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TECHNETIUM-99m RADIOLABELLING OF AN *N*-AMINO-ALKYL-BENZAMIDE NITRIDO- AND OXO-TECHNETIUM BIS(AMINOETHANETHIOL) DERIVATIVE SYNTHESIS AND BIOLOGICAL RESULTS. POTENTIAL MELANOMA TRACER AGENTS.

Philippe AUZELOUX[1], Marie-France MOREAU[1], Janine PAPON[1], Martine BAYLE[1], Michèle BOREL[1], Roberto PASQUALINI[2] and Jean-Claude MADELMONT[1]

[1] INSERM U 484, rue Montalembert, B.P. 184, 63005 Clermont-Ferrand CEDEX, France
[2] CIS bio international, 92192 Gif-sur-Yvette, France

SUMMARY

N-(2-diethylaminoethyl)-4-iodobenzamide has been reported to be an excellent agent for malignant melanoma diagnosis by SPECT. To obtain a ^{99m}Tc analog, we synthesized a new bis(aminothiol) (BAT) derivative. The benzamide function was conjugated onto a nitrogen of the BAT backbone. This ligand was successfully radiolabelled with both nitrido-technetium and oxo-technetium cores. These complexes were purified by HPLC. Tumour uptake was measured in intravenously injected mice bearing the B16 murine melanoma.

Key-Words : ^{99m}Tc, Benzamide, Bis(aminothiol), Melanoma, Nitrido-technetium complex, Oxo-technetium complex.

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INTRODUCTION

The development of technetium-99m-labelled small molecules recently met with encouraging results. It is now widely acknowledged that a technetium ligand can be conjugated to active structures to form radiopharmaceuticals of interest.[1]

N-alkyl-iodobenzamide series are reputed to form the best imaging agent classes for the scintigraphic detection of malignant melanoma and metastases.[2-5] A phase II clinical trial of *N*-(2-diethylaminoethyl)-4-[^{123}I]-iodobenzamide (Figure 1) has been successfully undertaken.[6, 7]

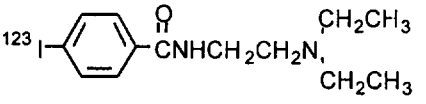


Figure 1. *N*-(2-diethylaminoethyl)-4-[^{123}I]-iodobenzamide ([^{123}I]-BZA).

To develop these structures, we began using technetium (^{99m}Tc, γ=140keV, T½=6h) in place of the iodine radionuclide (^{123}I, γ=159keV, T½=13h). Technetium is currently the most commonly used SPECT agent in nuclear medicine. However it has a metallic nature, therefore an organic ligand structure is necessary to form a stable complex. We followed a conjugate approach to introduce the metal core complex onto the benzamide pharmacophore. We recently reported successful a ^{99m}Tc-radiolabelling using a bis(dithiocarbamate) structure (Figure 2).[8] The weak tumour uptake we observed compelled us to look for a less lipophilic molecule.

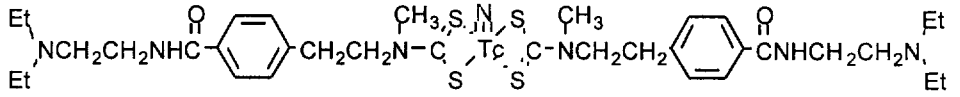


Figure 2. Structure of the bis(dithiocarbamate) nitrido-technetium derivative.

In this study the synthesis of a new bis(aminothiol) derivative, *N*-functionalized by the benzamide structure is reported. This ligand was radiolabelled with both

nitrido- and oxo-technetium cores (Figure 3). These two radiopharmaceuticals were evaluated in mice bearing the B16 murine melanoma.

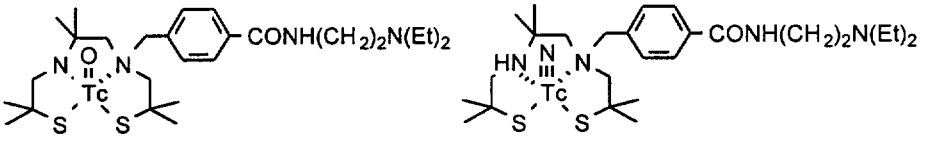


Figure 3. [^{99m}TcO]-BZA (left) and [^{99m}TcN]-BZA (right).

