Synthesis and evaluation of intercalating somatostatin receptor binding peptide conjugates for endoradiotherapy

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Dedicated to the memory of Prof. Antoine (Tony) A. Noujaim, in recognition of his outstanding contributions to radiopharmacy, diagnostic oncology and the immunotherapy of cancer.

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ABSTRACT – Purpose: Intercalators, planar aromatic compounds, are able to interact with DNA by sandwiching themselves between the stacked bases at right angles to the long axis of the helix. Under certain circumstances, Auger-electron-emitting radionuclides can be extremely radiotoxic and produce extensive DNA damage. Auger electron-emitting radioisotopes, are known to be highly cytotoxic when localized in cell nuclei due to highly localized energy deposition by low energy Auger electrons. In addition binding to the DNA might increase the retention in the receptor expressing tissues. **Methods:** In order to exploit the cytotoxic potential of intercalator-Auger-emitter conjugates, bis-benzimidazole dyes, Hoechst 33258 and 33342, were linked to a somatostatin receptor affine carrier peptide. For this purpose a bis-benzimidazole intercalating moiety was prepared using variations on the literature methods. The intercalating moieties were coupled under normal SPPS conditions to the carrier peptide, Tyr^3-octreotate. To attach the chelating agent (DOTA) to the intercalating moiety, a free amine derivative was prepared and coupled in solution to DOTA tris-*t*-butyl ester. The resulting chelator-intercalator conjugate was then coupled to a Tyr^3-octreotate carrying resin using SPPS. **Results:** The peptide conjugates were obtained in good yields after HPLC chromatography. The cellular uptake of the novel conjugates was determined using fluorescence microscopy. All intercalator-peptide conjugates revealed somatostatin receptor binding affinities in the nanomolar range. **Conclusions:** The novel chelator-intercalator derivatives of the somatostatin receptor binding Tyr^3-octreotate introduce a new scope to the range of tracers for therapeutic purposes.

INTRODUCTION

Small radiolabeled peptides have been proven as important tools in tumor diagnostic and endoradiotherapy for more than one decade. By specifically binding to receptors or other structures expressed on the surface of tumor cells, peptides are able to shuttle radionuclides to these tissues. Somatostatin receptor binding peptides labeled with a variety of gamma-, positron- and beta-emitters have been the prototypes for those constructs, as these receptors are frequently overexpressed in certain tumor types, such as neuroendocrine tumors and their metastases. When conjugated to 'therapeutic' nuclides such as ^{90}Y, somatostatin receptor binding peptides have been shown to be successful for the therapy of receptor expressing tumors. The most successful radiotherapeutic is ^{90}Y-DOTATOC. However, the therapeutic value of this treatment is limited by the side effects in non-target tissues such as the kidneys. The effectiveness of the treatment modality might be further increased with conjugates which enable the nuclear targeting of the low energy emitting radioactive isotopes. Intercalators, planar aromatic compounds, are able to interact with DNA by sandwiching themselves between the stacked bases at right angles to the long axis of the helix. The bis-benzimidazole dyes, Hoechst 33258 and 33342, have been shown to bind to the minor groove of DNA in A-T rich regions. The insertion of an intercalator changes the DNA conformation (1). Intercalators have thus thoroughly been examined with respect to their DNA-cleaving activity for anticancer therapy (2).

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Under certain circumstances, Auger-electron-emitting radionuclides can be extremely radiotoxic and produce extensive DNA damage. The degree of damage appears to depend upon the location of the decaying atom. Consequently, Auger electron-emitting radioisotopes, such as ^{195m}Pt, ^{114m}In, ^{111}In, ^{99m}Tc, ^{67}Ga and ^{51}Cr are known to be highly cytotoxic when localized in cell nuclei due to highly localized energy deposition by low energy Auger electrons (3). In addition, binding to the DNA might increase the retention in the receptor expressing tissues.

The cytotoxic capability of radioisotopes is determined by their tissue specific accumulation. Due to the necessity of the nuclear localization the prerequisites are yet higher in the case of Auger emitters. Consequently the combination of somatostatin receptor binding peptides that provide tumor specificity and internalization with intercalators that provides the nuclear localization of chelator-bound Auger emitters presents a novel strategy for the development of endoradio-therapeutic drugs (4). Recently, somatostatin receptor binding peptide conjugates that comprise an nuclear localization sequence and Auger emitting isotopes have been described (5). In the following, we present the multi step syntheses of novel intercalating radiotherapeutics and the evaluation of their somatostatin receptor binding affinities on rat cortex membranes.

EXPERIMENTAL SECTION

General comments

All reagents (Aldrich, Fluka, Lancaster, Merck, NovaBiochem, Macrocyclics Inc.) and solvents were of standard quality and used without further purification unless indicated. NMR spectra were recorded on a Bruker 250 MHz spectrometer with tetramethylsilane (^{1}H and ^{13}C) as internal standard.

Chemistry

A general scheme for the synthesis of substituted bis-benzimidazoles is presented in Figure 1.

2-(4-Cyanophenyl)benzimidazole-5-carboxylic acid (1)

To a suspension of 4-formylbenzonitrile (5.05 g, 38.5 mmol) and 3,4-diaminobenzoic acid (5.86 g, 38.5 mmol) in EtOH (250 mL) a solution of $Na_2S_2O_5$ (3.90 g, 20.5 mmol) in H_2O (50 mL) was added. The resulting suspension was heated under reflux conditions for 16 h. The reaction mixture was allowed to cool to RT, H_2O (100 mL) was added, and the reaction was left standing for 30 min. The solid was collected by filtration. The solid was washed with water (50 mL) and Et_2O (2 × 50 mL) and dried in vacuo for 20 h to give a brown powdery solid (10.11 g, 99.8%).

ESI: m/z = 262.1 ($[M-H]^-$)

^{1}H-NMR, 250 MHz, DMSO-D6: δ = 12.99 (s, broad signal, COOH or NH), 8.34 (d, 2H, H2'/H6'; *j* = 7.85 Hz), 8.2 (s, 1H, H4), 8.1 (d, 2H, H3'/H5'; *j* = 7.85 Hz), 7.8 (d, 1H, H6; *j* = 4.6 Hz), 7.6 (d, 1H, H7; *j* = 4.6 Hz)

^{13}C-NMR, 62 MHz, DMSO-D6: δ = 167.6 (COOH), 162.2 ($C3_a$), 151.6 (C2), 144.2 (C1'), 135.2 ($C7_a$), 133.7 (C5), 132.9 (C3', C5'), 129.7 (C6), 125.3 (C2', C6'), 124.0 (CN), 123.2 (C4), 118.4 (C7), 112.4 (C4')

Methyl N^3-(2-[4-cyanophenyl]benzimidazole-5-carbonyl)-3,4-diaminobenzoate (2)

To a stirred suspension of **(1)** (1.003 g, 3.67 mmol) in DMF (25 mL) HBTU (1.392 g, 3.67 mmol) was added, followed by DIPEA (0.712 g, 959 µl, 5.5 mmol). The solution was stirred for 20 minutes at RT and then methyl 3,4-diaminobenzoate (0.610 g, 3.67 mmol) was added. After 1 h a precipitate was observed. The reaction was stirred for another 24 h, poured into H_2O (80 mL), and the precipitate was collected. The solid was washed with Et_2O (2 × 25 mL) and dried in vacuo for 24 h to give **(2)** (1.412 g, 93.5%). ESI: m/z = 412.2 ($[M+H]^+$)

