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ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 4359–4366
Bioorganic & Medicinal Chemistry

Synthesis, in vitro pharmacology, and pharmacokinetic profiles of 2-[1-amino-1-carboxy-2-(9*H*-xanthen-9-yl)-ethyl]-1-fluoro-cyclopropanecarboxylic acid and its 6-heptyl ester, a potent mGluR2 antagonist

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Received 10 January 2008; revised 20 February 2008; accepted 21 February 2008
Available online 26 February 2008

Abstract—In this paper, we describe the synthesis of (+)-(1*R**,2*R**)-2-[(1*S**)-1-amino-1-carboxy-2-(9*H*-xanthen-9-yl)-ethyl]-1-fluoro-cyclopropanecarboxylic acid **(+)-16a**, a compound, that is, fluorinated at the alpha position of the carboxylic acid in the cyclopropane ring of a group II mGluRs antagonist, **1** (LY341495), using a previously reported stereoselective cyclopropanation reaction. The fluorinated compound **(+)-16a** exhibited almost the same affinity (IC_{50} = 3.49 nM) for mGluR2 as **1** but had a superior pharmacokinetic profile. Furthermore, a marked elevation of the plasma levels of **(+)-16a** was observed following the administration of a prodrug, **(+)-17**.

1. Introduction

L-Glutamate is a major excitatory neurotransmitter in the mammalian central nervous system (CNS).[1,2] Glutamate receptors are classified into two categories, ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs). iGluRs have an ion channel structure, whereas mGluRs belong to the family of G-protein coupled receptors. mGluRs have been further categorized into three groups (group I–III) comprising eight subtypes (mGluR1–8) based on similarities/dissimilarities in their coupling mechanisms, molecular structures, sequences homology, and the pharmacology of the receptors.[3–9] Group I mGluRs (mGluR1 and 5) activate phospholipase C, while both group II mGluRs (mGluR2 and 3) and group III mGluRs (mGluR4, 6, 7 and 8) inhibit adenylate cyclase.[9–11] mGluRs have received significant attention as therapeutic targets for the treatment of CNS disorders and conditions.[12–19] A number of antagonists of group II mGluRs have been identified, as shown in Figure 1.[20–23] Compounds **1**–**3** show very high affinities for mGluR2 and are potent functional antagonists of human mGluRs.

We previously reported the synthesis, in vitro pharmacological profiles, and structure–activity relationships of **2**–**3**, as well as the pharmacokinetic profiles of several typical compounds.[22,23] In particular, **2** (MGS0039) exhibited a high binding affinity for group II mGluRs,

Keywords: Group II mGluRs; Antagonist; Fluorinated compound; Prodrug.
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doi:10.1016/j.bmc.2008.02.066

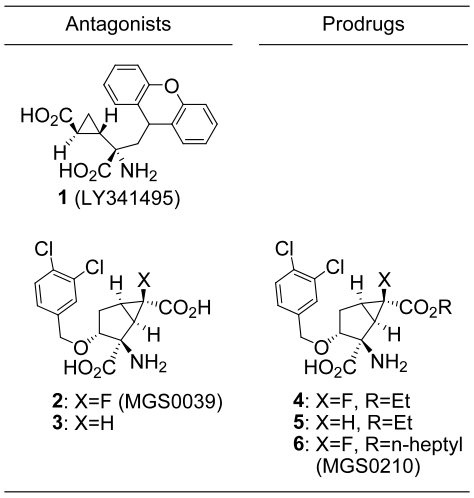


Figure 1. Group II mGluRs antagonists and their prodrugs.

but its oral bioavailability was 10.9%. However, we recently reported the pharmacokinetics of prodrugs of **2** and noted that the oral administration of ester-type prodrugs improved the bioavailability of **2**.[24] Compound **4** exhibited an approximately 10-fold enhancement in the plasma and brain concentrations of **2** as a result of the high reactivity of the ethyl ester during hydrolysis, arising from the electronegativity of the fluorine atom. In contrast, compound **5**, a non-fluorinated analogue, exhibited a poor pharmacokinetic profile. We also reported the results of both in vitro and in vivo studies indicating that heptyl ester **6** (MGS0210), a prodrug of **2**, exhibited an optimal pharmacokinetic profile. In these previous papers, we concluded that a fluorine atom at the C-6 position of the bicyclo[3.1.0] hexane ring played an important role in the binding affinity to mGluR2, strongly influencing the compounds' pharmacokinetic profiles.

With this background in mind, we focused on a compound fluorinated at the alpha position of carboxylic acid in the cyclopropane ring of the group II mGluRs antagonist **1** (LY341495). Although general methods for the mild and selective introduction of a fluorine atom into organic molecules are limited,[25] we previously reported a convenient stereoselective preparation method for 1-fluorocyclopropane-1-carboxylate.[26] Thus, we examined the synthesis of the fluorinated compound using our reported synthetic method for 1-fluorocyclopropane-1-carboxylate. In this paper, we report the synthesis, in vitro pharmacology, and pharmacokinetic profiles of the fluorinated compound and its prodrug, heptyl ester.

