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2-Methyl-3-furanyl-4*H*-1,2,4-triazol-3-ylthioamides: A new class of selective orexin 2 antagonists

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ABSTRACT

A new class of selective orexin 2 antagonist was identified among commercial products. Initial SAR was obtained using commercial derivatives only prior to starting ad hoc medicinal chemistry activities.

The discovery of the orexin (also known as hypocretin) neuropeptide system determined a major advancement in the understanding of sleep and arousal states. The orexin system is based on two peptides, orexin-A and orexin-B which are secreted by neurons located in the lateral hypothalamus. These excitatory neuropeptides bind, with different affinities, to two GPCR receptors, the orexin 1 (Ox1R) and 2 (Ox2R) receptor. This architecture seems to be highly conserved from rodents to humans.[1]

Preclinical data suggest that this pathway is critical for controlling different function related to arousal state and a positive proof of concept in humans was recently achieved.[2] Accordingly, the orexin field might be considered a new neuroscience area and a number of different actors (private and public groups) are working in this very attractive field. Different templates, able to interact selectively on one or both receptors have been disclosed and some of these structures (**1**–**4**) are reported in Figure 1. Recent reviews covering these and other interesting scaffolds are available.[3]

GlaxoSmithKline showed a long standing interest in the orexin field and results related to SB-649868 were recently disclosed.[4] In the quest to identify new potent and selective orexin antagonists, a substantial number of high throughput screening campaigns (HTS) was performed. During the retrospective analysis of the hits coming from one of these screenings, a potent structure (**5**, Fig. 2) was recently identified among the commercial compounds available in the corporate collection. Derivative **5** showed a functional pK_i (fpK_i)[5] = 6.1 versus the Ox1 receptor and a fpK_i = 8.8 versus the Ox2 receptor (Table 1), demonstrating a 500-fold selectivity for the latter one. Considering that a selective Ox2 derivative might potentially have different therapeutic applications from a mixed Ox1/Ox2 compound, additional steps were taken to further investigate this compound.

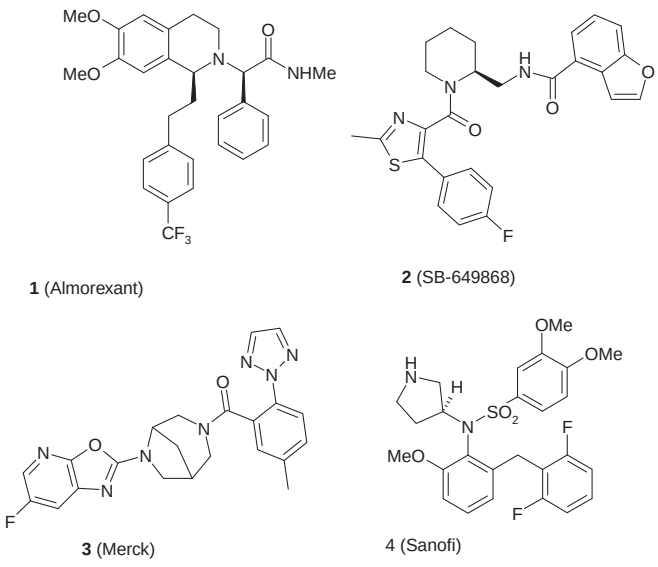

Figure 1. Some known orexin antagonists.

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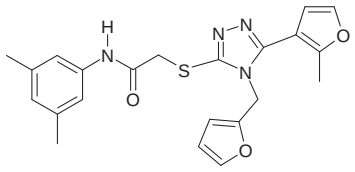

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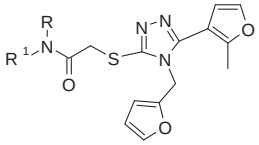
Figure 2. The newly discovered selective Ox2 antagonist hit.