^{1}H-NMR, 250 MHz, DMSO-D6: δ = 12.15 (s, 4H, broad signal, NH), 8.35 (d, 2H, H2'/H6'; *j* = 8.3 Hz), 8.15 (s, 1H, H4), 8.05 (d, 2H, H3',H5'; *j* = 8.42 Hz), 7.85 (d, 2H, H6, H3a; *j* = 6.75 Hz), 6.84 (m, 2H, H6a/H5a), 3.92 (s, 3H, -OCH_3)

^{13}C-NMR, 62 MHz, DMSO-D6: δ = 166.8 ($COOCH_3$), 166.2 (C=O), 148.3 (C2a), 144.0 (C2), 128.7 ($C3_a$), 128.2 ($C7_a$), 123.2 (C6), 123.3 (C1a), 122.8 (CN), 122.3 (C4a), 122.2 (C5), 116.8 (C4'), 116.3 (C2', C6'), 114.6 (C6a), 114.4 (C4, C7, C5a), 112.4 (C3', C5'), 55.3 ($COOCH_3$)

Methyl 2-(2-[4-cyanophenyl]benzimidazol-5-yl)-benz-imidazole-5-carboxylate (3)

A solution of **(2)** (1.022 g, 2.6 mmol) in glacial AcOH (3 mL) was heated at 120 °C for 4 h. After 30 mins a tan solid precipitate was observed. The reaction was diluted with AcOH (30 mL) and filtered. The brown solid was washed with glacial

AcOH (2 × 30 mL), Et_2O (2 × 30 mL) and dried in vacuo for 16 h to give **(3)** (0.895 g, 87.2%).
ESI: m/z = 394.2 ($[M+H]^+$)
^{1}H-NMR, 250 MHz, DMSO-D6: δ = 12.15 (s, 2H, broad signal, NH), 8.43 (s, 1H, H4b), 8.43 (d, 2H, H2'/H6'; *j* = 7.93 Hz), 8.11 (s, 1H, H4), 8.01 (d, 2H, H3'/H5'; *j* = 7.97 Hz), 7.75 (d, 2H, H6, H6b; *j* = 8.45 Hz), 7.64 (d, 2H, H7, H7b; *j* = 8.20 Hz) , 3.92 (s, 3H, -OCH_3)
^{13}C-NMR, 62 MHz, DMSO-D6: δ = 173.8 ($COOCH_3$), 165.7 (C1'), 150.8 (C2), 148.7 (C2b), 136.2 (C4'), 1343.2 ($C3_a$, $C3b_a$), 132.6 (C2', C6'), 127.4 (C3', C5'), 127.3 (C5), 125.2 (C6, C6b), 123.0 (C7, C7b), 122.8 ($C7_a$, $C7b_a$), 120.4 (CN), 118.4 (C6b), 114.4 (C4, C4b), 55.3 ($COOCH_3$)

2-(2-[4-cyanophenyl]benzimidazol-5-yl)benzimidazole-5-carboxylic acid (4)

A solution of **(3)** (0.428 g, 0.86 mmol) in 1M NaOH (20 mL) and MeOH (25 mL) was heated under reflux conditions for 2 h. Allowed to cool and concentrated to a third of the volume. The mixture was diluted with H_2O (50 mL) and extracted with EtOAc (50 mL). The aqueous layer was acidified with 1M $HCl_{(aq)}$ to pH 4, extracted with *n*BuOH (2 × 70 mL), washed with saturated $NaCl_{(aq)}$, dried over Na_2SO_4 and concentrated. The solid was subjected to column chromatography on silica gel ($CHCl_3$:MeOH:Et_3N, 7:2:1) to give a yellow solid (0.416 g, 92.2%).
ESI: m/z = 380.1 ($[M+H]^+$)
^{1}H-NMR, 250 MHz, DMSO-D6: δ = 12.15 (s, 2H, broad signal, NH), 8.43 (s, 1H, H4b), 8.43 (d, 2H, H2'/H6'; *j* = 7.93 Hz), 8.11 (s, 1H, H4), 8.01 (d, 2H, H3'/H5'; *j* = 7.97 Hz), 7.75 (d, 2H, H6, H6b; *j* = 8.45 Hz), 7.64 (d, 2H, H7, H7b; *j* = 8.20 Hz)
^{13}C-NMR, 62 MHz, DMSO-D6: δ = 173.8 (COOH), 165.7 (C1'), 150.8 (C2), 148.7 (C2b), 136.2 (C4'), 1343.2 ($C3_a$, $C3b_a$), 132.6 (C2', C6'), 127.4 (C3', C5'), 127.3 (C5), 125.2 (C6, C6b), 123.0 (C7, C7b), 122.8 ($C7_a$, $C7b_a$), 120.4 (CN), 118.4 (C6b), 114.4 (C4, C4b)

2-(2-[4-(tert-butoxyaminomethyl)phenyl]benzimidazol-5-yl)benzimidazole-5-carboxylic acid (5)

To a stirred solution of **(2)** (0.413 g, 1.05 mmol) in DMF (10 mL) and DIPEA (2 mL) Boc_2O (0.251 g, 265 µl, 1.15 mmol) was added followed by 10% palladium on charcoal (spatula end). The reaction vessel was flushed with H_2 three times and stirred at room temperature for 48 h. The solution was filtered and evaporated to dryness. The residue was subjected to silica gel chromatography (CH_2Cl_2:MeOH:Et_3N, 90:5:5) to give a powdery solid (0.364 g, 69.8%)
ESI: m/z = 484.3 ($[M+H]^+$)
^{1}H-NMR, 250 MHz, DMSO-D6: δ = 12.15 (s, 2H, broad signal, NH), 8.43 (s, 1H, H4b), 8.13 (d, 2H, H2'/H6'; *j* = 7.93 Hz), 8.11 (s, 1H, H4), 7.81 (d, 2H, H3'/H5'; *j* = 8.45 Hz), 7.75 (d, 2H, H6, H6b; *j* = 8.48 Hz), 7.67 (d, 2H, H7, H7b; *j* = 8.45 Hz), 4.23 (d, 2H, -CH_2; *j* = 5.94 Hz), 1.4 (s, 9H, Boc)
^{13}C-NMR, 62 MHz, DMSO-D6: δ = 166.8 (COOH), 158.3 (C=O), 140.8 (C2), 138.7 (C2b), 136.2 (C4'), 135.7 (C1'), 1343.2 ($C3_a$, $C3b_a$), 132.6 (C2', C6'), 127.4 (C3', C5'), 127.3 (C5), 125.2 (C6, C6b), 123.0 (C7, C7b), 122.8 ($C7_a$, $C7b_a$), 118.4 (C6b), 114.4 (C4, C4b), 78.5 (C-Boc), 44.6 (CH_2), 28.4 (CH_3-Boc)

2-(2-[4-(aminomethyl)phenyl]benzimidazol-5-yl)benzimidazole-5-carboxylic acid (6)

