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Anthranilic sulfonamide CCK1/CCK2 dual receptor antagonists II: Tuning of receptor selectivity and in vivo efficacy

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ABSTRACT

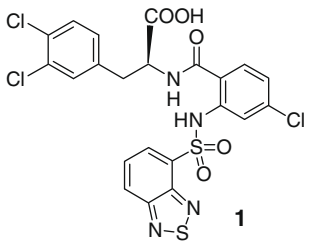
In the previous article we demonstrated how certain CCK2R-selective anthranilic amides could be structurally modified to afford high-affinity, selective CCK1R activity. We now describe our efforts at modulating and optimizing the CCK1R and CCK2R affinities aimed at producing compounds with good pharmacokinetics properties and in vivo efficacy in rat models of gastric acid and pancreatic amylase secretion.

While both CCK1 and CCK2 receptor hyperstimulation are proposed in the etiology of certain gastrointestinal diseases, no antagonist of either receptor has made it to market in the United States for the treatment of such diseases. This has not been for lack of effort on the part of a pharmaceutical industry that has brought small molecule CCKR antagonists into the clinic steadily over the past 20 years.[1] Failures in clinical phases of compound development have largely been associated with gallbladder stasis,[2] poor or variable pharmacokinetics,[3] lack of clinical efficacy for CNS indications,[4] and lack of perceived commercial viability in light of existing effective medications.[5]

In nearly every case, these compounds were designed to possess very high selectivities for one receptor subtype over the other. For the treatment of gastroesophageal reflux disease (GERD), which is characterized by both excessive and poorly regulated gastric acid production as well as lower esophageal sphincter (LES) dysfunction,[6] we hypothesized that antagonism of both CCK receptor subtypes would produce a unique mechanism of action for the control of GERD:CCK1R inhibition improving LES smooth muscle function and increasing the rate of gastric emptying,[7] and CCK2R inhibition moderating gastric acid secretion.[8] Due to the known actions of CCK1R antagonists on gallbladder contractility,[2] we sought a compound with significantly reduced activity at the CCK1R (i.e., ~10-fold higher activity at CCK2R).

We have discovered certain anthranilic amide-based CCK2 receptor antagonists that show high CCK2R selectivity with promising PK and in vivo activity in inhibiting pentagastrin-stimulated gastric acid secretion in the rat.[9]

In the previous Letter[10] we demonstrated that CCK1R affinity could be introduced into a novel CCK2R ligands by the manipulation of ring and alkyl side chain substitution. This is shown pictorially in Chart 1 for 24 analogs in this series in which affinity for CCK2R (pK_I) is plotted against the log ratio of CCK2R/CCK1R affinities for this series of compounds. We observed ca. 10 nM affinity for CCK2R with 20–30× selectivity over CCK1R in certain 3,4-dihalo phenylalanine-derived analogs. The point representing the best of these compounds is circled in Chart 1, corresponding to compound **1** (**2h** in Ref. 10).



1

As indicated in the preceding paper,[10] compound **1** displays good affinity for the CCK2 receptor with a selectivity factor over the CCK1 receptor that met our working hypothesis (CCK2R pK_I 7.8, and CCK2R/CCK1R affinity ratio of 16×).

Earlier studies had shown the [2,1,3]-benzothiadiazole ring system to be prone to CYP_{450}-mediated metabolism.[9,11] We therefore set about preparing analogs of **1** containing the less metabolically

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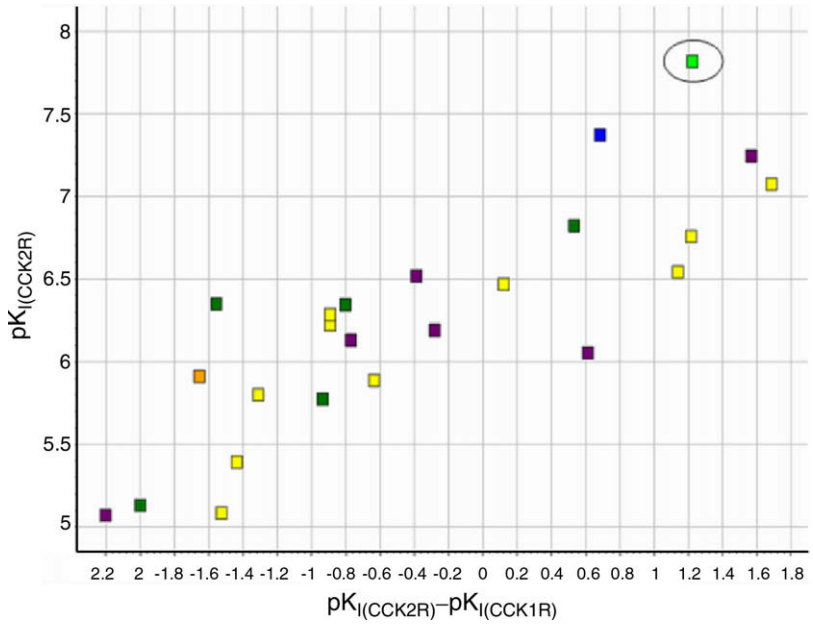


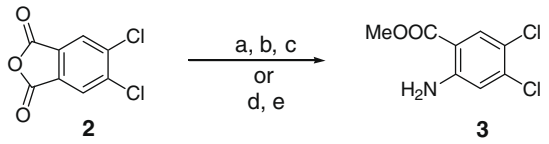
Chart 1. Plot of CCK2R affinity versus CCK2R/CCK1R selectivity.

labile quinoxaline ring system.[9] Additionally, we varied the pattern of halogenation of **1** and its quinoxaline congeners, in hope of further improving CCK2R affinity while maintaining the sought-after 10-fold selectivity over CCK1R.

