal. Calcd for $C_{26}H_{30}N_2O_3$·HCl: C, 68.63; H, 6.87; N, 6.16. Found: C, 68.48; H, 6.94; N, 5.95.

5.1.37. 8-{3-[1-(3-Hydroxybenzyl)piperidin-4-yl]propanoyl}-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one hydrochloride (9l). 36%, Mp 154–156 °C (EtOH–Et_2O). IR (KBr) 3182, 1667, 1592 cm^{-1}. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.30–1.92 (m, 7H), 2.15–2.36 (m, 2H), 2.71 (t, J = 7.8 Hz, 2H), 2.82–3.31 (m, 8H), 3.64 (s, 2H), 4.12 (t, J = 8.4 Hz, 2H), 6.68–7.18 (m, 5H), 7.65 (s, 1H), 7.69 (s, 1H). Anal. Calcd for $C_{26}H_{30}N_2O_3$·HCl·1.5H_2O: C, 64.79; H, 7.11; N, 5.81. Found: C, 64.67; H, 6.82; N, 5.65.

5.1.38. 8-{3-[1-(4-Hydroxybenzyl)piperidin-4-yl]propanoyl}-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one hydrochloride (9m). 11%, Mp 233–235 °C (EtOH–Et_2O). IR (KBr) 3432, 1652, 1591 cm^{-1}. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.22–1.52 (m, 3H), 1.58–1.84 (m, 4H), 1.90–2.18 (m, 2H), 2.66–2.76 (m, 2H), 2.80–3.10 (m, 6H), 3.21 (t, J = 8.4 Hz, 2H), 3.47 (s, 2H), 4.13 (t, J = 8.4 Hz, 2H), 5.98 (br, s, 1H), 6.64 (d, J = 8.4 Hz, 2H), 7.08 (d, J = 8.4 Hz, 2H), 7.65 (s, 1H), 7.70 (s, 1H). Anal. Calcd for $C_{26}H_{30}N_2O_3$·HCl·0.5H_2O:

C, 67.30; H, 6.95; N, 6.04. Found: C, 67.46; H, 7.20; N, 5.86.

5.1.39. 8-{3-[1-(2-Nitrobenzyl)piperidin-4-yl]propanoyl}-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one hydrochloride (9n). 85%, Mp 166–168 °C (EtOH–Et_2O). IR (KBr) 2934, 1669, 1597, 1532, 1493, 1342, 750 cm^{-1}. ^1H NMR (free base; 300 MHz, $CDCl_3$) δ 1.20–1.40 (m, 3H), 1.60–1.80 (m, 4H), 1.95–2.10 (m, 2H), 2.70–2.80 (m, 4H), 2.90–2.95 (m, 2H), 3.02 (t, J = 8.1 Hz, 2H), 3.23 (t, J = 8.4 Hz, 2H), 3.75 (s, 2H), 4.13 (t, J = 8.1 Hz, 2H), 7.37 (dt, J = 8.0, 1.2 Hz, 1H), 7.53 (dt, J = 7.5, 1.2 Hz, 1H), 7.62 (dd, J = 7.5, 1.2 Hz, 1H), 7.66 (s, 1H), 7.71 (s, 1H), 7.80 (dd, J = 8.0, 1.2 Hz, 1H). Anal. Calcd for $C_{26}H_{29}N_3O_4{\cdot}HCl{\cdot}H_2O$: C, 62.21; H, 6.43; N, 8.37. Found: C, 62.42; H, 6.54; N, 8.36.

