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Discovery of HTL6641, a dual orexin receptor antagonist with differentiated pharmacodynamic properties†

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A novel series of potent, selective, and orally efficacious dual antagonists of the orexin receptors has been investigated, resulting in the identification of lead compound 27 (HTL6641). Comprehensive data for 27 are presented, including *in vivo* PK parameters, confirmation of receptor occupancy through *ex vivo* binding and efficacy in a rat sleep model. A key feature of the series is a short dissociation half-life, measured by surface plasmon resonance (SPR) using stabilized receptors, and confirmed by radioligand-binding experiments. Based on a consideration of the requirements for a potential treatment for insomnia, compound 27 was identified as having the best balance of properties from the chemical series.

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The orexin neuropeptides orexin-A and orexin-B, derived from a precursor expressed in the hypothalamus, were independently revealed by two research groups in 1998.^{1,2} The orexins bind to two G protein-coupled receptors (GPCRs), OX₁ and OX₂, which are highly conserved across mammalian species, as are the neuropeptides themselves.^{1,3} The orexin system is a key regulator of behavioural arousal, sleep and wakefulness. Transgenic mice and rats in which the orexin neurons have been genetically ablated exhibit severe sleepiness, as do orexin peptide knockout mice. In each case a phenotype similar to human narcolepsy patients is observed.^{4–6} In humans, loss of the orexin neurons with intact receptor expression is linked to narcolepsy, a chronic sleep disorder characterised by excessive sleepiness during the day, fragmented sleep and cataplexy.⁷ In canines, it has been established that disruption of the OX₂ receptor gene results in narcolepsy, providing a clear genetic linkage between the orexin system and sleep modulation.⁸

Over the past decade, there has been a significant drive within the pharmaceutical industry to develop orexin

receptor antagonists, with both selective and dual profiles, to investigate the potential for treatment of insomnia, other sleep disorders and other diseases of the central nervous system.^{9–12} Current therapies for insomnia treatment centre on the use of benzodiazepines and related derivatives which have sedative, hypnotic and anxiolytic actions resulting from an enhancement of the effect of the neurotransmitter GABA at the GABA_A receptor. Due to a variety of side effects including depression, dependence and sexual dysfunction, and the potential for addiction, benzodiazepine therapies for insomnia are available only on prescription. A related treatment paradigm uses newer drugs such as zolpidem which are positive allosteric modulators of GABA_A receptors and share a similar risk-benefit profile.¹³ The strategy of insomnia treatment by modulation of GABA_A receptors is most prominently hampered by CNS-related side effects the morning after administration, including drowsiness, cognitive hang-over effects and a lack of coordination.^{14,15} Treatment of insomnia by antagonising orexin receptors presents an opportunity to more directly address the disorder than the existing GABA based therapies, as orexin receptor antagonists, which act to prevent wakefulness, should more selectively regulate the sleep/wake cycle and lack the general CNS depressant effects of current treatments. The majority of efforts in recent years have centred on the identification and progression of dual orexin receptor antagonists (DORAs), in line with observations that both receptors play a role in regulation of sleep and wakefulness.^{16,17} The precise role of the OX₁ receptor remains controversial, as it has been suggested by one group that antagonising the OX₂ receptor alone should have greater efficacy in the treatment of insomnia than a DORA,¹⁸

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however both receptors have been found to play different roles in sleep promotion.^{19,20} Selective antagonists of either receptor were reviewed in 2013,²¹ and more recently OX₂ selective molecules with efficacy in preclinical sleep or antidepressant models have been published by Merck and Eli Lilly respectively.^{22,23}

Several clinical studies have confirmed the therapeutic utility and safety of DORAs in the treatment of insomnia. Almorexant **1** (Fig. 1), developed by Actelion and subsequently licensed to GlaxoSmithKline, demonstrated efficacy in a phase II trial, increasing sleep efficiency in primary insomnia patients.²⁴ Secondary end points, dose-dependent decreases in latency to persistent sleep (LPS) and wake after sleep onset (WASO), were also met. Although in 2009 almorexant was reported to have achieved its primary end point in an initial phase III trial, development of the molecule was discontinued in 2011 after a review of additional studies conducted to further establish the clinical profile, including tolerability.²⁵ A second DORA from GSK, SB-649868 **2**, promoted sleep in male insomnia patients, but clinical development was subsequently stopped due to a preclinical safety observation in rats.^{26,27} Merck's suvorexant **3** is the most advanced DORA,²⁸ which was approved for use in adults with insomnia in August 2014. Additionally, Merck have progressed MK-6096 **4** (filorexant) into phase II trials in patients with primary insomnia,²⁹ and data from phase I trials of a further DORA from Actelion, ACT-462206 **5**, together with preclinical data, have recently been published.^{30,31} The clinical DORAs largely originate from high-throughput screening and it has been challenging to optimize properties such as lipophilicity, for example almorexant has *c* log *P* 5.9,³² which may be linked to the failure of several compounds due to safety issues unrelated to the mechanism of action.

