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NOVEL 1,5-BENZODIAZEPINDIONE GASTRIN/CCK$_B$ ANTAGONISTS

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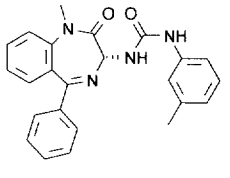
Abstract: The development of a potent, selective and orally bioavailable 1,5-benzodiazepindione gastrin/CCK$_B$ receptor antagonist (compound **7**, GR199114X) is described.

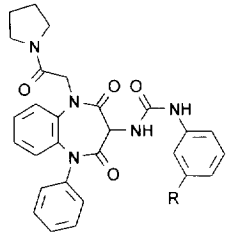
In seeking a novel mechanism for the control of gastric acid secretion we have focused on the peptide hormone gastrin which has a central role in the physiological cascade. It is thought that by blocking the gastrin receptor it may be possible to inhibit acid secretion profoundly without causing an increase in mucosal growth. Studies in the rat and dog have shown that inactivation of gastrin by antibodies results in a marked reduction of acid secretion induced by a range of physiological stimuli.[1]

Gastrin is a member of the family of peptides of which cholecystokinin, or CCK, (in various forms) is the most important in terms of its distribution as a neurotransmitter throughout the mammalian body. Indeed, the sulfated octapeptide (sCCK-8) is the most abundant neuropeptide in the central nervous system. In the periphery, CCK co-ordinates digestive function in the upper gastrointestinal tract mediating inhibition of gastric emptying, increased pancreatic secretion and contraction of the gall bladder. To avoid adverse effects with a gastrin receptor antagonist, it is necessary to find a compound which shows selectivity for the acid secretion pathway relative to these other physiological processes. There is evidence for at least two classes of CCK receptor;[2] the CCK$_A$ receptor is found mainly in the gallbladder, gastrointestinal tract, and discrete regions of the CNS. The gastrin/CCK$_B$ receptor is found in the gastrointestinal tract and is widespread in the CNS.

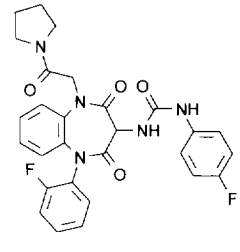
In 1989 Bock and colleagues published their work on the 1,4-benzodiazepinone CCK$_B$ antagonist L-365,260 (**1**) which was developed following the discovery of the natural product asperlicin.[3] The researchers working on the gastrin antagonist program, already running at Glaxo, decided to investigate further the use of 1,5-benzodiazepindione based ligands. The discovery of potent, selective and orally bioavailable 1,5-benzodiazepindione gastrin/CCK$_B$ antagonists forms the subject of this communication.



L-365,260 (**1**)



R = H, **2**; CO_2H, **3**; 5-Tetrazole, **4**



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One of the early compounds to emerge from the program was the 1,5-benzodiazepindione **2**. This compound was shown to be significantly more active than **1** at blocking pentagastrin stimulated acid secretion from isolated rat gastric mucosa (RGM)[4] (see Table 1). Furthermore **2** showed significant selectivity for the rat gastrin receptor over the guinea-pig CCK_A receptor as determined in the guinea-pig isolated ileum longitudinal muscle-myenteric plexus (GPI) assay.[4]

Table 1

Compound	RGM (pK_B)	GPI (pK_B)
1	7.6 (n=12)	7.7 (n=12)
2	8.5 (n=2)	7.3 (n=2)
3	8.6 (n=2)	5.7 (n=2)
4	10.3* (n=2)	7.4 (n=2)
5	8.6 (n=2)	6.8 (n=2)

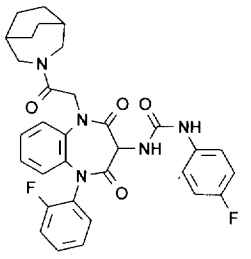
* Non-competitive inhibitor which may lead to an overestimate of pK_B

Further analogues were made in order to improve potency and selectivity. An area of the molecule that came under particular scrutiny was substitution on the phenyl ring of the urea. It was found that the ring was generally tolerant to substitution especially in the meta position.[5] However, particularly interesting, due to the enhanced levels of selectivity for the CCK_B receptor, were acidic substituents such as carboxylic acid **3** and tetrazole **4**. The tetrazole substituted compound **4** was both more selective and more potent (Table 1) than our lead **2** and merited further study. We examined the ability of **4** to inhibit acid secretion in the pentagastrin stimulated gastric fistula rat model,[6] by both *iv* and *ig* (*intra gastric*) administration routes. When introduced *iv* the tetrazole **4** is highly efficacious at blocking acid production (79% inhibition at 0.1 mg/kg) however, its relatively poor *ig* performance (45% inhibition at 1 mg/kg) suggests poor absorption and/or substantial first pass metabolism.

