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Synthesis of (3,4-dimethoxyphenoxy)alkylamino acetamides as orexin-2 receptor antagonists

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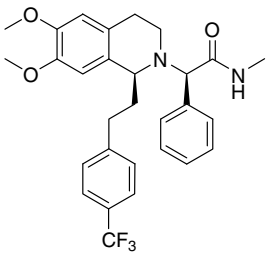
ABSTRACT

The discovery and synthesis of a series of (dimethoxyphenoxy)alkylamino acetamides as orexin-2 receptor antagonists from a small-molecule combinatorial library using a high-throughput calcium mobilization functional assay (HEK293—human OX2-R cell line) is described. Active compounds show a good correlation between high-throughput single concentration screening data and measured IC_{50}s. Specific examples exhibit IC_{50} values of ~20 nM using human orexin A as the peptide agonist for the orexin-2 receptor.

The orexins (hypocretins), orexin A and orexin B, are neuropeptides produced in the hypothalamus.[1,2] These peptides bind to and activate the two G protein-coupled receptors (GPCR's) orexin-1 (OX1-R/hypocretin R1) and orexin-2 (OX2-R/hypocretin R2). For OX1-R, orexin A has a 10-fold greater affinity than orexin B, whereas both peptides bind to OX2-R with similar affinity.[1] While the Orexin receptors share common ligands, they differ in several respects. Recombinantly-expressed OX1-R signals via Gq, and OX2-R via Gq or Gi/o, and OX1-R and OX2-R are also expressed in different regions of the brain.[3,4] The orexin receptor system has been implicated in feeding behavior and metabolism. Exogenous administration of orexin A increases arousal and food intake in rats and the stimulation of food intake has been shown to be blocked by a specific OX1-R antagonist.[1,8–12] Recently, the orexin receptor system has also been implicated in sleep and wakefulness. Knockout of the orexins, or a double knockout of OX1-R and OX2-R, results in sleep-onset REM periods, cataplexy, and short sleep latency in mice; deficiency in OX1-R alone leads only to fragmented sleep, whereas OX2-R deficiency leads to narcoleptic symptoms that are similar to but less severe than those manifested by the double-receptor knockout mice, with only mild cataplexy.[5–7]

Naturally-occurring mutations in OX2-R have been identified in narcoleptic dogs, whereas narcolepsy in humans has been associated primarily with decreased hypocretin levels in brain/cerebrospinal fluid and with the loss of hypocretin neurons.[13–18] Interestingly, low hypocretin levels in narcoleptic patients have also been associated with decreased metabolic rate and obesity despite the associated hypophagia. Experimental ablation of hypocretin neurons in mice has a similar effect.[19,20] The role of orexins acting at OX2-R suggests that this receptor might be a suitable target for pharmacological intervention for the treatment of narcolepsy (agonists) and insomnia (antagonists). There has been considerable interest in the discovery and development of hypocretin receptor antagonists such as ACT-078573 (an OX1-R/OX2-R antagonist)[21] which is currently the subject of clinical trials for the treatment of sleep disorders. We describe here the identification of novel small-molecule antagonists of OX2-R.



ACT-078573

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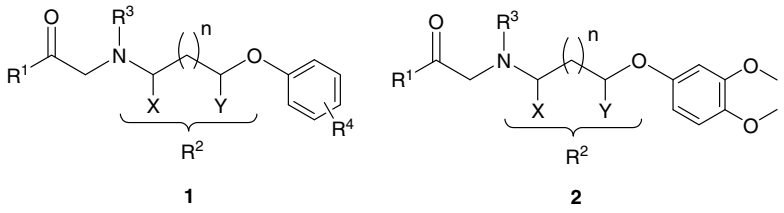
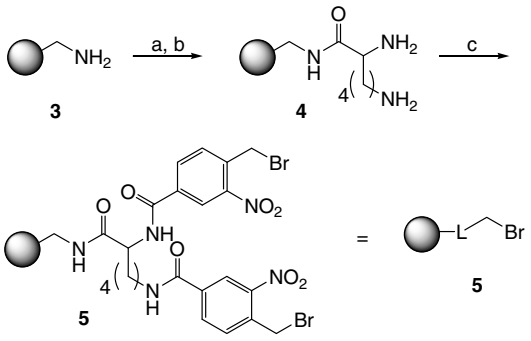


