Communications to the Editor

3-(1*H*-Indazol-3-ylmethyl)-1,5-benzodiazepines: CCK-A Agonists That Demonstrate Oral Activity as Satiety Agents

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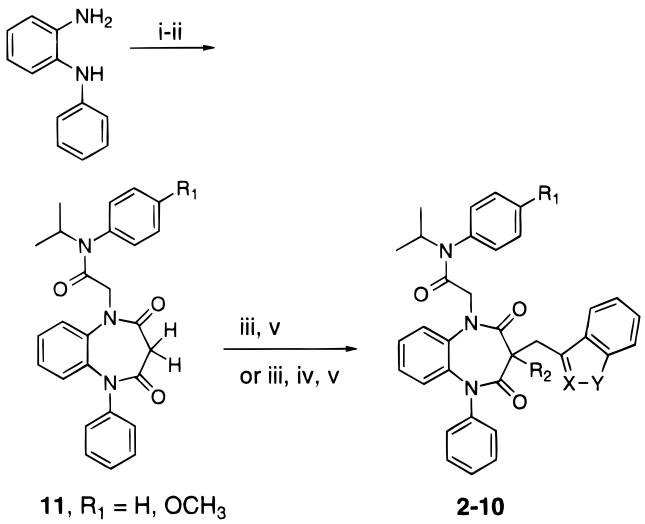
We recently reported the identification of a series of 3-(phenylureido)-1,5-benzodiazepine CCK-A agonists.[1] These compounds were the first reported nonpeptidyl CCK-A agonists. Unfortunately, they lacked binding selectivity for CCK-A vs CCK-B receptors and were not orally active as satiety agents in rodent feeding models. Subsequent systematic modification of the C-3 ureido substituent in this series led to the development of a CCK-A binding-selective partial agonist **1** (Figure 1).[2] While amide **1** showed CCK-A agonist activity in a mouse gallbladder-emptying assay following oral dosing, it was also not orally active in a rat feeding model. Reasoning that the partial agonist activity of **1** may be connected to its inability to inhibit feeding, studies were initiated to identify binding-selective CCK-A full agonists. This paper reports the biological profile of a series of isosteric replacements for the C-3 phenyl amide moiety.

Chemistry. Compounds **2**−**10** were synthesized using 1,5-benzodiazepine **11** (R = H or OCH_3) as a key intermediate (Scheme 1). Alkylation of *N*-phenyl-1,2-phenylenediamine with the appropriate bromoacetamide followed by condensation with malonyl dichloride afforded **11** in rapid fashion. The C-3 substituent was introduced via deprotonation using $KN(TMS)_2$ or $NaN(TMS)_2$ as the base followed by addition of the requisite alkyl halide. Deprotection, if necessary, was carried out under standard conditions. Formation of the C-3 quaternary analogs was achieved by a second deprotonation/alkylation sequence with the appropriate alkyl halide, followed by deprotection if necessary. The various alkyl halides used in construction of **2**−**10** were synthesized using established literature methods.

Results and Discussion. The strategy for modification of the C-3 pharmacophore was suggested by studying the structure of the selective CCK-A antagonist asperlicin,[3] which has been used by several groups as a template for the development of subtype-selective CCK antagonists.[4] Pioneering work at Merck resulted in a series of 1,4-benzodiazepine CCK-A selective antagonists,[5,6] suggesting that the benzodiazepine substructure provides a proper structural motif for interaction with the CCK receptor. Researchers at both Merck[5] and Lilly[4d,e] also demonstrated that the 3-indolinylmethyl group embedded within the structure of asperlicin was an important element for bioactivity. In addition, this moiety is structurally similar to L-tryptophan, a key amino acid required for agonist activity in the peptide sequence of CCK and various peptidomimetics of CCK.[7]

Scheme 1[a]



11, R_1 = H, OCH_3 **2-10**

[a] Reagents: (i) 2-bromo-*N*-isopropyl-*N*-phenylacetamide (R_1 = H) or 2-bromo-*N*-isopropyl-*N*-(4-methoxy)phenylacetamide (R_1 = OCH_3), K_2CO_3, DMF, 18 h; (ii) malonyl dichloride, THF, 0 °C to room temperature, 18 h; (iii) $NaN(TMS)_2$ or $KN(TMS)_2$, room temperature, DMF, 15 min; Ar-CH_2-Br, DMF, room temperature, 2−5 h; (iv) $KN(TMS)_2$, DMF, 0 °C, 15 min; MeI, DMF, room temperature, 3 h; (v) deprotection (if necessary).

