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Structure–Activity Relationships of Xanthene Carboxamides, Novel CCR1 Receptor Antagonists

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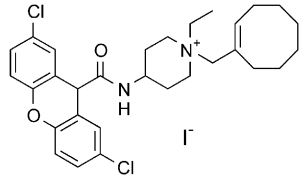
Abstract—The structure–activity relationships of xanthene carboxamide derivatives on the CCR1 receptor binding affinity and the functional antagonist activity were described. Previously, we reported a quaternarized xanthen-9-carboxamide **1** as a potent human CCR1 receptor antagonist that was derived from a xanthen-9-carboxamide lead **2a**. Further derivatization of **2a** focusing on installing an additional substituent into the xanthene ring resulted in the identification of **2b–1** with IC_{50} values of 1.8 nM and 13 nM in the binding assay using human CCR1 receptors transfected CHO cells and in the functional assay using U937 cells expressing human CCR1 receptors, respectively.

Introduction

Chemokines are a large family of 8–10 kDa chemotactic cytokines that play important roles in the recruitment of leukocytes from circulation to inflammatory lesions and may be pivotal proinflammatory mediators in various inflammatory diseases such as allergic diseases and ischemia.[1,2] Chemokines are divided into four subfamilies (CC, CXC, C and CX_3C) based on the position of the conserved cysteine residues.[3] In the past decade, more than 40 human chemokines have been discovered. The specific effects of chemokines are mediated by their receptors, which belong to a family of seven transmembrane-spanning G-protein-coupled receptors (GPCR) that are homologous to the family of chemotactic receptors. To date, 18 chemokine receptors (CCR1–11, CXCR1–5, XCR1 and CX_3CR1) have been cloned and characterized. Among CC chemokines, MIP-1α (macrophage inflammatory protein-1α) and RANTES (Regulated on Activation Normal T-cell Expressed and Secreted), which are known as representative ligands for CCR1 receptors, are suggested to play an important role in chronic inflammatory diseases, such as rheumatoid arthritis[4] and multiple sclerosis.[5] Therefore, selective blockade of CCR1 receptors may be an attractive therapeutic target for the treatment of rheumatoid arthritis[6] and multiple sclerosis.[7] Previously, we reported a quaternarized xanthen-9-carboxamide **1** as a potent human CCR1 receptor antagonist; however, it had poor oral activity due to its quaternarized structure.[8] Therefore, we attempted to identify a potent receptor antagonist without the quaternarized structure. We selected a xanthene carboxamide lead **2a**, that was reported to show the potent binding affinity in the tertiary amine class of the xanthene carboxamides,[8] and further modified it by installing an additional substituent into the xanthene ring. In this paper, we described the synthesis and structure–activity relationships (SARs) of new xanthene carboxamide derivatives on the binding affinity and functional antagonist activity for human CCR1 receptors.



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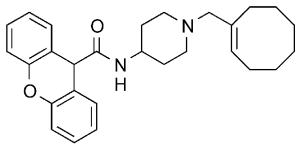

2a

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Results and Discussion

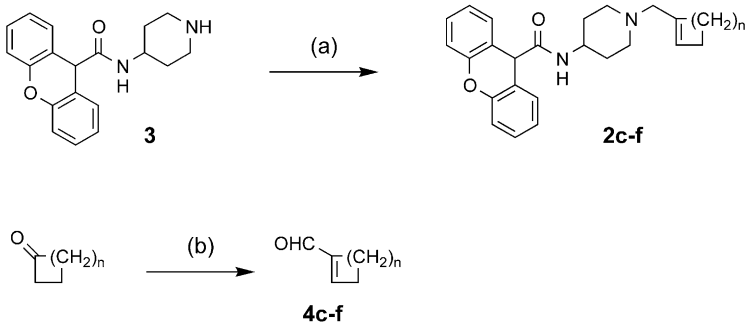
Chemistry

Compounds (**2c–f**) were prepared by reductive alkylation of a 4-aminopiperidine derivative **3**[8] with aldehydes (**4c–f**) as shown in Scheme 1. The six- to ten-membered cycloalkanone tosylhydrazones were treated with 4 equivalents of *n*-Butyl lithium (*n*-BuLi) in the presence of *N,N,N′,N′*-tetramethylenediamine (TMEDA) to generate the corresponding cycloalkenyl anions, which were reacted with DMF producing the aldehydes (**4c–f**) in 31–78% yields.

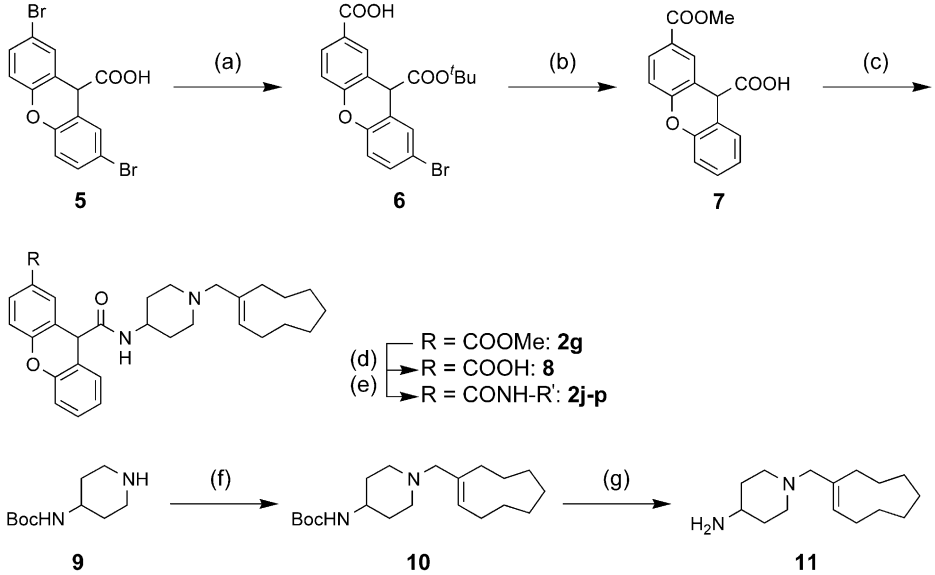
Synthesis of compounds (**2g**, **j–p**) was outlined in Scheme 2. Treatment of *t*-butyl dibromo-xanthene carboxylate with 4 equivalents of *n*-BuLi and the subsequent quench with dry ice afforded a carboxylic acid **6** exclusively in 89% yield. Esterification of **6** with diazomethane followed by debromination under a usual catalytic hydrogenation condition produced diester, in which the *t*-butyl ester group was deprotected under an acidic condition afforded **7** in quantitative yield. Condensation of this acid **7** with an amine **11** in the presence of 1,1′-carbonyldiimidazole produced **2g**, in which the methyl ester group was hydrolyzed under a basic condition, and the resulting carboxylic acid was condensed with appropriate amines to produce compounds (**2j–p**).

Scheme 3 summarizes the synthesis of 2-aminoxanthene derivatives (**2h** and **2i**). Curtius rearrangement of **8** using diphenylphosphoryl azide (DPPA) and allyl alcohol afforded allyl carbamate **2h** in 65% yield. Deprotection of the allyl carbamate group by utilizing tributyltin hydride in the presence of palladium catalyst gave an amine **2i** in 41% yield.

