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Discovery of potent cholecystokinin-2 receptor antagonists: Elucidation of key pharmacophore elements by X-ray crystallographic and NMR conformational analysis

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Abstract—A novel series of cholecystokinin-2 receptor (CCK-2R) antagonists has been identified, as exemplified by anthranilic sulfonamide **1** ($pK_i = 7.6$). Pharmacokinetic and stability studies indicated that this series of compounds suffered from metabolic degradation, and that both the benzothiadiazole and piperidine rings were rapidly oxidized by liver enzymes. A combination of synthesis, computational methods, ^{1}H NMR conformational studies, and X-ray crystallographic analyses were applied to elucidate key pharmacophore elements, and to discover analogs with improved pharmacokinetic profiles, and high receptor binding affinity and selectivity.

1. Introduction

The cholecystokinin-2 receptor (CCK-2R) is present in gastric tissue as well as the cortical region of the brain.[1] Recent interest in CCK-2R antagonists has been heightened by the implication of gastrin, a primary endogenous ligand for the CCK-2 receptor, as a potent trophic factor for gastrointestinal adenocarcinomas such as Barrett's metaplasia and pancreatic tumors.[2–4] The therapeutic potential of gastrin suppression has been demonstrated recently in clinical trials of the gastrin vaccine Gastrimmune™ as a treatment for stomach and pancreatic cancer.[5,6] Although numerous non-peptide CCK-2R antagonists have been forwarded as potential therapeutic agents for gastrointestinal disease, to date their promise remains unrealized.[7] This has been largely due to poor physicochemical properties, and the associated unfavorable and variable pharmacokinetic behavior.

Recently this laboratory disclosed a series of anthranilic sulfonamides that are potent and selective CCK-2R antagonists, and which exemplify a novel chemotype in this area of research.[8] Representatives of this series are benzo[1,2,5]thiadiazole-4-sulfonic acid, [5-bromo-2-(piperidine-1-carbonyl)phenyl] amide (**1**) and the 5-chloro congener (**2**), which display nanomolar binding affinity and high selectivity over the related CCK-1 receptor subtype (**1**: CCK-2R $pK_i = 7.6$; CCK-1R $pK_i < 5$; **2**: CCK-2R $pK_i = 7.4$; CCK-1R $pK_i < 5$; Fig. 1). Investigation of in vitro ADME predictors and in vivo pharmacokinetic properties show that **1** and **2** have good GI absorption, although these compounds have a high propensity for oxidative metabolism (Table 1). This was evidenced by poor stability toward human liver microsomes (HLM), with 15% and 3.9% of **1** and **2** remaining after 30 min, respectively.[8] These data correlate to an unfavorable

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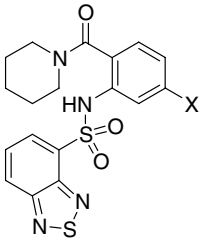


1: X = Br (pK_i = 7.6)
2: X = Cl (pK_i = 7.4)

Figure 1. Benzothiadiazole sulfonamides as CCK-2R antagonists.

Table 1. Pharmacokinetic and HLM parameters for **1**, **2**, and **28**

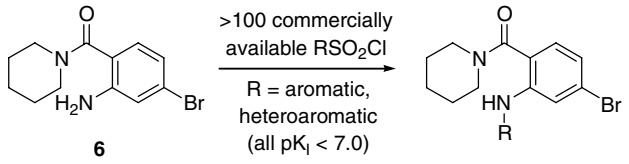
Compound	Rat pharmacokinetic parameters (±SE)[a]			HLM[b]
	CL (L/kg/h)	$t_{1/2}$(h)	%F	
1	0.70 ± 0.06	0.26 ± 0.03	68 ± 8	15
2	1.25 ± 0.26	0.16 ± 0.03	42 ± 17	3.9
17	0.83 ± 0.27	0.34 ± 0.11	50 ± 16	21
28	0.36 ± 0.05	0.62 ± 0.05	55 ± 13	60

[a] Calculated from at least three animals. Clearance values (CL) and half-lives ($t_{1/2}$) measured from iv doses of 2 μmol/kg. Relative fraction absorbed (%F) measured from 2 μmol/kg po doses.
[b] Percent remaining after 30 min in the presence of pooled human liver microsome preparations (n = 20).

in vivo pharmacokinetic profile, with rapid clearance and a half-life of only 16 and 10 min in the rat for **1** and **2**. Qualitative HPLC and mass spectral analysis of the HLM incubate of **1** suggested facile oxidation of the sulfur atom of the benzothiadiazole ring to the *S*-oxide (**3**), and subsequent hydration to the *S*,*S*-dioxide (**4**), in agreement with reported metabolic pathways of this ring system (Fig. 2).[9] In addition, oxidation of the carbon atom adjacent to the nitrogen atom in the piperidine ring was also observed (i.e., **5**, see Supplementary material).

2. Structural and conformational analysis

With these facts in evidence, replacement of the benzothiadiazole ring became a goal of paramount importance. Parallel synthesis and screening of a large number of analogs generated from a diverse set of commercially available sulfonyl chlorides and amine **6** failed to yield a compound with high CCK-2R binding affinity, demonstrating the extreme sensitivity to substitution in this region (Scheme 1).[10] Therefore, we undertook a more detailed examination of the physical properties and conformational preferences of this series, in order to help elucidate the role of the benzothiadiazole ring and identify the narrowly defined pharmacophore elements that are required for high binding affinity.



