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Synthesis and radiopharmacological characterization of [^{11}C]AL-438 as a nonsteroidal ligand for imaging brain glucocorticoid receptors

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Abstract—The radiosynthesis and the radiopharmacological characterization of **[^{11}C]AL-438** as a nonsteroidal ligand for the glucocorticoid receptor (GR) is described. Radiolabeling of the corresponding desmethyl precursor **10** with [^{11}C]MeI gave **[^{11}C]AL-438** in decay-corrected radiochemical yields of 30 ± 4% (based upon [^{11}C]CO_2) within 35 min at a specific radioactivity of 10–15 GBq/μmol at the end-of-synthesis. The radiopharmacological evaluation of **[^{11}C]AL-438** involved biodistribution and small animal PET imaging in rats, and autoradiography studies using rat brain sections. Biodistribution studies were performed in male Wistar rats and demonstrated high radioactivity uptake in pituitary and brain. However, the inability of high dose corticosterone to block binding would suggest that the radioactivity accumulation in the brain was not receptor-mediated.

Glucocorticoids which are appropriately labeled with the short-lived positron emitters ^{11}C ($t_{1/2}$ = 20.4 min) and ^{18}F ($t_{1/2}$ = 109.8 min) would allow the noninvasive in vivo imaging of brain glucocorticoid receptors (GRs) by means of positron emission tomography (PET). PET imaging of brain GRs would provide important information on the neurobiological basis of GR-mediated abnormalities of hypothalamo–pituitary–adrenocortical (HPA) axis function and regulation, which has been suggested to play a crucial role in the pathogenesis of depression.[1–5] The accumulating evidence of the important role of brain GRs in several neuropsychiatric disorders, such as severe depression and anxiety, has stimulated research on PET radiotracers for studying brain GRs in vivo over the last 15 years. Research was mainly focused on steroidal glucocorticoids, and numerous attempts have been made to synthesize GR-binding steroids labeled with the short-lived positron emitter ^{18}F[6–13] and, to a lesser extent, with ^{11}C.[14] Despite the excellent in vitro GR binding of some of the synthesized compounds, none of the investigated compounds are suitable for imaging brain GRs either due to their rapid in vivo defluorination and/or insufficient blood–brain-barrier penetration. Moreover, the steroidal GR ligands used (e.g. RU486) often show binding to other steroid hormone receptors, mainly to the progesterone receptor (PR). The lack of selectivity of some of the steroidal compounds has further limited their use as selective GR tracers.

In contrast, recently identified nonsteroidal compounds with high affinity and selectivity toward the GR provide an interesting alternative for the design and synthesis of PET radiotracers for GRs. Nonsteroidal GR-binding ligands comprise a broad structural variety. Prominent examples of nonsteroidal GR ligands are structurally based on dibenzyl anilines, *N*-arylpyrazolo-based ligands or benzopyrano-quinolines.[15]

A selection of structures for nonsteroidal GR ligands and the steroidal GR ligand RU486 is given in Figure 1.

Among the reported benzopyrano-quinolines, **AL-438** was identified as one of the first compounds showing both, high affinity and selectivity for the GR.[16] Moreover, the methoxy group at position 10 of **AL-438** makes this compound an attractive candidate for isotopic labeling with ^{11}C through a methylation reaction of

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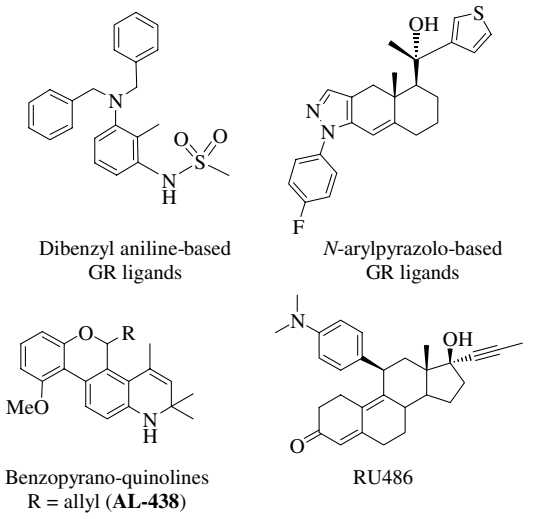

Figure 1. Structures of nonsteroidal GR ligands[15] and RU486.

the corresponding desmethyl precursor with [^{11}C]methyl iodide.

