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DESIGN, SYNTHESIS, AND BIOLOGICAL EFFECT OF (1-OXO-1,2,3,4-TETRAHYDRO-β-CARBOLIN-9-YL)-ACETIC ACIDS AS INHIBITOR OF ALDOSE REDUCTASE 2

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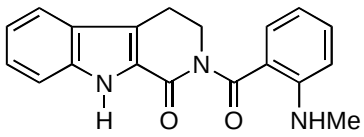
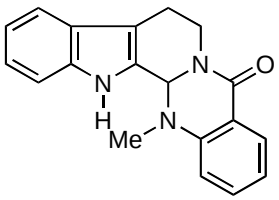
Abstract – Design and synthesis of (1-oxo-1,2,3,4-tetrahydro-β-carbolin-9-yl)acetic acids (**4a-e**) have been described. The acids (**4a-e**) synthesized here showed good to moderate inhibitory effects (IC_{50} = 21.7 ~ 45.3 μM) for the aldose reductase 2 (ALR2).

INTRODUCTION

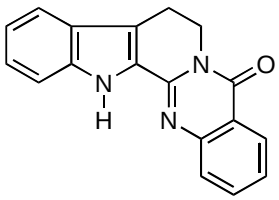
Diabetes mellitus and its disabling complications affect 150 million people worldwide. The glucose enters the polyol pathway and is reduced to sorbitol by ALR2. Under hyperglycemic conditions, the increased flux of glucose is metabolized in this pathway, and furthermore, conversion of sorbitol to fructose via aldehyde dehydrogenase (SDH) is pretty slow to result in the accumulation of sorbitol. Such metabolic disturbances eventually result in the progression of long-term diabetic complications. Inhibition of the key enzyme of ALR2 has been recognized as one possibility for preventing these complications, and also has received much attention as a target for therapeutic strategy in recent years.[1] Consequently, several synthetic works on the approach to this strategy have been reported to date.[2] Recently we found hot water extracts of *Evodia rutaecarpa* showed inhibitory activity against ALR2, and reported that rhetsinine, a minor alkaloidal component of *E. rutaecarpa*, significantly suppressed sorbitol accumulation in human erythrocytes by 79.3 % at 100 μM. On the other hand, major alkaloidal



