Discovery of 1,5-Benzodiazepines with Peripheral Cholecystokinin (CCK-A) Receptor Agonist Activity. 1. Optimization of the Agonist "Trigger"

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Directed screening of compounds selected from the Glaxo registry file for contractile activity on the isolated guinea pig gallbladder (GPGB) identified a series of 1,5-benzodiazepines with peripheral cholecystokinin (CCK) receptor agonist activity. Agonist efficacy within this series was modulated by variation of substituents on the *N*1-anilinoacetamide moiety. Remarkably, a single methyl group confers agonist activity, with an *N*-isopropyl substituent providing optimal efficacy. Hydrophilic substituents on the anilino nitrogen abolish agonist activity or produce antagonists of CCK. In contrast, hydrophilic electron-donating groups at the para-position of the anilino ring enhance or maintain *in vitro* and *in vivo* agonist activity. Despite decreased affinity for the human CCK-A receptor, relative to CCK-8, some of these compounds are equipotent to CCK as anorectic agents in rats following intraperitoneal administration.

Introduction

Cholecystokinin (CCK) is a gastrointestinal hormone and neurotransmitter involved in nutrient assimilation, including the secretion of bile and digestive enzymes and the regulation of enteric transit.[1] While a variety of endogenous molecular forms of CCK have been isolated, the C-terminal octapeptide (Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-PheNH_2, CCK-8) appears to be the minimum sequence required for bioactivity.[1] CCK-8 potently activates both peripheral (CCK-A) and central (CCK-B) receptor subtypes.[1] The utility of a CCK receptor agonist for the treatment of obesity is suggested by studies demonstrating that exogenous CCK can shorten meal duration and reduce meal size in several species, including lean[2] and obese[3] humans. Chronic administration of CCK-8 to patients on total parenteral nutrition has also demonstrated a role for CCK in the prevention of gallstones.[4] The relevant target for both effects is the CCK-A receptor.[5]

The high molecular weight, acid lability of the Tyr(SO_3H) residue, metabolic instability, lack of oral activity and lack of receptor selectivity appear to limit the therapeutic potential of CCK-8. Despite remarkable success in the design of metabolically stable, receptor selective peptide ligands, no orally active agonist has been reported.[6,7] Because nonpeptide ligands have historically offered greater opportunity for synthetic manipulation of both pharmacodynamic (selectivity and efficacy) and pharmacokinetic (oral bioavailability, duration) parameters,[8−10] we elected to search the Glaxo registry file for novel nonpeptidyl CCK-A agonist leads.

The 1,4-benzodiazepine CCK-A (devazepide, MK-329, L364,718)[11] and CCK-B (L-365,260)[12] receptor selective antagonists were optimized from a natural product lead which was originally identified by random screening in a receptor binding assay.[13] This same approach has provided numerous nonpeptidyl antagonist ligands in the past 10 years.[8−10] Although receptor binding assays are used routinely to screen for novel chemical leads, these assays only measure ligand affinity and secondary functional assays are required to fully characterize the pharmacological profile of any given lead. Since our goal was the identification of a CCK-A receptor agonist, we chose a functional assay as our primary screen. Compounds were selected for screening based on structural features of known CCK-A agonist peptides,[6] particularly the unique phenylurea moiety of the tetrapeptide agonist Boc-Trp-Lys(Tac)-Asp-MePheNH_2 (A-71623),[7] and evaluated at a single concentration (30 μM) for contractile activity on the isolated guinea pig gallbladder (GPGB).[14] Reversal of contraction with MK-329 (1 μM) was used to verify a CCK-A receptor mediated response. Compounds which did not produce contraction were evaluated for antagonist activity against a concentration−response curve for CCK-8 (10^{-11}−10^{-6} M). Of the 237 compounds screened, the majority were either inactive or antagonists of CCK-8. However, a series of 1,5-benzodiazepines was identified, **11c, 11d,** and **11k** (Table 1), in which agonist efficacy was modulated by appropriate substitution of the *N*1-anilinoacetamide moiety.

We now report our efforts to optimize the agonist "trigger" on these novel 1,5-benzodiazepines. In addition to exploring the potential for hydrophobic bulk at the anilino nitrogen, we have also attempted to improve the aqueous solubility of these compounds through the introduction of hydrophilic groups on the anilino nitrogen or at the para-position of the aromatic ring.

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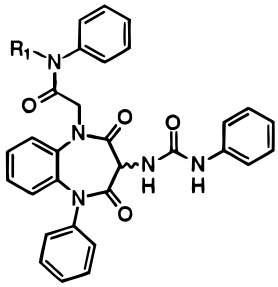
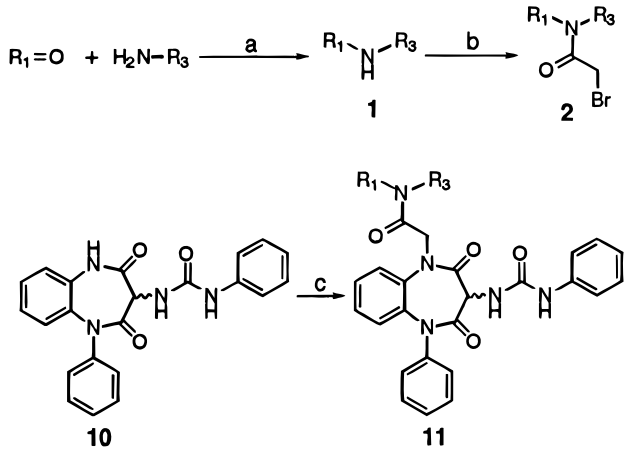
Table 1. Functional Activity of Benzodiazepine Ligands on the Isolated Guinea Pig Gallbladder



compd	R_1	GPGB[a] (30 μM) %CCK-8	GPGB[a] (30 μM) pK_B
11a	H	ia	6.33
11b	CH_3	ia	6.27
11c	CH_3CH_2	66	
11d	$CH_3CH_2CH_2$	50	
11e	$CH_3CH_2CH_2CH_2$	27	
11f	$NCCH_2CH_2$	32	
11g	$HOOCCH_2$	ia	6.33
11h	$EtOOCCH_2$	16	
11i	$H_2NCH_2CH_2$	7	5.88
11j	$CBZHNCH_2CH_2$	ia	5.34
11k	$(CH_3)_2CH$	86	
11l	$c(C_6H_{11})$	26	
11m	C_6H_5	43	

[a] Functional activity in the isolated guinea pig gallbladder following incubation with test ligand (30 μM) for 30 min at 37 °C; %CCK-8, percent contraction normalized to the percent contraction induced by 1 μM CCK-8; pK_B, single dose pA_2 calculated from the fold-shift of the CCK-8 concentration response curve in the presence of the test ligand (30 μM); ia, inactive.

Scheme 1[a]



[a] (a) $NaB(O_2CCH_3)_3H$, $ClCH_2CH_2Cl$; (b) $BrCH_2COBr$, TEA, DCM (c) NaH, DMF, **2**.

Methods

Compounds of general structure **11** were prepared by alkylation of intermediate phenylurea **10** with various bromoacetamides **2** (Scheme 1). Bromoacetamides **2** were prepared in high yield from the reaction of the corresponding secondary amines **1** with bromoacetyl bromide. Where suitable secondary amines could not be purchased from commercial suppliers, they were synthesized from the primary amine and aldehyde or ketone via reductive amination with sodium triacetoxyborohydride[15] in excellent yields and purity.

The convergent synthesis of intermediate phenylurea **10** is illustrated in Scheme 2. Condensation of ketomalonic acid with phenylhydrazine provided phenylhydrazone **3**, which was subsequently converted to the diacid chloride **4** by treatment with phosphorus pentachloride in carbon tetrachloride. Acylation of *N*-phenyl-1,2-phenylenediamine with anisoyl chloride in dichloromethane, followed by reduction with $LiAlH_4$, provided the *p*-methoxybenzyl-substituted diamine **6** in overall 81% yield. Condensation of diamine **6** with **4** provided benzodiazepine **7** in 83% yield. Following reduction of the phenylhydrazone with Zn/acetic acid and oxidative cleavage of the *p*-methoxybenzyl moiety, amine **9** was reacted with phenyl isocyanate to give the intermediate urea **10**. Where protecting groups were employed, these were removed as the final step by standard methods as described in the Experimental Section. Compounds **11a**−**v** were purified to homogeneity (>97%) by RP-HPLC chromatography and characterized by analytical RP-HPLC, ^{1}H-NMR spectroscopy, low- and high-resolution mass spectrometry, and combustion analysis.

