Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 3247–3252

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design and Parallel Synthesis of Piperidine Libraries Targeting the Nociceptin (N/OFQ) Receptor

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Received 27 March 2003; revised 10 June 2003; accepted 24 June 2003

Abstract—Based on literature structures, we proposed a pharmacophore for NOP receptor ligands and used it as a guide for the design of a focused piperidine library and an optimization library. Potent NOP receptor agonists and antagonists were obtained from these libraries as well as a few potent, mu selective agonists.

Combinatorial synthesis methodology has been widely utilized in modern drug discovery, especially in the lead generation and lead optimization stages. The combination of rational drug design and combinatorial synthesis has accelerated the process of lead generation and optimization. In this communication, we would like to report on our use of these techniques in the discovery of novel small molecule NOP receptor ligands.

The Nociceptin/orphanin FQ (N/OFQ) receptor (NOP, previously named ORL-1) was discovered by several research groups in 1994 through cDNA expression cloning techniques.[1] Subsequently its endogenous ligand nociceptin (N/OFQ), a novel heptadeca neuropeptide was isolated from brain and identified in 1995.[2] This discovery has generated considerable interest within the scientific community due to the important roles of classical opioid receptors in the CNS. Although NOP receptor is a member of the G-protein coupled receptor superfamily with about 47% identity to the classical opioid receptors [MOP (μ), DOP (δ) and KOP (κ)], native opioid peptides and synthetic agonists selective for MOP, DOP or KOP receptors do not show significant affinity for NOP receptor.[3] Using nociceptin (N/OFQ) and its peptide analogues, a number of in vivo experiments have demonstrated that N/OFQ modulates a variety of biological functions such as food intake, memory processes, cardiovascular functions, locomotor activity, and control of neurotransmitter release at peripheral and central sites.[4] Moreover, it was observed that N/OFQ is involved in modulating pain mechanisms at the level of the spinal cord and that N/OFQ might also be relevant to the treatment of CNS disorders such as anxiety and drug abuse.[4,5] It has been proposed that the identification of small molecule agonists and antagonists of nociceptin could lead to new medications for pain and anxiety.[6]

Recently, several research groups have reported their efforts in the search for small molecule N/OFQ agonists and antagonists.[7] These reports have prompted us to disclose our early efforts in the design and parallel synthesis of phenyl piperidine libraries targeting the NOP receptor.

Design of the Library

Our library design was based on several known agonists and antagonists that have been previously published (Fig. 1).[8] Compounds **1** and **2** were reported to have pK_i of 7.3 and 8.4 towards NOP receptor, respectively.[8] Compound **3** was also reported as an NOP ligand.[8] After a thorough analysis of the shared features of these compounds, we have developed a pharmacophore for the NOP receptor ligands (shown in Fig. 1). The pharmacophore contains a large hydrophobic group connected to a heteroatom-containing ring system through a 1–3 bond linker. A basic nitrogen was thought to be the preferred heteroatom on the ring since this basic

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doi:10.1016/S0960-894X(03)00665-6