RESULTS

Synthesis :

The ligand was obtained as shown in Figure 4. The cyclic diimine **1** was obtained following a published procedure.[9] Sodium cyanoborohydride was used to reduce **1** to the diaminodisulfide **2** to prevent bicyclic formation.[10] The mono *N*-substituted diaminodisulfide **3** was prepared by the nucleophilic substitution of **2** with methyl 4-(bromomethyl)benzoate. The position of this substitution was monitored by a complete NMR proton and carbon assignments (see table 1). The 8-N substitution was favored by steric effects. We subsequently used trimethylaluminium and *N,N*-diethylethylenediamine in CH_2Cl_2 to convert the ester function of **3** into the desired amide group.[11] The last step : the reduction of the disulfide bond could not be achieved with the usual reducing agents such as $LiAlH_4$ or $NaBH_4$ because of their lack of specificity. Eisenhut et al. reported the use of dithiothreitol (DTT) with BAT series as a specific reducing agent.[12] We successfully reduced compound **4** to dithiol **5** only under basic conditions (Et_3N). To prevent ring reformation, the BAT derivative **5** was isolated as its hydrochloride under an argon atmosphere.

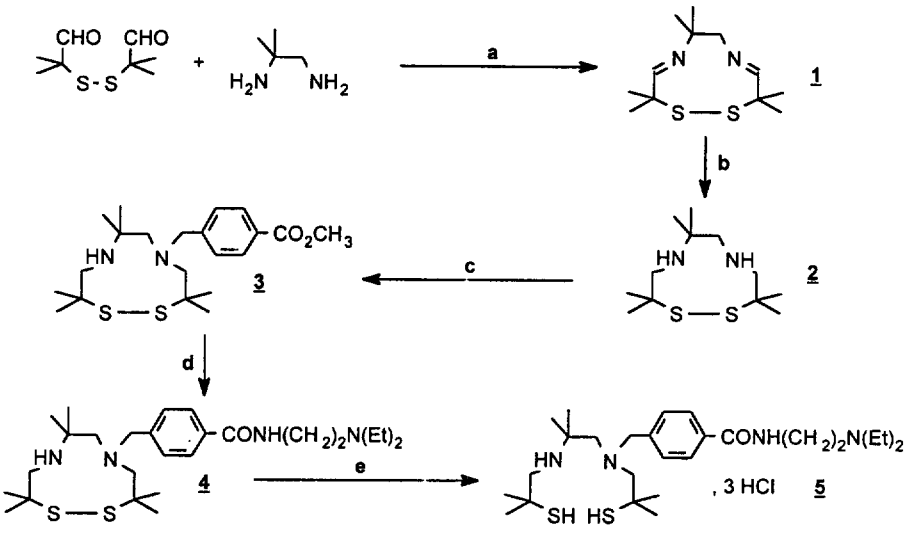
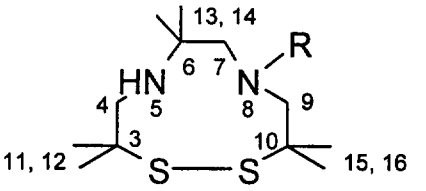


Figure 4. Synthesis of the BAT derivative ligand. (a) Δ, EtOH; (b) $NaBH_3CN$, Et_2O/HCl; (c) $BrCH_2C_6H_4CO_2Me$, K_2CO_3, CH_3CN; (d) $NH_2CH_2CH_2NEt_2$, $Al(CH_3)_3$, CH_2Cl_2; (e) DTT, Et_3N.

Table 1. NMR assignment of cyclic BAT precursors.



	2		3		4	
δ (ppm)	^1H	^{13}C	^1H	^{13}C	^1H	^{13}C
3	-	52.1	-	48.1	-	48.4
4	2.41	49.9	2.80, 3.00	57.8	2.78, 2.98	57.1
6	-	53.3	-	53.4	-	53.7
7	2.42, 2.64	57.8	2.42, 2.80	62.8	2.41, 2.83	62.5
9	2.64, 3.16	66.4	2.80, 3.20	65.2	2.78, 3.19	65.3
10	-	51.5	-	52.6	-	52.7
11, 12	1.23, 1.34	25.0, 29.1	1.25, 1.55	28.7, 29.4	1.23, 1.51	28.2, 28.9
13, 14	1.05, 1.10	26.6, 28.1	0.77, 0.81	23.4, 28.1	0.71, 0.77	24.0, 28.0
15, 16	1.21, 1.43	27.1, 27.8	1.24, 1.44	26.1, 30.0	1.23, 1.44	26.0, 30.0

Radiolabelling :

The oxo-technetium $[Tc=O]^{3+}$ core was obtained by the stannous reduction in basic conditions in the presence of the BAT ligand **5**. The crude oxo-technetium complex was purified by HPLC, and global yield from pertechnetate was 50 %. This complex is weakly lipophilic (octanol/PBS partition coefficient : log(P)=0.35).