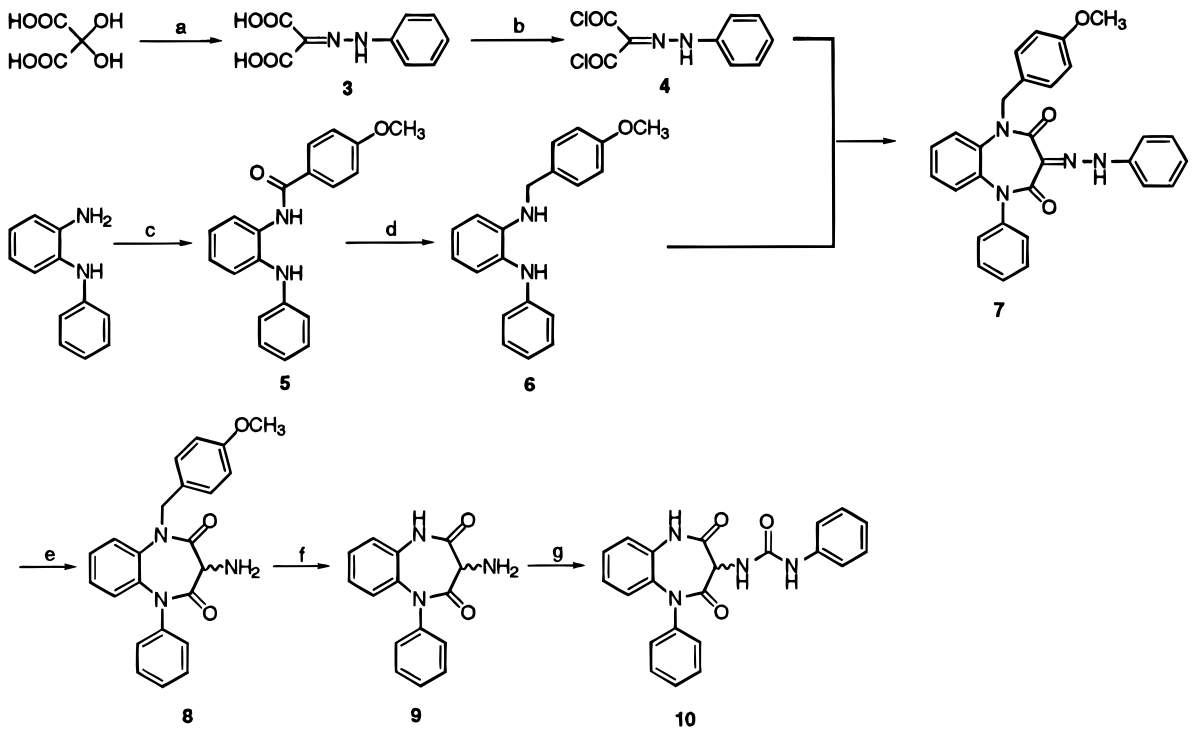
Analogs were evaluated for *in vitro* functional efficacy on the isolated guinea pig gallbladder at 30 or 1 μM concentrations (Tables 1−4).[14] The long incubation time (30−60 min) required for each compound to achieve maximal response precluded accurate measurement of potency through standard concentration−response curves. The contractile activity of all compounds reported was reversed completely with the CCK-A receptor selective antagonist MK-329.[11] This data is illustrated for **11k**, **11n**−**q**, and **11s** in Table 3. Compounds which did not produce contraction were evaluated for antagonist activity against a concentration−response curve for CCK-8 (10^{-11}−10^{-6} M). Receptor binding affinities were measured on membrane preparations from CHO-K1 cell lines stably transfected with cDNA from human CCK-A or CCK-B receptors (Table 2).[14] *In vivo* anorexia activity was assessed following intraperitoneal (IP) or oral (PO) administration to Long−Evans rats conditioned to a liquid diet and fasted for 2 h (Table 2).

Results

A directed functional screen of phenylurea-containing compounds from the Glaxo registry files identified a series of 1,5-benzodiazepines in which agonist efficacy was mediated by substitution of the *N*1-anilinoacetamide functionality (Table 1). Remarkably, while compounds with a hydrogen (**11a**) or methyl (**11b**) substituent were weak antagonists of CCK-8 on the isolated GPGB, the ethyl (**11c**), *n*-propyl (**11d**), *n*-butyl (**11e**), and cyanoethyl (**11f**) derivatives were agonists. Hydrophilic substituents produced either weak antagonists or inactive compounds (**11g**−**j**). Branching of the ethyl moiety to the isopropyl substituent (**11k**) further enhanced agonist efficacy. However, there was a limit to the hydrophobic bulk tolerated, as seen with the *n*-butyl (**11e**) or cyclohexyl (**11l**) derivatives. Finally, the diphenyl derivative (**11m**) retained reasonable agonist efficacy. The preferred substituent for maximal efficacy on the GPGB was the *N*-isopropyl derivative **11k**. This analog had equivalent affinity for the human CCK-A and CCK-B receptors (Table 2) and was anorectic in rats (ED_{50} = 0.95 μmol/kg) following ip administration (Table 2). Unfortunately, **11k** was not active following oral administration (up to 10 μmol/kg).

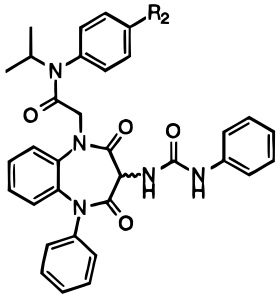
The poor aqueous solubility of **11k** and lack of tolerance for substitution of hydrophilic moieties on the anilino nitrogen prompted us to investigate substitution on the aromatic moiety. Related compounds identified in the original screen suggested that para-substitution would be preferred for maintaining agonist efficacy (data not shown). A range of substituents were prepared (Table 2). Except for the *p*-carboxyl derivative (**11r**), which was a weak antagonist of CCK-8, all of these compounds retained agonist efficacy. These para-substituted compounds had 60−3000-fold reduced affinity for the human CCK-A receptor, compared to CCK-8, with essentially equal affinity for both human CCK-A

Scheme 2[a]



[a] (a) $C_6H_5NHNH_2$, EtOH, H_2O; (b) PCl_5, CCl_4; (c) *p*-$CH_3OC_6H_4COCl$, TEA, DCM; (d) $LiAlH_4$, THF; (e) Zn dust, AcOH; (f) $(NH_4)_2Ce(NO_3)_6$, CH_3CN, H_2O; (g) C_6H_5NCO, DCM.

Table 2. Biological Profile of Functionalized Isopropylaniline Benzodiazepines



		%CCK-8[a]			pIC$_{50}$[b]			anorexia[c]	
compd	R_2	30 μM	1 μM	pK_B	hCCK-A	hCCK-B	A/B	ED$_{50}$	% max.
11k	H	86	ND		7.3 ± 0.3 (4)	7.6 ± 0.04 (3)	0.4	0.95	48
11n	OH	81	ND		7.4 ± 0.1 (4)	7.8 ± 0.4 (3)	0.5	0.05	74
11o	OCH_3	93	82		7.1 ± 0.1 (3)	7.3 ± 0.1 (3)	0.7	0.07	62
11p	$N(CH_3)_2$·TFA	75	46		7.2 ± 0.2 (4)	6.6 ± 0.1 (3)	3.4	0.09	54
11q	morpholino	ND	74		5.7 ± 0.1 (6)	6.8 ± 0.1 (3)	0.1	0.20	26
11r	COOH	10	ND	6.14	5.6 ± 0.01 (3)	7.4 ± 0.1 (2)	0.02	ND	ND
11s	F	ND	53		7.0 ± 0.1 (4)	7.5 ± 0.5 (3)	0.3	0.20	56
CCK-8			100		9.2 ± 0.1 (3)	9.5 ± 0.4 (9)	0.8	0.03	96[d]

[a] Functional activity in the isolated guinea pig gallbladder following incubation with test ligand (30 μM) for 30 min and/or (1 μM) for 60 min at 37 °C; %CCK-8, percent contraction normalized to the percent contractrion induced by 1 μM CCK-8; pK_B, single dose pA_2 calculated from the solid-shift of the CCK-8 concentration−response curve in the presence of the test ligand (30 μM). [b] pIC$_{50}$, −log of the concentration displacing 50% of [125-I]Bolton−Hunter CCK-8 from membrane preparations isolated from CHO-K1 cells stably transfected with cDNA of human CCK-A and CCK-B receptors ± SD (number of determinations); B/A, CCK-A receptor selectivity, IC$_{50}$ (B)/IC$_{50}$ (A). [c] Anorectic potency in rats conditioned to a palatable liquid diet and fasted for 2 h, ED$_{50}$, μmol/kg; % max, maximal response at 1 μmol/kg ip, t = 30 min. [d] % max., maximal response at 0.5 μmol/kg ip, t = 30 min; ND, not determined.

and CCK-B receptors. The functional activity (agonist or antagonist) associated with the CCK-B receptor affinity is unknown and needs to be evaluated.