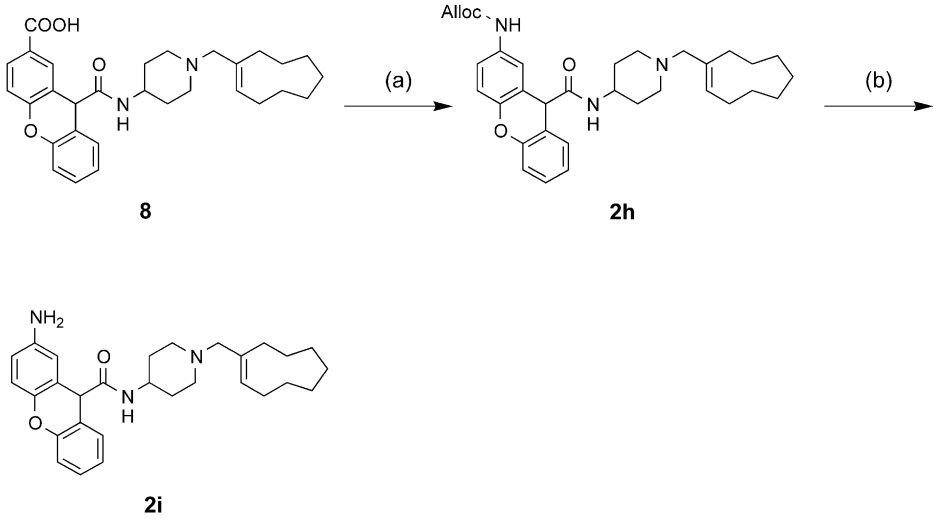
Synthesis of compounds **2b**, **q–w** was outlined in Scheme 4. Primary amines were condensed with acid **8** and deprotected under an acidic condition to afford **2q–t** in 4–60% yield. Since secondary amine was not condensed with acid **8**, target compounds were synthesized by an alternative route. Debromination of **6** under a usual catalytic hydrogenation condition produced acid **12**. Condensation of this acid **12** with piperidine produced **13**, in which the trifluoroactamide group was hydrolyzed under a basic condition (**2u**), or the azide group was reduced with triphenylphosphine to produce compounds (**2b**, **v–w**).



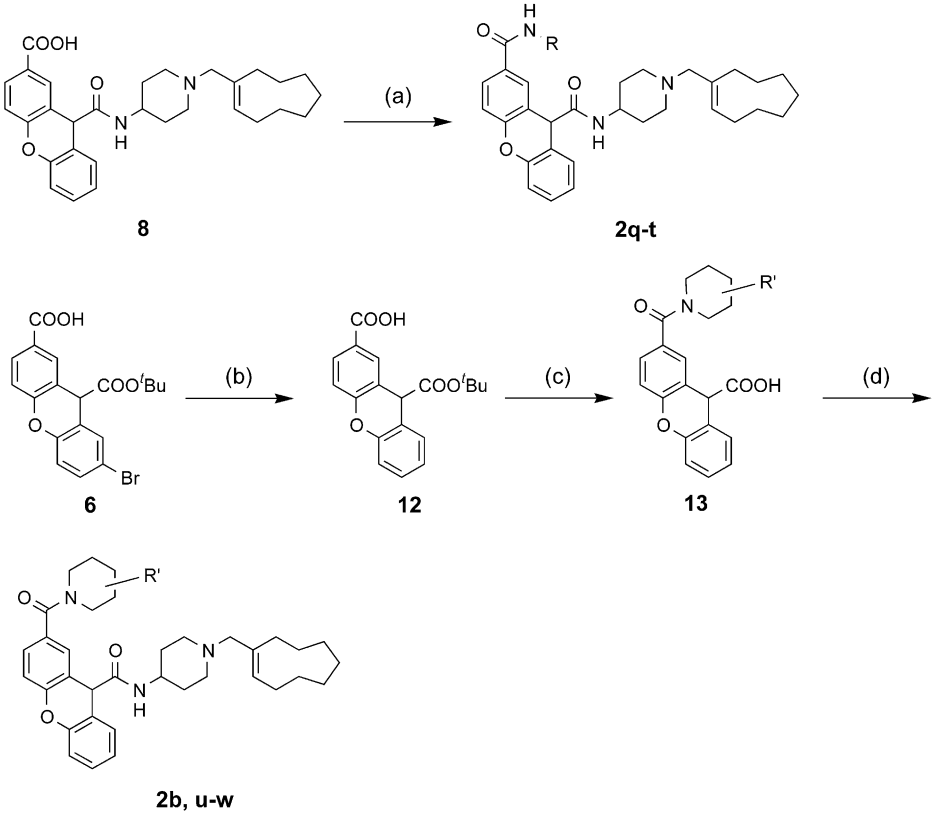
Scheme 1. Reagents: (a) **4c–f**, $NaBH(OAc)_3$, THF; (b) (**1**) *p*-$TsNHNH_2$, MeOH; (2) *n*-BuLi, TMEDA, then DMF.



Scheme 2. Reagents: (a) (1) *t*-Bu isourea, $CHCl_3$; (2) *n*-BuLi, THF, then CO_2; (b) (1) CH_2N_2, Et_2O; (2) H_2, Pd/C, *t*-BuOAc, EtOAc, *t*-BuOH; (3) TFA; (c) CDI, **11**, THF; (d) aq NaOH, THF; (e) R′-NH_2, CDI, THF; (f) **4e**, $NaBH(OAc)_3$, THF; (g) HCl–MeOH.



Scheme 3. Reagents: (a) allyl alcohol, DPPA, Et_3N, 1,4-dioxane; (b) Bu_3SnH, $PdCl_2(Ph_3P)_2$, AcOH, THF.



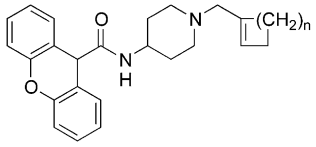
Scheme 4. Reagents: (a) (1) R-NH_2, WSC-HCl, HOBt, DMF; (2) TFA; (b) H_2, Pd/C, EtOAc, *t*-BuOH; (c) (1) (R′-)piperidine, WSC-HCl, HOBt, $CHCl_3$; (2) TFA; (d) (1) **11**, WSC-HCl, HOBt, $CHCl_3$; (2) NaOH aq (**2u**) or Ph_3P, THF–H_2O (**2b**, **v**–**w**).

Biological properties

Compounds (**2a**–**w**) were assessed for their binding activity against ^{125}I-MIP-1α binding to human CCR1 receptors. Selected compounds were examined for their functional antagonist activity in human CCR1 receptor-transfected U 937 cells.

Primarily, we optimized the ring size on the piperidine substituent of the lead compound **2a**. Compounds (**2c**–**2f**) with six- to ten-membered rings were prepared, and their affinity to human CCR1 receptors were examined (Table 1). These results suggested that the eight- or nine-membered ring size would be optimal for the human CCR1 receptor binding. In order to further improve the binding affinity, we assumed that exploration of an additional substituent on the xanthene ring of **2e** was needed. Since it is well known that the 2-position of the xanthene ring is easily functionalized via the dibromo-xanthene derivative (**5**), we prepared the carboxylic acid (**8**) and used as a key intermediate for further derivatization (Table 2).

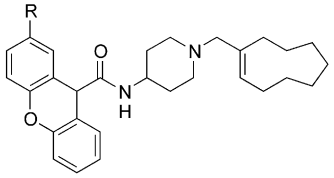
Table 1. Binding affinity to human CCR1 receptors[a]



Compd	*n*	IC_{50} (nM)
2c	3	1100±160
2d	4	570±120
2a	5	51±12
2e	6	48±8.8
2f	7	130±28

[a]The IC_{50} value of each compound was the mean and SD of three assays.

Table 2. Binding affinity to human CCR1 receptors[a]



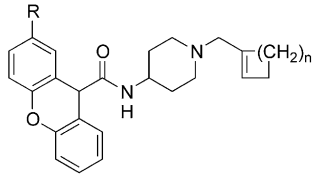
Compd	R	IC_{50} (nM)
2e	H	40±8.8
2g	CO_2Me	36±14
2h	$NHCO_2Allyl$	44±8.6
2i	NH_2	48±12
2j	$CONHCH_2Ph$	220±78
2k	$CONHCH_2Ph$(2-OMe)	120±29
2l	$CONHCH_2Ph$(3-OMe)	130±27
2m	$CONHCH_2Ph$(4-OMe)	32±8.7
2n	$CONHCH_2$(2-Py)	39±15
2o	$CONHCH_2$(3-Py)	37±12
2p	$CONHCH_2$(4-Py)	21±3.8

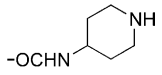
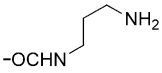
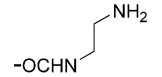
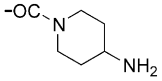
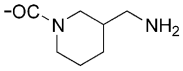
[a]The IC_{50} value of each compound was the mean and SD of three assays.

