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Non-basic ligands for aminergic GPCRs: The discovery and development diaryl sulfones as selective, orally bioavailable 5-HT$_{2A}$ receptor antagonists for the treatment of sleep disorders

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ABSTRACT

Scaffold hopping from a non-basic series of 5-HT$_{2A}$ receptor antagonists developed in-house that possessed reduced activity *in vivo* enabled the discovery of a novel series of diaryl sulfones that gave excellent occupancy on oral dosing. Not only does this work further demonstrate that oral bioavailability of a given series can be enhanced by improving physicochemical parameters such as log *P*, but it corroborates the growing evidence that a protonated amine is not essential for affinity at aminergic GPCRs.

G-protein Coupled Receptors (GPCRs) are membrane spanning proteins, consisting of seven transmembrane (7TM) domains, that elicit an intracellular signal transduction cascade through the activation of a G-protein. More than 30% of marketed drugs act on GPCRs, making them among the most important drug targets for the pharmaceutical industry.[1] Of these, the Rhodopsin (or Family A) class of GPCRs contain the largest number of receptors targeted by clinically used drugs[2] and include the biogenic amine GPCRs that are activated by the binding of small, basic amines such as dopamine, histamine, noradrenaline and serotonin.

For many years, aminergic GPCRs have been postulated to bind their endogenous ligands in a binding pocket within the 7TM region.[3] Mutagenesis studies have shown that amino acids in the TM5, TM6 and TM7 are involved in the binding of these ligands[4] and, in particular, the interaction between a conserved acidic amino acid (Asp 113 in the D_3 receptor: D3.32) on TM3 with the basic amine of the ligand has been shown crucial to the binding of both agonists and antagonists. The recent publication of the crystal structure of the inverse agonist carazolol bound to the β-2 adrenergic receptor has confirmed these early hypotheses.[5]

Among the aminergic GPCRs, the 5-HT$_{2A}$ receptor has enjoyed particular prominence due to its role in the efficacy of atypical antipsychotics such as risperidone and olanzapine (Fig. 1).[6] The number of structurally diverse compounds with affinity for the 5-HT$_{2A}$ receptor subtype has allowed the designation of a common pharmacophore for binding to this receptor.[7] Several groups have recognized the requirement for two aromatic rings and, in common with other aminergic GPCRs, a basic nitrogen for the binding of both 5-HT$_{2A}$ agonists and antagonists.[8] Furthermore, the optimal distance between the basic nitrogen and the two aromatic rings, as well as that between the two aromatic rings, has been defined.[9]

Contrary to established theory, however, work in these laboratories has shown that deletion of the basic nitrogen from at least one of the established series of 5-HT$_{2A}$ antagonists can result in compounds that retain high affinity for the receptor (e.g., **2** and **3**, Fig. 1).[10] This demonstration that a basic nitrogen is not crucial for binding allowed us to design structure types that have been hitherto not been considered in the development of SAR for 5-HT$_{2A}$ antagonists. Furthermore, this finding gave greater scope to avoid the off-target activities characteristic of many 5-HT$_{2A}$ receptor ligands. For instance, the pharmacophores for binding to the HERG channel, and dopamine and adrenergic receptors, have much similarity to the classical binding mode for 5-HT$_{2A}$ receptor ligands.[11]

Although we were able to identify low nanomolar compounds in the non-basic sulfonylpiperidine series, lead optimization based on compounds such as **2** and **3** did not result in achieving the

