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Rhenium and technetium tricarbonyl complexes anchored by 5-HT_{1A} receptor-binding ligands containing P,O/N donor atom sets

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Abstract

The (2-methoxyphenyl)piperazine pharmacophore, a part of the WAY 100635 structure, has been functionalized with phosphinoarylbenzylamide or phosphinoarylbenzylamine chelator groups using propylene or hexylene alkyl chains as linkers (**L2**–**L4**). These heterofunctionalized phosphines bearing an arylpiperazine moiety have been used to stabilize rhenium tricarbonyl complexes of the type $[Re(CO)_3Br(\kappa^2\text{-L})]$ (**4**, L = **L2**; **5**, L = **L3**; **6**, L = **L4**), which have been fully characterized, including by X-ray crystallographic analysis in the case of compounds **4** and **5**. These monomeric complexes are six-coordinate, displaying a distorted octahedral coordination geometry with a facial arrangement of the carbonyl groups. The other three remaining positions are occupied by a bromide and by the bidentate heterofunctionalized phosphine, which coordinates through the phosphorus and the oxygen atom or through the phosphorus and the nitrogen atom in **4** and **5**, respectively. The ^{99m}Tc complexes (**3a**–**6a**) were also prepared and their characterization established by comparative HPLC, using the Re complexes as surrogates. The in vitro binding affinity for the $5HT_{1A}$ receptor subtype and the selectivity against the $5HT_{2A}$ receptors for the rhenium complexes were determined. Compound **3** is the only one which presents a reasonable affinity and selectively towards $5HT_{1A}$ (IC_{50} = 20 nM) and $5HT_{2A}$ (IC_{50} = 4680 nM) receptors, respectively. When the spacer length between the chelate unit and receptor binding domain increased and/or the amide group in the chelator was replaced by a secondary amine unacceptable affinity values for $5HT_{1A}$ receptors (IC_{50} = 200–1100 nM) and lost of selectivity were observed.

Keywords: Rhenium; Technetium; Phosphine; Radiopharmaceuticals; $5HT_{1A}$ receptor-ligands

1. Introduction

The search for new radioligands as diagnostic tools for the visualisation and quantification of neuroreceptors in the central nervous system (CNS), either by positron emission tomography (PET) or single-photon emission tomography (SPECT), is a subject that has attracted considerable attention in the past years [1]. This interest arises mainly from the fact that several neurological diseases are directly related with changes in the density of specific receptors, which in the past could only be detected by post mortem binding studies [1]. The 5-HT_{1A} subtype of receptors for the neurotransmitter serotonin is involved in important physiological processes in the human brain, and changes in their number are associated with several neurological diseases, such as Alzheimer disease, schizophrenia, and depression [2]. Despite the great variety of 5-HT_{1A} radioligands

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with positron-emitting radionuclides for PET imaging, such as the successful radiopharmaceuticals [*carbonyl*-^{11}C] WAY-100635 (WAY) and *p*-[^{18}F]MPPF, there is still a scope for the development of technetium-labelled 5-HT_{1A} ligands for SPECT imaging [1,2]. In which concerns the development of ^{99m}Tc-based imaging agents selective for CNS receptors, [^{99m}Tc]TRODAT-1 represents up to now the most successful development for imaging DAT sites [3]. Since the introduction of the low-valent aquo-carbonyl complex *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ as a versatile precursor for the labelling of biomolecules, a number of biologically active peptides and CNS receptor ligands have been labelled making use of different bifunctional chelating agents (BFCA) [4–15]. Despite the recent advances in this area, further achievements still rely on the development of new ^{99m}Tc-organometallic complexes with adequate in vitro and in vivo behaviour, in order to obtain radiopharmaceuticals that fulfil the minimum requisites for CNS receptor imaging.

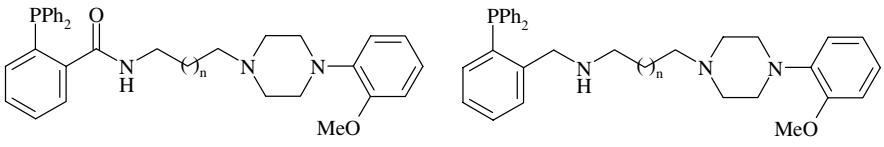
Recently, we have introduced several heterofunctionalized phosphines, which are able to stabilize Re and Tc in low and intermediate oxidation states [11,12,16–19]. For rhenium(I) we have described the complexes $[Re(CO)_3Br(\kappa^2\text{-}H_2PNO)]$ (**1**, H_2PNO = *N*-(2-hydroxyethyl)-2-(diphenylphosphanyl)benzamide), $[Re(CO)_3(\kappa^3\text{-}PN_2)]$ (**2**, HPN_2 = *N*-(2-aminoethyl)-2-(diphenylphosphanyl) benzamide), and $[Re(CO)_3Br(\kappa^2\text{-}\mathbf{L1})]$ (**3**) (**L1** = *N*-[4-(3-aminopropyl)-1-(2-methoxyphenyl)piperazine]-2-(diphenylphosphanyl)-benzamide), which are the first examples of *fac*-$[Re(CO)_3]^+$ anchored by heterofunctionalized phosphines containing PN_2 and PNO donor atom sets [11,12].