To develop novel antagonists of the orexin receptors we embarked upon a hit identification campaign using a combination of fragment, focused and virtual screening, selecting several relatively small sets of molecules to screen by surface plasmon resonance (SPR) and/or radioligand binding. A number of promising hits were identified from each approach and several were followed up by further purchasing or synthesis of close analogs. One particular screen of approximately 100 compounds contained molecules selected by medicinal chemists after consideration of how a number of known orexin antagonists were proposed to bind into a refined homology model (see ESI†) of the OX₂ receptor and then targeting molecules considered to have similarity in their pharmacophoric features. The effort uncovered several related hits exemplified by **6** (Fig. 2) which had encouraging binding affinity (OX₂ pK_i 6.6). Compound **6** and several other classes of molecules were selected to test the hypothesis that a central heterocyclic ring containing H-bond acceptors, flanked by two further aromatic substituents which might fold together into a hydrophobically collapsed conformation, would have the potential to bind to the orexin receptors.³³ A number of further commercially available analogs of **6** were available to rapidly build SAR, and synthetic routes were

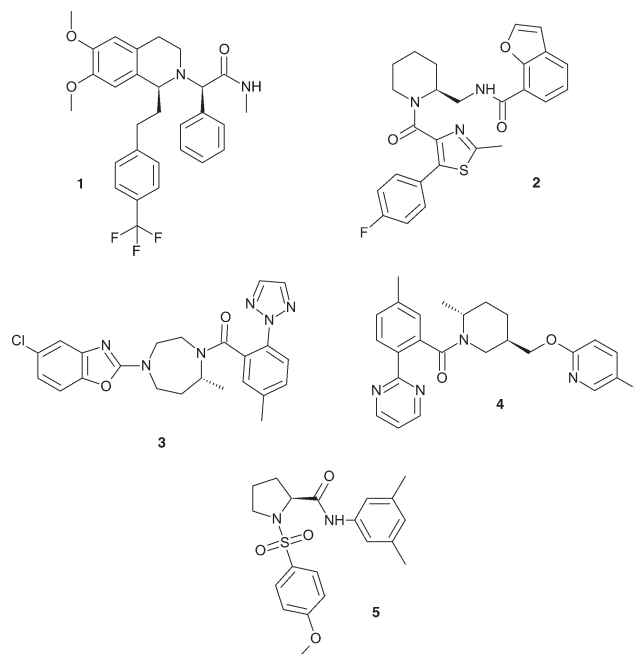


Fig. 1 Clinically evaluated DORAs 1–5.

readily amenable to further analog synthesis. An additional attraction of **6** was that the hit was largely dissimilar to known DORAs.^{9–12} Initially, a number of analogs which varied the benzyl substituent of **6**, but kept the 3,4-dimethoxyphenyl portion constant, were purchased or synthesized according to the general route in Fig. 3. Starting from 2-nitrobenzenesulfonyl chloride, sulfonamide coupling, nitro reduction and cyclisation with triphosgene or 1,1'-carbonyldiimidazole (CDI), yielded benzothiadiazin-3(4*H*)-one 1,1-dioxide intermediates which could then be readily *N*-substituted with benzyl halides or benzyl alcohols. As primary assays for establishing SAR, we used OX₁ and OX₂ radioligand binding assays (see ESI†), focusing our attention primarily on activity at the latter receptor. Data for key early compounds in the series are summarized in Table 1.

Removal of the methylenedioxyphenyl unit from **6**, which was deemed undesirable due to a potential bioactivation risk,³⁴ yielded an improvement in OX₂ affinity for the unsubstituted benzyl analog **7**. Fluorine substitution at the 2-position was favourable (**8**), whereas 3- and 4-fluorination

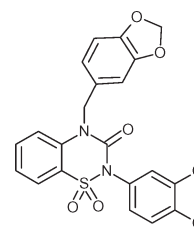


Fig. 2 Initial OX₂ hit **6**.

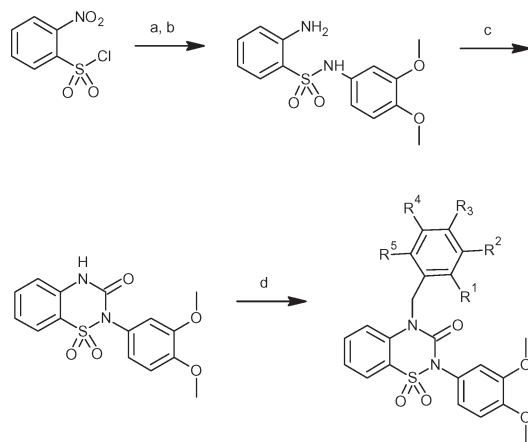


Fig. 3 General synthetic route for synthesis of analogs of **6**. Reagents and conditions: (a) 3,4-dimethoxyaniline, 1,4-dioxane, 80 °C; (b) SnCl_2 , EtOH, 100 °C or Pd/C, H_2 , EtOH/ H_2O , rt or Fe, AcOH, 60 °C; (c) triphosgene, 1,4-dioxane, 100 °C or CDI, Et_3N , DMF, 100 °C; (d) substituted benzyl alcohol, Ph_3P , diisopropyl azodicarboxylate (DIAD) or 1,1'-azobis(*N,N*-dimethylformamide) (TMAD), THF, rt or substituted benzyl halide, K_2CO_3 , DMF, 80 °C.

did not increase binding affinity significantly (data not shown). Substitution with chloro at the 2-position was also beneficial. 4-Substitution was envisaged to be advantageous for metabolic stability, and this was tolerated in combination with a 2-substituent (**9**, **10**). 2,6-Disubstitution (**11**) yielded a significant increase in affinity, which was largely maintained in the 2,4,6-trisubstituted analogs **12** and **13**, though not in 2-methoxy derivative **14**. Useful SAR emerged at the 4-position, methyl substitution (**15**) was tolerated in similar fashion to fluoro but installation of methoxy yielded further significant increases in OX_2 affinity (**16** vs. **12**; **17** vs. **13**). A range

of alkyl and cycloalkyl substitutions in place of the benzyl group were explored, as was replacement of the benzyl by substituted (pyridinyl)methyl groups, but neither strategy yielded sufficient affinity to be explored further (data not shown). In general, analogs exhibited a moderate preference for higher affinity at the OX_2 receptor than OX_1 .

