Bioorganic Chemistry 111 (2021) 104972

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Synthesis and evaluation of technetium-99m labelled 1-(2-methoxyphenyl) piperazine derivative for single photon emission computed tomography imaging for targeting 5-HT_{1A}

Neelam Kumari [a,b,c], Ankur Kaul [b], Raunak Varshney [b], Vinay Kumar Singh [d], Krishna Srivastava [e], Sunita Bhagat [c], Anil Kumar Mishra [b], Anjani Kumar Tiwari [a,b,*]

[a] Department of Chemistry, School of Physical & Decision Sciences (SPDS), Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow 226025, UP, India
[b] Division of Cyclotron and Radiopharmaceutical Sciences, Institute of Nuclear Medicine and Allied Sciences, Brig. S. K. Mazumdar Road, Delhi 110054, India
[c] Department of Chemistry, University of Delhi, Delhi 110054, India
[d] Department of Chemistry, Dr. S.M.N.R University, Lucknow 226018, India
[e] Faculty of Chemical Sciences, Shri Ramswaroop Memorial University, Lucknow 225003, UP, India

ARTICLE INFO

Keywords:
Serotonin
Molecular docking
Acetamidobenzoxazolone
5-HT_{1A}
SPECT/PET and Inflammation

ABSTRACT

Quantitative changes in expression level of $5HT_{1A}$ are somewhere related to common neurological disorders such as anxiety, major depression and schizophrenia. We have designed EDTA conjugated SPECT imaging probe for localization of $5HT_{1A}$ receptor in brain. For designing SPECT probe we have employed the concept of bivalent approach and a homodimeric system with desirable pharmacokinetics of $5HT_{1A}$ imaging. ^{99m}Tc-EDHT was also evaluated for its stability through serum stability assay and glutathione challenge experiment. Biodistribution study showed the highest accumulation of radioactivity in kidney which depicted the renal mode of excretion from the body. However in brain the uptake of 1.21% ID per gram was observed in initial 5 min of drug administration. On blocking the receptor this percent get decreased to 0.97% ID per gram. The regional distribution in brain was also performed which showed the accumulation of drug in cerebellum, cortex and hippocampus part, which are already known for $5HT_{1A}$ expression. Dynamic study in rabbit is also in support of results derived from biodistribution and blood kinetics experiment. These finding suggest that ^{99m}Tc-EDHT holds promising place for further optimization before nuclear medicine applications in different animal species.

1. Introduction

Enormous development in the field of molecular imaging has enabled neuroscientists to gain a deeper insight into the functioning of the central nervous system during the last few decades.[1–3] The discovery of high resolution imaging techniques having the capability to explore physiological, structural, and chemical properties in brain has really opened up the field of in-vivo brain mapping. New imaging techniques which are non-invasive in the nature can explore changes in receptor availability during neurological diseases and disorders involving the central nervous system including Parkinson's disease, schizophrenia, Alzheimer's disease, epilepsy and addiction.[4–6] These molecular imaging helps in understanding the changes in concentration and function of neurotransmitter receptors and transporters associated with disease onset and progression. Only a handful of established imaging agents exist for the imaging of CNS targets despite the fact that development of molecular tracers for in-vivo imaging of specific targets in the CNS began more than 25 years ago.[7–18] So, it's a great interest in molecular imaging field to develop ligands of high affinity and selectivity for the receptor to perform brain imaging (see Table 1).

In patients of psychiatric disorders including depression, anxiety and schizophrenia, there is reduction in serotonin receptor levels.[19–27] Only 1–2% of total body serotonin is found in central nervous system. $5HT_1$ family of receptors have a role in inhibiting cell firing. Out of all $5HT_1$ receptor family, $5HT_{1A}$ is the highest investigated serotonin receptor. It has also been found to have more control over the anxiety/

* Corresponding author at: Department of Chemistry, School of Physical & Decision Sciences (SPDS), abasaheb Bhimrao Ambedkar University (A Central University), Lucknow 226025, UP, India.
E-mail address: anjani7797@rediffmail.com (A.K. Tiwari).

https://doi.org/10.1016/j.bioorg.2021.104972
Received 26 November 2020; Received in revised form 17 April 2021; Accepted 4 May 2021
Available online 13 May 2021

Table 1
Hematological and Biochemistry Values data of animals treated with single dose of EDHT.

	Control	7 day	14 day
Haemoglobin(Hb)/ gm/dl	12.50	12.80	13.05
Neutrophil / %	32.16 ± 1.1	33.17 ± 0.12	36.15 ± 1.20
Lymphocyte / %	92.35 ± 2.18	93.67 ± 2.5	95.52 ± 1.20
Eosinophil / %	2.15 ± 0.51	2.82 ± 1.16	2.51 ± 1.50
Monocyte/ %	3.61 ± 0.11	1.98 ± 1.17	2.12 ± 0.19
ESR (Westegren's Method) /mm/ 1st hr.	0 ± 0	0 ± 0	0 ± 0
RBC Count / Millions/cmm	8.33 ± 0.51	7.51 ± 0.3.5	7.85 ± 0.8
P.C.V/Haematocrit / %	57.21 ± 0.95	55.27 ± 0.951	55.56 ± 2.50
M C V / fl.	61.53 ± 3.10	61.25 ± 1.76	62.52 ± 5.20
M C H / Picogram	21.15 ± 2.50	18.98 ± 1.61	17.26 ± 3.15
M C H C / gm/dl	32.13 ± 1.10	33.25 ± 2.15	35.53 ± 3.15
Platelet Count / Lakh/cmm	7.25 ± 3.50	8.87 ± 2.90	6.96 ± 1.30
AST (IU/L)	75.2 ± 16.10	68.2 ± 19.30	68.3 ± 9.30
ALT (IU/L)	25.7 ± 6.17	22.2 ± 5.30	24.7 ± 5.50
Protein (g/dL)	5.30 ± 1.53	5.02 ± 1.21	5.22 ± 1.32
Albumin (g/dL)	3.60 ± 1.30	3.65 ± 1.165	5.52 ± 1.32

anxiolytic action in comparison to the other members of the $5HT_1$ family. The hyperpolarisation of the neuron is caused because of $5HT_{1A}$ activation by triggering voltage gated K + channels. The most common location of postsynaptic $5HT_{1A}$ receptors is on GABAergic and glutaminergic neurons, which has high concentrations in several cortical and subcortical areas mainly in limbic system.[28–29]

Molecular imaging techniques using PET or SPECT are the tools used in the investigation of changes in $5HT_{1A}$ receptor expression. During past decades, there is a rise of interest of medicinal chemist for providing integrating molecular medicine solutions using new radiolabeled ligands with dedicated and optimized imaging systems to investigate in-vivo changes of $5HT_{1A}$ receptors.[30–32]

Metal complexes are not generally found suitable for normal brain imaging probes due to their low BBB permeability but it can be utilized in diseased state. Despite the exponential growth in the number of nuclear medicine hospitals equipped with SPECT, ^{99m}Tc is one of the most common radionuclide owing to its good imaging characteristics and easy availability of $^{99m}Tc/^{99}Mo$ generators. There are many reviews which have been written for the development of metal coordinated imaging probes in CNS applications.[33–36]

In general, imaging addresses two main things: structure and function. With the aid of different imaging modalities we can image anatomy or examine physiological functions of the brain. Arylpiperazines is one of the most promising classes to design $5HT_{1A}$ receptor ligands. The most common skeletons are gepirone, buspirone, WAY-100135/ WAY-100635 and others.[37–44] WAY-100635 (N-(2-(4-(2-methoxyphenyl)-1-piperazinyl) ethyl)-N-(2-pyridyl)-cyclohexane carboxamide) was the first successful compound (selective antagonists) in this category. The most important pharmacophore present in WAY-100635 is 1-(2-methoxyphenyl)piperazine which have high affinity for the $5HT_{1A}$ subreceptors.[45] The amalgamation of aromatic ring and basic nitrogen containing piperazine is the primary requisite scaffold for $5HT_{1A}$ receptor binding. The basic nitrogen in the piperazine can further exploited for functionalization with a long chain alkyl linker.

Three different mixed ligand complex approaches (3 + 1, 4 + 1, 2 + 1 + 1) were used to attach the technetium to the pharmacophore moiety of WAY 100635.[46–57]Besides that tetradentate N_2S_2 chelates and the Tc-tricarbonyls were also used to attach this pharmacophore.[58–61] Out of these approaches, the most successful was the 3 + 1 concept for the synthesis of neutral, lipophilic, small size oxotechnetium complexes. This approach uses the simultaneous action of a dianionic tridentate ligand (having SSS, SOS or SN(R)S donor atom set) and a monodentate thiol as co-ligand on a suitable oxotechnetium precursor.[54–56,58,62] The best part of these complexes are their appropriate capability to cross blood–brain barrier due to its neutrality and diverse aspects of conjugation with pharmacologically relevant groups.

Fig. 1 represents the representative compounds of all category. Compound **1** and **2** shows 3 + 1 approach based mixed ligand complex in which the requisite pharmacophore moiety has been placed in two opposite direction. Compound **3** and **4** represents 4 + 1 and 2 + 1 + 1 complexes. The other two approaches tetradentate N_2S_2 chelates and the Tc-tricarbonyl approaches have been shown by compound **5** and **6**. Compound **7** represent nonmetallic PET ligand labelled with carbon-11 isotope having 1-(2-methoxyphenyl) piperazine moiety.