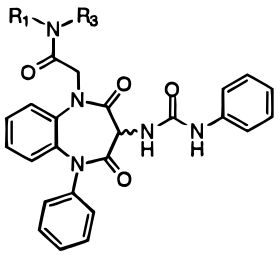
Despite decreased CCK-A receptor affinity, some (**11n**−**p**) of these compounds were potent anorectics in the rat following ip administration, with potency and efficacy comparable to CCK-8. The *p*-OH (**11n**), *p*-OCH_3 (**11o**), and *p*-$N(CH_3)_2$ (**11p**) derivatives were more potent and more efficacious than **11k** at the 1 μmol/kg dose. Again, none of these compounds were orally active (up to 10 μmol/kg) in the rat.

Finally, an additional subset of compounds was identified which demonstrate that the anilino moiety is not required for agonist activity (Table 4). Substitution of the *N*1-acetamide moiety with simple aliphatic substituents also provided agonist leads. To date, no

Table 3. GPGB Contraction in the Absence or Presence of MK-329

		GPGB tension, g		
compd	concn, μm	compd	compd + MK329	% reversal
11k	30	2.53	−0.43	117
11n	30	2.25	0	100
11o	30	2.88	−0.2	107
11p	1	2.55	0.01	100
11q	1	1.88	−0.43	123
11s	1	2.14	−0.34	116

Table 4. Agonist Activity of Atypical Benzodiazepine Ligands on the Isolated Guinea Pig Gallbladder



compd	R_1	R_3	GPGB[a] (30 μM) %CCK-8
11k	$(CH_3)_2CH$	C_6H_5	86
11t	$(CH_3)_2CH$	$CH_2C_6H_5$	34
11u	$(CH_3)_2CH$	$CH(CH_3)_2$	21
11w	CH_3CH_2	CH_2CH_3	38

[a] Functional activity in the isolated guinea pig gallbladder following incubation with test ligand (30 μM) for 30 min at 37 °C; %CCK-8, percent contraction normalized to the percent contraction induced by 1 μM CCK-8.

attempt has been made to optimize the efficacy of this series of compounds and the data are presented here only to demonstrate the nonrigid requirements for agonist activity in this series of compounds and to underline the general necessity for bulk and lipophilicity at this position.

Discussion

The mechanism for the remarkable conversion from antagonist to agonist activity in this series of compounds is unknown. Incorporation of small, bulky, lipophilic groups on the anilino nitrogen can introduce steric, electronic, and conformational effects. In addition to simply binding a lipophilic pocket within the receptor, these groups might also perterb the *E* to *Z* conformational distribution of the amide bond or affect the interplanar angle between the phenyl ring and amide group.[16] Both the spacial geometry of the acetamide as well as the ability of the phenyl ring to conjugate with the amide could be influenced by substitution. Likewise, incorporation of para-substituents on the phenyl ring might not merely optimize a direct spacial or electronic interaction with the receptor, but could also inductively alter the hydrogen-bonding ability of the amide carbonyl or the partial double bond character of the N−C bond.

While efforts continue to optimize these benzodiazepines as potential drug candidates, an equally important goal is to understand how they bind and activate the CCK-A receptor. Historically, the design of nonpeptidyl agonist ligands for peptide receptors has focused on iterative structural modifications of the endogenous peptide directed toward stabilizing the bioactive conformation.[17] This approach assumes that receptor activation depends on a single unique interaction with the agonist ligand. Recent efforts to identify the amino acid residues required for receptor recognition of nonpeptide antagonists of the neurokinin,[18,19] angiotensin II (AII),[18,19] opioid,[20−22] and CCK[23,24] receptor families, however, suggest that the binding site(s) for nonpeptide antagonists are distinct from that for peptide ligands. Moreover, the few nonpeptide agonists reported to date resemble nonpeptide antagonists more than the endogenous peptide they mimic, with minor structural modifications providing the "trigger" for interconversion between antagonist and agonist functional activity. This

Table 5. Analytical Data for Target Analogs

					HRMS molecular ion[c]		
compd	yield, %	method	formula[a]	% purity[b]	theory	found	analysis
11a	95	A	$C_{30}H_{25}N_5O_4$	95	520.1985	520.1987	
11b	59	A	$C_{31}H_{27}N_5O_4$	>99	534.2141	534.2143	C, H, N
11c	56	A	$C_{32}H_{29}N_5O_4 \cdot 0.4H_2O$	98	548.2298	548.2299	C, H, N
11d	47	A	$C_{33}H_{31}N_5O_4$	>99	562.2454	562.2451	C, H, N
11e	34	A	$C_{34}H_{33}N_5O_4 \cdot 0.6H_2O$	>99	576.2511	576.2612	C, H, N
11f	47	A	$C_{33}H_{28}N_6O_4 \cdot 0.6H_2O$				C, H, N[d]
11g	45	B	$C_{32}H_{27}N_5O_6$	98	578.2040	578.2045	
11h	59	A	$C_{34}H_{31}N_5O_6 \cdot 0.5H_2O$	>99	606.2353	606.2348	C, H, N
11i	35	C	$C_{32}H_{30}N_6O_4 \cdot TFA \cdot 2H_2O$	98	563.2407	563.2407	C, H, N
11j	72	A	$C_{40}H_{36}N_6O_6 \cdot 1.5H_2O$	>99	697.2775	697.2764	C, H, N
11k	45	A	$C_{33}H_{31}N_5O_4$	98	562.2454	562.2456	C, H, N
11l	68	A	$C_{36}H_{35}N_5O_4 \cdot 1.2H_2O$	96	602.2767	602.2769	C, H, N[e]
11m	69	A	$C_{36}H_{29}N_5O_4 \cdot 0.5EtOH$	96	596.2298	596.2296	C, H, N
11n	59	D	$C_{33}H_{31}N_5O_5 \cdot H_2O$	95	578.2403	578.2401	C, H, N
11o	47	A	$C_{34}H_{33}N_5O_5$	>99	592.2560	592.2563	C, H, N
11p	57	A	$C_{35}H_{36}N_6O_4 \cdot 0.5TFA \cdot 0.5H_2O$	95	605.2876	605.2875	C, H, N
11q	64	A	$C_{37}H_{38}N_6O_5 \cdot H_2O$	96	647.2982	647.2982	C, H, N
11r	54	E	$C_{34}H_{31}N_5O_6$	96	606.2354	606.2354	
11s	87	A	$C_{33}H_{30}N_5O_4F$	97	580.2360	580.2366	C, H, N
11t	39	A	$C_{34}H_{33}N_5O_4 \cdot 1.6H_2O$	97	576.2611	576.2617	C, H, N[f]
11u	38	A	$C_{30}H_{33}N_5O_4 \cdot 0.4TFA$	>99	528.2611	528.2607	C, H, N
11v	50	A	$C_{28}H_{29}N_5O_4$	99	500.2298	500.2298	C, H, N

[a] Satisfactory ^{1}H-NMR and low-resolution MS were obtained for all compounds. [b] Purity as determined by reversed phase HPLC. [c] FAB MS, $[M + H]^+$. [d] H: calcd 5.04, found 4.73; N: calcd 14.40, found 13.93. [e] H: calcd 6.05, found 6.56; N: calcd 11.24, found 10.04. [f] H: calcd 6.04, found 5.60.

was first demonstrated in the opioid peptide family with the conversion of the potent agonist 14-hydroxydihydromorphinone into the antagonists naloxone or naltrexone through simple replacement of a methyl group with allyl or cyclopropylmethyl, respectively.[25] More recently, substitution of an isobutyl moiety for hydrogen in a series of potent nonpeptidyl angiotensin II (AII) antagonists provided a partial AII agonist.[26,27] Finally, with the 1,5-benzodiazepine CCK-A agonists reported here, a minor chemical modification (CH_3 to CH_3CH_2, Table 1) converts an antagonist into an agonist. Thus, although multiple structural alignments between **11k** and CCK-8 could be proposed, it remains to be determined whether these structurally diverse ligands share similar receptor binding determinants.

