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Structure–activity relationship studies and sleep-promoting activity of novel 1-chloro-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyrazine derivatives as dual orexin receptor antagonists. Part 2

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ABSTRACT

Replacement of the dimethoxyphenyl moiety in the core skeleton of almorexant by appropriately substituted imidazoles afforded novel 1-chloro-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyrazine derivatives as potent dual orexin receptor antagonists. We describe in this Letter our efforts to further optimize the potency and brain penetration of these derivatives by fine-tuning of the pivotal phenethyl motif, and we comment on the sleep-promoting activity of selected compounds in a rat electroencephalographic (EEG) model.

The neuropeptides orexin-A and B (or hypocretin-1 and 2) are produced by a small population of neurons in the lateral hypothalamus. Both peptides are endogenous ligands for two G-protein-coupled receptors (GPCRs) known as orexin-1 (OX_1R) and orexin-2 (OX_2R) receptors.[1,2] Since the discovery of the orexin neuropeptides in 1998, numerous studies have highlighted their implication in the regulation of major biological functions including feeding[2] and the sleep–wake cycle.[3,4] We have disclosed that almorexant (ACT-078573), an orally-administered dual orexin receptor antagonist (DORA),[5] promoted robust sleep responses without evidence of cataplexy in rats, dogs, and humans.[6] In the meantime, additional clinical proof-of-concept studies have substantiated the finding that orexin receptor antagonists promote sleep and therefore offer potential as a novel therapy for the treatment of primary insomnia.[7] Since the discontinuation in 2011 of the clinical development of almorexant, there is a need for new molecules devoid of adverse effects. In search of non-peptide, low molecular weight orexin receptor antagonists, we investigated the heterocyclic replacement of the dimethoxyphenyl unit in almorexant.[8] Thus, we reported that the replacement of this moiety by a substituted pyrazole afforded the corresponding pyrazolo-tetrahydropyridines as potent DORAs.[9] Our laboratory also described that replacement of the dimethoxyphenyl unit by appropriately substituted imidazoles led to 1-chloro-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyrazines as a novel series of DORAs.[10] We now describe in this Letter (i) our efforts to further optimize the potency and brain penetration of these derivatives by fine-tuning of the substituted phenyl moiety (Fig. 1), and (ii) the sleep-promoting activity of selected compounds in a rat electroencephalographic (EEG) model.

Initial structure–activity relationship (SAR) studies in the 5,6,7,8-tetrahydroimidazo[1,5-*a*]pyrazine series allowed to identify appropriate substituents for the imidazole moiety in order to obtain DORAs. Thus, these preliminary investigations have emphasized that a chloro substituent was required for carbon C1, and that ethyl or cyclopropyl represented the two preferred substituents for carbon C3 (Fig. 1).[10] However, oral administration to male Wistar rats of these two novel DORAs, as direct analogues of almorexant containing the *p*-CF_3-phenethyl, afforded five to sixfold lower brain concentrations than with almorexant.[10] Therefore, in a next step, we envisaged to evaluate the influence of the substituted phenyl moiety on brain penetration with the goal to improve brain exposure for these novel 1-chloro-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyrazines.[11] In order to reach this goal, diversely substituted 1-chloro-3-ethyl-5,6,7,8-tetrahydroimidazo[1,5-*a*]-pyrazines **9** were synthesized (Scheme 1) starting from the key tri-substituted imidazole **4** which was efficiently prepared according to our previously described route of synthesis.[10] Thus, diiodination of commercially available 2-ethyl-1*H*-imidazole **1** afforded smoothly

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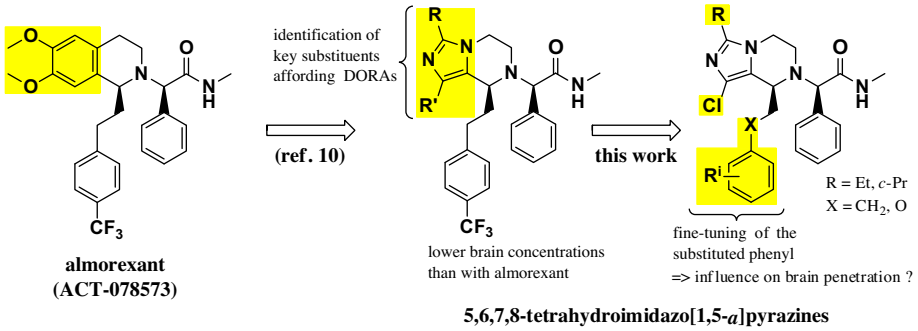
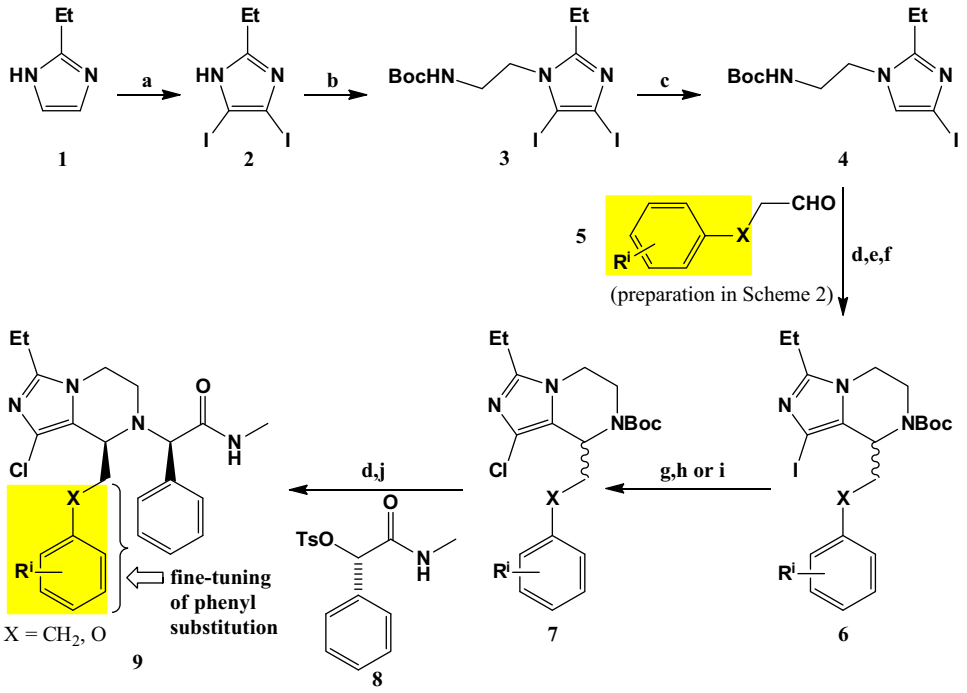


