Bioorganic & Medicinal Chemistry Letters 21 (2011) 5562–5567

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery process and pharmacological characterization of a novel dual orexin 1 and orexin 2 receptor antagonist useful for treatment of sleep disorders

Romano Di Fabio [a,*], Annalisa Pellacani [a], Stefania Faedo [a], Adelheid Roth [a], Laura Piccoli [a], Philip Gerrard [a], Rod A. Porter [b], Christopher N. Johnson [b], Kevin Thewlis [b], Daniele Donati [a], Luigi Stasi [a], Simone Spada [a], Geoffrey Stemp [b], David Nash [b], Clive Branch [b], Leanda Kindon [c], Mario Massagrande [a], Alessandro Poffe [a], Simone Braggio [a], Elisabetta Chiarparin [a], Carla Marchioro [a], Emiliangelo Ratti [a], Mauro Corsi [a]

[a] GlaxoSmithKline Medicine Research Centre, Via A. Fleming, 4, 37135 Verona, Italy
[b] GlaxoSmithKline New Frontiers Science Park, Third Avenue, Harlow, Essex CM19 5AW, UK
[c] GlaxoSmithKline Medicine Research Centre, Particles Generation Control and Engineering, Product Development, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY, UK

ARTICLE INFO

Article history:
Received 20 April 2011
Revised 15 June 2011
Accepted 18 June 2011
Available online 30 June 2011

Keywords:
Orexin receptor antagonist
G-Protein coupled receptor (GPCR)
Central nervous system (CNS)
Sleep disorders
Hypnotic agent
Conformational analysis
Structure–activity relationship (SAR)

ABSTRACT

The hypothalamic peptides orexin-A and orexin-B are potent agonists of two G-protein coupled receptors, namely the OX_1 and the OX_2 receptor. These receptors are widely distributed, though differentially, in the rat brain. In particular, the OX_1 receptor is highly expressed throughout the hypothalamus, whilst the OX_2 receptor is mainly located in the ventral posterior nucleus. A large body of compelling evidence, both pre-clinical and clinical, suggests that the orexin system is profoundly implicated in sleep disorders. In particular, modulation of the orexin receptors activation by appropriate antagonists was proven to be an efficacious strategy for the treatment of insomnia in man. A novel, drug-like bis-amido piperidine derivative was identified as potent dual OX_1 and OX_2 receptor antagonists, highly effective in a pre-clinical model of sleep.

The neuropeptides orexin-A (OX-A) and orexin-B (OX-B), also called hypocretin 1 and hypocretin 2, respectively, were originally isolated in the rat hypothalamus and derive from the proteolytic cleavage of prepro-orexin,[1] a common 133 aminoacids (AA) polypeptide precursor. Orexins were originally identified as natural ligands of the former orphan G-protein coupled receptor (GPCR) HFGAN72, now known as the orexin-1 receptor (OX_1).[1,2] Neuroanatomical studies revealed that orexin containing neurons originate exclusively in the lateral and posterior hypothalamus[1–3] projecting mainly in the brain stem nuclei (e.g., locus coeruleus and raphe nuclei), the hypothalamic nuclei (e.g., the arcuate nucleus and the tuberomammillary nucleus)[3–5] and throughout the cerebral cortex, hence suggesting a major role in the regulation of sleep and arousal states. The projection of orexin neurons throughout the entire neuroaxis, with the exception of cerebellum, is paralleled by the distribution of the orexin receptors.[6–8] In particular, the action of OX-A and OX-B is mediated via two class A GPCRs, namely OX_1 and OX_2 receptors, which are 64% homologous and, like the peptides themselves, are highly conserved across mammalian species. OX-A binds similarly to OX_1 and OX_2 receptors, whereas OX-B recognises preferentially the OX_2 receptor.

In addition, these receptors have been associated with different signal transduction pathways, which may account for differential biological functions.[3] A great deal of evidence suggests that the orexin system is critical in the regulation of arousal and sleep. In particular, it has been observed that: (a) intracerebroventricular (icv) injection of OX-A in the rat caused increases in locomotor activity, rearing and grooming behavior, findings which were supported by the evidence that OX-A was able to increase locus coeruleus (LC) firing;[9,10] (b) electroencephalogram (EEG) and electromiogram (EMG) recordings in the rat reveal that icv OX-A administered at the beginning of the normal sleep period increased time spent awake and decreased both rapid eye movement (REM) sleep and slow wave (deep) sleep;[11] (c) mice devoid of the orexin precursor prepro-orexin and the OX_1/OX_2 dual KO mouse exhibit a behavioral phenotype similar to that of narcolepsy;[12] (d) an OX_2 receptor mutation, producing a non-functional receptor, was associated with narcolepsy in dog;[13] in addition, narcolepsy in man was associated with reduced orexin levels in the cerebrospinal fluid (CSF);[14] (f) the dual OX_1 and OX_2 receptor antagonist almorexant[15,16] as well as, more recently, MK-4305,[17] another dual

* Corresponding author. Tel.: +39 045 8218879; fax: +39 045 8218196.
E-mail address: romano.difabio@aptuit.com (R. Di Fabio).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.06.086

