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Research Article

Preparation of a technetium-99m SPECT agent for imaging the sigma-2 receptor status of solid tumors

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Summary

The synthesis and *in vitro* binding of a novel ^{99m}Tc-labeled radiotracer for imaging the sigma-2 (σ_2) receptor status of breast tumors is described. Structural characterization and *in vitro* binding studies were conducted using the corresponding rhenium surrogate, **Re-2**. X-ray crystallographic studies revealed that the complexation reaction gave exclusively the *syn* isomer. *In vitro* binding studies indicated that this complex has a high affinity for σ_2 ($K_i = 13.7$ nM) versus σ_1 receptors ($K_i = 1125$ nM). These data indicate that

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[^{99m}Tc]**2** may be a promising agent for imaging the σ_2 receptor status of tumors *in vivo* with the functional imaging technique, single photon emission computed tomography (SPECT).

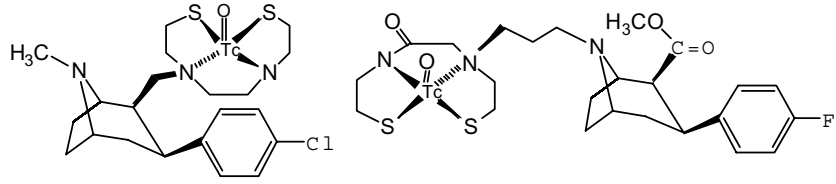
Key Words: sigma receptors; breast cancer; SPECT

Introduction

Sigma receptors represent a class of proteins that were initially classified as a subtype of the opiate receptor.[1] Subsequent studies revealed that sigma receptors are a distinct class of proteins having a widespread distribution in the CNS and peripheral tissues.[1,2] Radioligand binding and biochemical studies have revealed that there are two types of sigma receptors, σ_1 and σ_2 receptors. The σ_1 receptor has been cloned and displays a 30% sequence homology with the enzyme, yeast sterol isomerase.[3] The σ_2 receptor has not been cloned, but evidence suggests that this receptor is linked to potassium channels in NCB-20 cells.[1,2]

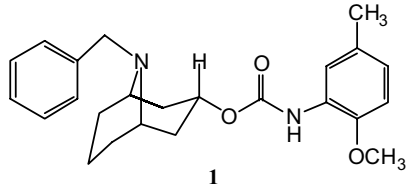
A number of studies have reported an overexpression of sigma receptors in a variety of human and murine tumors.[4,5] The observation that the density of σ_2 receptors is greater than that of σ_1 receptors in a wide variety of tumor cells grown under cell culture conditions[5] suggests that the σ_2 receptor is a suitable target for developing receptor-based radiotracers for noninvasive imaging procedures such as single photon emission computed tomography (SPECT) and positron emission tomography (PET). In addition, the recent observation that the density of σ_2 receptors is 10-fold higher in proliferating versus quiescent mouse mammary adenocarcinoma cells both *in vitro*[6,7] and *in vivo*[8] suggests that radioligands possessing a high affinity and selectivity for σ_2 receptors have the potential to measure the proliferative status of breast tumors using noninvasive imaging procedures.

Technetium-99m (^{99m}Tc) is the radionuclide of choice for nuclear medicine imaging procedures. The popularity of this radionuclide stems from the low cost and widespread availability of the ^{99}Mo/^{99m}Tc generator, its medium gamma ray energy (140 keV) which is suitable for gamma camera detection, and its physical half-life ($t_{1/2} = 6$ h) which is convenient for image acquisition. A number of recent studies have indicated that it is possible to prepare ^{99m}Tc-labeled imaging agents possessing a high affinity for a receptor. For example, [^{99m}Tc]TRO-



[^{99m}Tc]TRODAT-1

[^{99m}Tc]Technepine



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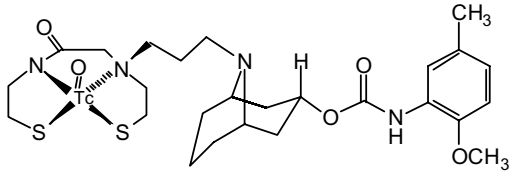


[^{99m}Tc]2

Figure 1. Structures of [^{99m}Tc]TRODAT-1, [^{99m}Tc]technepine, lead compound 1, and [^{99m}Tc]2

DAT-1[9] and [^{99m}Tc]technepine[10] have been shown to possess a high affinity for the dopamine transporter (Figure 1). These studies suggest the feasibility of preparing a small molecule based ^{99m}Tc-labeled radiotracer for imaging σ_2 receptors provided that there is a suitable lead compound for radiotracer development.

