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Synthesis, characterization and biological activity of ^{99m}Tc-labeled piperidine analogues targeting sigma receptors

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Sigma receptors are expressed in high density in various types of cancer cells including brain tumours and are also involved in various diseases of central nervous system. This makes ligands that bind to these receptors, attractive molecular vectors for targeting radiation to the specific sites with the purpose of imaging and therapy of neurological disorders. We report synthesis of three derivatives of 4-amino-*N*-benzylpiperidine namely, 4-dithiocarbamato-*N*-benzylpiperidine, 4-iminodiacetato-*N*-benzylpiperidine and 4-(*N*-benzylpiperidine)-pyridin-2-ylmethyl-amino)-acetic acid and their radiolabeling with technetium-99m. The *in vivo* evaluation of these radiolabeled compounds has been carried out in mice, for assessment of their binding affinity with sigma receptors. Of the three complexes, [^{99m}TcN]-4-dithiocarbamato-*N*-benzylpiperidine, [^{99m}TcN]Pip-DTC exhibited the most promising characteristics with brain uptake of 0.6% ID/g at 5 min.p.i. that reduced to 0.3% ID/g after 2 h.p.i. Competition experiment carried out with [^{99m}TcN]Pip-DTC complex, using (+)-pentazocine showed its specificity towards sigma receptors, as was found to be evident from reduction in the brain uptake of this complex. Introduction of iminodiacetate and pyridine moieties and subsequent radiolabeling did not result in complexes with significant potential of targeting and binding with sigma receptors.

Keywords: benzylpiperidine; sigma receptors; $[^{99m}Tc(CO)_3(H_2O)_3]^+$; $[^{99m}TcN]^{2+}$

Introduction

The study of the central nervous system (CNS) and its related neuropsychiatric disorders using diagnostic techniques of nuclear medicine is a rapidly expanding area of research.[1,2] Towards this, the specific receptors, their subtypes, transmitters and enzymes expressed on the CNS can serve as targets for development of radiolabeled receptor-selective molecules. Among the various neuroreceptors identified, sigma receptors belong to a unique class of proteins different from opioid, *N*-methyl-D-aspartate (NMDA) phencyclidine, dopaminergic and other known receptors acting as neurotransmitter agents.[3] Sigma receptors having high affinity for neuroleptic drugs are known to be involved in several disorders of the CNS like schizophrenia, depression, dementia, etc.[4,5] Besides being present in brain, sigma receptors are also over-expressed in high density in different tumours like those of breast, prostate, melanoma, non-small cell lung carcinoma and other tumours of neural origin making them favourable targets for developing radiotracers to be used in nuclear medicine for imaging as well as subsequent therapy.[6] On the basis of their pharmacological action, sigma receptors have been classified into two subtypes as sigma-1 and sigma-2. Of the two subtypes, sigma-1 receptors are well studied and characterized. While sigma-1 receptors exhibit high binding affinity for [^{3}H](+)-pentazocine and other (+)-benzomorphans, di-o-tolyl guanidine (DTG) shows high affinity towards both sigma-1 and sigma-2 sites.[7,8]

Radiopharmaceuticals designed to target sites expressing sigma receptors may therefore serve as potential tools for imaging and subsequent therapeutic intervention in various psychiatric diseases and various types of cancers. Positron emission tomography (PET) as well as single photon emission computed tomography (SPECT) has been used for imaging of sigma receptors. In this respect, various radiolabeled agents have been prepared using ^{11}C, ^{18}F, ^{99m}Tc and ^{123}I as radioisotopes.[9–13] Though PET radioligands studied out-number those of SPET ligands, wide accessibility and attractive nuclear-physical properties of technetium-99m provide strong impetus to efforts in improving the availability and research for new suitable SPET imaging agents. Technetium-99m is the ideal diagnostic radionuclide in nuclear medicine due to its favourable decay characteristics ($t_{1/2}$ = 6 h, E_γ = 140 keV), easy availability and cost effectiveness.[14] In the present studies, novel $[^{99m}TcN]^{2+}$ and $[^{99m}Tc(CO)_3(H_2O)_3]^+$ cores have been chosen as ideally suited precursors for designing of receptor-specific radiotracers. $[^{99m}TcN]^{2+}$ core forms stable complexes with N and S donors in a tetradentate array. In this respect, dithiocarbamates are ideally suited for complexation, leading

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to formation of neutral complexes as a result of coordination of two molecules of dithiocarbamate moiety.[15] $[^{99m}Tc(CO)_3(H_2O)_3]^+$ core containing d^6 Tc(I) center forms stable low spin complexes with N-containing ligands such as histidine, histamine, imidazole, iminodiacetic acid, diethylene triamine, Schiff's bases.[16] The use of $[^{99m}TcN]^{2+}$ and $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursors give high specific activity complexes emerging from the requirement of low ligand concentration, thereby offering a promising advantage towards designing agents for targeting receptors.

