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Rapid Communication

Benzotriazonine as a New Core Structure for the Design of CCK-Receptor Antagonists

ACHIM ESCHERICH[a], CHANTAL ESCRIEUT[b], DANIEL FOURMY[b] and LUIS MORODER[a,*]

[a] Max-Planck-Institut für Biochemie, Am Klopferspitz 18A, D-82152 Martinsried, Germany
[b] INSERM U 151, Bat L, CHU de Rangueil, 31054, Toulouse Cedex, France

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Abstract: The search for heterocyclic scaffolds for the design of non-peptidic and highly selective agonists or antagonists of peptide hormone receptors led to 4-*N*-benzyl-2,3,4,5,6,7-hexahydro-1*H*-1,4,7-benzotriazonin-2,6-dione with a 9-membered core structure as a new low mass lead compound that exhibits submicromolar antagonistic activity at the CCK-A receptor with a 54-fold selectivity over the CCK-B/gastrin receptor. Copyright © 1999 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: benzotriazonine; peptide hormone; CCK; gastrin; CCK-receptor antagonists

Peptidic hormones and neurotransmitters are involved in many pathophysiological processes in the central nervous system as well as in the periphery, and thus their receptors represent promising targets for the drug design of low mass, non-peptidic and highly selective agonists or antagonists. The identification of ligands for CCK-receptors has been of particular interest over the past years, and the central issue of pharmacological discrimination between the peripheral CCK-A and the central CCK-B receptor subtype is still the focus of intensive research (for a recent review see Reference [1]).

CCK-A and CCK-B/gastrin receptors belong to the family of G protein-coupled receptors (GPCR), which are characterized by seven transmembrane domains connected by intracellular and extracellular loops with an extracellular N-terminal and an intracellular C-terminal extension. Mechanisms of ligand binding and activation of GPCRs are particularly important due to their ubiquitous expression and potential use as drug targets. Molecular interaction between ligands and these receptors are best defined for small molecule ligands that bind within the transmembrane helices, whereas extracellular domains seem to be more important for peptide ligands as determined by the effects induced upon receptor mutagenesis.

Subtype specific CCK-receptor antagonists are still the focus of intensive research and provide useful tools for the pharmacological discrimination between the peripheral CCK-A and the central CCK-B receptor subtypes. Based on asperlicine as a natural lead compound with antagonistic activity at CCK-receptors, mainly the 7-membered heterocyclic 1,4-benzodiazepine (**2**) [2] and 1,5-benzodiazepine (**3**) [3] templates were used for the development of CCK-receptor ligands, among which L-364,718 and L-365,260 have emerged as highly potent and selective antagonists of the CCK-A and the CCK-B receptor subtypes, respectively [4,5]. As shown in Figure 1, besides the 6-membered quinazolinones (**1**) [6], even larger ring systems, such as the 8- and 9-membered conformationally constrained phenylalanine analogues **4** and **5**, have been used to display two aromatic moieties in a spatial array that mimics the tetrapeptide amide H-Trp-Met-Asp-Phe-NH_2 as the common message

* Correspondence to: Max-Planck-Institut für Biochemie, Am Klopferspitz 18A, D-82152 Martinsried, Germany.
E-mail: moroder@biochem.mpg.de

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sequence of gastrin and CCK. These studies clearly revealed that the size of the restricting ring plays a decisive role in the biological activity by conferring a more or less favourable disposition of the aromatic rings [7].

The search for new, more or less rigid scaffolds led us to analyze the 9-membered benzotriazonine compound **6** that strongly resembles the 1,5-benzodiazepines (**2**). It was reported that 9-membered benzotriazonines could have favourable pharmacological properties in the central nervous system and potential applicability in radiopharmacy as technetium 99m carriers for the diagnosis of the biliary tract [8]. This inspired our approach to investigate the potential binding affinities of the benzotriazonine derivative **6** to the CCK-A and the CCK-B/gastrin receptor.