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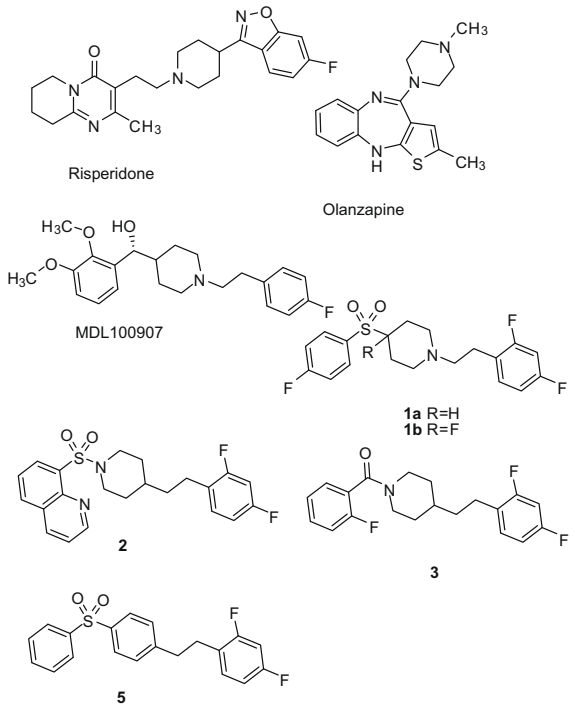


Figure 1. 5-HT_{2A} receptor antagonists.

sub-nanomolar affinity that is characteristic of bench-mark compounds such as MDL100907 (Table 1). Moreover, one of the compounds from the non-basic series, **3**, achieved only 40% occupancy of 5-HT_{2A} receptors in the CNS after oral dosing in an *in vivo* occupancy assay at 10 mg/kg. The relatively low occupancy of this compound compared to **1b** could be a function both of its lower affinity for 5-HT_{2A} and its low oral bioavailability. *In vitro* metabolism studies on **3** showed extensive oxidation of the piperidine ring, the phenethyl side chain and cleavage of the amide bond.

The microsomal instability of the piperidine core of **3** prompted us to examine potential replacements. This communication describes this work which has led to the identification of a novel series of 5-HT_{2A} ligands with subnanomolar affinity and introduces, to our knowledge for the first time, non-basic 5-HT_{2A} receptor antagonists with high oral bioavailability and *in vivo* occupancy comparable to bench-mark compounds. In keeping with other 5-HT_{2A} antagonists such as **1b** reported by these laboratories[12], a compound from this class (**11**) was also shown to enhance Slow Wave Sleep (SWS) and reduce the number of awakenings in rats. On the basis of its excellent *in vitro* and *in vivo* profile, **11** was selected as a clinical candidate for the treatment of insomnia and other sleep disorders (*Vide Infra*).

In the absence of the basic nitrogen, key features in compounds such **2** and **3** that may be considered important for activity are the H-bond acceptor (sulfone or amide) and the two aryl rings. Hence, a number of ways of linking these key binding elements were explored. One of the first groups considered as a replacement for the metabolically labile piperidine was a phenyl ring, giving a compound such as structure **5** (Fig. 1). A phenyl ring has the advantage that metabolic oxidation may be controllable by appropriate substitution. Furthermore, this modification removes the potential for one of principal routes of metabolism of **3** which involves cleavage of the piperidine amide.

The synthesis of the diarylsulfones was initially accomplished using the route shown in Schemes 1 and 2.[13]

