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ARTICLE *in* BIOORGANIC & MEDICINAL CHEMISTRY LETTERS · DECEMBER 2002

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Synthesis and Structure–Activity Relationships of Aminoalkylazetidines as ORL1 Receptor Ligands

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Received 23 May 2002; accepted 24 July 2002

Abstract—A series of aminoalkylazetidines has been discovered as novel ORL1 receptor ligands. Structure–activity relationships have been investigated at the azetidine N and the alkyl side chain sites. Several potent and selective analogues have been identified.

The ORL1 receptor is a G-protein coupled receptor with high homology to the opiate receptors.[1] Its endogenous ligand is called Nociceptin (or Orphanin FQ) and is a 17-aminoacid neuropeptide that is structurally related to the opiate peptide dynorphin A. Nociceptin, however, shows little affinity for the other opiate receptors.[2] Nociceptin has been shown to have many different behavioral effects, including interfering with motor performance, stimulating feeding, producing hyperalgesia, reversing stress-induced analgesia, producing analgesia and anxiolytic-like effects.[3] Recently, the discovery of non-peptidic small molecule ORL1 ligands has been disclosed.[4] In this communication, we would like to report our discovery and SAR investigations of a novel series of ORL1 ligands.

Our aminoalkylazetidine lead compound **1** (Fig. 1) was identified as an agonist from high-throughput screening of our chemical library, as it showed high affinity for ORL1 with a K_i of 31 nM and modest selectivity over μ-, κ, and δ receptors. Optical resolution was achieved by using a chiral column (Chiracel OJ, eluting with 95:5:0.2 hexane, EtOH, Et_2NH). The absolute stereochemistry of the less active enantiomer **1a** was determined by X-ray crystallography of its L-tartaric acid salt.[5] In a GTPγS assay both the racemate and its two enantiomers behave as agonists at the ORL1 receptor.

The synthesis of racemic aminoalkylazetidine analogues with various side chains is outlined in Scheme 1.[6] Thus condensation of the commercially available ethyl dibromobutyrate and benzhydrylamine followed by DIBAL reduction of the resulting azetidine ester gave the *N*-benzhydrylazetidine-2-carboxaldehyde. Grignard addition gave the aminoalcohols as a mixture of erythro and threo isomers (erythro-**4b**/threo-**4a** 3:1) in >50% yield. Because initial screening results demonstrated that the threo aminoalkylazetidines are consistently more potent than their erythro counterparts, we focused our efforts on the threo diamine analogues. Taking advantage of this natural selectivity, we subsequently inverted the stereochemistry of the major erythro aminoalcohol via a Mitsunobu reaction giving the desired threo aminoazide product.[7] Reduction of the azide generated the desired diamine analogues as racemates.[8,9]

The compounds described were evaluated in radioligand binding assays (Table 1). K_i values against the human ORL1 receptor were determined from competition binding assays using [^{125}I]Nociceptin and h-ORL1 receptor expressing Chinese hamster ovary (CHO) cell membranes as described.[10] K_i values for human μ-, κ-, and δ-opioid receptors were determined using [^{3}H]diprenorphine and CHO cell membranes expressing the opioid receptors as described.[10b] Functional [^{35}S]GTPγS binding assays for h-ORL1 receptor were carried out in CHO cell membranes expressing the receptor as described, using a 1 h incubation prior to initiation of assays.[10] Compounds that induce an increase in [^{35}S]GTPγS binding were identified as agonists, while compounds that have no effect on basal [^{35}S]GTPγS binding, but inhibit Nociceptin induced increase in the binding were identified as antagonists.