The synthesis of compound **6** was carried out essentially by the method reported previously [8], as shown in Figure 2. However, by this procedure the benzotriazonine derivative **6** was obtained only in moderate yields, but was readily purified by recrystallization and RP-HPLC. This synthetic approach bears some similarity to the synthesis of the 7-membered 1,5-benzodiazepines, which are obtained in satisfactory yields upon reaction of aromatic 1,2-diamines with a dicarboxylic acid derivative [3,9]. Attempts to improve the yields by reacting the *o*-phenylendiamine with the bis-chloroacetyl derivative in analogy to the methods reported for the preparation of benzotriazonine-2,5-dione [10] failed.

Assays of the binding affinities of the benzotriazonine derivative **6** led to IC_{50} values of 0.36 μM for the CCK-A and of 19.5 μM for the CCK-B/gastrin receptor, respectively. The full absence of functional binding as assessed by inositol triphosphate accumulation in intact CHO cells, indicates the derivative **6** as a promising new lead structure for the synthesis of highly potent and selective CCK-receptor antagonists (see Table 1). Its constrained ring system could provide new structural information about the mode of ligand binding to the CCK-receptors.

EXPERIMENTAL

Solvents and reagents used in the synthesis were of the highest quality commercially available. TLC was carried out on silica gel-60 plates. Analytical HPLC was performed with Waters equipment (Eschborn, Germany) on Nucleosil 300/C18 (Machery and Nagel, Duren, Germany) with a linear gradient of acetonitrile/2% H_3PO_4 from 5:95 to 80:20 in 30 min and preparative HPLC with Abimed equipment (Langenfeld, Germany) on Nucleosil 250/C18. FAB-MS spectra were recorded on a Finnigan MAT 900 and NMR spectra on a Bruker AMX 400 spectrometer.

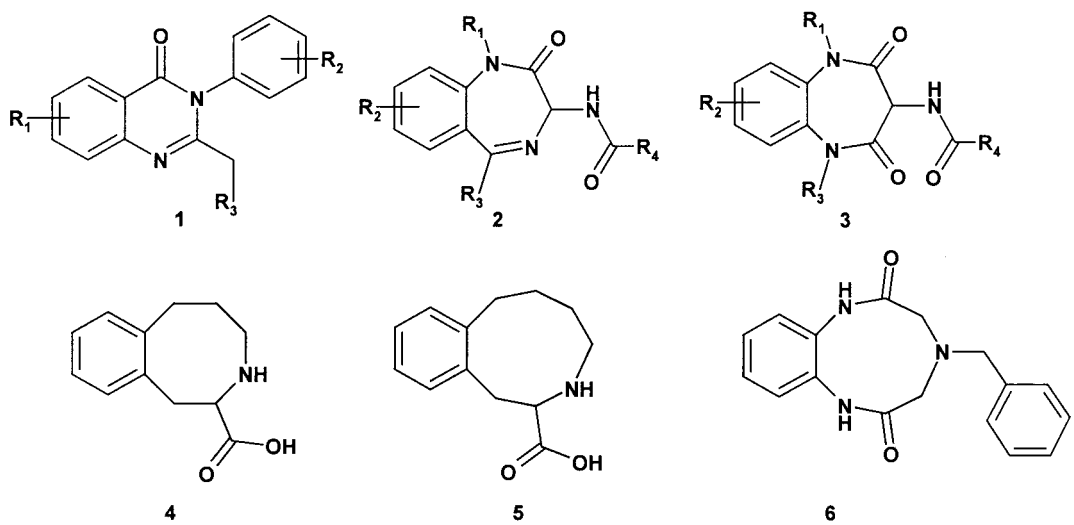


Figure 1 Heterocyclic systems of different ring size used so far as templates for the design of CCK-A and CCK-B/gastrin receptor antagonists.