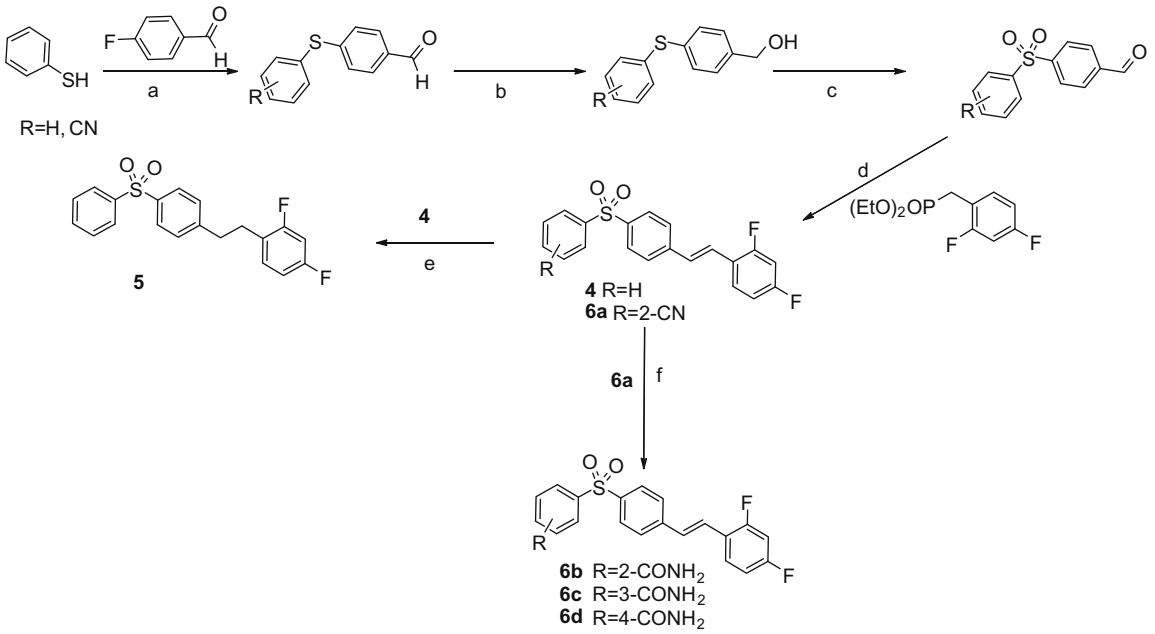
S_NAr displacement of the fluorine in 4-fluorobenzaldehyde was followed by reduction of the aldehyde to prevent further oxidation of this group to the acid during the oxidation of the sulfide to the sulfone. Hence oxidation of the sulfide, followed by oxidation of the primary alcohol to the aldehyde and Wittig reaction with the requisite phosphonate gave the trans stilbene as the major isomer. The reduced compound **5** was obtained by hydrogenation of **4** under standard conditions. The substituted compounds **6b–f** were obtained by manipulation of nitrile functionality in **6a** according to the route shown in Schemes 1 and 2.

A convergent route to these molecules was also developed by synthesizing the aryl sodium sulfinate incorporating the 4-fluorophenyl stilbene (Scheme 3). The coupling of sodium 4-bromophenyl sulfinate directly with 4-fluorophenylstyrene gave the coupled product in only 44% yield. Purification of **7**, either by recrystallisation or chromatography, was also difficult hence compromising the viability of this approach for the large scale synthesis of this intermediate. Utilising the β-keto protecting group for sulfinates developed by Baskin and Wang[14] gave **8a** which was subjected to the Heck conditions and once again gave the sulfinate **7** in moderate yield due to cleavage of the protecting group. Reasoning that the