OX_1 and OX_2 receptor antagonist, have shown clinical efficacy in primary insomnia studies. All these findings, confirm that a dual orexin receptor antagonist has the potential to treat sleep disorders; in addition, exploiting a different biochemical pathway than both benzodiazepins and non-benzodiazepine hypnotic agents, the orexin antagonists were expected to be devoid of typical side effects associated to zolpidem, the current gold standard drug for insomnia. As part of a wide drug discovery program aimed at identifying drug-like dual OX_1 and OX_2 receptor antagonists, an HTS was performed in house in a chinese hamster ovary (CHO) cell line transfected with the recombinant human OX_1 receptor, using a FLIPR calcium based functional assay protocol. Selectivity screening was performed against CHO cell line transfected with the recombinant human OX_2 receptor. From this screening activity the series of compounds of type A, shown in Figure 1, were identified and selected for further investigation based upon the encouraging observed target activity.

In particular, racemic compounds **2** and **3**, shown in Table 1, exhibited signs of in vitro potency at both the OX_1 and OX_2 receptors (compound **2**, pK_i = 6.9 and 6.3, compound **3**, pK_i = 6.4 and 6.2 at the OX_1 and OX_2 receptor, respectively). Hence, the general investigative program highlighted in Table 1, was initiated to elaborate the key structural features for maximizing the in vitro activity at both receptors and optimize pharmacokinetic properties, namely the investigation of the SAR of the disubstituted amide bottom region and the role of the substitution of the right-hand side (RHS) part of the chemical series selected.

As shown in Table 1, an initial key observation was the positive effect of the presence of an additional aromatic moiety in the bottom region of the molecule, that is, introducing a 2-biaryl or a 1-naphyl moiety, and concomitant substitution at the *para* position by a fluorine atom of the RHS phenyl ring. In particular, compound **5** showed good target activity at both receptors (pK_i = 8.3 and 7.7 at the OX_1 and OX_2 receptor, respectively), while **6** featured good OX_1 activity and was moderately selective over OX_2 (pK_i = 8.2 and 6.9 at the OX_1 and OX_2 receptor, respectively). From an additional iteration focused on the exploration of the effect of substituents of the RHS phenyl ring, the 2,4-difluoro substituted derivative **7** was identified as the most potent dual orexin receptor antagonist belonging to this sub-series (pK_i = 9.8 and 9.1 at the OX_1 and OX_2 receptor, respectively).[18] However, this class of compounds was characterized by high in vitro clearance (Cl_i) in both human and rat liver microsomes; in particular, compound **5** exhibited a Cl_i >50 ml/min/g of total liver. The high metabolic instability was hypothesized to be due either to the high lipophilicity and/or the presence of the ether functionality in the RHS, potentially responsible for an easy oxidation at the α position to the oxygen atom. Later on, in vitro metabolite identification studies confirmed the metabolic liability of the aryl ether moiety. Hence, to stabilize this class of molecules, while maintaining high in vitro activity at both the OX_1 and OX_2 receptors, attention was drawn on modifying the ether linkage. Among the functional groups investigated, the secondary amide **8** (Table 1, pK_i = 8.1 and 7.7 at the OX_1 and OX_2 receptor, respectively) showed very encouraging in vitro potency.

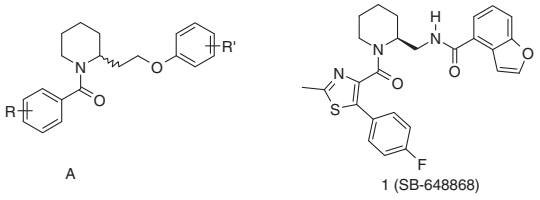
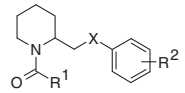


Figure 1. Chemical series A identified by HTS and chemical structure of compound **1** (SB-649868).

Table 1
In vitro functional activity (pK_i) at *h*-OX_1 and *h*-OX_2 receptors



Entry	R^1	R^2	X	OX_1 pK_i[a]	OX_2 pK_i[a]	*n*
1[b]	—	—	—	9.5	9.4	20
2[b]	3-OCH_3-C_6H_4	3,4-Cl	CH_2O	6.9	6.3	4
3[b]	3-CN-C_6H_4	3,4-Cl	CH_2O	6.4	6.2	4
4[b]	3,4-Cl-C_6H_3	3,4-Cl	CH_2O	5.6	<5.5	4
5[b]	2-C_6H_5-C_6H_4	4-F	CH_2O	8.3	7.7	4
6[c]	1-$C_{10}H_7$	4-F	CH_2O	8.2	6.9	4
7[c]	2-C_6H_5-C_6H_4	2,4-F	CH_2O	9.8	9.1	4
8[c]	2-C_6H_5-C_6H_4	H	NHCO	8.1	7.7	4
9a[c]	2-C_6H_5-C_6H_4	4-F	NHCO	9.9	9.6	8
9b[d]	2-C_6H_5-C_6H_4	4-F	NHCO	7.4	7.5	8
10[c]	2-C_6H_5-C_6H_4	4-F	N(CH_3)CO	6.3	5.9	4
11[c]	2-C_6H_5-C_6H_4	H	CONH	7.3	7.5	4
12[c]	2-C_6H_5-C_6H_4	H	CH_2CO	8.0	7.7	4
13[c]	2-C_6H_5-C_6H_4	H	NHCONH	9.0	8.3	6

[a] pK_i values are the mean of *n* experiments each performed in duplicate.
[b] Racemate.
[c] (*S*)-Enantiomer.
[d] (*R*)-Enantiomer.