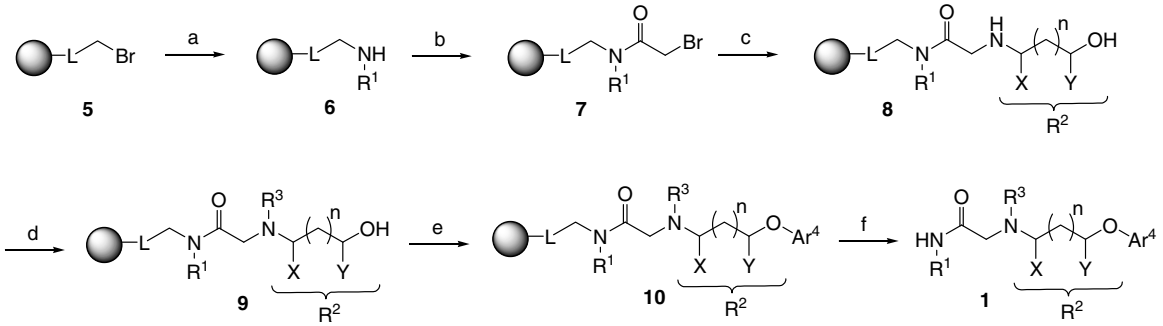
Figure 1. General structure of OX2-R active library (**1**) and subset of OX2-R antagonists (**2**).



Scheme 1. Reagents and conditions: (a) N-α-N-ε-bis-Fmoc-Lys, HOBt monohydrate, DIC, CH_2Cl_2, DMF, 25 °C; (b) piperidine, DMF, 25 °C; (c) 4-(bromomethyl)-3-nitrobenzoic acid, HOBt monohydrate, DIC, CH_2Cl_2, DMF, 25 °C.

A high-throughput screen of 90 distinct ECLiPS™ (Encoded Combinatorial Libraries on Polymeric Support)[22] libraries comprising >4 million compounds was conducted employing a 384-well FLIPR-based calcium flux assay designed to identify small-molecule antagonists of the human OX2-R receptor. A library of ~140,000 compounds, synthesized through the incorporation of four points of diversity (R^1–R^4) and based on a substituted phenoxyalkylaminoacetamide motif (**1**), resulted in the identification of a set of (3,4-dimethoxyphenoxy) alkylaminoacetamides (**2**) as potent OX2-R antagonists (Fig. 1).

The combinatorial library was synthesized utilizing aminomethyl-terminated Tentagel® (**3**) as the polymeric support (Scheme 1). Initial derivatization of **3** with *N*-α-*N*-ε-bis-Fmoc-lysine followed by Fmoc deprotection to generate **4** increases the loading capacity of the resin by doubling the number of amino groups for further functionalization. This was followed by acyla-



Scheme 2. Reagents and conditions: (a) R^1-NH_2, THF, 25 °C; (b) bromoacetic acid, DIC, DMF, 25 °C; (c) R^2-NH_2, DMSO, 25 °C; (d) $R^{3'}$-CHO, $NaCNBH_3$, 1% AcOH/MeOH, 25 °C; (e) Ar^4OH, TMAD, PBu_3, THF/CH_2Cl_2, 25 °C; (f) hυ (365 nm), 2% v/v TFA/MeOH, 50 °C.