A solution of **(5)** (0.099 g, 0.2 mmol) in TFA (2 mL) was stirred at room temperature for 2h. Diluted Et_2O and the white precipitate collected by centrifugation. The solution was removed by decanting and the remaining solid was subjected to preparative RP-HPLC to give a white fluffy solid (0.046 g, 58.6%).
ESI: m/z = 384.2 ($[M+H]^+$)
^{1}H-NMR, 250 MHz, DMSO-D6: δ = 8.58 (s, 1H, H4b), 8.35 (d, 2H, H2',H6'; *j* = 8.28 Hz), 8.33 (s, 1H, H4), 7.71 – 8.28 (m [4d], 4H, H6, H6b, H7, H7b, j_1 = 8.48 Hz, j_2 = 8.58 Hz), 7.72 (d, 2H, H3',H5'; *j* = 8.33 Hz), 5.35 (broad signal, 4H, NH_2), 4.72 (d, 2H, -CH_2;*j* = 5.18 Hz)
^{13}C-NMR, 62 MHz, DMSO-D6: δ = 166.8 (COOH), 158.3 (C=O), 140.8 (C2), 138.7 (C2b), 136.2 (C4'), 135.7 (C1'), 1343.2 ($C3_a$, $C3b_a$), 132.6 (C2', C6'), 127.4 (C3', C5'), 127.3 (C5), 125.2 (C6, C6b), 123.0 (C7, C7b), 122.8 ($C7_a$, $C7b_a$), 118.4 (C6b), 114.4 (C4, C4b), 44.6 (CH_2)

2-(2-[4-ButylDOTA-aminomethylphenyl]benz-imidazol-5-yl)benzimidazole-5-carboxylic acid (7)

A solution of HBTU (20.7 mg, 5.5×10^{-5} mol) in DMF (0.4 mL) was added to tris-butyl DOTA ester (31.4 mg, 5.5×10^{-5} mol) in a 1.5 mL Eppendorf. To this solution DIPEA (35.4 mg, 47.7 µl, 2.7×10^{-4} mol) was added. The solution was shaken for 15 min. To this solution was added a solution of **(6)** (21 mg, 5.5×10^{-5} mol) in DMF (0.2 mL). The reaction was shaken a t room temperature for 3h

and subjected to preparative RP-HPLC to give a white fluffy solid (42.1 mg, 81.9%).
ESI: m/z = 469.8 ($[M+2H]^+$)
^{1}H-NMR, 250 MHz, DMSO-D6: δ = 12.15 (s, broad signal, NH), 8.43 (s, 1H, H4b), 8.13 (d, 2H, H2'/H6'; *j* = 7.93 Hz), 8.11 (s, 1H, H4), 7.81 (d, 2H, H3'/H5'; *j* = 8.45 Hz), 7.75 (d, 2H, H6, H6b; *j* = 8.48 Hz), 7.67 (d, 2H, H7, H7b; *j* = 8.45 Hz), 4.43 (s, 2H, -ArCH$_2$;), 3.64 (s, 16H, -CH$_2$CH$_2$-), 3.40 (s, 8H, -CH$_2$-), 1.4 (s, 27H, Boc)
^{13}C-NMR, 62 MHz, DMSO-D6: δ = 176.7 (COO*t*Bu), 167.8 (COOH), 166.2 (CONH), 164.3 (C=O), 140.8 (C2), 138.7 (C2b), 136.2 (C4'), 135.7 (C1'), 1343.2 (C3$_a$, C3b$_a$), 132.6 (C2', C6'), 127.4 (C3', C5'), 127.3 (C5), 125.2 (C6, C6b), 123.0 (C7, C7b), 122.8 (C7$_a$, C7b$_a$), 118.4 (C6b), 114.4 (C4, C4b), 58.2 (CH$_2$-CO), 52.4 (CH$_2$), 44.6 (Ar-CH$_2$), 28.7 (Boc)

2-(2-[4-DOTA-aminomethylphenyl]benzimidazol-5-yl)benzimidazole-5-carboxylic acid (8)
A solution of **(7)** (25.2 mg, 2.7 × 10^{-5} mol) in TFA (3 mL) was stirred at room temperature for 5h and concentrated. The residue was subjected to Prep RP-HPLC to give a white fluffy solid (8.4 mg, 40.6%).
^{1}H-NMR, 250 MHz, DMSO-D6: δ = 12.15 (s, broad signal, NH), 8.43 (s, 1H, H4b), 8.13 (d, 2H, H2'/H6'; *j* = 7.93 Hz), 8.11 (s, 1H, H4), 7.81 (d, 2H, H3'/H5'; *j* = 8.45 Hz), 7.75 (d, 2H, H6, H6b; *j* = 8.48 Hz), 7.67 (d, 2H, H7, H7b; *j* = 8.45 Hz), 4.43 (d, 2H, -ArCH$_2$; *j* = 7.08 Hz), 3.35 (s, 16H, -CH$_2$CH$_2$-), 3.40 (s, 8H, -CH$_2$-)
^{13}C-NMR, 62 MHz, DMSO-D6: δ = 176.7 (COOH), 167.8 (Ar-COOH), 166.2 (CONH), 164.3 (C=O), 140.8 (C2), 138.7 (C2b), 136.2 (C4'), 135.7 (C1'), 1343.2 (C3$_a$, C3b$_a$), 132.6 (C2', C6'), 127.4 (C3', C5'), 127.3 (C5), 125.2 (C6, C6b), 123.0 (C7, C7b), 122.8 (C7$_a$, C7b$_a$), 118.4 (C6b), 114.4 (C4, C4b), 58.2 (CH$_2$-CO), 52.4 (CH$_2$), 44.6 (Ar-CH$_2$)

2-(4-Benzyloxyphenyl)benzimidazole-5-carboxylic acid (9)
To a suspension of 4-benzyloxybenzaldehyde (3.20 g, 15 mmol) and 3,4-diaminobenzoic acid (2.30 g, 15 mmol) in EtOH (250 mL) a solution of $Na_2S_2O_5$ (1.50 g, 8 mmol) in H$_2$O (50 mL) was added. The resulting suspension was heated under reflux conditions for 16 h. The reaction was allowed to cool, H$_2$O (100 mL) was added and the reaction was left standing for 30 min. The solid was collected by filtration, washed with water (50 mL) and Et$_2$O (2 × 50 mL) and dried in vacuo for 20 h to give a brown powdery solid (3.51 g, 67.5%). ESI: m/z = 345.0 ($[M+H]^+$)
^{1}H-NMR, 250 MHz, DMSO-D6: δ = 8.21 (s, 1H, H4), 8.16 (d, 2H, H2',H6'; *j* = 8.7 Hz), 7.95 (d, 1H, H6, *j* = 8.52), 7.73 (d, 1H, H7, *j* = 8.48), 7.28 (d, 2H, H3',H5'; *j* = 8.72 Hz), 7.2-7.4 (m, 5H, Ph), 5.23 (s, 2H, -CH$_2$)
^{13}C-NMR, 250 MHz, DMSO-D6: δ = 166.8 (COOH), 161.8 (C4'), 142.7 (C3$_a$), 136.8 (C1Ph), 134.5 (C2Ph, C3Ph, C5Ph), 130.8 (C4Ph, C6Ph), 128.0 (C2), 126.3 (C2', C6'), 122.2 (C5), 118.2 (C7$_a$), 123.2 (C6), 115.4 (C3', C5'), 114.4 (C4, C7), 70.0 (CH$_2$)