Although the methyl ester (**2g**), carbamate (**2h**), and amine (**2i**) almost retained the potent binding affinity comparable to that of **2e** for CCR1 receptors, the benzylamide (**2j**) showed a 5-fold reduction in the binding affinity. However, introduction of a methoxy group (**2m**) into the 4-position on the benzyl group in **2j** led to improvement of the binding affinity that was comparable to that of **2e** for CCR1 receptors. We speculated that the methoxy group on the phenyl ring of **2m** worked as an additional hydrogen bonding acceptor and improved the binding affinity. Based on this speculation, we tried to replace the benzyl group with a 2- (**2n**), 3- (**2o**), or 4-pyridylmethyl group (**2p**). As expected, these compounds showed increased binding affinity when compared with **2j**. Among them, the 4-pyridylmethyl derivative (**2p**) showed approximately 2-fold increased potency as compared to that of **2e**.

The 4-pyridylmethyl derivative (**2p**) was measured for its ability to inhibit the MIP-1α-induced Ca^{2+} response

Table 3. Biological properties of **2e**, **p**–**v**

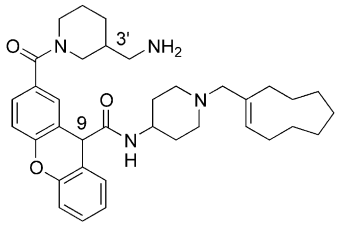


Compd	*n*	R	Binding affinity IC_{50} (nM)[a]	Ca^{2+} Response IC_{50} (nM)[a]	Metablic stability[c] Remaining (%)[b]		
					Rat	Dog	Human
2p	6	$CONHCH_2$(4-Py)	21±3.8	>1100	9	8	8
2q	6		13±0.7	82±2.0	69	73	76
2r	5		42±4.1	550±4.1	—	—	—
2s	6		15±1.0	120±31	—	—	—
2t	6		14±2.1	150±41	—	—	—
2u	6		11±1.0	170±50	—	—	—
2v	6		4.8±0.3	29±8.0	—	—	—
2e	6	H	40±8.8	NT	10	17	28

[a]The IC_{50} value of each compound was the mean and SD of three assays.
[b]Values represent the mean of three incubations.
[c]Compounds (1 μM) were incubated with liver microsomes at a concentration of 1 mg/mL for 30 min at 37 °C.

in U937 cells expressing human CCR1 receptors, however, 4-pyridylmethyl derivative (**2p**) was not a potent CCR1 receptor antagonist (IC_{50}: >1100 nM), probably due to its high lipophilicity (Table 3). With regard to metabolic stability, **2p** was not stable in rat, dog, or human hepatic microsomes.[9] It was assumed that a decrease in the lipophilicity of **2p** would result in the improvement of the functional inhibitory activity and metabolic stability. Therefore, the 4-pyridyl moiety in **2p** was replaced with a more hydrophilic substituent, a 4-piperidinylamine group. The binding affinity of the resulting compound (**2q**) was improved to some extent (IC_{50}: 13 nM), and its functional activity was dramatically improved (IC_{50}: 82 nM). In addition, metabolic stability of the resulting compound (**2q**) in the hepatic microsomes significantly improved. In contrast, a cyclooctenylmethyl derivative (**2r**) exhibited a 3-fold reduction in the binding affinity and a 7-fold decrease in the functional activity as compared with those of the cyclononenylmethyl derivative **2q**. Replacement of the 4-aminopiperidine moiety with an ethylenediamine (**2t**) or a propylenedimaine (**2s**) did not affect the binding affinity but reduced their functional activity. In particular, **2s** showed more potent functional activity than **2t**, suggesting that the propylenedimaine moiety seemed more favorable than the ethylenediamine moiety.

Table 4. Optical resolution of **2v**



Compd	R	Binding affinity IC_{50} (nM)[a]	Ca^{2+} Response IC_{50} (nM)[a]	Metablic stability[c] Remaining (%)[b]		
				Rat	Dog	Human
2v	Racemate	48±0.3				
2W	3′*R*	3.9±0.5				
2b	3′*S*	2.5±0.2				
2b-1	3′*S*, 9**R*[d]	1.8±0.2	13±0.1	56	35	28
2b-2	3′*S*, 9**S*[d]	120±12				

[a]The IC_{50} value of each compound was the mean and SD of three assays.
[b]Values represent the mean of three incubations.
[c]Compounds (1 μM) were incubated with liver microsomes at a concentration of 1 mg/mL for 30 min at 37 °C.
[d]The configuration of 9-position was not determined, named R* or S* for convenience.

Considering the structures of the amide part at the 2-position in **2q** and **2r**, we designed **2u** with an 4-aminopiperidinamide and **2v** with an 3-aminomethylpiperidinamide. Interestingly, **2v** showed a 2-fold improvement both in the binding affinity and the functional activity as compared with those of **2q**. Since this compound was a mixture of 4 enantiomers at the 3′-position and the 9-position, we decided to separate them to identify the most potent enantiomer and to determine the stereochemical structure (Table 4). Primarily, **2w** and **2b** were derived from a commercially available (3*R*)- and (3*S*)-3-aminopiperidine, respectively. Their binding data suggested that both diastereomers (**2w** and **2b**) were potent toward human CCR1 receptors, while **2b** was 2-fold more active than was **2w**. Optical resolution of **2b** revealed that the active principle was **2b–1** with an IC_{50} value of 1.8 nM. This compound also showed an IC_{50} value of 13 nM in the functional assay, which that **2b–1** would be one of the most potent CCR1 receptor antagonists in this series. The metabolic stability of **2b–1** had improved to some extent when compared with that of the original lead compound **2p**, however, further improvements are needed.

Conclusion

Derivatization of the lead compound **2a** aiming at improving the inhibitory activity against human CCR1 receptors led to a compound **2p** in which an additional amide group was installed into the 2-position of the xanthene ring. Further derivatization by replacing the amine moiety of this amide in **2p** with a basic amine resulted in the identification of **2b–1**, which had potent binding affinity (IC_{50} value: 1.8 nM) toward human CCR1 receptors and functional activity (IC_{50} value: 13 nM) against MIP-1α-induced Ca^{2+} response in U937 cells expressing human CCR1 receptors. These results suggested that the configuration at the 9-position played a role in the binding affinity and functional activity. Since the metabolic stability of **2b–1** in the rat, dog, and human microsomes was moderate, we need to address ways to improve the metabolic stability and to identify orally-active CCR1 receptor antagonists.

Experimental

Materials and methods

All reagents and solvents were of commercial quality and were used without further purification unless otherwise noted. ^{1}H NMR spectra were recorded on a Varian VXR 300 spectrometer with tetramethylsilane as an internal standard. Mass spectrometry was performed with a JEOL JMS-SX102A spectrometer. TLC was done with Merck Kieselgel F_{254} pre-coated plates. Silica gel column chromatography was carried out on Merck silica gel 60 (mesh 63–200 nm).

