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A NON-PEPTIDE LIGAND FOR THE SOMATOSTATIN RECEPTOR HAVING A BENZODIAZEPINONE STRUCTURE

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Abstract: The 1,3,4-trisubstituted-1,4-benzodiazepin-2-one **2** is a non-peptide mimic of somatostatin **1**. It displaces the radioligand [^{125}I-Tyr^{3}]-octreotide with an IC_{50} of 7 μM.

Somatostatin (SRIF) is a cyclic peptide occuring in two major physiologically active forms (SRIF-14) **1** and SRIF-28 which are expressed in a tissue specific manner in several organs including small intesting, stomach, pancreas and brain. SRIF inhibits the release of many hormones such as growth hormone, insulin, gastrin, glucagon and it acts as a neurotransmitter in the brain.[1] Due to the rapid proteolytic degradation of somatostatin *in vivo* (plasma $T_{1/2}$< 3min), analogues with increased metabolic stability have been obtained which are highly useful for the treatment of various endocrine and malignant disorders.[2] In an attempt to obtain non-peptide mimetics of SRIF-14 **1**, compounds utilizing sugars as scaffolding and carrying the side chains of **1** important for biological activity have been reported.[3,4] These compounds have IC_{50} values of approximately 20 μM for the SRIF-receptor as measured by means of radioligand binding on rat cortex membranes.[4,5] The low affinity of these ligands might be attributed to the high number of degrees of rotational freedom and therefore to the large change in entropy needed to attain the "stacking" association between the indolyl and the aminoalkyl substituents thought to be necessary for binding.[6,7] In this paper, we report the design, synthesis, and receptor binding affinity of a new SRIF-mimetic **2** having restricted conformational freedom (Figure 1).

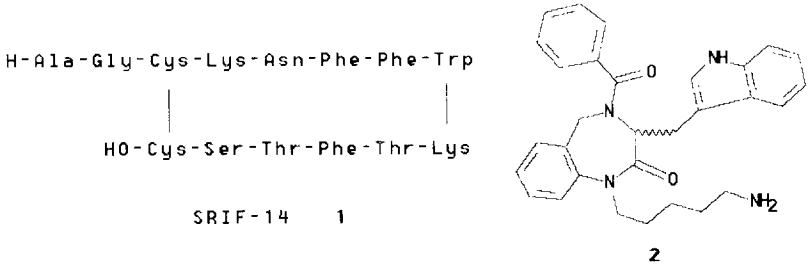


Figure 1 . Structures of SRIF-14 **1** and mimetic **2**

In the absence of experimental data about the bioactive conformation of somatostatin, ideally a receptor bound conformation of the peptide, we decided on rational de novo design. Our approach is based on SRIF's β-sheet bioactive conformation deduced empirically from structure-activity relationships[2,3,4,8] as well as molecular modelling studies where SRIF-14 was docked into its transmembrane receptor.[9] In addition, an X-ray crystallographic analysis of the synthetic somatostatin analogue octreotide showed a type-II' β-turn at D-Trp-Lys.[10] In order to minimize the loss of the binding energy due to entropic factors, we directed our efforts in finding a conformationally restricted organic template sitting within the backbone of the peptide and from which pharmacophores can radiate. Our attention was drawn to the 1,4-benzodiazepine skeleton because it binds several different peptidergic receptors depending on the substitution pattern and because of its inherent oral bioavailability. Potent ligands for the opiate[11], CCK[12], gastrin[13] and GPIIb/IIIa[14] receptors have been obtained on a benzodiazepine scaffold.

After extensive structure-based molecular modelling investigations,[15] the trisubstituted -1,4-benzodiazepin-2-one **2** was chosen since it offers three functional groups corresponding to Phe-Trp-Lys of the essential β-turn sequence of SRIF as well as a carbonyl mimicking the carbonyl of the amide bond between Trp and Lys. Moreover, the fused phenyl ring of the benzodiazepinone template may protrude partly into the pocket occupied by Phe[6] and Phe[11] of SRIF-14 (Figure 2).

