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Asymmetric Synthesis and In Vitro and In Vivo Activity of Tetrahydroquinolines Featuring a Diverse Set of Polar Substitutions at the 6 Position as Mixed Efficacy μ Opioid Receptor/ δ Opioid Receptor Ligands

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Abstract

We have previously reported a small series of mixed efficacy μ opioid receptor (MOR) agonist/ δ opioid receptor (DOR) antagonist opioid peptidomimetics featuring a tetrahydroquinoline (THQ) scaffold, and showed the promise of this series as effective analgesics after intraperitoneal administration in mice. We report here expanded SAR of the pendant region of these compounds and focus in particular on the incorporation of heteroatoms into this side chain. These analogues provide new insight into the binding requirements for this scaffold at MOR, DOR, and the κ opioid receptor (KOR), and several (**10j,k,m,n**) significantly improve upon the overall MOR agonist/DOR antagonist profile of our previous compounds. *In vivo* data for **10j,k,m,n** are also reported, and show the antinociceptive potency and duration of action of compounds **10j** and **10m** to be comparable to morphine.

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Author Contributions

Synthesis was carried out by A.M.B. Research project was designed by A.M.B, H.I.M and J.R.T. *In vitro* assays were carried out by N.W.G. *In vivo* studies were carried out by J.P.A and designed by J.P.A and E.M.J.

Conflict of Interest

The authors declare no conflict of interest.

Supporting Information Available

Experimental procedures, ¹H and ¹³C-NMR data, MS(EI) and HPLC data, X-ray crystallography data, and copies of NMR spectra are available free of charge at <http://pubs.acs.org>.

Keywords

Opioid; mixed efficacy; intraperitoneal; dependence; tolerance; tetrahydroquinoline

Introduction

Opioid analgesics have long been the standard for the treatment of severe pain. Unfortunately, the use of opioids can lead to the development of a number of undesirable side effects, such as respiratory depression, constipation, and perhaps most problematically, dependence and tolerance [1]. There is therefore a great unmet need to develop agents that act as potent analgesics, but without the development of these side effects [2-3].

There is a growing body of evidence to suggest that the δ opioid receptor (DOR) plays a significant role in the modulation of the side effects related to the chronic use of opioid analgesics. Although the analgesic effects of traditional opioid agents such as morphine are associated with stimulation of the μ opioid receptor (MOR), the co-administration of DOR antagonists has been shown to maintain the desired antinociceptive activity but with a reduced side effect profile as compared to a MOR agonist alone [4-7].

Because of these findings, our group and others have sought to develop bifunctional ligands that bind to both MOR and DOR, while only stimulating MOR. Several classes of compounds have been employed in this pursuit, including peptides [8], pseudopeptides [9,10] and small molecules [11,12]. MOR/DOR bivalent ligands developed by Portoghesi and colleagues have been demonstrated to be effective analgesics with a diminished tolerance profile (Figure 1d) [13], and recently the MOR/DOR heteromer biased agonist CYM51010 was also shown to display reduced antinociceptive tolerance as compared to morphine (Figure 1c) [14]. MOR agonist/DOR antagonist compounds are also being developed clinically for the treatment of irritable bowel syndrome [15]. We have recently reported two MOR agonist/DOR antagonist compounds that are effective and bioavailable analgesics: a glycosylated cyclic pentapeptide [16] and a small molecule with a tetrahydroquinoline core (Figure 1a) [17-18]. Additionally, UMB425, a small molecule MOR agonist/DOR antagonist derived from thebaine, was also reported to display analgesia after subcutaneous administration, with reduced tolerance compared to morphine (Figure 1b) [19].

The compounds reported in this paper build on the limited initial SAR done on our bioavailable THQ lead compound (*S*)-2-amino-N-((*R*)-6-benzyl-1,2,3,4-tetrahydroquinolin-4-yl)-3-(4-hydroxy-2,6-dimethylphenyl)propanamide (Figure 1a).