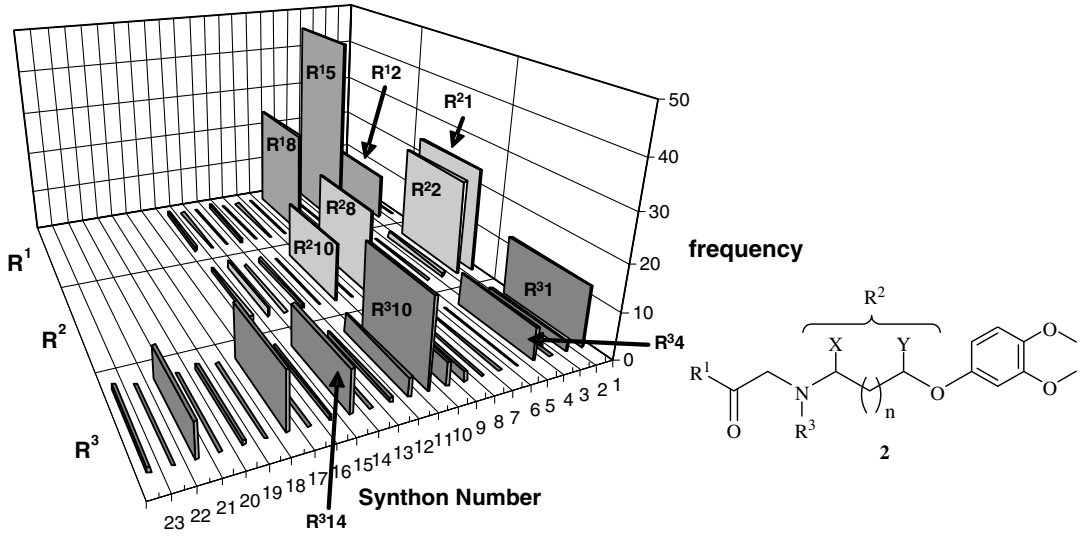


Figure 2. Synthon frequency of active compounds (**2**) identified in OX2-R screening of the combinatorial library.

tion with 4-(bromomethyl)-3-nitrobenzoic as a linker to generate **5**. This linker allows photo-mediated cleavage of the final compounds from solid phase, and is compatible with the chemistry required to conduct the library synthesis.

Synthesis of the library, using commercially available reagents, was initiated by the generation of a resin-bound secondary amine **6** via alkylation of a series of primary amines (R^1NH_2) with **5** (Scheme 2). Subsequent bromoacetylation of **6** provided **7**, which was used to alkylate a series of aminoalcohols (R^2-NH_2) to yield **8**. Incorporation of the third point of diversity was achieved via reductive alkylation of **8** with a series of aromatic aldehydes ($R^{3'}$-CHO) to give **9**.

Library synthesis was completed through Mitsunobu coupling of the resin-bound alcohol **9** with a series of substituted phenols (Ar^4-OH) utilizing *N*,*N*,*N*′,*N*′-tetramethylazodicarboxamide (TMAD) and tri-*n*-butyl phosphine (PBu_3) to generate **10**. The use of TMAD/PBu_3 in the Mitsunobu coupling allowed efficient conversion of both primary and secondary alcohols to the corresponding aryl ethers. Photo-mediated cleavage of **10** to release **1** from the solid support was achieved by irradiating a suspension of **10** in 2% v/v trifluoroacetic acid/methanol at 365 nm.

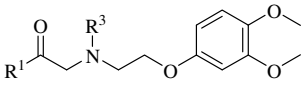
High-throughput screening was conducted by monitoring human orexin A-stimulated calcium release in a HEK293/OX2-R cell line following stimulation with 20 nM (EC_{80}) of human orexin-A. No agonist activity was observed for the compounds described. As a specificity control, compounds were also tested in the presence of 8 μM ATP in place of orexin-A for effect on activation of endogenous purinergic receptors in the same HEK293/OX2-R cell line. Compounds derived from the portion of the combinatorial library in which Ar^4 was present as the 3,4-dimethoxyphenyl substituent were shown to exhibit good OX2-R antagonist activity and showed no effect on challenge with ATP. Structural determination of the active compounds (>60% reduction in calcium release) present within this portion of the library was conducted (defining the R^1–R^3 substituents) via analysis of the encoding molecules incorporated during ECLiPS™ library synthesis.