The nitrido-technetium $[Tc\equiv N]^{2+}$ core was introduced using the procedure of Pasqualini R. *et al.*[13-15] as shown in Figure 5. In this reaction, the tertiary phosphine was the reducing agent and *N*-methyl-*S*-methyl-dithiocarbazate (MDTCZ) acted as nitrogen (N^{3-}) donor. The ligand exchange reaction was followed by thin layer chromatography. The radiolabelling yield, measured by TLC, was better than 45 %. The complex purification was done by HPLC, collecting the 12 to 15 min fractions. To minimize the HPLC injected volume, the exchange solvent was first evaporated and the complex **[^{99m}TcN]-BZA** was dissolved in a minimum volume of dichloromethane. The overall yield from the pertechnetate was 52 % after correction for radioactivity decrease. This complex is slightly hydrophilic (octanol/PBS partition coefficient : log(P)=-0.11).

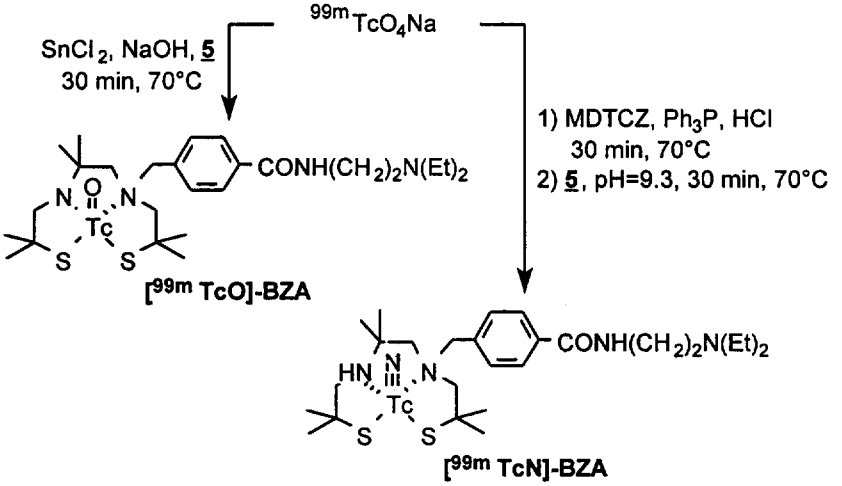


Figure 5. The two Technetium cores synthesised from **5**.

The injected solutions were prepared with 10 % ethanol in PBS buffer, to allow radiopharmaceutical miscibility.

Biological Results:

Tumour uptake was studied in mice bearing the B16 murine melanoma by injection of 0.2 mL of the previous solution (0.74 MBq). Results of the biodistribution studies are summarised in the following four tables.

Table 2. Biodistribution in mice of [^{99m}TcO]-BZA (% ID/g ; mean ± s.e.).

Organ	5 min	1 h	3 h	6 h	24 h
Blood	6.32±0.14	2.04±0.17	0.97±0.01	0.65±0.04	0.22±0.01
Liver	24.6±0.5	14.2±0.3	8.02±0.07	6.24±0.90	2.86±0.23
Kidney	19.0±1.2	8.30±0.46	4.21±0.13	3.09±0.27	1.14±0.07
Lung	23.6±0.74	9.61±1.06	4.65±0.16	3.08±0.33	1.06±0.16
Muscle	1.92±0.08	0.73±0.07	0.29±0.01	0.16±0.01	0.08±0.01
Brain	0.76±0.06	0.25±0.03	0.13±0.01	0.12±0.01	0.09±0.00
Eye	1.65±0.11	1.62±0.18	1.26±0.15	1.24±0.05	1.34±0.11
Tumour	2.94±0.61	1.51±0.24	1.42±0.29	0.81±0.05	0.53±0.02

Table 3. Tumour-to-organ radioactivity ratios of [^{99m}TcO]-BZA (mean ± s.e.).

