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Novel thioamide derivatives as neutral CB1 receptor antagonists

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ABSTRACT

A novel class of cannabinoid-1 (CB1) receptor antagonists for the treatment of obesity is presented. The carboxamide linker in a set of 5,6-diaryl-pyrazine-2-amide derivatives was transformed into the corresponding thioamide, by using a one-pot synthesis. The structural series of thioamides not only showed retained CB1 potency (below 10 nM), but also showed improved solubility. In addition, the neutral antagonist **2c** significantly reduced body weight in cafeteria diet obese mice.

Obesity is a predisposing factor for the development of type-2 diabetes, hypertension, and cardiovascular disease.[1] Currently available anti-obesity therapeutics includes Orlistat[2] and Sibutramine.[3] Orlistat works by inhibiting gastric and pancreatic lipases, and Sibutramine is a CNS acting serotonin-norepinephrine re-uptake inhibitor. The body weight reduction Orlistat and Sibutramine offer is modest, and both drugs are associated with adverse effects.[4] Alternative treatments are thus desirable. Blocking the cannabinoid-1 (CB1) receptor presents an interesting approach for treating obesity.[5] While the clinical effectiveness of this mechanism was indeed borne out by the CB1 receptor antagonist Rimonabant, the compound was rejected before launch by the FDA in June 2007,[6] and was withdrawn from all other markets late 2008 due to an increased risk of psychiatric disorders.[7]

In our quest for novel CB1 receptor antagonists as anti-obesity agents we, and others, have previously described scaffold-hopping activities. That is, the pyrazole ring-scaffold in Rimonabant has been replaced by other scaffolds, such as pyrazine,[8] thiazole,[9] pyrrole,[10] and pyridine[11,12] rings. All these classes include ligands with high potencies for the CB1 receptor. It is thus believed that the central scaffold is mainly essential for geometrical reasons, since these fragments are electronically quite different.[8,13] In contrast, modifications made on the carboxamide linker have shown more influential effects. In an interesting study by Reggio,[14] a vinyl cyclohexyl analog (VCHSR) of Rimonabant was presented (Fig. 1). Results indicated that the analog, lacking hydrogen bonding ligand-receptor interaction possibilities in the (carboxamide) linker region, was significantly less potent at CB1. Intriguingly, the vinyl cyclohexyl analog was also reported to act as neutral antagonists at CB1, while Rimonabant acts as an inverse agonist at this receptor. Moreover, there are a number of examples where differentiation between neutral and inverse agonism has been shown to have clinical consequences.[15] This is of particular interest since CB1 neutral antagonists and inverse agonists may cause different degrees of unwanted side-effects at clinically relevant doses.[16] We hypothesized that, by modifying the hydrogen bonding strength in the linker region, potent and neutral CB1 antagonists that suppress appetite, while avoiding undesired side-effects, could be designed.

The frequently occurring carboxamide linker[8–13] was replaced by a thioamide linker, using a one-step reaction, see Figure 2. Molecular alignments showed that the carboxamide linker in the pyrazine-2-carboamide derivatives could be exchanged to a thioamide, while retaining the proposed bioactive conformation.[8] Figure 3 illustrates a shape-based overlay of compound **1b** over **2b**. The geometries of the proposed bioactive conformations are virtually identical. The ROCS program was used for the shape overlay.[17]

Thioamides are typically prepared by treating amides with reagents such as phosphorus pentasulfide. In the current work

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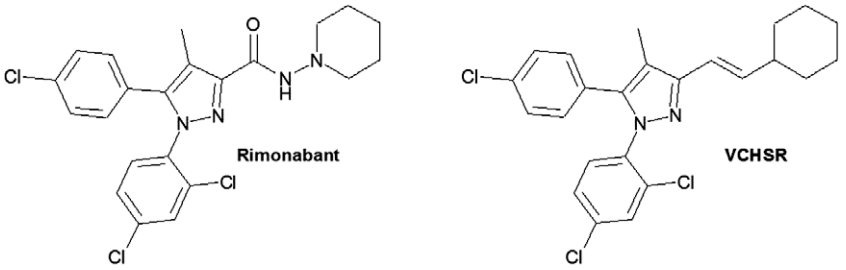


Figure 1. Structures of Rimonabant and VCHSR.