In this paper we describe the radiosynthesis and radiopharmacological evaluation of **[^{11}C]AL-438** as a first example of a nonsteroidal PET radiotracer for imaging brain glucocorticoid receptors.

The synthesis of labeling precursor **10** and **AL-438** as reference compound is depicted in Figure 2.

A suitable starting material for synthesis of compounds **10** and **AL-438** is tetracyclic lactone **6**, which was prepared according to literature procedure.[16] The phenolic OH-group at position 10 was protected as silyl ether using TBDMSCl and KO^{t}Bu as the base in DMF in 65% yield. The TBS-protected tetracyclic compound **7** was further functionalized at position 5. For this purpose lactone **7** was subjected to a controlled reduction using DIBAL in toluene followed by acid-catalyzed methanolysis affording methyl acetal **8** in 58% yield for both steps.

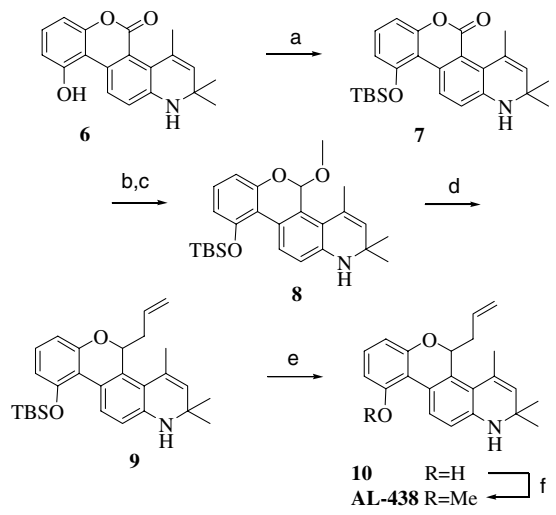

Figure 2. Reagents: (a) TBDMSCl, KO^t-Bu, DMF, 65%; (b) DIBALH, toluene; (c) MeOH, TsOH·H_2O, 58% for two steps; (d) allyltrimethylsilane, BF_3·Et_2O, 95%; (e)TBAF, 68%; (f) Cs_2CO_3, MeI, 73%.

Treatment of methyl acetal **8** with allytrimethylsilane in the presence of the Lewis acid BF_3·Et_2O afforded allylated product **9** in excellent 95% yield. Removal of the TBS-ether protecting group in **9** succeeded through treatment with TBAF in 68% yield to give compound **10** as labeling precursor. Subsequent treatment of desmethyl precursor **10** with Cs_2CO_3 as the base in the presence of an excess of MeI afforded **AL-438** in 73% yield. The total yields of desmethyl precursor **10** and reference compound **AL-438** were 24% and 18%, respectively, based upon compound **6**.

The radiosynthesis of **[^{11}C]AL-438** is shown in Figure 3.

The radiolabeling was accomplished by a methylation reaction of desmethyl precursor **10** with [^{11}C]methyl iodide. The radiosynthesis of **[^{11}C]AL-438** was performed in a remotely controlled synthesis apparatus (Nuclear Interface, Münster). [^{11}C]Methyl iodide was prepared starting from [^{11}C]CO_2 according to Crouzel and coworkers.[17] [^{11}C]Methyl iodide was transferred in a stream of nitrogen into the reaction vessel containing the sodium salt of desmethyl precursor **10** (1 mg) in DMF (400 μl) and 5 N NaOH (30 μl) at −20 °C. The formation of the sodium salt of **10** could easily be monitored by the yellow color of the DMF solution. After completion of the [^{11}C]MeI transfer, the reaction vessel was sealed and heated at 100 °C for 5 min. The reaction mixture was diluted with eluent (1 ml) and the mixture was transferred from the reaction vessel onto a semi-preparative C-18 column (83:17 acetonitrile/water containing 0.1% ammonium formate, 4 ml/min). The fraction eluting at 9–11 min was collected, diluted with water (10 ml), and passed through a C-18 Sep-Pak Light cartridge. The cartridge was washed with water (5 ml) and the product was eluted with ethanol (0.5 ml). Addition of 0.9% saline (9.5 ml) gave 5% EtOH solution in saline suitable for the animal experiments.