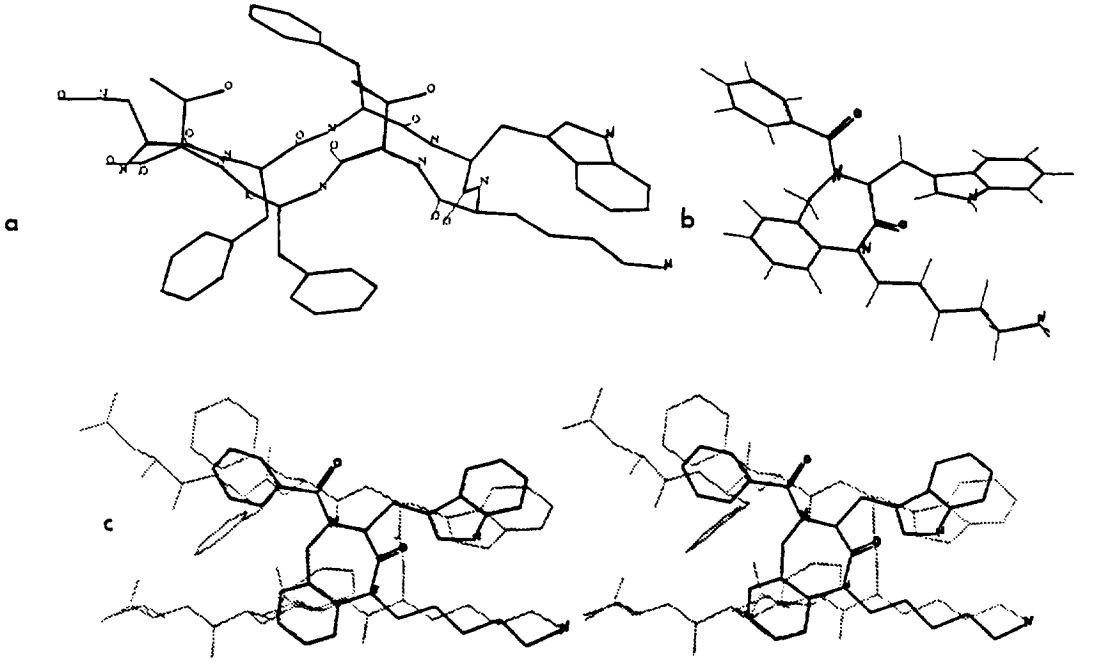
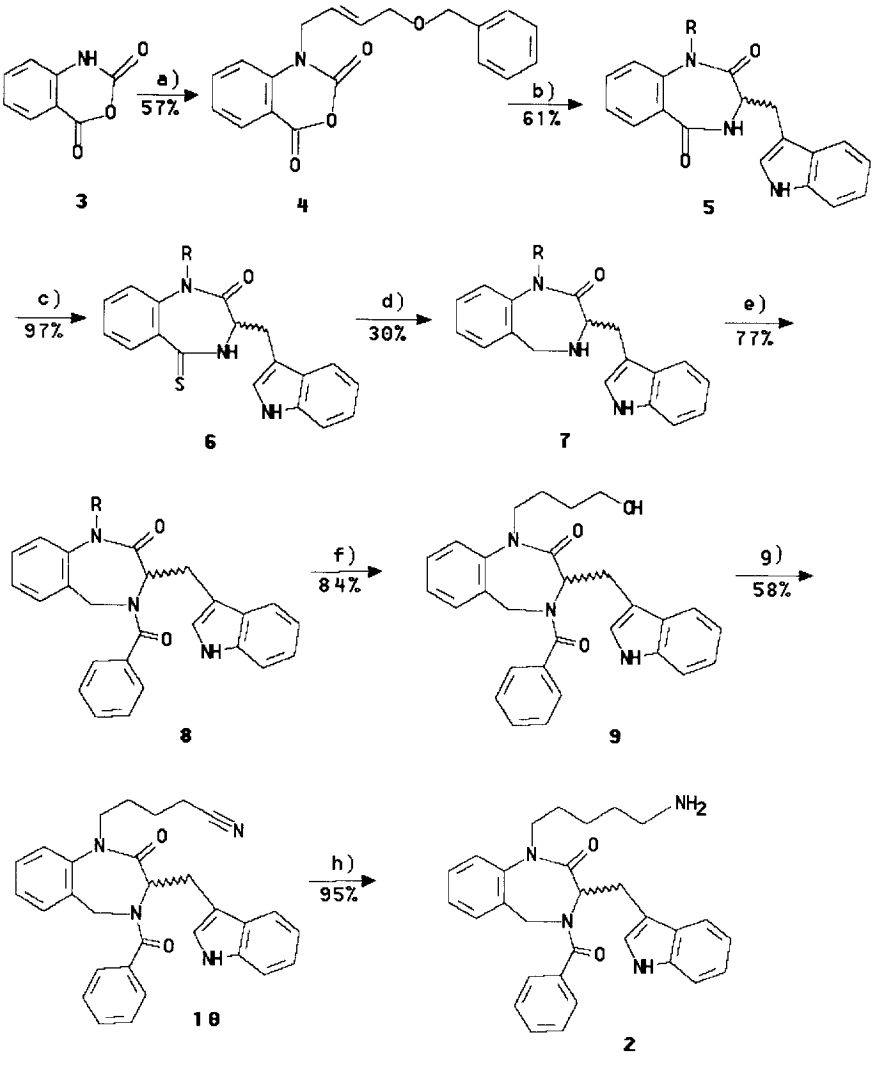


