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1,4-Benzodiazepine Peripheral Cholecystokinin (CCK-A) Receptor Agonists

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Dedicated to the memory of Marc Rodriguez, a pioneer in CCK research, an exceptional scientist and a dear friend

Abstract—A series of 1,4-benzodiazepines, *N*-1-substituted with an *N*-isopropyl-*N*-phenylacetamide moiety, was synthesized and screened for CCK-A agonist activity. In vitro agonist activity on isolated guinea pig gallbladder along with in vivo induction of satiety following intraperitoneal administration in a rat feeding assay was demonstrated.

Obesity, defined by the World Health Organization as a body mass index exceeding 30 kg m^{-2}, is the most prevalent metabolic disorder in western nations. Increased relative risk of coronary heart disease, type 2 diabetes mellitus, hypertension, and cholelithiasis is positively correlated with excess adiposity.[1] Current pharmacotherapeutic intervention strategies seek to either regulate nutrient intake, absorption or metabolism. Cholecystokinin (CCK) is a gastrointestinal peptide hormone and neurotransmitter that regulates satiety, biliary and pancreatic secretion, gallbladder contraction, and gut motility through the peripheral CCK-A seven transmembrane G-coupled protein receptor.[2] Administration of CCK to obese humans has been shown to reduce meal duration and size suggesting this mechanism as a means of drug intervention in the obese population.[3]

We have previously described[4] several series of 1,5-benzodiazepine CCK ligands (**1a**–**d**), which all incorporated an *N*-isopropyl-*N*-phenylacetamide agonist 'trigger' attached to *N*-1 to modulate an agonist functional response at the peripheral CCK-A receptor (Fig. 1). These analogues were potent fully efficacious agonists with anorectic efficacy in rat feeding models. As both the 1,5- and 1,4-benzodiazepine scaffolds have been utilized as CCK-A antagonists,[5] we envisioned that these same modifications, which imparted agonist functional activity in the former series, could be adopted onto the 1,4-benzodiazepine pharmacophore (**2**). The results of these studies are detailed below.

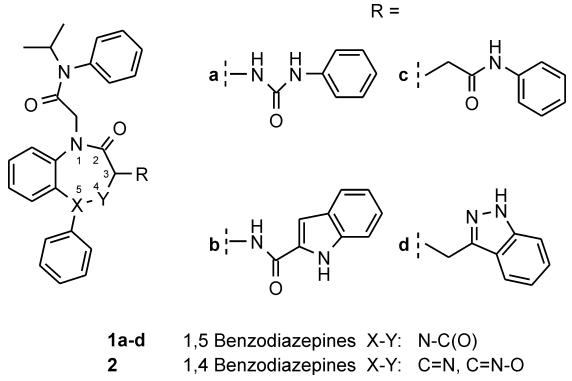

Figure 1.

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The prerequisite 3-amino-1,4-benzodiazepine core and agonist trigger were constructed from the previously published intermediates: 3-Cbz-amino-1,4-benzodiazepines, **3**;[6] and bromoacetamides, **4**[4a] (Scheme 1). The isopropyl anilide 'trigger' was installed by alkylation of *N*-1 of benzodiazepine **3** with **4** to generate intermediate **5**. Deprotection and acylation of the *C*-3 amino group provided analogues **2a**,**b**,**e**,**f**,**h**,**j**. *N*-Oxide analogues (**2g**,**i**,**k**,**n**) were prepared by oxidation of **5** with *m*-CPBA followed by catalytic removal of the Cbz protecting group and acylation.

Ureas **2a**,**e**,**h**–**k**,**n** were prepared from either treatment with phenyl isocyanate (**2a**,**h**–**k**) or the *p*-nitrophenylcarbamate of 3-*t*-butylcarboxy-aniline followed by TFA deprotection (**2e**,**n**). Amides (**2b**,**f**,**g**) were prepared through carbodiimide coupling with indole-2-carboxylic acid.