rhetsinine



evodiamine



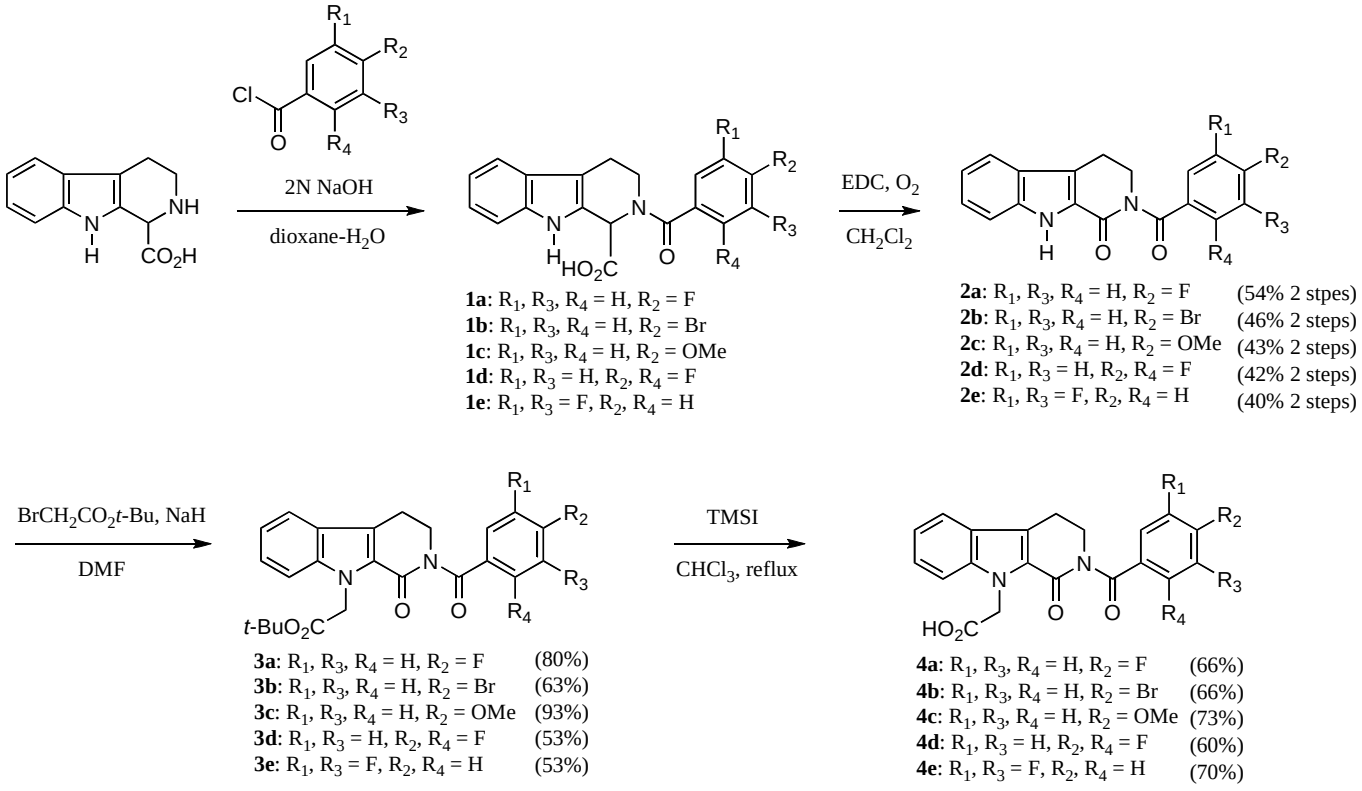
rutaecarpine

components of *E. rutaecarpa*, evodiamine, rutaecarpine, and the bitter principle limonin exhibited less than 50 % inhibition, even at concentrations of 5 mg/mL.[3]

Here we would like to report the design, synthesis and evaluations to ALR2 inhibitory effects of novel 1-oxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acids.

RESULTS AND DISCUSSION

The key 1-oxo-1,2,3,4-tetrahydro-β-carboline derivatives (**2a-e**) were prepared by utilizing the Kawase's method.[4] Thus, the oxidation of carboxylic acids (**1a-e**) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) in stead of DCC reported in the original Kawase's procedure provided the oxidized products (**2a-e**). Alkylation of **2a-e** with *t*-butyl bromoacetate gave the corresponding *t*-butyl esters (**3a-e**), which was converted to carboxylic acids (**4a-e**) by treatment with TMSI in refluxing $CHCl_3$. (**Scheme 1**)



Scheme 1

The mono-fluoro derivative **4a** was a good inhibitor of ALR2 (IC_{50} = 21.7 μM). Replacement of F-group in **4a** by Br- (**4b)** or OCH_3- (**4c**) group resulted in lower inhibition on ALR2 with IC_{50} values of 34.6 and 35.8 μM, respectively. Moreover, introduction of 2,4- or 3.5-difluorobenzoyl unit (**4d** and **4e)** also reduced their inhibition activities (IC_{50} = 45.3 and 34.5 μM, respectively).

In summary, we synthesized the five new carboxylic acids (**4a-e**), and found the mono-fluoro derivative (**4a**) was a good inhibitor of ALR2. Further structure-activity relationship (SAR) studies are under investigations, and these results will be published in due course.

EXPERIMENTAL

General Melting points are uncorrected. NMR spectra were recorded at 500 or 300 MHz (^{1}H), 75 MHz (^{13}C). Chemical shifts are given in parts per million (ppm, δ) downfield from TMS and referenced to $CHCl_3$ (7.26 ppm) or DMSO (2.50 ppm) as internal standard. Mass spectra were obtained by EI mode. Column chromatography was performed on Kanto Kagaku silica gel 60N.

General procedure for the preparation of carboxylic acids (1a-e)

To a stirred solution of 1,2,3,4-tetrahydro-β-carboline-1-carboxylic acid[5] (8 mmol) in 2N NaOH (aq) (4.2 mL) and dioxane (4.2 mL) were added at the same time acyl chloride (8 mmol) and 2N NaOH (aq) (6.2 mL) over 20 min. at 0 °C, then the resulting mixture was stirred at rt for 20 h. The reaction mixture was acidified with conc. HCl to give a solid, which was corrected, washed with H_2O, and dried to afford the carboxylic acid. The carboxylic acids (**1a-e**) were used in the next step directly.

