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Research Article

Synthesis and evaluation of [^{11}C]RU40555, a selective glucocorticoid receptor antagonist

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Summary

We demonstrated the synthesis of carbon-11 labeled 17-α-hydroxy-11-*β*-/4-/[methyl]-[1-methylethyl]-aminophenyl/-17α-[prop-1-ynyl]esta-4-9-diene-3-one (RU40555), a selective glucocorticoid receptor (GR) antagonist, and examined the *in vivo* profile of [^{11}C]RU40555. [^{11}C]RU40555 was synthesized by direct *N*-methylation with [^{11}C]CH_3OTf at 60°C for 5 min and an injectable solution of [^{11}C]RU40555 was obtained in 31 min at the end of bombardment. The decay-corrected radiochemical yield was 19%, the specific radioactivity was 57.5 ± 14.0 GBq/μmol, and the radiochemical purity was more than 99% as determined by HPLC. In rat experiments, the effects of adrenalectomy (ADX) on brain accumulation of [^{11}C]RU40555 were examined. ADX significantly decreased plasma corticosterone levels, and significantly increased brain accumulation of [^{11}C]RU40555. We succeeded in developing a rapid automated synthesis method for [^{11}C]RU40555, a GR antagonist, and showed [^{11}C]RU40555 had a potential as a PET tracer for mapping GR. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: [^{11}C]RU40555; glucocorticoid receptor antagonist; PET

Introduction

It is well known that stress exposure activates the hypothalamic–pituitary–adrenal (HPA) axis, and adrenal glucocorticoids secretion plays a role in

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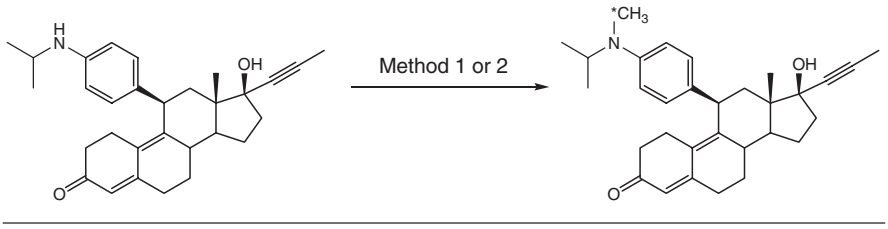

Method 1. ^{11}C-CH_3I, DMI, 150 °C, 10 min
Method 2. ^{11}C-CH_3OTf, acetone, 60 °C, 5 min

Figure 1. The synthetic route for [^{11}C]RU40555. Asterisk denotes position of ^{11}C labeling

negative feedback control.[1,2] In this negative feedback regulation, the hippocampus, which is rich in glucocorticoid and mineralcorticoid receptors,[3] plays a central role. Abnormality in this negative feedback control system contributes to several kinds of psychiatric conditions, such as depression and post-traumatic stress disorder (PTSD).[4,5] Imaging of the glucocorticoid receptor (GR) in the central nervous system is useful in pathological and physiological studies in these psychiatric conditions. In order to perform GR imaging, several PET tracers have been developed.[6–10] However, these tracers have not satisfied all the requirements as a PET tracer such as permeability and/or metabolic stability,[7–10] and none of them could achieve *in vivo* imaging of the glucocorticoid receptor. Recently a selective GR antagonist, RU40555 (17-α-hydroxy-11-β-/4-/[methyl]-[1-methylethyl]-aminophenyl/-17α-[prop-1-ynyl]esta-4-9-diene-3-one) has been described[11,12] (Figure 1). It was reported that RU40555 blocked the suppressive effect of dexamethasone on acute stress-induced corticosterone secretion in the feedback system, and that it did not decrease corticosterone secretion when rats undergo stress.[13,14] Therefore, it was suggested that RU40555 could penetrate the blood–brain barrier, and it may be a suitable candidate for a PET tracer for GR in the brain. In the present study, we developed a synthesis method of carbon-11 labeled RU40555 as a PET tracer for GR, and we examined its brain accumulation in rats.