The presented compound **8** of this work comprises benzoxazolone conjugated with MPP system having EDTA chelator to evaluate the $5HT_{1A}$ expression in brain. Idea behind using EDTA in place of other vehicle, is to improve the lipophilicity of the ligand towards brain regions.

2. Experimental

2.1. Synthesis (Chemistry)

2.1.1. Material and methods

All chemicals and solvents required for the synthesis have been purchased from Sigma Aldrich, Alfa Aesar and Spectrochem Pvt. Ltd. Buchi M−560 instrument is used for determining the melting point and IR spectra has been drawn from Perkin-Elmer FT-IR spectrometer. For some compounds KBr disc was prepared and some compounds were analyzed in form of thin film. NMR spectra were recorded at Jeol alpha-400 spectrometer. Chemical shift values in case of ^1H NMR are reported on δscale and coupling constants (J) are reported in Hz unit. Mass spectra's were obtained on a 6530 Q-TOF instrument from Agilent Technologies.

3. Animals

Young healthy male Sprague Dawley Rats matured for 2–3 months, weight 150–200 g were acquired from the Experimental animal facility (EAF) of Institute of Nuclear Medicine and Allied Sciences (INMAS), Delhi. The take care of animals were regulated as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of the institute (INM/DASQA/IAEC/09/015). All the animals were administered food (Hindustan Lever Ltd, Mumbai, India) and water *ad libitum*. Management and husbandry conditions were identical in all groups of experimental animals with 12/12 h light/dark cycle at 22 ± 2 °C. The animals were acclimatized to laboratory conditions for at least one week before experimentation. The animals were kept under observation during the entire experimental period.

3.0.1. General procedure for synthesis of 2,2′-(ethane-1,2-diylbis((2-((3-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-2-oxo-2,3-dihydrobenzo[d]oxazol-5-yl)amino)-2-oxoethyl)azanediyl))diacetic acid (EDHT)

- Synthesis of 5-nitro-1,3-benzoxazol-2(3*H*)-one (2)

To a solution of 2-amino-4-nitrophenol derivative **1** (0.8 g, 5.19 mmol) in THF (30 mL) was added 1,10-carbonyldiimidaziole (1.42 g, 6.23 mmol) at room temperature. The mixture was stirred at reflux for 3 hrs and cooled to room temperature. The reaction was then quenched by adding 2 M HCl solution, and the mixture was extracted with EtOAc. The organic layer was washed with brine and dried over anhydrous sodium

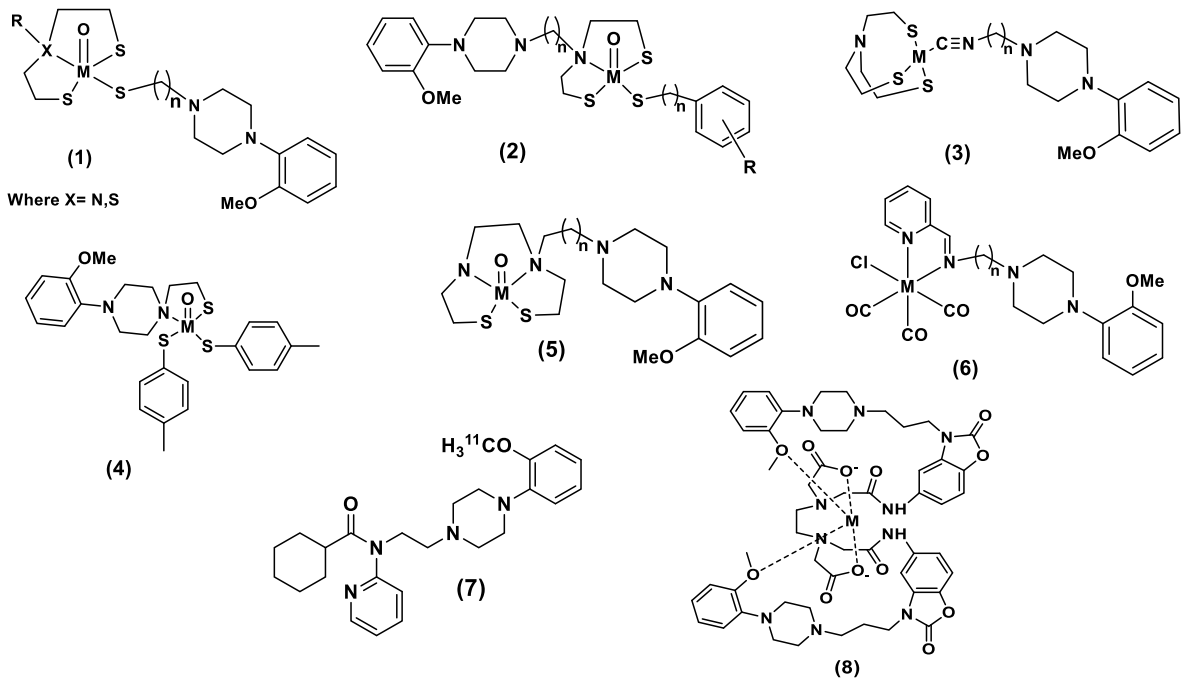


Fig. 1. Representative systems having 1-(2-methoxyphenyl) piperazine moiety for $5HT_{1A}$ receptor imaging.

sulphate. After filtration, the solvent was removed in vacuo to give **2**

Physical state and yield: Yellow solid, 99% yields.

$\mathbf{R_f}$ = 0.7 (40% ethyl acetate in hexane)

M. Pt.: 98–99 °C

IR (thin film) ν_{max}: 3127, 1785, 1678, 1522, 1473 and 1344 cm^{-1}

^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.92 (1H, dd, J = 2.4 and 8.8 Hz), 7.76 (1H, d, J = 2.8 Hz) and 7.36 (1H, d, J = 9.2 Hz)

^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 154.7, 148.2, 144.2, 131.5, 119.2, 110.35 and 105.6;

HR-ESI-TOF-MS: *m/z* 179.0083 ($[M-H]^+$), calcd. for $[C_7H_4N_2O_4\text{-}H]^+$ 179.0087.

- Synthesis of 3-(4-bromobutyl)-5-nitrobenzo[*d*]oxazol-2(3H)-one (3)

1, 4-dibromobutane (2.4 g, 11.1 mmol) in acetone (10 mL) was added dropwise to the solution of 5-nitrobenzo[*d*]oxazol-2(3H)-one (0.5 g, 2.7 mmol) and potassium carbonate (0.76 g, 5.5 mmol) in acetone (30 mL) at room temperature followed by the refluxing for 4 h. The mixture was filtered for eliminating the inorganic material and the solvent was removed through rotary evaporator to obtain a residue. The solution was extracted with dichloromethane (3 × 50 mL) and the resultant extract were washed, dried and evaporated. The solution was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel by using ethyl acetate in hexane as gradient solvent system to afford **3** as white solid.

Physical state and Yield: yellow oil, 88%

$\mathbf{R_f}$ = 0.7 (20% EtOAc in Hexane)

IR (KBr) ν_{max}: 3744, 2876, 1787, 1617, 1529, 1344, 1264 and 750 cm^{-1}

^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.27 (1H, d, J = 9.6 Hz), 8.068 (1H, d, J = 8.0 Hz), 7.57 (1H, d, J = 8.4 Hz), 3.95–3.89 (2H, m), 3.58–3.53 (2H, m) and 1.87–1.71 (4H, m)

^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 153.6, 146.5, 144.1, 132.0, 118.9, 110.1, 104.9, 60.20, 34.6, 29.4 and 26.0.

HR-ESI-TOF-MS: *m/z* 315.004 ($[M+H]^+$), calcd. for $[C_{11}H_{11}BrN_2O_4+H]^+$ 315.00.

- Synthesis of 3-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-5-nitrobenzo[*d*]oxazol-2(3H)-one (4)

To an ice-cool solution of **3** (0.4 g, 1.2 mmol) in DMF (20 mL) anhydrous K_2CO_3 (0.35 g, 2.5 mmol) was added followed by the addition of 1-(2-methoxyphenyl)piperazine (0.227 g, 1.18 mmol). The mixture was stirred at 0 °C for 3 hrs. Reaction mixture was cooled and poured into ice water which results into formation of precipitate. The collected precipitate was further purified by silica gel column chromatography using ethyl acetate in hexane as gradient solvent system to afford **4.**

Physical state and Yield: pale yellow solid, 92%

$\mathbf{R_f}$ = 0.3 (10% MeOH in $CHCl_3$)

IR (KBr) ν_{max}: 2942, 2816, 1787, 1673, 1492, 1342, 1238, 1141, 1023 and 749 cm^{-1}

^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.26, (1H, s), 8.09 (1H, d, J = 9.2 Hz), 7.58 (1H, d, J = 9.2 Hz), 6.90–6.83 (4H, m), 3.94 (2H, t, J = 6.3 Hz), 3.75 (3H, s), 2.88 (4H, m), 2.50–2.49 (4H, m), 2.34 (2H, t, J = 6.3 Hz), 1.78 (2H, s) and 1.52 (2H, s).