Initially, bulky, hydrophobic modifications to the side chain at position R₁ were examined (Table 1) [17], as these substitutions were directly comparable to a previously reported series of MOR/DOR cyclic peptides [20-21], and were hypothesized to interact favorably with residues in the MOR active pocket to act as full MOR agonists while simultaneously behaving as DOR antagonists. These initial compounds are highly lipophilic, which is desirable for blood brain barrier penetration, but less than optimal for both aqueous solubility and metabolic stability [22]. We therefore explored a variety of polar side chains on this scaffold, for the purpose of improving these parameters and further probing the chemical space in this region of each of the receptors. Not only did simple heteroatom replacements have profound effects on receptor selectivity, these modifications also led to compounds that improved upon the *in vivo* profile of our initial THQ analogue. Compounds **10j** and **10m** both produced a maximum antinociceptive response in the mouse warm water tail withdrawal (WWTW) assay, and both improved significantly upon the duration of action of our lead peptidomimetic. These findings highlight the promise of this scaffold *in vivo*, and show that our SAR strategy focusing on polar side chains was effective for improving bioavailability. *In vitro* data for both binding affinity and efficacy are presented for all three opioid receptor types (MOR, DOR and the κ opioid receptor KOR).

Chemistry

The synthesis of compounds **10a-c**, **10f-o** began with the acylation of *p*-toluidine with 3-bromopropionyl chloride (Scheme 1). The resulting alkyl bromide then underwent an intramolecular cyclization, followed by triflic acid mediated β -lactam rearrangement to give ketone **1** [23-24] which was subsequently Boc-protected on the THQ nitrogen to give **2**. Ketone **2** was then brominated on the aryl methyl group as described previously [25] to give **3**, onto which could be added the pendant of choice, either through a Suzuki coupling (**4a**, **4f-i**) or through substitution with the appropriate secondary amine (**4b,c** **4j-o**). It is important to note that **3** could be synthesized on a multi-gram scale, and all substitutions on this intermediate were high yielding. For the synthesis of compounds **10d** and **10e**, commercially available para-substituted anilines were then carried forward in a similar manner as in the synthesis of compound **2** to give intermediates **4d** and **4e** (Scheme 2).

Ketones **4a-o** were converted to the corresponding imines with (*R*)-(+)-2-methyl-2-propanesulfinamide and Ti(OEt)₄, and could then be reduced asymmetrically with NaBH₄ in situ to give *tert*-butanesulfinyl-protected amines **9a-o** as single diastereomers, as previously described for analogous scaffolds [26,27]. Deprotection with concentrated HCl gave the corresponding primary, enantiomerically pure (*R*) amines as HCl salts. Stereochemistry of the HCl salt was verified by X-ray crystallography of 6-benzyl-1-(*tert*-butoxycarbonyl)-1,2,3,4-tetrahydroquinolin-4-aminium chloride, which was prepared by an identical synthetic route (Figure 2).

Boc-protected L-2,6-dimethyltyrosine could then be coupled to the chiral HCl salt, and deprotected with TFA (**10a-l**, Scheme 3). Analogues were then purified by RP-HPLC to provide enough material for *in vitro* and *in vivo* pharmacological evaluation (~5-10 mg). In the case of *N*-acetylated analogues **10m-o**, Boc deprotection of the THQ nitrogen was performed prior to coupling to Boc-L-Dmt. After the amide coupling, the acetyl group was

introduced by stirring the crude material in excess pyridine/acetic anhydride (1:1) overnight, followed by a second Boc deprotection and RP-HPLC purification. TFA content of final analogues was estimated by ^{19}F -NMR as described previously, using a fluorinated analogue of **10m** (see supporting information) and was found to be approximately 2.5 TFA molecules per final compound [28].

In Vitro Assays

Binding affinity (K_i) was measured by the competitive displacement of [^3H]-diprenorphine (a non-selective opioid antagonist) in C6 cells stably expressing MOR or DOR, or Chinese Hamster Ovary (CHO) cells stably expressing KOR. *In vitro* potencies (EC_{50}) and efficacies (as maximal % stimulation) were obtained by agonist-stimulated [^{35}S]-GTP γ S binding in the same cell types using previously described protocols [21, 29].

