Design, Radiosynthesis, and Biodistribution of a New Potent and Selective Ligand for in Vivo Imaging of the Adenosine A_{2A} Receptor System Using Positron Emission Tomography

Sergio Todde,[†] Rosa Maria Moresco,[†] Pasquale Simonelli,[†] Pier Giovanni Baraldi,[‡] Barbara Cacciari,[‡] Giampiero Spalluto,[§] Katia Varani,[∥] Angela Monopoli,[⊥] Mario Matarrese,[†] Assunta Carpinelli,[†] Fulvio Magni,[†] Marzia Galli Kienle,[†] and Ferruccio Fazio*[,†]

CNR-INB, University of Milano−Bicocca, Scientific Institute H San Raffaele, Via Olgettina 60, I-20132 Milano, Italy, Dipartimento di Scienze Farmaceutiche and Dipartimento di Medicina Clinica e Sperimentale−Sezione di Farmacologia, Università degli Studi di Ferrara, Via Fossato di Mortara 17-19, I-44100 Ferrara, Italy, Dipartimento di Scienze Farmaceutiche, Università degli Studi di Trieste, Piazzale Europa 1, I-34127 Trieste, Italy, and Schering-Plough Research Institute, San Raffaele Scientific Park, Via Olgettina 58, I-20132 Milano, Italy

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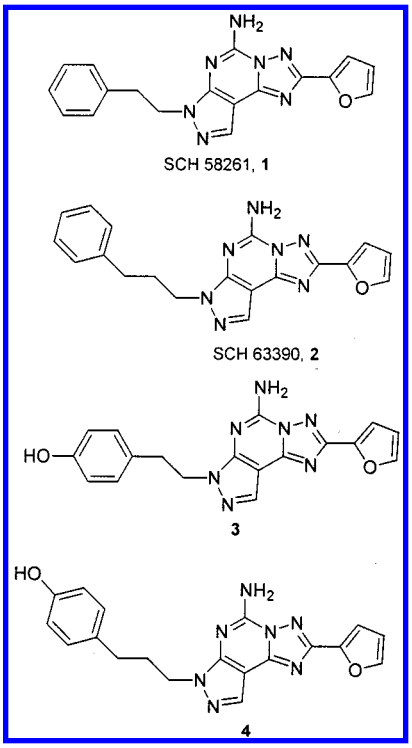
Introduction. Adenosine is an endogenous modulator of neurotransmission in both the central and peripheral nervous systems. This neuromodulator interacts with four different G-protein-coupled receptors classified as A_1, A_{2A}, A_{2B}, and A_3[1] located on cell membranes. In particular, the A_{2A} adenosine receptor subtype, which is positively coupled with adenylyl cyclase, is highly expressed in the striatum,[2] where it is functionally linked to dopamine D_2 receptors. Experimental evidence indicates that in striato-pallidal neurons the administration of A_{2A} adenosine receptor agonists decreases the affinity of D_2 dopamine receptor agonists.[3,4] These findings suggest that adenosine A_{2A} receptor antagonists may be useful in the treatment of Parkinson's disease.