General method: *N*-[1-(1-cyclohexenylmethyl)piperidin-4-yl] xanthen-9-carboxamide (2c). (i) 1-Cyclohexenylcarboxaldehyde (4c). To a stirred suspension of *p*-tolylsulfonylhydrazine (5.4 g, 28.9 mmol) and cyclohexanone (3.0 mL, 28.9 mmol) in MeOH (20 mL) was added concd HCl (0.1 mL) at room temperature. After the mixture was stirred for 16 h, the precipitated cycloalkanone *p*-tolylsulfonylhydrazone (4.1 g, 53%) was collected by filtration. To a stirred suspension of the solid (1.5 g, 5.64 mmol) in TMEDA (20 mL) was added 1.6 M of *n*-BuLi in hexane (9.0 mL, 22.5 mmol) at −55 to −45 °C under N_2. The resulting deep red solution was stirred at −45 °C for 0.5 h, and then allowed to warm to room temperature over a period of 1 h. When N_2 evolution had ceased, the mixture was cooled at 0 °C, and DMF (2.2 mL, 28.4 mmol) was added. After the mixture was stirred for 1 h, the reaction was quenched by adding water. The mixture was extracted with EtOAc, and the organic layer was washed with 2 N HCl and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel column chromatography (3% EtOAc in hexane) to give **4c** (360 mg, 58%) as a colorless oil:^{1}H NMR ($CDCl_3$) δ 1.50–1.80 (m, 4H), 2.10–2.40 (m, 4H), 6.80 (t, J=8.2 Hz, 1H), 9.40 (s, 1H).

(ii). To a solution of *N*-(piperidin-4-yl)xanthen-9-carboxamide (**3**)[8] (80 mg, 0.260 mmol) in THF (3.0 mL) was added **4c** (35 mg, 0.318 mmol) and $NaBH(OAc)_3$ (80 mg, 0.377 mmol) at room temperature, and the mixture was stirred for 20 h. After the addition of a saturated $NaHCO_3$ solution, the mixture was extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by preparative TLC (5% MeOH in $CHCl_3$) to give **2c** (9.0 mg, 9%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.19–2.10 (m, 14H), 2.50–2.69 (m, 2H), 2.74 (s, 2H), 3.60–3.76 (m, 1H), 4.84 (s, 1H), 5.16 (d, J=8.0 Hz, 1H), 5.47–5.55 (m, 1H), 7.10 (t, J=7.7 Hz, 2H),

7.13 (d, $J=7.7$ Hz, 2H), 7.30 (t, $J=7.7$ Hz, 2H), 7.38 (d, $J=7.7$ Hz, 2H); HRMS calcd for $C_{26}H_{31}N_2O_2$ $(M+H)^+$: 403.2386, found 403.2380.

The following compounds (**2d–f**) were prepared in a similar manner to the procedure described for **2c** by using an appropriate ketone.

***N*-[1-(1-Cycloheptenylmethyl)piperidin-4-yl]xanthen-9-carboxamide (2d).** This compound was prepared from cycloheptanone (10%): ^{1}H NMR ($CDCl_3$) δ 1.15–1.67 (m, 18H), 2.72 (s, 2H), 3.58–3.75 (m, 1H), 4.84 (s, 1H), 5.12 (d, $J=7.9$ Hz, 1H), 5.62 (t, $J=6.2$ Hz, 1H), 7.10 (t, $J=7.6$ Hz, 2H), 7.13 (d, $J=7.6$ Hz, 2H), 7.30 (t, $J=7.6$ Hz, 2H), 7.38 (d, $J=7.6$ Hz, 2H); HRMS calcd for $C_{27}H_{33}N_2O_2$ $(M+H)^+$: 417.2546, found 417.2542.

***N*-[1-(1-Cyclononenylmethyl)piperidin-4-yl]xanthen-9-carboxamide (2e).** This compound was prepared from cyclononanone (10%): ^{1}H NMR ($CDCl_3$) δ 1.00–1.68 (m, 22H), 2.74 (s, 2H), 3.59–3.75 (m, 1H), 4.84 (s, 1H), 5.11 (d, $J=7.8$ Hz, 1H), 5.36 (t, $J=8.4$ Hz, 1H), 7.10 (t, $J=7.6$ Hz, 2H), 7.13 (d, $J=7.6$ Hz, 2H), 7.30 (t, $J=7.6$ Hz, 2H), 7.38 (d, $J=7.6$ Hz, 2H); HRMS calcd for $C_{29}H_{37}N_2O_2$ $(M+H)^+$: 445.2845, found 445.2855.

***N*-[1-(1-Cyclodecenylmethyl)piperidin-4-yl]xanthen-9-carboxamide (2f).** This compound was prepared from cyclodecanone (17%): ^{1}H NMR ($CDCl_3$) δ 1.10–2.15 (m, 24H), 2.74 (s, 2H), 3.58–3.77 (m, 1H), 4.84 (s, 1H), 5.11 (d, $J=8.4$ Hz, 1H), 5.25 (t, $J=6.0$ Hz, 1H), 7.11 (t, $J=7.7$ Hz, 2H), 7.13 (d, $J=7.7$ Hz, 2H), 7.30 (t, $J=7.7$ Hz, 2H), 7.38 (d, $J=7.7$ Hz, 2H); HRMS calcd for $C_{30}H_{39}N_2O_2$ $(M+H)^+$: 459.3009, found 459.3012.

General method: N^9-[1-(1-cyclononenylmethyl)piperidin-4-yl]-N^2-benzylxanthen-2,9-dicarboxamide (2j). (i) *t*-Butyl 2-bromo-7-carboxyxanthene-9-carboxylate (6). To a stirred suspension of 2,7-dibromoxanthene-9-carboxylic acid[10] (6.0 g, 15.6 mmol) in $CHCl_3$ (100 mL) was added *N,N′*-diisopropyl-*O*-*t*-butylisourea (13.1 g, 65.5 mmol) in $CHCl_3$ (50 mL) at room temperature. After the mixture was stirred for 14 h, the precipitated urea was removed by filtration, and the filtrate was evaporated. The residue was purified by silica gel column chromatography (30–50% EtOAc in hexane) to give *t*-butyl 2,7-dibromoxanthene-9-carboxylate (6.10 g, 89%) as a colorless solid. To a stirred solution of *t*-butyl 2,7-dibromoxanthene-9-carboxylate (6.60 g, 15.0 mmol) in THF (150 mL) was added 1.6 M of *n*-BuLi in hexane (25.0 mL, 62.5 mmol) at −78 °C under N_2. After the solution was stirred at −78 °C for 0.5 h, CO_2 was added and then allowed to warm to room temperature. When CO_2 evolution had ceased, the reaction was quenched by adding 10% citric acid solution. The mixture was extracted with EtOAc, and the organic layer was washed with water, dried ($MgSO_4$), and concentrated in vacuo to give **6** (3.64 g, 60%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.38 (s, 9H), 4.86 (s, 1H), 7.05 (d, $J=8.6$ Hz, 1H), 7.18 (d, $J=8.6$ Hz, 1H), 7.41 (dd, $J=2.1$, 8.6 Hz, 1H), 7.47 (d, $J=2.1$ Hz, 1H), 8.03 (dd, $J=2.1$, 8.6 Hz, 1H), 8.10 (d, $J=2.1$ Hz, 1H); HRMS calcd for $C_{19}H_{16}O_5Br$ $(M-H)^-$: 403.0182, found 403.0168;

(ii) Methyl 9-carboxyxanthene-2-carboxylate (7). A solution of **6** (3.64 g, 8.99 mmol) in MeOH (50 mL) was treated with excess amount of CH_2N_2 in ether until the evolution of N_2 ceased. The solvent was removed, and the residue was purified by silica gel column chromatography (5–10% EtOAc in hexane) to give *t*-butyl 2-bromo-7-methoxycarbonylxanthene-9-carboxylate (3.78 g, quant) as a slightly yellowish solid. The solid (3.78 g, 9.02 mmol) was dissolved in *t*-BuOH (50 mL) and EtOAc (50 mL), 10% Pd/C was added, and the mixture was vigorously stirred under H_2 for 2 h at ambient temperature. The mixture was filtered, and the filtrate was concentrated in vacuo to give *t*-butyl 2-methoxycarbonylxanthene-9-carboxylate (2.94 g, quant) as a yellowish solid. The solid (2.94 g, 9.02 mmol) was dissolved in TFA (10 mL), and the mixture was stirred for 15 h at room temperature. The mixture was concentrated in vacuo to give **7** (2.56 g, quant) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 3.89 (s, 3H), 4.99 (s, 1H), 7.05–7.20 (m, 3H), 7.25–7.39 (m, 2H), 7.98 (dd, $J=2.1$, 8.2 Hz, 1H), 8.01 (d, $J=2.1$ Hz, 1H); HRMS calcd for $C_{15}H_{11}O_3$ $(M-H-CO_2)^-$: 239.0708, found 239.0706;