Methyl N^3-(2-[4-benzyloxyphenyl]benzimidazole-5-carbonyl)-3,4-diaminobenzoate (10)
To a stirred suspension of **(9)** (1.004 g, 2.9 mmol) in DMF (20 mL) was added HBTU (1.106 g, 2.9 mmol), followed by DIPEA (0.565 g, 762 µl, 4.4 mmol). The solution was stirred for 20 minutes at room temperature, and then methyl 3,4-diaminobenzoate (0.485 g, 2.9 mmol) was added. The reaction mixture was stirred for 24 h, poured into H$_2$O (60 mL) and the precipitate was collected. The solid was washed with Et$_2$O (2 × 50 mL) and dried in vacuo for 24 h to give **(10)** (1.208 g, 84.1%). ESI: m/z = 417.2 ($[M+H]^+$)
^{1}H-NMR, 250 MHz, DMSO-D6: δ = 9.63 (s, 1H, H3a), 8.23 (s, 1H, H4), 8.14 (d, 2H, H2',H6'; *j* = 8.81 Hz), 7.82 (m, 2H, H6, H5a), 7.73 (m, 2H, H7, H6a), 7.17 (d, 2H, H3',H5'; *j* = 8.76 Hz), 7.25-7.55 (m, 5H, Ph), 5.20 (s, 2H, -CH$_2$), 3.75 (s, 3H, -OCH$_3$)
^{13}C-NMR, 62 MHz, DMSO-D6: δ = 166.8 (COOCH$_3$), 166.2 (C=O), 160.8 (C4'), 148.3 (C2a), 144.0 (C2), 134.5 (C2Ph, C3Ph, C5Ph), 130.8 (C4Ph, C6Ph), 128.7 (C3$_a$), 128.2 (C7$_a$), 123.2 (C6), 123.3 (C1a), 122.3 (C4a), 122.2 (C5), 116.3 (C2', C6'), 114.6 (C6a), 114.4 (C4, C7), 112.4 (C3', C5'), 70.0 (CH$_2$), 52.3 (COOCH$_3$)

Methyl 2-(2-[4-benzyloxyphenyl]benzimidazol-5-yl)benzimidazole-5-carboxylate (11)
A solution of **(10)** (0.104 g, 0.2 mmol) in glacial AcOH (3 mL) was heated at 120 °C for 2 h. After 30 min, a tan solid precipitate was observed, concentrated to dryness, and subjected to silica gel chromatography (CH$_2$Cl$_2$:MeOH:Et$_3$N, 90:5:5) to give a tan powdery solid (0.097 g, 96.8%). ESI: m/z = 475.1 ($[M+H]^+$)

^{1}H-NMR, 250 MHz, DMSO-D6: δ = 8.40 (s, 1H, H4b), 8.15 (d, 2H, H2',H6'; *j* = 8.82 Hz), 8.13 (s, 1H, H4), 7.72 – 8.08 (m [4d], 4H, H6, H6b, H7, H7b, j_1 = 8.54 Hz, j_2 = 8.75 Hz), 7.25 (d, 2H, H3',H5'; *j* = 8.89 Hz), 7.23-7.51 (m, 5H, Ph), 5.22 (s, 2H, -CH_2), 3.87 (s, 3H, -CH_3)
^{13}C-NMR, 62 MHz, DMSO-D6: δ = 171.8 ($COOCH_3$), 166.7 (C4'), 160.8 (C1'), 134.5 (C2Ph, C3Ph, C5Ph), 130.8 (C4Ph, C6Ph), 128.7 (C2, C2b), 128.2 ($C3_a$, $C3b_a$), 128.1 ($C7_a$, $C7b_a$), 125.2 (C6, C6b), 123.3 (C5), 123.0 (C5b), 122.6 (C2', C6'), 122.4 (C6b), 121.4 (C3', C5'), 114.4 (C4, C4b, C7, C7b), 70.0 (CH_2), 55.3 ($COOCH_3$)

2-(2-[4-Benzyloxyphenyl]benzimidazol-5-yl)benz-imidazole-5-carboxylic acid (12)

A solution of **(11)** (0.124 g, 0.26 mmol) in 1M NaOH (10 mL) and MeOH (10 mL) was heated under reflux conditions for 2 h, allowed to cool and concentrated to a third of the volume. The mixture was diluted with H_2O (50 mL) and extracted with EtOAc (50 mL). The aqueous layer was acidified with 1M $HCl_{(aq)}$ to pH 4, extracted with *n*BuOH (2 × 70 mL), washed with saturated $NaCl_{(aq)}$, dried over Na_2SO_4 and concentrated. The solid was subjected to silica gel chromatography ($CHCl_3$:MeOH:Et_3N, 8:1:1) to give a yellow solid (0.105 g, 87.6%). ESI: m/z = 461 ($[M+H]^+$)
^{1}H-NMR, 250 MHz, DMSO-D6: δ = 10.82 (s, 1H, broad signal, COOH), 8.51 (s, 1H, H4b), 8.25 (d, 2H, H2',H6'; *j* = 8.91 Hz), 8.18 (s, 1H, H4), 7.84 (m, 2H, H6, H6b), 7.71 (m, 2H, H7, H7b), 7.15 (d, 2H, H3',H5'; *j* = 8.95 Hz), 7.28-7.61 (m, 5H, Ph), 5.23 (s, 2H, -CH_2)
^{13}C-NMR, 62 MHz, DMSO-D6: δ = 171.8 (COOH), 166.7 (C4'), 160.8 (C1'), 134.5 (C2Ph, C3Ph, C5Ph), 130.8 (C4Ph, C6Ph), 128.7 (C2, C2b), 128.2 ($C3_a$, $C3b_a$), 128.1 ($C7_a$, $C7b_a$), 125.2 (C6, C6b), 123.3 (C5), 123.0 (C5b), 122.6 (C2', C6'), 122.4 (C6b), 121.4 (C3', C5'), 114.4 (C4, C4b, C7, C7b), 70.0 (CH_2)

2-(4-Methoxyphenyl)benzimidazole-5-carboxylic acid (13)

To a suspension of *p*-anisaldehyde (3.80 g, 27.9 mmol) and 3,4-diaminobenzoic acid (4.25 g, 27.9 mmol) in EtOH (250 mL) a solution of $Na_2S_2O_5$ (2.83 g, 14.9 mmol) in H_2O (50 mL) was added. The resulting suspension was heated under reflux conditions for 60 h, allowed to cool, and H_2O (100 mL) was added. The reaction was left standing for 30 min. The solid was collected by filtration, washed with water (50 mL) and Et_2O (2 × 50 mL) and dried in vacuo for 20 h to give a brown powdery solid (5.63 g, 75.3%)
ESI: m/z = 267.1 ($[M-H]^-$)
^{1}H-NMR, 250 MHz, DMSO-D6: δ = 8.15 (d, 2H, H2'/H6'; *j* = 8.75 Hz), 8.13 (s, 1H, H4), 7.81 (d, 1H, H6; *j* = 8.5 Hz), 7.6 (d, 1H, H7; *j* = 8.4 Hz), 7.10 (d, 2H, H3'/H5'; *j* = 8.75 Hz), 3.83 (s, 3H, -OCH_3)
^{13}C-NMR, 250 MHz, DMSO-D6: δ = 166.8 (COOH), 161.8 (C4'), 128.7 ($C3_a$), 128.0 (C2), 126.3 (C2', C6'), 122.2 (C5), 118.2 ($C7_a$), 123.2 (C6), 115.4 (C3', C5'), 114.4 (C4, C7), 55.2 (OCH_3)