Experimental Section

General. All chemical and solvents are reagent grade unless otherwise specified. CCK-8 was purchased from Sigma (St. Louis, MO). MK-329[11] was obtained from Merck & Co. (Rahway, NJ). The following solvents and reagents have been abbreviated: tetrahydrofuran (THF), ethyl ether (Et_2O), dimethyl sulfoxide (DMSO), ethyl acetate (EtOAc), dichloromethane (DCM), trifluoroacetic acid (TFA), dimethylformamide (DMF). Reactions were monitored by thin-layer chromatography on 0.25 mm silica gel plates (60F-254, E. Merck) and visualized with UV light. Final compounds were typically purified by preparative reversed phase high-pressure liquid chromatography (RP-HPLC) using a Waters Model 3000 Delta Prep equipped with a Delta-pak radial compression cartridge (C-18, 300 A, 15 μm, 47 mm × 300 mm) as the stationary phase. The mobile phase employed 0.1% aqueous TFA with acetonitrile as the organic modifier. Linear gradients were used in all cases and the flow rate was 100 mL/min (t_o = 5 min). Appropriate fractions were combined and lyophilized to obtain the target analogs. Analytical purity was assessed by RP-HPLC using a Waters 600E system equipped with a Waters 990 diode array spectrometer (λ range 200−400 nm). The stationary phase was a Vydac C-18 column (5 μm, 4.6 mm × 200 mm). The mobile phase was the same as above, and the flow rate was 1.0 or 1.5 mL/min (t_o = 3 min). Analytical data is reported as retention time, t_R, in minutes (% acetonitrile, time, flow rate).

^{1}H-NMR spectra were recorded on either a Varian VXR-300 or a Varian Unity-300 instrument. Chemical shifts are reported in parts per million (ppm, δ units). Coupling constants are reported in units of hertz (Hz). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad. Low-resolution mass spectra (MS) were recorded on a JEOL JMS-AX505HA, a JEOL SX-102, or a SCIEX-APIiii spectrometers. High-resolution mass spectra were recorded on a AMD-604 (AMD Electra GmbH) high-resolution double-focusing mass spectrometer (Analytical Instrument Group, Raleigh, NC). Mass spectra were acquired in the positive ion mode under electrospray ionization (ESI) or fast atom bombardment (FAB) methods. Combustion analyses were performed by Atlantic Microlabs, Inc., Norcross, GA.

General Method for Reductive Amination of Primary Amines with Ketones/Aldehydes To Obtain Compounds of General Formula 1. Where appropriate secondary amines could not be procured from commercial sources, a primary amine was converted to the desired secondary amine via reductive amination with an appropriate aldehyde or ketone employing the method of Abdel-Magid.[15] Spectral data for representative compounds are as follows.

Isopropyl(4-methoxyphenyl)amine: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.18 (d, 6H, J = 6.1 Hz), 2.92 (br s, 1H), 3.55 (m, 1H), 3.75 (s, 3H), 6.57 (d, 2H, J = 9.1 Hz), 6.78 (d, 2H, J = 8.8 Hz); TLC R_f = 0.72 (EtOAc/hexanes, 2:3).

(4-(Benzyloxy)phenyl)isopropylamine: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.21 (d, 6H, J = 6.4 Hz), 2.84 (br s, 1H), 3.54 (m, 1H), 5.00 (s, 2H), 6.65 (d, 2H, J = 9.0 Hz), 6.86 (d, 2H, J = 9.0 Hz), 7.31−7.43 (m, 5H); TLC R_f = 0.77 (CH_2Cl_2/CH_3OH, 19:1).

General Procedure for Acylation of Secondary Amines To Give 2-Bromoacetamides of General Formula 2. A solution of amine (152 mmol) in DCM (250 mL) and triethylamine (152 mmol) was cooled with stirring in an ice bath (<3 °C). Bromoacetyl bromide (152 mmol) dissolved in DCM (100 mL) was added dropwise over 45 min with stirring. The reaction mixture was stirred overnight at ambient temperature, washed successively with 0.3 N HCl (300 mL) and brine (300 mL), dried over sodium sulfate, filtered, and evaporated *in vacuo*. The resultant oil was filtered through a pad of silica gel (150 g), the silica was washed with EtOAc/hexanes (1:1, 900 mL), and the combined filtrates were evaporated *in vacuo* to give the corresponding 2-bromoacetamide in good yield (>85% typical) and high purity. Spectral data for representative compounds are as follows.

2-Bromo-*N*-isopropyl-*N*-(4-methoxyphenyl)acetamide: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.04 (d, 6H, J = 6.8 Hz), 3.53 (s, 2H), 3.84 (s, 3H), 4.93 (m, 1H), 6.93 (d, 2H, J = 9.1 Hz), 7.10 (d, 3H, J = 9.1 Hz); TLC R_f = 0.18 (EtOAc/hexane, 3:17).

***N*-(4-(Benzyloxy)phenyl)-2-bromo-*N*-isopropylacetamide:** ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (d, 6H, J = 6.7 Hz), 3.55 (s, 2H), 4.94 (m, 1H), 5.08 (s, 2H), 7.01 (d, 2H, J = 8.7 Hz), 7.11 (d, 2H, J = 8.6 Hz), 7.36−7.47 (m, 5H); TLC R_f = 0.16 (EtOAc/hexane, 3:17).

2-(Phenylhydrazono)malonic Acid (3). Phenylhydrazine (23.3 g, 215 mmol) was added dropwise over 40 min to a vigorously stirred solution of ketomalonic acid monohydrate (29.33 g, 215 mmol) in ethanol (140 mL) and water (300 mL) at ambient temperature. The resultant slurry was stirred overnight at ambient temperature. The precipitate was separated by filtration, washed sucessively with cold water (100 mL) and ethanol (25 mL), air-dried, and dried at 75 °C overnight in a vacuum oven to give **3** (42.38 g, 204 mmol, 95%) as a yellow solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.12 (t, 1H), 7.35−7.48 (m, 4H); mp 155−157 °C dec.

2-(Phenylhydrazono)propanedioyl Dichloride (4). Phosphorus pentachloride (36.84 g, 177 mmol) was added portionwise over a 20 min period to a stirred slurry of **3** (14.73 g, 70.8 mmol) in chloroform (90 mL) at 5 °C. After complete addition, the solution was warmed to room temperature, stirred for 1 h, and then heated to reflux for 3 h. The solution was cooled in an ice bath and the resultant preciptate filtered, washed with cold hexane (50 mL), and dried under high vacuum overnight to give **4** (13.4 g, 54.7 mmol, 77%) as a bright yellow solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.12 (t, 1H), 7.20−7.56 (m, 4H); mp 135−138 °C dec.

4-Methoxy-*N*-(2-(phenylamino)phenyl)benzamide (5). *p*-Anisoyl chloride (18.66 g, 109 mmol) in dichloromethane (100 mL) was added dropwise with stirring over 20 min to a solution of *N*-phenyl-1,2-phenylenediamine (20.15 g, 109 mmol) and triethylamine (11.07 g, 109 mmol) in dichloromethane (325 mL). The reaction mixture was maintained at < 5 °C with an ice/acetone bath during the addition, then warmed to ambient temperature, and stirred 2 h. The organic mixture was washed successively with water (200 mL), 2 N HCl (80 mL), and brine (160 mL), dried over sodium sulfate, and filtered through a pad of silica (150 g), further eluting with ethyl acetate (1 L). The eluants were combined and concentrated *in vacuo*. The resultant solid was triturated overnight with Et_2O (350 mL), cooled, filtered, and dried *in vacuo* to give **5** (21.67 g, 88.4 mmol, 81%) as a light pink solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.82 (s, 3H), 5.75 (br s, 1H), 6.80−6.91 (m, 5H), 7.12−7.29 (m, 5H), 7.62 (d, 2H, J = 8.8 Hz), 8.15 (dd, 1H, J = 1.7, 7.8 Hz), 8.36 (s, 1H); TLC R_f = 0.24 (EtOAc/Hex, 1:4); mp 148−150 °C.

