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Bioorganic & Medicinal Chemistry Letters 15 (2005) 3665–3669
Bioorganic & Medicinal Chemistry Letters

4-Fluorosulfonylpiperidines: Selective 5-HT$_{2A}$ ligands for the treatment of insomnia

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Received 31 March 2005; revised 9 May 2005; accepted 26 May 2005
Available online 1 July 2005

Abstract—Incorporation of fluorine at the 4-position of an existing series of sulfonyl piperidine 5-HT$_{2A}$ antagonists gave compounds with increased selectivity over the IKr potassium channel. This work led to the identification of **3b**, a compound that gave no increase in QT$_c$ in the anesthetized dog up to plasma levels as high as 148 μM. Furthermore, **3b** has been shown to increase slow-wave sleep bout duration and to decrease the number of awakenings in rats, indicating the potential utility of 5-HT$_{2A}$ antagonists in the treatment of insomnia.

Chronic insomnia affects about 10% of the global population, with between 30% and 50% of people affected by poor sleep (of any duration or severity) at any time.[1] Insomnia is associated with significant impairments in physical and mental well-being, it can have a major negative impact on quality of life, and leads to a higher incidence of psychiatric disturbances including depression and anxiety.[2] The leading prescription drugs for the treatment of insomnia, including Ambien,® are sedative hypnotics that act at the benzodiazepine site on the GABA-A ion channel. Unresolved issues with these drugs include abuse potential[3] and the risk of fractures in elderly patients, due to an increased incidence of falls.[4] Hence, there is a need for new therapeutics that will provide improvements in quality of sleep without the adverse effects of existing treatments.

Serotonin (5-hydroxytryptamine, 5-HT) was one of the first neurotransmitters associated with the regulation of the sleep-wake cycle.[5] It is known that ritanserin, a 5-HT$_{2A}$ antagonist, increases the amount of slow-wave sleep (SWS), one of the components of deep sleep, in both humans and rats.[6] Furthermore, in clinical trials, several agents that antagonize 5-HT$_{2A}$ receptor activity, including ritanserin,[7] nefazodone,[8] and trazodone,[9] have been shown to reduce sleep disturbance in patients who suffer from a primary disorder other than sleep disturbance, for example, schizophrenia, depression, and anxiety. While these agents have potent activity at several G-protein-coupled receptors (GPCRs), preliminary (unpublished) information indicates that the selective 5-HT$_{2A}$ antagonist M100907 (Fig. 1) increases SWS

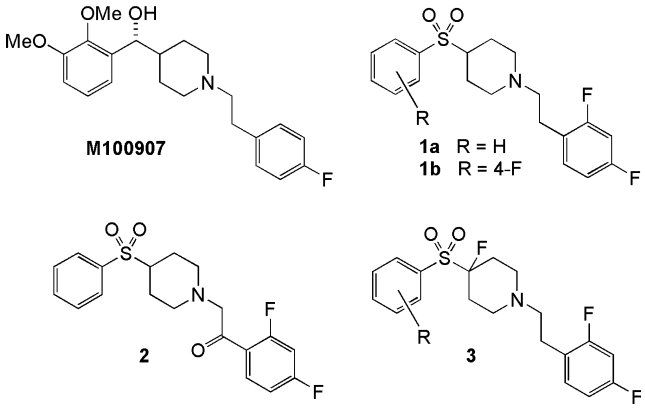

Figure 1. Selected 5-HT$_{2A}$ antagonists.

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doi:10.1016/j.bmcl.2005.05.104

and total sleep time, as well as reducing time spent awake after sleep onset in elderly subjects.[10]