(iii) *N*-[1-(1-Cyclononenylmethyl)piperidin-4-yl]-2-methoxycarbonylxanthen-9-carboxamide (2g). A solution of **7** (0.62 g, 2.31 mmol) and 1,1′-carbonyldiimidazole (0.45 g, 2.78 mmol) in THF (30 mL) was stirred at room temperature for 1 h. To this mixture was added **11** (0.55 g, 2.33 mmol) in THF (5 mL), and the mixture was stirred for 14 h. The mixture was diluted with EtOAc, washed with saturated $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel column chromatography (3% MeOH in $CHCl_3$) to give **2g** (0.60 g, 57%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.10–1.15 (m, 20H), 2.48–2.53 (m, 2H), 2.71 (s, 2H), 3.59–3.75 (m, 1H), 3.91 (s, 3H), 4.85 (s, 1H), 5.11 (d, $J=8.1$ Hz, 1H), 5.35 (t, $J=8.5$ Hz, 1H), 7.10–7.19 (m, 2H), 7.13 (d, $J=8.5$ Hz, 1H), 7.29–7.40 (m, 2H), 7.99 (dd, $J=2.0$, 8.5 Hz, 1H), 8.14 (d, $J=2.0$ Hz, 1H); HRMS calcd for $C_{31}H_{39}N_2O_4$ $(M+H)^+$: 503.2910, found 503.2910;

(iv) *N*-[1-(1-Cyclononenylmethyl)piperidin-4-yl]-2-carboxyxanthen-9-carboxamide (8). To a solution of **2g** (270 mg, 0.54 mmol) in THF (5.0 mL) and MeOH (5.0 mL) was added 1 N NaOH at room temperature. After being stirred for 20 h, the mixture was adjusted to pH 4 with 1 N HCl and extracted with EtOAc, dried ($MgSO_4$), and concentrated in vacuo to give **8** (260 mg, 99%) as a colorless solid: ^{1}H NMR (CD_3OD) δ 1.30–1.60 (m, 10H), 1.75–1.99 (m, 2H), 2.00–2.15 (m, 2H), 2.15–2.35 (m, 4H), 2.82–3.03 (m, 2H), 3.32–3.46 (m, 2H), 3.57 (s, 2H), 3.75–3.92 (m, 1H), 4.97 (s, 1H), 5.82 (t, $J=8.6$ Hz, 1H), 7.05–7.21 (m, 3H), 7.21–7.36 (m, 2H), 7.90–8.00 (m, 1H), 7.96 (s, 1H); HRMS calcd for $C_{30}H_{37}N_2O_4$ $(M+H)^+$: 489.2753, found 489.2740;

(v). A solution of **8** (60 mg, 0.12 mmol) and 1,1′-carbonyldiimidazole (24 mg, 0.15 mmol) in THF (2.0 mL) was stirred at room temperature for 1 h. To this mixture

was added benzylamine (50 μL, 0.46 mmol), and the mixture was stirred for 11 h. The mixture was diluted with EtOAc, washed with saturated $NaHCO_3$ solution and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by preparative TLC (5% MeOH in $CHCl_3$) to give **2j** (22 mg, 36%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.10–2.20 (m, 20H), 2.50–2.56 (m, 2H), 2.73 (s, 2H), 3.55–3.74 (m, 1H), 4.61 (dd, J = 5.6, 14.4 Hz, 1H), 4.67 (dd, J = 5.6, 14.4 Hz, 1H), 4.83 (s, 1H), 5.16 (d, J = 7.8 Hz, 1H), 5.36 (t, J = 8.5 Hz, 1H), 6.48 (t, J = 5.6 Hz, 1H), 7.10–7.21 (m, 3H), 7.24–7.41 (m, 7H), 7.81 (dd, J = 2.1, 8.6 Hz, 1H), 7.86 (d, J = 2.1 Hz, 1H); HRMS calcd for $C_{37}H_{44}N_3O_3$ $(M+H)^+$: 578.3383, found 578.3370.

The following compounds (**2k–p**) were prepared in a similar manner to the procedure described for **2j** by using **8** and an appropriate amine.

***N*^{9}-[1-(1-Cyclononenylmethyl)piperidin-4-yl]-*N*^{2}-(2-methoxybenzyl)xanthen-2,9-dicarboxamide (2k).** This compound was prepared from 2-methoxybenzylamine (13%): ^{1}H NMR ($CDCl_3$) δ 1.18–2.25 (m, 20H), 2.52–2.72 (m, 2H), 2.75 (s, 2H), 3.58–3.75 (m, 1H), 3.90 (s, 3H), 4.61 (dd, J = 5.7, 14.5 Hz, 1H), 4.66 (dd, J = 5.7, 14.5 Hz, 1H), 4.84 (s, 1H), 5.31 (d, J = 7.3 Hz, 1H), 5.37 (t, J = 8.4 Hz, 1H), 6.75 (t, J = 5.7 Hz, 1H), 6.85–7.00 (m, 2H), 7.09–7.19 (m, 3H), 7.21–7.39 (m, 4H), 7.76 (dd, J = 1.8, 8.6 Hz, 1H), 7.83 (d, J = 1.8 Hz, 1H); HRMS calcd for $C_{38}H_{46}N_3O_4$ $(M+H)^+$: 608.3488, found 608.3479.

***N*^{9}-[1-(1-Cyclononenylmethyl)piperidin-4-yl]-*N*^{2}-(3-methoxybenzyl)xanthen-2,9-dicarboxamide (2l).** This compound was prepared from 3-methoxybenzylamine (27%): ^{1}H NMR ($CDCl_3$) δ 1.11–2.19 (m, 20H), 2.45–2.69 (m, 2H), 2.73 (s, 2H), 3.55–3.75 (m, 1H), 3.81 (s, 3H), 4.59 (dd, J = 5.6, 14.0 Hz, 1H), 4.62 (dd, J = 5.6, 14.0 Hz, 1H), 4.83 (s, 1H), 5.13 (d, J = 7.3 Hz, 1H), 5.36 (t, J = 8.8 Hz, 1H), 6.40 (t, J = 5.6 Hz, 1H), 6.84 (d, J = 8.2 Hz, 1H), 6.91 (s, 1H), 6.95 (d, J = 7.4 Hz, 1H), 7.10–7.38 (m, 6H), 7.81 (dd, J = 2.1, 8.4 Hz, 1H), 7.86 (d, J = 2.1 Hz, 1H); HRMS calcd for $C_{38}H_{46}N_3O_4$ $(M+H)^+$: 608.3488, found 608.3475.

***N*^{9}-[1-(1-Cyclononenylmethyl)piperidin-4-yl]-*N*^{2}-(4-methoxybenzyl)xanthen-2,9-dicarboxamide (2m).** This compound was prepared from 4-methoxybenzylamine (8%): ^{1}H NMR ($CDCl_3$) δ 1.18–2.22 (m, 20H), 2.52–2.71 (m, 2H), 2.76 (s, 2H), 3.57–3.75 (m, 1H), 3.80 (s, 3H), 4.54 (dd, J = 5.5, 14.5 Hz, 1H), 4.59 (dd, J = 5.5, 14.5 Hz, 1H), 4.82 (s, 1H), 5.28 (d, J = 9.2 Hz, 1H), 5.37 (t, J = 8.6 Hz, 1H), 6.42–6.53 (m, 1H), 6.88 (d, J = 8.6 Hz, 2H), 7.05–7.38 (m, 7H), 7.79 (dd, J = 2.1, 8.5 Hz, 1H), 7.85 (d, J = 2.1 Hz, 1H); HRMS calcd for $C_{38}H_{46}N_3O_4$ $(M+H)^+$: 608.3488, found 608.3479.