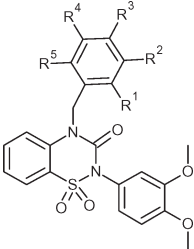
Having gained confidence that high affinity could be achieved through optimization of the benzyl group, our attention turned to modification of the 3,4-dimethoxyphenyl unit and the central heterocyclic scaffold, with the primary aim of reducing lipophilicity and obtaining better developability properties within the series. An additional benefit in moving away from the dimethoxyphenyl unit was that the potential for reactive metabolite risks associated with this moiety,²² or indeed with the presence of a masked aniline, would be removed.

Synthesis in an analogous fashion to the route depicted in Fig. 3 allowed replacement of the dimethoxyphenyl group by a variety of dimethoxypyridines. In general this was tolerated (Table 2), with pyridine variants having broadly comparable affinity (compare **18** and **19** to **12**; **20** to **13**). As with the phenyl variants (data not shown), two methoxy substituents were required for high affinity, for example **21** is more than 30-fold less active at OX_2 than the analogous **12**, though as in the phenyl series (data not shown) both 3,4- and 3,5-dimethoxy substitution patterns were well tolerated (**18**, **23** and **19**, **22** respectively). Installation of the pyridine nitrogen at the 2-position was also well tolerated (**24**). Benzyl position SAR was in general highly transferable, and by selection of the most active substituents, OX_2 binding could be readily tuned to high levels, for example **22**, **23** and **24** have affinities in excess of pK_i 9 ($K_i < 1$ nM).

In an exploration of close analogs of the highest affinity compounds, we observed that one or both methoxy groups could be replaced, for example by methyl (data not shown) or methylamino (**25**). In general, when compared directly to the methoxy analogs (data not shown), up to a 10-fold loss of OX_2 affinity was observed, and the majority of these changes also resulted in a significant loss of *in vitro* metabolic stability. Changes to the thiazine-3(4*H*)-one 1,1-dioxide portion of the central scaffold were poorly tolerated, for example **28**, Table 2, whereas modification of the fused phenyl portion was more encouraging. Most notably, installation of a nitrogen atom at the 5-position allowed the lipophilicity of the series to be decreased whilst maintaining high levels of OX_1 and OX_2 binding affinity (**25**–**27**, Table 2). 5-Aza analogs of this type could be readily accessed from 2-chloropyridine-3-sulfonyl chloride using the synthetic route depicted in Fig. 4, with sulfonamide coupling preceding $\text{S}_\text{N}\text{Ar}$ reaction with a substituted benzylamine, followed by ring closure with triphosgene or CDI.

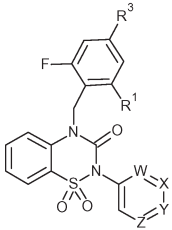
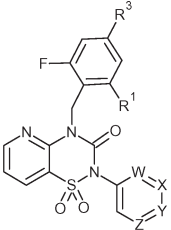
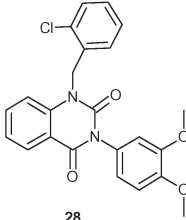
An important consideration that we were aware of during the course of the project is that the duration of action *in vivo* of orexin antagonists will be dependent upon both the pharmacokinetic and receptor–ligand kinetic parameters of the molecule. Pharmacokinetic parameters are an important

Table 1 OX_1 and OX_2 SAR of **7**–**17** (R^1 – R^5 = H unless specified)

			
	R ¹ –R ⁵	OX ₁ pK _i ^a	OX ₂ pK _i ^a
7	—	5.7	7.1
8	R ¹ = F	6.5	8.2
9	R ¹ = F, R ³ = Cl	7.2	8.0
10	R ¹ = Cl, R ³ = F	<5.6	7.6
11	R ¹ = Cl, R ⁵ = F	8.1	9.1
12	R ¹ , R ³ , R ⁵ = F	7.3	8.7
13	R ¹ = Cl, R ³ , R ⁵ = F	7.6	9.0
14	R ¹ = OMe, R ³ , R ⁵ = F	7.2	8.2
15	R ¹ = Cl, R ³ = Me, R ⁵ = F	8.2	9.1
16	R ¹ = F, R ³ = OMe, R ⁵ = F	8.7	9.3
17	R ¹ = Cl, R ³ = OMe, R ⁵ = F	9.4	10.0

^a Data represent geometric means of two or more measurements.

