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Anthranilic sulfonamide CCK1/CCK2 dual receptor antagonists I: Discovery of CCKR1 selectivity in a previously CCKR2-selective lead series

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ABSTRACT

A series of CCK2R-selective anthranilic amides is shown to derive CCK1R affinity via selective substitution of the amide side chain. Thus, extending the length of the original benzamide side chain by a single methylene unit imparts CCK1R affinity to the series, and further fine tuning of the affinity results in CCK1R selectivity of greater than 100-fold.

Small molecule cholecystokinin type 1 receptor (CCK1R) antagonists have been known in the literature since the discovery and biological profiling of the natural product asperlicin.[1] Since then, high affinity ligands selective for CCK1R over the related cholecystokinin type 2 receptor (CCK2R) have been described.[2–4] In practice, high receptor subtype selectivity has been readily realized via optimization of affinity for just one receptor (i.e., pharmacophore determinants for the two receptor subtypes are typically distinct such that improvement in the affinity of a small molecule series for just one of the subtypes typically results in no improvement of affinity for the other). However, important examples in which subtype selectivity has been reversed through SAR studies have been reported, notably in benzodiazepine[5] and glutamic acid based series.[4,6,7]

We have previously reported the discovery and initial optimization of a novel series of anthranilic amides as high affinity CCK2R selective antagonists.[8] Few compounds in this series were found to have measurable CCK1R affinities below 10 μM IC_{50}, in contrast to that demonstrated in a related indole amide series.[9] The best compounds in the series were shown to have favorable pharmacokinetic profiles in the rat and dog and have high potency for the reversal of gastrin stimulated gastric acid secretion upon oral dosing. Researchers from our labs have also reported the discovery of novel, highly selective CCK1R antagonists in the pyrazole carboxylic acid class.[10,11] Selectivity for the respective receptor subtypes was always found to be extremely high, with early compounds in our benzamide series showing little to no affinity for the CCK1 receptor (e.g., **1**; CCK1R pK_i = 5.6).

We discovered that by simply extending the amide side chain by a single methylene unit to give phenethyl amides such as **2a**, high CCK1R affinity and selectivity was achieved (CCK1R pK_i 6.8, CCK2R pK_i 5.2, Fig. 1, Table 1). Further extension of this side chain (e.g., **3**) resulted in a significant loss of CCK1R affinity (CCK1R pK_i 5.7). Interestingly, the high CCK1R affinity and selectivity of the phenethylamides could be reversed by the simple N-methylation of compound **2** (i.e., **4**, CCK1R pK_i 5.4, CCK2R pK_i 6.5).

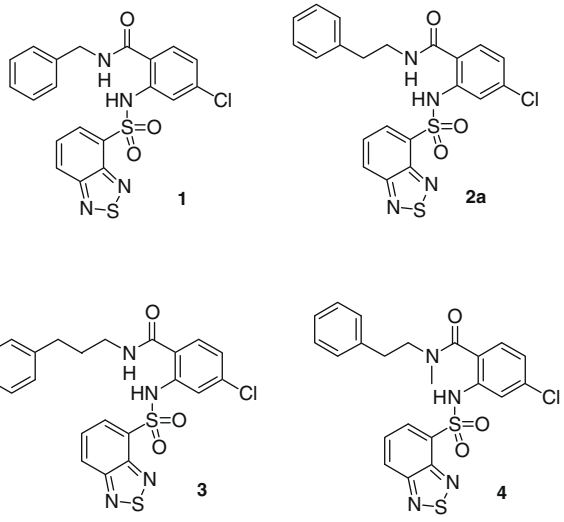

Figure 1. Selected early compounds.