Several derivatives of piperidine, piperazine,[17,18] benzamides[19] and alkylamine[20] radiolabeled with suitable isotopes have been reported in literature as potential radiopharmaceuticals for imaging of sigma receptors. In the present studies, 4-amino-*N*-benzylpiperidine has been chosen as the parent molecule with the aim of preparing an agent for specific targeting of sigma receptors. The *N*-benzyl piperidine template is an integral unit of a host of ligands such as 2-[^{125}I]BP, 2-[^{125}I] *N*-(*N*-benzylpiperidin-4-yl)-2-iodobenzamide, for use as CNS receptor-specific agents.[13,19] As $-NH_2$ group of piperidine in the 4-amino-*N*-benzylpiperidine template is a pendant moiety, its facile yet versatile modes of derivatization to incorporate suitable functional groups provide possible routes for syntheses of different ligands without affecting the receptor binding properties. The strategy in designing of the ligands were envisaged keeping in mind that the net charges on the resultant radiolabeled complexes would be determining factors governing their biological specificity as receptor-targeting agents. This paper reports the efforts to prepare three different derivatives of 4-amino-*N*-benzylpiperidine **1** namely, 4-dithiocarbamato-*N*-benzylpiperidine (Pip-DTC) **L1**, 4-(*N*-benzylpiperidine)-pyridin-2-ylmethyl-amino)-acetic acid (PPAA) **L2** and 4-iminodiacetato-*N*-benzylpiperidine (Pip-IDA) **L3.** Ligand **L1** has been radiolabeled with $[^{99m}TcN]^{2+}$ core, whereas ligands **L2** and **L3** have been radiolabeled with$[^{99m}Tc(CO)_3(H_2O)_3]^+$ core. *In vivo* evaluation of ^{99m}Tc-complexes has been carried out to assess the uptake in brain, which, in turn, reflects the ability to cross the blood–brain barrier and bind to the receptors. Competition studies with sigma receptor-specific ligand, (+)-pentazocine, have also been carried out to study the receptor specificity of these complexes.

Results and discussion

Chemistry

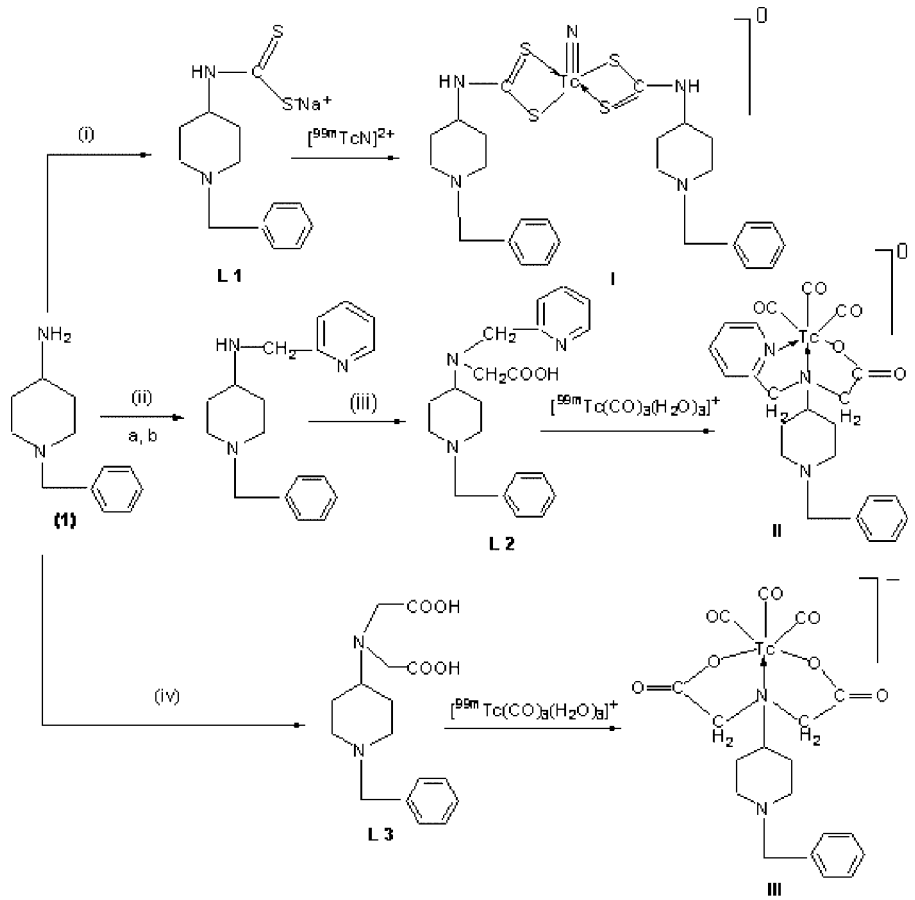
Several molecular modelling studies have been reported for the determination of the biologically active molecular unit responsible for binding with sigma receptors. It is proposed that the primary binding requires presence of a hydrophobic site offered by a phenyl group and a hydrophilic site represented by the nitrogen atom and its lone pair. The secondary binding site requires the presence of an electronegative atom like oxygen or sulphur.[21] With the aim of preparing a ^{99m}Tc-labeled agent specific for sigma receptors, three different derivatives of 4-amino-*N*-benzylpiperidine have been prepared by carrying out functionalization of the amino group. (Scheme 1) Ligand **L1,** a dithiocarbamate, was prepared by reaction of the parent molecule, 4-amino-*N*-benzylpiperidine, with an equimolar amount of carbon disulphide in the presence of sodium hydroxide at room temperature. Formation of **L1** could be confirmed by ^{1}H-NMR, ^{13}C-NMR and mass spectra. A peak at δ213.6 in ^{13}C-NMR spectra indicates the incorporation of an additional carbon atom corresponding to the carbon atom of the dithiocarbamate group. **L2** was prepared in two steps as depicted in Scheme 1. Refluxing **1** with pyridine 2-carboxaldehyde resulted in the formation of imine, which was subsequently reduced to the corresponding secondary amine using sodium borohydride as the reducing agent. The formation of the reduced product was confirmed by disappearance of the imine proton at δ8.0 observed in the ^{1}H-NMR spectra of the imine and subsequent appearance of a two-proton singlet corresponding to pyridyl methyl protons at δ2.9 and pyridine protons between δ8.54 and 7.18 in the aromatic region. The amine formed was subsequently reacted with an equimolar amount of bromoacetic acid to yield the final product, **L2**. Formation of final product could be confirmed by the appearance of a peak at δ3.55 in ^{1}H-NMR spectra corresponding to amino acetic acid group. Mass and ^{13}C-NMR spectral characterization were also carried out for the ligand **L2**. Ligand **L3** was prepared by dialkylation of the amino group and was carried out by reaction of **1** with two equivalents of bromoacetic acid under alkaline conditions. The iminodiacetate formation was confirmed by ^{1}H-NMR where a two-proton singlet at δ3.34 corresponding to $-NCH_2COOH$ group was observed. The compound was further characterized by mass and ^{13}C-NMR spectra.