Organ	5 min	1 h	3 h	6 h	24 h
Blood	0.46±0.11	0.73±0.06	1.46±0.53	1.26±0.18	2.38±0.07
Liver	0.12±0.03	0.11±0.02	0.18±0.05	0.13±0.01	0.19±0.03
Kidney	0.15±0.04	0.18±0.02	0.33±0.07	0.26±0.03	0.47±0.04
Lung	0.13±0.04	0.16±0.01	0.31±0.09	0.27±0.05	0.54±0.15
Muscle	1.51±0.31	2.05±0.19	4.93±1.46	4.91±0.30	7.38±1.66
Brain	4.04±1.42	5.95±0.37	10.9±3.5	6.61±0.80	6.08±0.52
Eye	1.76±0.33	0.93±0.07	1.19±0.45	0.66±0.08	0.40±0.02

For the oxo-technetium complex, rapid organ clearances were observed. Blood activity at 6 hours post-injection was 10 % of the initial 5-minute activity (6.32 at 5 min and 0.65 % ID/g at 6h p.i.). Liver, lung and kidney displayed high radioactivity levels at 5 minutes (respectively 24.6, 23.6 and 19.0 % DI/g). No particular tropism for muscle or brain was observed, and a fairly constant radioactivity level in eyes

Table 4. Biodistribution in mice of [^{99m}TcN]-BZA (% ID/g ; mean ± s.e.).

Organ	5 min	1 h	3 h	6 h	24 h
Blood	7.00±0.57	0.47±0.01	0.23±0.01	0.13±0.01	0.03±0.00
Liver	27.90±1.10	4.57±0.52	2.02±0.07	1.50±0.11	0.70±0.05
Kidney	12.08±0.56	0.95±0.08	0.57±0.01	0.37±0.03	0.09±0.01
Lung	6.20±0.15	0.83±0.09	0.50±0.06	0.28±0.03	0.13±0.02
Muscle	1.12±0.04	0.18±0.05	0.05±0.01	0.04±0.01	0.01±0.00
Brain	0.17±0.02	0.03±0.00	0.02±0.00	0.03±0.01	0.01±0.00
Eye	0.99±0.05	0.24±0.01	0.16±0.00	0.14±0.01	0.14±0.01
Tumour	2.63±0.12	0.71±0.13	0.21±0.01	0.18±0.03	0.06±0.01

Table 5. Tumour-to-organ radioactivity ratios of [^{99m}TcN]-BZA. (mean ± s.e.).

Organ	5 min	1 h	3 h	6 h	24 h
Blood	0.38±0.04	1.49±0.37	0.94±0.04	1.39±0.35	2.08±1.07
Liver	0.09±0.00	0.16±0.05	0.11±0.00	0.12±0.04	0.09±0.03
Kidney	0.22±0.02	0.77±0.25	0.38±0.03	0.50±0.15	0.65±0.10
Lung	0.43±0.04	0.91±0.33	0.44±0.06	0.63±0.09	0.45±0.04
Muscle	2.34±0.06	4.94±2.20	4.61±1.23	5.26±2.03	4.95±1.76
Brain	15.4±1.12	24.3±7.1	8.76±0.55	8.30±3.47	4.98±1.78
Eye	2.67±0.09	3.02±0.87	1.34±0.11	1.28±0.33	0.45±0.12

was recorded. The tumour uptake was almost 3 % ID/g at 5 minutes, followed by slow elimination: the radioactivity level was constant between 1 and 3 hours post-injection (see table 2). Eyes excepted, all t/o ratios increased during the experiment, especially for blood and muscle (see table 3).

For the nitrido-technetium complex, a very rapid clearance was noted in all the organs. At 1 hour post injection, only the liver value was above 1 % ID/g (see table 4). The tumour radioactivity was 2.63 % ID/g at 5 minutes but rapidly fell below 1 % ID/g. For to the tumour-to-organ ratios, only the blood value showed a significant rise in time (see table 5).

DISCUSSION

In this study, we did not use the bicyclic intermediate usually employed.[9, 16] The two methylene groups at position 6 of the cyclic diamine compound **2** switched the substitution reaction to the 8-N monosubstitution. This was confirmed by the complete NMR assignments of **2**, **3** and **4**. The radiochemical yields of the oxo- and nitrido-technetium purified complexes were similar, and no instability was noted for 24 hours. In preliminary biological results, a faster clearance was observed for the nitrido-technetium complex in blood and in all organs. The tumour uptakes were near 3 % ID/g at 5 minutes post-injection, but for [^{99m}TcN]-BZA, the 24-hour value became very weak. A comparison between these two technetium complexes and the iodo-benzamide tumour uptakes[17] is shown in table 6. Two comments can be made. First, the 24-hour values for [^{99m}TcO]-BZA and [^{125}I]-BZA are quite similar, which demonstrates the possibility of conjugating the benzamide structure and a technetium complex. Second, the radiopharmaceutical lipophilicity seems to be a major parameter for the biodistribution of the molecule. In conclusion, we demonstrate tumour tropism for two new technetium radiopharmaceutical derivatives of a benzamide compound.