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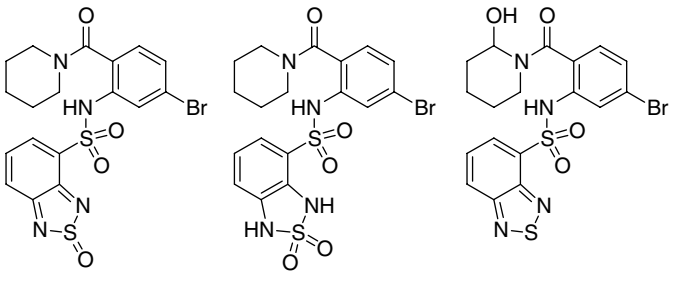
Scheme 1. Parallel synthesis of benzothiadiazole replacements.

The structure of **1** obtained by single crystal X-ray diffraction clearly shows a cup-shaped conformation in the solid state (Fig. 3A), where the carbonyl oxygen of the piperidine amide and N3 of the benzothiadiazole ring are engaged in a proximal relationship with the acidic proton of the sulfonamide group, in agreement with the known conformational preferences of anthranilic amides.[11–13] In order to arrive at a geometry more representative of the solution state than the crystal structure, **1** was energy minimized (B3LYP/6-31++G(2d,2p)) beginning with the solid state conformation. The resulting coordinates are very similar to the crystal structure, as shown in Figure 3B. Although NMR studies of **1** were complicated by rotameric broadening, NOESY experiments conducted on the related benzylamide **7** demonstrated that the conformation placing the carbonyl oxygen atom of the amide in a proximal position to the sulfonamide NH is highly populated in solution (i.e., H_a–H_b NOE, Fig. 4), in agreement with the solid state findings.[11–13] In addition, a small reciprocal NOE enhancement observed between H_c and H_d suggests that the phenyl portion of the benzothiadiazole ring is preferentially neighboring the phenyl ring of the anthranilic core, with the ring nitrogen atoms oriented toward the sulfonamide nitrogen. This is in agreement with the solid state structure (Fig. 3) and provides further evidence that



3 **4** **5**

Figure 2. Major metabolites of **1** identified by HPLC/MS analysis of HLM incubate.

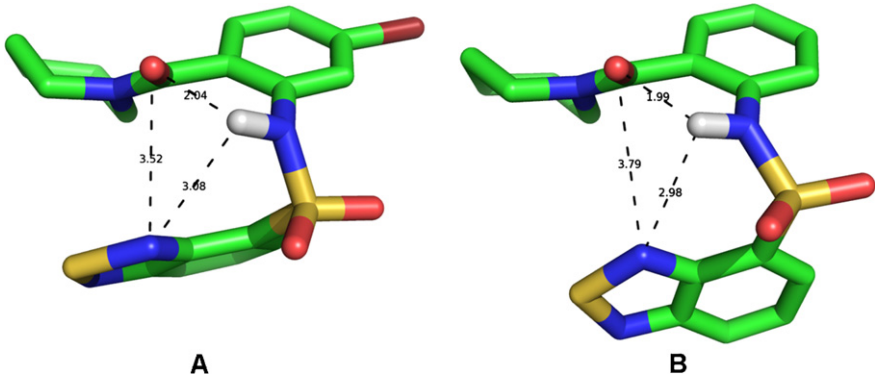


Figure 3. (A) Solid state conformation of **1** as determined by X-ray crystallography and (B) energy minimized conformation (B3LYP/6-31++G(2d,2p), bromide removed to simplify calculation). Distances are in angstroms.

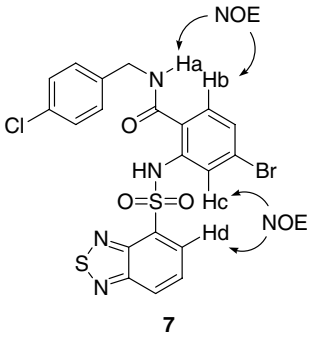


Figure 4. Diagnostic NOE enhancements observed during NMR conformational analysis of **7** (600 MHz, DMSO-d_6).

the solid state conformation is a reasonable representation of the solution state conformation.[11] The clear conformational preference demonstrated by these crystallographic, computational, and NMR data provided hypotheses for the pharmacophoric requirements of high binding affinity. One possible scenario is that the amide carbonyl group, sulfonamide NH atom, and benzothiadiazole N3 atom may form a three-point intramolecular interaction, which enforces a conformation that is possibly representative of the binding conformation. Although the observed geometry is not completely optimal for a formal three-point hydrogen bonding array, an energy minimized structure in which the benzothiadiazole ring was initially rotated 180° about the carbon–sulfur bond (and thus unable to participate in this putative three-point interaction) was energetically less favored by 0.8 kcal/mol (B3LYP/6-311++G(3d,3p)//B3LYP/6-31++G(2d,2p), not shown), which is consistent with the formation of a weak interaction (Fig. 3). A second possibility is that one or both benzothiadiazole nitrogen atoms are involved in direct receptor interactions that are critical for high binding affinity. Although the validity of either possibility was uncertain at the outset, in searching for benzothiadiazole replacements it seemed reasonable to use this conformational guide as a template. The following describes the synthesis, biological evaluation, and physical properties of compounds which address both the issue of metabolic stability and the questions raised by the above structural analysis.

