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Selective Non-Peptide Ligands for an Accommodating Peptide Receptor. Imidazobenzodiazepines as Potent Cholecystokinin Type B Receptor Antagonists

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Abstract—A series of imidazobenzodiazepines, non-peptide antagonists of the peptide hormone cholecystokinin (CCK), are described. Derived by chemical modification of the benzodiazepine ring system embedded within the CCK-B antagonist L-365,260, these compounds display CCK-B/CCK-A selectivity and some analogs have receptor binding affinities in the subnanomolar range. This group of novel imidazobenzodiazepines, among which *N*-[(2*S*,4*R*)-methyl-6-phenyl-2,4-dihydro-1*H*-imidazo[1,2-*a*][1,4]benzodiazepin-4-yl]-*N*′-[3-methylphenyl]-urea (**12**) is the principal compound, expands the structural diversity of the collection of non-peptide CCK-B antagonists and will be useful in further delineating the function of CCK in the central nervous system.

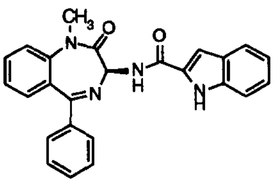
Introduction

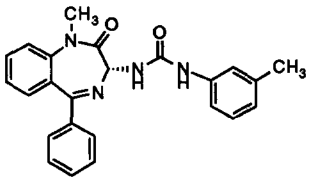
The attempted mimicry and replacement of bioactive peptides with low molecular weight non-peptide constructs is undergoing a surge in development.[1] Progress using this approach is being fueled by the quest toward a more explicit understanding of the structure and function of peptide receptors and by the potential for novel therapeutic applications in enzyme inhibition,[2] hormone agonism, and hormone antagonism.[1,3] While a principal goal of this endeavor remains the *de novo* design of orally bioavailable drug candidates, our present incomplete grasp of peptide receptor–ligand interactions makes this an arduous undertaking.

The employment of isolated receptor preparations and sensitive bioassays affords a practical and productive means for identifying selective nonpeptide ligands of novel structure.[4] Practice of these techniques led to the discovery of the natural product asperlicin which was subsequently characterized as a micromolar affinity antagonist of the brain–gut peptide, cholecystokinin (CCK).[5]

CCK itself is a member of a family of homologous peptides which exert effects on a variety of gut functions including control of pancreatic secretion and bile ejection. CCK is also one of the most widely distributed of brain peptides where it is postulated to modulate the dopaminergic and enkephalinergic systems.[6-8] The diverse biological functions attributed to CCK infer that more than one receptor type subserves these processes. Indeed, evidence that more than one form of CCK receptor might exist was advanced nearly 15 years ago.[9] At present, CCK receptors have been differentiated into primarily two subtypes designated as CCK-A and CCK-B.[10] The latter receptor subtype also displays ligand specificities similar to the stomach gastrin receptor.[11,12]

The keystone of the development program which yielded the CCK-A and CCK-B selective agents, MK-329 (**1**) and L-365,260 (**2**), respectively, was the discovery of asperlicin.[13] The CCK-A antagonist **1** has been under clinical investigation as a potential therapeutic agent for the treatment of irritable bowel syndrome,[14] whereas **2** has advanced through the early stages of clinical development as a palliative for anxiety disorders.[15]


1, MK-329

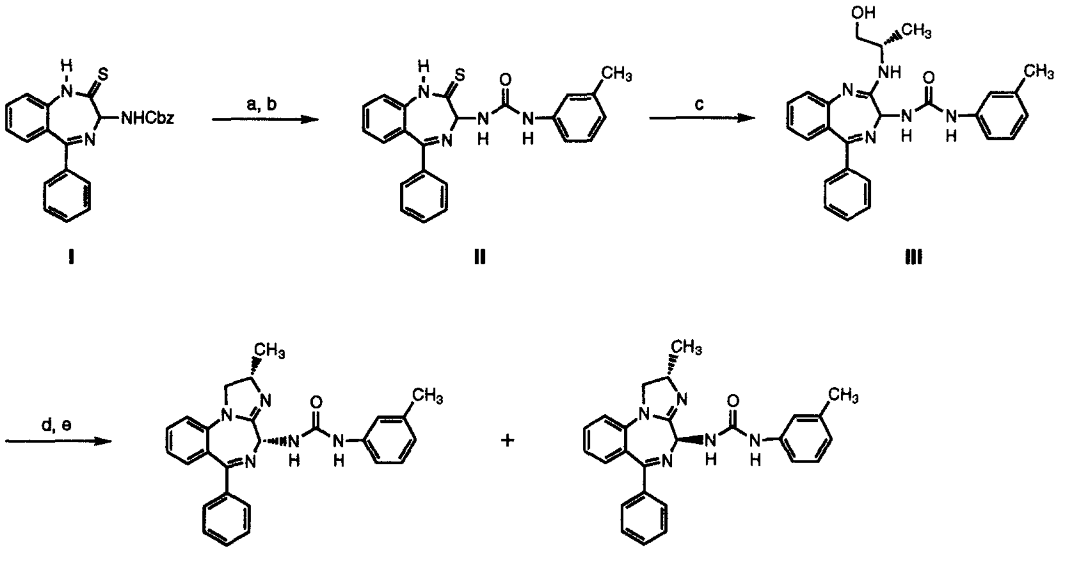

2, L-365,260

These compounds are distinct from the collection of CCK antagonists which preceded them by virtue of their potency, receptor selectivity, oral bioavailability, duration of action, and their unique non-peptidal structures. Following the disclosures of these agents, additional examples of nonpeptide CCK receptor antagonists have appeared, thereby expanding the structural diversity of compounds binding to the G protein-coupled CCK receptors, especially those of the CCK-B subtype.[16-18] The most striking feature among this newer generation of CCK-B antagonists is their lack of semblance not only to the native substrate(s) but also to one another. The CCK-B receptor's seeming absence of fidelity toward a given peptide assembly or non-peptide template has thus provided researchers with the opportunity to continue optimizing the pharmacological and physicochemical profiles of CCK-B antagonist candidates by circumventing disadvantages (e.g. bioavailability, metabolism, solubility) inherent in a particular structural class. It is within this context that we elaborate on the core structural motif of L-365,260 and reveal imidazobenzodiazepines to be an alternative structural class of CCK-B antagonists.

Chemical Methods

The compounds which comprise this study were synthesized according to the strategies presented in Schemes I–III. The methodology outlined in Scheme I illustrates the approach by which the majority of analogs were prepared and specifically exemplifies the preparation of **12** and **13**. Thus, 2-thiono-1,4-benzodiazepine **I**[19] was initially deprotected with HBr gas to afford the corresponding 3-amino derivative; the latter compound was then reacted in the free base form with *p*-tolyl isocyanate to yield the urea **II** in the usual manner. Conversion of the thioamide function in **II** to afford the amidine **III** was accomplished via the mercury salt assisted displacement of sulfur with chiral 2-amino-1-propanol.[20]

