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Effects of the Incorporation of IBTM β-turn Mimetics Into the Dipeptoid CCK_1 Receptor Agonist PD 170292

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Abstract—Replacement of the 2-Adoc-D-αMeTrp residue in the non-selective CCK_1 receptor agonist PD 170292 by the Z-(2*R*,5*R*,11b*S*)-IBTM skeleton, able to fix a type II β-turn-like conformation, led to a conformationally restricted dipeptoid analogue, namely **3a**, which exhibited a notable increase in the CCK_1 selectivity and antagonist properties.

In order to overcome the number of deficiencies which limit the utility of peptides as drugs, considerable efforts have been devoted toward the development of non-peptide ligands for neuropeptide receptors. In the field of cholecystokinin (CCK), this research effort led to the discovery of a great number of non peptide antagonists for both CCK_1 and CCK_2 receptors.[1–3] However, there are relatively few reports in the literature of non peptide-based small molecules that are agonists at these receptor subtypes.[4,5] Thus, Semple et al. have described the discovery of two series of peptoids, derived from the structure of CCK_4, that mimic the actions of CCK at the CCK_2 receptors.[6] More recently, a series of compounds bearing 1-methylbenzimidazole-5,6-dicarboxylate and 1-methylindole-5,6-dicarboxylate as scaffolds have been described to show agonist actions which could be blocked by the CCK_2 antagonist L-365,260.[7] Among the non peptide CCK_1 receptor agonists we can highlight two main families: the benzodiazepine-based compounds developed by Glaxo-Wellcome[8] and the dipeptoid hybrids mainly disclosed by Parke-Davis.[9–12] The approach for the dipeptoid agonists was based upon appending phenylurea-substituted Lys residues, the key feature of a series of peptide CCK_1 receptor selective agonists onto different peptoid motifs.[13] Within these peptoid CCK_1 agonists, PD 170292 (**1**) behaves as a potent agonist at the high affinity and as antagonist at the low affinity CCK_1 binding sites.[12] However, this derivative shows lack of selectivity, and it has also been shown to be a high affinity CCK_2 receptor antagonist (Fig. 1). [12]

We have recently demonstrated that replacement of the α-MeTrp residue of both CCK_1 and CCK_2 selective dipeptoid antagonists with type II, and specially type II′ β-turn mimetics, namely (2*R*,5*R*,11b*S*)- and (2*S*,5*S*,11b*R*)-2-amino-3-oxohexahydroindolizino[8,7-*b*]indole-5-carboxylate [(2*R*,5*R*,11b*S*)- and (2*S*,5*S*,11b*R*)-IBTM],[14,15] respectively, led to constrained dipeptoid antagonists **2a** and **2b** with improved CCK_1 selectivity. This led us to hypothesize that β-turn like conformations could facilitate the binding of dipeptoids to CCK_1 receptors. In order to gain further insight into the utility of the IBTM skeletons for getting selective CCK_1 ligands, we studied the effects of the incorporation of the (2*R*,5*R*,11b*S*)- and (2*S*,5*S*,11b*R*)-IBTM frameworks into the structure of the non selective dipeptoid agonist PD 170292. This paper deals with the synthesis, conformational behaviour in solution, and biological profile of the new conformationally constrained hybrid dipeptoid analogues **3a** and **3b**.

The synthesis of compound **3a** and **3b** involved the condensation of Z-IBTM derivatives **4a** and **4b**[16–18] with H-βHly(Tac)-TMSE,[12] previously prepared, followed by removal of the C-terminal carboxylate protecting group under acidic conditions (Scheme 1).[19]