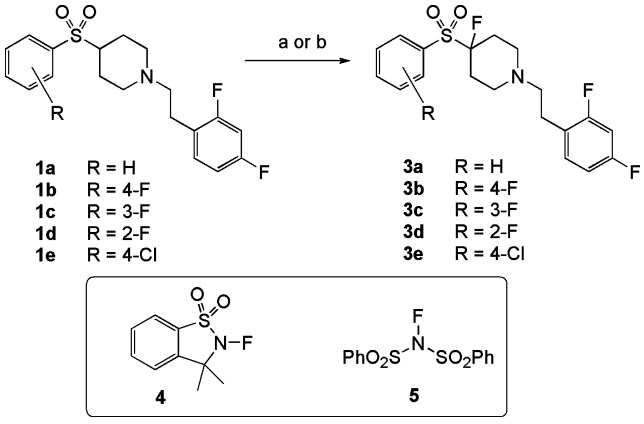
Previous work from our laboratories[11] identified a series of sulfonylpiperidines of general structure **1**, with high affinity for the 5-HT_{2A} receptor and good selectivity over other GPCR targets, including 5-HT_{2C} and D_2. However, in common with many related structures,[12] compounds of this class have moderate affinity for the hERG (IKr) potassium channel. It is well known that IKr blockade can lead to prolongation of the QT_c interval in vivo, which in turn can lead to the episodes of Torsade de Pointe that may result in death. Compound **1a** gave rise to a significant increase in QT_c interval after i.v. infusion to an anesthetized ferret.[11] However, we found that the acetophenone **2** has reduced affinity for the IKr channel and subsequently demonstrated that **2** did not significantly increase the QT_c interval in ferret.

One possible reason that acetophenone **2** has reduced affinity for the IKr channel is its lower basicity compared to the parent phenethyl compound **1a** (Table 1). However, a drawback with acetophenones such as **2** is that compounds of this class chemically degrade in solution, particularly in polar solvents such as methanol and DMSO. Hence, we sought other means of reducing the basicity of the sulfonylpiperidines. Fluorine is commonly incorporated into biologically active compounds since it gives rise to minimal steric impact, whilst its electronegativity influences pK_a.[13] Moreover, it is known that the presence of a fluorine at the 4-position of a piperidine ring can substantially reduce its pK_a.[14] We therefore set out to synthesize α-fluorosulfones, of general structure **3**, as an alternative approach to reduce the pK_a of the piperidine ring.

To gain rapid access to this class of compounds, we sought to harness the intrinsic reactivity of the alkylsulfonyl moiety and incorporate a fluorine atom by an electrophilic quench onto an alkylsulfonyl anion, thus allowing us to use available advanced fragments. Hence, deprotonation of sulfones **1a** and **1b** with NaHMDS, followed by reaction with *N*-fluoro compound **4**, successfully afforded the desired 4-fluorosulfonylpiperidines **3a** and **3b**, respectively, albeit in low yield (Scheme 1).

Some improvement was made by changing both the base, to *n*-butyllithium, and the electrophilic fluorine source, to *N*-fluorobenzenesulfonamide **5**. However, given that the yields of fluorinated products **3c–e** were still low, we switched to using the *N*-Boc intermediate **6** (Scheme 2). In this case, deprotonation with *n*-butyllithium, followed by *N*-fluorobenzenesulfonamide quench, afforded the α-fluorosulfone **7** in good yield.[15] The synthesis of the core was then completed by removal of the Boc-protecting group and alkylation using standard protocols.

To further vary the 4-aryl substituent, we again used the intrinsic reactivity of the system, this time by nucleophil-

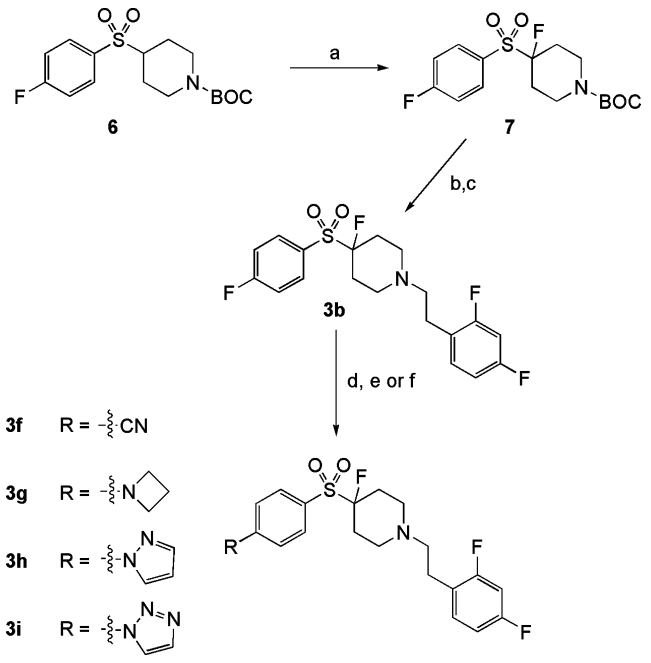


Scheme 1. Reagents and conditions: (a) **1a** or **1b**, NaHMDS, THF, −78 °C to room temperature, then −78 °C, **4**, to room temperature, 25% (**3a**), 18% (**3b**); (b) **1c–e**, ^{n}BuLi, THF, −78 °C, then **5**, to room temperature, 25–35% (**3c–e**).