Results and Discussion

In previous reports, we described a mixed efficacy MOR agonist/DOR antagonist opioid peptidomimetic featuring a tetrahydroquinoline (THQ) scaffold and a benzyl pendant at ring position 6 [17,18] (Figure 1a). This compound was shown to be an effective analgesic in the warm water tail withdrawal (WWTW) assay after intraperitoneal administration, with a duration of action slightly shorter than morphine [17]. The initial SAR done on this lead compound was focused on several additional hydrophobic, aromatic substitutions at the 6 position, including 1-methylnaphthyl, 2-methylnaphthyl, 2-methylindanyl, and ethylphenyl. The side chains of these 4 compounds were chosen to mirror modifications made in a peptide series upon which the peptidomimetic scaffold was based [20] and as expected, modifications featuring a more extended pendant (2-methylnaphthyl, 2-methylindanyl, ethylphenyl) were compatible with the larger DOR *inactive* binding pocket, but not the smaller DOR *active* pocket, explaining the observed low efficacy at DOR. While these compounds displayed the desired MOR agonist/DOR antagonist efficacy profile, their binding profile was not optimal. Affinity for MOR for all 4 compounds was at least an order of magnitude higher than affinity for DOR, and the 2-methylnaphthyl compound showed over 2 orders of magnitude preference for MOR. Ligands with more balanced binding affinities at MOR and DOR would provide a better starting point for further development of this type of mixed efficacy opioid ligand [9,30]. Additionally, although we showed that an extended hydrophobic pendant translates to low DOR efficacy, changes in the electronic characteristics and polarity of the pendant were left unexplored.

To begin our expanded SAR, we first replaced the phenyl pendant of our lead compound (Fig. 1a) with a 3-pyridine (**10a**, Table 1). We observed not only a slight loss in binding affinity at both MOR and DOR (Table 1), but a significant loss in MOR efficacy and potency (Table 2). Although **10a** adopts a similar conformation in the MOR active site to our lead compound, this loss in MOR binding and efficacy can be attributed to loss of hydrophobic contacts in this region of the receptor binding pocket (see Figure 3). Although this analogue did not improve upon the MOR agonist/DOR antagonist profile of our previous compounds, we were intrigued by the drastic consequences that a simple change in pendant electronics had on both binding and efficacy, and wished to explore this further.

Compared to **10a** and our lead compound, replacement with piperidine in analogue **10b** widened the binding affinity preference for MOR over DOR even further, although this compound behaved as a moderately potent, full agonist at MOR, improving upon the MOR efficacy profile of **10a**. Expansion of the piperidine ring in **10b** to azepane (**10c**) resulted in improved binding at DOR and KOR. In contrast, morpholine analogue **10d** displayed diminished binding affinities at DOR and KOR, and also decreased potency at MOR as compared to **10b**. We next turned our attention to smaller aromatic systems, including a 1,2,4-triazole substitution (**10e**) and a 3-furan (**10f**). While the overall binding profile of **10f** was comparable to the previous substitutions, **10e** displayed a marked loss in binding affinity for MOR and KOR, and displayed no efficacy at MOR.

In the initial series [17], the 2-methylnaphthyl modification resulted in the highest MOR efficacy, but the MOR/DOR binding balance favored MOR by over 2 orders of magnitude. To see if changes in electronics to the naphthyl system could improve DOR binding while maintaining low DOR efficacy, we first synthesized quinoline analogue **10g**. Interestingly, the binding affinity of 6-quinoline analogue **10g** at all three receptors was considerably lower than the previous bicyclic analogues. This finding suggests that both an extended pendant and pendant electronic characteristics are important for maintaining binding for this series at DOR. Using our previously published models of interactions of opioid ligands with the active states of the three receptors [20, 21], **10g** was docked into the MOR active binding pocket. The quinoline nitrogen of **10g** was found to extend much deeper into the hydrophobic pocket of the MOR active pocket, disrupting important contacts with hydrophobic residues W133, V143, and I144 as shown in Figure 3.

