Second Generation "Peptoid" CCK-B Receptor Antagonists: Identification and Development of *N*-(Adamantyloxycarbonyl)-α-methyl-(*R*)-tryptophan Derivative (CI-1015) with an Improved Pharmacokinetic Profile

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We have previously described the design and development of CI-988, a peptoid analogue of CCK-4 with excellent binding affinity and selectivity for the CCK-B receptor. Due to its anxiolytic profile in animal models of anxiety, this compound was developed as a clinical candidate. However, during its development, it was determined that CI-988 had low bioavailability in both rodent and nonrodent species. In the clinic, it was further established that CI-988 had poor bioavailability. Thus, there was a need to identify an analogue with an improved pharmacokinetic (PK) profile. The poor bioavailability was attributed to poor absorption and efficient hepatic extraction. We envisaged that reducing the molecular weight of the parent compound (**5**, MW = 614) would lead to better absorption. Thus, we synthesized a series of analogues in which the key α-methyltryptophan and adamantyloxycarbonyl moieties, required for receptor binding, were kept intact and the C-terminus was extensively modified. This SAR study led to the identification of tricyclo[3.3.1.1^{3,7}]dec-2-yl [1*S*-[1α(*S**)2β]-[2-[(2-hydroxycyclohexyl)amino]-1-(1*H*-indol-3-ylmethyl)-1-methyl-2-oxoethyl]carbamate (CI-1015, **31**) with binding affinities of 3.0 and 2900 nM for the CCK-B and CCK-A receptors, respectively. The compound showed CCK-B antagonist profile in the rat ventromedial hypothalamus assay with a K_e of 34 nM. It also showed an anxiolytic like profile orally in a standard anxiety paradigm (X-maze) with a minimum effective dose (MED) of 0.1 μg/kg. Although the compound is less water soluble than CI-988, oral bioavailability in rat was improved nearly 10 times relative to CI-988 when dosed in HPβCD. The blood−brain permeability of CI-1015 (**31**) was also enhanced relative to CI-988 (**5**). On the basis of the overall improved pharmacokinetic profile as well as enhanced brain penetration, CI-1015 (**31**) was chosen as a development candidate.

Cholecystokinin (CCK) has been implicated in the neurobiology of anxiety and panic disorder. CCK-4, the tetrapeptide, has been shown to produce panic-like attacks in humans and produces anxiogenesis in animal models of anxiety.[1] CCK-B receptor antagonists have been shown to produce anxiolytic-like activity in preclinical studies. Due to continued interest in developing novel therapeutic agents, which interact selectively with the cholecystokinin receptors for the treatment of anxiety, panic disorder, etc., several different classes of antagonists have been developed.[2,3] We previously reported a series of peptoid analogues with excellent affinity and selectivity for the CCK-B receptor.[4] From this series of compounds, CI-988 (Figure 1) was developed as a clinical candidate due to anxiolytic activity observed in an established in vivo paradigms such as X-maze in rat.[5] However, during the preclinical and clinical development of this compound, it was determined that its bioavailability was very low (1−3%) in rat,[6] monkey,[7,8] and humans.[9] The low bioavailability

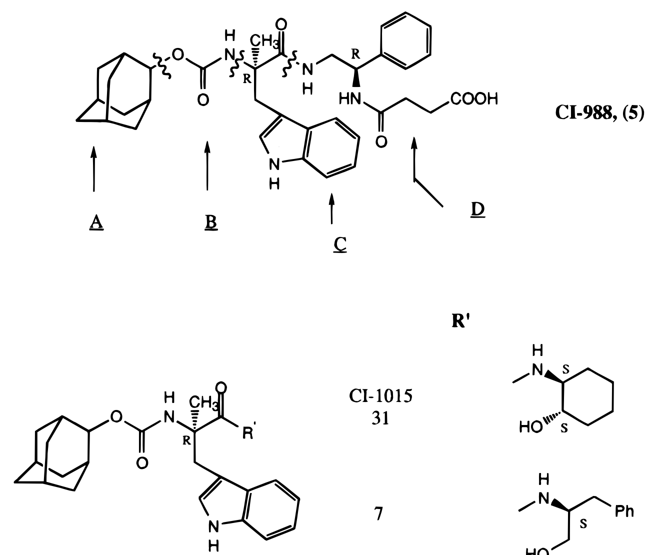

Figure 1. Chemical structures of CI-988 (**5**), CI-1015 (**31**), and **7**.

in preclinical studies was attributed to inefficient absorption as well as high biliary excretion in part due

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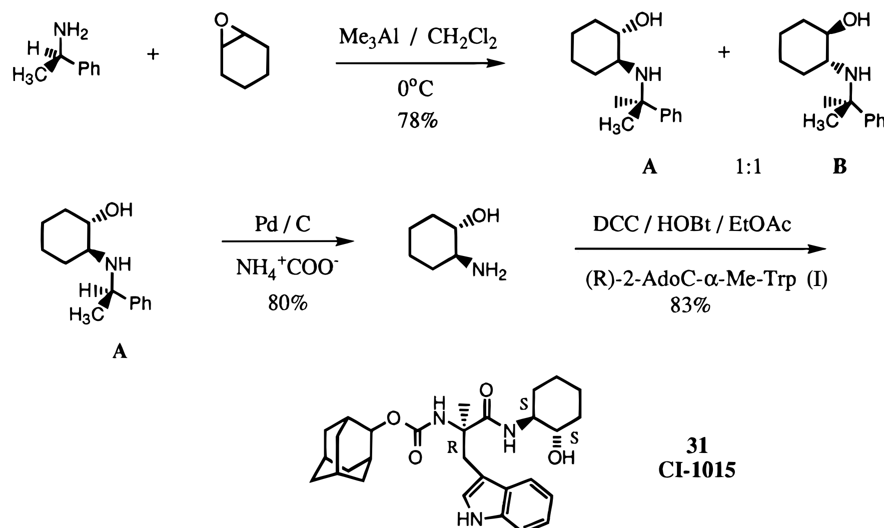
Scheme 1. Synthesis of **31** [CI-1015]



to the high molecular weight (MW = 614) of the compound. Thus, there was a need to identify an analogue with an improved pharmacokinetic profile. It is of interest to note that similar issues faced the development of L-365260, a benzodiazepine-based CCK-B receptor antagonist developed by Merck Laboratories, which was also shown to have low oral bioavailability in preclinical studies.[10] The lack of oral bioavailability was attributed in part to the poor aqueous solubility (<0.002 mg/mL) of the compound. Thus, attempts were made to improve solubility and absorption of the compound by incorporation of polar ionic functionalities into the molecule to enhance aqueous solubility.[11,12]