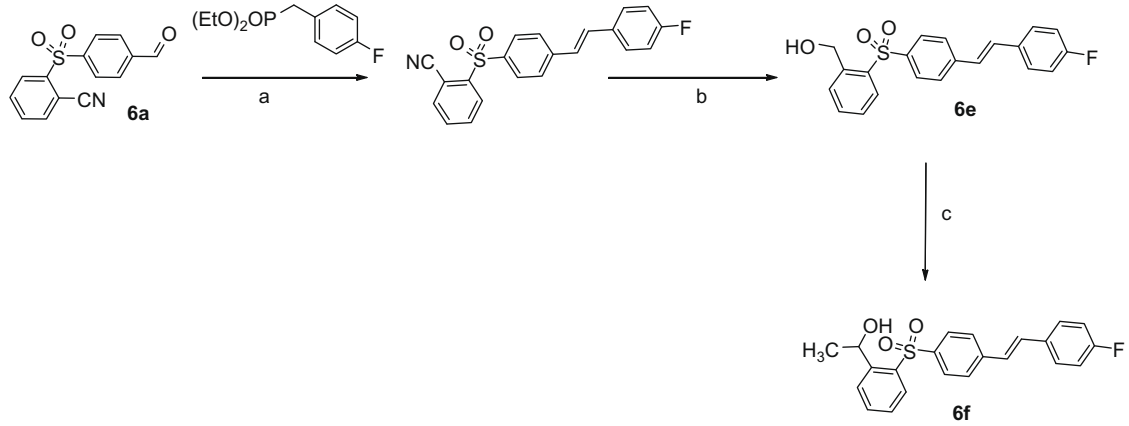
Table 1
In vitro and occupancy data

Compound	h5-HT_{2A} K_i/nM[a]	h5-HT_{2C} K_i/nM[a]	Selectivity[b]	hIK$_r$ K_i/nM[a]	% Turnover (HLM)[c]	% Occupancy (dose mg/kg)[d]
M1009013	0.31	13	42	1100	ND	ND
1a	0.42	39	92	710	ND	ND
1b	0.39	69	176	5561	ND	98 (10)
2	2.10	184	84	>9000	ND	ND
3	3.90	100	28	>9000	99	ND
4	1.32	65	49	4342	33	40 (10)
5	1.43	526	368	>9000	84	ND
6b	0.65	127	195	>9000	15.8	58 (10)
6c	0.20	21	106	>9000	3	50 (10)
6d	0.65	41	63	7919	0	>100 (10)
6f	0.07	7	100	5953	28	99 (5)
6f	0.10	13	130	4000	<10	85 (10)
11	0.22	47	213	8252	45	83 (1)

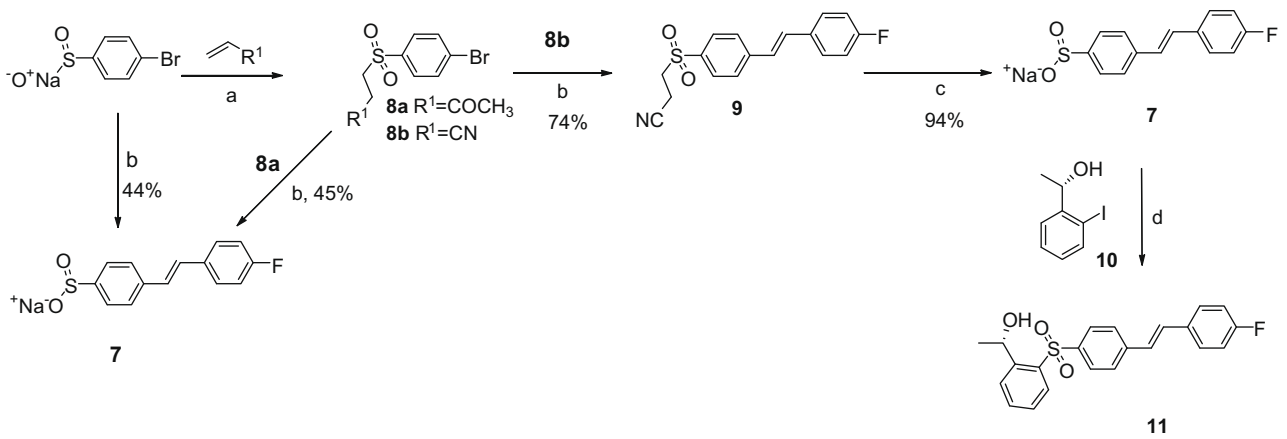
[a] h5-HT_{2A}, h5-HT_{2C} and hIK$_r$ were determined as described in Ref. [6] ($n = 2$).
[b] h5-HT_{2C}/h5-HT_{2A}.
[c] Microsomal turnover data were obtained by incubation of the test compound (and diazepam as a control compound) at 1 M concentration with liver microsomes pooled from a number of individuals, at a protein concentration of 0.5 mg/mL for 0.25 h. Concentration of test compound remaining after incubation was determined by LC/MS/MS analysis. Turnover values are the mean of three determinations, with a standard deviation of </) 5% for the values quoted.
[d] Determined after oral dosing at 0.5 h time point. See Ref. [12] for details of protocol



Scheme 1. Reagents and conditions: (a) DMSO, 120 °C; (b) $NaBH_4$, EtOH; (c) (i) AcOH, H_2O_2; (ii) ClCOCOCl, DMSO, CH_2Cl_2, −78 °C; (d) NaH, 15-Crown-5, THF, 0–25 °C; (e) H_2, 10% Pd/C, EtOH; (f) 4 N NaOH, EtOH, 80 °C; (g) (i) DIBAL-H, CH_2Cl_2, −78 °C; (ii) $NaBH_4$, EtOH; (h) TPAP, CH_2Cl_2, (ii) MeMgBr, THF.