In a typical experiment, 30 GBq of [^{11}C]CO_2 could be converted into 3.0 GBq of **[^{11}C]AL-438** (30 ± 4% decay-corrected radiochemical yield) after HPLC purification at a specific radioactivity of 10–15 GBq/μmol at the end-of-synthesis within 35 min. The radiochemical purity exceeded 95%.

The biodistribution results[18] of **[^{11}C]AL-438** in normal and corticosterone pretreated male Wistar rats are depicted in Table 1.

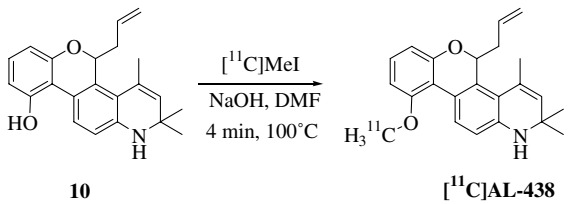

Figure 3. Radiosynthesis of compound **[^{11}C]AL-438**.

Table 1. Radioactivity expressed as percent injected dose per gram tissue in different organs after single intravenous injection of 20 MBq of **[^{11}C]AL-438** in 0.5 ml saline with 2% ethanol

%ID/g tissue (time p.i.)	Control		Blocked	
	5 min	60 min	5 min	60 min
Blood	0.52 ± 0.10	0.60 ± 0.19	0.52 ± 0.07	0.74 ± 0.30
Rest of brain	1.52 ± 0.41	0.56 ± 0.10	1.46 ± 0.21	0.61 ± 0.14
Cortex	1.67 ± 0.32	0.60 ± 0.18	1.55 ± 0.23	0.74 ± 0.11
Cerebellum	1.39 ± 0.37	0.53 ± 0.06	1.46 ± 0.26	0.64 ± 0.11
Hippocampus	1.30 ± 0.34	0.61 ± 0.09	1.35 ± 0.29	0.68 ± 0.11
Pituitary	2.49 ± 0.69	1.77 ± 1.04	2.45 ± 0.93	1.76 ± 0.82
Adrenals	7.63 ± 1.38	4.40 ± 1.07	8.68 ± 1.19	3.80 ± 0.34
Pancreas	2.79 ± 0.55	1.99 ± 0.48	2.81 ± 0.38	1.43 ± 0.19
Spleen	0.97 ± 0.16	1.06 ± 0.26	1.21 ± 0.18	0.91 ± 0.28
Kidneys	1.72 ± 0.32	1.19 ± 0.29	2.06 ± 0.11	1.13 ± 0.08
Fat	1.19 ± 0.42	3.11 ± 1.30	1.51 ± 0.32	2.43 ± 0.37
Brown fat	5.22 ± 2.50	8.54 ± 1.43	5.00 ± 1.09	7.44 ± 1.57
Muscle	0.94 ± 0.29	0.59 ± 0.08	0.90 ± 0.11	0.52 ± 0.06
Heart	1.70 ± 0.38	0.75 ± 0.15	1.91 ± 0.43	0.67 ± 0.06
Lungs	1.44 ± 0.35	1.11 ± 0.21	1.44 ± 0.11	1.03 ± 0.20
Thymus	1.57 ± 0.47	1.48 ± 0.32	1.46 ± 0.38	1.60 ± 0.43
Harderian glands	1.68 ± 0.32	3.35 ± 0.92	1.65 ± 0.0.35	2.87 ± 0.63
Liver	2.65 ± 0.38	2.54 ± 0.54	3.04 ± 0.54	3.84 ± 1.46
Femur	0.90 ± 0.20	0.88 ± 0.15	0.85 ± 0.07	0.70 ± 0.12
Testis	0.47 ± 0.14	0.52 ± 0.15	0.43 ± 0.11	0.50 ± 0.08

$n = 4$ for each time point; p.i., post injection.