^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 153.6, 151.9, 146.5, 144.0, 141.2, 132.0, 122.3, 120.8, 118.9, 117.8, 111.8, 110.5, 104.8, 57.1, 55.3, 53.0, 50.0, 42.1, 24.9 and 23.0.

HR-ESI-TOF-MS: *m/z* 427.1968 ($[M+H]^+$), calcd. for $[C_{22}H_{26}N_4O_5+H]^+$ 427.20.

- Synthesis of 5-amino-3-(4-(4-(2-methoxyphenyl)piperazin-1-yl) butyl)benzo[*d*]oxazol-2(3H)-one (5)

To a solution of purified compound **4** (0.3 g, 0.75 mmol) in methanol (20 mL) was added 10% palladium on charcoal (97 mg). The flask was supplied with hydrogen balloon and the mixture was stirred for 2 hrs at room temperature. At finishing of the reactant, the catalyst was celite filtered, the filtrate was evaporated under reduced pressure to afford amine derivative **5**

Physical state and Yield: White solid, 74%

$\mathbf{R_f}$ = 0.1 (20% MeOH in $CHCl_3$)

IR (KBr) ν_{max}: 3744, 3356, 2946, 2880, 2823, 1760, 1628, 1497, 1366, 1240, 1023 and 754 cm^{-1}

^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.97–6.85 (5H, m), 6.44 (1H, s),

6.28 (1H, s), 5.07 (2H, brs), 3.78–3.71 (5H, m), 3.37–3.36 (2H, m), 2.97 (4H, s), 2.60 (4H, s), 1.72–1.65 (2H, m) and 1.51 (2H, s).

^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 154.5, 151.9, 145.9, 133.2, 131.41, 122.6, 120.8, 117.9, 111.9, 109.9, 107.0, 94.9, 55.3, 52.6, 41.0 and 24.9.

HR-ESI-TOF-MS: *m/z* 397.2230 ($[M+H]^+$), calcd. for $[C_{22}H_{28}N_4O_3+H]^+$ 297.22.

- Synthesis of 2,2′-(ethane-1,2-diylbis((2-((3-(4-(4-(2-methoxyphenyl) piperazin-1-yl)butyl)-2-oxo-2,3-dihydrobenzo[*d*]oxazol-5-yl) amino)-2-oxoethyl)azanediyl))diacetic acid (EDHT)

A solution of compound **5** (0.35 g, 0.89 mmol) and Et_3N (0.19 g, 1.9 mmol) in anhydrous DMF (10 mL) was stirred at room temperature. EDTA dianhydride (0.10 g, 0.39 mmol) was added slowly to the reaction mixture and was continued to stir at 50 °C for next 20 h. After finishing of the starting material in reaction mixture, solvent was removed and residue was redissolved in methanol and diethyl ether to get the precipitate. Achieved crude product was further dried under reduce vacuum to afford compound **EDHT.**

Physical state and Yield: white solid, 80%

IR (KBr) ν_{max}: 3394, 2361, 1593, 1435, 769 cm^{-1}

^{1}H NMR (DMSO-d_6, 400 MHz): δ 10.48 (2H, brs), 7.61 (2H, s), 7.30–6.79 (12H, m), 4.24 (25H, m), 3.73(12H, s), 3.41–3.35 (10*H*, m), 2.96–2.49 (6H, m) and 1.67–1.54 (8H, m).

^{13}C NMR (DMSO-d_6, 100.6 MHz): δ 174.5, 170.0, 1154.5, 153.2, 141.2, 138.3, 136.1, 132.7, 134.4, 122.4, 122.6, 119.2, 110.3, 112.2, 109.7, 101.22, 66.20, 58.2, 55.3, 53.2, 49.6, 40.2, 25.6 and 23.2

HR-ESI-TOF-MS: *m/z* 1071.4904 ($[M+Na]^+$), calcd. for $[C_{54}H_{68}N_{10}O_{12}+Na]^+$ 1071.49.

Reaction conditions: (i) CDI, THF, reflux, 3hrs; (ii) 1,4-dibromobutane, K_2CO_3, acetone, reflux, 4hrs; (iii) arylpiperazine, K_2CO_3, acetonitrile, stirred at 0 °C, 3hrs (iv) Pd/C, MeOH, rt; (v) 4,4′-(ethane-1,2-diyl) bis(morpholine-2,6-dione), Et_3N, DMF, 50 °C

3.1. In vitro receptor binding assay

In vitro receptor binding assay was performed through competitive binding experiments by using [3H]-8-OH-DPAT as radioligand in duplicate. Aliquots of volume 50 μL of rat hippocampal homogenates were mixed with 50 μL [^{3}H]-8-OH-DPAT (0.180 nM) tris-HCl buffer (50 mM tris-HCl, 0.1% ascorbic acid, 2 mM $CaCl_2$ at pH7.5) and 50 μL of increasing concentrations of competing EDHT and its Re complex. Nonspecific binding was defined with 10 μM 8-OH-DPAT. The mixture was incubated for 20 min at 37 °C and then bound and free radioligand was separated by filtration and resoaked with 1% bovine serum albumin (BSA). It was washed three times with 3 mL of ice cold tris-HCl buffer (50 mM tris-HCl, 150 mM NaCl) and dried over for 10 min, then placed in a 2 mL scintillation cocktail. The results of competitive experiments were analyzed to obtain the IC_{50} values.

3.2. Radiolabeling of EDHT

The radiolabelling of EDHT is performed by using sodium pertechnate as labeling reagent and $SnCl_2$ as a reducing agent. In summary, EDHT was dissolved in water, then 1 N HCl solution of 50 mg $SnCl_2{\cdot}2H_2O$ was added to the solution and the pH was adjusted to 7.0. After filtration through a 0.22 μm millipore filter and lyophilized. An eluate of 1 mL $Na^{99m}TcO_4$ was added to a lyophilized kit. The labelling efficiency was more than 98%. The radiochemical purity of ^{99m}Tc-EDHT was determined by ascending instant thin layer chromatography (ITLC) using silica gel coated fibre glass sheets (*Pall Corporation*) and dual solvent systems, namely (a) 100% acetone and (b) a solvent mixture of Ethanol, Ammonia and Water (2:1:5 v/v) as mobile phases. The radioactive contaminants were identified as reduced/hydrolyzed (R/H) ^{99m}Tc (R_f = 0.0) and free ^{99m}Tc-pertechnetate (R_f = 1.0) and the labeled product remained at the point of application when 100% acetone alone was used as mobile phase.

The characterization of 99mTc-EDHT was accomplished by comparative studies after co-injection of the Re complexes and corresponding 99mTc complexes at tracer level with HPLC analysis with UV–vis and gamma detection. HPLC analysis was performed with a reverse phase C18 column. The isocratic solvent CH_3CN:H_2O were used in the ratio of 70:30 for ^{99m}Tc- EDHT.

3.3. Glutathione challenge

To test the ^{99m}Tc-EDHT stability, it's challenged with Glutathione within concentration range of 25 mM- 100 mM. Labelled compound was incubated with solution mixture of glutathione in saline and percentage of transcomplexation and labelled compound was determined at different time interval by instant thin layer chromatography (ITLC).

3.4. In-Vitro serum stability of ^{99m}Tc-EDHT

The radiolabeled drug ^{99m}Tc-EDHT was tested for its in-vitro serum stability by ascending instant thin layer chromatography. For in-vitro stability in physiological saline and serum, 100 μL of the radiolabel was mixed with 2 mL each of 0.9% saline and serum. Instant thin layer chromatography was carried out to assess the labeling efficiency after incubating at 37 °C for 30 min. For in-vitro protein binding, 37 MBq of ^{99m}Tc-EDHT in 0.1 mL was mixed with 2 mL of plasma (n = 6). Aliquots were taken at various time intervals and proteins were precipitated by adding equal volumes of 12.5% trichloroacetic acid (TCA) and plasma. The radioactivity in the precipitate and supernatant was measured in a well type gamma counter.

3.5. Blood kinetics

For in-vivo studies, 74 MBq of radiotracer was administered to the rabbit and blood samples were withdrawn at various time intervals. Drug was injected through the ear vein and blood samples were collected at different time intervals post administration (n = 3). The radioactivity in blood samples was measured in a well type gamma counter and was calculated as percentage of the injected dose.

Formula used for Blood-clearance:

%Radioactivity in blood = (Counts *100 * Volume of blood)
(Weight of blood * Total counts injected)

3.6. Biodistribution

The animals were used from the experimental animal facility at the Institute. The animals were fed laboratory chow pellets, in *ad libitum* with free access to food and water. The animals were in the room with daytime light and no light after 1900 h until morning with the temperature of approx. 25 °C.

In-vivo distribution of ^{99m}Tc-EDHT was studied in 2 months old BALB/c mice (n = 3; each weighing approximately 22 g). An aliquot of 100 μL of ^{99m}Tc-EDHT (3.7 MBq) was administered intravenously to each mouse, weighing about 25 g, through the tail vein. The animals were sacrificed at different time intervals and different organs were removed, collected into preweighed tubes. The radioactivity in each organ was counted using well-type gamma spectrometer and Counts per minute (CPM) values were decay-corrected and results were calculated as % ID per gram of wet tissue. All animal studies were approved by the institutional Experimental Animal Ethical Committee and performed in accordance with their guidelines.