Further work with acidic compounds similar to **4** showed that despite excellent potency we could not achieve the *ig/iv* ratio (ratio of ED_{50}s) that we were seeking. In order to address this general problem we reasoned that absorption may be improved by removing the polar and ionisable tetrazole. At the same time we wished to block potential sites of metabolism. It is reported in the literature that some anxiolytic benzodiazepines are hydroxylated on the aromatic rings[7] and therefore fluorine substitution was incorporated to help deflect this route of clearance. The difluorinated compound **5** proved to be as potent as **2** and slightly more selective. On progression to the *in vivo* gastric fistula rat model **5** showed good activity *iv* (86% inhibition at 1 mg/kg) and, more importantly, was almost equi-effective when administered *ig* (73% inhibition at 1 mg/kg). With such an encouraging *ig/iv* ratio this compound was studied pharmacokinetically in the rat.[8] Gratifyingly, the tactics proved fruitful with **5** showing a very good level of oral bioavailability (F 68%) together with low plasma clearance (Clp 15 ml/min /kg).

Soon after this, work in molecular biology lead to the development of CCK_A and CCK_B binding assays based on the human receptor. The human CCK_B receptor was isolated from a human temporal cortex cDNA library and stably transfected into a HeLa cell line.[9] The human CCK_A receptor was isolated from a human gallbladder cDNA library[10] and transiently transfected into a COSM6 cell line.

Compound **5** was retested in the new human screen[11] and the results showed a decrease of both potency and selectivity (Table 2). This led us to explore further modifications of both the N-1 and N-5 substituents in order to correct these new found deficiencies.



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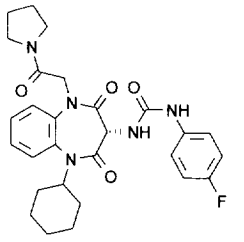

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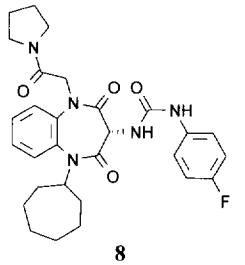

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Table 2

Compound	CCK_B (pK_i)*	CCK_A (pK_i)*	F (%)†	Clp (ml/min/kg)†
(±)-5	7.9 (n=1)	7.3 (n=1)	68	15
(±)-6	9.4 (n=1)	8.0 (n=1)	7	28
(*S*)-7	8.6 (n=9)	7.1 (n=6)	23	36
(*S*)-8	8.8 (n=1)	7.1 (n=1)	<10	ND

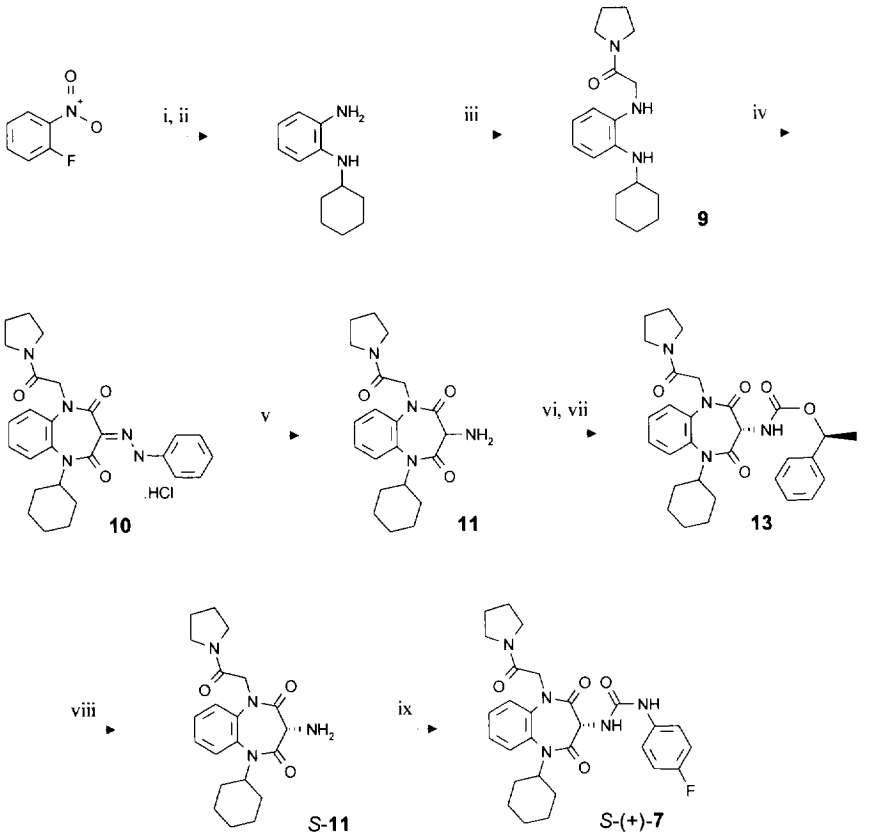
* Human receptor † Pharmacokinetics in rat