General procedure for the oxidation of carboxylic acids (1a-e)

To a stirred solution of carboxylic acid (**1a-e**, 2 mmol) in CH_2Cl_2 (10 mL) was added EDC (403 mg, 2.1 mmol), and the resulting mixture was stirred under the oxygen atmosphere (1 atm) at rt for 24 h. The reaction was quenched with AcOH (0.41 mL), and stirring was continued for 0.5 h. The reaction mixture was diluted with CH_2Cl_2, and the organic layer was washed with satd. $NaHCO_3$ (aq) solution and brine, dried over $MgSO_4$, and evaporated to give the residue, which was chromatographed on silica gel (CH_2Cl_2/EtOAc 10:1) to give oxidized product (**2a-e**).

2-(4-Fluorobenzoyl)-1-oxo-1,2,3,4-tetrahydro-β-carboline (**2a**)

yield 54%; mp 255-256 °C (EtOAc); IR (KBr) 3281, 1672 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.21 (2H, t, J = 6.4 Hz), 4.21 (2H, t, J = 6.4 Hz), 7.13 (1H, t, J = 7.7 Hz), 7.25 (2H, t, J = 8.5 Hz), 7.32 (1H, t, J = 7.7 Hz), 7.42 (1H, d, J = 7.7 Hz), 7.66 (2H, m), 7.71 (1H, d, J = 7.7 Hz), 11.79 (1H, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.6 (t), 46.6 (t), 112.7 (d), 114.7 (d), 115.0 (d), 120.0 (d), 120.9 (d), 123.2 (s), 124.2 (s), 125.5 (s), 125.8 (d), 130.7 (d), 130.8 (d), 132.9 (s), 138.4 (s), 160.9 (s), 171.8 (s); MS 308 (M^+), 123 (100); HRMS Calcd. for $C_{18}H_{13}FN_2O_2$: 308.0961, Found: 308.0990.

2-(4-Bromobenzoyl)-1-oxo-1,2,3,4-tetrahydro-β-carboline (**2b**)

yield 46%; mp 241-243 °C (EtOAc); IR (KBr) 3318, 1666 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.21 (2H, t, J = 6.4 Hz), 4.22 (2H, t, J = 6.4 Hz), 7.13 (1H, t, J = 7.7 Hz), 7.33 (1H, t, J = 7.7 Hz), 7.42 (1H, d, J = 7.7 Hz), 7.52 (2H, d, J = 8.5 Hz), 7.63 (2H, d, J = 8.5 Hz), 7.71 (1H, d, J = 7.7 Hz), 11.78 (1H, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.6 (t), 46.4 (t), 112.7 (d), 120.0 (d), 120.9 (d), 123.4 (s), 124.2 (s), 124.6

(d), 125.4 (s), 125.9 (s), 129.9 (d), 130.9 (d), 135.6 (s), 138.5 (s), 160.8 (s), 171.9 (s); MS 368 (M^+), 185 (100); HRMS Calcd. for $C_{18}H_{13}BrN_2O_2$: 368.0160, Found: 368.0120.

2-(4-Metoxybenzoyl)-1-oxo-1,2,3,4-tetrahydro-β-carboline (**2c**)

yield 43%; mp 200~201 °C (EtOAc); IR (KBr) 3311, 1667 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 3.18 (2H, t, *J* = 6.4 Hz), 3.81 (3H, s), 4.16 (2H, t, *J* = 6.4 Hz), 6.96 (2H, d, J = 9.0 Hz), 7.13 (1H, t, *J* = 7.7 Hz), 7.32 (1H, t, *J* = 7.7 Hz), 7.43 (1H, d, *J* = 7.7 Hz), 7.59 (2H, d, *J* = 9.0 Hz), 7.71 (1H, d, *J* = 7.7 Hz), 11.77 (1H, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.7 (t), 47.0 (t), 55.4 (q), 112.7 (d), 113.2 (d), 119.9 (d), 120.8 (d), 122.8 (s), 124.3 (s), 125.66 (d), 125.72 (s), 128.1 (s), 130.6 (d), 138.3 (s), 138.5 (s), 161.1 (s), 161.8 (s), 172.6 (s); MS 320 (M^+), 135 (100); HRMS Calcd. for $C_{19}H_{16}N_2O_3$: 320.1161, Found: 320.1159.