Table 6. Partition coefficient compared with tumour uptake of nitrido-, oxo-technetium complexes and ^{125}I-BZA (% ID/g ; mean ± s.e.).

		% ID/g				
Compound	Log P	5 min	1 h	3 h	6 h	24 h
[^{99m}TcN]-BZA	-0.11	2.63±0.12	0.71±0.13	0.21±0.01	0.18±0.03	0.06±0.01
[^{99m}TcO]-BZA	0.35	2.94±0.61	1.51±0.24	1.42±0.29	0.81±0.05	0.53±0.02
[^{123}I]-BZA	1.34	-	5.98±1.47	4.24±1.50	3.47±1.76	0.55±0.32

MATERIALS and METHODS

General :

Chemistry: All reagents and solvents were from commercial suppliers and used with no further purification. Analytical thin layer chromatography (TLC) was conducted on pre-coated silica gel plates (SDS, plastic sheet 60 F_{254}, layer thickness 0.25 mm), on aluminium oxide plates (Merck,

plastic sheet 60 F_{254}, neutral type E, layer thickness 0.20 mm). subsequently visualised under UV light (254 nm) and exposed to iodine vapour. Medium pressure chromatography was performed using silica gel (SDS, Chromatogel 60A 40-60 μm). Solvent mixture was expressed as volume-to-volume ratio (v:v). Melting points (mp) were measured with a Reichert-Jung Kofler apparatus. Infrared (IR) spectra were recorded on a Vector 22 Bruker instrument (ν expressed in cm^{-1}). ^{1}H-NMR and ^{13}C-NMR were recorded at respectively 200.1 MHz and 50.3 MHz on a Bruker AM 200 (4.5 T) instrument. Chemical shifts (δ) are reported in parts per million relative to the internal standard $(CH_3)_4Si$ or using the deuterated solvents ($CDCl_3$, δ = 7.26 ppm for ^{1}H-NMR and δ=77.0 ppm for ^{13}C-NMR).

Radiochemistry: [^{99m}Tc] sodium pertechnetate as no-carrier-added solution was purchased from the Jean Perrin Cancer Hospital (Clermont-Ferrand). All solvents were degassed under argon before use. TLC radioactive spots were scanned and recorded by an AMBIS 4000 detector (a computer-controlled multi-wire proportional counter). HPLC purifications were performed on a Shimadzu HPLC system (LC6A pump, SCL6B system controller and CR5A integrator) equipped with a semi-preparative reverse phase column (Merck, Lichroprep RP 18, 12×200 mm), connected to a Shimadzu SPD6AV UV spectrophotometric detector (254 nm) in series with a Raytest NaI (Tl) gamma detector.

Synthesis :

3,3,6,6,10,10-hexamethyl-1,2-dithia-5,8-diazacyclo-deca-4,8-diene (1).

2-methyl-1,2-diaminopropane (8.82 g, 100 mmol) was added dropwise to a solution of 2,2,5,5-tetramethyl-3,4-dithiahexa-1,6-dial[18] (20.63 g, 100 mmol) in absolute ethanol (200 mL). The reaction mixture was stirred at 55°C for 4 hours. The solution was concentrated four times, to afford after recrystalling 19.38g of white crystals of the desired diimine **1** (75 mmol, 75% yield). Mp : 100°C; ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.26-1.43 (6s, 18H, 6xCH_3); 2.92 (d, 1H, 7-H, *J*=9.3 Hz); 3.78 (dd, 1H, 7'-H, *J*=1.3, 9.3 Hz); 6.84 (s, 1H, 4-H); 6.90 (d, 1H, 9-H, *J*=1.2 Hz); ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 21.1-28.8 (6C, 6xCH_3); 52.7 and 52.8 (2C, 3-C and 10-C); 63.2 (1C, 6-C); 72.0 (1C, 7-C); 161.2 (1C, 4-C); 167.9 (1C, 9-C); IR (KBr) ν: 2950-2825, 1650, 1440 and 1380-1340.

3,3,6,6,10,10-hexamethyl-1,2-dithia-5,8-diazacyclodecane(2).