A wide range of N-1 amide analogues were made *inter alia*: 3,3-dimethylpiperidine, 2,6-dimethylpiperidine, 4-piperidinylpiperidine, 4-hydroxypiperidine, 2,6-dimethylmorpholine, homopiperidine, 2-phenylpyrrolidine, and it became clear that the receptor pocket occupied by this fragment of the molecule is essentially lipophilic in nature. 7-Membered ring and bicyclic amides were among the best substituents in terms of potency.[12] The [3.3.2]-azabicyclononyl amide group (as seen in **6**) resulted in a 30 fold increase in potency with respect to the pyrrolidinyl amide **5**. However, the oral bioavailability of **6** is low (7%). As the systemic plasma clearance is moderate (28 ml/min/kg), it is likely that the low bioavailability is in part due to poor absorption.

A similar range of substituents at N-5 revealed a broadly similar pattern of activity; polar functionality (eg alcohol, ether) led to a loss of potency whereas lipophilic substituents led to gains in binding affinity. Thus saturation of the N-5 phenyl ring led to the cyclohexyl substituted compound **7** which was resolved into its separate enantiomers to improve selectivity for the CCK_B receptor. Clearly, the cyclohexyl group at N-5 leads to a useful increase in both potency and selectivity[13] (over **5**). On progression to the *in vivo* pharmacodynamic model **7** showed a very good level of activity both *iv* (60% inhibition at 0.3 mg/kg) and *ig* (67% inhibition at 0.3 mg/kg). With an *ig/iv* ratio of 100% **7** was quickly progressed to pharmacokinetic studies in the rat. These showed a diminution of bioavailability and metabolic stability with respect to **5** (Table 2) but the levels were still very encouraging.

To see if we could increase potency and selectivity still further the N-5 cycloheptyl compound **8** was prepared, again in enantiomerically pure form. This compound showed no significant increase in potency and selectivity but was evaluated in pharmacokinetics. As seen with **6** (and to a lesser extent **7**) by increasing lipophilicity

bioavailability was reduced. A large number of compounds were made in this area and it became clear that **7** represented the best compromise between potency and selectivity on the one hand and pharmacokinetic properties on the other.



(i) Cylcohexylamine, 97%; (ii) H_2 Pd/C 100%; (iii) $BrCH_2CON(CH_2)_4$, K_2CO_3, DMF, 74%; (iv) $PhNHNC(COCl)_2$, THF, 93%; (v) Zn, glacial acetic acid, 81%; (vi) (*S*)-$PhCH(Me)OCO_2$(*p*-NO_2)Ph, **12**, 92%; (vii) Chromatography, 40-45%; (viii) H_2, Pd/C, 89%; (ix) *p*-fluorophenylisocyanate, 82%.