These initial data suggested that superior MOR efficacy (and low DOR efficacy) may result from a fused ring pendant in which the six-membered, non-heteroatom containing aromatic moiety is located in the most distal position from the THQ core. To test this hypothesis, analogues **10h-i** were synthesized. **10h** showed high efficacy at MOR, and improved DOR binding approximately tenfold as compared to the lead compound and **10g**. **10j** and **10k** both behaved as potent, full MOR agonists that improved upon the efficacy of our original lead, with no efficacy at DOR. On the other hand, MOR efficacy is reduced in the case of 3,4-(methylenedioxy)phenyl analogue **10i**. This is again consistent with the observation that distal electronegative substitutions adversely affect MOR efficacy. Reduction of the aromatic ring of **10k** to give decahydroisoquinoline analogue **10l** maintained a comparable, if slightly inferior *in vitro* profile as compared to **10k**. While **10j** and **10k** showed potent stimulation at MOR (while exhibiting no efficacy at DOR), we still wished to improve the binding affinities of each at DOR. We reasoned that the THQ aniline was synthetically accessible and amenable to substitutions, and would be the next logical site for diversification. Preliminary studies in related analogues suggested that *N*-acetylation at the THQ core improved DOR affinity without increasing DOR efficacy (manuscript in preparation), so we likewise explored the effect of an acetyl substituent here, giving final analogues **10m-o**. We were pleased to see that this modification not only improved DOR binding relative to the un-acetylated counterpart compounds (**10j-l**) but also that **10m** showed similarly high affinity for MOR and DOR, and interestingly for KOR as well. As expected from its high binding affinity and lack of stimulation of [³⁵S]GTPγS binding, **10m**

acted as an antagonist of DPDPE. **10m** afforded a 7.8-fold rightward shift in the agonist concentration-response curve for DPDPE, giving an antagonist affinity constant (K_e) for **10m** of 4.6 nM.

An overlay of **10k** docked into the active site of all three receptors is shown in Figure 4. The compound fits nicely into the MOR active site, but clashes with M199 and L125 in the DOR active site. It is interesting to note that **10k** and **10m**, both featuring the 1,2,3,4-tetrahydroisoquinoline (THIQ) pendant, behave as partial KOR agonists. As shown in Figure 4, **10k** fits in the KOR active site, but clashes slightly with I294 (and thus displays lower efficacy as compared to MOR). Additionally, the THIQ nitrogen of **10k** is positioned to make a polar contact with Y312, a residue unique to the KOR binding pocket at this position, which may account for the high affinity of **10k** and **10m** for KOR. The MOR agonist/KOR agonist mixed efficacy profile has shown promise as a treatment for drug dependence, specifically cocaine addiction [31-33], and additional SAR on MOR/KOR agonist peptides has recently been reported [34]. Further substitutions on the THIQ pendant will have to be explored to fully optimize this profile, particularly for the purpose of improved potency at KOR.

On the basis of their favorable *in vitro* profiles, compounds **10j**, **10k**, **10m**, and **10n** were chosen for *in vivo* studies. Effects of **10j**, **10k**, **10m**, and **10n** were compared with the lead compound by two-way ANOVA with Tukey's multiple comparisons post hoc test. There was a significant interaction ($F(12,76)=8.7$, $p<0.0001$) as well as significant main effects of dose ($F(3,76)=82.7$, $p<0.0001$) and compound ($F(4,76)=24.6$, $p<0.0001$). In the mouse warm water tail withdrawal (WWTW) assay (Figure 5), our benzyl pendant lead compound and compounds **10j** and **10m** were fully efficacious and produced dose-dependent increases in latency to tail flick, with 3.2 (at least $p<0.05$) and 10 mg/kg ($p<0.001$) significantly increasing latency times as compared with baseline. **10m** was not statistically different from the lead compound, but **10j** produced slight higher tail flick latencies at 3.2 ($p<0.001$) and 10 ($p<0.05$) mg/kg as compared with the lead compound. It is interesting to note that **10k**, which lacks only the *N*-acetyl group of **10m**, and **10n**, which is the *N*-acetylated counterpart to **10j**, did not significantly increase tail flick latency above baseline levels up to a dose of 10 mg/kg. To determine the duration of action of compounds **10j** and **10m**, tail withdrawal latencies were measured at intervals following the administration of the cumulative 10 mg/kg dose (Figure 6). Compounds **10j** and **10m** showed a full antinociceptive response for 200 minutes before returning to baseline. Compared with the lead compound (Figure 1a), these compounds both displayed a much longer duration of action after intraperitoneal injection.