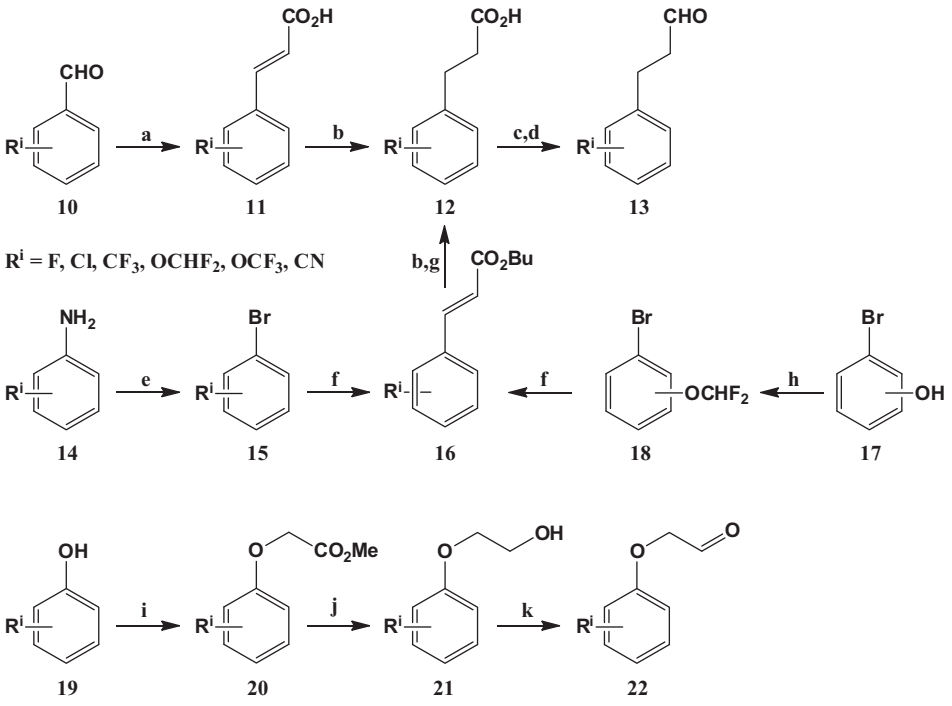
Figure 1. Almorexant and the investigated 1-chloro-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyrazines as novel DORAs.



Scheme 1. Preparation of novel 1-chloro-3-ethyl-5,6,7,8-tetrahydroimidazo[1,5-*a*]-pyrazines **9**. Reagents and conditions: (a) I_2, Na_2CO_3, dioxane, H_2O, rt (99%); (b) NaH, DMF, rt, then $Br(CH_2)_2NHBoc$, 100 °C (75%); (c) (i) 3 M EtMgBr in Et_2O, THF, −40 °C, (ii) H_2O (95%); (d) 4 M HCl in dioxane, CH_2Cl_2, 0 °C to rt (100%); (e) aldehyde **5**, DIPEA, EtOH, microwave (50 W; 140 °C; 6 bar; 10 min); (f) Boc_2O, DIPEA, CH_2Cl_2, rt (38–87% over two steps); (g) H_2 (1 atm), 10% Pd/C (10% in weight), K_2CO_3, MeOH, rt (72–100%); (h) *N*-chlorosuccinimide (1.0 equiv), MeCN, 70 °C (31–85%); (i) *n*-BuLi, hexachloroethane, THF, −78 °C (28–66%); (j) tosylate **8**, DIPEA, 3-methyl-2-butanone, 80 °C (25–60%).