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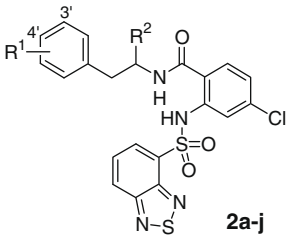
Table 1
Selectivity and affinity analysis of selected early compounds and Phe-derived amides



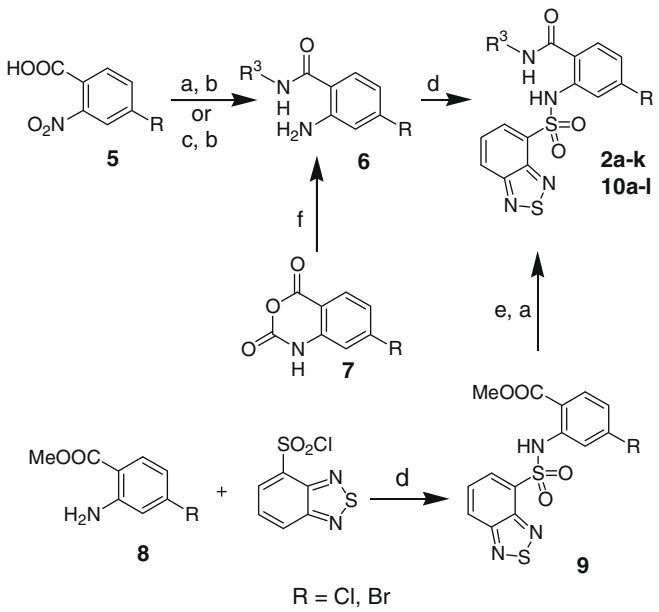
2a-j

Compds	R^1	R^2	CCK1R pK_i[a]	CCK2R pK_i[a]	Log ratio[b]
1	—	—	<5	5.6	<−0.6
2a	H	H	6.8	5.2	1.6
2b	4′-Cl	H	6.0	<5	>1
2c	3′,4′-Cl_2	H	7.1	5.1	2.0
2d	H	(*S*)-COOH	5.4	7.1	−1.7
2e	H	(*R*)-COOH	5.5	6.8	−1.3
2f	4′-Cl	(*S*)-COOH	5.7	7.2	−1.5
2g	4′-Cl	(*R*)-COOH	6.5	6.2	0.3
2h	3′,4′-Cl_2	(*S*)-COOH	6.6	7.8	−1.2
2i	3′,4′-Cl_2	(*R*)-COOH	7.1	6.3	0.8
2j	3′-Br, 4′-Cl	(*S*)-COOH	6.7	7.4	−0.7
3	—	—	5.7	6.0	−0.3
4	—	—	5.4	6.5	−0.9

[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.[16] All values are ±0.3 log units unless otherwise stated.
[b] pK_{iCCK1R}−pK_{iCCK2R}.

In this Letter we report our efforts at capitalizing on this discovery and identifying compounds exhibiting high CCK1R selectivity within the anthranilic amide series. In the accompanying Letter,[12] we describe the tuning of CCK1R and CCK2R affinities in this series to produce dual receptor antagonists for the potential treatment of gastroesophageal reflux disease (GERD).

The preparation of test compounds is depicted in Scheme 1. Appropriately halogenated 2-nitrobenzoic acids **5** were coupled with side chain amine groups R^3NH_2 to provide, after reduction, anthranilic amides **6**. Sulfonylation with 4-chlorosulfonyl-2,1,3-benzothiadiazole afforded final compounds **2** or **10**. Alternately, anthranilic amides **6** were prepared directly from the corresponding isatoic anhydrides **7** via direct condensation with R^3NH_2. In a second route, methyl anthranilates **8** were sulfonylated to afford intermediates **9**, which were then saponified and coupled as described above.



Scheme 1. Reagents and conditions: (a) R^3NH_2, HATU, pyr, DMF, 1 h (DIPEA added when the HCl salt of R^3NH_2, was used); (b) $SnCl_2{\cdot}2H_2O$, EtOAc, DCM; (c) (i) $SOCl_2$; (ii) R^3NH_2, DMF; (d) 4-chlorosulfonyl-2,1,3-benzothiadiazole, pyr, DCM, 16 h; (e) $LiOH{\cdot}H_2O$, THF, water, 3–16 h; (f) R^3NH_2, DMAP, DMF, 50 °C, 12 h.