Positron emission tomography (PET) enables the in vivo study of several physiological and neurochemical variables using methods originally developed for quantitative autoradiography.[5] Radioligands suitable for PET studies should fulfill several criteria, such as receptor affinity in the nanomolar range, high selectivity for the target receptor subtype, minimal metabolism in tissues, permeability across the blood−brain barrier (BBB), and low nonspecific binding.[6] Owing to the short half-life of positron-emitting radioisotopes, ligand design should also guarantee an easy and fast chemical approach to the radiosynthesis of the labeled form and a high specific radioactivity of the final product. To date, several xanthine derivatives with antagonist activity for A_{2A} receptors have been labeled with positron-emitting radioisotopes: (*E*)-8-(3-chlorostyryl)-1,3-dimethyl-7-[^{11}C]-methylxanthine ([^{11}C]CSC),[7] (*E*)-8-(3,4-dimethoxystyryl)-1,3-dipropyl-7-[^{11}C]methylxanthine ([^{11}C]KF17837),[8] (*E*)-8-(3,4,5-trimethoxystyryl)-1,3-dimethyl-7-[^{11}C]methylxanthine ([^{11}C]KF18446),[9] and (*E*)-1,3-diallyl-7-[^{11}C]methyl-8-(3,4,5-trimethoxystyryl)xanthine ([^{11}C]-KF19631).[9] [^{11}C]CSC is highly selective, but its affinity for adenosine A_{2A} receptors is relatively low (K_i = 54 nM). [^{11}C]KF17837 has higher affinity (K_i = 1 nM) for adenosine A_{2A} receptors, but a low striatum-to-cerebellum ratio was found when tested in vivo with PET.[10,11] Among the ^{11}C-labeled ligands for adenosine A_{2A} receptors prepared to date, the most suitable ligand for PET application appears to be [^{11}C]KF18446, which shows good in vivo selectivity and specificity for the target tissues.[9] However, the above compounds are xanthine derivatives and are subject to photoisomerization, which is a specific drawback of this class of molecules.

In recent years efforts have been made to synthesize novel non-xanthine compounds with antagonistic properties toward the adenosine A_{2A} receptor subtype. In particular, the synthesis of a large group of pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines behaving as potent and selective A_{2A} antagonists has recently been reported.[12−14] In general, this class of compounds shows a good binding profile in terms of in vitro affinity and receptor selectivity. The two prototypes of this family, SCH58261 (**1**) and SCH63390 (**2**), together with their 4-hydroxyphenyl derivatives (**3**, **4**), proved to be the best derivatives in terms of both affinity and selectivity for human A_{2A} adenosine receptors.



Out of this family, we selected for the development of a new PET ligand the derivative 5-amino-7-(3-(4-methoxyphenyl)propyl)-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (SCH442416, **5**), whose chemi-

* To whom correspondence should be addressed. Phone: +39 02 2153056. Fax: +30 02 26415202. E-mail: fazio@mednuc.hsr.it.
[†] University of Milano−Bicocca.
[‡] Dipartimento di Scienze Farmaceutiche, Università degli Studi di Ferrara.
[§] Università degli Studi di Trieste.
[∥] Dipartimento di Medicina Clinica e Sperimentale−Sezione di Farmacologia, Università degli Studi di Ferrara.
[⊥] Schering-Plough Research Institute.

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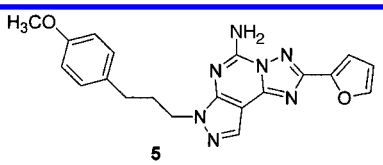

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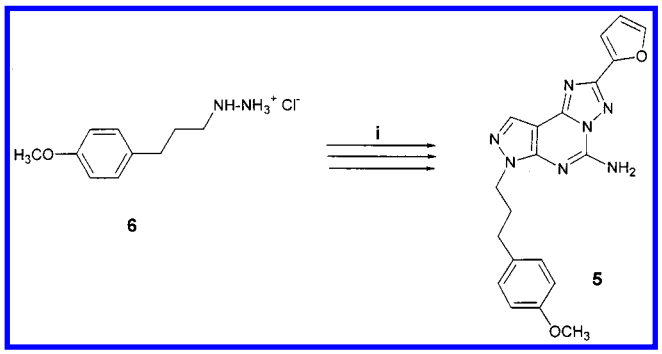
Species	A_1 (K_i, nM)	A_{2A} (K_i, nM)	A_{2B} (K_i, nM)	A_3 (K_i, nM)	A_1/A_{2A}	A_{2B}/A_{2A}	A_3/A_{2A}
human	1,111[a] (905-1,365)	0.048[b] (0.032-0.070)	> 10,000[c]	> 10,000[d]	23,145	> 200,000	> 200,000
rat	1,815[e] (1,681-1,960)	0.50[f] (0.41-0.60)	N.D.[g]	> 10,000[h]	3,630	N.D.	> 20,000

