Controlled Modification of Acidity in Cholecystokinin B Receptor Antagonists: *N*-(1,4-Benzodiazepin-3-yl)-*N*′-[3-(tetrazol-5-ylamino)phenyl]ureas

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The design, synthesis, and biological activity of a novel series of CCK-B receptor antagonists (**1**) which incorporate a tetrazol-5-ylamino functionality attached to the phenyl ring of the arylurea moiety of L-365,260 are described. In these compounds, the acidity of the tetrazole was gradually modified by utilization of simple conformational constraints, and X-ray crystallographic data were obtained to support the conformational dependence of the pK_a of the aminotetrazoles. Compounds to emerge from the present work such as **1f** and **2c**,**d** are among the highest affinity and, in the case of **1f**, most selective (CCK-A/CCK-B, 37 000) antagonists so far reported for this receptor. The C_5-cyclohexyl compound **2c** (L-736,380) dose-dependently inhibited gastric acid secretion in anesthetized rats (ID_{50}, 0.064 mg/kg) and *ex vivo* binding of [^{125}I]CCK-8S in BKTO mice brain membranes (ED_{50}, 1.7 mg/kg) and is one of the most potent acidic CCK-B receptor antagonists yet described.

Introduction

The polypeptide hormone cholecystokinin (CCK) is a member of a family of peptides which was first isolated from the gastrointestinal tract and subsequently identified in the central nervous system (CNS). Several biologically active forms of CCK exist, with CCK-58, CCK-33, CCK-8, and CCK-4 predominating in the periphery and CCK-8 being the prevailing form in the CNS.[1] Two CCK receptor subtypes, CCK-A and CCK-B, which mediate the diverse biological functions of CCK have been identified so far. The human CCK-A[2] and CCK-B[3−5] receptors have been cloned and shown to belong to the G-protein-coupled receptor (GPCR) superfamily. CCK-A (alimentary) receptors are primarily located in the gut where they mediate pancreatic enzyme secretion, gallbladder contraction, gastric emptying, and intestinal motility.[6] Peripheral CCK-A receptors have also been implicated in satiety,[7] gastrointestinal cancer,[6] and neuroprotection.[8] Additionally, CCK-A receptors have been identified in discrete areas of the CNS[9] and might play a significant role in neuropsychiatric disorders.[10] CCK-B (brain) receptors are more widely distributed in the CNS, and they are thought to be involved in the modulation of anxiety,[11] panic disorder,[12] depression,[13] nociception,[14] and satiety.[10,15] CCK-B receptors have also been found in the periphery and might be identical with the gastrin receptor.[5]

This wealth of potential therapeutic utilities for CCK receptor agonists and antagonists has provided the impetus for intensive research in the area, and over the past decade, a variety of selective nonpeptidic CCK-A and CCK-B receptor antagonists have been disclosed by several laboratories.[16,17] From a pioneering program of work at Merck,[18] sparked by the isolation of the natural product asperlicin, series of benzodiazepine-based CCK-A and CCK-B receptor antagonists were developed, and first-generation compounds such as MK-329 (CCK-A) and L-365,260 (CCK-B) have been evaluated in the clinic. The latter compound, however, had to be specially formulated in order to achieve adequate levels of oral bioavailability, mainly as a consequence of the low aqueous solubility of its crystalline form (<0.002 mg/mL, pH 7.4).[19] Comprehensive structure−affinity relationship (SAR) studies on L-365,260[20] revealed regions of the molecule which permitted the modulation of its physicochemical properties and often resulted in improved CCK-B receptor affinity and selectivity. Most notably, the evolution of second-generation benzodiazepine-based CCK-B receptor antagonists which incorporate basic[21] or acidic[22] solubilizing functionalities has been recently disclosed. Prominent compounds to emerge from the latter series include the tetrazole L-368,730 and the 1,2,4-oxadiazolone L-369,466 (Chart 1) which, despite excellent potency, selectivity, and solubility, not