the corresponding 2-ethyl-4,5-diiodo-1*H*-imidazole **2** that was converted to the related derivative **3** by *N*-alkylation. A totally regioselective iodine/magnesium exchange of the 5-iodo moiety in **3** led to the desired 4-iodoimidazole **4**. After quantitative Boc-deprotection of **4**, the resulting primary amine was allowed to react in a microwave-assisted Pictet–Spengler reaction[12] with a variety of aldehydes **5** (preparation described in Scheme 2). A subsequent Boc-protection delivered racemic 5,6,7,8-tetrahydroimidazo[1,5-*a*]pyrazines **6**. The iodo-substituent allowed the straightforward introduction of diverse substituents as illustrated in our previous communication.[10] For the purpose of our planned investigations, the pivotal chloro substituent was advantageously incorporated in **7** via hydrogenolytic cleavage of the iodo-substituent in **6** (H_2, 10% Pd/C, K_2CO_3, MeOH) followed by regioselective C1-chlorination of the imidazole moiety (*N*-chlorosuccinimide, MeCN). Alternatively, this chlorination was performed by iodine/lithium exchange with **6** and subsequent trapping of the intermediate carbanion with hexachloroethane. Boc-deprotection of **7** and *N*-alkylation with the chiral (*S*)-tosylate **8** delivered a diastereoisomeric mixture of 1-chloro-3-ethyl-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyrazines **9** that were finally separated into the pure enantiomers via chromatography.

Substituted aldehydes **5** were required for the investigation of the phenyl moiety, and several pathways allowed their convenient preparation (Scheme 2). Thus, Knoevenagel–Doebner condensation between benzaldehydes **10** and malonic acid gave the corresponding cinnamic acids **11**. A subsequent catalytic hydrogenation, under standard conditions (H_2, 10% Pd/C, MeOH) or under improved reaction conditions with chloro-substituted derivatives (H_2, 10% Pd/C, $ZnBr_2$, AcOEt),[13] afforded the related hydrocinnamic acids **12**. The target aldehydes **13** were then obtained after a reduction/oxidation sequence. An alternative preparation of the hydrocinnamic acids **12** was based on a palladium-catalyzed Heck reaction (aryl bromides **15**, *n*-butyl acrylate, DABCO, K_2CO_3, $Pd(OAc)_2$, DMF)[14] that was then followed by a palladium-catalyzed hydrogenation of **16** and a subsequent saponification. For this last approach, the required aryl bromides **15** were prepared from the corresponding anilines **14** via Sandmeyer reaction (*tert*-butyl ni-

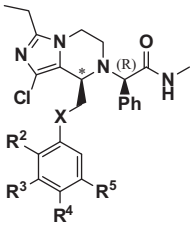


Scheme 2. Synthetic routes towards substituted aldehydes **13** and **22**. Reagents and conditions: (a) malonic acid, pyridine, piperidine, 75 °C (84–96%); (b) standard conditions: H_2 (1 atm), 10% Pd/C (10% in weight), MeOH, rt (86–100%); for chloro-substituted aryl derivatives: H_2 (1 atm), 10% Pd/C (6% in weight), $ZnBr_2$ (0.2 equiv), AcOEt, rt (85–92%); (c) 1 M BH_3 in THF, THF, 0 °C to rt (88–99%); (d) pyridinium chlorochromate, CH_2Cl_2, 0 °C to rt (52–93%); (e) $CuBr_2$, *tert*-butyl nitrite, MeCN, 45 °C (70–86%); (f) *n*-butyl acrylate, DABCO, K_2CO_3, $Pd(OAc)_2$, DMF, 120 °C (53–97%); (g) 1 M NaOH, MeOH, H_2O, rt (94–100%); (h) $ClCF_2CO_2Na$, K_2CO_3, DMF, H_2O, 100 °C (74%); (i) methyl bromoacetate, K_2CO_3, acetone, reflux (98–100%); (j) $LiAlH_4$, THF, 0 °C (93–99%); (k) oxalyl chloride, DMSO, Et_3N, CH_2Cl_2, −78 °C (95–99%).

trite, $CuBr_2$, MeCN).[15] Difluoromethoxy-substituted aldehydes **13** were efficiently prepared by heating the bromo-phenols **17** in presence of sodium chlorodifluoroacetate.[16] Finally, substituted 2-phenoxyacetaldehydes **22** were obtained from the related phenols **19** after *O*-alkylation with methyl bromoacetate, reduction of the methyl ester, and subsequent oxidation of the resulting primary alcohol under Swern conditions.[17]