Results and discussion

Chemistry

The methyl carbon bound to the aniline nitrogen of RU40555 was selected as the position to be labeled because the labeling reagent, [^{11}C]methyl iodide ([^{11}C]CH_3I), is easily prepared and the sterically hindered methyl group was expected to have sufficient metabolic stability. The precursor (**4**) and RU40555 were synthesized using examples from the European patent[15]

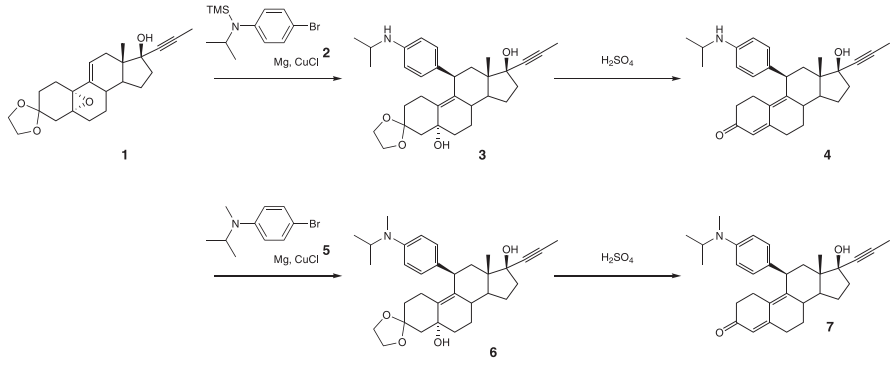

Figure 2. The synthetic route for precursor and RU40555

(Figure 2). Compound (**1**) was reacted with a compound, which was prepared from compound (**2**), Mg and CuCl, to give compound (**3**) in a yield of 75%. Compound (**3**) was unprotected by the use of 6 N H_2SO_4, resulting in the precursor (**4**) in a yield of 90%. At first, the reaction of the precursor (**4**) with [^{11}C]CH_3I in dimethylimidazolidinone was performed and purified by HPLC. Total synthesis time was 40 min from the end of bombardment (EOB). The decay-corrected radiochemical yield was 7.8 ± 1.1%, calculated from EOB, and the specific radioactivity was 13.1 ± 5.5 Bq/μmol. The radiochemical yield was not enough for animal studies. The reason for this low yield was considered to result from the low reactivity of the amino group of the precursor (**4**). The bulky isopropyl group may have further decreased the reactivity of the aniline nitrogen and prevented the nucleophilic reaction with the methylating agent. This information led us to use [^{11}C]methyl triflate ([^{11}C]CH_3OTf). Reaction of the precursor (**4**) with [^{11}C]CH_3OTf in acetone gave [^{11}C]RU40555 with better radiochemical yields. Total synthesis time was 31 min after EOB. The decay-corrected radiochemical yield was 19%, the radioactivity was 2.5 ± 0.3 GBq, radiochemical purity was more than 99%, and the specific radioactivity was 57.5 ± 14.0 GBq/μmol ($n = 4$). The radiochemical purity and specific radioactivity were enough to perform several kinds of animal experiments. Recently, Wilson *et al.* reported that the aromatic amine function reacted much slower than the aliphatic function, and that [^{11}C]CH_3OTf was superior to [^{11}C]CH_3I for alkylation of aromatic amine.[16] These results corresponded with our results. It was found that [^{11}C]CH_3OTf could be applied to the methylation of a much hindered nucleophile, such as *N*-isopropylaniline derivatives.

Whole body distribution and brain accumulation in rat

Typical time course images of whole body distribution following [^{11}C]RU40555 intravenous injection into rat is shown in Figure 4. Accumula-

tion of radioactivity in the liver and then the small intestine was clearly demonstrated. There was little accumulation in the bladder. Therefore, it was considered that RU40555 and/or its metabolites were eliminated in the bile acid. We hypothesized that [^{11}C]RU40555 could penetrate the blood–brain barrier and accumulate in the brain. The maximum brain accumulation of [^{11}C]RU40555 was observed 20 min after injection. Therefore, further *ex vivo* experiments using rats were performed 20 min after injection.

