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Novel pyrazolo-tetrahydropyridines as potent orexin receptor antagonists

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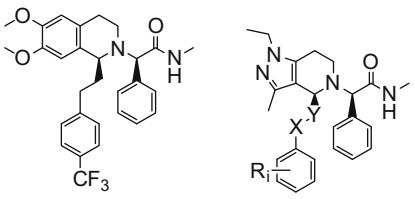
ABSTRACT

A novel series of dual orexin receptor antagonists was prepared by heteroaromatic five-membered ring system replacement of the dimethoxyphenyl moiety contained in the tetrahydroisoquinoline core skeleton of almorexant. Thus, replacement of the dimethoxyphenyl by a substituted pyrazole and additional optimization of the substitution pattern of the phenethyl motif allowed the identification of potent antagonists with low nanomolar affinity for hOX_1R and hOX_2R. The synthesis and structure–activity relationship of these novel antagonists will be discussed in this communication. These investigations furnished several suitable candidates for further evaluation in in vivo studies in rats.

In 1998, two independent research groups discovered two neuropeptides, orexin A and orexin B (or hypocretin-1 and hypocretin-2), which are produced exclusively by neurons in the lateral hypothalamus.[1,2] Two orphan G protein-coupled receptors (GPCR) for these endogenous ligands were as well identified and are known as orexin 1 (OX_1R) and orexin 2 (OX_2R) receptors. Orexin A shows potent agonistic activity toward both subtypes of orexin receptors,[2] whereas, orexin B exhibited greater affinity for OX_2R as compared to the OX_1R.[2] Several studies highlighted the potential role of the orexin neuropeptides in the regulation of a variety of biological functions including feeding,[2] and the sleep/wake cycle.[3,4] Several groups have developed orexin antagonists to explore the physiological role of the orexin receptors.[5] We have recently reported that a dual orexin receptor antagonist, almorexant (Fig. 1) enabled somnolence without cataplexy in rats, dogs, and humans.[6] In the course of our efforts toward the identification of small molecule orexin antagonists, we were interested in the heterocyclic replacement of the dimethoxyphenyl moiety contained in the tetrahydroisoquinoline core skeleton.[7] Here, we describe the efforts in optimizing a novel series of nonpeptidic, dual orexin antagonists structurally related to almorexant where the dimethoxyphenyl part was replaced by a substituted pyrazole (Fig. 1).

The preparation of the planned pyrazolo-tetrahydropyridine derivatives began with the synthesis of the trisubstituted pyrazole **9** (Scheme 1). Acylation of *Meldrum's* acid **1** with diketene **2** quantitatively afforded intermediate **3**.[8] Subsequent reaction of a methanolic solution of **3** with ethylhydrazine in presence of triethylamine regioselectively led to the formation of the pyrazolo-derivative **4**.[9] Hydrolysis under acidic conditions (*p*-TsOH in methanol) resulted in the formation of methyl ester **5** which was quantitatively converted to the corresponding primary amide **6** by treatment with a solution of ammonia in methanol. Dehydration of amide **6** (trifluoroacetic anhydride, pyridine) smoothly delivered the expected nitrile **7**.[10] Reduction of **7** ($LiAlH_4$, THF) followed by protection of the resulting primary amine with a benzyloxycarbonyl moiety gave after purification the protected compound **8**. A final deprotection of the amino function by hydrogenolysis using palladium over charcoal delivered the expected primary amine **9**.

For the preparation of the homologous primary amine **13**, deviation from the first synthetic route began with methyl ester **5** (Scheme 2). Reduction of methyl ester **5** ($LiAlH_4$) quantitatively led to the corresponding primary alcohol **10**. Activation of the alcohol **10** as the mesylate and subsequent nucleophilic displacement mediated by tetrabutylammonium cyanide gave the expected nitrile **11**. Borane reduction of **11** followed by protection of the resulting primary amine with a benzyloxycarbonyl moiety afforded



Almorexant
(ACT-078573)

Figure 1. Almorexant and the corresponding pyrazolo-tetrahydropyridines.