The antagonistic activity of the novel 1-chloro-3-ethyl-5,6,7,8-tetrahydroimidazo[1,5-*a*]-pyrazines **9** with both orexin receptors was determined with a cell-based FLIPR assay (fluorometric imaging plate reader) measuring Ca^{2+} flux as a functional determinant of orexin binding.[18] The results of the structure–activity relationship studies of the substituted phenyl derivatives are summarized in Table 1. Preliminary investigations were devoted towards derivatives containing a mono-substituted phenyl ring. Thus, moving the electron-withdrawing trifluoromethyl moiety present in almorexant from the *para*-position (stereoisomer **23**) to the *meta*-position (**24**) induced an almost twofold loss in hOX_1R potency while maintaining the affinity for hOX_2R. The importance of the linker between the 1-chloro-3-ethyl-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyrazine and the trifluoromethylphenyl ring was as well investigated. Thus, the CH_2O linker led to a substantial loss of affinity for hOX_1R as illustrated with the potent selective orexin 2 receptor antagonists **25** and **26**. Additional electron-withdrawing substituents were evaluated for comparison with trifluoromethyl. A *para*-cyano group (stereoisomer **27**) resulted in strongly decreased activity for the hOX_1R (100-fold loss in potency) while high potency at hOX_2R was maintained. Compared to *para*-trifluoromethyl, a *para*-difluoromethoxy moiety resulted in an almost twofold loss in hOX_1R potency while allowing a ninefold increase in potency for hOX_2R (**28**). The electron-withdrawing *para*-trifluoromethoxy residue as well resulted in a significant loss of activity towards the hOX_1R as shown with the preferential orexin 2 receptor antagonist **29**. After this initial exploration of derivatives containing mono-substituted phenyl rings, the influence of poly-substitution was scrutinized. The addition of a *meta*-fluorine atom on the *para*-trifluoromethylphenyl was beneficial, with a twofold increase in hOX_1R potency, and afforded derivative **30** which is one of the most potent DORA identified in this series. The influence of the stereochemistry was investigated with the potent (*S*;*R*) stereoisomer **30** in comparison to the corresponding (*R*;*R*) isomer **31**, which was significantly less potent against both orexin receptors (30- and 32-fold decrease, respectively, in hOX_1R and hOX_2R potency with **31**). For potent affinities with the orexin receptors, it is mandatory that the compounds possess the (R)-configuration at the phenylglycine moiety (see structure associated to Table 1). Thus, the two additional stereoisomers corresponding to **30** and **31**, and that contain the (S)-configured phenylglycine moiety, were essentially inactive (IC_{50} >1 μM with both orexin receptors). Compared to *meta*-fluorine in **30**, a *meta*-chloro substituent on the *para*-trifluoromethylphenyl (derivative **32**) was equally beneficial for the hOX_1R potency but chlorine generated an almost threefold drop in hOX_2R affinity. The association of an *ortho*-fluorine atom and a *para*-trifluoromethyl group did not alter the interactions with the orexin receptors as shown with the equipotent DORAs **23** and **33**. Difluorination of the *para*-trifluoromethylphenyl was well tolerated (stereoisomers **34**–**36**) as shown with compound **36**. A trifluorinated phenethyl moiety (stereoisomer **37**) allowed potent interactions with both orexin receptors, and the trifluoromethyl group is therefore not a mandatory structural feature for obtaining potent DORAs. In a next step, additional substitution of the *meta*-trifluoromethylphenyl was further investigated. Thus, the addition of a *para*-fluorine atom resulted in lower potency for the hOX_1R as shown for the selective orexin 2 receptor antagonist **38**. On the other hand, the insertion of an *ortho*-fluorine atom on the *meta*-trifluoromethylphenyl proved to be beneficial as shown with the significant increase in hOX_1R potency for DORA **39** as compared to compound **38**.

Table 1
SAR studies of 1-chloro-3-ethyl-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyrazines: influence of the substituted phenyl on potency



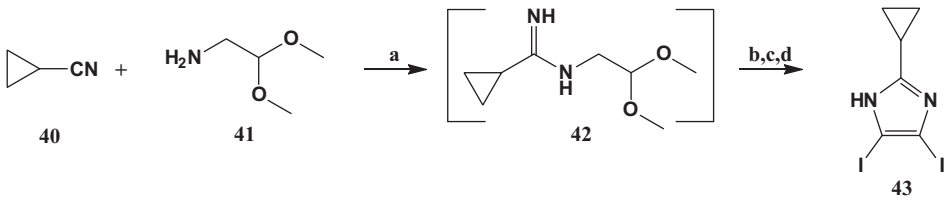
Compound	X	Stereochemistry	R^2	R^3	R^4	R^5	hOX_1R[a]	hOX_2R[a]
Almorexant							13	8
23	CH_2	(*S*;*R*)	H	H	CF_3	H	40	9
24	CH_2	(*S*;*R*)	H	CF_3	H	H	72	10
25	O	(*R*;*R*)	H	H	CF_3	H	431	9
26	O	(*R*;*R*)	H	CF_3	H	H	312	4
27	CH_2	(*S*;*R*)	H	H	CN	H	3902	16
28	CH_2	(*S*;*R*)	H	H	$OCHF_2$	H	74	1
29	CH_2	(*S*;*R*)	H	H	OCF_3	H	203	18
30	CH_2	(*S*;*R*)	H	F	CF_3	H	18	7
31	CH_2	(*R*;*R*)	H	F	CF_3	H	547	227
32	CH_2	(*S*;*R*)	H	Cl	CF_3	H	20	18
33	CH_2	(*S*;*R*)	F	H	CF_3	H	45	12
34	CH_2	(*S*;*R*)	F	F	CF_3	H	17	14
35	CH_2	(*S*;*R*)	F	H	CF_3	F	32	17
36	CH_2	(*S*;*R*)	H	F	CF_3	F	16	8
37	CH_2	(*S*;*R*)	F	F	F	H	30	5
38	CH_2	(*S*;*R*)	H	CF_3	F	H	378	16
39	CH_2	(*S*;*R*)	F	CF_3	H	H	16	9

[a] IC_{50} values in nM (FLIPR assay).