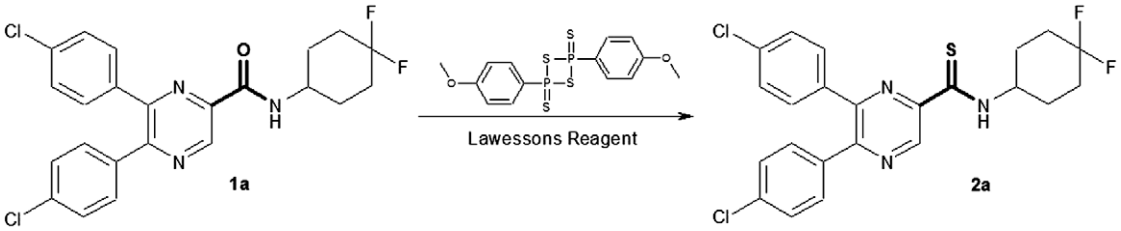


Figure 2. Structures of the carboxamide **1a** and the corresponding thioamide analog **2a**.

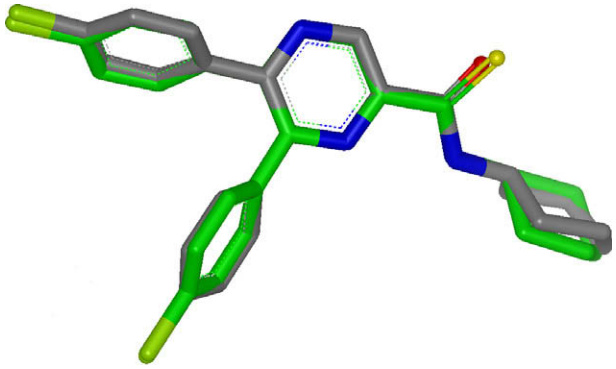


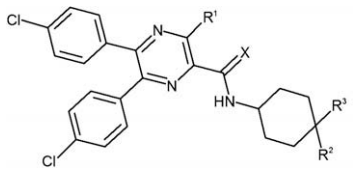
Figure 3. A molecular overlay of **1b** and **2b**.

Lawesson's reagent[18] was used to prepare compounds **2a–c** from compounds **1a–c**, see Figure 2. All compounds showed ^{1}H NMR spectra consistent with their structure and were >95% pure by HPLC.[19]

A GTPγS CB1 functional assay was used to assess the potency of the pyrazine derivatives described above.[8] Receptor inhibition potencies for compounds **1a–c** and **2a–c**, are reported in Table 1. For all carboxamide thioamide pairs (**1a** vs **2a**, **1b** vs **2b** and **1c** vs **2c**) the CB1 affinity was found to be equipotent.

CB1 receptor antagonists are in general very lipophilic compounds, with low solubilities. The compounds in Table 1 all show experimentally determined log *D* values greater than 6.5.[20] Unexpectedly, when considering the thioamide analogs of the carboxamide structures it was observed that two of the three pairs showed improved solubility relative to the parent carboxamide compound.[21] To establish if this was a general effect, a search of the AstraZeneca corporate database identified seven additional such pairs which differed only by the substitution of a thioamide for a carboxamide. In all such cases the measured solubility for these 'matched-pairs'[22] showed that the carboxamide derivative was the most soluble partner. Thus the solubilities of the pyrazine structures reported in Table 1 appear to be anomalous in their behavior with respect to substitution of carboxamide with thioamide.

