JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Labelled Cpd Radiopharm 2001; **44**: 849–858.
DOI: 10.1002/jlcr.508

Research Article

Synthesis and in vitro evaluation of iodine labelled pyrazolo[1,5-*a*]pyridines as highly selective dopamine D4 receptor ligands

O. Prante[1,*], S. Löber[2], H. Hübner[2], P. Gmeiner[2] and T. Kuwert[1]
[1] *Clinic of Nuclear Medicine, Friedrich-Alexander University, D-91054 Erlangen, Germany*
[2] *Department of Medicinal Chemistry, Emil Fischer Center, Friedrich-Alexander University, D-91052 Erlangen, Germany*

Summary

Two ^{131}I-labelled radioligands, namely 3-(4-[^{131}I]iodophenylpiperazin-1-ylmethyl)-pyrazolo[1,5-*a*]pyridine and 3-(4-chlorophenylpiperazin-1-ylmethyl)-7-[^{131}I]iodopyrazolo[1,5-*a*]pyridine, which are required for studies or binding of these ligands to the D4 receptor, have been synthesized in 80% and 32% radiochemical yields, respectively; the radiochemical purity in each case was $>97\%$. For the second radioligand a kit preparation, based on iododestannylation followed by solid-phase extraction, was possible. *In vitro* characterization using CHO-cells expressing different dopamine receptor subtypes gave K_i values of 3.1 and 2.6 nM. Both radioligands are highly selective for the D4 subtype as compared to other dopamine subtypes. Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: dopamine D4 receptor; Pyrazolo[1,5-*a*]pyridine

*Correspondence to: O. Prante, Universität Erlangen-Nürnberg, Nuklearmedizinische Klinik mit Poliklinik, Teílbereich Kopfklinik, Schwabachanlage 6, 91054 Erlangen, Germany.
E-mail: olaf.prante@nuklear.imed.uni-erlangen.de

Contract/grant sponsor: ELAN-Fonds of the Friedrich-Alexander University Erlangen-Nürnberg.

Received 21 May 2001
Revised 25 June 2001
Accepted 10 July 2001

Introduction

The non-invasive investigation of neurotransmission by single photon emission computer tomography (SPECT) or positron emission tomography (PET) is a very important tool for understanding the neurochemical basis and pathology of neuropsychiatric diseases.[1] The D4 subtype of the dopamine receptors has been recently cloned;[2] its precise function and exact distribution in the central nervous system are of great interest, as disturbances of this receptor subtype have been implicated in the genesis of a broad range of psychotic disorders such as schizophrenia.[3,4] Moreover, an enhanced D4 receptor density has been found in postmortem brain tissue of schizophrenic patients; this, however, has not been supported by other studies.[5–7] These discrepancies have been interpreted in terms of a lack of an effective dopamine D4 receptor radioligand with high affinity and selectivity relative to other dopamine receptors. Currently, dopamine receptor concentrations *in vitro* can only be estimated indirectly by using complex binding assays involving subtraction of a number of binding sites defined with [^{3}H]raclopride (D2/D3 antagonist) from the total binding ([^{3}H]nemonapride) and the use of masking agents for other non-specific sites.[8,9] Recently, ^{11}C-labelled compounds such as [^{11}C]SDZ GLC 756,[10] a substituted methoxybenzamide[11] or SB-235753[12] have been synthesized for the exploration of D4 receptor density *in vivo* by PET; however, none has proved suitable, due to lack of specificity or undesirable pharmacological properties.

Recently, Löber *et al*. have described azaindole derivatives with high affinity for the dopamine D4 receptor.[13] As a result of this work, the highly potent dopamine D4 receptor ligand FAUC 113 was found to have superior subtype selectivity when compared to the corresponding indole derivative. The aim of the present study was the radiosynthesis of iodine labelled azaindole derivatives using FAUC 113 as lead compound in order to overcome the lack of a selective D4-radioligand for SPECT (Figure 1). Here we present the radiosynthesis of piperazinylmethyl-substituted pyrazolo[1,5-*a*]pyridines as a novel class of highly selective D4 receptor radioligands. This work includes the radiosyntheses and *in vitro* characterization of 3-(4-[^{131}I]iodophenylpiperazin-1-ylmethyl)-pyrazolo[1,5-*a*]pyridine (**[^{131}I]1**) and 3-(4-chlorophenylpiperazin-1-ylmethyl)-7-[^{131}I]iodopyrazolo [1,5-*a*]pyridine (**[^{131}I]2**) as model compounds for their ^{123}I-labelled analogues. We also