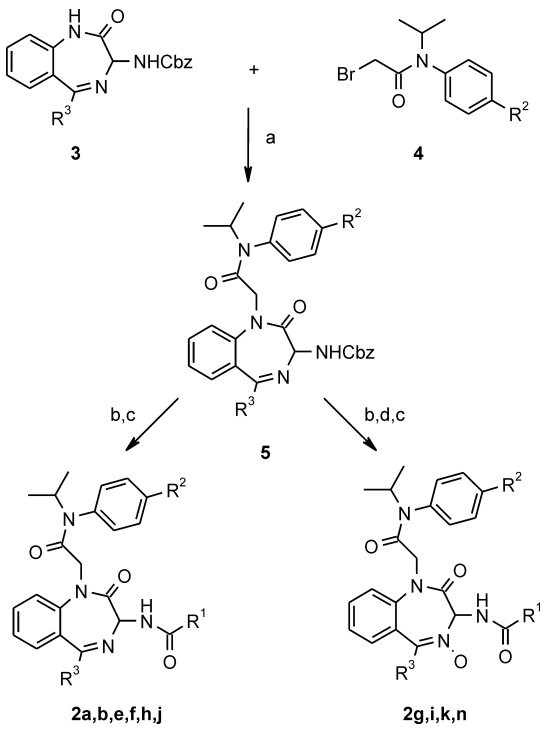
C-3 alkyl analogues were derived from benzodiazepine intermediate **7**, which in turn was prepared by alkylation of known benzodiazepine[7] **6** with bromide **4** (Scheme 2). Alkylation of **7** with *t*-butyl bromoacetate, TFA deprotection and PyBroP mediated coupling of aniline provided analogue **2c**. Indazole (**2l**,**m**) and indole (**2d**) derivatives were prepared by alkylation of **7** with the corresponding *N*-Boc-protected bromomethyl aryl intermediate[4d,e] followed by TFA deprotection. *N*-Oxide analogue **2m** was prepared by oxidation with *m*-CPBA, prior to Boc deprotection.

In vitro CCK-A functional efficacy was measured through contraction of isolated guinea pig gallbladder at 1 or 30 μM concentration.[8] Full dose–response curves were obtained for those compounds that induced >40% of the response produced by CCK-8. Agonist activity of all compounds was reversed by the selective CCK-A antagonist MK-329.[9] The satiety effect of several compounds was measured in a rat feeding model[4a] following intraperitoneal (ip) administration in fasted animals. ED_{50} values reflect the dose of test compound demonstrating 50% of the maximal response observed at 10 μmol/kg. '%Max' refers to the percentage reduction in food intake at 30 min dosed at 10 μmol/kg (0.5 μmol/kg for CCK-8) relative to control animals.

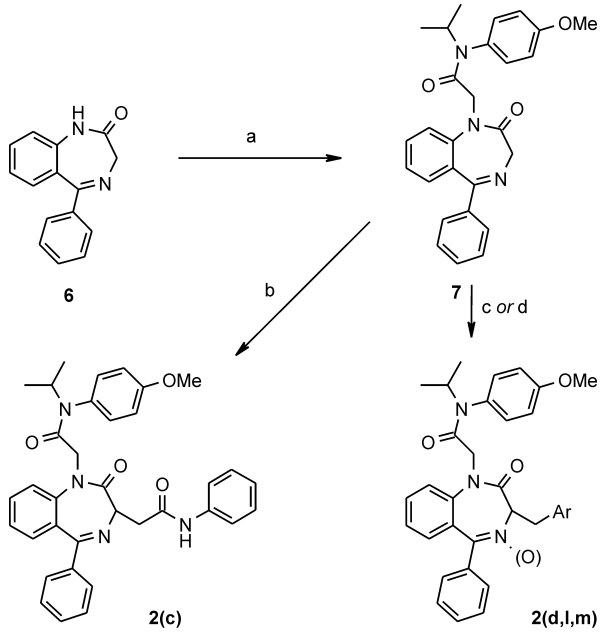
We had previously optimized the substituents necessary to modulate the conversion of 1,5-benzodiazepine CCK-A antagonists to CCK-A agonists.[4] Accordingly, we sought to incorporate the identical pharmacophores into a 1,4-benzodiazepine scaffold. Therefore, all of our analogues contain the isopropyl anilinoacetamide that served as the prerequisite agonist 'trigger' in the 1,5-series.[4a] At the 3-position, phenyl ureas,[4a,b] anilino acetamides[4c] and indazolylmethyl[4d,e] or indolylmethyl[4d] all served as optimal substituents when combined with the agonist 'trigger'.