Table 1. Binding affinities, in vivo occupancy data, and pK_a values

Compound	h5-HT_{2A} K_i/nM[a]	r5-HT_{2A} K_i/nM[b]	h5-HT_{2C} K_i/nM[a]	hD_2 K_i/nM[a]	hIKr K_i/nM[a]	In vivo occupancy[c]	pK_a[d]
M100907[e]	0.31	0.10	13	1300	1100		
1a[e]	0.33	0.18	25	>1500	1000		7.3
1b[e]	0.42		92	>1500	710		7.2
2[e]	2.4	0.51	130	>1500	6800		6.3
3a	0.68		49	>1000	2446		
3b	0.39	0.63	176	313	5561	98 ± 5%	6.3
3c	0.30		49	791	1525		
3d	0.32		14	266	398		
3e	0.64		398	>1000	397		
3f	2.7	1.33	1491	863	2702	80 ± 4%	6.4
3g	1.0	0.30	844	>2000	9971	49 ± 8%[f]	
3h	2.0	1.6	>4000	>2000	>9000	37 ± 8%	
3i	0.66		>4000	>2000	>9000	62 ± 7%	

[a] h5-HT_{2A}, h5-HT_{2C}, hD_2, and hIKr were determined as described in Ref. 11 (n = 2).
[b] r5-HT_{2A} data were determined as described in Ref. 16 (n = 2).
[c] Occupancy is quoted at 30 min after dosing at 10 mg/kg po as a mean ± SEM (%), measured using the protocol described in Ref. 17.
[d] pK_a values were determined using a Sirius SGA apparatus.
[e] Data from Ref. 11.
[f] At 3 mg/kg.



Scheme 2. Reagents and conditions: (a) ^{n}BuLi, THF, −78 °C, then **5**, to room temperature, 76%; (b) 11 N HCl (aq), EtOH, 80 °C, >95%; (c) 2,4-difluorophenylethyl bromide, K_2CO_3, MeCN, 85 °C, 84%; (d) NaCN, DMSO, 80 °C, 72%; (e) azetidine, DMSO, 150 °C, 10 min (Smith® Personal Synthesiser), >95%; (f) pyrazole or 1,2,3-triazole, Cs_2CO_3, DMSO, 150 °C, 10 min (Smith® Personal Synthesiser), 70–80%.

ic displacement of the fluorine atom of the 4-fluorophenylsulfone moiety (Scheme 2). Hence, **3b** was reacted with NaCN, azoles (as Cs salts), or cyclic amines, in DMSO, either thermally or by fixed temperature microwave irradiation, to give the desired products **3f–i** in high yield.

These novel compounds were screened for activity at the 5-HT_{2A} receptor, together with related GPCRs (5-HT_{2C} and D_2) and the hIKr channel (Table 1). Initial results with **3a** were disappointing in that there is little difference in ion channel activity compared to **1a**. However, it became clear that IKr channel affinity is more sensitive to incorporation of the fluorine atom on the piperidine ring in instances when there is also substitution on the phenylsulfone moiety. Thus, the 4-fluorophenyl compound **3b** shows significantly less IKr affinity compared with its parent, **1b**. Comparison of **3b** with the 3- and 2-fluoro-substituted compounds (**3c**,**d**) demonstrates that there is also a degree of positional dependence in activity at the IKr channel. Finally, the significance of the nature of the 4-substituent is further illustrated by compounds **3e–i**, with chlorine (**3e**) leading to increased IKr channel affinity, whereas reduced affinity was obtained with the azetidine (**3g**) and polar heterocycles, such as pyrazole (**3h**) and triazole (**3i**).

To prioritize the compounds for further profiling, in vivo CNS activity of the selective compounds, **3b** and **3f–i**, was measured using a competitive 5-HT_{2A} receptor occupancy assay, after oral dosing in rat (Table 1).[17] On the basis of superior pharmacodynamic activity, **3b** was selected for further evaluation.