Scheme 2. Reagents and conditions: (a) NaH, 15-Crown-5, THF, 0–25 °C; (b) (i) DIBAL-H, CH_2Cl_2, −78 °C; (ii) $NaBH_4$, EtOH; (c) TPAP, CH_2Cl_2, (ii) MeMgBr, THF.



Scheme 3. Reagents and conditions: (a) AcOH, H_2O, 100 °C, 1.5 h; (b) $Pd(OAc)_2$, NaOAc, NMP, 135 °C, 35 min; (c) NaOMe, THF, MeOH, 25 °C, 1 h; (d) CuI (1.3 equiv.), DMSO, 90 °C, 88 h.

Table 2
Comparison of dosing vehicles for **4**[a]

Dosing vehicle	Hpv AUC (μM h)	Plasma AUC (μM h)	Brain AUC (μM h)
Methocel suspension	0.46	0.09	0.38
Imwittor/Tween	6.39	0.79	1.96

[a] Concentration of compound in rat plasma obtained from hepatic portal vein (Hpv), cardiac (systemic) blood samples and brain homogenate following po administration at 5 mg/kg (four animals per group) and measured by electrospray mass spectrometry. See Ref. [6] for details.

Table 3
Comparison of occupancy, brain and plasma levels for **6b**, **6c** and **6d**

	6b	6c	6d
Dose (mg/kg)	10	10	10
Occupancy (% inhibition)[a]	100	50	53
Plasma concentration (nM)	2727	40	300
Brain concentration (nM)	5770	40	620

[a] Determined after oral dosing a methocel suspension at the 0.5 time point. See Refs. [6] and [12] for details of protocol.

Table 4
In vitro and *in vivo* pharmacokinetic properties for **6b** and **11**[a].

Compound	Species	% Turnover	$T_{1/2}$ (h)	Cl_p[b]
6b	Rat	0	28	3
	Dog	<10	134	0.3
	Rhesus	23	85	0.13
11	Rat	50	3.8	95[c]
	Dog	34	3.15	8
	Rhesus	44	2.13	32
	Human	45		

[a] Determined in 15 male Sprague–Dawley rats, 3 dosed i.v. (0.1 mg/kg) and 3 dosed p.o. (1 mg/kg). See Ref. [6] for details of protocol.
[b] mL/min/kg.
[c] V_{dss} 19L/kg.

cleavage occurred most probably due to the basicity of the reaction conditions, we decided to synthesise the nitrile analogue of **8a** since it may be more stable to the Heck conditions, the p*K*a of the protons adjacent to a nitrile being higher compared to the ketone (p*K*a CH_2CN 25 vs. CH_2COCH_3 20). Nucleophilic addition of sodium-4-bromophenylsulfinate to acrylonitrile gave the protected sulfinate **8b** which was coupled with 4-fluorophenylstyrene to give the stilbene **9** in good yield, after a single recrystallisation. The pure sulfinate **7** could be obtained by deprotection of the sulfone. The substituted sulfinates were coupled under copper catalysis with aryl iodides utilizing conditions developed by Suzuki and Hajime[15] as shown for the synthesis of **11**, the (*S*)-enantiomer of **6f**.

The first compound in this series, **5**, gave nanomolar affinity at the 5-HT_{2A} receptor with moderate selectivity over 5-HT_{2C} (Table 1). Metabolism in human liver microsomes (HLM), however, was high. The stilbene analogue **4** gave significantly improved selectivity over 5-HT_{2C} as well as reduced turnover on HLM. However, dosing **4** orally as a methocel suspension at 10 mg/kg once again gave only moderate occupancy (40%) of 5-HT_{2A} receptors. An *in vivo* absorption study with **4** dosed orally as a methocel suspension and, separately, as a solution in Imwittor/Tween revealed that significantly improved plasma levels were obtained when it was formulated as a solution (Table 2).