We also planned to evaluate the influence on brain penetration of a cyclopropyl substituent on carbon C3 of the 5,6,7,8-tetrahydroimidazo[1,5-*a*]pyrazines. A set of 1-chloro-3-cyclopropyl-5,6,7,8-tetrahydroimidazo[1,5-*a*]-pyrazines was prepared according to the previously described synthetic route (Scheme 1) but starting from 2-cyclopropyl-4,5-diiodo-1*H*-imidazole **43**. The trisubstituted imidazole **43** was obtained via a copper(I)-induced addition of 2,2-dimethoxyethanamine **41** to cyclopropanecarbonitrile **40**, and subsequent cyclisation of the resulting amidine **42** under acidic conditions followed by diiodination (Scheme 3).[19]

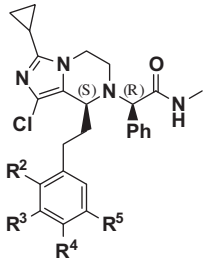
The antagonistic activities of the cyclopropyl-substituted derivatives are collected in Table 2. Shifting the electron-withdrawing trifluoromethyl moiety from the *para*-position (DORA **44**) to the *meta*-position (**45**) induced an almost 3-fold loss in hOX_1R potency while not affecting the potency at hOX_2R. In contrast to the ethyl-substituted analogue **30**, the addition of a *meta*-fluorine atom on the *para*-trifluoromethylphenyl unit resulted in an almost twofold drop in hOX_1R potency (stereoisomer **46**). The insertion of an *ortho*-fluorine atom (derivative **47**) negatively affected the interactions with both orexin receptors. Replacement of the *para*-trifluoromethyl in **46** by a *para*-difluoromethoxy moiety resulted in an almost threefold decrease in hOX_1R potency, and a fivefold increase in potency at hOX_2R as shown with the potent selective orexin 2 receptor antagonist **48**. A general trend after evaluation of this set of compounds is that the cyclopropyl-containing derivatives are less potent towards hOX_1R than the corresponding ethyl-substituted analogues while the hOX_2R potency remained fairly similar for both series.

The potential for blood–brain barrier penetration of 10 leading DORAs of the 1-chloro-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyrazine series was evaluated, and concentrations of the dual orexin receptor antagonists in brain and plasma sampled 3 h following oral administration (100 mg/kg, po) to male Wistar rats were measured (Table 3). For this study, reference compounds for comparison of brain exposure are **23** and **44**, two direct analogues of almorexant containing the *para*-CF_3-phenethyl moiety, which exhibited five to sixfold lower brain concentrations than almorexant.[10] It was particularly noteworthy to observe that the potent DORA **30**, carrying an additional *meta*-fluorine atom on the *para*-trifluoromethylphenyl unit, afforded a fourfold increase in brain concentration (1222 nM) as compared to **23**. The corresponding brain to plasma ratio ([B]/[P] = 0.12) was also twofold improved. A similar increase in CNS penetration due to the presence of a *meta*-fluorine atom could not be observed for the related cyclopropyl-substituted derivative **46**, presenting a fairly comparable brain concentration and brain to plasma ratio as parent compound **44**. Replacement of the *meta*-fluorine atom in **30** by a *meta*-chloro substituent led to a 10-fold decrease in brain concentration for compound **32**



Scheme 3. Synthesis of 2-cyclopropyl-4,5-diiodo-1*H*-imidazole **43**. Reagents and conditions: (a) CuCl, neat, 0–85 °C; (b) thioacetamide, MeOH, 0–45 °C; (c) 12 M HCl, MeOH, 0–80 °C; (d) I_2, Na_2CO_3, dioxane, H_2O, rt (overall yield: 26%).

Table 2
SAR studies of 1-chloro-3-cyclopropyl-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyrazines: influence of the substituted phenyl moiety on potency



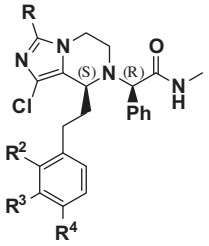
Compound	R^2	R^3	R^4	R^5	hOX_1R[a]	hOX_2R[a]
44	H	H	CF_3	H	30	9
45	H	CF_3	H	H	91	7
46	H	F	CF_3	H	53	10
47	F	H	CF_3	H	94	26
48	H	F	$OCHF_2$	H	170	2

[a] IC_{50} values in nM (FLIPR assay).