3. Chemistry

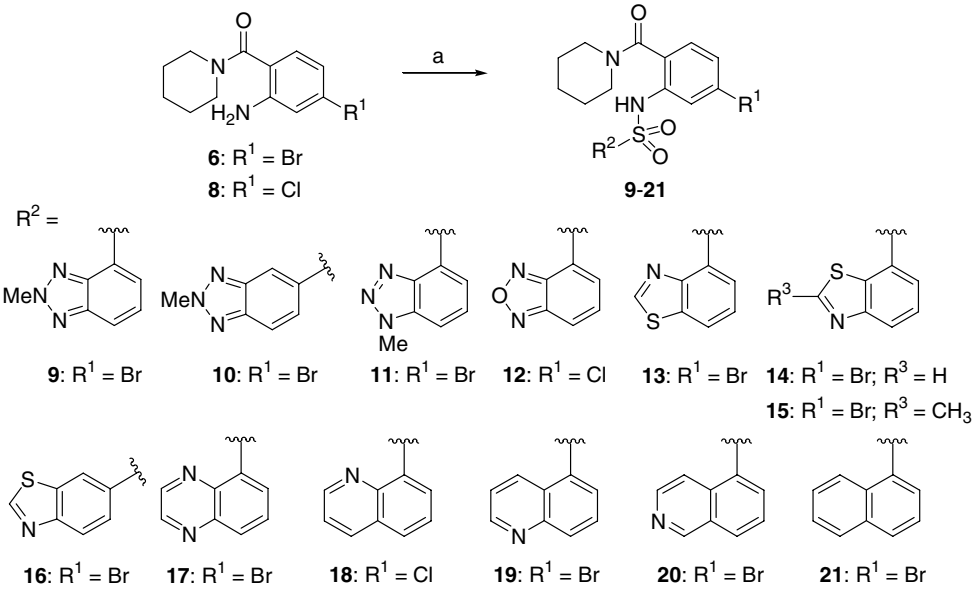
We designed a series of heterocyclic sulfonamides as potential replacements for the benzothiadiazole ring system, including 4-benzoxadiazolyl, 4-benzotriazolyl, 5-quinoxalyl, in order to test our hypotheses about the conformational requirements for high CCK-2R binding affinity. For compounds **12**–**21**, aniline **6** or **8** was coupled with known or commercially available sulfonyl chlorides (Scheme 2).[8]

For compounds **9**–**11**, the required sulfonyl chlorides **22**–**24** were obtained by direct chlorosulfonation of the parent heterocycle, directly followed by coupling with amine **6** (Scheme 3). Any sulfonamide regioisomers present were separated at this stage by chromatography or crystallization, and the regiochemisty was established by ^{1}H NMR. In the case of triazole **11**, the regiochemistry was further established by observation of reciprocal NOE enhancements between the *N*-methyl group of the triazole and the neighboring aromatic proton. Benzimidazole analog **26** was prepared by reduction of benzothiadiazole **1**with zinc metal in acetic acid, and treating the resulting crude phenylenediamine intermediate **25** with $CH(OMe)_3$,[14] while benzylamide **7** was obtained by HATU mediated amide formation from acid **27** (Scheme 4).[8]

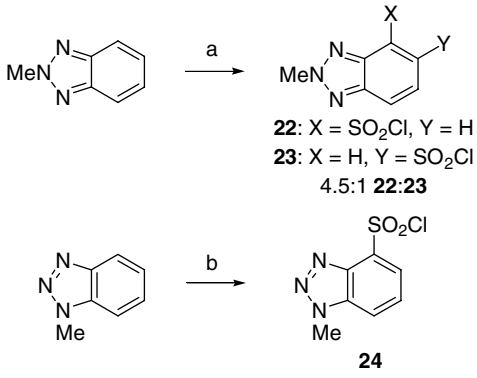
4. Results and discussion

4.1. Structure–activity relationship

The structure–activity relationship (SAR) of the targeted analogs was evaluated using a human CCK-2R radioligand competition binding assay (Table 2).[16–18] Initially we examined analogs in which the [4.3.0]-fused heterocyclic motif was preserved. Replacement of the sulfur atom of the benzothiadiazole ring of **1** with an *N*-methyl group resulted in the 2-methylbenzotriazole-4-yl (**9**) and -5-yl (**10**) analogs with over 15- and 100-fold loss in binding affinity, respectively. A similar loss of activity was observed for the 1-methylbenzotriazole-4-yl (**11**) substitution pattern. Strikingly, the benzoxadiazole-4-yl congener (**12**) displayed a 50-fold loss in binding affinity relative to benzothiadiazole **2**. This unexpected



Scheme 2. Reagents and condition: (a) R^2SO_2Cl, pyridine, CH_2Cl_2, 23 °C, 3–16 h.

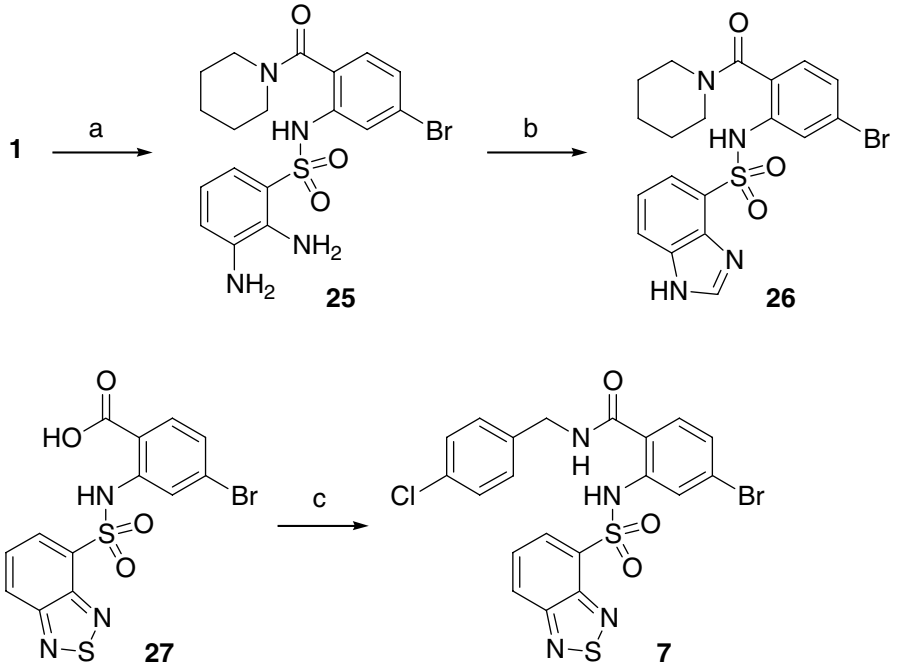


Scheme 3. Reagents and conditions: (a) $ClSO_3H$, reflux, 20 min; (b) $ClSO_3H$, reflux, 6 h.