***N*^{9}-[1-(1-Cyclononenylmethyl)piperidin-4-yl]-*N*^{2}-(2-pyridylmethyl)xanthen-2,9-dicarboxamide (2n).** This compound was prepared from 2-picolylamine (32%): ^{1}H NMR ($CDCl_3$) δ 1.16–2.21 (m, 20H), 2.50–2.69 (m, 2H), 2.75 (s, 2H), 3.58–3.75 (m, 1H), 4.68–4.82 (m, 2H), 4.88 (s, 1H), 5.23 (d, J = 8.4 Hz, 1H), 5.37 (t, J = 8.4 Hz, 1H), 7.09–7.75 (m, 9H), 7.87 (dd, J = 2.0, 8.6 Hz, 1H), 7.95 (d, J = 2.0 Hz, 1H), 8.58 (dd, J = 0.9, 5.0 Hz, 1H); HRMS calcd for $C_{36}H_{43}N_4O_3$ $(M+H)^+$: 579.3335, found 579.3320.

***N*^{9}-[1-(1-Cyclononenylmethyl)piperidin-4-yl]-*N*^{2}-(3-pyridylmethyl)xanthen-2,9-dicarboxamide (2o).** This compound was prepared from 3-picolylamine (20%): ^{1}H NMR ($CDCl_3$) δ 1.19–2.22 (m, 20H), 2.58–2.74 (m, 2H), 2.77 (s, 2H), 3.55–3.74 (m, 1H), 4.58 (dd, J = 5.8, 14.7 Hz, 1H), 4.63 (dd, J = 5.8, 14.7 Hz, 1H), 4.82 (s, 1H), 5.38 (t, J = 8.4 Hz, 1H), 5.60–5.85 (m, 1H), 7.05–7.41 (m, 7H), 7.70 (ddd, J = 1.6, 2.2, 7.9 Hz, 1H), 7.76 (dd, J = 2.1, 8.5 Hz, 1H), 7.90 (d, J = 2.1 Hz, 1H), 8.50 (dd, J = 1.6, 4.8 Hz, 1H), 8.59 (d, J = 2.2 Hz, 1H); HRMS calcd for $C_{36}H_{43}N_4O_3$ $(M+H)^+$: 579.3335, found 579.3329.

***N*^{9}-[1-(1-Cyclononenylmethyl)piperidin-4-yl]-*N*^{2}-(4-pyridylmethyl)xanthen-2,9-dicarboxamide (2p).** This compound was prepared from 4-picolylamine (25%): ^{1}H NMR ($CDCl_3$) δ 1.20–2.75 (m, 22H), 2.78 (s, 2H), 3.56–3.74 (m, 1H), 4.57 (dd, J = 6.1, 16.0 Hz, 1H), 4.63 (dd, J = 6.1, 16.0 Hz, 1H), 4.83 (s, 1H), 5.38 (t, J = 8.4 Hz, 1H), 5.58–5.71 (m, 1H), 7.09–7.42 (m, 6H), 7.24 (d, J = 5.9 Hz, 2H), 7.79 (dd, J = 2.0, 8.5 Hz, 1H), 7.92 (d, J = 2.0 Hz, 1H), 8.53 (d, J = 5.9 Hz, 2H); HRMS calcd for $C_{36}H_{43}N_4O_3$ $(M+H)^+$: 579.3335, found 579.3334.

***N*-[1-(1-Cyclononenylmethyl)piperidin-4-yl]-2-allyloxycarbonylaminoxanthen-9-carboxamide (2h).** To a stirred suspension of **8** (40 mg, 0.082 mmol) in 1,4-dioxane (2.0 mL) were added diphenylphosphoryl azide (26.5 μL, 0.123 mmol), allyl alcohol (20.0 μL, 0.294 mmol) and Et_3N (17.0 μL, 0.122 mmol) at room temperature. After being refluxed for 17 h, the reaction mixture was cooled and diluted with brine, extracted with $CHCl_3$, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by preparative TLC (9% MeOH in $CHCl_3$) to give **2h** (29 mg, 65%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.15–2.22 (m, 20H), 2.50–2.68 (m, 2H), 2.75 (s, 2H), 3.68–3.78 (m, 1H), 4.67 (d, J = 5.7 Hz, 2H), 4.79 (s, 1H), 5.12–5.43 (m, 4H), 5.86–6.04 (m, 1H), 6.70 (s, 1H), 7.02.18 (m, 3H), 7.21–7.48 (m, 4H); HRMS calcd for $C_{33}H_{42}N_3O_4$ $(M+H)^+$: 544.3175, found 544.3165.

***N*-[1-(1-Cyclononenylmethyl)piperidin-4-yl]-2-aminoxanthen-9-carboxamide (2i).** To a stirred solution of **2h** (20 mg, 0.037 mmol) in CH_2Cl_2 (2.0 mL) was added $PdCl_2(Ph_3P)_2$ (2.9 mg, 0.004 mmol), AcOH (5.0 μL, 0.087 mmol) and n-Bu_3SnH (11.0 μL, 0.041 mmol) at room temperature. After being stirred for 1 h, the reaction mixture was diluted with brine, extracted with $CHCl_3$, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by preparative TLC (9% MeOH in $CHCl_3$) to give **2i** (7.0 mg, 41%) as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 1.08–2.22 (m, 20H), 2.48–2.65 (m, 2H), 2.73 (s, 2H), 3.40.75 (m, 3H), 4.74 (s, 1H), 5.14 (d, J = 7.5 Hz, 1H), 5.36 (t, J = 8.4 Hz, 1H), 6.66 (dd, J = 2.6, 8.6 Hz, 1H), 6.69 (d, J = 2.9 Hz, 1H), 6.93 (d, J = 8.6 Hz, 1H), 7.00–7.15 (m, 2H), 7.21–7.40 (m, 2H); HRMS calcd for $C_{29}H_{38}N_3O_2$ $(M+H)^+$: 460.2964, found 460.2964.

General method: *N*^{2}-(2-aminoethyl)-*N*^{9}-[1-(1-cyclononenylmethyl)piperidin-4-yl]xanthen-2,9-dicarboxamide (2t). To a stirred solution of **8** (30 mg, 0.061 mmol) and t-butyl

N-(2-aminoethyl)carbamate (20 mg, 0.125 mmol) in DMF (1.0 mL) were added 1-hydroxybenzotriazole (15 mg, 0.111 mmol) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (20 mg, 0.104 mmol) at room temperature. After stirring for 17 h, the reaction mixture was diluted with EtOAc, washed with saturated $NaHCO_3$ solution and water, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by preparative TLC (5% MeOH in $CHCl_3$) to give N^2-(2-*t*-butoxycarbonylaminoethyl) - N^9 - [1 - (1 - cyclononenylmethyl)-piperidin-4-yl]xanthen-2,9-dicarboxamide (25 mg, 76%) as a colorless solid. A solution of the solid (25 mg, 0.040 mmol) in TFA (1.0 mL) was stirred at room temperature for 1 h. The mixture was concentrated in vacuo, and the residue was purified by preparative TLC (5% MeOH in $CHCl_3$) to give **2t** (17 mg, 79%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.05–2.65 (m, 22H), 2.71 (s, 2H), 2.96 (t, J = 5.7 Hz, 2H), 3.51 (q, J = 5.7 Hz, 2H), 3.55–3.71 (m, 1H), 4.85 (s, 1H), 5.17 (d, J = 8.2 Hz, 1H), 5.35 (t, J = 8.4 Hz, 1H), 6.93 (t, J = 5.7 Hz, 1H), 7.10–7.40 (m, 5H), 7.82 (dd, J = 2.1, 8.6 Hz, 1H), 7.90 (d, J = 2.1 Hz, 1H); HRMS calcd for $C_{32}H_{43}N_4O_3$ $(M+H)^+$: 531.3335, found 531.3322.