2. Chemistry

Scheme 1 illustrates the synthesis of fluorinated compounds **(±)-16a**, **(±)-16b**, **(+)-16a**, **(−)-16a**, and its prodrug **(+)-17**. We previously reported a stereoselective preparation for 1-fluorocyclopropane-1-carboxylate through the iodo-atom transfer radical addition of fluoroiodoacetate to alkenes, followed by an intramolecular substitution reaction. According to our procedure for the preparation of the cyclopropane ring system, the radical addition of fluoroiodoacetate to **7** in the presence of triethyl borane produced **8** as a mixture of the two diastereomers. The cyclization of **8** using lithium hexamethyldisilazane (LHMDS) as a base produced **9**, in a highly *cis*-selective manner in 98% yield.[26] The deprotection of the *tert*-butyldiphenylsilyl group of **9** using tetrabutylammonium fluoride (TBAF) gave **10** in 92% yield. We adopted a convergent method for the coupling of the cyclopropane and xanthene moiety using a palladium-catalyzed reaction of the corresponding zinc reagent with acid chloride to obtain **14**. A carboxylic acid obtained from **10** by Jones oxidation was allowed to react with $SOCl_2$ to yield acid chloride **11**. According to the reported method for the preparation of zinc reagents,[27] zinc reagent **13** was synthesized from iodide **12**[20] using zinc powder in the presence of cuprous chloride. Then, the zinc reagent **13** was reacted with **11** in the presence of $Pd(PPh_3)_4$ to give **14** in 68% yield (three steps from compound **10**). Compound **14** was allowed to react under Bucherer–Bergs conditions[28] to yield two hydantoin diastereomers. To separate the diastereomeric mixtures, we converted the two hydantoin diastereomers to *p*-methoxybenzylhydantoins **15**. Then the two diastereomers **15a** (a highly polar isomer) and **15b** were separated by silica gel column chromatography.

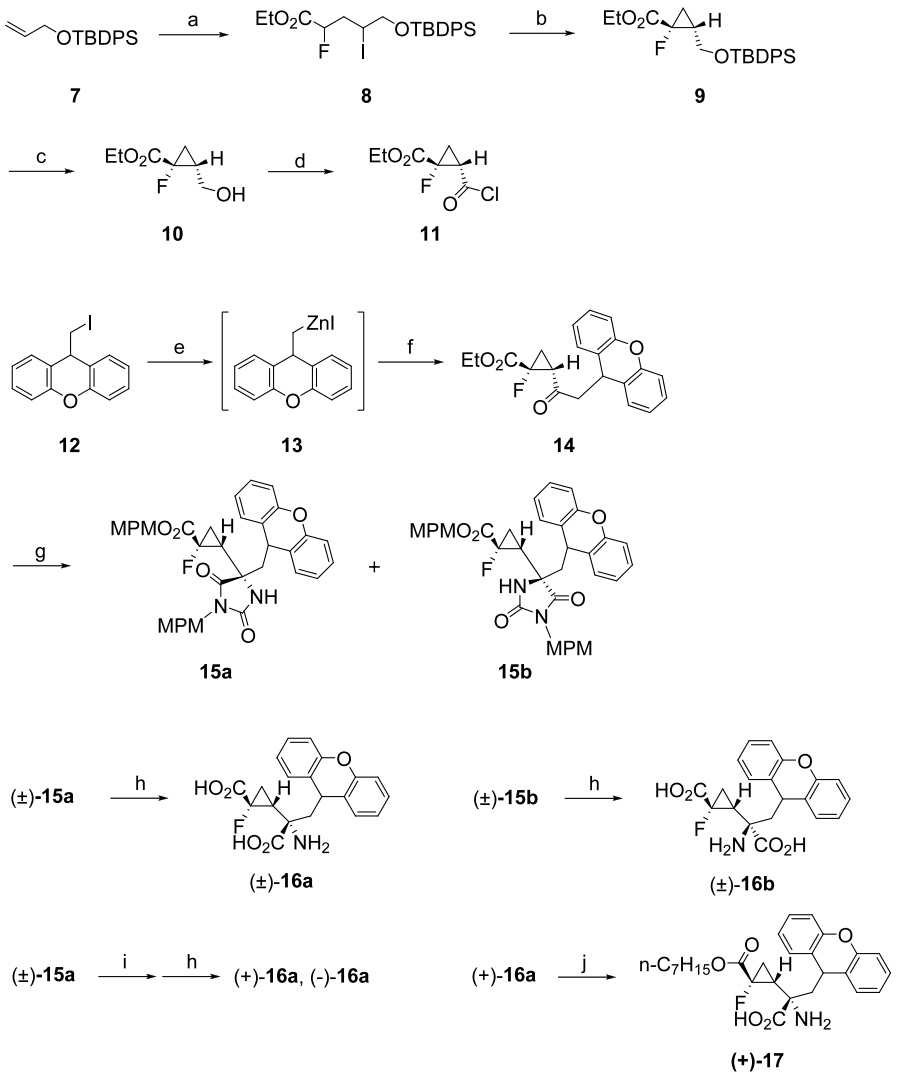
The hydrolysis of **(±)-15a** and **(±)-15b** with 1 M NaOH at 200 °C in a sealed apparatus for between 44 h and 5.5 days gave **(±)-16a** and **(±)-16b**. The harsh conditions required to complete these reactions gave a complicated mixture, resulting in a low yield. Compound **(±)-15a** was resolved into two enantiomers, **(+)-15a** and **(−)-15a**, using HPLC. Finally, the hydrolysis of **(+)-15a** and **(−)-15a** under the above-mentioned conditions yielded **(+)-16a** and **(−)-16a**, respectively. The selective esterification of **(+)-16a** at the C-1 carboxy group in the cyclopropane ring with *n*-heptanol was performed in the presence of $SOCl_2$ at 70 °C to obtain **(+)-17** in 78% yield.

The relative configurations of the two diastereomers were determined as follows. X-ray diffraction analysis of carboxylic acid **18**, which was obtained from **15b** by hydrolysis with $LiOH{\cdot}H_2O$ (Scheme 2), showed that **18** is (1*RS*,2*RS*)-1-fluoro-2-[(4*RS*)-1-(4-methoxybenzyl)-2,5-dioxo-4-(9*H*-xanthen-9-ylmethyl)-imidazolidin-4-yl]cyclopropanecarboxylic acid (Fig. 2). Both **15a** and **15b** have the same relative configuration for their cyclopropane ring system; therefore, the X-ray diffraction analysis of **18** suggested that the stereochemistry of **15a**, a diasteromer of **15b**, was 1*RS*, 2*RS*, 4*SR*.

