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SYNTHESIS, CHARACTERIZATION AND BLOOD CELL LABELLING EVALUATION OF NEW ^{99m}Tc NITRIDO RADIOPHARMACEUTICALS WITH THIOAMIDE [$R^1C(=S)NHR^2$] DERIVATIVES

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SUMMARY

The synthesis of a series of new ^{99m}Tc-thioamide complexes with the $[Tc{\equiv}N]^{2+}$ nitrido core, in which the thioamide ligand substituents were varied to include different lengths of aliphatic alkyl chains or a phenyl group is reported. TLC analysis shows that the complexes are neutral and lipophilic compounds. The ^{99m}Tc complexes have a low blood cell labelling efficiency in whole blood compared to the reference complex of dithiocarbamate $^{99m}TcN(Et(EtO)NCS_2)_2$.

Key words : Blood cell labelling / radiopharmaceutical / Technetium-99m / nitrido complexes / Thioamide ligands.

INTRODUCTION

The recent development by Pasqualini *et al.*[1] of an efficient method for the preparation of $[^{99m}Tc{\equiv}N]^{2+}$ radiopharmaceutical kits, under sterile and apyrogenic conditions has prompted the synthesis of new nitridotechnetium-99m complexes and subsequent evaluation of their biological distribution. The procedure is based on the initial reaction of pertechnetate ($^{99m}TcO_4^-$) with N-methyl-S-methyl dithiocarbazate (H_2N-N(Me)C(=S)SMe) as

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nitride ion donor, in the presence of a reducing agent ($SnCl_2$. H_2O or PR_3/HCl), followed by addition of the ligand. The method has been successfully applied to a range of ligands including dithiocarbamates[2], xanthates[3] and dithiocarboxylates[4]. Among them, N-ethoxy-N-ethyl dithiocarbamate (NOET) induces a high myocardial uptake and the radiopharmaceutical [^{99m}TcN(NOET)$_2$] is currently under investigation as a heart imaging agent[5].

There is growing interest in blood cell labelling in order to study immune response mechanisms or visualize inflammatory lesions[6]. The method has been successfully applied for 10 years with ^{111}In-oxine and ^{99m}Tc-hexamethylpropyleneamine oxime (HMPAO). These well-known blood cell labelling agents suffer from several disadvantages and above all their lack of selectivity, which results in a necessary first separation step of the target blood cells before labelling. Current efforts focus on the design and preparation of more specific blood cell labelling agents. In our laboratory, we found that nitridotechnetium-99m complexes demonstrate promising results. The dithiocarbamate NOET ligand allows specific polynuclear cell labelling in whole blood *in vitro*[7] whereas dithiocarboxylates with a linear aliphatic chain induce a high lymphocyte selectivity under the same conditions[8].

In this paper, we report the synthesis and blood cell labelling capacities of nitrido ^{99m}Tc complexes [TcN(TAD)$_2$] with thioamides [$R^1C(=S)NHR^2$] = TAD-H. The NS coordination of the $[Tc{\equiv}N]^{2+}$ core has been previously reported at the macroscopic scale with various ligands[9].

EXPERIMENTAL

Materials

Toluene and tetrahydrofuran (THF) were distilled twice under nitrogen from sodium/benzophenone. Dichloromethane and diethyl ether were distilled from calcium chloride. Bromoalkanes, magnesium turnings, carbon disulfide, methyl iodide, primary amines, ammonia and dimethylformamide (DMF) were obtained from Aldrich (St Quentin Fallavier, France). Culture medium RPMI 1640 with L-glutamine was from OSI (78312 Maurepas Cédex, France). ^{99}Mo/^{99m}Tc generator (ELU III, CIS bio international, Gif sur Yvette, France) was purchased from Centre Eugène Marquis (Centre de Médecine Nucléaire, Rennes, France). The kits for nitridotechnetium-99m radiopharmaceutical preparation were kindly provided by Cis bio international. All other chemicals were of laboratory grade and used without further purification.

Thioamides 1-7 ; general procedure

Magnesium bromides were prepared in THF according to the previously described method[10,11]. Methyl dithioesters were synthesized from a Grignard reagent following the previously reported method[12, 13, 14]. Primary amines (4.5 mmol) were added to a solution of methyl ester (4.5 mmol) in dichloromethane (30 mL). The stirring was

continued for 24 hours before the solvent was removed under reduced pressure. Thioamides **1-7** were obtained in yields of 57 % to 100 %.

