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Research Article

Synthesis, radio-LC-MS analysis and biological evaluation of ^{99m}Tc-techmazenil

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Summary

A ^{99m}Tc-labelled compound with the biological characteristics of flumazenil would be useful for determination of neuronal viability after the onset of a stroke. Therefore, we have derivatized Ro-15-3890 (a flumazenil metabolite bearing a carboxylic acid group instead of an ethyl ester) by coupling it with a bisamino bisthiol tetraligand bearing a 3-hydroxypropyl side chain (3-hydroxypropyl-BAT) to enable labelling with technetium-99m. After purification by RP-HPLC, the ligand was deprotected and labelled in a 'one pot' reaction, yielding a ^{99m}Tc-BAT-propylester of Ro-15-3890 (^{99m}Tc-techmazenil). Radio-LC-MS analysis of the isolated main peak showed the molecular ion mass (608.0618) of the expected ^{99m}Tc-techmazenil. The biodistribution of ^{99m}Tc-techmazenil was investigated in normal mice and indicated that the tracer is cleared from plasma mainly by the hepatobiliary system and shows a very low uptake in brain. *In vitro* binding studies on mice brain slices indicated that techmazenil does not bind to benzodiazepine receptors.

Key Words: flumazenil; radio-LC-MS; technetium-99m; stroke

Introduction

Therapeutic (i.e. thrombolytic) interventions in patients having suffered an acute ischemic stroke can only be successful if they are initiated when viable tissue still exists within the area affected by the blood flow disturbance. Aggressive treatment aimed at reperfusion may however be harmful if morphological integrity has not been preserved. Therefore, clear distinction between necrotic and potentially viable tissue, known as penumbra, is essential.[1,2]

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Although X-ray computed tomography (CT), T_2-weighed and diffusion weighed MRI are excellent tools to determine the full extent of an infarction at later time points (2–3 weeks after onset), they appear to be inadequate in the early hours after stroke onset.[3,4] At present, a complex multitracer study, requiring quantitative measurements of blood flow and oxygen consumption by positron emission tomography (PET), is the only reliable method to detect irreversible damage in an early stage.[5,6] Due to the short half-life of the PET isotopes, this technique is difficult to apply in an acute setting. Therefore, a neuronal viability marker, labelled with technetium-99m, allowing distinction between necrotic and ischemic tissue in an acute setting would be helpful.

Because of their abundance in the cortex and their sensitivity to ischemic damage, visualization of the distribution of γ-aminobutyric acid (GABA) receptors can be used as an indicator of neuronal integrity.

It has been suggested that radiolabelled central benzodiazepine receptor ligands can detect neuronal damage since they mark intact cortical neurons.[1,2,7–11] Several studies using ^{11}C-flumazenil have shown that reduction of flumazenil binding is proportional to the actual infarction size as determined by later MRI-scans.[7–9,11] The main advantage of the carbon-11 label is preservation of the chemical structure and the biological characteristics of flumazenil. Unfortunately the short half-life of ^{11}C and the need for a cyclotron and a PET-camera for production and visualization of these derivatives limit the use of ^{11}C-flumazenil in an acute clinical setting.

On the other hand iodine-123 labelled iomazenil has been proposed and used for the same purpose.[12] ^{123}I has several advantages such as its half-life of 13 h and SPECT-camera visualization. However, clinical routine use of this tracer agent is limited due to its high cost and its non-continuous availability.

A number of fluorescent derivatives of flumazenil have been described in the literature. These molecules showed high affinity for the benzodiazepine receptor in spite of the introduction of a bulky fluorescent group.[13] This indicates that conjugation of flumazenil with a technetium-99 m chelate (e.g. a neutral ^{99m}Tc-bisamino bisthiol complex, ^{99m}Tc-N_2S_2 or ^{99m}Tc-BAT) could be feasible without loss of affinity for the benzodiazepine receptor. Bearing in mind the availability and relatively low cost of technetium-99m and the high resolution visualization of ^{99m}Tc-tracers by widely available SPECT-cameras, a technetium-99m labelled compound with the biological characteristics of flumazenil would be an interesting alternative for *in vivo* visualization of the benzodiazepine receptors in an acute setting analogous to the application of ^{99m}Tc-ECD for the visualization of epileptic foci.[14]

The aim of this study was the synthesis and the biological evaluation in mice of ^{99m}Tc-techmazenil (Figure 1), a ^{99m}Tc-BAT-propyl ester of Ro-15-3890, the acid derivative of flumazenil.