Methyl N^3-(2-[4-methoxyphenyl]benzimidazole-5-carbonyl)-3,4-diaminobenzoate (14)

To a stirred suspension of **(13)** (1.899 g, 7.1 mmol) in DMF (60 mL) HBTU (2.685 g, 7.1 mmol) was added, followed by DIPEA (2.5 mL). The solution was stirred for 20 minutes at room temperature, and then methyl 3,4-diamino-benzoate (1.176 g, 7.1 mmol) was added. The reaction was stirred for 72 h, poured into H_2O (150 mL), and the precipitate was collected. The solid was washed with Et_2O (2 × 50 mL) and dried in vacuo for 24 h to give **(14)** (1.677 g, 56.8%). ESI: m/z = 417.2 ($[M+H]^+$)
^{1}H-NMR, 250 MHz, DMSO-D6: δ = 12.95 (s, broad signal, NH or NH_2), 12.00 (s, broad signal, NH or NH_2), 8.15 (d, 2H, H2'/H6'; *j* = 8.75 Hz), 8.13 (s, 1H, H4), 7.81 (s, 1H, H3a), 7.66 (d, 2H, H6, H5a; *j* = 2.7 Hz), 7.15 (d, 2H, H3'/H5'; *j* = 8.72 Hz), 6.84 (d, 2H, H7'/H6a; *j* = 2.5 Hz), 3.85 (s, 3H, -$COOCH_3$), 3.76 (s, 3H, -OCH_3)
^{13}C-NMR, 62 MHz, DMSO-D6: δ = 166.8 ($COOCH_3$), 166.2 (C=O), 160.8 (C4'), 148.3 (C2a), 144.0 (C2), 128.7 ($C3_a$), 128.2 ($C7_a$), 123.2 (C6), 123.3 (C1a), 122.3 (C4a), 122.2 (C5), 116.3 (C2', C6'), 114.6 (C6a), 114.4 (C4, C7), 112.4 (C3', C5'), 55.3 ($COOCH_3$), 51.2 (OCH_3)

Methyl 2-(2-[4-methoxyphenyl]benzimidazol-5-yl)benz-imidazole-5-carboxylate (15)

A solution of **(14)** (0.991 g, 2.4 mmol) in glacial AcOH (10 mL) was heated at 120 °C for 2 h, concentrated to dryness and subjected to silica gel chromatography (CH_2Cl_2:MeOH:Et_3N, 90:5:5) to give a tan powdery solid (0.845 g, 89.1%).
ESI: m/z = 399.2 ($[M+H]^+$)
^{1}H-NMR, 250 MHz, DMSO-D6: δ = 13.14 (s, broad signal, NH or NH_2), 12.97 (s, broad signal, NH or NH_2), 8.16 (d, 2H, H2'/H6'; *j* = 8.81 Hz),

8.12 (s, 1H, H4), 7.81 (s, 1H, H4b), 7.66 (m, 2H, H6, H6b), 7.24 (m, 2H, H7', H7b), 7.13 (d, 2H, H3'/H5'; *j* = 8.45 Hz), 3.87 (s, 3H, -$COOCH_3$), 3.82 (s, 3H, -OCH_3)
^{13}C-NMR, 62 MHz, DMSO-D6: δ = 171.8 ($COOCH_3$), 166.7 (C4'), 160.8 (C1'), 128.7 (C2, C2b), 128.2 ($C3_a$, $C3b_a$), 128.1 ($C7_a$, $C7b_a$), 125.2 (C6, C6b), 123.3 (C5), 123.0 (C5b), 122.6 (C2', C6'), 122.4 (C6b), 121.4 (C3', C5'), 114.4 (C4, C4b, C7, C7b), 55.3 ($COOCH_3$), 51.8 (OCH_3)

2-(2-[4-Methoxyphenyl]benzimidazol-5-yl)benz-imidazole-5-carboxylic acid (16)

A solution of **(15)** (0.100 g, 0.25 mmol) in 1M NaOH (15 mL) and MeOH (20 mL) was heated under reflux conditions for 2 h, allowed to cool and concentrated to a third of the volume. The mixture was diluted with H_2O (50 mL) and extracted with EtOAc (50 mL). The aqueous layer was acidified with 1M $HCl_{(aq)}$ to pH 4, extracted with *n*BuOH (2 × 30 mL), washed with saturated $NaCl_{(aq)}$, dried over Na_2SO_4 and concentrated. The solid was subjected to silica gel chromatography ($CHCl_3$:MeOH:Et_3N, 8:1:1) to give a yellow solid (0.077 g, 79.8%). ESI: m/z = 385.0 ($[M+H]^+$)
^{1}H-NMR, 250 MHz, DMSO-D6: δ = 8.69 (s, 1H, H4b), 8.38 (d, 2H, H2',H6'; *j* = 8.88 Hz), 8.28 (s, 1H, H4), 7.64 – 8.38 (m [4d], 4H, H6, H6b, H7, H7b, j_1 = 8.43 Hz, j_2 = 8.54 Hz), 7.16 (d, 2H, H3',H5'; *j* = 8.91 Hz), 5.30 (broad signal, 2H, NH_2), 3.82 (d, 3H, -CH_3)
^{13}C-NMR, 62 MHz, DMSO-D6: δ = 168.8 (COOH, 162.7 (C4'), 155.8 (C1'), 130.7 (C2, C2b), 128.2 ($C3_a$, $C3b_a$), 128.1 ($C7_a$, $C7b_a$), 125.2 (C6, C6b), 123.3 (C5), 123.0 (C5b), 122.6 (C2', C6'), 122.4 (C6b), 121.4 (C3', C5'), 114.4 (C4, C4b, C7, C7b), 57.8 (OCH_3)

2-(2-[4-Hydroxyphenyl]benzimidazol-5-yl)benz-imidazole-5-carboxylic acid (17)

A suspension of **(16)** (217 mg, 0.54 mol) in 48% $HBr_{(aq)}$ was heated at 150 °C for 24 h (a precipitate formed after 1h) and allowed to cool. The solid collected by filtration, washed with water, dried in vacuo, and subjected to silica gel chromatography ($CHCl_3$:MeOH:Et_3N, 6:3:1) to give a yellow solid (188 mg, 93.1%).
ESI: m/z = 371.0 ($[M+H]^+$)
^{1}H-NMR, 250 MHz, DMSO-D6: δ = 8.48 (s, 1H, H4b), 8.09 (d, 2H, H2',H6'; j = 8.73 Hz), 8.25 (s, 1H, H4), 8.22 (d, 1H, H7b; *j* = 8.33 Hz), 7.91 (m, 2H, H6, H6b), 7.04 (d, 2H, H3',H5'; *j* = 8.75 Hz), 7.78 (d, 1H, H7; *j* = 8.40 Hz)
^{13}C-NMR, 62 MHz, DMSO-D6: δ = 168.8 (COOH, 162.7 (C4'), 155.8 (C1'), 130.7 (C2, C2b), 128.2 ($C3_a$, $C3b_a$), 128.1 ($C7_a$, $C7b_a$), 125.2 (C6, C6b), 123.3 (C5), 123.0 (C5b), 122.6 (C2', C6'), 122.4 (C6b), 121.4 (C3', C5'), 114.4 (C4, C4b, C7, C7b)

General procedure for coupling of the Hoechst derivatives to Tyr^3-octreotate

The resin preloaded with Tyr^3-octreotate was swelled in DCM for a minimum of 2h and washed thoroughly with DMF. To the swelled resin was added a pre-activated solution of the Hoechst derivative (2 eq.), HATU (1.95 eq.) and DIPEA (4 eq.) in DMF (300µl). The resin was shaken for 2-4 h, washed with DMF and then DCM and dried in vacuo for 16 h. The Hoechst derivatised Tyr^3-octreotates were then cleaved from the resin using TFA:triisopropylsilane:H_2O (95:2.5:2.5) and purified using Prep RP-HPLC.