Chart 1

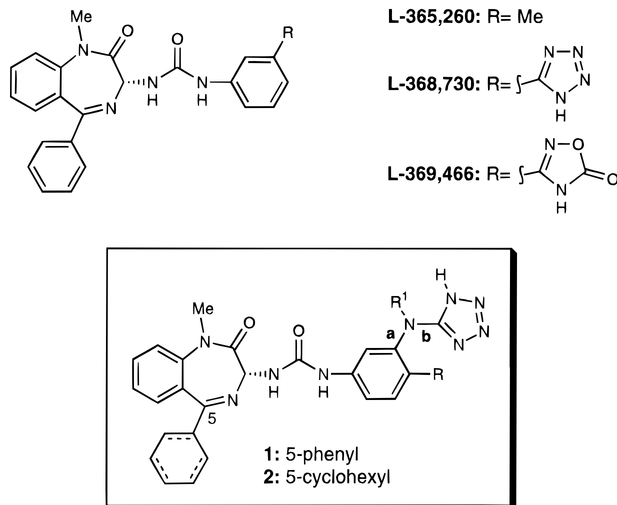

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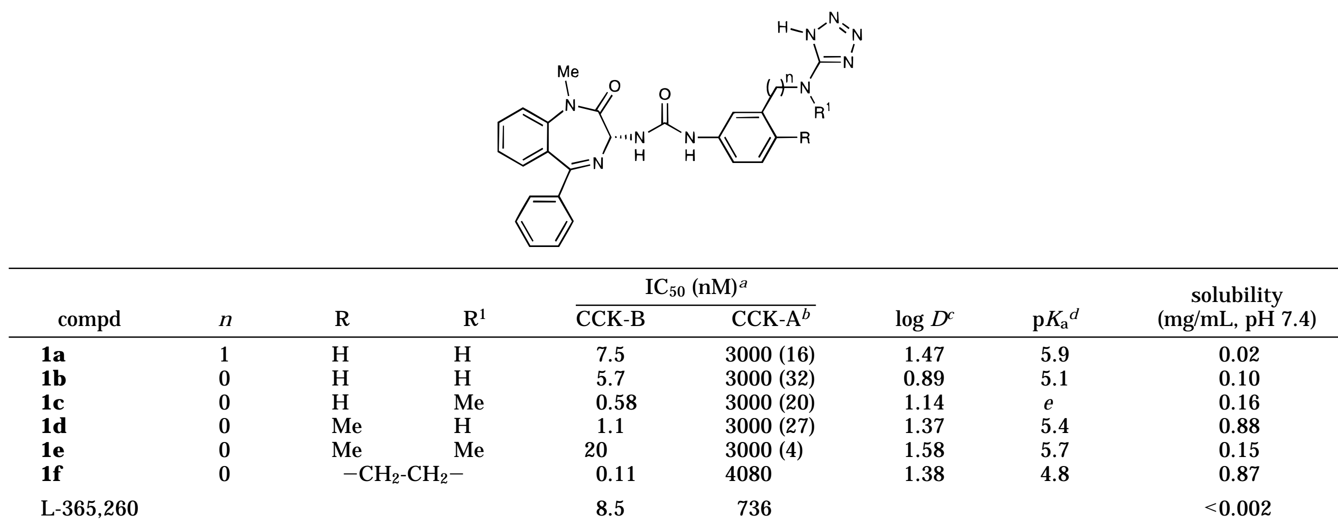
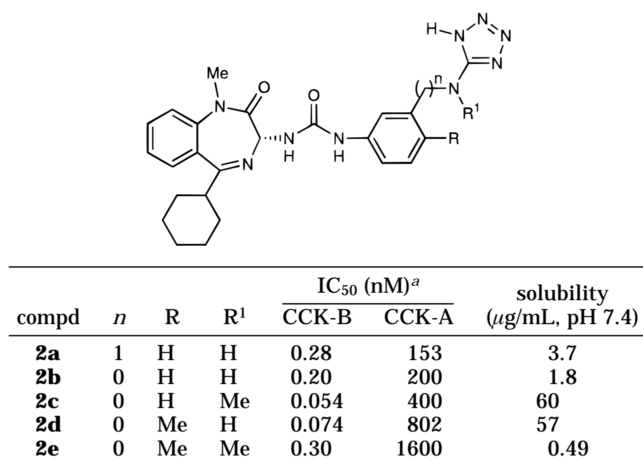
Table 1. CCK Receptor Binding Affinities and Physicochemical Properties of *N*-(5-Phenyl-1,4-benzodiazepin-3-yl)−*N*′-[3-(tetrazol-5-ylamino)phenyl]ureas



compd	*n*	R	R[1]	IC50 (nM)[a] CCK-B	CCK-A[b]	log *D*[c]	p*K*a[d]	solubility (mg/mL, pH 7.4)
1a	1	H	H	7.5	3000 (16)	1.47	5.9	0.02
1b	0	H	H	5.7	3000 (32)	0.89	5.1	0.10
1c	0	H	Me	0.58	3000 (20)	1.14	*e*	0.16
1d	0	Me	H	1.1	3000 (27)	1.37	5.4	0.88
1e	0	Me	Me	20	3000 (4)	1.58	5.7	0.15
1f	0	−CH2-CH2−		0.11	4080	1.38	4.8	0.87
L-365,260				8.5	736			<0.002

[a] Receptor binding is expressed as IC_{50}, the concentration of compound required for half-maximal inhibition of the binding of [^{125}I]BH CCK-8S to receptors in pancreatic tissue (CCK-A) or guinea pig cortical membranes (CCK-B). The results represent the geometric mean of two to three separate experiments. [b] Full IC_{50} not obtained, percentage of inhibition at a concentration of 3000 nM given in parentheses. [c] Log *P* measured at pH 7.4. [d] Determined by nonlinear regression analysis of pH-dependent partition measurements.[32] [e] This pK_a could not be reliably determined due to poor solubility of the compound at low pHs.

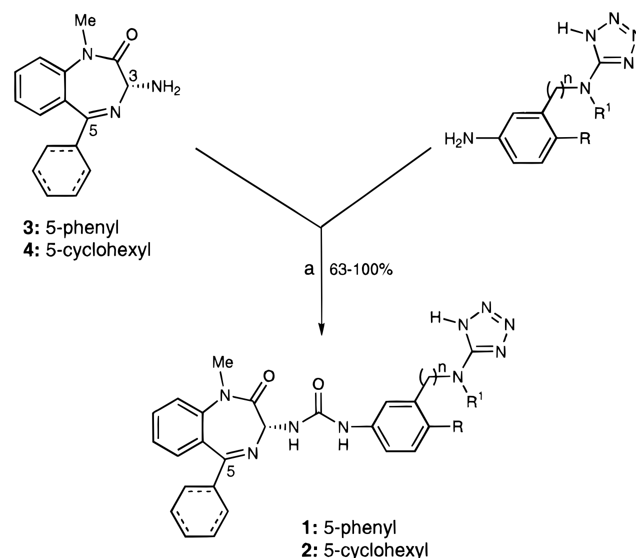
Table 2. CCK Receptor Binding Affinities and Solubilities of *N*-(5-Cyclohexyl-1,4-benzodiazepin-3-yl)-*N*′-[3-(tetrazol-5-ylamino)phenyl]ureas



compd	*n*	R	R[1]	IC50 (nM)[a] CCK-B	CCK-A	solubility (μg/mL, pH 7.4)
2a	1	H	H	0.28	153	3.7
2b	0	H	H	0.20	200	1.8
2c	0	H	Me	0.054	400	60
2d	0	Me	H	0.074	802	57
2e	0	Me	Me	0.30	1600	0.49

[a] See Table 1, footnote a. The results represent the geometric mean of two to five separate experiments.