Formula for biodistribution studies:

% Injected Dose per Gram Organ = (Counts * 100)
(Wt of organ* Total counts injected)

3.7. SPECT imaging of ^{99m}Tc-EDHT

SPECT whole body scintigraphic scan was carried out after intravenous administration of 40 MBq of radiolabeled EDHT in normal New Zealand rabbit through the dorsal ear vein. The animal was anaesthetized by intramuscular injection of diazepam 10 min before imaging. The animal was fixed on a board in supine position and imaging was performed using Single Photon Emission Computerized Tomography

(Symbia® Siemens,USA).

3.8. In-vivo safety evaluation

For safety evaluation, the animals were injected with a single dose of 0.5 mg/ kg body weight of tested compound, intravenously and left for seven days and fourteen days. After the experimental period, animals were sacrificed and blood sample was collected through cardiac puncture into tubes containing EDTA for hematological estimations. The hematological parameters were evaluated using hematological automatic analyzer. The parameters determined were as follows:White blood count, WBC (10^3/μl), Red blood cell, RBC count (10^6/μl), Hemoglobin, Hb (g/dL), Hematocrit HCT (%), Mean Corpuscular Volume, MCV (fL), Mean Cell Hemoglobin, MCH (pg), Mean Cell Hemoglobin Concentration, MCHC (g/dL), Platelet (10^3/μl), Clotting time (s), Lymphocyte (%), Neutrophil (%), Monocyte (%), Basophil (%), Eosinophil (%).

4. Result and discussion

4.1. Synthesis (Chemistry)

In first step 2-amino-4-nitrophenol was cyclised by using CDI reagent in THF solvent at refluxing for 3 h to afforded 5-nitrobenzo[*d*]oxazol-2 (3H)-one (**2**) in 90% yield. In next step 1, 4 dibromobutane is conjugated to compound **2** in K_2CO_3 and acetone on refluxing for 4 h to produce 3-(4-bromobutyl)-5-nitrobenzo[*d*]oxazol-2(3H)-one (**3**). The bromo group in compound **3** is further utilized for 1-(2-methoxyphenyl)piperazine conjugation to afford compound 4 with 92% yield. Subsequently nitro group of the compound is reduced to amine group (**5**) as a reducing agent. Now this amine group can be utilized to conjugate with EDTA dianhydride to afford EDHT (**6**) and can be conjugated to isocynate group of FITC to form FIHT (**7**). The structures of all synthesized intermediates and compounds were unambiguously established on the basis of their spectral data (^{1}**H** NMR, ^{13}C NMR spectra, IR spectra and HRMS) analysis (Scheme 1).

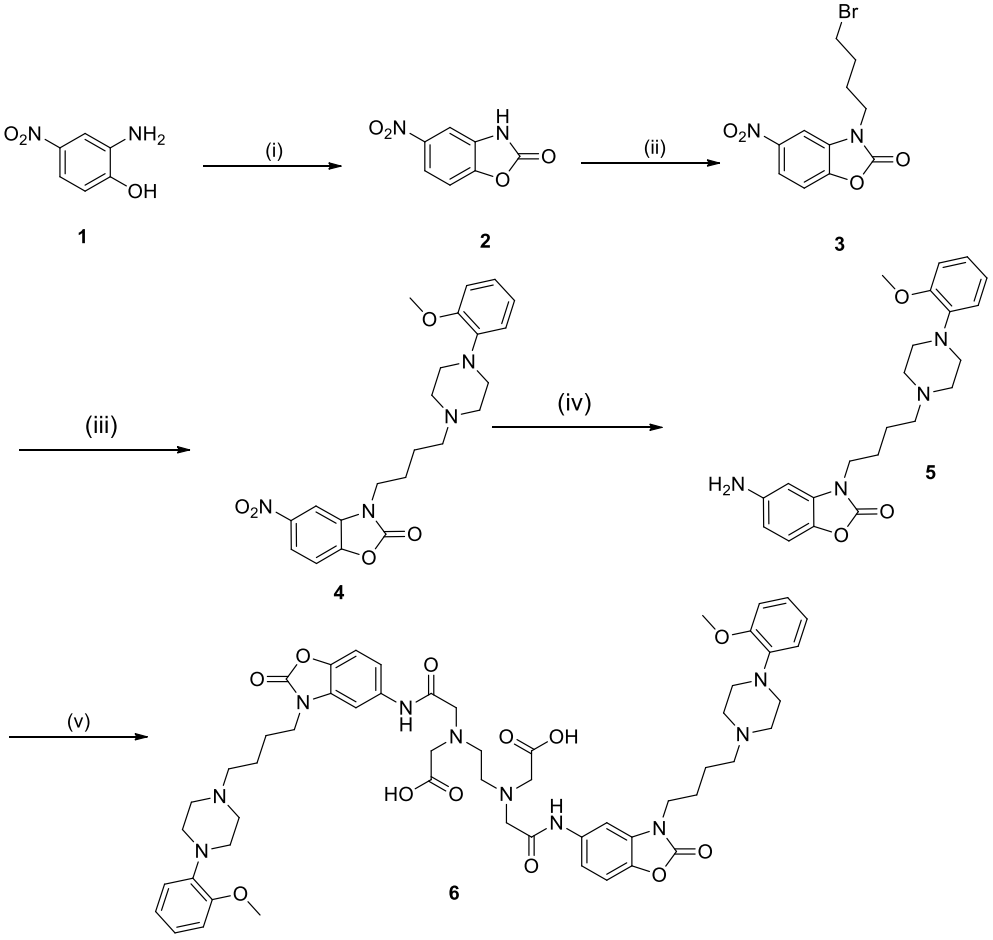
4.2. Radiolabeling of EDHT

The radiolabeling efficiency of the compounds and stability are controlled by several factors including temperature, type of complexing ligand, pH, reducing agent and the incubation time. Prior to in-vivo studies, by keeping all parameters constant and the labeling efficiency was measured through ITLC. It was observed that the radiolabeling efficiency was considerably high for the complex at a pH 7. So, it qualifies the complexing agent for in-vivo administration and evaluation. The labeling efficiency in solvent system (b) is shown in Fig. 2.

4.3. Glutathione challenge and in vitro binding assay

99mTc-EDHT showed only 0.72% of transcomplexation of 99mTc on glutathione concentration of 0.25 mM and 95.31% of the radiiolabelled compound remain associated at 1 mM concentration of 37 °C even after 2 h of glutathione challenge (Fig. 3).

The analogous Re compounds were synthesized as a surrogate for the ^{99m}Tc complexes for use in receptor binding assays. During in vitro binding assays, the Re analogues were tested with a competition assay against [3H]-8-OH-DPAT. The ligand and its metal complex showed



Scheme 1. Synthesis of 2,2′-(ethane-1,2-diylbis((2-((3-(4-(4-(2-methoxyphenyl)piperazin-1-yl)butyl)-2-oxo-2,3-dihydrobenzo[*d*]oxazol-5-yl)amino)-2-oxoethyl) azanediyl))diacetic acid (EDHT).

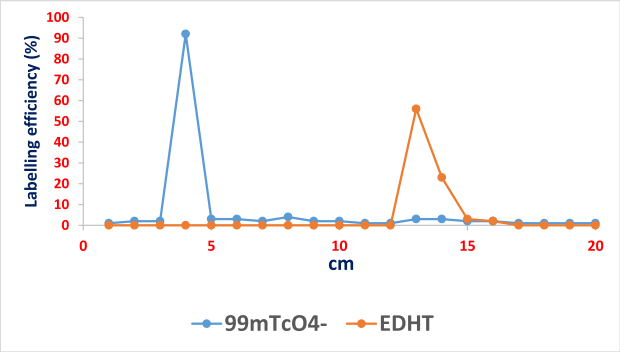


Fig. 2. Radiochromatogram of ^{99m}Tc-EDHT using ITLC-SG in Ethanol, Ammonia and Water (2:1:5 v/v) at room temperature (25 °C).

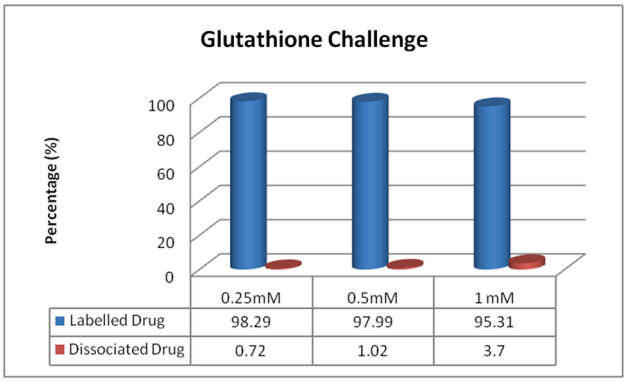


Fig. 3. Transcomplexation of ^{99m}Tc-EDHT by glutathione challenge.

nanomolar affinities to the target receptor. The binding affinity of the EDHT displayed appropriate affinity for the 5-HT1A receptor (IC_{50} = 11.3 nM) in a competition assay against [^{3}H]-8-OH-DPAT. No significant impact was found after introduction of a Re metal into EDHT (IC50 = 38.7 nM for Re-EDHT).