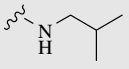
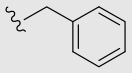
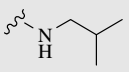
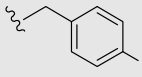
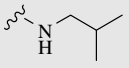
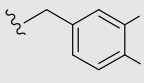
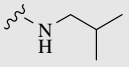
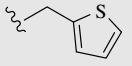
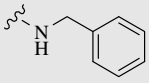
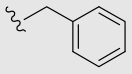
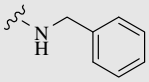
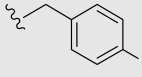
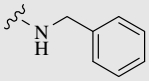
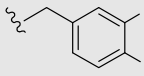
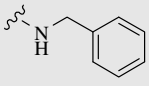
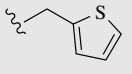
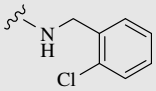
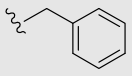
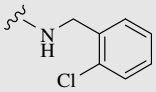
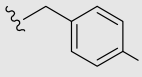
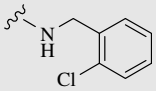
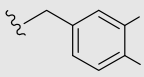
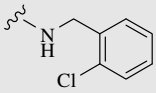
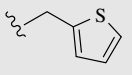
Analysis of the frequency of the individual combinatorial synthons that were present in the active compounds is shown graphically in Figure 2. Preference for specific synthons was observed at each of the combinatorial variants. Synthon R^1 corresponds to incorporation of a secondary amino substituent at the glycinyl carboxy group. A strong preference is observed for synthons $R^1$2, $R^1$5 and $R^1$8, which correspond to isobutyl, benzyl and 2-chlorobenzyl substitutions, respectively. Analysis of the activities in the screen for the active compounds reveals that fragment $R^1$2 (isobutyl) is only present in active structures that demonstrated inhibition of calcium release by 60–70% in the screen, whereas $R^1$5 and $R^1$8 are identified in compounds with superior activity. Synthon R^2 defines the aminoether core of the library. Within this combinatorial element, a strong preference is observed for synthons $R^2$1, $R^2$2, $R^2$8 and $R^2$10, corresponding to ethylene, α-methyl ethylene, β-methyl ethylene and propylene-based amino ether cores. Screening activity data suggested comparable potencies for the ethylene-based systems and slightly weaker potency for the propylene-based aminoether systems. Synthon R^3 corresponds to the reductive amination performed to generate the tertiary amino center of the aminoether and a selection of substituents are present within the data set (Fig. 2), indicating that a variety of substitutions will be tolerated within this position of the molecule. The most frequently occurring synthons at the R^3 position are $R^3$1 (benzyl), $R^3$10 (3,4-difluorobenzyl), $R^3$14 (2-thiophene methyl) and $R^3$17 (3-thiophene methyl).

To correlate percent activity derived from library screening with accurate IC_{50} values,[23] a selection of compounds were synthesized in multi-milligram quantities by parallel synthesis following the solid-phase synthetic route outlined in scheme 2. The measured potencies are detailed in Table 1 (ethylene-based systems) and Table 2 (propylene-based systems).

Analysis of the potencies for the synthesized compounds indicated that the isobutyl carboxamide (**11**–**14**) is the least potent of the three most frequently occurring R^1 substituents. This observation is in good agreement with the screening activities observed in library screening. Incorporation of the unsubstituted benzylic component ($R^1$5, **15**–**18**) at R^1 appears to provide, on average, a fivefold increase in potency in comparison to the 2-chloro-substituted benzylic component ($R^1$8, **19**–**22**), potentially indicating a preference for an electron-neutral aromatic system or an adverse steric or conformational effect provided by the 2-chloro substitution. A moderate preference for the ethylene-based amino ether (**15**–**18**) in compari-

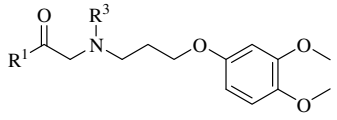
Table 1
Ethylene-based OX2-R antagonists

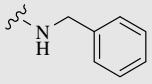
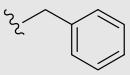
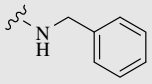
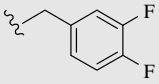
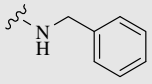
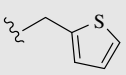


Compound	R^1	R^3	OX2-R pIC_{50} ± SEM[a]
11 ($R^1$2, $R^3$1)			6.52 ± 0.04
12 ($R^1$2, $R^3$4)			6.25 ± 0.06
13 ($R^1$2, $R^3$10)			6.64 ± 0.10
14 ($R^1$2, $R^3$14)			6.49 ± 0.01
15 ($R^1$5, $R^3$1)			7.60 ± 0.06
16 ($R^1$5, $R^3$4)			7.71 ± 0.01
17 ($R^1$5, $R^3$10)			7.73 ± 0.08
18 ($R^1$5, $R^3$14)			6.96 ± 0.14
19 ($R^1$8, $R^3$1)			6.82 ± 0.02
20 ($R^1$8, $R^3$4)			6.86 ± 0.01
21 ($R^1$8, $R^3$10)			7.09 ± 0.08
22 ($R^1$8, $R^3$14)			6.71 ± 0.06

[a] pIC_{50} ± standard error mean upon challenge with 20 nM Ox-A, based on ⩾3 independent determinations.