Radiochemistry

$[^{99m}TcN]^{2+}$ known to form stable complexes with dithiocarbamates was used to radiolabel **L1**. Ligands **L2** and **L3** behaving as tridentate ligands with N_2O and NO_2 as donor atoms, respectively, were radiolabeled with the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ core.

$[^{99m}TcN]^{2+}$ core was prepared using the kit following a procedure reported earlier.[22] The complex was then prepared by incubation of **L1** with the $[^{99m}TcN]^{2+}$ core. The core as well as the complex [^{99m}TcN]Pip-DTC **I** could be obtained in >98% radiochemical yield as determined by HPLC. In HPLC gradient system, the nitrido core exhibited the retention time of 4 min (Figure 1(a)), whereas the complex was eluted as a single species with retention time of 15 min (Figure 2(a)). Additional evidence showing formation of the ^{99m}Tc-nitrido complex in good yields (>98%) were obtained from TLC and paper electrophoresis. TLC was carried out in ethanol:chloroform:toluene:0.5 M ammonium acetate mixture (6:3:3:0.5 v/v) as well as in saline. In the former solvent system, ^{99m}Tc-nitrido precursor as well as reduced technetium remained at the origin (R_f = 0–0.1) corresponding to >98% of activity whereas $^{99m}TcO_4^-$ moved to R_f = 0.4–0.6. However, in saline, both $^{99m}TcO_4^-$ and ^{99m}Tc-nitrido intermediate corresponding to >98% of activity moved with the solvent front (R_f = 0.8–1.0) with reduced technetium remaining at the origin. Radiochemical purity of the complex (>98%) could be determined by TLC in saline wherein the complex remained at the origin (R_f = 0). In paper electrophoresis studies, while the ^{99m}Tc-nitrido intermediate showed a movement of 5 cm towards the anode, the complex did not show any movement indicating possible neutrality of the complex. Lipophilicity of the radiolabeled complex was determined by distribution in octanol and water and the partition coefficient (log *P*) was found to be 1.7 ± 0.2.

The ^{99m}Tc-tricarbonyl synthon used for complexation was prepared in-situ according to the reported procedure.[23] It could be prepared in >98% yield as determined by C-18 reverse phase HPLC system. $^{99m}Tc(CO)_3$-PPAA **II** and $[^{99m}Tc(CO)_3$-Pip-IDA$]^-$ **III** complexes were prepared by incubation of ligands with



Syntheses of ligands: (i) CS_2, diethylether, NaOH, 24 h (ii) (a) Pyridine 2-carboxaldehyde, MeOH, reflux, 24 h (b) $NaBH_4$, MeOH, reflux, 5 h
(iii) $BrCH_2COOH$, MeOH, K_2CO_3, 5 d (iv) $BrCH_2COOH$, MeOH, K_2CO_3, 5 d

Scheme 1. Syntheses of 4-amino-*N*-benzylpiperidine ligands.