Preliminary studies at the no carrier added level revealed that the ligands H_2PNO, HPN_2 and the 5-HT_{1A} receptor ligand **L1** could be labelled with the *fac*-$[^{99m}Tc(CO)_3]^+$ moiety in good yields (80–85%), being found for complex **3** an encouraging biological activity [12]. As part of our ongoing research on the development of technetium-labelled receptor-specific agents, and trying to establish structure biological activity relationships, we report herein on the derivatization of the pharmacophore (2-methoxyphenyl)piperazine, part of the WAY 100635 structure, with phosphinoarylbenzylamide (PO donor atom set) or phosphinoarylbenzylamine (PN donor atom set) chelator groups, using different spacer lengths between the chelator unit and the receptor binding domain (**L2**–**L4**). We also describe the synthesis and characterization of tricarbonyl complexes of the type $[M(CO)_3Br(\kappa^2\text{-}L)]$ (M = ^{99m}Tc, L = **L1** (**3a**); M = Re, ^{99m}Tc and L = **L2** (**4**, **4a**), L = **L3** (**5**, **5a**), L = **L4** (**6**, **6a**)) and the in vitro binding affinity of the Re complexes for 5-HT_{1A} and 5-HT_{2A} receptors, cf. Scheme 1.

2. Materials and methods

2.1. General procedures

All Chemicals and solvents were of reagent grade and were used without further purification. *N*-[2-(diphenylphosphanyl)benzoyloxy]succinimide, $(NEt_4)_2[ReBr_3(CO)_3]$, *N* -[4-(3-amino-propyl)-1-(2-methoxyphenyl)piperazine]-2-(diphenylphosphanyl)benzamide (**L1**), 4-(6-aminohexyl)-1-(2-methoxyphenyl)piperazine, *fac*-$[Re(CO)_3Br(\kappa^2\text{-}\mathbf{L1})]$ (**3**) and *fac*-$[M(OH_2)_3(CO)_3]^+$ (M = Re, ^{99m}Tc) were prepared according to published methods [4,11,16–19]. ^{1}H and ^{31}P NMR spectra were recorded on a Varian Unity 300 MHz spectrometer; ^{1}H chemical shifts were referenced with the residual solvent resonances relatively to tetramethylsilane and the ^{31}P NMR chemical shifts with external 85% H_3PO_4 solution. NMR spectra were run in $CDCl_3$ and CD_3CN. IR spectra were recorded as KBr pellets on a Perkin–Elmer 577 spectrometer. Carbon, hydrogen and nitrogen analysis were performed on a Perkin–Elmer automatic analyser. Na[$^{99m}TcO_4$] was eluted from a ^{99}Mo/^{99m}Tc generator using 0.9% saline. HPLC analyses were performed on two different HPLC systems: (a) Merck–Hitachi L-7000-system equipped with an L-7400 tunable absorption detector, a Berthold LB-508 radiometric detector; (b) Perkin–Elmer LC pump 200 (binary) coupled to a LC 290 tunable UV/Vis detector, a Berthold LB-507A radiometric detector. For the radiochemical analyses two different Macherey–Nagel C-18 reversed-phase columns were used: nucleosil (5 μm, 250 × 3 mm – flow rate: 0.5 ml/min) and nucleosil (10 μm, 250 × 4 mm – flow rate 1 ml/min). HPLC solvents consisted of 0.1% CF_3COOH in H_2O (solvent A) and methanol (solvent B). The HPLC system started with 100% of A from 0 to 3 min. The eluent switched at 3 min to 75% A and



L1, n = 1
L2, n = 4

L3, n = 1
L4, n = 4

Scheme 1. Receptor-binding ligands **L1**–**L4**.

25% B and at 9 min to 66% A and 34% B followed by a linear gradient 66% A/34% B to 100% B from 9 to 20 min. The gradient remained at 100% B for 2 min before switching back to 100% A.

2.2. Synthesis of N-[4-(6-Aminohexyl)-1-(2-methoxyphenyl)piperazine]-2-(diphenylphosphanyl)benzamide (*L2*)

N-[2-(Diphenylphosphanyl)benzoyloxy]-succinimide (500 mg, 1.24 mmol) dissolved in dry dichloromethane (4 ml) was added dropwise to a stirred solution of 4-(6-aminohexyl)-1-(2-methoxyphenyl)piperazine (360 mg, 1.24 mmol) in the same solvent (10 ml). After 18 h at room temperature, the solvent was evaporated to dryness. The obtained residue was chromatographed on an appropriate column of silica gel with 1–4% methanol/chloroform (gradient) to afford a yellow oil. Yield: 79%, 570 mg. Anal. Calc. (found) for $C_{36}H_{42}N_3O_2P$: C, 74.59 (74.36); H, 7.30 (7.25); N, 7.25 (7.32)%. IR (KBr, ν/cm^{-1}): 1630 (C=O), 1235, 740, 690. ^1H NMR ($CDCl_3$): δ 7.58 (m, aromatic, 1H), 7.38–7.23 (m, aromatic, 12H), 7.01–6.82 (m, aromatic, 5H), 5.92 (t br, NH, 1H), 3.83 (s, OCH_3, 3H), 3.23 (q, CH_2, 2H), 3.13 (s br, CH_2, 4H), 2.72 (s, br, CH_2, 4H), 2.45 (t, CH_2, 2H), 1.54 (m, CH_2, 2H), 1.36 (m, CH_2, 2H), 1.26 (m, CH_2, 4H). ^{31}P NMR ($CDCl_3$): δ −9.7.

2.3. Synthesis of N-[4-(3-aminopropyl)-1-(2-methoxyphenyl) piperazine]-2-(diphenyl-hosphanyl)benzylamine (*L3*) and N-[4-(6-aminohexyl)-1-(2-methoxyphenyl)piperazine]-2-(diphenylphosphanyl)benzylamine (*L4*)

To a solution of **L1** (300 mg, 0.57 mmol) or **L2** (290 mg, 0.50 mmol) in dry toluene, 1 M borane-dimethylsulfide solution in dichloromethane (**L1**, 1.14 ml, 1.14 mmol; **L2**, 1.5 ml, 1.50 mmol) was added at room temperature, under a nitrogen atmosphere with stirring. The solution was allowed to reflux during 24 h under a nitrogen atmosphere.