We previously reported that *O*-(9-benzyl-9-azabicyclo[3.3.1]nonan-3α-yl)-*N*-(2-methoxy-4-methylphenyl)carbamate, **1**, has a high affinity and moderate selectivity for σ_2 versus σ_1 receptors.[11] The goal of the current study was to determine if it is possible to prepare a ^{99m}Tc-labeled analogue of **1** using the strategy described previously for the development of [^{99m}Tc] technepine (Figure 1). In this communication, we describe the synthesis, *in vitro* binding, and preliminary *in vivo* evaluation of [^{99m}Tc] **2**, a potential radiotracer for measuring the σ_2 receptor status of solid tumors in vivo with SPECT.

Results

Chemistry

The first step in the development of [^{99m}Tc]**2** involved the synthesis of the rhenium complex, **Re-2**. Since the chemistry of technetium and rhenium are similar, the corresponding rhenium complex is generally used in the structural characterization and *in vitro* binding studies of receptor-based radiotracers using technetium as the radionuclide. Reduction of 9-benzyl-9-azabicyclo[3.3.1]nonan-3-one, **3**, with *L*-Selectride gave the corresponding 3-α hydroxy granatane, **4**, in 90% yield.[11] The NMR signal confirming the formation of the 3α-hydroxy granatane product, **4**, involved the interaction between the C1, C5 bridgehead protons and the equatorial protons on C2 and C4, which produced the characteristic doublet with a large coupling constant ($J \sim 10$ Hz). This splitting pattern is similar to that observed with the 3α-amino granatane analogues.[12,13] Treatment of **4** with 2-methoxy-5-methylisocyanate gave the corresponding carbamate derivative, **1**, in 70% yield. Removal of the *N*-benzyl group via catalytic dehydrogenation afforded the secondary amine, **5**, in quantitative yield. Treatment of amine **5** with [*N*-[2-[(3′-chloropropyl)(2-mercaptoethyl)-amino]acetyl]2-aminoethanethiolato]rhenium (V) oxide[10] afforded the corresponding rhenium complex, **Re-2**, in an overall yield of 45% that was exclusively the *syn* isomer. This is consistent with the results obtained by Meltzer *et al*. for the synthesis of the rhenium-complex of technepine.[10] The *syn* relationship between the Re=O core and the *N*-alkyl substituent was confirmed by X-ray crystallography (Figure 2).

In vitro binding studies. In vitro binding studies were conducted in order to measure the affinity of the **Re-2** for σ_1 and σ_2 receptors using established assays for these receptors.[14,15] The affinity of **Re-2** for the σ_2 receptor was 13.7 ± 0.9 nM. The affinity of **Re-2** for σ_1 receptors was 1125 ± 35 nM, indicating that this compound has $\sim$80-fold selectivity for σ_2 versus σ_1 receptors.

Radiolabeling studies. The N_2S_2 chelate, **6**, was prepared using the procedure outlined in Scheme 1.[10] Radiolabeling with ^{99m}Tc was accomplished using acidic conditions with Sn-glucoheptonate and [^{99m}Tc]sodium pertechnetate at 120°C for 30 min. The prolonged heating at this temperature was required to remove the trityl protecting