Table 1
Structure and activity for compounds **1a–c** and **2a–c**



Compound	X	R^1	R^2	R^3	GTPγS IC_{50} values[a] (nM)	Solubility[b] (μM)
Rimonabant					2.1	<1.1
1a	O	H	F	F	5.3	<0.1
2a	S	H	F	F	4.5	6.6
1b	O	H	H	H	16.6	<0.1
2b	S	H	H	H	5.8	6.1
1c	O	CH_2OCH_3	F	F	2.9	<0.1
2c	S	CH_2OCH_3	F	F	2.4	<0.1

The experimental potency data are given as IC_{50} values.
[a] The experimental potency data are given as IC_{50} values, for the CB1 antagonists.
[b] The aqueous solubility data was measured in a consistent way from stored DMSO solutions.

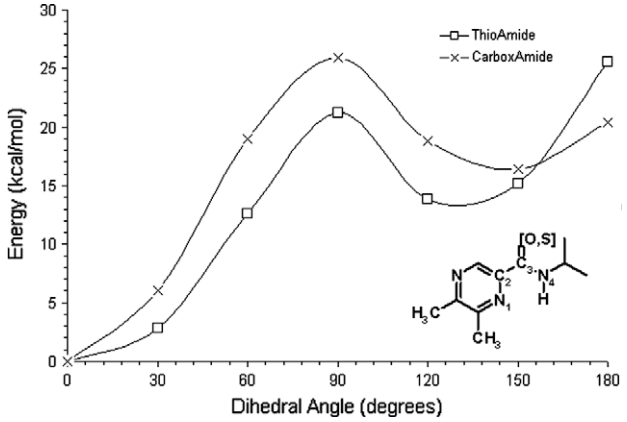


Figure 4. Two torsional scans using density functional theory (B3LYP/6-31G**). The thioamide (□) and the carboxamide (×) structures both show a strong preference for an internal hydrogen bond. That is, they both reach a geometry optimum at zero degrees (N_1–C_2–C_3–N_4 = 0).

Thioamide derivatives are analogous to carboxamides, but they exhibit greater multiple bond character along the amide (C–N) bond, resulting in a larger rotational barrier.[23] We performed detailed density functional theory calculations to investigate if the geometry preference for thioamides and carboxamides attached to 2-pyrazines differs. The relaxed coordinate scan implemented in the Jaguar program[24] was employed for this purpose, using

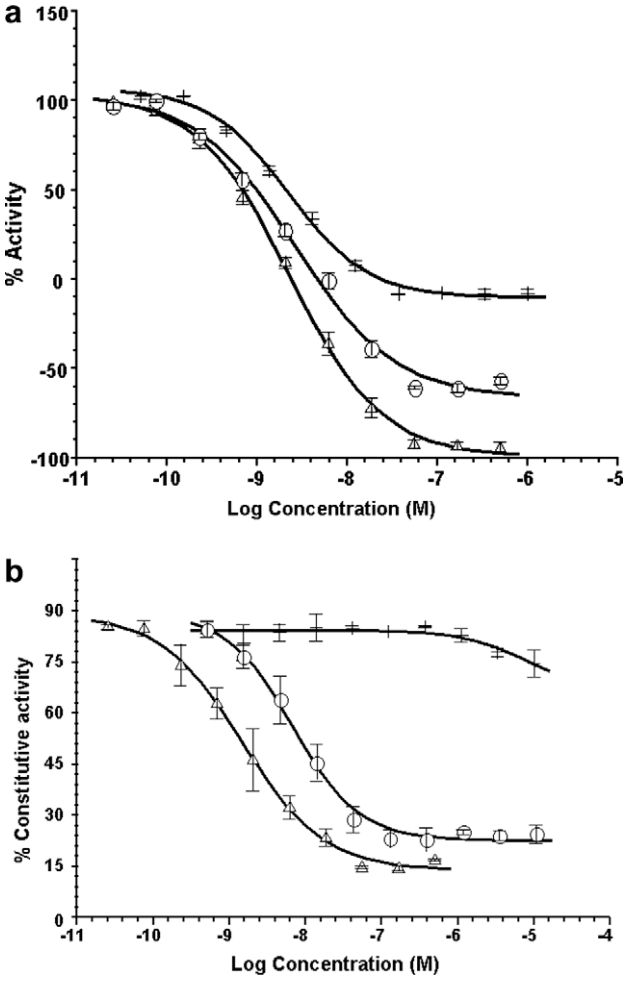
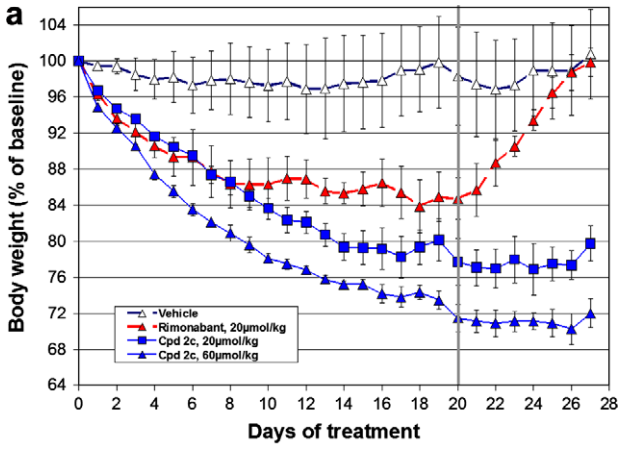