Table 2 OX₁ and OX₂ SAR and rat microsomal stability of **18–28**

										
	R ¹	R ³	W	X	Y	Z	OX ₁ pK _i ^a	OX ₂ pK _i ^a	RLM <i>t</i> _{1/2} (min)	<i>c</i> log <i>P</i> ^b
18	F	F	CH	N	COMe	COMe	7.9	8.3	28	2.9
19	F	F	CH	COMe	N	COMe	7.8	8.9	16	3.1
20	Cl	F	CH	N	COMe	COMe	7.4	8.8	23	2.5
21	F	F	CH	COMe	N	CH	6.4	7.2	23	2.7
22	F	OMe	CH	COMe	N	COMe	9.0	9.6	16	3.0
23	F	OMe	CH	N	COMe	COMe	8.6	9.4	9	2.8
24	F	OMe	N	COMe	COMe	CH	nd	9.7	8	2.8
25	F	OMe	CH	N	COMe	CNHMe	8.9	9.2	7	0.3
26	F	F	N	COMe	COMe	CH	7.6	8.8	16	1.4
27	F	F	CH	N	COMe	COMe	7.7	8.6	23	1.4
28	—	—	—	—	—	—	<5.2	<5.0	nd	4.0

^a Data represent geometric means of two or more measurements, nd = not determined. ^b Ref. 32.

consideration during medicinal chemistry optimization, however whilst these measurements are routine it is not always the case that pharmacodynamic effects are measured at the level of the receptor.^{35,36} At Heptares we use biochemical and/or biophysical approaches to routinely probe structure-kinetic relationships (SKR) to enable us to select molecules with appropriate attributes for progression. A notable feature of the advanced DORAs suvorexant and almorexant is that they exhibit slow kinetics at the orexin receptors.^{37,38} In the treatment of sleep disorders, a potential issue for compounds with slow receptor kinetics is that they may exhibit a prolonged duration of action which could potentially promote next-day residual effects. Therefore, as part of our progression strategy we sought to identify molecules with appropriate parameters, both in terms of moderate pharmacokinetic half-lives and a fast receptor off-rate.

To address these twin challenges, we first examined examples of our series in biophysical and biochemical assays to determine receptor binding kinetics. Wild-type GPCRs are rarely stable enough to be successfully captured on to biosensor chips for evaluation by SPR,³⁹ a limitation which can be successfully overcome by increasing thermostability through protein engineering.^{40,41} Introduction of a small number of mutations, which do not affect the binding site characteristics of the receptor but dramatically increase thermostability, yield modified GPCRs known as StaR® proteins. The stabilized receptors can be successfully immobilized on biosensor chips and used for fragment screening,⁴² Biophysical Mapping™,⁴³ and for evaluation of receptor-ligand kinetic parameters,⁴⁴ as we have previously described. OX₁ and OX₂ StaR proteins were generated which had significantly increased thermostability and retained the ligand binding

characteristics of the wild-type receptors.⁴⁰ The enhanced stability of the purified proteins allowed capture on SPR sensor chips (see ESI†) and examination of the kinetics of receptor-ligand binding interactions of standard molecules and multiple compounds in our series. Dilution series of each compound were injected, and blank-subtracted data were fitted to a 1:1 interaction model to obtain kinetic and affinity constants. Our series, exemplified by **18** and **27**, displayed fast OX₂ receptor kinetics, with **18** (see ESI†) having an on rate (*k*_a) of $2.8 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$, an off rate (*k*_d) of $2.7 \times 10^{-3} \text{ s}^{-1}$ and a dissociation half-life (*t*_{1/2} = (ln 2/*k*_d)/60) of 4.3 min. The close

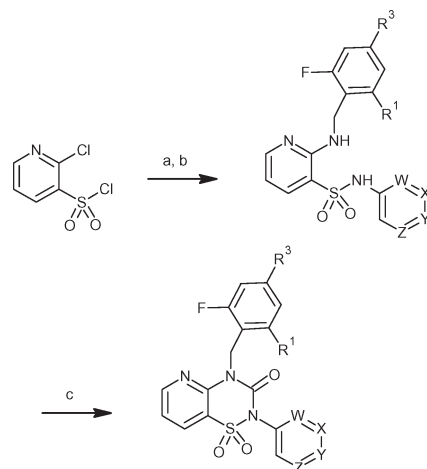


Fig. 4 Synthesis of **25–27**. Reagents and conditions: (a) heteroaryl amine, pyridine, DCM, 0 °C or heteroaryl amine, 1,4-dioxane, 0 °C; (b) substituted benzylamine, MeCN, microwave heating, 180 °C, 1–2 h; (c) triphosgene, 1,4-dioxane, 100 °C or CDI, Et₃N, DMF, 100 °C.

analog 27 (Fig. 5) was profiled at both the OX_1 (k_a 2.0×10^4 $M^{-1} s^{-1}$, k_d $1.6 \times 10^{-2} s^{-1}$, $t_{1/2} = 0.8$ min) and OX_2 receptors (k_a 3.3×10^4 $M^{-1} s^{-1}$, k_d $2.8 \times 10^{-3} s^{-1}$, $t_{1/2} = 4.2$ min). Suvorexant by comparison displayed much slower receptor kinetics with dissociation half-lives of 24 and 210 min for the OX_1 and OX_2 receptors respectively. Evaluation of OX_2 receptor kinetics using Motulsky–Mahan radioligand-binding studies was also undertaken,^{38,45} and yielded comparable data: 18 $t_{1/2} = 3.0$ min; 27 $t_{1/2} = 7.6$ min; suvorexant $t_{1/2} = 79$ min; the latter figure is additionally in line with that reported in the literature by workers from Merck.³⁷ In OX_2 radioligand-binding studies almorexant has a dissociation half-life of 242 min.³⁸ The significantly faster receptor kinetics observed with members of our series including 18 and 27 should facilitate rapid re-equilibration with changing orexin levels *in vivo*, to help reduce the potential risk for next day somnolence effects.³⁷