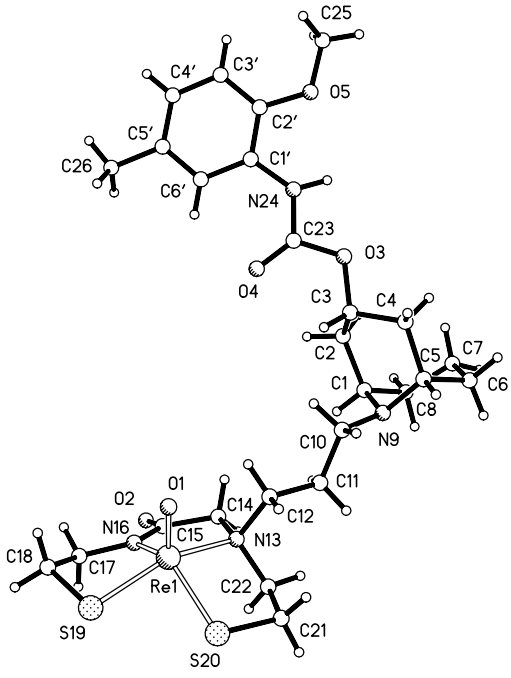
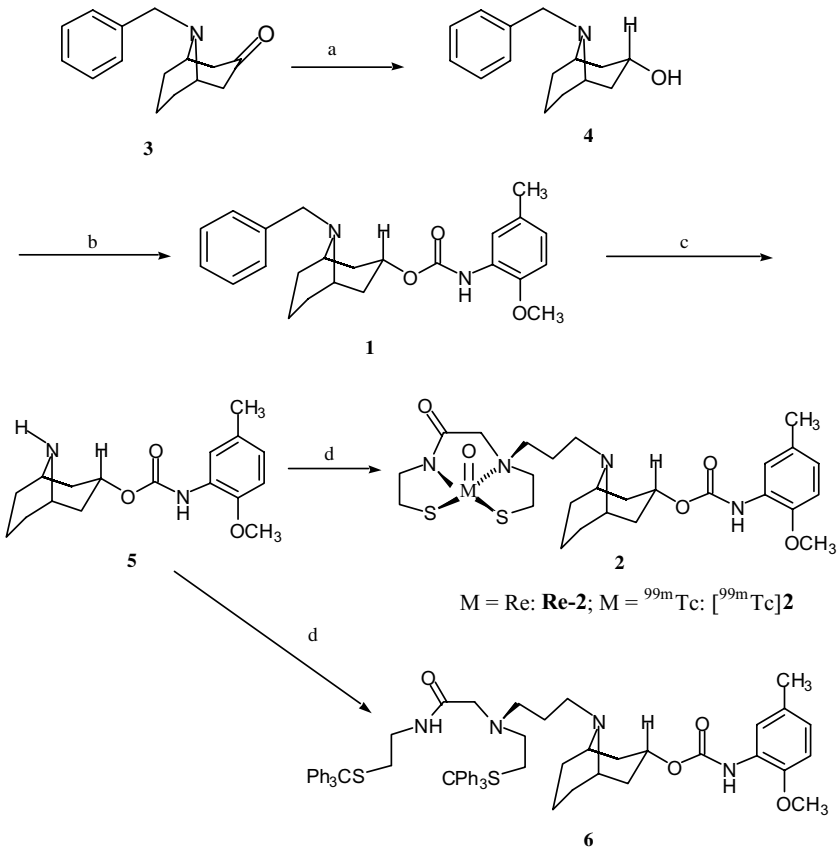


Figure 2. Crystal structure for the rhenium complex, Re-2

groups prior to complexation with ^{99m}Tc. The reaction mixture was diluted with saturated aqueous sodium bicarbonate and the product was extracted into ethyl acetate. The yield of the radiolabeling reaction ranged from 10 to 57%. Reversed-phase HPLC analysis showed that the *syn* isomer was obtained in >99% radiochemical purity (Figure 3). The labeled compound, [^{99m}Tc]**2**, was stable at room temperature for at least 18 h (data not shown).

In conclusion, we report in this communication the synthesis and structural characterization of a Re–N_2S_2 complex possessing a nanomolar affinity and high (80-fold) selectivity for σ_2 versus σ_1 receptors. The high density of σ_2 receptors in a wide variety of human tumors grown under cell culture conditions[4,5] indicates that [^{99m}Tc]**2** may be a useful radiotracer for imaging a wide variety of solid tumors. Furthermore, *in vivo* biodistribution studies indicate that there is a high uptake of [^{99m}Tc]**2** in mouse mammary adenocarcinoma xenografts.[16] Additional studies are currently being conducted in order to determine if this radiotracer is suitable for SPECT studies aimed at determining the σ_2 receptor status of solid tumors.