effect may be attributed to the lowered basicity of the ring nitrogens due to the greater electronegativity of the oxygen atom, and the concomitant lowering of the ability of these atoms to participate in the putative hydrogen bonding interactions. This is supported by the calculated pK_a values of −2.29 and −3.81 for the conjugate acid of the ring nitrogen atoms of **2** and **12**, respectively. Furthermore, the greater atomic radius of sulfur leads to a larger ring size, and the resulting combination of bond angles and nitrogen lone-pair orientation may favor high receptor binding affinity. The effect of ring size is explored in greater detail in the [6,6]-fused ring analogs (vide infra).

Elimination of the central heteratom in the five-membered ring is illustrated by the benzimidazole and benzothiazole sulfonamides. The benzimidazole-4-yl compound (**26**) possessed poor CCK-2R affinity (pK_i = 5.8), which may be attributable to the contraction of ring size due to replacement of sulfur with a carbon atom having a smaller atomic radius, as well as the change in nitrogen hybridization state. In the benzothiazole series, the 4-yl linkage (**13**) allowed for a pK_i value of 7.1. This result was encouraging since the CCK-2R affinity was, for the first time, only somewhat diminished from the parent benzothiadiazole **1** ($\Delta pK_i = -0.5$). Further examination of the SAR of the benzothiazole ring showed that transposing the nitrogen and sulfur atoms (i.e., 7-yl, **14**) led to a 10-fold reduction in affinity, while the 6-yl regioisomer (**16**) displayed an additional deterioration in binding affinity of similar magnitude. This further demonstrates the importance of the proximal relationship between the ring nitrogen atom and the sulfonamide linker. The introduction of additional steric bulk in the form of a methyl group in the 2-position of the benzothiazole (**15**) led to a 10-fold decrease in receptor affinity compared to the des-methyl analog **14**.

The above results demonstrate that the sulfur atom in the benzothiadiazole and benzothiazole ring systems plays an important role in CCK-2R binding affinity. We infer from these data that the role may be primarily steric rather than electronic, since sulfur and carbon have approximately equal Pauling electronegativity values. Therefore, a key difference between high affinity compounds such as benzothiadiazole **1**, where a larger sulfur atom bridges the two nitrogens, and lower affinity analogs such as benzimidazole **26** with a smaller carbon bridge, is the size of the five-membered heterocyclic ring. To examine this, as well as positional effects of the nitrogen atoms, we assessed the isosteric ethylene group as a sulfur replacement. Toward this end, we were pleased to find that the quinoxalin-5-yl sulfonamide **17**, which is isosteric to **1**, possesses high CCK-2R binding affinity, with a pK_i value of 7.2. This was a particularly encouraging result, as **17** was the first compound with this level of receptor affinity that lacked the divalent sulfur atom. We then assessed the contribution of each individual nitrogen atom of the quinoxaline ring to the overall receptor binding affinity. Replacement of either nitrogen atom with a methylene group gave the related quinolin-



Scheme 4. Reagents and conditions: (a) Zn, AcOH, 50 °C, 20 min; (b) $CH(OEt)_3$, $TsOH{\cdot}H_2O$, 100 °C, 15 min, 44% (two steps); (c) 4-chlorobenzylamine, HATU, pyridine, DMF, 23 °C, 67%.

Table 2. Human CCK-1 and CCK-2 receptor binding affinities

Compound	CCK-1R[a] (pK_i)	CCK-2R[a] (pK_i)
1	<5	7.6
2	<5	7.4
7	<5	6.3
9	<5	6.1
10	<5	<5
11	<5	5.7
12	<5	5.8
13	<5	7.1
14	<5	6.1
15	<5	5.3
16	<5	5.5
17	<5	7.2
18	<5	<5
19	<5	6.1
20	<5	<5
21	<5	<5
26	<5	5.8
28	<5	7.5

[a] pK_i, negative logarithm of the antagonist equilibrium dissociation constant calculated from the antagonist concentration required to displace 50% of [125]I-CCK-8S binding (pIC_{50}) by the method of Cheng and Prussoff.[19] All values are ±0.3 log units unless otherwise stated.

8-yl (**18**) and quinolin-5-yl (**19**) sulfonamides, while elimination of both provided the napthyl-1-yl sulfonamide (**21**). As expected from our pharmacophore hypotheses, removal of either quinoxaline nitrogen was detrimental to CCK-2R binding affinity, and **18** and **19** had pK_i values of <5 and 6.1, respectively. Moving the nitrogen atom of **19** to the adjacent position gave the isoquinolin-5-yl (**20**), resulting in total loss of receptor affinity. Removal of both nitrogens (i.e., **21**) also led to complete abolition of binding affinity. These results clearly demonstrate that the quinoxaline nitrogen atom proximal to the sulfonamide group is advantageous for receptor binding, while a distal heteroatom (cf. **13**, **14**, **17**, **19**) is absolutely required.