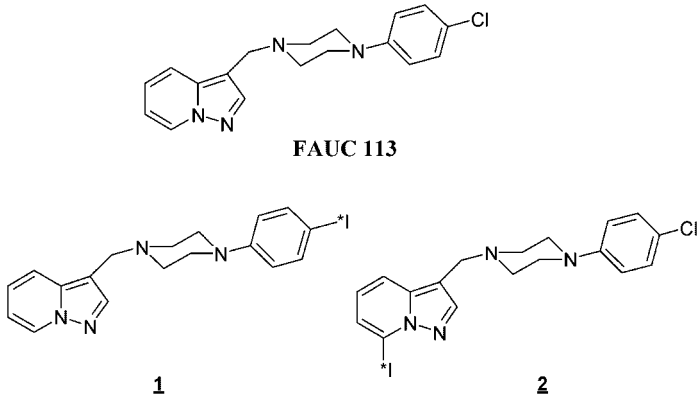
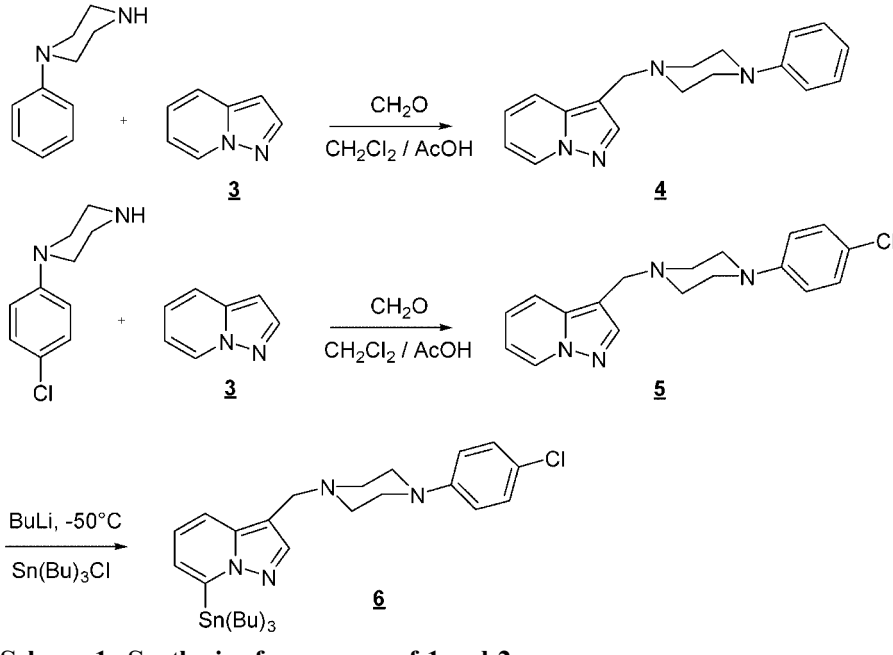


Figure 1. Structures of the various compounds

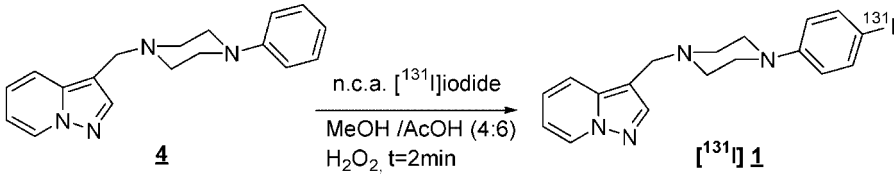
developed a synthesis for two precursor substances allowing a regiospecific iodine-labelling procedure.

Results and discussion

The precursor synthesis was carried out as depicted in Scheme 1. The pyrazolo[1,5-*a*]pyridine framework (**3**) was synthesized by 1,3-dipolar



Scheme 1. Synthesis of precursors of 1 and 2



Scheme 2. Radiosynthesis of 3-(4-[^{131}I]iodophenylpiperazin-1-ylmethyl)-pyrazolo[1,5-*a*]pyridine (1)

cycloaddition followed by decarboxylation as described in paper[13] and references cited therein. Precursor **4** was prepared directly from the readily available phenylpiperazine and **3** by *Mannich* reaction. Compound **4** was obtained after purification by flash chromatography (EtOAc) in 76% yield. Following this pathway, coupling of **3** with 4-chlorophenylpiperazine yielded compound **5** easily after crystallization (ethanol) in 82% yield. Recently, we synthesized various 7-substituted pyrazolo[1,5-*a*]pyridines starting from the corresponding 7-iodo derivatives.[14]