2-(2,4-Difluorobenzoyl)-1-oxo-1,2,3,4-tetrahydro-β-carboline (**2d**)

yield 42%; mp 195~197 °C (EtOAc); IR (KBr) 3291, 1678 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 3.18 (2H, t, *J* = 6.4 Hz), 4.31 (2H, t, *J* = 6.4 Hz), 7.14 (1H, t, *J* = 7.7 Hz), 7.34 (1H, t, *J* = 7.7 Hz), 7.42 (1H, d, *J* = 7.7 Hz), 7.63 (1H, m), 7.71 (1H, d, *J* = 7.7 Hz), 7.76 (1H, m), 11.84 (1H, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.6 (t), 45.4 (t), 103.9 (d), 111.5 (d), 112.8 (d), 120.1 (d), 121.0 (d), 123.7 (s), 124.2 (s), 125.3 (s), 126.1 (d), 131.0 (d), 138.6 (s), 160.3 (s), 166.0 (s); MS 326 (M^+), 141 (100); HRMS Calcd. for $C_{18}H_{12}F_2N_2O_2$: 326.0867, Found: 326.0864.

2-(3,5-Difluorobenzoyl)-1-oxo-1,2,3,4-tetrahydro-β-carboline (**2e**)

yield 40%; mp 204~206 °C (EtOAc); IR (KBr) 3310, 1676 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.18 (2H, t, *J* = 6.4 Hz), 4.31 (2H, t, *J* = 6.4 Hz), 7.14 (1H, t, *J* = 7.7 Hz), 7.34 (1H, t, *J* = 7.7 Hz), 7.42 (1H, d, *J* = 7.7 Hz), 7.63 (1H, m), 7.71 (1H, d, *J* = 7.7 Hz), 7.76 (1H, m), 11.84 (1H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2 (t), 46.8 (t), 106.7 (d), 111.0 (d), 111.3 (d), 112.6 (d), 120.9 (d), 121.0 (d), 124.3 (s), 124.6 (s), 125.1 (s), 126.9 (d), 138.6 (s), 139.5 (s), 161.3 (s), 171.1 (s); MS 326 (M^+), 326 (100); HRMS Calcd. for $C_{18}H_{12}F_2N_2O_2$: 326.0867, Found: 326.0843.

General procedure for the alkylation of β-carbolines (2a-e)

To a stirred solution of β-carboline (**2a-e**, 1.0 mmol) in DMF (10 mL) was added NaH (60%, 60 mg, 1.5 mmol) at 0 °C, and the resulting solution was stirred at rt for 30 min. To the reaction mixture was added $BrCH_2CO_2t$-Bu (0.22 mL, 1.5 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was diluted with Et_2O, and the organic mixture was washed with H_2O and separated. The aqueous layer was extracted with Et_2O (3 times), and the organic layer and extracts were combined, dried over $MgSO_4$ and evaporated to give the residue, which was chromatographed on silica gel (hexane/acetone 15:1~10:1) to give the corresponding *t*-butyl ester (**3a-e**).

t-Butyl [2-(4-Fluorobenzoyl)-1-oxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetate (**3a**)

yield 80%; mp 82~84 °C ; IR (KBr) 3056, 1746, 1686 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.38 (9H, s),

3.25 (2H, t, *J* = 6.4 Hz), 4.32 (2H, t, *J* = 6.4 Hz), 5.14 (2H, s), 7.04 (2H, t, *J* = 8.5 Hz), 7.20-7.30 (2H, m), 7.43 (1H, t-like, *J* = 8.2 Hz), 7.63-7.70 (3H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.3 (t), 27.9 (q), 46.4 (t), 46.5 (t), 82.1 (s), 109.8 (d), 114.8 (d), 115.1 (d), 121.0 (d), 121.1 (d), 123.8 (s), 124.7 (s), 126.7 (d), 130.6 (d), 130.7 (d), 132.2 (s), 140.0 (s), 161.9 (s), 167.5 (s), 172.4 (s); MS 422 (M^+), 199 (100); HRMS Calcd. for $C_{24}H_{23}FN_2O_4$: 422.1642, Found: 422.1620.

