Synthesis, Characterization, and Biological Evaluation of M(I)(CO)$_3$(NNO) Complexes (M = Re, ^{99m}Tc) Conjugated to 2-(4-Aminophenyl)benzothiazole as Potential Breast Cancer Radiopharmaceuticals

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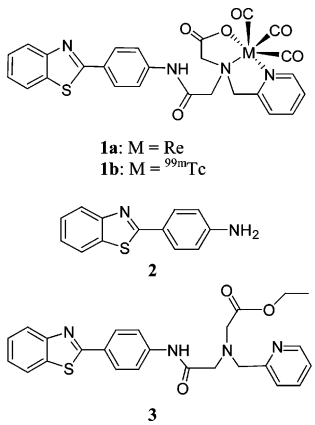
Abstract: The synthesis and biological evaluation of new M(I)(CO)$_3$(NNO) (M = Re, ^{99m}Tc) complexes attached to the antitumor agent 2-(4-aminophenyl)benzothiazole are reported. The fluorescent rhenium complex enters MCF-7 breast cancer cells but does not enter normal HFFF-2 and MRC-5 cells. The analogous radioactive ^{99m}Tc complex produces fast blood and soft tissue clearance when administered to healthy mice. These complexes are promising candidates for developing radiopharmaceuticals for imaging (^{99m}Tc) and targeted radiotherapy (^{186}Re, ^{188}Re) of breast cancer.

Breast cancer is the most frequent malignancy among women (31% in the U.S.[1] and 27% in Europe[2]) and one of the leading causes of cancer-related death. Early diagnosis and treatment remain the key to surviving breast cancer. While mammography is the most effective tool for screening and early detection of breast cancer in women,[3] its sensitivity is limited by dense breasts, the presence of implants and scars, and the use of estrogen replacement therapy. As a result, other noninvasive imaging techniques such as single-photon-emission computed tomography (SPECT), positron-emission tomography (PET), and magnetic resonance imaging (MRI) complement mammography in the characterization and staging of breast tumors, the evaluation of high-risk patients, the detection of axillary lymph node metastatic involvement and the assessment of tumor response to chemotherapy.[4,5]

The most widely used radionuclide for diagnostic imaging with SPECT is the metastable isotope of technetium, ^{99m}Tc, because of its favorable physical properties ($t_{1/2}$ = 6 h, E_γ = 140 keV), low cost, and widespread availability.[6] Three ^{99m}Tc complexes, ^{99m}Tc-sestamibi,[7] ^{99m}Tc-tetrofosmin,[8] and ^{99m}Tc-methylene diphosphonate,[9] are already employed in the clinical practice of breast cancer diagnosis as adjuncts to mammography, and research in this area is active aiming at improving sensitivity and specificity in the detection of breast tumors. The development of technetium complexes as potential radiopharmaceuticals is facilitated by the use of rhenium, the group VIIB congener of technetium. Rhenium, a mixture of the ^{185}Re and ^{187}Re isotopes, generally produces complexes with similar physical and biodistribution properties to those of technetium and is often used as a nonradioactive alternative to technetium for large-scale synthesis and structural characterization.[10] Furthermore, the β-emitting radioisotopes of rhenium,^{186}Re ($t_{1/2}$ = 3.8 days, E_{max} = 1.07 MeV) and ^{188}Re ($t_{1/2}$ = 0.7 day, E_{max} = 2.12 MeV), have been introduced in the field of nuclear medicine for radiotherapy applications aiming at the delivery of therapeutically significant radiation doses to malignant lesions without adversely affecting normal tissue.[11] Attempts toward the development of analogous complexes of ^{99m}Tc and ^{186}Re, ^{188}Re as "matched pairs" for cancer imaging and targeted radiotherapy have been reported in the literature.[12]

