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Oxorhenium and oxotechnetium [SNS/S] mixed ligand complexes having a pendant diisopropylaminoethyl- group. Synthesis, characterization and biodistribution studies.

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SUMMARY

A novel SNS tridentate ligand, the N,N-bis(2-mercaptoethyl)-N',N'-diisopropylethylenediamine was synthesized and the corresponding oxorhenium and oxotechnetium "mixed ligand" complexes using 3 monodentate aromatic thiols as coligands were prepared and characterized.

Rhenium complexes were obtained as crystals with high yield by substitution of ligand and coligand on the trichlorobis(triphenylphosphine)- rhenium(V) oxide, using a molar ratio ligand:coligand:precursor of 1. Characterization was performed by HPLC analysis, UV-vis and IR spectra, elemental analysis and X-ray diffraction. Results were consistent with the ReOLC structure. The complexes adopted a distorted trigonal bipiramidal geometry and were neutral due to ionization of all sulphur atoms upon complexation.

Consequently ^{99m}Tc complexes were also prepared and evaluated in mice as potential brain imaging agents. Labelling was performed by substitution using ^{99m}Tc-glucoheptonate as precursor. The labelling yield was over 85% and the radiochemical purity of the complexes over 90%. Biodistribution in mice demonstrated high uptake and retention in brain.

However comparison with previously reported "mixed ligand" complexes showed that the incorporation of the diisopropylaminoethyl group in the SNS/S backbone is not enhancing the biological characteristics of this type of complexes.

Key words: SNS/S mixed ligand complexes, technetium and rhenium lipophilic complexes, chemical and biological evaluation

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INTRODUCTION

Radionuclides of the group 7 elements have useful physical characteristics for medical applications. ^{99m}Tc is the ideal radionuclide for diagnosis, while ^{186}Re and ^{188}Re have been regarded as appropriate for systemic radiotherapy (1,2).

The "mixed ligand " concept, based on the simultaneous action of two different ligands on an adequate precursor, has lately been applied in the design of new radiopharmaceuticals (3,4,5,6). The advantage of this concept lies in the possibility of tailoring essential properties of the complexes through changes introduced in either of the ligands.

Synthesis and characterization of a series of SNS/S mixed ligand technetium and rhenium complexes, MOL^1L^2 (M=Tc or Re), where L^1H_2 is an N-substituted bis-(2-mercaptoethyl)amine[N,N-bis(2-mercaptoethyl)-N',N'-diethylethylendiamine] and L^2H is a monodentate aromatic thiol, have recently been reported (7). The coordination of the tridentate ligand L^1H_2 on the $(MO)^{3+}$ core leaves an open coordination site cis to the oxo group that is occupied by the monodentate thiol. The final complex is neutral due to ionization of both ligand and coligand . ^{99m}Tc complexes penetrate the intact blood brain barrier and some of them exhibit prolonged brain retention, which demonstrates the potentiality of the SNS/S donor atom set in the design of brain blood flow imaging agents(8).

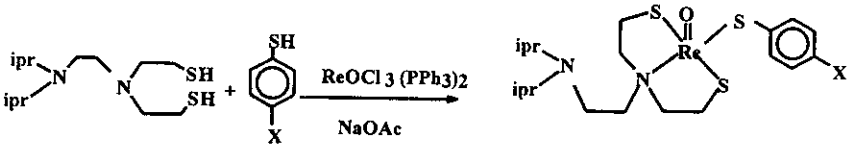
In order to investigate the effect of the structure of the tridentate ligand on the chemistry and biodistribution of the Re and Tc mixed ligand complexes, a novel SNS tridentate ligand, the N,N-bis(2-mercaptoethyl)-N',N'diisopropyl-ethylenediamine, has been synthesized. Mixed ligand rhenium complexes with this ligand and different monothiols as coligands have been prepared and characterized. Biological behaviour of the corresponding ^{99m}Tc complexes is also evaluated in mice.

RESULTS AND DISCUSSION

Simultaneous action of the tridentate ligand N,N-bis(2-mercaptoethyl)-N',N'-diisopropylethylenediamine and the monodentate aromatic thiols on the

$[Re(V)O]^{3+}$precursor in a molar ratio 1:1:1 readily yielded the expected mixed ligands complexes 1, 2 and 3 in high yield (Scheme 1).