the ^{99m}Tc-carbonyl core. Retention time of the precursor on HPLC was 13.7 min (Figure 1(b)), whereas that of complexes **II** and **III** were 15.8 min and 11.6 min, respectively (Figure 2(b) and (c)). The radiochemical yield of the complexes as determined by HPLC analyses was $>98\%$. Optimization of the pH for complexation was required for radiolabeling of the two ligands with $[^{99m}Tc(CO)_3(H_2O)_3]^+$. Under optimized conditions it was found that pH of highly alkaline ^{99m}Tc-carbonyl precursor had to be adjusted to 7 and 4 using PO_4^{3-} buffer (pH 7.5, 0.5 M):HCl (1.0 M) (1:3, v/v) before addition of ligands **L2** and **L3,** for preparation of complexes **II** and **III**, respectively. Logarithm of partition coefficient (log *P*) is a parameter used to provide an indication of the lipophilicity of the complexes. Log *P* values as determined by extractions in octanol/saline were found to be 1.56 ± 0.18 and 0.95 ± 0.1 for the complexes **II** and **III**, respectively. The log *P* value between 0.9 and 2.5 has been suggested optimal for BBB crossing.[24] As values of log *P* for complexes under study lie within this range, they are expected to bind to CNS receptors.

In vitro stability of the complexes was determined by incubation of the complexes in water bath at 37°C for 6 h. The radiochemical purity of the complexes were checked after 6 h using HPLC analyses where no observable change in the retention times were found thereby indicating the stability of the three complexes.

Challenge studies performed by incubation of complexes with excess amount of histidine and cysteine also did not lead to alterations in the retention time and pattern of the peak obtained in HPLC system, indicating their kinetic inertness and stability towards transchelation.

Biodistribution studies

Pharmacokinetic pattern of the three complexes were studied by carrying out biodistribution studies in normal Swiss mice. (Table 1) The uptake of complex **I** in brain was higher than those of the other complexes, being 0.6% ID/g at 5 min.p.i. with retention till 30 min. The uptake reduced to 0.3% ID/g at 2 h.p.i. The apparently low brain uptake of 0.6% ID/g, however, compares well with many of the other reported agents for brain receptor imaging.[25] Results of competition studies at 30 min.p.i. for complexes **I** and **II** are given in Figure 3. *In vivo* blocking studies wherein (+)-Pentazocine, a drug known to bind specifically to sigma receptors, was administered 1 h prior to the injection of the radiolabeled complex were carried out in Swiss mice models. No radioactivity was observed in the mice brain

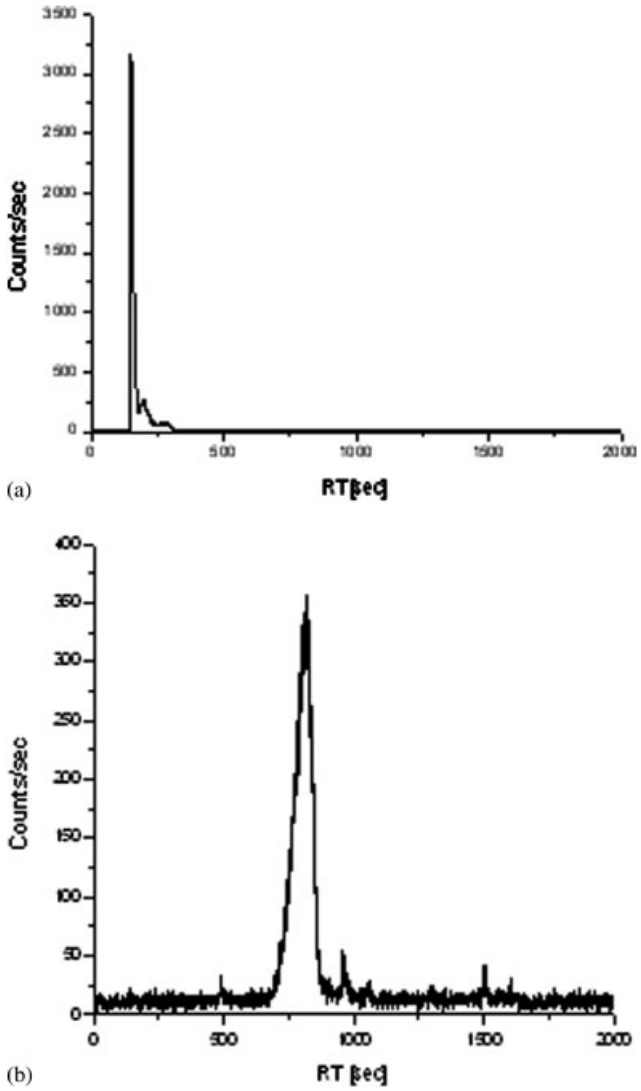


Figure 1. HPLC pattern of precursors (a) $[^{99m}TcN]^{2+}$ and (b) $[^{99m}Tc(CO)_3(H_2O)_3]^+$.

pre-administered with (+)-pentazocine in case of studies with complex **I** indicating complete blockage and hence the probable specificity of complex **I** for binding with sigma receptors. Uptake in liver and lungs was also reduced as these organs are also known to express sigma receptors. These observations are suggestive of receptor-mediated uptake of the complex.