Reaction conditions suitable for transforming the benzodiazepin-2-amine **III** to an annulated imidazobenzodiazepine were then briefly examined. Activation of the hydroxyl group in **III** with *p*-toluenesulfonyl chloride, followed by further reaction in pyridine, was an effective method. However, conversion of **III** to the corresponding mesylate and concomitant cyclization to give the desired imidazobenzodiazepines **12** and **13** proved to be the optimal cyclization conditions. The diastereomeric mixture of **12** and **13** was readily separable, on an analytical scale, by TLC, reverse phase HPLC, and flash chromatography. Unfortunately, the unanticipated high affinity of **12** and **13** for silica gel (a property common to all imidazobenzodiazepines examined) made standard chromatographic separation impractical on a preparative scale (< 40 % recovery). A further complicating factor was the apparent susceptibility of these compounds toward epimerization, at the 4-position, on prolonged exposure to silica gel. For these reasons, the diastereomers **12** and **13** were purified employing a preparative, covalent Pirkle L-leucine column with hexane/2-propanol/triethylamine as eluant (isochratic conditions). For all sets of diastereomers examined (including also enantiomeric mixtures of benzodiazepines not part of this study), the 4*R*-configured isomer had a shorter retention time. This observation served as a

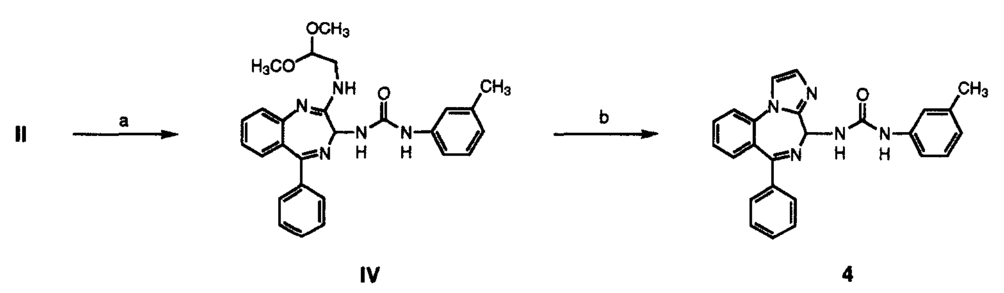


I II III

12 13

[a] Reagent and conditions: (a) HBr(g), CH_2Cl_2, HOAc, 0–25 °C, 2 h; Na_2CO_3 (10 %); (b) *p*-tolylisocyanate, THF, 0–23 °C, 30 min; (c) (S)-(+)-2-amino-1-propanol, $HgCl_2$, THF, 55 °C, 3 h; (d) MsCl, DIEA, CH_2Cl_2, 0–25 °C, 1.5 h; (e) HPLC, Pirkle L-leucine column.

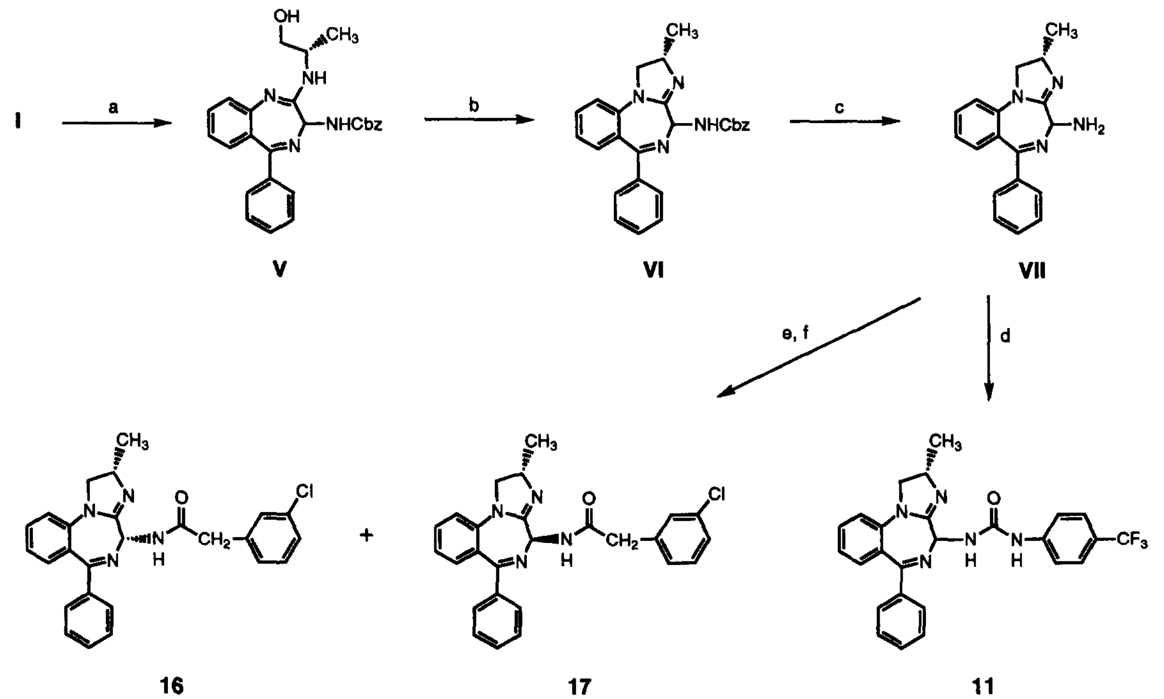
Scheme I.



IV 4

[a] Reagents and conditions: (a) aminoacetaldehyde dimethylacetal, $HgCl_2$, THF, 55 °C, 2 h; (b) $BF_3 \cdot OEt_2$, CH_2Cl_2, 50 °C, 2.5 h.

Scheme II.



[a]Reagents and conditions: (a) (S)-(+)-2-amino-1-propanol, $HgCl_2$, THF, 55 °C, 3 h; (b) MsCl, DIEA, CH_2Cl_2, 0–25 °C, 1.5 h; (c) H_2, (1 atm.) 10 % Pd/C, CH_3OH, 25 °C, 1.5 h; (d) *p*-trifluoromethylphenyl isocyanate, THF, 0–23 °C, 15 min; (e) *m*-chlorophenylacetic acid, EDC, HBT, NEt_3, DMF, 25 °C, 18 h; (f) HPLC, Pirkle L-leucine column.

Scheme III.

preliminary means of assigning the configuration at the 4-position of new analogs. The absolute configuration of the resolved imidazobenzodiazepines like **12** and **13** was further corroborated based on the comparison of circular dichroism spectra to other benzodiazepine CCK-B receptor ligands where the absolute configuration had been unambiguously established by X-ray crystallographic or chemical methods.

The fully unsaturated imidazobenzodiazepine **4** was prepared according to the sequence of reactions shown in Scheme II. The penultimate 2-amino-1-acetaldehyde dimethylacetal **IV**, obtained from **II** in the standard fashion, was initially subjected to acidic hydrolytic conditions in an attempt to unmask the aldehyde and effect dehydrative cyclization.[21] These probe reactions employing a variety of protic acids were uniformly unsuccessful. However, exposure of **IV** to Lewis acids yielded favorable results. The first successful preparation of **4** was carried out by reacting a solution of **IV** in methylene chloride with iodotrimethylsilane (55 °C, 4 h). Further improvements in this transformation were made by employing boron trifluoride etherate as the Lewis acid.

For the synthesis of some compounds in Table 1, it proved expedient to reverse the order in which the 3-amino group in **I** is elaborated and the imidazo ring is installed. This alternative reaction sequence is summarized in Scheme III whereby the 2-thiono-1,4-benzodiazepine **I** was converted to **V** and thence to **VI** in the customary fashion. Removal of the Cbz-protecting group in **VI** was subsequently accomplished via catalytic hydrogenation under neutral conditions. Without purification, the amine **VII** was then reacted with *p*-trifluoromethylphenyl isocyanate, for the synthesis of **11**, or coupled with *m*-chlorophenylacetic acid to give **16** and **17**. The latter mixture of diastereomers was separated similarly to the above described ureas **12** and **13**.