We approached this problem in the following manner. Since these analogues were easily accessible from optically active 2-Adoc-α-methyltrp acid, we chose to prepare analogues modified at the C-terminus. Since the molecular weight seemed to have contributed to the lack of oral absorption, we dissected the molecule in four different parts (Figure 1). We analyzed each part of the molecule and concluded that fragments A and B, required for high affinity, contributed minimally toward the molecular weight. Fragment C, which contributes 30% of the MW, was critical for affinity. However, fragment D, which represented 38% of the MW, could be further manipulated since earlier SAR studies had suggested that binding affinity was maintained while changing the C-terminus. Additionally, we were also interested in identifying requisite key structural features of compound **3** for high receptor affinity, which had been identified as one of the most potent ligands for the CCK-B receptor.[13] Thus, we began a systematic study of the SAR at the C-terminus.

Chemistry

Synthesis of these analogues was carried out using previously described methods.[4,13] Thus, coupling of an amine with *N*-2-Adoc-α-methyl-(*R*)-tryptophan (**I**) using dicyclohexylcarbodiimde (DCC) and hydroxy benzotriazole (HOBt) or pentafluorophenol (PFP) in ethyl acetate provided targeted analogues. Example **31** (CI-1015) and **32** were synthesized as shown in Scheme 1. The requisite optically active *trans*-2(*S*)-hydroxy-1(*S*)-aminocyclohexane and the corresponding *R*,*R* enantiomer were synthesized by first reacting (*R*)-α-methylbenzylamine with cyclohexane epoxide in the presence of trimethylaluminum in CH_2Cl_2.[14,15] Separation of the diastereomers using column chromatography followed by hydrogenation provided both chiral amino alcohols. Coupling of these amino alcohols with the acid **I** provided corresponding amides **31** and **32**. The absolute stereochemistry for both the amino alcohols was previously assigned.[14,15] On the basis of the comparative physicochemical data (i.e. melting point, rotation), we assigned the absolute stereochemistry to the amino alcohols. This was further corroborated by obtaining an X-ray crystal structure[16] for compound **31** (Figure 2) which confirmed the relative (trans) and absolute stereochemistry at both the amine and alcohol centers being *S*,*S*. Thus, we assigned the *R*,*R* stereochemistry for the other isomer (**32**). Similarly, compounds **33** and **34** were also synthesized.

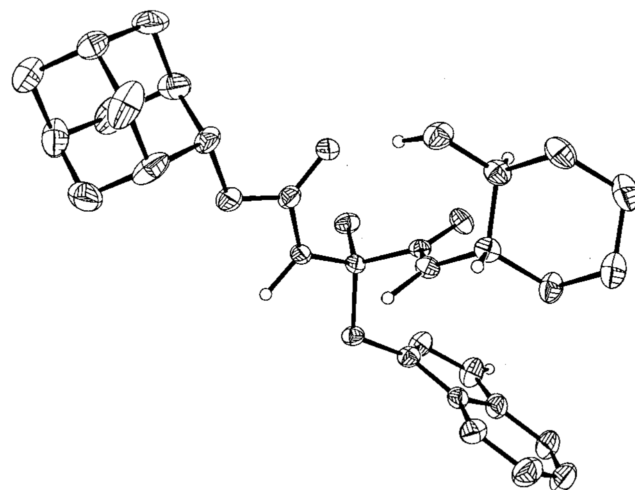


Figure 2. X-ray of CI-1015 (**31**).

Results and Discussion

Earlier studies on this class of compounds had revealed that replacement of the C-terminus of CI-988 with a phenethylamine moiety provided a compound with 32 nM affinity for the CCK-B receptor. Interestingly, incorporation of an acetic acid side chain α to the

amine provided compound **3** with 200-fold improvement in binding affinity with a K_i of 0.15 nM. This is one of the most potent antagonists from the "dipeptoid" series. To define which functional group was critical for potency in **3**, we chose to prepare analogues in which the carboxylate moiety of **3** was first replaced with a phenyl group (**8**). Insertion of an additional phenyl provided a compound with improved affinity and selectivity (**8** vs **1**); however, it was 100-fold less potent than compound **3**. We then prepared a compound in which the phenyl moiety of **3** was replaced with a carboxylate function (**10**). It was anticipated that if the acid functionality in this part of molecule is rendering potency via H-bonding interactions, then compound **10** would provide such an interaction as well. This would provide us the opportunity to synthesize analogues with significantly increased hydrophilicity and, thus, improve oral absorption. However, compound **10**, having two carboxylic acid moieties at the C-terminus, lost all of the binding affinity whereas the corresponding ester (**9**) showed modest binding affinity. These data suggest that having an additional ionic function in this part of the molecule is detrimental to binding. Additionally, it confirms that both a polar carboxylate and a hydrophobic phenyl moieties are necessary for high affinity for the CCK-B receptor.

We then prepared a series of compounds in which we incorporated a variety of small amine side chains at the C-terminus (Table 1). The binding data suggested that for this series of compounds, a nonplanar lipophilic moiety was preferred over a planar hydrophobic function (**12** vs **11**). Additionally, N-alkylation of **12** provided a compound with loss in binding affinity and selectivity, suggesting that the free NH was required for potency. On the basis of these preliminary observations, we focused our efforts toward the synthesis of a series of analogues having a variety of cycloalkylamines and hydrazines. A series of hydrazides were prepared (**14**–**19**). Interestingly, the binding affinity improved with the increase in lipophilicity. Thus, the azabicyclo octane analogue **17** was 20-fold more potent than **14** with a binding affinity of 6.5 nM for the CCK-B receptor. Incorporation of a methoxymethyl functionality on to 1-aminopyrrolidine gave the corresponding *S* (**18**) and *R* (**19**) (methoxymethyl)pyrrolidine derivatives. These analogues showed stereospecific interactions at the receptor, and thus, **19** was more potent and selective with binding affinities of 2.5 and 3430 nM for CCK-B and CCK-A receptors, respectively. It is also of interest to note that, for the first time, the C-terminus side chain was lacking a carboxylic acid moiety which was earlier thought to be critical for potency.

