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Synthesis and evaluation of 4-substituted piperidines and piperazines as balanced affinity μ opioid receptor (MOR) agonist/δ opioid receptor (DOR) antagonist ligands

Aaron M. Bender[1,2], **Mary J. Clark**[3], **Michael P. Agius**[1,2], **John R. Traynor**[3], and **Henry I. Mosberg**[1,2,*]

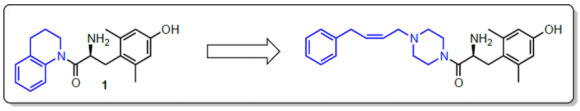
[1]Department of Medicinal Chemistry, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109

[2]Interdepartmental Program in Medicinal Chemistry, University of Michigan, Ann Arbor, MI 48109

[3]Department of Pharmacology, Medical School, University of Michigan, Ann Arbor, MI 48109

Abstract

In this report, we describe a series of 4-substituted piperidine and piperazine compounds based on tetrahydroquinoline **1**, a compound that shows balanced, low nanomolar binding affinity for the mu opioid receptor (MOR) and the delta opioid receptor (DOR). We have shown that by changing the length and flexibility profile of the side chain in this position, binding affinity is improved at both receptors by a significant degree. Furthermore, several of the compounds described herein display good efficacy at MOR, while simultaneously displaying DOR antagonism. The MOR agonist/DOR antagonist has shown promise in the reduction of negative side effects displayed by selective MOR agonists, namely the development of dependence and tolerance.



Although opioid analgesics represent the gold standard for the treatment of acute and chronic pain, their usage is often accompanied by undesirable side effects such as the development of dependence and tolerance. A considerable amount of research has thus been done to find a potent analgesic that does not display these negative attributes. In general, clinically used opioid analgesics such as morphine evoke both the desired and undesired effects through activation of the mu opioid receptor (MOR). Numerous reports have indicated that the undesired MOR-related side effects may be ameliorated by concomitant ligand interaction with the delta opioid receptor (DOR). It has been shown that the co-administration of DOR-selective agonists[1] or antagonists[2] with a MOR agonist can attenuate the dependence and tolerance typically associated with the latter.

A ligand displaying good binding affinity for both MOR and DOR represents a significant advantage over the co-administration of multiple drugs, due to both increased

*To whom correspondence should be addressed him@med.umich.edu.

pharmacokinetic simplicity as well as improved patient compliance. For these reasons, the development of small molecule MOR/DOR bifunctional opioid ligands has attracted much attention. MOR agonist/DOR antagonist compounds have been shown to be effective analgesics with a diminished tolerance and dependence profile[3,4] and have found use in other areas, such as for the treatment of irritable bowel syndrome.[5] Recently, we showed that a MOR agonist/DOR antagonist compound was an effective analgesic after interperitoneal administration, with a duration of action comparable to morphine.[6]

In an effort to further develop drug-like MOR/DOR bifunctional ligands, we turned our attention to compound **1** (Figure 1) a compound previously synthesized by our lab that displays equal binding affinity for both MOR and DOR, as well as for the kappa opioid receptor (KOR) (K_i = 25.8 nM (MOR); 33.0 nM (DOR); 36.5 nM (KOR), unpublished observations). Given the relative simplicity of the compound and its nonselective binding profile, we reasoned it would be a good starting point for derivatization. Computational modeling suggested that position 4 would be the optimal point for diversification, as an aromatic moiety at this position would be ideally situated to interact with Asn^{125}, Thr^{218}, and Lys^{303} in the MOR active site, and the resulting compound would thus function as a MOR agonist.[7].