Biological Methods

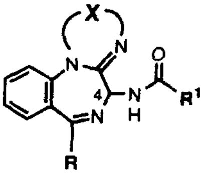
Radioreceptor binding protocol

Radioligand binding to guinea pig cortical membranes was conducted using 50 pM [^{125}I] Bolton Hunter CCK-8s in a 20 mM HEPES buffer, pH 6.5, containing 1 mM EGTA, 5 mM $MgCl_2$, 150 mM NaCl, and 0.025 % bacitracin. For rat pancreatic membranes, the 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 wet weight to 120 mL. Rat pancreatic membranes were prepared in 10 mM HEPES/0.01 % trypsin inhibitor, pH 7.4, centrifugation, and resuspension of the pellet in assay buffer at a 1 to 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 gamma counter. The IC_{50} values thus obtained are the means of triplicate determinations.

VMH slice assay

Coronal sections containing the VMH were cut from the brains of male Sprague–Dawley rats, 350 μm thick, and placed in a chamber where they were continuously

Table 1. Receptor binding affinities of imidazobenzodiazepine ureas[a]



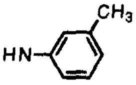
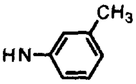
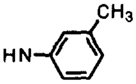
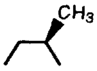
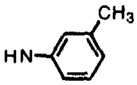
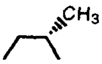
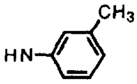
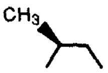
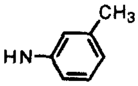
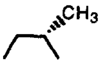
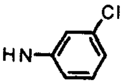
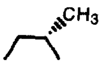
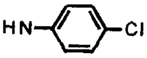
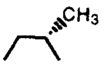
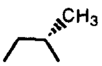
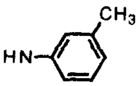
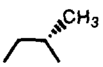
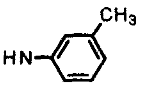
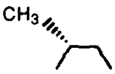
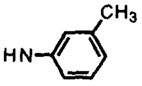
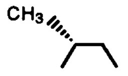
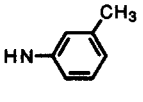
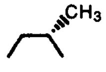
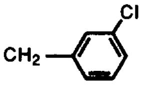
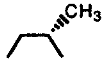
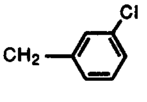
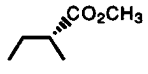
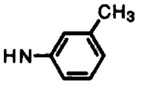
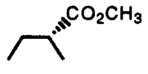
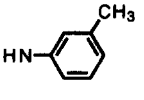
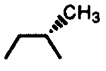
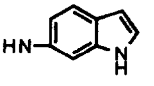
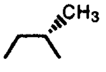
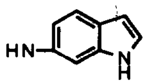
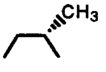
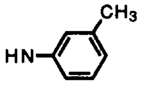
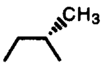
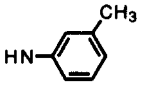
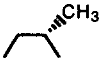
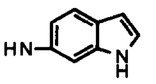
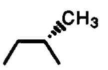
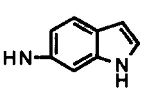
No.	X	R	R1	4-Stereo	IC50 (nM) CCK-B	IC50 (nM) CCK-A
3	CH_2CH_2	phenyl		R,S	9.3 (8.1, 10.7)	38.4 (35.1, 42)
4	CH=CH	phenyl		R,S	2.1 (1.1, 4)	121 (113, 129)
5	$CH_2CH_2CH_2$	phenyl		R,S	272 (181,410)	649 (582,723)
6		phenyl		R,S	61.8 (42.9, 89)	142 (115, 175)
7		phenyl		R,S	2.3 (1.2, 4.7)	695 (641, 754)
8		phenyl		R,S	36.6 (24.4, 54.8)	50.7 (48.5, 53)
9		phenyl		R,S	7.1 (5.6, 9.1)	783 (734, 836)
10		phenyl		R,S	8.2 (4.9, 13.7)	3112 (2791, 3470)
11		phenyl		R,S	41.6 (19.9, 86.9)	3451 (2970, 4009)
12		phenyl		R	1.81 (1.2, 2.7)	1559 (1119, 2171)
13		phenyl		S	3.1 (2.3, 4)	1021 (556, 1874)
14		phenyl		R	3.2 (1.7, 5.9)	572 (510, 642)

Table 1. *Continued.*

No.	X	R	R^1	4-Stereo	IC_{50} (nM)	
					CCK-B	CCK-A
15		phenyl		S	50.7 (39.4, 65.3)	64.3 (60.9, 67.8)
16		phenyl		R	419 (405,434)	>3000
17		phenyl		S	1512 (1412, 1620)	>3000
18		phenyl		R	2.4 (1.8, 3.2)	213 (203, 224)
19		phenyl		S	11.1 (10.7, 11.6)	136 (131, 141)
20		phenyl		R	0.49 (0.27, 0.9)	175 (157, 194)
21		phenyl		S	1.7 (0.87, 3.1)	269 (221, 328)
22		cyclohexyl		R	0.06 (0.02, 0.16)	130 (100, 168)
23		cyclohexyl		S	1.5 (1.5, 1.6)	173 (158, 189)
24		cyclohexyl		R	0.57 (0.37, 0.89)	114 (111, 116)
25		cyclohexyl		S	2.3 (1.2, 4.5)	23.8 (21, 26.9)

[a]Receptor binding is expressed as IC_{50}, the concentration (nM) of compound required for half-maximal inhibition of the binding of $[^{125}I]$BH CCK-8s to receptors in rat pancreatic tissue (CCK-A) or guinea pig cortical membranes (CCK-B). The results represent the geometric mean of between 2 and 6 separate experiments. Statistical limits are given in parentheses.

Ex vivo *binding protocol*

Male BKTO mice (25–30 g) were administered with either vehicle or drug (iv). After 30 min, animals were anesthetized with isoflurane and transcardially perfused with 0.9 % heparinized saline for 20 s. The brains were removed and homogenized in assay buffer (20 mM HEPES, 1 mM EGTA, 5 mM $MgCl_2$, 150 mM NaCl, 0.025

% bacitracin, pH 6.5) in a final dilution of 40 mg wet weight/mL. For the binding assay, 100 µL of tissue homogenate was incubated with 50 pM [^{125}I] BH-CCK-8s (50 µL), assay buffer (300 µL), and either saline, 1 µM CCK-8s (non specific) or drug (calibration curve), for 120 min at ambient temperature. The assay was terminated by rapid filtration and radioactivity determined by counting filters in an LKB gamma counter. Results were expressed as %-inhibition of specific binding of [^{125}I] BH-CCK-8s for each dose. A plot of %-inhibition vs log dose allowed calculation of ED_{50} values for each sample.

Results and Discussion

The initial disclosure of the selective CCK-B receptor antagonist L-365,260 (**2**) was followed by a flurry of activity resulting in the identification of a variety of other, potent CCK-B selective receptor ligands.[16–18] These agents, several of which are independent in genesis and structurally distinct from one another, have aided in defining the binding prerequisites of non-peptide ligands to the CCK-B peptide receptor. With the clinical fate of CCK-B antagonists yet to be established, it seemed to us that the next generation of CCK-B antagonists should, at least, continue to expand the structural diversity of the current collection of CCK-B antagonists, thereby adding to our understanding of ligand/CCK-B receptor interaction and possibly circumventing potential pharmacokinetic problems associated with a given structural motif.