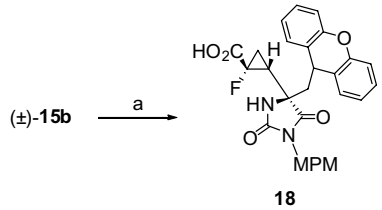
3. Results and discussion

3.1. Pharmacological profile studies

The mGluR2 binding affinities of the fluorinated compounds **(±)-16a**, **(±)-16b**, **(+)-16a** and **(−)-16a** were evaluated by examining [^{3}H]-(1*S*,2*S*,3*S*,5*R*,6*S*)-2-amino-3-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid binding



Scheme 1. Reagents and conditions: (a) $ICFHCO_2Et$, Et_3B, CH_2Cl_2, rt, 6.5 days, 98%; (b) LHMDS, THF, −66 °C; 4.5 h, 98%; (c) TBAF, THF, rt, 1 h, 92%; (d) i—Jones reagent, acetone, 4 °C, 40 min, ii—$SOCl_2$, rt, 18 h; (e) Zn, CuCl, DMF, benzene, 60 °C, 3 h; (f) **11**, $PdCl_2(PPh_3)_2$, benzene, rt, 3 h, 68% (three steps, from **10**); (g) i—1 M NaOH, EtOH, rt; 30 min, ii—KCN, $(NH_4)_2CO_3$, 60 °C, 5 days, 77%, iii—*p*-methoxybenzyl chloride, K_2CO_3, DMF, 125 °C, 12 h, **15a** (29%), **15b** (47%); (h) 1 M NaOH, 200 °C (in a stainless sealed apparatus), **(±)-16a** (44 h, 18%), **(±)-16b** (5.5 days, 20%), **(+)-16a** (3 days, 51%), **(−)-16a** (3 days, 14%); (i) chiral HPLC; (j) *n*-heptanol, $SOCl_2$, 70 °C, 4 h, 78%.



Scheme 2. Reagents and conditions: (a) $LiOH{\cdot}H_2O$, THF, H_2O, rt, 30 min, 89%.

in CHO cells that stably expressed mGluR2. The IC_{50} values obtained for mGluR2 are shown in Table 1. Compound **(±)-16a** exhibited an approximately 47-fold higher binding affinity than its diastereomer **(±)-16b**. Furthermore, enantiomer **(+)-16a** also exhibited a high binding affinity for mGluR2 (IC_{50} = 3.49 nM). The enantiomer **(+)-16a** showed almost the same affinity to mGluR2 as **1**, and the IC_{50} was 2.90 nM. We successfully synthesized **(+)-16a**, a compound, that is, fluorinated at the alpha position of the carboxylic acid in the cyclopropane ring of the group II mGluRs antagonist **1**, and found that **(+)-16a** exhibited a high affinity for mGluR2.

3.2. Pharmacokinetic evaluation

Table 2 shows the pharmacokinetic parameters of **(+)-16a** and its prodrug **(+)-17** following the oral administration to male Crl:CD(SD) rats at a dose of 10 mg/kg. Compound **(+)-16a** exhibited higher plasma concentrations ($AUC_{0-\infty}$: 3310 ng h/mL) than **1** ($AUC_{0-\infty}$; 284 ng h/mL).[21] Furthermore, the plasma levels of the parent compound **(+)-16a** following the oral administration of the prodrug **(+)-17** to rats were much higher ($AUC_{0-\infty}$; 78,000 ng h/mL) than those observed following the oral administration of **(+)-16a**. The introduction of a fluorine atom to **1** (LY341495) allowed the high

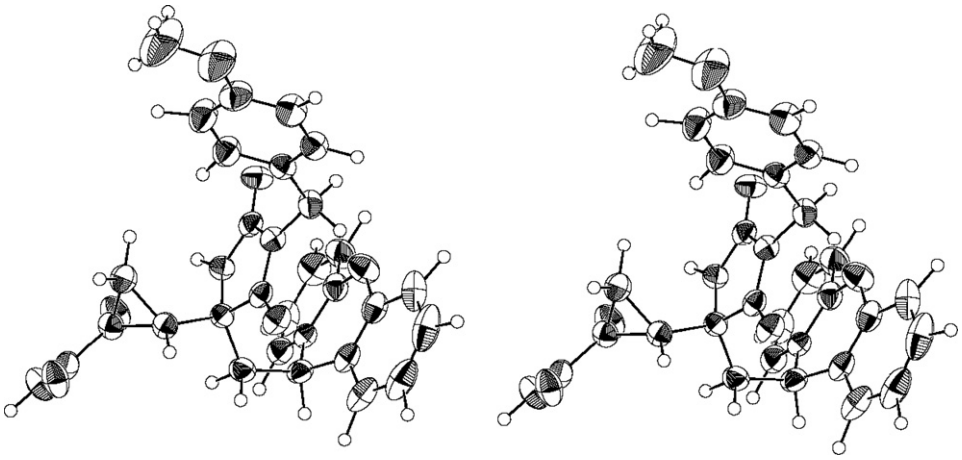


Figure 2. Stereoview of **18** from the X-ray crystallograph.

Table 1. Binding affinity for mGluR2

Compound	Affinity[a] IC_{50} (nM)
(±)-16a	4.45
(±)-16 b	211
(+)-16a	3.49
(−)-16a	>10,000
LY341495[21]	2.90

[a] Affinity for mGluR2 was determined by the binding study utilizing [^{3}H]-(1*S*,2*S*,3*S*,5*R*,6*S*)-2-amino-3-fluorobicyclo[3.1.0]hexane-2,6-dicarboxylic acid. IC_{50} values were calculated from the concentration–response curves obtained in duplicate.