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0960-894X/02/$ - see front matter
PII: S0960-894X(02)00652-2

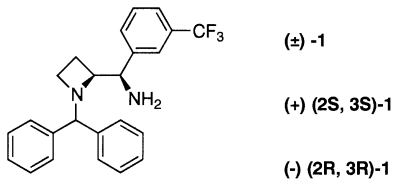
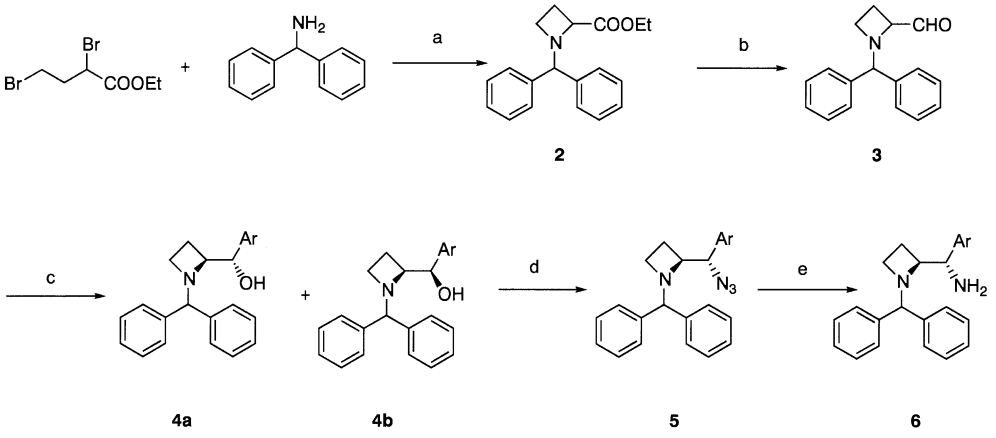


Figure 1. Aminoalkylazetidine lead compounds.

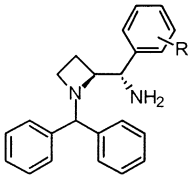
Nociceptin shows an EC_{50} of 1.11 ± 0.08 nM (mean ± SEM, $n = 10$) and a 100% increase in [^{35}S]GTPγS binding over basal level in this assay. In the meta substituted series, the methoxy and methyl moieties are poorly tolerated, while simply substituting the *m*-trifluoromethyl substituent with *m*-chloro or *m*-fluoro yields compounds with significant affinity and agonist functional properties. In the para position, chloro, methyl and methoxy groups are reasonably well tolerated, but *p*-phenoxy substitution dramatically reduces affinity.

Derivatizations at the azetidine N were carried out using a parallel synthesis approach as shown in Scheme 2. The *N*-allylazetidine carboxaldehyde was prepared as above starting with allylamine. Addition of commercially available 3-chlorophenylmagnesium bromide to the aldehyde gave a mixture of diastereomers (erythro/threo 3:1). After the threo isomer was transformed to the desired erythro one by a two-step procedure,[11] the erythro amino alcohol **9b** was converted to the threo aminoazide **10**. Reduction and protection gave the primary amine as an advanced intermediate for parallel synthesis. The azetidine nitrogen was revealed by removal of the allyl group[12] and then alkylated with various substituents. In cases where two diastereomers were generated, chromatographic separation gave pure