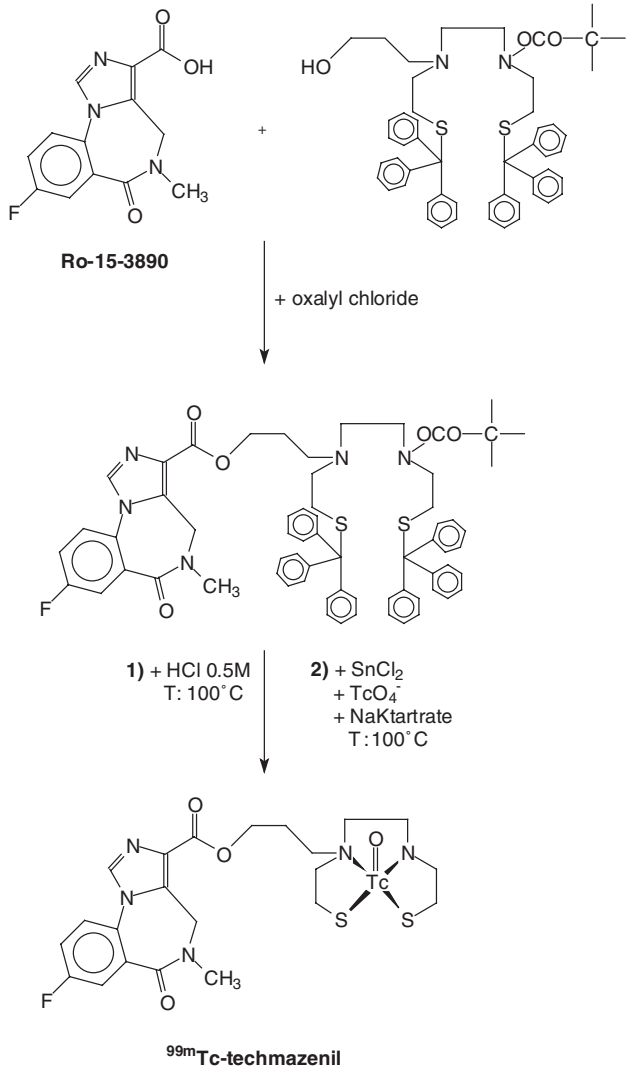

Figure 1. Synthesis of *bis*-*S*-trityl-*N*-Boc-*N*′-(3-propyl)-BAT ester of Ro-15-3890 and ^{99m}Tc-techmazenil (proposed structure)

Results and discussion

To enable the preparation of a conjugate of a flumazenil derivative such as Ro-15-3890 (Figure 1) with a neutral ^{99m}Tc-chelate such as a ^{99m}Tc-BAT complex, a hydroxypropyl group was introduced on one of the amines, yielding bis-*S*-trityl-*N*-Boc-*N*′(3-hydroxypropyl)-BAT.

Several methods were explored for esterification of Ro-15-3890 with bis-*S*-trityl-*N*-Boc-*N*′-(3-hydroxypropyl)-BAT. In a first method, the protected hydroxypropyl substituted BAT was reacted with Ro-15-3890 in the presence of methanesulfonylchloride. However, analysis using thin layer

chromatography (TLC) and mass spectrometry (MS) did not show the desired esterified product (results not shown).

In another approach, addition of the protected BAT-propanol after activation of desethylflumazenil (Ro-15-3890) using DCC and DMAP was attempted. The desired product was obtained in very low yields (5%).

The best results were obtained by conversion of Ro-15-3890 to the acid chloride using oxalyl chloride on a disposable solid phase column followed by reaction with the protected hydroxypropyl-BAT in CH_2Cl_2 (20% yield).