The following compounds (**2q–s**) were prepared in a similar manner to the procedure described for **2t** by using **8** and an appropriate protected amine.

N^9-[1-(1-Cyclononenylmethyl)piperidin-4-yl]-N^2-(4-piperidinyl)xanthen-2,9-dicarboxamide (2q). This compound was prepared from 4-amino-1-*t*-butoxycarbonylpiperidine[11] (4%): ^{1}H NMR ($CDCl_3$) δ 0.80–3.00 (m, 31H), 3.23–3.38 (m, 2H), 3.58–3.78 (m, 1H), 4.05–4.25 (m, 1H), 4.85 (s, 1H), 5.31–5.49 (m, 1H), 5.62–5.90 (m, 1H), 6.75–6.92 m, 1H), 7.08–7.40 (m, 5H), 7.80 (d, J = 8.9 Hz, 1H), 7.92 (s, 1H); HRMS calcd for $C_{35}H_{47}N_4O_3$ $(M+H)^+$: 571.3646, found 571.3648.

N^9-[1-(1-Cyclooctenylmethyl)piperidin-4-yl]-N^2-(4-piperidinyl)xanthen-2,9-dicarboxamide (2r). This compound was prepared in a similar manner to the procedure described for **2q** (9%): ^{1}H NMR ($CDCl_3$) δ 0.80–3.20 (m, 31H), 3.55–3.72 (m, 1H), 4.00–4.18 (m, 1H), 4.85 (s, 1H), 5.27 (d, J = 8.0 Hz, 1H), 5.42 (t, J = 7.9 Hz, 1H), 6.31 (d, J = 7.4 Hz, 1H), 7.10–7.40 (m, 5H), 7.79 (dd, J = 2.2, 8.4 Hz, 1H), 7.86 (d, J = 2.2 Hz, 1H); HRMS calcd for $C_{34}H_{45}N_4O_3$ $(M+H)^+$: 557.3492, found 557.3483.

N^2-(3-Aminopropyl)-N^9-[1-(1-cyclononenylmethyl)piperidin-4-yl]xanthen-2,9-dicarboxamide (2s). This compound was prepared from *t*-butyl *N*-(2-aminopropyl)carbamate (6%): ^{1}H NMR ($CDCl_3$) δ 1.10–2.22 (m, 22H), 2.48–2.65 (m, 2H), 2.71 (s, 2H), 2.92 (t, J = 6.0 Hz, 2H), 3.50–3.75 (m, 3H), 4.84 (s, 1H), 5.25–5.42 (m, 2H), 7.07–7.95 (m, 8H); HRMS calcd for $C_{33}H_{45}N_4O_3$ $(M+H)^+$: 545.3492, found 545.3483.

General method: 2-([(3-aminomethyl)piperidino]carbonyl)-*N*-[1-(1-cyclononenylmethyl)piperidin-4-yl]xanthen-9-carboxamide (2v). (i) *t*-Butyl 2-carboxyxanthen-9-carboxylate (12). To a solution of **6** (4.0 g, 9.88 mmol) in *t*-BuOH (150 mL) and EtOAc (40 mL) was added 10% Pd/C (1.5 g), and the mixture was vigorously stirred under H_2 for 12 h at room temperature. The mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (5–10% MeOH in $CHCl_3$) to give **12** (3.2 g, quant) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 9H), 4.91 (s, 1H), 7.05–7.22 (m, 3H), 7.25–7.39 (m, 2H), 8.05 (dd, J = 2.0, 8.6 Hz, 1H) 8.12 (d, J = 2.0 Hz, 1H); HRMS calcd for $C_{19}H_{17}O_5$ $(M-H)^-$: 325.1076, found 325.1073;

(ii) 2-([(3-Azidomethyl)piperidino]carbonyl)xanthen-9-carboxylic acid (13). To a stirred solution of **12** (400 mg, 1.23 mmol) and 3-(azidomethyl)piperidine[12] (220 mg, 1.25 mmol) in DMF (5.0 mL) were added 1-hydroxybenzotriazole (250 mg, 1.85 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (350 mg, 1.83 mmol) and Et_3N (0.50 mL, 3.59 mmol) at room temperature. After being stirred for 17 h, the reaction mixture was diluted with EtOAc, washed with saturated $NaHCO_3$ solution, 10% citric acid solution, and water; dried ($MgSO_4$); and concentrated in vacuo. The residue was purified by silica gel column chromatography (30–50% EtOAc in hexane) to give *t*-butyl 2-([(3-azidomethyl)piperidino]carbonyl)xanthen-9-carboxylate (530 mg, 96%) as a colorless oil. A solution of the oil (530 mg, 1.18 mmol) in TFA (6.0 mL) was stirred at room temperature for 0.5 h. The mixture was concentrated in vacuo to give **13** (460 mg, quant) as a colorless solid: ^{1}H NMR (CD_3OD) δ 1.20–2.10 (m, 5H), 2.60–2.80 (m, 2H), 2.81–3.15 (m, 2H), 3.49–4.00 (m, 1H), 4.00–4.58 (m, 1H), 5.05 (s, 1H), 7.08–7.15 (m, 3H), 7.18–7.51 (m, 4H); HRMS calcd for $C_{20}H_{19}N_4O_2$ $(M-H-CO_2)^-$: 347.1508, found 347.1523;

(iii). To a stirred solution of **13** (460 mg, 1.17 mmol) and **11** (430 mg, 1.39 mmol) in DMF (8.0 mL) were added 1-hydroxybenzotriazole (240 mg, 1.78 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (340 mg, 1.78 mmol) and Et_3N (1.0 mL, 7.18 mmol) at room temperature. After being stirred for 14 h, the reaction mixture was diluted with EtOAc, washed with saturated $NaHCO_3$ solution and water, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel column chromatography (3% MeOH in $CHCl_3$) to give 2-([(3-azidomethyl)piperidino]carbonyl) - *N* - [1 - (1 - cyclononenylmethyl)piperidin-4-yl]xanthen-9-carboxamide (600 mg, 83%) as a colorless solid. A solution of the above solid (600 mg, 0.98 mmol) and Ph_3P (260 mg, 0.99 mmol) in THF (15 mL) and MeOH (15 mL) was stirred at 90 °C for 1.5 h. The mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography (10% MeOH in $CHCl_3$–1% NH_4OH and 10% MeOH in $CHCl_3$) to give **2v** (570 mg, quant) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.10–2.88 (m, 34H), 2.71 (s, 2H), 3.53–3.75 (m, 2H), 4.82 and 4.83 (s, 1H), 5.10–5.20 (m, 1H), 5.35 (t, J = 8.3 Hz, 1H), 7.03–7.72 (m, 7H); HRMS calcd for $C_{36}H_{49}N_4O_3$ $(M+H)^+$: 585.3805, found 585.3793.

The following compounds (**2b**, **u**, **w**) were prepared in a similar manner to the procedure described for **2v** by using **12** and an appropriate amine.

2-[(4-Aminopiperidino)carbonyl]-*N*-[1-(1-cyclononenylmethyl)piperidin-4-yl]xanthen-9-carboxamide (2u). This compound was prepared from 4-(trifluoroacetamido)-piperidine[13] and deprotected by 4 N NaOH aq in MeOH (41%): ^{1}H NMR ($CDCl_3$) δ 1.15–2.25 (m, 26H), 2.54–3.12 (m, 4H), 2.76 (s, 2H), 3.58–3.75 (m, 2H), 4.84 (s, 1H), 5.37 (t, J = 8.6 Hz, 1H), 5.23–5.45 (m, 1H), 7.08–7.58 (m, 7H); HRMS calcd for $C_{35}H_{47}N_4O_3$ $(M+H)^+$: 571.3648, found 571.3638.

