Brief Articles

Melanoma Uptake of ^{99m}Tc Complexes Containing the *N*-(2-Diethylaminoethyl)benzamide Structural Element†

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On the basis of the avid uptake of radioiodinated benzamides by melanoma cells, ^{99m}Tc complexes containing the structural elements of *N*-(dialkylaminoalkyl)benzamide pharmacophores have been synthesized and evaluated in vitro and in vivo for melanoma uptake. One of the complexes **Tc-12** containing the ligand 4-(*S*-benzoyl-2-thioacetyl-glycyl-glycylamido)-*N*-(2-diethylaminoethyl)benzamide (**11**) displayed the highest melanoma uptake. The 1-h melanoma uptake values and the corresponding blood counts indicate an interdependence of tumor uptake and bioavailability of the ^{99m}Tc complexes.

Introduction

Based on the melanoma-affinity characteristics of radioiodinated benzamides,[1] the development of ^{99m}Tc complexes containing structural elements of *N*-(dialkylaminoalkyl)benzamide as pharmacophores has been pursued. Thus, a series of '3 + 1' mixed-ligand [^{99m}Tc]-oxotechnetium complexes [TcO(SN(R)S)(SNX_2)] were synthesized and studied in B16 murine melanoma cells as well as in the C57Bl/6-B16 mouse melanoma model. In biodistribution studies with the compound in which R = Me and X = Bu, up to 5% ID/g and an average melanoma/nontarget tissue ratio (M/NTT) of 12.6 were obtained at 1 h postinjection.[2] When the [^{99m}Tc]oxotechnetium(V) complexes of tetradentate amine-amide-dithiol (AADT) chelates containing only tertiary amine substituents, AADT-CH_2-$[CH_2]_n$-NR_2 (n = 1, 2; R = Et, *n*-Bu), were tested, melanoma uptake of 7.6% ID/g (M/NTT 9.5) was obtained at 1 h after administration.[3] In other approaches the complete benzamide pharmacophore *N*-(2-diethylaminoethyl)benzamide was connected to bis(aminothiol) ligands (BAT), resulting in [^{99m}Tc]-oxotechnetium [TcO(BAT)] and [^{99m}Tc]nitridotechnetium [TcN(BAT)] complexes that displayed melanoma uptake between 0.43 and 1.51%ID/g (M/NTT 4.5 and 1.6) 1 h postinjection in the C57Bl/6-B16 mouse model[4] (see also Supporting Information).

Herein, we report the synthesis and in vivo evaluation in the C57Bl/6-B16 mouse melanoma model of ^{99m}Tc complexes formed from ligands containing the *N*-(2-diethylaminoethyl)benzamide structural element using (1) a chelate design that integrates the phenyl ring of the pharmacophore within the complex, (2) a bis-(aminothiol) ligand connected via a butylene spacer, and (3) a hydrazinonicotinamide ligand with tricine as ancillary coligand.

Results and Discussion

Chemistry. The syntheses of the four complexes **Tc-7**, **Tc-12**, **Tc-15**, and **Tc-19** were performed as outlined in Scheme 1. Ligand **6** was synthesized by *N*-BOC protection of commercially available 3,4-diaminobenzoic acid (**1**), using di-*tert*-butyl dicarbonate followed by conjugation with 2-(diethylamino)ethylamine using HATU as a coupling agent to give the *N*-BOC-protected 3,4-diamino-*N*-(2-diethylaminoethyl)benzamide (**3**). BOC was removed from **3** with 25% HCl. Then **4** was coupled with the *S*-benzoyl-protected thioglycolic acid (**5**)[5] using DCC as the coupling agent to afford ligand **6**. Ligand **11** was synthesized by activating **5** with *N*-hydroxysuccinimide (NHS) to give **9**, which was then conjugated to Gly-Gly in an aqueous CH_3CN solution at 90 °C to provide **10** in 93% yield. Equimolar amounts of **10** and 4-amino-*N*-(2-diethylaminoethyl)benzamide (**8**) were reacted with DCC to form **11**. Technetium-99m-labeled complexes **Tc-7** and **Tc-12** were obtained in 70% radiochemical yield by reduction of [^{99m}Tc]pertechnetate in the presence of ligand **6** and ligand **11**, respectively, with $SnCl_2$ at 80 °C for 10 min followed by HPLC purification.