(128 nM). The insertion of an *ortho*-fluorine atom on the *para*-trifluoromethylphenyl moiety for DORAs **33** and **47** did not influence CNS exposure, and the resulting brain concentrations remained similar to those measured for the reference compounds **23** and **44**. A beneficial increase of CNS penetration associated with a *meta*-fluorine atom could again be observed for the difluorinated derivative **34** that allowed an almost twofold increase of brain concentration compared to compounds **23** and **33**. On the other hand, shifting the trifluoromethyl group from the *para*-position in **33** to the *meta*-position (**39**) appeared to result in an almost threefold drop in brain concentration. Finally, replacement of the *para*-trifluoromethyl moiety in **34** by a *para*-fluorine atom (**37**) generated a dramatic 40-fold reduction of brain concentration, and this observation underlined the pivotal role of the trifluoromethyl group for brain penetration in the 1-chloro-5,6,7,8-tetrahydroimidazo[1,5-*a*]-pyrazine series. Specific observations from this structure-brain exposure relationship study and values of [B]/[P] ratio may be rationalized by the fact that some 1-chloro-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyrazines have affinities for the rat P-glycoprotein (P-gp) transporter system.[20] The simple addition of a fluorine substituent on the phenethyl moiety can mitigate the rat P-glycoprotein-mediated efflux of compounds (DORA **30** vs **23**) and fluorination can therefore represent a viable strategy to reduce P-gp recognition in this series of DORAs. In order to further evaluate permeability and human P-glycoprotein-mediated efflux properties for DORA **30**, two crucial parameters for blood–brain barrier penetration, we conducted a MDR1-MDCK permeability experiment. [20c] The apparent passive permeability measured bi-directionally across MDR1-MDCK cell monolayers was 1.98×10^{-6} cm/s ($Papp_{A\text{-}to\text{-}B}$) in the A-to-B direction and 68.8×10^{-6} cm/s ($Papp_{B\text{-}to\text{-}A}$) in the B-to-A direction. The resultant high human P-gp-mediated efflux ratio (ER = 35) underlined the strong affinity of compound **30** for the human P-gp transporter system and, as a consequence, DORA **30** should exhibit low brain penetration in humans.

The potent DORA **30** was evaluated in an in vivo BBB-experiment.[21] Brain penetration was estimated in male Wistar rats at 3 h following oral administration of 100 mg/kg formulated in PEG-400. At this time point, total brain concentration reached 639 ng/g (1.22 μM). These concentrations were sufficient to impact sleep and wake states. The effects of DORA **30** on sleep and wake cycles were evaluated in freely moving adult male Wistar rats implanted with radiotelemetric probes recording electroencephalographic (EEG) and electromyographic (EMG) signals as well as locomotor activity.[22] DORA **30** showed comparable sleep-promoting activity as almorexant when tested under the same experimental conditions, that is when drugs were administered at the beginning of the dark active phase when the orexin level rises (Fig. 2). Behaviourally, DORA **30**, at 100 mg/kg po, decreased significantly the home cage activity by 36% over the 12 h night period following administration compared to vehicle treated animals (−43% for almorexant 100 mg/kg po vs vehicle, p <0.001, paired *t*-test for both DORA **30** and almorexant; Fig. 2A). It translated electrophysiologically by a significant decrease of the time spent in active wake (−13% and −20% vs vehicle for DORA **30** and almorexant, respectively, p <0.001 for both compounds, paired *t*-test, Fig. 2B). Homeostatically, the time spent in non-REM (rapid eye movement) sleep was significantly increased (+16% and +20% vs vehicle for

Table 3
Brain and plasma concentrations of DORAs from the 1-chloro-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyrazine series after oral administration to male Wistar rats



Compound	R	R^2	R^3	R^4	[Brain][a] (nM)	[Plasma][a] (nM)	[B]/[P] ratio	hOX_1R[b]	hOX_2R[b]
23	Et	H	H	CF_3	303	4210	0.07	40	9
44	*c*-Pr	H	H	CF_3	385	6398	0.06	30	9
30	Et	H	F	CF_3	1222	9815	0.12	18	7
46	*c*-Pr	H	F	CF_3	450	6804	0.07	53	10
32	Et	H	Cl	CF_3	128	1596	0.08	20	18
33	Et	F	H	CF_3	363	6425	0.06	45	12
47	*c*-Pr	F	H	CF_3	338	6760	0.05	94	26
34	Et	F	F	CF_3	695	6766	0.10	17	14
39	Et	F	CF_3	H	136	1855	0.07	16	9
37	Et	F	F	F	18	387	0.05	30	5

[a] Concentrations determined 3 h following oral administration to male Wistar rats (100 mg/kg).
[b] IC_{50} values in nM (FLIPR assay).