The introduction of a fluorine atom in the *para* position of the RHS aromatic ring further increased the in vitro affinity (compound **9a**, Table 1, pK_i = 9.9 and 9.6 at the OX_1 and OX_2 receptor, respectively) along with a partial reduction of the intrinsic clearance (Cl_i = 19 ml/min/g and 23 ml/min/g in rat and human liver microsomes, respectively). Notably, the corresponding (*R*)-enantiomer **9b** was significantly less active (pK_i = 7.4 and 7.5 at the OX_1 and OX_2 receptor, respectively). A substantial drop of in vitro affinity was observed for the *N*-methyl derivative **10**, the reverse amide **11** and the ketone derivative **12**, giving an initial insight into the required spatial orientation of the aromatic moiety present in the RHS region of the molecule to maximize the in vitro affinity. Conversely, the urea derivative **13**, demonstrated good in vitro affinity, particularly at the OX_1 receptor (pK_i = 9.0 and 8.3 at the OX_1 and OX_2 receptor, respectively).

Following these initial results, a wide exploration of the bottom region of the molecule was undertaken with the specific objective of replacing the lipophilic biaryl moiety by more hydrophilic heterocycles. A series of five-membered and six-membered ring substituted heterocycles was introduced, replacing the central aryl ring (namely, pyrazoles, triazoles, thiazoles, isoxazoles, pyrazines); then, an attempt to replace the 'pendant' terminal phenyl ring mainly by pyridines was performed.[19] This exploration led to the identification of the 2-phenyl-5-methyl thiazole 'motif' as the preferred piperidine amide substituent offering best balance among in vitro activity, OX_1 versus OX_2 receptor selectivity ratio and reduction of the general lipophilicity. Having optimized the bottom region of the molecule, the optimization of the secondary amide aryl moiety present in the RHS region of the compounds was undertaken managing to replace the amide phenyl moiety with a focus set of heterocycles.[19] The 4-carboxyamidebenzofuran group[20] was selected as an instrumental moiety to maximize the in vitro activity at both the OX_1 and OX_2 receptor. *N*-[((2*S*)-1-{[5-(4-Fluorophenyl)-2-methyl-1,3-thiazol-4-yl]carbonyl}-2-piperidinyl)methyl]-1-benzofuran-4-carboxamide **1** (SB-649868)[19] was identified as one the most in vitro potent dual OX_1 and OX_2 receptor antagonist known at that time (pK_i = 9.4 and 9.5 at the OX_1 and OX_2 receptor, respectively), featuring signs of reduction of the in Cl_i both in the rat and human liver microsomes (Cl_i = 8.7 mL/min/g and 15.1 mL/min/g, respectively). This data, when corrected with

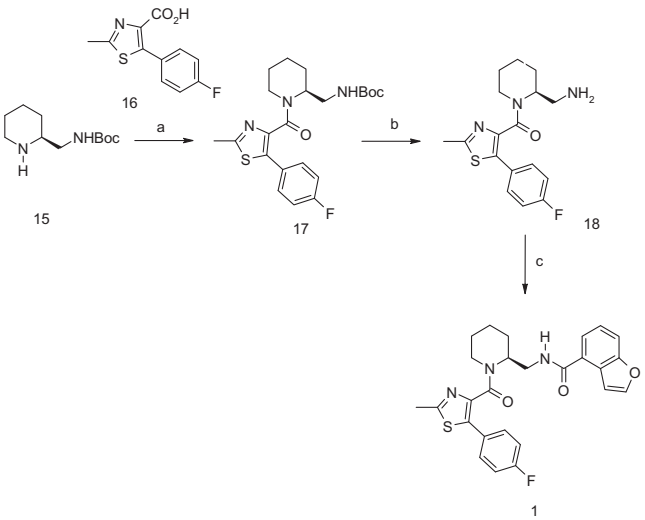
the fraction unbound,[21] suggested a low to moderate in vivo clearance, therefore accelerating its in vivo pharmacokinetic characterization. Compound **1** was smoothly prepared as shown in Scheme 1, by two sequential amidation reactions from the known chiral diamine template 15.[22]

From an in depth analysis of its ^{1}H NMR spectrum, it was observed that the chemical shifts of the protons present in α position to the disubstituted amide nitrogen were associated with unexpected values, suggesting the presence in solution of a mixture of the *E* and *Z* rotamers, as depicted Figure 2, originating from the slow rotation around the disubstituted amide N–C═O bond. The conformation of those rotamers was postulated by rotating frame Overhauser 2D ROESY experiments on the equilibrium mixture. This data suggested that in both rotamers the six membered piperidine core adopts a twisted-chair conformation, as shown in Figure 2, while the exocyclic CH_2NHCOR group occupies an axial position. In the *Z* conformation the presence of weak ROE effects between the aromatic rings suggests a π-stacking interaction, while in the E conformation the methyl group present in the thiazole ring shows several spatial correlations with the RHS aromatic ring, calling for a close proximity of these moieties.