The removal of the basic nitrogen resulted in reduced solubility of the non-basic compounds and so consideration was given to re-incorporation of a basic group in another part of the molecule, this time not to enhance 5-HT_{2A} binding, but as a solubilising group. One disadvantage in this approach is that HERG channel affinity may be become an issue again. Hence, other approaches to improving the absorption in this series were explored. The propertyl profile of the lead **4** (M_W= 356, H-bond donor = 0, H-bond acceptor = 2, *c* log *P* = 5.1) prompted us to examine whether the high log *P* is a contributory factor to its sub-optimal *in vivo* profile. Therefore incorporation of a primary carboxamide, a polar group shown to be tolerated in the piperidine series exemplified by **1a**[15], at the 2-, 3- and 4-positions of the aryl sulfone was undertaken with the view to reduce log *P*.

All three carboxamide analogues gave subnanomolar affinity at the 5-HT_{2A} receptor, with excellent selectivity over both 5-HT_{2C} and HERG. Interestingly, there were significant differences in both plasma and brain levels for the three regioisomers when they were dosed orally in an *in vivo* occupancy assay (Table 3). Intriguingly, the receptor occupancies for **6c** and **6d** did not reflect the differences in their brain concentrations. The 2-carboxamide analogue **6b** gave significantly higher plasma and brain levels than both of its regioisomers, the high brain levels being reflected in the significantly higher occupancy of 5-HT_{2A} receptors in the CNS. There is no obvious explanation for this difference in the *in vivo* profiles of the carboxamide isomers. We speculate the proximity of the amide to the sulfone in **6b** may affect ease of solvation/desolvation of both functional groups and influence the ability of the molecule to cross membranes, including the blood–brain barrier.

Further development of **6b** was disappointingly prevented by its low microsomal turnover which was reflected in the exceptionally long half-lives in all three preclinical species (Table 4). Compound **6b** was also shown to be an activator of the nuclear pregnane X (PXR) receptor[16] which is indicative of its potential to induce CYP3A4 enzymes (PXR activation 33–158% @ 10 μM relative to the positive control Rifampicin). The effect of the 2-carboxamido group on improving the absorption in the diaryl sulfone series prompted us to examine other polar groups at this position that would also have the effect of lowering the log *P* of the original lead **6b**. We examined the primary alcohol **6e** which, like **6b**, gave excellent *in vitro* and *in vivo* profiles (Table 1). The CYP induction potential assay showed that unlike **6b**, **6e** had a negligible effect at the same concentration (0.9% @ 10 μM). In contrast, its CYP inhibition profile indicated that **6e** was a potent inhibitor of the CYP2C9 P450 isozyme (IC_{50} CYP2C9 100 nM).

Fortunately it was not necessary to make significant changes to the structure of **6e** to balance the CYP induction and inhibition profiles whilst retaining the excellent affinity and selectivity of this series towards the 5-HT_{2A} receptor. The secondary alcohol **6f** was synthesised and resolved. The more active (*S*)-enantiomer **11**[17] was shown to have subnanomolar 5-HT_{2A} affinity with selectivity over 5-HT_{2C}, HERG, dopamine and adrenergic receptors. In a MDS Pharma screen **11** was also shown to have no significant off target activities against any other enzymes, ion channels or GPCRs.

A rising dose study with **11** showed that plasma level of 60 nM (at a dose of <1 mg/kg) in rat resulted in 80% occupancy of 5-HT_{2A}

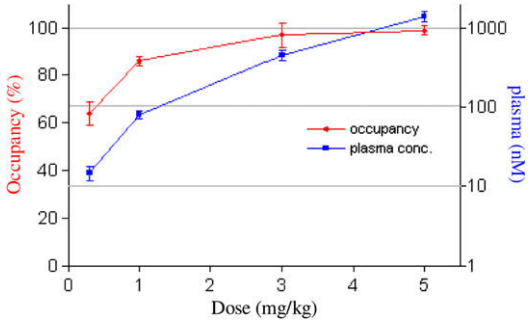


Figure 2. Oral dose–response for **11** 2h post-dose.