We then prepared analogues in which we incorporated additional hydrophilicity by incorporating carboxylic acid and primary alcohol into the molecules. Geminal substitution of such functionalities provided analogues with only modest binding affinity and selectivity (**23**–**26**). However, vicinal substitutions provided analogues with significantly improved binding affinity. Thus, compound **27**, a mixture of diastereomers, showed an excellent binding affinity of 0.99 nM for the CCK-B receptor and showed 700-fold selectivity. Removal of the acid with a cyano (**28**) or a methyl group (**29**) significantly reduced binding affinity. Both compounds were 4–40-fold less potent than **27**.

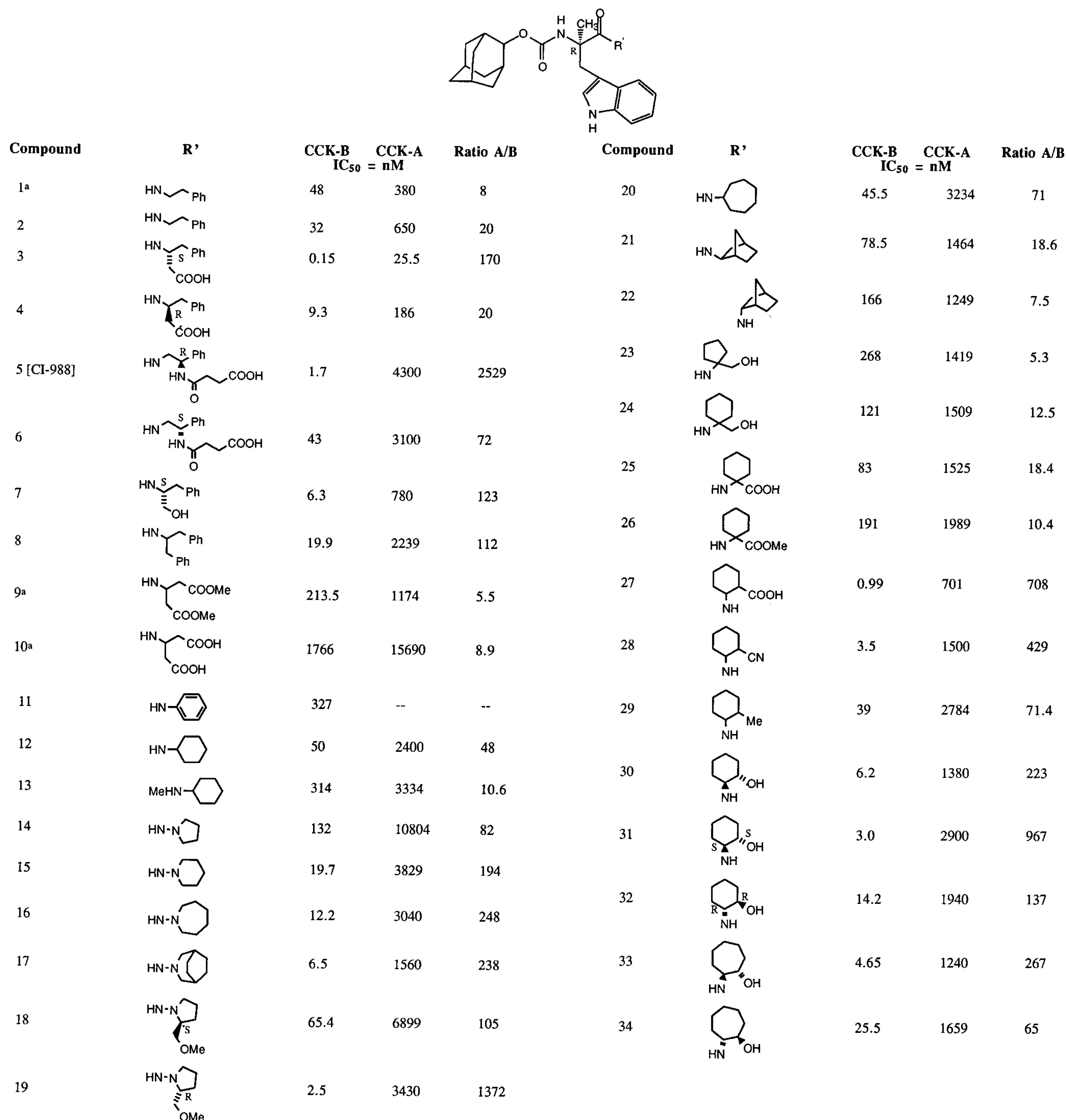
Further exploration of the SAR revealed that incorporation of a hydroxyl moiety on the vicinal carbon (**30**) provided a compound with 6.2 nM binding affinity for the CCK-B receptor. Separation of the individual diastereomers provided us with compound **31** with binding affinities of 3.0 and 2900 nM for the CCK-B and CCK-A receptors, respectively. The other diastereomer (**32**) was less potent and selective. Increasing the size of the cycloalkyl functionality once again provided us with a set of diastereomers (**33, 34**) which showed stereoselective interactions at the receptor. However, compound **33** was less selective compared to the corresponding cyclohexyl derivative (**31**). Similar observations of stereselective interactions for a series of benzodiazepine analogues at the CCK-A and CCK-B receptors have been reported.[17,18]

From this in vitro SAR study a few compounds were further evaluated in the secondary functional assays. In particular, we compared extensively two compounds with a polar hydroxyl group (**7, 31**). On the basis of our earlier observations that for this class of peptoids compounds having a free carboxyl group were poorly absorbed, we chose not to pursue similar compounds (**27**) despite their high potency. Thus, compounds **7** and **31** were first evaluated in an in vitro ventromedial hypothalamus (VMH) assay.[19] These analogues showed K_e values of 1.8 and 34 nM, respectively. These analogues were also assayed (iv) for their ability to block pentagastrin-induced acid secretion in the Ghosh and Schild test in rats. The ED_{50} values for **7** and **31** were 0.5 and 0.3 mg/kg, respectively. These values compared well with the ED_{50} of 0.2 mg/kg for CI-988.