Then, ^{1}H NMR exchange experiments were carried out to measure thermodynamics and kinetics of the interconversion process. The *E* rotamer was found to be the thermodynamically most stable in all solvents, and the interconversion half-life values of the two rotamers, at $T = 25$ °C, were less than 1 s. The $\Delta G^{\#}_{ZE}$ and $\Delta G^{\#}_{EZ}$ activation energy values for rotation of the amide bond in DMSO-d_6 were 18.0 ± 0.2 kcal/mol and 17.8 ± 0.2 kcal/mol, respectively.[23]

The solid-state structure of compound **1**, determined by X-ray crystallography, as depicted in Figure 3, shown that the most stable *E* conformation in solution, was the only one present in the crystalline state. Notably, the energy stabilization deriving from postulated weak π-stacking in the *Z* conformation does not seem to drive the crystal packing while the preferred orientation of the C═O of the disubstituted amide looks responsible for the conformation adopted by compound **1**.

In summary, the information generated by NMR experiments suggested the preferential presence in solution of a 'U-shaped' *E* conformer in rapid equilibrium with the *Z* conformer, whereas the former one was the only conformer observed in the crystalline state by X-ray analysis. Recently, this finding was confirmed independently by Merck's scientists investigating a different class of dual orexin receptor antagonists,[24] giving specific insights on the spatial pharmacophore requirements to design novel series of compounds.



a) i. **16**, $(COCl)_2$, DMF c.a., CH_2Cl_2, room temp, 6 h; ii. 15, CH_2Cl_2, TEA, room temp, overnight; b) TFA, CH_2Cl_2, room temp, 3 h; c) i. 1-benzofurane-4-carboxylic acid, $(COCl)_2$, DMF c.a., CH_2Cl_2, room temp, 5 h; ii. 18, TEA, room temp, overnight.

Scheme 1. Synthesis of compound **1**.

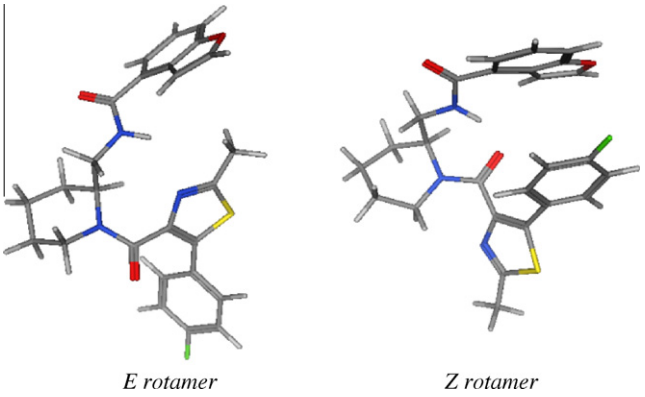


Figure 2. Low energy conformations predicted for compound **1** using systematic conformational search using parameterized force field for gas phase organic molecules. These structures are consistent with the ROEs constraints derived from NMR measurements.

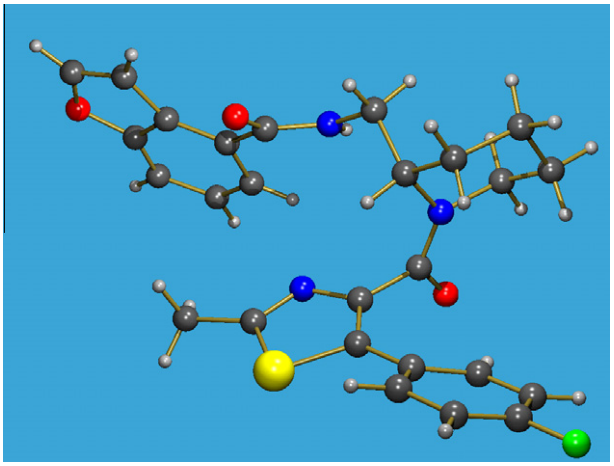


Figure 3. X-ray crystal structure of compound **1**.