Figure 5. The antagonistic (a) and inverse agonistic (b) effects of compounds **1c** (o), **2c** (+) and Rimonabant (Δ), as measured in a GTPγS CB1 functional assay.



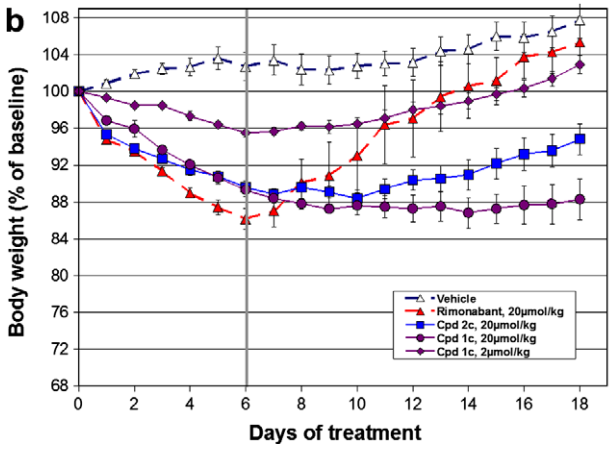


Figure 6. (a–b) The in vivo treatment effect on body weight in cafeteria diet fed mice of **1c**, **2c** and Rimonabant is shown. Vertical grey lines depicts end of treatment/start of washout phase.

B3LYP[25] and basis set 6-31G**, in PBF solvent. The energy profile for torsion N_1–C_2–C_3–N_4 is shown in Figure 4. To speed up the calculations they were performed on a reduced system.

It is clear that both pyrazine-2-carboxamides and pyrazine-2-thioamide will, without a doubt,[26] adopt planar geometries. Thus, both systems show a preferred energy minimum at zero degrees (N_1–C_2–C_3–N_4 = 0). The main reason for the steep energy profile is the stabilizing internal hydrogen bond (N_4–H–N_1). It is worth noting that the thioamide N–H is a stronger hydrogen bond donor, as compared to the carboxamide N–H, but the sulfur it is a weaker hydrogen bond acceptor than the carboxamide oxygen.[27]

A few neutral CB1 antagonists (i.e., ligands that lack the ability to attenuate CB1 receptor constitutive activity) have been reported in the literature.[16] For example, the high affinity CB1 antagonist O-2050 is devoid of agonist effects. Nevertheless, O-2050 still shows anorectic effects similar to Rimonabant.[28] Figure 5a shows the antagonistic effects of compounds **1c**, **2c** and Rimonabant, as measured using antagonism of CP-55940 activation in GTPγS CB1 functional assay whilst Figure 5b demonstrates their inverse agonist properties. The thioamide **2c** display the typical characteristics of a neutral antagonist; inhibiting agonist mediated activity whilst not effecting the intrinsic activity of the CB1 receptor, whereas its carboxamide partner **1c**, as well as Rimonabant, shows typical inverse agonist behavior.