Scheme 1[a]



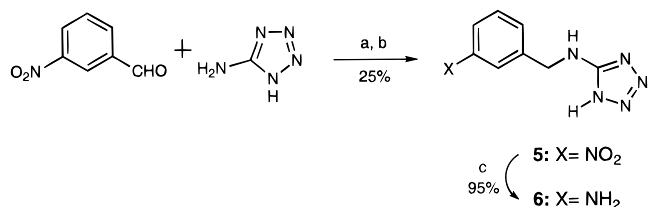
[a] Reagents: (a) triphosgene, Et_3N, THF.

unexpectedly showed a much reduced ability to cross the blood−brain barrier (BBB) when compared to the uncharged lead L-365,260. It proved, therefore, of interest to investigate the possibility of influencing the brain penetrability of related compounds by modulating the pK_a of a constant acid moiety. Here we report on the design, synthesis, and biological activity of a series of CCK-B receptor antagonists (**1**) that incorporate a 5-aminotetrazole unit, the pK_a of which could be gradually modified by rationally controlling the torsion angles around bonds a and b (see structure **1**) using simple conformational constraints. X-ray crystallographic evidence was obtained to support the conformational dependence of the pK_a of the aminotetrazoles.

Synthetic Chemistry

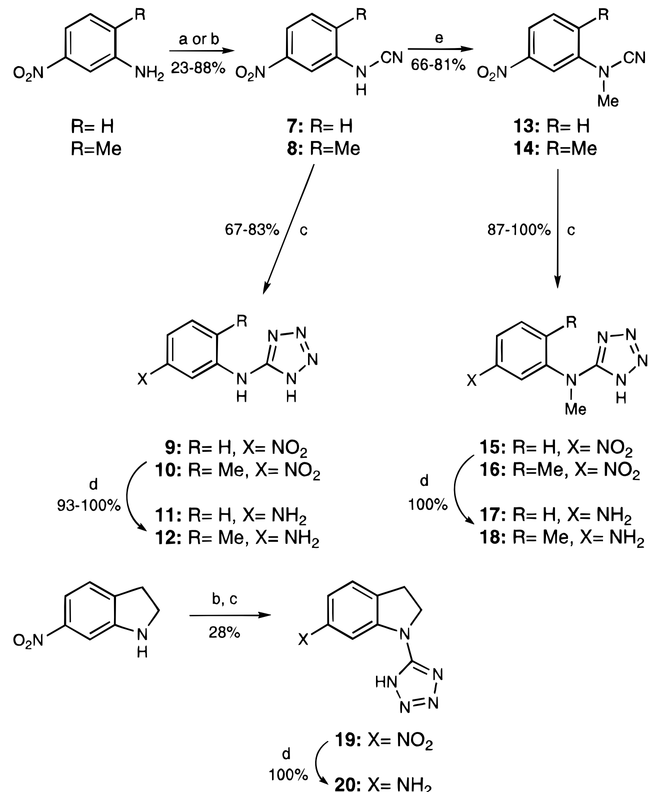
The compounds shown in Tables 1 and 2 were synthesized from the previously described (3*R*)-amino-1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one (**3**),[23] or its C_5-cyclohexyl analogue **4**,[24] and the corresponding 3-(tetrazol-5-ylamino)anilines utilizing a triphosgene-promoted coupling (Scheme 1). 3-[(Tetrazol-5-ylamino)methyl]aniline (**6**) was prepared by reductive alkylation of 5-aminotetrazole with 3-nitrobenzaldehyde followed by catalytic hydrogenation (Scheme 2). Synthesis of the remaining anilines was achieved as shown in Scheme 3. Thus, reaction of 3-nitroaniline or 2-methyl-5-nitroaniline with cyanogen bromide using a modified literature procedure[25] afforded cyanamides **7** and **8**, respectively, which were converted to the corresponding tetrazoles **9** and **10** by reaction with sodium azide.[26] Alternatively, cyanamides **7** and **8** were N-methylated to give **13** and **14** and subsequently transformed into tetrazoles **15** and **16**, as above. A similar sequence was followed to prepare 6-nitro-1-tetrazol-5-ylindoline (**19**). The anilines were obtained

Scheme 2[a]



[a] Reagents: (a) EtOH, AcOH, reflux; (b) $NaBH_4$, EtOH; (c) H_2, Pd−C, MeOH−H_2O.

Scheme 3[a]



[a] Reagents: (a) BrCN, NaOH, AcOH−H_2O; (b) BrCN, NaOH, AcOH−EtOH−H_2O; (c) NaN_3, NH_4Cl, DMF, 165 °C; (d) H_2, Pd−C, MeOH−H_2O; (e) NaH, MeI, THF−DMF.

from the appropriate nitro compounds by catalytic hydrogenation.

Results and Discussion

Modulation of pK_a. The data presented in Tables 1 and 3 clearly show that the pK_a of this novel series of CCK-B receptor antagonists can be controlled through the use of minor structural modifications around the aminotetrazole group. This acid moiety was chosen because its pK_a (6.0)[27] is substantially higher than that for tetrazole itself (4.9),[27] and it was anticipated that the acidity of the former could be gradually increased by modifying the degree of charge delocalization from the amino group into the tetrazole ring. Here we describe in detail the implementation of this idea utilizing the model compounds **5**, **9**, **10**, **15**, **16**, and **19**, which are themselves intermediates in the synthesis of the final products **1a**−**f** and **2a**−**e**. In this regard, attachment of 5-aminotetrazole through its exocyclic nitrogen atom to a 3-nitrophenyl ring using a methylene spacer results in a compound (**5**) which has a measured pK_a of 5.53 (Table 3).[28] Removal of the insulating methylene group of **5** to give **9** leads to a substantial decrease in pK_a (4.23), presumably as a direct consequence of electron delocalization from the amino group to both the tetrazole nucleus and the phenyl ring. This would effectively reduce the amount of electron density that the amino group is able to donate to the tetrazole moiety resulting in a reduction in pK_a compared to 5-[(3-nitrobenzyl)amino]tetrazole (**5**). It would, therefore, be reasonable to anticipate that by utilizing conformational constraints to adjust the degree of the delocalization of the amino group electron lone pair to these two rings, it should be possible to further modulate the pK_a. In this context, introduction of a methyl group ortho to the aminotetrazole as in **10** should reduce the delocalization into the phenyl ring and increase its pK_a compared to **9**. Incorporation of a second methyl group to give **16** should continue this deconjugation process and raise the pK_a even further. This is, indeed, the case, and the measured pK_as for **10** and **16** were 4.46 and 5.02, respectively. Structural evidence in support of the above notion was obtained from single-crystal X-ray analysis of the nitro compounds **10** and **16** (Tables 3 and 4). In both of these compounds the exocyclic amino group has a planar configuration and is in full conjugation with the tetrazole ring (N lone pair almost orthogo-