***N*-(4-Methoxybenzyl)-*N*-phenylbenzene-1,2-diamine (6).** A solution of **5** (5.0 g, 15.7 mmol) in THF (30 mL) was added over 45 min to a stirred solution of lithium aluminum hydride (1.0 g, 25.1 mmol) in THF (40 mL) cooled to 5 °C and then heated to reflux for 1.5 h. The solution was cooled to room temperature, and excess lithium aluminum hydride was quenched with ethanol until hydrogen evolution ceased.

Saturated aqueous sodium hydrogen carbonate (100 mL) was added and the resultant solution extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were dried with sodium sulfate, filtered through a pad of silica gel, and further eluted with ethyl acetate (500 mL), and the combined filtrates were concentrated *in vacuo* to give **6** (4.78 g, 15.7 mmol, 100%) as a brown oil which solidified on standing: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.79 (s, 3H), 4.27 (s, 2H), 4.52 (br s, 1H), 5.08 (s, 1H), 6.67−6.74 (m, 4H), 6.79−6.86 (m, 3H), 7.04−7.24 (m, 6H); TLC R_f = 0.57 (EtOAc/Hex, 1:4).

1-(4-Methoxybenzyl)-5-phenyl-3-(phenylhydrazono)-1,5-dihydrobenzo[*b*][1,4]diazepine-2,4-dione (7). Solutions of **6** (4.86 g, 15.7 mmol) in THF (40 mL) and **4** (5.58 g, 22.8 mmol) in THF (40 mL) were added concomitantly dropwise with cooling in an ice/methanol bath over 30 min. The solution was allowed to warm to room temperature and stirred for 16 h. A yellow precipitate was separated by filtration, washed with cold THF (40 mL), and dried under high vacuum overnight to give the HCl salt of **7** (6.23 g, 13.1 mmol, 83%) as a yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.78 (s, 3H), 4.69 (d, 1H, J = 14.7 Hz), 5.76 (d, 1H, J = 14.9 Hz), 6.80−6.87 (m, 3H), 7.02−7.12 (m, 4H), 7.19−7.40 (m, 11H), 11.19 (s, 1H); LRMS (FAB) m/z 477.0 $[M + H]^+$; TLC R_f = 0.18 (EtOAc/Hex, 1:4).

3-Amino-1-(4-methoxybenzyl)-5-phenyl-1,5-dihydrobenzo[*b*][1, 4]diazepine-2,4-dione (8). To a vigorously stirred slurry of zinc dust (6.49 g, 99.2 mmol) in acetic acid (50 mL) cooled to 10 °C was added a slurry of **7** (5.75 g, 12.1 mmol) in acetic acid (30 mL) over 15 min. After complete addition, the solution was warmed to room temperature and stirred for 3 h. The zinc was separated by filtration and the cake washed with ethyl acetate (75 mL). The filtrate was concentrated *in vacuo* and partitioned between water (60 mL) and ethyl acetate (100 mL). The pH was adjusted to 9 with saturated sodium hydrogen carbonate, and the phases were separated. The aqueous phase was extracted with ethyl acetate (2 × 75 mL), the organic layers were combined, dried with magnesium sulfate, filtered, and concentrated in vacuo to give a yellow oil which was dried under high vacuum to give **8** (4.59 g, 11.9 mmol, 98%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.05 (s, 2H), 3.75 (s, 3H), 4.35 (s, 1H), 4.64 (d, 1H, J = 14.7 Hz), 5.82 (d, 1H, J = 14.7 Hz), 6.59−6.85 (m, 6H), 7.06−7.29 (m, 6H), 7.51 (d, 1H, J = 7.4 Hz); LRMS (FAB) m/z 388.2 $[M + H]^+$; TLC R_f = 0.50 (CH_2Cl_2/CH_3OH, 9:1).

3-Amino-1-phenyl-1,5-dihydrobenzo[*b*][1,4]diazepine-2,4-dione (9). Ceric ammonium nitrate (1.84 g, 3.35 mmol) was added portionwise over 10 min to a stirred solution of **8** (0.50 g, 1.29 mmol) in acetonitrile/H_2O (9:1, 12 mL) at ambient temperature and the solution stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo* and the resultant solid partitioned between saturated aqueous potassium carbonate (40 mL) and ethanol (60 mL). The phases were separated and the aqueous phase extracted with ethanol (4 × 50 mL). The ethanol portions were combined, dried over sodium sulfate, and concentrated *in vacuo* to a tan solid. This solid was extracted exhaustively with boiling DCM (10 × 60 mL), and the organics were combined, dried over sodium sulfate, filtered, and concentrated to give **9** (0.30 g, 1.12 mmol, 87%) as a tan solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.98 (br s, 2H), 4.08 (s, 1H), 6.86 (d, 1H, J = 8.4 Hz), 7.11−7.46 (m, 8H), 10.78 (br s, 1H); ^{13}C (75.429 MHz, DMSO-d_6) δ 56.98, 123.41, 126.22, 126.51, 127.34, 128.30, 128.89, 130.15, 132.29, 134.42, 142.36, 168.13, 169.39; LRMS (FAB) m/z 268.10 $[M + H]^+$; TLC R_f = 0.21 (CH_2Cl_2/CH_3OH, 15:1).

1-(2,4-Dioxo-1-phenyl-2,3,4,5-tetrahydro-1*H*-benzo[*b*]-[1,4]diazepin-3-yl)-3-phenylurea (10). To a slurry of **9** (0.398 g, 1.49 mmol) in dichloromethane (5 mL) was added phenyl isocyanate (0.177 g, 1.49 mmol) with stirring at ambient temperature. The reaction mixture was stirred 2 h, and the cream precipitate was separated by filtration to afford **10** (0.413 g, 1.07 mmol, 72%) as a white solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.97 (d, 1H, J = 7.5 Hz), 6.88−6.97 (m, 3H), 7.13−7.47 (m, 12H), 9.16 (s, 1H), 10.78 (br s, 1H); TLC R_f = 0.21 (CH_2Cl_2/CH_3OH, 19:1).

General Procedure for the Preparation of Compounds of General Formula 11. To a solution of **10** (0.256 mmol) in DMF (2 mL) was added sodium hydride (0.0104 g, 60% suspension in mineral oil, 0.256 mmol) with stirring and cooling in an ice bath (<3 °C). The mixture was stirred for 30 min followed by addition of the appropriate 2-bromoacetamide (general formula **2**) (0.256 mmol). The reaction mixture was stirred at ambient temperature for 16 h, the solvent removed *in vacuo*, and the crude product purified by preparative RP-HPLC chromatography on a C-18 column with linear gradient elution typically from 40 to 60% acetonitrile in water with 0.1% TFA buffer over 30 min at a rate of 100 mL/min. Fractions containing the desired material were combined, frozen, and lyophilized to afford products of general formula **11** as white lyophiles. Spectral data and HPLC conditions for compounds from Tables 1−3 are given below.

2-[2,4-Dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*-phenylacetamide (11a): ^{1}H NMR (250 MHz, DMSO-d_6) δ 4.76 (d, 1H, J = 16 Hz), 5.08 (d, 1H, J = 16 Hz), 5.18 (d, 1H, J = 8 Hz), 6.90−7.73 (m, 20H), 9.20 (s, 1H), 10.40 (s, 1H); HPLC t_R = 18 min (30−60% CH_3CN, 30 min, 1.5 mL/min); mp 250−252 °C.

2-[2,4-Dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*-methyl-*N*-phenylacetamide (11b): ^{1}H NMR (250 MHz, DMSO-d_6) δ 3.25 (s, 3H), 4.48 (d, 1H, J = 16 Hz), 4.76 (d, 1H, J = 16 Hz), 5.13 (d, 1H, J = 8 Hz), 6.90−7.70 (m, 20H), 9.28 (s, 1H); HPLC t_R = 16.5 min (30−60% CH_3CN, 30 min, 1.5 mL/min); mp 248−252 °C.

2-[2,4-Dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*-ethyl-*N*-phenylacetamide (11c): ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.05 (t, 3H, J = 7 Hz), 3.72 (q, 2H, J = 7 Hz), 4.40 (d, 1H, J = 16 Hz), 4.70 (d, 1H, J = 16 Hz), 5.11 (d, 1H, J = 8 Hz), 6.90−7.70 (m, 20 H), 9.22 (s, 1H); HPLC t_R = 22 min (30−60% CH_3CN, 30 min, 1.5 mL/min); mp 242 °C.