Here we have used this methodology for the direct regiodirected deprotonation of **5** in 7-position and subsequent stannylation of the intermediate by $ClSnBu_3$. This reaction procedure yielded the stannane precursor **6** after flash chromatography in excellent yield (92%). Unlabelled **1**, which was used as standard substrate for radio-HPLC, was prepared as previously described.[13] The standard for derivative **2** was prepared using a standard literature procedure using reaction of **6** and I_2 in THF.

The radiosynthesis of ^{131}I-labelled **1** was performed with hydrogen peroxide (3%) as oxidant using 7.5 mM of **4** in methanol/acetic acid (4:6, v/v) as reaction medium (Scheme 2). The radiochemical yield after a minimized reaction period (1 min) was determined to be 80% yielding the *para*-iodophenylpiperazinylmethyl substituted product exclusively (HPLC).

As shown in Table 1 and Figure 2, other solvent systems and oxidants such as trifluoromethane sulfonic acid/NCS, which was evaluated by Mennicke *et al.*,[14] resulted in decreased radiochemical yields of about 20%. For an optimized incorporation of [^{131}I]iodide, the precursor concentration should not be less than 7.5 mM (Figure 2). After semi-preparative HPLC purification, **[^{131}I]1** was obtained in >97% radiochemical purity. The 7-tributylstannyl substituted compound **6** was labelled with [^{131}I]iodine using either chloroamine-T or hydrogen

Table 1. Effect of the solvent system on the radiochemical yield (RCY) of 1

Reaction medium	RCY (%)
TFA	7 ± 4
MeOH/H_2O	9 ± 3
TFMSA/NCS	19 ± 4
MeOH/AcOH (9:1)	34 ± 1
MeOH/AcOH (4:6)	61 ± 3

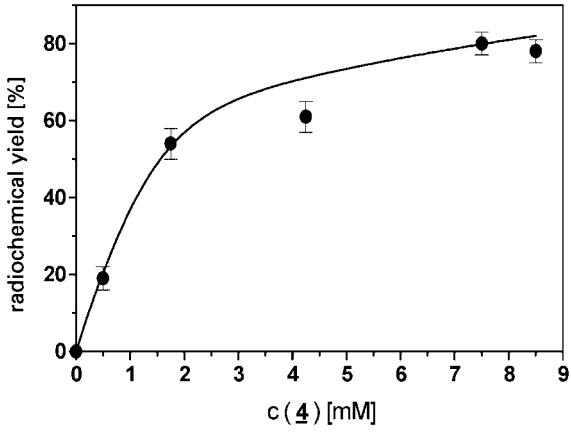
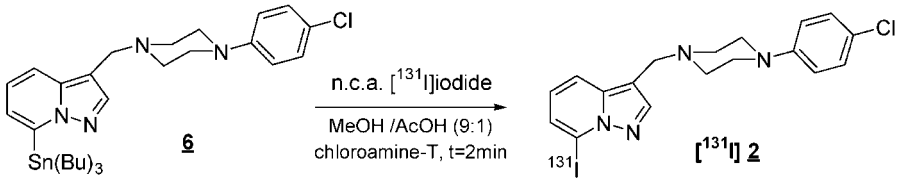


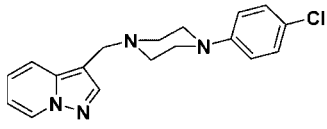
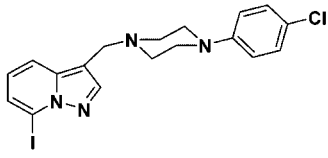
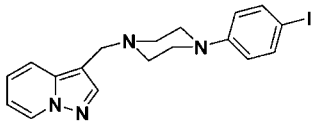
Figure 2. Precursor dependence of the radiochemical yield (RCY) of 3-(4-[^{131}I]iodophenylpiperazin-1-ylmethyl)-pyrazolo[1,5-*a*]pyridine (1) ($V = 110\,\mu l$, H_2O_2 (3%), MeOH/AcOH (4 : 6), $t = 2$ min, n.c.a. [^{131}I]iodide)