Having discovered the quinoxaline ring system as a suitable replacement for the metabolically labile benzothiadiazole ring of **1**, we were free to address the piperidine amide portion of this molecule, which had also been identified as a site of oxidative degradation. We reasoned that insertion of an electron withdrawing atom into this ring should decrease the propensity for oxidation. This rationale led to morpholine analog **28** ($pK_i = 7.5$, Fig. 5), which has a markedly improved pharmacokinetic profile relative to **1** (Table 1), while achieving receptor affinity slightly higher than the related chloro and bromo analogs (data not shown). The synthesis and biological properties of this molecule have been described elsewhere.[8]

4.2. X-ray crystallographic and conformational analysis

In order to further interpret our results and elucidate the critical relationship of the heterocyclic sulfonamide moi-

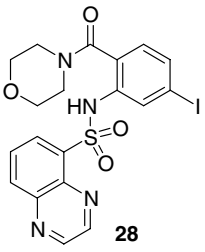


Figure 5. Quinoxaline sulfonamide **28**.

ety to binding affinity, we subjected select compounds to similar structural and conformational analyses that we applied to **1**. As a high affinity CCK-2R ligand, quinoxoline **28** was subjected to single crystal X-ray diffraction studies. For comparison, the X-ray crystal structure of quinoline **19** was obtained, which is a ligand of much lower affinity. Quinoline **19** lacks the nitrogen atom proximal to the sulfonamide group, which we believe is required to either interact directly with the receptor, or conversely to form the intramolecular interaction that enforces a preference for the postulated cup-shaped binding conformation. Examining the superimposed solid state structures of **1**, **19,** and **28** reveals conformations that are qualitatively similar to that which was observed for **1**, yet which differ somewhat in the placement of the quinoline and quinoxaline heterocycles (Fig. 6A). However, on allowing these structures to relax by energy minimization (B3LYP/6-31++G(2d,2p)), the geometries of **19** and **28** more closely align with **1** with respect to the heterocycles (Fig. 6B). A deviation is observed for **19**, where the quinoline ring deviates about the dihedral angle aNSCC by 12° relative to **1** and **28**, and has twisted such that the carbon atom analogous to the nitrogen atom in **28** points outwards relative to the conformations of **1** and **28** (Table 3). Energy minimized geometries of **19** and **28** were also obtained in which the respective heterocycles were initially rotated 180° about the carbon–sulfur bond, as was done in the case of **1** (vide supra). This alternate geometry of **28** was less favored by 1.4 kcal/mol, while, surprisingly, the alternate geometry of **19** was also less favored by 1.0 kcal/mol. Thus, it is questionable as to how much an intramolecular hydrogen bond contributes to the stabilization of the low-energy conformations observed, and a more likely explanation for the importance of the nitrogen atom proximal to the sulfonamide group is that it makes a direct interaction with the receptor that is favorable, but not required, for CCK-2R binding affinity. The SAR data clearly indicates that distal nitrogen atom is involved in an interaction that is crucial for binding.

5. Conclusion

We have described the identification of a series of potent CCK-2R antagonists based on an anthranilic sulfonamide structural motif. Metabolic instability of the benzothiadiazole and piperidine rings was found to be a major liability of this series, and parallel synthesis techniques failed to discover a suitable replacement for the benzothiadiazole ring.[10] Extensive characterization of the physical and conformational properties of sulfonamide **1** was carried out using X-ray crystallography, NMR spectroscopy, and computational methods, and the synthesis and evaluation of compounds suggested

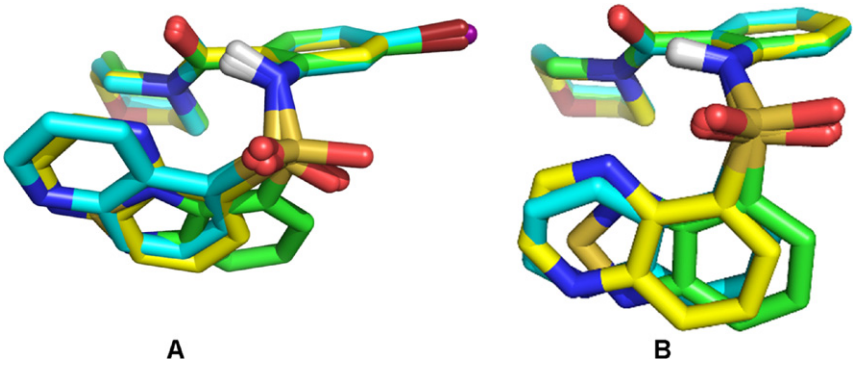
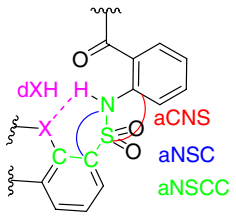


Figure 6. Superposition of **1** (green), **19** (cyan), and **28** (yellow): (A) X-ray crystal structures and (B) energy minimized structures (B3LYP/6-31++G(2d,2p), halide atoms removed to simplify calculation).

Table 3. Bond angles and distances for X-ray and minimized structures of **1**, **19**, and **28**



Compound	aCNS		aNSC		aNSCC		dXH (Å)	
	X-ray	Min	X-ray	Min	X-ray	Min	X-ray	Min
1	119.4	126.8	103.8	106.4	−72.5	−61.1	3.1	3.0
19	122.0	125.1	107.8	106.5	−73.6	−73.1	3.1	3.2
28	119.0	126.1	107.2	107.2	−64.2	−61.3	2.5	2.6

by this analysis was carried out, leading to the discovery of quinoxaline sulfonamide **28**. Structural analysis of **28**, along with related compounds **13** and **19**, was performed using X-ray crystallography similar to that used for **1**. Comparison of the conformations of these compounds revealed subtle differences in the orientation of the heterocyclic ring of the sulfonamide relative to the anthranilic amide core that can help explain the remarkable differences in CCK-2R affinity observed with relatively minor structural changes. The design and synthesis of conformationally-restricted analogs to further explore the structural and pharmacophoric requirements for high levels of CCK-2R binding, as well as further optimization toward reducing oxidative metabolism and improving in vivo efficacy, will be the subject of future disclosures from these laboratories.