Figure 5 showed the accumulation of [^{11}C]RU40555 in the cortex, striatum, hippocampus, cerebellum, and plasma 20 min after injection. The radioactivity of plasma was the same as that of brain regions. It was widely distributed in the brain, and the percentage of injected dose (%ID/kg/g) was about 0.03% in the cortex, striatum, hippocampus, and cerebellum in normal rat. Percentages of unchanged [^{11}C]RU40555 in the plasma were 50.8% at 20 min. We examined the effect of adrenalectomy (ADX) to avoid the effect of endogenous corticosteroid in the present study. The plasma corticosterone level was significantly decreased from 423 ± 23 to 23 ± 3 ng/mg by adrenalectomy ($n = 5$). These accumulations in the brain were significantly increased by about 20% by ADX. It was reported that endogenous corticosterone occupied 28% of the GR of the hippocampus in normal conditions.[17] Therefore, the increase by ADX might result from avoidance of endogenous corticosterone occupation.

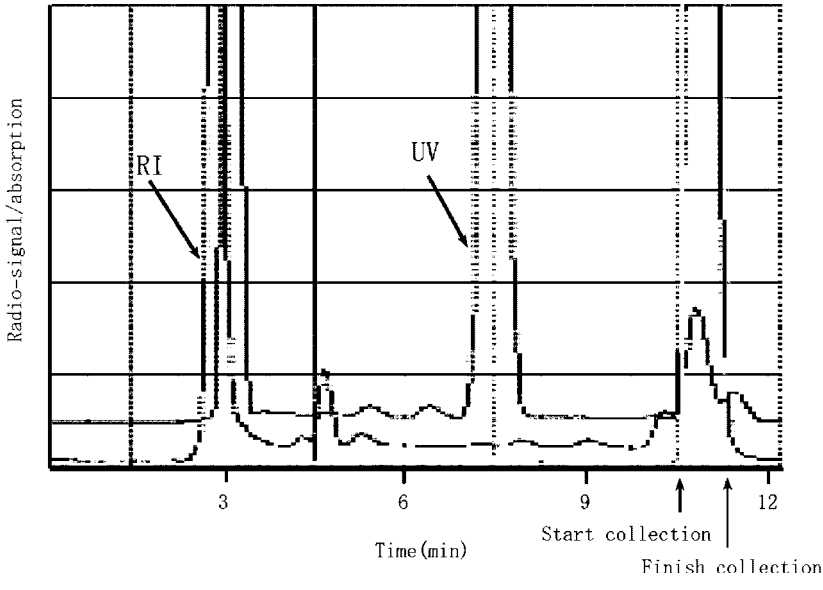

Figure 3. A typical preparative HPLC chromatogram of the purification of [^{11}C]RU40555 obtained using a UV-detector ($\lambda = 254$ nm) and a radiodetector

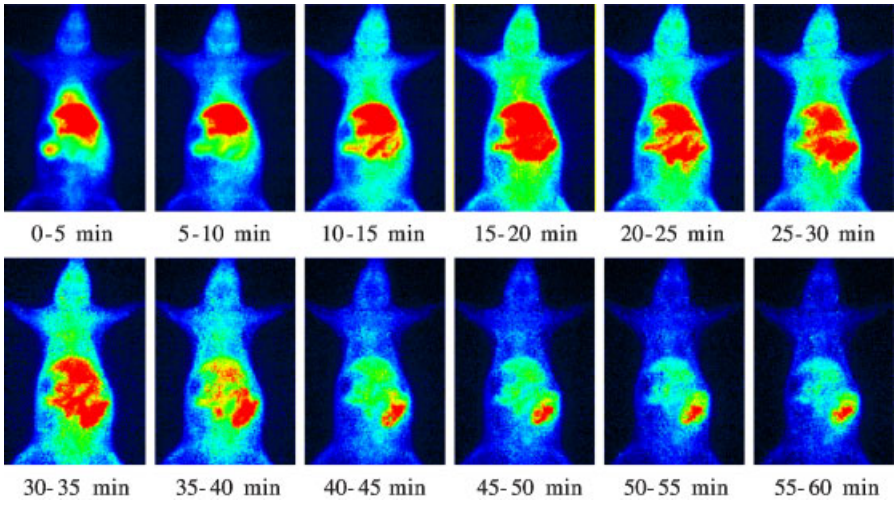

Figure 4. A typical dynamic image of whole body distribution of [^{11}C]RU40555 following intravenous injection ($n = 3$). Each image represents an accumulative image for 5 min. The upper left image represents 0–5 min, and the bottom right image represents 55–60 min