We have continued to regard the benzodiazepine ring system embedded within **2** as a unique template whose utility as a source for novel CCK-B antagonists was again recently demonstrated.[22–24] One observation from these investigations is that judicious modification of three key structural domains on the periphery of the 3-(arylureido)-1,4-benzodiazepine core of **2** can lead to more potent analogs with greater CCK-B receptor subtype selectivity and with improved physicochemical properties. Based on these findings, we felt it meaningful to examine derivatives of **2**, containing modest structural perturbations of the benzodiazepine nucleus itself, which would lead to compounds with slightly altered electronic, conformational, and by inference, pharmacokinetic properties.

An analog of **2** which meets the structural prerequisites enumerated above is the imidazobenzodiazepine **3** (Table 1). Its CCK-B receptor affinity is roughly equivalent to that of **2** justifying further exploration of this compound class. Since it is racemic, it is understandable that **3** does not show a larger preference for the CCK-B versus the CCK-A receptor subtypes;[25,26] nonetheless, the degree of CCK-B selectivity was viewed with encouragement. The fully unsaturated and presumably more rigid homolog **4** is 4-fold more potent than **2** and also displays better CCK-B selectivity, whereas the conformationally more flexible 1,2,3,5-tetrahydropyrimido[1,2-*a*][1,4]benzodiazepine **5** shows evidence of an opposite trend. The disparity in receptor affinity among the closely related homologs **3**, **4**, and **5** is of interest since it confirms that conformational restriction of the 1,4-benzodiazepine ring does influence receptor binding. In this regard, it should be noted that the immediate precursors of the imidazobenzodiazepines **3**, **5**, and **6** (Table 1) were also assayed. In each case, these 2-aminobenzodiazepines were at least an order of magnitude less potent than their cyclized products and displayed selectivity for the CCK-A receptor subtype (data not shown).

In earlier analog work with **2** we showed that replacement of the N^1-methyl group with more lipophilic substituents, specifically *i*-butyl, yielded compounds with enhanced CCK-B receptor affinity and selectivity.[24] This tactic was used with similar effect with the imidazobenzodiazepines. Appending a methyl group onto the imidazo ring of **3** gave **7**, resulting in a 4-fold increase in CCK-B receptor affinity; **7** also shows CCK-B/CCK-A selectivity which is enhanced 70-fold compared with **3**. For the purpose of model building, the imidazo ring in **7** can be considered as an analog of **2** wherein a hypothetical N^1-*i*-butyl group has been cyclized onto the C^2-carbonyl oxygen. It is intriguing that the chirality of the C^2-carbon (imidazobenzodiazepine numbering) bearing the methyl group in **7** has such a marked influence on CCK-B receptor affinity and selectivity (*c.f.* **6**). In this regard, it is therefore not surprising that the regiochemistry of the methyl substituent also plays a role in the CCK receptor binding of these compounds (*c.f.* **6**, **7**, and **8**).

In concert with previous findings, the stereochemistry of the C^4-appendage of **7** also affects CCK receptor selectivity.[25,26] The resolution of **7** gave **12** and **13**, wherein the (4*R*)-configured diastereomer **12** displays a clear preference for binding to the CCK-B receptor. Unlike the precedent set with structures like **2**, inversion of the C^4-configuration in **12** is not a sufficient structural change to reverse its sense of selectivity since the corresponding (4*S*)-configured derivative **13** still shows a bias for the CCK-B receptor subtype. However, given the susceptibility of these compounds toward epimerization (*vide supra*) and the level of homogeneity of the resolved isomers (Table 2), the conclusion that all (4*S*)-diastereomers listed in Table 1 are also CCK-B selective is tenuous. What is clear is that (4*R*)-configured diastereomers in Table 1 have higher affinity for the CCK-B receptor than their 4S-configured counterpart and that the quoted CCK-B receptor binding affinities of the former are conservative.

Products **12** and **14** are hybrid structures which appear to combine optimum binding features of the imidazobenzodiazepine scaffold with the arylurea side chain of **2**. Attempts to further tune the CCK-B binding and selectivity profile of these compounds led to the generation of closely related homologs, for example amides **16** and **17**. Though both isomers appear to be CCK-B selective, their binding affinities are diminished, underscoring the pertinence of the urea linkage (*c.f.* **9** and **16**). Similarly, substituting the imidazo ring of **3** with a polar methyl ester group yields **18** and **19** and no measurable improvement in affinity and selectivity versus **12** and **14**. Modest gains in CCK-B receptor affinity are realized by exchanging the *m*-tolylurea in **12** with a 6-indolylurea side chain giving **20**; however, a

Table 2. Physicochemical data for imidazobenzodiazepines

No.	Scheme	Purification Method[a]	yield[b]	% purity HPLC;[c] (%ee)[d]	Physical form,[e] mp, °C	FAB Mass Spec	C,H,N Analysis
3	I	A	19	92.5	A, 147(d)	410 (M^++H)	$C_{25}H_{23}N_5O\cdot0.15CHCl_3\cdot0.75CH_3OH$ Calc'd: C, 68.90; H, 5.84; N, 15.52. Found: C, 68.92; H, 5.67; N, 15.18.
4	II	A	51	100	A, 144(d)	408 (M^++H)	$C_{25}H_{21}N_5O\cdot0.4CHCl_3$ Calc'd: C, 67.01; H, 4.74; N, 15.39. Found: C, 66.94; H, 4.87; N, 15.35.
5	I	A,D	82	100	C, 172-174(d)	424 (M^++H)	$C_{26}H_{25}N_5O\cdot0.5CHCl_3\cdot1.35CH_3OH$ Calc'd: C, 63.53; H, 5.92; N, 13.30. Found: C, 63.51; H, 5.94; N, 13.55.
6	I	A,D	54	95	C, 141(d)	424 (M^++H)	$C_{26}H_{25}N_5O\cdot0.65Et_2O\cdot0.10$ Hexane Calc'd: C, 73.01; H, 6.90; N, 14.58. Found: C, 72.99; H, 6.52; N, 14.20.
7	I	A	84	95	A, 146(d)	424 (M^++H)	$C_{26}H_{25}N_5O\cdot0.4CHCl_3\cdot0.2CH_3OH$ Calc'd: C, 66.88; H, 5.53; N, 14.66 Found: C, 66.90; H, 5.53; N, 14.60.
8	I	A,D	64	98	A, 140(d)	424 (M^++H)	$C_{26}H_{25}N_5O\cdot0.05CHCl_3\cdot0.10$ Hexane Calc'd: C, 73.06; H, 6.09; N, 15.99. Found: C, 72.88; H, 6.00; N, 15.71.
9	III	A,E	17	95	A, 143-146(d)	444 (M^++H)	$C_{25}H_{22}ClN_5O\cdot0.15CHCl_3\cdot0.95CH_3OH$ Calc'd: C, 63.67; H, 5.31; N, 14.23. Found: C, 63.69; H, 5.22; N, 13.91.
10	III	A,B	48	96	A, 164(d)	444 (M^++H)	$C_{25}H_{22}ClN_5O$ Calc'd: C, 67.64; H, 5.00; N, 15.78. Found: C, 67.44; H, 5.13; N, 13.14.
11	III	A	38	97	A, 148-152(d)	478 (M^++H)	$C_{26}H_{22}F_3N_5O\cdot0.45$ i-Propanol Calc'd: C, 65.10; H, 5.11; N, 13.88. Found: C, 65.28; N, 4.88; N, 13.85.
12	I	A,C,F	27	99.7 (94.2)	A, 149(d)	424 (M^++H)	$C_{26}H_{25}N_5O\cdot0.1CHCl_3\cdot0.05CH_3OH$ Calc'd: C, 71.86; H, 5.83; N, 16.03. Found: C, 71.84; H, 5.88; N, 15.86.
13	I	A,C,F	34	99.5 (97.0)	A, 155(d)	424 (M^++H)	$C_{26}H_{25}N_5O\cdot0.5CHCl_3\cdot0.1$Hexane Calc'd: C, 66.18; H, 5.51; N, 14.24. Found: C, 66.40; H, 5.44; N, 14.24.
14	I	F	49	99.1 (>99)	A, 145-148(d)	424 (M^++H)	$C_{26}H_{25}N_5O\cdot0.35CHCl_3\cdot0.10$ i-Propanol Calc'd: C, 67.91; H, 5.59; N, 14.86. Found: C, 68.15; H, 5.58; N, 14.59.
15	I	F	49	97.2 (>99)	A, 135-138(d)	424 (M^++H)	$C_{26}H_{25}N_5O\cdot0.25CHCl_3\cdot0.15$ Hexane Calc'd: C, 69.93; H, 5.91; N, 15.02. Found: C, 69.95; H, 5.73; N, 15.04.