Scheme 3. Radiosynthesis of 3-(4-chlorophenylpiperazin-1-ylmethyl)-7-[^{131}I]iodopyrazolo[1,5-*a*]pyridine (2)

peroxide as oxidants (Scheme 3). The use of hydrogen peroxide leads to the formation of 5 as a by-product. However, after the labelling procedure assisted by chloroamine-T, compound 5 was not detected by HPLC. By comparison to the radiosynthesis of **[^{131}I]1**, ^{131}I-labelled 2 can be isolated by solid-phase extraction (Sep-Pak^{TM} C-18

Table 2. K_i values of highly selective D4 receptor ligands containing the pyrazolo [1, 5-*a*]pyridine framework ((*) bovine D2 receptor))

	K_i-values (nM)	bD1	hD2	hD3	hD4.4
FAUC 113 (13)		12000	3200	5000	3.6
2 (this work)		2400	7600	1900	2.6
1 (13)		17000	1400(*)	6700	3.1

plus-cartridge) with physiological saline solution (5% ethanol). After a reaction period of 2 min, pure **[^{131}I]2** was isolated in a radiochemical yield of 32% under the reaction conditions described here (Scheme 3).

For receptor binding studies we used compounds **1** and **2** and CHO cells expressing human dopamine D2, D3 and D4.4 receptors with [^{3}H]spiperone and bovine striatal membrane preparations containing D1 receptors with [^{3}H]SCH 23390 for competition binding analysis[16–19] in order to determine receptor affinity and subtype selectivity. Here, we compare K_i values determined for the cold compound **2** to the binding characteristics of FAUC 113 and to those of compound **1** which were already published.[13] The results are presented in Table 2. For compound **2**, D1 and D3 binding is increased in comparison to the lead compound FAUC 113, but D4/D2 selectivity is reduced. The iodine substituent in the 7-position of the pyrazolo[1,5-*a*]pyridine moiety is tolerated by D4-receptors without substantial loss of D4-selectivity and affinity (cf. dissertation[20]).

Conclusion

Considering the promising K_i values of **1** (3.1 nM) and **2** (2.6 nM) and their high selectivities over other dopamine receptors *in vitro*, we plan to further characterize both radioligands *in vitro* and *in vivo* by

autoradiography using rat brain slices. Following the *in vitro* characterization, biodistribution and metabolism studies for **1** and **2** are planned. Furthermore, chemical studies in order to develop an efficient ^{18}F-labelled radioligand with azaindole substructure are in progress in our laboratory.

Experimental

Materials and methods

All reagents were obtained from commercial sources. Na^{131}I, product code IBSSO, was obtained from Amersham Buchler (Braunschweig, Germany). Sep-Pak^{TM} C-18 plus-cartridges were purchased from Waters. Flash silica gel column chromatography was carried out on silica gel (230–400 mesh). Thin layer chromatography (TLC) was carried out on silica gel-coated aluminium plates (60F254, Merck); for radio-TLC 'Baker-flex'© sheets (silica gel IB-F) were used. Compounds were visualized by UV light (254 nm). Analytical HPLC was performed on the following system: HPLC Hewlett Packard (HP 1100) with a quarternary pump and variable wavelength detector (HP 1100) and radio-hplc-detector D505TR (Canberra Packard). Computer analysis of the HPLC data was performed using FLO-One software (Canberra Packard). Electronic autoradiography (Instant Imager^{TM}, Canberra Packard) was used to analyse radio-TLC data. ^{1}H NMR spectra were recorded on a Bruker AM 360 spectrometer operating at 360 MHz. All spectra were measured in $CDCl_3$ using TMS as internal standard. Melting points are uncorrected and determined on a Büchi melting point apparatus. Mass spectra were measured on a Finnigan MAT TSQ 70 instrument.

Cell culture

Receptor-binding studies were performed as described before.[19]