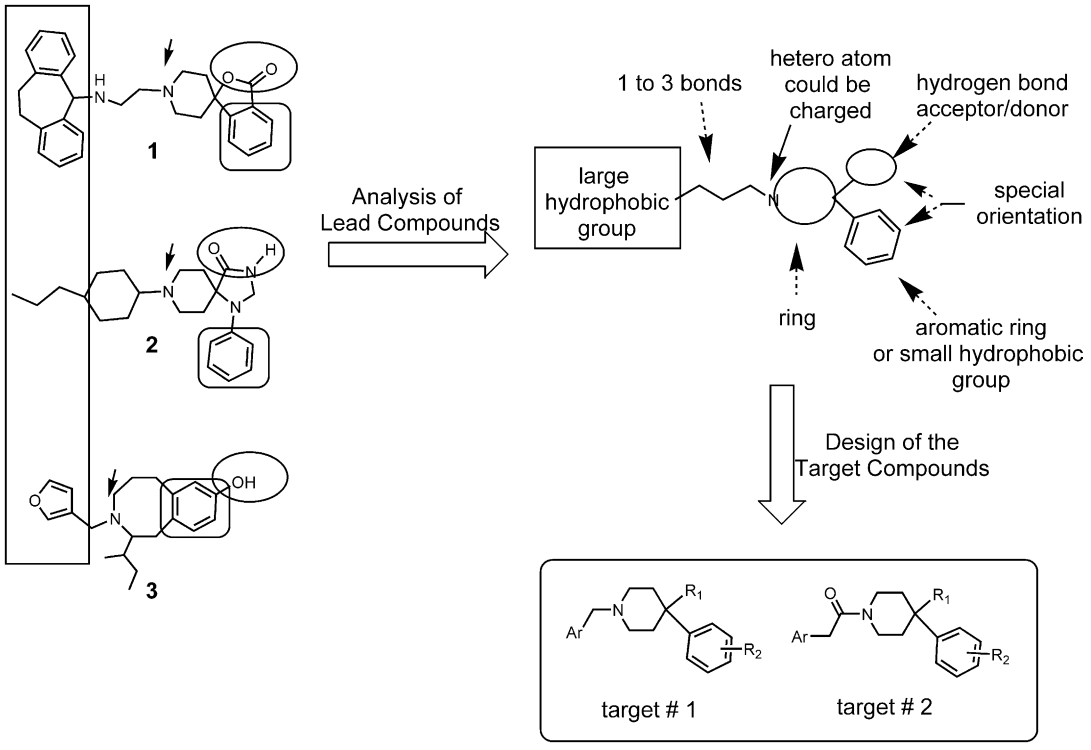


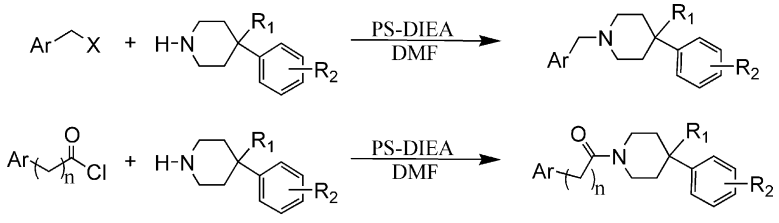
Figure 1. Design of phenylpiperidine libraries.

nitrogen might interact with the ASP[268] residue in the NOP receptor in analogy to the way the nitrogen atom of morphine binds to the μ receptor. On the right side of the pharmacophore, there are a medium size hydrophobic group and a hydrophilic group attached to the ring and these two groups could be separated by a specific distance or dihedral angle. Using this 2D pharmacophore as a guide, through a similarity search of the ACD database, we identified a group of commercially available piperidines with a high degree of similarity to the right portion of the pharmacophore and proposed to synthesize functionalized piperidines (target library **1** and **2**) as a starting point for our search of novel NOP receptor agonists and antagonists. Herein, R_1 is an hydrophilic group such as hydroxyl group while Ar is an hydrophobic group such as an aromatic or heteroaromatic ring.

Parallel Synthesis of a Phenyl-piperidine Library

The library of piperidines was synthesized by alkylation or acylation of selected piperidines (Scheme 1). These piperidine building blocks are listed in Table 1. P1–P7 are 4-phenylpiperidines with a hydroxyl, nitrile or acetyl group at the 4-position. P8–P12 are other piperidines with a high degree of similarity to the pharmacophore in Figure 1. The building blocks for alkylating and acylating reagents are listed in Table 2(a and b).