Chart 1



Within this context and aiming at the development of analogous technetium and rhenium complexes with affinity for breast tumors as potential target-specific breast cancer radiopharmaceuticals, we report herein the synthesis of new M(I)(CO)$_3$(NNO) complexes **1a** (M = Re) and **1b** (M = ^{99m}Tc) conjugated to the antitumor agent 2-(4-aminophenyl)benzothiazole (**2**, Chart 1).[13] Compound **2** elicits pronounced inhibitory activity in vitro on the nanomolar scale against a panel of human breast cancer cell lines with a biphasic dose response and is the lead compound in the production of a series of potent and selective antitumor benzothiazole agents that culminated in the identification of a clinical candidate, Phortress.[14]

The active molecule **2** was joined (through an acetyl group) to the ethyl ester of the metal chelator (*N*-(2-pyridylmethyl)-aminoacetic acid (**5**) to generate the appropriate ligand **3**, as outlined in Scheme 1.[15] Standard literature procedures were used in order to obtain **2** and **5**.[16] All synthesized products were characterized by NMR and IR spectroscopies and elemental analyses.

The synthesis of complex **1a**[15] was effected by ligand exchange reaction using the organometallic Re tricarbonyl precursor [NEt$_4$]$_2$[*fac*-Re(CO)$_3$(Br)$_3$].[17] Equimolar quantities of the precursor and ligand **3** were stirred under reflux for 3 h in acetonitrile to afford **1a** as a white precipitate in 80% yield. Basic hydrolysis of the ethyl ester group of **3** with aqueous NaOH preceded its reaction with the precursor. Complex **1a** was fully characterized by IR, NMR, elemental analysis, and mass spectrometry. Detailed ^{1}H and ^{13}C NMR chemical shift data in DMSO-d_6 at 25 °C are in Supporting Information.

Biological evaluation of complex **1a** was accomplished by cell uptake experiments monitored by fluorescence microscopy.[15] Complex **1a** can be used as a fluorescent probe because it absorbs light in the UV region with a peak absorbance at 332 nm and emits fluorescence with maximum intensity at 407 nm. Cellular entry of **1a** was tested in the MCF-7 human breast cancer cell line, which is particularly sensitive in **2**,[13] and in two human normal fibroblast cell lines, the fetal lung fibroblast,

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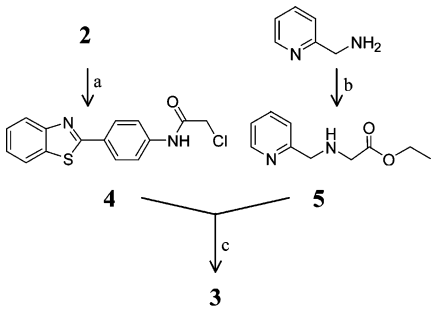
Scheme 1[a]



[a] Reagents and conditions: (a) NEt_3 (1.5 equiv), dry CH_2Cl_2, 0 °C, chloroacetyl chloride (1.1 equiv), under nitrogen, 0 °C, 30 min, followed by room temp, 60 min, yield 40%; (b) $BrCH_2COOEt$ (1 equiv), dry THF, 0 °C, 30 min, followed by room temp, 48 h, yield 75%; (c) **4** (1 equiv), **5** (1.1 equiv), Hunig's base (1.5 equiv), CH_3CN, 80 °C, 24 h, yield 56%.