Scheme 1.- Synthesis of the rhenium complexes



X = H Complex I
X = CH_3 Complex II
X = Cl Complex III

The lipophilic complexes were extracted by dichloromethane and isolated as crystalline products. The complexes are soluble in acetone, dichloromethane and chloroform, slightly soluble in methanol and ethanol and insoluble in ether or water. They are stable in solid state as well as in solution.

Elemental analysis performed for C, H, N, S, for all complexes was consistent with the proposed structure. IR spectra showed the expected ReO stretching band for monoxocomplexes in the region of 950cm^{-1}. Electronic absorptions were determined from the HPLC eluent using the PDA detector. They are characterized by an intense band in the region of 410 - 420 nm , probably due to S-----> Re charge transfer transition. Additional absorptions at shorter wavelength correspond to ligand and coligand.

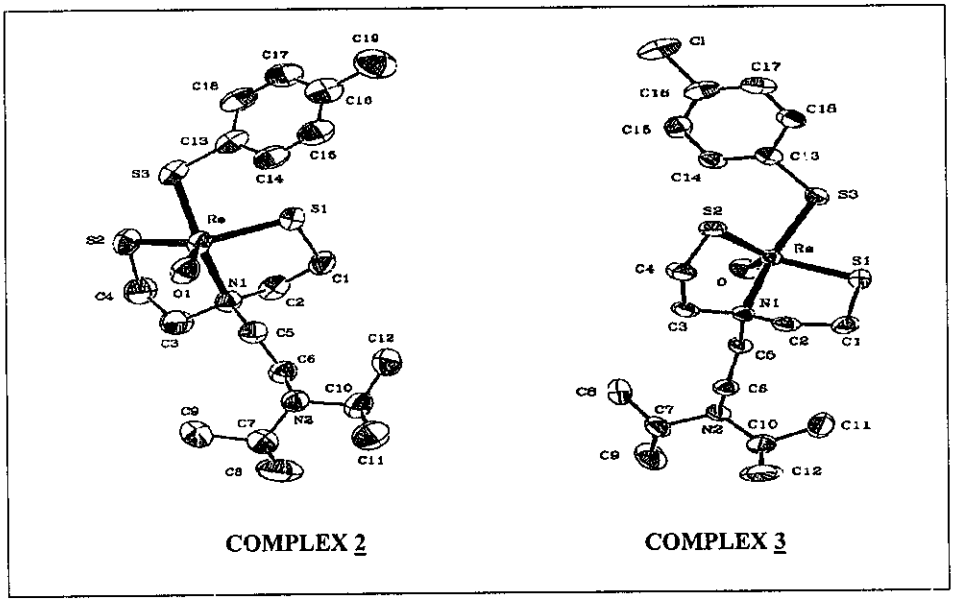
X-ray crystallographic studies were also performed to complexes 2 and 3. ORTEP diagrams are shown in Figures 1.

The coordination geometry about rhenium is distorted trigonal bipyramidal with the basal plane defined by the sulphur atoms of the tridentate ligand and the oxo-group and the apical positions occupied by the nitrogen atom of the ligand and the sulphur atom of the monodentate thiol.

Analysis of Addison et al (9) for the shape determining angles gave a value for the trigonality index τ, 0.65 and 0.61 for complexes 2 and 3 respectively.

The complexes are the syn isomers with the N-substituent in cis configuration to the oxo-group.

Figure 1 - ORTEP diagrams of complexes 2 and 3.



COMPLEX 2 **COMPLEX 3**

Selected bond distances and angles for both complexes are listed in Table 1.

Table 1. Selected bond distances (Å) and angles (°)

	Complex 2	*Complex 3*
Distances		
Re-S(1)	*2.279(2)*	*2.287(1)*
Re-S(2)	*2.277(2)*	*2.270(1)*
Re-S(3)	*2.305(2)*	*2.307(1)*
Re-N(1)	*2.209(5)*	*2.208(3)*
Re-O(1)	*1.691(4)*	*1.690(3)*
Angles		
S(1)-Re-S(2)	*121.6(1)*	*122.0(1)*
S(1)-Re-S(3)	*88.9(1)*	*83.6(1)*
S(2)-Re-S(3)	*85.3(1)*	*89.2(1)*
N(1)-Re-S(1)	*82.9(1)*	*83.6(1)*
N(1)-Re-S(2)	*83.9(1)*	*83.3(1)*
N(1)-Re-S(3)	*160.4(1)*	*158.7(1)*
O(1)-Re-S(1)	*120.5(2)*	*118.1(1)*
O(1)-Re-S(2)	*117.1(2)*	*119.5(1)*
O(1)-Re-S(3)	*105.1(2)*	*103.7(1)*
O(1)-Re-N(1)	*94.4(2)*	*97.3(2)*

