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POTENT, SELECTIVE, WATER-SOLUBLE BENZODIAZEPINE-BASED CCK_B RECEPTOR ANTAGONISTS THAT CONTAIN LIPOPHILIC CARBOXYLATE SURROGATES

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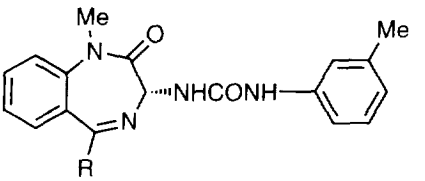
Abstract. Acylsulphonamide analogues of the meta-tolylurea L-708,474 have been synthesised and evaluated as CCK_B receptor antagonists. Such derivatives retain very high affinity and subtype selectivity for the CCK_B receptor, and have good aqueous solubility. The ortho-tolyl acylsulphonamide L-736,309 is orally bioavailable and brain penetrant in rat.

Cholecystokinin type B (CCK_B) receptors have been implicated in the pathophysiology of panic disorder and anxiety[1]. Indeed, injection of CCK tetrapeptide (CCK-4) precipitates persuasive panic-like symptoms in normal volunteers[2]. Furthermore, panic disorder patients demonstrate an increased sensitivity to CCK-4. Recent double-blind, placebo-controlled clinical trials show that the benzodiazepine-based CCK_B receptor antagonist, L-365,260, is capable of blocking the panicogenic effects of CCK-4 in panic disorder patients[3].



L-365,260; R=Ph
L-708,474; R=cyclohexyl

We recently reported "second generation" families of benzodiazepine-based CCK_B receptor antagonists that had high affinity and high subtype (ie, CCK_B/CCK_A) selectivity, and that were water-soluble[4,5]. The search for these compounds was initiated to remedy the perceived limitations of the prototypical 5-phenyl-1,4-benzodiazepine CCK_B receptor antagonist, L-365,260[6,7] - ie, low aqueous solubility, slow rate of dissolution of crystalline drug, insufficient receptor subtype selectivity. One of those families comprised a series in which the methyl group of the tolylurea fragment present in L-365,260 had been successfully replaced by simple acidic, water-solubilising groups: specifically, carboxylate, tetrazole and oxadiazolone were reported[5] The present report complements and extends those findings in the C5-cyclohexyl series. Remarkably, the acidic "eastern" chain can be considerably extended into previously unexplored regions of the CCK_B receptor, while retaining high affinity and subtype selectivity.

The knowledge[8] that replacing the C5-phenyl ring in L-365,260 by a cyclohexyl ring (L-708,474) markedly improved CCK_B receptor affinity (IC_{50}: 0.28 nM, L-708,474; 8.5 nM, L-365,260) and subtype selectivity (CCK_A/CCK_B selectivity: 6500-fold, L-708,474; 82-fold, L-365,260), made L-708,474 an ideal starting point for the discovery of water-soluble compounds. Novel eastern chains were investigated that use the N-acylsulphonamide group[9] (and its "reversed" N-sulphonylamide isomer) in a three-fold capacity as i) an acidic moiety to encourage aqueous solubility; ii) a linking group to explore the reaches of the CCK_B receptor; and iii) a molecular handle by means of which physicochemical properties could be modulated.

N-Acylsulphonamides have been widely recognised and utilised as surrogates for the (often metabolically-susceptible) carboxylic acid in several therapeutic areas[10,11]. The acylsulphonamide group generally has a pKa closely comparable to that of a carboxylic acid or tetrazole (pKa ~ 4-5), but, unlike the latter, its structural characteristics permit further molecular modification through substitution at the carbon or sulphur atom. By a judicious choice of substituents, additional regions of a receptor or enzyme surface can therefore be accessed and physicochemical properties can be controlled. In attempting to design potent and selective analogues of L-708,474 which would be brain-penetrant yet water-soluble, we investigated acylsulphonamides which had lipophilic groups flanking both ends of the ionisable function, a manoeuvre whose simplistic intent was to shield the anionic charge and thereby encourage CNS penetration. The ortho-tolyl acylsulphonamide, L-736,309, was orally bioavailable and met these criteria.