1 was initially substituted with a benzyl group at the 4 position (**2**, Table 1). The synthesis of **2** began by subjecting ketone **13** to a Wittig reaction to yield alkene **14**, which was subsequently hydrogenated and deprotected to give amine **15**, to which was coupled Boc-protected L-2,6-dimethyltyrosine (Boc-L-Dmt) and deprotected (Scheme 1). Binding affinity (K_i) was obtained by competitive displacement of radiolabeled [^{3}H]diprenorphine in C6 cells stably expressing MOR or DOR or CHO cells stably expressing KOR. Efficacy was assessed by agonist-stimulated [^{35}S] GTPγS binding in the same cells.[6,8,9]

Compared to **1**, the resulting compound **2** displayed no significant change in binding affinity for MOR and DOR, but showed decreased affinity for KOR (Table 1). Unfortunately, **2** also displayed no notable efficacy at MOR as determined by the [^{35}S] GTPγS assay. Because the synthesis of **2** proved somewhat laborious, and the resulting diastereomers could not be resolved by RP-HPLC, we reasoned that synthesis of further analogues could be simplified by the replacement of the tetrahydroquinoline (THQ) core of **2** with a piperidine, effectively eliminating a stereocenter. The resulting compound **3** displayed roughly a tenfold increase in binding affinity for MOR and DOR, but still lacked any efficacy at MOR. The remainder of our SAR campaign was focused on changing the length and flexibility profile of the side chain in an attempt to not only retain strong binding affinity for both MOR and DOR, but to increase efficacy at MOR. For purposes of synthetic utility as well as increased solubility, the piperidine core was also replaced with a piperazine for most of the analogues, the results of which are summarized in Table 1.

Compounds **3–5**, and **9** were synthesized by coupling a commercially available piperidine or piperazine derivative with Boc-L-Dmt, followed by TFA-mediated deptrotection and HPLC purification to yield the final compounds. In the case of **8** and **12**, a commercially available primary alcohol was first mesylated and refluxed with excess piperazine to give intermediates **16** and **17**, which were then coupled with Boc-L-Dmt and deprotected under similar conditions (Scheme 2). The remainder of the compounds were synthesized as shown in Scheme 3. The appropriate commercially available aldehyde was subjected to a Horner-Wadsworth-Emmons type olefination to give alkenes **18**, **19** and **27**, which were then reduced to the corresponding alcohols using either DIBAL (for the formation of allylic alchols) or LAH (for the formation of saturated alcohols). Before reduction, alkene **19** was first hydrogenated to give saturated ester **24**. All intermediates were then carried forward in a similar manner as in Scheme 2 to give finished products.

The synthesized analogues in Table 1 display a broad range of binding affinities for MOR (29 nM to 0.29 nM), and to a lesser extent, DOR (150 nM to 6.6 nM). Extension of the side chain of **3** from 1 to 3 methylene units did little to change binding at MOR or DOR, but encouragingly, the resulting compound (**4**) behaved as a weak partial agonist at MOR. Replacement of the piperidine core of **4** with a piperazine (**5**) proved inconsequential, and the continued balanced MOR/DOR binding profile of this analogue led us to pursue other aromatic moieties separated by three methylene units from the piperazine core. Analogue **6** in particular showed an improved balanced MOR/DOR binding profile, and also displayed a partial agonist profile at MOR. Interestingly, compound **7**, in which the 1-naphthyl side chain of **6** is constrained with an additional double bond, showed no efficacy in the [^{35}S]GTPγS assay at all three receptors, with an additional loss of binding affinity for KOR. The insertion of an extra aromatic moiety as in the case of the diphenylmethyl analogue **8** did little to increase binding affinity for either MOR or DOR. Further extension of the distance between the aromatic side chain and the piperazine core (**9**) resulted in a boost in MOR binding, without drastically affecting DOR. Although these 4 carbon analogues (**9–11**) suffered a slight loss of MOR/DOR affinity balance, all displayed good efficacy at MOR, particularly the unsaturated analogues **10** and **11** (20 and 41 nM, respectively). Side chain extension to 5 methylene units (**12**) did little to improve upon the profile of **10** or **11**.