2-[2,4-Dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*-propyl-*N*-phenylacetamide (11d): ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.85 (t, 3H, J = 7 Hz), 1.50 (m, 2H), 3.65 (m, 2H), 4.25 (d, 1H, J = 16 Hz), 4.58 (d, 1H, J = 16 Hz), 5.40 (d, 1H, J = 8 Hz), 6.40 (d, 1H, J = 8 Hz), 6.89 (s, 1H), 6.95−7.50 (m, 19H); HPLC t_R = 27.5 min (30−60% CH_3CN, 30 min, 1.5 mL/min); mp 237 °C.

***N*-Butyl-2-[2,4-dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*-phenylacetamide (11e):** ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (m, 3H), 1.25 (m, 2H), 1.45 (m, 2H), 3.66 (m, 2H), 4.26 (d, 1H, J = 16.8 Hz), 4.55 (d, 1H, J = 17.0 Hz), 5.38 (d, 1H, J = 7.6 Hz), 6.43 (d, 1H, J = 7.3 Hz), 7.00 (m, 3H), 7.13−7.49 (m, 17H); LRMS (FAB) m/z 576.1 $[M + H]^+$; TLC R_f = 0.23 (EtOAc/hexanes, 2:3); HPLC t_R = 24.0 min (47−57% CH_3CN, 30 min, 1.0 mL/min).

***N*-(2-Cyanoethyl)-2-[2,4-dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*-phenylacetamide (11f):** ^{1}H NMR (250 MHz, DMSO-d_6) δ 2.75 (t, 2H, J = 6 Hz), 3.95 (t, 2H, J = 6 Hz), 4.45 (d, 1H, J = 16 Hz), 4.72 (d, 1H, J = 16 Hz), 5.13 (d, 1H, J = 8 Hz), 6.90−7.70 (m, 20H), 9.22 (s, 1H); mp 291 °C dec.

[[2-[2,4-Dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]acetyl]phenylamino]acetic acid (11g). A solution of **11h** (22 mg, 0.036 mmol), methanol (5 mL), and 1 N NaOH (0.190 mL, 0.190 mmol) was stirred for 16 h at ambient temperature. The solvent was removed *in vacuo* and the residue dissolved in aqueous sodium hydrogen carbonate (25 mL) and washed with EtOAc (25 mL). The pH of the aqueous phase was adjusted to 1.6 with 6 N HCl and extracted with EtOAc (2 × 25 mL). The organic phases were dried over sodium sulfate, filtered, and concentrated. The crude product was purified by preparative RP-HPLC with linear gradient elution from 32 to 42% CH_3CN in H_2O with 0.1% TFA buffer over 30 min with a flow rate of 100 mL/min. The fraction containing the desired material was frozen and lyophilized to give **11g** (6.6 mg, 11.4 mmol, 31%) as a white lyophile: ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.29 (s, 2H), 4.48 (d, 1H, J = 16.8 Hz), 4.73 (d, 1H, J = 16.8 Hz), 5.05 (d, 1H, J = 7.8 Hz), 6.92 (m, 3H), 7.18−7.55 (m, 17H), 9.15 (s, 1H); LRMS (FAB) m/z 578.0 $[M + H]^+$; TLC R_f = 0.32 (CH_2-

Cl_2/CH_3OH, 9:1); HPLC t_R = 27.0 min (32−42% CH_3CN, 30 min, 1.0 mL/min).

[[2-[2,4-Dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]acetyl]phenylamino]acetic acid ethyl ester (11h): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.23 (t, 3H), 4.16 (q, 2H), 4.30 (d, 1H, J = 17.4 Hz), 4.42 (d, 2H, J = 18.3 Hz), 4.61 (d, 1H, J = 16.9 Hz), 5.41 (d, 1H, J = 7.8 Hz), 6.60 (d, 1H, J = 7.8 Hz), 6.97 (m, 2H), 7.14−7.48 (m, 20H); LRMS (ESI) m/z 606.1 $[M + H]^+$; TLC R_f = 0.25 (CH_2Cl_2/CH_3OH, 19:1); HPLC t_R = 15.5 min (47−57% CH_3CN, 30 min, 1.0 mL/min).

***N*-(2-Aminoethyl)-2-[2,4-Dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*-phenylacetamide (11i).** A mixture of **11j** (202 mg, 0.290 mmol) and 10% Pd/C (40 mg) in ethanol/EtOAc (35 mL, 5:2) was stirred under hydrogen (1 atm) at ambient temperature for 16 h. The catalyst was separated by filtration and the solvent removed *in vacuo*. The crude product was purified by preparative RP-HPLC with linear gradient elution from 30 to 37% CH_3CN in H_2O with 0.1% TFA buffer over 30 min with a flow rate of 100 mL/min. The fraction containing the desired material was frozen and lyophilized to give **11i** (57 mg, 0.101 mmol, 35%) as a white lyophile: ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.88 (m, 2H), 3.87 (m, 2H), 4.34 (d, 1H, J = 16.8 Hz), 4.66 (d, 1H, J = 16.8 Hz), 5.06 (m, 1H), 6.86−6.98 (m, 3H), 7.18−7.60 (m, 15H), 7.75 (m, 2H), 9.15 (m, 1H); LRMS (ESI) m/z 563.0 $[M + H]^+$; HPLC t_R = 15.0 min (30−37% CH_3CN, 30 min, 1.0 mL/min).

***N*-[2-[(Carbobenzyloxy)amino]ethyl]-2-[2,4-Dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]-diazepin-1-yl]-*N*-phenylacetamide (11j):** ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.36 (m, 2H), 3.81 (m, 2H), 4.23 (d, 1H, J = 16.6 Hz), 4.53 (d, 1H, J = 16.6 Hz), 5.02 (m, 2H), 5.36 (m, 2H), 6.44 (d, 1H, J = 7.6 Hz), 6.96−7.46 (m, 25H); LRMS (FAB) m/z 697.2 $[M + H]^+$; TLC R_f = 0.24 (CH_2Cl_2/CH_3OH, 19:1); HPLC t_R = 19.0 min (47−57% CH_3CN, 30 min, 1.0 mL/min).

2-[2,4-Dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*-isopropyl-*N*-phenylacetamide (11k): ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.95 (d, 3H, J = 7.3 Hz), 0.98 (d, 3H, J = 7.3 Hz), 4.19 (d, 1H, J = 16.6 Hz), 4.48 (d, 1H, J = 16.9 Hz), 4.79 (m, 1H), 5.04 (d, 1H, J = 7.8 Hz), 6.87−6.92 (m, 1H), 6.95 (d, 1H, J = 7.6 Hz), 7.18−7.57 (m, 17H), 9.14 (s, 1H); LRMS (FAB) m/z 562.0 $[M + H]^+$; TLC R_f = 0.21 (CH_2Cl_2/CH_3OH, 19:1); HPLC t_R = 16.5 min (51−60% CH_3CN, 30 min, 1.0 mL/min).

2-[2,4-Dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*-cyclohexyl-*N*-phenylacetamide (11l): ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.81−1.01 (m, 3H), 1.22−1.33 (m, 2H), 1.49 (m, 1H), 1.64−1.79 (m, 4H), 4.18 (d, 1H, J = 16.6 Hz), 4.37 (m, 1H), 4.44 (d, 1H, J = 16.6 Hz), 5.03 (d, 1H, J = 7.8 Hz), 6.88−6.96 (m, 3H), 7.18−7.56 (m, 17H), 9.14 (s, 1H); LRMS (FAB) m/z 602.1 $[M + H]^+$; HPLC t_R = 18.0 min (47−57% CH_3CN, 30 min, 1.0 mL/min).

2-[2,4-Dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*,*N*-diphenylacetamide (11m): ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.58 (d, 1H, J = 16.6 Hz), 4.81 (d, 1H, J = 16.6 Hz), 5.08 (d, 1H, J = 7.9 Hz), 6.86−6.99 (m, 1H), 7.19−7.64 (m, 22H), 9.16 (s, 1H); LRMS (ESI) m/z 596.5 $[M + H]^+$; TLC R_f = 0.23 (EtOAc/hexanes, 2:3); HPLC t_R = 9.5 min (51−60% CH_3CN, 30 min, 1.5 mL/min).