Table 2. *Continued.*

No.	Scheme	Purification Method[a]	yield[b]	% purity HPLC;[c] (%ee)[d]	Physical form,[e] mp, °C	FAB Mass Spec	C,H,N Analysis
16	III	F	5	(>99)	A, 120-124(d)	443, 445	$C_{26}H_{23}ClN_4O \cdot 0.15CHCl_3 \cdot 0.3H_2O$ Calc'd: C, 67.36; H, 5.13; N, 12.02. Found: C, 67.37; H, 5.13; N, 11.97.
17	III	F	4	(>99)	A, 116-118(d)	443, 445	$C_{26}H_{23}ClN_4O \cdot 0.2CHCl_3 \cdot 0.4H_2O$ Calc'd: C, 66.39; H, 5.10; N, 11.82. Found: C, 66.42; H, 5.11; N, 11.72.
18	I	C	15	95.3(54)	A, 137-140(d)	468 (M^++H)	$C_{27}H_{25}N_5O_3 \cdot 0.4CHCl_3$ Calc'd: C, 63.86; H, 4.97; N, 13.59. Found: C, 63.74; H, 5.13; N, 13.32.
19	I	C	37	93(76)	A, 152(d)	468 (M^++H)	$C_{27}H_{25}N_5O_3 \cdot 0.25CHCl_3 \cdot 0.1EtOAc$ Calc'd: C, 65.60; H, 5.19; N, 13.84. Found: C, 65.73; H, 5.25; N, 13.77.
20	III	C,F	8	99(10)	A, 180-185(d)	449 (M^++H)	$C_{27}H_{24}N_6O \cdot 0.4EtOAc \cdot 0.65H_2O$ Calc'd: C, 69.33; H, 5.80; N, 16.96. Found: C, 69.27; H, 5.47; N, 16.98.
21	III	C,F	8	99(10)	A, 176-180(d)	449 (M^++H)	$C_{27}H_{24}N_6O \cdot 0.4EtOAc \cdot 0.45H_2O$ Calc'd: C, 69.98; H, 5.73; N, 17.24. Found: C, 70.02; H, 5.53; N, 17.22.
22	I	C,D	10	92(95.5)	C, 166(d)	430 (M^++H)	$C_{26}H_{31}N_5O \cdot 0.2CHCl_3$ Calc'd: C, 69.39; H, 6.94; N, 15.45. Found: C, 69.23; H, 6.82; N, 15.20.
23	I	C,D	18	99 (40)	C, 142(d)	430 (M^++H)	$C_{26}H_{31}N_5O \cdot 0.4CHCl_3 \cdot 0.15EtOAc$ Calc'd: C, 66.11; H, 6.70; N, 14.28. Found: C, 66.38; H, 6.46; N, 14.24.
24	III	C,F	28	98.1 (98)	A, 191(d)	455 (M^++H)	$C_{27}H_{30}N_6O \cdot 0.35CHCl_3 \cdot 0.75CH_3OH$ Calc'd: C, 64.85; H, 6.46; N, 16.15. Found: C, 64.83; H, 6.15; N, 15.79.
25	III	C,F	34	95.3 (99)	A, 180 (d)	455 (M^++H)	$C_{27}H_{30}N_6O \cdot 0.35CHCl_3 \cdot 0.75CH_3OH$ Calc'd: C, 64.85; H, 6.46; N, 16.15. Found: C, 64.84; H, 6.09; N, 15.81.

[a] A = Preparative thick layer chromatography on E. Merck silica gel; 2 mm, 1 mm, or 0.5 mm plate thickness.
B = Preparative thin layer chromatography on E. Merck silica gel; 0.25 mm plate thickness.
C = Flash chromatography on 230–400 Mesh E. Merck silica gel.
D = Crystallization or trituration.
E = Semi-preparative reverse phase HPLC using a Vydac C-18 column (1" I.D.).
Conditions: 45 minute gradient elution, H_2O (95 %) containing 0.1 % TFA/acetonitrile (5 %) containing 0.1 % TFA to H_2O (5 %) containing 0.1 % TFA/acetonitrile (95 %) containing 0.1 % TFA; 8 mL/min flow rate.
F = Semi-preparative chiral HPLC using a Pirkle covalent L-leucine column (21 mm I.D.)
Conditions: 60 minute isochratic elution hexane (50 %) containing 0.2 % triethylamine/2-propanol (50 %) containing 0.2 % triethylamine; 6 mL/min flow rate.

[b] Yield refers to final synthetic step, is unoptimized, and represents analytically pure material. For compounds **12–25**, theoretical yield is 50 % based on separation of diastereomers.

[c] 214 nm wavelength detection.

[d] Assayed employing an analytical Pirkle covalent L-leucine column (4.6 mm I.D.), isochratic elution with hexane (50 %) containing 0.2 % triethylamine/-2-propanol (50 %) containing 0.2 % triethylamine; 2 mL/min flow rate.

[e] A = amorphous solid; C = crystalline solid.

commensurate increase in receptor subtype selectivity could not be achieved with this modification. Finally, interchange of the 6-phenyl ring in **12** and **20** with a cyclohexyl ring was also examined. Since the affinity enhancing attributes of the cyclohexyl ring had been demonstrated in an earlier analog study of **2**,[22] the high CCK-B affinity of analogs **22–25** is not unanticipated.

The imidazobenzodiazepine **12** remains one of the most attractive compounds to emerge from this study. To follow up on the high CCK-B receptor affinity and selectivity exhibited by this material, its functional activity was assessed *in vitro* by carrying out electrophysiological studies in rat brain slices.[27] The pentagastrin-induced single cell firing rate of rat ventromedial hypothalamic (VMH) neurons was potently blocked by **12** (**12**, K_b 2.7 ± 0.5 nM, n = 7; **2**, K_b = 41 nM, n > 5). Since excitatory effects in the VMH by pentagastrin are mediated through CCK-B receptors,[28] these results indicate that **12** is a potent and selective CCK-B receptor antagonist.

As CCK-B receptors are widely distributed throughout the central nervous system (CNS), the facility with which **12** crosses the blood–brain barrier is a key issue regarding potential clinical efficacy. Accordingly, the ability of **12** to penetrate into the CNS after systemic administration and determination of its *in vivo* potency was assessed employing an *ex vivo* binding model in the mouse.[29] Using this model, **12** dose-dependently inhibits *ex vivo* binding (**12**, ED_{50} = 2.9 mg/kg (iv); **2**, ED_{50} = 13 mg/kg (iv)). The difference in *in vivo* potency between **12** and **2** compares nicely with the relative difference between their *in vitro* binding (4.7-fold). This result suggests that the brain penetration of **12** is comparable to that observed for the clinical compound L-365,260 (**2**).