[a]Displacement of specific [^{3}H]DPCPX in CHO cells transfected with the human recombinant adenosine A_1 receptor, expressed as K_i in nM (n = 3). [b]Displacement of specific [^{3}H]SCH58261 in HEK-293 cells transfected with the human recombinant A_{2A} adenosine receptors, expressed as K_i in nM (n = 3). [c]Displacement of specific [^{3}H]DPCPX in HEK-293 cells transfected with the human recombinant A_{2B} adenosine receptors, expressed as K_i in nM (n = 3). [d]Displacement of specific [^{125}I]AB-MECA in HEK-293 cells transfected with the human recombinant A_3 adenosine receptors, expressed as K_i in nM (n = 3). [e]Displacement of specific [^{3}H]DPCPX in rat brain membranes, expressed as K_i in nM (n = 3). [f]Displacement of specific [^{3}H]SCH58261 in rat striatal membranes, expressed as K_i in nM (n = 3). [g]Not determined. [h]Displacement of specific [^{125}I]AB-MECA in CHO cells transfected with the rat recombinant A_3 adenosine receptors, expressed as K_i in nM (n = 3).

Figure 1. Structure and biological profile of selected compound **5**.

Scheme 1[a]



[a] (i) See Baraldi et al. *J. Med. Chem.* **1998**, *41*, 2126−2133.

cal structure allows an easy introduction of a methyl group by direct O-alkylation of the phenolic function with [^{11}C]CH_3I under alkaline conditions (Figure 1).

The aim of this study was to use [^{11}C]SCH442416 as a new ligand for the in vivo imaging of A_{2A} receptors using PET. This report describes the in vitro binding profile of "cold" SCH442416, the radiosynthesis of [^{11}C]SCH442416, and its in vivo evaluation in rats.

Results and Discussion. Compound **5** was prepared following the general synthetic strategy for the synthesis of the pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines previously reported,[14] starting from the 3-(4-methoxyphenyl)propyl hydrazine **6** (Scheme 1).

The reaction of (ethoxymethylene)malononitrile with **6** afforded the pyrazole **11**, which was then transformed to the corresponding imidate **12** by refluxing in triethyl orthoformate. The imidate was reacted with 2-furoic acid hydrazide in refluxing 2-methoxyethanol to provide the pyrazolo[4,3-*e*]pyrimidine intermediate **13** which, without any purification, was converted through a thermally induced cyclization in diphenyl ether to the derivative **14** in good yield. Treatment of **14** with dilute hydrochloric acid at reflux temperature induced pyrimidine ring opening to furnish the 5-amino-4-(1*H*-1,2,4-triazol-5-yl)pyrazole **15** in quantitative yield. This derivative was converted into the final compound **5** by reaction with an excess of cyanamide in 1-methyl-2-pyrrolidone at 140 °C. Purification procedures yielded **5** as a white solid (Scheme 2).