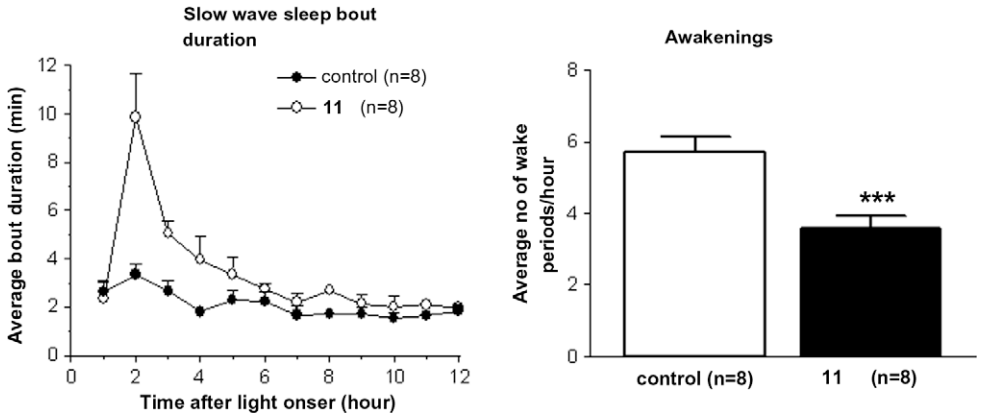


Figure 3. Telemetry studies in rat for compound **11** (3 mg/kg, p.o.). See Ref. [12] for details of protocol.

receptors in the CNS, with full occupancy being achieved at a dose of 3 mg/kg[18] (Fig. 2). Its CYP induction (8% of control @ 10 μM) and CYP inhibition (CYP2C9 6 μM, CYP1A 4.8 μM, other CYPs >30 μM) profiles were acceptable in light of the plasma levels needed for complete occupancy of 5-HT_{2A} receptors. The cardiovascular profile of **11** in dog was benign with no effect seen on QT_c or heart rate after *i.v.* infusion of up to 10 mg/kg (cumulative mean plasma level 80.9 μM). Consistent with other 5-HT_{2A} antagonists developed in these laboratories, **11** was shown to significantly increase Slow Wave Sleep (SWS) and decrease the number of awakenings in rats over a 15 h period, as measured by EEG and EMG recordings and compared to vehicle, at a dose of 3 mg/kg (Fig. 3). No effects on Rapid Eye Movement (REM) sleep or sleep onset were observed indicating **11** to be devoid of sedative side effects. Compound **11** was also shown to be bioavailable in three pre-clinical species (F% rat 44%, dog 45%, rhesus 10%) with acceptable half-lives that would enable its development as a treatment for sleep disorders in man (Table 4).[19]

This account described our work on utlising a series of piperidine sulfonamide/amide non-basic 5-HT_{2A} ligands, which did not have optimal *in vitro* and *in vivo* profiles, in order to identify a series of novel, non-basic bis-aryl sulfones with subnanomolar affinity for the 5-HT_{2A} receptor and selectivity over 5-HT_{2C}, HERG and a broad panel of other GPCRs and ion channels. Our work culminated in the identification of **11**, a compound that was demonstrated to be bioavalable in three preclinical species to give 80% receptor occupancy of central 5-HT_{2A} receptors at doses <1 mg/kg whilst giving a benign cardiovascular profile in dog up to plasma levels well above those required for a therapeutic effect. On the basis of its excellent *in vitro* and *in vivo* profile, **11**[20] was selected as a clinical candidate for the treatment of sleep disorders.

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17. The *in vitro* profile for the less active enantiomer of **6f** was 5-HT_{2A} 1 nM, 5-HT_{2C} 150 nM, selectivity for 5-HT_{2A} 150-fold.
18. A PET study showed that an Occ80 was achieved at plasma levels of 170 nM in rhesus monkey. The authors would like to thank the Imaging Group at Merck & Co., West Point, PA for these results.
19. Good dose proportional increases in plasma levels after oral dosing at 1–100 mg/kg in rats was seen although some variability was observed at the top dose.
20. Compound **11** was shown to be stable both as a solid and in solution in acetonitrile/water (pH 1–10, 1500 °C, 5 days). In a phototoxicity assay, after oral dosing of 15 to mice at 10–2000 mg/kg for 13 days showed slight erythema at the top dose on day 1 only.