Ligand **14** was synthesized by reacting the heterobifunctional chelator *N*-hydroxysuccinimide ester of 6-(4′-(4″-carboxyphenoxy)butyl)-2,10-dimercapto-2,10-dimethyl-4,8-diazaundecane (**13**), described previously,[6] with a 5-fold excess of 2-(diethylamino)ethylamine. Complexation of **14** with technetium-99m was performed with [^{99m}Tc]pertechnetate and $SnCl_2$ at ambient temperature

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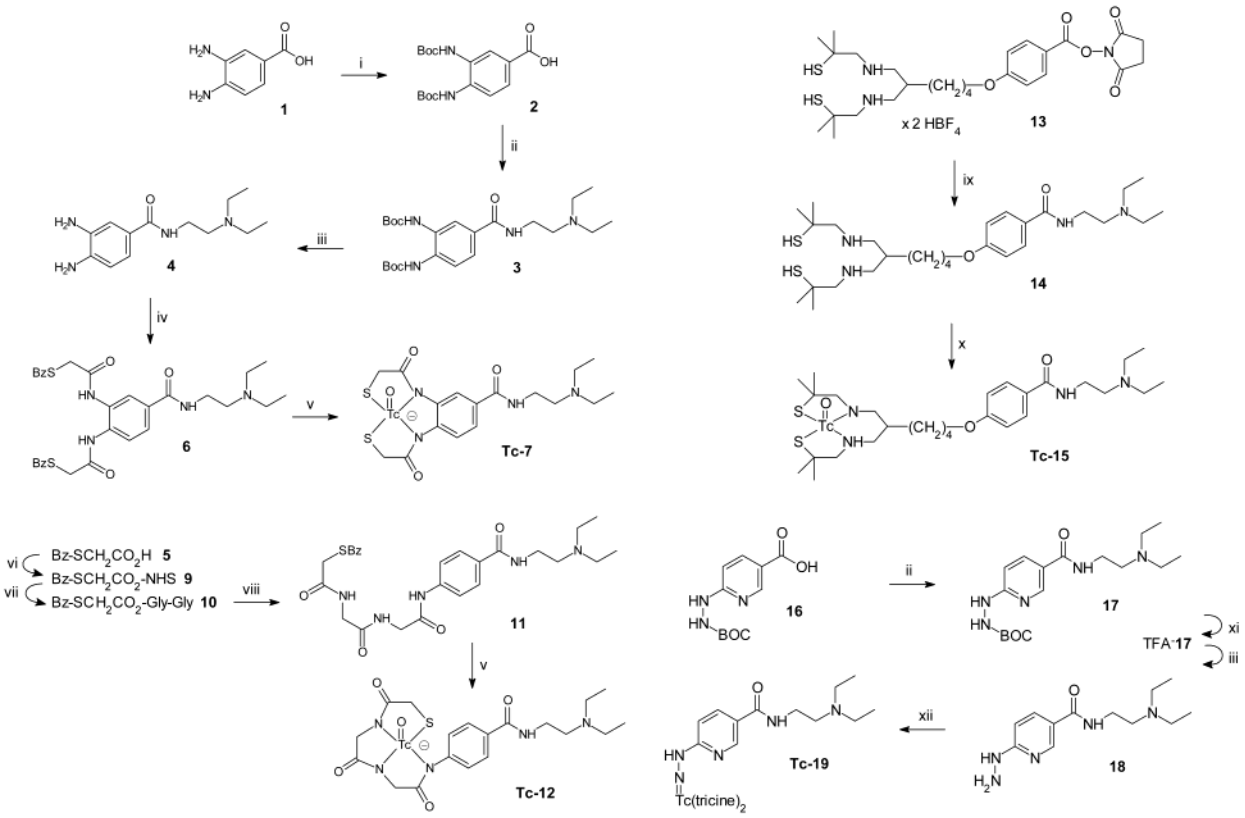
Scheme 1[a]