Table 2
Propylene-based OX2-R antagonists



Compound	R^1	R^3	OX2-R pIC_{50} ± SEM[a]
23 ($R^1$2, $R^3$1)			7.29 ± 0.03
24 ($R^1$2, $R^3$10)			6.97 ± 0.06
25 ($R^1$2, $R^3$14)			6.88 ± 0.07

[a] pIC_{50} ± standard error mean upon challenge with 20 nM Ox-A, based on ⩾3 independent determinations.

son to the propylene-based amino ether (**23**–**25**) is also evident from the synthesized compounds. Alkylation to provide the tertiary amino system as determined by R^3 gives compounds of excellent potency (IC_{50} < 30 nM) for $R^3$1 (benzyl, **15**), $R^3$4 (4-fluorobenzyl, **16**) and $R^3$10 (3,4-difluorobenzyl, **17**). However, based on the screening data, a number of substitutions appear to be tolerated at the R^3 position, providing the potential for further optimization and the investigation of components that may provide increased antagonist activity. It is noteworthy that the 3,4-dimethoxyphenyl substituent (Ar^4), was present in all active compounds. Minor deviation from the 3,4-dimethoxy phenyl system to provide the 3-methoxy, 4-methoxy and 3,4-methylenedioxy analogs resulted in compounds exhibiting IC_{50} values of > 2 μM.

In conclusion, potent antagonists of OX2-R were identified in a ~140,000 member combinatorial library using a 384-well high-throughput functional calcium-mobilization screen. Correlation between library screening data (% inhibition) and measured IC_{50} values was excellent. Analysis of the specific substituents present in the active compounds identifies areas of the molecules where key functionality is required. The toleration of multiple tertiary amine substituents in conjunction with varying chain lengths and substitutions of the aminoalcohol-based spacer provides the opportunity for a number of cyclic constraints to be applied to the molecules. The optimization of this series of compounds as OX2-R antagonists and the scope of constraint applications will be reported elsewhere.

References and notes

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23. HEK293/OX2-R cells were seeded at 20,000 cells (50 μL) per well in poly-D-lysine)-treated 384-well plates (Costar, black clear-bottom cell culture-treated) in culture medium (Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal bovine serum, 1% Gluta-Max (Invitrogen), 1% nonessential amino acids, 1% 1 U/mL Penicillin/1 μg/mL Streptomycin, 1 mM sodium pyruvate, 10 mM HEPES, and 200 μg/mL G418). After 24 h at 37 °C, 5% CO_2, culture medium was removed and replaced with 25 μL per well of dye/buffer mix (equal parts of 2× concentrated Molecular Devices Calcium 3 no-wash dye and buffer consisting of Hanks' Balanced Salt Solution, 20 mM HEPES, pH 7.5, 0.2% BSA), with probenecid to a final concentration of 2.5 mM. Plates were incubated at 37 °C, 5% CO_2 for 30 min, and then equilibrated to room temperature for 30–90 min. Test compounds were diluted in Hanks' Balanced Salt Solution (HBSS), 20 mM HEPES, pH 7.5, 0.1% BSA, 2.5 mM probenecid (assay buffer), with 2% DMSO. Prior to each assay run, FLIPR 384 tips (Molecular Devices) were presoaked in 1% BSA/HBSS/20 mM HEPES, pH 7.5. For the antagonist assay, 12.5 μL test compound was added to dye-loaded cells in the $FLIPR^{384}$, followed within 30 min by addition of 12.5 μL of human orexin A (Bachem) in assay buffer to a final concentration of 20 nM. Fluorescence was monitored for 125 s after additions.