During the medicinal chemistry progression of the series described herein, in addition to the studies presented in this manuscript, multiple co-crystal structures of compounds from the benzothiadiazine series, another novel series from our laboratories, and literature compounds such as suvorexant and SB-334867,⁴⁶ were solved in complex with OX_1 and/or OX_2 StaR proteins. These crystal structures revealed, in fine detail, the binding modes of the various series and how they relate to one another, as well as giving some insight into the binding kinetics of the receptors. In particular the X-ray structures provided a detailed understanding of the differences between the two receptors and have been used extensively to inform further iterations of our medicinal chemistry project leading to the design of uniquely selective OX_1 antagonists which have potential in the treatment of addiction disorders.⁴⁷ These data are beyond the scope of this communication and will be the subject of future publications in specialized journals. During the preparation

of this manuscript, the crystal structure of suvorexant bound to an OX_2 fusion protein construct was disclosed.⁴⁸

In parallel with profiling key compounds through standard *in vitro* assays such as the hERG ion channel and P450 inhibition we routinely monitored *in vitro* stability in rat-liver microsomal preparations (RLM $t_{1/2}$, Table 2). For the reasons detailed above, we needed to strike a delicate balance between having sufficient metabolic stability to not compromise oral bioavailability through high first-pass metabolism, yet having a relatively short pharmacokinetic half-life in rat as an initial pre-clinical species. Profiling of early molecules in PK experiments in male Sprague Dawley rats by both iv and po routes allowed us to identify a half-life range in the RLM assay of 15–35 minutes as an appropriate criteria for progression; data for key advanced compounds are summarized in Table 3. Compound 18 and the regioisomeric 19 have low *in vivo* clearance (18 and 17 $mL min^{-1} kg^{-1}$ respectively) with 18 having a shorter half-life by virtue of a lower volume of distribution than 19. In line with its higher measured plasma protein binding (PPB) in rat, an additional *in vitro* parameter which we closely monitored during series progression, 19 additionally compares poorly to its regioisomer in terms of the unbound fraction in the brain (approximated as the ratio of drug concentration in the cerebrospinal fluid (CSF) to that in brain at the same time point);⁴⁹ in common with the majority of compounds profiled, both molecules had very good brain penetration. Chloro derivative 20, with both higher clearance and volume of distribution than 18, had a similar half-life and a higher free fraction in brain. All three molecules, together with the aza-examples 26 and 27, had acceptable oral bioavailability (29–66%). Selected compounds were additionally progressed to PK studies in male beagles; 18 had low clearance, moderate volume of distribution, long half-life and excellent bioavailability. Compound 27, the aza-variant of 18, also had a good profile in beagle with moderate clearance and volume of distribution, good bioavailability and a shorter half-life than 18. The mean residence time (MRT) of 27 was 1.9 h and 4.0 h in rat and beagle, respectively. Overall, these PK parameters suggest a short to moderate half-life in human.

After consideration of the overall profiles of 27 and related close analogs, 27 was selected for extensive further profiling as summarized below and in Table 4. The dual-antagonist profile of 27 was confirmed in OX_1 and OX_2 antagonist functional cell assays measuring receptor-stimulated ERK1/2 phosphorylation. A clean *in vitro* profile was apparent, with no issues identified with inhibition of P450 isoforms or the hERG ion channel. Evaluation of bidirectional apparent permeability (P_{app}) across a Caco-2 cell monolayer indicated that the molecule had high passive permeability with low efflux. Binding to human, dog and mouse plasma proteins was high, in common with the measured value in rat (99.5%), and the latter is consistent with the CSF concentrations observed in rat PK experiments. Evaluation of suvorexant as a benchmark in these assays returned comparable data (99.4, 98.3, 99.3% for human, dog and mouse respectively).

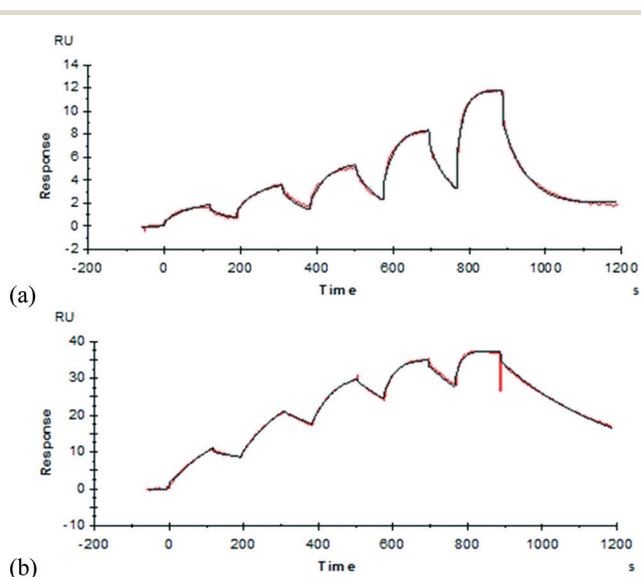


Fig. 5 (a) OX_1 and (b) OX_2 surface plasmon resonance sensorgrams of 27.