L3: The mixture was cooled, 15 ml of a 10% $NaHCO_3$ solution was added, and refluxed again for 30 minutes. The solvents were evaporated in vacuum to dryness, and the residue was treated with 15 ml water and extracted with dichloromethane (3×25 ml). The organic phases were collected, dried over $MgSO_4$, filtered and the solvent evaporated. The residue was chromatographed on a silica gel column with 1–3% methanol–chloroform (gradient) to afford a colorless oil. Yield: 28%, 160 mg. Anal. Calc. (found) for $C_{33}H_{38}N_3OP \cdot CH_3OH$: C, 73.49 (73.83); H, 7.62 (7.99); N, 7.56 (7.59)%. IR (KBr, ν/cm^{-1}): 1589, 1500, 1453, 1240, 747, 698 cm^{-1}. ^1H NMR (CD_3CN): δ 7.53 (m, aromatic, 1H), 7.40–7.14 (m, aromatic, 12H), 6.96–6.84 (m, aromatic, 5H), 3.94 (s, CH_2, 2H), 3.77 (s, OCH_3, 3H), 2.92 (s br, CH_2, 4H), 2.55 (t, CH_2, 2H), 2.47 (s, br, CH_2, 4H), 2.32 (t, CH_2, 2H), 1.50 (m, CH_2, 2H). ^{31}P NMR (CD_3CN): δ −15.4.

L4: The mixture was cooled, and methanol was added until liberation of H_2 stops. The solvents were evaporated in vacuum to dryness, and the residue was suspended in MeOH and refluxed for further 30 min. The solvents were evaporated in vacuum to dryness, and the residue chromatographed on a silica gel column with 0.5–4% methanol–chloroform (gradient) to afford a colorless oil. Yield: 53%, 0.15 g. Anal. Calc. (found) for $C_{36}H_{44}N_3OP$: C, 76.43 (76.38); H, 7.84 (7.90); N, 7.43 (7.40)%. IR (KBr, ν/cm^{-1}): 1495, 1240, 740, 695. ^1H NMR ($CDCl_3$): δ 7.47 (m, aromatic, 1H), 7.34–7.21 (m, aromatic, 11H), 7.14 (m, aromatic, 1H), 7.01–6.82 (m, aromatic, 5H), 3.98 (s, CH_2, 2H), 3.84 (s, OCH_3, 3H), 3.10 (s br, CH_2, 4H), 2.65 (s, br, CH_2, 4H), 2.48 (t, CH_2, 2H), 2.38 (t, CH_2, 2H), 1.46 (m, CH_2, 2H), 1.36–1.16 (m, CH_2, 6H). ^{31}P NMR ($CDCl_3$): δ −15.6.

2.4. General procedure for the preparation of the complexes fac-[Re(CO$_3$)(κ^2-L)] (*4*, L = *L2*; *5*, L = *L3*; *6*, L = *L4*)

A solution of the appropriate ligand **L2**–**L4** (**L2**: 38 mg, 0.065 mmol; **L3** 25 mg, 0.048 mmol; **L4**: 47 mg, 0.083 mmol) in methanol (ca. 2 ml) was added, with stirring, to a solution of $(NEt_4)_2[ReBr_3(CO_3)]$ (**L2**: 50 mg, 0.065 mmol; **L3** 37 mg, 0.048 mmol; **L4**: 64 mg, 0.083 mmol) in the same solvent (ca. 3 ml). After refluxing for 4 h, the reaction mixture was centrifuged to separate the solid formed in the case of **5**, and in the case of complexes **4** and **6**, the solvent was evaporated to dryness. The precipitate (**5**) or the oily residues (**4**, **6**) were thoroughly washed with water and dried under vacuum.

2.5. fac-[Re(CO$_3$)(k^2-*L2*)] (*4*)

The complex, which was obtained as an yellow oil, could be recrystallized from CH_2Cl_2/Hexane giving pale-yellow crystals suitable for X-ray diffraction analysis. Yield: 38 mg, 69%. Anal. Calc. (found) for $C_{39}H_{42}N_3O_5PRe \cdot HBr$: C, 46.34 (46.03); H, 4.29 (4.35); N, 4.16 (4.32)%. IR (KBr, ν/cm^{-1}): 2010, 1910, 1870, 1590 (C=O), 1235, 740 and 690 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.68–7.55 (m, aromatic, 4H), 7.46–7.43 (m, aromatic, 6H), δ (7,37–7.26, m, aromatic, 3H), 7.17–6.78 (m, aromatic, 5H), 3.86 (s, OCH_3, 3H), 3.58–3.47 (m, CH_2, 6H), 3.18–3.04 (m br, CH_2, 2H), 1.86 (s br, CH_2, 4H), 1.62 (s br., CH_2, 8H). ^{31}P NMR ($CDCl_3$): δ 14.9.

2.6. fac-[Re(CO$_3$)(κ^2-*L3*)] (*5*)

The complex was obtained in the form of a white solid, which could be recrystallized from CH_2Cl_2/hexane yielding pale-yellow crystals suitable for X-ray diffraction analysis. Yield: 19 mg, 46%. Anal. Calc. (found)

for $C_{36}H_{38}N_3O_4PBrRe \cdot H_2O$: C, 48.48 (48.53); H, 4.52 (4.48); N, 4.71 (4.96)%. IR (KBr, ν/cm^{-1}): 2010, 1925, 1890, 1260, 800 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.54–7.28 (m, aromatic, 13H), 6.95 (t, aromatic, 2H), 6.88–6.79 (m, aromatic, 3H), 4.28 (d, CH, 1H, J = 12 Hz), 4.05 (d, CH, 1H, J = 11 Hz), 3.82 (s, OCH_3, 3H), 3.30 (s br, CH_2, 3H), 3.12 (s br, CH_2, 2H), 2.86–2.65 (m, CH_2, 7H), 2.05 (s br, CH, 1H), 1.58 (s br, CH, 1H). ^{31}P NMR ($CDCl_3$): δ 11.7.