Figure 2. a)Conformation of the fragment 5-12 of SRIF-14; b)Conformation of the mimetic **2**; c)Stereoview of the superimposition of SRIF 5-12 and **2** .



SCHEME

a) NaH (1.1equiv.), $BrCH_2CH{=}CHCH_2OCH_2Ph$ **11** (1.5equiv.), DMF, 0°C ; b) D,L-Trp (1.1equiv.), AcOH, Δ, 4h ; c) Lawesson's reagent (0.55equiv.), PhMe, Δ, 3h ; d) Raney-Ni (excess), EtOH, Δ, overnight e) PhCOCl (1.5equiv.), CH_2Cl_2, 0°C ; f) H_2, 5% Pd/C (5%equiv.), MeOH, 2days ; g) CH_3SO_2Cl (4equiv.), pyridine, 3h then Bu_4NCN (1.1equiv.), THF, r.t.; h) Raney-Ni (excess), $MeOH/NH_3$, Δ, 3h.

Our synthetic strategy for preparing **2** was based on the premise that it could be derived via selective thionation of the benzodiazepine-1,5-dione **6** followed by desulfurization. Further retrosynthetic analysis[16] suggested that **6** could be simplified to substituted isatoic anhydride **3** and D,L-Trp (Scheme). Indeed, alkylation of the anion of isatoic anhydride **3** with the known allylic bromide **11**[17] led to **4** in 57% yield. Reaction of **4** with racemic tryptophan in refluxing acetic acid afforded 61% of key intermediate **5**. This diamide was treated with Lawesson's reagent in toluene at 80°C giving regioselectively and quantitatively the thionamide **6**. The regioselectivity of the thionation of diamides depends heavily on steric factors since an analogous benzodiazepinone led to a 1:1 mixture of thionamides.[18] Desulfurization with concomitant reduction of the olefin was brought about by the treatment of **6** with Raney nickel in refluxing ethanol. The modest yield of this reaction (30%) prompted us to examine the alternative $NaBH_4/NiCl_2$ dethionation system[19] but **7** was obtained again with the same 30% yield. With both methods extensive decomposition occurred. The remainder of the synthesis was accomplished via benzoylation of the basic nitrogen (77%) followed by a three step homologation of the butyloxybenzyl chain. The resulting cyanide **10** was cleanly reduced to the desired trisubstituted-1,4-benzodiazepin-2-one **2** by hydrogenation over Raney nickel in refluxing ethanol/ammonia solution.[20,21]