Table 3 RLM and PK parameters of 18–20, 26 and 27 in rat and beagle

	RLM $t_{1/2}$ (min)	Species	iv				po			
			Cl (mL min ⁻¹ kg ⁻¹)	V_{ss} (L kg ⁻¹)	Brain : plasma (0.5 h)	CSF : brain (0.5 h)	$t_{1/2}$ (po) (h)	AUC _{inf} (ng h ⁻¹ mL ⁻¹)	T_{max} (h)	F_{po} (%)
18 ^a	28	Rat	18	2.2	0.77	0.01	1.6	558	1.5	29
		Beagle	5	3.6	—	—	10.6	6957	0.4	100
19 ^a	15	Rat	17	3.9	1.51	BQL ^c	3.3	705	0.4	36
20 ^a	23	Rat	34	3.5	0.79	0.02	1.8	300	0.8	29
26 ^a	16	Rat	29	2.3	0.84	0.01	1.3	401	0.5	34
27 ^b	23	Rat	18	2.1	0.49	0.01	1.8	1217	0.5	66
		Beagle	13	3.0	—	—	7.1	2442	0.4	70

^a Dosed at 1 & 2 mg kg⁻¹ iv/po respectively in rat (2 & 2 mg kg⁻¹ in beagle), using 10% DMA, 10% solutol HS15, 80% saline as vehicle. ^b Dosed at 1 & 2 mg kg⁻¹ iv/po respectively in the rat PK studies (1 & 1 mg kg⁻¹ in beagle), using 10% DMA, 10% solutol HS15, 80% (10% aqueous (2-hydroxypropyl)- β -cyclodextrin) as vehicle. ^c BQL = below quantifiable limit of 1.00 ng mL⁻¹.

Screening in an *in vitro* cytotoxicity assay in HepG2 cells, determining cell viability by ATP measurements, revealed no issues, and the molecule was inactive in a 5-strain AMES test with and without S9 fractions. Stability in human, cynomolgus monkey, dog and rat hepatocytes was high, and subsequently the major metabolites arising from incubation with hepatocytes from these four species were characterized. In all species mono-demethylation of 27 was the major metabolite, with subsequent glucuronide conjugation also observed. Low levels of mono-oxidation, with and without sulfation or glucuronidation, were also noted. The two regioisomeric de-methylated metabolites of 27 were synthesized and profiled in binding assays, and in each case a significant drop in affinity (approximately 80-fold) compared to the parent was observed, suggesting a low risk of active metabolite formation *in vivo*. No metabolites that were unique to human were observed, and there was no evidence of addition of glutathione to the parent molecule or metabolites, which would be indicative of a risk of formation of a reactive metabolite. Cross-screening of 27 in a small number of in-house GPCR radioligand binding assays (M_1 , M_2 , M_3 , M_4 , CGRP, GLP1, mGlu₅) provided initial confidence that the molecule had good selectivity for the OX receptors. Wider profiling in an external panel of 14 GPCR, kinase, ion-channel and nuclear receptor targets indicated that 27 had at least 1000 fold selectivity against the panel members. Compound 18, a close analog of 27, had been earlier screened against an extended panel of 68 targets and also had excellent selectivity, providing overall confidence in the selectivity of the chemotype.

With these data in hand, 27 was advanced to a rat *ex vivo* autoradiography experiment (RenaSci Ltd, Nottingham, UK) to evaluate OX₂ receptor occupancy in the CNS from an oral dose. Male Sprague Dawley rats were dosed orally with vehicle alone (10% DMA, 10% solutol HS15, 80% (10% aqueous (2-hydroxypropyl)- β -cyclodextrin)) or 27 (1 mg kg⁻¹, po) and sacrificed 1 h post-dose. Frontal cortex sections were prepared and incubated with [³H]EMPA,⁵⁰ with levels of bound radioactivity in the sections determined using a beta imager. Receptor occupancy was determined from mean specific binding with the vehicle treated control taken as 100%. In this study 27 demonstrated a mean receptor occupancy of 57% from an oral dose of 1 mg kg⁻¹ (Fig. 6); by comparison, suvorexant from a 1 mg kg⁻¹ oral dose achieved 31% receptor occupancy. Plasma exposures at this time point were 72 ng mL⁻¹ and 22 ng mL⁻¹ for 27 and suvorexant respectively. The study confirmed that despite the fast receptor kinetics of 27, high occupancy of the OX₂ receptors could be achieved from an oral dose.

The effect of 27 treatment on rat sleep architecture *in vivo* was investigated using a telemetered CT18 sleep study (Aptuit (Verona) Srl., Italy), a model which has previously been used to support orexin lead optimisation programmes prior to positive clinical proof of concept in insomnia patients.⁵¹ Adult male CD rats were implanted with telemetric probes to record electroencephalogram (EEG) and neck electromyogram (EMG) readings. The study employed a balanced cross-over design in which all animals were alternatively treated with drug and vehicle. Rats were dosed with vehicle (80%

Table 4 Profile of 27

OX ₁ pK _i 7.7 ^a	Permeability ^c 52 × 10 ⁻⁶ cm s ⁻¹ (efflux ratio 0.7)
OX ₂ pK _i 8.6 ^a	
OX ₁ pK _b 6.6 ^a	PPB ^d 99.3, 98.1, 99.2
OX ₂ pK _b 7.3 ^a	
OX ₁ kinetics: $t_{1/2}$ 0.8 min (SPR)	Hepatocyte $t_{1/2}$: >110 min (rat, dog), >250 min (cyno, human)
OX ₂ kinetics: $t_{1/2}$ 4.2 min (SPR), 7.6 min (biochemical)	
P450 inhibition pIC ₅₀ < 5 ^b	No reactive metabolites
hERG pIC ₅₀ < 5	Ames negative ^e

^a See ESI for assay details. ^b 1A2, 2C8, 2C9, 2C19, 2D6 and 3A4 isoforms. ^c Caco-2 monolayer, P_{app} A–B. ^d Binding to human, canine and murine plasma proteins respectively. ^e 5 strains, with and without S9 fractions.