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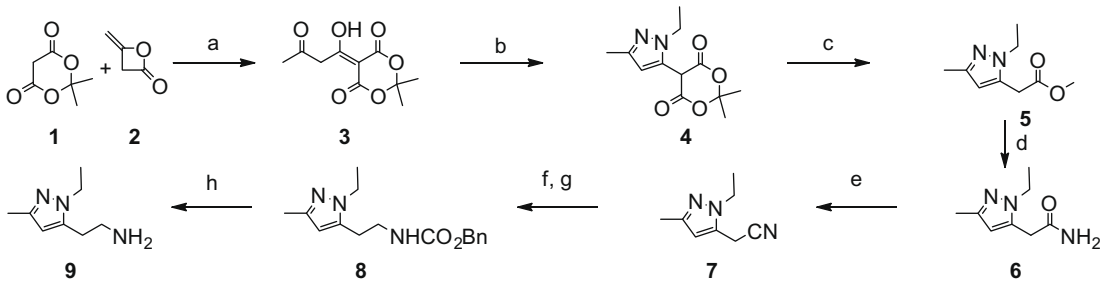
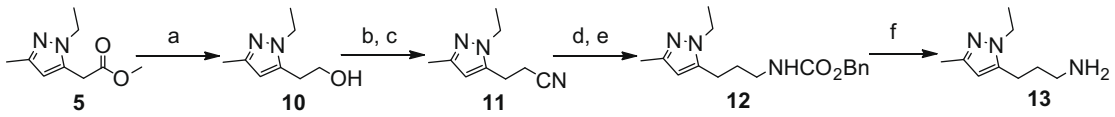


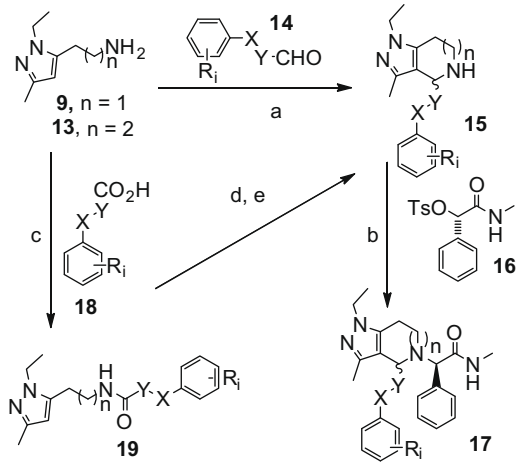
Scheme 1. Reagents and conditions: (a) Et_3N, CH_2Cl_2, room temperature (100%); (b) $EtNHNH_2$, Et_3N, MeOH, 60 °C; (c) *p*-TsOH, MeOH, 60 °C, (70% for two steps); (d) 7 M NH_3 in MeOH, room temperature (100%); (e) trifluoroacetic anhydride, pyridine, dioxane, room temperature (88%); (f) $LiAlH_4$, THF, 0 °C; (g) $ClCO_2Bn$, $NaHCO_3$, THF, H_2O, room temperature (29% for two steps); (h) H_2, 10% Pd/C, MeOH, room temperature (94%).



Scheme 2. Reagents and conditions: (a) $LiAlH_4$, THF, 0 °C (100%); (b) $MeSO_2Cl$, Et_3N, THF, 0 °C; (c) *n*-Bu_4NCN, THF, 75 °C (86% for two steps); (d) 1 N BH_3·THF, THF, 75 °C; (e) $ClCO_2Bn$, K_2CO_3, THF, H_2O, room temperature (74% for two steps); (f) H_2, 10% Pd/C, MeOH, room temperature (100%).

12. Finally, deprotection of the amino function by hydrogenolysis using palladium over charcoal delivered the expected primary amine **13**.

The target pyrazolo-tetrahydropyridine derivatives could be prepared according to two convenient synthetic routes (Scheme 3). Primary amine **9** was allowed to react with diversely substituted aldehydes **14** in a microwave-assisted *Pictet–Spengler* reaction (AcOH, EtOH, microwave) affording with good yields a racemic mixture of pyrazolo-tetrahydropyridine derivatives **15**. Finally, N-alkylation with the chiral (*S*)-tosylate **16** yielded, after purification, the target pyrazolo-tetrahydropyridine derivatives **17**. According to an alternative synthetic route, primary amines **9** and **13** were acylated with diversely substituted carboxylic acid derivatives **18** (PyBOP, DIPEA, DMF) resulting in the amides **19** which were transformed into the pyrazolo-tetrahydropyridine derivatives **15** via *Bischler–Napieralski* reaction ($POCl_3$, MeCN) and subsequent imine reduction ($NaBH_4$, MeOH). Again, a final alkylation of the resulting racemic mixture of secondary amines **15** with the chiral (*S*)-tosylate **16** gave after purification the target pyrazolo-tetrahydropyridine derivatives **17**.

