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Communications to the Editor

A New Class of Non-peptidic Cholecystokinin-B/Gastrin Receptor Antagonists Based on Dibenzobicyclo[2.2.2]octane

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There have been a number of recent reports about compounds which are antagonists at cholecystokinin-B (CCK_B)/gastrin receptors.[1] Some of these were found as a result of optimizing leads generated from broadly based cross-screening exercises.[2-5] Other approaches have concentrated on working from the amino-acid sequence of tetragastrin, the minimum active fragment of the hormone gastrin,[6] and have led to a number of potent compounds,[7,8] but some of these have been prone to retain some of the agonist features of the parent hormone when examined in certain assays.[9,10] Here we wish to report our discovery of a new series of potent, selective, non-peptide CCK_B/gastrin receptor antagonists.

Peptidomimetic approaches to provide antagonists for peptide hormone receptors are being used increasingly to overcome problems such as poor oral bioavailability which are associated with the use of peptides as therapeutic agents.[11] In the absence of structural information regarding the receptor, our approach to the search for CCK_B/gastrin receptor antagonists was to start from a model shape of the tetragastrin molecule derived from molecular mechanics calculations and constrained by the use of some data from fluorescence studies.[12] From this basis, we proposed that BOC-tetragastrin could exist in a 3_{10} helix with the two aromatic rings of the tryptophan and the phenylalanine side chains interacting in a π-stacking arrangement with a separation of 5–7 Å. The electrostatic field of the molecule in this conformation was essentially dipolar. Therefore, we decided to look for rigid skeletons that could be used to replace the peptide backbone of tetragastrin while maintaining the stereoelectronic features outlined above.

We considered a number of possibilities but met with little success until we focused on the dibenzobicyclo-[2.2.2]octane (BCO) skeleton and designed the inital targets of general formula **1** (Scheme **1**). In this, the fused aromatic ring, endo to the nonfused substituents, represented one of the two peptide side chain aromatic groups. The second aryl substituent was introduced as part of substituent X. These kinds of structures, when examined by molecular modeling, had dipolar electrostatic fields which were dominated by the carboxylic acid and the amide bond in the molecule. Hence, they satisfied the stereoelectronic requirements for the putative pharmacophore of tetragastrin.

Scheme **1** shows the preparation of a number of examples of this type of molecule (representative examples are compounds **1a–d** in Table **1**) in which the nature of the aryl group and the length of the chain linking it to the rest of the molecule were varied. These compounds were made in two steps starting with a Diels–Alder reaction between anthracene and maleic anhydride[13] followed by ring opening of the anhydride with the appropriate amine. Given the well-documented congruence in the behavior of ligands at CCK_B/gastrin receptors located in the periphery and the CNS, our minimum screening tactic was to look for affinity in a CCK_B radioligand binding assay.[14] As shown in Table **1**, compounds **1a–d** have low affinity at CCK_B receptors in mouse cortical membranes. However, these compounds were inactive in a functional gastric acid secretion assay involving the isolated, immature, lumen-perfused rat stomach.[15] Evidently the compounds were not sufficiently soluble in 1:400 DMSO/buffer, pH 7.4 (limit of their solubility, $\sim 1 \times 10^{-5}$ M), to enable the bath concentrations to be achieved which would have been required to show significant inhibition of pentagastrin-stimulated acid secretion in this assay.

Further progress was made when the side chain aromatic group was changed for an alicyclic group. This decision was based on a number of considerations, principally that it is known[16] that an alicyclic group,