Against the background of previously described non-peptide CCK-B receptor antagonists, the compounds disclosed in this work not only extend their SAR's but also expand our understanding of the receptor itself. Among the most promising analogs discovered is **12**, which displays improved CCK-B receptor binding affinity, selectivity, and *in vivo* activity, relative to the benchmark compound **2**. Moreover, it can be expected that **12** will display different pharmacokinetic properties since one of the primary metabolic sites in **2**,[30] the N^1-methyl group, has been modified. Presumably, the metabolic pathway leading to the N^1-demethylation of **2** has been impeded in **12**. While several related imidazobenzodiazepines in this study gave indication of fickle physicochemical properties (e.g. propensity for epimerization), the stability of **12** is enduring.[31] The imidazobenzodiazepine **12** therefore represents an attractive addition to the collection of non-peptide CCK antagonists with which to study CCK-B receptor pharmacology.

Summary

We have presented the synthesis and biological properties of a new class of benzodiazepine CCK-B receptor antagonists. This is a continuation of our efforts to customize the benzodiazepine ring toward interacting with multiple receptor types with high affinity and specificity. The parent imidazobenzodiazepine, **3**, displays CCK-B receptor affinity equivalent to the clinical compound, L-365,260 (**2**). Optimization of the potency and selectivity of **3** by substituting the imidazo ring and by resolving stereomeric mixtures led to the identification of *N*-[(2*S*,4*R*)-methyl-6-phenyl-2,4-dihydro-1*H*-imidazo[1,2-*a*][1,4]benzodiazepin-4-yl]-*N*'-[3-methylphenyl]-urea (**12**) as a high affinity CCK-B receptor antagonist with greater than 800-fold selectivity versus the CCK-A receptor. The addition of these imidazobenzodiazepines to the burgeoning list of non-peptide CCK-B antagonists will add to our understanding of the binding prerequisites of the CCK-B receptor—mandatory information for the *de novo* design of non-peptide drugs interacting with peptide receptors.

Experimental Section

Biological methods

Radioligand binding assays, *in vitro*, and *in vivo* antagonist studies were performed as described in the text. Detailed experimental procedures for these assays have been previously reported.[25,27,29]

Chemical methods

Melting points were determined in open capillaries on either an Electrothermal® or a Thomas–Hoover melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian XL-300, Nicolet NT-360, or Varian VXR-400S spectrometer, each instrument with an internal lock on the deuterium resonance of the solvent. Data are reported as follows: chemical shift (multiplicity, integrated intensity). Mass spectra were obtained with a VG MM/ZAB-HF spectrometer. All compounds exhibited spectra consistent with their structure. HPLC was carried out on a Spectra Physics Model SP8800 liquid chromatograph using a Vydac C-18 column and a Pirkle covalent L-leucine reversible HPLC column. Elemental combustion analyses were performed on a Perkin–Elmer 240 elemental analyzer. All final products prepared for biological studies were dried *in vacuo* over P_2O_5 at temperatures ranging from ambient to 110 °C, depending on the melting point of the sample and showed acceptable (± 0.4 %) elemental analysis; the occurrence of solvation which is extensive for this class of compounds (*c.f.* Table 2) was confirmed independently by ^1H NMR spectroscopy.

Flash chromatography was performed on silica gel (E. Merck 230–400 mesh). Thin layer chromatography (TLC) and preparative thick-layer chromatography (PLC) were carried out on E. Merck 60F-254 precoated silica gel plates (0.25, 0.5, and 2 mm thickness) using UV_{254} light, iodine vapors, or 5 % phosphomolybdic acid reagent in 95 % ethanol to visualize the developed plates. All reactions, except those performed in aqueous solvents, were carried out with use of standard techniques for the exclusion of moisture. Commercial chemicals were used as obtained without purification, except for solvents, which were purified and dried, where appropriate, before use by standard methods.

(R,S)-N-(2,3-Dihydro-2-thiono-5-phenyl-1H-1,4-benzodiazepin-3-yl)-N′-(3-methylphenyl)-urea (II). 1,3-Dihydro-3-(*R,S*)-amino-5-phenyl-2*H*-1,4-benzodiazepin-2-thione (400 mg, 1.5 mmol) was dissolved in 40 mL of dry tetrahydrofuran at 0 °C and treated with 194 μL (1.5 mmol) of *m*-tolylisocyanate. After 10 min, an additional 10 μL of *m*-tolylisocyanate was added to drive the reaction to completion. The reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The residual material was chromatographed on silica gel ($CHCl_3$:CH_3OH:conc. NH_4OH, 96:4:0.4 elution) to afford 480 mg (80 %) of **II**. Trituration with ether–petroleum ether yielded the analytical material: mp 164 °C (d); R_f = 0.33 ($CHCl_3$:CH_3OH:conc. NH_4OH, 95:5:0.5); ^1H NMR (DMSO-d_6) δ 2.24 (s, 3H), 5.42 (d, 1H, C*H*NH), 6.75 (d, 1H), 7.13 (dd, 1H), 7.18 (d, 1H, CHN*H*), 7.25 (br s, 1H), 7.38 (d, 1H), 7.46 (m, 5H), 7.53 (m, 4H), 7.71 (ddd, 1H), 9.19 (s, 1H, Ar–NH–C(=S)); FAB-MS: 401 (M^+ + H); anal. calcd for $C_{23}H_{20}N_4OS$: C, 68.98; H, 5.03; N, 13.99; found: C, 68.96; H, 5.07; N, 13.74.

N-{(S)-(+)-2-Amino-[(R,S)-3-(((3-methylphenyl) amino)-carbonyl)amino]-5-phenyl-2H-1,4-benzodiazepin-2-yl}-1-propanol (III). The thionobenzodiazepinylurea **II** (382 mg, 0.954 mmol) was dissolved in 10 mL of dry tetrahydrofuran and treated in succession with (*S*)-(+)-2-amino-1-propanol (297 μL, 3.82 mmol) and mercuric chloride 337 mg (1.24 mmol). The resulting suspension was then heated at 55 °C for 2 h. The reaction mixture was filtered and the filter cake was washed with tetrahydrofuran. The filtrate was concentrated *in vacuo* and the residue was flash chromatographed on silica gel (2 % CH_3OH in $CHCl_3$) to yield 415 mg (95 % yield) of the analytical product as a mixture of diastereomers: mp 137–140 °C; R_f = 0.23 and 0.28 ($CHCl_3$:CH_3OH, 92:8); ^1H NMR ($CDCl_3$) (all envelopes doubled) δ 1.05 and 1.18 (s, 3H), 2.22 and 2.23 (s, 3H, Ar-CH_3), 3.44 and 3.47 (d, 1H), 3.56 (m, 2H), 3.80 and 3.13 (br d, 1H), 4.05 (br s, 2H), 5.02 and 5.16 (d, 1H, C*H*NH), 5.88 and 6.12 (br d, 1H), 6.67–7.45 (m, 12H), 7.74 and 8.06 (s, 1H); FAB-MS: 442 (M^+ + H); anal. calcd for $C_{26}H_{27}N_5O_2 \cdot 0.5CHCl_3 \cdot 0.35CH_3OH$: C, 62.93; H, 5.68; N, 13.67; found: C, 62.97; H, 5.50; N, 13.45.