Both alkylation and acylation reactions used solid supported DIEA as a base and DMF as solvent. The reactions were run at 0.2 mMol scale in parallel in 1-dram vials under vortex shaking. After 12 h at rt or 80 °C (heating was needed for alkylation reactions), the vials were cooled to room temperature, the solution was filtered into a new set of vials and an aliquot was taken for LC/MS. For about half of the reactions, the crude products were >70% pure. Our library purity cutoff for the initial high throughput screening was set to be greater than 70% (determined by LC/MS). The largest percent impurity was unreacted amine or decomposition of the alkylating or acylating reagents in the reaction condition. It is noteworthy that three alkylating building blocks (AL3, AL7 and AL13) and one acylating building blocks (AC10) gave substantially more by-products due to their instability or over-reactivity. For those less than 70% pure, an aqueous acid/base wash was done to purify the products. After this purification, >90% of compounds passed the 70% purity cutoff line.



Scheme 1. Synthesis of target libraries 1 and 2.

Table 1. Piperidine building blocks P1–P12

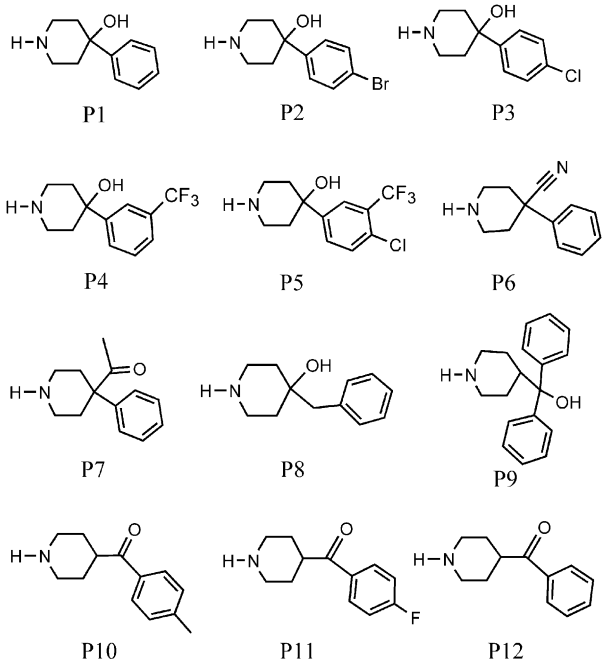
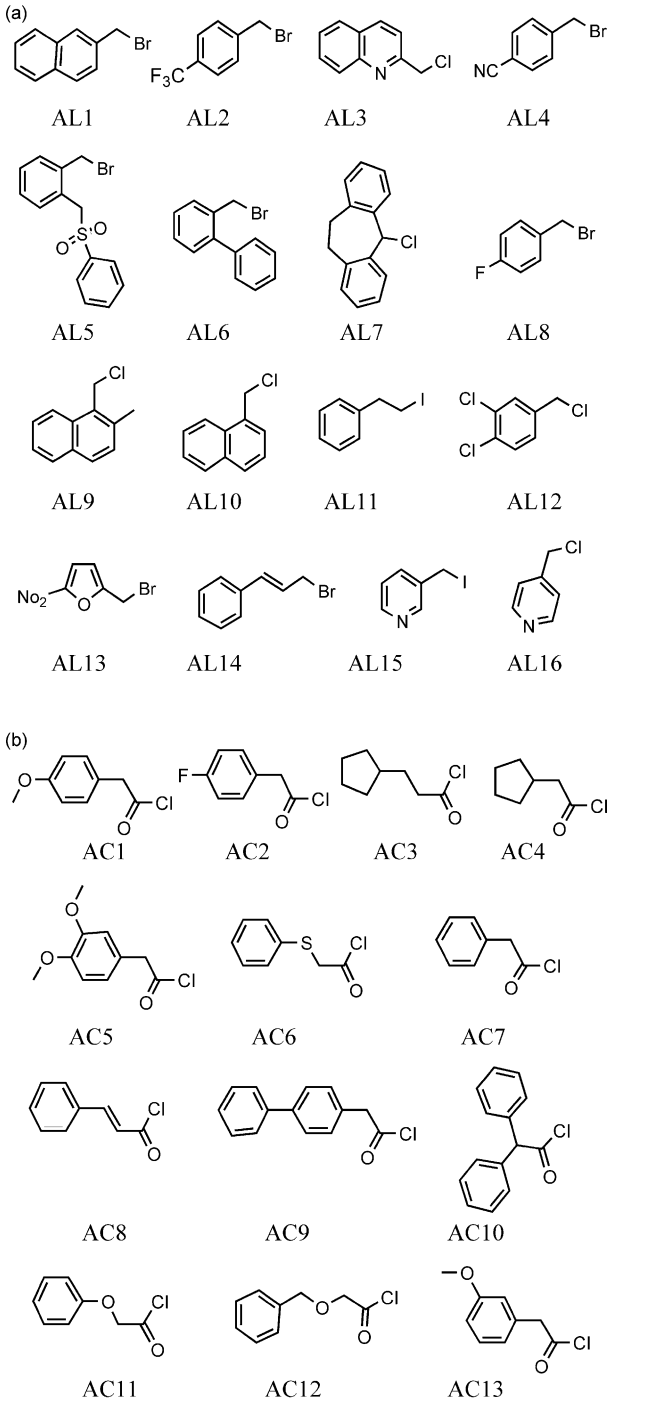