t-Butyl [2-(4-Bromobenzoyl)-1-oxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetate (**3b**)

yield 63%; mp 60~62 °C; IR (KBr) 3057, 1747, 1682 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.15 (9H, s), 3.01 (2H, t, *J* = 6.4 Hz), 4.09 (2H, t, *J* = 6.4 Hz), 4.89 (2H, s), 7.00-7.06 (6H, m), 7.19 (1H, t-like, *J* = 8.1 Hz), 7.45 (1H, d, *J* = 8.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2 (t), 27.9 (q), 46.1 (t), 46.4 (t), 82.2 (s), 109.8 (d), 121.1 (d), 121.2 (d), 123.9 (s), 124.7 (s), 124.9 (s), 125.9 (s), 126.96 (d), 129.7 (d), 131.2 (d), 135.2 (s), 140.2 (s), 161.9 (s), 167.7 (s), 172.7 (s); MS 482 (M^+), 199 (100); HRMS Calcd. for $C_{24}H_{23}BrN_2O_4$: 482.0841, Found: 482.0852.

t-Butyl [2-(4-Methoxybenzoyl)-1-oxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetate (**3c**)

yield 93%; mp 74~75 °C; IR (KBr) 3059, 1746, 1678 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.39 (9H, s), 3.23 (2H, t, *J* = 6.4 Hz), 3.83 (3H, s), 4.29 (2H, t, *J* = 6.4 Hz), 5.19 (2H, s), 6.86 (2H, d, *J* = 8.5 Hz), 7.22 (1H, t, *J* = 7.1 Hz), 7.27 (1H, d, *J* = 7.1 Hz), 7.41 (1H, t-like, *J* = 7.1 Hz), 7.64-7.70 (3H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2 (t), 27.9 (q), 46.3 (t), 46.5 (t), 55.1 (q), 81.9 (s), 109.7 (d), 113.1 (d), 120.8 (d), 123.8 (s), 124.2 (s), 124.9 (s), 126.4 (d), 128.0 (s), 130.6 (d), 139.8 (s), 162.0 (s), 162.1 (s), 167.5 (s), 172.9 (s); MS 434 (M^+), 199 (100); HRMS Calcd. for $C_{25}H_{26}N_2O_5$: 434.1842, Found: 434.1824.

t-Butyl [2-(2,4-Difluorobenzoyl)-1-oxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetate (**3d**)

yield 53%; mp 75~76 °C; IR (KBr) 3059, 1746, 1683, 1656 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.37 (9H, s), 3.22 (2H, t, *J* = 6.4 Hz), 4.39 (2H, t, *J* = 6.4 Hz), 5.14 (2H, s), 6.73 (1H, t-like, *J* = 8.5 Hz), 6.95 (1H, t-like, *J* = 8.5 Hz), 7.22 (1H, t, *J* = 7.7 Hz), 7.27 (1H, d, *J* = 8.5 Hz), 7.42 (1H, t, *J* = 7.7 Hz), 7.60 (1H, q, *J* = 8.5 Hz), 7.67 (1H, d, *J* = 7.7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2 (t), 27.9 (q), 45.5 (t), 46.4 (t), 82.0 (s), 103.4 (d), 103.8 (d), 104.1 (d), 109.8 (d), 111.2 (d), 111.5 (d), 120.9 (d), 121.1 (d), 123.8 (s), 124.5 (s), 125.3 (s), 126.8 (d), 130.8 (d), 131.0 (d), 140.1 (s), 161.1 (s), 166.8 (s), 167.3 (s); MS 440 (M^+), 141 (100); HRMS Calcd. for $C_{24}H_{22}F_2N_2O_4$: 440.1548, Found: 440.1542.