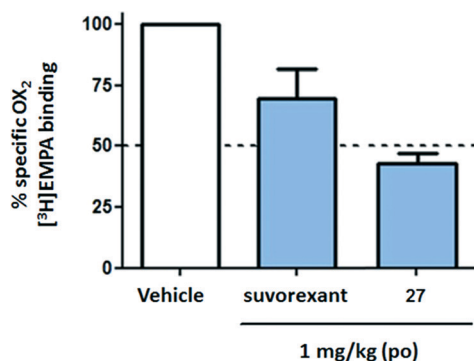


Fig. 6 $[^3\text{H}]\text{EMPA}$ *ex vivo* autoradiography in rat frontal cortex 1 hour following oral administration of 27 and suvorexant at 1 mg kg^{-1} .

PEG-400 and 20% Cremophor® EL) or with 27 (3 and 10 mg kg^{-1}) po, administered at circadian time 18h (CT18, six hours after lights off). The effect of 27 on sleep patterns was evaluated starting the recording 6 hours in to the dark phase. EEG and neck EMG readings were recorded and analysed for 5 hours after treatment to determine time spent awake, NREM and REM sleep (Fig. 7A–C). Sleep and wake stages in rats were altered by 27 compared to vehicle, with the hypnotic effect of 27 starting to decline after 3 hours. Peak effects on sleep parameters occurred at approximately 2 h, in contrast to a T_{max} of approximately 0.5 h in the rat oral PK study, and a clear dose response in several of the sleep parameters examined was not apparent. Given the limitations of this initial study further *in vivo* efficacy experiments are warranted to better understand the properties of 27. During episodes of insomnia, it is important to decrease the time spent awake during the night, a clinical end point measured during a sleep trial, as well as increasing the duration of sleep. At 3 and 10 mg kg^{-1} , 27 promoted sleep and reduced the time spent awake by 17.4 and 18.5% ($p < 0.05$; Fig. 7D),

respectively, over 3 hours. The time spent asleep was also increased by both doses of 27, which promoted sleep primarily by increasing REM, with smaller effects on NREM. Over 3 hours, the time spent in REM sleep was increased by 70.6 and 69.6% ($p < 0.05$; Fig. 7F) by 3 and 10 mg kg^{-1} 27. The time spent in NREM sleep was significantly increased by 28.8 and 30.7% ($p < 0.01$; Fig. 7E) at 3 and 10 mg kg^{-1} 27, respectively. Consistent with a smaller effect on NREM, there was no significant effect on the latency to the first NREM episode following treatment with 27 at the doses tested (Fig. 7G). However, the latency to the first REM episode was reduced by treatment with 10 mg kg^{-1} 27, reducing the time taken by 35.0% from 70.9 ± 7.7 to 46.1 ± 6.6 min ($p < 0.05$; Fig. 7H) compared to vehicle. In a similar rat sleep model, suvorexant demonstrated significant changes in sleep architecture from a 30 mg kg^{-1} dose with greater effects on REM than NREM, providing overall confidence that 27 demonstrates encouraging efficacy *in vivo*.⁵²

Conclusions

In summary, the series described herein, exemplified by compounds such as 18 and 27, represents a novel, high affinity dual orexin receptor antagonist chemotype, with a good overall *in vitro* and *in vivo* developability profile. Based on a consideration of the requirements for a potential treatment for insomnia, compound 27 (HTL6641), was identified as having the best balance of properties in the chemical series. Key factors which warrant the further investigation of 27 are its appropriate pre-clinical PK profile, high CNS receptor occupancy, fast off-rate kinetics and relevant *in vivo* efficacy in a rat sleep study.

Experimental details

All studies involving live animals were performed in compliance with national laws and institutional guidelines, following approval by the relevant committees of the study centres.

Synthetic methods

The purity of the final compounds was determined by LC/MS analysis to be $\geq 95\%$. Supplier information for purchased compounds, LCMS QC data for all compounds, experimental details and ^1H NMR data for all synthesized compounds in Tables 1 and 2 are described in the ESI.† Suvorexant was synthesized in accordance with literature conditions.⁵³ J values are given in Hz. Synthesis of 27 is described below.

2-Chloro-*N*-(5,6-dimethoxypyridin-3-yl)pyridine-3-sulfonamide.