6. Experimental

6.1. General

Except where indicated, materials and reagents were used as supplied. Reaction monitoring was performed with EMD Silica Gel 60 F_{254} 250 μm pre-coated TLC plates and visualized with UV light. Routine chromatographic purifications were performed via semi-automated MPLC using prepacked RediSep 35–60 μm silica gel columns on ISCO Sg100 systems with UV peak detection. Semipreparative HPLC purification was performed on a Gilson automated HPLC system running Gilson Unipoint LC software with UV peak detection at 220 nm and fitted with a reverse phase YMC-Pack ODS-A 250 × 30 mm column; mobile phase gradients consisted of mixtures of MeCN and water with 0.05% TFA and flow rates of 10–20 mL/min. Mass spectra were recorded on an Agilent single quadrupole electrospray MSD mass detection. Proton NMR spectra were recorded at either 400 or 500 MHz using Bruker DPX-400 and DPX-500 spectrometers. Melting point determinations were made using a Stanford Research Systems OptiMelt system and are uncorrected. Combustion analyses were performed by NuMega Resonance Labs, Inc., San Diego, CA. Bioanalytical quantitation was performed via mass spectrometry using either a Finnigan Quantum Ultra system (Thermo Electron Corp.), or an API-4000 system (Applied Biosystems), unless otherwise indicated. The syntheses and characterization of compounds **12**, **17**–**21**, and **28** have been previously described.[8]

6.2. 2-(Benzo[1,2,5]thiadiazole-4-sulfonylamino)-4-bromo-*N*-(4-chlorobenzyl) benzamide (7)

A solution of HATU (0.038 g, 0.10 mmol) and DMF (0.20 mL) was added to a solution of acid **27**[8] (0.021 g, 0.050 mmol), pyridine (0.012 mL, 0.15 mmol), and DMF (0.20 mL), with stirring. The mixture was maintained for 1 h, then 4-chlorobenzylzamine (0.012 mL, 0.10 mmol) was added. The reaction was allowed to proceed for 1 h, then TFA (0.050 mL) and DMF (1.0 mL) was added. The crude reaction mixture was directly subjected to preparative HPLC purification, providing **7** as a solid (0.018 mg, 67%). MS (ESI): *m/z* 536.8 $[M-H]^-$. ^1H NMR (400 MHz, $CDCl_3$, rotameric broadening): δ 11.56 (s, 1H), 8.37 (d, J = 7.0 Hz, 1H), 8.22 (d, J = 8.8 Hz, 1H), 7.90 (d, J = 1.7, 1H), 7.72 (dd, J = 8.8, 7.1 Hz, 1H), 7.34 (d, J = 8.4 Hz, 2H), 7.24 (d, partially obscured by $CHCl_3$, 2H), 7.16 (d, J = 8.4 Hz, 1H), 7.07 (dd, J = 8.4, 1.7 Hz, 1H), 6.25 (br t, 1H), 4.53 (d, J = 5.8 Hz, 2H).

6.3. General procedure for sulfonamide formation (9–11, 13–16)

The appropriate sulfonyl chloride was added to a solution of anthranilic piperidine amide **6** or **8**,[8] pyridine (ca. 1.5 equiv) and DCM (0.1 M final concentration), and the reaction mixture was maintained overnight at ambient temperature. The volatiles were removed in vacuo, then the residue was taken up in EtOAc and washed with 1 N HCl. The organic layer was dried (Na_2SO_4), filtered, concentrated, and purified as indicated to provide the titled sulfonamides.

6.3.1. 2-Methyl-2H-benzotriazole-4-sulfonic acid [5-bromo-2-(piperidine-1-carbonyl)phenyl]amide (9) and 2-methyl-2H-benzotriazole-5-sulfonic acid [5-bromo-2-(piperidine-1-carbonyl)phenyl]amide (10). The titled compounds were prepared from a crude mixture of sulfonyl chlorides **22** and **23** (0.29 g, 1.3 mmol, 4.5:1 **22**:**23**) and amine **6** (0.30 g, 1.1 mmol),[8] according to the above general procedure. The crude products were separated by silica gel chromatography (hexane/EtOAc 20:80 to 70:30). The more rapidly eluting fractions were collected to provide **10** as a solid (0.031 g, 6%). Continued elution provided **9** as a solid (0.14 g, 28%). Compound **9**: MS (ESI): *m/z* 476.0 $[M-H]^-$. ^1H NMR (400 MHz, $CDCl_3$, rotameric broadening): δ 8.62 (s, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 6.8 Hz, 1H), 7.80 (d, J = 2.0 Hz, 1H), 7.39 (dd, J = 8.8, 7.6 Hz, 1H), 7.10 (dd, J = 8.4, 2.0 Hz, 1H), 6.85 (d, 8.0 Hz, 1H), 4.55 (s, 3H), 2.70–3.45 (br s, 4H), 1.10–1.50 (br m, 6H). Anal. ($C_{19}H_{20}BrN_5O_3S{\cdot}0.4\ C_6H_{14}$) C, H, N. Compound **10**: MS (ESI): *m/z* 476.0 $[M-H]^-$. ^1H NMR (400 MHz, $CDCl_3$, rotameric broadening): δ 8.84 (s, 1H), 8.46 (s, 1H), 7.94 (d, J = 8.8 Hz, 1H), 7.89 (d, J = 2.0 Hz, 1H), 7.77 (dd, J = 8.8, 1.6 Hz, 1H), 7.19 (dd, J = 8.4, 2.0 Hz, 1H), 6.95 (d, J = 8.4 Hz, 1H), 4.56 (s, 3H), 2.52–3.52 (br m, 4H), 1.15–1.55 (br m, 6H). Anal. ($C_{19}H_{20}BrN_5O_3S$) C, H, N.