t-Butyl [2-(3,5-Difluorobenzoyl)-1-oxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetate (**3e**)

yield 53%; mp 57~58 °C; IR (KBr) 3052, 1748, 1678 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.37 (9H, s), 3.26 (2H, t, *J* = 6.4 Hz), 4.32 (2H, t, *J* = 6.4 Hz), 5.11 (2H, s), 6.89 (1H, t-like, *J* = 8.5 Hz), 7.10 (2H, m), 7.23 (1H, t, *J* = 8.1 Hz), 7.29 (1H, d, *J* = 8.5 Hz), 7.43 (1H, t, *J* = 8.1 Hz), 7.68 (1H, d, *J* = 8.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.1 (t), 27.8 (q), 46.1 (t), 46.4 (t), 82.3 (s), 106.1 (d), 106.3 (d), 106.5 (d), 109.9 (d), 110.9 (d), 111.1 (d), 121.2 (d), 121.3 (d), 123.9 (s), 124.5 (s), 125.2 (s), 127.1 (d), 140.3 (s),

161.7 (s), 167.6 (s), 171.2 (s); MS 440 (M^+), 339 (100); HRMS Calcd. for $C_{24}H_{22}F_2N_2O_4$: 440.1548, Found: 440.1532.

General procedure for the preparation of carboxylic acids (4a-e)

To a stirred solution of TMSI (prepared from NaI (2.0 mmol) and TMSCl (2.0 mmol) in $CHCl_3$ (3 mL) at 0 °C for 1 h) in $CHCl_3$ (3 mL) was added a solution of *t*-butyl esters (**3a-e**, 0.5 mmol) in $CHCl_3$ (2 mL) via a double-tipped stainless steel needle at 0 °C, and then the resulting mixture was refluxed for 16 h. The reaction was quenched with 10% HCl (aq) solution, and the aqueous mixture was extracted with $CHCl_3$ (6 times). The organic extracts were combined, dried over $MgSO_4$, and evaporated to give the residue, which was chromatographed on silica gel (hexane/acetone 20:1~2:1) to give the corresponding carboxylic acid (**4a-e**).

[2-(4-Fluorobenzoyl)-1-oxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid (**4a**)

yield 66%; mp 240~242 °C ; IR (KBr) 3058, 2661, 2571, 1729, 1671 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 3.25 (2H, t, *J* = 6.0 Hz), 4.18 (2H, t, *J* = 6.0 Hz), 5.19 (2H, s), 7.19-7.26 (3H, m), 7.41 (1H, t, *J* = 7.7 Hz), 7.62-7.66 (2H, m), 7.77 (1H, d, *J* = 7.7 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.6 (t), 45.8 (t), 46.4 (t), 110.8 (d), 114.8 (d), 115.1 (d), 120.6 (d), 121.1 (d), 123.3 (s), 124.4 (s), 124.6 (s), 126.3 (d), 130.7 (d), 130.6 (d), 130.7 (d), 132.7 (s), 139.8 (s), 161.4 (s), 169.9 (s), 172.0 (s); MS 366 (M^+), 199 (100); HRMS Calcd. for $C_{20}H_{15}FN_2O_4$: 366.1016, Found: 366. 1041.

[2-(4-Bromobenzoyl)-1-oxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid (**4b**)

yield 66%; mp 260~262 °C; IR (KBr) 3054, 2660, 2574, 1726, 1666 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 3.25 (2H, t, *J* = 6.3 Hz), 4.19 (2H, t, *J* = 6.3 Hz), 5.18 (2H, s), 7.21 (1H, t, *J* = 7.7 Hz), 7.41 (1H, t, *J* = 7.7 Hz), 7.50 (2H, d, *J* = 8.5 Hz), 7.60 (2H, d, *J* = 8.5 Hz), 7.61 (1H, d, J = 7.7 Hz), 7.77 (1H, d, *J* = 7.7 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.6 (t), 45.8 (t), 46.2 (t), 110.8 (d), 120.6 (d), 121.1 (d), 123.3(s), 124.3 (s), 124.7 (s), 124.8 (s), 126.4 (d), 129.8 (d), 130.9 (d), 135.5 (s), 139.8 (s), 161.3 (s), 169.9 (s), 172.0 (s); MS; HRMS Calcd. for $C_{20}H_{15}BrN_2O_4$: 426.0215, Found: 426.0243.