2-[2,4-Dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*-(4-hydroxyphenyl)-*N*-isopropylacetamide (11n). A mixture of 2-[2,4-dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*-isopropyl-*N*-(4-phenoxyphenyl)-acetamide (170 mg, 0.254 mmol) in ethanol/EtOAc/methanol (25 mL, 3:1:1) and 10% Pd/C (100 mg) was stirred under hydrogen (1 atm) at ambient temperature for 16 h. The catalyst was separated by filtration and the solvent removed *in vacuo*. The crude product was purified by preparative RP-HPLC with linear gradient elution from 42 to 52% CH_3CN in H_2O with 0.1% TFA buffer over 30 min with a flow rate of 100 mL/min. The fraction containing the desired material was frozen and lyophilized to give **11n** (44.4 mg, 0.077 mmol, 30%) as a white lyophile: ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.94 (m, 6H), 4.20 (d, 1H, J = 16.9 Hz), 4.50 (d, 1H, J = 16.9 Hz), 4.74 (m, 1H), 5.04 (d, 1H, J = 7.8 Hz), 6.86−6.97 (m, 4H), 7.11 (m, 2H), 7.21−7.52 (m, 12H), 9.15 (s, 1H), 9.80 (s, 1H); LRMS (FAB) m/z 578.1 $[M + H]^+$; TLC R_f = 0.48 (CH_2Cl_2/CH_3OH, 9:1); HPLC t_R = 14.0 min (42−52% CH_3CN, 30 min, 1.0 mL/min).

2-[2,4-Dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*-isopropyl-*N*-(4-methoxyphenyl)-acetamide (11o): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (m, 6H), 3.83 (s, 3H), 4.17 (d, 1H, J = 16.8 Hz), 4.45 (d, 1H, J = 16.8 Hz), 4.95 (m, 1H), 5.35 (m, 1H), 6.97 (m, 3H), 7.16 (m, 5H), 7.30 (m, 6H), 7.41 (m, 4H); LRMS (FAB) m/z 592.0 $[M + H]^+$; TLC R_f = 0.15 (EtOAc/hexanes, 2:3); HPLC t_R = 27.5 min (42−60% CH_3CN, 30 min, 1.5 mL/min).

***N*-[4-(Dimethylamino)phenyl]-2-[2,4-dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*-isopropylacetamide (11p):** ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.01 (m, 6H), 3.01 (s, 6H), 4.20 (d, 1H, J = 16.4 Hz), 4.41 (d, 1H, J = 16.4 Hz), 4.93 (m, 1H), 5.38 (d, 1H, J = 7.6 Hz), 6.61 (d, 1H, J = 7.3 Hz), 6.96−7.40 (m, 19H); LRMS (FAB) m/z 605.1 $[M + H]^+$; TLC R_f = 0.13 (EtOAc/hexanes, 2:3); HPLC t_R = 14.5 min (38−48% CH_3CN, 30 min, 1.0 mL/min).

2-[2,4-Dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*-isopropyl-*N*-(4-morpholin-4-ylphenyl)acetamide (11q): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.98 (m, 6H), 3.12 (s, 2H), 3.84 (s, 2H), 4.18 (d, 1H, J = 16.1 Hz), 4.42 (d, 1H, J = 16.1 Hz), 4.92 (m, 1H, J = 7.1 Hz), 5.38 (d, 1H, J = 7.8 Hz), 6.51 (d, 1H, J = 7.8 Hz), 6.80−7.50 (m, 18H); LRMS (FAB) m/z 647.3 $[M + H]^+$; TLC R_f = 0.22 (CH_2Cl_2/CH_3OH, 9:1); HPLC t_R = 22.5 min (30−60% CH_3CN, 30 min, 1.5 mL/min).

4-[[2-[2,4-Dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]acetyl]isopropylamino]benzoic Acid (11r). A mixture of of 4-[[2-[2,4-dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*]-[1,4]diazepin-1-yl]acetyl]isopropylamino]benzoic acid benzyl ester (30 mg, 0.043 mmol) and 10% Pd/C (35 mg) in ethanol/EtOAc (20 mL, 3:1) was stirred under hydrogen (1 atm) at ambient temperature for 16 h. The catalyst was separated by filtration and the solvent removed *in vacuo*. The crude product was purified by preparative RP-HPLC with linear gradient elution from 40 to 50% CH_3CN in H_2O with 0.1% TFA buffer over 30 min with a flow rate of 100 mL/min. The fraction containing the desired material was frozen and lyophilized to give **11r** (14.1 mg, 0.023 mmol, 54%) as a white lyophile: ^{1}H NMR (300 MHz, CD_3OD) δ 1.08 (m, 6H), 4.26 (d, 1H, J = 16.6 Hz), 4.60 (d, 1H, J = 16.6 Hz), 4.80 (m, 1H), 6.96 (t, 1H), 7.04 (dd, 1H, J = 8.2, 1.1 Hz), 7.20−7.51 (m, 16H), 8.19 (d, 2H, J = 8.5 Hz); LRMS (FAB) m/z 606.2 $[M + H]^+$; HPLC t_R = 12.0 min (40−50% CH_3CN, 30 min, 1.0 mL/min).

2-[2,4-Dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*-(4-fluoro-phenyl)-*N*-isopropylacetamide (11s): ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.96 (m, 6H), 4.20 (d, 1H, J = 16.6 Hz), 4.47 (d, 1H, J = 16.6 Hz), 4.79 (m, 1H), 5.04 (d, 1H, J = 7.6 Hz), 6.86−6.97 (m, 3H), 7.21−7.54 (m, 16H), 9.15 (s, 1H); LRMS (FAB) m/z 580.2 $[M + H]^+$; TLC R_f = 0.20 (EtOAc/hexanes, 2:3); HPLC t_R = 28.0 min (30−60% CH_3CN, 30 min, 1.5 mL/min).

***N*-Benzyl-2-[2,4-dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[b][1,4]diazepin-1-yl]-*n*-isopropylacetamide (11t):** ^{1}H NMR (300 MHz, acetone-d_6) δ 1.06−1.22 (m, 6H), 4.40−5.35 (m, 6H), 6.56 (m, 1H), 6.89−7.72 (m, 19H), 8.56 (m, 1H); LRMS (FAB) m/z 576.1 $[M + H]^+$; TLC R_f = 0.12 (EtOAc/hexanes, 2:3); HPLC t_R = 24.5 min (43−53% CH_3CN, 30 min, 1.0 mL/min).

2-[2,4-Dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*,*N*-diisopropylacetamide (11u): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.25−1.37 (m, 12H), 3.55 (m, 1H), 3.91 (m, 1H), 4.67 (d, 1H, J = 15.8 Hz), 4.77 (d, 1H, J = 15.8 Hz), 5.40 (d, 1H, J = 7.1 Hz), 6.54 (d, 1H, J = 7.1 Hz), 7.03 (m, 2H), 7.15−7.43 (m, 13H); LRMS (FAB) m/z 528.0 $[M + H]^+$; TLC R_f = 0.24 (CH_2Cl_2/CH_3OH, 19:1); HPLC t_R = 17.0 min (45−55% CH_3CN, 30 min, 1.0 mL/min).

2-[2,4-Dioxo-5-phenyl-3-(3-phenylureido)-2,3,4,5-tetrahydrobenzo[*b*][1,4]diazepin-1-yl]-*N*,*N*-diethylacetamide (11v): ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.09 (t, 3H, $J = 7$ Hz), 1.25 (t, 3H, $J = 7$ Hz), 3.38 (m, 4H), 4.62 (d, 1H, $J = 15$ Hz), 4.94 (d, 1H, $J = 15$ Hz), 5.45 (d, 1H, $J = 7$ Hz), 6.45 (d, 1H), 6.95−7.48 (m, 14 H); HPLC $t_R = 22$ min (30−60% CH_3-CN, 30 min, 1.5 mL/min).

Anorexia Assays. Male Long−Evans rats (225−300 g) were conditioned for 2 weeks to consume a palatable liquid diet (Bio-Serve F1657, Frenchtown, NJ) after a 2 h fast. On pretreatment day, rats were fasted (100 min) and injected ip with drug vehicle (propylene glycol, PG, 1 mL/kg) and an oral preload of saline (0.9% NaCl, 8 mL/kg). Liquid diet access was provided 20 min later, and consumption was measured at 30, 90, and 180 min. To qualify for the drug treatment study, rats had to consume at least 8 mL of liquid diet within the first 30 min on the pretreatment day. The next day, following the 100 min deprivation, rats (8−10 animals per dose) were treated ip or po with vehicle (PG, 1 mL/kg) or various doses (0.01−10 μmol/kg) of test compound dissolved in PG (1 mL/kg), immediately followed by the saline oral preload. Food access was again provided 20 min later, and food intake was measured at 30, 90, and 180 min. All food intake data were normalized for each rat to the respective values from the pretreatment day. Potency was determined at 30 min and efficacy at the 30 min, 1 μmol/kg dose.