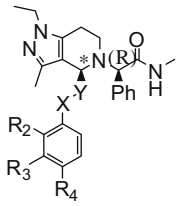


Scheme 3. Reagents and conditions: (a) AcOH, EtOH, microwave (100 W; 130 °C; 14 bar; 6 min); (b) DIPEA, 3-methyl-2-butanone, 90 °C; (c) PyBOP, DIPEA, DMF, room temperature; (d) $POCl_3$, MeCN, 90 °C; (e) $NaBH_4$, MeOH, 0 °C.

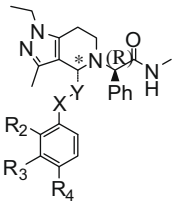
A cell-based FLIPR assay (fluorometric imaging plate reader) measuring Ca^{2+} flux as a functional determinant of orexin binding allowed to evaluate the antagonistic activity of the synthesized pyrazolo-tetrahydropyridines with both orexin receptors.[11] Preliminary structure–activity relationship (SAR) studies explored the potency against both receptors of derivatives containing a monosubstituted phenyl ring. A variety of substituents representing different electronic properties were investigated. Moving an electron-donating methyl substituent from the *ortho* to the *meta*-position allowed a sixfold increase in hOX_1R potency as illustrated with the two mixtures of diastereoisomers **20** and **21** (Table 1). A *para*-methyl group allowed an additional gain of potency toward both orexin receptors (diastereoisomers **22**). Compared to methyl, a *para*-methoxy moiety was detrimental and resulted in a complete loss of potency toward hOX_1R (**23**). Previous investigations in the tetrahydroisoquinoline series (X–Y = CH_2CH_2) indicated that the (*S*;*R*)-stereoisomer was the most active one (Fig. 1). The influence of the stereochemistry was investigated with pyrazolo-tetrahydropyridine derivatives containing an electron-donating *para*-ethyl or *para*-isopropyl substituent. Evaluation of the antagonistic activity of the separated diastereoisomers highlighted that the (*R*;*R*) isomers were significantly less potent against both receptors (stereoisomers **24**–**27**). Derivatives with a *para*-ethyl or a *para*-methyl group are almost equipotent (**22** and **24**). A larger *para*-isopropyl residue induced a threefold decrease in hOX_1R potency (**26**). Similar effects on potency could be observed with electron-withdrawing substituents. Thus, moving a fluorine atom from the *ortho* to the *meta*-position allowed an enhancement in potency toward both orexin receptors (diastereoisomeric mixtures **28** and **29**). A larger *meta*-trifluoromethyl moiety afforded an almost equipotent compound (**30**), and a *para*-CF_3 was even more beneficial (**31**). Substitution of the *para*-position with a chlorine atom resulted in decreased potency toward hOX_1R as shown with diastereoisomers **32**. The importance of the linker between the pyrazolo-tetrahydropyridine and the phenyl ring was as well investigated. Replacement of the CH_2CH_2 linker with the CH_2O linker gave a complete loss of affinity for hOX_1R as illustrated with the selective orexin 2 receptor antagonist **33**, a direct analogue of **22**. Shortening of the C_2-linker as in **31** resulted in reduced potency as exemplified with **34**.

After this first exploration of derivatives containing a monosubstituted phenyl ring, it was apparent that a *para*-methyl or a *para*-trifluoromethyl moiety afforded the most potent compounds.

Table 1
SAR studies of the phenyl-X–Y moiety



X-Y = CH_2CH_2 or CH_2: (S;R)-stereoisomer
X-Y = OCH_2: (R;R)-stereoisomer



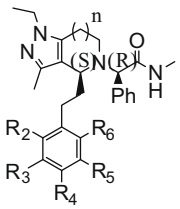
X-Y = CH_2CH_2 or CH_2: (R;R)-stereoisomer
X-Y = OCH_2: (S;R)-stereoisomer