Structurally, these analogues exhibit some similarities to the class of *trans*-3,4-dimethyl- 4-(3-hydroxyphenyl)piperidine opioid antagonists originally described by Zimmerman[10] and explored by others.[11] In our series, the 3-hydroxyphenyl moiety is replaced by 2,6-L-dimethyltyrosine, and the piperidine (or piperazine) core is left unsubstituted. In both series, receptor selectivity is modulated by the nature of the lipophilic side chain attached para to the phenolic component of the molecule. The 11 piperidine and piperazine analogues of tetrahydroquinoline **1** described here display a favorable balance between binding affinity at MOR and DOR, and several (**4–6**, **9–12**) display improved potency at MOR as compared to morphine (K_i (MOR) = 6.3 nM, (DOR) = 171 nM; EC50 (MOR) = 194 nM).[12,13] These analogues are therefore promising leads for further derivatization and *in vivo* studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Diminished Tolerance and Dependence Side Effects. J. Med. Chem. 2012; 55:8350–8363. [PubMed: 23016952]

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8. Radioactive compounds were purchased from Perkin-Elmer (Waltham, MA, U.S.). Opioid ligand-binding assays were performed using competitive displacement of 0.2 nM [^{3}H]diprenorphine (50Ci/mmol) by the test compound from membrane preparations containing opioid receptors. The assay mixture, containing membrane suspension (20 μg protein/ tube) in 50 mM Tris-HCl buffer (pH 7.4), [^{3}H]diprenorphine, and various concentrations of test peptide, was incubated at room temperature for 1 h to allow binding to reach equilibrium. The samples were rapidly filtered through Whatman GF/C filters using a Brandel