[2-(4-Methoxybenzoyl)-1-oxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid (**4c**)

yield 73%; mp 235~236 °C ; IR (KBr) 3076, 2662, 2571, 1707, 1667 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 3.22 (2H, t, *J* = 6.4 Hz), 3.82 (3H, s), 4.13 (2H, t, *J* = 6.4 Hz), 5.20 (2H, s), 6.95 (2H, d, J = 8.6 Hz), 7.20 (1H, t, *J* = 7.7 Hz), 7.40 (1H, t, *J* = 7.7 Hz), 7.62 (1H, d, *J* = 7.7 Hz), 7.56 (2H, d, *J* = 8.6 Hz), 7.75 (1H, d, *J* = 7.7 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.7 (t), 45.8 (t), 46.7 (t), 55.4 (q), 110.8 (d), 113.2 (d), 120.6 (d), 121.0 (d), 123.3 (s), 124.2 (s), 124.6 (s), 126.2 (d), 128.0 (s), 130.5 (d), 139.7 (s), 161.6 (s), 161.8 (s), 170.0 (s), 172.6 (s); MS 378 (M^+), 199 (100); HRMS Calcd. for $C_{21}H_{18}N_2O_5$: 378.1216, Found: 378.1226.

[2-(2,4-Difluorobenzoyl)-1-oxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid (**4d**)

yield 60%; mp 237~239 °C; IR (KBr) 3056, 2661, 2575, 1711, 1681 cm^{-1}; ^1H NMR (500 MHz,

DMSO-d_6) δ 3.21 (2H, t, *J* = 6.4 Hz), 4.27 (2H, t, *J* = 6.4 Hz), 5.18 (2H, s), 7.12-7.31 (3H, m), 7.41 (1H, t, *J* = 7.4 Hz), 7.61 (2H, ABq-like, *J* = 15.4 Hz), 7.76 (1H, d, *J* = 7.4 Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.5 (t), 45.3 (t), 45.7 (t), 104.1 (d), 110.9 (d), 111.4 (d), 111.7 (d), 120.7 (d), 121.1 (d), 123.2 (s), 124.1 (s), 125.1 (s), 126.5 (d), 130.8 (d), 139.9 (s), 160.6 (s), 166.2 (s), 169.7 (s); MS 384 (M^+), 199 (100); HRMS Calcd. for $C_{20}H_{14}F_2N_2O_4$: 384.0922, Found: 384.0909.

[2-(3,5-Difluorobenzoyl)-1-oxo-1,2,3,4-tetrahydro-β-carbolin-9-yl]acetic acid (**4e**)

yield 70%; mp 240~241 °C; IR (KBr) 3057, 2661, 2573, 1713, 1679 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.27 (2H, t, *J* = 6.4 Hz), 4.20 (2H, t, *J* = 6.4 Hz), 5.18 (2H, s), 7.21 (1H, t, *J* = 7.7 Hz), 7.28 (2H, d-like, J = 6.8 Hz), 7.41 (2H, m), 7.62 (1H, d, *J* = 7.7 Hz), 7.77 (1H, m), 7.71 (1H, d, *J* = 7.7 Hz), 7.76 (1H, m), 11.84 (1H, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ 20.4 (t), 45.8 (t), 46.1 (t), 106.1 (d), 110.6 (d), 110.9 (d), 111.0 (d), 120.7 (d), 121.1 (d), 123.2 (s), 124.1 (s), 125.2 (s), 126.5 (d), 139.9 (s), 140.2 (s), 160.1 (s), 160.9 (s), 169.9 (s), 170.4 (s); MS 384 (M^+), 199 (100); HRMS Calcd. for $C_{20}H_{14}F_2N_2O_4$: 384.0922, Found: 384.0910.

Assay of Enzyme activity.

Recombinant aldose reductase 2 (ALR2), which retains the same properties exhibited by human muscle and retina, was purchased from Wako Pure Chemical Industries (Osaka, Japan). ALR2 activity was spectrophotometrically measured at 37 ˚C by using 100 mM D,L-glyceraldehyde as the substrate (Cappiello et al., 1994).[6]

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