Compounds **2**−**10** were evaluated for *in vitro* functional efficacy in inducing contraction of isolated guinea pig gallbladder (Table 1).[8] Compounds were tested at 30 μM concentration and their efficacy normalized to CCK-8 at 1 μM. The contractile activity of all compounds was reversed with the CCK-A selective antagonist MK-329.[5] To investigate the viability of our strategy in the 1,5-benzodiazepine CCK-A agonist series, the phenyl amide moiety in **1** was replaced by a 3-indolylmethyl group. This modification maintains agonist activity, with the 3-indolyl analog **2** having equal efficacy to that of amide **1** (Table 1). Previous work[1] had demonstrated that replacement of the *p*-hydrogen in the anilide "trigger" portion of the molecule with a methoxy group provided a modest increase in efficacy. However, in the indole series this substitution led to a small decrease in efficacy (**2** vs **3**, Table 1). Removal of the C-3 methyl substituent provided a slight increase in efficacy (**3** vs **4**). Efforts were then directed toward replacement of the indole moiety. The 3-indazole moiety was initially chosen on the basis of its capability to function as an indole bioisostere in other systems.[9] Replacement of the indole group with a

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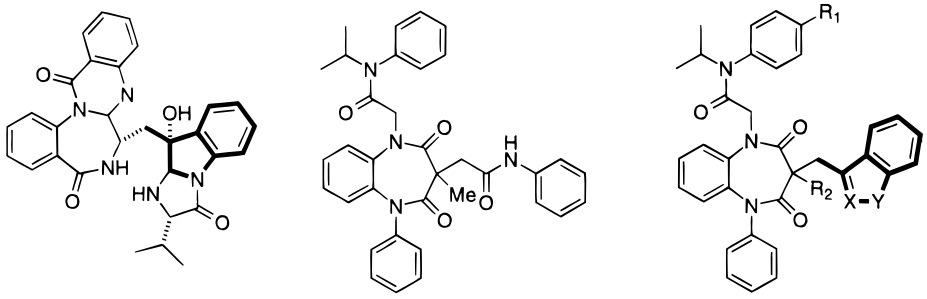

Figure 1.

Table 1. *In Vitro* Activity of 1,5-Benzodiazepine CCK-A Agonists

	structures[a]				functional assay[b]		binding assay[c]		
no.	R_1	R_2	X	Y	ED_{50} (nM)	% max	CCK-A (pIC_{50})	CCK-B (pIC_{50})	A/B sel
CCK-8	–	–	–	–	2 ± 1 (5)	100	8.88 ± 0.22 (8)	9.46 ± 0.04 (8)	0.3
1	–	–	–	–	190 ± 20 (4)	80 (4)	7.12 ± 0.02 (3)	5.08 ± 0.04 (3)	110
2	H	CH_3	CH	NH	470 (1)	80 (1)	6.97 ± 0.09 (3)	–	–
3	OCH_3	CH_3	CH	NH	530 (1)	60 (3)	8.08 ± 0.24 (4)	4.76 ± 0.26 (5)	2090
4	OCH_3	H	CH	NH	340 (1)	70 (4)	7.96 ± 0.14 (3)	5.28 ± 0.08 (3)	480
5	H	CH_3	N	NH	20 (1)	80 (2)	6.92 ± 0.12 (3)	5.58 ± 0.05 (3)	20
6	OCH_3	CH_3	N	NH	320 (1)	80 (3)	7.88 ± 0.02 (3)	4.16 ± 0.23 (3)	5100
7	OCH_3	H	N	NH	109 ± 60 (4)	100 (6)	7.64 ± 0.12 (5)	6.00 ± 0.23 (4)	50
8	H	H	N	NH	140 (1)	90 (2)	7.06 ± 0.05 (3)	6.75 ± 0.10 (3)	2
9	OCH_3	H	N	NCH_3	70 ± 10 (2)	90 (3)	7.37 ± 0.43 (3)	5.62 ± 0.20 (3)	60
10	OCH_3	H	N	NCH_2Ph	340 (1)	40 (2)	6.51 ± 0.16 (5)	4.00 ± 0.0 (2)	320