Thioamides 8 and 9 ; procedure

Ammonia (4.15 mmol; solution in water) was added to a solution of methyl dithioester (4.15 mmol) in a mixture of DMF/Toluene (50/50 : 30 mL). The stirring was continued for 48 hours before the solvent was removed under reduced pressure. The mixture was poured onto water, extracted with diethyl ether and the organic layer was washed with water, dried over $MgSO_4$ and the solvent removed under reduced pressure. Thioamides **8** and **9** were afforded in yields of 42 % and 45 % respectively.

Preparation of $^{99m}TcNL_2$ complexes

The reference radiopharmaceutical [$TcN(NOET)_2$] was prepared according to the kit method described earlier[15, 16]. The radiopharmaceuticals [$^{99m}TcN(TAD)_2$] were prepared using a freeze-dried formulation previously described[15, 16]. The lyophilized formulation was carried out as follows : Pertechnetate (0.4-0.8 GBq) was added to a vial containing 1.0 mg of S-methyl-N-methyl dithiocarbazate [H_2N-N(Me)C(=S)SMe], 0.1 mg of $SnCl_2.H_2O$ and 10 mg of 1,2-diaminopropane-N,N,N',N'-tetraacetic acid (DTPA) in a freeze-dried form (CIS bio international). The mixture was heated at 100°C for 15 min and then cooled at room temperature. 10 mg of thioamide [$R^1C(=S)N(H)R^2$, TAD-H] dissolved in 1 mL of carbonate buffer (pH=10.2, 0.05M) was finally added to the intermediate nitridotechnetium-99m obtained in the reaction medium and the solution heated at 80°C for 20 min. The radiochemical purity (RCP) of the products was characterized by thin layer chromatography (TLC) as the ratio of migrated radiactivity to total radioactivity. The complex migrated as a single radioactive spot near the solvent front [EtOH/Toluene/$CHCl_3$/aqueous ammonium acetate 0.5 M (6 :3 :3 :1)].

Analytical methods

Ligands and complexes

All prepared compounds were characterized by ^1H and ^{13}C NMR, recorded on a Bruker ARX 400 . Elemental analysis of the ligands was performed by I.C.S.N. on a Carlo Erba-1106 analyser as well as mass spectroscopy (E.I., 70 eV spectrometer Varian) (91198 Gif sur Yvette, France) for some representative ligands (see table 3).

All products were stored at −12°C under a nitrogen atmosphere (new ligands **1-5,8,9** are listed in table 1, phenylthioamides **6** and **7** are reported the literature[12-14]).

Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} aluminium backed plates and with a mixture of EtOH/toluene/$CHCl_3$/aqueous ammonium acetate 0.5 M (6 :3 :3 :1) as elueant. Areas of known R_f were cut and the radioactivity counted on a KONTRON GAMMAmatic counter.

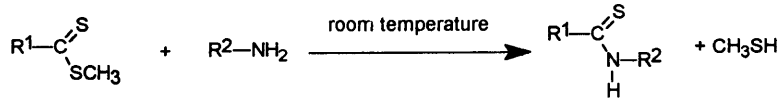
Blood cell labelling experiments

Whole blood from normal volunteers (2-3 mL) was labelled with 74 MBq (2 mCi) of [^{99m}TcN(TAD)$_2$] with gentle stirring for 10 min. The non-bonded radioactivity was eliminated by washing with a culture medium (2 x 10 mL RPMI, 10 min at 600 g). Bound and free radioactivities were determined by counting the pellet and the supernatants respectively in a CAPINTEC CRC 120 activimeter. The labelling yield was calculated as 100 x µCi pellet / (µCi pellet + µCi supernatants).

RESULTS AND DISCUSSION

Characterization

Thioamide ligands were synthesized at room temperature in CH_2Cl_2 (24h) for TAD-H **1-7** or DMF/Toluene (48h) for TAD-H **8** and **9**. The yield and R_f value are summarized in Table 1. Spectral data and elemental analysis/mass spectrometry are reported in Tables 2-3 respectively.



