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Synthesis and evaluation of a ligand targeting the somatostatin-2 receptor for drug delivery to neuroendocrine cancers ☆

Guo Li, Philip S. Low *

Department of Chemistry, Purdue University, 560 Oval Drive, West Lafayette, IN 47907-2084, United States

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ABSTRACT

Over-expression of the somatostatin-2 (SST2) receptor on plasma membranes of neuroendocrine cancer cells renders it attractive for use in targeting both imaging and therapeutic agents to neuroendocrine tumors. Peptide analogs of somatostatin have dominated this approach to date, however, many peptide analogs are either unstable in vivo or exhibit unwanted non-specific uptake in the liver and kidneys. The purpose of this Letter is to describe the preparation and evaluation of a non-peptide SST2 agonist for use in targeting drugs to neuroendocrine cancers.

A non-peptide ligand for the SST2 receptor was identified from the literature as a candidate for development of targeted pharmaceuticals for neuroendocrine tumors, based on its SST2 binding affinity and selectivity for SST2 over other somatostatin receptors. It also offered a multiplicity of possible conjugation sites. Rhodamine conjugates in two positions were used for optical imaging and two compounds were internalized in an SST2 receptor transduced cell line (C6-SST2) via SST2 receptor-mediated endocytosis. Radionuclide conjugates were prepared for in vivo imaging and biodistribution studies in mice. The in vitro binding affinity of ^{99m}Tc conjugates ranged from a K_d of 37–494. Of these, one ^{99m}Tc conjugate was selected and dosed by IV injection into mice bearing C6-SST2 tumor xenografts. The highest uptake was into tumor, intestine and skin four hours after IV injection. Competition studies with octreotide, a synthetic peptide and SST2 agonist, confirmed that uptake was SST2 receptor mediated. While relatively high uptake in intestine, liver, kidneys and skin discouraged further development of the conjugate for delivery of chemotherapeutic agents, the conjugate may still be worthy of further development for neuroendocrine tumor imaging.

The somatostatin-2 (SST2) receptor is one of five specialized somatostatin receptors found in human tissues and cancers.[1] Somatostatin is an inhibitory hormone with multiple sites of action in the gastrointestinal tract, pituitary gland and various regions of the brain. It participates in the regulation of gastrin, glucagon and growth hormone (GH), to name a few of many physiological roles. SST2 receptor over-expression occurs predominantly in neuroendocrine tumors[1,2] which are among the most lethal of common human cancers. For unknown reasons, the incidence of neuroendocrine cancers is increasing in the United States.[3]

Neuroendocrine tumors derive from those cells that respond to neural stimulation by secreting hormones. Neuroendocrine tumors retain their ability to produce hormones, adding to the severity of their pathology. Because of its increased potency and 38-fold increase in half-life versus natural somatostatin,[4] the synthetic peptide octreotide, a somatostatin mimetic drug, is used to treat the symptoms of flushing and diarrhea that are associated with metastatic carcinoid tumors and vasoactive intestinal peptide

Abbreviations: ATCC, American Type Culture Collection; CDI, *N*,*N*′-carbonyldiimidazole; Dde, 2-acetyldimedone; DIPEA, diisopropylethylamine; Dmab, 4-{*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methyl-butyl]-amino}benzyl ester; DMF, dimethylformamide; DSC, *N*,*N*′-disuccinimidyl carbonate; EDT, ethylanedithiol; Fmoc, fluorenylmethyloxycarbonyl; GH, growth hormone; HBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HOBt, hydroxybenzotriazole; LC–MS, liquid chromatography–mass spectrometry; NMP, *N*-methyl-2-pyrrolidone; ^{1}H NMR, proton nuclear magnetic resonance; *i*-PrOH, isopropyl alcohol; ivDde, 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl; PET, Positron Emission Tomography; PyBOP, benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate; RBITC, rhodamine B isothiocyanate; RP-HPLC, reverse-phase high performance liquid chromatography; SPPS, solid phase peptide synthesis; SST2, Somatostatin Receptor Subtype 2; 5-TAMRA, 5-carboxytetramethylrhodamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TIPS, triisopropylsilane.

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* Corresponding author. Tel.: +1 765 494 5273.
E-mail address: plow@purdue.edu (P.S. Low).

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(VIP) secreting adenomas. Somatostatin agonists, such as octreotide, function as inhibitors of multiple endocrine systems.

The prevalence of SST2 receptor is especially notable in growth hormone producing pituitary adenomas, gut carcinoids, gastrinomas, paragangliomas, pheochromocytomas, neuroblastomas, medulloblastoma and meningioma,[1] but it also occurs in lower density in small cell lung cancers, lymphomas and carcinomas of the breast, kidney, ovary and gastric tissue.[1,5] The increasing incidence of gastroenteropancreatic neuroendocrine tumors[3,6] and the scarcity of treatment options which demonstrate prolonged remission[6] are further justification for ongoing research into new methods to target these pathologies.

Exploitation of somatostatin analogs for targeted drug delivery to neuroendocrine tumors is based on (i) the aforementioned overexpression of SST2 receptors on this tumor type and (ii) the ability of SST2 receptors to avidly bind and internalize somatostatin analogs and their conjugates.[1,2,5–8] While conjugates of somatostatin analogs have been primarily exploited for radioimaging applications, they have also been used in therapeutic applications when conjugated with β-emitting radionuclides such as Yttrium-90 and Lutetium-177.[7]

While many of the somatostatin analogs have proven effective in the selective targeting of SST2 receptors[1,7–9] many of these same peptides have displayed unwanted uptake in the liver and the kidneys,[10,11] possibly as a consequence of the expression of peptide scavenger receptors in these tissues.[12,13] The goal of this study was to develop a non-peptide targeting ligand for SST2 overexpressing tumors that would exhibit reduced uptake in the liver and kidneys. In this paper, we explore the utility of a previously synthesized SST2 agonist for use in selective delivery of drugs to neuroendocrine tumors. Although we observe significantly reduced kidney uptake compared to competition with octreotide, we find no improvement in liver uptake.