Compound **3b** has good bioavailability (F 51%) in rat and, despite having a high i.v. clearance (Cl_p 69 mL/min, V_d 11 L/kg), the compound has a moderate half-life ($t_{1/2}$ 3.8 h) in this species.[18]

We assessed the cardiovascular impact of **3b** using rising dose studies in anesthetized dogs ($n = 3$; Fig. 2A).[19] Hence, at cumulative doses of 3, 10, and 30 mg/kg (each dose infused i.v. over 30 min), there was no observed QT_c prolongation or significant change in blood pressure and heart rate. The maximum plasma levels reached in this study were 148 μM. In contrast, M100907 gave significant changes in QT_c interval at plasma levels as low as 0.49 μM in dogs ($n = 2$) following intravenous infusion at increasing dose levels (1, 3, and 10 mg/kg; Fig. 2B). The completely benign profile of **3b** in the dog cardiovascular study is intriguing, considering that the difference in IKr binding between **3b** and M100907 is only ca. fivefold. Clearly, factors such as plasma pro-

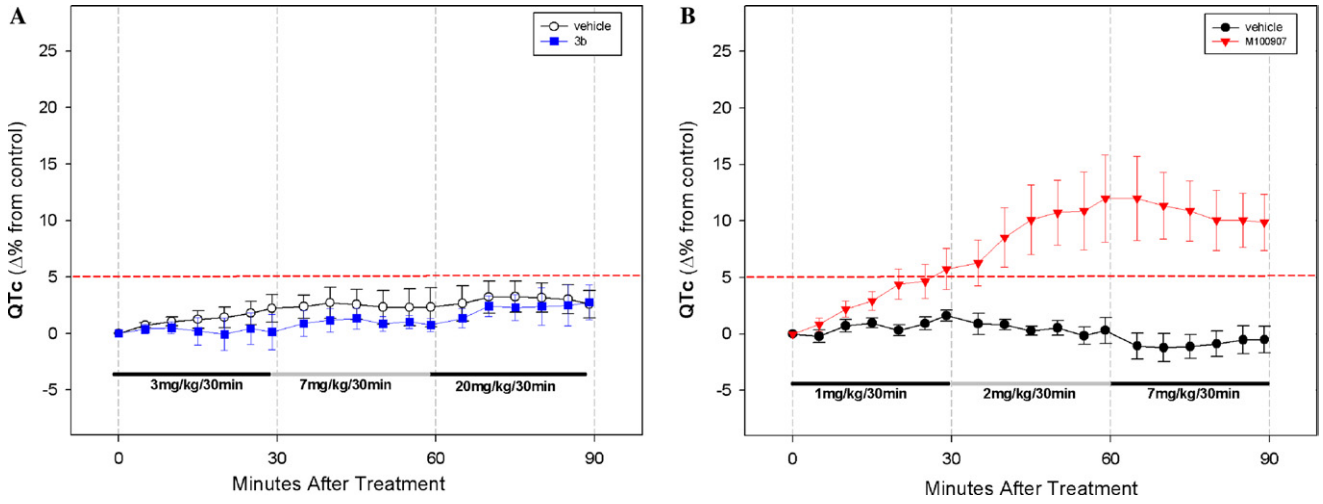


Figure 2. Cardiovascular effects of **3b** (A) and M100907 (B) in anesthetized dogs.

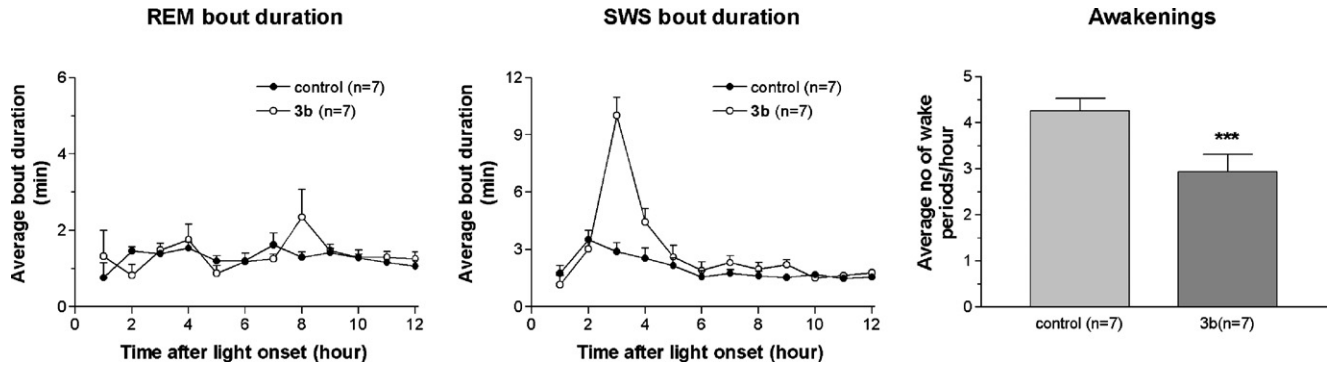


Figure 3. Effect of **3b** on sleep patterns in rat.

tein binding and the functional IKr activities of these two compounds may have further influenced the outcome in this assay.