TAD-H : **1-9**

Table 1 : Ligand synthesis

TAD-H	R^1	R^2	$R_f(CH_2Cl_2)$	yield(%)
1	CH_3CH_2	CH_3CH_2	0.5	92
2	CH_3CH_2	$(CH_2)_7CH_3$	0.9	100
3	$CH_3(CH_2)_3$	$(CH_2)_3CH_3$	0.7	87
4	$CH_3(CH_2)_8$	CH_3	0.5	57
5	$CH_3(CH_2)_8$	$(CH_2)_3CH_3$	0.8	95
6	C_6H_5	CH_3	0.5	100
7	C_6H_5	$(CH_2)_3CH_3$	0.7	85
8	CH_3CH_2	H	0.2	42
9	$CH_3(CH_2)_8$	H	0.3	45

Table 2 : ^{1}H NMR and ^{13}C NMR of TAD-H : 1-9

TAD-H	^{1}H NMR [ppm, peak multiplicity, (number of hydrogens)][a), b)]		
1	CH_3:1.27,3(3H);1.30,3(3H)	CH_2:2.67,4(2H);3.69,4(2H)	
2	CH_3:0.88,3(3H);	CH_3+CH_2:1.33,m(13H)+CH_2:1.66,5(2H);2.67,4(2H);3.63,3(2H)	NH:7.40,1(1H)
3	CH_3:0.92,3(3H);0.95,3(3H)	CH_2:1.32, m(4H);1.63,6(2H);1.74,5(2H);2.64,3(2H);3.65,4(2H)	NH:7.46,1(1H)
4	CH_3:0.87,3(3H);3.17,1(3H)	CH_2:1.25,m(12H);1.76,5(2H);2.66,3(2H)	
5	CH_3:0.87,3(3H);0.95,3(3H)	CH_2:1.29,m(12H);1.40,6(2H);1.63,5(2H);1.76,5(2H);2.63,3(2H);3.66,5(2H)	
6	CH_3:3.30,1(3H)	Harom.:7.36,3(2H);7.45,3(1H);7.72,2(2H)	NH:7.90,(1H)
7	CH_3:1.00,3(3H)	CH_2:1.47,6(2H);1.75,5(2H);3.81,4(2H)	
		Harom.: 7.38,3(2H);7.46,3(1H);7.73,2(2H)	NH:7.64,1(1H)
8	CH_3:1.23,3(3H)	CH_2:2.62,4(2H)	NH_2:7.11,1(1H);7.88,1(1H)
9	CH_3:0.87,3(3H)	CH_2:1.26,m(12H);1.76,5(2H);2.65,3(2H)	NH_2::6.95,1(1H);7.68,1(1H)
		^{13}C NMR (ppm)[b)]	
1	CH_3 : 13.18 ;13.57	CH_2 : 40.04 ; 40.89	C=S : 206.34
2	CH_3 + CH_2 : 13.64 to 46.19 ($2CH_3$ + $8CH_2$)		C=S : 206.41
3	CH_3 + CH_2 : 13.66 to 47.07 ($2CH_3$ + $6CH_2$)		C=S : 205.47
4	CH_3 : 14.09 ; 46.91	CH_2 :22.66 to 32.81 ($8CH_2$)	C=S : 206.50
5	CH_3 + CH_2 : 13.77 to 47.43 ($2CH_3$ + $11CH_2$)		C=S : 205.52
6	CH_3 : 33.67	Carom :126.61 ; 128.45 ; 131.03 ; 145.55	C=S : 200.04
7	CH_3 : 21.45 CH_2 : 27.92 ; 37.82 ; 54.28	Carom : 134.23 ; 136.12 ; 138.60 ; 149.68	C=S : 206.76
8	CH_3 : 12.30	CH_2 : 37.39	C=S : 211.16
9	CH_3 : 14.12	CH_2 : 22.67 to 45.58 ($8CH_2$)	C=S : 211.27

[a] m = multiplet, [b] solvent = $CDCl_3$

The chemical identity of all the [^{99m}TcN(TAD)$_2$] products prepared at tracer level was determined from their chromatographic behaviour. The results are reported in table 4. All preparations are neutral and lipophilic complexes with a radiochemical purity (RCP) depending on the substituents of the thioamide ligand used.