Table 1
Functional pK_i (fpK_i) at orexin 1 and 2 receptors (FLIPR assay)



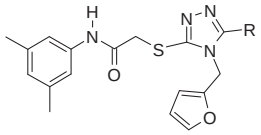
Entry	Ox1 fpK_i	Ox2 fpK_i	R	R^1
2	9.5	9.4	N.A.	N.A.
5	6.1	8.8	3,5-Dimethylphenyl	H
6	6.2	8.6	3,4-Dimethylphenyl	H
7	5.9	6.3	2,4-Dimethylphenyl	H
8	<4.8	6.4	2,5-Dimethylphenyl	H
9	<4.8	<4.8	2,6-Dimethylphenyl	H
10	<4.8	<4.8	2,3-Dimethylphenyl	H
11	5.6	7.8	2-Methoxy, 5-methylphenyl	H
12	6.0	7.4	3-Chloro, 4-fluorophenyl	H
13	<4.8	6.0	Phenyl	H
14	<4.8	7.4	4-Methylphenyl	H
15	5.6	7.3	3-Methylphenyl	H
16	<4.8	<4.8	2-Methylphenyl	H
17	<4.8	6.8	4-Fluorophenyl	H
18	<4.8	6.3	3-Fluorophenyl	H
19	<4.8	5.7	2-Fluorophenyl	H
20	5.7	7.0	4-Chlorophenyl	H
21	5.7	7.2	3-Chlorophenyl	H
22	<4.8	6.3	2-Methoxy phenyl	H
23	<4.8	6.7	4-Methoxy phenyl	H
24	<4.8	6.6	1,3-Benzodioxol-5-yl-phenyl	H
25	5.6	7.5	4-Acetylphenyl	H
26	5.5	6.5	3-Acetylphenyl	H
27	6.1	6.8	4-(Dimethylamino)phenyl	H
28	<4.8	6.0	1,3-Thiazol-2-yl	H
29	<4.8	7.0	4-Methyl-1,3-thiazol-2-yl	H
30	<4.8	6.4	5-Methyl-1,3,4-thiadiazol-2-yl	H
31	<4.8	5.8	Phenethyl	H
32	<4.8	6.0	2,3-Dihydro-1*H*-indolyl	
33	<4.8	<4.8	2-Methylbutyl	H
34	<4.8	<4.8	H	H

[a] SEM data sets is ±0.1; N.A. = not applicable.

After potency and structure confirmation from a solid sample, it was decided to follow an approach that would have considered only commercially available derivatives analogs of compound **5** before investing synthetic resources on the new hit. These structures were fished out from the corporate database with the use of substructure searches and 2D- and 3D-similarity searches. Luckily enough, about 30 structures were present in the screening collection and were requested for immediate testing. The results of this preliminary exercise are reported in Tables 1 and 2. It is worth noting, however, that some of the results presented here are on the lower scale of affinity at the Ox2 receptor as not all the commercially available compounds were available in our hands.

The dimethyl pattern on the aromatic ring (**6**–**10**) seemed to play an important role for the activity of the hits. Actually, while the 3,4-dimethyl derivative **6** showed almost a comparable fpK_i profile to derivative **5**, all the remaining compounds showed a decrease in the affinity at the Ox2 receptor. The introduction of the

Table 2
Functional pK_i (fpK_i) at orexin 1 and 2 receptors (FLIPR assay)



Entry	Ox1 fpK_i	Ox2 fpK_i	R
35	<4.8	6.4	2-Pyridyl
36	<4.8	5.7	3-Pyridyl
37	<4.8	6.2	2-Pyrazinyl

[a] SEM data sets is ±0.1.

methyl group in position 2 of the aromatic ring seemed to have a negative effect on the affinity (**7**–**10**) even if this might be specifically related to the characteristics of the methyl group itself. The presence of a 2-methoxy group (**11**), actually, did not show such a dramatic decrease in the affinity at the Ox2 receptor.