To assess the effects of **3b** on the sleep patterns in rats, we developed an EEG telemetry assay in this species.[20] Hence, rats were dosed orally with either **3b** at 10 mg/kg or vehicle (0.5% methyl cellulose) during the first 15 min of the light phase in a cross-over design. EEG and EMG recordings were then collected for the whole of the light phase (12 h) and at every 12 s, the data were scored as either awake, SWS, or rapid eye movement (REM) sleep. This assay demonstrated that **3b** significantly decreases the number of awakenings (Fig. 3) and increases SWS bout duration. In contrast, **3b** showed no significant effect on REM bout duration.

In conclusion, we have described our work on a novel series of high affinity, selective 4-fluorosulfonylpiperidine 5-HT_{2A} antagonists and examined the effect of fluorine incorporation on affinity for the IKr channel. We found that modulating the pK_a of the piperidine nitrogen alone does not significantly affect IKr affinity in vitro. However, incorporation of the fluorine on the piperidine ring together with substitution at the 4-position of the phenyl ring, as in **3b**, does significantly reduce this ion channel activity. In contrast to the flagship compound in this class, M100907, **3b** shows no cardiovascular effects in dogs. Furthermore, **3b** was assessed in rat sleep studies and shown to significantly increase SWS bout duration and decrease the number of awakenings, thus supporting the use of 5-HT_{2A} antagonists as a potential treatment for insomnia.

Acknowledgments

We thank Paul Scott-Stevens for in vivo pharmacokinetic work, Richard Woltmann and Kevin Fitzgerald for carrying out the dog CV studies, and Steve Thomas and Helen Atherton for carrying out pK_a determinations.

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15. In a typical example, *n*-butyllithium (1.6 M in isohexanes; 13 mL, 20.8 mmol) was added dropwise to a stirred solution of *N*-BOC 4-(4-fluorophenylsulfonyl)piperidine (6 g, 17.4 mmol) in THF (70 mL) at −78 °C. After 1 h, a solution of *N*-fluorobis(phenylsulfonyl)amine (6.04 g, 19 mmol) in THF (17 mL) was added dropwise and the mixture was brought to ambient temperature, stirred for 1 h, quenched by the addition of water (1 mL), and then partitioned between saturated aqueous NH_4Cl (80; mL) and EtOAc (80 mL). The organic fraction was washed with brine (30 mL), dried over Na_2SO_4, filtered, and

concentrated in vacuo. Purification by column chromatography (silica, 20% EtOAc/isohexane) afforded *N*-BOC 4-fluoro-4-(4-fluorophenylsulfonyl)piperidine as an off-white solid (4.8 g, 76%): δ_H (360 MHz, $CDCl_3$) 1.46 (9H, s), 1.80–1.95 (2H, m), 2.05–2.30 (2H, m), 2.90–3.00 (2H, m), 4.12–4.22 (2H, m), 7.28 (2H, t, $J = 8.5$ Hz), 7.93–7.96 (2H, m). *m*/*z* (ES^+) 261 $[(M\text{-}BOC)H]^+$.