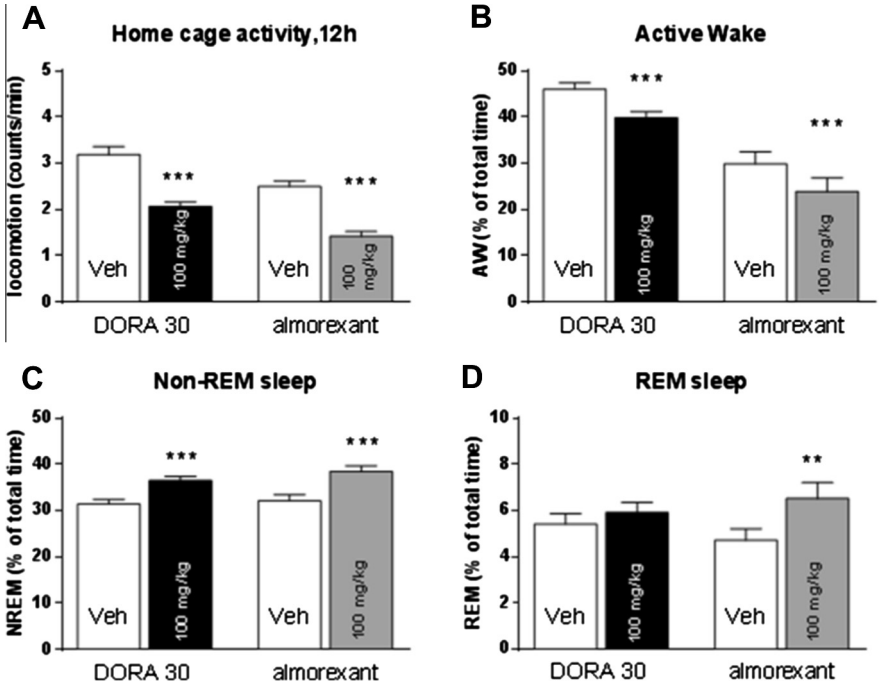


Figure 2. Effects of DORA **30** and almorexant on home cage activity (A), time spent in active wake (AW, B), time spent in non-REM sleep (NREM, C) and time spent in REM sleep (D). Effects evaluated over the 12 h night period following oral administration. Data are presented as means ± SEM. $^{**}p$ <0.01, $^{***}p$ <0.001 (n = 16 for DORA **30** and n = 12 for almorexant).

Table 4
Relative proportion of non-REM and REM sleep over the total sleep time of the 12 h night period following administration

	Non-REM sleep (% of total time)	REM sleep (% of total time)
Vehicle (DORA **30**)	85.4	14.6
DORA **30**	86.2	13.8
Vehicle (almorexant)	87.3	12.7
Almorexant	85.8	14.2

DORA **30** and almorexant respectively, p <0.001 for both, paired t-test, Fig. 2C). Finally, the time spent in REM sleep was also increased compared to vehicle treated rats, non-significantly with DORA **30** (+9%) and significantly for almorexant (+38%, p <0.01, paired t-test, Fig. 2D).

As already observed with other DORAs,[7b,23] the general architecture of the sleep, that is the relative proportion of time spent in non-REM and REM sleep over the total sleep time was maintained with 85–87% of non-REM sleep and 13–15% of REM sleep (Table 4).

In conclusion, we described additional structure–activity relationship studies concerning the phenethyl motif in the 1-chloro-5,6,7,8-tetrahydroimidazo[1,5-*a*]pyrazine series. We identified several potent dual orexin receptor antagonists in the low nanomolar inhibitory activity range which are suitable for further in vivo evaluation. Fine-tuning of the phenethyl part also underlined the substantial influence of this moiety on brain concentration. Finally, evaluation of 1-chloro-3-ethyl-5,6,7,8-tetrahydroimidazo[1,5-*a*]-pyrazine **30** in a rat EEG model indicated that sleep-promoting activity comparable to almorexant can be obtained in this novel series of DORAs. The issue of human P-gp recognition needs to be addressed and further optimization work is required in this series in order to improve brain exposure in humans by increasing membrane permeability and decreasing human P-gp-mediated efflux.

More details on the synthesis and analytical data for the compounds described herein are disclosed in the patent literature.[24]

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References and notes

1. de Lecea, L.; Kilduff, T. S.; Peyron, C.; Gao, X.; Foye, P. E.; Danielson, P. E.; Fukuhara, C.; Battenberg, E. L.; 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.
2. Sakurai, T.; Amemiya, A.; Ishii, M.; Matsuzaki, I.; Chemelli, R. M.; Tanaka, H.; Williams, S. C.; Richardson, J. A.; Kozlowski, G. P.; Wilson, S.; Arch, J.; 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.
3. 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.
4. 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* **1999**, *98*, 437.
5. A dual orexin receptor antagonist (DORA) is defined in this communication as having less than 20-fold selectivity for either hOX1R or hOX2R.
6. 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**, *13*, 150.
7. For recent and comprehensive reviews on the medicinal chemistry of orexin antagonists: (a) Roecker, A. J.; Coleman, P. J. *Curr. Top. Med. Chem.* **2008**, *8*, 977; (b) Boss, C.; Brisbare-Roch, C.; Jenck, F.; Aissaoui, H.; Koberstein, R.; Sifferlen, T.; Weller, T. *Chimia* **2008**, *62*, 974; (c) Boss, C.; Brisbare-Roch, C.; Jenck, F. *J. Med. Chem.* **2009**, *52*, 891; (d) Gatfield, J.; Brisbare-Roch, C.; Jenck, F.; Boss, C. *ChemMedChem* **2010**, *5*, 1197; (e) Coleman, P. J.; Renger, J. J. *Expert Opin. Ther. Patents* **2010**, *20*, 307; (f) Coleman, P. J.; Cox, C. D.; Roecker, A. J. *Curr. Top. Med. Chem.* **2011**, *11*, 696; (g) Christopher, J. A. *Pharm. Pat. Analyst* **2012**, *1*, 329.
8. Koberstein, R.; Aissaoui, H.; Bur, D.; Clozel, M.; Fischli, W.; Jenck, F.; Mueller, C.; Nayler, O.; Sifferlen, T.; Treiber, A.; Weller, T. *Chimia* **2003**, *57*, 270.
9. Sifferlen, T.; Boss, C.; Cottreel, E.; Koberstein, R.; Gude, M.; Aissaoui, H.; Weller, T.; Gatfield, J.; Brisbare-Roch, C.; Jenck, F. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1539.