The preparation of test compounds followed synthetic schemes previously described[10] with the addition that methyl 2-amino-4,5-dichlorobenzoate (**3**, used in the preparation of **4i** and **4j**) was prepared in 54–65% overall yield from 4,5-dichlorophthalic anhydride (Scheme 1).

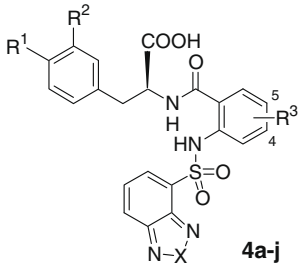
Ten carboxylic acids (and one methyl ester) were evaluated for their CCK1R and CCK2R binding affinities in previously described binding assays.[9] We evaluated only compounds possessing anthranilic R^3 chloro, bromo, iodo or dichloro substitutions. As is shown in Table 1, all of the compounds but one (**4a**) display improved CCK2R affinity compared to **1** with the most significant increase coming from compounds tested containing the 3-bromo-4-fluoro halogenation pattern around the aromatic phenylalanine (Phe) ring. Amongst these 3-bromo-4-fluoro compound, changes in halogenation of the 4-position of the central anthranilic ring had a modest effect on affinity for either receptor (cf. **4d**, **4f**, **4h**). This is in contrast to some of our earlier, non-Phe based CCK2R antagonists, which possessed significantly improved receptor affinity as a function of increasing halogen size (i.e., Cl to Br to I).[9]

The data in Table 1 also confirm our earlier finding[9] that, with respect to CCK2 receptor binding, the quinoxaline ring system is useful replacement for the [2,1,3]-benzothiadiazole system with respect to maintaining high receptor affinity. In addition we found for the current compounds that replacement of the [2,1,3]-benzothiadiazole ring system with quinoxaline shows little loss in CCK1 receptor affinity (cf. **4d** and **4e**, **4f** and **4g**). This was not evaluable in our earlier investigations due to very low affinities observed for the CCK1 receptor for compounds containing the quinoxaline ring system.



Scheme 1. Reagents and conditions: (a) NaOMe, MeOH, reflux; (b) $SOCl_2$, reflux; (c) NaN_3, acetone, 0 °C; then AcOH, H_2O, reflux, (54%, three steps); or, (d) $TMSN_3$, toluene, reflux; then EtOH; (e) MeOH (65%, two steps).

Table 1
Selectivity and affinity analysis of halogenated phenylalanine analogs



4a-j

Compd	R^1	R^2	R^3	X	CCK1R pK_i[a]	CCK2R pK_i[a]	Log ratio[b]
4a	Cl	Cl	4-I	S	6.7	7.6	0.9
4b	Cl	Br	4-Br	S	6.9	7.9	1.0
4c	Cl	Br	4-Br	CH=CH	7.0	8.0	1.0
4d	F	Br	4-Cl	S	6.6	8.0	1.4
4e	F	Br	4-Cl	CH=CH	6.4	8.0	1.6
4f	F	Br	4-Br	S	6.6	8.2	1.6
4g	F	Br	4-Br	CH=CH	6.5	8.3	1.8
4h	F	Br	4-I	S	6.8	8.3	1.5
4i	F	Br	4,5-Cl_2	CH=CH	6.8(6.7)[c]	8.2(6.6)[c]	1.4(0.1)[c]
4j	F	Br	4,5-Cl_2	S	6.8	8.0	1.2

[a] Negative logarithm of the antagonist equilibrium dissociation constant calculated from the concentration required to displace 50% ^{125}I-CCK-8S (pIC_{50}) by the method of Cheng and Prussoff.[14] All values are ±0.3 log units unless otherwise stated.
[b] pK_{iCCK1R}–pK_{iCCK2R}.
[c] Numbers in parentheses are for the corresponding methyl ester.

We next evaluated certain analogs for liver microsome stability and membrane permeability. Incubation in the presence of human liver microsome preparations showed consistently good stability toward oxidation under these assay conditions (Table 2). The fact that no meaningful difference in stability was observed for compounds that possessed either the [2,1,3]-benzothiadiazole or the

Table 2
Human liver microsomal stability, Caco-2 permeability values of selected compounds

Compd	HLM %rem 15 min[a]	P_{app} A to B[b]	P_{app} B to A[c]
4b	61	0.1	0.19
4c	69	0.1	1.97
4d	65	0.04	1.25
4e	50	0.04	4.76
4f	47	0.05	0.34
4g	54	0.03	4.36
4i	65	0.1	1.05
4j	76	0.02	0.37

[a] Percent remaining after 15 min in the presence of pooled human liver microsomes and NADPH.
[b] Apparent compound permeability (10^{-6} cm/s) from apical to basolateral side of Caco-2 monolayer grown on transwell plates.
[c] Apparent compound permeability (10^{-6} cm/s) from basolateral to apical side of Caco-2 monolayer grown on transwell plates.