{2-(2-[4-(aminomethyl)phenyl]benzimidazol-5-yl)benz-imidazole-5-carbonyl}-D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Thr-OH (18)

ESI-MS: *m/z* 1415.1 $[M + H]^+$, 707.8 $[M + 2H]^{2+}$

{2-(2-[4-DOTA-aminomethylphenyl]benz-imidazol-5-yl)benzimidazole-5-carbonyl}-D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Thr-OH (19)

ESI-MS: *m/z* 901.1 $[M + H]^{2+}$, 601.1 $[M + 2H]^{3+}$

{2-(2-[4-Benzyloxyphenyl]benzimidazol-5-yl)benz-imidazole-5-carbonyl}-D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Thr-OH (20)

ESI-MS: *m/z* 1492.6 $[M + H]^+$, 746.3 $[M + 2H]^{2+}$

{2-(2-[4-Methoxyphenyl]benzimidazol-5-yl)benz-imidazole-5-carbonyl}-D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Thr-OH (21)

ESI-MS: *m/z* 1416.6 $[M + H]^+$, 708.4 $[M + 2H]^{2+}$

{2-(2-[4-Hydroxyphenyl]benzimidazol-5-yl)benz-imidazole-5-carbonyl}-D-Phe-c[Cys-Tyr-D-Trp-Lys-Thr-Cys]-Thr-OH (22)

ESI-MS: *m/z* 1401.8 $[M + H]^+$, 701.3 $[M + 2H]^{2+}$

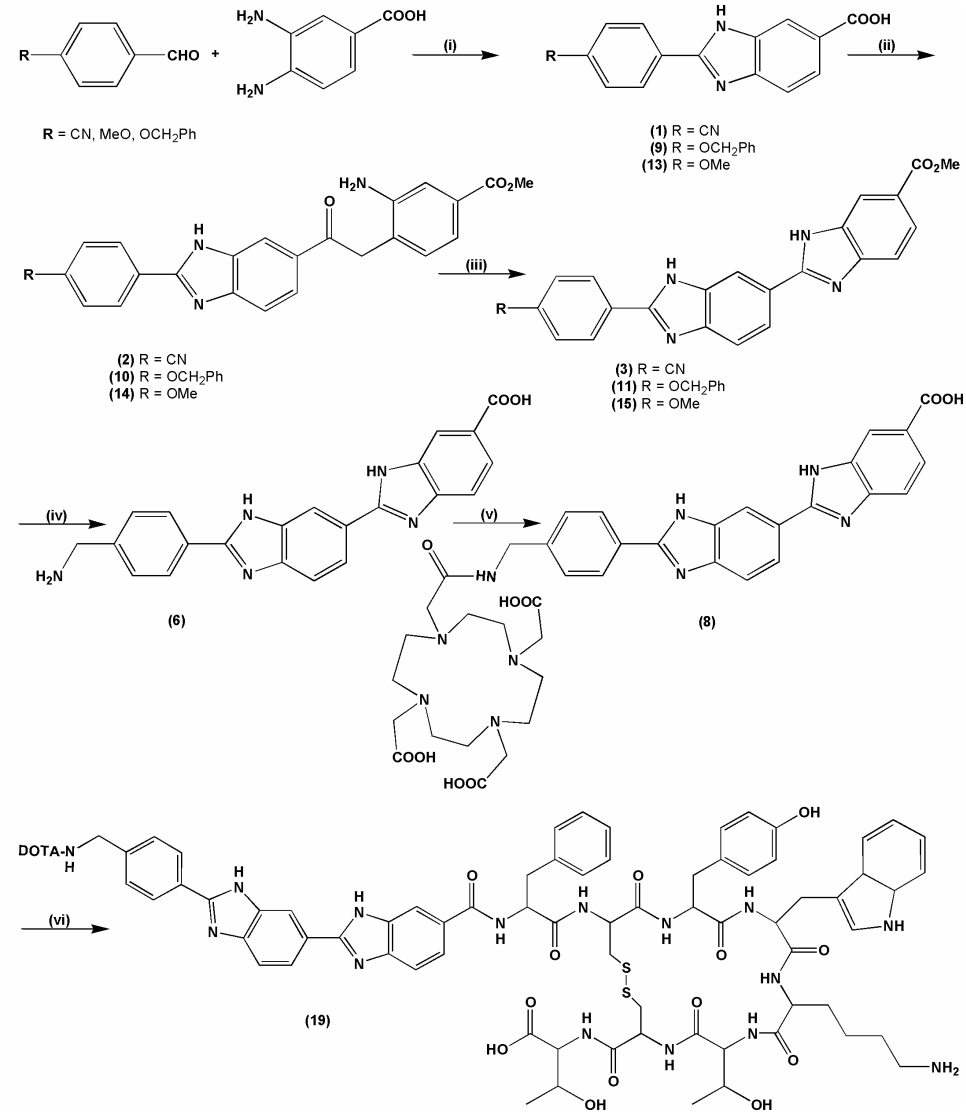

Figure 1. General scheme for the synthesis of substituted bis-benzimidazoles.

Receptor binding assay

The competition receptor binding assay of all compounds and octreotide was conducted as described previously with a few modifications (6). In brief, 200 μL rat cortex membrane fractions

containing 70 μg of protein were mixed with [^{125}I]-Tyr3-octreotide (ca. 20,000 cpm) in 10 mM HEPES with 5% BSA fraction V, 1% $MgCl_2$, and 1% bacitracin, pH 7.6 with increasing concentrations of the competitor octreotide (10^{-6} - 10^{-12} M). The final incubation volume in each tube was 300 μLl. The mixture was incubated at ambient temperature on a shaker. The binding experiments were performed in triplicate on at least two occasions. After 1 h, the incubation was terminated by rapid filtration through Whatman (GF/B glass fiber) filters soaked in the incubation medium with 1% BSA. The filters were washed with ice-cold buffer (0.9% NaCl, 0.121% TRIS, pH 7.5) 5 × 3 mL. Then the filter bound radioactivity was measured in a γ-counter (Multi-crystal counter, LB 2104, Berthold, Germany). The IC_{50} values and non-specific binding from the displacement experiments were analyzed using SigmaPlot 2001. Data were expressed as mean ± S.E.M.

Cell lines

The AR42J cell line was cultured in DMEM medium supplemented with vitamins (2%),

essential and nonessential amino acids (2%, respectively), 100,000 IU/L penicillin, 100 mg/L streptomycin, and 10% FCS as described (7), and the MH3924A cell line was cultured in RPMI 1640 medium supplemented with 100,000 IU/L penicillin, 100 mg/L streptomycin, and 20% FCS as previously reported by Haberkorn et al. (8). The cells were routinely plated at 2×10^5 cells /mL into 80-cm^2 flasks at 37 °C, 5% CO_2, 95% humidity environment.