6.3.2. 1-Methyl-1H-benzotriazole-4-sulfonic acid [5-bromo-2-(piperidine-1-carbonyl) phenyl]amide (11). Compound **11** was prepared from crude sulfonyl chloride **24** (0.15 g, 0.65 mmol) and amine **6**[8] (0.12 g, 0.43 mmol), as described in the general procedure for sulfonamide formation. The crude product was purified by recrystallization (hexanes/EtOAc) to provide **11** (0.040 g, 19%). Mp = 208–209 °C. MS (ESI): *m/z* 479.8 $[M+H]^+$. ^1H NMR (400 MHz, $CDCl_3$, rotameric broadening): δ 8.96 (s, 1H), 7.96 (dd, J = 7.3, 0.8 Hz, 1H), 7.86 (d, J = 1.8 Hz, 1H), 7.75 (dd, J = 8.4, 0.8 Hz, 1H), 7.56 (dd, J = 8.4, 7.6 Hz, 1H), 7.12 (dd, J = 8.2, 1.8 Hz, 1H), 6.92 (d, J = 8.2 Hz, 1H), 4.34 (s, 3H), 3.10–3.70 (br s, 4H), 1.20–1.90 (br m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, rotomeric broadening): δ 167.1, 140.7, 136.7, 134.5, 130.0,

129.9, 126.7, 126.6, 125.1, 124.6, 124.3, 123.8, 114.9, 34.7, 25.7, 24.5. Anal. ($C_{19}H_{20}BrN_5O_3S$) C, H, N.

6.3.3. Benzothiazole-4-sulfonic acid [5-bromo-2-(piperidine-1-carbonyl)phenyl]amide (13). The titled compound was prepared from benzothiazole-4-sulfonyl chloride[15] (0.12 g, 0.53 mmol) and **6**[8] (0.075 g, 0.27 mmol), as described in the general procedure for sulfonamide formation. Silica gel chromatography (EtOAc/hexanes 5:95 to 75:25) provided **13** (0.12 g, 91%). MS (ESI): *m/z* 482.0 $[M+H]^+$. ^1H NMR (500 MHz, $CDCl_3$, rotameric broadening): δ 9.26 (s, 1H), 8.87 (s, 1H), 8.19 (dd, $J = 8.1$, 0.9 Hz, 1H), 8.14 (dd, $J = 7.6$, 0.9 Hz, 1H), 7.81 (d, $J = 1.2$ Hz, 1H), 7.56 (t, $J = 7.8$ Hz, 1H), 7.14 (dd, $J = 8.2$, 1.8 Hz, 1H), 6.92 (d, $J = 8.2$ Hz, 1H), 2.80–3.65 (br m, 4H), 1.35–1.65 (br m, 6H). Anal. ($C_{19}H_{18}BrN_3O_3S_2$) C, H, N.

6.3.4. Benzothiazole-7-sulfonic acid [5-bromo-2-(piperidine-1-carbonyl)phenyl]amide (14). Compound **14** was prepared from benzothiazole-7-sulfonyl chloride[15] (0.12 g, 0.53 mmol) and **6**[8] (0.075 g, 0.27 mmol), as described in the general procedure for sulfonamide formation. Silica gel chromatography (EtOAc/hexanes 5:95 to 75:25) provided a solid (0.13 g, 100%). MS (ESI): *m/z* 482.0 $[M+H]^+$. ^1H NMR (500 MHz, $CDCl_3$, rotameric broadening): δ 9.13 (s, 1H), 9.03 (s, 1H), 8.30 (dd, $J = 8.1$, 0.7 Hz, 1H), 7.91-7.92 (m, 2H), 7.60 (t, $J = 7.8$ Hz, 1H), 7.22 (dd, $J = 8.2$, 1.9 Hz, 1H), 6.89 (d, $J = 8.2$ Hz, 1H), 2.55–3.45 (br m, 4H), 1.10–1.55 (br m, 6H). Anal. ($C_{19}H_{18}BrN_3O_3S_2$) C, H, N.

6.3.5. 2-Methyl-benzothiazole-7-sulfonic acid [5-bromo-2-(piperidine-1-carbonyl)-phenyl]-amide (15). The titled compound was prepared from 2-methylbenzothiazole-7-sulfonyl chloride[15] (0.10 g, 0.35 mmol) and **6**[8] (0.15 g, 0.53 mmol), as described in the general procedure for sulfonamide formation. Silica gel chromatography (EtOAc/hexanes 0:100 to 75:25) provided **15** as a solid (0.13 g, 75%). MS (ESI): *m/z* 495.8 $[M+H]^+$. ^1H NMR (500 MHz, $CDCl_3$, rotameric broadening): δ 9.00 (s, 1H), 8.10 (dd, $J = 8.1$, 0.9 Hz, 1H), 7.90 (d, $J = 1.9$ Hz, 1H), 7.80 (dd, $J = 7.7$, 1.0 Hz, 1H), 7.51 (t, $J = 7.9$ Hz, 1H), 7.21 (dd, $J = 8.2$, 1.9 Hz, 1H), 6.90 (d, $J = 8.2$ Hz, 1H), 2.87 (s, 3H), 2.55–3.55 (br m, 4H), 1.05–1.60 (br m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$, rotomeric broadening): δ 170.2, 167.1, 154.9, 137.1, 133.2, 133.0, 128.7, 127.4, 126.9, 126.9, 125.9, 125.1, 124.8, 124.5, 25.7–25.9 (broad), 24.2, 9.9. Anal. ($C_{20}H_{20}BrN_3O_3S_2$) C, H, N.