Compound **1** (5.00 g, 19.3 mmol) in absolute ethanol (50 mL) was placed in a three-necked round-bottomed flask under an argon atmosphere. The solution was stirred and cooled in an ice bath at 0°C, and $NaBH_3CN$ (1.46 g, 23.2 mmol) in ethanol (5 mL) was slowly added. Ether/HCl (2 *M*, 10 mL) was added and the mixture was stirred 3 h at room temperature. The solution was quenched with water (5 mL) and 1 *M* aq NaOH (20 mL). The organic solvents were evaporated under reduced

pressure. Three CH_2Cl_2 extractions afforded crude product **2** (4.90 g, 18.7 mmol, 97% yield). Purification was by flash chromatography ethyl acetate/cyclohexane 30:70 (2.20 g, 8.38 mmol, 43 % yield). Colourless oil; TLC R_f 0.75 (aluminium oxide, CH_2Cl_2/EtOH 95:5). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.05, 1.10 (2s, 6H, 6-C(CH_3)$_2$); 1.21, 1.43 (2s, 6H, 10-C(CH_3)$_2$); 1.23, 1.34 (2s, 6H, 3-C(CH_3)$_2$); 2.0 var. (broad, 2H, 2xNH); 2.41 (s, 2H, 4-H); 2.42 (d, 1H, 7-H, *J*=12.6 Hz); 2.64 (mu, 2H, 7'-H and 9-H); 3.16 (d, 1H, 9'-H, *J*=12.3 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 25.1, 29.1 (2C, 3-C($\underline{C}H_3$)$_2$); 26.6, 28.1 (2C, 6-C($\underline{C}H_3$)$_2$); 27.1, 27.8 (2C, 10-C($\underline{C}H_3$)$_2$); 49.9 (1C, 4-C); 51.5 (1C, 10-C); 52.1 (1C, 3-C); 53.3 (1C, 6-C); 57.8 (1C, 7-C); 66.4 (1C, 9-C).

8-(4-methylbenzoate)methyl-3,3,6,6,10,10-hexamethyl-1,2-dithia-5,8-diazacyclodecane (3).

Methyl 4-(bromomethyl)benzoate (2.51 g, 10.96 mmol) was added to a solution of diamine **2** (1.94 g, 7.39 mmol) and potassium carbonate (1.52 g, 10.96 mmol) in acetonitrile (50 mL). The resulting mixture was refluxed for 24 hours and the reaction was quenched with water (10 mL). After dichloromethane extraction, purification was carried out by flash chromatography on alumina gel (dichloromethane/ethanol 99.5:0.5) to afford 1.91 g of ester **3** (4.66 mmol, 63 % yield). TLC R_f 0.50 (aluminium oxide, dichloromethane/ethanol 99:1). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 0.77, 0.81 (2s, 6H, 6-C(CH_3)$_2$); 1.24, 1.44 (2s, 6H, 10-C(CH_3)$_2$); 1.25, 1.55 (2s, 6H, 3-C(CH_3)$_2$); 2.1 (broad, 1H, NH); 2.42 (d, 1H, 7-H, *J*=14.5 Hz); 2.75-2.88 (m, 3H, 4-H, 7'-H, 9-H), 3.00 (d, 1H, 4'-H, *J*=11.3 Hz); 3.20 (d, 1H, 9'-H, *J*=15.5 Hz); 3.73, 3.92 (2d, 2H, CH_2Ph, *J*=14.5 Hz); 3.92 (s, 3H, OCH_3); 7.48, 7.92 (2d, 4H, Harom, *J*=8.2 Hz). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 23.4, 28.1 (2C, 6-C($\underline{C}H_3$)$_2$); 26.1, 30.1 (2C, 10-C($\underline{C}H_3$)$_2$); 28.7, 29.4 (2C, 3-C($\underline{C}H_3$)$_2$); 48.1 (1C, 3-C); 52.0 (1C, OCH_3); 52.6 (1C, 6-C); 53.4 (1C, 10-C); 57.8 (1C, 4-C); 62.8 (1C, 7-C); 63.4 (1C, CH_2Ph); 65.2 (1C, 9-C); 128.9, 129.4, 129.6, 144.3 (6C, Carom); 166.9 (1C, C=O).

8-(*N*-diethylaminoethyl-4-methylenebenzamide)-3,3,6,6,10,10-hexamethyl-1,2-dithia-5,8-diazacyclodecane(4).