Compound	X–Y	Stereochemistry	R_2	R_3	R_4	hOX_1R[a]	hOX_2R[a]
20	CH_2CH_2	(*S*;*R*) + (*R*;*R*)	Me	H	H	1031	21
21	CH_2CH_2	(*S*;*R*) + (*R*;*R*)	H	Me	H	180	19
22	CH_2CH_2	(*S*;*R*) + (*R*;*R*)	H	H	Me	108	6
23	CH_2CH_2	(*S*;*R*) + (*R*;*R*)	H	H	OMe	10,000	219
24	CH_2CH_2	(*S*;*R*)	H	H	Et	63	3
25	CH_2CH_2	(*R*;*R*)	H	H	Et	1424	174
26	CH_2CH_2	(*S*;*R*)	H	H	*i*-Pr	161	9
27	CH_2CH_2	(*R*;*R*)	H	H	*i*-Pr	1919	179
28	CH_2CH_2	(*S*;*R*) + (*R*;*R*)	F	H	H	669	22
29	CH_2CH_2	(*S*;*R*) + (*R*;*R*)	H	F	H	402	10
30	CH_2CH_2	(*S*;*R*) + (*R*;*R*)	H	CF_3	H	358	7
31	CH_2CH_2	(*S*;*R*)	H	H	CF_3	128	3
32	CH_2CH_2	(*S*;*R*) + (*R*;*R*)	H	H	Cl	993	12
33	OCH_2	(*R*;*R*)	H	H	Me	10,000	19
34	CH_2	(*S*;*R*) + (*R*;*R*)	H	H	CF_3	769	25

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

In an attempt to further improve potency in this series of orexin receptor antagonists, additional substituents were added on the phenyl moiety (Table 2).[12] Having a *para*-methyl substituent, the addition of an *ortho*-fluorine atom was unfavorable mainly for hOX_1R potency (**35**) whereas a *meta*-fluorine was well tolerated as illustrated with compound **36**. Difluorination of the *para*-tolyl motif was also nicely tolerated as shown with derivative **37**, and an almost threefold increase in hOX_1R potency could be even achieved with the isomeric and potent dual orexin receptor antagonist **38**. The addition of a second methyl substituent in the *para*-tolyl moiety was then investigated and like with fluorine, an additional *ortho*-methyl group was unfavorable mainly for hOX_1R potency (**39**). The addition of a *meta*-methyl residue was beneficial due to an additive effect on orexin activity which allowed for a substantial eightfold increase of the affinity for the hOX_1R resulting in compound **40**, the most potent dual orexin receptor antagonist identified so far in this series. In a next step, the addition of fluorine substituents was explored with the derivative containing a *para*-trifluoromethyl group on the phenyl ring. An additional *ortho*-fluorine atom induced a twofold increase in hOX_1R potency (**41**). Difluorination of the *para*-trifluoromethylphenyl allowed for a significant gain in potency as shown with **42**, and the affinity for the hOX_1R could be even increased with the isomeric derivative **43** which is one of the most potent dual orexin receptor antagonist identified in this series. Finally, investigations of seven-membered analogues (**44** and **45**) showed that the corresponding six-membered derivatives (**40** and **43**) were more potent toward both orexin receptors. In vivo effects of compounds **40** and **43** on sleep and wake cycles were evaluated in adult male wistar rats implanted with radiotelemetric probes.[13] Compounds were orally administrated at 100 mg/kg at the beginning of the dark active phase and electroencephalographic (EEG) and electromyographic (EMG) signals, home cage activity and body temperature recorded. Both compounds showed the same pattern of activity on sleep and wake cycles as almorexant (Fig. 1)[6] but to a lesser extent. Over the 12 h dark period following administration, the compounds decreased the home cage activity (p <0.05 for compound **40** and p <0.001 for compound **43**, paired t-test, Fig. 2A) and the time spent in active wake (p <0.01 for compound **40** and p <0.05 for compound **43**, paired t-test, Fig. 2B). Time spent in non-REM (rapid eye movement) sleep was increased significantly (p <0.05 for both compound, paired t-test). We also observed a non-significant increase of the time spent in REM sleep (15% and 8% increase for compounds **40** and **43**, respectively). Like almorexant, the two dual orexin receptor antagonists showed a very particular profile, that is, they increased both non-REM and REM sleep in physiological proportions.

