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Sugar Amino Acid Containing Somatostatin Analogues that Induce Apoptosis in Both Drug-Sensitive and Multidrug-Resistant Tumor Cells†

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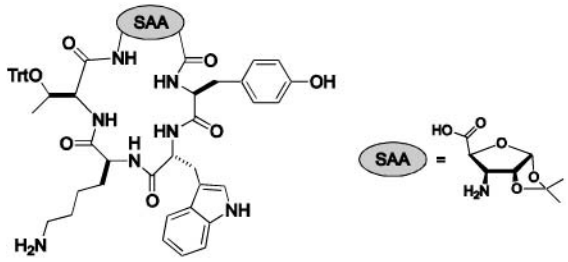
ABSTRACT



Resistance to chemotherapy has become a major problem in cancer therapy. The new sugar amino acid (SAA) containing somatostatin analogues presented possess antiproliferative and apoptotic activity against both multidrug-resistant and drug-sensitive hepatoma carcinoma cells. Synthesis, design, and biological evaluation of the cyclic analogues and of the furanoid SAA used will be discussed. Four analogues have IC_{50} values in the low μM range, making them promising leads for chemotherapeutic drugs against multidrug-resistant carcinoma.

One of the major problems of cancer treatment is the development of resistance to chemotherapy. Primary liver cancer, hepatocellular carcinoma, is essentially refractory to chemotherapy. In this work we present several novel somatostatin analogues containing the new furanoid sugar amino acid (SAA) f-SAA **1** as a key structure-inducing template. f-SAA **1** was designed to have structure-inducing properties, to be easily accessible, and to introduce the possibility of varying the functional groups on the carbohydrate moiety and thereby fine-tune pharmacokinetic properties. The synthesis of f-SAA **1** is fast and straightforward and starts from cheap, commercially available diacetone glucose. The novel cyclic compounds **7**−**14** are part of a small library of 25 analogues containing f-SAA **1**, which were built up with a combination of solid phase and solution chemistry techniques. Analogues **11** and **12** possess strong antiproliferative activity (suppression of the rapid multiplication of tumor cells) as well as trigger apoptosis (programmed suicidal cell death) on both multidrug-resistant (MDR) and drug-sensitive (DS) hepatoma carcinoma cells. The only other compound active against MDR hepatoma carcinoma cells is TT232: cyclo[2,6]D-Phe-Cys-Tyr-D-Trp-Lys-Cys-Thr-NH_2 **15**.[1]

In contrast to most other SAAs so far described, synthesis of f-SAA **1** is simple and direct. We have optimized the synthesis of the azide precursor **5**, which has been reported

† Dedicated to Joachim Thiem on the occasion of his 60th birthday.

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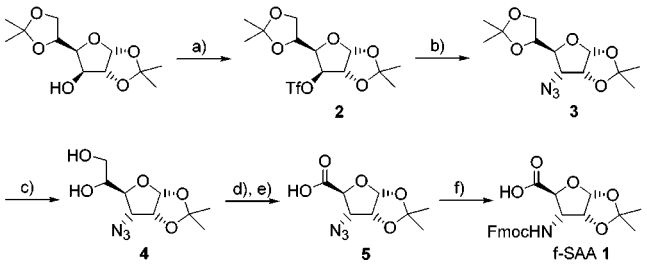
before.[2] Compound **5** was used as a precursor for the total synthesis of chryscandin, an antifungal antibiotic.[2c] However, protected or unprotected f-SAA **1** has not so far been reported in the literature, nor has f-SAA **1** or a derivative thereof been introduced into a peptide backbone. The key step of the synthesis of f-SAA **1** is the azidolysis of the triflate activated diacetone glucose **2 (**Scheme 1).

Scheme 1. Synthesis of Fmoc-Protected f-SAA **1**[a]



[a] (a) Tf_2O, py, −10 °C, CH_2Cl_2; (b) NaN_3, Bu_4NCl (cat), 50 °C, DMF, 70%; (c) 77% HOAc, 3 h, 65 °C, quant; (d) $NaIO_4$, 5 h, 10 °C, MeOH; (e) $KMnO_4$, 50% HOAc, rt; (f) H_2, Pd/C, MeOH, FmocCl, $NaHCO_3$, pH 8−9, THF, MeOH, rt, 70%.

Compound **2** was brought to reaction with NaN_3 at 50 °C in DMF in the presence of catalytic Bu_4NCl for 5 h to yield the azide **3**. By addition of catalytic amounts of Bu_4NCl, considerably higher yields (70% instead of 48%) than in ref 3 were achieved. The exocyclic hydroxyl groups of **3** are quantitatively and selectively deprotected to **4** using concentrated acetic acid.[2a] Subsequently, the diol **4** is oxidatively cleaved using $NaIO_4$, followed by $KMnO_4$ oxidation to yield **5**.[2a] In a one-pot reaction the azide **5** was reduced and simultaneously Fmoc-protected to yield about 70% of the f-SAA **1**. Because the acetonide is fused to a second five-membered ring, it is stable against conventional acids such as pure trifluoroacetic acid or 1 N HCl. The overall yield of f-SAA **1** starting from diacetone glucose was 44%.