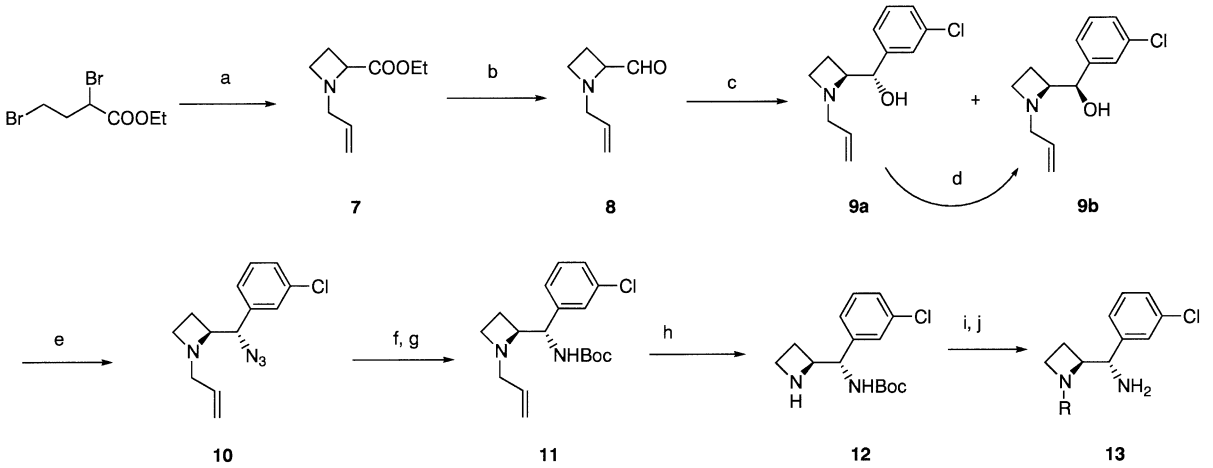
Scheme 1. (a) $NaHCO_3$, CH_3CN, 55%; (b) DIBAL-H, 50%; (c) ArMgBr, THF, >50%; (d) $Zn(N_3)_2(Pyr)_2$, Ph_3P, DIAD, toluene, ~75%; (e) $NiCl_2(H_2O)_6$, $NaBH_4$, MeOH, ~70%.

Table 1. Receptor binding of azetidine substituted phenyl analogues[a]



Compd	R	ORL1 K_i (nM)	μ K_i (nM)	κ K_i (nM)	δ K_i (nM)	Functional assay
(±)-1	CF_3	31	1967	1634	6088	Agonist
(+)-1a	CF_3	236	6088	3565	10960	Agonist
(−)-1b	CF_3	14	516	641	3312	Agonist
6a	3-F	191	1766	997	2923	Agonist
6b	3-Cl	37	1654	1232	8344	Nd
6c	3-Me	3% @ 10 μM	Nd	Nd	Nd	Nd
6d	3-MeO	45% @ 10 μM	Nd	Nd	Nd	Nd
6e	4-Cl	152	586	607	1439	Agonist
6f	4-Me	94	488	660	1856	Nd
6g	4-MeO	198	865	1341	2919	Nd
6h	4-PhO	7222	5401	2772	7722	Nd
6i	3-F-4-Me	58	152	150	634	Agonist
6j	3,5-F_2	105	4245	4015	9533	Agonist

[a]Results shown are mean values of 2–3 independent determinations. All assays were performed in duplicates. K_d values used for radiolabeled ligands were: [^{125}I]Nociceptin 23 pM for hORL1 receptor; [^{3}H]Diprenorphine 0.8 nM for hδ-, 0.31 nM for hκ-, and 0.12 nM for hμ-opioid receptors.



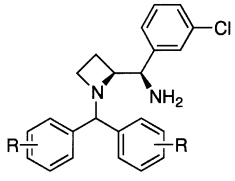
Scheme 2. Reagents and conditions: (a) allylamine, CH_3CN, 50%; (b) DIBAL, ether; (c) *m*-chlorophenylmagnesium bromide, THF, 68% two steps; (d) Ph_3P, DIAD, 4-nitrobenzoic acid; K_2CO_3, MeOH, 72%; (e) $Zn(N_3)_2(Pyr)_2$, Ph_3P, DIAD, toluene, 70%; (f) Ph_3P, THF–H_2O, 87%; (g) $(Boc)_2O$, TEA, 71%; (h) $Pd_2(dba)_3$, dppb, thiosalicylic acid, 73%; (i) RX, K_2CO_3; (j) TFA.

racemic isomers. Finally, the desired azetidine diamine analogues were obtained by removal of the Boc groups.