harvester (Brandel, Gaithersburg, MD, U.S.) and washed three times with 50 mM Tris-HCl buffer. The radioactivity retained on dried filters was determined by liquid scintillation counting after saturation with EcoLume liquid scintillation cocktail in a Wallac 1450 MicroBeta (Perkin-Elmer, Waltham, MA, U.S.). Nonspecific binding was determined using 10 μM naloxone. K_i values were calculated using nonlinear regression analysis to fit a logistic equation to the competition data using GraphPad Prism, version 5.01, for Windows (GraphPad Software Inc., La Jolla, CA). The results presented are the mean ± standard error from at least three separate assays performed in duplicate.
9. Membranes (10–20 μg of protein/tube) were incubated 1 h at room temperature in GTPγS buffer (50 mM Tris-HCl, 100 mM NaCl, 5 mM MgCl, pH 7.4) containing 0.1 nM [^{35}S]GTPγS, 30 μM guanosine diphosphate (GDP), and varying concentrations of test peptides. Peptide stimulation of [^{35}S]GTPγS was compared with 10 μM standard compounds [D-Ala2,N-MePhe4,Gly-ol]enkephalin (DAMGO) at MOR, D-Pen2,5-enkephalin (DPDPE) at DOR, or U69,593 at KOR. The reaction was terminated by rapidly filtering through GF/C filters and washing 10 times with GTPγS buffer, and retained radioactivity was measured as described above. The results presented are the mean ± standard error from at least three separate assays performed in duplicate; maximal stimulation was determined using nonlinear regression analysis with GraphPad Prism (GraphPad Software Inc., La Jolla, CA).
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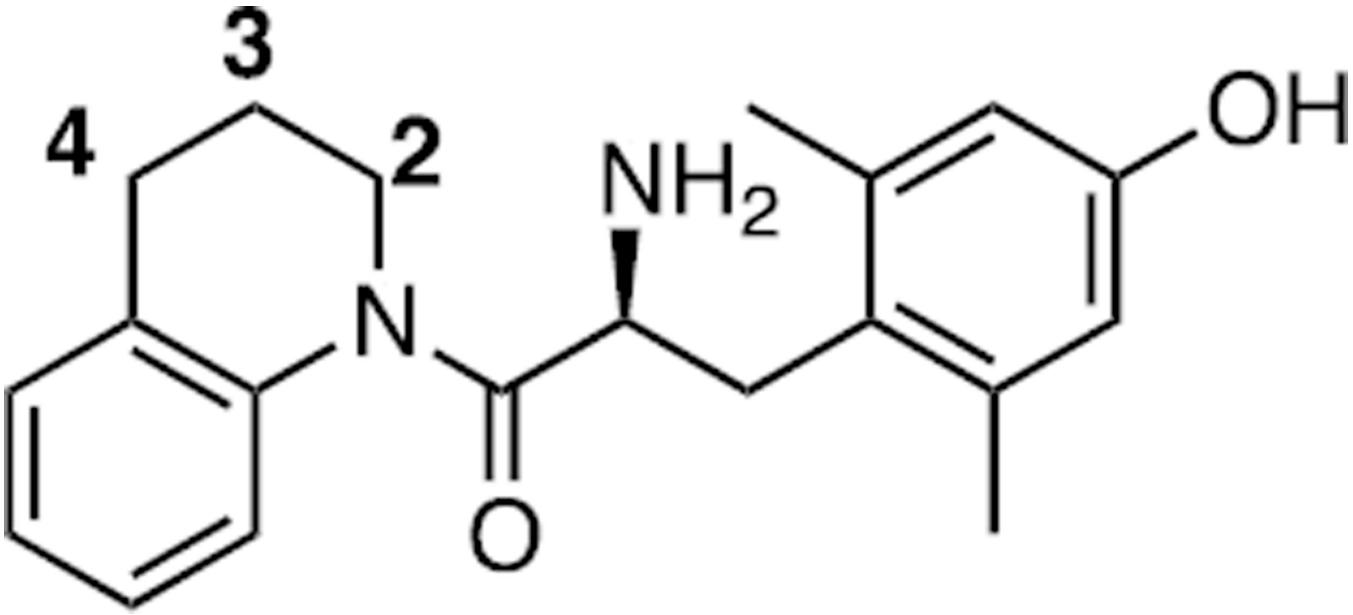
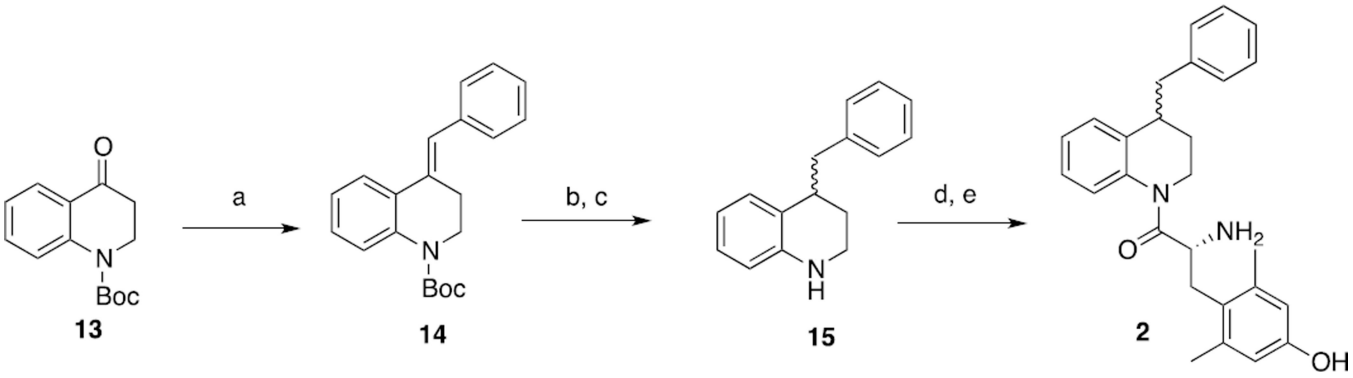
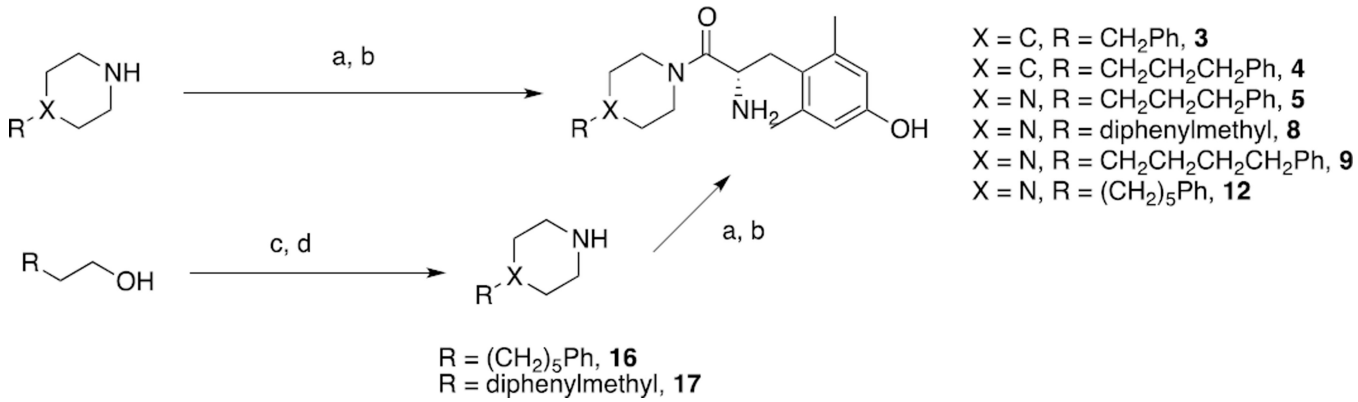