Table 3. Solid State Conformations and pK_as of Substituted 5-Aminotetrazoles

compd	n	R	R[1]	angle (deg)[b] PhPlane − AmPlane	angle (deg)[b] HetPlane − AmPlane	pK_a[a]
5	1	H	H			5.53
9	0	H	H			4.23
10	0	Me	H	40	13	4.46
15	0	H	Me	34	9	4.46
16	0	Me	Me	70	13	5.02
19	0	−CH_2CH_2−				4.02

[a] Determined by potentiometric titration.[28] [b] PhPlane: plane containing the carbon atoms of the phenyl ring. AmPlane: plane containing the linking nitrogen atom and its three substituents. HetPlane: plane containing the non-hydrogen atoms of the tetrazole.

Table 4. X-ray Crystallographic Data for 5-[(3-Nitrophenyl)amino]tetrazoles **10**, **15**, and **16**

	compound **10**	compound **15**	compound **16**
formula	$C_8H_8N_6O_2$	$C_9H_{10}N_6O_2$	$C_8H_8N_6O_2$
fw	220.19	234.21	220.19
a (Å)	6.082(3)	11.634(1)	18.333(1)
b (Å)	4.932(2)	9.738(2)	30.222(2)
c (Å)	15.864(2)	9.749(2)	7.066(1)
Z	2	4	16
space group	$P2_1$	$P2_1$	$Fdd2$
radiation	Cu Kα	Cu Kα	Cu Kα
temperature (K)	294	294	294
reflections measured	1142[a]	2347[a]	939[a]
reflections used	853	2347	876
R	0.038[b]	0.047[c]	0.043[b]
residual peak (e Å^{-3})	0.02	0.18	0.20

[a] Difractometer used: AFC5R. [b] Solved with SHELXS and refined with SDP-PLUS on F. [c] Solved with SHELXS and refined with SHELXL on F^2.

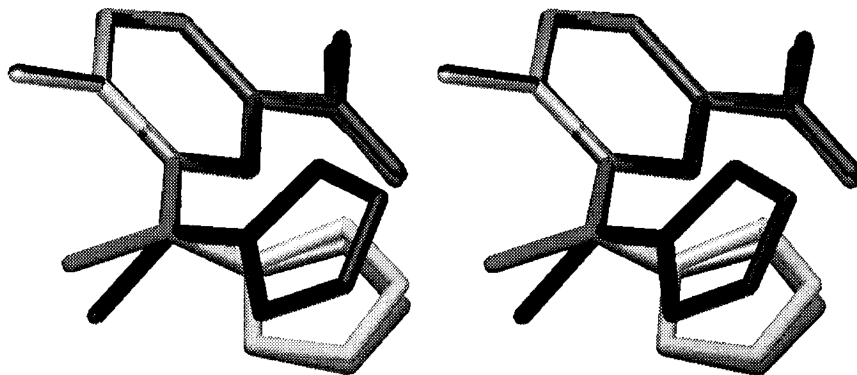


Figure 1. Computer-generated stereoview of the superimposed crystal conformations for 5-[(3-nitrophenyl)amino]tetrazoles **10** (light gray), **15** (medium gray), and **16** (black).

nal to the plane of the tetrazole) (Figure 1 and Table 3). Both compounds differ, however, in the degree of conjugation with the phenyl ring, which can be numerically expressed by considering the angles between the plane containing the carbon atoms of the phenyl ring (PhPlane) and the plane containing the linking amino group and its three substituents (AmPlane). As can be seen in Table 3 and Figure 1, this angle is much larger for **16** (70°) than for **10** (40°), and it is reflected in the higher pK_a of the former. Gratifyingly, the planarity of the nitrogen atom and the conformations seen in the solid state for **10** and **16** could be reproduced in *ab initio* calculations at the 6-31G* level[29-31] and would, therefore, not appear to be a consequence of crystal packing. Translocation of the methyl group from the ortho position of **10** to the adjacent exocyclic nitrogen atom, to give **15**, had no effect on the pK_a, and not surprisingly, the conformation of this compound in the crystal was very similar to that of **10** (Figure 1 and Table 3). Having been able to modulate the pK_a of **9** from 4.23 to 5.02, it was now of interest to increase the acidity of **9.** In order to achieve such a decrease in pK_a, the degree of delocalization of the nitrogen lone pair should be maximized toward the phenyl ring and minimized toward the tetrazole. Both of these requirements could be realized by constraining the nitrogen atom in a rigid 5-membered ring to afford a 1-(tetrazol-5-yl)indoline (**19**), which had a measured pK_a of 4.02. The pK_a modulation discussed above was corroborated in the final compounds **1a**,**b**,**d**–**f** (Table 1) which, indeed, followed a very similar acidity pattern to the model system.[32]