16. r5-HT_{2A} affinities were measured by the same protocol as described for h5-HT_{2A} receptor binding in Ref. 11, except HEK cells stably expressing rat 5-HT_{2A} receptor were used.
17. The assay was carried out as follows: drug compound (10 mg/kg; 1 mg/mL in 0.5% methylcellulose), vehicle, or **2** (to determine non-specific binding, 10 mg/kg; 1 mg/mL in 0.5% methylcellulose) was dosed to male CD1 rats at a volume of 1 mL/kg, p.o. After 5 min (for test compounds/vehicle) or 1.5 h (for **2**), 6 μCi [^{3}H] radioligand was administered by tail vein injection. After a further 30 min, rats were culled by stun and decapitation and the brain was removed. The frontal cortex was dissected, weighed, and homogenized in 50 volumes of ice-cold buffer (50 mM Tris, pH 7.4, HCl) using a Teflon homogenizer (10–15 strokes, 1000 rpm). Aliquots of homogenate (1 mL) were filtered over Whatmann GF/B filters, which were presoaked in 0.1% PEI. The filters were washed with 10 mL ice-cold buffer and filters placed in vials. Scintillation fluid was added and radioactivity was counted using a beta liquid scintillation counter. Occupancy at the 5-HT_{2A} receptor was calculated using the following equation: Total binding (TB) = Vehicle counts − Non-specific binding counts; Compound binding (CB) = Compound counts − Non-specific binding counts; Occupancy (%) = 100 − [100 × (CB/TB)].
18. Dosed 1 mg/kg i.v. to rat as a 1 mg/mL solution in 3:1 PEG300:water ($n = 1$). Dosed 5 mg/kg p.o. to rat ($n = 1$) as a 1 mg/mL suspension in 0.5% methylcellulose.
19. Conditioned, male or female mixed-breed or beagle dogs (5–14 kg) were anesthetized with sodium pentobarbital (35 mg/kg i.v.) following approximately 18 h of food deprivation. Anesthesia was maintained during the study with a constant infusion of sodium pentobarbital (6 mg/kg/10 mL/h). Normal body temperature (~37 °C) was maintained with circulating-water warming pad and infrared lamp monitored through a controller unit with an esophageal thermistor. Each dog had an endotracheal tube inserted and was ventilated with ambient room using a large animal ventilator (tidal volume, ~140–160 mL; breaths/min, ~15–19; %inspiration/expiration ratio, 40:60). Animals were vagotomized bilaterally, then the femoral arteries and veins isolated through incisions in the right and left inguinal regions. Polyethylene catheters (ID ~1.67 mm, prefilled with heparinised saline; 20 U/mL) were implanted into both the right and the left femoral vein and one femoral artery. One venous catheter was used for drug administration and the contralateral for blood sampling. Arterial blood pressure was recorded from the catheterized femoral artery with a disposable-type pressure transducer. Heart rate was derived from pulsatile arterial pressure or the electrocardiogram (ECG). ECG activity from Leads II and V was captured simultaneously from subcutaneous limb and precordial needle electrodes, respectively. After surgery, the animals were allowed to stabilize for a minimum of 45 min prior to collecting pretreatment control data. A computerized data acquisition system (CARecorder; DISS, Inc.) was used to collect and analyze raw input signals. Mean arterial pressure (mmHg), heart rate (beats/min), and cardiac intervals (QT, QTc, PR, and QRS; s) were recorded continuously throughout the study and averaged at 1 min intervals. Baseline values (pretreatment) were taken at time 0 min (control) and compared to values measured at 5 min intervals during each 30 min i.v. infusion of vehicle or test agent. Bazett's formula [$QT_C = QT/\sqrt{(R - R)}$] was used to correct the QT interval. Vehicle or test agent were infused in a rising dose paradigm and each dose was delivered in a volume of 10 mL over 30 min using a programmable syringe infusion pump. Cumulative doses of 1, 3, and 10 mg/kg of each agent were obtained by infusing doses of 1, 2, and 7 mg/kg i.v., respectively, and cumulative doses of 3, 10, and 30 mg/kg by infusing doses of 3, 7, and 20 mg/kg, respectively. Whole blood samples (2 mL) were taken at the 10, 20, and 29 min time points of each infusion for determination of plasma drug levels.
20. Animals were dosed with either drug or vehicle at light onset in a cross-over design. Recording of EEG and EMG signals began immediately after dosing, and EEG and EMG data were continuously sampled with Data Sciences International hardware and software. The vigilance states were automatically scored with software developed in house. The automatic analysis is based on the changes of EEG and EMG activity that defines the different vigilance states in rat—WAKE is characterized by desynchronized, low-amplitude EEG activity with increased EMG activity; NREM displays synchronized, high-amplitude EEG activity accompanied by low-muscle activity; REM shows desynchronized low-amplitude EEG activity with low or absent EMG activity (Timo-Iaria, C.; Negrao, N.; Schmidek, W. R.; Hoshino, K.; Lobato de Menezes, C. E.; Leme da Rocha, T. *Physiol. Behav.*, **1970**, *5*, 1057–1061). After rejection of epochs with artifacts, every 12 s epoch was assigned to one of the three vigilance states: WAKE, NREM, or REM.