Fluorescent microscopy studies

Fluorescence microscopy was conducted as described by Nouel et al. (9) with some modifications. AR42J cells or MH3924A cells (10^5 cells/well in 0.9 mL medium) were grown to subconfluence. In order to settle and flatten out on the cover slips as monolayers, they were seeded in culture 24-well plates containing circular glass coverslips and allowed to grow for 24 hours. The fluorescent dyes (10^{-4} to 10^{-5} M) were added to the wells and incubated at 37 °C for 1 hour. Propidium iodide (2 or 10 µg/mL) was used as positive nucleus stain control. The cells were washed with ice-cold 1 PBS thrice. Then the cells were fixed with 4% formaldehyde on ice for 20 min. The cells were washed once with ice-cold PBS. Finally, the cells were fixed with methanol for 2 min on ice. The cover slips containing the cells were mounted on a slice with DAKO fluorescent mounting medium. Cells were analyzed and photographed using an Axiophot microscope and the digital high resolution imaging system (AxioCam/AxioVision) as described by Schober et al. (10).

RESULTS

Chemistry

The synthesis of a Hoechst 32258 analogue amino acid building block has been previously reported (11). The construction of the 2-(2-aryl-benzimidazol-5-yl)benzimidazole framework was repeated, and the yields for some the steps were found to be inconsistent. The initial formation of the 2-arylbenzimidazole-5-carboxylic acid used the oxidation conditions of nitrobenzene at high temperatures. Obviously, nitrobenzene should be avoided due to its extremely toxic nature. Other methods for the formation of 2-arylbenzimidazoles from the reaction between diaminobenzenes and benzaldehydes have been investigated. These the oxidative conditions include 1,4-benzoquinone (12), 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (13), DMSO impregnated on silica gel (14), catalytic Fe(III)/Fe(II) redox cycling (15) and sodium bisulfite (16; 17).

The formation of 2-(4-cyanophenyl)benz-imidazole-5-carboxylic acid **(1)**, 2-(4-methoxy-phenyl)benzimidazole-5-carboxylic acid **(13)** and 2-(4-benzyloxyphenyl)-benzimidazole-5-carboxylic acid **(9)** from 3,4-diaminobenzoic acid and the corresponding benzaldehyde were investigated (16) and the sodium bisulfite method was found to the best, giving the desired products in high purity as precipitates, thus, avoiding laborious chromatographic purifications. The second benz-imidazole moiety was introduced in two steps. First, the benzimidazole-5-carboxylic acid s **(1), (9)** and **(13)** were converted to their activated 1,2,3-hydroxybenzotriazole ester (HOBt ester) and reacted with methyl 3,4-diaminobenzoic acid to give the corresponding amides, methyl N^3-(2-[4-cyanophenyl]benzimidazole-5-carbonyl)-3,4-diaminobenzoate **(2)**, methyl N^3-(2-[4-methoxy-phenyl]benzimidazole-5-carbonyl)-3,4-diamino-benzoate **(14)** and methyl N^3-(2-[4-benzyloxy-phenyl]benzimidazole-5-carbonyl)-3,4-diamino-benzoate **(10)**. The second step was the intramolecular acid catalyzed ring closure. In the original method this step used sulphuric acid in nitrobenzene under high temperatures. Other methods were investigated and the best method was found heated a suspension of the amide in glacial acetic acid at 120 °C and the desired product would precipitate out on cooling (18).

The cyano group of **(2)** was reduced and Boc protected in one pot carrying out by catalytic hydrogenation with 10% palladium on charcoal in the presence of di-tert-butylpyrocarbonate and DIPEA to give 2-(2-[4-(*tert*-butoxyaminomethyl-phenyl]benzimidazol-5-yl)benzimidazole-5-carboxylic acid **(4)**.

The hydrolysis of the methyl esters **(3)**, **(11)** and **(15)** were carried out using 1M NaOH in MeOH to afford the corresponding 2-(2-[4-(*tert*-butoxy-aminomethylphenyl]benzimidazol-5-yl)-benz-imidazole-5-carboxylic acid **(4)**, 2-(2-[4-methoxy-phenyl]benzimidazol-5-yl)benz-imidazole-5-carboxylic acid **(16)** and 2-(2-[4-benzyloxyphenyl]benz-imidazol-5-yl)benzimidazole-5-carboxylic acid **(12)**. The methyl group of **(16)** was removed by

treatment with 48% HBr under reflux conditions to afford the free phenol **(17).**

The Hoechst amino acid analogue was functionalized further with the chelator ligand DOTA. This was achieved by removing the Boc protecting group of **(4)** with TFA the yield the free amine **(6)**. This amine was reacted with the activated 1,2,3-hydroxybenzotriazole ester (HOBt ester) of DOTA tris tert-butyl ester. This DOTA-Hoechst derivative **(8)** along with **(6)**, **(12)**, **(16)** and **(17)** could now be coupled using a Solid Phase Peptide Synthesis (SPPS) protocol to resins preloaded with Tyr^3-octreotate.

Solid Phase Peptide Synthesis

The Tyr^3-octreotate was synthesized using standard Fmoc protocol starting with Fmoc-Thr(tBu)-Wang Resin, and disulphide bridge formation with $Tl(TFA)_3$ to afford H-D-Phe-c[Cys-Tyr(tBu)-D-Trp(Boc)-Lys(Boc)-Thr(tBu)-Cys]-Thr(tBu)-Wang Resin. The Hoechst derivatives were coupled using HATU and the desired Hoechst derivatised Tyr^3-octreotates **(18) - (22)** were cleaved from the resin and purified by RP-HPLC.

Fluorescence microscopy imaging

The question whether or not the synthesized bis-benzimidazole conjugates are taken up into the cell is a fundamental one to define their clinical applicability for diagnosis and endoradiotherapy of somatostatin receptor-expressing tumors. Fluorescence microscopy imaging was used to determine if the novel intercalating compounds reach their projected target, the cell nucleus. The cells were incubated with the fluorescent dye Amino-Hoechst-TATE, and the fluorescence was recorded one hour after incubation. Figure 2 shows a distinct staining of the round cell nuclei. Proteins and other structures in the oval AR42J cells, structures around the nuclei or on the cell surface are not fluorescent at all and have thus not been stained by bis-benzimidazole Amino-Hoechst-TATE.

The fluorescent dye propidium iodide cannot penetrate cell membranes by mere diffusion or a specific transport system; the cells have to be lysed prior to intercalation. In a permeated cell, propidium iodide readily enters the nucleus and intercalates with DNA. In Figure 2, at least three discrete cells can be seen. In one of them (on the upper right side), cell lysis was not successful; the cell is thus stained with Amino-Hoechst-TATE only, not with propidium iodide. In two cells (the single cell on the left and the large cell on the bottom end of the cell cluster) a co-localization of propidium iodide and the Hoechst conjugate can be observed. The areas of fluorescent bis-benzimidazole dye and propidium iodide are almost congruent due to the fact that they both exclusively stain structures in the cell nucleus.