Structure–Affinity Relationships. The affinities of the compounds **1a**–**f** and **2a**–**e** for CCK receptors (Tables 1 and 2) were assessed by radioligand binding techniques as previously reported.[21] The 3*R*-enantiomers were synthesized and evaluated because this absolute stereochemistry usually confers CCK-B over CCK-A receptor selectivity.[20-22,24] It can be seen from the data in Table 1 that an aminotetrazole functionality is well tolerated at the CCK-B receptor, either directly attached to the phenyl ring (**1b**) or linked through a methylene spacer (**1a**). The CCK-B receptor affinity of **1b** (IC_{50}, 5.7 nM) was increased 1 order of magnitude by introduction of an ortho methyl group to give **1c** (IC_{50}, 0.58 nM). This agrees with previous SAR studies on L-365,260 which showed that methylation of the phenyl ring at position 4′ or 3′ results in some 5-fold increase in receptor affinity compared to the unsubstituted phenyl.[20a] A similar (5-fold) improvement in affinity was observed by N-methylation of the exocyclic nitrogen atom (**1d**; IC_{50}, 1.1 nM). These two contributions to lipophilic binding are not additive in improving the CCK-B receptor affinity, and in fact, the combination of ortho and N-methylation (**1e**; IC_{50}, 20 nM) gives rise to a 34-fold decrease in affinity compared to the most active compound **1c**. The lipophilic advantage of both substitutions could be exploited, however, in the constrained indoline analogue **1f**, the most active compound in this series (IC_{50}, 0.11 nM). This remarkable 180-fold difference in affinity between **1e** and **1f** might suggest that the preferred bioactive conformation of these molecules has a more coplanar arrangement of the aminotetrazole moiety with respect to the phenyl ring (**1f**),[33] rather than the highly twisted disposition seen in the crystal structure of **16** (model system analogue of **1e**).[34]

Compounds **1c**,**d**,**f** (Table 1) showed a much improved *in vitro* profile (CCK-B affinity, CCK-B/CCK-A receptor selectivity) and aqueous solubility compared to that of L-365,260. In agreement with previously described findings,[24] the more lipophilic cyclohexyl derivatives **2a**–**e** (Table 2) all showed improved affinity for CCK-B receptors compared to the C_5-phenyl analogues, but their aqueous solubility at physiological pH was substantially reduced. The 10–50-fold increase in affinity for **2a**–**e** compared to **1a**–**e** has been speculated to arise from improved interactions between the cyclohexyl ring and Val[349] in the human and rat CCK-B receptors. The indolinyltetrazole **1f** (L-738,425) and the C_5-cyclohexyl compounds **2c** (L-736,380) and **2d** (L-737,481) are extremely high affinity ligands for the CCK-B receptor, and **1f** has exceptional selectivity (CCK-A/CCK-B, 37 000) over the CCK-A receptor. Compounds such as **1c** and **2c** were also shown to be devoid of affinity for the $GABA_A$ benzodiazepine receptor ([^{3}H]Ro15-1788: IC_{50}, >10 000; rat cortical membranes). Not surprisingly,[5] however, **1b**,**c** and **2c** were unable to differentiate between CCK-B and gastrin receptors, and indeed, their affinities for these two receptors were almost identical ([^{125}I]gastrin: IC_{50}s, 3.9, 0.47, and 0.13 nM, respectively; guinea pig gastric glands).[35]

Functional Activity and Brain Penetration. The similar affinities of the compounds for CCK-B and gastrin receptors were utilized to assess their functional antagonist activity *in vivo*. Thus, L-736,380 (**2c**) showed a dose-related inhibition of pentagastrin-induced gastric acid secretion in anesthetized rats,[35] with an ID_{50} of 0.064 mg/kg, following dosing by the intraperitoneal route. L-365,260 is also active in this model, but it is

some 15-fold weaker (ID_{50}, 1.0 mg/kg). Because L-365,260 is known to be a CCK-B receptor antagonist, and CCK-B and gastrin receptors appear to be identical, it is reasonable to conclude that the compounds described above are also CCK-B antagonists. Further evidence to support this conclusion was gained from an *in vitro* electrophysiological model of CCK-B receptor activation carried out in rat brain slices.[35,21] Thus, compound **1c** blocked the pentagastrin-induced excitation of single neurons in an *in vitro* slice preparation of the rat ventromedial hypothalamic nucleus (VMH slice) with a K_b of 0.52 ± 0.06 nM (n = 5), indicating that it is a potent and selective CCK-B antagonist.

The prevalent CNS distribution of CCK-B receptors makes almost mandatory that clinically useful antagonists for this receptor should have the ability to cross the BBB. In this regard, the CNS penetration of several of these novel aminotetrazolyl-based CCK-B receptor antagonists, after systemic (iv) administration, was assessed using an *ex vivo* binding assay in BKTO mice.[36] In this model **1c**,**f**, **2c**, and the racemate of **2a** (IC_{50}, 0.82 nM) dose-dependently inhibited *ex vivo* binding of [^{125}I]BH-CCK-8S to mouse brain membranes with ED_{50}s of 14, 10, 1.7, and 16 mg/kg, respectively.[37] The extent of inhibition of *ex vivo* binding for **1d** was only 19% at 10 mg/kg iv. For reference, L-365,260 has an ED_{50} of 13 mg/kg in this assay. Because the CCK-B affinity of L-365,260 is 15-, 77-, and 157-fold lower than that of **1c**,**f** and **2c**, respectively, the above data show that these acidic compounds are significantly less brain penetrant than the former. Additionally, the increase in lipophilicity on going from the C_5-phenyl compound **1c** (log *D*, 1.14) to its cyclohexyl analogue **2c** (log *D*, 1.85) did not affect brain penetration, and the 8-fold difference in ED_{50}s (14 vs 1.7) simply reflects the 10-fold improved affinity of **2c**. To date, **2c** is one of the most potent acidic CCK-B receptor antagonists reported. The present data suggest that pK_a in this series of compounds does not influence their brain uptake.[38]

Conclusions

A novel series of CCK-B receptor antagonists (**1**) which incorporate an aminotetrazole unit attached to the phenylurea moiety of L-365,260 was designed and synthesized. In these compounds, the acidity of the tetrazole functionality was gradually modified by utilization of simple constraints, and X-ray crystallographic data were obtained to support the conformational dependence of the aminotetrazole pK_as. The structural changes introduced for the modulation of the pK_a affect CCK-B receptor affinity and selectivity and appear to suggest that the preferred bioactive conformation of these molecules has a rather coplanar arrangement of the aminotetrazole and the phenyl ring of the arylurea moiety. Compounds such as **1c**,**f** and **2c**,**d** showed a much improved *in vitro* profile compared to that of L-365,260 and are among the most potent and, in the case of **1f**, most selective (CCK-A/CCK-B, 37 000) CCK-B receptor antagonists so far reported.