Table 2. Pharmacokinetic parameters for **(+)-16a** and **(+)-17** following the oral administration to rats at the dose of 10 mg/kg

Compound	**(+)-16a**	**(+)-17**[a]	LY341495[21]
T_{max} (h)	2.67	2.00	0.50
C_{max} (ng/mL)	308	8640	64.1
$t_{1/2}$ (h)	5.05	3.87	2.64
$AUC_{0-\infty}$ (ng h/mL)	3310	78,000	284

[a] Pharmacokinetic parameters of the parent compound **(+)-16a** after oral administration **(+)-17** to rats.

affinity for mGluR2 to be retained and improved the oral bioavailability. Furthermore, the plasma levels of **(+)-16a** were significantly elevated following the administration of the prodrug.

We next investigated the in vitro metabolic stabilities of prodrug **(+)-17** using rat, monkey, and human liver S9 fractions. Table 3 shows the transformation ratio of **(+)-17** to **(+)-16a**. Prodrug **(+)-17** yielded the carboxylic acid form **(+)-16a** when it was incubated with rat, monkey, or human liver S9 fractions. However, the conversion ratios of **(+)-17** in the liver S9 fractions were relatively low, with the exception of that in the rat liver S9 fractions. It would, therefore, be necessary to examine some prodrugs of **(+)-16a** for further progression.

Table 3. Transformation ratio (%) from prodrug **(+)-17** to carboxylic acid **(+)-16a** by liver S9 fractions

Rat S9	Monkey S9	Human S9
68.1	7.30	12.1

4. Conclusions

In summary, we successfully synthesized **(+)-16a**, which contains a fluorine atom at the C-1 position of **1**, using a previously reported stereoselective cyclopropanation reaction.[26] Compound (+)-**16a** exhibited almost the same affinity for mGluR2 as **1**.

The introduction of the fluorine atom at the C-1 position of the cyclopropane ring in **1** improved the oral bioavailability. Furthermore, the plasma levels of **(+)-16a** were elevated following the administration of prodrug **(+)-17**. However, further structural conversions of prodrug **(+)-17** are required to obtain a favorable pharmacokinetic profile in humans because of the low transformation ratio of **(+)-17** to the parent compound **(+)-16a**, as observed in the in vitro study using liver S9 fractions.

5. Experimental

Melting points were determined on a Yanaco MP-500D melting point apparatus, and are uncorrected. ^{1}H and ^{19}F NMR spectra were obtained using a Varian Gemini 2000, Varian Unity Inova 300, JEOL Alpha500, Lambda500, or ECA500. Chemical shifts are reported in parts per million relative to tetramethylsilane (TMS), sodium 3-trimethylsilylpropionate-2,2,3,3-d_4 (TMSP) or trichlorofluoromethane as the internal standard. Mass spectra (MS) were obtained on a Micromass Platform LC (ESI). High-resolution spectra were recorded on a Micromass GCT instrument or Micromass Q-TOF2 instrument. Optical rotations were determined with a JASCO DIP-360 polarimeter and are reported at the sodium D-line (589 nm). Elemental analyses were performed on a Perkin-Elmer 2400. Silica gel [C-200, 100–200 mesh (Wako Pure Chemical)] was used for the column chromatography using the solvent systems (volume ratios) indicated below.

5.1. 5-(*tert*-Butyldiphenylsilanyloxy)-2-fluoro-4-iodopentanoic acid ethyl ester (8)

Triethylborane (1.01 M in hexanes, 88.8 mL, 89.7 mmol) was added to a mixture of alloxy-*tert*-butyldiphenylsilane **7** (26.6 g, 89.7 mmol) and ethyl fluoroiodoacetate (52.0 g, 224 mmol) in CH_2Cl_2 (380 mL), and the reaction mixture was stirred for 6 h. To the reaction mixture was added triethylborane (1.01 M hexane soln, 44.4 mL, 44.8 mmol), and stirred for 3 h. To the reaction mixture was added triethylborane (1.01 M hexane soln, 44.4 mL, 44.8 mmol), and stirred for 3 days. To the reaction mixture was added triethylborane (1.01 M hexane soln, 44.4 mL, 44.8 mmol), and stirred for 3 days. The reaction mixture was washed with H_2O and brine, dried over $MgSO_4$, concentrated under reduced pressure, and chromatographed on silica gel (hexane/EtOAc = 15:1) to yield **8** (46.3 g, 98% as a diastereomeric mixture) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.06–1.10 (9H, m), 1.32 (3H, t, J = 7.2 Hz), 2.14–2.79 (2H, m), 3.80–3.94 (2H, m), 4.19–4.38 (3H, m), 4.93–5.24 (1H, m), 7.33–7.51 (6H, m), 7.56–7.74 (4H, m); HRMS (ESI) *m*/*z* 551.0895 $(M+Na)^+$, calcd for $C_{23}H_{30}FINaO_3Si$: 551.0891.

5.2. (1*RS*,2*RS*)-2-(*tert*-Butyldiphenylsilanyloxymethyl)-1-fluorocyclopropancarboxylic acid ethyl ester (9)

LHMDS (29% in THF, 171 mL, 297 mmol) was added to a solution of **8** (52.3 g, 99.0 mmol) in THF (600 mL) at −66 to −61 °C, and the mixture was stirred at −66 °C for 4.5 h. 1 M HCl was added to the reaction mixture, and extracted with Et_2O. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, concentrated under reduced pressure, and chromatographed on silica gel (hexane/EtOAc = 20:1) to yield **9** (38.7 g, 98%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.05 (9H, s), 1.07–1.19 (1H, m), 1.31 (3H, t, J = 7.1 Hz), 1.47 (1H, ddd, J = 10.5, 8.7, 6.5 Hz), 1.86–1.98 (1H, m), 3.75 (1H, ddd, J = 11.2, 7.9, 1.2 Hz), 3.90 (1H, ddd, J = 11.2, 5.8, 1.3 Hz), 4.27 (2H, q, J = 7.1 Hz), 7.33–7.47 (6H, m), 7.63–7.71 (4H, m); HRMS (ESI) *m*/*z* 401.1967 $(M+H)^+$, calcd for $C_{23}H_{30}FO_3Si$: 401.1948.