Scheme 1. Reagents: (a) *L*-Selectride/THF/-78°C; (b) 2-methoxy-5-methylisocynate/CH_2Cl_2/dibutyltin diacetate; (c) H_2/Pd-charcoal/methanol; (d)[10]

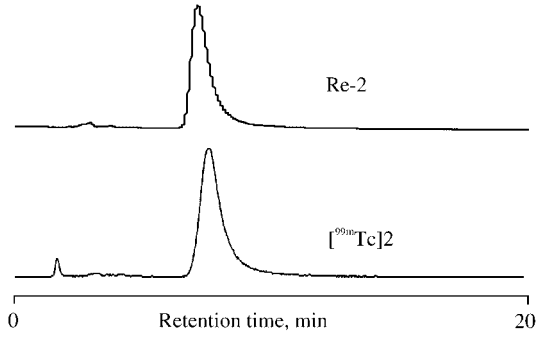


Figure 3. Reversed-phase HPLC chromatogram of Re-2 and [^{99m}Tc]2. Conditions: PRP-1 column, 250 × 4.1 mm; CH_3CN/3,3-dimethylglutarate buffer, 5 mM, pH 7, volume ratio 9 : 1; flow rate 1 ml/min. Re-2 was monitored at 254 nm

Materials and methods

Experimental section

^{1}H NMR spectra were recorded on a Bruker 300 MHz NMR spectrometer. Chemical shifts are reported in δ values (parts per million, ppm) relative to an internal standard of tetramethylsilane (TMS). The following abbreviations are used for multiplicity of NMR signals: br s = broad singlet, d = doublet, dd = doublet of doublets, m = multiplet, q = quartet, s = singlet, t = triplet, td = triplet of doublets. Melting points were determined on a Fischer-Johns melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA and were within $\pm$ 0.4% of the calculated values. All reactions were carried out under an inert atmosphere of nitrogen. Mass spectrometry studies were conducted by the Washington University Resource for Biomedical and Bio-organic Mass Spectrometry, St. Louis, MO. The radiosynthesis of [^{99m}Tc]**2**, *in vitro* stability studies, and detailed *in vivo* biodistribution studies will be published separately.[16]

[N-[2[-[3′-N′-Propyl-3″α(2-methoxy-5-methylphenylcarbamate)-granatane]-(2-mercaptoethyl)amino]acetyl]-2-aminoethanethiolato]-rhenium(V) oxide (Re-2). To a solution of amine **5** (340 mg, 1.13 mmol) in dry acetonitrile (10 ml) was added [*N*-[2-[(3′-chloropropyl)(2-mercaptoethyl)amino]acetyl]-2-aminoethane-thiolato]rhenium(V) oxide[10] (530 mg, 1.13 mmol), potassium iodide (187 mg, 1.13 mmol), and potassium carbonate (1.6 g, 11.3 mmol). The reaction mixture was refluxed overnight. Upon completion of reaction (as shown by TLC), the mixture was cooled to room temperature. Silica gel (3 g) was added, and the solvent was evaporated. The resulting solid was layered onto a silica gel column and eluted with ethyl acetate : methanol : ammonium hydroxide (200 : 4 : 1). Compound **Re-2** was isolated in 45% yield (375 mg): mp 193–194°C (dec); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.14 (s, 1H), 6.73–6.80 (m, 2H), 5.05–5.14 (m, 1H), 4.69 (d, 1H, J = 16 Hz), 4.55–4.61 (m, 1H), 3.91–4.13 (m, 3H), 3.85 (s, 3H), 3.61–3.67 (m, 1H), 3.38–3.46 (m, 1H), 3.18–3.27 (m, 1H), 3.03 (br s, 2H), 2.85–2.91 (m, 1H), 2.62–2.73 (m, 2H), 2.38–2.48 (m, 2H), 2.30 (s, 3H), 2.10–2.17 (m, 1H), 1.78–1.87 (m, 4H), 1.51–1.67 (m, 4H), 1.24–1.33 (m, 2H); HRFAB calculated for $C_{26}H_{39}N_4O_5S_2Re$ $[M]^+$ 738.1920, found