N,N-diethylethylenediamine (363 mg, 3.12 mmol) was diluted with CH_2Cl_2 (5 mL) in a three-necked, round-bottomed flask equipped with reflux condenser under an argon atmosphere. The solution was stirred and cooled in an ice bath at 0°C and trimethylaluminium in hexane (1.8 mL, 2*M* solution, 3.6 mmol) was slowly added. Fifteen minutes after complete addition, the cooling bath was removed and ester **3** (1.16 g, 2.83 mmol) in CH_2Cl_2 (10 mL) was added. The resulting solution was heated under reflux for 40 hours, cooled to room temperature and slowly hydrolyzed with water to prevent foam formation. The mixture was extracted three times with CH_2Cl_2, and the organic layers were combined and dried ($MgSO_4$). The solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (silica gel, ethyl acetate/EtOH) to afford 604 mg of benzamide **4** (1.22 mmol, 43% yield). TLC R_f 0.55 (aluminium oxide, CH_2Cl_2/MeOH 95:5);

^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.71, 0.77 (2s, 6H, 6-C(CH_3)$_2$); 1.10 (t, 6H, 2x$CH_2C\underline{H}_3$, *J*=7.1 Hz); 1.23, 1.44 (2s, 6H, 10-C(CH_3)$_2$); 1.23, 1.51 (2s, 6H, 3-C(CH_3)$_2$); 2.2 (broad, 1H, NH); 2.40 (d, 1H, 7-H, *J*=14.4 Hz); 2.60 (q, 4H, 2x$C\underline{H}_2CH_3$, *J*=7.1 Hz); 2.66 (t, 2H, $C\underline{H}_2NEt_2$, *J*=6.3 Hz); 2.78 (m, 2H, 4-H, 9-H), 2.83 (d, 1H, 7'-H, *J*=14.4 Hz); 2.95 (d, 1H, 4'-H, *J*=11.2 Hz); 3.19 (d, 1H, 9'-H, *J*=15.6 Hz); 3.48 (q, 1H, CONHC$\underline{H}_2$, *J*=6.3 Hz); 3.69, 3.87 (2d, 2H, CH_2Ph, *J*=14.5 Hz); 7.09 (m, 1H, CONH); 7.37, 7.76 (2d, 4H, Harom, *J*=8.2 Hz). ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 11.9 (2C, 2x$CH_2\underline{C}H_3$); 24.0, 28.0 (2C, 6-C($\underline{C}H_3$)$_2$); 26.0, 30.0 (2C, 10-C($\underline{C}H_3$)$_2$); 28.2, 28.9 (2C, 3-C($\underline{C}H_3$)$_2$); 37.1 (1C, CONH$\underline{C}H_2$); 46.9 (2C, 2x$\underline{C}H_2CH_3$); 48.4 (1C, 3-C); 51.4 (1C, $\underline{C}H_2NEt_2$); 52.7 (1C, 10-C); 53.7 (1C, 6-C); 57.1 (1C, 4-C); 62.4 (1C, 7-C); 63.2 (1C, CH_2Ph); 65.3 (1C, 9-C); 126.8, 129.8, 133.3, 142.5 (6C, Carom); 167.0 (1C, C=O).