5.3. (1*RS*,2*RS*)-1-Fluoro-2-hydroxymethyl-cyclopropanecarboxylic acid ethyl ester (10)

TBAF (1 M in THF, 472 mL, 472 mmol) was added to a mixture of **9** (126 g, 315 mmol) and THF (780 mL), and the mixture was stirred at room temperature for 1 h. The mixture was concentrated under reduced pressure, and the residue was dissolved with EtOAc. The organic layer was wash with saturated aqueous NH_4Cl. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, concentrated under reduced pressure, and chromatographed on silica gel (hexane/EtOAc = 2:1–1:2) to yield **10** (46.7 g, 92%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.19–1.29 (1H, m), 1.32 (3H, t, J = 7.2 Hz), 1.51–1.64 (2H, m), 1.90–2.07 (1H, m), 3.67 (1H, dd, J = 11.1, 8.9 Hz), 3.93–4.07 (1H, m), 4.27 (2H, q, J = 7.15 Hz); MS (ESI) *m*/*z* 185 $(M+H)^+$.

5.4. (1*RS*,2*RS*)-1-Fluoro-2-(2-9*H*-xanthen-9-yl-acetyl)-cyclopropanecarboxylic acid ethyl ester (14)

8 M Jones reagent (130 mL) was added to a mixture of **10** (21.2 g, 131 mmol) and acetone (260 mL) with ice-cooling, and the mixture was stirred for 40 min. Saturated aqueous $NaHSO_3$ was added to the reaction mixture and extracted with EtOAc three times. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. $SOCl_2$ (180 mL) was added to the residue and the mixture was stirred at room temperature for 18 h. The mixture was concentrated under reduced pressure to give crude **11**.

A mixture of 9-iodomethyl-9*H*-xanthene[20] (66.4 g, 206 mmol), zinc powder (67.4 g, 1.03 mmol), CuCl (5.10 g, 51.5 mmol), DMF (70 mL), and benzene (640 mL) was stirred at 60 °C for 3 h under nitrogen atmosphere. After cooling, a mixture of crude **11**, $Pd(PPh_3)_4$ (14.5 g, 20.6 mmol), and benzene (160 mL) was added to the reaction mixture and stirred at room temperature for 3 h. The reaction mixture was diluted with EtOAc, and filtered through Celite® pad. The filtrate was washed with 10% aqueous $NaHSO_4$, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$, concentrated under reduced pressure, and chromatographed on silica gel (hexane/EtOAc = 10:1–8:1) to yield **14** [31.7 g, 68% (three steps, from **10**)] as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.29 (3H, t, J = 7.2 Hz), 1.59 (1H, dt, J = 9.5, 6.3 Hz), 2.16 (1H, ddd, J = 18.4, 8.1, 6.3 Hz), 2.49 (1H, ddd, J = 9.5, 8.1, 2.8 Hz), 2.96 (1H, dd, J = 17.1, 6.1 Hz), 3.04 (1H, dd,J = 17.1, 7.0 Hz), 4.23 (2H, q, J = 7.2 Hz), 4.67 (1H, dd, J = 7.0, 6.1 Hz), 7.02–7.32 (8H, m); HRMS (ESI) *m*/*z* 377.1184 $(M+Na)^+$, calcd for $C_{21}H_{19}FNaO_4$: 377.1165.

5.5. (1*RS*,2*RS*)-1-Fluoro-2-[(4*SR*)-1-(4-methoxybenzyl)-2,5-dioxo-4-(9*H*-xanthen-9-ylmethyl)-imidazolidin-4-yl]-cyclopropanecarboxylic acid 4-methoxybenzyl ester (15a) and (1*RS*,2*RS*)-1-fluoro-2-[(4*RS*)-1-(4-methoxybenzyl)-2,5-dioxo-4-(9*H*-xanthen-9-ylmethyl)-imidazolidin-4-yl]-cyclopropanecarboxylic acid 4-methoxybenzyl ester (15b)

A mixture of **14** (31.6 g, 89.2 mol), 1 M NaOH (98.1 mL, 98.1 mmol), and EtOH (200 mL) was stirred at room temperature for 30 min. Ammonium carbonate (77.1 g, 803 mmol), KCN (29.0 g, 446 mmol), and H_2O (102 mL) were added to the reaction mixture, and the mixture was stirred at 60 °C for 5 days. The reaction mixture was washed with Et_2O, and adjusted with 6 M HCl to pH 1. After stirring the mixture with ice-cooling for 1 h, the precipitation was filtered and washed with H_2O and IPE to yield a mixture of two hydantoin diastereomers (27.3 g, 77%). A mixture of the precipitation (2.37 g, 5.98 mmol), $KHCO_3$ (1.67 g, 16.7 mmol), 4-methoxybenzyl chloride (1.87 mL, 13.8 mol), DMF (18 mL) was stirred at 125 °C for 12 h. The reaction mixture was diluted with Et_2O, and washed with H_2O. The aqueous layer was extracted with Et_2O twice. The combined organic layer was dried over $MgSO_4$, concentrated under reduced pressure, and chromatographed on silica