The binding affinity of **2** was measured by means of radioligand binding studies on rat cortex membrane using [^{125}I-Tyr^3]-octreotide ligand.[4] Complete displacement of the radioligand from its receptor occured at 10^{-4} μM and the calculated IC_{50} value was 7 μM (pK_i=5.1±0.08 n=4). The shape of the binding curve was consistent with competition for a single set of binding sites. Employing the above route, the optically active derivatives of **2** were synthesized starting from D- and L-Trp. The two isomers had comparable affinity for the somatostatin receptor with IC_{50} values of 8.2 μM (pK_i=5.1±0.01 n=3) and 6.5 μM (pK_i=5.2±0.05 n=4) for (+)-**2** and (-)-**2** respectively. The lack of significant difference in the affinity of the two enantiomers is in agreement with the observed affinity for D-Trp^8 and L-Trp^8 diastereomeric somatostatins (IC_{50}=0.43 nM and 0.43 nM respectively) and indicates that the indolyl substituent of mimetic **1** is indeed a replacement for Trp at the i+1 position of the β-turn.

A comparison of the IC_{50} values obtained with **2**, with those obtained for the D-glucose and D-xylose based mimetics (Figure 3) in the same binding assay,[4] shows that the 1,4-benzodiazepin-2-one has a two to three fold higher affinity (7 μM, 16 μM and 23 μM respectively). This can be attributed to the difference in the degrees of rotational freedom between the two substance classes. Importantly, **2** has higher affinity for the somatostatin receptor even though it lacks lipophilic substituents capable of filling the pocket normally occupied by Phe^6 and Phe^{11} of SRIF-14. These lipohilic substituents are already present in the sugar-based mimetics. A replacement for the two Phe moieties can potentially be obtained through the substitution of the aromatic nucleus of **2** with benzylic groups and may lead to highly active peptidomimetics.

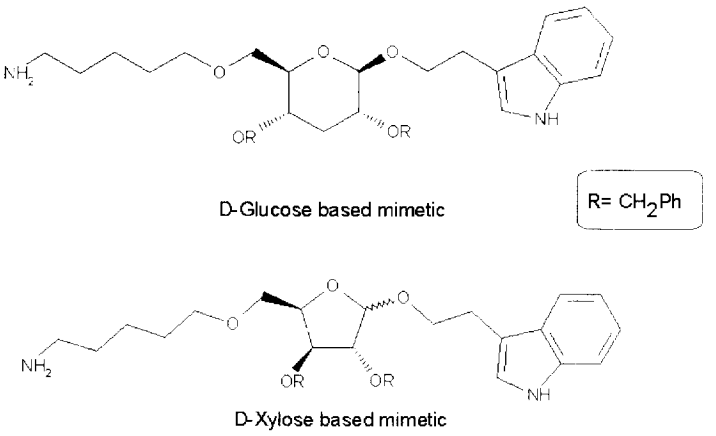


Figure 3. Structure of somatostatin peptidomimetics (references 3 and 4).

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20)Spectral data (^{1}H-NMR and FAB-MS) consistent with the structures were obtained for all compounds.

21)Compound **2**: ^{1}H-NMR (DMSO-d_6, D_2O, 120°C) : 1.43-1.78 (m, 8H), 2.80 (m, 2H), 3.62-3.94 (m, 2H), 4.35 and 4.85 (AB, 2H, J=12.5, J=5.8), 5.70 (dd, 2H, J=4.6, J=6.6), 6.78-7.10 (m, 9H), 10.38 (m,1H)

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