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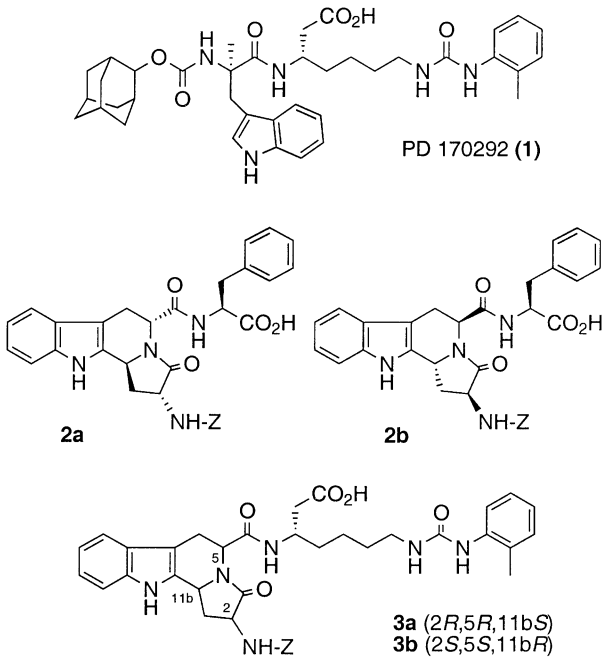
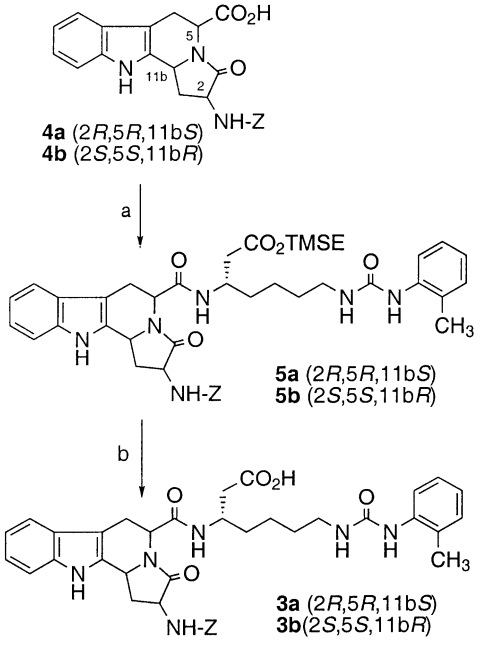


Fig. 1. Structure of the peptoid agonist PD 170292, the β-turned dipeptoid antagonists **2**, and the novel designed hybrids **3**.



Scheme 1. Reagents and conditions: (a) H-βHLy(Tac)-TMSE, BOP, Et_3N, CH_2Cl_2; (b) TFA, CH_2Cl_2. (TMSE: trimethylsilylethyl).

Table 1. Temperature coefficients for amide protons in compounds **3a** and **3b** [$\Delta\delta/\Delta T$ (10^{-3} ppm/K)][a]

Compd	2-NH	1′-NH
3a	−4.76	−0.20
3b	−4.44	+3.16

[a]Determined by least-squares linear regression analysis from measurements over the temperature range 30–60 °C (seven points), DMSO-d_6.

The ability of compounds **3a** and **3b** to adopt β-turn like conformations in solution was evaluated by ^{1}H NMR. The spectra of both compounds showed no coupling between the downfield H-6 proton and the vicinal H-5 proton of the IBTM moiety. This agrees with an axial disposition of the 5-carboxylate group, as required for the adoption of β-turn conformations in IBTM-containing analogues.[17,18]

It has been described that the temperature coefficients of the amide protons indicate their accessibility to solvent. Thus, small absolute values ($\Delta\delta/\Delta T \leq 3 \leq 10^{-3}$ ppm/K) indicate their protection from solvent, whereas values higher than 4×10^{-3} ppm/K indicate exposure to the solvent.[20] The values of the temperature coefficients for the 2-NH and 1′-NH amide protons of compounds **3a** and **3b** are shown in Table 1. These values indicated that the 2-NH protons of both derivatives were accessible to solvent, whereas the 1′-NH proton of compound **3a** showed a very small variation, indicative of the existence of a hydrogen-bonded type II β-turn conformation. On the other hand, the value of the $\Delta\delta/\Delta T$ for the 1′-NH proton in compound **3b** is not conclusive regarding the existence or not of a β-turn type II′.