4.4. *In-Vitro serum stability of ^{99m}Tc-EDHT*

In-vitro studies like Serum-stability, glutathione challenge test are done to confirm the strength of the radio conjugation when administered into systemic circulation. In presence of serum, the radiolabeled EDHT showed good stability upto 24 h post labelling which is important for further in vivo studies (Fig. 4).

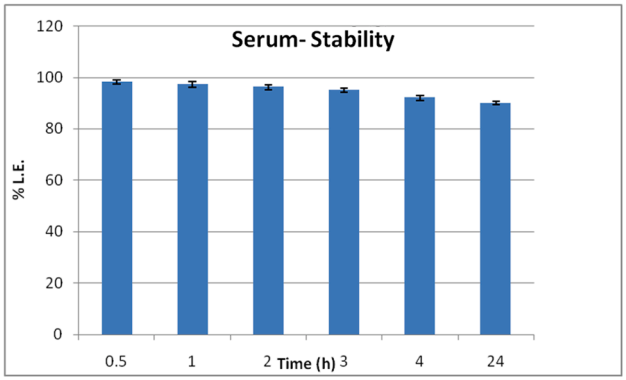


Fig. 4. In-vitro serum stability studies of ^{99m}Tc-EDHT.

4.5. *Blood kinetics*

New Zealand albino rabbits weighing 2.5 to 3.0 kg were used for blood clearance studies. Blood Kinetics of ^{99m}Tc-EDHT in whole blood after intravenous administration in rabbits exhibited bi-exponential clearance pattern.

The % radioactivity associated with blood at 1 h was found to be 8.97% and this value decreased to 2.70% at 24 h post administration. Fig. 5 shows that radiolabelled EDHT showed initial fast elimination followed by slow clearance from systemic circulation.

4.6. *Biodistribution*

Biodistribution of ^{99m}Tc-EDHT in various organs in mice at various time intervals post administration is shown in Fig. 6.

Among all the organs studied, kidneys showed maximum uptake of 9.21% of injected dose per whole organ at 5 min and liver showed the uptake of 7.28% of injected dose per whole organ at 5 min. Significant radioactivity in kidney and liver suggest its excretion through both the hepatobiliary and renal routes. Low uptake by blood was observed and low protein binding justifies for its low activity in blood pool. Furthermore, absence of radioactivity in the stomach confirms the in-vivo stability of the radiolabel throughout the duration of the study. The targeted organ in the studies i.e. brain showed the uptake of 1.21% injected dose per gram in initial 5 min of drug administration followed by the slow wash out in next 2hr. Biodistribution in blocking case was also performed to analyze the specificity. In Fig. 7 the radiolabelled EDHT uptake is shown when the receptor is already pre-blocked by the cold substitute. The reduction of % ID in brain after blocking, in comparison to the control case is expected due to the specificity of the ligand toward serotonin receptor. Preblocked brain showed the highest uptake of 0.97% ID at 5 min and at the same time point in control case the uptake is 1.21% ID.

Biodistribution pattern is further observed in various region of brain also to determine the accumulation of radioactivity in $5HT_{1A}$ rich region. In control case at 5 min, 10 min, 30 min, 60 min and 120 min of time point the % ID of drug is determined for cerebellum, cortex, striatum, and hippocampus. The highest accumulation in the hippocampus and cortex was because of expression of $5HT_{1A}$ receptor. One of the way to see the regional brain uptake in Hippocampus (HP) Cerebellum region (CB). The ratio of HP/CB increased till 10 min but decreases in between 30 and 60 min and further increases 60–120 min. With the time the decline in radioactivity is observed (Fig. 8). On comparing the activity we found that the radioactive accumulation is reduced in case of cerebellum, cortex and hippocampus but remain unaffected in case of stratium. It indicates that the uptake of radioactivity in hippocampus, cerebellum and cortex is specifically because of $5HT_{1A}$ receptor.

During blocking experiment the upatake of radioactivity in the

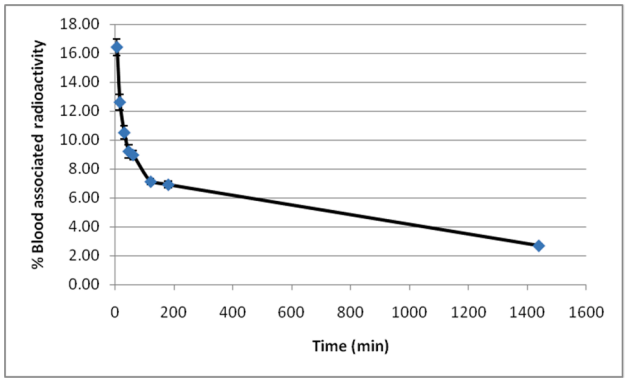
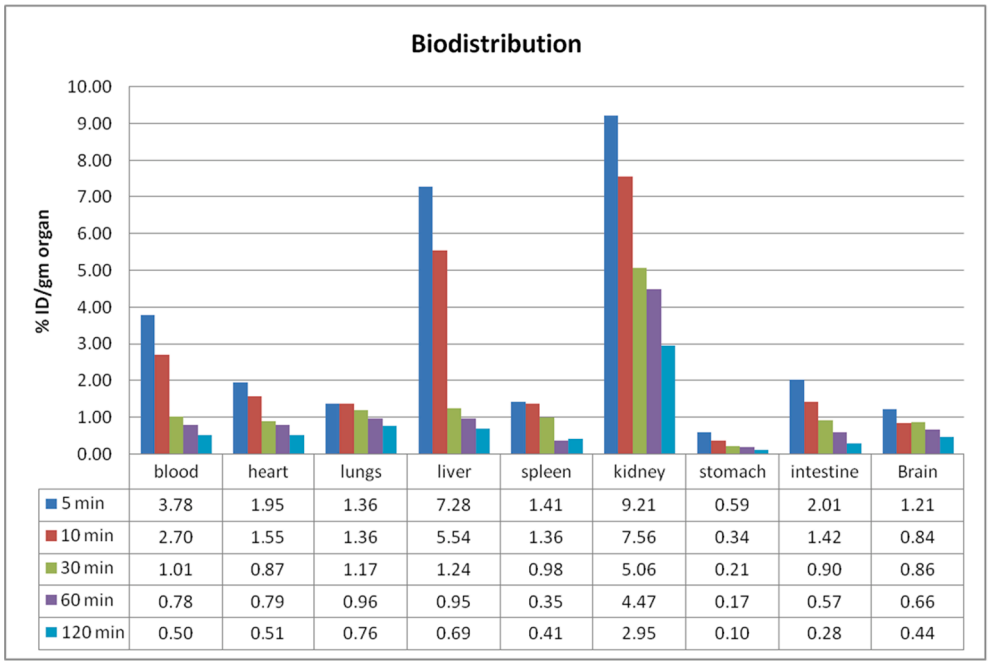
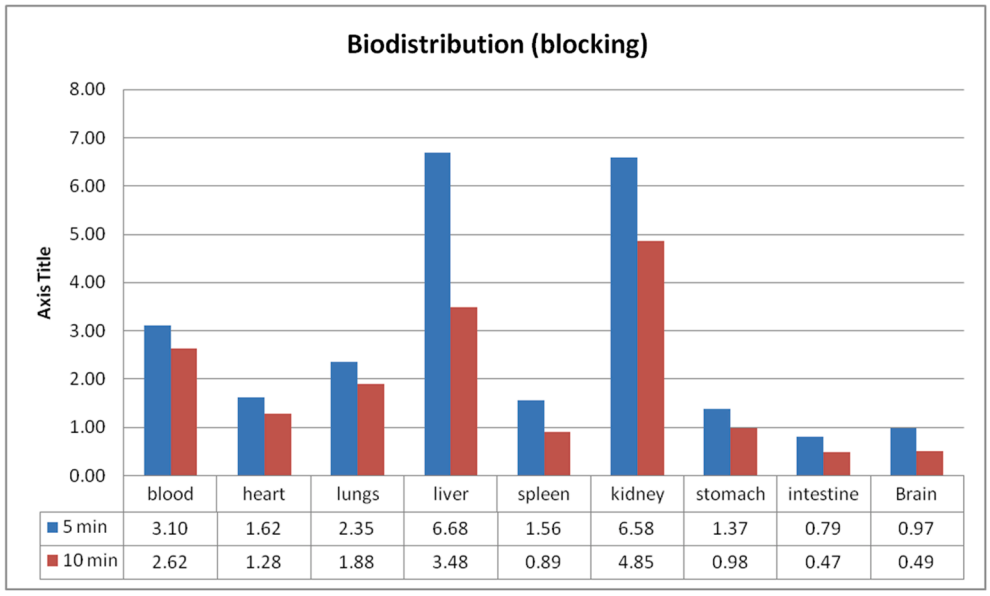


Fig. 5. Blood clearance of ^{99m}Tc-EDHT in rabbits.



	blood	heart	lungs	liver	spleen	kidney	stomach	intestine	Brain
5 min	3.78	1.95	1.36	7.28	1.41	9.21	0.59	2.01	1.21
10 min	2.70	1.55	1.36	5.54	1.36	7.56	0.34	1.42	0.84
30 min	1.01	0.87	1.17	1.24	0.98	5.06	0.21	0.90	0.86
60 min	0.78	0.79	0.96	0.95	0.35	4.47	0.17	0.57	0.66
120 min	0.50	0.51	0.76	0.69	0.41	2.95	0.10	0.28	0.44

Fig. 6. Bio-distribution-study of radiolabeled EDHT in BALB/c mice.



	blood	heart	lungs	liver	spleen	kidney	stomach	intestine	Brain
5 min	3.10	1.62	2.35	6.68	1.56	6.58	1.37	0.79	0.97
10 min	2.62	1.28	1.88	3.48	0.89	4.85	0.98	0.47	0.49

Fig. 7. Bio-distribution-study of ^{99m}Tc-EDHT in blocked (PK11195) BALB/c mice.

blocked and non blocked mice as been dipected in form of graph in Fig. 9.