2.7. *fac*-[Re(CO_3)(k^2-**L4**)] (**6**)

The complex was obtained as an yellow oil. Yield: 52 mg, 68%. Anal. Calc. (found) for $C_{39}H_{44}N_3O_4PBrRe$: C, 51.15 (51.22); H, 4.84 (4.79); N 4.59 (4.55)%. IR (KBr, ν/cm^{-1}): 2020, 1920, 1870, 1245, 750, 695. ^{1}H NMR ($CDCl_3$): 7.55–7.31 (m, aromatic, 13H), 7.05 (m, aromatic, 1H), 6.94–6.76 (m, aromatic, 4H), 4.21 (d, CH, 1H, J = 12 Hz), 4.08 (d, CH, 1H, J = 12 Hz), 3.84 (s, OCH_3, 3H), 3.49 (m, CH_2, 6H), 3.32 (m, CH_2, 2H), 3.09–2.90 (m, CH_2, 6H), 2.00 (s br, CH_2, 2H), 1.77 (s br, CH_2, 2H), 1.44 (m, CH_2, 2H). ^{31}P NMR ($CDCl_3$): δ 10.7.

2.8. X-ray crystallographic analysis

Pale-yellow crystals of **4** and **5**, suitable for X-ray diffraction analysis, were obtained from a mixture of CH_2Cl_2/hexane and were fixed inside thin-walled glass capillaries. Data were collected at room temperature on an Enraf–Nonius CAD4-diffractometer with graphite-monochromatized Mo Kα radiation, using a -2θ scan mode. Unit cell dimensions were obtained by least-squares refinement of the setting angles of 25 reflections with $14.0 < 2\theta < 28.0°$ for **4** and $16.8 < 2\theta < 27.9°$ for **5**. A summary of the crystallographic data is given in Table 1. Data were corrected for Lorentz and polarization effects and for absorption by empirical corrections based on Ψ scans [20]. The heavy atom positions were located by Patterson methods using SHELXS-97 [21]. The remaining atoms were located by sucessive difference Fourier techniques and refined by least-squares refinements on F^2 using SHELXL-97 [22]. All the non-hydrogen atoms were refined with anisotropic thermal motion parameters and the contribution of the hydrogen atoms were included in calculated positions. Atomic scattering factors and anomalous dispersion terms were taken from [20]. The drawings were made with ORTEP-3 [23].

Table 1
Crystal data for [Re(CO_3)Br(κ^2-**L2**)] (**4**) and [Re(CO_3)Br(κ^2-**L3**)] (**5**)

Complex	**4**	**5**
Empirical formula	$C_{39}H_{42}BrN_3O_5PRe$	$C_{36}H_{38}BrN_3O_4PRe$
Crystal size, mm^3	$0.55 \times 0.16 \times 0.16$	$0.76 \times 0.54 \times 0.36$
Formula weight	929.84	873.77
Crystal system	Triclinic	Triclinic
Space group	$P\bar{1}$	$P\bar{1}$
a (Å)	8.8133(14)	11.238(3)
b (Å)	15.893(4)	11.656(3)
c (Å)	15.968(4)	14.4143(15)
α (°)	61.225(19)	91.818(15)
β (°)	74.324(16)	92.986(11)
γ (°)	84.612(18)	112.03(2)
Volume (Å^3)	1885.8(8)	1745.2(6)
Z	2	2
$\rho_{calc.}$ (g cm^{-3})	1.638	1.663
μ(Mo Kα) (mm^{-1})	4.370	4.714
No. reflections measured	7047	7349
No. unique reflections	6575 [R_{int} = 0.0352]	6939 [R_{int} = 0.0493]
R_1[a]	0.0846	0.0470
wR_2[a]	0.1920	0.1031

[a] $R_1 = \sum ||F_o| - |F_c|| / \sum |F_o|$ and $wR_2 = [\sum(w(F_o^2 - F_c^2)^2)/\sum(w(F_o^2)^2)]^{1/2}$. The values were calculated for data with $I > 2\sigma(I)$.

2.9. Synthesis at tracer level, ^{99m}Tc-complexes (**3a**–**6a**) – general method

To a solution of the organometallic precursor *fac*-[^{99m}Tc$(OH_2)_3(CO)_3$]$^+$ (900 μl; 3–4 mCi) was added 100 μl of a 10^{-3} M stock solution of the ligands (**L1**–**L4**) in EtOH was added. The vial was then heated (**L1**, 100 °C/30 min; **L2** 100 °C/60 min; **L3** 100 °C/30 min; **L4** 100 °C/60 min), and the final solution analysed by HPLC.

2.10. Receptor biding assays on rat brain homogenates

The $5HT_{1A}$ receptor binding assays were performed using [^{3}H]-8-OH-DPAT as radioligand and rat hippocampus homogenate. The binding assay was carried out in a final volume of 2.5 ml Tris–HCl buffer (50 mM, pH 7.4, 0.1% ascorbic acid, 2 mM $CaCl_2$) containing 0.10 nM [^{3}H]-8-OH-DPAT, membran homogenate (about 20 μg/ml protein), and various concentration of the rhenium complexes **3**–**6**. The complexes were dissolved in DMSO up to 1 nM, then diluted with buffer. Non-specific binding was defined as the amount of [^{3}H]-8-OH-DPAT bound in the presence of serotonin (Sigma). The samples were incubated in triplicates at 20°C for 120 min. The incubation was terminated by rapid filtration through GF/B glass fiber filters (Whatman) using a 30-port Brandel Cell Harvester. The filters were rapidly washed with four 4 ml portions of ice-cold buffer, transferred into 10 ml scintillation fluid (Ultima-Gold, Packard) and analysed for radioactivity.