The restricted dipeptoid analogues **3a** and **3b** were evaluated for their ability to displace [^{3}H]propionyl-CCK_8 from rat pancreatic (CCK_1) and cerebral cortex homogenates (CCK_2) following a previously described procedure.[21] Binding affinities are depicted in Table 2, along with those for IBTM-dipeptoid CCK_1 antagonists **2a** and **2b**, and those described for the model dipeptoid CCK_1 agonist PD 170292.[12]

The substitution of the α-MeTrp residue of dipeptoid PD 170292 by the β-turn type II mimetic, as in compound **3a**, reduced by almost three orders of magnitude the affinity for CCK_2 receptors relative to the parent dipeptoid **1**, whereas the incorporation of the type II′ β-turn mimetic, as in **3b**, was not tolerated at all by the CCK_2 binding sites. As shown, these results are in agreement with those obtained for the constraint dipeptoid analogues **2**.[15] At the CCK_1 receptors, compound **3a** showed similar binding affinity to that previously described for PD 170292, but a decrease by two orders of magnitude in the affinity of compound **3b** was observed with respect to compounds **1** and **3a**. We can

Table 2. Inhibition of the [3H]pCCK_8-specific binding to rat pancreas (CCK_1) and cerebral cortex membranes (CCK_2) by IBTM dipeptoid analogues **3a** and **3b**

Compd	CCK_1 IC_{50} (nM)[a]	CCK_2 IC_{50} (nM)[a]
PD170292 (**1**)[b]	1.2	6.7
2a[c]	1.7±0.3	202.0±99.0
2b[c]	4.7±1.3	>10,000
3a	8.2±2.1	5799±1376
3b	324.2±82.4	>10,000

[a]Values are the mean±SEM of at least three experiments, performed with seven concentrations of test compounds in duplicate.
[b]From ref 12.
[c]From ref 15.

speculate that the different affinities found for compounds **3a** and **3b** could be related to the difference in the ability of these derivatives to adopt β-turn like conformations. In fact, constrained dipeptoids **2a**, **2b** and **3a**, all able to adopt β-turn secondary structures, showed nanomolar affinities at the CCK_1 receptors, while compound **3b**, for which the ^1H NMR conformational analysis indicated a diminished ability to fix the corresponding hydrogen bonded β-turn conformation, is unable to efficiently interact with the CCK_1 receptors. Compared to the corresponding dipeptoid **2b**, with a Phe residue, it seems that the incorporation of the βHly(Tac) residue into **3b** disrupts to some extent the ability of type II′ IBTM β-turn mimetic to adopt the expected β-turn-like conformation. On the whole, these new biological data support our previous hypothesis of the preference of β-turn like conformations during the binding of dipeptoids to CCK_1 receptors, whereas this secondary structure is not appropriate to interact with the CCK_2 receptor binding sites. Therefore, the IBTM-containing dipeptoid analogues are, in general, more selective ligands toward CCK_1 receptors than the parent dipeptoids, for which the higher conformational flexibility could facilitate the adoption of suitable conformations for binding to both CCK_1 and CCK_2 receptors binding sites.

The functional activity of derivative **3a** was evaluated in the amylase release assay.[22] This compound was able to antagonize the CCK_8 induced amylase secretion from pancreatic acini with a IC_{50} value of 196 nM, while it did not show any intrinsic effect on the amylase release at a 1μM concentration. Therefore, compound **3a** is a new potent and selective CCK_1 receptor antagonist. A possible explanation for the change in the functional activity of **3a** when compared to PD 170292 could be the lower conformational flexibility of the new restricted dipeptoid that would hinder the side chain of the homolysine from adopting the precise orientation required for receptor activation. In this sense, previous works on peptide agonists have shown a dependence of the activity on the length of the methylene bridge between the urea moiety and the peptoid backbone. Thus, the substitution of the Lys residue in A-71623 with ornithine and homolysine, shortened and lengthened side chain analogues, respectively, led to a loss in the pancreatic affinity, although they maintained certain ability to stimulate pancreatic amylase release.[13b] However, the substitution with aromatic linkers produced moderately potent CCK_1 antagonist.[23] On the other hand, it has been described that seemingly minor structural changes in different families of non peptide CCK ligands give rise to fundamental changes in the nature of the ligand, converting antagonists to agonists, and vice versa.[7,8]