These analogues were further examined for their ability to antagonize CCK induced behavioral response in different animal models in vivo. They were compared with CI-988 in the elevated rat X-maze test where CI-988 had previously been shown to have an anxiolytic-like profile.[20] Oral administration of **7** (0.01–100 μg/kg) and CI-988 (1–10000 μg/kg) 40 min before test increased time spent by rats on the end sections of the X-maze with minimum effective doses (MED) of 0.1 and 10 μg/kg, respectively (Figure 3). A reduction in activity was observed for **7** at the high dose. Similarly, administration of **31** (0.1–100 μg/kg) 30 min before test also increased the time spent on the end sections of the X-maze with a MED of 1.0 μg/kg. The increase in the percent time and entries onto the end sections of the X-maze suggests that these compounds possess anxiolytic-like activity. None of these analogues altered the total number of entries, suggesting a lack of effect on spontaneous locomotor activity. Similarly, compounds **7** and **31** were compared in the light–dark box test in mice in which standard anxiolytics have been shown to be active. In this test (Figure 4), these compounds showed an increase in time spent by mice in the illuminated side, suggesting anxiolytic-like activity with MED of 1 and 10 μg/kg, respectively.

Encouraged by the in vivo activity observed for these analogues, we then evaluated compounds **7** and **31** for their overall pharmacokinetic profile in rat (Table 2). These compounds were administered orally at 20 mg/kg and were delivered in a variety of vehicles. A marked

Table 1. CCK Receptor Binding Affinities

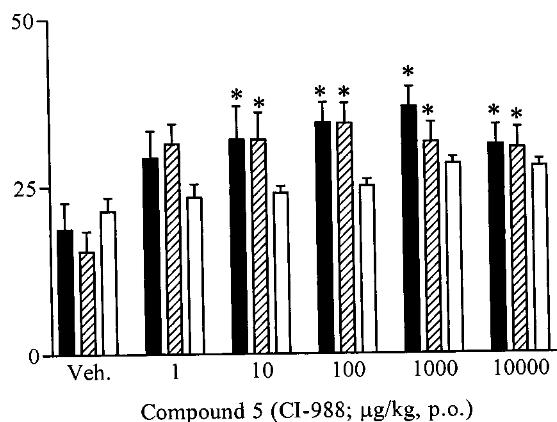


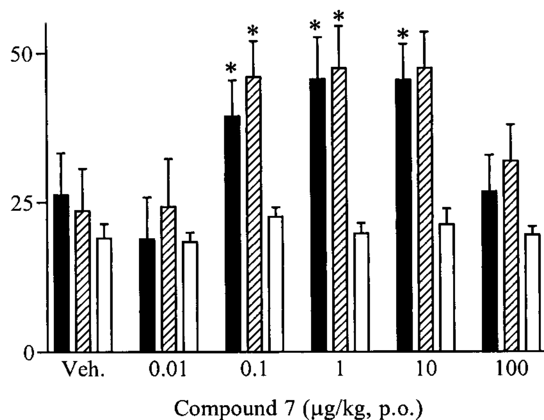
Compound	R'	CCK-B IC_{50} = nM	CCK-A IC_{50} = nM	Ratio A/B
1[a]		48	380	8
2		32	650	20
3		0.15	25.5	170
4		9.3	186	20
5 [CI-988]		1.7	4300	2529
6		43	3100	72
7		6.3	780	123
8		19.9	2239	112
9[a]		213.5	1174	5.5
10[a]		1766	15690	8.9
11		327	--	--
12		50	2400	48
13		314	3334	10.6
14		132	10804	82
15		19.7	3829	194
16		12.2	3040	248
17		6.5	1560	238
18		65.4	6899	105
19		2.5	3430	1372
20		45.5	3234	71
21		78.5	1464	18.6
22		166	1249	7.5
23		268	1419	5.3
24		121	1509	12.5
25		83	1525	18.4
26		191	1989	10.4
27		0.99	701	708
28		3.5	1500	429
29		39	2784	71.4
30		6.2	1380	223
31		3.0	2900	967
32		14.2	1940	137
33		4.65	1240	267
34		25.5	1659	65

[a] (*R*,*S*)-α-methyltrp acid. [b] IC_{50} values are average of triplicate determinations for each compound.

improvement in the oral bioavailability (%F) of **31** relative to that for CI-988 (**5**) was observed with different formulations studied. The optimal bioavailability (28%) was achieved with **31** dosed as a solution in (hydroxypropyl)-β-cyclodextrin [50% (w/v) HPβCD in saline:0.1 N HCl in ethanol (7:3, v/v)]. This formulation offered nearly 10-fold improvement over CI-988. The other three formulations had lower yet similar bioavailability of about 10%, which represented about 2−3-fold improvement relative to CI-988. Oral bioavailability of **7** was also better than that for CI-988 for three of the four formulations tested. When dosed as a solution in HPβCD, the highest bioavailability of 16% was achieved for **7**. As a solution in either PEG 400 or dimethylacetamide:water (3:7), bioavailability was comparable at nearly 10%. However, low bioavailability similar to that of CI-988 was observed when dosed as an aqueous suspension in 5% methylcellulose.