Table 3
Rat pharmacokinetics[a]

Compd	$T_{1/2}$[b] (h)	V_{SS}[c] (mL/kg)	Cl[d] (mL/kg/min)	F[e] (%)
4d	1.3 ± 0.1	105 ± 9	3.1 ± 0.2	11 ± 3
4e	1.5 ± 0.1	145 ± 36	4.1 ± 0.7	4 ± 1
4h	6.4 ± 0.6	215 ± 85	3.1 ± 1.1	14 ± 6
4i	7.0 ± 0.7	129 ± 12	2.0 ± 0.1	9 ± 2
4j	6.2 ± 0.4	203 ± 55	2.6 ± 0.1	21 ± 4

[a] Calculated from at least 3 animals at iv and po doses of 2 μmol/kg.
[b] Elimination half-life from the iv dose.
[c] Steady-state volume of distribution.
[d] Clearance from the iv dose.
[e] Fraction absorbed from the oral dose ($(AUC_{po}/AUC_{iv}) \times 100$).

quinoxaline heterocycles was surprising and contrasts our earlier findings in a related series that the quinoxaline ring system was less prone to oxidative metabolism than was the [2,1,3]-benzothiadiazole ring system.[9] We attribute this difference to the presence of a net negative charge in the present compounds and consequent resistance to CYP-mediated oxidation. In addition, Caco-2 permeability measurements (P_{app}) showed that all of the compounds tested possessed limited apical (A) to basolateral (B) permeability, while displaying pronounced efflux (B to A).

The pharmacokinetic parameters of selected compounds were then evaluated in the rat (Table 3). Following oral administration of solutions **4d**, **4e**, **4h**, **4i**, and **4j** showed low to moderate absorption (%F = 4–28), in line with results from the Caco-2 assay. Plasma half-life values were high for analogs containing the anthranilic R^3 = I or R^3 = Cl_2 core (**4h**–**j**, $T_{1/2}$ = 6.2–7.0 h). As predicted by liver microsome studies, benzothiadiazole analog **4d** showed an elimination half-life that was not significantly different from its quinazolinone congener **4e**. Plasma clearance values were also observed to be low (⩽10% hepatic blood flow) for all compounds tested (see Table 3).

Lastly, compounds were evaluated for their pharmacodynamic effects in vivo (Table 4). Thus, the potential for inhibition of the release of pancreatic amylase[12] (a CCK1R mediated effect measured as amylase released into the plasma) and the inhibition of gastric acid secretion[13] (CCK2R mediated) were evaluated in the rat. Most compounds tested produced significant (45–67%) inhibition of

Table 4
Effect of CCK1/CCK2 dual receptor antagonists on CCK8s-stimulated plasma amylase and pentagastrin-stimulated gastric acid secretion in the rat

Compd	Inhibition of amylase[a]	Inhibition of acid secretion, pED_{50} ± sd (ED_{50})[b]
4a	45 ± 10%	5.1 ± 0.1 (7.9 μmol/kg)
4c	65 ± 11%	ND
4g	67 ± 7%	ND
4i	−5 ± 31%	ND
4j	67 ± 8%	5.8 ± 0.1 (1.6 μmol/kg)

[a] Inhibition of CCK8s-stimulated plasma amylase (10 nmol/kg s.c. dosed 60 min following oral administration of 30 μmol/kg test compounds). Data are expressed at percentage of reduction in the plasma amylase AUC of the concentration–time curve over 6 h (plasma sample time points: 1, 2, 4, 6 h following administration of CCK8s).
[b] Inhibition of gastric acid secretion stimulated by intravenous infusion of pentagastrin (100 nmol/kg/h) in the anesthetized Ghosh and Schild rat model.[11] Test compounds were administered by intravenous bolus administration and responses measured as % decrease in the pH of the gastric lumen perfusate.

CCK8s-stimulated pancreatic amylase secretion (Table 4). In addition, compounds **4a** and **4j** also potently inhibited pentagastrin-stimulated gastric acid secretion, with oral pED_{50} values of 5.1 (ED_{50} = 5.3 mg/kg, 7.9 μmol/kg) for **4a**, and 5.8 (ED_{50} = 1.0 mg/kg, 1.6 μmol/kg) for **4j.**

In conclusion, we have shown for the first time that good CCK1R/CCK2R dual affinity can be imparted to certain formerly CCK2R selective anthranilic amides. Subsequently, compounds were designed with ∼10× selectivity for CCK2R/CCK1R that manifested in potent in vivo inhibition of gastric acid secretion along with moderate inhibition of pancreatic amylase production. It is hypothesized that the moderate antagonism of the CCK1 receptor may providing significant benefit for GERD patients from improved lower esophageal sphincter function and gastric prokinesis while still allowing periodic gall bladder contraction.

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