[a] Figure 1. [b] Functional activity in the isolated guinea pig gallblader following incubation with the test ligand for 30 min at 37 °C; ED_{50}, concentration at which 50% of the maximal contraction was observed ± SE (number of determinations); % max (number of determinations), relative efficacy as determined by the maximal contraction observed at 30 μM standardized to CCK-8 (1 μM) = 100%, all values ±5%. Reversal of contraction with MK-329 (1 μM) was used to verify a CCK-A receptor mediated response. [c] Binding affinity for human CCK-A and CCK-B receptors; pIC_{50}, −log of the concentration that displaced 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 ± SE (number of determinations); −, not determined; A/B sel, CCK-A receptor selectivity calculated from IC_{50} (B)/IC_{50} (A).

3-indazolyl moiety tended to provide an increase in efficacy (**3** vs **6**, **4** vs **7**, Table 1). Notably, indazole **7** was a full agonist, being as efficacious as CCK-8 in this assay. In parallel to the indole series, removal of the *p*-methoxy group resulted in a slight decrease in efficacy (**7** vs **8**). Methylation of the indazole N-1 nitrogen decreased efficacy slightly (**7** vs **9**), while *N*-benzyl analog **10** had substantially reduced agonist activity. Importantly, compounds **2**−**10** also displayed submicromolar potencies ranging from 20 to 500 nM in the guinea pig gallbladder assay (Table 1). Interpretations on potency differences within this series are precluded due to the lack of a significant number of full dose−response experiments for most entries.

Receptor binding affinities for compounds **1**−**10** were measured on membrane preparations from CHO-K1 cell lines stably transfected with cDNA from either human CCK-A[10] or CCK-B[11] receptors. IC_{50} values were determined using competitive radioligand binding with labeled CCK-8. Replacement of the *N*-phenylamide with the 3-indazolyl moiety resulted in a 5-fold drop in selectivity (**1** vs **5**, Table 1). Removal of the C-3 methyl group resulted in a decrease in A/B selectivity in both the indole and indazole series (**3** vs **4**, **6** vs **7**). Previous work[1] had shown that incorporation of a *p*-methoxy group in the anilide "trigger" portion of the molecule provided a slight increase in A/B binding selectivity. Incorporation of this moiety in the indole series led to increased affinity for the CCK-A receptor (**2** vs **3**) and provided a substantial improvement in the indazole series (**5** vs **6**, **8** vs **7**), with compound **6** displaying >5000-fold selectivity for the CCK-A receptor. In general, optimal A/B selectivity in this series is achieved by incorporation of both a *p*-methoxy group in the anilide trigger and a methyl group at C-3 of the 1,5-benzodiazepine ring.

Indazole **7** was chosen for *in vivo* evaluation based on *in vitro* efficacy, as it was the only compound which demonstrated full agonist activity and moderate CCK-A binding selectivity. Compound **7** was tested in a mouse gallbladder-emptying assay[1] to examine whether the increased *in vitro* efficacy translated to increased CCK-A agonist activity *in vivo*. This assay measures the extent of CCK-mediated gallbladder emptying 30 min after compound is administered[12] and provides a direct physiological reading of peripheral CCK-A agonist activity. Indazole **7** was both more potent and more efficacious than amide **1** in this assay (Table 2), showing efficacy equal to that of CCK-8 when administered intraperitoneally. This increase in bioactivity also held true upon oral administration, with **7** demonstrating full agonist activity and a substantial improvement in potency over amide **1**.