Experimental Section

Biological Methods. Detailed procedures for the assessment of antagonist properties of CCK-B ligands using *in vitro* pentagastrin-induced excitation of VMH neurons and by *in vivo* pentagastrin-induced gastric acid secretion in rats have been previously reported.[21,35] Measurement of CNS penetration by *ex vivo* binding has also been described.[36] Radioligand binding to guinea pig cortical membranes was performed using 50 pM ^{125}I-labeled Bolton Hunter CCK-8S in 20 mM HEPES buffer, pH 6.5, containing 150 mM NaCl, 5 mM $MgCl_2$, 1 mM EGTA, and 0.025% bacitracin. For rat pancreatic membranes, assay buffer was supplemented with 0.01% trypsin inhibitor and 0.2% BSA. Guinea pig cortical membranes were prepared by homogenization in 0.32 M sucrose, centrifugation, and resuspension of the P2 pellet in assay buffer at 1 g of wet weight in 120 mL. Rat pancreatic membranes were prepared in 10 mM HEPES/0.01% trypsin inhibitor, pH 7.4, and centrifuged, and the pellet was resuspended in assay buffer at a 1:2000 dilution. Specific binding in all cases was defined using 1 μM CCK-8S, and the reaction was terminated by filtration through Whatman GF/C filters, using a Brandel cell harvester with 3 × 3 mL washes in ice-cold 100 mM saline wash buffer. Filters were counted on a LKB γ counter.

pK_a Determinations. Potentiometric pK_a determinations for aminotetrazoles **5**, **9**, **10**, **15**, **16**, and **19** were performed using a Sirius PCA-101 titrator (Sirius Analytical Instruments Ltd., East Sussex, England) equipped with a Ross type combination glass electrode calibrated for mixed solvent titrations. The mixed solvent approach was employed because of the limited aqueous solubility of the compounds across the pH range. A cosolvent of 1,4-dioxane/water (60:40, v/v), ionic strength adjusted with 0.15 M KCl, was used. Three separate titrations were performed for each compound with different water/cosolvent ratios to obtain pK_as in the presence of cosolvent (psK_a values). Aqueous pK_a values were calculated by extrapolation to 0% cosolvent using the Yasuda–Shedlovsky relationship:[28] a linear plot of psK_a + log [H_2O] versus $1/\epsilon$, where ϵ is the dielectric constant of the water cosolvent mixture. pK_as for final compounds **1a**,**b**,**d**–**f** were determined by nonlinear regression analysis of pH-dependent partition measurements.[32]

Chemical Methods. General Directions. Unless otherwise stated, all ^{1}H NMR spectra were recorded at 360 MHz on a Bruker AM 360 spectrometer or at 250 MHz on a Bruker AC250 instrument. Mass spectra were obtained with a VG70-250 spectrometer. Melting points are uncorrected. Anhydrous THF, DMF, Et_2O, MeOH, and toluene were purchased from Aldrich Chemical Co., Sureseal. Et_3N was distilled from CaH_2. All solutions were dried over Na_2SO_4 or $MgSO_4$ and concentrated on a Büchi rotary evaporator. Flash chromatography was performed on silica gel (Fluka Art. No. 60738). Log *D*s were determined using 1-octanol and pH 7.4 buffer by the shake flask method.

General Procedure for the Preparation of (1,4-Benzodiazepin-3-yl)ureas 1a–f and 2a–e: (+)-*N*-[(3*R*)-5-Cyclohexyl-2,3-dihydro-1-methyl-2-oxo-1*H*-1,4-benzodiazepin-3-yl]-*N'*-[3-(*N*-methyl-*N*-tetrazol-5-ylamino)phenyl]urea (2c). To a cooled (0 °C) and stirred milky solution of 3-(*N*-methyl-*N*-tetrazol-5-ylamino)aniline (**17**) (230 mg, 1.21 mmol) in anhydrous THF (30 mL) was added solid triphosgene (120 mg, 0.40 mmol) in one portion, under a nitrogen atmosphere. After 5 min of stirring, anhydrous Et_3N (505 μL, 3.63 mmol) was added dropwise, and the mixture was allowed to warm to 17 °C over 15 min. The reaction mixture was recooled to 0 °C, and a solution of **4**[24] (225 mg, 0.83 mmol) in anhydrous THF (5 mL) was added dropwise *via* cannula over 4 min. After the mixture was stirred at 0 °C for 15 min and at room temperature for 2 h, a white precipitate was removed by filtration and the solvent was evaporated under reduced pressure. The remaining residue was dissolved in EtOAc (200 mL), washed with 10% aqueous citric acid (2 × 40 mL) and brine (40 mL), dried, and concentrated. Flash chromatography of the crude material (CH_2Cl_2–MeOH, 90:10) afforded 336 mg (83%) of **2c** as a white solid: mp 222–225 °C (MeOH); $[\alpha]^{23}_D$ 15.9° (*c* 0.71, DMF); ^{1}H NMR (DMSO-d_6) δ 15.2 (1H, br s), 9.11 (1H, s), 7.74 (1H, d, J = 7.7 Hz), 7.63 (1H, t, J = 8.4 Hz), 7.54 (1H, d, J = 8.2 Hz), 7.48 (1H, br s), 7.37 (1H, t, J = 7.2 Hz), 7.29 (1H, d, J = 8.4 Hz), 7.25 (1H, t, J = 8.0 Hz), 7.12 (1H, br d, J = 8.9 Hz), 6.94 (1H, br d, J = 8.0 Hz), 5.06 (1H, d, J = 8.4 Hz), 3.39 (3H, s), 3.34 (3H, s), 2.93 (1H, m), 1.90 (1H, m), 1.78 (1H, m), 1.68–1.08 (7H, m),