Compound **1** was selected for further biological and pharmacokinetic characterization based on the high in vitro potency at both orexin receptors and the reduced Cl_i observed. As reported in Table 2, it exhibited pK_i values of 9.50 and 9.40 on human OX_1 and OX_2 receptors, respectively. Slightly higher functional potencies were found for the rat receptors (pK_i of 9.93 and 10.11 on OX_1 and OX_2 receptors, respectively). This similarity was not wholly unexpected as it is well established that sequence homologies of the orexin peptides and receptors are highly conserved across

Table 2
Functional potency and receptor binding affinity values (*fp*K_i) obtained using recombinant cell lines expressing human and rat OX_1 and OX_2 receptors

	fpK_i ± SEM[a]	*n*	*fp*K_i ± SEM[a]	*n*
h-OX_1	9.50 ± 0.02	38	9.12 ± 0.12[b]	3
h-OX_2	9.40 ± 0.05	23	8.89 ± 0.08[b]	3
r-OX_1	9.93 ± 0.08	6	—	—
r-OX_2	10.11 ± 0.11	6	—	—

[a] *fp*K_i values are the mean of *n* experiments each performed in duplicate.
[b] Displacement binding study using [^{3}H]almorexant as radioligand.

mammalian species.[3] In addition, no agonism was observed with compound **1** when tested per se up to the concentration of 1 μM.

Compound **1** displaced, in CHO cells overexpressing both the human recombinant OX_1 and OX_2 receptors, [^{3}H]almorexant[25] in a concentration dependent manner. The calculated receptor affinity values as reported in Table 2, were pK_i = 9.12 and 8.89 for the human OX_1 and OX_2 receptor, respectively, confirming a similar potency for compound **1** at both receptor subtypes. When tested in the CEREP selectivity screen, compound compound **1**, at 1 μM concentration, produced less than 50% inhibition of binding in the CEREP test battery, which included GPCRs, ion channels and enzymes, resulting in >1000 time selectivity for the orexin target receptors.

Pharmacokinetic studies in the male CD rat, performed at 1 mg/kg, iv and 3 mg/kg, po,[26] demonstrated an excellent pharmacokinetic profile for a hypnotic agent featuring moderate clearance in plasma (Cl_p = 24 mL/min/kg), short half-life of (<0.6 h) and a low volume of distribution (V_{ss} = 1.1 l/kg), coupled with excellent oral bioavailability (F = 85%) and good exposure in plasma (C_{max} = 333 ng/mL). A brain to blood ratio (B/B) of 0.1:1 was observed 1 h after iv administration, a value in line with the expected partition between the two compartments based on the lower tissue binding observed in vitro in brain tissues (fraction unbound/brain = 5.28%) with respect to plasma proteins (fraction unbound/plasma = 1.34%).[27] These data suggest that compound **1** is brain penetrant and is not substrate for brain efflux transporters (e.g., P-glycoprotein).

The ability of compound **1** to antagonise in vivo OX receptors has been determined in the OX-A evoked grooming model in the rat. Intracerebroventricular (icv) injection of Ox-A (3 μg/5 μl) induced a robust grooming response in the male CD rat. OX_1 receptor antagonists are able to attenuate this behaviour and the model has been used as a pharmacodynamic (PD) readout for the investigation of in vivo effects mediated via the OX_1 receptor.[28,29]

Data are expressed as time spent in grooming and numbers of bouts (mean ± SEM, n = 8) over 1 h of observation. Statistically significant differences compared to vehicle treated animals are indicated as $^{**}p$ <0.01 and statistically significant reversal of OX-A induced grooming is indicated as $^{\#\#}p$ <0.01 (ANOVA followed by Dunnett's t test).

The compound effect was assessed over 1 h on rat grooming as time and frequency (bouts), starting immediately after OX-A injection. Compound **1**, administered orally 3 h before OX-A injection at doses of 1, 3 and 10 mg/kg, caused a dose-dependent reduction of OX-A induced grooming as measured by total time spent grooming and number of grooming bouts (p <0.01 at 3 and 10 mg/kg po, Fig. 4).

Following these positive results, the hypnotic profile of compound **1** was assessed in the Circadian Time (CT) model in male CD rats,[30] by using telemetric recording of electroencephalogram (EEG) and electrooculogram (EOG). Simultaneous recording of EEG and EOG allows the accurate derivation of the key sleep parameters, namely: awake, non-REM (NREM) sleep and REM sleep. These effects were assessed at 3, 10 and 30 mg/kg, po dose, over a 5 h period in the active phase of the rat, starting the recording phase at CT 18 (light off at CT 12) of the rat light–dark cycle. CT18 was specifically chosen to give a maximum window to assess the hypnotic effects of the compound. In these experimental conditions, as shown both in Table 3 and Figure 5, vehicle treated rats showed a latency time to sleep (time required for the first sleep episode of a duration of 1 min) of 46 ± 10 min and a total sleep time (sum of NREM and REM sleep time) of 81 ± 11 min, recorded over a 5 h period. In addition, compound **1** produced a dose related reduction in sleep latency, with a maximal effect at 30 mg/kg (8.3 min ± 1.4, as shown in Fig. 6). The reduction of latency to sleep was statistically significant at all doses tested (p <0.01 for all doses).