In conclusion for biodistribution of ^{99m}Tc-EDHT was not homogeneous in the brain. 5-HT$_{1A}$ rich regions like hippocampus, showed low concentrations of radioactivity and somehow in other regions does not bind specifically to 5-HT$_{1A}$ receptors in vivo.

4.7. SPECT imaging of ^{99m}Tc-EDHT

Dynamic imaging was performed on rabbit and the images were acquired on gamma camera for 60 min (Fig. 10). The early phase showed the high uptake in the brain and the wash out was observed in 30 min. The highest accumulation in brain is seen at till 15 min of post injection followed by slow wash out. The uptake is also observable in kidney and urinary bladder which indicates its renal mode of exrection. This scan facilitated in explaining the in-vivo delivery of radiocomplex to different organs and correlated with the findings of the tissue distribution. A representative static image at 30 min has been shown in Fig. 11.

4.8. Hematology and biochemistry evaluation

No mortality was observed in experimental animal models till 14 days.

5. of controlled and treated mice was recorded and no deviation was found till 14 day.

The variation in the clinical biochemistry parameters in the control

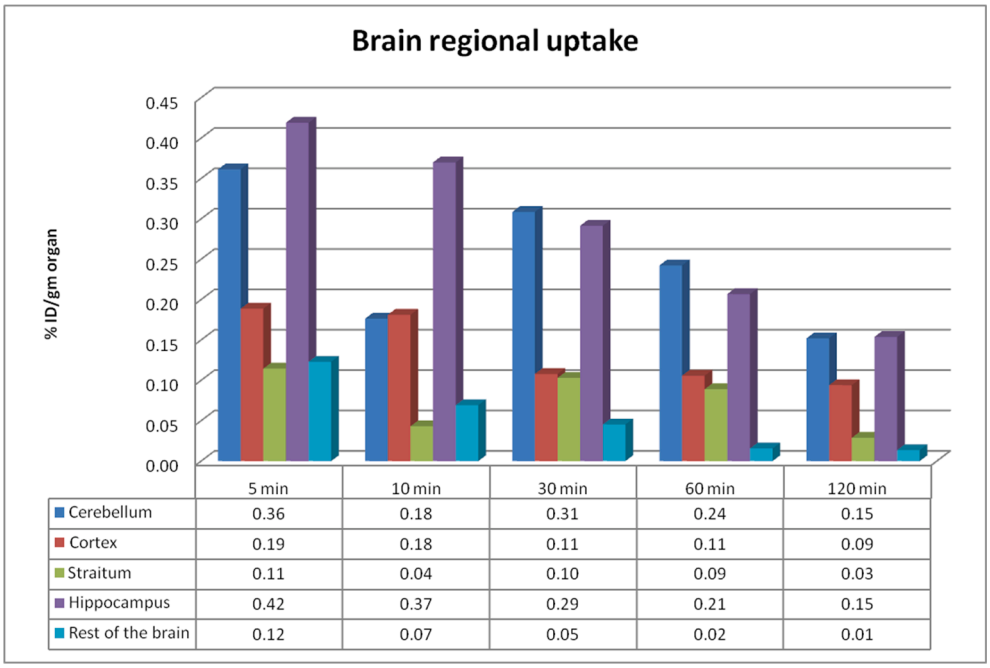


Fig. 8. Regional brain uptake of ^{99m}Tc-EDHT in BALB/c mice.

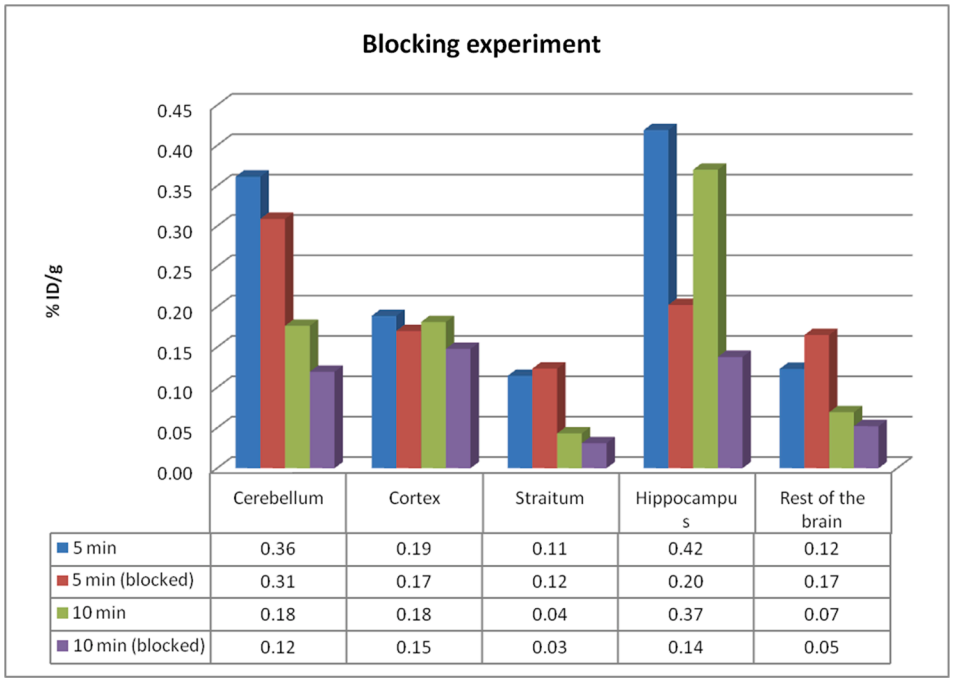


Fig. 9. Regional brain uptake of ^{99m}Tc-EDHT in PK11195 pre-treated (blocking) condition.

and test groups were less than 10%. There was no uniformity in increase/decrease pattern of parameters in the studied animals in the same or different groups. Any increase and decrease in particular group could be considered as incidental.

6. Conclusion

In this work, we have designed EDTA conjugated SPECT imaging probe for localization of 5HT$_{1A}$ receptor in brain. Our results demonstrate the potential of ^{99m}Tc-EDHT with the ability to bind 5HT$_{1A}$ receptor. However, substantial imaging of 5-HT$_{1A}$ with ^{99m}Tc requires further development of complexes with improved biological profiles of ligands.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

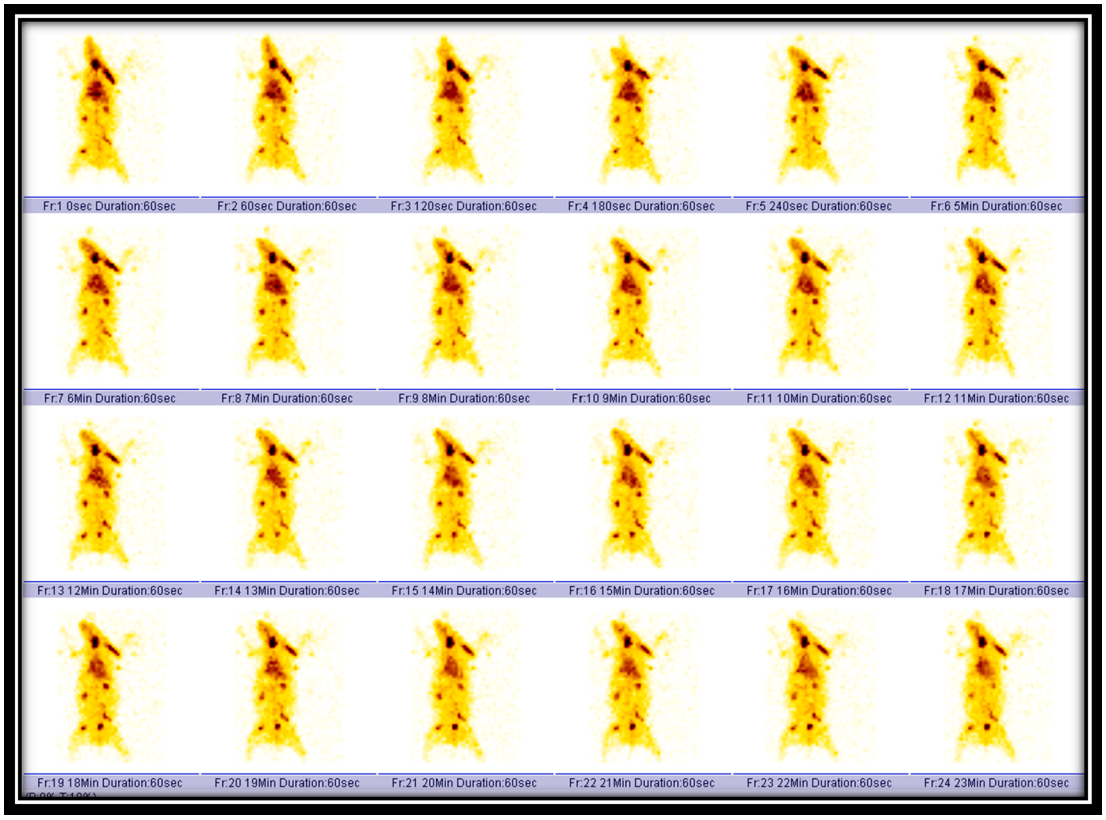


Fig. 10. Dynamic SPECT imaging in New Zealand rabbit (0–30 min).