The radioactivity concentration in the blood remains stable over time reaching the maximum 5 min post injection (p.i.). The highest initial radioactivity uptake was observed in the adrenals (7.63 ± 1.38% ID/g at 5 min) which decreased to 4.40 ± 1.07% ID/g at 60 min. Compared to other tissues and organs relatively high radioactivity concentration was further found in the pancreas, liver, and brown fat. The pituitary as target organ known to express high levels of GRs showed high radioactivity uptake of 2.49 ± 0.69% ID/g at 5 min which was slightly reduced to 1.77 ± 1.04 ID/g after 60 min. In all organs, excluding the adrenals, changes of the activity concentration within the first hour after injection were relatively low. The compound readily penetrates the blood–brain barrier as shown by the relatively high radioactivity concentration in selected brain regions, like cortex, hippocampus, and cerebellum. However, radioactivity was steadily washed out from the brain over time reaching comparable radioactivity levels in the cortex (0.60 ± 0.18% ID/g), hippocampus (0.61 ± 0.09% ID/g) and cerebellum (0.53 ± 0.06% ID/g) at 60 min p.i.

The blocking experiments with corticosterone were performed to study glucocorticoid binding site-mediated uptake. However, no change of radioactivity concentration was observed for the GR-rich target organs (adrenals, thymus), the pituitary and selected brain regions (cortex and hippocampus). Thus, it can be concluded that the observed radioactivity uptake is not receptor-mediated and the biodistribution reflects predominantly nonspecific distribution of compound **[^{11}C]AL-438**. This is in keeping with the calculated high lipophilicity of this compound (log P 6.13).

This finding may be explained by the fact that in this study normal nonadrenalectomized rats were used. Consequently, the endogenous adrenal steroid capacity of the animals was high during the experiments which might have prevented sufficient blocking of radiotracer uptake. It is known that under normal conditions the GRs are occupied through endogenous corticosterone by 28%.[19] The effect of plasma corticosterone level in normal rats (423 ± 23 ng/mg) and in adrenalectomized rats (23 ± 3 ng/mg) was discussed recently.[14] The almost 30% occupancy of the brain GR binding sites by endogenous corticosterone in addition to the rather low specific activity of **AL-438** (5 GBq/μmol at the time of injection) probably caused a substantial saturation of the binding sites which may have prevented a measurable blocking effect.

Our data on the competition binding of **AL-438** and the potent antiprogestin RU486 for GR and PR (Table 2) are in agreement with the proposed GR binding affinity and selectivity properties of **AL-438**. The binding affinity of **AL-438** is lower but still in a comparable range to that of the high affinity GR/PR ligand RU486. However, **AL-438** demonstrates lower binding to the PR compared to RU486 resulting in an improved GR selectivity profile toward the PR. The calculated log P values (6.13 for **AL-438** and 4.84 for RU486, respectively) reflect a high lipophilicity for both compounds, being especially high for the nonsteroidal compound **AL-438**. The high log P value for compound **AL-438** agrees with the biodistribution profile of a highly lipophilic compound.

Figure 4 shows an autoradiogram of the in vivo distribution of **[^{11}C]AL-438** in the median horizontal plane of the rat brain at 5 min after injection.[20] High radioactivity concentration was detected in the cortex and thalamic region. The observed regional radioactivity accumulation is in agreement with the results reported

Table 2. Competition binding of AL-438 or RU486 at GR and PR. Binding data were generated using fluorescence polarization assay kits available from Invitrogen (PolarScreen Glucocorticoid Receptor Competitor Assay Kit, Green and PolarScreen Progesterone Receptor Competitor Assay Kit, Green)

Compound	GR	PR	log *P*
AL-438	8.1 ± 0.21	6.9 ± 0.17	6.13
RU486	8.6 ± 0.01	7.8 ± 0.16	4.84

Data are expressed as pEC50 with 95% confidence interval of the mean of $N = 3$ determinations. RU 486 was purchased from Sigma. Calculated log *P* values are based on ACDLab predictions.

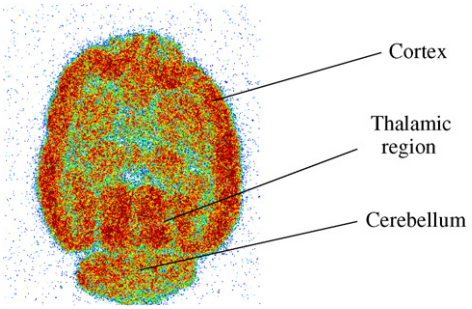

Figure 4. Digital autoradiograph of a rat brain section at 5 min after single iv administration of **[^{11}C]AL-438**.

by Ahima et al. using ^{3}H-labeled corticosteroids. In this study the authors report high densities of GRs in all regions of the cerebellar cortex.[21]