Table 3
Sleep promoting effects of compound **1** administered at 3, 10 and 30 mg/kg, po in rat during active phase (starting time CT18)

	Vehicle	3 mg/kg	10 mg/kg	30 mg/kg
Awake	218 ± 11.32	194 ± 10.8	117 ± 4.6**	87 ± 6.7**
NREM latency	46.1 ± 10.0	19.0 ± 6.2**	11.6 ± 1.62**	8.3 ± 1.4**
NREM sleep	78.1 ± 11.3	99.3 ± 10.9	165.2 ± 5.4**	186.6 ± 8.0**
REM latency	146.3 ± 54.2	30.1 ± 17.7**	26.6 ± 7.7**	13.8 ± 2.2**
REM sleep	3.22 ± 0.73	6.5 ± 1.0	17.7 ± 2.2**	24.1 ± 12.1**

Values are expressed as time post-dosing in minutes as mean ± SEM (n = 8).
** p <0.01 for each dose versus corresponding vehicle as determined by one-way Anova followed by Dunnett's test.

The hypnotic effect observed was marked and demonstrates that a dual OX_1 and OX_2 receptor antagonist is able to facilitate sleep induction in the rat. In addition, compound **1** produced a robust dose dependent increase in total sleep time over the 5 h testing period, effect statistically significant at both 10 mg/kg and 30 mg/kg doses (81 ± 11 min for vehicle; 182 ± 4.6 min for 10 mg/kg, p <0.01; 213 ± 6.7 p <0.01 for 30). A more detailed analysis of the sleep patterns showed that compound **1** induced a robust dose dependent effect in terms of significant increase of both NREM and REM sleep time (Table 3).

Finally, to determine if compound **1** caused impairment in motor coordination when administered orally at 1, 3 and 10 mg/kg or in combination with ethanol, motor performance in male CD rats was investigated using an accelerated rotarod apparatus. In order to differentiate results obtained, zolpidem was included in the study as positive control. Results obtained (Fig. 6) showed that zolpidem reduced ability of rats to remain on rotarod. As expected, when administered in combination with ethanol the motor impairing effects of zolpidem were potentiated. Conversely, compound **1**,

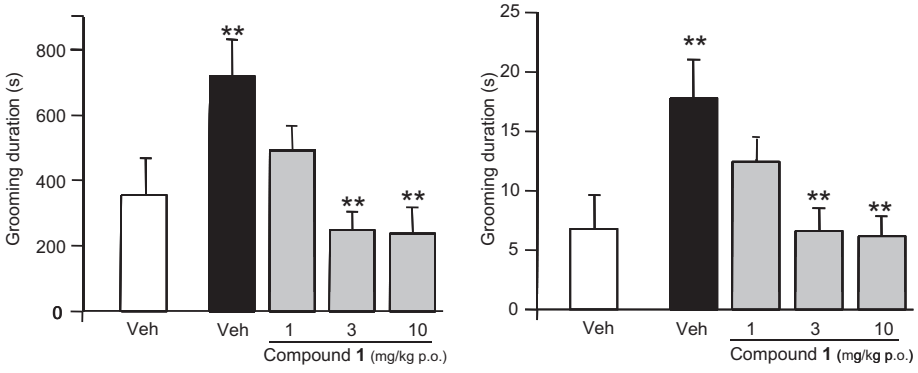


Figure 4. Effects of compound **1** (1, 3 and 10 mg/kg po) on time spent in grooming (a) and frequency (b) in the OX-A evoked grooming model in the rat.

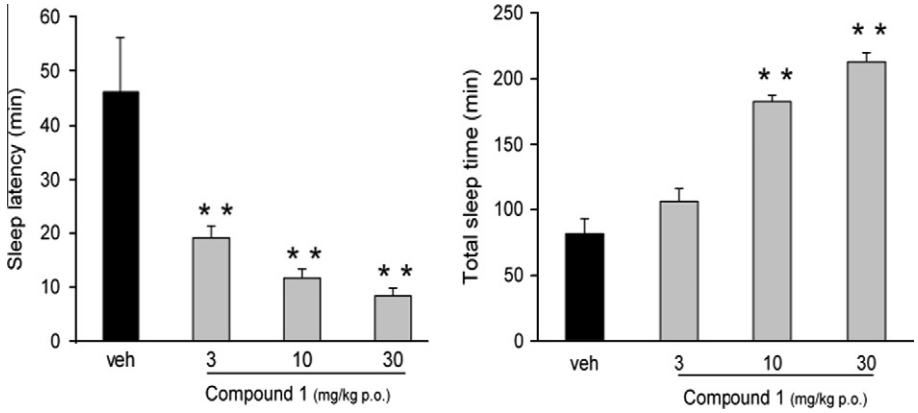


Figure 5. Effects of compound **1** (3, 10 and 30 mg/kg po) on sleep latency and total sleep time in the rat CT18 model.

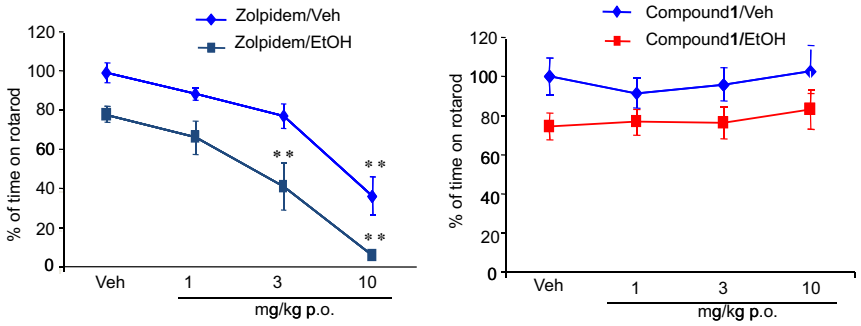


Figure 6. Assessment of potential for induction of locomotor deficits by compound **1** and zolpidem and potentiation by ethanol (1 g/kg, ip) using an accelerated rotarod apparatus.

when given alone or combined with ethanol, failed to cause motor coordination impairment in the rotarod model in the rat at all dose tested.