Labelling of the conjugate with technetium-99 m was achieved after deprotection of the thiol groups and the BOC-protected amines using a mixture of trifluoroacetic acid, anisole and methanesulfonic acid. The deprotected conjugate was then isolated, purified and stored for labelling. Alternatively, a more convenient method was a one-pot two-step reaction to deprotect and radiolabel the *S*,*S*′-bis-trityl-*N*-Boc protected conjugate.[15] In a first step the protected conjugate was heated under acidic conditions in a boiling water bath to obtain the deprotected complex which was subsequently labelled with technetium-99m in a second heating step after addition of solutions of phosphate buffer pH 7, NaKtartrate, $SnCl_2$ and $^{99m}TcO_4^-$. Using this one-pot method, the ^{99m}Tc-techmazenil complex was obtained in a radiochemical yield of 80%, which is comparable to the yields of the more traditional two-pot labelling procedure.

Confirmation of the identity of ^{99m}Tc-techmazenil was obtained using liquid chromatography in combination with mass spectrometry using a radiometric detector (radio-LC-MS). A preparation reconstituted using an eluate with a relatively high technetium content (Monday morning eluate, containing 0.07 ng Tc per GBq) was purified by reversed phase high performance liquid chromatography (RP-HPLC). Radio-LC-MS analysis of the isolated main peak showed the molecular ion mass of the expected ^{99m}Tc-techmazenil complex (608.0618 Da, Figure 2) on the mass spectrometer channel at the time of elution of a peak in the radiometric channel (retention time (R_t): 14 min) with a relative error of 10 ppm.

Before proceeding to animal testing of ^{99m}Tc-techmazenil, a number of biological parameters were determined. In the literature, a parabolic relationship is suggested for the passive diffusion of neutral molecules into the central nervous system (CNS) with an optimal passage over the blood–brain barrier found for compounds with a logarithm of the partition coefficient in 1-octanol/water ($\log P_{oct}$) of about 2. The $\log P_{oct}$ of ^{99m}Tc-techmazenil is 1.49, compatible with reasonable brain uptake. In addition, the logarithm of the partition coefficient in cyclohexane/water ($\log P_{cyh}$) was found to be -2.51. From these values, $\Delta \log P$, defined as $\log P$ (1-octanol/water) $-$ $\log P$ (cyclohexane/water) and related to the overall hydrogen-bonding ability, was calculated and found to be 4.0, which predicts a brain/blood ratio in rat

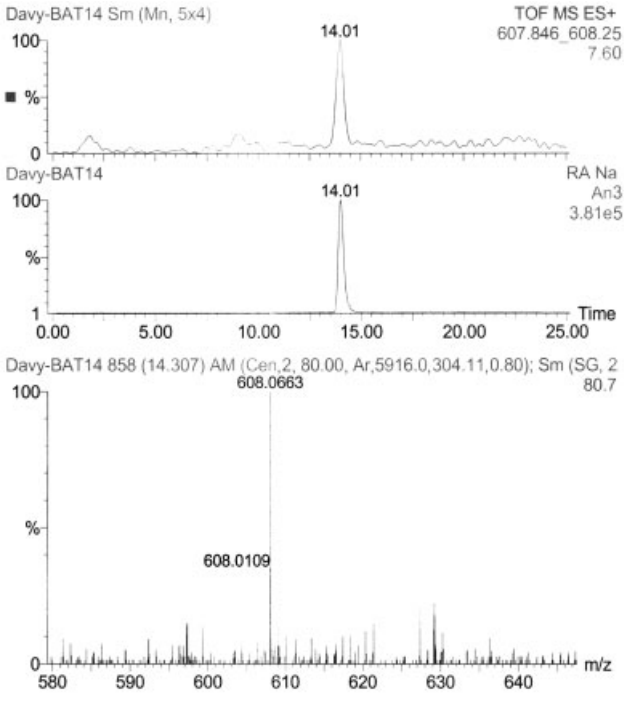

Figure 2. Radio-LC-MS analysis of Tc-techmazenil (A) Single mass chromatogram (607.846–608.250): peak with R_t: 14.01′. (B) Radiometric channel: peak with identical R_t as peak in A. (C) Experimental mass spectrum (relative error: 7.4 ppm)

brain of 0.065 according to the formula: log $(C_{brain}/C_{blood}) = -0.604*\Delta \log P + 1.23$.[16]