N-[(2S,4R)-Methyl-6-phenyl-2,4-dihydro-1H-imidazo[1,2-a][1,4]benzodiazepin-4-yl]-N′-[3-methylphenyl]-urea (12) and N-[(2S,4S)-methyl-6-phenyl-2,4-dihydro-1H-imidazo-[1,2-a][1,4]benzodiazepin-4-yl]-N′-[3-methylphenyl]-urea (13). An ice cold solution of 430 mg (0.974 mmol) of *N*-{(*S*)-(+)-2-amino-[(*R,S*)-3-(((3-methylphenyl) amino)-carbonyl)amino]-5-phenyl-2*H*-1,4-benzodiazepin-2-yl}-1-propanol (**III**) in 17 mL of methylene chloride was protected from moisture and treated with methanesulfonyl chloride (91 μL, 1.17 mmol) and diisopropyl ethylamine (356 μL, 2.04 mmol). After 15 min, the reaction mixture was allowed to warm to room temperature and stirring was continued for 1.5 h more. All volatiles were removed under reduced pressure and the residue was dissolved in 190 mL of ethyl acetate. The organic phase was washed once with 10 % citric acid solution containing brine (50 % by volume), with 10 % sodium carbonate solution (2 × 20 mL), and then with brine. The organic extracts were then dried (sodium sulfate) and concentrated to yield 427 mg of crude product as a mixture of diastereomers. This material was purified to homogeneity via HPLC employing a Pirkle covalent L-leucine column (5 μ, 100 A), (Regis Chemical Co.); chromatography conditions: 1:1 hexane:isopropanol eluant containing 0.2 % triethylamine; 2 mL/min flow rate. The product-containing fractions with retention time 6.95 min were pooled, concentrated, and transferred with a chloroform–methanol solvent mixture to yield **12** in 99.7 % chemical purity: mp 149 °C (d); R_f = 0.19 (ethyl acetate:hexane, 7:3); ^1H NMR ($CDCl_3$) δ 1.31 (d, 3H), 2.20 (s, 3H), 3.46 (m, 1H), 4.34 (m, 2H), 5.95 (d, 1H, C*H*NH), 6.70 (dd, 1H), 7.0–7.4 (m, 10H), 7.5 (m, 2H), 7.88 (m, 2H); FAB-MS: 424 (M^+ + H); anal. calcd for $C_{26}H_{25}N_5O \cdot 0.5CHCl_3 \cdot 0.05CH_3OH$: C, 71.86; H, 5.83; N, 16.03; found: C, 71.84; H, 5.88; N, 15.86.

The product-containing fractions with retention time 11.47 min were pooled, concentrated, and transferred with a chloroform–hexane solvent mixture to yield **13** in 99.7 % chemical purity: mp 155 °C (d); R_f = 0.33 (ethyl acetate:hexane, 7:3); ^1H NMR ($CDCl_3$) δ 1.54 (d, 3H), 2.18 (s, 3H), 3.72 (m, 1H), 3.87 (dd, 1H), 4.26 (m, 1H), 5.94 (d, 1H, C*H*NH), 6.79 (dd, 1H), 7.05 (m, 4H), 7.13 (s, 1H), 7.23 (d, 1H), 7.31 (dd, 2H), 7.39 (dd, 1H), 7.5 (m, 4H), 8.18 (d, 1H); FAB-MS: 424 (M^+ + H); anal. calcd for $C_{26}H_{25}N_5O \cdot 0.5CHCl_3 \cdot 0.1$hexane: C, 66.18; H, 5.51; N, 14.24; found: C, 66.40; H, 5.44; N, 14.24.

N-2-Amino-{[(R,S)-3-(((3-methylphenyl)amino)carbonyl)-amino]-5-phenyl-2H-1,4-benzodiazepin-2-yl}-1-acetaldehyde dimethylacetal (IV). Synthesized from (*R,S*)-*N*-(2,3-dihydro-2-thiono-5-phenyl-1*H*-1,4-benzodiazepin-3-yl)-*N*-(3-methylphenyl)-urea (153 mg, 0.382 mmol) and aminoacetaldehyde dimethylacetal 167 μL (1.528 mmol) in quantitative yield according to the procedure used to prepare **III**: amorphous solid; R_f = 0.38 (ethyl acetate:hexane, 1:1); ^1H NMR ($CDCl_3$) δ 2.22 (s, 3H), 2.26 (s, 3H), 3.30 (s, 3H), 3.43 (m, 1H), 3.75 (m, 1H), 4.43 (dd, 1H), 5.17 (d, 1H, C*H*NH), 6.4 (br s, 1H), 6.79 (d, 1H), 6.85 (d, 1H), 6.95 (m, 2H), 7.05 (dd, 1H), 7.2–7.5 (m, 8H), 7.6 (d, 2H).

N-[6-Phenyl-2,4-dihydro-4H-imidazo[1,2-a][1,4]benzodiazepin-4-yl]-N′-[3-methylphenyl]-urea (4). The dimethyl acetal **IV** (180 mg) was dissolved in 5 mL of methylene chloride and treated with 1.5 mL of borontrifluoride etherate. The reaction mixture was stirred at 50 °C for 2.5 h, cooled, and diluted with 250 mL of ethyl acetate. The resulting solution was washed with 10 % sodium carbonate solution and brine, then dried and concentrated to give 150 mg of the title compound in crude form. The analytical material was obtained after preparative thick layer chromatography on silica gel ($CHCl_3$:MeOH elution, 96:4): mp 144 °C (d); R_f = 0.53 ($CHCl_3$:MeOH, 9:1); ^1H NMR (DMSO-d_6) δ 2.25 (s, 3H), 6.12 (d, 1H, C*H*NH), 6.78 (d, 1H), 7.08 (dd, 1H), 7.12 (br s, 1H), 7.14 (d, 1H), 7.28 (br s, 1H), 7.3–7.54 (m, 9H), 7.62 (m, 2H), 8.20 (br s, 1H); FAB-MS: 408 (M$^+$ + H); anal. calcd for $C_{25}H_{21}N_5O{\cdot}0.4CHCl_3$: C, 67.01; H, 4.74; N, 15.39; found: C, 66.94; H, 4.87; N, 15.35.

N-{(S)-(+)-2-Amino-[(R,S)-3-(benzyloxycarbonyl)amino]-5-phenyl-2H-1,4-benzodiazepin-2-yl}-1-propanol (V). 1,3-Dihydro-3-(*R,S*)-(benzyloxycarbonyl)amino-5-phenyl-2*H*-1,4-benzodiazepin-2-thione (**I**) (500 mg, 1.24 mmol) was converted to the amidine **V** with (*S*)-(+)-2-amino-1-propanol (386 µL, 4.96 mmol) and mercuric chloride (543 mg, 2.0 mmol) employing reaction conditions analogous to those described for the preparation of **III**. In this way, **V** was obtained as a mixture of diastereomers in 70 % yield after flash chromatography on silica gel (EtOAc:hexane, 1:1): amorphous solid; R_f = 0.52 and 0.63 ($CHCl_3$:CH_3OH:conc. NH_4OH, 95:5:0.5); ^{1}H NMR (DMSO-d_6) (all envelopes doubled) δ 0.99 and 1.18 (d, 3H), 3.18 and 3.24 (m, 1H), 3.47 (m, 1H), 3.96 (m, 1H), 4.70 and 4.90 (dd, 1H, O*H*), 4.81 and 4.83 (d, 1H, C*H*NH), 5.10 (AB q, 2H, CH_2–Ar), 6.63 (m, 1H), 6.98 (dd, 1H), 7.14–7.2 (m, 2H), 7.34–7.52 (m, 11H), 8.6 and 8.62 (br d, 1H); FAB-MS: 443 (M$^+$ + H).