The $5HT_{2A}$ receptor binding assays: the cortex of rat brain was homogenized in ice-cold buffer (50 mM Tris–HCl, pH 7.6) with an Ultra-Turrax T 25. The homogenate was centrifuged at 20,000g for 10 min. The resulting pellet was resuspended and centrifuged under above conditions. After repeating the same procedure the

pellet was resuspended in 10 ml of buffer and stored at −20 °C.

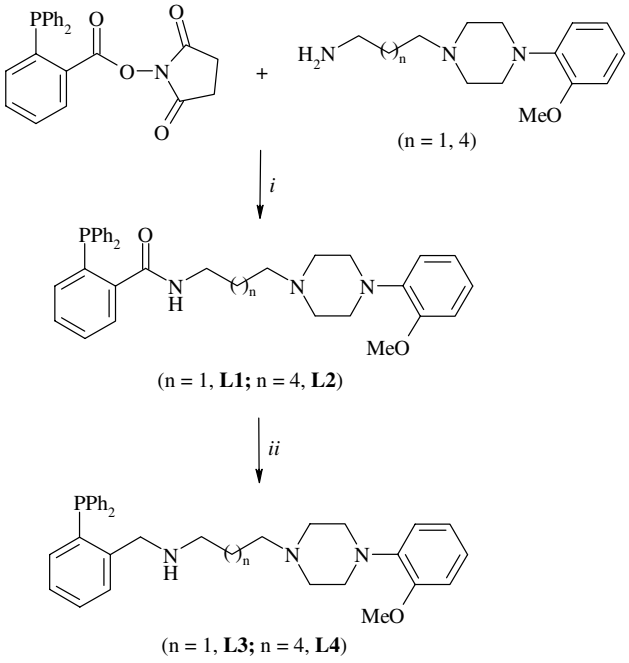
[^{3}H]ketanserin (from NEN) was used as a radioligand. The binding assay was carried out in a final volume of 5 ml Tris–HCl buffer, pH 7.6, containing 0.12 nM [^{3}H]ketanserin, membrane homogenate (about 20 μg/ml protein), and various concentrations of the Re complexes. The complexes were dissolved in DMSO up to 1 nM, then diluted with buffer. Non-specific binding was defined as the amount of [^{3}H]ketanserin bound in the presence of 1 μM mianserin (Sigma). The samples were incubated in triplicates at 20 °C for 60 min. Incubation, filtration and counting of the samples were the same as described above.

3. Results and discussion

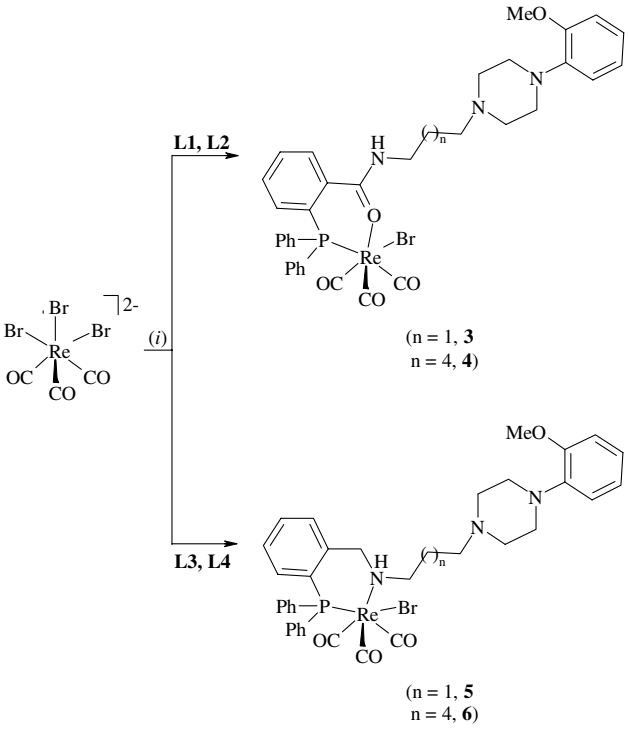
The bifunctional chelating agents (BFCA) **L1** and **L2** have been prepared, similarly to a previously published method, by reaction of *N*-[2-(diphenylphosphanyl)-benzoyloxy]succinimide with 4-(3-aminopropyl)-1-(2-methoxyphenyl)-piperazine and 4-(6-aminohexyl)-1-(2-methoxyphenyl)piperazine, respectively [11,12]. The BFCA **L3** and **L4** were obtained by reduction of the amide groups in **L1** and **L2** with $BH_3 \cdot SMe_2$ in refluxing toluene, respectively (Scheme 2).

The compounds **L2**–**L4** were characterized by ^{31}P and ^{1}H NMR spectroscopy, elemental analysis and IR spectroscopy. In the IR spectra of **L3** and **L4** no stretching bands in the range 1630–1640 cm^{-1} were found, confirming the absence of the carbonyl amide function. As expected, the ^{31}P NMR spectra show only one signal for the phosphorus atom for all compounds (**L1**, δ −8,3; **L2**, δ −9.7; **L3**, δ −5.4; **L4**, δ −15.6). The rhenium tricarbonyl complexes *fac*-[Re(CO)$_3$Br- (κ^2-L)] (**3**, L = **L1**; **4**, L = **L2**; **5**, L = **L3**; **6**, L = **L4**), were obtained by reacting **L1**–**L4** with the precursor $(NEt_4)_2[ReBr_3(CO)_3]$ under refluxing methanol, as depicted in Scheme 3. These compounds have been used as surrogates for ^{99m}Tc-complexes (**3a**–**6a**).