Thus, for both the compounds (**7** and **31**), solution formulations in general achieved better systemic bioavailability than did suspension formulations. These results suggested that the oral absorption of these neutral compounds was solubility limited. Consequently with the right formulation, overall systemic exposure of these second-generation "peptoids" could be markedly improved relative to CI-988. Use of HPβCD





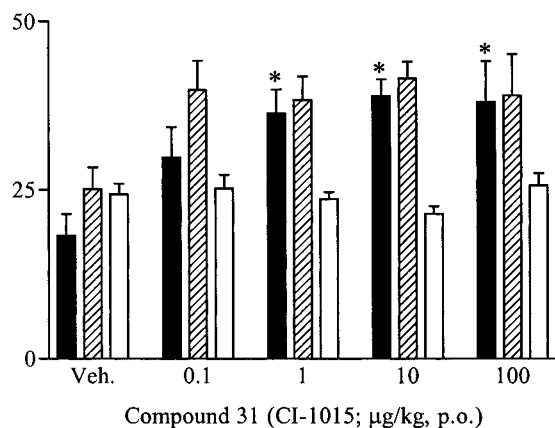
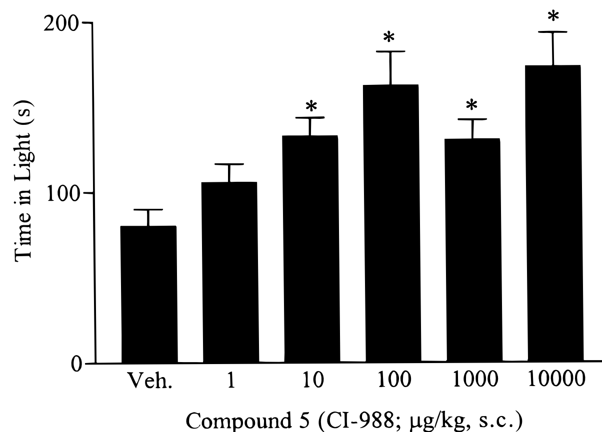
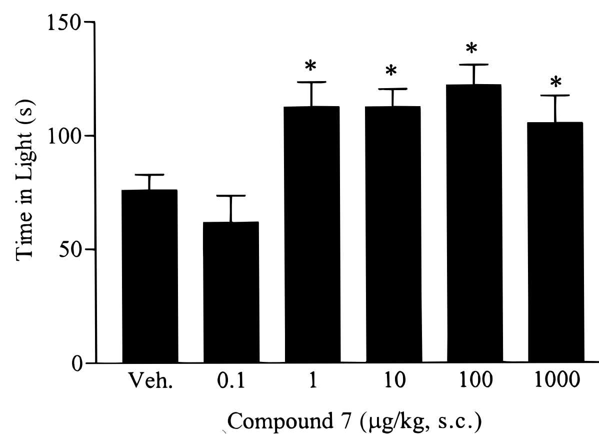


Figure 3. Effects of CI-988 (**5**), **7**, and CI-1015 (**31**) in the rat elevated X-maze. CI 988 and **7** were administered po in PEG 400, 40 min before test. CI-1015 (**31**) was administered po 30 min before test. The percent time spent (%TOEA) and entries made (%EOEA) onto the end half sections of the open arms and the total number of entries (TE) were measured for each animal during the 5 min test. Results are shown as the mean (verticle bars represent ± SEM) of 10 animals per group. *Significantly different from vehicle treated controls, $P < 0.05$ (ANOVA followed by Dunnett's *t*-test).





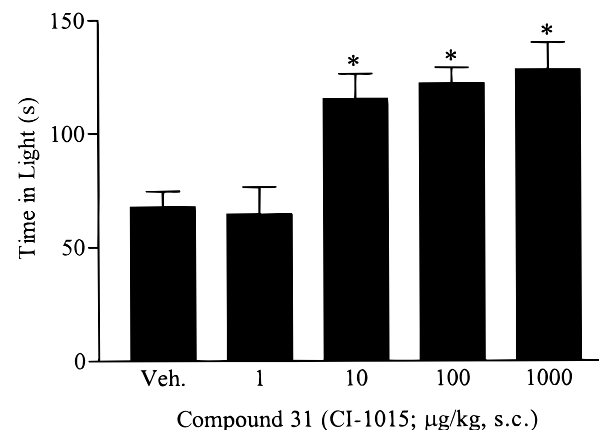


Figure 4. Effects of CI-988 (**5**), **7**, and CI-1015 (**31**) in the mouse light–dark box. CI 988 and **7** were administered sc in PEG 400, 40 min before test. CI-1015 (**31**) was administered sc 30 min before measuring the time spent in the light side during the 5 min observation period. Results are shown as the mean (verticle bars represent ± SEM) of 10 animals per group. *Significantly different from vehicle treated controls, $P < 0.05$ (ANOVA followed by Dunnett's *t*-test).

in oral formulations has been found to be safe for preclinical studies with only about 2% systemic availability.[21,22] Indeed with the decrease in molecular weight and absence of any ionizable group, the overall bioavailability was noticeably improved relative to CI-988.

These new analogues were compared to CI-988 (**5**) for their ability to penetrate the blood–brain barrier in an ex vivo experiment. Following the methods described, brain levels of each drug were determined at 5, 10, and 20 min post iv dose (Table 3). Interestingly, CI-988 (**5**) even after administration of 10 mg/kg iv dose showed only marginal drug levels whereas L-365260, a benzodiazepine-based CCK-B antagonist, showed modest brain levels after 5 min (115 pmol/forebrain) which were significantly reduced after 20 min (33 pmol/forebrain).

Table 2. Summary of Mean (% RSD) Pharmacokinetic Parameters from Fasted Wistar Rats following a Single Oral 20 mg/kg Dose of Different CCK-B Antagonists[a]

compound	formulation	physical form	C_{max} (ng/mL)	t_{max} (h)	AUC(0−∞) (ng·h/mL)	$t_{1/2}$ (h)	%F
5 [CI-988]	saline	solution	32 (19)	2.3 (140)	134 (34)	ND	3.2 (44)
	HPβCD[b]	solution	5 (51)	0.25 (0)	2.3 (80)	ND	<1
31 [CI-1015]	0.5% MC[c]	suspension	185 (56)	3.0 (38)	673 (47)	1.0 (10)	8.9 (47)
	10% PEG[d]	suspension	203 (35)	3.5 (75)	953 (35)	1.1 (41)	13 (35)
	100% PEG[e]	solution	219 (14)	1.0 (71)	724 (26)	1.3 (22)	9.6 (25)
	HPβCD[f]	solution	756 (35)	1.8 (89)	2130 (12)	0.9 (24)	28 (12)
7	0.5% MC[c]	suspension	42 (20)	2.9 (45)	188 (32)	ND	2.0 (35)
	DMA:H_2O[g]	solution	212 (45)	1.2 (42)	890 (26)	2.3 (39)	9.6 (30)
	100% PEG[d]	solution	167 (54)	2.8 (54)	886 (64)	1.1 (18)	9.6 (66)
	HPβCD[f]	solution	416 (31)	0.9 (88)	1440 (26)	1.5 (13)	16 (30)

[a] %RSD = percent relative standard deviation; C_{max} = maximum plasma concentration; t_{max} = time to C_{max}; AUC(0−∞) = area under the plasma concentration−time curve from 0 to infinity; $t_{1/2}$ = terminal elimination half-life; %F = absolute oral bioavailability. [b] HPβCD = 1:2 mmolar ratio of CI-988 to HPβCD in water. [c] 0.5% MC = 0.5% methylcellulose in water. [d] 10% PEG = 10% PEG400 in water. [e] 100% PEG = 100% PEG400. [f] HPβCD = 50% (w/v) (hydroxypropyl)-β-cyclodextrin (HPβCD) in saline:ethanol (7:3, v/v). [g] DMA:H_2O = dimethylacetamide:water (3:7, v/v).