Table 2. Alkylating and acylating building blocks



The solvents in vials were removed under vacuum at rt by using a speedvac (Genevac HT8). The residue in the vessels was dissolved in DMSO, diluted to ca. 10 μM and then sent for preliminary high throughput screening.

Preliminary screening of the library identified 20 active ligands, which showed greater than 50% inhibition against NOP receptor. Statistical analysis of the hit compounds shows: (1) the hit compounds were centered around a few piperidines: six hits from P5, four hits from P4 and P6, two hits from P2 and P3, one hit from P1 and P9 each (see Table 1 for structures). (2) Of the 20 hits, 19 compounds come from the alkylation reactions, only one hit from the acylation reactions. This suggested that the basic amine on the piperidine ring is necessary for better ligands. (3) 12 different alkylating agents provided one or more hit compound. This indicated that the large hydrophobic pocket in the receptor could accommodate a variety of different groups.

Hit confirmation

To further characterize the hits, the 20 hit compounds were selected for resynthesis via traditional synthetic methods and their K_i's measured via binding to the human NOP receptor expressed in recombinant HEK-293 cells.[9] To confirm our preliminary screening results, we also synthesized four inactive compounds and tested them as well. Table 3 shows some representative data from this library. The K_i's of the hit compounds ranged from 153 nM to 6.5 μM. Three inactive compounds (AL4P5, AL13P5, and AL9P6) are inactive in our radioligand binding assay while one inactive compound (AL2P4) shows marginal activity. We also identified 2 false positives (AL8P6 and AC12P2). The false hit from the acylation product AC12P2 further confirms the importance of the basic nitrogen on the piperidine ring that could serve as a binding point to the NOP receptor.

Optimization Library

The data in Table 3 suggested that building blocks P3 to P6 (Table 1) confer good affinity at the NOP receptor. However, all the hits generated from the alkylation library are only moderately active in our N/OFQ assay. To increase the affinity in this novel series, it was proposed that cycloalkyl groups attached to the piperidine nitrogen could be better ligand for the NOP receptor due to their greater conformational flexibility than the aromatic groups.[10] Based on this assumption, an optimization library was synthesized using four ketones, one aldehyde and one alkyl bromide (Scheme 2) via reductive amination reactions and alkylation reactions.