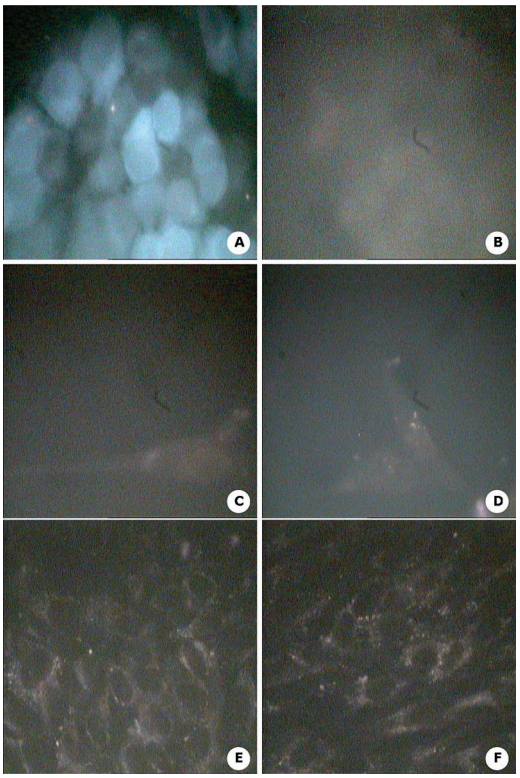


Figure 1. Fluorescence microscopy photos showing the selective cellular entrance of complex **1a** into human breast cancer cells (MCF-7) and not into healthy human foreskin (HFFF-2) and healthy human lung (MRC-5) fibroblast cell lines along with the control experiment for each cell line: (A) MCF-7 cells after 24 h of exposure to 100 μM **1a**; (B) MCF-7 cells cultivated in growth medium free of **1a**; (C) HFFF-2 cells after 24 h of exposure to 100 μM **1a**; **(**D) HFFF-2 cells cultivated in growth medium free of **1a**; (E) MRC-5 cells after 24 h of exposure to 100 μM **1a**; (F) MRC-5 cells cultivated in growth medium free of **1a**.

MRC-5, and fetal foreskin fibroblast, HFFF-2. After a 24 h incubation, satisfactory cellular entry of **1a** was evident (for concentrations of **1a** ranging from 100 to 20 μM)[15] in the MCF-7 breast cancer cells but not in the HFFF-2 or MRC-5 normal cells (Figure 1).

The stability of **1a** in cell culture supernatant at 37 °C was checked by NMR to ensure that the fluorescent signal is due to complex **1a** and not to a product of its potential decomposition.[15] No peaks were present in the NMR spectra that could be attributed to any decomposition products.

The corresponding ^{99m}Tc complex **1b** was prepared at tracer level by ligand exchange reaction[15] employing the precursor

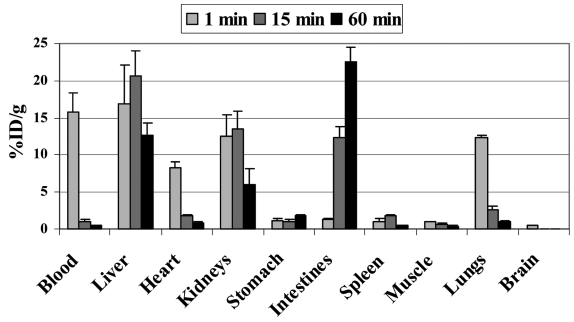


Figure 2. Summary of the biodistribution data (% ID/g) of the ^{99m}Tc complex **1b** in healthy mice.

fac-$[^{99m}Tc(CO)_3(H_2O)_3]^+$.[18] The concentration of ligand **3** was approximately 10^{-4} M. The mixture was analyzed by reversed-phase HPLC, and the analysis demonstrated that a single complex was formed (radiochemical yield of >90%) that was stable for at least 2 half-lives of the isotope (12 h). The identity of the complex was established by comparative HPLC studies using a sample of the well-characterized complex **1a** as reference. When samples of the complexes **1a** and **1b** were co-injected on a reversed-phase HPLC column, they eluted with practically identical retention times as witnessed by simultaneous UV (**1a**) and radioactivity (**1b**) detection, revealing their structural analogy.[15] Complex **1b** was stable against the histidine and cysteine challenge.[19,15]

In vivo biological evaluation of complex **1b** was effected by biodistribution studies in Swiss albino mice following intravenous administration of the HPLC purified complex (1−2 μCi).[15] The tissue biodistribution data shown in Figure 2 demonstrate rapid blood and soft tissue clearance. The low stomach and spleen values are indicative of the lack of in vivo oxidation of technetium to pertechnetate or Tc colloid. The radioactivity was mainly excreted via the hepatobiliary system while tissues in the thoracic cavity remained practically free of radioactivity, leaving a clear background suitable for a possible application in breast cancer imaging.