The bond distances in the coordination sphere are in the ranges observed in analogous rhenium complexes having trigonal bipyramidal geometry. The Re-S(3) bond distances are slightly longer than the other two, because of the apical arrangement of the monodentate thiol. The angles between the atoms of the basal plane are close to the ideal value of 120°, but the S(3)-Re-N(1) is away from the ideal 180° (160.4(1)° and 158.7(1) for **2** and **3** respectively) showing a degree of distortion. Rhenium lies 0.108 and 0.074 Å out of the basal plane for complex **2** and **3** respectively, in the direction of the thiol.

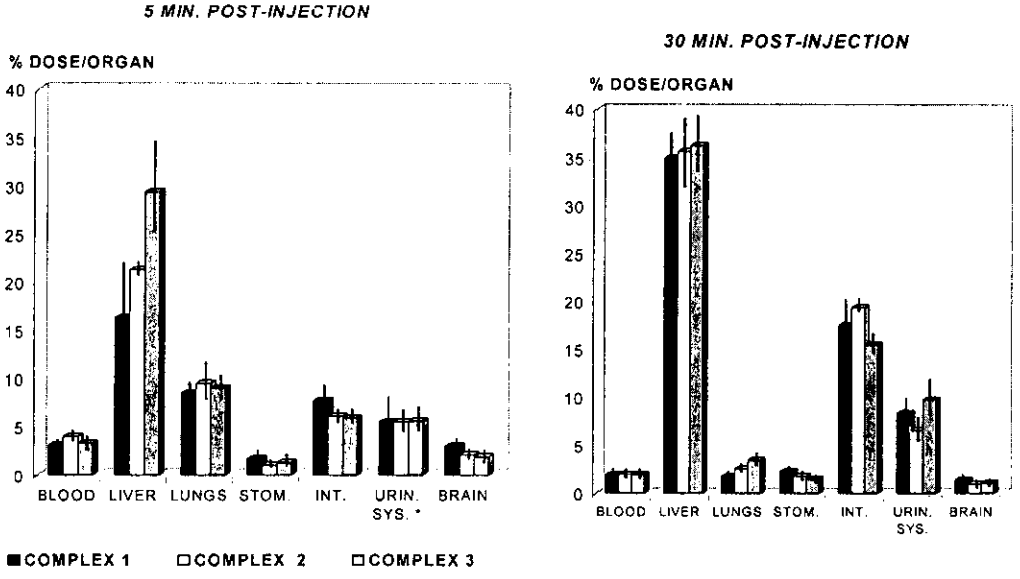
The two five membered rings defined by the atoms Re, S(1), C(1), C(2), N(1) and Re, S(2), C(3), C(4), N(1) are in the envelope form. In both complexes, the carbon atoms adjacent to N(1) are the "flap" atoms and are displaced by 0.65 Å (C(2)) and 0.59 Å (C(3)) from the mean plane defined by the remaining four atoms. The torsion angles of the tridentate backbone, S1-C1-C2-N1 and N1-C3-C4-S2, are 45.0 and -46.1° for complex **2** and 51.5 and -52.5° for complex **3**. The interatomic distance C(5)....O(1) is close to 3 Å (2.91 and 2.96 Å for complex **2** and **3** respectively) and is consistent with those observed in the syn isomers of analogous oxorhenium complexes.

Tracer level preparation was achieved using ^{99m}Tc-glucoheptonate as precursor. The labelling yield, calculated as the percentage of dichloromethane extraction from the reaction mixture, was above 85% for all compounds. The radiochemical purity of the organic extract was controlled by HPLC. A major peak with a retention time of about 5 min. was obtained for all complexes. The radioactivity of the peak was more than 90%. The by-products could not be identified as they were not formed at "carrier level".