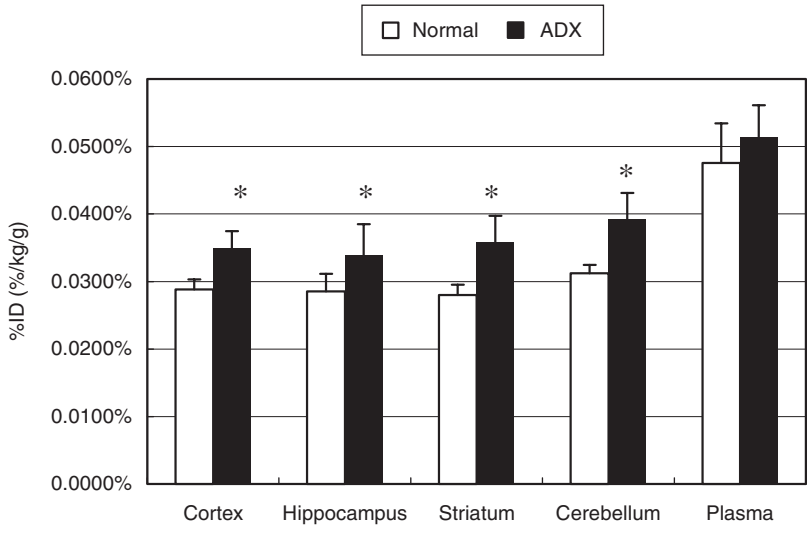

Figure 5. The accumulation of [^{11}C]RU40555 in the cortex, striatum, hippocampus, cerebellum, and plasma 20 min after injection. ADX = adrenalectomy. Each column represents the mean of five animals and the bar represents SD. $^{*}P < 0.05$ versus normal rats

Reul *et al.* reported the distribution of GR in rats.[3] According to their *in vitro* autoradiography study, high levels of GR were in the cortical,

amygdaloid, lateral, medial thalamic and hypothalamic regions, and low levels of GR were in the hindbrain and cerebellum. These observations were different from the present results in that the distribution of [^{11}C]RU40555 was widespread in all regions in this study. This discrepancy may be due to the differences between *in vitro* and *in vivo* results, because it is known that GR shows internalization when agonist and antagonist bind to the receptor.[18]

Though further experiments are necessary to evaluate the ability of [^{11}C]RU40555 to act as a GR imaging agent, [^{11}C]RU40555 showed some potential as a PET tracer.

Experimental

Materials and reagents

Acetone, tetrahydrofuran (THF), hexane, and ethyl acetate were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Silver triflate was purchased from Aldrich Japan (Tokyo, Japan). Graphpac GC material was purchased from Alltech Japan (Tokyo, Japan). Sterile filters were purchased from Millipore Japan (Tokyo, Japan). Hydroxypropyl-β-cyclodextrine was purchased from TOKYO KASEI KOGYO Co., Ltd. (Tokyo, Japan). 5α, 10α-epoxy-17α-ethynyl-17β-hydroxy-estr-9(11)-ene-3-one 3-(cyclic 1,2-ethanediyl acetal) was synthesized according to the literature.[15]

Animals

All animals were kept under a natural light/dark cycle and had unlimited access to water and food. In the case of ADX rats, 0.9% saline was substituted for water. All experiments using rats were performed in accordance with the institutional guidelines of The Medical and Pharmacological Research Center Foundation.

Male SD rats (8 weeks old) were used in the present study. Normal and ADX rats were purchased from Japan SLC (Shizuoka, Japan). ADX was performed at 5 weeks of age.

Analysis

HPLC analysis was performed with the Agilent 1100 HPLC system (Agilent Technologies Japan, Tokyo) and an Aloka positron detector RLC-700 (Aloka Co., Ltd., Tokyo, Japan).

HPLC analysis of RU40555

Column: YMC-Pack C18 Pro (4.6 $\times$ 150 mm, 5 μm) (YMC Co., Ltd., Kyoto, Japan).

Mobile phase: (acetonitrile:THF = 6 : 1): 0.05 M NH_4OAc-0.1% AcOH = 65:35 (v/v).

Flow rate: 1 ml/min.

Wavelength: 254 nm.