While *fac*-[Re(CO)$_3$Br(κ^2-**L1**)] (**3**) and *fac*-[Re(CO)$_3$Br(κ^2-**L3**)] (**5**) were isolated as white solids, which precipitate during reaction course or work-up, complexes *fac*-[Re(CO)$_3$Br(κ^2-**L2**)] (**4**) and *fac*-[Re(CO)$_3$Br-(κ^2-**L4**)] (**6**) were obtained as viscous colorless oils after evaporation of the solvent and washing of the residues with water. The neutral complexes **3**–**6** are air and moisture stable, being soluble in halogenated solvents. All the complexes have been fully characterized by ^{1}H/^{31}P NMR and IR spectroscopy and, in the cases of **4** and **5**, by X-ray diffraction analysis. The **L2** receptor-binding ligand in complex **4** coordinates as neutral and bidentate through the phosphorus and the oxygen atom of the carbonyl, in a similar way to what has been previously observed for **L1** in complex **3** [11]. The receptor-binding



Scheme 2. Synthesis of the ligands **L1**–**L4**. (i) Dichloromethane, room temperature; (ii) $BH_3 \cdot SMe_3$, toluene, reflux.



Scheme 3. Synthesis of the complexes **3**–**6**. (i) Methanol, reflux.

ligands **L3** and **L4** coordinate also as neutral and bidentate, through the phosphorus and the nitrogen atom of the secondary amine. IR spectra were consistent with the *fac*-[M(CO)$_3$] geometry of compounds **4**–**6**, as

previously observed for complex **3** [11]. Very strong ν(CO) stretching bands appear at 2010, 1910 and 1870 cm^{-1} for **4**; 2010, 1925 and 1890 cm^{-1} for **5**; 2020, 1920 and 1870 cm^{-1} for **6**. The ν(C=O) stretching vibration of the carbonyl group of the receptor-binding ligand **L2** in complex **4** appears at 1590 cm^{-1}, i.e. 40 cm^{-1} lower in energy relatively to the corresponding free ligand (1630 cm^{-1}), which is a strong evidence for metal coordination through the carbonyl function. Complexes **4–6** present only one signal in the ^{31}P NMR spectra (**4**, δ 14.9; **5**, δ 11.7; **6**, δ 10.7 ppm), strongly downfield shifted relatively to the corresponding free ligands (**L2**, δ −9.7; **L3**, δ −15.4; **L4**, δ −15.6 ppm), confirming a strong σ-donor character for the phosphorus atom.

The most important feature of the ^{1}H NMR spectra of complexes **5** and **6** is related with the diastereotopic character of the two methylenic protons near to the phenyl ring in **L3** and **L4** upon coordination to the metallic centre (**5**: δ 4.28, d, J = 12 Hz, 1H; δ 4.05, d, J = 11 Hz, 1H; **6**: δ 4.21, d, J = 12 Hz, 1H; δ 4.08, 1H, d, J = 12 Hz).

We have evaluated the possibility of preparing the complexes **3–6** at the no carrier added level, using *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$ as precursor. Thus, the neutral ^{99m}Tc(I) tricarbonyl complexes, analogues of the Re complexes with the receptor-binding ligands **L1–L4**, $[^{99m}Tc(CO)_3Cl(\kappa^2$-**L1**$)]$ (**3a**), $[^{99m}Tc(CO)_3Cl(\kappa^2$-**L2**$)]$ (**4a**), $[^{99m}Tc(CO)_3Cl(\kappa^2$-**L3**$)]$ (**5a**), and $[^{99m}Tc(CO)_3Cl(\kappa^2$-**L4**$)]$ (**6a**) have been prepared. These complexes have been obtained in relatively high yields (87–98%), using a 10^{-4} M final ligand concentration (Table 2).

The characterization of **3a–6a** was made by comparing their radioactive traces on the HPLC with the UV traces (monitored at 254 nm) of the analytically pure Re analogues (Table 2).

4. Description of the structures

The structures of complexes $[Re(CO)_3Br(\kappa^2$-**L2**$)]$ (**4**) and $[Re(CO)_3Br(\kappa^2$-**L3**$)]$ (**5**) consist of discrete mononuclear units. The ORTEP views of the molecular structures of **4** and **5** are given in Figs. 1 and 2, respectively. The bond distances and angles are unexceptional, as shown in Tables 3 and 4. The Re atom in the neutral complexes **4–5** is six-coordinated, exhibiting a slightly distorted octahedral coordination geometry. The three carbonyl ligands occupy one triangular face of the coordination polyhedron, being the other three remaining coordination sites defined by a bromide and by the bidentate **L2** and **L3** ligands in **4** and in **5**, respectively.

Deviations from the idealized octahedral geometry can be seen on the bond angles around the Re atom (Tables 3 and 4). The *cis* and *trans* bond angles range between 80.4(3)–99.7(6)° and 169.8(5)–179.5(6)°, and between 82.84(15)–94.3(3)° and 174.2(2)–177.1(3)° in **4** and **5**, respectively. In these complexes the bidentate chelators form non-planar six-membered rings which present distorted half-boat and boat conformations in **4** ([Re, O4,C4, C111, C110, P1]) and **5** ([Re, N1, C4, C111, C110, P1]), respectively. All the above referred parameters indicate for **4** an higher distortion from the octahedral geometry than for **5,** which can certainly be due to the sp^2 hybridization of the C4 atom in complex **4**.