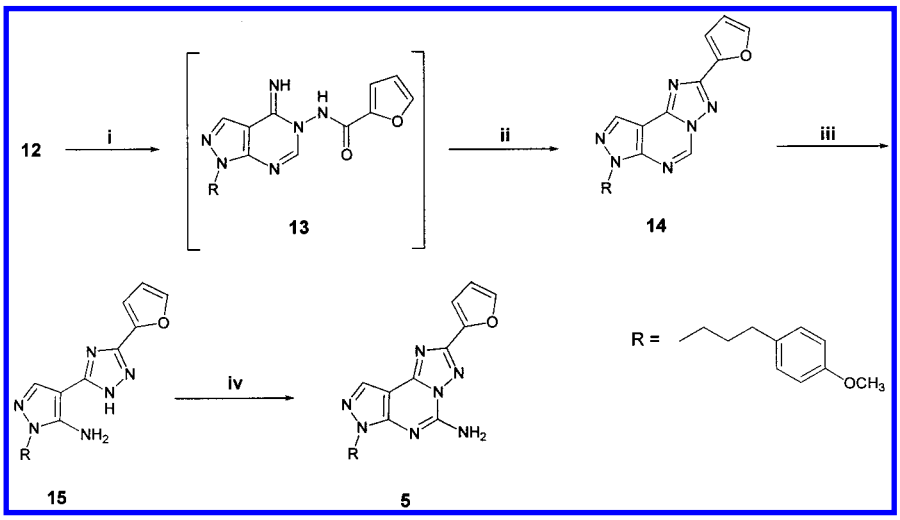
The in vitro binding profile of compound **5** was evaluated on rat cerebral membranes and on CHO human cells transfected with A_1 adenosine receptors and [^{3}H]DPCPX as a radioligandand, on HEK-293 cells transfected with A_{2A}, A_{2B}, and A_3 adenosine receptors using [^{3}H]SCH58261, [^{3}H]DPCPX, and [^{125}I]AB-MECA, respectively. Compound **5** showed high affinity and selectivity for both human and rat adenosine A_{2A} receptor subtypes. In particular, for the human A_{2A} adenosine receptor subtypes we found an in vitro affinity in the picomolar range, indicating that compound **5** is one of the most potent antagonists ever reported.

The synthesis of [^{11}C]SCH442416 (**7**) was carried out via the direct alkylation of compound **4** with [^{11}C]CH_3I. The labeled methyl iodide **8** was prepared starting from [^{11}C]CO_2 (obtained by irradiating a mixture of $^{14}N_2$ and 1% O_2 with a cyclotron proton beam of 11 MeV) which, after reduction with lithium aluminum hydride and subsequent hydrolysis with HI, afforded [^{11}C]CH_3I with high yield (80−90% decay corrected) (Scheme 3).[15]

Compound **7** was prepared by dissolving **4** in dimethylformamide, followed by the addition of 5 M NaOH as a base; **8** was bubbled into the reaction vessel in a helium stream at room temperature, and the mixture was allowed to react at 90 °C for 4 min. The final product **7** was purified by preparative HPLC and solid-phase extraction (SPE) techniques. The method proved reliable, and the overall synthesis lasted 40 min, thus minimizing the loss of activity due to decay.[16] The average radiochemical yield, calculated from the starting [^{11}C]CO_2 and decay corrected, was 28.7 ± 7.2% (n = 18); the specific activity of the final [^{11}C]SCH442416 ranged 400−3390 mCi/μmol, with a mean of 1490 ± 978 mCi/μmol (n = 18). This variability was probably due to the different quality of the lithium aluminum hydride/tetrahydrofuran solution, which is known to be a major source of "cold" CO_2 contamination. **7** was consistently obtained with radiochemical purity >96% and chemical purity >95%.

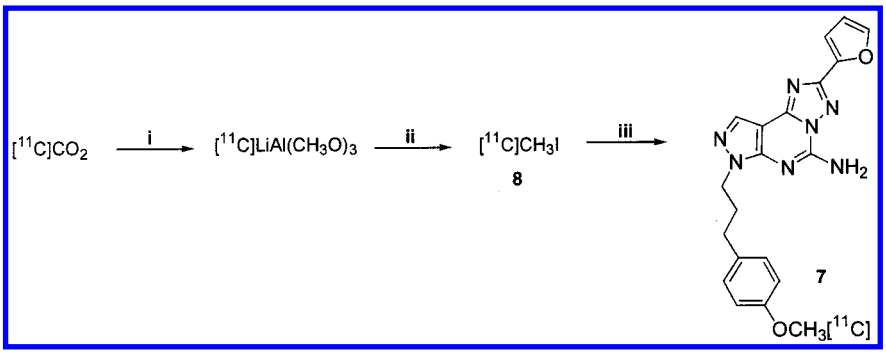
Biodistribution studies indicated that [^{11}C]SCH442416 preferentially accumulates not only in adrenal glands and kidneys, where A_{2A} receptors are highly expressed,

Scheme 2[a]



[a] Reagents: (i) 2-furoic hydrazide, $MeO(CH_2)_2OH$; (ii) Ph_2O, 260 °C, flash chromatography; (iii) HCl, reflux; (iv) NH_2CN, 1-methyl-2-pyrrolidone, pTsOH, 140 °C.