*Preparation of 4-(N-isopropyl-N-trimethylsilylamino)phenyl bromide (**2**).* A solution of *N*-isopropyl-4-bromoaniline (2.47 g, 11.5 mmol) in THF (7 ml) was cooled in a dry ice bath. A solution of *n*-BuLi (1.58 M hexane solution, 7.23 ml, 11.4 mmol) was added dropwise with stirring at −78°C for 15 min. TMSCl (1.38 g, 12.7 mmol) was added at the same temperature. The reaction mixture was stirred at −78°C for 30 min and the solution was allowed to warm to room temperature. The mixture was concentrated *in vacuo*. The residue was subjected to vacuum distillation (bp 81–83°C at 0.55 mmHg) to yield (**2**) (1.55 g, 47%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ0.00 (9H, s), 0.96 (6H, d), 2.45–2.48 (1H, m), 6.88 (2H, d, J= 8.8 Hz), 7.38 (2H, d, J= 8.8 Hz).

*Preparation of 4-(N-isopropyl-N-methylamino)phenyl bromide (**5**).* The reaction mixture of *N*-isopropyl-4-bromoaniline (20 g, 93.4 mmol) and HCHO (28.1 g, 934 mmol) in formic acid (35.2 ml, 934 mmol) was stirred at 120°C for 2 h. The mixture was concentrated *in vacuo*. The mixture was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution and brine. The extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The desired product was obtained by distillation (108–115°C, 0.2 mmHg) (19 g, 89%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ1.51 (6H, d, J= 6.6 Hz), 2.69 (3H, s), 4.03 (1H, q, J= 6.6 Hz), 6.64 (2H, d, J= 8.8 Hz), 7.28 (2H, d, J= 8.8 Hz); MS (ES+) m/e 228.2.

*Preparation of 5α-17β-dihydroxy-11β-[4[(N-isopropyl-N-trimethylsilylamino)-phenyl]-17α-ethynyl-esta-9-ene-3-one 3-(cyclic 1,2-ethanediyl acetal) (**3**).* A mixture of magnesium (129 mg, 5.31 mmol) in THF (3 ml) was stirred at room temperature. A solution of 4-(*N*-isopropyl-*N*-trimethylsilylamino)phenyl bromide (1.552 g, 5.42 mmol) in THF (10 ml) was added dropwise with stirring at room temperature. The reaction mixture was stirred and heated to reflux for 25 min. The Grignard solution was added to a solution of CuCl (35.2 mg, 0.355 mmol) in THF (5 ml) at 6–7°C. After stirring for 30 min, a mixture of 5α, 10α-epoxy-17α-ethynyl-17β-hydroxy-estr-9(11)-ene-3-one 3-(cyclic 1,2-ethanediyl acetal) (658 mg, 1.78 mmol) in THF (15 ml) was added dropwise at 6–7°C. After stirring at 6–7°C for 30 min, the mixture was concentrated *in vacuo*. The residue was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution and brine. The extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluted with a mixture of hexane and ethyl acetate (7:3) to give the desired product as an

amorphous solid (671 mg, 75%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ0.48 (3H, s), 1.16–1.37 (12H, m), 1.41–1.81 (4H, m), 1.82–1.93 (7H, m), 1.89 (3H, s), 1.92–2.40 (2H, m), 2.44–2.54 (1H, m), 3.58 (1H, q, J= 5.8 Hz), 3.87–4.06 (4H, m), 4.20–4.27 (1H, m), 4.43 (1H, s), 6.54 (2H, brs), 7.00 (2H, d, J= 8.4 Hz); MS (ES^+) m/e 506.5.