gel (hexane/AcOEt = 3:1–1:1) to yield **15a** (1.12 g, 29%) and **15 b** (1.79 g, 47%) as a colorless solid. **(±)-15a**; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.34–1.62 (2H, m), 1.74 (1H, ddd, J = 10.5, 8.9, 4.4 Hz), 2.33 (1H, dd, J = 14.9, 7.2 Hz), 2.44 (1H, dd, J = 14.9, 4.7 Hz), 3.71 (3H, s), 3.81 (3H, s), 4.25 (1H, dd, J = 7.2, 4.7 Hz), 4.32 (1H, d, J = 14.5 Hz), 4.40 (1H, d, J = 14.5 Hz), 4.49 (1H, s), 5.06 (1H, d, J = 12.4 Hz), 5.11 (1H, d, J = 12.4 Hz), 6.70–6.92 (4H, m), 7.03–7.29 (12H, m); HRMS (ESI) m/z: 637.2343 $(M+H)^+$, calcd for $C_{37}H_{34}FN_2O_7$: 637.2350. **(±)-15b**; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.17–1.30 (2H, m), 1.93–2.06 (1H, m), 2.24 (1H, dd, J = 14.8, 5.4 Hz), 2.67 (1H, dd, J = 14.8, 6.0 Hz), 3.74 (3H, s), 3.81 (3H, s), 4.10 (1H, dd, J = 6.0, 5.4 Hz), 4.19 (1H, d, J = 14.5 Hz), 4.38 (1H, d, J = 14.5 Hz), 4.54 (1H, s), 5.07 (1H, d, J = 11.8 Hz), 5.12 (1H, d, J = 11.8 Hz), 6.76–6.90 (4H, m), 6.97–7.15 (6H, m), 7.17–7.29 (6H, m); HRMS (ESI) m/z 637.2338 $(M+H)^+$, calcd for $C_{37}H_{34}FN_2O_7$: 637.2350.

5.6. (1*RS*,2*RS*)-2-[(1*SR*)-1-amino-1-carboxy-2-(9*H*-xanthen-9-yl)ethyl]-1-fluorocyclopropancarboxylic acid ((±)-16a)

A mixture of **(±)-15a** (278 mg, 0.437 mol) and 1 M NaOH (8.4 mL) was stirred at 200 °C for 44 h in a stainless sealed apparatus. After cooling, the mixture was adjusted to pH 1 with 1 M HCl. The mixture was chromatographed on AG50W-X8 cation-exchange resin (Bio-Rad) (H_2O ~ 50% aqueous THF ~ 10% aqueous pyridine) yield to crude product (61 mg). THF (1 mL) was added to the crude product and stirred at room temperature for 6 h. The solid was collected and washed with THF to give **(±)-16a** (29 mg, 18%) as a white solid: mp > 270 °C (decomposed). ^{1}H NMR (300 MHz, D_2O) δ: 1.34–1.55 (2H, m), 1.84–2.00 (1H, m), 2.10 (1H, dd, J = 15.0, 7.7 Hz), 2.29 (1H, dd, J = 15.0, 5.8 Hz), 4.23 (1H, dd, J = 7.7, 5.8 Hz), 7.15–7.50 (8H, m); ^{19}F NMR (470 MHz, D_2O) δ: −197.1; MS (ESI) m/z 370 $(M-H)^-$. Anal. Calcd for $C_{20}H_{18}FNO_5 \cdot 0.3H_2O$: C, 63.76; H, 4.98; N, 3.72; F, 5.04. Found: C, 63.88; H, 4.89; N, 3.77; F, 5.02.

5.7. (1*RS*,2*RS*)-2-[(1*RS*)-1-amino-1-carboxy-2-(9*H*-xanthen-9-yl)ethyl]-1-fluorocyclopropancarboxylic acid ((±)-16b)

In a manner similar to the preparation of **(±)-16a** from **(±)-15a**, **(±)-16b** (40 mg, 20%, as a white solid) was obtained from **(±)-15b** (343 mg, 0.539 mmol). Mp > 295 °C (decomposed); ^{1}H NMR (300 MHz, D_2O) δ: 1.39–1.53 (1H, m), 1.69–1.88 (2H, m), 2.23 (1H, dd, J = 15.1, 8.0 Hz), 2.48 (1H, dd, J = 15.1, 5.8 Hz), 4.26 (1H, dd, J = 8.0, 5.8 Hz), 7.18–7.47 (8H, m); ^{19}F NMR (470 MHz, D_2O) δ: −197.4; MS (ESI) m/z 370 $(M-H)^-$; Anal. Calcd for $C_{20}H_{18}FNO_5 \cdot H_2O$: C, 61.69; H, 5.18; N, 3.60; F, 4.88. Found: C, 61.41; H, 4.98; N, 3.54; F, 4.78.

5.8. (+)-(1*R**,2*R**)-1-Fluoro-2-[(4*S**)-1-(4-methoxybenzyl)-2,5-dioxo-4-(9*H*-xanthen-9-ylmethyl)-imidazolidin-4-yl]-cyclopropanecarboxylic acid 4-methoxybenzyl ester ((+)-15a) and (−)-(1*S**,2*S**)-1-fluoro-2-[(4*R**)-1-(4-methoxybenzyl)-2,5-dioxo-4-(9*H*-xanthen-9-ylmethyl)-imidazolidin-4-yl]-cyclopropanecarboxylic acid 4-methoxybenzyl ester ((−)-15a)

Compound **(±)-15a** (720 mg, 1.13 mmol) was resolved using CHIRALPAK AD (DAICEL CHEMICAL IND., LTD, 2.0 × 25 cm, eluent; hexane/2-propanol = 1:2, flow rate; 4.0 mL/min., temperature; room temperature, detect; UV 220 nm) to **(+)-15a** (294 mg) and **(−)-15a** (299 mg).

(+)-15a; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.34–1.62 (2H, m), 1.74 (1H, ddd, J = 10.5, 8.9, 4.4 Hz), 2.33 (1H, dd, J = 14.9, 7.2 Hz), 2.44 (1H, dd, J = 14.9, 4.7 Hz), 3.71 (3H, s), 3.81 (3H, s), 4.25 (1H, dd, J = 7.2, 4.7 Hz), 4.32 (1H, d, J = 14.5 Hz), 4.40 (1H, d, J = 14.5 Hz), 4.49 (1H, s), 5.06 (1H, d, J = 12.4 Hz), 5.11 (1H, d, J = 12.4 Hz), 6.70–6.92 (4H, m), 7.03–7.29 (12H, m); MS (ESI) m/z 635 $(M-H)^-$; $[\alpha]_D^{28}$ +66.0° (c = 0.577, $CHCl_3$); t_R = 17.5 min (CHIRALPAK AD, 0.46 × 25 cm, eluent; hexane/2-propanol = 1:2, flow rate; 1.0 mL/min, temperature; room temperature, detect; UV 220 nm).