In **4** and **5** the metrical parameters are comparable to those that we have previously reported for other rhenium tricarbonyl complexes also anchored by heterofunctionalized phosphines [11,12]. The average Re–CO bond distances in **4** (average 1.92(2) Å) and **5** (average 1.93(1) Å) are similar and in the range found for other neutral Re(I) tricarbonyl compounds containing monodentate, bidentate or tridentate ligands (1.89–

Table 2
Labelling conditions of ligands **L1–L4**; HPLC retention times (R_t) of complexes **3a–6a** (^{99m}Tc) and of the analogues Re compounds **3–6**

Ligands	Labelling conditions			Yields (%)[a]
	Reaction time (min)	R_t (min)		
		Re[b]	^{99m}Tc[c]	
L1	30	**3** – 22.7	**3a** – 22.5	**3a** – 95
L2	60	**4** – 21.5	**4a** – 23.3	**4a** – 98
L3	30	**5** – 21.7	**5a** – 22.7	**5a** – 87
L4	60	**6** – 22.05	**6a** – 23.6	**6a** – 92

[a] Addition of 1% SDS after labelling; [L] = 10^{-4} M, 100 °C.
[b] UV traces monitored at 254 nm.
[c] Radioactive traces.

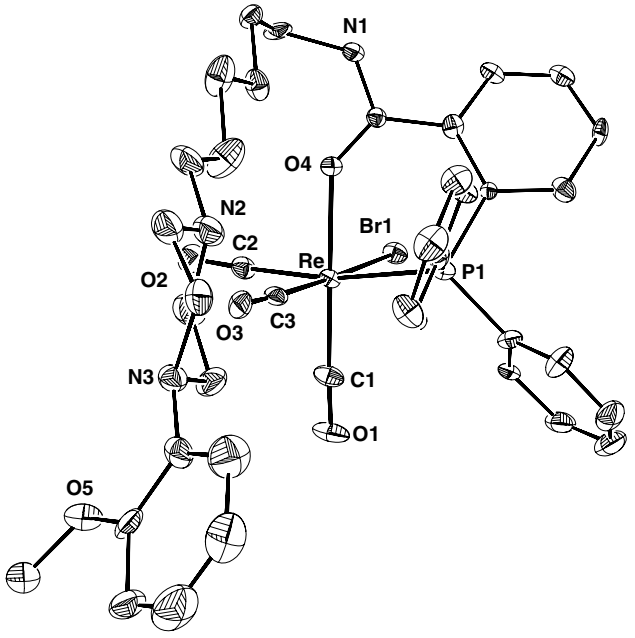


Fig. 1. ORTEP view of the neutral complex *fac*-$[Re(CO)_3Br(\kappa^2$-**L2**$)]$ (**4**). Vibrational ellipsoids are drawn at the 20% probability level.

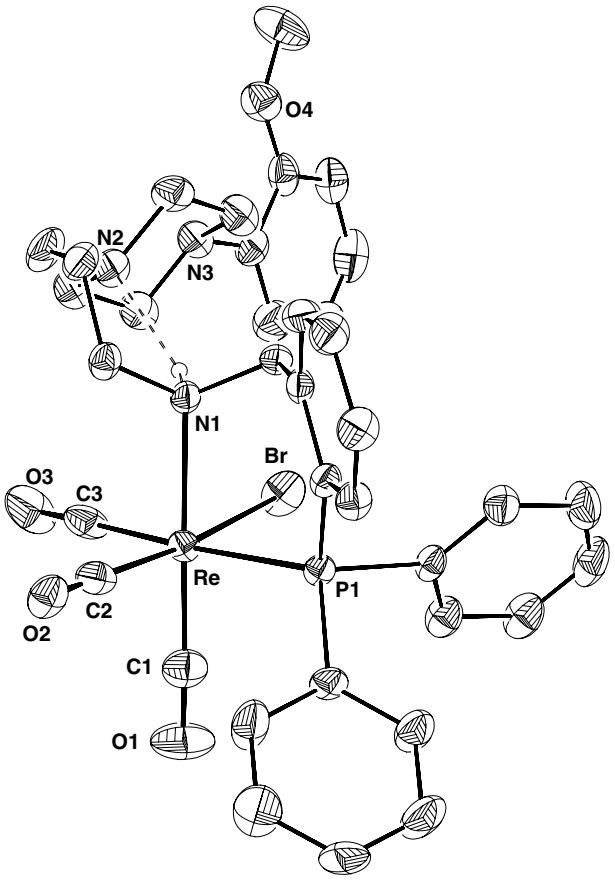


Fig. 2. ORTEP view of the neutral complex *fac*-[Re(CO)$_3$Br(κ^2-**L3**)] (**5**). Vibrational ellipsoids are drawn at the 30% probability level.

Table 3
Selected bond lengths (Å) and angles for (°) for complex [Re(CO$_3$)Br(κ^2-**L2**)] (**4**)

Re–C(1)	1.913(17)	Re–Br	2.6382(19)
Re–C(2)	1.914(18)	Re–O(4)	2.162(10)
Re–C(3)	1.94(2)	Re–P	2.444(4)
C(1)–Re–C(2)	90.5(8)	C(3)–Re–P(1)	92.2(5)
C(1)–Re–C(3)	89.5(7)	O(4)–Re–P(1)	80.4(3)
C(2)–Re–C(3)	87.8(7)	C(1)–Re–Br(1)	92.0(5)
C(1)–Re–O(4)	179.5(6)	C(2)–Re–Br(1)	90.9(5)
C(2)–Re–O(4)	89.4(6)	C(3)–Re–Br(1)	178.0(5)
C(3)–Re–O(4)	91.0(5)	O(4)–Re–Br(1)	87.5(3)
C(1)–Re–P(1)	99.7(6)	P(1)–Re–Br(1)	88.82(11)
C(2)–Re–P(1)	169.8(5)		

2.03 Å) [11]. Likewise, the Re–P bond distances found in **4** (2.444(4) Å) and **5** (2.470(2) Å) and the Re–Br bond lengths are normal (**4**, 2.6382(19) Å; **5**, 2.6118(11) Å), comparing well with the values described previously for other tricarbonyl rhenium complexes [11]. The Re–N bond distance of 2.237(6) Å in **5** is typical of a single bond and agrees with the presence of an sp^3 hybridized amine nitrogen [24].