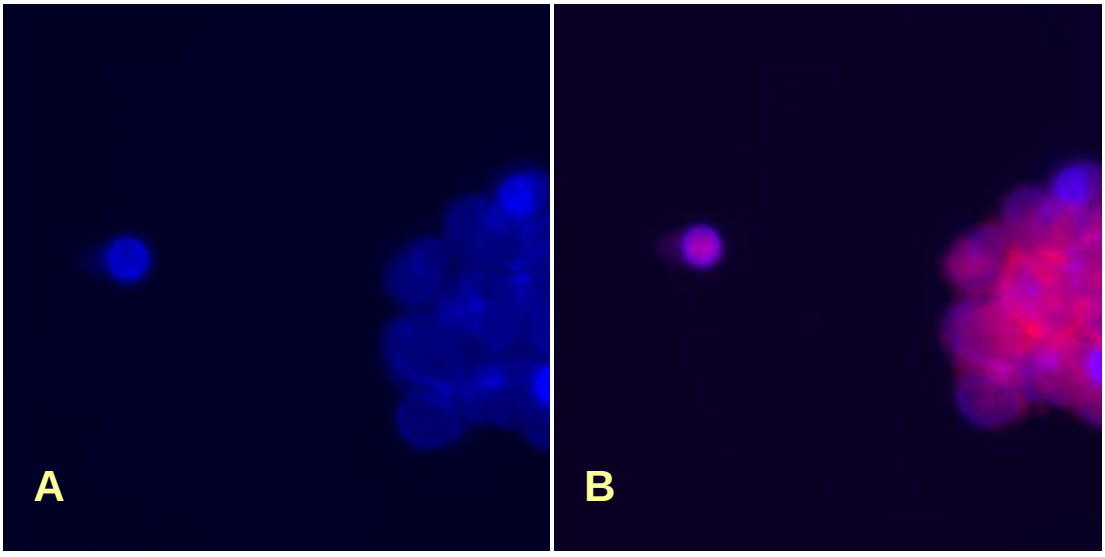

Figure 2. [A] Fluorescence microscopy of (6) (Amino-Hoechst-TATE); in the somatostatin receptor-expressing AR42J cells [B] dual staining with propidium iodide**.**

This picture thus confirms the uptake of the modified bis-benzimidazoles into the cell nucleus and, as both propidium iodide and the bis-benzimidazoles are intercalating agents, strongly suggests interaction of the novel compounds with DNA.

To further confirm these findings the cellular localization of two compounds, Amino-Hoechst-TATE-OH and DOTA-Amino-Hoechst-TATE-OH, was compared to a sulforhodamine B-conjugated octreotate (which has no intercalating properties) in the SSTR-positive AR42J and MH3924 cells as SSTR-negative control. Both Hoechst derivatives showed positive blue fluorescent labeling in AR42J cells after 1 hour incubation at 37 °C. No uptake was found in receptor negative MH3924A cells (data not shown). As exemplified for Amino-Hoechst-TATE (see Figure 2), dual staining with propidium iodide in fluorescence microscopy images revealed the selective uptake of the intercalator conjugates in the receptor-expressing AR42J cells. No uptake was found in receptor negative MH3924A cells (data not shown).

Somatostatin receptor binding affinities

The receptor affinities of the compounds were determined in a competition binding assay, as described before (6). The affinities of the compounds tested were in the nanomolar range. The comparison with [Tyr^3]-octreotate and octreotide revealed that the large substituents coupled at the N-terminus do not significantly reduce the receptor affinities. The receptor affinities of the synthesized compounds and known standards can be seen in Table 1.

DISCUSSION

For targeted radionuclide therapy (endo-radiotherapy) radioactively labeled carrier molecules, such as monoclonal antibodies that possess high specificity for target antigens on the surface of tumor cells, are applied (19). The specific binding of peptides to receptors expressed on tumor cells meets the essential requirement in tumor targeting. Receptor binding peptides coupled to highly toxic radioisotopes or radionuclides that allow a diagnostic imaging are becoming a significant component of anticancer treatments. By combining the exquisite targeting specificity of peptides with the tumor-killing power of radioisotopes, peptide conjugates permit sensitive discrimination between target and normal tissue, resulting in fewer toxic side effects than most conventional chemotherapeutic drugs.
The next generation of advances in peptide engineering will permit greater control of targeting, clearance and pharmacokinetics, resulting in significantly improved tumor delivery of radioisotopes (20).

In conclusion the use of tumor-specific peptides in the therapy and diagnosis of malignant disease is a modality that has recently shown rapid progress. Chelator-conjugated peptides, such as the somatostatin analogues Octreoscan® and DOTATOC that can be labeled with ß-emitting radiometals such as ^{90}Y, ^{111}In and ^{177}Lu (Figure 3) have shown high efficiency in the treatment of disseminated occult disease.

Table 1: Receptor affinities of selected compounds tested. Competition binding experiments with ^{125}I-TOC on rat cortex membrane preparations (IC_{50}, mean ± S.E.M. n = 3).

Compound	**IC_{50} [nM]**
[Tyr^3]-Octreotate (= TATE)	0.40 ± 0.07
Octreotide	2.78 ± 0.13
Amino-Hoechst-TATE	0.39 ± 0.02
Methoxy-Hoechst-TATE	1.73 ± 0.24
DOTA-Amino-Hoechst-TATE	1.19 ± 0.25
DOTA-Hoechst-6-aminohexylic acid-TATE	2.80 ± 0.04

In order broaden the spectrum of therapeutic application of carrier substances based on tumor specific peptides the application of targeted tracers that enable the application of Auger emitting isotopes that exert their cytotoxic effect in the nucleus is warranted. The novel chelator-intercalator derivatives of Tyr^3-octreotate introduce a new scope to the range of octreotate derivatives for therapeutic purposes. The data from this study reveal that the intercalating moiety does not interfere with both the receptor affinities and cellular uptake. In order to investigate the potential of increased retention of the targeted compounds, the kinetics of the tumor localization and the metabolic fate needs to be studied in detail.

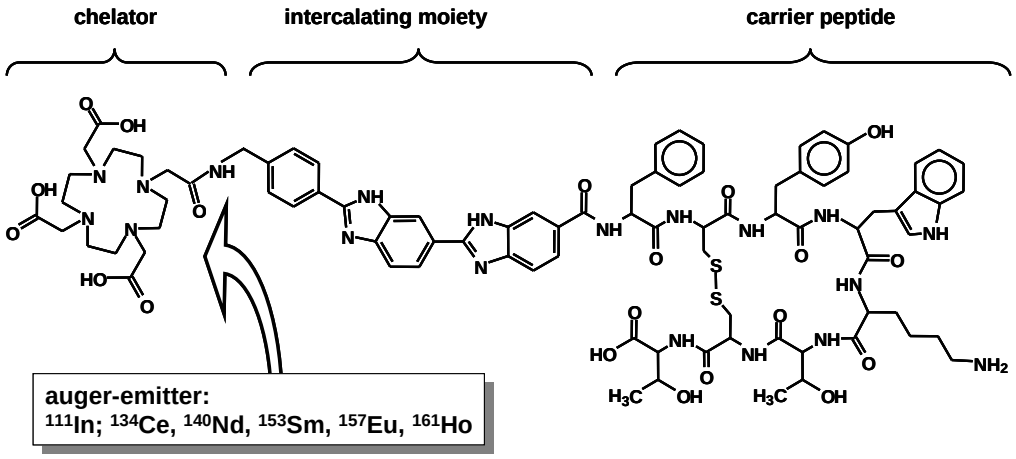

Figure 3: Chemical structure of the compounds investigated as exemplified for a DOTA-bisbenzimide-Tyr^3-octreotate derivative

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