5.1.40. 8-{3-[1-(3-Nitrobenzyl)piperidin-4-yl]propanoyl}-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one hydrochloride (9o). 99%, Mp 214–216 °C (EtOH–Et_2O). IR (KBr) 2924, 1669, 1525, 1491, 731 cm^{-1}. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.26–1.44 (m, 3H), 1.62–1.83 (m, 4H), 1.86–2.12 (m, 2H), 2.72 (t, J = 7.6 Hz, 2H), 2.80–3.12 (m, 6H), 3.23 (t, J = 8.5 Hz, 2H), 3.57 (s, 2H), 4.13 (t, J = 8.5 Hz, 2H), 7.48 (dd, J = 8.0, 7.8 Hz, 1H), 7.64–7.76 (m, 3H), 8.10 (t, J = 8.0 Hz, 2H), 8.19 (s, 1H). Anal. Calcd for $C_{26}H_{29}N_3O_4{\cdot}HCl{\cdot}0.5H_2O$: C, 63.34; H, 6.34; N, 8.52. Found: C, 63.05; H, 6.40; N, 8.32.

5.1.41. 8-{3-[1-(4-Nitrobenzyl)piperidin-4-yl]propanoyl}-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one hydrochloride (9p). 78%, Mp 230–232 °C (EtOH–Et_2O). IR (KBr) 2920, 1670, 1518, 1491, 735 cm^{-1}. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.26–1.43 (m, 3H), 1.64–1.78 (m, 4H), 1.92–2.08 (m, 2H), 2.72 (t, J = 7.9 Hz, 2H), 2.77–3.06 (m, 6H), 3.23 (t, J = 8.5 Hz, 2H), 3.57 (s, 2H), 4.14 (t, J = 8.3 Hz, 2H), 7.50 (d, J = 8.8 Hz, 2H), 7.69 (d, J = 8.8 Hz, 2H), 8.15 (s, 1H), 8.19 (s, 1H). Anal. Calcd for $C_{26}H_{29}N_3O_4{\cdot}HCl{\cdot}0.5H_2O$: C, 63.34; H, 6.34; N, 8.52. Found: C, 63.66; H, 6.33; N, 8.52.

5.1.42. 2-({4-[3-Oxo-3-(4-oxo-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-8-yl)propyl]piperidin-1-yl}methyl)-benzonitrile hydrochloride (9q). 84%, Amorphous. IR (KBr) 2291, 1668, 1596, 1156 cm^{-1}. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.21–1.45 (m, 3H), 1.61–1.83 (m, 4H), 1.99–2.18 (m, 2H), 2.72 (t, J = 7.8 Hz, 2H), 2.82–3.07 (m, 6H), 3.23 (t, J = 8.4 Hz, 2H), 3.67 (s, 2H), 4.13 (t, J = 8.4 Hz, 2H), 7.26–7.40 (m, 1H), 7.49–7.68 (m, 4H), 7.71 (s, 1H). Anal. Calcd for $C_{27}H_{29}N_3O_2{\cdot}HCl{\cdot}2.5H_2O$: C, 63.71; H, 6.73; N, 8.25. Found: C, 64.00; H, 6.54; N, 8.23.

5.1.43. 3-({4-[3-Oxo-3-(4-oxo-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-8-yl)propyl]piperidin-1-yl}methyl)-benzonitrile hydrochloride (9r). 76%, Amorphous. IR (KBr) 2926, 2627, 2232, 1667, 1597, 1493, 1383, 1159, 754 cm^{-1}. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.20–1.40 (m, 3H), 1.60–2.10 (m, 6H), 2.71 (t, J = 7.8 Hz, 2H), 2.80–2.90 (m, 4H), 3.02 (t, J = 7.8 Hz, 2H), 3.22 (t, J = 8.4 Hz, 2H), 3.51 (s, 2H), 4.13 (t, J = 8.4 Hz, 2H), 7.40 (t, J = 7.8 Hz, 1H), 7.50–7.65 (m, 3H), 7.67 (s, 1H), 7.71 (s, 1H). Anal. Calcd for $C_{27}H_{29}N_3O_2{\cdot}HCl{\cdot}0.8H_2O$: C, 67.78; H, 6.66; N, 8.78. Found: C, 67.79; H, 6.97; N, 8.51.