Once again, the presence of the 2-methyl group had a detrimental effect on the desired Ox2 affinity (compound **16** vs derivatives **14** and **15**), while such an effect was not so heavily observed for the 2-methoxy derivative (compound **22** vs derivative **23**). The effect of the 4-Ac group (derivative **25**) was comparable to the 4-methyl derivative **14**, while the introduction of the 4-*N*-dimethyl amino (derivative **27**) lead to a slight decrease of affinity.

The replacement of the phenyl group (**13**) with a five-membered heteroaromatic ring (**28**) proved to be feasible and the introduction of a further substituent on the system (**29**) provided a slight increase in the Ox2 affinity. The nature of the five-membered scaffold, however, seemed to be critical: actually, the only other compound available in the collection (the thiadiazole derivative **30**) showed that the introduction of an extra nitrogen was slightly detrimental for the achievement of good affinity at the desired receptor. The introduction of a 'spacer' between the aromatic portion and the amidic nitrogen (**31** vs **13**) had no major effect. Similarly, the removal of the amide hydrogen (**32** vs **13**) seemed to have no major effect on affinity. Once again, it is important to consider—as a caveat—that these were preliminary observations drawn on weakly active compounds: accordingly, appropriately designed derivatives have to be tested to further refine these initial results.

The complete removal of the aromatic portion (**33**, **34**) led to the complete loss of affinity at both receptors, suggesting that this moiety might play a fundamental role in the interactions with the receptor.

Computational chemistry calculations were performed to analyze the orientation of the three rings in the space to understand their ability to interact with the Ox2 receptor.

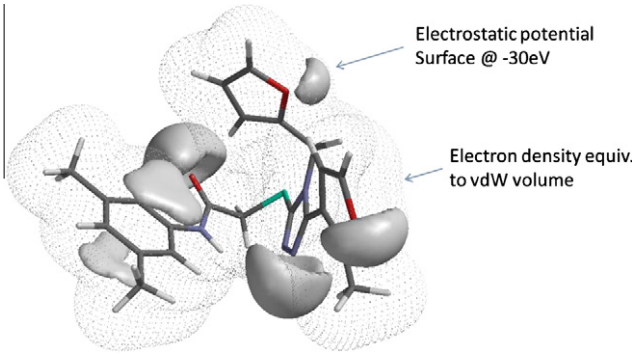


Figure 3. Global minimum structure for derivate **5**. The conformation depicted is partially folded. Solid gray volumes represent points with higher (negative) electrostatic potential.

A minimum energy conformation[6] which depicts the 3D structure of derivative **5** is reported in Figure 3. It should be noted that various attempt were made to superimpose (using shape and pharmacophoric approaches) derivative **5** with molecules **1–4**. Furthermore, no fitting was achieved into the in house available selective- and mixed-pharmacophoric models of know Ox-antagonists. Considering that no reasonable superimposition was obtained either with these derivatives or with other known templates, it is possible to infer that that derivative **5** might represent a novel class of Ox2 binders.

The last three compounds examined in this preliminary exercise (**35–37**, Table 2) showed a single point variation of the furane moiety directly linked to the thiotriazole scaffold.

All of them are basic six-membered heteroaromatic and their introduction seemed to have a negative outcome on Ox2 affinity when compared to derivative **5**. No commercially available compound with a replacement of the 'benzylic' furane was unfortunately present in the collection at the moment of the exercise.

To confirm that derivative **5** was a good starting point for a proper medicinal chemistry exploration, further experiments were run. The selectivity of the compound was tested as complete concentration–response curve (CRC) towards a relatively small panel of GPCRs and the compound show at least 1000-fold selectivity over dopaminergic (D_2/D_3) and serotoninergic (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}) receptors; the compound also showed more than 1000-fold selectivity over the hERG channel. Moreover, it was tested in single shot in a panel of about 300 receptors, enzymes and ion channels with no remarkable activity.