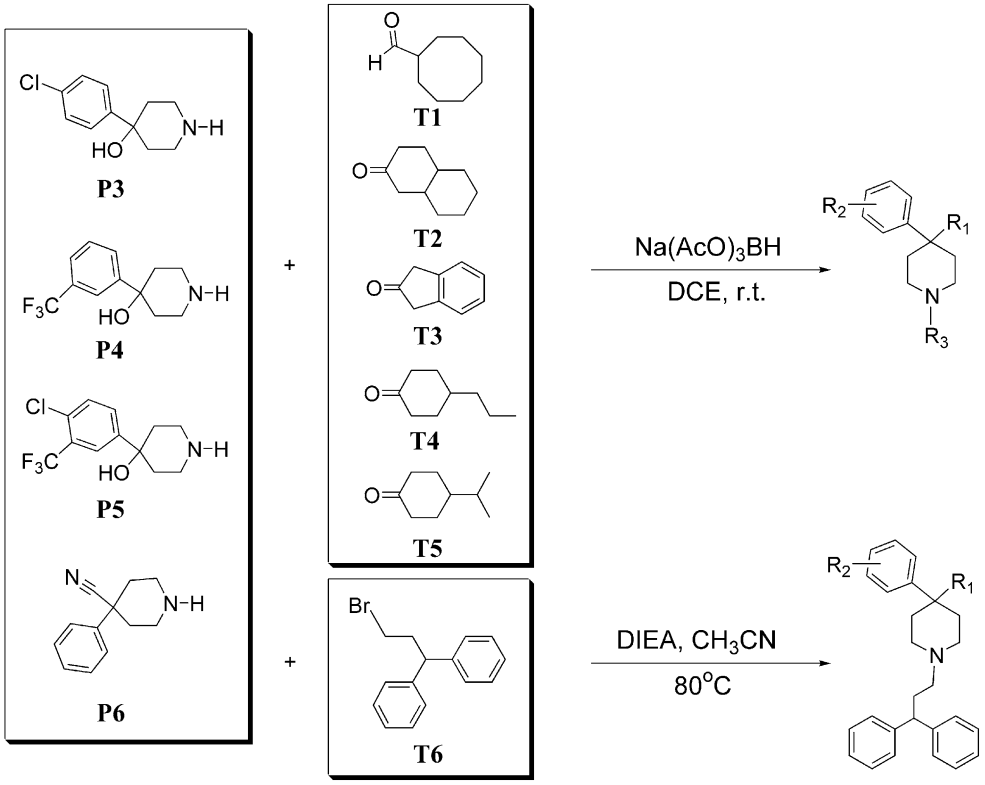
Table 3. Hit conformation

Compd	Piperidine	% Inhibition	K_i (nM)[a]
AL1P3	P3	90	153 (±47)
AL2P3	P3	85	307 (±114)
AL1P4	P4	85	715 (±100)
AL2P4	P4	Inactive	6520 (±2484)
AL8P4	P4	80	692 (±134)
AL1P5	P5	70	879 (±260)
AL2P5	P5	55	1194 (±448)
AL4P5	P5	Inactive	>10,000
AL8P5	P5	90	234 (±147)
AL13P5	P5	Inactive	>10,000
AL1P6	P6	70	1106 (±360)
AL8P6	P6	55	>10,000
AL9P6	P6	Inactive	>10,000
AL10P6	P6	55	827 (±236)
AL12P6	P6	65	1270 (±303)
AC12P2	P2	50	>10,000

[a]Values are means of three experiments, standard deviation is given in parentheses (>10,000 = not active).

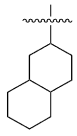
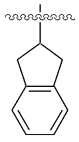
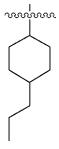
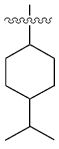
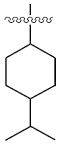
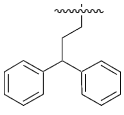
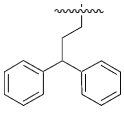
The 20 reductive amination reactions and four alkylation reactions were run in parallel fashion in 10 mL vials at 0.5 mMole scale. An aliquot of the crude reaction mixture was injected directly onto an LC/MS instrument to access purity. All reactions were found to have a crude purity of 75–90%. The reactions were worked up by adding water and EtOAc in sequence. The EtOAc layers were pipetted out into a new set of vials and solvent was removed in a speedvac. The residue was dissolved in a small amount of DCM and loaded into a 20-mL tube containing 5 g of silica gel. The tubes were eluted in parallel first by a mixture of EtOAc/hexanes (1:9) and followed by a mixture of Et_3N/EtOAc/hexane (5, 25, 70%). The fractions were collected in 10 mL vials and analyzed by LC/MS. The solvents were removed in a speedvac. The pure fractions (>97% pure by LC/MS and NMR) were collected and submitted for binding and functional assays.