[a] Reaction conditions: (i) $(BOC)_2O$, DIPEA; (ii) 2-(diethylamino)ethylamine, HATU; (iii) 25% HCl_{aq}; (iv) *S*-benzoylthioglycolic acid **5**, DCC; (v) $Na^{99m}TcO_4$, 0.9% NaCl, tartaric acid, $SnCl_2$, 80 °C; (vi) NHS, DCC, 0 °C; (vii) GlyGly, H_2O, 90 °C; (viii) 4-amino-*N*-(2-diethylaminoethyl)benzamide **8**, DCC; (ix) 2-(diethylamino)ethylamine; (x) $Na^{99m}TcO_4$, 0.9% NaCl, tartaric acid, $SnCl_2$; (xi) TFA/thioanisol/H_2O (92/6/2); (xii) tricine and (x). If not stated otherwise the reactions were performed in DMF and at ambient temperature.

to yield **Tc-15** in 59% isolated radiochemical yield after HPLC purification.

Ligand **18** was prepared using a previously described 6-BOC-hydrazinopyridine-3-carboxylic acid (**16**) precursor, which was synthesized via the reaction of 6-hydrazinonicotinic acid with BOC anhydride.[7] The resulting compound **16** without further purification was coupled with 2-(diethylamino)ethylamine using HATU, affording the 6-hydrazinonicotinamide **17**. Unexpectedly BOC deprotection with TFA yielded almost exclusively the trifluoroacetylated product TFA-**17**. TFA cleavage was performed by overnight treatment of TFA-**17** with 25% HCl resulting in ligand **18** as an HCl salt (23% yield). Complexation of **18** was achieved by reduction of [^{99m}Tc]pertechnetate in the presence of the coligand tricine with $SnCl_2$ followed by complexation with **18** to give complex Tc-**19** in 75% radiochemical yield following HPLC purification.

While the tracer concentrations of the ^{99m}Tc complexes **Tc-7**, **Tc-12**, **Tc-15**, and **Tc-19** (Scheme 1) preclude macroscopic characterization, the proposed structures are in accordance with DADS and MAG_3 ^{99m}Tc complexes of similar ligands.[8,9] Similarly, the structure of the ^{99m}Tc complexes **Tc-15** and **Tc-19** are analogous to the neutral technetium complexes formed with the bis(aminoethanethiol)[10] and HYNIC[7] ligands.

Electrophoresis was employed to determine the net charge of the complexes at pH 7.4. At this physiological pH, the electrophoretic mobility of complex **Tc-7** and **Tc-12** was zero while that of complex **Tc-15** and **Tc-19** displayed migration of the spots toward the cathode. Control measurements with distinctly dianionic and monocationic complexes, [^{99m}Tc-MAG_3]$^{2-}$ and [^{99m}Tc-MIBI]$^+$, indicated migration of the radioactive spots toward the anode and cathode, respectively. The behavior of **Tc-7** and **Tc-12** complexes can be viewed as zwitterionic as the negative charge resulting from the complexation of ^{99m}Tc by the chelate is also accompanied by the protonation of the tertiary amino group at pH 7.4 resulting in a net neutral charge. The neutral complexes **Tc-15** and **Tc-19** become positively charged under these conditions and hence display migration toward the cathode.

The octanol−buffer partition coefficients for ^{99m}Tc complexes were measured at pH 7.4. With respect to log $D_{(pH\ 7.4)}$, the four complexes can be divided into two groups: (1) the negatively charged complexes **Tc-7** (log $D_{(pH\ 7.4)}$ 1.81 ± 0.01) and **Tc-12** (1.89 ± 0.01) with medium lipophilicity, and (2) the neutral **Tc-15** (2.53 ± 0.02) and **Tc-19** (2.68 ± 0.03) complexes exhibiting high lipophilicity. Although the log $D_{(pH\ 7.4)}$ of **Tc-7** and **Tc-12** was similar to values found for the radioiodinated benzamides,[11,12] there is no meaningful correlation of log $D_{(pH\ 7.4)}$ with melanoma accumulation in these compounds.