Table 4
Selected bond lengths (Å) and angles (°) for [Re(CO$_3$)Br(κ^2-**L3**)] (**5**)

Re–C(1)	1.899(10)	Re–N(1)	2.237(6)
Re–C(2)	1.940(10)	Re–P(1)	2.4703(19)
Re–C(3)	1.951(9)	Re–Br(1)	2.6118(11)
C(1)–Re–C(2)	89.3(4)	C(3)–Re–P(1)	176.3(2)
C(1)–Re–C(3)	90.5(4)	N(1)–Re–P(1)	88.94(15)
C(2)–Re–C(3)	90.6(3)	C(1)–Re–Br(1)	94.3(3)
C(1)–Re–N(1)	177.1(3)	C(2)–Re–Br(1)	174.2(2)
C(2)–Re–N(1)	93.5(3)	C(3)–Re–Br(1)	84.9(3)
C(3)–Re–N(1)	88.8(3)	N(1)–Re–Br(1)	82.84(15)
C(1)–Re–P(1)	91.7(3)	P(1)–Re–Br(1)	92.00(5)
C(2)–Re–P(1)	92.4(2)		

In complex **5** an intramolecular hydrogen bridge between N1 and N2 was observed (N1···N2, 2.932 Å; N1–H···N2, 144.01°), forcing a folding of the pharmacophore towards the metal center.

4.1. Receptor binding assays on rat brain homogenates

The rhenium (I) tricarbonyl complexes **3**–**6** have been used for determination of the receptor-binding affinity and selectivity in rat brain homogenates. The IC$_{50}$ values of these compounds for 5-HT$_{1A}$ and 5-HT$_{2A}$ receptores, summarized in Table 5, were determined by displacement studies.

From the values found we can conclude that complex **3** is the only one which presents a relatively interesting affinity and specificity toward the 5HT$_{1A}$ receptors for further biological evaluation (5HT$_{1A}$, IC$_{50}$: 20 nM; 5HT$_{2A}$, IC$_{50}$: 4680 nM).

For all the other complexes the affinity and specificity towards 5HT$_{1A}$ decrease significantly. The fit of the molecule into the binding pocket of the receptors was significantly affected when an amide group in the chelate unit (**3**, 5HT$_{1A}$, IC$_{50}$: 20 nM) was replaced by a secondary amine (**5**, IC$_{50}$: 5HT$_{1A}$, 285 nM). This effect is even more pronounced when the alkyl chain length increases (**3** versus **4** and **5** versus **6**).

Taking into account previous results on structure/activity relationships and also considering that what is rational and predictable in the design of organic receptor ligands cannot simply be extended to technetium compounds, we think that the somehow encouraging results

Table 5
Inhibition constants (IC$_{50}$) of **3**–**6** for 5HT$_{1A}$ and 5HT$_{2A}$ receptors

Complex	IC$_{50}$ (nM)		
	5-HT$_{1A}$ [^{3}H]–OH–DPAT	5-HT$_{2A}$ [^{3}H]ketanserin	5-HT$_{2A}$/5-HT$_{1A}$
3	20 ± 0.1 nM	4680 ± 0.1 nM	234
4	200 ± 4 nM	340 ± 9 nM	1.7
5	285 ± 4 nM	490 ± 8 nM	1.7
6	1100 ± 4 nM	1190 ± 0.5 nM	1.0

obtained for compound **3** can be due to a certain rigidity imposed by the presence of the amide group, which decreases when the coordination to the metal is done through a secondary amine. On contrary, increasing the alkyl chain length a higher mobility of the pendant arm is observed with a folding towards the metal center. In the case of compound **5** this folder seems to be favoured due to an intramolecular hydrogen bridge between the secondary amine of the piperazine (Figs. 1 and 2). Whether this type of interactions are maintained in solution is difficult to say, although the mobility of the pendant arm seems to exist in solution as indicated by the broadness of the piperazine resonances observed in the ^{1}H NMR spectra of **5**.

5. Conclusions

This work describes the synthesis and characterization of several heterofunctionalized phosphines carrying the bioactive fragment (2-methoxyphenyl)piperazine (**L**) and different donor atom sets for metal stabilization. For the same donor atom set different alkyl chains were introduced between the coordinating atoms and the bioactive fragment. Using these chelators several rhenium complexes of the type *fac*-[Re(CO)$_3$-Br(κ^2-L)] have been isolated and fully characterized, including by X-ray structural analysis. The corresponding ^{99m}Tc complexes have also been prepared in good yields and their identity confirmed by HPLC comparison with the corresponding rhenium complexes used as surrogates. The IC50 values of the synthesized and characterized complexes for 5HT$_{1A}$ and 5HT$_{2A}$ receptors are not encouraging, indicating a significant effect of the chelate donor atom set and also of the linker used to bind covalently the technetium chelate unit to the (2-methoxyphenyl)piperazine pharmacophore. In fact, this effect can be rationalized in terms of the structural features of the complexes, namely the folding of the pharmacophore towards the metal center when the alkyl chain length increases. These results confirm the importance of structure/activity relationships, indicating that compound **3** is the only one which would be interesting to evaluate in vivo, being very much important to decrease its molecular weight to eventually promote brain uptake.

6. Supplementary material

Crystallographic data without structure factors for the two structures reported in this paper have been deposited with Cambridge Crystallographic Data Center as supplementary publication nos. CDCC 235823 (**4**) and CDCC 235824 (**5**). Copies of the data can be obtained free of charge from the CDCC, 12 Union Road, Cambridge CB2 1EZ, UK; Tel.: +44-1223-336408; fax: +44-1223-336003; e-mail: deposit@cdcc.cam.ac.uk; www: http://ccdc.cam.ac.uk

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