Radiochemistry

*3-(4-[^{131}I]Iodophenylpiperazin-1-ylmethyl)-pyrazolo[1,5-a]pyridine [^{131}**I**] **1***. In a conical reaction vessel equipped with a magnetic stirrer and closed with a Teflon septum, 0.9 µmol of **4** in dry CH_2Cl_2 was placed. The reactor was evaporated to dryness and filled with Argon. Hundred

microlitres of MeOH/AcOH (4:6, v/v) was added. 2–5 µl of a no-carrier-added (n.c.a.) $Na^{131}I$ solution containing approximately 30 µCi was added by using a microlitre syringe. After 2 min 10 µl of H_2O_2 (30%) was introduced. The reaction mixture was stirred for 2 min at room temperature; for HPLC analysis, a 10 µl aliquot of the reaction mixture was transferred into an Eppendorf reaction vial and quenched by dilution with 100 µl of methanol and subsequent addition of 100 µl 50 mM sodium bisulphite in 2 N NaOH. The k' value for **1** was 2.6 using a HP-RP-8 (125 × 4 mm) column and methanol/0.1 M ammonium formiate (pH 6.8) (70:30) as eluant (0.6 ml/min). For semi-preparative HPLC the reaction mixture was diluted with 1 ml of MeOH/H_2O (70:30, v/v) after addition of the sodium bisulphite solution. Separation by HPLC was performed by using a Lichrosorb RP-18 Select B 5 µm (250 × 4 mm) column with methanol/0.1 M ammonium formiate (pH 6.8) (70:30) and a flow rate of 1 ml/min. The product fraction was collected and concentrated on a rotary evaporator. **1** was isolated by solid phase extraction on a Sep-Pak™ C-18 plus-cartridge using PBS (5% ethanol) as eluant. **1** was obtained in a radiochemical yield of 80% and a radiochemical purity of >96% (radio-TLC:$R_f = 0.39$ (CH_2Cl_2/MeOH: 95/5))

***3**-(4-Chlorophenylpiperazin-1-ylmethyl)-7-[^{131}I]iodo-pyrazolo[1,5-a] pyridine [^{131}**I**]**2**.* Into an Eppendorf reaction vial, 0.5 µmol of **6** in dry CH_2Cl_2 was transferred and the solvent was slowly removed by heating at 30°C. Hundred microlitres of MeOH/AcOH (9:1) and typically 30 µCi (2–5 µl) n.c.a. $Na^{131}I$ solution was added. The tube was placed on an automatic shaker and mixed at 600 oscillations/min. A solution of chloroamine-T (15 µl, 23 mg/ml) was introduced via a microlitre syringe to start the reaction. The reaction mixture was stirred for 2 min at room temperature. For HPLC analysis a 10 µl aliquot of the reaction mixture was transferred and quenched as described for the radiosynthesis of **1**. The *k*' value for **2** was 3.8 using a HP-RP-8 (125 × 4 mm) column and methanol/0.1 M ammonium formiate (pH 6.8) (70 : 30) as eluant (0.6 ml/min). **2** can easily be separated by solid-phase extraction using a Sep-Pak™ C-18 plus-cartridge. The reaction mixture was diluted with 2 ml of water and passed through the pre-conditioned cartridge. Washing was performed with a further 5 ml of water. The Sep-Pak™-cartridge was dried in an Argon stream. **2** was isolated from the solid phase by extration with PBS (5% ethanol). **2** was obtained in a radiochemical yield of 32%. After solid-phase extraction the product fraction

contained **2** with a radiochemical purity of 98% (HPLC; radio-TLC: $R_f = 0.42$ (CH_2Cl_2/MeOH: 95/5)).

Chemistry

Compounds **1**, **3**, **4** and **5** were prepared as described in the literature.[13]

***3**-(4-Chlorophenylpiperazin-1-ylmethyl)-7-(tributylstannyl)pyrazolo-[1,5-a]pyridine* **6**. To a solution of **5** (6 g, 18.5 mmol) in dry THF (80 ml) was added, at −60°C, a solution of *n*-BuLi (11.6 ml, 1.6 M in *n*-hexane, 18.62 mmol). After stirring for 60 min, a solution of tributyltin chloride (5.0 ml, 18.6 mmol) in dry THF was added at −50°C. The reaction mixture was stirred for a further 30 min and then treated with saturated $NaHCO_3$ (100 ml) and extracted with CH_2Cl_2 (200 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (EtOAc) to give 10.4 g (16.9 mmol) of **6** as a pale yellow oil (TLC: $R_f = 0.62$ (CH_2Cl_2/MeOH: 95/5)).