Brain uptake of complex **II** was found to be 0.5% ID/g 5 min.p.i, which reduced to 0.2% ID/g 2 h.p.i. There was no decrease in the radioactivity in the brain on carrying out blocking studies with (+)-pentazocine which could be attributed possibly to its less specific binding to sigma receptors as compared with that of complex **I** (Figure 3). The presence of the additional aromatic moiety (pyridine ring) besides the benzyl group leading to steric hindrance may be the probable cause for decrease in the binding affinity.

Complex **III** showed negligible brain uptake of 0.18% ID/g at 5 min.p.i. Blood uptake was less compared with other lipophilic derivatives. Within 30 min of injection of the complex, the radioactivity cleared from all the major organs whereby studies at 2 h.p.i were not carried out with this complex. Brain uptake studies are consistent with the observed log *P* values.

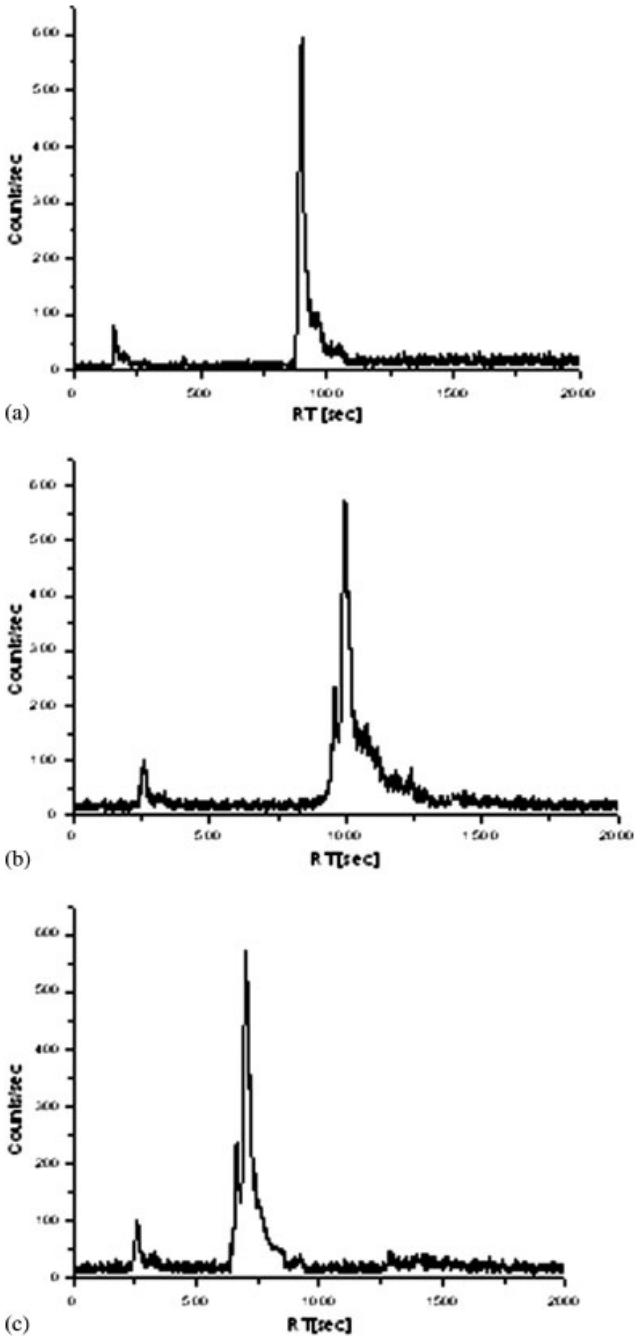


Figure 2. HPLC pattern of ^{99m}Tc-complexes (a) $[^{99m}TcN]$Pip-DTC (I), (b) $^{99m}Tc(CO)_3$-PPAA (II), (c) $[^{99m}Tc(CO)_3$-PipIDA$]^-$ (III).

The proposed structure of the complex **I** has been shown in scheme 1 where two dithiocarbamate molecules each behaving as a bidentate ligand coordinates with technetium resulting in the formation of a neutral complex. Such a speculation can be substantiated by earlier reports where the involvement of two DTC moieties have been proposed.[26] Ligand **L2** acts as a tridentate ligand for complexation with the $^{99m}Tc(CO)_3$-precursor with pyridine nitrogen, carboxyl oxygen and iminodiacetate nitrogen acting as coordinating groups. Ligand **L3** has a tridentate array of donors in the iminodiacetate moiety as the chelating groups which replace the three labile aqua molecules of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ by a nitrogen and two carboxyl oxygen