The optimization library resulted in potent NOP agonists and antagonists. Compounds **7** and **10** are potent agonists (12 nM, Table 4) for NOP receptor.[11] However, the changes from aromatic groups to cycloalkyl groups also enhanced the mu activity. Overall, both compounds only show 2–3 fold selectivity over the μ receptor, but they are 400+-fold selective against the δ and κ receptors. Compounds **7**, **9**, **10** and **11** were



Scheme 2. Optimization libraries.

Table 4. Selected data from optimization library

Compd	R1	R2	R3	ORL1 K_i (nM)	μ K_i (nM)	Selectivity μ/ORL1	δ K_i (nM)	κ K_i (nM)	ORL-1 functional
4	OH	4-Cl		502	626	1.25	N/A	N/A	**Antagonist**
5	OH	3-CF3		102	113	1.11	N/A	4240	**Antagonist**
6	OH	3-CF3, 4-Cl		47	124	2.64	8779	1653	**Antagonist**
7	OH	3-CF3		12	21	1.75	10,000	N/A	**Agonist**
8	OH	3-CF3, 4-Cl		82	19	0.23	10,000	N/A	**Agonist**
9	OH	3-CF3		135	46	0.34	N/A	6000	**Agonist**
10	OH	3-CF3		12	36	3.00	5674	4153	**Agonist**
11	OH	3-CF3, 4-Cl		33	8	0.24	10,000	N/A	**Agonist**
12	OH	3-CF3		116	1	0.01	9385	N/A	**Agonist**
13	OH	3-CF3, 4-Cl		225	4	0.02	N/A	N/A	**Agonist**

screened as a mixture of *cis*- and *trans*-isomers, since the *cis*- and *trans*-isomers could not be separated by regular column chromatography.

Compounds **4**, **5** and **6** are NOP antagonists with K_i's ranging from 47 to 502 nM. They are also partial μ agonists (μ functional data not shown). Compound **6** shows good selectivity against δ and κ receptors (186- and 35-fold, respectively).

It is also interesting to note that compounds **12** and **13** are potent and selective μ agonists (1 and 4 nM, respectively, μ functional data not shown) with selectivities against NOP receptor at 116- and 56-fold, respectively.

Compound **12** also showed a high degree of selectivity against the δ receptor (9000-fold).

Based on literature structures, we have proposed a pharmacophore for NOP receptor ligands and have used it as a guide for the design of a focused piperidine library. The preliminary library has provided us with 20 hits of which 18 were confirmed hits. A second optimization library has improved the potency of the lead compounds by 10- to 100-fold and provided us potent NOP agonists and antagonists with a few potent mu selective agonists. Future work in this series will be focused on the selectivity between the MOP receptor and NOP receptor.

Acknowledgements

We thank Drs. Donald J. Kyle, R. Richard Goehring, Qun Sun for suggestions and helpful discussions, and Drs. Sam F. Victory, Yakov Rotshteyn and Jeffrey M. Marra for technical assistance.

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11. Values are means of three experiments. Human μ, κ and δ opioid receptor dose displacement binding assays were conducted according to the product inserts (Perkine Elmer Life Sciences). A compound was considered to be a full N/OFQ agonist when its ability to stimulate GTPγS binding was $>75\%$ in comparison to N/OFQ. An N/OFQ antagonist stimulated GTPγS binding with efficiency $<25\%$ in comparison to N/OFQ.