10. For the first part describing our efforts in the 5,6,7,8-tetrahydroimidazo[1,5-*a*]pyrazine series: Sifferlen, T.; Koberstein, R.; Cottreel, E.; Boller, A.; Weller, T.; Gatfield, J.; Brisbare-Roch, C.; Jenck, F.; Boss, C. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2212.
11. Previous investigations in the corresponding tetrahydroisoquinoline and pyrazolo-tetrahydropyridine series have demonstrated that brain penetration can be substantially influenced by the substitution of the phenethyl motif.
12. For a comprehensive review on the synthesis of pharmaceutically relevant heterocycles based on Pictet–Spengler reactions: Pulka, K. *Curr. Opin. Drug Discov. Devel.* **2010**, *13*, 669.
13. Wu, G.; Huang, M.; Richards, M.; Poirier, M.; Wen, X.; Draper, R. W. *Synthesis* **2003**, *11*, 1657.
14. Li, J.-H.; Wang, D.-P.; Xie, Y.-X. *Synthesis* **2005**, *13*, 2193.
15. Doyle, M. P.; Siegfried, B.; Dellaria, J. F., Jr. *J. Org. Chem.* **1977**, *42*, 2426.
16. O'Shea, P. D.; Chen, C.-Y.; Chen, W.; Dagneau, P.; Frey, L. F.; Grabowski, E. J. J.; Marcantonio, K. M.; Reamer, R. A.; Tan, L.; Tillyer, R. D.; Roy, A.; Wang, X.; Zhao, D. *J. Org. Chem.* **2005**, *70*, 3021.
17. Mancuso, A. J.; Swern, D. *Synthesis* **1981**, *3*, 165.
18. *FLIPR assay:* Chinese hamster ovary (CHO) cells expressing the human orexin receptors (hOX_1R or hOX_2R) were seeded into 96-well plates and incubated at 37 °C in 5% CO_2 with the cytoplasmic fluorescent calcium indicator fluo-3 AM (Molecular Probes). After washing the cells, Ca^{2+} mobilization assays were performed by FLIPR (Molecular Devices): Differing concentrations of orexin receptor antagonists were added to the plates prior to addition of an approximate EC_{80} of orexin A. For each antagonist, the IC_{50} (the concentration of compound needed to inhibit 50% of the orexin A-induced calcium response) was calculated.
19. (a) Frutos, R. P.; Rodriguez, S.; Patel, N.; Johnson, J.; Saha, A.; Krishnamurthy, D.; Senanayake, C. H. *Org. Process Res. Dev.* **2007**, *11*, 1076; (b) Frutos, R. P.; Gallou, I.; Reeves, D.; Xu, Y.; Krishnamurthy, D.; Senanayake, C. H. *Tetrahedron Lett.* **2005**, *46*, 8369.
20. For articles discussing the influence of P-glycoprotein-mediated efflux of compounds in medicinal chemistry: (a) Hitchcock, S. A. *J. Med. Chem.* **2012**, *55*, 4877; (b) Mahar Doan, K. M.; Humphreys, J. E.; Webster, L. O.; Wring, S. A.; Shampine, L. J.; Serabjit-Singh, C. J.; Adkison, K. K.; Polli, J. W. *J. Pharmacol. Exp. Ther.* **2002**, *303*, 1029; (c) Feng, B.; Mills, J. B.; Davidson, R. E.; Mireles, R. J.; Janiszewski, J. S.; Troutman, M. D.; de Morais, S. M. *Drug Metab. Dispos.* **2008**, *36*, 268.
21. In vivo experiments were done on male Wistar rats. Rats are maintained on a 12 h light/12 h dark cycle. For BBB penetration studies, we measured the concentration of the orexin receptor antagonist in plasma and brain sampled 3 h following oral administration. Plasma and brain are collected from the same animal at the same time (±5 min). Blood is sampled from the vena cava caudalis into containers with EDTA as anticoagulant and centrifuged to yield plasma. Brain is sampled after cardiac perfusion of 10 mL NaCl 0.9% and homogenized into one volume of cold phosphate buffer (pH 7.4).
22. For pharmacodynamic sleep studies, animals were implanted with miniature radiotelemetric implants (Data Sciences International) under general anesthesia. Those implants consist of two pair of differential leads; one pair for cranial placement to record the electroencephalogram (EEG) and one pair placed in either side of the muscular neck to record the electromyogram (EMG). This technology allows stress-free acquisition of vigilance and sleep stages, spontaneous activity and body temperature from freely moving rats in their home cage environment. Compounds were administered orally at the beginning of the night dark cycle and formulated in 100% PEG-400. In each experiment, we used groups of 12 or 16 rats, in a crossover design, with at least 4-days washout periods separating consecutive administrations.
23. Aissaoui, H.; Koberstein, R.; Zumbrunn, C.; Gatfield, J.; Brisbare-Roch, C.; Jenck, F.; Treiber, A.; Boss, C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5729.
24. (a) Aissaoui, H.; Boss, C.; Gude, M.; Koberstein, R.; Sifferlen, T.; WO2008/078291, 2008, Actelion Pharmaceuticals Ltd.; (b) Aissaoui, H.; Boss, C.; Koberstein, R.; Siegrist, R.; Sifferlen, T.; WO2009/156951, 2008, Actelion Pharmaceuticals Ltd.