Scheme 3[a]



[a] Reagents: (i) LAH, THF; (ii) HI (57%), 145 °C; (iii) compound **4**, DMF, NaOH, 90 °C.

Table 1. Biodistribution of [^{11}C]SCH442416 in Rats

	% ID/g				
organs	5 min	15 min	30 min	60 min	90 min
blood	0.13 ± 0.01	0.09 ± 0.02	0.06 ± 0.01	0.03 ± 0.02	0.05 ± 0.01
plasma	0.19 ± 0.01	0.13 ± 0.02	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
heart	0.26 ±0.03	0.15 ± 0.02	0.09 ± 0.03	0.04 ± 0.01	0.04 ± 0.01
lung	0.38 ± 0.11	0.52 ± 0.21	0.20 ± 0.13	0.37 ± 0.28	0.23 ± 0.18
trachea	0.17 ± 0.03	0.12 ± 0.02	0.09 ± 0.05	0.04 ± 0.01	0.03 ± 0.01
liver	0.94 ± 0.17	0.74 ± 0.14	0.52 ± 0.06	0.36 ± 0.03	0.37 ± 0.08
adrenal	0.85 ± 0.33	0.44 ± 0.10	0.25 ± 0.09	0.13 ± 0.00	0.14 ± 0.03
kidney	0.69 ± 0.09	0.49 ± 0.09	0.24 ± 0.06	0.13 ± 0.02	0.11 ± 0.04
spleen	0.18 ± 0.03	0.12 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
testes	0.12 ± 0.02	0.13 ± 0.02	0.23 ± 0.27	0.03 ± 0.00	0.03 ± 0.00
intestine	0.37 ± 0.13	0.28 ± 0.07	0.25 ± 0.10	0.10 ± 0.02	0.16 ± 0.07
muscle	0.15 ± 0.04	0.11 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.02 ± 0.01

Table 2. Biodistribution of [^{11}C]SCH442416 in Rat Brain

	% ID/g				
brain areas	5 min	15 min	30 min	60 min	90 min
pituitary	0.18 ± 0.03	0.06 ± 0.01	0.05 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
hypothalamus	0.16 ± 0.01	0.07 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
hippocampus	0.17 ± 0.02	0.09 ± 0.02	0.04 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
striatum	0.43 ± 0.19	0.46 ± 0.10	0.20 ± 0.03	0.06 ± 0.01	0.04 ± 0.02
thalamus	0.20 ± 0.04	0.10 ± 0.02	0.05 ± 0.02	0.02 ± 0.00	0.02 ± 0.01
pons	0.20 ± 0.04	0.11 ± 0.02	0.06 ± 0.01	0.02 ± 0.00	0.02 ± 0.01
cerebellum	0.19 ± 0.04	0.10 ± 0.02	0.05 ± 0.01	0.02 ± 0.00	0.02 ± 0.01
anti-CTX	0.18 ± 0.03	0.12 ± 0.03	0.06 ± 0.02	0.02 ± 0.00	0.02 ± 0.00
post-CTX	0.19 ± 0.03	0.10 ± 0.02	0.05 ± 0.02	0.02 ± 0.01	0.02 ± 0.01

but also in lung and liver, reaching its maximum uptake 5 min after injection. Tracer accumulation was lower in the heart, where adenosine receptors are mainly represented by the A_1 subtype (Table 1). In the remaining organs radioactivity concentration ranged between 0.1% and 0.2% ID/g. When injected in rats with an intravenous pulse, **7** was rapidly cleared from plasma, at 15 min after tracer injection, radioactivity concentration being 0.13 ± 0.020% ID/g. The tracer preferentially distributed in plasma, as indicated by its plasma-to-blood ratio which was always >1 during the experimental time.