***N*-diethylaminoethyl-4-[2-(2-methyl-2-thiopropane)-4,4,7-trimethyl-7-thio-2,5-diazaoctane] benzamide (5).**

102 mg of cyclic compound **4** (0.206 mmol) were stirred under an argon atmosphere with diethylamine (0.1 mL) in 2.0 mL of degassed methanol. 158 mg of dithiothreitol DTT (1.02 mmol) were rapidly added to this solution. The reaction mixture was stirred for six days. The BAT derivative was extracted three times with minimum degassed chloroform. Organic layers were combined, dried ($MgSO_4$) and the solvent was evaporated under reduced pressure. The desired thiol was stirred in dry ether/HCl (2*N*) to afford 97 mg of compound **5** as hydrochloride (0.150 mmol, 78 % yield). TLC R_f 0.67 (aluminium oxide, CH_2Cl_2/MeOH 94:6); ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1,05 (s, 6H, NHC($C\underline{H}_3$)$_2$); 1.06 (t, 6H, 2x$CH_2C\underline{H}_3$, *J*=7.1 Hz); 1.33, 1.34 (2s, 12H, 2x($C\underline{H}_3$)$_2$CSH); 2.43, 4.44 (2s, 2H, 2xSH); 2.55 (s, 2H, CH_2NH); 2.59 (q, 4H, 2xCH_2CH_3, *J*=7.1 Hz); 2.66 (t, 2H, CH_2NEt_2, *J*=5.6 Hz); 2.69 (2s, 4H, PhCH$_2$N($C\underline{H}_2$)$_2$); 3.49 (q, 2H, CONHC$\underline{H}_2$, *J*=5.6 Hz); 3.89 (s, 2H, CH_2Ph); 6.97 (m, 1H, CONH); 7.45, 7.73 (2d, 4H, Harom, *J*=8.2 Hz). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 11.9 (2C, 2x$CH_2\underline{C}H_3$); 26.3 (2C, NHC($\underline{C}H_3$)$_2$); 30.5, 31.8 (4C, 2x($\underline{C}H_3$)$_2$CSH); 37.1 (1C, CONH$\underline{C}H_2$); 45.5, 45.8 (2C, 2x(CH_3)$_2\underline{C}$SH); 46.8 (2C, 2x$\underline{C}H_2CH_3$); 51.3 (1C, $\underline{C}H_2NEt_2$); 53.9 (1C, (CH_3)$_2\underline{C}$NH); 55.8 (1C, CH_2NH); 62.2 (1C, CH_2Ph); 67.8, 69.9 (2C, PhCH$_2$N($\underline{C}H_2$)$_2$); 126.8, 129.3, 133.3, 143.5 (6C, Carom); 167.1 (1C, CO).

Radiolabelled with ^{99m}Tc :

Oxo-technetium complex: In a reaction vial under an argon atmosphere were added 1 mg of ligand **5** (1,6 μmol) in 0.2 mL 95° ethanol, 0.4 mL of water, 0.1 mL of 1*N* sodium hydroxide solution, 0.5 to 1.0 mL of the sodium pertechnetate solution (activity ranging from 0.2 to 0.74 GBq) and 0.25 mL of a $SnCl_2$ solution (10 mg/mL in water). This solution was heated to 70°C for 30 min and then cooled to room temperature. The solvents were evaporated under reduced pressure. The residue was dissolved in 1.0 mL of dichloromethane before HPLC separation (0.4 % NH_4OH/MeOH 15:85).

Flow rate : 5 mL/min. Rt 12.7 min. Radiochemical yield : 50%. HPLC solvents were evaporated under reduced pressure, and the complex was dissolved in PBS buffer with 10% ethanol.

Nitrido-technetium complex: In a reaction vial were placed 0.4 mL of *N*-methyl-*S*-methyl-dithiocarbazate solution (2.5 mg/mL in 95° ethanol), 0.2 mL of triphenylphosphine solution (5.0 mg/mL in 95% ethanol) and 0.1 mL of 1*M* hydrochloric acid under an argon atmosphere. 0.5 to 1.0 mL of the sodium pertechnetate solution (activity ranging from 0.2 to 0.74 GBq) was added. The resulting mixture was heated to 70°C for 30 min and then cooled to room temperature. The pH was adjusted by adding 0.1 mL of 1*M* sodium hydroxide solution followed by 0.9 mL of 0.5*M* bicarbonate buffer (pH 9.4). 1.0 mL of this solution was added to a second reaction vial containing the ligand **5** as hydrochloride (1 mg, 1,6 μmol) in 1.0 mL 95° ethanol under an argon atmosphere. The solution was heated to 70°C for 30 min and the solvents then evaporated under reduced pressure. The residue was dissolved in 1.0 mL of dichloromethane before HPLC separation (0.4 % NH_4OH/CH_2Cl_2 33:67). Flow rate: 5 mL/min. Rt = 12.6 min. Radiochemical yield: 52%. HPLC solvents were evaporated under reduced pressure, and the complex was dissolved in PBS buffer with 10% ethanol.

Biological :

Tumour uptake was studied in C57BL/6 J1 co male mice bearing the B16 murine melanoma. Transplantable B16 mouse melanotic melanoma was originally obtained from ICIG (Villejuif, France). 5×10^5 viable cells were injected subcutaneously. Ten days later, the tumours became palpable. Following the intravenous injection in the tail vein of 0.74 MBq ^{99m}Tc-labelled complex, mice (n = 3) were sacrificed by exsanguination after set time intervals of 5 min, 1, 3, 6 and 24 h. Aliquots of different tissues were weighed, and radioactivity was immediately measured. Samples were counted in a γ-counter (Packard Autogamma® A 5530). The fractional accumulation of radioactivity in the tissue was expressed as % injected dose/g of tissue (% ID/g).

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