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References

(1) Crawley, J. N.; Corwin, R. L. Biological Actions of Cholecystokinin. *Peptides* **1994**, *15*, 731−755.
(2) Kissileff, H. R.; Pi-Sunyer, F. X.; Thornton, J.; Smith, G. P. C-Terminal Octapeptide of Cholecystokinin Decreases Food Intake in Man. *Am. J. Clin. Nutr.* **1981**, *34*, 154−160.
(3) Pi-Sunyer, X.; Kissileff, H. R.; Thornton, J.; Smith, G. P. C-Terminal Octapeptide of Cholecystokinin Decreases Food Intake in Obese Men. *Physiol. Behav.* **1982**, *29*, 627−630.
(4) Sitzmann, J. V.; Pitt, H. A.; Steinborn, P. A.; Pasha, Z. R.; Sanders, R. C. Cholecystokinin Prevents Parenteral Nutrition Induced Biliary Sludge in Humans *Surg. Gynacol. Obstetrics* **1990***170*, 25−31
(5) Dourish, C. T.; Ruckert, A. C.; Tattersall, F. D.; Iversen, S. D. Evidence That Decreased Feeding Induced by Systemic Injection of Cholecystokinin is Mediated by CCK-A Receptors. *Eur. J. Pharmacol.* **1989**, *173*, 233−234.
(6) Hermkens, P. H. H.; Ottenheijm, H. C. J.; van der Werf-Pieters, J. M. L.; Broekkamp, C. L. E.; de Boer, T.; van Nispen, J. W. CCK-A Agonists: Endeavours Involving Structure-Activity Relationship Studies. *Recl. Trav. Chim. Pays-Bas* **1993**, *112*, 95−106.
(7) Shiosaki, K.; Lin, C. W.; Kopecka, H.; Craig, R.; Wagenaar, F. L.; Bianchi, B.; Miller, T.; Witte, D.; Nadzan, A. M. Development of CCK-Tetrapeptide Analogues as Potent and Selective CCK-A Receptor Agonists. *J. Med. Chem.* **1990**, *33*, 2950−2952.
(8) Freidinger, R. M. Toward Peptide Receptor Ligand Drugs: Progress on Nonpeptides. *Prog. Drug Res.* **1985**, *40*, 33−98.
(9) Giannis, A.; Kolter T. Peptidomimetics for Receptor Ligands Discovery, Development, and Medicinal Perspectives. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244−1267.
(10) Rees, D. C. Non-Peptide Ligands for Membrane-Bound Peptide Receptors. *Curr. Med. Chem.* **1994**, *1*, 145−158.
(11) Evans, B. E.; Bock, M. G.; Rittle, K. E.; Dipardo, R. M.; Whitter, W. L.; Veber, D. L.; Anderson, P. S.; Freidinger, R. M. Design of Potent, Orally Effective, Nonpeptidyl Antagonists of the Peptide Hormone Cholecystokinin. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4918−4922.
(12) Bock, M. G.; Dipardo, R. M.; Evans, B. E.; Rittle, K. E.; Whitter, W. L.; Veber, D. F.; Anderson, P. S.; Freidinger, R. M. Benzodiazepine Gastrin and Brain CCK Receptor Ligands: L365,260. *J. Med. Chem.* **1989**, *32*, 13−16.
(13) Chang, R. S.; Lotti, V. J.; Monaghan, R. L.; Birnbaum, J.; Stapley, E. O.; Goetz, M. A.; Albers-Schonberg, G.; Patchett, A. A.; Liesch, J. M.; Hensens, O. D. A Potent Nonpeptide Cholycystokinin Antagonist Selective for Peripheral Tissues Isolated from Aspergillus Alliaceus. *Science* **1985**, *230*, 177−179.
(14) Sugg, E. E.; Kimery, M. J.; Ding, J. M.; Kenakin, D. C.; Miller, L. J.; Queen K. L.; Rimele, T. J. CCK-A Receptor Selective Antagonists Derived from the CCK-A Receptor Selective Tetrapeptide Agonist Boc-Trp-Lys(Tac)-Asp-MePhe-NH_2 (A-71623). *J. Med. Chem.* **1995**, *38*, 207−211.
(15) Abdel-Magid, A. F.; Maryanoff, C. A.; Carson, K. G. Reductive Amination of Aldehydes and Ketones by Using Sodium Triacetoxyborohydride. *Tetrahedron Lett.* **1990**, *31*, 5595−5598.
(16) Koetsch, C. F.; Britain, J. W. Effect of N-Alkyl on Formation of Quinolines from N-Alkylbenzoylacetanilides. *J. Org. Chem.* **1959**, *24*, 1551−1553.
(17) Marshall, G. R. A Hierarchical Approach to Peptidomimetic Design *Tetrahedron* **1993**, *17*, 3547−3558.
(18) Strader, C. D.; Fong, T. M.; Tata, M. R.; Underwood, D. Structure and Function of G Protein-Coupled Receptors. *Ann. Rev. Biochem.* **1994**, *63*, 101−132.
(19) Schwartz, T. W. Locating Ligand-Binding Sites in 7TM Receptors by Protein Engineering. *Curr. Opin. Biotech.* **1994**, *5*, 434−444.
(20) Heerding, J.; Raynor, K.; Kong, H.; Yu, L.; and Reisine, T. Mutagenesis Reveals that Agonists and Peptide Antagonists Bind in Fundamentally Distinct Manners to the Rat mu Receptor than Do Nonpeptide Antagonists. *Regulatory Peptides* **1994**, *54*, 119−120.
(21) Wang, J. B.; Imai, Y.; Johnson, P. S.; Walther, D.; Stein, C.; Schaefer, M.; Wu, J. M.; Wang, W. F.; Moriwaki, A.; Uhl, G. R. Human μ Receptor: Gene Structure, Expression, and μ/κ chimeras That Define Nontransmembrane Domains Influencing Peptide Binding Affinities. *Ibid.* **1994**, *54*, 317−320.
(22) Onogi, T.; Minami, M.; Katao, Y.; Nakagawa, T.; Aoki, Y.; Toya, T.; Katsumata, S.; and Satoh, M. DAMGO, A μ-Opioid Receptor Selective Agonist, Distinguishes Between μ- and δ-opioid receptors Around their First Extracellular Loops. *FEBS Lett.* **1995**, *357*, 93−97.
(23) Beinborn, M.; Lee, Y. M.; McBride, E. W.; Quinn, S. M.; Kopin, A. S. A Single Amino Acid of the Cholecystokinin-B/Gastrin Receptor Determines Specificity for Non-Peptide Antagonists. *Nature* **1993**, *362*, 348−350.
(24) Mantamadiotis, T.; Baldwin, G. S. The Seventh Transmembrane Domain of Gastrin/CCK Receptors Contributes to Nonpeptide Antagonist Binding. *Biochem. Biophys. Res. Commun.* **1994**, *201*, 1381−1389.
(25) Furst, S.; Hosztafi, S; Friedmann, T. Structure Activity Relationships of Synthetic and Semisynthetic Opioid Agonists and Antagonists. *Curr. Med. Chem.* **1995**, *1*, 423−440.
(26) Perlman, S.; Schambye, J. T.; Rivero, R. A.; Greenlee, W. J.; Hjorth, S. A.; Schwartz, T. W. Nonpeptide Angiotensin Agonist. *J. Biol. Chem.* **1995**, *270*, 1493−1496.
(27) Kivlign, S. D.; Huckle, W. R.; Zingaro, G. J.; Rivero, R. A.; Lotti, V. J.; Chang, R. S. L.; Schorn, T. W.; Kevin, N.; Johnson, R. G., Jr.; Greenlee, W. J.; Siegl, P. K. S. Discovery of L-162,313: A Nonpeptide That Mimics the Biological Actions of Angiotensin II. *Am. J. Physiol.*, **1995**, *268*, R820−R823.

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