^{1}H-NMR ($CDCl_3$, 360 MHz) δ(ppm): 0.87 (t, $J = 7.5$ Hz, 9 H), 1.22 (t, $J = 8.0$ Hz, 6 H), 1.28–1.38 (m, 6 H), 1.52–1.61 (m, 6 H), 2.61–2.64 (m, 4 H), 3.41–3.17 (m, 4 H), 3.73 (s, 2 H), 6.76 (dd, $J = 7.5$ Hz, $J = 1.5$ Hz, 1 H), 6.79–6.84 (m, 2 H), 7.00 (dd, $J = 9.0$ Hz, $J = 7.5$ Hz, 1 H), 7.16–7.20 (m, 2 H), 7.50–7.53 (dd, $^3J = 9.0$ Hz, $^4J = 1.5$ Hz, 1 H), 7.82 (s, 1 H)

EI-MS: m/z 615 (M^+)

***3**-(4-Chlorophenylpiperazin-1-ylmethyl)-7-iodopyrazolo[1,5-a] pyridine* **2**. To a solution of 0.2 g **6** (0.32 mmol) in 5 ml of dry THF 4 ml of a 0.1 M iodine solution in THF was slowly added. After stirring for 2 h at room temperature the reaction mixture was concentrated under reduced pressure. The residue was diluted with 5 ml of saturated $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Purification of the crude product was performed by flash chromatography on silica gel (EtOAc) yielding 0.1 g (0.25 mmol) of **2** as a white solid (TLC: $R_f = 0.35$ (CH_2Cl_2/MeOH: 95/5)).

Mp.: 177°C; ^{1}H-NMR ($CDCl_3$, 360 MHz) δ(ppm): 2.58–2.63 (m, 4 H), 3.12–3.19 (m, 4 H), 3.73 (s, 2 H), 6.79–6.89 (m, 3 H), 7.16–7.22 (m, 2 H), 7.34 (dd, $^3J = 7.0$ Hz, $^4J = 1.0$ Hz, 1 H), 7.68 (dd, $^3J = 9.0$ Hz, $^4J = 1.0$ Hz, 1 H), 8.02 (s, 1 H)

EI-MS: m/z 452 (M^+)

Acknowledgements

This work was supported by a grant from the ELAN-Fonds of the Friedrich-Alexander University Erlangen-Nürnberg.

References

1. Kuwert T, Bartenstein P, Grünwald F, *et al*. *Nervenarzt* 1998; **69**: 1045–1060.
2. Van Tol HHM, Bunzow JR, Guan H-C, *et al*. *Nature* 1991; **350**: 610–614.
3. Benes FM. *Neuroscientist* 1995; **1**: 104–115.
4. Weinberger DR. *Arch Gen Psychiatry* 1987; **44**: 660–669.
5. Seeman P, Guan H-C, van Tol HHM. *Nature* 1993; **365**: 441–445.
6. Reynolds GP, Mason SL. *Eur J Pharmacol* 1995; **281**: R5–R6.
7. Lahti RA, Cochrane EV, Primus RJ, Gallager DW, Roberts RC, Tammiga CA. *Am Soc Neurosci* 1996; **22**: 614–621.
8. Tarazi FI, Kula NS, Baldessarini RJ. *NeuroReport* 1997; **8**: 3423–3426.
9. Tarazi FI, Campbell A, Yeghiayan SK, Baldessarini RJ. *Neuroscience* 1998; **83**: 169–176.
10. Boy C, Klinke A, Holschbach M, *et al*. *Synapse* 1998; **30**: 341–350.
11. Langer O, Halldin C, Sandell J, *et al*. *J Labelled Cpd Radiopharm* 1999; **42** (suppl. 1): 182–184.
12. Matarrese M, Soloviev D, Moresco RM, *et al*. *J Labelled Cpd Radiopharm* 2000; **43**: 359–374.
13. Löber S, Hübner H, Gmeiner P. *Bioorg Med Chem Lett* 1999; **9**: 97–102.
14. Mennicke E, Holschbach M, Coenen HH. *J Labelled Cpd Radiopharm* 2000; **43**: 721–737.
15. Aboul-Fadl T, Löber S, Gmeiner P. *Synthesis* 2000; **12**: 1727–1732.
16. Hayes G, Biden TJ, Selbie LA, Shine J. *Mol Endocrinol* 1992; **6**: 920–926.
17. Sokoloff P, Andrieux M, Besancon R, *et al*. *Eur J Pharmacol* 1992; **225**: 331–337.
18. Asghari V, Sanyal S, Buchwaldt S, Paterson A, Jovanovic V, van Tol HHM. *J Neurochem* 1995; **65**: 1157–1165.
19. Hübner H, Kraxner J, Gmeiner P. *J Med Chem* 2000; **43**: 4563–4569.
20. Löber S. *Dissertation*, University of Erlangen-Nürnberg, 2000.