Table 3. Brain Levels of Selected Antagonists Following iv Administration Determined by Ex Vivo Binding

compound dose [1 mg/kg]	brain level (pmol/forebrain) after injection			mice (n)
	5 min	10 min	20 min	
5 [CI-988][a]	10 ± 5	BLD[b]	8 ± 4	3−6
L-365260	115 ± 21	67 ± 14	33 ± 3	6
31 [CI-1015]	177 ± 46	154 ± 39	166 ± 35	9
7	40 ± 13	68 ± 19	43 ± 17	11−13

[a] Dose of 10 mg/kg. [b] BLD = below level of detection (approximately 1 pmol/forebrain).

Compound **7** produced only modest brain levels over 20 min. However, **31** showed highest levels in the brain and maintained those levels throughout the experiment. Thus, it suggests that the higher lipophilicity of **31** (log P = 4.3) relative to CI-988 (log P = 2.0 at pH 7) may in part contribute to the high degree of brain penetration observed for this compound.

Conclusion

In summary, the strategy of lowering molecular weight of the parent compound CI-988 (**5**) to address the issue of low bioavailability has provided **31** with enhanced bioavailability. Interestingly, this peptoid molecule, which lacks the carboxylic acid function, earlier thought to be required for high affinity, maintains an excellent binding affinity and selectivity for the CCK-B receptor. Additionally, it shows antagonist profile both in vitro as well as in vivo. In two separate paradigms it shows an anxiolytic profile. Furthermore, **31** not only shows better bioavailability in rodents than **5**, but also has enhanced blood−brain penetration. Thus, on the basis of its overall improved profile, **31** (CI-1015) has been chosen for further preclinical and clinical evaluation. It is anticipated that CI-1015 will serve as a better tool to assess the clinical utility of CCK-B receptor antagonist as an anxiolytic agent.

Experimental Section

Chemistry. Materials used were obtained from commercial suppliers and used without purification, unless otherwise noted. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. NMR spectra were determined on a Varion Unity 400 or Bruker AM300 or 250 MHz instrument or a Varion XL-200 spectrometer. Chemical shifts are expressed as parts per million downfield from internal tetramethylsilane. Elemental analyses were determined on a Perkin-Elmer Model 240C elemental analyzer or were determined by CHN Analysis Limited, Leicester, U.K., and are within 0.4% of theory, unless otherwise noted. Optical rotations were determined with the use of a Perkin-Elmer 241 polarimeter.

***N*-[2-[(2-hydroxycyclohexyl)amino]-1-(1*H*-indol-3-ylmethyl)-1-methyl-2-oxoethyl]carbamic Acid Tricyclo[3.3.1.1^{3,7}]dec-2-yl Ester (30) and Separation of Diastereomers 31 and 32.** A solution of α-methyl-*N*-[(tricyclo[3.3.1.1^{3,7}]dec-2-yloxy)carbonyl]-(*R*)-tryptophan (0.79 g, 2.0 mmol) in ethyl acetate (60 mL) was treated with *N*,*N*-dicyclohexylcarbodiimide (0.49 g, 2.0 mmol) and 1-hydroxybenzotriazole hydrate (0.3 g, 2.0 mmol). After 2 h of stirring at room temperature, the precipitated solid was filtered and washed with EtOAc. To the clear filtrate were added *trans*-2-aminocyclohexanol hydrochloride (0.3 g, 2.0 mmol) and triethylamine (0.2 g, 2.0 mmol), and the reaction mixture was stirred overnight. It was washed with 5% HCl, 5% $NaHCO_3$, and brine. The organic extract was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by flash column chromatography over silica gel using hexanes−ethyl acetate (1:1) to give 0.65 g of **30**.

Individual diastereomers of **30** were separated by Waters Model 46K HPLC using silica column (6 μm, Prep Nova Pak). The mobile phase was ethyl acetate−hexane (6:4), and the flow rate was 15 mL/min. Compound **31** (mp 125−130 °C) had a retention time of 11.92 min, and compound **32** (mp 130−137 °C) had a retention time of 13.87 min.

Tricyclo[3.3.1.1^{3,7}]dec-2-yl [1*S*-[1α(*S)2β]-[2-[(2-Hydroxycyclohexyl)amino]-1-(1*H*-indol-3-ylmethyl)-1-methyl-2-oxoethyl]carbamate (31). (a) (1*S*,2*S*)-*trans*-2-[(*R*)-(α-Methylbenzyl)amino]cyclohexanol (A) and (1*R*,2*R*)-*trans*-2-[(*R*)-(α-Methylbenzyl)amino]cyclohexanol (B).** To a well-stirred solution of (*R*)-α-methylbenzylamine (6.06 g, 5.0 mmol) in CH_2Cl_2 (60 mL) was added dropwise a solution of trimethylaluminum (27.5 mL, 2.0 M solution in toluene, 55 mmol) at 0−5 °C. The resulting solution was stirred for additional 45 min, and a solution of cyclohexane epoxide in methylene chloride (15 mL) was added dropwise at 0 °C. The reaction mixture was stirred overnight at room temperature. The aluminum complex was decomposed by adding NaF (8.82 g, 210 mmol) at 0 °C followed by water (6 mL). After 1 h of stirring, the reaction mixture was passed through Celite and concentrated. The residue was purified by flash column chromatography on silica gel using EtOAc−hexane−Et_3N (1:1:0.1) to provide compound A (4.5 g, 39.%, R_f = 0.5) and compound B (4.5 g, 39%, R_f = 0.32).