Peroral administration of **1c**, **2c** and Rimonabant reduced body weight relative to vehicle in cafeteria diet induced obese mice.[29] The body weight after 1–3 weeks dropped to about 85% of the initial body weight for Rimonabant (20 μmol/kg; Fig. 6a–b), as compared to 85–90% for **2c** (20–60 μmol/kg; Fig. 6a–b) after 1 week

Table 2
Plasma and brain tissue levels of **1c** and **2c** in diet induced obese mice at different time points, after the last administration of **2c**[a]

Time	Compound					
	2c		**1c**		Rimonabant	
	Plasma (μmol/L)	Brain (μmol/g)	Plasma (μmol/L)	Brain (μmol/g)	Plasma (μmol/L)	Brain (μmol/g)
0.5 h	0.403	0.18	0.136	0.05	1.36	1.11
2 h	0.604	0.32	0.153	0.08	1.95	1.69
6 h	0.137	0.14	0.148	0.07	0.90	0.68
7d	0.032	0.05	0.024	ND[b]	ND[b]	ND[b]

[a] 20 μmol/kg, PO administration (see Fig. 6a).
[b] ND stands for not determined; below limit of quantification.

and approximately 75% after 3 weeks. Treatment with **1c** (2–20 μmol/kg) resulted in body weight reductions to 90–95% after 6 days (Fig. 6b). Interestingly, the body weight reduction effect remains for **1c** and **2c** after a 1–2 week washout period. This is not the case for Rimonabant (Fig. 6a–b).

While pharmacokinetic factors may contribute, available data are consistent with the suggestion that the body weight-decreasing effect of **2c** in the diet induced obese mouse model stems primarily from its neutral antagonist properties. It might be argued that bioconversion of **2c** to **1c** accounts for the observed in vivo effect. However, following repeat administration of compound **2c** (20 μmol/kg, PO; Fig. 6a), the observed plasma and brain concentrations of this agent clearly exceeds that of compound **1c** in the same samples (Table 2). Notably, no levels of **1c** were detectable in the 7 day washout samples from **2c**-treated mice despite the sustained in vivo effect on body weight at this time point (Fig. 6a). Moreover, the neutral CB1 antagonist **2c** is expected to block the effects of agonists as well as inverse agonists at CB1. Given the equipotency and the relative concentrations of **1c** versus **2c** in the biophase, the neutral antagonism of **2c** would therefore be expected to prevail over any inverse agonist effects of **1c**.

To conclude, a linker hopping approach has been exploited for the design of a novel class of CB1 antagonists for the treatment of obesity. The synthesis and CB1 antagonistic activities of a new series of 5,6-diaryl-pyrazine-2-thioamide derivatives have been described. Several compounds showed in vitro potency below 10 nM for the CB1 receptor. This approach not only showed retained CB1 activity, but also, of particular interest in the CB1 area, is the improved solubility of the thioamide derivates, as compared to the corresponding carboxamides. A significant reduction in body weight for cafeteria fed mice was observed for compound **2c**. It is hypothesized that the anorectic effects of **2c** stems primarily from its neutral antagonist properties. In future work, the identified carboxamide/thioamide pairs can be used to better understand what different mode of actions mean.

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19. In a typical experiment compound **1a** (0.15 mmol) and Lawesson's reagent (0.31 mmol) were mixed in toluene (5 mL) and allowed to react at 150 °C for 10 min in the microwave reactor (single node heating). Toluene was removed under reduced pressure and the residue was diluted with dichloromethane (30 mL) and then washed with water (2 × 10 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel eluting with 25–80% ethyl acetate in heptane. The product fraction was concentrated under reduced pressure to give **2a** in 54% yield.
20. All compounds in Table 1 were found to be outside the calibration range (i.e., log *D* >6.5) at pH 7.4. The lipophilicity (log *D*) values were determined by a HPLC LC/MS method described by Valko et al. (Valko, K.; Bevan, C.; Reynolds, D. *Anal. Chem.* **1997**, *69*, 2022).
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