The biological affinity for the benzodiazepine receptor was assessed by *in vitro* autoradiography on mice brain sections after incubation with the tracer agent. Pre-incubation was the same in all the experiments, but two different incubation times (30 and 40 min) and three different wash times (15, 30 and 60 s) were assessed. However, for all tested conditions no binding of ^{99m}Tc-techmazenil to a brain structure was found. This was unexpected in view of the affinity for benzodiazepine receptors observed for conjugates of Ro-15-3890, which were esterified via the same carboxyl group with bulky fluorescent groups.[13]

Biodistribution studies in normal mice showed no uptake of ^{99m}Tc-techmazenil in the brain at 10 and 60 min p.i. (respectively 0.05 and 0.03% of injected dose (ID), Table 1). Furthermore, extensive hepatobiliary excretion (68.1% of ID in liver and intestines at 60 min p.i.) was observed. The absence of significant radioactivity in the stomach, even after 60 min, indicates that no pertechnetate is reformed *in vivo*.

Table 1. Biodistribution of ^{99m}Tc-TECHMAZENIL in mice

	% of ID ($n=4$)		% of ID/g ($n=4$)	
	10 min p.i. mean ± sd	60 min p.i. mean ± sd	10 min p.i. mean ± sd	60 min p.i. mean ± sd
Urine	0.1 ± 0.1	5.6 ± 0.8	—	—
Kidneys	13.5 ± 1.3	1.3 ± 0.5	20.4 ± 2.2	1.9 ± 0.8
Liver	45.1 ± 3.9	18.9 ± 3.2	17.8 ± 2.8	8.1 ± 0.9
Lungs	0.7 ± 0.1	0.1 ± 0.0	2.2 ± 0.6	0.4 ± 0.0
Heart	0.4 ± 0.1	0.0 ± 0.0	2.3 ± 0.3	0.2 ± 0.1
Intestines	14.2 ± 4.0	68.1 ± 3.8	3.4 ± 0.6	18.5 ± 3.7
Stomach	0.6 ± 0.4	0.9 ± 0.2	0.9 ± 0.8	1.5 ± 0.4
Cerebrum	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0
Cerebellum	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.0	0.1 ± 0.1
Blood	4.9 ± 0.7	0.9 ± 0.7	1.7 ± 0.2	0.3 ± 0.1

The low brain uptake of ^{99m}Tc-techmazenil is surprising, in view of its molecular weight of 608 the fact that a molecular weight limit and between 400 and 700 is accepted for significant entry into the brain.[16]

Experimental

Materials

Ro-15-3890 (desethylflumazenil) was a gift from Hoffman-La Roche (Basel, Switzerland). All other reagents were ACS or HPLC grade and purchased commercially. $Na^{99m}TcO_4$ was obtained from a $^{99}Mo/^{99m}Tc$ generator (Ultratechnekow FM, Mallinckrodt, Petten, The Netherlands).

^{1}H-NMR spectra were recorded using a Varian 200 MHz spectrometer (Varian, Palo Alto, CA, USA). Chemical shifts are reported in ppm relative to the internal standard tetramethylsilane (TMS, $\delta = 0$).

Reversed phase HPLC was carried out using a system consisting of a Merck Hitachi L-7100 separation module connected to a RP C18 column (Xterra™ RP18 5 µm 250 mm × 4.6 mm, Waters Milford, USA). The column eluate was analysed using a radiometric detector (3-in NaI(Tl) detector connected to a radiation analyser module, Canberra Packard, Meriden, Conneticut, USA).