In an effort to explain the unpredictable *in vivo* results for these structurally similar analogues, compounds **10j**, **10k**, and **10m** were screened to determine their plasma stability, and were all found to be completely stable after 30 minutes at 37 °C (99.6, 100.3, and 95.3 mean % remaining respectively after 30 minutes as compared to positive control Eucatropine, 43.2%). There also appears to be no correlation between the predicted clogP values (Table 1) of these analogues with the observed *in vivo* data. While the explanation of the disparate *in vivo* results is unclear, differences in first pass metabolism after intraperitoneal administration [35] may be a contributing factor. To this end, additional

synthetic and pharmacokinetic studies will need to be performed to shed light on how these subtle structural differences can have such a profound effect on the bioavailability of this scaffold.

Conclusions

We have described a series of opioid peptidomimetics with chemically diverse substitutions at the 6 position of the THQ scaffold, and have shown that changes in both pendant sterics and electronic characteristics can have profound impacts on receptor selectivity for both binding and efficacy. Intermediate **3**, which is relatively simple to synthesize and can be done so on a multi-gram scale, represents a valuable building block for the expedient synthesis of a wide range of opioid small molecules, and makes possible the incorporation of diverse and readily available side chains (either through Suzuki coupling or SN2 substitution) that have not yet been explored in traditional opioid ligands. Compounds **10j** and **10m** also display promise *in vivo*, with efficacy and duration of action comparable to morphine, and improve upon the duration of action of our original lead.

Methods

General Synthetic Methods

All reagents and solvents were obtained from commercial sources and used without additional purification. Reactions were carried out in anhydrous solvents under an inert atmosphere unless otherwise specified. Suzuki couplings were performed on a Discover S-class (CEM) microwave in a closed vessel with maximum power input of 300 W. Flash column chromatography was carried out using P60 silica gel (230–400 mesh). Purification of final compounds was performed using a Waters semipreparative HPLC with a Vydac protein and peptide C18 reverse phase column, using a linear gradient of 10% solvent B (0.1% TFA in acetonitrile) in solvent A (0.1% TFA in water) to 60% solvent B in solvent A at a rate of 1% per minute. UV absorbance was monitored at 230 nm. Purity of synthesized compounds was determined on a Waters Alliance 2690 analytical HPLC instrument and a Vydac protein and peptide C18 reverse phase column, using a linear gradient of 0% solvent B in solvent A to 45% solvent B in solvent A in 45 min, measuring UV absorbance at 230 nm. Purities of the final compounds used for testing were >95% as determined by HPLC. ¹H-NMR, ¹³C-NMR, and ¹⁹F-NMR data were obtained on either a 400 or 500 MHz Varian instrument. In chloroform-d, shifts are referenced to TMS. If TMS peak was not visible in ¹³C-NMR spectra, shifts were referenced to the solvent peak (δ 77.16). Samples in CD₃OD are unreferenced. Mass spec analysis was performed using an Agilent 6130 LC–MS mass spectrometer in positive mode.

In Vitro Assays

Binding affinity (K_i) was measured by the competitive displacement of [³H]-diprenorphine (a non-selective opioid antagonist) in C6 cells stably expressing MOR or DOR, or Chinese Hamster Ovary (CHO) cells stably expressing KOR. *In vitro* potencies (EC₅₀) and efficacies (as maximal % stimulation) were obtained by agonist-stimulated [³⁵S]-GTP γ S binding in the same cell types using previously described protocols [21, 29, 36]. To determine the DOR

antagonist activity of **10m** a concentration response curve for DPDPE was obtained using the [³⁵S]GTP γ S binding assay in C6 cells expressing DOR in the presence or absence of 30 nM compound **10m**, as previously described [21]. The ratio of potency (EC₅₀) values of DPDPE in the presence and absence of **10m** was determined to provide the dose-ratio. The antagonist affinity constant (K_e) for **10m** was calculated using the equation $K_e = [10m] / DR - 1$. All concentration response curves were analyzed using GraphPad Prism (La Jolla, CA).