Figure 1.
Compound **1**



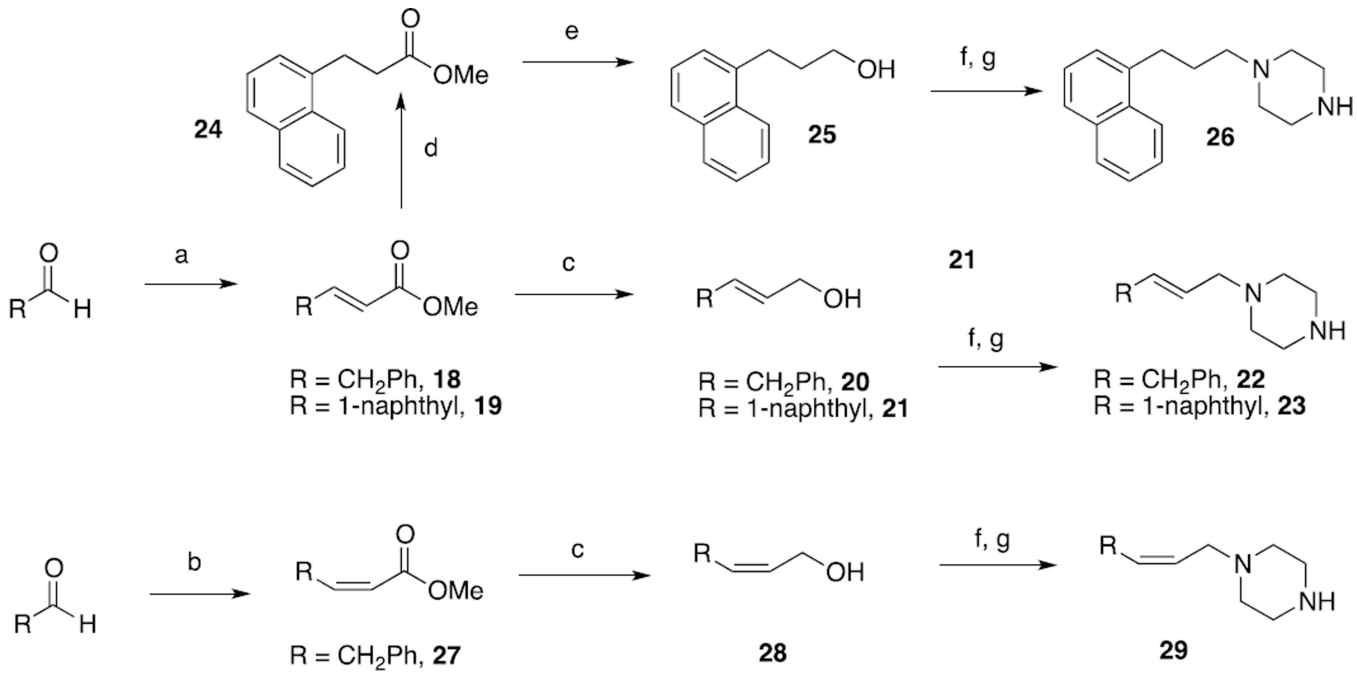
Scheme 1. Synthesis of compound 2a

a. Reagents and conditions: (a) triphenylphosphinebenzyl bromide, n-BuLi, THF, reflux; (b) H_2, 10% Pd/C, MeOH, 50 psi; (c) TFA, DCM; (d) Boc-L-Dmt, HATU, HOBt-Cl, DIEA, DMF, 4Å molecular sieves, 40°C; (e) TFA, DCM.