(b) (1*S*,2*S*)-*trans*-2-Aminocyclohexanol. A mixture of (1*S*,2*S*)-*trans*-2-[(*R*)-(α-methylbenzyl)amino]cyclohexanol (2.2 g, 0.01 mol), 10% Pd/C, and ammonium formate (0.38 g, 0.06 mol) in methanol (75 mL) was stirred overnight at room temperature. The mixture was passed through Celite and concentrated. The residue was diluted with ethyl acetate, washed with water, dried over $MgSO_4$, filtered, and concentrated to yield 0.92 g (80%) of (1*S*,2*S*)-*trans*-2-aminocyclohexanol.

(c) Tricyclo[3.3.1.1^{3,7}]dec-2-yl [1*S*-[1α(*S)2β]-[2-[(2-hydroxycyclohexyl)amino]-1-(1*H*-indol-3-ylmethyl)-1-methyl-2-oxoethyl]carbamate (31).** A solution of (*R*)-2-(adamantyloxycarbonyl)-α-methyltryptophan (0.79 g, 2.0 mmol) in ethyl acetate (60 mL) was treated with dicyclohexylcarbodiimide (0.495 g, 2.0 mmol) and 1-hydroxybenzotriazole hydrate (0.35 g, 2.2 mmol). After 2 h of stirring at room temperature, the precipitated urea was filtered. To the clear filtrate was added (1*S*,2*S*)-*trans*-2-aminocyclohexanol (0.23 g, 2.0 mmol). The reaction mixture was stirred at room temperature overnight. The solution was washed with 5% HCl, followed by 5% $NaHCO_3$ and brine. The organic phase was dried over $MgSO_4$ and concentrated. The residue was purified by flash column chromatography over silica gel using ethyl acetate−hexane (1:1) to yield 0.72 g of material which was repurified on Waters HPLC (Model 46K) using silica column (6 μm, Prep Nova Pak). The mobile phase was ethyl acetate−hexane (1:1), and the flow rate was 15 mL/min. Compound **31** was obtained as a solid (0.4 g, 39.5%, retention time of 9.54 min) having a melting point of 137−142 °C: NMR ($CDCl_3$) 0.8−2.1 (25H, m), 2.76 (1H, br s), 3.22 (1H, dt, J = 4.1 and 5.7 Hz), 3.33 (1H, d, J = 14.75 Hz), 3.48 (1H, d, J = 14.76 Hz), 3.6−3.8 (1H, m), 4.84 (1H, br s), 5.21 (1H, s), 6.0 (1H, d, J = 8.3 Hz), 7.0−7.23 (3H, m), 7.38 (1H, d, J = 8 Hz), 7.61 (1H, d, J = 7.6 Hz), 8.32 (1H, s). Anal. C, H, N.

Biological Assays. CCK-A and CCK-B receptor binding assays were performed as previously described.[4,13] The pharmacological activity of these antagonists was evaluated in the rat elevated X-maze and the mouse light−dark box as described by Singh et al.[20]

The pharmacokinetic studies were performed as follows: The oral bioavailability and pharmacokinetics of these analogues were evaluated in fasted Wistar rats. Groups of three to four male wistar rats received either a single oral (po) or iv dose of a given compound at 20 mg/kg. Due to aqueous solubility limitations, different vehicles were used for dosing. Heparinized plasma samples were collected from an implanted cannula in the jugular vein at serial time out to 24 h. Plasma samples were frozen until analysis and assayed with validated HPLC-fluorescence methods for each compound.[23] Pharmacokinetic parameters were determined by standard noncompartmental methods. Absolute oral bioavailability was calculated as the dose normalized ratio of the area under the curve following po dose to the area under the curve following iv dose.

Ex vivo binding studies were performed as follows: Mice were dosed intravenously with 1 mg/kg test compound or vehicle (saline for **5** and 10% EtOH−60% propylene glycol−30% saline for **31** and L-365260). Animals were sacrificed at various times after dosing and brains removed. The forebrain was dissected from the cerebellum and homogenized in 5 mL assay buffer (10 mM Hepes, 130 nM NaCl, 4.7 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, containing 0.25 mg/mL bacitracin, pH 7.2 at 21 °C). Aliquots (300 μL) of tissue from animal dosed with either test compound or vehicle were incubated with 50 pM [^{125}I]BH-CCK8s (total assay volume = 1 mL) for 2 h at 22 °C before filtering under vacuum onto GF\B filters. The amount of specifically bound radioligand was determined using a gamma counter. Calibration curves to each compound were run in parallel using tissue from vehicle-treated animals and used to determine the amount of test compound present in the brain homogenates. The limit of detection using this methodology was approximately 1 pmol/forebrain.

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Supporting Information Available: Tables of physical and chemical data of **8**−**34** and crystallographic data (14 pages). Ordering information is given on any current masthead page.

References

(1) Bourine, M.; Malinge, M.; Vasar, E.; Bradwejn, J. Two faces of cholecystokinin: anxiety and schizophrenia. *Fundam. Clin. Pharmacol.* **1996**, *10*, 116−126.

(2) Trivedi, B. K. Ligands for cholecystokinin receptors: recent developments. *Curr. Opin. Ther. Pat.* **1994**, *4* (1), 31−44.

(3) Trivedi, B. K. Cholecystokinin Receptor Antagonists: Current Status. *Curr. Med. Chem.* **1994**, *1*, 313−327.

(4) Horwell, D. C.; Hughes, J.; Hunter, J. C.; Pritchard, M. C.; Richardson, R. S.; Roberts, E.; Woodruff, G. N. Rationally designed 'dipeptoid' analogues of CCK. Methyltryptophan derivatives as highly selective and orally active gastrin and CCK-B antagonists with potent anxiolytic properties. *J. Med. Chem.* **1991**, *34*, 404−414.

(5) Hughes, J.; Boden, P.; Costall, B.; Domeney, A.; Kelly, E.; Horwell, D. C.; Hunter, J. C.; Pinnock, R. D.; Woodruff, G. N. Development of a class of selective cholecystokinin type B receptor antagonists having potent anxiolytic activity. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *87*, 6728.