Compound affinity was evaluated in CCK1R and CCK2R radioligand binding assays that have been previously described.[13–15] Table 1 shows selected initial compounds aimed at improving both CCK1R affinity and selectivity in the series. Starting with the unsubstituted phenethyl amide **2a** (CCK1 pK_i 6.8; CCK2R pK_i 5.2), CCK1R affinity was significantly reduced by the addition of a chlorine atom at the aryl C4′ position of the phenethyl side chain (**2b**). Interestingly, good CCK1R affinity returned by the addition of a second chlorine atom at the aryl C3′ position (**2c**, pK_i 7.1).

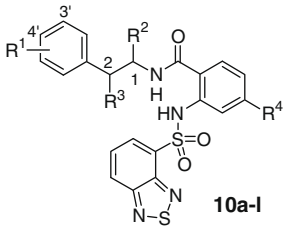
In order to help increase the aqueous solubility of this series, and recognizing that a common motif in known CCK receptor ligands is the carboxylic acid (or equivalent), we examined amides of phenylalanine (Phe) and their halogenated derivatives (**2d–j**, Table 1). The addition of an (*S*)-COOH group to **2a** (**2d**, derived from L-Phe) resulted in a reversal of affinity with an unexpectedly high CCK2R affinity of pK_i 7.1 and selectivity over CCK1R (pK_i 5.4). This result is in contrast to that observed in literature reports in which addition of a COOH group in a related, racemic series resulted in poor CCK2R affinity, with high selectivity for CCK1R.[9] This demonstrates an important pharmacophoric difference between the present benzothiadiazole sulfonamides and the literature indole amide anthranilates. The enantiomer (**2e**, derived from D-Phe) showed a somewhat reduced CCK2R affinity but still 20× selectivity over CCK1R.

As we observed for the phenethyl-derived amides **2a–c**, the receptor affinity of the Phe-derived amides also showed a pronounced sensitivity to the pattern of halogenation of the aryl ring. Simple mono-chlorination of the aryl C4′ position in the (*S*) enantiomer showed little change in receptor affinity or selectivity.

Chlorination at the same position in the (*R*) series resulted in a substantial improvement of CCK1R affinity (ca. 10×) and a concomitant reduction in CCK2R affinity to provide a compound (**2g**) with near equal receptor affinities of about 0.5 μM (K_i). Further ring chlorination in the (*S*) series (**2h**) resulted in a 4–10× improvement in affinity at both receptors with respect to the mono-chlorinated analog, while maintaining CCK2R selectivity. In contrast, applying the same analysis to the (*R*) series (i.e., **2e**→**2g**→**2i**) we see a complete reversal in receptor subtype selectivity. Finally, this series could be refined for high CCK2R affinity through further manipulation of the halogenation pattern of the aryl ring (**2j**). We found that substitution of this ring by polar or highly electron-donating substituents resulted in a marked reduction in affinity for both receptors (data not shown).

In a second line of investigation, we examined the effect of increased hydrophobicity and conformational restriction on the alkyl portion of the phenethyl side chain. Placing a methyl group at the C2 alkyl position (R^3, Table 2) of **2b** resulted in racemate **10a** that retained CCK1 selectivity while increasing affinity with respect to **2b**. Quarternization of this center results in *gem*-dimethyl analog **10b** that shows a further improvement in CCK1R affinity (pK_i 7.3) and 160× (log ratio 2.2) selectivity over CCK2R. An investigation into the effect of the absolute stereochemistry in the methylated series on receptor affinity was performed in the bromo anthranilic acid series (**10c–f**). Compared with the racemic material (**10d**), the single enantiomers (**10e** and **10f**) fail to reveal a eutomeric/distomeric pair at CCK1R, but the (*S*) enantiomer (**10e**) appears to have a small preference for CCK2R binding with the result that the (*R*) enantiomer (**10f**) shows greater overall receptor selectivity (log ratio 1.3 vs 0.9).