The radio-LC-MS system consisted of a Waters 2690 separation module connected to a RP C18 column (XTerra™ MS C18 3.5 µ 2.1 mm × 50 mm). The column eluate was monitored for radioactivity using a radiometric detector (3-in NaI(Tl) detector connected to a radiation analyser module, The Nucleus, Oak Ridge, USA). Finally the column eluate was directed to a time-of-flight mass spectrometer (Micromass LCT, Waters) equipped with an orthogonal ESI probe.

For the *in vitro* autoradiography studies a phosphor imaging system was used (Cyclone^{TM}, Canberra Packard).

S,S′-bis-trityl-N-Boc-N′-(3-hydroxypropyl)-1,2-ethanedicysteamine (S,S′-bis-trityl-N-Boc-N′-(3-hydroxypropyl)BAT)

S,S′-bis-trityl-*N*-Boc-BAT, (3.53 g; 4.6 mmol) was synthesized according to the described method[17] and was dissolved in a mixture of 20 ml acetonitrile and 1.9 ml diisopropylethylamine. 3-Bromo-1-propanol (1.4 g; 10 mmol) was added and the mixture was refluxed overnight. After solvent and organic base evaporation, the residue was dissolved in 20 ml CH_2Cl_2. After several consecutive washing steps using respectively water, 0.1 M citric acid, 1 M sodium bicarbonate and saturated saline solution, the organic layer was dried using magnesium sulphate and evaporated.

Purification was performed with column chromatography using a solvent system of hexane–ether–triethanolamine (50–45–5) to yield 35% of a colourless, viscous oil.

^{1}H-NMR ($CDCl_3$) δ 1.38 (s, 9 H, $C(CH_3)_3$), 1.48 (m, 2 H, $CH_2CH_2CH_2OH$), 2.2–2.4 (m, 10H, $CH_2NHCH_2 + 2 \times SCH_2CH_2N$, $NCH_2CH_2CH_2OH$), 2.93 (m, 4 H, $CH_2NBocCH_2$), 3.61 (m, 2H, CH_2OH), 7.2–7.4 (m, 30 H, ArH).

MS ESI+: m/z $(M+H)^+$ found 823 (calcd, $C_{52}H_{58}N_2O_3S_2$, 823).

Bis-S-trityl-N-Boc-N′-(3-propyl)-BAT ester of Ro-15-3890

Ro-15-3890 (27.5 mg; 0.1 mmol), was dissolved in 5 ml 0.5 M phosphate buffer pH 8. This solution was applied on a disposable PRP-column (Chromafix HR-P 700 mg, Macherey-Nagel, Düren, Germany) after which it was rinsed with water (10 ml). The column was dried for 1 h with a flow of N_2 gas (1 bar). A vial containing oxalyl chloride (100 µl, 1 mmol) was inserted between the N_2 source and the PRP column. Again, a N_2 flow of 1 bar was applied for 1 h after which the column was eluted with 5 ml of CH_2Cl_2. The solvent, along with the excess of oxalyl chloride, was evaporated. *S,S′*-bis-trityl-*N*-Boc-*N′*-(3-hydroxypropyl)BAT (82.3 mg; 0.1 mmol), dissolved in a mixture of 5 ml of CH_2Cl_2 and 15 µl of pyridine was added to the residue. The mixture was stirred for 2 days after which it was purified using RP-HPLC, using as the mobile phase a mixture of acetonitrile, tetrahydrofuran and 0.1 M ammonium acetate (76.5:8.5:15), eluted in an isocratic way at a flow of 1 ml/min. The appropriate fraction was evaporated under reduced pressure and the residue was led over a Sep-Pak C18 high capacity cartridge (Alltech, Deerfield, Illinois, USA) that was then rinsed with 50 ml water. Afterwards, the product was eluted from this column using 50 ml pure MeOH (3 times) and a slightly yellow oil was obtained after evaporation (yield: 15%).

MS ESI+: *m/z* $(M+H)^+$ found 1080 (calcd, $C_{65}H_{66}FN_5O_5S_2$, 1080).