Table 1. Biodistribution studies of complexes I, II and III in normal Swiss mice

Complex	Time (p.i.)	Blood	Liver	Intestine	Kidney	Heart	Brain	Lungs	Spleen
I	5 min	8±0.6	31±2.0	2.6±0.4	22±3.0	10±1.0	0.6±0.05	23±2.0	10±0.7
	30 min	3.3±0.6	25.4±4.0	5.6±1.0	11±0.8	7.4±1.0	0.43±0.03	15.6±2.8	9±0.6
	2 h	2±0.06	13±0.5	7±1.0	7.8±1.0	5.7±1.5	0.3±0.05	7.2±0.8	8±0.5
II	5 min	8.3±0.8	20.3±6.0	5.3±0.6	24.6±4.9	9.6±0.2	0.5±0.02	14.7±1.4	5.6±1.6
	30 min	3.6±0.5	16±3.3	8.8±0.4	19.6±3.2	6.8±0.5	0.46±0.2	9.2±3.7	5.4±.1.7
	2 h	1.8±0.2	16.4±1.0	10.8±1.7	12.2±2.5	5.4±0.9	0.21±0.12	5.3±0.5	3.9±0.8
III	5 min	2.2±0.25	34±1.5	2.8±0.3	6±0.4	1.4±0.17	0.18±0.03	2.3±0.54	0.9±0.2
	30 min	1.4±0.6	9±2.0	20±4.0	2±0.7	0.4±0.2	0.06±0.02	0.7±0.06	0.4±0.05

%ID/g of organ (mean±standard deviation), $n=3$.

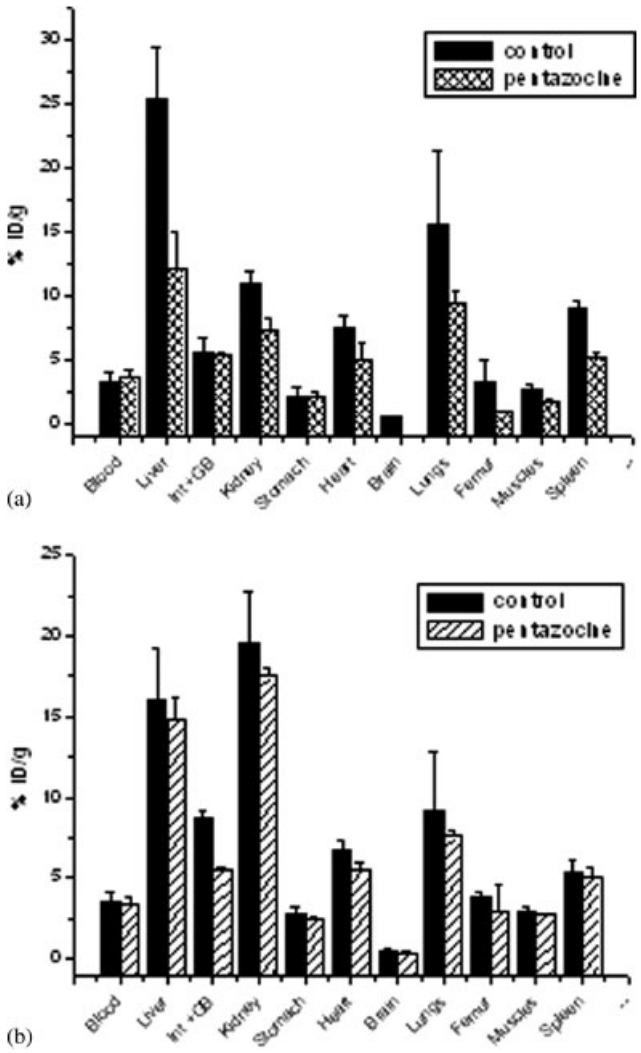


Figure 3. Effect of (+)-pentazocine on distribution of radioactivity (30 min.p.i.) of (a) [^{99m}TcN]Pip-DTC (I) and (b) ^{99m}Tc(CO)$_3$-PPAA (II).

to form the complex. Complexes **I** and **II** are neutral with similar lipophilicity thus showing similar brain uptake. However, no significant blocking was observed in competition studies for complex **II** indicating lower receptor affinity of the complex. The insignificant brain uptake of complex **III** was expected due to its residual negative charge and lower lipophilicity. Besides this feature no uptake in other organs expressing sigma receptors could be seen implying that introduction of the iminodiacetic acid moiety strongly affects the receptor affinity.

Experimental

General

All chemical reagents were of commercial grade. Carbon monoxide in 0.5 L refillable canisters was obtained from M/s Alchemie Gases & Chemicals, Mumbai, India. $^{99m}TcO_4^-$ was eluted from an in-house ^{99}Mo/^{99m}Tc column generator using normal saline. 4-amino-*N*-benzylpiperidine was purchased from Fluka Chemie GmbH. Commercial kit for preparation of ^{99m}Tc-nitrido species was obtained from CIS Bio International. Electrophoresis experiments were carried out using 0.025 M phosphate buffer (pH 7.5) at 300 V/cm for 1 h. HPLC analyses were performed on a Jasco PU 1580 system with a Jasco 1575 tunable absorption detector and a radiometric detector system. A C-18 reversed phase HiQ Sil (5 μ, 250 × 4 mm) column has been used. About 25 μL of the test solution was injected into the column and the elution was monitored by observing the radioactivity profile. The flow rate was maintained at 1 mL/min. The gradient system consisting of eluting solvents H_2O (solvent A) and acetonitrile (solvent B) with 0.1% trifluoroacetic acid was used (0–28 min, 90% A-10% A; 28–30 min, 10% A; 30–32 min, 10% A-90% A). NMR spectra were obtained using Varian VXR 300S spectrophotometer operating at 300 MHz for ^{1}H and at 75 MHz for ^{13}C. Mass spectra were recorded on QTOF Micromass Instrument using electron spray ionization (ESI) in positive mode. All the animal experiments were carried out in compliance with the relevant National laws as approved by the local committee on the conduct and ethics of animal experimentation.