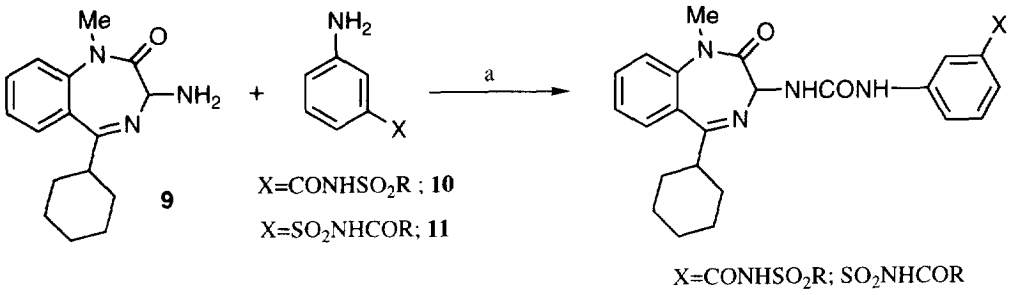
Synthesis

The acylsulphonamides (**1-8**) shown in Table 1 were prepared by reacting either the (3R)- or (3RS)-3-amino-5-cyclohexyl-1,4-benzodiazepine[8] (**9**) with the required aniline (**10** or **11**) and triphosgene (Scheme 1). The anilines (**10** and **11**) were synthesised according to Scheme 2. 3-Nitrobenzoic acid (**12**) was coupled with the sulphonamide (**13**) to produce the acylsulphonamide (**14**) in high yield. Palladium-catalysed hydrogenation of (**14**) gave the aniline (**10**). The "reversed" acylsulphonamide (**11**) was constructed in a similar manner starting from 3-nitrobenzenesulphonamide (**15**). After a carbodiimide coupling with the appropriate acid (**16**), reduction of the resultant acylsulphonamide (**17**) afforded the desired aniline (**11**).

Discussion

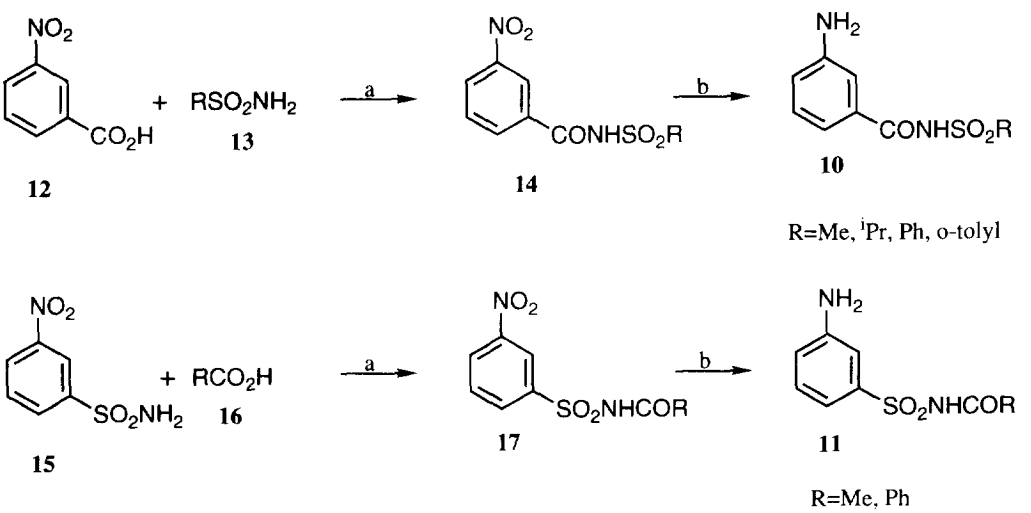
The (racemic) carboxylic acid (**18**) had expectedly[5] high affinity (IC_{50} 1.4nM) at the CCK_B receptor[12] The methyl (IC_{50} 0.58nM), isopropyl (IC_{50} 0.3nM) and phenyl (IC_{50} 0.59nM) acylsulphonamides (Table 1) derived from this acid all showed high CCK_B receptor affinity. The similar affinities of the methyl (**1**) and phenyl (**4**) acylsulphonamides suggest that the intrinsic binding potential of the phenyl ring is not being fully expressed and thus that the phenyl ring is not tightly bound at the receptor. As anticipated from the profile of L-708,474, the (R)-enantiomers were highly selective for the CCK_B receptor over the CCK_A receptor[12] (Table 1). The ortho-tolyl acylsulphonamide (**6**; L-736,309; IC_{50} 0.27nM; CCK_B/CCK_A selectivity 5900) likewise was a nanomolar- affinity ligand with excellent subtype selectivity. This pleasing combination of