In conclusion, the isostructural Re and ^{99m}Tc complexes **1a** and **1b** are amenable to fluorescent and radioimaging studies and offer the opportunity of directly correlating their in vitro and in vivo biological behavior.[20] Their preliminary evaluation data show selective uptake of **1a** by the MCF-7 breast cancer cells and no specific tissue accumulation of **1b** in healthy mice. These results are encouraging for further exploration of their properties toward the development of ^{99m}Tc, ^{186}Re, and ^{188}Re complexes for breast imaging and therapy. Determination of the cytotoxicity of **1a**, quantitative assessment of the uptake of **1b** into MCF-7 cells, and biodistribution study of **1b** in tumor bearing mice are currently in progress.

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Supporting Information Available: Synthetic procedures for **1a**,**b** and **2**−**5**, ^{1}H and ^{13}C NMR assignments for **1a** and **2**−**5**, procedures for stability tests of **1a** and **1b**, cell uptake experiments of **1a**, and biodistribution of **1b** in healthy mice. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) The American Cancer Society Web page: Cancer Statistics 2006. http://www.cancer.org/downloads/STT/Cancer_Statistics_2006_Presentation.ppt#4.

(2) Boyle P.; Ferlay J. Cancer Incidence and Mortality in Europe, 2004. *Ann. Oncol.* **2005**, *16*, 481−488.
(3) The American Cancer Society Web page: Breast Cancer Facts & Figures 2005−2006. http://www.cancer.org/downloads/STT/CAFF2005BrF.pdf.
(4) (a) Benard, F.; Turcotte, E. Imaging in Breast Cancer: Single-Photon Computed Tomography and Positron-Emission Tomography. *Breast Cancer Res.* **2005**, *7*, 153−162. (b) Schillaci, O.; Buscombe, J. R. Breast Scintigraphy Today: Indications and Limitations. *Eur. J. Nucl. Med. Mol. Imaging* **2004**, *31*, S35−45.
(5) Schnall, M. D.; Orel, S. G. Breast MRI. *Breast Dis.* **1998**, *10*, 97−111.
(6) Jurisson, S. S.; Lydon, J. D. Potential Technetium Small Molecule Radiopharmaceuticals. *Chem. Rev.* **1999**, *99*, 2205−2218.
(7) JurKhalkhali, I.; Mena, I.; Diggles, L. Review of Imaging Techniques for the Diagnosis of Breast Cancer: A New Role for Prone Scintimammography, Using Technetium-99m Sestamibi. *Eur. J. Nucl. Med.* **1994**, *21*, 357−362.
(8) Rambaldi, P. F.; Mansi, L.; Procaccini, E.; Di-Gregorio, F.; Del-Vecchio, E. Breast Cancer Detection with Tc-99m Tetrofosmin. *Clin. Nucl. Med.* **1995**, *20*, 703−705.
(9) Turhal, N. S.; Dane, F.; Dede, F.; Gumus, M.; Yumuk, P. F.; Cakalagaoglu, F.; Savci, D.; Karaman, M.; Gulluoglu, B.; Turoglu, H. T. 99m-Tc-MDP-Scintimammography in the Evaluation of Breast Masses or Tumor Angiogenesis. *Anticancer Res.* **2004**, *24*, 1999−2006.