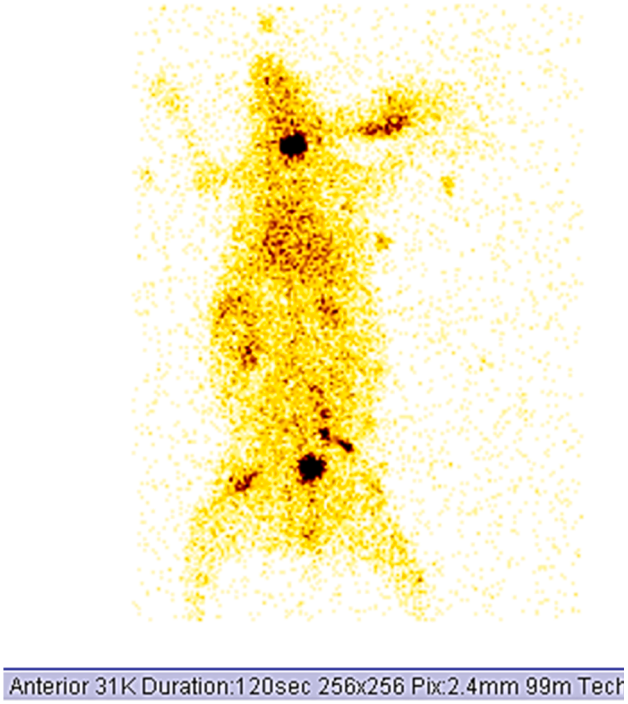


Fig. 11. Static SPECT imaging in New Zealand rabbit after injection of ^{99m}Tc-EDHT.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2021.104972.

References

[1] T.B. Ustun, J.L. Ayuso-Mateos, S. Chatterji, C. Mathers, C.J. Murray, Global burden of depressive disorders in the year 2000, Br. J. Psychiatry. 184 (2004) 386–392.
[2] N.M. Barnes, T. Sharp, A review of central 5-HT receptors and their function, Neuropharmacology. 38 (8) (1999) 1083–1152.
[3] J.M. Monti, The role of dorsal raphe nucleus serotonergic and non-serotonergic neurons, and of their receptors, in regulating waking and rapid eye movement (REM) sleep, Sleep Med. Rev. 14 (5) (2010) 319–327.
[4] G. Remington, Alterations of dopamine and serotonin transmission in schizophrenia, Prog. Brain Res. 172 (2008) 117–140.
[5] M. Garcia-Alloza, F.J. Gil-Bea, M. Diez-Ariza, et al., Cholinergic-serotonergic imbalance contributes to cognitive and behavioral symptoms in Alzheimer's disease, Neuropsychologia. 43 (3) (2005) 442–449.
[6] K.A. Michelsen, J. Prickaerts, H.W. Steinbusch, The dorsal raphe nucleus and serotonin: implications for neuroplasticity linked to major depression and Alzheimer's disease, Prog. Brain Res. 172 (2008) 233–264.
[7] P.C. Jobe, J.W. Dailey, J.F. Wernicke, A noradrenergic and serotonergic hypothesis of the linkage between epilepsy and affective disorders, Crit. Rev. Neurobiol. 13 (4) (1999) 317–356.
[8] R. Varshney, P.P. Hazari, A.K. Tiwari, et al., Synthesis and biological evaluation of modified laminin peptide (N_2S_2-KDP) with enhanced affinity for neuronal growth and targeted molecular imaging (SPECT), Bioorg. Chem. 107 (2021), 104516.
[9] A.K. Tiwari, Y. Zhang, T. Yamasaki, et al., 'Radiosynthesis and evaluation of acetamidobenzoxazolone based radioligand N′-MPB for visualization of 18 kDa TSPO in brain, New J. Chem. 44 (19) (2020) 7912–7922.
[10] P. Srivastava, D. Kakkar, P. Kumar, A.K. Tiwari, Modified benzoxazolone (ABO-AA) based single photon emission computed tomography (SPECT) probes for 18 kDa translocator protein, Drug Dev. Res. 80 (6) (2019) 741–749.
[11] P. Srivastava, N. Kumari, D. Kakkar, A. Kaul, P. Kumar, A.K. Tiwari, 'Comparative evaluation of 99m Tc-MBIP-X/11 [C] MBMP for visualization of 18 kDa translocator protein, New J. Chem. 43 (2019) 11288–11295.
[12] P. Srivastava, A. Kaul, H. Ojha, P. Kumar, A.K. Tiwari, Design, synthesis and biological evaluation of methyl-2-(2-(5-bromo benzoxazolone) acetamido)-3-(1 H-indol-3-yl) propanoate: TSPO ligand for SPECT, RSC Adv. 6 (115) (2016) 114491–114499.
[13] N. Chadha, D. Sinha, A.K. Tiwari, K. Chuttani, A.K. Mishra, Synthesis, Biological Evaluation and Molecular Docking Studies of High-Affinity Bone Targeting N, N'-Bis (alendronate) Diethylenetriamene-N, N'-Triacetic Acid, Chem. bio & Drug Des. 82 (4) (2013) 468–476.
[14] D. Sinha, G. Shukla, A.K. Tiwari, et al., 99mTc-DTPA-amino acids conjugate as specific SPECT pharmaceuticals for tumor imaging, Chem. Biol. Drug Des. 74 (2) (2009) 159–164.

[15] H.D. Hansen, M.M. Herth, A. Ettrup, et al., Radiosynthesis and in vivo evaluation of novel radioligands for PET imaging of cerebral 5-HT7 receptors, J. Nucl. Med. 55 (4) (2014) 640–646.

[16] Y. Shimoda, J. Yui, L. Xie, et al., Synthesis and evaluation of 1-[2-(4-[(11)C] methoxyphenyl) phenyl]piperazine for imaging of the serotonin 5-HT7 receptor in the rat brain, Bioorg. Med. Chem. 21 (17) (2013) 5316–5322.

[17] H.D. Hansen, E. Lacivita, P. Di Pilato, et al., Synthesis, radiolabeling and in vivo evaluation of [(11)C](R)-1-[4-[2-(4-methoxyphenyl)phenyl]piperazin-1-yl]-3-(2-pyrazinyloxy)-2-propanol, a potential PET radioligand for the 5-HT(7) receptor, Eur. J. Med. Chem. 79 (2014) 152–163.

[18] D. Baldwin, S. Rudge, The role of serotonin in depression and anxiety, Int. Clin. Psychopharmacol. 9 (Suppl 4) (1995) 41–45.

[19] M.C. Buhot, S. Martin, L. Segu, Role of serotonin in memory impairment, Ann. Med. 32 (3) (2000) 210–221.

[20] W.C. Drevets, E. Frank, J.C. Price, et al., PET imaging of serotonin 1A receptor binding in depression, Biol. Psychiatry. 46 (10) (1999) 1375–1387.

[21] C.C. Meltzer, J.C. Price, C.A. Mathis, et al., Serotonin 1A receptor binding and treatment response in late-life depression, Neuropsychopharmacology. 29 (12) (2004) 2258–2265.

[22] M.J. Owens, C.B. Nemeroff, The serotonin transporter and depression, Depress Anxiety. 8 (Suppl 1) (1998) 5–12.

[23] C.A. Stockmeier, Involvement of serotonin in depression: evidence from postmortem and imaging studies of serotonin receptors and the serotonin transporter, J. Psychiatr. Res. 37 (5) (2003) 357–373.

[24] S. Kasper, Depression and anxiety–separate or continuum? World J. Biol. Psychiatry. 2 (4) (2001) 162–163.

[25] A. Neumeister, E. Bain, A.C. Nugent, et al., Reduced serotonin type 1A receptor binding in panic disorder, J Neurosci. 24 (3) (2004) 589–591, https://doi.org/10.1523/JNEUROSCI.4921-03.2004.

[26] S. Kasper, J. Tauscher, M. Willeit, et al., Receptor and transporter imaging studies in schizophrenia, depression, bulimia and Tourette's disorder–implications for psychopharmacology, World J. Biol. Psychiatry. 3 (3) (2002) 133–146.

[27] M. Hamon, L. Lanfumey, S. el Mestikawy, et al., The main features of central 5-HT1 receptors, Neuropsychopharmacology. 3 (5–6) (1990) 349–360.

[28] P.R. Albert, L. Robillard, G protein specificity: traffic direction required, Cell Signal. 14 (5) (2002) 407–418.