Scheme 1



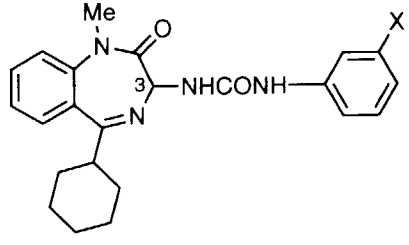
Reagents: a) Triphosgene, Et_3N, THF.

Scheme 2



Reagents: a) DMAP, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, CH_2Cl_2; b) H_2 (40psi), Pd on C, EtOH.

Table 1



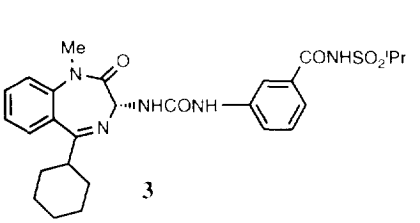
Number[a]	X	C3-Stereo.	CCK_B (IC_{50}, nM)[b]	CCK_B/CCK_A[c]	log D[d]	Sol.@pH 7.4 (mg/mL)
L-708,474	CH_3	R	0.28	6500	>4	(<10ng/mL)
18	CO_2H	RS	1.4	2	+0.89	1.05
1	$CONHSO_2CH_3$	RS	0.58	3	+0.34	1.3
2	$CONHSO_2{}^iPr$	RS	0.3	40	+0.85	0.74
3	$CONHSO_2{}^iPr$	R	0.78	1700	-	1.16
4	$CONHSO_2Ph$	RS	0.59	19	+1.42	-
5	$CONHSO_2Ph$	R	0.73	>8000	-	0.37
6 (L-736,309)	$CONHSO_2$-*o*-tolyl	R	0.27	5900	+2.0	0.41
7	$SO_2NHCOCH_3$	RS	0.36	53	+0.72	0.26
8	$SO_2NHCOPh$	RS	0.24	3100	+1.78	0.05

[a]All new compounds gave satisfactory analytical data in full agreement with the proposed structure. [b]CCK_B binding was measured by displacement of [^{125}I]-CCK from guinea pig cortical membranes as described in reference 12. [c]CCK_A binding was measured by displacement of [^{125}I]-CCK from rat pancreatic tissues as described in reference 12. [d]See reference 17.

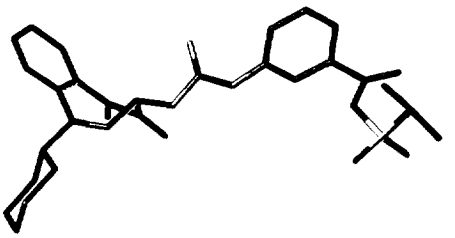
affinity and selectivity could not have been predicted at the outset and demonstrates the tolerance of the CCK_B receptor to these considerably extended acidic substituents. The aqueous solubilities of these compounds (Table 1) are dramatically improved compared to the tolylurea L-708,474. The ability to modulate lipophilicity is illustrated by comparing the log D values in Table 1. Interestingly, the isomeric ("reversed") acylsulphonamides (Table 1) also proved to have high receptor affinities, despite the fact that the geometries of these two forms of the acylsulphonamide are quite distinct. The sp_2 hybridisation of the carbonyl carbon as opposed to the sp_3 hybridisation of the sulphur atom is the factor influencing the disposition of acidic chains. This can be clearly seen from an inspection of Dreiding molecular models. The crystallographically[13] determined conformation of the iso-propyl acylsulphonamide (**3**) is illustrated in the Figure. The "reversed" acylsulphonamides were also much more selective for the CCK_B receptor than the analogous "normal" acylsulphonamides (compare **7** and **8** with **1** and **4**). In particular, the phenyl acylsulphonamide **8**, despite being a **racemic** compound, was 3100-fold subtype selective. The log D measurements reveal a physicochemical difference between the two types of acylsulphonamide, the "reversed" variants being somewhat more lipophilic than their "normal" counterparts (compare **7** and **1** in the Table).