The parent compound (Fig. 1) is a known SST2 agonist[9] and was selected on the basis of its binding affinity (K_i = 0.1 nM) to the SST2 receptor. This compound was also chosen because of its relative ease of conjugation to desired drugs (see functional groups at positions A and B in Fig. 1).

Literature reports have established that the availability of the lysine and tryptophan moiety in this molecule are essential for SST2 receptor binding,[2,14–16] leaving the benzo-imidazole ring and *tert*-butyl ester as the best remaining sites for conjugation. On position A of compound **1**, we replaced the *tert*-butyl group with an amino-cadaverine group, so that the resulting molecule would retain the hydrophobicity of the parent while preserving the amino group for further modifications. Modifications to position A are outlined in Scheme 1. On position B, we deprotonated the hydrogen in the imidazole ring, followed by alkylation of the nitrogen atom. Modifications to position A are outlined in Scheme 2.

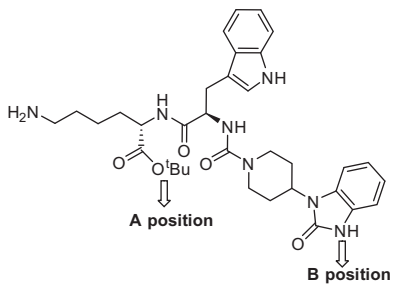

Figure 1. Structure of SST2 agonist selected for targeted delivery of drugs to neuroendocrine tumors.

Compound **5** was prepared by protection of the carboxylic acid on Fmoc-Lys(ivDde)-OH with a tert butyl group.[19] Fmoc deprotection and amide coupling reaction of compound **5** with Fmoc-D-tryptophan gave intermediate **6**. Then following the same DSC condensation procedure outlined in Scheme 1, compound **7** was prepared. Treatment of compound **7** with sodium hydride and an alkylation reaction with methyl 4-iodobutanoate at room temperature gave compound **8**. Selective hydrolysis of the methyl ester using lithium hydroxide completed the synthesis of compound **9**. Compound **9** was conjugated with rhodamine per Figure 2 to form conjugate **11**.

Rhodamine conjugate **10** was prepared by reaction between the amine intermediate labeled as compound **4** (Scheme 1) and rhodamine B isothiocyanate (RBITC) in tetrahydrofuran (THF) and *N*,*N*-diisopropylethylamine (DIPEA). Rhodamine conjugate **11** was prepared by coupling the acid intermediate labeled as compound **9** (Scheme 2) with 5-TAMRA cadaverine. In the final step, the ivDde protecting group for lysine was removed with hydroxylamine/imidazole in NMP.[20]

The preparation of technetium chelating conjugate **12** was achieved with solid phase synthesis by adopting the Fmoc-Boc SPPS strategy (Scheme 3). Compound **4** was coupled with linker in step **e** and the ivDde protecting group was removed in step **f**.

In Scheme 4, the weak acid labile H-Cys(Trt)-2-chlorotrityl-resin was applied using a solid phase peptide synthesis (SPPS) strategy to avoid cleaving the *tert*-butyl group in the original molecule. Next, the Fmoc-Asp(ODmab)-OH and Dde-diaminopropionic (Fmoc) acid were used to prepare the ^{99m}Tc chelating moiety. The Dde, ivDde and Dmab protecting groups were all removed during solid phase synthesis by bubbling with 2% hydrazine in DMF. Lastly, the final technetium chelating conjugate **13** was cleaved from the resin using a cocktail of 1% TFA/2% TIPS in CH_2Cl_2, leaving the *tert*-butyl group intact.

Using reported procedures[21] these conjugates were formulated and chelated with ^{99m}Tc for the in vitro saturation binding assay and the in vivo biodistribution study using a mouse model.

Both rhodamine conjugates **10** and **11** were observed to internalize into C6-SST2 cells. After one hour of incubation, the majority of dye conjugates displayed a punctate distribution within the cultured cancer cells, indicating their trafficking into endosomal compartments (photograph B in both Figs. 3 and 4). Both conjugates were administered to the same cultured cells at the same concentration (100 nM) and for the same incubation period. Based on the appearance of some residual uptake in the presence of excess unlabeled ligand in Figure 3c, we conclude that uptake was somewhat more SST2 receptor specific for conjugate **11** than conjugate **10**. Importantly, competition studies with octreotide, a synthetic peptide and SST2 agonist, confirmed that uptake is SST2 receptor mediated. Octreotide was administrated at a 100 fold molar excess versus the dye conjugates. The observation of rapid internalization is consistent with published literature.[5]

Saturation binding curves for the ^{99m}Tc conjugates of the same targeting constructs are presented in Figure 5. The technetium chelating conjugate **12** (K_i = 494 nM) shows less binding affinity than conjugate **13** (K_i = 37 nM). This suggests that the derivatization at position B on the parent molecule constitutes a better site for conjugation of this compound.

Based on the confocal microscopy data and the results of the in vitro binding studies, the conjugates stemming from derivatization at position B on the parent molecule seemed to better retain SST2 receptor binding specificity. Therefore, we decided to further explore development of ^{99m}Tc-conjugate **13** and determine its in vivo distribution profile in our mouse model (Figure 6).

Conjugate **13** showed specific (competable) uptake in the implanted C6-SST2 tumor, as well as in the blood, intestine and skin. Unfortunately, the conjugate also showed high nonspecific