The chemistry to prepare **7** and its analogues has been developed over several years and now represents an efficient route to these types of structures. 2-Fluoronitrobenzene was heated with an excess of cyclohexylamine in chloroform to give an excellent yield of recrystallised N-cyclohexyl-2-nitroaniline as bright orange plates. Catalytic hydrogenation gave a quantitative yield of N-cyclohexylphenylenediamine which was selectively alkylated on the primary nitrogen with bromoacetylpyrrolidine (itself prepared in 84% yield from pyrrolidine and bromoacetylbromide). The pure monoalkylated aniline was isolated by a single recrystallisation from hexane/*tert*-butylmethyl ether in 74% yield. The known phenylhydrazone of malonyl dichloride (a yellow crystalline solid, prepared from the diacid and phosphorous pentachloride in around 70% yield) has proved to be a very convenient source of protected aminomalonate. Simply mixing the aniline **9** with the acid chloride in THF gave the bisamide **10** as its hydrochloride salt which was isolated in excellent yield by precipitation from the reaction mixture with hexane and filtration. Reduction of the hydrazone to liberate the free C-3 amine **11**

was effected by zinc dust in glacial acetic acid and the product isolated by silica gel chromatography in acceptable yield.

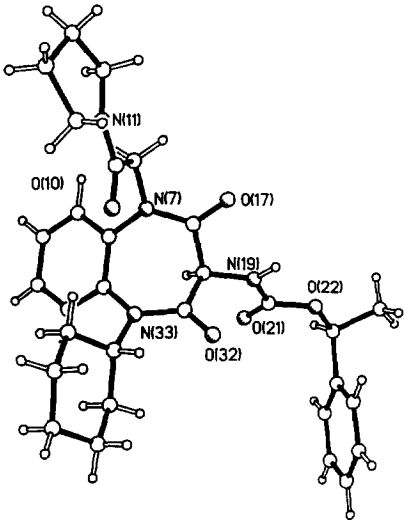


Figure 1. X-Ray crystal structure of compound **14**.

Although the enantiomers of the final compound **7**, and those of the amine **11**, are separable by preparative chiral HPLC (Chiralcel OD) it is more convenient (on a multi-gram scale) to prepare a diastereomeric derivative of **11** and separate the resultant diastereomers by chromatography. The amine **11** was reacted with homochiral carbonate[14] **12**, derived from *S*-(+)-1-phenylethanol, and the resultant diastereomers **13** and **14** (92% combined yield) were separated by silica gel chromatography. Pure diastereomer **13** was isolated in 40-45% yield (80-90% theory) and the carbamate subsequently cleaved by hydrogenolysis to give the homochiral C-3 amine *S*-**11** in 89% yield. *S*-**11** was treated with *p*-fluorophenylisocyanate and the crude product purified by silica gel chromatography and recrystallisation from aqueous ethanol to give **7** in 82% yield.[15] The optical purity was checked by HPLC (Chiralcel OD-H) and shown to be 99.5% ee. Using this route batches of (+)-**7** of larger than 20g have been prepared. The unwanted isomer **14** crystallised from ethyl acetate/hexane in a form amenable to single crystal x-ray analysis and the structure was duly solved. The unit cell contains two slightly different conformations of the side chains one of which is shown in Figure 1. From this the absolute stereochemistry of **14** can be determined as *R* which allows the stereochemistry of (+)-**7** to be unequivocally assigned as *S*. This result is in line with the analogous compounds in the 1,4-benzodiazepinone series.[3]

Further evaluation of compound **7** is currently being actively pursued.

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[8] **Pharmacokinetic Protocol:** Each rat received a single oral or intravenous dose equivalent to 3 mg/kg bodyweight. Blood was collected under anaesthesia at 0, 5, 15, 30, 45 min, and 1, 1.5, 2, 4, 6, 8, 10, 12 and 24 h post dosing (two rats per time point). The blood samples were placed in heparinised tubes and centrifuged to separate the plasma. Plasma samples were extracted by solid phase extraction with C8 cartridges. The extracts were analysed by HPLC using an Inertsil ODS column with UV detection.

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[11] Except for **7**, pKis were determined in triplicate in a single experiment.

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[13] The same observation was made independently by workers at Merck in the 1,4-benzodiazepinone series: Chambers, M.S., Hobbs, S.C., Fletcher, S.R., Matassa, V.G., Mitchell, P.J., Watt, A.P., Baker, R., Freedman, S.B., Patel, S. and Smith, A.J. *Bioorg. and Med. Chem. Lett.* **1993**, *3*, 1919.

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[15] mp 159-164°C, $[\alpha]_D$ +37.65 (c 0.53, $CHCl_3$).

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