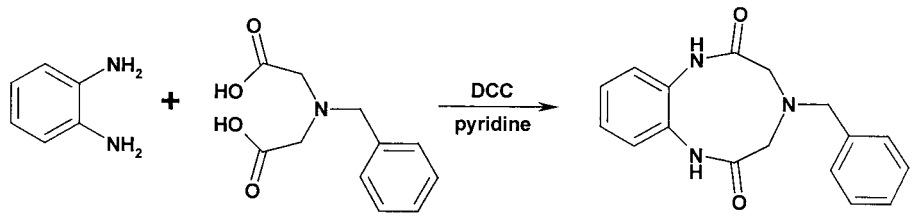
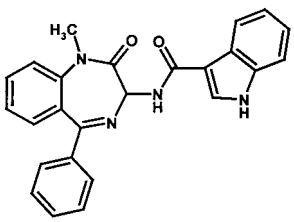
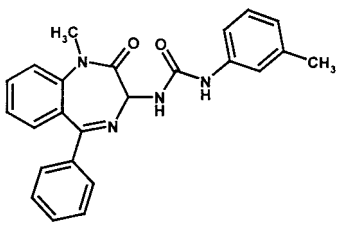
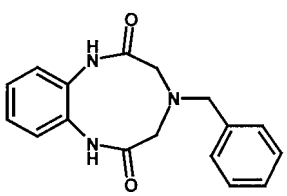


Figure 2 Synthesis of 4-*N*-benzyl-2,3,4,5,6,7-hexahydro-1*H*-1,4,7-benzotriazonin-2,6-dione.

Synthesis of 4-*N*-benzyl-2,3,4,5,6,7-hexahydro-1*H*-1,4,7-benzotriazonin-2,6-dione (6)

To a solution of *o*-phenylendiamine (0.48 g; 4.48 mmol) and *N*-benzyliminodiacetic acid (1 g; 4.48 mmol) in 50 ml pyridine DCC (1.85 g; 8.96 mmol) was added, and the mixture was stirred at room temperature overnight. The dicyclohexylurea was filtered off, the solvent removed *in vacuo* and the residue taken up in ethyl acetate. Upon addition of hexane, the product was collected and recrystallized from 2-propanol; yield: 0.1 g (8%). For analytical characterization, a sample was reverse-phase chromatographed on Nucleosil 250 C18 using a linear gradient of water/acetonitrile from 85:15 to 15:85 in 90 min; homogenous on HPLC (linear gradient from 5:95 to 80:20 acetonitrile/2% H_3PO_4; $t_R = 21.75$ min) and TLC (cyclohexane/$CHCl_3$/AcOH, 45:45:10; $R_f = 0.33$); ^{1}H-NMR (DMSO-d_6): $\delta = 3.45$ (s, 4H, 2 × CH_2), 3.85 (s, 2H, CH_2-benzyl), 7.15 (m, 2H, CH-aromatic), 7.25–7.35 (br m, 5H, CH-aromatic), 7.50 (m, 2H, CH-aromatic), 10.0 (s, 2H, 2 × NH); FAB-MS: $m/z = 591.2$ $[2M + H]^+$, 886.4 $[3M + H]^+$; $M_r = 295.3$. Calc. for: $C_{17}H_{17}N_3O_2$; as previously reported [8], the molecular ion $[M + H]^+$ could not be detected.

Table 1 Binding Affinities of **6** for CCK-A and CCK-B Receptor Overexpressed in CHO Cells and Comparison with those of the Asperlicine-derived Antagonists

Compound	CCK-A receptor IC_{50} (μM)	CCK-B/gastrin receptor IC_{50} (μM)
 Devazepide [4]	1.04×10^{-4}	0.14
 L-365 260 [5]	0.74	8.5×10^{-3}
 **6**	0.36	19.5

Binding affinities

The binding affinities of compound **6** for the CCK-A and CCK-B receptors were determined with plasma membrane preparations of CHO cells using ^{125}I-BH-(Thr, Nle)-CCK-9 as tracer according to procedures described elsewhere [11].

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