The functional characterization[7] of the mechanism of action of derivative **5** was preliminary determined in recombinant cells expressing rat Orexin receptors by using the [^{3}H]-inositol phosphates (IPs) accumulation method as described by Brandish.[8] Competitive sourmontable behavior was observed at both Ox1 and Ox2 receptor.

Finally, to ensure that the selected template was characterized by appropriate developability characteristics from the beginning of the exploration, the compound went through generic developability screens such as CYPEX bactosome P450 inhibition and rat and human in vitro clearance in liver microsomes. IC_{50} values for all major P450 isoforms tested (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) were greater than 5 μM with the exception of 3A4 DEF (2 μM), while intrinsic clearance (Cli) values both in human and in rat resulted high (>50 ml/min/g of protein). This might be related to the presence of two unsubstituted furane rings and of a 'benzylic' position in the template and need to be addressed with an appropriately designed medicinal chemistry exploration.

In summary, a new and selective class of Orexin 2 antagonists was identified. The initial SAR was achieved using commercially available derivatives[11] only, delaying the start of the synthetic medicinal chemistry approach until a solid starting point was identified. This objective was further strengthened by the introduction of developability screens in an early phase the screening cascade. Such a strategy allowed to immediately targeting potential issues in a future lead optimization process on the new scaffold.

The approach here reported has the potential to be used by organizations which have limited chemical resources for their exploratory targets or do not want to dissipate time and money on potentially poor tractable hits.