In conclusion, the incorporation of an IBTM type II β-turn mimetic into the structure of the non selective dipeptoid CCK_1 agonist PD 170292 led to the constrained analogue **3a**, that maintains the nanomolar affinity at the CCK_1 receptors, but notably increased its selectivity. This increase in selectivity is accompanied by a change in the functional activity of compound **3a**, that behaves as an antagonist in the amylase release assay. These results might be considered as an additional indication that a β-turn-like conformation within the dipeptoid tridimensional structure could favour the CCK_1 receptor recognition, while the reversal in the expression of efficacy could be related to a reduced mobility of the βHly(Tac) side chain in **3a** with respect to PD 170292.

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19. Significant analytical and spectroscopic data: Compound **3a**: HPLC: $t_R = 6.58$ min. CH_3CN/H_2O (0.05% TFA) 40/60. ^1H NMR (300 Mhz, DMSO-d_6): δ 11.01 (s, 1H, NH^i), 8.35 (d, 1H, 2-NH, $J = 7.7$ Hz), 7.81 (m, 1H, α-NH), 7.58 (s, 1H, NHAr), 7.81–6.80 (m, 13 H, Ph, Tol, In), 6.51 (t, 1H, ε-NH, $J = 5.19$ Hz), 5.15 (t, 1H, H-11b, $J = 7.45$ Hz), 5.08 [m, 2H, CH_2(Z)], 4.97 (d, 1H, H-5, $J = 7.45$ Hz), 4.02 (m, 2H, H-2, α-Lys), 3.43 (d, 1H, H-6, $J = 15.13$ Hz), 2.96 (m, 2H, ε-Lys), 2.78 (m, 1H, H-6), 2.35 (m, 2H, H-1), 2.22 (m, 2H, β′-Lys), 2.14 [s, 3H, CH_3(Tol)], 1.48–1.15 (m, 6H, β-Lys, γ-Lys, δ-Lys). Anal. calcd for $C_{38}H_{42}N_6O_7$: C, 65.69; H, 6.09; N, 12.10. Found: C, 65.37; H, 6.25; N, 12.05. Compound **3b**: HPLC: $t_R = 7.11$ min. CH_3CN/H_2O (0.05% TFA) 40/60. ^1H NMR (300 Mhz, DMSO-d_6): δ 11.03 (s, 1H, NH^i), 8.27 (d, 1H, 2-NH, $J = 7.4$ Hz), 7.76 (m, 1H, α-NH), 7.62 (s, 1H, NHAr), 7.78–6.81 (m, 13 H, Ph, Tol, In), 6.55 (t, 1H, ε-NH, $J = 5.37$ Hz), 5.17 (m, 1H, H-11b), 5.09 [m, 2H, CH_2(Z)], 4.95 (d, 1H, H-5, $J = 7.2$ Hz), 4.01 (m, 2H, H-2, α-Lys), 3.45 (m, 1H, H-6), 2.95–2.76 (m, 3H, H-6, ε-Lys), 2.42–2.16 (m, 4H, H-1, β′-Lys), 2.13 [s, 3H, CH_3(Tol)], 1.52–1.22 (m, 4H, β-Lys, δ-Lys), 1.10 (m, 2H, γ-Lys). Anal. calcd for $C_{38}H_{42}N_6O_7$: C, 65.69; H, 6.09; N, 12.10. Found: C, 65.41; H, 6.29; N, 11.94.
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