A mixture of 2-chloropyridine-3-sulfonyl chloride (530 mg, 2.50 mmol), 5,6-dimethoxypyridin-3-amine (424 mg, 2.75 mmol) and pyridine (0.60 mL, 7.42 mmol) in DCM (10 mL) was stirred at 0°C for 3 h before concentration *in vacuo*. Purification by gradient column chromatography, eluting with 12–60% EtOAc in iso-hexane yielded the title compound (542 mg, 1.64 mmol, 66%) as a pale orange solid. m/z 328.2, 330.2 ($\text{M} - \text{H}^-$); δ_{H} (400 MHz; $\text{d}_6\text{-DMSO}$) 3.70 (3 H, s),

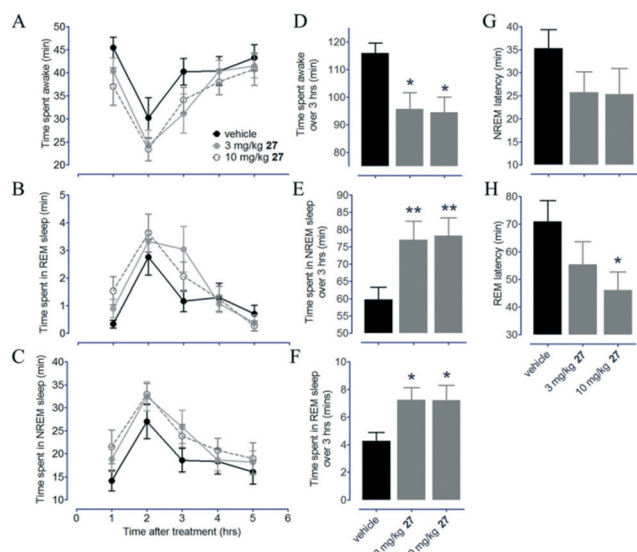


Fig. 7 Telemetered rat circadian time (CT) 18 sleep study of 27.

3.77 (3 H, s), 7.02 (1 H, d, *J* 2.3), 7.39 (1 H, d, *J* 2.3), 7.61 (1 H, dd, *J* 7.9, 4.8), 8.38 (1 H, dd, *J* 7.9, 1.8), 8.64 (1 H, dd, *J* 4.8, 1.8), 10.71 (1 H, s).

***N*-(5,6-Dimethoxypyridin-3-yl)-2-[(2,4,6-trifluorobenzyl)-amino]pyridine-3-sulfonamide.** A mixture of 2-chloro-*N*-(5,6-dimethoxypyridin-3-yl)pyridine-3-sulfonamide (165 mg, 0.50 mmol) and 2,4,6-trifluorobenzylamine (0.18 mL, 1.47 mmol) in MeCN (3 mL) was heated in a microwave reactor at 180 °C for 1 h. After concentration *in vacuo* purification by gradient column chromatography, eluting with 7–60% EtOAc in iso-hexane yielded the title compound (218 mg, 0.48 mmol, 96%) as a yellow oil. *m/z* 453.2 (*M* – *H*)⁺; δ_{H} (400 MHz; *d*₆-DMSO) 3.62 (3 H, s), 3.73 (3 H, s), 4.60 (2 H, d, *J* 5.5), 6.49–6.72 (2 H, m), 6.83 (1 H, d, *J* 2.3), 7.14 (2 H, t, *J* 8.7), 7.21 (1 H, d, *J* 2.3), 7.78 (1 H, dd, *J* 7.8, 1.8), 8.22 (1 H, dd, *J* 4.8, 1.6), 10.27 (1 H, s).

2-(5,6-Dimethoxypyridin-3-yl)-4-(2,4,6-trifluorobenzyl)-2*H*-pyrido[2,3-*e*][1,2,4]thiadiazin-3(4*H*)-one 1,1-dioxide (27, HTL6641). A mixture of *N*-(5,6-dimethoxypyridin-3-yl)-2-[(2,4,6-trifluorobenzyl)amino]pyridine-3-sulfonamide (218 mg, 0.48 mmol), 1,1'-carbonyldiimidazole (311 mg, 1.92 mmol) and triethylamine (0.13 mL, 0.93 mmol) in DMF (1.5 mL) in a sealed tube was heated at 100 °C for 2 h. After concentration *in vacuo* purification by gradient column chromatography, eluting with 10–80% EtOAc in iso-hexane yielded the title compound (159 mg, 0.33 mmol, 69%) as a white solid. *m/z* 481.1 (*M* + *H*)⁺; δ_{H} (400 MHz; *d*₆-DMSO) 3.77 (3 H, s), 3.93 (3 H, s), 5.59 (2 H, s), 7.19 (2 H, t, *J* 8.8), 7.35 (1 H, d, *J* 2.0), 7.53 (1 H, dd, *J* 7.8, 4.8), 7.75 (1 H, d, *J* 2.0), 8.51 (1 H, dd, *J* 7.8, 1.8), 8.85 (1 H, dd, *J* 4.9, 1.6); δ_{F} (376 MHz; *d*₆-DMSO, C₆H₅CF₃) –111.0 (1 F), –113.6 (2 F); δ_{C} (101 MHz; CD₃CN) 36.8 (1 C), 54.5 (1 C), 56.7 (1 C), 101.3 (2 C, ddd, ²*J*_{CF} 26.1, ²*J*_{CF} 26.1, ⁴*J*_{CF} 2.3), 110.4 (1 C, td, ²*J*_{CF} 18.4, ⁴*J*_{CF} 4.6), 119.9, 120.8, 122.1, 122.9, 133.7 (1 C), 138.8 (1 C), 145.6 (1 C), 147.9 (1 C), 151.0 (1 C), 154.4 (1 C), 156.3 (1 C), 162.9 (2 C, ddd, ¹*J*_{CF} 249.6, ³*J*_{CF} 15.3, ³*J*_{CF} 11.1), 163.3 (1 C, dt, ¹*J*_{CF} 247.7, ³*J*_{CF} 16.1).

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