Acknowledgment

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References and notes

1. (a) De Lecea, L.; Kilduff, T. S.; Peyron, C. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 322; (b) Sakurai, T.; Amemiya, A.; Ishii, M. *Cell* **1998**, *92*, 573.
2. Brisbare-Roch, C.; Dingemanse, J.; Koberstein, R. *Nat. Med.* **2007**, *13*, 150.
3. (a) Coleman, P. J.; Renger, J. J. *Expert Opin. Ther. Pat.* **2010**, *20*, 307; (b) Roecker, A. J.; Coleman, P. J. *Curr. Top. Med. Chem.* **2008**, *8*, 977; (c) Gatfield, J.; Brisbare-Roch, C.; Jenck, F.; Boss, C. *ChemMedChem Early View*, doi: 10.1002/cmdc.201000132.
4. Di Fabio, R.; Gerrard, P.; Porter, R.; Stemp, G.; Nash, D.; Branch, C.; Massagrande, M.; Poffe, A.; Piccoli, L.; Braggio, S.; Ratti, E. *Abstracts of Papers, 238th ACS National Meeting*, Aug 16–20, 2009; Washington, DC, United States, 2009.
5. Human and rat orexin 1/2 antagonist FLIPR assays.
Materials: For cell culturing, the following media and supplements were used: alpha MEM, DMEM/F12, fetal bovine serum (FBS), non-essential aminoacids (NEAA) and pen/strep obtained from Gibco. Fluorimetric imaging plate reader assay (FLIPR) was performed in 384-well, black wall, clear bottom, cell treated microtiter plates from Greiner. Dimethyl sulfoxide (DMSO), HEPES, NaCl, KCl, $CaCl_2$, $MgCl_2$, glucose, probenecid, pluronic acid F-127 and bovine serum albumin (BSA) were purchased from Sigma. Fluo-4 AM was from Molecular Probes. hOrexin-A was purchased from California Peptide.
Methods: Calcium mobilization assays.
CHO cells stably expressing human orexin 1 or orexin 2 receptor and RBL cells stably expressing rat orexin 1 or orexin 2 receptor were stored in ready-to-use frozen aliquots in liquid nitrogen vapor. The day before the experiment, frozen cell aliquots were thawed and seeded in black walled clear-base 384-well plates (Greiner cat. no. 781091) at a density of 20,000 cells/well in culture medium (except for hOrexin 1 CHO seeded at a density of 10,000 cells/well) and maintained overnight (95%:5% air:CO_2 at 37 °C). FLIPRTM experiments ran 16–24 h after plating. On the experimental day, cell plates were washed two-times with the assay buffer and then batch-loaded for 1 h with 2 μM Fluo-4 AM at 37 °C in the dark. After the loading incubation, cell plates were washed two-times to remove unloaded Fluo-4 test compounds, appropriately diluted in the buffer, were added and cell plates were stored in the dark at 37 °C to equilibrate for 10 min. Cell plates were then placed into the FLIPRTM (molecular devices) to monitor cell fluorescence (λ_{ex} = 488 nm, λ_{em} = 540 nm) before and after the addition of a submaximal concentration (EC_{80}) of orexin-A peptide.
Functional responses using FLIPR™ were measured as peak fluorescence intensity minus basal fluorescence and expressed as a percentage of a non-inhibited agonist-induced response on the same plate. Iterative curve fitting and parameter estimations were carried out using a four parameter logistic model by ActivityBase software (IDBS). Results are expressed as pIC_{50} and transformed in fpK_i by the modified Cheng–Prusoff equation.
6. Initial global minimum-energy structures for molecule **5** were generated using Spartan '02 (from Wavefunction Inc.) using the conformer distribution module and the merck force field (MMFF). The global minimum structures was then fully optimized at the SCF level with the 6-31G* basis set. Successively density and electrostatic potential were calculated for the global minimum.
7. *Method*: [^{3}H]-inositol phosphates (IPs) accumulation assay. Functional characterization of mechanism of action was determined in recombinant cells expressing rat orexin receptors by using the [^{3}H]-inositol phosphates (IPs) accumulation method as described by Brandish [6]. Briefly, RBL cells stably expressing either rat OX1 or rat OX2 receptor were seeded at 3×10^4 or 1.5×10^4 cells per well, respectively, in 96-well plates. Sixteen hours before the experiment, cells were supplemented with medium containing 1 μCi per well of [3H]-myo-inositol (Perkin–Elmer). Cells were then washed with the assay buffer (1× HBSS buffer, 20 mM HEPES pH 7.4, 0.1% BSA, 10 mM LiCl) and pre-incubated for 30 min at 37 °C with four different concentrations of antagonist. Orexin-A concentration–response curves (CRC, 10 μM–0.1 nM) were determined in the presence of LiCl 10 mM and 0.1% BSA for 60 min of challenge. After cells lysis with 0.1 M ice-cold formic acid, 20 μl of the supernatant were placed in 96-well Optiplate (Perkin–Elmer) with 1 mg/well Ysi RNA beads (Amersham) and shacked for 1 h at RT. After 2 h at 4 °C, plates were read with Packard TopCount (Perkin–Elmer). *Data analysis*: Baseline was subtracted from CPM values and then data were normalized to the maximal effect produced by orexin-A stimulation within each plate. Data were analyzed with GraphPad prism (v.5.0). The negative logarithmic of the orexin-A concentration producing 50% of maximal response (pEC_{50}) was calculated by non-linear regression analysis, Sigmoidal dose–response equation. Surmountable antagonist that produces parallel dextral displacement of agonist concentration–response curves with no concomitant diminution of the maximal response to the agonist were analyzed using classical Gaddum equation[9] and Schild's analysis.[10] fpK_i values of test drug were calculated from the IC_{50} using Cheng–Prusoff equation: $fK_i = IC_{50}/1 + ([A]/EC_{50})$ where: [A] is the concentration of the agonist in the assay and EC_{50} is the agonist EC_{50} value obtained in the same experiment. fpK_i is defined as $-\log fK_i$.
8. Brandish, P. E.; Hill, L. A.; Zheng, W.; Scolnick, E. M. *Anal. Biochem.* **2003**, *313*, 311.
9. Gaddum, J. H. *Pharmacol. Rev.* **1957**, *9*, 211.
10. Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol.* **1959**, *14*, 48.
11. All the compounds reported (**5**–**37**) are commercially available by different vendors including Asinex (www.asinex.com).