Table 5. Physical Data for *N*-(1,4-Benzodiazepin-3-yl)-*N*'-[3-(tetrazol-5-ylamino)phenyl]-ureas

compd	empirical formula	mp, °C (solvent)[a]	$[\alpha]_D$, deg (c, g/100 mL)[b]
1a	$C_{25}H_{23}N_9O_2 \cdot H_2O$	190–195 (A)	+75.4 (0.50)
1b	$C_{24}H_{21}N_9O_2 \cdot 0.1H_2O$	233–243 (A)	+69.6 (0.50)
1c	$C_{25}H_{23}N_9O_2 \cdot 0.5H_2O$	205–210 (A)	+65.2 (0.50)
1d	$C_{25}H_{23}N_9O_2 \cdot 0.5H_2O$	198–201 (A)	+63.4 (0.50)
1e	$C_{26}H_{25}N_9O_2 \cdot 0.2H_2O$	217–225 (B)	+68.6 (0.50)
1f	$C_{26}H_{23}N_9O_2 \cdot 1.6H_2O$	215–220 (C)	+64.8 (0.50)
2a	$C_{25}H_{29}N_9O_2 \cdot 0.05H_2O$	175–178 (D)	+17.4 (0.50)
2b	$C_{24}H_{27}N_9O_2 \cdot 0.6H_2O$	190–193 (A)	+8.4 (0.50)
2c	$C_{25}H_{29}N_9O_2$	222–225 (A)	+15.9 (0.71)
2d	$C_{25}H_{29}N_9O_2$	190–193 (B)	+12.2 (0.29)
2e	$C_{26}H_{31}N_9O_2$	223–225 (B)	+20.3 (0.34)

[a] A, MeOH; B, MeOH–CH_2Cl_2; C, MeOH–H_2O; D, MeOH–EtOAc. [b] DMF solutions.

0.89 (1H, m); MS (CI) m/z 488 (M^- + 1). Anal. ($C_{25}H_{29}N_9O_2 \cdot 0.25H_2O$) C, H, N.

The enantiomeric purity of **2c** was shown to be >99% ee by HPLC analysis using a PIRKLE (*S*)-DNBL column (250 mm × 4.6 mm i.d., 5 μm particle size) and eluting with CH_2Cl_2–MeOH–AcOH (94:2.5:0.8) (flow, 1 mL/min; retention time, 5.66 min for the *R*-enantiomer and 10.21 min for the *S*-enantiomer).

3-[(Tetrazol-5-ylamino)methyl]nitrobenzene (5). A mixture of 3-nitrobenzaldehyde (1.51 g, 10 mmol) and 5-aminotetrazole monohydrate (1.03 g, 10 mmol) in absolute EtOH (30 mL) and glacial AcOH (0.57 mL, 10 mmol) was stirred at room temperature for 40 min and then refluxed for 5.5 h under nitrogen. Solvents were removed under high vacuum, the remaining solid was suspended in absolute EtOH (50 mL), and $NaBH_4$ (1.2 g) was added at room temperature over 20 min. After a further 15 h of stirring, the solvent was evaporated, and the remaining residue was dissolved in H_2O (100 mL) and extracted with Et_2O (2 × 30 mL). The basic aqueous phase was acidified to pH 2 with 2 N HCl, and the precipitated solid was collected by filtration, washed with water and Et_2O, and finally recrystallized from absolute EtOH to give 420 mg (19%) of **5** as white needles: mp 208–10 °C; ^{1}H NMR (DMSO-d_6) δ 8.21 (1H, br s), 8.12 (1H, br d, J = 9 Hz), 7.80 (1H, d, J = 8.0 Hz), 7.70 (1H, br t, J = 6.3 Hz), 7.64 (1H, t, J = 8.0 Hz), 4.53 (2H, d, J = 6.3 Hz); MS (CI) m/z 220 (M^-).

General Procedure for the Preparation of Cyanamides 7 and 8: (2-Methyl-5-nitrophenyl)cyanamide (8). To a cooled (4 °C) and stirred suspension of 2-methyl-5-nitroaniline (15.2 g, 100 mmol) in a mixture of AcOH (145 mL) and water (47 mL) was added cyanogen bromide (15.9 g, 150 mmol) followed by 1 N NaOH (110 mL), over 10 min. After 2 h, the mixture was diluted with EtOH (160 mL) and stirred for a further 1.5 h before 2 N NaOH (10 mL) was added, and stirring was continued at 15 °C for 16 h. Solvents were removed under vacuum (bath temperature, <45 °C), 2 N NaOH (500 mL) was added, and the resulting mixture was filtered to remove a fine precipitate. The clear filtrate was acidified to pH 1 with 5 N HCl, and the solid was collected, washed with water, and dried over P_2O_5 to give 15.5 g (88%) of **8**: ^{1}H NMR (DMSO-d_6) δ 7.84 (1H, dd, J = 8.3, 2.4 Hz), 7.77 (1H, d, J = 2.4 Hz), 7.49 (1H, d, J = 8.3 Hz), 2.30 (3H, s); MS (CI) m/z 177 (M^-).

General Procedure for the Methylation of Cyanamides 7 and 8: *N*-Methyl-(3-nitrophenyl)cyanamide (13). To a cooled (−20 °C) and stirred solution of (3-nitrophenyl)cyanamide (**7**) (1 g, 6.1 mmol) in a mixture of anhydrous THF and anhydrous DMF (3:1, 20 mL) was added NaH (60% dispersion in oil, 294 mg) in one portion, under nitrogen. After 8 min of stirring at −20 °C, iodomethane (1.14 mL, 18.4 mmol) was added, the red mixture was allowed to warm to room temperature, and it was diluted with anhydrous DMF (5 mL). After a further 45 min, H_2O (75 mL; CAUTION! hydrogen evolution) was added and products were extracted with EtOAc (2 × 100 mL). The combined organic solutions were washed with brine (50 mL), dried, and concentrated. The residue was dissolved in EtOAc (30 mL), and hexane (100 mL) was added to give 880 mg (81%) of **13** as pale yellow needles: ^{1}H NMR (DMSO-d_6) δ 7.98 (1H, m), 7.85 (1H, t, J = 2.2 Hz), 7.73 (1H, t, J = 7.7 Hz), 7.60 (1H, m), 3.45 (3H, s); MS (CI) m/z 177 (M^-).