^{99m}Tc-BAT-propyl ester of Ro-15-3890 (^{99m}Tc-techmazenil, Figure 1)

1 Mg of protected 3-propyl-BAT ester of Ro-15-3890 was dissolved in 1 ml ethanol and this solution was added to a labelling vial containing 0.5 ml HCl 0.1 M. The solution was heated for 10 min in a boiling water bath. The mixture was neutralized using 1 ml of phosphate buffer 0.5 M pH 7 before addition of NaKtartrate (1 mg), $SnCl_2$ (100 µg in 25 µl HCl 0.1 M) and generator eluate (370 MBq ^{99m}Tc in the form of pertechnetate in 1 ml). The vial was heated again for 10 min in a boiling water bath.

The labelling reaction mixture was analysed and purified using RP-HPLC on an Xterra^{TM} RP_{18} column (5 µm, 4.6 mm × 250 mm) which was eluted at a flow rate of 1 ml/min using a mixture of CH_3CN and 0.1 M NH_4OAc (65:35).

Radio-LC-MS analysis

To enable accurate mass calculations, the column eluate was mixed with a lock mass solution (flumazenil 0.01 mg/ml) infused at a flow rate of 5 µl/min. The mass difference between the theoretical mass and the measured accurate mass of ^{99m}Tc-techmazenil was divided by the theoretical mass, yielding the relative error expressed as ppm. The HPLC column was eluted at a flow rate of 300 µl/min with linear gradient mixtures of CH_3CN and 0.1 M NH_4OAc ($t = 0$ min 0% CH_3CN; $t = 20$ min 90% CH_3CN V/V).

Determination of the partition coefficients: log P_{oct}, log P_{cyh} and Δ log P

To a test tube containing 2 ml phosphate buffer (0.025 M, pH 7.4) and 2 ml 1-octanol, 25 µl of HPLC purified ^{99m}Tc-techmazenil solution was added. The test tube was shaken for 5 min, followed by centrifugation for 10 min at 6000 rpm (Centrifuge 4226, ALC, Milano, Italy). Finally 50 mg of the octanol phase and 500 mg of the water phase was withdrawn and the radioactivity of both samples was measured using a gamma counter equipped with a 3-in. NaI(Tl) scintillation detector (1480 Wizard 3″, Wallac, Finland). The results were used to calculate the $\log P_{\mathrm{oct}}$. This experiment was performed in triplicate.

A similar procedure was followed for the determination of $\log P_{\mathrm{cyh}}$ using cyclohexane instead of 1-octanol. The $\Delta \log P$ value was calculated by subtraction of $\log P_{\mathrm{cyh}}$ from $\log P_{\mathrm{oct}}$.

In vitro autoradiography

For determination of the binding affinity of ^{99m}Tc-techmazenil for the benzodiazepine receptor, a male NMRI mouse was sacrificed by decapitation; the brain was removed and quick-frozen in isopentane cooled over dry ice to −40°C. Twenty-micrometer sections were cut at −20°C and thaw-mounted

onto gelatine-coated slides, desiccated in a stream of air and frozen at −80°C until processing.

Tissue sections were pre-incubated for 10 min at room temperature in Tris–HCl buffer (50 mM, pH 7.4) to remove any endogenous ligand. Sections were then incubated at room temperature with 300 µl of ^{99m}Tc-techmazenil solution (3.7 kBq/ml) for 30 or 40 min. Three different intervals of rising in distilled water were assessed (15, 30 and 60 s) after which the sections were exposed to a high performance storage phosphor screen (Canberra, Packard). After 24 h, the screen was analysed using a phosphor imaging system.

Biodistribution in mice

RP-HPLC purified ^{99m}Tc-techmazenil (15 kBq/100 µl) was injected in 8 normal male NMRI mice (body mass 30–40 g) via a tail vein. Four of them were sacrificed at 10 min p.i. and the other four at 60 min p.i. and the organs and body parts were dissected and weighed. The activity in the dissected organs and body parts was measured using a gamma counter. Results are expressed as percentage of injected dose (% of ID) and percentage of injected dose per gram (% of ID/g). For calculation of total blood radioactivity, blood mass was assumed to be 7% of the body mass.[18]

Conclusion

The formation of the ester of *S*,*S*′-bis-trityl-*N*-Boc-*N*′-(3-hydroxypropyl)BAT with desethylflumazenil (Ro-15-3890) was accomplished by means of solid phase activation of Ro-15-3890 using oxalyl chloride. The traditional two-pot two-step labelling method was simplified to a one-pot two-step labelling method with similar high yields of ^{99m}Tc-techmazenil. The identity of the expected Tc-complex was confirmed using radio-LC-MS.