[29] V.W. Pike, C. Halldin, H.V. Wikström, Radioligands for the study of brain 5-HT1A receptors in vivo, Prog. Med. Chem. 38 (2001) 189–247.

[30] R.A. Bantick, J.F. Deakin, P.M. Grasby, The 5-HT1A receptor in schizophrenia: a promising target for novel atypical neuroleptics? J. Psychopharmacol. 15 (1) (2001) 37–46.

[31] M.J. Millan, Improving the treatment of schizophrenia: focus on serotonin (5-HT) (1A) receptors, J. Pharmacol. Exp. Ther. 295 (3) (2000) 853–861.

[32] K. Chen, M. Cui, Recent progress in the development of metal complexes as β-amyloid imaging probes in the brain, Medchemcomm. 8 (7) (2017) 1393–1407.

[33] H. Liu, Y. Qu, X. Wang, Amyloid β-targeted metal complexes for potential applications in Alzheimer's disease, Future Med. Chem. 10 (6) (2018) 679–701.

[34] X. Zhang, P. Yu, Y. Yang, et al., ^{99m}Tc-Labeled 2-Arylbenzothiazoles: Aβ Imaging Probes with Favorable Brain Pharmacokinetics for Single-Photon Emission Computed Tomography, Bioconjug. Chem. 27 (10) (2016) 2493–2504.

[35] S. Chaturvedi, A. Kaul, P.P. Hazari, A.K. Mishra, Mapping neuroreceptors with metal-labeled radiopharmaceuticals, Medchemcomm. 8 (5) (2017) 855–870.

[36] F. Yasuno, T. Suhara, T. Ichimiya, A. Takano, T. Ando, Y. Okubo, Decreased 5-HT1A receptor binding in amygdala of schizophrenia, Biol. Psychiatry. 55 (5) (2004) 439–444.

[37] J. Passchier, A. van Waarde, Visualisation of serotonin-1A (5-HT1A) receptors in the central nervous system, Eur. J. Nucl. Med. 28 (1) (2001) 113–129.

[38] C.Y. Shiue, G.G. Shiue, P.D. Mozley, et al., P-[18F]-MPPF: a potential radioligand for PET studies of 5-HT1A receptors in humans, Synapse. 25 (2) (1997) 147–154.

[39] L. Lang, E. Jagoda, B. Schmall, et al., Development of fluorine-18-labeled 5-HT1A antagonists, J. Med. Chem. 42 (9) (1999) 1576–1586.

[40] D.K. Maiti, P.K. Chakraborty, D.C. Chugani, O. Muzik, T.J. Mangner, H.T. Chugani, Synthesis procedure for routine production of [carbonyl-11C]desmethyl-WAY-100635, Appl. Radiat. Isot. 62 (5) (2005) 721–727.

[41] V.W. Pike, C. Halldin, H. Wikström, et al., Radioligands for the study of brain 5-HT (1A) receptors in vivo–development of some new analogues of way, Nucl. Med. Biol. 27 (5) (2000) 449–455.

[42] M. Karramkam, F. Hinnen, M. Berrehouma, et al., Synthesis of a [6-pyridinyl-18F]-labelled fluoro derivative of WAY-100635 as a candidate radioligand for brain 5-HT1A receptor imaging with PET, Bioorg. Med. Chem. 11 (13) (2003) 2769–2782.

[43] R.K. Hom, J.A. Katzenellenbogen, Technetium-99m-labeled receptor-specific small-molecule radiopharmaceuticals: recent developments and encouraging results, Nucl. Med. Biol. 24 (6) (1997) 485–498.

[44] A.H. Newman, P. Grundt, M.A. Nader, Dopamine D3 receptor partial agonists and antagonists as potential drug abuse therapeutic agents, J. Med. Chem. 48 (11) (2005) 3663–3679.

[45] D. Papagiannopoulou, I. Pirmettis, T. Maina, et al., Development of novel mixed-ligand oxotechnetium [SNS/S] complexes as potential 5-HT1A receptor imaging agents, J. Biol. Inorg. Chem. 6 (3) (2001) 256–265.

[46] I. Heimbold, A. Drews, R. Syhre, M. Kretzschmar, H.J. Pietzsch, B. Johannsen, A novel Tc-99m radioligand for the 5-HT1A receptor derived from Desmethyl-WAY-100635 (DWAY), Eur. J. Nucl. Med. 29 (2002) 82–87.

[47] I. Pirmettis, G. Patsis, M. Pelecanou, C. Tsoukalas, A. Papadopoulos, C. P. Raptopoulou, A. Terzis, M. Papadopoulos, E. Chiotellis, Synthesis of oxorhenium (V) and oxotechnetium(V) [SN(R)S/S] mixed ligand complexes containing a phenothiazine moiety on the tridentate SN(R)S ligand, Bioorg. Med. Chem. Lett. 11 (2001) 1859–1862.

[48] R. Geetha, et al., Synthesis of SNS tridentate ligand and mixed ligand technetium complex (3 + 1 combination) for CNS serotonin receptor imaging, Ind. J. Nucl. Med. 15 (2001) 133–134.

[49] Rey, A., Synthesis and characterization of novel '2 + 1 + 1' rhenium complexes, Technetium, Rhenium and other Metals in Chemistry and Nuclear Medicine (Nicolini, M., Mazzi, U., Eds), SG Editoriali, Padua,1999, 213–216.

[50] A. Rey, I. Pirmettis, M. Pelecanou, M. Papadopoulos, C.P. Raptopoulou, L. Mallo, C. I. Stassinopoulou, A. Terzis, E. Chiotellis, A. León, Synthesis and characterization of mixed-ligand oxorhenium complexes with the SNN type of ligand. Isolation of a novel ReO[SN][S][S] complex, Inorg. Chem. 39 (19) (2000) 4211–4218.

[51] Ioannis C. Pirmettis, Minas S. Papadopoulos, Efstratios Chiotellis, Novel 99mTc aminobis-thiolato/monothiolato "3 + 1" mixed ligand complexes: Structure–activity relationships and preliminary in vivo validation as brain blood flow imaging agents, J. Med. Chem. 40 (1997) 2539–2546.

[52] R. Alberto, R. Schibli, August P. Schubiger, Ulrich Abram, H.-J. Pietzsch, B. Johannsen, First application of fac-[99mTc(OH2)3(CO)3]+ in bioorganometallic chemistry: design, structure, and in vitro affinity of a 5-HT1A receptor ligand labelled with 99mTc, J. Am. Chem. Soc., 121 (25) (1999), 6076–6077.

[53] L. Lang, E. Jagoda, B. Schmall, B.K. Vuong, H.R. Adams, D.L. Nelson, R.E. Carson, W.C. Eckelman, Development of fluorine-18-labeled 5-HT1A antagonists, J. Med. Chem. 42 (9) (1999) 1576–1586.

[54] J. Passchier, A. van Waarde, Visualisation of serotonin-1A (5-HT1A) receptors in the central nervous system, Eur. J. Nucl. Med. 28 (2001) 113–129.

[55] M. Papadopoulos, C. Tsoukalas, I. Pirmettis, B. Nock, T. Maina, Z. Abedin, P. Catherine, R, Synthesis and characterization of five-coordinated rhenium (V) and technetium (V) mixed ligand bifunctional complexes carrying the SNS/S or the SNN/S donor atom set, Inorg. Chim. Acta 285 (1999) 97–106.

[56] I.C. Pirmettis, M.S. Papadopoulos, S.G. Mastrostamatis, C.P. Raptopoulou, A. Terzis, E. Chiotellis, Synthesis and Characterization of Oxotechnetium(V) Mixed-Ligand Complexes Containing a Tridentate N-Substituted Bis(2-mercaptoethyl)amine and a Monodentate Thiol, Inorg. Chem. 35 (6) (1996) 1685–1691.

[57] P. Bouziotis, I. Pirmettis, M. Pelecanou, C.P. Raptopoulou, A. Terzis, M. Papadopoulos, E. Chiotellis, Novel oxorhenium and oxotechnetium complexes from an aminothiol[NS]/thiol[S] mixed-ligand system, Chemistry. Chem. J. Eur. 7 (2001) 3671–3680.

[58] B. Johannsen, H. Pietzsch, Development of technetium-99m-based CNS receptor ligands: Have there been any advances? Eur. J. Nucl. Med. 29 (2002) 263–275.

[59] B. Johannsen, R. Berger, P. Brust, H.J. Pietzsch, M. Scheunemann, S. Seifert, H. Spies, R. Syhre, Structural modification of receptor-binding technetium-99m complexes in order to improve brain uptake, Eur. J. Nucl. Med. 24 (1997) 316–319.

[60] E.A. Forster, I.A. Cliffe, D.J. Bill, A pharmacological profile of the selective silent 5-HT1A receptor antagonist, WAY-100635, Eur. J. Pharmacol. 1995 (25) (1995) 81–88.

[61] C. Bolzati, A. Mahmood, E. Malagò, et al., The [(99m)Tc(N)(PNP)](2+) metal fragment: a technetium-nitrido synthon for use with biologically active molecules. The N-(2-methoxyphenyl)piperazyl-cysteine analogues as examples, Bioconjug. Chem. 14 (6) (2003) 1231–1242.

[62] Development of 99mTc agents for imaging central neural system receptors, Technical reports series no. 426, International Atomic Energy Agency.