Table 3 : Elemental analysis and mass spectrometry of ligands

TAD-H	Found values (%)			Calculated values (%)		
	C	H	N	C	H	N
1	50.91	9.42	27.12	51.02	9.47	27.31
2	65.52	11.51	15.61	65.62	11.52	15.89
3	62.37	11.05	8.08	62.42	11.07	8.15
4	65.22	11.44	15.45	65.62	11.52	15.89
5	68.82	11.87	13.39	69.08	12.02	13.15
6	63.54	6.00	9.26	63.64	6.03	9.32
7	68.35	7.82	7.25	68.44	7.83	7.32
8	40.42	7.91	15.71	40.48	7.91	15.79
9	64.11	11.30	7.48	64.21	11.32	7.53
			Mass spectroscopy			
TAD-H	Formula		Molecular weight	$M^{+\cdot}$ (Intensity)		$[M\text{-}SH]^{+}$ (Intensity)
1	$C_5H_{11}NS$		117.06122	117.0617 (100)		84.0 (63)
2	$C_{11}H_{23}NS$		201.15511	201.1548 (56)		168.0 (100)
4	$C_{11}H_{23}NS$		201.15511	201.1548 (3)		168.0 (67)
5	$C_{14}H_{23}NS$		243.20206	243.2019 (7)		210.0 (100)
9	$C_{10}H_{21}NS$		187.34944	no peak		154.1 (37)

Table 4 : Influence of the chemical nature of the [^{99m}TcN(TAD)$_2$] on blood cell labelling

TAD-H	R_f [a]	RCP (%) [b]	Labelling yield (± 2%)
1	0.3	57	21
2	0.7	57	19
3	1.0	44	10
4	1.0	41	5
5	1.0	73	15
6	0.9	63	13
7	1.0	66	8
8	0.6	72	3
9	1.0	30	4
NOET	0.9	95	60

(a) : TLC analysis on SiO_2 normal plates eluted with EtOH/Toluene/$CHCl_3$/aqueous ammonium acetate 0.5 M (6 :3 :3 :1)

(b) : 100 x µCi migrated radioactive spot/µCi sample

The RCP values of the complexes are satisfactory (between 60-70%) for the majority of the thioamide ligands TAD-H (table 4). However, lower RCPs (30% and 40%) are observed with a primary thioamide bearing a nonyl chain **9** and also for ligands **3** and **4**. Generally, we observe that the RCP is always lower than that of our reference compound [^{99m}TcN(NOET)$_2$]. A different reactivity of the NS ligands by comparison to S_2 ligands at the microscopic scale can explain the decreased results. Moreover, the kits for nitridotechnetium-99m radiopharmaceuticals for a total reaction between the intermediate complexes and thioamide ligands, were not optimized, but the migration of radioactive spots (as found by TLC analysis) is in good agreement with the formation of lipophilic entities ($0.3 < R_f < 1.0$). However, the new radiopharmaceuticals were tested *in vitro* in whole blood for leucocyte labelling.

Blood cell labelling

Bis(dithiocarbamato)nitridotechnetium-99m complexes [^{99m}TcN(S_2CNRR')$_2$] have been reported to label polynuclears in whole blood *in vitro* [7]. The choice of the ethyl and ethoxy substituant (R=Et, R'=OEt) gives the best results for this purpose. Recently we have shown that bis(dithiocarboxylato)nitridotechnetium-99m complexes [^{99m}TcN(S_2CR)$_2$] label specifically lymphocytes *in vitro* in whole blood when the dithiocarboxylate ligand has an aliphatic linear chain of 8 to 10 atoms of carbon (R= $CH_3(CH_2)_n$ $7 < n < 9$). The choice of thioamide ligand can take advantage of both, the presence of a nitrogen atom α to the thiocarbonyl moiety together with the large possibility of substituent variations to modulate the lipophilicity.

We have tried a great number of substituents but no thioamide ligands demonstrate an important leucocyte labelling in whole blood (< 21%). We have not observed selectivity or affinity for polynuclears/lymphocytes in contrast to dithiocarbamate or dithiocarboxylate ligands. The different coordination of the NS ligands may explain these results. Nevertheless, this study provided new information towards the better understanding of the origin of the selectivity between ^{99m}Tc-radiopharmaceuticals and white blood cells.

Following the poor results for the thioamide ligands, we are currently investigating a new series of ligands based on thiourea and hope to obtain new nitridotechnétium 99m complexes which provide significant labelling in whole blood.

CONCLUSION

This study has contributed towards the development of new nitridotechnetium-99m radiopharmaceuticals for leucocyte labelling in whole blood. Though the different hydrocarbonated parts and lipophilicities of thioamide ligands used, we have shown the satisfactory synthesis of bis-(thioamido) nitrido Tc-99m complexes but poor labelling yields are obtained because the structure of complexes offers no selectivity for leucocytes.

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