5.1.44. 4-({4-[3-Oxo-3-(4-oxo-1,2,5,6-tetrahydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-8-yl)propyl]piperidin-1-yl}methyl)-benzonitrile hydrochloride (9s). 78%, Mp 220–222 °C (EtOH–Et_2O). IR (KBr) 2930, 2627, 2230, 1669, 1597, 1493, 1383, 754 cm^{-1}. ^1H NMR (free base; 200 MHz, $CDCl_3$) δ 1.20–1.40 (m, 3H), 1.60–2.10 (m, 6H), 2.71 (t, J = 7.8 Hz, 2H), 2.80–3.10 (m, 6H), 3.22 (t, J = 8.4 Hz, 2H), 3.49 (s, 2H), 4.13 (t, J = 8.4 Hz, 2H), 7.40 (t, J = 7.8 Hz, 1H), 7.50–7.65 (m, 3H), 7.67 (s, 1H), 7.71 (s, 1H). Anal. Calcd for $C_{27}H_{29}N_3O_2{\cdot}HCl{\cdot}0.5H_2O$: C, 68.56; H, 6.61; N, 8.88. Found: C, 68.68; H, 6.80; N, 8.91.

5.2. AChE inhibition activity

AChE activity was measured by an acetylthiocholine method using human-erythrocyte-derived AChE. 30 μL of 80 mM Tris–HCl (pH 7.4), 50 μL of AChE solution (0.2 IU/mL), 50 μL of 5 mM 5,5-dithio-bis(2-nitrobenzoic acid), 20 μL of drug solution, and 50 μL of 4 mM acetylthiocholine iodide were added to the microplate wells. Absorbance at 412 nM was read every 30 s for 10 min using a microplate reader (Spectra rainbow thermo, Tecan, Switzerland) followed by the calculation of the reaction velocities using analysis software (Biolise 2.01). The IC_{50} value and 95% confidence interval of each drug was calculated by least-squares regression analysis.

5.2.1. AUC200 in Guinea pigs. Male 5-week old Hartley Guinea pigs were used. The animals were anesthetized with an intraperitoneal injection of urethane (1.2 g/kg). The urinary bladder was exposed through an incision in the abdomen, and the urethra was ligated. A needle (20-gauge) connected to a polyethylene tube (PE-100) was inserted into the bladder dome to record intravesical pressure using a pressure transducer (AP461G, Nihon koden, Tokyo). Pressure signals were recorded via multiple unit data acquisition system (MP-100A-CE, Biopac systems, Santa Barbara, CA, USA) by a personal computer at a sampling rate of 5 Hz. Warmed physiological saline (38 °C) was injected until regular isovolumetric bladder contractions appeared. Drugs were administered intravenously after confirming stable rhythmic contractions. The effects of the drugs were estimated by the area under the curve of the vesical contractions about 5 min after the drug-administration. AUC200 value was calculated for each compound as the dose increasing the AUC to twice the pre-drug AUC.

5.2.2. AUC200 in rats. Male Sprague–Dawley rats weighing 210–250 g were anesthetized with an intraperitoneal injection of urethane (1.2 g/kg). A lower abdominal midline incision was made to expose the bladder, and two 23-gauge needles connected to a polyethylene tube (PE-50) were inserted into the bladder dome, for recording the intravesical pressure and for intravesical infusion of physiological saline at the rate of 0.1 mL/

min, respectively. After confirming successive micturition reflexes induced by intravesical infusion of physiological saline, the urethra was ligated not to leak the urine. The bladder was completely drained off, and infusion was then restarted. Bladder filling was continued until micturition was observed, and the AUC of the vesical contractions induced by micturition reflex versus time were calculated. The post-drug values of the AUC were measured 30 min after the intraduodenal drug administration. The ratio of the change of the AUC after the drug administration, and AUC200 value was calculated as for the Guinea pig study.

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