Table 2
SAR studies of derivatives containing a poly-substituted phenyl ring



Compound	*n*	R_2	R_3	R_4	R_5	R_6	hOX_1R[a]	hOX_2R[a]
35	1	F	H	Me	H	H	147	8
36	1	H	F	Me	H	H	49	2
37	1	F	F	Me	H	H	57	5
38	1	H	F	Me	F	H	16	5
39	1	Me	H	Me	H	H	131	7
40	1	H	Me	Me	H	H	7	3
41	1	F	H	CF_3	H	H	52	8
42	1	F	F	CF_3	H	H	23	8
43	1	H	F	CF_3	F	H	5	4
44	2	H	Me	Me	H	H	36	5
45	2	H	F	CF_3	F	H	22	12

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

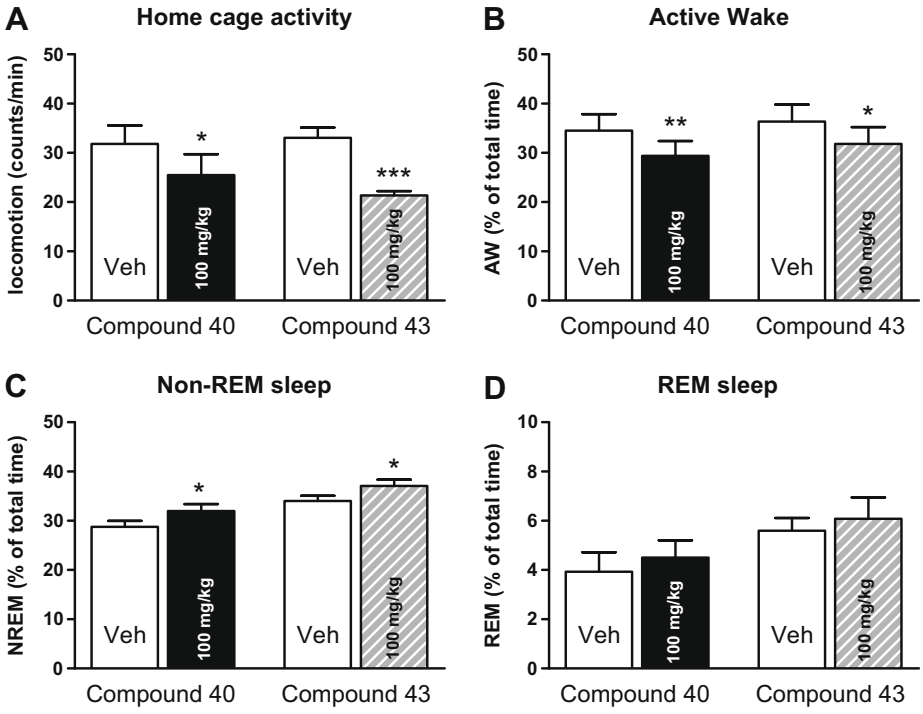


Figure 2. Effects of compound **40** and compound **43** on home cage activity (A), time spent in AW (AW, B), time spent in non-REM sleep (NREM, C) and time spent in REM sleep (D). Effects measured and integrated over 12-h night period following administration. Data are presented as means ± SEM. $^{*}p$ <0.05, $^{**}p$ <0.01, $^{***}p$ <0.001 (n = 7 for compound **40** and n = 8 for compound **43**).

In conclusion, we have described a novel series of dual orexin receptor antagonists based on the heterocyclic replacement of the dimethoxyphenyl moiety present in the tetrahydroisoquinoline series around almorexant. Additional structure–activity relationship (SAR) studies of the phenethyl motif allowed the identification of potent dual receptor antagonists with low nanomolar potency for hOX_1R and hOX_2R. This series demonstrated excellent in vitro cell-based activity and exhibited activity in the in vivo sleep-model.

Acknowledgments

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11. *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, intercellular Ca^{2+} mobilization was monitored as a change in cell fluorescence intensity by FLIPR (Molecular Devices). Differing concentrations of orexin antagonists were added to the plates prior to addition of orexin A. For each antagonist, IC_{50} (the concentration of compound needed to inhibit 50% of the agonistic response) is calculated.
12. For the purposes of this communication, a dual antagonist is defined as having less than 20-fold selectivity for either OX_1R or OX_2R.
13. For pharmacodynamic sleep studies, adult male wistar rats were implanted with radiotelemetric probes (Data Sciences International) under general anesthesia. Those implants allow the recording of the electroencephalogram (EEG), the electromyogram (EMG), home cage activity and body temperature in freely moving animals. Compounds were administrated orally at the beginning of the dark active phase and formulated in 100% PEG-400. We used groups of 7 or 8 animals, in a cross over design, with at least 4-day washout periods separating consecutive administration.