General Procedure for the Preparation of Tetrazoles 9, 10, 15, and 16: 3-(*N*-Methyl-*N*-tetrazol-5-ylamino)-nitrobenzene (15). To a stirred suspension of NaN_3 (352 mg, 5.4 mmol) and NH_4Cl (2.37 g, 44.3 mmol) in anhydrous DMF (2 mL) was added dropwise, *via* cannula, a solution of **13** (800 mg, 4.5 mmol) in anhydrous DMF (4 mL). The resulting yellow mixture was heated at 165 °C for 3 h 45 min, under nitrogen. After cooling, water (60 mL) and 2 N NaOH (8 mL) were added, and the clear solution was extracted once with Et_2O (30 mL). The aqueous phase was acidified with 5 N HCl, and the pale yellow precipitate was collected, washed with water (2 × 15 mL), and recrystallized from H_2O–EtOH (3:1, 30 mL) to give 863 mg (86.9%) of **15** as pale yellow needles: mp 196–198 °C; ^{1}H NMR (DMSO-d_6) δ 8.43 (1H, t, J = 2.3 Hz), 7.94 (2H, m), 7.68 (1H, t, J = 8.3 Hz), 3.56 (3H, s); MS (CI) m/z 220 (M^-).

9: mp 228–230 °C (EtOH–H_2O, 2:1); ^{1}H NMR (DMSO-d_6) δ 15.8 (1H, br s), 10.43 (1H, s), 8.57 (1H, t, J = 2.1 Hz), 7.90 (1H, m), 7.80 (1H, m), 7.61 (1H, t, J = 8.2 Hz); MS (CI) m/z 206 (M^-).

10: mp 245–249 °C (MeOH–H_2O); ^{1}H NMR (DMSO-d_6) δ 9.34 (1H, s), 8.80 (1H, d, J = 2.4 Hz), 7.82 (1H, dd, J = 8.3, 2.4 Hz), 7.49 (1H, d, J = 8.3 Hz), 2.41 (3H, s); MS (CI) m/z 220 (M^-).

16: mp 201–205 °C (H_2O); ^{1}H NMR (DMSO-d_6) δ 8.24 (1H, d, J = 2.4 Hz), 8.15 (1H, dd, J = 8.4, 2.4 Hz), 7.65 (1H, d, J = 8.4 Hz), 3.40 (3H, s), 2.24 (3H, s); MS (CI) m/z 234 (M^-).

6-Nitro-1-(tetrazol-5-yl)indoline (19). To a cooled (4 °C) and stirred solution of 6-nitroindoline (3.04 g, 18.5 mmol) in a mixture of glacial AcOH (22 mL), H_2O (11 mL), and absolute EtOH (23 mL) was added solid cyanogen bromide (2.93 g, 27.7 mmol) followed by 1 N NaOH (25 mL), over 3 min. The mixture was allowed to warm to room temperature and stirred for 21 h, and the solid was collected by filtration, washed with water, and dried over P_2O_5 to give 2.94 g (84%) of 1-cyano-6-nitroindoline as a yellow solid: ^{1}H NMR (DMSO-d_6) δ 7.90 (1H, dd, J = 8.1, 2.2 Hz), 7.55–7.50 (2H, m), 4.23 (2H, t, J = 8.4 Hz), 3.30 (2H, t, J = 8.4 Hz); MS (CI) m/z 189 (M^-).

Reaction of 1-cyano-6-nitroindoline with sodium azide, using a similar method to that described for **15**, afforded **19** in 33% isolated yield: mp 262–270 °C (MeOH–H_2O); ^{1}H NMR (DMSO-d_6) δ 8.60 (1H, d, J = 2.2 Hz), 7.83 (1H, dd, J = 8.1, 2.2 Hz), 7.50 (1H, d, J = 8.1 Hz), 4.21 (2H, t, J = 8.5 Hz), 3.40 (2H, t, J = 8.5 Hz); MS (CI) m/z 232 (M^-).

General Procedure for the Preparation of Anilines 6, 11, 12, 17, 18, and 20: 3-(Tetrazol-5-ylamino)aniline (11). A solution of **9** (410 mg) in a mixture of MeOH (50 mL) and H_2O (5 mL) was hydrogenated at 35 psi over 10% Pd–C (170 mg) for 4 min. The catalyst was filtered off and washed with MeOH (2 × 10 mL), and solvents were removed under vacuum. The residue was azeotroped with MeOH (20 mL) and further dried under high vacuum to give 324 mg (93%) of **11** as a brown solid: ^{1}H NMR (DMSO-d_6) δ 9.44 (1H, s), 6.92 (1H, t, J = 8.0 Hz), 6.76 (1H, s), 6.57 (1H, d, J = 8.0 Hz), 6.17 (1H, d, J = 8.0 Hz).

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Supporting Information Available: ^{1}H NMR and microanalytical data for novel compounds and X-ray crystallographic data for **10**, **15**, and **16** (16 pages). Ordering information is given on any current masthead page.

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(37) In this *ex vivo* binding assay, other acidic benzodiazepine-based CCK-B receptor antagonists such as oxadiazolone L-369,466, tetrazole L-368,730,[22a] and the recently disclosed acylsulfonamide L-736,309[22b] had ED_{50} values of 6.5, 18, and 4.8 mg/kg, respectively. The ED_{50}/IC_{50} ratios for these compounds (25, 18, and 17, respectively) were comparable to that observed for **2c** (30).[38]
(38) Comparisons of the above brain penetration data have to be taking cautiously, however, because in this model the extent of binding to central CCK-B receptors is measured after 30 min of dosing, and different pharmacokinetic behavior of the compounds might influence the end result.

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