In Vitro Melanoma-Uptake Studies. Tumor-cell uptake of the ^{99m}Tc complexes was perfomed with cultured murine B16 melanoma cells which were also used for the in vivo studies. For comparison, cell uptake of [^{131}I]IMBA was determined as well. As shown in Figure 1a, two of the benzamide complexes, **Tc-12** and **Tc-15**, display high uptake which exceeded the [^{131}I]-

Table 1. Biodistribution of ^{99m}Tc-Complexes **Tc-7**, **Tc-12**, **Tc-15**, and **Tc-19** in C57Bl/6 Mice with B16 Melanoma

		mean tissue concentrations (%ID/g, $n = 3$)[a]										
compd	time (h, pi)	M/NTT[b]	melanoma	blood	heart	lungs	spleen	liver	kidneys	muscle	brain	adrenals
Tc-7	1	4.7	0.16 ± 0.01	0.26 ± 0.20	0.11	0.30	0.08	1.12	1.84	0.02	0.01	0.17
	6	11.0	0.09 ± 0.02	0.03 ± 0.01	0.02	0.05	0.04	0.39	0.39	0.00	0.00	0.04
Tc-12	1	2.6	3.42 ± 0.30	3.44 ± 0.38	1.55	1.96	1.30	1.55	11.90	0.90	0.52	1.22
	6	7.3	4.35 ± 0.92	3.20 ± 0.73	1.32	1.73	0.88	1.31	1.93	0.60	0.15	1.19
Tc-15	1	3.2	0.84 ± 0.17	0.55 ± 0.13	0.86	1.96	1.73	25.59	8.74	0.26	0.05	1.97
	6	5.2	0.45 ± 0.15	0.28 ± 0.12	0.33	0.67	0.46	6.65	4.92	0.07	0.02	1.36
Tc-19	1	5.4	1.24 ± 0.31	0.75 ± 0.20	0.48	0.97	0.25	3.11	2.84	0.28	0.05	0.89
	6	4.0	0.89 ± 0.26	0.68 ± 0.21	0.47	1.01	0.48	2.16	2.49	0.20	0.05	0.87

[a] To simplify the table, standard deviations are shown only for melanoma and blood data. [b] M/NTT: average melanoma/nontarget tissue ratio (excluding adrenals).

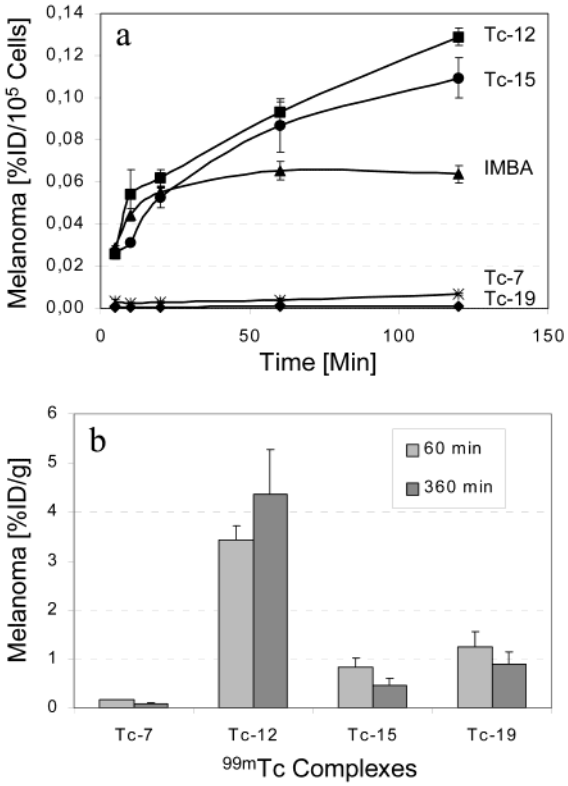


Figure 1. (a) In vitro **c**ell uptake of complexes **Tc-7**, **Tc-12**, **Tc-15, Tc-19**, and [^{131}I]IMBA in B16 melanoma cells. (b) In vivo melanoma uptake values of **Tc-7**, **Tc-12**, **Tc-15**, and **Tc-19**.

IMBA values, while **Tc-7** and **Tc-19** accumulated much less in melanoma cells.

With the exception of complex **Tc-15**, these results are in accordance with the melanoma uptake values obtained from biodistribution studies (Figure 1b). An explanation for the low in vivo melanoma uptake of **Tc-15** cannot be given.

Biodistribution Studies. The animal experiments were performed to assess organ uptake, target/nontarget ratios and pharmacokinetics of the ^{99m}Tc complexes, **Tc-7**, **Tc-12**, **Tc-15**, and **Tc-19**. Following HPLC separation, approximately 5 MBq of complex was intravenously injected into C57Bl/6 mice bearing subcutaneously transplanted B16 melanoma tumors. The animals were sacrificed 1 and 6 h after injection, and ^{99m}Tc activity in selected organs and tissues was analyzed. The biodistribution data are summarized in Table 1.