Having identified **7** as an orally active full agonist *in vivo* in a mouse gallbladder emptying assay, the satiety effects of this compound were evaluated in a rat feeding model. The *in vivo* effect of **7** on feeding was assessed in Long−Evans rats conditioned to a liquid diet and fasted for 2 h prior to oral administration of drug. Drug administration was followed immediately by a saline oral preload. Food access was provided 20 min later, and food intake was measured at 30, 90, and 180 min. *d*-Amphetamine was used as a positive control. This model provides measurement of the anorectic activity

Table 2. *In Vivo* Activity of 1,5-Benzodiazepine CCK-A Agonists **1** and **7**

	mouse gallbladder emptying[a]			
	ip		po	
no.	ED_{50} (nmol/kg)	% empty[b]	ED_{50} (nmol/kg)	% empty[b]
CCK-8	0.03	95	–	–
1	30	70	1000	70
7	0.2	90	8.0	90

[a] Following overnight food deprivation, male CD-1 mice (10 animals per dose) were treated (ip or po) with vehicle (ethanol/propylene glycol/water, 2:3:5, 1 mL/kg) or test compound dissolved in vehicle (1 mL/kg). Animals were sacrificed (CO_2) 30 min after drug treatment, and the gallbladders were removed and weighed. Gallbladder wet weights of the treated animals were normalized to the vehicle control group; –, not determined. [b] % empty = percent emptying, $p < 0.05$ at 0.1 μmol/kg (ip) or 10 μmol/kg (po) relative to CCK-8 at 1 nmol/kg, ip.

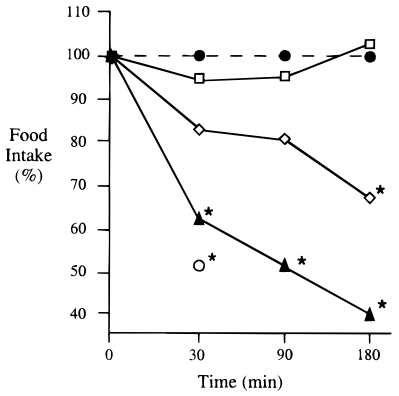

Figure 2. Key: (●) vehicle; (□) **7**, 0.1 μmol/kg; (◇) **7**, 1.0 μmol/kg; (▲) **7**, 10 μmol/kg; (○) *d*-Amphetamine, 2 mg/kg. Male Long–Evans rats (225–300 g) were conditioned for 2 weeks to consume a palatable liquid diet (Bio-Serve F1657, Frenchtown, NJ) after a 2 h fast. On pretreatment day, rats were fasted (100 min) and injected po 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/dose) were treated po with vehicle (PG, 1 mL/kg) or various doses (0.1–10 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. *$p < 0.05$ using Dunnett's multiple comparison test.

of CCK-A agonists, taking into account both vagally-mediated behavioral satiety effects in combination with effects through inhibition of gastric emptying.[13] The results (Figure 2) demonstrate indazole **7** was effective at reducing food intake to 40% of vehicle controls when given orally at a dose of 10 μmol/kg, with statistically significant reduction in food intake occurring at a dose of 1 μmol/kg after 180 min. At the higher dose an effect on food intake could be seen up to 24 h after dosing (data not shown). The chronic effectiveness of compound **7** in this model has not been evaluated.

Conclusion. Previous work[1,2] has identified 1,5-benzodiazepine CCK-A agonists whose efficacy and binding selectivity are dependent on the structure of the C-3 pharmacophore. This report illustrates that replacement of the C-3 urea or amide pharmacophore with a 3-indolylmethyl group maintains agonist activity. Subsequent structure–activity studies have identified indazole **7** as a binding-selective CCK-A full agonist which is orally active in a mouse gallbladder emptying assay. *In addition, compound **7** is the first CCK-A agonist which has demonstrated suppression of food intake when given orally in a rat feeding model.* This compound, GW 5823, shows promise as an orally active satiety agent which may be useful for the treatment of obesity.

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Supporting Information Available: Experimental, spectral, and analytical data for compound **7** (4 pages). Ordering information is given on any current masthead page.

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