Table 2
Selectivity and affinity analysis of selected alkyl- and aryl-substituted phenethyl amides



10a-l

Compound	R^1	R^2	R^3	R^4	CCK1R pK_i[a]	CCK2R pK_i[a]	Log ratio[b]
10a	4-Cl	H	(*R*/*S*)-Me	Cl	7.0	6.4	0.6
10b	4-Cl	H	Me_2	Cl	7.3	5.1	2.2
10c	H	H	H	Br	6.4	6.2	0.2
10d	H	H	(*R*/*S*)-Me	Br	7.1	6.2	0.9
10e	H	H	(*S*)-Me	Br	7.2	6.3	0.9
10f	H	H	(*R*)-Me	Br	7.1	5.8	1.3
10g	4-Cl	H	4-Cl-Ph	Cl	7.5	<5	>2.5
10h	3,4-Cl_2	H	(*R*/*S*)-Me	Cl	7.9	6.3	1.6
10i	3,4-Cl_2	(*S*)-COOH	(*R*)-Me	Cl	6.3	6.8	−0.5
10j	3,4-Cl_2	(*R*)-COOH	(*R*)-Me	Cl	6.7	5.8	0.9
10k	4-Cl	(*S*)-COOH	Me_2	Cl	5.4	6.0	−0.6
10l	4-Cl	(*S*)-COOH	4-Cl-Ph	Cl	<5	5.9	<−0.9
10m	3-Br	(*R*)-Me	(*S*)-OH	Cl	7.6	5.9	1.7

[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.[16] All values are ±0.3 log units unless otherwise stated.
[b] $pK_{iCCK1R}-pK_{iCCK2R}$.

Substitution was added to the alkyl C2 position without chirality by the addition of a 4-chlorophenyl group to **2b**. The resulting analog, **10g**, showed very good affinity for the CCK1 receptor with no measurable affinity for CCK2R, making this the most selective compound we have discovered to date in the anthranilic sulfonamide series.

As with previous compounds, the presence of aryl 3,4-dihalogenation in the C2-methylated series produced an increase in CCK1R affinity with little change in CCK2R affinity (cf. **10h** and **10a**). Unfortunately for the sake of improving aqueous solubility, the combination of C2 substitution and C1 carboxylation did not result in compounds with useful affinities for either receptor subtype (**10i–l**). The combination of (*S*)-COOH at C1 and (*R*)-methyl group at C2 resulted in a compound, **10i**, showing significant loss of CCK1R affinity coupled with only a modest improvement in CCK2R affinity (at least with respect to the non-carboxylated analog **10h**). The addition of an (*R*)-COOH in the same manner provided stereoisomer **10j**, which, unlike the trend observed for the compounds in Table 1, failed to provide improved affinity for CCK1R. The addition of an (*S*)-COOH group to *gem*-dimethyl analog **10b** gave **10k** a compound exhibiting poor CCK1R affinity with only modest improvement in CCK2R affinity. Likewise, addition of an (*S*)-COOH group to benzhydryl analog **10g** produced a compound, **10l**, with no measurable CCK1R affinity and little improvement in CCK2R affinity.

We also evaluated a series of ephedrine-based derivatives. The result is that compounds bearing the (1*R*,2*S*) stereochemistry were the only ones of the three stereoisomers investigated (the (1*R*,2*R*) analog was not prepared) showing affinity of <100 nM for either receptor. A representative example is given in Table 2 (**10m**, CCK1 pK_i 7.6).

In conclusion, we have discovered for the first time that within a very narrow structural subclass of anthranilic sulfonamide CCK receptor antagonists (phenethyl amides), one can observe CCK1R selectivity. The level of the CCK1R affinity as well as selectivity over CCK2R was found to be enhanced by appropriate halogenation of the phenethyl ring as well as by alkylation at the C2 position of the side chain. Armed with the knowledge of how to adjust both CCK1R and CCK2R affinities within this series, we were then able to design CCK1/CCK2 dual receptor antagonists with affinities modulated toward inhibiting both receptors in the periphery with the aim of providing treatment for GERD. The preliminary in vivo work in support of this effort is described in the following article in this series.

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