We were pleased to observe that addition of the agonist 'trigger' to a 3-phenylureido substituted 1,4-benzodiazepine (**2a**) indeed yielded a CCK-agonist, albeit a partial one with only 39% (30 μM) of the maximal CCK-8 response (Table 1). Incorporating the 2-indoleamide (**2b**) to the 3-position yielded a weak partial agonist with only 12% (30 μM) of the maximal CCK response.

Introduction of a 4-methoxy substituent to the aniline present in the 'trigger' had produced potent agonists in the 1,5-benzodiazepine series.[4a,d,e] Combination of the

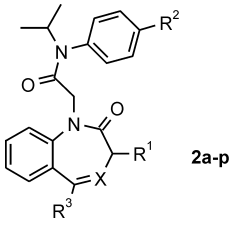


Scheme 1. (a) NaH, DMF; (b) HBr, AcOH or Pd/C, H_2, EtOH; (c) (i) 3-*t*-Bu-CO_2PhNHCO_2-*p*-NO_2-Ph, CH_3CN; (ii) TFA or PhNCO, CH_2Cl_2 or EDC, indole-2-carboxylic acid, CH_2Cl_2; (d) *m*-CPBA, CH_2Cl_2.

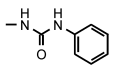
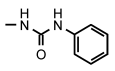
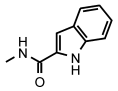
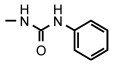
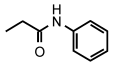
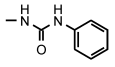
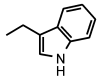
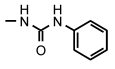
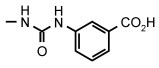
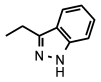
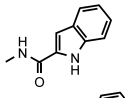
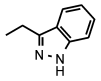
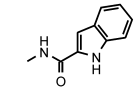
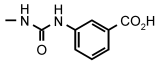


Scheme 2. (a) NaH, **4**, DMF; (b) (i) KHMDS, *t*-Bu-bromoacetate, THF; (ii) TFA; (iii) PyBroP; $PhNH_2$, CH_2Cl_2; (c) (i) LiHMDS, $BrCH_2Ar$,[4d,e] THF; (ii) TFA (d) for **2m** (i) LiHMDS, $BrCH_2Ar$,[4d,e] THF; (ii) *m*-CPBA, CH_2Cl_2; (iii) TFA, CH_2Cl_2.

Table 1. In vitro activity of 1,4-benzodiazepine CCK-A agonists



2a-p

2	R^1	R^2	R^3	X	GPGB % CCK[a] (μM)	ED_{50}[b] (nM)	2	R^1	R^2	R^3	X	GPGB % CCK (μM)	ED_{50} (nM)
a		H	Ph	N	39 (30)	ND	**h**		OMe	C_6H_{11}	N	56 (30)	233
b		H	Ph	N	12 (30)	ND	**i**		OMe	C_6H_{11}	N-O	87 (30)	342
c		OMe	Ph	N	43 (30)	1100	**j**		OMe	Me	N	90 (30)	296
d		H	Ph	N	94 (30)	41	**k**		OMe	Me	N-O	100 (30)	202
e		OMe	Ph	N	27 (1)	ND	**l**		OMe	Ph	N	27 (1)	ND
f		OMe	C_6H_{11}	N	0 (30)	ND	**m**		OMe	Ph	N-O	100 (30)	13
g		OMe	C_6H_{11}	N-O	43 (30)	131	**n**		OMe	C_6H_{11}	N-O	98 (30)	3

[a]Functional activity in the isolated guinea pig gallbladder following incubation with the test ligand for 30 min at 37 °C; relative efficacy as determined by the maximal contraction observed at 1 or 30 μM standardized to CCK-8 (1 μM) = 100%.
[b]ED_{50}, concentration at which 50% of the maximal contraction was observed for % CCK.

4-methoxy trigger modification with the *C*-3 anilinoacetamide (**2c**) and indolylmethyl (**2d**) groups produced partial agonists in the 1,4-benzodiazepine series (43 and 24%, respectively; 30 μM). We were initially somewhat disappointed with the efficacy demonstrated in the 1,4-benzodiazepine template relative to the 1,5-benzodiazepines. The analogous 1,5-benzodiazepine homologues[4] of analogues **2a**–**d** demonstrated 86, 49, 78, and 70% of the maximal CCK response at 30 μM, respectively; essentially 2–3 times more efficacious. However, addition of the 3-carboxy functionality to the phenyl urea (**2e**) produced a full CCK-agonist with 40 nM potency in our in vitro assay. The 1,5-homologue[4b] of analogue **2e** had demonstrated identical potency and efficacy (100% at 30 uM, ED_{50} = 40 nM).