(10) (a) Deutsch, E.; Libson, K.; Vanderheyden, J.-L.; Ketring, A. R.; Maxon, H. R. The Chemistry of Rhenium and Technetium as Related to the Use of Isotopes of These Elements in Therapeutic and Diagnostic Nuclear-Medicine. *Nucl. Med. Biol.* **1986**, *13*, 465−477. (b) Dilworth, J.; Parrot, S. The Biomedical Chemistry of Technetium and Rhenium. *Chem. Soc. Rev.* **1998**, *27*, 43−55.
(11) (a) Hashimoto, K.; Yoshihara, K. Rhenium Complexes Labelled with Re-186, Re-188 for Nuclear Medicine. *Top. Curr. Chem.* **1996**, *176*, 275−291. (b) Schubiger, P. A.; Alberto, R.; Smith, A. Vehicles, Chelators, and Radionuclides: Choosing the "Building Blocks" of an Effective Therapeutic Radioimmunoconjugate. *Bioconjugate Chem.* **1996**, *7*, 165−179.
(12) (a) Zamora, P. O.; Gulhke, S.; Bender, H.; Diekmann, D.; Rhodes, B. A.; Biersack, H. J.; Knapp, F. F. Experimental Radiotherapy of Receptor-Positive Human Prostate Adenocarcinoma with Re-188-RC-160, a Directly-Radiolabeled Somatostatin Analogue. *Int. J. Cancer* **1996**, *65*, 214−220. (b) Zamora, P. O.; Marek, M. J.; Knapp, F. F. Preparation of Re-188-RC-160 Somatostatin Analog: A Peptide for Local/Regional Radiotherapy. *Appl. Radiat. Isot.* **1997**, *48*, 305−309. (c) Safavy, A.; Khazaeli, M. B.; Qin, H.; Buchsbaum, D. J. Synthesis of Bombesin Analogues for Radiolabeling with Rhenium-188. *Cancer* **1997**, *80*, 2354−2359. (d) Volkert, W. A.; Hoffman, T. J. Therapeutic Radiopharmaceuticals. *Chem. Rev.* **1999**, *99*, 2269−2292. (e) Heeg, M. J.; Jurisson, S. S. The Role of Inorganic Chemistry in the Development of Radiometal Agents for Cancer Therapy. *Acc. Chem. Res.* **1999**, *32*, 1053−1060. (f) Heppeler, A.; Froidevaux, S.; Eberle, A. N.; Maecke, H. R. Receptor Targeting for Tumor Localization and Therapy with Radiopeptides. *Curr. Med. Chem.* **2000**, *7*, 971−994. (g) Gali, H.; Hoffman, T. J.; Sieckman, G. L.; Owen, N. K.; Katti, K. V.; Volkert, W. A. Synthesis, Characterization, and Labelling with Tc-99m/Re-188 of Peptide Conjugates Containing a Dithia-Bisphosphine Chelating Agent. *Bioconjugate Chem.* **2001**, *12*, 354−363. (h) Miao, Y.; Owen, N. K.; Whitener, D.; Gallazzi, F.; Hoffman, T. J.; Quinn, T. P. In Vivo Evaluation of (188)Re-Labeled Alpha-Melanocyte Stimulating Hormone Peptide Analogs for Melanoma Therapy. *Int. J. Cancer.* **2002**, *101*, 480−487. (i) Miao Y.; Owen, N. K.; Fisher, D. R.; Hoffman, T. J.; Quinn, T. P. Therapeutic Efficacy of a Re-188-Labelled Alpha-Melanocyte-Stimulating Hormone Peptide Analog in Murine and Human Melanoma-Bearing Mouse Models. *J. Nucl. Med.* **2005**, *46*, 121−129.
(13) (a) Shi, D.-F.; Bradshaw, T.; Wrigley, S.; McCall, C. J.; Lelieveld, P.; Fichtner, I.; Stevens, M. F. G. Antitumor Benzothiazoles. 3. Synthesis of 2-(4-Aminophenyl)benzothiazoles and Evaluation of Their Activities against Breast Cancer Cell Lines in Vitro. *J. Med. Chem.* **1996**, *39*, 3375−3384. (b) Bradshaw, T. D.; Wrigley, S.; Shi, D.-F.; Schultz, R. J.; Paull, K. D.; Stevens, M. F. G. 2-(4-Aminophenyl)benzothiazoles: Novel Agents with Selective Profiles of in Vitro Anti-Tumour Activity. *Br. J. Cancer* **1998**, *77*, 745−752.