Data are expressed as mean ± SEM (n = 7–9). $^{*}p$ <0.01 refer to treated group versus respective vehicle; ## refer to each single dose of compound/ethanol versus respective compound/vehicle group (ANOVA followed by Dunnett's test).

In conclusion, a large body of evidence suggests that the orexin system plays a key role in the control of arousal and sleep/wake behavior and that dual OX_1 and OX_2 receptor antagonists may provide innovative hypnotics of truly novel mechanism of action.[31,32] Compound **1** was identified as a in vitro potent and selective dual OX_1 and OX_2 receptor antagonist, able to revert in the rat the grooming behavior induced by icv administration of OX-A. Pharmacokinetic analysis and in vivo activity in a pre-clinical sleep model showed an appropriate profile of compound **1** as a hypnotic agent, without inducing animal motor impairment at the active dose. A comparative characterization of compound **1** with respect to almorexant in animal models of insomnia has recently been published,[33] confirming the excellent in vivo efficacy observed in house. Based on these characteristics, compound **1** was selected as a drug candidate and successfully progressed to clinical phase studies.

Acknowledgment

Professor William Clegg and Dr. Sophie Dale (University of Newcastle) are thanked for the GSK funded X-ray crystallographic analysis of compound **1**.

References and notes

1. Sakurai, T.; Amemiya, A.; Ishii, M.; Matsuzaki, I.; Chemelli, R. M.; Tanaka, H.; Williams, S. C.; Richarson, J. A.; Kozlowski, G. P.; Wilson, S.; Arch, J. R. S.; Buckingham, R. E.; Haynes, A. C.; Carr, S. A.; Annan, R. S.; Mcnulty, D. E.; Liu, W.; Terrett, J. A.; Elshourbagy, N. A.; Bergsma, D. J.; Yanagisawa, M. *Cell* **1998**, *92*, 573.
2. De Lecea, L.; Kilduff, T. S.; Peyron, C.; Gao, X.-B.; Foye, P. E.; Danielson, P. E.; Fukuhara, C.; Battenberg, E. L. F.; Gautvik, V. T.; Bartlett, F. S., II; Frankel, W. N.; Van Den Pol, A. N.; Bloom, F. E.; Gautvik, K. M.; Sutcliffe, J. G. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 322.
3. Sakurai, T. *Nat. Rev. Neurosci.* **2007**, *8*, 171.
4. Nambu, T.; Sakurai, T.; Mizukami, K.; Hosoya, Y.; Yanagisawa, M.; Goto, K. *Brain Res.* **1999**, *827*, 243.
5. Peyron, C.; Tighe, D. K.; van den Pol, A. N.; de Lecea, L.; Heller, H. C.; Sutcliffe, J. G.; Kilduff, T. S. *J. Neurosci.* **1998**, *18*, 9996.
6. Date, Y.; Ueta, Y.; Yamashita, H.; Yamaguchi, H.; Matsukura, S.; Kangawa, K.; Sakurai, T.; Yanagisawa, M.; Nakazato, M. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 748.
7. Backberg, M.; Hervieu, G.; Wilson, S.; Meister, B. *Eur. J. Neurosci.* **2002**, *15*, 315.
8. Marcus, J. N.; Aschkenasi, C. J.; Lee, C. E.; Chemelli, R. M.; Saper, C. B.; Yanagisawa, M.; Elmquist, J. K. *J. Comp. Neurol.* **2001**, *435*, 6.
9. Hagan, J. J.; Leslie, R. A.; Patel, S.; Evans, M. L.; Wattam, T. A.; Holmes, S.; Benham, C. D.; Taylor, S. G.; Routledge, C.; Hemmati, P.; Munton, R. P.; Ashmeade, T. E.; Shah, A. S.; Hatcher, J. P.; Hatcher, P. D.; Jones, D. N. C.; Smith, M. I.; Piper, D. C.; Hunter, A. J.; Porter, R. A.; Upton, N. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 10911.
10. Soffin, E. M. *Neuropharmacology* **2002**, *42*, 127.
11. Piper, D.; Upton, N.; Smith, M.; Hunter, J. A. *Eur. J. Neurosci.* **2000**, *12*, 726.
12. Chemelli, R. M.; Willie, J. T.; Sinton, C. M.; Elmquist, J. K.; Scammell, T.; Lee, C.; Richardson, J. A.; Williams, S. C.; Xiong, Y.; Kisanuki, Y.; Fitch, T. E.; Nakazato, M.; Hammer, R. E.; Saper, C. B.; Yanagisawa, M. *Cell* **1998**, *98*, 437.
13. Lin, L.; Faraco, J.; Li, R.; Kadotani, H.; Rogers, W.; Lin, X.; Qiu, X.; de Jong, P. J.; Nishino, S.; Mignot, E. *Cell* **1999**, *98*, 365.
14. Mignot, E.; Lammers, G. J.; Ripley, B.; Okun, M.; Nevsimalova, S.; Overeem, S.; Vankova, J.; Black, C.; Harsh, H.; Bassetti, C.; Shrader, H.; Nishino, S. *Arch. Neurol.* **2002**, *59*, 1553.