2-[([(3*S*)-3-Aminomethyl]piperidino)carbonyl]-*N*-[1-(1-cyclononenylmethyl)piperidin-4-yl]xanthen-9-carboxamide (2b). This compound was prepared from (3*R*)-3-(azidomethyl)piperidine (51%): ^{1}H NMR ($CDCl_3$) δ 1.10–4.60 (m, 38H), 4.82 and 4.84 (s, 1H), 5.12–5.25 (m, 1H), 5.36 (t, J = 8.3 Hz, 1H), 7.01–7.55 (m, 7H); HRMS calcd for $C_{36}H_{49}N_4O_3$ $(M+H)^+$: 585.3805, found 585.3812.

2-[([(3*R*)-3-Aminomethyl]piperidino)carbonyl]-*N*-[1-(1-cyclononenylmethyl)piperidin-4-yl]xanthen-9-carboxamide (2w). This compound was prepared from (3*S*)-3-(azidomethyl)piperidine (84%): ^{1}H NMR ($CDCl_3$) δ 1.12–2.90 (m, 34H), 2.71 (s, 2H), 3.55–3.78 (m, 2H), 4.82 and 4.83 (s, 1H), 5.32–5.57 (m, 1H), 5.35 (t, J = 8.3 Hz, 1H), 7.03–7.55 (m, 7H); HRMS calcd for $C_{36}H_{49}N_4O_3$ $(M+H)^+$: 585.3805, found 585.3793.

Chiral HPLC separation of 2b-1 and 2b-2. To a solution of **2b** (0.57 g, 0.98 mmol) in $CHCl_3$ (5 mL) was added di-*t*-butyl dicarbonate (0.25 mL, 1.09 mmol), and the mixture was stirred for 14 h. The mixture was concentrated in vacuo, and the residue was purified by silica gel column chromatography (3–5% MeOH in $CHCl_3$) to give Boc-protected **2b** (0.48 g, 72%) as a colorless solid. Boc-protected **2b** (0.48 g/11 times) was separated on a Daicel Chiralcel OD column (2 cm × 25 cm), eluting with 25% *i*-propanol and 0.1% Et_2NH in *n*-hexane at a flow rate of 20 mL/min. Column effluent was monitored at λ = 254 nm. The early and late isomers eluted at 7 and 12 min, respectively, and 208 mg of each was isolated. After separation, their enantiomeric purity was determined by chiral HPLC (Daicel Chiralcel OD column (0.46 cm × 25 cm), eluting with 25% *i*-propanol and 0.1% Et_2NH in *n*-hexane at a flow rate of 1.0 mL/min and monitored at λ = 254 nm) and was found to be 99.5% for the early and 98.7% for the late isomer. Each of the Boc-protected enantiomers was deprotected by 4 N HCl–dioxane, and 170 mg of the early isomer (**2b-2**) and 175 mg of the late isomer (**2b-1**) were isolated.

2b-1. ^{1}H NMR ($CDCl_3$) δ 1.10–4.60 (m, 38H), 4.84 (s, 1H), 5.12–5.25 (m, 1H), 5.36 (t, J = 8.3 Hz, 1H), 7.01–7.55 (m, 7H); HRMS calcd for $C_{36}H_{49}N_4O_3$ $(M+H)^+$: 585.3805, found 585.3812.

2b-2. ^{1}H NMR ($CDCl_3$) δ 1.10–4.60 (m, 38H), 4.82 (s, 1H), 5.12–5.25 (m, 1H), 5.36 (t, J = 8.3 Hz, 1H), 7.01–7.55 (m, 7H); HRMS calcd for $C_{36}H_{49}N_4O_3$ $(M+H)^+$: 585.3805, found 585.3812.

4-*t*-Butoxycarbonylamino-1-(1-cyclononenylmethyl)piperidine (10). This compound was prepared in a similar manner to the procedure described for **2e** by using 4-*t*-butoxycarbonylaminopiperidine and **4e** (81%, colorless oil): ^{1}H NMR ($CDCl_3$) δ 1.28–1.56 (m, 12H), 1.44 (s, 9H), 1.81–2.03 (m, 4H), 2.05–2.25 (m, 4H), 2.65–2.85 (m, 2H), 2.79 (s, 2H), 3.30–3.55 (m, 1H), 4.29–4.55 (m, 1H), 5.40 (t, J = 8.6 Hz, 1H); HRMS calculated for $C_{20}H_{37}N_2O_2$ $(M+H)^+$: 337.2855, found 337.2857.

4-Amino-1-(1-cyclononenylmethyl)piperidine (11). **10** (2.55 g, 7.59 mmol) was dissolved in 10% HCl-MeOH (10 mL) and the resulting mixture was stirred for 14 h. The mixture was concentrated in vacuo, and the residue was basified with 1 N NaOH, extracted by $CHCl_3$, dried ($MgSO_4$), and concentrated in vacuo to give **11** (1.5 g, 84%) as a colorless solid: ^{1}H NMR ($CDCl_3$) δ 1.25–1.63 (m, 12H), 1.72–1.82 (m, 2H), 1.82–1.97 (m, 2H), 2.08–2.25 (m, 4H), 2.55–2.70 (m, 1H), 2.72–2.88 (m, 2H), 2.79 (s, 2H), 5.40 (t, J = 8.5 Hz, 1H); HRMS calcd for $C_{15}H_{29}N_2$ $(M+H)^+$: 237.2332, found 237.2343.

^{125}I-Chemokine binding study. The cell-based binding assays were performed in 96-well microplates in a total volume of 400 μL. CHO cells transfected with human CCR1 receptors were detached by PBS (—) containing 2 mM EDTA and resuspended in binding buffer (Krebs-Linger Phosphate Buffer containing 0.1% BSA and 0.1% glucose). CHO cells (1×10^5 cells) were incubated with 50 pM ^{125}I-MIP-1α in the presence of increasing concentrations of the antagonist for 1 h at 37 °C in the binding buffer to reach equilibrium. Non-specific binding was determined in the presence of 100 nM of unlabeled MIP-1α. After incubation, the ice-cold binding buffer was added to the binding reaction. Then, the binding reaction was filtered by GF/C glass fiber filter (Whatman International Ltd., Maidstone, UK) presoaked with 1% polyethylenimine to reduce nonspecific binding to the glass filter. The radioactivity on the glass filter was determined with a gamma counter (COBRA 5002, Packard, Downers Grove, IL, USA).

Measurement of intracellular Ca^{2+}. U937 cells transfected with human CCR1 receptors were loaded with 1 μM Fura-2 acetoxymethyl ester (Molecular Probes Inc.: Eugene, OR, USA) for 30 min at 37 °C. After two washings, the cells were resuspended at a concentration of 1×10^6 cells/mL in Krebs–Henseleit–Hepes buffer containing 0.1% BSA. The cell suspension (500 μL) was transferred into cuvettes with constant stirring, pretreated with the various concentrations of the antagonist for 5 min, and challenged with 10 nM of MIP-1α. Changes in fluorescence were monitored at 37 °C using a spectrophotometer (CAF-110; JASCO Corp. Tokyo, Japan) at excitation wavelengths of 340 and 380 nM and an emission wavelength of 510 nM. Calculation of Ca^{2+} concentration was performed using a K_d for Ca^{2+} binding of 224 nM. An antagonist was added to the cuvette 5 min prior to the addition of a chemokine.

Metabolic stability in hepatic microsomes. Hepatic microsomes were prepare from liver samples of male rats and female dogs. Human liver microsomes were obtained from Pennsylvania Regional Tissue Bank via

KAC. The test compound (10 μM) was incubated at 37 °C for 30 min with the hepatic microsomes (1 mg protein/mL) in the presence of an NADPH-generating system. The reaction was terminated by mixing 4 volumes of EtOH to the medium. After centrifugation, the supernatant was analyzed for concentration of the test compound by HPLC (GL-Science Inertsil ODS.5 μm, 1.5×150 mm, eluent CH_3CN/H_2O (55:45) containing 10 mM $AcONH_4$, flow rate = 0.1 mL/min, oven temperature 40 °C, electrochemical detection 950 mV).

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