*Preparation of 5α-17β-dihydroxy-11β-[4[(N-isopropyl-N-methylamino)phenyl]-17α-ethynyl-esta-9-ene-3-one 3-(cyclic 1,2-ethanediyl acetal) (**6**).* A solution of 4-(*N*-isopropyl-*N*-methylamino)phenyl bromide (10.5 g, 46 mmol) in THF (10 ml) was added to a suspension of Mg (1.09 g, 44.6 mmol) in THF (3 ml) dropwise at room temperature. The reaction mixture was stirred at reflux for 25 min. The Grignard solution was added to a solution of CuCl (294 mg, 2.97 mmol) in THF (50 ml), at 6–7°C. After stirring for 30 min, a solution of 5α, 10α-epoxy-17α-ethynyl-17β-hydroxy-estr-9(11)-ene-3-one 3-(cyclic 1,2-ethanediyl acetal) (5.51 g, 14.9 mmol) in THF (50 ml) was added dropwise at 6–7°C. After stirring at 6–7°C for 30 min, the mixture was concentrated *in vacuo*, the residue was diluted with ethyl acetate and washed with aqueous 1 N hydrogen chloride solution, saturated aqueous sodium bicarbonate solution and brine. The extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was filtered and washed with IPE to give the desired product as a white powder (11.6 g, 100%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ0.48–2.07 (3H, s), 1.09–1.17 (6H, m), 1.25–1.40 (2H, m), 1.40–1.60 (2H, m), 1.60–1.64 (1H, m), 1.64–1.78 (3H, m), 1.80 (1H, brs), 1.82–1.92 (2H, m), 1.88 (3H, s), 1.93–2.07 (4H, m), 2.07–2.39 (5H, m), 2.44–2.55 (1H, m), 2.69 (3H, s), 3.88–4.10 (3H, m), 4.21–4.28 (1H, m), 4.43 (1H, s), 6.69 (2H, d, J= 7.7 Hz), 7.04 (2H, d, J= 7.7 Hz); MS (ES^+) m/e 520.5.

*Preparation of 17-β-hydroxy-11-β-/4-/[1-methylethyl]-aminophenyl/-17α-[prop-1-ynyl]est a-4-9-diene-3-one (**4**).* A solution of 6 N H_2SO_4 (4.42 ml, 26.5 mmol) was added to a solution of ketal (**3**) (670 mg, 1.32 mmol) in acetone (26 ml) dropwise with cooling on an ice bath. The reaction mixture was stirred at 0°C for 1 h. The mixture was concentrated *in vacuo*. The residue was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution and brine. The extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluted with a mixture of hexane and ethyl acetate (1:1) to give a gum. The desired product was obtained by freeze-drying from benzene (530 mg, 90%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ0.56 (3H, s), 1.10–2.09 (18H, m), 2.15–2.52 (6H, m), 2.53–2.63 (2H, m), 2.72–2.84 (1H, m), 3.58 (1H, q, J= 6.2 Hz), 4.33 (1H, d, J= 7.0 Hz), 5.75 (1H, s), 6.52 (2H, d, J= 8.4 Hz), 6.94 (2H, d, J= 8.4 Hz); MS (ES+) m/e 444.5.

Preparation of RU40555 (7). A solution of 6N H_2SO4 (50 ml, 300 mmol) was added to a solution of ketal (**6**) (7.8 g, 15 mmol) in acetone (300 ml) dropwise while cooling on an ice bath. The reaction mixture was stirred at 0°C for 1 h. The mixture was concentrated *in vacuo*. The residue was diluted with ethyl acetate and washed with saturated aqueous sodium bicarbonate solution and brine. The extracts were dried over magnesium sulfate, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography eluted with a mixture of hexane and ethyl acetate (7:3) to give a gum. The desired products were recrystallized from ethanol and water to give a light yellow powder (6.0 g, 87%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ0.56 (3H, s), 1.14 (6H, m), 1.19–1.55 (2H, m), 1.62–1.82 (3H, m), 1.89 (3H, s), 1.91–2.06 (2H, m), 2.18–2.53 (7H, m), 2.54–2.61 (2H, m), 2.70 (3H, s), 2.72–2.84 (1H, m), 3.98–4.10 (1H, m), 4.35 (1H, d, J= 6.6 Hz), 5.76 (1H, s), 6.70 (2H, d), 6.99 (2H, d, J= 8.8 Hz); MS (ES^+) m/e 458.5.

Preparation of [^{11}C]CH_3I and [^{11}C]CH_3OTf

Production of [^{11}C]CO_2 was accomplished via the ^{14}N(p, α)^{11}C nuclear reaction by proton bombardment (12 MeV, 35 μA) of a nitrogen target using a cyclotron-target system (OSCAR, JFE Plant & Service Corporation, Yokohama, Japan). [^{11}C]CH_3I was made from reduction with $LiAlH_4$ and reaction with HI. Then [^{11}C]CH_3I was converted to [^{11}C]CH_3OTf by passing through a glass column (6 cm length, 5 mm internal diameter) heated to 200°C and containing silver triflate-impregnated graphitized carbon (300 mg).