Chemical synthesis

*4-dithiocarbamato-N-benzylpiperidine (**L1**)*

To a solution of 4-amino-*N*-benzylpiperidine (**1**) (50 mg, 0.26 mmol) in diethyl ether (10 mL), carbon disulphide (15 μL, 0.25 mmol) was added along with sodium hydroxide (4.8 mg, 0.12 mmol). A white precipitate was obtained immediately on addition of carbon disulphide. The reaction mixture was stirred at room temperature overnight. After 24 h, solvent was removed under reduced pressure and the product was purified using ammonia:methanol (5:95) as eluting solvent on a silica gel column.

^{1}H-NMR (δ ppm, $CDCl_3$): 7.40 (s, 5H, aromatic), 4.18 (s, 2H, benzyl), 2.21 (m, 1H, piperidine), 2.09 (m, 4H, piperidine), 1.48

(m, 4H, piperidine); ^{13}C-NMR (δ ppm, CD_3OD) 31.78 (2C, piperidine), 35.58 (2C, piperidine), 53.43 (C, piperidine), 64.03 (C, benzyl), 128.47 (C, phenyl), 129.36 (2C, phenyl), 130.82 (2C, phenyl), 138.58 (C, phenyl), 213.6 (C, $NHCS_2$); MS (ESI): mass calculated for $C_{13}H_{17}N_2S_2Na$; 288, found 289 $[M+H]^+$.

*{4-(N-benzylpiperidine)}-pyridin-2-ylmethyl-amine (**2**)*

To a solution of **1** (190 mg, 1 mmol) in dry methanol (10 mL), 2-pyridine carboxaldehyde (100 mg, 1 mmol) was added and the reaction mixture was refluxed for 24 h. Formation of the product was confirmed by TLC in 15% MeOH/$CHCl_3$ R_f (product) = 0.3. $NaBH_4$ was added to the above mixture and refluxed for 5 h. After completion of the reaction, methanol was evaporated in a rotary evaporator. The product was extracted in $CHCl_3$ (3 × 10 mL), washed with water thrice and dried over anhydrous Na_2SO_4. The solution was filtered and $CHCl_3$ was evaporated to yield a yellow solid. The product was characterized by TLC in 1% NH_3/methanol. R_f (product) = 0.7.

^{1}H-NMR (δ ppm, $CDCl_3$): 8.54 (d, 1H, pyridine), 7.64 (t, 1H, pyridine), 7.33 (s, 5H, phenyl), 7.33 (d, 1H, pyridine), 7.18 (t, 1H, pyridine), 3.94 (s, 2H, benzyl), 2.98 (s, 2H, pyridylmethyl), 2.58 (m, 1H, piperidine), 2.20 (m, 2H, piperidine), 1.54 (t, 2H, piperidine).

*{4-(N-benzylpiperidine)}-pyridin-2-ylmethyl-amino)-acetic acid (**L2**)*

To a solution of **2** (195 mg, 0.7 mmol) in methanol (10 mL), bromoacetic acid (97 mg, 0.7 mmol) was added along with potassium carbonate (0.1 mg, 0.07 mmol) and stirred at room temperature for 5 days. The solvent was removed under vacuum. The residue was extracted in chloroform (3 × 10 mL) and the combined extracts were washed with water and dried over anhydrous sodium sulphate. TLC (silica): 20% MeOH/$CHCl_3$ R_f (product) = 0.5.

^{1}H-NMR (δ ppm, $CDCl_3$): 8.54 (d, 1H, pyridine), 7.64 (t, 1H, pyridine), 7.33 (s, 5H, phenyl), 7.32 (d, 1H, pyridine), 7.18 (t, 1H, pyridine), 3.94 (s, 2H, benzyl), 3.55 (s, 2H, $-CH_2COOH$), 2.90 (s, 2H, pyridylmethyl), 2.58 (m, 1H, piperidine), 2.09 (m, 4H, piperidine), 1.54 (t, 4H, piperidine); ^{13}C-NMR (^{1}H-decoupled δ ppm, CD_3OD) 28.3 (2C, piperidine), 32.90 (2C, piperidine), 53.8 (C, piperidine), 59.5 (C, pyridylmethyl), 58.8 (C, $-CH_2COOH$) 62.9 (C, benzyl), 123.6 (C, pyridine), 125 (C, pyridine), 128.6 (C, phenyl), 129.3 (2C, phenyl), 130.9 (2C, phenyl), 138.5 (C, phenyl), 138.6 (C, pyridine), 149.6 (C, pyridine), 161 (C, pyridine), 177.7 (C, $-CH_2COOH$); MS (ESI) mass calculated for $C_{20}H_{25}N_3O_2$; 339, found 340 $[M+H]^+$.