The tolyl acylsulphonamide L-736,309 has been evaluated in some detail. L-736,309 is a potent antagonist of single-cell firing in a slice preparation of the rat ventromedial hypothalamic nucleus *in vitro* (K_b 1.2nM), a model of CCK_B receptor activation[14] but has no affinity for the (rat) benzodiazepine receptor (IC_{50} > 10uM). Following a 3mg/kg dose (po and iv) in an aqueous vehicle, L-736,309 has an oral bioavailability[15] of 14% in rat. In a separate experiment, significant levels of parent compound were detected in rat brain 1 min (222 ng/g), 10 min (120 ng/g) and 30 min (72 ng/g) after an iv dose of 3mg/kg[16]. While these levels are rather lower than those for the substantially more lipophilic molecule L-365,260 (836 ng/g, 1 min; 344 ng/g, 10 min; 91 ng/g, 30 min) under the same protocol, these data suggest that L-736,309 significantly penetrates the blood-brain barrier.

Figure 1. X-Ray Crystal Structure of the Iso-propyl Acylsulphonamide (3)



3



In summary, acylsulphonamide analogues of the meta-tolylurea L-708,474 have been designed, synthesised and evaluated as CCK_B receptor antagonists. Such derivatives retain high affinity and subtype selectivity for the CCK_B receptor, and are water soluble. The ortho-tolyl acylsulphonamide L-736,309 is orally bioavailable

and brain-penetrant in rat. This series of acidic compounds complements the cation-based amidine series reported earlier from our laboratories .

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9. Acylsulphonamides were concurrently prepared in the C5-phenyl series of Reference 5 and will be reported in due course.
10. Y. K. Yee *et al* pioneered the utility of arylsulphonamides as carboxylic acid replacements in the LTD_4 antagonist field, work which led to a clinically-effective anti-asthma drug. For leading references, see Shaw, A.; Krell, R.D. *J. Med. Chem.*, **1991**, *34*, 1235-1242.
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12. CCK_B and CCK_A binding was carried out as described by Chang, R. S. L.; Lotti, V. J. *Proc. Natl. Acad. Sci. USA*, **1986**, *83*, 4923-4926.
13. Crystal structure details: $C_{27}H_{33}N_5O_5S$, M_r = 539.659, monoclinic, *C*2, a = 30.579 (5), b = 13.909 (4), c = 7.047 (2) Å; β = 95.73 (2)°, V = 2982 (2) Å^3, Z = 4, monochromatized radiation λ(Cu K_α) = 1.541838 Å, $F(000)$ = 1144, T = 294 K. Diffractometer data; 4215 reflections measured to 72.5° in θ; 3260 observed reflections at $I \geq 3\sigma(I)$. Full-matrix least-squares refinement on F using 342 parameters. The final agreement statistics are: R = 0.087, wR = 0.102, S = 4.52, $(\Delta/\sigma)_{max}$ = 2.74 with a weighting scheme of $1/\sigma^2(F)$. The maximum peak height in final difference Fourier map is 0.59(6) eÅ^{-3} and it has no chemical significance.
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15. L-736,309 was suspended in 0.2% methylcellulose for oral dosing and dissolved in propylene glycol for intravenous dosing. Concentrations were 3mg/mL in both formulations and the doses were 3mg/kg. Extracts of plasma (separated from blood samples collected up to 8h after dosing) were analysed by reverse phase HPLC using a Spherisorb ODS2 column.
16. L-736,309 was dissolved (2mg/mL) in 87% propylene glycol and dosed (3mg/kg) to nine rats. Extracts of rat brain tissue (collected at 1, 10 and 30 min after dosing) were analysed by reverse phase HPLC using a Spherisorb ODS column.
17. Log D is the log of the ratio of concentrations in each phase obtained from partitioning between octanol and 100mM KH_2PO_4 buffer at pH 7.4.

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