738.1944. Anal. ($C_{26}H_{39}N_4O_5S_2Re$) calcd. C, 42.27; H, 5.32; N, 7.59. Found C, 42.63; H, 5.40; N, 7.44.

O-(9-Azabicyclo[3.3.1]nonan-3α-yl)-N-(2-methoxy-5-methylphenyl) carbamate (5). The solution of *N*-benzylcarbamate **1** (3.0 g) in methanol (200 ml) was added to 10% Pd/C (0.3 g). The suspension was connected to a hydrogenation apparatus at 50 p.s.i. overnight. The catalyst was filtered through a pad of celite and the solvent was removed to give the carbamate **5** in a quantitative yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (s, 1H), 7.16 (s, 1H), 6.73–6.80 (m, 2H), 4.95–5.05 (m, 1H), 3.85 (m, 3H), 3.34–3.37 (m, 2H), 2.34–2.41 (m, 2H), 2.30 (s, 3H), 2.01–2.19 (m, 1H), 1.63–1.75 (m, 2H), 1.43–1.54 (m, 6H). Compound **5** was converted to the hydrochloride for elemental analysis. Anal. ($C_{16}H_{24}N_2O_3$.HCl) calcd. C, 59.97; H, 7.41; N, 8.23. Found C, 59.71; H, 7.46; N, 8.15.

3′-α(2-methoxy-5-methylphenylcarbamate)-9-[3-[N-2-[[[2-[[triphenyl-methyl-thio]ethyl]amino]carbonyl]methyl-N-[S-(triphenylmethyl)-thio] ethyl]amino]-propyl]granatane (6). To a solution of amine **5** (304 mg, 1.0 mmol) in dry acetonitrile (20 ml) was added [*N*-[[[2-[(triphenyl-methyl)-thio]ethyl]amino]carbonyl]methyl]-*N*-(3′-chloropropyl)-*S*-tri-phenylmethyl)-2-aminoethanethiol[10] (755 mg, 1.0 mmol), potassium iodide (166 mg, 1.0 mmol), and potassium carbonate (1.38 g, 10 mmol). The reaction mixture was refluxed overnight. The reaction mixture was cooled to room temperature, filtered, and concentrated in vacuo. The resulting solid was dissolved in ethyl acetate and purified by silica gel column chromatography (5% triethylamine in ethyl acetate). Compound 6 was isolated in 68% yield (740 mg): mp 83–84°C (dec); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.13–7.48 (m, 31H), 6.76 (m, 2H), 5.07 (m, 1H), 3.85 (s, 3H), 2.97–3.01 (m, 6H), 2.25–2.85 (m, 11H), 1.12–1.83 (m, 15H).

X-ray crystallography. Single crystals of purified **Re-2** were obtained by slow growth of the interface of a hexane/anhydrous ethanol suspension maintained at room temperature. The crystals are, at 228 K, triclinic, space group P-1 (No. 2) with $\underline{a} = 7.166(2)$ Å, $\underline{b} = 11.812(3)$ Å, $\underline{c} = 17.241(4)$ Å, $\alpha = 103.297(9)°$, $\beta = 91.82(2)°$, $\gamma = 93.33(3)°$, $V = 1416.4(6)$ Å^3, and $Z = 2\{d_{calcd} = 1.730\ g\ cm^{-3}; \mu_a(MoK\alpha) = 4.480\ mm^{-1}\}$. A total of 5919 psi-scan corrected reflections having 2Θ (MoKα) $< 50.76°$ (the equivalent of 0.8 limiting CuKα sphere) were collected on a

computer-controlled Bruker P4 autodiffractometer using ω scans and graphite-monochromated MoKα radiation of which 4721 were independent. The structure was solved using 'Direct Methods' techniques with the Bruker SHELXTL-PC (vers 5.1) software package. The resulting structural parameters have been refined to convergence {$\underline{R}_1$ (unweighted, based on F) = 0.0477 for 3596 independent reflections having 2Θ (MoKα) < 50.76° and $F^2 > 2\sigma(F^2)$} {$\underline{R}_1$ (unweighted, based on F) = 0.0802 and w$\underline{R}_2$ (weighted, based on F^2) = 0.0906 for all 4721 reflections} using counter-weighted full-matrix least-squares techniques and a structural model which incorporated anisotropic thermal parameters for all nonhydrogen atoms. Hydrogen atom H_{24} was located from a difference Fourier map and was refined as an independent isotropic atom. The two methyl groups (C_{25}, C_{26} and their hydrogens) were refined as rigid rotors with idealized sp^3-hybridized geometry and a C–H bond length of 0.97 Å. The remaining hydrogen atoms were included in the structure factor calculations as idealized atoms (assuming sp^2-or sp^3-hybridization of the carbon atoms and C–H bond lengths of 0.94–0.99 Å) 'riding' on their respective carbon atoms. The isotropic thermal parameter for H_{24} refined to a final value of 0.06(4) Å^2. The isotropic thermal parameters of the remaining hydrogen atoms were fixed at values 1.2 (nonmethyl) or 1.5 (methyl) times the equivalent isotropic thermal parameters of the carbon atoms to which they are covalently bonded.