(6) Feng, R.; Hinton, J. P.; Hoffman, K.; Parker, T. D.; Wright, D. S. Pharmacokinetics and oral bioavailability of CI-988 ester prodrugs in wistar rats. *Pharm. Res.* **1993**, *10* (10), S-346.

(7) Hinton, J. P.; Rutkowski, K.; Johnson, E. L.; Wright, D. S. Single dose pharmacokinetics and absolute bioavailability of the anxiolytic CI-988 in fasted and fed cynomolgus monkeys. *Pharm. Res.* **1991**, *8* (10), S-267.

(8) Hinton, J. P.; Hoffman, G.; Poisson, A.; Klemisch, W.; Wright, D. S. Mass Balance and disposition of [^{14}C]CI-988 in cynomolgus monkeys. *Pharm. Res.* **1993**, *10* (10), S-330.

(9) Bradwejn, J.; Koszycki, D.; Paradis, M.; Reece, P.; Hinton, J. P.; Sedman, A. Effect of CI-988 on cholecystokinin tetrapeptide-induced panic symptoms in healthy volunteers. *Biol. Psychiatry* **1995**, *38*, 742−746.

(10) Chen, I−W.; Dorley, J. M.; Ranjit, H. G.; Pitzenberger, S. M.; Lin, J. H. Physiological disposition and metabolism of L-365260, a potent antagonist of brain cholecystokinin receptor in laboratory animals. *Drug Metab. Dispos.* **1992**, *20(3)*, 390−395.

(11) Bock, M. J.; Mellin, E. C.; Newton, R. C.; Veber, D. F.; Freedman, S. B.; Smith, A. J.; Patel, S.; Kemp, J. A.; Marshall, G. R.; Fletcher, A. E.; Chapman, K. L.; Anderson, P. S.; Freidinger, R. M. Second-generation benzodiazepine CCK-B antagonists. Development of subnanomolar analogues with selectivity and water solubility. *J. Med. Chem.* **1994**, *37*, 722−724.

(12) Showell, G. A.; Bourrain, S.; Neduvelil, J. G.; Fletcher, S. R.; Baker, R.; Watt, A. P.; Fletcher, A. E.; Freedman, S. B.; Kemp, J. A.; Marshall, G. R.; Patel, S.; Smith, A. J.; Matassa, V. G. High affinity and potent, water soluble 5-amino-1,4-benzodiazepine CCK-B/gastrin receptor antagonists containing a cationic solubilizing group. *J. Med. Chem.* **1994**, *37*, 719−721.

(13) Boden, P. R.; Higginbottom, M.; Hill, D. R.; Horwell, D. C.; Hughes, J.; Rees, D. C.; Roberts, E.; Singh, L.; Suman-Chauhan, N.; Woodruff, G. N. Cholecystokinin dipeptoid antagonists: design, synthesis and anxiolytic profile of some novel CCK-A and CCK-B selective and mixed antagonists. *J. Med. Chem.* **1993**, *36*, 552−565.

(14) Overman, L. E.; Sugai, S. A convenient method for obtaining trans-2-amino cyclohexanol and trans-2-amino cyclopentanol in enantiomerically pure form. *J. Org. Chem.* **1985**, *50*, 4154−4155.

(15) Aubé, J.; Wolfe, M. S.; Yantiss, R. K.; Cook, S. M.; Takusagawa, F. Synthesis of enantiopure N-tert-butoxycarbonyl-2-aminocycloalkanones. *Synth. Commun.* **1992**, *22* (20), 3003−3012.

(16) Andre Michel, personal communication. X-ray coordinates will be deposited in Cambridge Database.

(17) Evans, B. E.; Rittle, K. E.; Bock, M. G.; DiPardo, R. M.; Whitter, W. L.; Veber, D. F.; Anderson, P. S.; Freidinger, R. M.; Gould, N. P.; Lundell, G. F.; Homnick, C. F.; Chang, R. S. L.; Lotti, V. J.; Cerino, D. J.; Chen, T. B.; Kling, P. J.; Kunkel, K. A.; Springer, J. P.; Hirshfield, J. Design of nonpeptidal ligands for a peptide receptor: cholecystokinin antagonists. *J. Med. Chem.* **1987**, *30*, 1229.

(18) Evans, B. E.; Rittle, K. E.; Bock, M. G.; DiPardo, R. M.; Friedinger, R. M.; Whitter, W. L.; Lundell, G. F.; Veber, D. F.; Anderson, P. S.; Chang, R. S. L.; Lotti, V. J.; Cerino, D. J.; Chen, T. B.; King, P. J.; Kunkel, K. A.; Springer, J. P.; Hirshfield, J. Methods for drug discovery: development of potent, selective, orally effective cholecystokinin antagonists. *J. Med. Chem.* **1988**, *31*, 2235.

(19) Boden, P. R.; Pinnock, R. D.; Pritchard, M. C.; Woodruff, G. N. *Br. J. Pharmacol.* **1994**, *112*, 666−670.
(20) Singh, L.; Field, M. J.; Hughes, J.; Menzies, R.; Oles, R. J.; Vass, C. A.; Woodruff, G. N. The behavioral prperties of CI-988, a selective cholecystokinin B receptor antagonist. *Br. J. Pharmacol.* **1991**, *104*, 239−245.
(21) Szejtli, J. Cyclodextrins: Properties and Applications. *Drug Invest.* **1990**, *2* (4), 11−21.
(22) Frijlink, H. W.; Visser, J.; Hefting, N. R.; Oosting, R.; Meijer, D. K. F.; Lerk C. F. The Pharmacokinetics of β-cyclodextrin and hydroxypropyl-β-cyclodextrin in the rat. *Pharm. Res.* **1990**, *7* (12), 1248−1252.
(23) Hinton, J. P.; Pablo, J.; Bjorge, S.; Hoffman, K.; Jennings, K.; Wright, D. S. Three complementary liquid chromatographic methods for determination of the peptoid cholecystokinin-B antagonist, CI-988, in rat plasma. *J. Pharm Biomed. Anal.* **1996**, *14*, 815−824.

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