6.3.6. Benzothiazole-6-sulfonic acid [5-bromo-2-(piperidine-1-carbonyl)phenyl]amide (16). Compound **16** was prepared from benzothiazole-6-sulfonyl chloride[15] (0.12 g, 0.53 mmol) and **6**[8] (0.075 g, 0.27 mmol), as described in the general procedure for sulfonamide formation. Silica gel chromatography (EtOAc/hexanes 5:95 to 75:25) afforded a solid (0.12 g, 93%). MS (ESI): *m/z* 482.0 $[M+H]^+$. ^1H NMR (500 MHz, $CDCl_3$, rotameric broadening): δ 9.20 (s, 1H), 8.87 (s, 1H), 8.51 (d, $J = 1.8$ Hz, 1H), 8.19 (d, $J = 8.6$ Hz, 1H), 7.93 (dd, $J = 8.6$, 1.8 Hz, 1H), 7.91 (d, $J = 1.9$ Hz, 1H), 7.22 (dd, $J = 8.2$, 1.9 Hz, 1H), 6.95 (d, $J = 8.2$ Hz, 1H), 2.65–3.60 (br m, 4H), 1.00–1.55 (br m, 6H). Anal. ($C_{19}H_{18}BrN_3O_3S_2$) C, H, N.

6.4. 2-Methyl-2H-benzotriazole-4-sulfonyl chloride (22) and 2-methyl-2H-benzotriazole-5-sulfonic acid (23)

A solution of 2-methylbenzotriazole (0.75 g, 5.6 mmol) and chlorosulfonic acid (10 mL) was heated to reflux for 30 min. The reaction mixture was allowed to cool to 23 °C, and was carefully poured over ice (*caution! highly exothermic!*). The resulting mixture was extracted with DCM, dried, and concentrated, providing 1.2 g of a 4.5:1 mixture of **22** and **23** as a solid (1.2 g, 93%). This mixture was used directly without purification.

6.5. 1-Methyl-1H-benzotriazole-4-sulfonyl chloride (24)

A mixture of 1-methyl-1H-benzotriazole (1.0 g, 7.5 mmol) and chlorosulfonic acid (2.5 mL) was heated to reflux for 6 h, then was allowed to cool to rt and poured over ice. The mixture was extracted with EtOAc, dried, and concentrated to a tan oil (1.05 g, 61%). This crude material was used directly without purification.

6.6. 2,3-Diaminobenzenesulfonic acid [5-bromo-2-(piperidine-1-carbonyl)-phenyl]-amide (25)

A mixture of **1**[8] (0.10 g, 0.21 mmol), zinc powder (0.15 g, 2.1 mmol), and acetic acid (2 mL) was heated to 50 °C for 20 min. The mixture was allowed to cool to rt, and was then filtered through Celite, rinsing with MeOH. The resulting solution was concentrated to a solid. This mixture of **25** and zinc acetate was used directly in the subsequent step. An analytical sample was prepared by partitioning a portion of the crude material between EtOAc and aqueous $NaHCO_3$. The organic layer was dried and concentrated, and the residue was chromatographed (5:95 MeOH/DCM). MS (ESI): *m/z* 455.0 $[M+H]^+$. ^1H NMR (500 MHz, $CDCl_3$, rotameric broadening): δ 8.60–8.90 (br s, 1H), 7.81 (d, $J = 1.9$ Hz, 1H), 7.17–7.22 (m, 2H), 6.96 (d, $J = 8.2$ Hz, 1H), 6.82 (dd, $J = 7.6$,1.3 Hz, 1H), 6.60 (t, $J = 7.9$, 1H), 4.65–4.90 (br s, 2H), 2.9–3.8 (br m, 6H), 1.4–1.65 (br m, 6H).

6.7. Benzimidazole-4-sulfonic acid [5-bromo-2-(piperidine-1-carbonyl)phenyl]amide (26)

Crude **25** (0.12 g, 0.21 mmol based on amount of **1** from previous step, vide supra), triethyl orthoformate (1.0 mL), and *p*-toluenesulfonic acid monohydrate (2.0 mg, 0.011 mmol) was heated to 100 °C for 15 min. The solution was allowed to cool to rt, and then was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, dried, and concentrated. The residue was chromatographed (MeOH/DCM 0:100 to 5:95) to provide the titled compound as a solid (0.043 g, 44% over two steps). MS (ESI): *m/z* 464.9 $[M+H]^+$. ^1H NMR (500 MHz, $CDCl_3$, rotameric broadening): δ 11.12 (br s, 1H), 9.07 (br s, 1H), 8.01 (br s, 2H), 7.92 (d, $J = 1.6$ Hz, 1H), 7.60 (d, $J = 7.5$ Hz, 1H), 7.25–7.30 (m, 2H), 6.93 (d, $J = 8.2$ Hz, 1H), 3.05–3.40 (br m, 2H), 2.40–2.75 (br m, 2H), 1.15–1.55 (br m, 6H). Anal. ($C_{19}H_{19}BrN_4O_3S$) C, H, N.

6.8. Human CCK-1 and CCK-2 radioligand binding assays

Human CCK-1 and CCK-2 receptor radioligand binding assays were performed as previously described.[16–18]

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Supplementary data

Protocols for HLM metabolite identification, tabular combustion analysis data, and X-ray crystallographic data are available online at www.sciencedirect.com. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2008.01.059.

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