(14) (a) Bradshaw, T. D.; Stevens, M. F. G.; Westwell, A. D. Discovery of the Potent and Selective Antitumour Agent 2-(4-Amino-3-methylphenyl)benzothiazole (DF 203) and Related Compounds. *Curr. Med. Chem* **2001**, *8*, 203−210. (b) Bradshaw, T. D.; Westwell, A. D. The Development of the Antitumor Benzothiazole Prodrug, Phortress, as a Clinical Candidate. *Curr. Med. Chem.* **2004**, *11*, 1009−1021.
(15) For further information, see Supporting Information.
(16) (a) Phoon, C. W.; Ng, P. Y.; Ting, A. E.; Yeo, S. L.; Sim, M. M. Biological Evaluation of Hepatitis C Virus Helicase Inhibitors. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1647−1650. (b) Policar, C.; Lambert, F.; Cesario, M.; Morgenstern-Badarau, I. An Inorganic Helix [Mn(IPG)(MeOH)]n[PF6]n: Structural and Magnetic Properties of a syn−anti Carboxylate-Bridged Manganese(II) Chain Involving a Tetradentate Ligand. *Eur. J. Inorg. Chem.* **1999**, *12*, 2201−2207.
(17) Alberto, R.; Egli, A.; Abram, U.; Hegetschweiler, K.; Gramlich, V.; Schubiger, P. A. Synthesis and Reactivity of $[NEt_4]_2[ReBr_3(CO)_3]$. Formation and Structural Characterization of the Clusters $[NEt_4][Re_3(\mu\text{-}3\text{-}OH)(\mu\text{-}OH)_3(CO)_9]$ and $[NEt_4][Re_2(\mu\text{-}OH)_3(CO)_6]$ by Alkaline Titration. *J. Chem. Soc., Dalton Trans.* **1994**, *19*, 2815−2820.
(18) Alberto, R.; Schibli, R.; Egli, A.; Schubiger, A. P.; Abram, U.; Kaden, T. A. A Novel Organometallic Aqua Complex of Technetium for the Labeling of Biomolecules: Synthesis of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ from $[^{99m}TcO_4]^-$ in Aqueous Solution and Its Reaction with a Bifunctional Ligand. *J. Am. Chem. Soc.* **1998**, *120*, 7987−7988.
(19) Schibli, R.; La Bella, R.; Alberto, R.; Garcia-Garayoa, E.; Ortner, K.; Abram, U.; Schubiger, P. A. Influence of the Denticity of Ligand Systems on the in Vitro and in Vivo Behavior of ^{99m}Tc(I)-Tricarbonyl Complexes: A Hint for the Future Functionalization of Biomolecules. *Bioconjugate Chem.* **2000**, *11*, 345−351.
(20) (a) Stephenson, K. A.; Banerjee, S. R.; Besanger, T.; Sogbein, O. O.; Levadala, M. K.; McFarlane, N.; Lemon, J. A.; Boreham, D. R.; Maresca, K. P.; Brennan, J. D.; Babich, J. W.; Zubieta, J.; Valliant, J. F. Bridging the Gap between in Vitro and in Vivo Imaging: Isostructural Re and ^{99m}Tc Complexes for Correlating Fluorescence and Radioimaging Studies. *J. Am. Chem. Soc.* **2004**, *126*, 8598−8599. (b) James, S.; Maresca, K.; Babich, J.; Valliant, J.; Doering, L.; Zubieta, J. Isostructural Re and ^{99m}Tc Complexes of Biotin Derivatives for Fluorescence and Radioimaging Studies. *Bioconjugate Chem.* **2006**, *17*, 590−596.

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