All calculations were performed using the SHELXTL-PC (Version 5.1) interactive software package (G. Sheldrick, Bruker-AXS, Madison, WI).

Crystal data for **Re-2**: triclinic, P-1, $a = 7.166(2)$ Å, $b = 11.812(3)$ Å, and $c = 17.241(4)$ Å, $\alpha = 103.297(9)°$, $\beta = 91.82(2)°$, $\gamma = 93.33(3)°$, $V = 1416.4(6)$ Å^3, $Z = 2$, $D_x = 1.730$ g/cm^3, $T = 228(2)$ K. θ range 2.37–25.38°; reflections collected/unique: 5919/4721.

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References

1. Walker JM, Bowen WD, Walker FO, Matsumoto RE, De Costa B, Rice KR. *Pharmacol Rev* 1990; **42**: 355–402.

2. Hellewell SB, Bruce A, Feinstein G, Orringer J, Williams W, Bowen WD. *Eur J Pharmacol Mol Pharmacol Sec* 1994; **268**: 9–18.
3. Hanner M, Moebius FF, Flandorfer A, Knaus H-G, Striessnig J, Kempner E, Glossmann H. *Proc Natl Acad Sci USA* 1996; **93**: 8072–8077.
4. Bem WT, Thomas GE, Mammone JY, Homan SM, Levy BK, Johnson FE, Coscia CJ. *Cancer Res* 1991; **51**: 6558–6562.
5. Vilner BJ, John CS, Bowen WD. *Cancer Res* 1995; **55**: 408–413.
6. Mach RH, Smith CR, Al-Nabulsi I, Whirrett BR, Childers SR, Wheeler KT. *Cancer Res* 1997; **57**: 156–161.
7. Al-Nabulsi I, Mach RH, Sten K, Childers SR, Wheeler KT. *Brit J Cancer* 1999; **81**: 925–933.
8. Wheeler KT, Wang L-M, Wallen CA, Childers SR, Cline JM, Keng PC, Mach RH. *British J Cancer* 2000; **82**: 1223–1232.
9. Meegalla S, Plössl K, Kung M-P, Stevenson DA, Kushner SA, McElgin WT, Mozley PD, Kung HF. *J Med Chem* 1997; **40**: 9–17.
10. Meltzer PC, Blundell P, Jones AG, Mahmood A, Garada B, Zimmerman RE, Davison A, Holman BL, Madras BK. *J Med Chem* 1997; **40**: 1835–1844.
11. Mach RH, Yang B, Wu L, Kuhner RJ, Whirrett BR, West T. *Med Chem Res* 2001; **10**: 339–355.
12. Bermudez J, Fake CS, Joiner GF, Joiner KA, King FD, Miner WD, Sanger GJ. *J Med Chem* 1990; **33**, 1924–1929.
13. Mach RH, Luedtke RR, Unsworth CD, Boundy VA, Nowak PA, Scripko JG, Elder ST, Jackson JR, Hoffman PL, Evora PH, Rao AV, Molinoff PB, Childers SR, Ehrenkaufer RL. *J Med Chem* 1993; **36**, 3707–3720.
14. Huang YH, Hammond PS, Whirrett BR, Kuhner RJ, Wu L, Childers SR, Mach RH. *J Med Chem* 1998; **41**: 2361–2370.
15. Mach RH, Huang Y, Buchheimer N, Kuhner R, Wu L, Morton TE, Wang L-M, Ehrenkaufer RL, Wallen CA, Wheeler KT. *Nucl Med Biol* 2001; **28**: 451–458.
16. Choi S-R, Yang B, Plössl K, Chumpradit S, Wey S-P, Acton PD, Wheeler KT, Mach RH, Kung HF. *Nucl Med Biol*, in press.