Small animal PET imaging with **[^{11}C]AL-438** was performed using a microPET® P4 primate model scanner (CTI Concorde Microsystems Inc., Knoxville, TN). The raw data were sorted into 3D sinograms followed by Fourier re-binning and two-dimensional ordered subsets expectation maximization (OSEM) image reconstruction. No correction for recovery and partial volume effects was applied. General anesthesia of the animal was induced and maintained by inhalation of halothane (3%) and N_2O (65%) in O_2. The animal was positioned with the brain in the center of the 22 cm transaxial and 8 cm axial field of view (FOV) of the scanner. The rat was injected with 30 MBq of **[^{11}C]AL-438** via the tail vein and sacrificed at 2 h p.i. by intravenous application of KCl. The rat was scanned for 50 min, and the raw data were sorted into 30 frames with photon attenuation correction. Two-dimensional projections of sagittal, transversal, and coronal small animal PET images are displayed in Figure 5.

The brain is clearly visible in the microPET images, which is consistent with the obtained regional brain distribution data. The initial high radioactivity uptake in the brain declined over time. Summation of frames between 30 and 60 min shows local accumulation of radioactivity consistent with the regional radioactivity distribution found in the ex vivo autoradiography.

In summary, ^{11}C-labeled nonsteroidal glucocorticoid **[^{11}C]AL-438** as novel potential ligand for studying brain GRs could be conveniently synthesized in sufficient radiochemical yield via ^{11}C-methylation of the corresponding desmethyl precursor **10**. Compound **[^{11}C]AL-438** represents the first example of a nonsteroidal glucocorticoid ligand labeled with a short-lived positron emitter. The radiopharmacological investigation of **[^{11}C]AL-438** showed promising brain uptake and accumulation in brain region known to express high levels of GRs. However, the failure to demonstrate effective blockade by corticosterone pre-treatment suggests that the uptake of **[^{11}C]AL-438** into target tissues may not be GR-mediated. For further experiments, optimization of the benzopyrano-quinolines-based lead structure will be necessary to reduce the lipophilicity. For this purpose several recently described novel ligands bearing a sulfonamide moiety[22] should be envisaged for further ^{11}C and ^{18}F radiolabeling experiments to provide compounds with high affinity and selectivity for the GR while showing a significantly reduced lipophilicity.

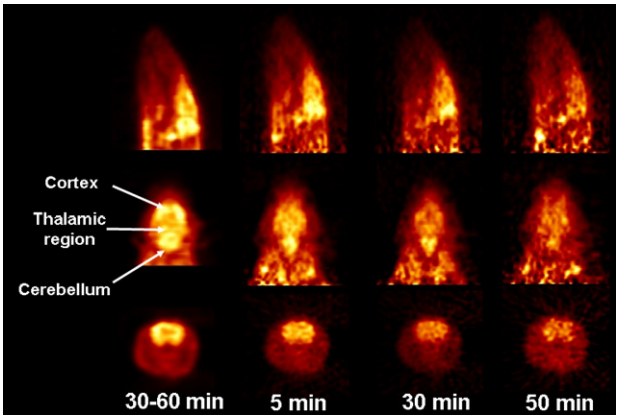

Figure 5. Sagittal, coronal, and transaxial images of a male Wistar rat brain after intraveneous injection of 30 MBq of **[^{11}C]AL-438**.

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18. All animal experiments were carried out according to the guidelines of the German Regulations for Animal Welfare. The protocol was approved by the local Ethical Committee for Animal Experiments. The male Wistar rats (Kyoto-Wistar strain, aged 6–8 weeks, 160–180 g) were housed under standard conditions with free access to standard food and tap water. The animals were intravenously injected with 20 MBq of **[^{11}C]AL-438** in 0.5 ml saline with 5% ethanol. Animals were sacrificed at 5 and 60 min post injection. Blood, pituitary, adrenals, and the major organs were collected, wet-weighed, and counted in a γ counter (Packard). The percent injected dose per gram (%ID/g) was determined for each sample. For each animal, radioactivity of the tissue samples was calibrated against a known aliquot of injectate. Values are quoted as means ± standard deviation (SD) for a group of four animals. The specific localization of the radiotracer on glucocorticoid binding sites was investigated by injecting 10 mg/kg of corticosterone dissolved in 0.5 ml of 10% ethanol/saline (blocked) 1 h before the application of the radiotracer.
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