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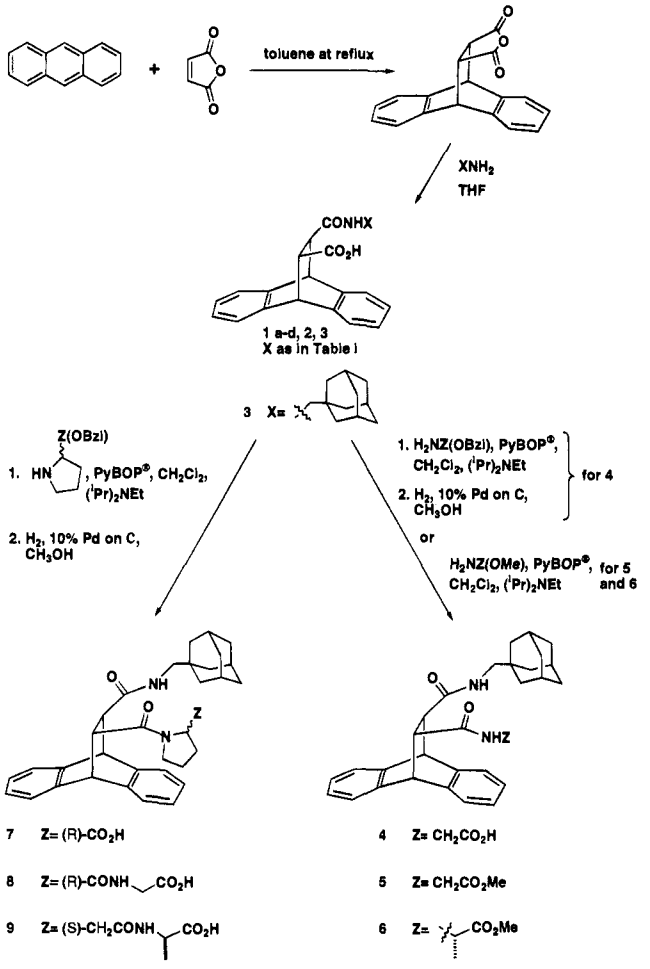
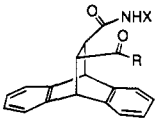
Scheme 1

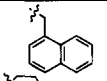
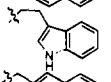
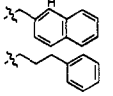
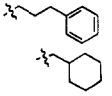
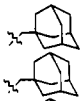


such as cyclohexyl, can replace the aromatic group of phenylalanine in BOC-tetragastrin without any apparent loss of activity. In our series, the cyclohexylmethyl analogue **2** showed a similar affinity to some of the aromatic derivatives in the CCK_B assay, and in addition, this material was sufficiently soluble, under the immature rat stomach assay conditions, to show some modest activity. The 1-adamantylmethyl analogue **3** expressed higher affinity, showing sub-micromolar potency at the CCK_B receptor. Thus it was clear that the integrity of the active structure could be maintained by other interactions (such as hydrogen bonds) in the absence of π stacking.

A number of logical changes to compound **3** were now made, of which the most successful involved derivatization of the acid group. Glycine and its methyl ester were introduced as shown in Scheme 1. The resulting compounds, **4** and **5**, showed no appreciable change in activity relative to compound **3**. Constraining the polar function of the glycine-extended moieties by use of L- and D-proline and L- and D-alanine, together with their methyl esters, yielded the more potent L-alanine methyl ester **6** and the D-proline carboxylic acid derivative **7**. In both cases, the CCK_B/gastrin receptor affinity, as measured in the functional assay, increased with the affinity at cholecystokinin-A (CCK_A) receptors, as judged in a guinea-pig pancreatic radioligand binding assay,[17] staying at the low values found for the majority of compounds in this series. Thus the CCK_B/gastrin receptor selectivity for these more potent compounds was of the order of 30-fold. The similarity in the behavior of these two compounds was rather surprising and could not be readily explained.

Table 1. Receptor Affinity Values for CCK_B/Gastrin Antagonists



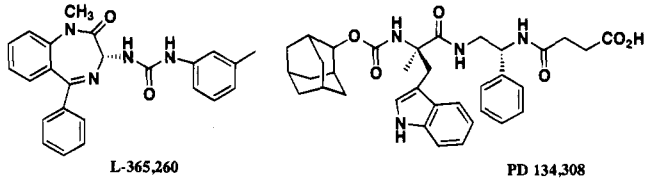
no.[a]	X	R	CCK_B/gastrin functional[b,c]	CCK_B/gastrin binding[d]	CCK_A[e,f,g]
1a		OH	IA (1.10^{-5}M)	5.60±0.07	NT
1b		OH	IA (1.10^{-5}M)	5.12±0.04	NT
1c		OH	IA (1.10^{-5}M)	5.33±0.04	NT
1d		OH	IA (1.10^{-5}M)	5.05±0.07	NT
2		OH	5.43±0.27	5.00±0.07	5.07±0.05
3		OH	5.85±0.05	6.27±0.08	5.08±0.12
4		$NHCH_2CO_2H$	5.62±0.25	5.72±0.07	4.69±0.08
5		$NHCH_2CO_2Me$	5.71±0.41	5.81±0.13	4.70
6		HN CO_2Me	6.51±0.18	6.72±0.15	<5.0[h]
7		N CO_2H	6.44±0.28	6.05±0.03	5.09±0.13
8		N H CO_2H O	6.90±0.18	6.67±0.05	4.83±0.11
9[i]		N O H CO_2H	7.08±0.13	6.82±0.11	4.89
10[i]		N O H CO_2H	7.25±0.22	7.39±0.14	4.62
11[i]		N O H CO_2H	6.91±0.29	6.11±0.10	5.13
L-365260			7.61±0.12	8.43±0.09	6.33±0.11