Preparation of [^{11}C]RU40555 from [^{11}C]CH_3I

Desmethyl-RU40555 was prepared as previously described. [^{11}C]RU40555 was synthesized by bubbling [^{11}C]CH_3I into a reaction vessel containing 3.0 mg desmethyl-RU40555 in dimethylimidazolidinone (0.22 ml) at 10°C (Figure 1). The solution was heated at 150°C for 10 min.

Preparation of [^{11}C]RU40555 from [^{11}C]CH_3OTf

The synthesis route of [^{11}C]RU40555 is shown in Figure 1. Desmethyl-RU40555 was prepared as previously described. [^{11}C]RU40555 was synthesized by bubbling [^{11}C]CH_3OTf into a reaction vessel containing 1.0 mg desmethyl-RU40555 in acetone (0.22 ml) at −20°C (Figure 1). The solution was heated at 60°C for 5 min.

Separation and preparation of [^{11}C]RU40555

The reaction mixture was purified by reversed-phase HPLC. The fraction containing [^{11}C]RU40555 was collected (Figure 3) and concentrated under reduced pressure. The residue was dissolved in 3% hydroxypropyl-β-

cyclodextrine saline solution and passed through a sterile membrane filter (MILLEX-GV, 0.22 μm) into a sterile vial. The retention time of the product (7 min) was identical to that of the authentic sample.

Preparative HPLC was performed with a Lab-Quatec IP-7500 pump system (Lab-Quatec. Co., Ltd., Tokyo, Japan).

Column: YMC-Pack C18Pro (10 × 250 mm, 5 μm)

Mobile phase: Acetonitrile: 0.1% acetic acid-0.05 M NH_4OAc = 70 : 30m > (v/v)

Flow late: 6 ml/min

Wavelength: 254 nm

Retention time: RU40555 (11 min)

Whole body distribution of [^{11}C]RU40555 in rat

Whole body distribution was examined with a new planar positron imaging system (PPIS) with a spatial resolution less than 2.1 mm FWHM. This system has been developed for studies in small animals and plants (Hamamatsu Photonics, Shizuoka, Japan).[19] Rats ($n = 3$) were anesthetized with intraperitoneal injection of pentobarbital (25 mg/kg), and fixed on an acrylic plate, which was the center of the detector units. A 60-min emission scan consisting of 12 frames was obtained following [^{11}C]RU40555 (15 MBq) intravenous injection (Figure 4).

Accumulation of [^{11}C]RU40555 in the rat brain

Normal ($n = 5$) and ADX ($n = 5$) rats were anesthetized with intraperitoneal injection of pentobarbital (25 mg/kg), and [^{11}C]RU40555 (50 MBq) was intravenously injected. Twenty minutes after injection, arterial blood was corrected for plasma corticosterone level measurement from the aorta abdominalis, and then the rats were quickly decapitated. The cerebral cortex, striatum, hippocampus, and cerebellum were removed, and their radioactivity was counted using an auto well gamma counter (1480 WIZARD; Wallac Oy, Turku, Finland). Data are represented as the percentage of injected dose (%ID/kg/g) (Figure 5).

Analysis of plasma contents of unchanged [^{11}C]RU40555 was performed 20 min after injection. Acetonitrile (0.1 ml) was added to the plasma sample (0.1 ml), mixed at room temperature, and centrifuged for 3 min at 12 000*g*. The supernatant was spotted onto TLC plates, and developed (MeOH: H_2O = 80 : 20). After drying, the TLC plate was exposed to an imaging plate (Fuji Film, Tokyo, Japan) for 45 min, and was analyzed for the contents of nonmetabolites and metabolites of [^{11}C]RU40555 using an imaging plate reader (BAS1800, Fuji Film, Tokyo, Japan).

Conclusion

A selective GR antagonist, [^{11}C]RU40555, was successfully synthesized by using [^{11}C]MeOTf. It was found that [^{11}C]RU40555 could cross the blood brain barrier. Though further experiments are necessary, we showed that [^{11}C]RU40555 has the potential to be used as a PET tracer for GR.

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