Corroboration of the structure of the ^{99m}Tc complexes was achieved by comparing their HPLC profiles with the corresponding to the Re complexes upon coinjection. Radioactivity and UV detectors showed identical chromatographic profiles suggesting that the same chemical structure was formed under both chelating conditions.

The biological behaviour of the corresponding ^{99m}Tc complexes was evaluated in mice. Figure 3 shows results expressed as % Dose/organ in the most significant organs as a function of time.

FIGURE 3. Biodistribution results at 5 and 30 minutes post-injection



*URIN. SYST. = KIDNEYS + BLADDER + URINE

All complexes demonstrated high brain uptake (3.01, 2.06 and 1.85 % dose per organ, for ^{99m}**Tc-1**, ^{99m}**Tc-2**, and ^{99m}**Tc-3**, respectively, 5 min post injection) while about 50% of the radioactivity was retained in the brain at 30 min post injection (1.35, 1.01 and 1.08 for ^{99m}**Tc-1**, ^{99m}**Tc-2**, and ^{99m}**Tc-3**, respectively). Blood clearance was quite fast for all complexes and this is reflected in brain to blood ratio which was above 2 at 5 and 30 min post injection for all complexes. The initial brain uptake of complexes ^{99m}**Tc-1**, and ^{99m}**Tc-2** can be considered comparable with the brain uptake of the diethylaminoethyl analogues (compounds 7 and 10, reference 8). However, complexes ^{99m}**Tc-1**, and ^{99m}**Tc-2** showed faster clearance of the radioactivity from the brain.

Significant accumulation of radioactivity was observed in the lungs, ranging between 8.47 - 9.54 % dose per organ, as it is expected for derivatives carrying a pendant alkylamino group.

The radioactivity from the novel technetium complexes was excreted mainly through the hepatobiliary system. Excretion through hepatobiliary tract determined also the high intestinal activity (15 - 20% of the injected dose at 30 min. post injection). On the other hand, urinary excretion was low (2.66 - 6.98 % in urine 30 min post injection).

Stomach and thyroid values were within acceptable levels (aprox 2.0% and 0.1% respectively at 30 min.) demonstrating no "in vivo" reoxidation.

EXPERIMENTAL

All laboratory chemicals were reagent grade and used without further purification. Solvents used for chromatographic analysis were HPLC grade.

[^{99m}Tc]$NaTcO_4$ was obtained from Elumatic III generator, Cis Bio-international. Thiophenol, thiocresol and p-chlorothiophenol used as coligands were commercially available.

HPLC analysis was developed using normal phase Spherisorb-Si column, methanol:dicloromethane 95:5, 1 ml/min and a LC-10 AS Shimadzu Liquid Chromatograph. Detection was either accomplished with a photodiode array detector (SPD-M10A, Shimadzu) that recorded UV-vis spectra on flux or a 3"x3" NaI (Tl) crystal scintillation detector.

IR spectrum was obtained from KBr pellets in the range 4000 to 200 cm^{-1} on a Bomen MB-102 FT-IR spectrophotometer. Elemental analysis was performed on a Carlo Erba EA 1108 analyzer.

Activity measurements were performed either in a Dose Calibrator, Carpintec CRC- 5R or in a scintillation counter, 3"x3" NaI (Tl) crystal detector associated to an ORTEC monochanel analyzer.

Synthesis of the SNS ligand

The ligand, N,N-bis(2-mercaptoethyl)-N',N'-diisopropylethylenediamine, was synthesized by reacting N,N-diisopropylethylenediamine with ethylene

sulphide in toluene at 110°C, following a previously described method (10) . Purification was achieved by high vacuum distillation.

Yield: 44%; b.p.: 120-122 °C/0.2 mmHg; IR cm^{-1}(KBr) 985, 1060, 1119, 1206, 1295, 1361, 1387, 1457, 2360, 2512, 2817, 2965; ^1H NMR: δ: 1,0 (d, 6H, CH_3), 1.9 (s, 2H, SH), 2.5 (m, 12H, CH_2), 2.9 (m, 2H, CH). All analytical data were consistent with the assigned structure.