Scheme 2. Synthesis of analogues 3–5, 8, 9, 12a

a. Reagents and conditions: (a) Boc-L-Dmt, PyBOP or HATU, HOBt-Cl, DIEA, DMF; (b) TFA, DCM; (c) MsCl, Et_3N, DCM, 0°C; (d) piperazine, THF, reflux.

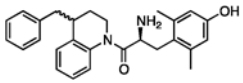
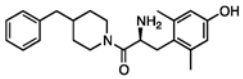
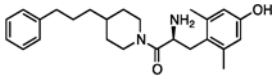
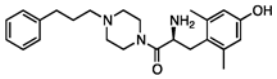
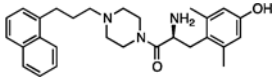
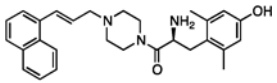
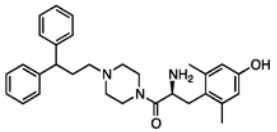
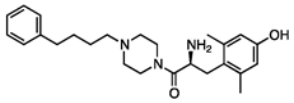


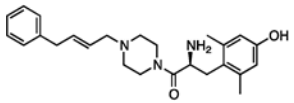
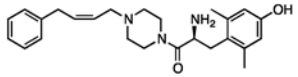
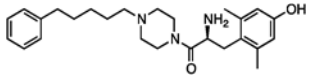
Scheme 3. Synthesis of analogues 6, 7, 10, 11a

a. Reagents and conditions: (a) $O=P(CH_3CH_2O)_2CH_2CO_2CH_3$, NaH, THF, 0°C; (b) $O=P(CF_3CH_2O)_2CH_2CO_2CH_3$, NaH, THF, 0°C; (c) DIBAL, DCM, −78°C; (d) H_2, 10% Pd/C, MeOH, 15 psi; (e) LAH, THF, 0°C; (f) MsCl, Et_3N, DCM, 0°C; (g) piperazine, THF, reflux.

Table 1

Binding affinity and efficacy data for analogues 2–12[a]

	Structure	K_i (nM)			EC_{50} (nM)			% stimulation		
		MOR	DOR	KOR	MOR	DOR	KOR	MOR	DOR	KOR
2		29±9	14±2	310±50	dns	dns	dns	11±6	dns	dns
3		1.4±0.1	7.8±0.9	140±24	dns	dns	dns	dns	dns	dns
4		2.0±0.8	12±2	110±11	119±39	dns	dns	26±2	dns	dns
5		3.8±0.8	36±4	250±51	150±49	dns	dns	23±2	dns	dns
6		1.1±0.3	6.6±2	78±15	85±10	dns	dns	17±3	dns	dns
7		6.4±0.1	11±0.65	330±92	dns	dns	dns	dns	dns	dns
8		1.1±0.2	21±7	150±42	dns	dns	dns	dns	dns	dns
9		0.45±0.3	6.6±2	78±15	64±3	dns	dns	43±6	dns	12±1

	Structure	K_i (nM)			EC_{50} (nM)			% stimulation		
		MOR	DOR	KOR	MOR	DOR	KOR	MOR	DOR	KOR
10		0.29±0.07	23±8.9	89±23	20±3.6	dns	1500±120	36±4	dns	16±3
11		0.3±0.1	28±6.8	54±3.2	41±15	dns	dns	49±5	dns	11±7
12		0.42±0.15	6.9±1.7	39±15	53±23	dns	660±80	42±1	dns	12±5

[a] All values are expressed as the mean ± SEM of three separate assays performed in duplicate. dns: does not stimulate. Binding affinities (K_i) were obtained by competitive displacement of radiolabeled [^{3}H]diprenorphine in membrane preparations. Efficacy data were obtained using agonist induced stimulation of [^{35}S]GTPγS binding assay. Efficacy is represented as EC_{50} (nM) and percent maximal stimulation relative to standard agonist DAMGO (MOR), DPDPE (DOR), or U69,593 (KOR) at 10 μM.