The complex **Tc-12** exhibited the highest melanoma uptake at both time points. As illustrated in Figure 1b, the 1-h uptake values of compounds **Tc-7**, **Tc-15**, and **Tc-19** were lower, with **Tc-7** showing the lowest value. In this context it is noteworthy that only the melanoma uptake of complex **Tc-12** displayed an increase in accumulation between 1 and 6 h while all other complexes washed out from the tumor. Prominent uptake in nontarget tissues was observed for **Tc-12** (kidneys) and **Tc-15** (kidneys, liver). Low values throughout all tissues were observed after injection of **Tc-7**, and this may be the result of its rapid excretion.

The differences in the biodistribution data reflect differences in the route of metabolic degradation and the rate of tissue and renal clearance of metabolites, which competes with melanoma uptake by reducing intravascular concentrations.[11] A correlation of the 1-h melanoma uptake values with the corresponding blood counts shows that the degree of melanoma uptake is proportional to the concentration of the ^{99m}Tc complex circulating in the blood. Figure 2 (Supporting Information) demonstrates a linear correlation ($r = 0.977$) of both parameters indicating that the melanoma uptake of these complexes is distinctly related to their bioavailability. A similar bioavailability relationship has recently been described in a series of radioiodinated benzamides.[11] The results obtained earlier with [^{99m}Tc]-oxotechnetium(V) complexes of tetradentate amine-amide-dithiol (AADT) chelates[3] also fit this bioavailability/melanoma uptake model, displaying significant correlation between the 1-h blood and melanoma values ($r = 0.951$). The '3 + 1' mixed-ligand [^{99m}Tc]oxotechnetium complexes,[2] however, do not follow this pattern, with the correlation points scattered.

In addition to the net accumulation of the ^{99m}Tc complex in melanoma tissue, another important parameter for an imaging agent is the melanoma/nontarget-tissue ratio (M/NTT), which is comprehensively expressed here as the average M/NTT of eight organs (adrenals were excluded because of their small size). Aside from **Tc-19 (**M/NTT = 5.4 and 4.0 at 1 and 6 h postinjection, respectively), all complexes displayed ratios that increased from 1 to 6 h postinjection indicating specific melanoma binding. Although the highest M/NTT ratio at 6 h was obtained with **Tc-7** (4.0 and 11.0), this ligand exhibits the lowest melanoma uptake. The M/NTT ratio for **Tc-12** (2.6 and 7.3) was the second highest, and this compound displayed the highest melanoma uptake value among these complexes, indicating more favorable characteristics as an imaging agent.

Conclusions

Four new ^{99m}Tc complexes derived from *N*-(2-diethylaminoethyl)benzamide have been evaluated in the

C57Bl/6-B16 mouse model. Two of theses complexes, **Tc-12** and **Tc-15**, showed high melanoma uptake in vitro. For complex **Tc-12** this result was confirmed in vivo with M/NTT ratios (imaging contrast) increasing with time. A comparison with earlier results indicates that the melanoma uptake values of **Tc-12** are (1) well above those obtained with ^{99m}TcO and ^{99m}TcN bis(aminothiol) complexes carrying the *N*-(2-diethylaminoethyl)benzamide pharmacophore,[4,13,14] (2) comparable with '3 + 1' mixed-ligand TcO complexes,[2] and (3) lower than the amine-amide-di-thiol (AADT) ^{99m}TcO complexes both of which contain dialkylamino-alkyl substituents.[3] Similar relationships were found for the melanoma/nontarget-tissue ratios with the exception of ^{99m}TcO-AADT complexes.[3] Especially at 6 h after injection ^{99m}TcO-AADT complexes have higher M/NTT ratios indicating faster excretion from background tissue. The in vitro and in vivo melanoma uptake of **Tc-12** also suggests that appropriate incorporation of the benzamide structure within the ^{99m}Tc-complexes can result in ^{99m}Tc-complexes that display significant melanoma uptake.

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Supporting Information Available: Experimental Section including syntheses, electrophoresis, determination of octanol−buffer partition coefficients, and biological investigations. This material is available free of charge via the Internet at http://pubs.acs.org.

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