Extensive structure−activity relationship studies of somatostatin revealed that the structural reduction retaining the cyclic nature of somatostatin led to a β-turn about the native Phe[7]-Trp[8]-Lys[9]-Thr[10] sequence of somatostatin.[4] Hence, replacement of Trp[8] with D-Trp stabilizes the β-turn in various cyclic somatostatin analogues. Activity is thus increased, while at the same time enzymatic stability is enhanced.[5]

The "Veber−Hirschmann" peptide cyclo[-Phe-Pro-Phe[7]-d-Trp[8]-Lys[9]-Thr[10]-] **6** showed similar activity as somatostatin.[6] However, its clinical development has been halted due to steatorrhea caused as a side effect. In 1994 we reported the incorporation of glucosyluronic acid methylamine (Gum) as a dipeptide isoster as replacement of Phe-Pro into the sequence of **6**.[7]

One of the aims of this work was to develop a new SAA as a structure-inducing template, which is considerably easier, cheaper, and faster to synthesize than the previously described ones.[8] Our new f-SAA **1** was designed to meet the above required demands. We incorporated it in the novel cyclic structures **7**−**14** (Figure 1).

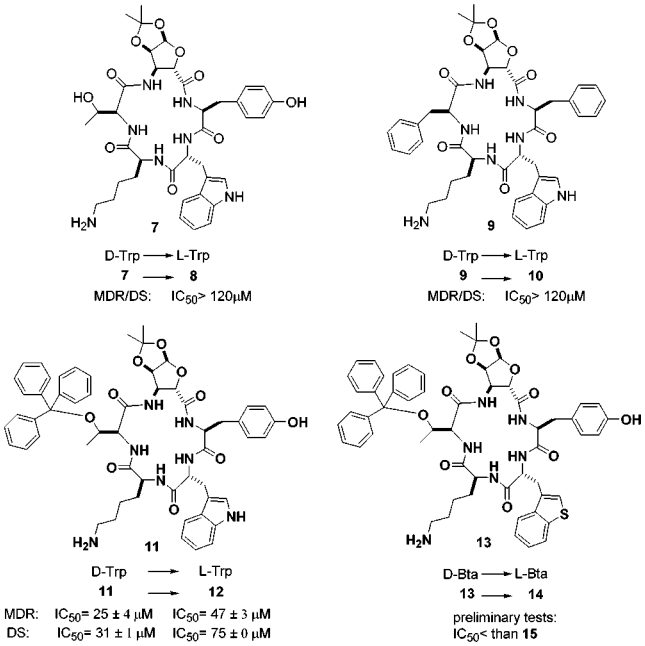


Figure 1. Structures and activities of compounds **7**−**14**. Cell lines used for IC_{50} determination. MDR: multidrug-resistant hepatoma cell line clone 2(10 × 80)T1.[9] DS: drug-sensitive hepatoma cell line clone 2.[10] Preliminary tests on A431[11] and HT-29[12] cell lines; **15** was internal reference for all tests: MDR 9 ± 0.5 μM; DS 10 ± 0.7 μM.

The peptides were assembled on solid phase on trityl chloride-polystyrene (TCP) resin. Standard Fmoc-protocol was employed, using 2 equiv of the amino acid, 2 equiv of

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HATU ([*O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate]), and 2 equiv HOAt (1-Hydroxy-7-azabenzotriazol) as coupling reagents and 2,4,6-collidine (20 equiv) as base.[13] Side-chain-unprotected tyrosine and tryptophane were used. Lysine was orthogonally protected with the hydrazine labile ivDde-protecting group (1-(4,4-dimethyl-2,6-dioxo-cyclo-hexylidene)3-methylbutyl).[14] After final Fmoc-deprotection the linear peptides were cleaved from the resin with hexafluoro 2-propanol (20% in dichloromethane, 3 × 20 min),[15] before being cyclized using DPPA (diphenylphosphoryl azide) and $NaHCO_3$ in DMF (0.1 mM).[16] Deprotection with 3% hydrazine in DMF led to the crude cyclic peptides. rp-HPLC purification yielded 8−41 mg of the cyclic compounds **7**−**14** in over 99% HPLC purity and good overall yields between 20% and 41%.

Antitumor activity of the TFA salts of the compounds **7**−**14** was tested on drug-sensitive rat hepatoma carcinoma cell line Clone 2^{10} and multidrug-resistant subclone Clone 2(10 × 80)T1.[9] The concentrations of compounds **7**−**14** that reduced the viability of the treated cells by 50% compared to controls (IC_{50} values) were determined from at least two independent experiments for each compound tested, using the XTT assay.[17] Compound **12** showed an IC_{50} of 75 μM for the drug-sensitive and 47 μM for the MDR cell line. Compound **11** containing D-Trp, was even more active with an IC_{50} of 31 μM for the drug-sensitive and 25 μM for the drug-resistant cell line. Compound **15**[1] was used as internal reference, with IC_{50} values of 10 μM for the drug-sensitive clone and 9 μM for the MDR one. Compounds **7** and **8**, with Thr in the Thr^{10} position, and compounds **9** and **10**, with the comparably small aromatic amino acid Phe in the 10 position, show IC_{50} values over 120 μM, whereas compounds **11** and **12**, with the big aromatic trityl ether in the corresponding position, show high activities.

The studies presented here demonstrate that a big aromatic residue in the Thr^{10} position seems to be imperative for high antiproliferative and apoptotic activity. Compounds **11** and **12** with IC_{50} values in the low μM range are very promising leads for potential chemotherapeutic drugs against multidrug-resistant hepatoma carcinoma. Preliminary results show that replacement of the D-Trp with D-Bta (D-benzothienylalanin) resulting in compound **13** or L-Bta resulting in **14** enhances activity. Those Bta-containing compounds are more active than **15**, the only other compound known to show apoptotic and antiproliferative activity against MDR carcinoma cell lines. Preliminary studies suggest that our compounds selectively exert their apoptotic and antiproliferative activity against cancer cells. The nonproteinogenic properties of SAAs should make the compounds physiologically more stable.

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Supporting Information Available: Characterization of key compounds and detailed descriptions of biological tests and cell lines. This material is available free of charge via the Internet at http://pubs.acs.org.

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