Our results clearly showed that [^{11}C]SCH442416 permeates the BBB and accumulates in the brain where the maximum uptake was reached between 5 and 15 min after injection. In agreement with the known regional distribution of A_{2A} adenosine receptors within the brain, **7** highly accumulated in the striatum, whereas in the remaining brain regions examined the tracer retention was definitely lower and fairly homogeneous (Table 2).

At the time of maximum uptake, the radioactivity concentration ratio between striatum (which is rich in A_{2A} adenosine receptors) and cerebellum (where the expression of A_{2A} adenosine receptors is low or negligible) was 4.6 ± 0.17. A similar distribution was observed on coronal brain slices obtained with a phosphor-imaging technique, indicating that **7** is mainly retained at the level of basal ganglia (Figure 2).

HPLC chromatograms of plasma extracts showed the presence of three main radioactive compounds: one

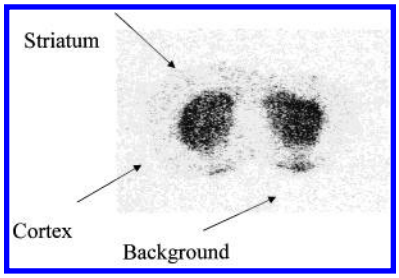


Figure 2. Coronal brain slice of radioactivity distribution in rat brain sampled at 15 min after the injection of [^{11}C]-SCH442416.

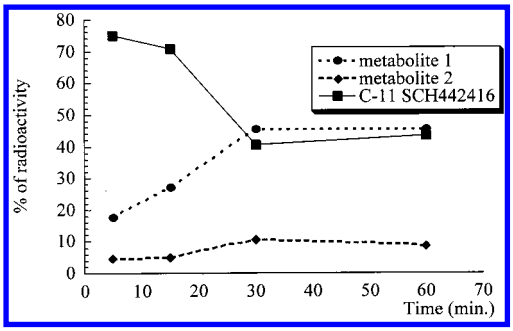


Figure 3. Time course of radioactive metabolites and parent compound measured in plasma of rats injected with [^{11}C]-SCH442416.

more lipophilic compound (t_R = 9.5 min) that corresponds to unmetabolized **7**, as confirmed by the HPLC chromatogram of the cold SCH442416 used as a reference standard, and two more hydrophilic compounds (t_R = 4.5 and 2.5 min, respectively) probably attributable to metabolites. The metabolism of [^{11}C]SCH442416 is slow, and the plasma concentration of the injected tracer accounted for more than 40% of total plasma activity after 60 min (Figure 3).

At the time of the higher cerebral uptake (15 min after injection), HPLC chromatograms of acetonitrile brain extracts demonstrated the presence of only one metabolite with a retention time similar to that of the first plasma metabolite (t_R = 2 min) which represented only 6% of the total extractable activity in the cerebellum and less than 1% in the striatum.

Conclusions. In conclusion, the regional distribution in brain and periphery, the good signal-to-noise ratio observed between 5 and 15 min after injection, and the low occurrence of radioactive metabolites all suggest that [^{11}C]SCH442416 is applicable as the first non-xanthine ligand suitable for the in vivo imaging of A_{2A} adenosine receptors using PET. In addition, the data obtained from the binding experiments showed a higher affinity of the title compound for human A_{2A} adenosine receptors vs rat receptors (0.048 nM vs 0.5 nM). Due to the lower K_i values found in human cell lines, a higher striatum-to-cerebellum ratio should be expected for the in vivo measurement of the A_{2A} receptor in human brain using PET.

Supporting Information Available: Experimental details. This information is available free of charge via the Internet at http://pubs.acs.org.

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