The SAR of the azetidine *N*-substituted analogues is shown in Tables 2 and 3. While the 3,3′-difluoro benzhydryl analogue **13a** gave comparable potency as the lead compound, other substituents on the benzhydryl moiety generally decreased the K_i dramatically (Table 2). Replacement of one phenyl ring of the benzhydryl group with acyclic alkyl groups generated compounds with high affinity and improved selectivity versus other opioid receptors. In each example the '**B**' diastereomer was more potent than the '**A**' diastereomer. These designations were assigned by their chromatographic mobility upon SiO_2 chromatography and no effort has been made to determine the relative stereochemistry. The potency peaked at incorporation of a butyl group (**13k**, $n=3$). Other analogues with a butyl chain demonstrated similar binding profiles. Replacement of the benzhydryl with a 5-nonanyl group resulted in an inactive compound. These results may suggest that the two phenyl groups of the benzhydryl probably interact differently with the receptor. Pyrrolidine analogues of this series had remarkably reduced affinity for ORL1.

In summary, we have developed a practical, stereoselective synthetic route to threo aminoalkylazetidines to explore the ORL1 structure–activity relationships around the lead compound **1**. These compounds show stereoselective binding to the ORL1 receptor and have modest to good potency. Selectivity over other opiate receptors is generally good. In particular compound **13k–b** shows high affinity ($K_i=30$ nM) and good selectivity (μ/ORL1 = 61, κ/ORL1 = 114, and δ/ORL1 = 268). Compound **13k–b** is an agonist at the ORL1 receptor. Although both agonist and antagonists of this receptor have been observed, the series typically yields agonists when high affinity is present. Further work in this area will be presented in due course.

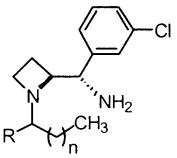
Table 2. Receptor binding of azetidine *N*-substituted benzhydryl analogues[a]



Compd	R	ORL1 K_i (nM)	μ K_i (nM)	κ K_i (nM)	δ K_i (nM)	Functional assay
13a	3,3′-F_2	64	4317	3533	4787	Agonist
13b	3,3′-Cl_2	420	7159	6037	2709	Agonist
13c	3,3′-$(CF_3)_2$	2593	Nd	Nd	Nd	Nd
13d	4,4′-F_2	684	2176	2240	7430	Nd
13e	4,4′-Cl_2	3221	3452	3251	5013	Nd
13f	4,4′-Br_2	2532	2502	1484	4987	Nd
13g	4,4′-Me_2	1135	801	1841	5011	Nd

[a]See Table 1 notes.

Table 3. Receptor binding of azetidine *N*-alkylsubstituted analogues[a]



Compd	R	n	ORL1 K_i (nM)	μ K_i (nM)	κ K_i (nM)	δ K_i (nM)	Functional assay
13h	Ph	0	1062	10620	4099	39120	Nd
13i–a	Ph	1	570	8067	13130	22410	Nd
13i–b	Ph	1	94	7217	7718	22370	Agonist
13j–a	Ph	2	367	4130	2938	10090	Nd
13j–b	Ph	2	79	6015	7172	16460	Agonist
13k–a	Ph	3	745	2248	3684	8819	Nd
13k–b	Ph	3	30	1815	3432	8048	Agonist
13l–a	Ph	4	772	2148	3911	7579	Nd
13l–b	Ph	4	34	1939	2993	5958	Nd
13m–a	Ph	5	1016	1591	1945	4978	Nd
13m–b	Ph	5	185	1494	2855	3913	Antagonist
13n–a	3-F-Ph	3	680	4705	4125	12270	Nd
13n–b	3-F-Ph	3	26	1599	2134	8710	Agonist
13o–a	3,5-F_2-Ph	3	347	5551	6488	9372	Nd
130–b	3,5-F_2-Ph	3	49	3975	4219	11240	Agonist
13p	*n*-Bu	3	1345	9666	4262	31780	Nd

[a]See Table 1 notes.

Acknowledgements

We would like to thank Dr. William Greenlee, Dr. Michael Czarniecki, and Dr. Dean Tulshian for their support and encouragement of this work.

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