15. Brisbare-Roch, C.; Dingemanse, J.; Koberstein, R.; Hoever, P.; Aissaoui, H.; Flores, S.; Mueller, C.; Nayler, O.; Van Gerven, J.; De Haas, S. L.; Hess, P.; Qiu, C.; Buchmann, S.; Scherz, M.; Weller, T.; Fischli, W.; Clozel, M.; Jenck, F. *Nat. Med.* **2007**, *3*, 150.
16. Neubauer, D. N. *Curr. Opin. Invest. Drugs* **2010**, *11*, 101.
17. Cox, C. D.; Breslin, M. J.; Whitman, D. B.; Schreier, J. D.; McGaughey, G. B.; Bogusky, M. J.; Roecker, A. J.; Mercer, S. P.; Bednar, R. A.; Lemaire, W.; Bruno, J. G.; Reiss, D. R.; Harrell, C. M.; Murphy, K. L.; Garson, S. L.; Doran, S. M.; Prueksaritanont, T.; Anderson, W. B.; Tang, C.; Roller, S.; Cabalu, T. D.; Cui, D.; Hartman, G. D.; Young, S. D.; Koblan, K. S.; Winrow, C. J.; Renger, J. J.; Coleman, P. J. *J. Med. Chem.* **2010**, *53*, 5320.
18. Gailland, P.; Johnson, C. N.; Novelli, R.; Porter, R. A.; Stemp, G.; Thewlis, K. M. WO 02/089800 A2.
19. Branch, C. L.; Johnson, C. N.; Stemp, G.; Thewlis, K. WO 01/96302.
20. A1Eissenstat, M. A.; Bell, M. R.; D'Ambra, T. E.; Alexander, E. J.; Daum, S. J.; Ackerman, J. H.; Gruett, M. D.; Kumar, V.; Estep, K. G. *J. Med. Chem.* **1995**, *38*, 3094.
21. Chao, P.; Uss, A. S.; Cheng, K. C. *Exp. Opin. Drug Metab. Toxicol.* **2010**, *6*, 189.
22. Froelich, O.; Desos, P.; Bonin, M.; Quirion, J. C.; Husson, H. P.; Zhu, J. *J. Org. Chem.* **1996**, *61*, 6700.
23. For the barriers of rotation of tertiary aromatic amides see: Clayden, J.; Pink, J. H. *Angew. Chem., Int. Ed.* **1998**, *37*, 1937.
24. Cox, C. D.; McGaughey, G. B.; Bogusky, M. J.; Whitman, D. B.; Ball, R. G.; Winrow, C. J.; Renger, J. J.; Coleman, P. J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2997.
25. Malherbe, P.; Borroni, E.; Pinard, E.; Wettstein, J. G.; Knoflach, F. *Mol. Pharmacol.* **2009**, *76*, 618.
26. Compound **1** was administered iv as a bolus [H_2O, 2% DMSO (v/v) and 25% PEG400 (v/v)] and po as a suspension [H_2O, 1% (w/v) methocel].
27. Read, K. D.; Braggio, S. *Exp. Opin. Drug Metab. Toxicol.* **2010**, *6*, 337.
28. Duxon, M. S.; Stretton, J.; Starr, K.; Jones, D. N. C.; Holland, V.; Riley, G.; Jerman, J.; Brough, S.; Smart, D.; Johns, A.; Chan, W.; Porter, R. A.; Upton, N. *Psychopharmacology* **2001**, *153*, 203–209.
29. All the works involving animals were carried out in accordance with European Directive 86/609/EEC governing animals welfare and protection, which is acknowledged by Italian Legislative Decree number 116, 27 January 1992 and were reviewed and approved by GlaxoSmithKline Committee on Animal Research & Ethics (CARE) according to the company policy on the Care and Use of Laboratory Animals.
30. Weiergräber, M.; Henry, M.; Hescheler, J.; Smyth, N.; Schneider, T. *Brain Res. Prot.* **2005**, *14*, 154.
31. For a recent review on orexin receptor antagonists see: Gatfield, J.; Brisbare-Roch, C.; Jenck, F.; Boss, C. *Chem. Med. Chem.* **2010**, *5*, 1197.
32. Boss, C.; Brisbare-Roch, C.; Jenck, F. *J. Med. Chem.* **2009**, *52*, 891.
33. Boss, C.; Brisbare-Roch, C.; Jenck, F.; Aissaoui, H.; Koberstein, R.; Sifferlen, T.; Weller, T. *Chimia* **2008**, *62*, 974.