*4-iminodiacetato-N-benzylpiperidine (**L3**)*

To a solution of **1** (38 mg, 0.18 mmol) in methanol (3 mL), bromoacetic acid (50 mg, 0.36 mmol) was added along with potassium carbonate (0.07 mg, 0.5 mmol). The reaction mixture was stirred at room temperature for 5 days. Subsequently, solvent was removed under rotary evaporation. TLC (silica): 20% MeOH/$CHCl_3$ R_f (product) = 0.7.

^{1}H-NMR (δ ppm, D_2O): 7.39 (s, 5H, phenyl), 3.86 (s, 2H, benzyl), 3.34 (s, 4H, $-CH_2COOH$), 2.28 (m, 1H, piperidine), 1.98 (m, 4H, piperidine), 1.62 (t, 4H, piperidine); ^{13}C-NMR (δ ppm, CD_3OD) 27.92 (2C, piperidine), 30.0 (2C, piperidine), 52.62 (C, piperidine), 58.8 (2C, $-CH_2COOH$), 62.83 (C, benzyl), 128.60 (C, phenyl), 129.45 (2C, phenyl), 130.73 (2C, phenyl), 137.94 (C, phenyl), 177.87 (2C, $-CH_2COOH$); MS (ESI): mass calculated for $C_{16}H_{22}N_2O_4$; 306, found 307 $[M+H]^+$.

Radiochemistry

$[^{99m}Tc(CO)_3(H_2O_3)]^+$ precursor

The synthon was prepared by using modified procedure reported by Alberto *et al.*[23] An aqueous solution of $NaBH_4$ (5.5 mg), Na_2CO_3 (4 mg) and Na/K tartrate (15 mg), in 0.5 mL double distilled water was prepared. Carbon monoxide gas was purged through this solution for 5 min and after adding $^{99m}TcO_4^-$ (1 mL, 37 MBq) to the solution, it was heated at 80°C for 15 min. The reaction mixture was then cooled on an ice bath for 10 min. and pH of the reaction mixture was adjusted to 8 with 300 μL of 0.5 M phosphate buffer (pH 7.5): 1 M HCl (1:3 v/v). The synthon was characterized by HPLC.

$[^{99m}TcN]^{2+}$ core

The kit vial, containing succinic dihydrazide (5.0 mg), stannous chloride dihydrate (100 μg), 1,2-diaminopropane–N,N,N′,N′-tetra-acetic acid (5 mg), sodium dihydrogen phosphate (0.5 mg) and disodium hydrogen phosphate (5.8 mg) in freeze dried form, was used for preparing the precursor. The kit vial stored at 4°C was allowed to attain ambient temperature. The freshly eluted $^{99m}TcO_4^-$ (1 mL, 37 MBq) was added to the vial, vortexed and allowed to stand at room temperature for 20 min. The ^{99m}Tc-nitrido intermediate thus prepared was characterized by TLC.

*$[^{99m}TcN]$Pip-DTC (**I**)*

To 0.1 mL solution of **L1** (50 μg, 0.2 mM) in methanol, 1 mL of $[^{99m}TcN]^{2+}$ precursor prepared using the kit vial was added. The reaction mixture was incubated at room temperature for 30 min. The complex was characterized by TLC and HPLC.

*$^{99m}Tc(CO)_3$-PPAA (**II**)*

To 0.1 mL solution of **L2** (400 μg, 0.6 mM) in methanol, 0.4 mL of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor was added. pH was adjusted to 7 and the reaction mixture was heated at 100°C for 20 min. The complex was characterized by HPLC.

*$[^{99m}Tc(CO)_3$-PipIDA$]^-$ (**III**)*

To 0.1 mL solution of **L3** (500 μg, 0.83 mM) in methanol, 0.4 mL of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor was added. pH was adjusted to 4 and the reaction mixture was heated at 100°C for 20 min. The complex was characterized by HPLC.

Biodistribution and blocking studies

Biodistribution studies were carried out in normal Swiss mice (20–25 g, 4–5 weeks old). The radiolabeled complex (0.1 mL, 3–7 MBq) was injected via the tail vein. After 5 min, 30 min and 2 h post-injection, animals (n = 3) were sacrificed. All major organs were excised, weighed and counted for radioactivity in a NaI(Tl) flat geometry detector. Radioactivity has been expressed as percentage of injected dose per gram of tissue. To determine the receptor specificity, blocking studies were carried out where animals were administered intraperitoneal injection of 25 μg of (+)-pentazocine 1 h prior to the administration of radiolabeled complex. After 5 min post-injection of the complex, animals were sacrificed and percent radioactivity associated with each organ was estimated.

Conclusion

Three different ligands derived from the parent molecule 4-amino-*N*-benzylpiperidine have been radiolabeled with different Tc-99m cores and studied for their capability to target receptors *in vivo*. The three ^{99m}Tc-complexes could be prepared in $>$98% radiochemical yields. However, the significant uptake in the brain was observed only for one of the complex, [^{99m}TcN]Pip-DTC which could be completely blocked on injection of sigma-1 receptor specific drug, (+)-pentazocine. [^{99m}TcN]Pip-DTC has also shown promising results towards *in vivo* binding with sigma receptor albeit with uptake in liver.

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