Preparation of rhenium complexes

To a stirred suspension of trichlorobis(triphenylphosphine)rhenium(V) oxide (11)(166 mg, 0.2 mmol) in methanol (10 mL), a 1 N solution of CH_3COONa in methanol (2 mL, 2 mmol) was added. A mixture of N,N-bis(2-mercaptoethyl)-N',N'-diisopropylethylenediamine (L^1H_2, 0.2 mmol) and 0.2 mmol of the appropriate thiophenol (L^2H) was added under stirring. The solution was refluxed until the green-yellow colour of the precursor turned to dark-green. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 (30 mL) and then washed with water. The organic layer was separated from the mixture and dried over $MgSO_4$. The volume of the solution was reduced to 5 mL and then 5 mL of methanol were added. Slow evaporation of the solvents at room temperature afforded the products of the reaction as a green solids.

(Benzenethiolate) [N,N - bis - (2 - ethylsulphide) - N',N' - diisopropyl-ethylene-diamine] -oxorhenium(V) (Complex 1): Green crystals, Yield 41.1%, Anal. Calcd. for $C_{18}H_{31}N_2S_3ReO$: C 37.68%, H 5.45%, N 4.88%, S 16.76%; Found: C 38.57%,H 5.62%,N 5.03%, S 17.52%; IR(KBr): 951.7 cm^{-1}; (Re=0); UV-vis: 243, 414 nm.

(4-methyl-benzenethiolate)[N,N-bis-(2-ethylsulphide)-N',N'-diisopropyl-ethylene-diamine]-oxorhenium(V)(Complex 2): Green crystals, Yield 42.31%, Anal. Calcd. for $C_{19}H_{33}N_2S_3ReO$: C 38.82%, H 5.66%, N 4.77%, S 16.36%; Found: C 39.27%,H 5.36%,N 4.80%,S 15.802%;IR(KBr): 950.0 cm^{-1}; (Re=0); UV-vis: 245, 420 nm.

(4-chloro-benzenethiolate)[N,N-bis- (2-ethylsulphide)- N',N'- diisopropyl-ethylene-diamine]-oxorhenium(V)(Complex3): Green crystals, Yield 44.8%, Anal. Calcd.for $C_{18}H_{30}N_2S_3ClReO$:C35.54%,H 4.97%, N 4.61%, S 15.81%; Found: C 35.72%, H 4.84% , N 4.64%,S 16.29%; IR(KBr): 942.3cm^{-1}; (Re=0); UV-vis:241,412 nm.

X-ray crystal structural determination of complexes 2 and 3.

Crystals suitable for X-ray crystallography were obtained by recrystallization from $MeOH/CH_2Cl_2$.

A green crystal of complex **2** (0.20 x 0.40 x 0.60 mm) and a dark green crystal of complex **3** (0.40 x 0.40 x 0.40 mm) were mounted in air. Diffraction measurements were made on a Crystal Logic Dual Goniometer diffractometer using graphite monochromated Mo radiation. Unit cell dimensions were determined and refined by using the angular settings of 25 automatically centred reflections in the range $11 < 2\theta < 23°$ and they appear in Table 2. Intensity data were recorded using a θ-2θ scan. Three standard reflections monitored every 97 reflections showed less than 3% variation and no decay. Lorentz, polarization and psi-scan absorption corrections were applied using Crystal Logic software. The structures were solved by direct methods using SHELXS-86 (12) and refined by full-matrix least-squares techniques on F^2 with SHELXL-93 (13).

Table 2. Summary of Crystal, Intensity Collection and Refinement Data

Complex	**2**	**3**
Formula	$C_{19}H_{33}N_2OS_3Re$	$C_{18}H_{30}ClN_2OS_3Re$
fw	587.85	608.97
a, Å	11.509(5)	12.796(4)
b, Å	8.575(4)	13.883(4)
c, Å	12.912(6)	14.045(5)
α, (°)	89.28(2)	
β, (°)	76.65(2)	113.016(9)
γ, (°)	72.44(2)	
V, ($Å^3$)	1179.8(9)	2296.5(1)
Z	2	4
D_{calcd}/D_{measd}, ($Mg\ m^{-3}$)	1.655/1.63	1.759/1.74
Space Group	*P1*	$P2_1/n$
Temp, K	298	298
Radiation, λ (Å)	Mo Ka 0.71073	Mo Ka 0.71073
Abs.Coeff (μ), cm^{-1}	5.426	5.691
Octants Collected	±h,k,±l	±h,-k,l
GOF on F^2	1.109	1.089
R*1*	0.0381 [b]	0.0317 [a]
wR2	0.0954 [b]	0.0819 [a]