Although physicochemical parameters of this Tc-complex ($\log P_{oct}$, $\log P_{cyh}$ and $\Delta \log P$) were found to be compatible with brain uptake, *in vivo* biodistribution studies in mice indicated absence of brain uptake. This is probably due to the rather large size of techmazenil. Unexpectedly, *in vitro* binding studies with mice brain slices showed no affinity of ^{99m}Tc-techmazenil for the benzodiazepine receptors.

In view of these negative results, ^{99m}Tc-techmazenil is not useful for the *in vivo* visualization of cerebral benzodiazepine receptors.

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References

1. Heiss WD, Graf R, Wienhard K. *Cerebrovasc Dis* 2001; **11**: 73–81.

2. Heiss WD, Graf R, Löttgen J, Ohta K, Fujita T, Wagner R, Grond M, Wienhard K. *J Cerebr Blood F Met* 1997; **17**: 388–400.
3. Kummer Rv, Meyding-Lamade U, Forsting M, Rosin L, Rieke K, Hacke W, Sartor K. *Am J Neuroradiol* 1994; **15**: 9–15.
4. Warach S, Gaa J, Siewert B, Wielopolski P, Edelman RR. *Ann Neurol* 1995; **37**: 231–241.
5. Baron JC, Rougemont D, Soussaline F, Bustany P, Crouzel C, Bousser MG, Comar D. *J Cerebr Blood F Met* 1984; **4**: 140–149.
6. Baron JC. *Cerebrovasc Dis* 1999; **9**: 193–201.
7. Heiss WD, Grond M, Thiel A, Ghaemi M, Sobesky J, Rudolf J, Bauer B, Wienhard K. *Stroke* 1998; **29**: 454–461.
8. Heiss WD, Kracht L, Grond M, Rudolf J, Bauer B, Wienhard K, Pawlik G. *Stroke* 2000; **31**: 366–369.
9. Heiss WD, Kracht LW, Thiel A, Grond M, Pawlik G. *Brain* 2001; **124**: 20–29.
10. Heiss WD, Graf R, Fujita T, Ohta K, Bauer B, Löttgen J, Wienhard K. *Stroke* 1997; **28**: 2045–2051.
11. Thiel A, Löttgen J, Grond M, Pietrzyk, Heiss WD. *J Comput Assist Tomo* 2001; **25**: 446–451.
12. Nakagawara J, Sperling B, Lassen NA. *Stroke* 1997; **28**: 124–132.
13. Janssen MJ, Hulst R, Kellogg RM, Hendriks MM, Ensing K, De Zeeuw A. *Pharmazie* 2000; **55**: 42–48.
14. Oliveira AJ, da Costa JC, Hilario LN, Anselmi OE, Palmini A. *Epilepsia* 1999; **40**: 693–702.
15. Cleynhens BJ, Bormans GM, Vanbilloen HP, Vanderghinste DV, Kieffer DM, de Groot TJ, Verbruggen AM. *Tetrahedron Lett* 2003; **44**: 2597–2600.
16. Young RC, Mitchell RC, Brown TH, Ganellin CR, Griffiths R, Jones M, Rana KK, Saunders D, Smith IR, Sore NE, Wilks TJ. *J Med Chem* 1998; **31**: 656–671.
17. Bormans G, Cleynhens B, de Groot TJ, Mortelmans L, Moretti JL, Verbruggen A. *J Label Compd Radiopharm* 2003; **46**: 575–585.
18. Fritzberg AR, Whitney W, Kuni C, Klingensmith W. *Int J Nucl Med Biol* 1982; **9**: 79–82.