(−)-15a; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.34–1.62 (2H, m), 1.74 (1H, ddd, J = 10.5, 8.9, 4.4 Hz), 2.33 (1H, dd, J = 14.9, 7.2 Hz), 2.44 (1H, dd, J = 14.9, 4.7 Hz), 3.71 (3H, s), 3.81 (3H, s), 4.25 (1H, dd, J = 7.2, 4.7 Hz), 4.32 (1H, d, J = 14.5 Hz), 4.40 (1H, d, J = 14.5 Hz), 4.49 (1H, s), 5.06 (1H, d, J = 12.4 Hz), 5.11 (1H, d, J = 12.4 Hz), 6.70–6.92 (4H, m), 7.03–7.29 (12H, m); MS (ESI) m/z 635 $(M-H)^-$; $[\alpha]_D^{27}$ −65.8° (c = 0.975, $CHCl_3$); t_R = 12.3 min (CHIRALPAK AD, 0.46 × 25 cm, eluent; hexane: 2-propanol = 1: 2, flow rate; 1.0 mL/min., temperature; room temperature, detect; UV 220 nm).

5.9. (+)-(1*R**,2*R**)-2-[(1*S**)-1-Amino-1-carboxy-2-(9*H*-xanthen-9-yl)ethyl]-1-fluorocyclopropancarboxylic acid ((+)-16a)

In a manner similar to the preparation of **(±)-16a** from **(±)-15a**, **(+)-16a** (679 mg, 51%, as a white solid) was obtained from **(+)-15a** (1.21 mg, 1.90 mmol). Mp > 270 °C (decomposed); ^{1}H NMR (300 MHz, D_2O) δ: 1.34–1.55 (2H, m), 1.84–2.00 (1H, m), 2.10 (1H, dd, J = 15.0, 7.7 Hz), 2.29 (1H, dd, J = 15.0, 5.8 Hz), 4.23 (1H, dd, J = 7.7, 5.8 Hz), 7.15–7.50 (8H, m); ^{19}F NMR (470 MHz, D_2O) δ: −197.1. MS (ESI) m/z 370 $(M-H)^-$; Anal. Calcd for $C_{20}H_{18}FNO_5 \cdot 1.7\ H_2O$: C, 59.76; H, 5.37; N, 3.48; F, 4.73. Found: C, 59.87; H, 5.36; N, 3.54; F, 4.77; $[\alpha]_D^{26}$ +27.1° (c = 0.335, 1 M NaOH).

5.10. (−)-(1*S**,2*S**)-2-[(1*R**)-1-Amino-1-carboxy-2-(9*H*-xanthen-9-yl)ethyl]-1-fluorocyclopropancarboxylic acid ((−)-16a)

In a manner similar to the preparation of **(±)-16a** from **(±)-15a**, **(−)-16a** (22 mg, 14%, as a white solid) was obtained from **(−)-15a** (266 mg, 0.418 mmol). Mp > 270 °C (decomposed); ^{1}H NMR (300 MHz, D_2O) δ: 1.34–1.55 (2H, m), 1.84–2.00 (1H, m), 2.10 (1H, dd, J = 15.0, 7.7 Hz), 2.29 (1H, dd, J = 15.0, 5.8 Hz), 4.23 (1H, dd, J = 7.7, 5.8 Hz), 7.15–7.50 (8H, m); ^{19}F NMR (470 MHz, D_2O) δ: −197.1. MS (ESI) m/z 370 $(M-H)^-$;

Anal. Calcd for $C_{20}H_{18}FNO_5 \cdot 0.1\ H_2O$: C, 64.37; H, 4.92; N, 3.75; F, 5.09. Found: C, 64.26; H, 4.92; N, 3.58; F, 5.06; $[\alpha]_D^{24}$ −25.8° (c = 0.329, 1 M NaOH).

5.11. (+)-(1*R**,2*R**)-2-[(1*S**)-1-Amino-1-carboxy-2-(9*H*-xanthen-9-yl)ethyl]-1-fluorocyclopropanecarboxylic acid heptyl ester ((+)-17)

A mixture of **(+)-16a** (281 mg, 0.757 mmol), $SOCl_2$ (220 μL, 3.03 mmol), and 1-heptanol (2.8 mL) was stirred at 70 °C for 4 h. $SOCl_2$ was removed under reduced pressure at 80 °C. EtOH (2.2 mL) and propylene oxide (2.2 mL) was added to the residue, and the mixture was refluxed for 1 h. After removing the solvent, Et_2O was added and stirred at room temperature for 12 h. The precipitation was collected and washed with Et_2O and IPE to yield **(+)-17** (277 mg, 78%) as a white solid. ^{1}H NMR (300 MHz, CD_3OD) δ: 0.90 (3H, t, J = 6.7 Hz), 1.26–1.53 (10H, m), 1.57–1.69 (2H, m), 2.05 (1H, dd, J = 14.6, 6.5 Hz), 2.10–2.17 (1H, m), 2.22 (1H, dd, J = 14.6, 6.5 Hz), 4.07–4.21 (2H, m), 4.30 (1H, t, J = 6.45 Hz), 7.06–7.19 (4H, m), 7.21–7.33 (2H, m), 7.44–7.53 (2H, m); ^{19}F NMR (470 MHz, CD_3OD) δ: −206.4; HRMS (ESI) m/z 468.2201 $(M-H)^-$, calcd for $C_{27}H_{31}FNO_5$: 468.2186; $[\alpha]_D^{27}$ +10.6° (c = 0.537, pyridine).