We then chose to modify the 5-position of our series substituting cyclohexyl and methyl for the C-5 phenyl group of our earlier analogues. However, to our surprise, the combination of the 5-cyclohexyl group along with the *C*-3 indole amide (**2f**) produced a CCK-A antagonist as evidenced by inhibition of the CCK-8 functional response to the test tissue (data not shown). This was particularly unexpected considering the analogous 1,5-benzodiazepine analogue had demonstrated CCK-A agonist activity in the guinea pig gallbladder assay (34% at 1 μM, unpublished data).

We hypothesized that the carbonyl functionality present at the 4-position in the 1,5-series, but absent in the 1,4-series, played a critical role in regulating the functional activity of the ligand within the receptor. Oxidation of the 1,4-benzodiazepine to its corresponding *N*-oxide might approximate the carbonyl pharmacophore present in the 1,5-series. We were pleased to find that oxidation of analogue **2f** to **2g** restored the CCK-A agonist

Table 2. Anoretic profile[a] of 1,4-benzodiazepine CCK-A agonists

Compounds	ED_{50} (nM)[b]	%Max[c]
2i	25	75
2m	150	79
2n	80	69
CCK-8	27	98

[a]Anoretic potency in Long–Evans rats conditioned to a palatable liquid diet and fasted for 2 h.
[b]ED_{50}, dose of test compound that produces a half-maximal response (nM/kg).
[c]%Max, maximal response at 0.5 μmol/kg, ip for CCK-8 and 10 μmol/kg, ip for test compound.

activity (43% at 30 μM, 131 nM). This result suggested that the *N*-oxide moiety might be an essential pharmacophore for optimal agonist response. However, 5-cyclohexyl phenyl urea analogue **2h** and its corresponding *N*-oxide **2i** were similarly efficacious (56 and 87% at 30 μM, respectively) and equipotent (233 vs 342 nM, respectively). Further, 5-methyl phenyl urea analogue **2j** and its corresponding *N*-oxide **2k** were also essentially equally efficacious (90 and 100% at 30 μM, respectively) and equipotent (296 vs 202 nM, respectively). We had already shown the indazolylmethyl substituent at *C*-3 to be an optimal CCK-A agonist in the 1,5-series.[4d,e] The analogous 1,4-analogue (**2l**) was a weak partial agonist (27% at 1 μM). However, oxidation to **2m** provided an extremely potent fully efficacious agonist (98% at 30 μM, 13 nM). The potency of this 1,4-analogue actually exceeded the analogous 1,5-homologue[4d] (109 nM). From the SAR available, the nature of the functional group attached to *C*-3 of the benzodiazepine ring dictated whether oxidation to the *N*-oxide was required for agonist activity. Analogue **2n** incorporated all of the functional groups which had demonstrated superior efficacy and potency in this series: the 5-cyclohexyl group, the 4-methoxy substituted 'trigger' and the 3-carboxyphenyl urea at the 3-position. As expected, this compound was a full agonist (98% at 30 μM) with exceptional potency (3 nM).

Finally, we wished to evaluate the in vivo anoretic efficacy of these agents in a rat conditioned-feeder assay.[10] (Table 2). Analogues **2i**, **2m**, and **2n** were selected for further screening based on potency and efficacy demonstrated in the in vitro assay. Following intraperitoneal (ip) administration, all three agents demonstrated dramatic reduction in food intake, similar in both potency and efficacy to CCK-8.

In summary, we have described a novel series of potent and efficacious 1,4-benzodiazepine and 1,4-benzodiazepine-4-*N*-oxide CCK-A agonists that incorporate an *N*-isopropyl-*N*-phenylacetamide agonist 'trigger' at the *N*-1 ring position. Modifications to the *C*-3 position, which had demonstrated agonist activity in the 1,5-benzodiazepine series, were succesfully adapted to this template to give compounds whose potency, in some cases, equalled or exceeded their 1,5-homologues. These agents demonstrated efficacy in an in vitro guinea pig gallbladder assay and anoretic activity in a rat feeding model.

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