[a] Compounds **3**–**4** and **7**–**11** were tested as their *N*-methyl-D-glucamine salts. All new compounds gave satisfactory microanalytical and ^{1}H NMR data. [b] $pK_B \pm$ SEM values were estimated from single shifts of pentagastrin concentration–effect curves in the isolated, lumen-perfused immature rat stomach. [c] IA indicates an inactive compound at the concentration stated in parentheses. [d] $pK_i \pm$ SEM competition with 20 pM [^{125}I]BH-CCK-8S for CCK_B binding sites in mouse cortical homogenates from at least three separate experiments. [e] $pK_i \pm$ SEM competition with 20 pM [^{125}I]BH-CCK-8S at CCK_A binding sites on guinea-pig pancreatic cells from at least three separate experiments. [f] Values without SEM were obtained from one to two separate experiments. [g] NT indicates that the compound was not tested in this assay. [h] This compound gave 37% inhibition at 1×10^{-5} M, the highest soluble dose under assay conditions. [i] Compound **9** is a mixture of diastereomers of which compound **10** is the more polar diastereomer by normal phase TLC and compound **11** the less polar diastereomer.

When the carboxylic acid of compound **7** was coupled with glycine, the resulting compound **8** had greater affinity at CCK_B/gastrin receptors ($pK_B \simeq 7.0$) and was about 100-fold selective for these receptors over CCK_A receptors. Some improvements were made to the potency and selectivity of compound **8**, by making a series of systematic changes to the structure. This resulted in compound **9** in which the D-proline had been replaced by an L-(carbonylmethyl)pyrrolidine group and the glycine replaced by D-alanine.

The homologation strategy which led to compounds **4** and **5** and later to **8** and **9** was prompted in part by the work of Parke-Davis[18] and Rorer[19] who both showed that molecules could be made more potent at CCK_B/

Chart 1. Structures of L-365,260 and PD 134,308



L-365,260 PD 134,308

gastrin receptors by adding appropriately located carboxylic acids.

Compounds **1**–**5** in Table 1 are all mixtures of enantiomers, whereas compounds **6**–**9** are all mixtures of diastereomers. We found that the diastereomers could be separated when required, either at the final step by HPLC, or by recrystallization of a suitable intermediate such as the benzyl ester. Compounds **10** and **11** are the individual diastereomers of compound **9**, and their diastereomeric purity, when assessed by ^{1}H NMR and HPLC, was greater than 90%. As can be seen from the data, the majority of the CCK_B/gastrin activity appears to be present in one diastereomer, namely compound **10**, the more polar of the two compounds. However, it has not yet been possible to make an assignment of the absolute stereochemistry of this material.

The model used as the initial stimulus for our thinking has now been significantly modified in light of our SAR studies. In summary, we now believe that the important features of our molecules are the adamantyl group which finds a hydrophobic pocket in the receptor, as well as the presence of a correctly positioned polar group, most usually a carboxylic acid. The BCO framework is responsible for holding these important functional groups in a bioactive orientation at the receptor as well as for contributing some binding affinity of its own. These features are also reflected in the structure of the peptoid CCK_B/gastrin ligand PD 134,308 which relies on hydrogen bonding to hold the backbone rigid and thus the important functional elements in the correct spatial disposition. The evolution of this model and our model for BOC-tetragastrin will be discussed in more detail in forthcoming publications.

In conclusion, compound **10** is one of a series of potent, non-peptidic CCK_B/gastrin receptor ligands, based on the rigid BCO skeleton, which exhibits a 500-fold selectivity for these receptors over CCK_A receptors. It is comparable in its potency as an antagonist to the CCK_B/gastrin ligand L-365,260 which, both in our hands and in the literature,[20] has expressed a pK_B value of about 7.5 in the isolated, immature rat stomach assay, although in the cerebral cortex binding assay the Merck ligand is still some 10-fold more potent. In contrast to our compound, L-365,260 is significantly less selective for CCK_B/gastrin receptors over CCK_A receptors. The synthetic routes to our compounds are extremely concise, relatively simple, and flexible. Further details of structure–activity relationships and *in vivo* studies relating to these ligands will be reported in due course.

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