5.12. (1*RS*,2*RS*)-1-Fluoro-2-[(4*RS*)-1-(4-methoxybenzyl)-2,5-dioxo-4-(9*H*-xanthen-9-ylmethyl)-imidazolidin-4-yl]-cyclopropanecarboxylic acid (18)

A mixture of **(±)-15b** (940 mg, 1.48 mol), $LiOH \cdot H_2O$ (93 mg, 2.21 mmol), THF (11 mL), and H_2O (5.5 mL) was stirred at room temperature for 30 min. The reaction mixture was washed with EtOAc, and adjusted with 1 M HCl to pH 2. The aqueous layer was extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure to yield **18** (682 mg, 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.27–1.40 (m, 2H), 1.96–2.09 (m, 1H), 2.27 (dd, J = 14.8, 5.4 Hz, 1H), 2.71 (dd, J = 14.7, 6.0 Hz, 1H), 3.75 (s, 3H), 4.12 (t, J = 5.8 Hz, 1H), 4.20 (d, J = 14.6 Hz, 1H), 4.38 (d, J = 14.6 Hz, 1H), 5.39 (s, 1H), 6.76–6.84 (m, 2H), 6.97–7.30 (m, 10H); HRMS (ESI) m/z: 517.1786 $(M+H)^+$, calcd for $C_{29}H_{26}FN_2O_6$: 517.1775.

5.13. [^{3}H]-(1*S*,2*S*,3*S*,5*R*,6*S*)-2-Amino-3-fluorobicyclo-[3.1.0]hexane-2,6-dicarboxylic acid ([^{3}H]MGS0008) binding

The binding of [^{3}H]MGS0008 (34 Ci/ mmol) was performed according to the method previously described.[12] CHO cells stably expressing mGluR2 were collected by centrifugation at 1000 rpm for 5 min. The cells were homogenized with 50 mM Tris–HCl buffer (pH 7.4) and centrifuged at 48,000g for 20 min at 4 °C. The pellet was suspended in 50 mM Tris–HCl buffer (pH 7.4), and incubated at 37 °C for 15 min, after which the pellet was washed twice with 50 mM Tris–HCl buffer (pH 7.4) by resuspension and recentrifugation. The pellet obtained was then suspended in 50 mM Tris–HCl buffer (pH 7.4) containing 2 mM $MgCl_2$ and served as a crude membrane preparation.

For a typical binding experiment, the reaction was initiated by incubating 0.5 mL of the crude membrane preparation with [^{3}H]MGS0008. The reaction mixture was incubated for 1 h at 25 °C. The reaction was terminated by rapid filtration through Whatman GF/C glass fiber filters presoaked with 0.3% polyethyleneimine, after which the filters were washed three times with 3 mL of ice-cold 50 mM Tris–HCl buffer (pH 7.4), using a multicell harvester M-48 (Perkin-Elmer Life and Analytical Science Inc., Wellesly, MA, USA). Aquazol-2 scintillator (Perkin-Elmer Life and Analytical Science Inc., Wellesly, MA, USA) (10 mL) was added, and filter-bound radioactivity was counted in a liquid scintillation spectrometer (LS6000TA, Perkin-Elmer Life and Analytical Science Inc., Wellesly, MA, USA). Nonspecific binding was determined in the presence of 10 μM LY354740.

In the competition binding assay, the reaction was carried out using 3.0 nM [^{3}H]MGS0008. The concentration of the test compound that caused 50% inhibition of specific binding of [^{3}H]MGS0008 (IC_{50} value) was determined from each concentration–response curve.

5.14. Pharmacokinetic evaluation

Each compound was administered to male Crl:CD(SD) rats (Charles River, Japan) orally at a dose of 10 mg/kg. Blood samples were taken from the tail vein at 0.5, 1, 2, 4, 6, 8, and 24 h post-dose into EDTA-containing tubes. Immediately plasma was separated by centrifugation (11,200g, 4 °C, 2 min), and then plasma was mixed with 5 M HCl (plasma/5 M HCl = 50:1). The plasma samples were frozen with liquid N_2 immediately and stored at −80 °C until bioanalysis.

To 50-μL aliquot of plasma sample, 400 μL of acetonitrile/methanol (9:1) solution containing internal standard was mixed, and centrifuged at 11,200g, 4 °C, 15 min. The resulting supernatant was analyzed by liquid chromatography tandem mass spectrometry (LC–MS/MS) on an Agilent ZORBAX SB-C18 column (5 μm, 50 × 2.1 mm). The analytes were eluted with mobile phase in linear gradient by increasing acetonitrile concentration in 0.1% acetic acid from 5% to 95% over 4 min at flow rate of 250 μL/min. Tandem mass spectrometric detection was carried out using TurboIonSpray (AB/MDS Sciex API3000) in positive ion mode.

5.15. Metabolic stability study

Liver S9 fractions (1 mg protein/mL) from rat (BD Bioscience), monkey (Daiichi), and human (Xenotech) were incubated with 3 μM prodrug in the presence of an NADPH generating system (0.16 mM $NADP^+$, 2.4 mM $MgCl_2$, and 1.5 mM glucose-6-phosphate) in 0.25 M phosphate buffer containing 69 mM KCl (pH 7.4) for 60 min at 37 °C. All experiments were performed in triplicate. After incubation, a twofold volume of DMSO was added to incubation medium, and the tube was vortexed and centrifuged at 2150g, 4 °C, 10 min. The resulting supernatant was subjected to LC–MS/MS analysis.

Acknowledgments

The authors thank Professor Sigetada Nakanishi of the Department of Biological Sciences, Faculty of Medicine, Graduate School of Biostudies, Kyoto University, for providing us with the CHO cell line that stably expressed rat mGluR2.

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