[a]*for 3719 reflections with I>2□(I);* [b]*for 3789 reflections with I>2□(I).*

For complex **2**: $2\theta_{max} = 50°$, scan speed 2.2°/min, scan range 2.5 plus $\alpha_1\alpha_2$ separation, reflections collected/unique/used = 4468/4162(R_{int}=0.0145)/4162, 330

parameters refined, R1/wR2 (for all data) = 0.0427/0.0996, $[\Delta\rho]_{max}/[\Delta\rho]_{min}$ = 1.664/-1.714 e/Å^3, $[\Delta/\sigma]_{max}$ = 0.085. Hydrogen atoms of C3, C9, C11 and C19 were introduced at calculated positions as riding on bonded atoms, the rest were located by difference maps and refined isotropically. All non-hydrogen atoms refined anisotropically.

For complex **3** : $2\theta_{max}$ = 50°, scan speed 4.0°/min, scan range 2.4 plus $\alpha_1\alpha_2$ separation, reflections collected/unique/used = 4217/4046(R_{int}=0.0138)/4046, 303 parameters refined, R1/wR2 (for all data) = 0.0348/0.0843, $[\Delta\rho]_{max}/[\Delta\rho]_{min}$ = 1.539/-1.406 e/Å^3, $[\Delta/\sigma]_{max}$ = 0.018. Hydrogen atoms of C4, C8, C9, C10, C11, C12 and C17 were introduced at calculated positions as riding on bonded atoms, the rest were located by difference maps and refined isotropically. All non-hydrogen atoms refined anisotropically.

Preparation of ^{99m}Tc complexes

Preparation of the complexes at tracer level was accomplished by using ^{99m}Tc-glucoheptonate as precursor (14). A vial containing a lyophilized mixture of 200 mg calcium glucoheptonate and 0.2 mg $SnCl_2.2H_2O$ was reconstituted with 5 mL water and 0.5 mL of this solution was mixed with [^{99m}Tc] $NaTcO_4$ (0.5-1 mL with an activity of 5 - 50 mCi (185 - 1850 MBq). Substitution was performed at coligand/ligand molar ratio of 1 for all complexes, by adding the precursor (with radiochemical purity > 95%) to a centrifuge tube containing the ligand (0.02 mmoles) and the coligand (0.02mmoles) . The mixture was agitated in a vortex mixer and left to react at room temperature for 10 minutes. The lipophilic species were extracted with CH_2Cl_2 and the organic layer dried with $MgSO_4$, filtered and analyzed by HPLC.

The characterization of the ^{99m}Tc complexes was accomplished by chromatographic correlation (HPLC) with the corresponding rhenium complexes.

Biodistribution studies

Normal mice (Swiss Albino, 19-34 g, 3-6 animals per group) were injected via tail vein with of the HPLC purified ^{99m}Tc complex, reconstituted with 30% methanol, 70% saline (0.1 ml, 2-3 μCi). At 5 and 30 minutes after injection the animals were sacrificed by cardiectomy under light ether anesthesia. Whole organs of

interest, total urine volume and samples of blood and muscle were collected, weighted and assayed for radioactivity. Bladder and excreted urine were not weighted. Results were expressed as % dose/organ (calculated by comparison of sample radioactivity to standard solutions containing 1% of the injected dose) and %Dose/g. For blood and muscle the calculation was based upon the measured activity, the sample weight and body composition data (7% and 43% of the body weight, respectively). The brain/blood ratio was calculated from the corresponding percent dose/g values.

CONCLUSIONS

In this study a novel tridentate aminodithiol ligand carrying the diisopropylaminoethyl group has been synthesized. Three neutral mixed-ligand oxorhenium complexes and their technetium-99m analogues have been synthesized and characterized using this ligand and different monodentate thiols. The identity of the ^{99m}Tc complexes was determined by comparative HPLC analysis.

These complexes were found capable of penetrating the intact blood brain barrier and a significant amount of radioactivity maintains into the brain over the time. However the incorporation of the diisopropylaminoethyl group in the SNS/S backbone is not enhancing the biological characteristics of this type of complexes.

ACKNOWLEDGEMENTS

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