[(2S,4S,4R)-Methyl-6-phenyl-2,4-dihydro-1H-imidazo[1,2-a][1,4]benzodiazepin-4-yl]carbamic acid benzyl ester (VI). The *N*-(*S*)-(+)-2-amino-1,4-benzodiazepin-2-yl-1-propanol (V) (865 mg, 1.96 mmol) was reacted with methanesulfonyl chloride (182 µL, 2.35 mmol) and diisopropylethylamine (683 µL, 3.92 mmol) in 35 mL of methylene chloride according to the procedure used to prepare **12** and **13**. In this way, **VI** was obtained in 81 % yield as a mixture of diastereomers: amorphous solid; R_f = 0.42 (ethyl acetate:hexane, 3:2); ^{1}H NMR (DMSO-d_6) (some envelopes doubled) δ 1.04 and 1.35 (d, 3H), 3.39 and 3.65 (dd, 1H), 3.87 and 4.25 (dd, 1H), 4.0 and 4.14 (m, 1H), 5.08 (m, 2H, CH_2–Ar), 5.38 (m, 1H, C*H*NH), 7.11 (dd, 1H), 7.19 (d, 1H), 7.22 (dd, 1H), 7.3–7.46 (m, 9H), 7.49 (m, 1H), 7.6 (dd, 1H), 8.25 (d, 1H, N*H*); FAB-MS: 425 (M$^+$ + H).

(4S,4R)-Amino-(2S)-methyl-6-phenyl-2,4-dihydro-1H-imidazo[1,2-a][1,4]benzodiazepine (VII). A solution of 25 mL of dry methanol containing 120 mg (0.28 mmol) of the Cbz-protected 4-aminobenzodiazepine **VI** was treated with 55 mg of 10 % palladium/carbon catalyst and hydrogenated at room temperature and atmospheric pressure for 1.5 h. An additional 50 mg of catalyst was added and the reaction was continued for 2 h more. The reaction mixture was filtered through Celite and the filtrate was rotoevaporated. The residual material (81 mg) was azeotropically dried with toluene and used in the next reaction without further purification: R_f = 0.33 and 0.42 ($CHCl_3$:CH_3OH:conc.NH_4OH, 90:10:1).

N-[(2S,4S,4R)-Methyl-6-phenyl-2,4-dihydro-1H-imidazo[1,2-a][1,4]benzodiazepin-4-yl]-N'-[4-trifluoromethylphenyl]-urea (11). An ice cold solution of tetrahydrofuran (6.5 mL) containing 68.5 mg (0.24 mmol) of **VII** was treated with 41 µL (0.28 mmol) of 4-trifluoromethylphenyl isocyanate. After 15 min, the reaction mixture was warmed to room temperature and concentrated *in vacuo*. The residual material was purified via preparative TLC on silica gel ($CHCl_3$:CH_3OH, 94:6) to afford 88 mg of **11**. Re-chromatography on silica gel ($CHCl_3$:*i*-PrOH, 95:5) yielded 43 mg (38 %) of the analytical product as a mixture of diastereomers: mp 148–152 °C (d); R_f = 0.66 ($CHCl_3$:CH_3OH, 90:10); ^{1}H NMR (DMSO-d_6) (some envelopes doubled) δ 1.08 and 1.38 (d, 3H), 3.42 and 3.7 (m, 1H), 3.91 and 4.29 (m, 1H), 4.08 and 4.20 (m, 1H), 5.5 (d, 1H, C*H*NH), 7.14 (dd, 1H), 7.22 (d, 1H), 7.25 (dd, 1H), 7.4–7.66 (m, 11H), 9.55 (m, 1H); FAB-MS: 478 (M$^+$ + H); anal. calcd for $C_{26}H_{22}FN_5O{\cdot}0.45$*i*-PrOH: C, 65.10; H, 5.11; N, 13.88, found: C, 65.28; H, 4.88; N, 13.85.

N-[(2S,4R)-Methyl-6-phenyl-2,4-dihydro-1H-imidazo[1,2-a][1,4]benzodiazepin-4-yl]-3-chlorophenyl acetamide (16) and N-[(2S,4S)-methyl-6-phenyl-2,4-dihydro-1H-imidazo[1,2-a][1,4]benzodiazepin-4-yl]-3-chlorophenyl acetamide (17). Into a stirred solution of DMF (3 mL) containing 74 mg (0.25 mmol) **VII** were combined 48 mg (0.28 mmol) 3-chlorophenylacetic acid, 38 mg (0.28 mmol) 1-hydroxybenzotriazole hydrate, 54 mg (0.28 mmol) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, and 39 µL (0.28 mmol) of triethylamine. The reaction mixture was protected from moisture and after 15 min the pH of the reaction medium was adjusted to 8 with the addition of more triethylamine (20 µL). The reaction mixture was then stirred at ambient temperature overnight. All volatiles were removed under reduced pressure and the residue was partitioned between ethyl acetate and water. The organic phase was washed twice with water and 10 % citric acid solution, and then with brine. The organic extracts were dried (Na_2SO_4) and concentrated to yield 60 mg of crude product as a mixture of diastereomers. This material was purified via HPLC employing a Pirkle covalent L-leucine column (21 mm, ID), (Regis Chemical Co.); chromatography conditions: 20 % isopropanol in hexane containing 0.2 % triethylamine; 6 mL/min flow rate. The product-containing fractions were pooled, concentrated and re-chromatographed on a Pirkle covalent L-leucine column (10 mm, ID), (Regis Chemical Co.); chromatography conditions: 40 % isopropanol in hexane containing 0.2 % triethylamine; 2 mL/min flow rate. The fractions with retention time 7.09 min were pooled, concentrated, and transferred with chloroform to yield **16**: mp 120–124 °C (d); R_f = 0.53 ($CHCl_3$:MeOH, 95:5); ^{1}H NMR ($CDCl_3$) δ 1.19 (d, 3H), 3.46 (d, 1H), 3.73 (s, 2H), 4.3 (m, 2H), 5.84 (d, 1H, C*H*NH), 7.07 (m, 2H), 7.26 (m, 4H), 7.34–7.52 (m, 7H), 7.80 (br s, 1H); FAB-MS: 443 (M$^+$ + H); anal. calcd for $C_{26}H_{23}ClN_4O{\cdot}0.15CHCl_3{\cdot}0.3H_2O$: C, 67.36; H, 5.13; N, 12.02; found: C, 67.37; H, 5.13; N, 11.97.

The product-containing fractions with retention time 8.58 min were pooled, concentrated, and transferred with chloroform to yield **17**: mp 116–118 °C (d); R_f = 0.59 ($CHCl_3$:MeOH, 95:5); ^{1}H NMR ($CDCl_3$) δ 1.47 (d, 3H), 3.70 (m, 1H), 3.73 (s, 2H), 3.90 (dd, 1H), 4.20 (m, 1H), 5.84 (d, 1H, C*H*NH), 7.02 (d, 1H), 7.07 (dd, 1H), 7.26 (m, 4H), 7.36 (m, 2H), 7.4–7.52 (m, 5H), 7.82 (br s, 1H); FAB-MS: 443 (M$^+$ + H); anal. calcd for $C_{26}H_{23}ClN_4O{\cdot}0.2CHCl_3{\cdot}0.4H_2O$: C, 66.39; H, 5.10; N, 11.82; found: C, 66.42; H, 5.11; N, 11.72.

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31. Analysis of **12** by analytical chiral HPLC reveals no diminution of % *e.e.* over a 12 month period.

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