Animals

Adult male C57BL/6 mice, purchased from Harlan Laboratories (IN, USA) and weighing between 20-30g at 8-16 weeks old, were used for the described experiments. Mice were group-housed and had free access to food and water at all times. Experiments were conducted in the housing room, which was maintained on a 12h light/dark cycle (with lights on at 0700). Each mouse was used only once and experiments were conducted between 9 am and 5 pm. Studies were performed in accordance with the University of Michigan Committee on the Use and Care of Animals and the Guide for the Care and Use of Laboratory Animals [37].

Antinociception

All compounds were dissolved in sterile saline and administered by intraperitoneal (i.p.) injection in a volume of 10 mL/kg of body weight. Antinociceptive effects were evaluated in the warm water tail withdrawal (WWTW) assay. Tail withdrawal latencies were determined by briefly placing a mouse into a plastic, cylindrical restrainer and putting 2-3 cm of the tail tip into a water bath maintained at 50°C. The latency to tail withdrawal or rapidly flicking the tail back and forth was recorded with a maximum cutoff time of 20 sec. If the mouse did not remove its tail by the cutoff time, the experimenter removed its tail from the water to prevent tissue damage.

Acute antinociceptive effects were determined using a cumulative dosing procedure. Each animal received an injection of saline ip and then 30 min later, baseline withdrawal latencies (3-6 sec) were recorded. Following baseline determinations, increasing cumulative doses of the test compound were given ip at 30 min intervals. Thirty min after each injection, the tail withdrawal latency was measured as described above.

Plasma Stability

Plasma stability was assessed by Quintara Discovery (San Francisco, CA) Mouse plasma (K2 EDTA) was obtained from BioreclamationIVT. The assay was carried out in 96-well microtiter plates. Compounds were diluted into 200 μ M in DMSO, then spiked into the plasma. After mixing, samples were immediately aliquoted into three 96-well plates. The time 0 plate was quenched immediately. The other two plates were incubated at 37°C. Reaction mixtures (20 μ L) contained a final concentration of 1 μ M test compound. The extent of metabolism was calculated as the disappearance of the test compound, compared to the 0-min control reaction incubations. Eucatropine was included as a positive control to verify assay performance.

At each of the time points, 150 μ L of quench solution (100% acetonitrile with 0.1% formic acid) with internal standard was transferred to each well. Plates were sealed, vortexed, and centrifuged at 4°C for 15 minutes at 4000 rpm. The supernatant was transferred to fresh plates for LC/MS/MS analysis.

All samples were analyzed on LC/MS/MS using an AB Sciex API 4000 instrument, coupled to a Shimadzu LC-20AD LC Pump system. Analytical samples were separated using a Waters Atlantis T3 dC18 reverse phase HPLC column (10 mm $\times$ 2.1 mm) at a flow rate of 0.5 mL/min. The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in 100% acetonitrile (solvent B).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Bifunctional MOR/DOR Ligands^a

a. Compound **a** from Ref. 17

Figure 2.
Crystal structure of (*R*)-6-benzyl-1-(tert-butoxycarbonyl)-1,2,3,4-tetrahydroquinolin-4-aminium chloride

Figure 3. Docking of 10g in the MOR active site.^a

a. Key hydrophobic contacts are highlighted in red (I144, V143, W133).

Figure 4. Overlay of 10k in the MOR, DOR and KOR active site.^a

^a. Grey residues = MOR, Yellow = DOR, Purple = KOR.

Figure 5. Dose response curve for compounds 10j,k,m,n.^a

^a Cumulative antinociceptive dose response curves in the mouse WWTW assay for 10j,k,m,n after intraperitoneal administration (n = 3-6) plotted as average $\pm$ SEM.

Figure 6. Time course for compounds 10j and 10m.^a

^a. Time course of antinociceptive response in the mouse WWTW assay after a cumulative dose of 10 mg/kg ip administration over time for **10j** and **10m**. Plotted as average $\pm$ SEM.

Scheme 1. Synthesis of intermediates 4a-c, f-l^a

^a Reagents and conditions: (a) 3-bromopropionyl chloride, K₂CO₃, DCM, r.t.; (b) NaOtBu, DMF, r.t.; (c) TfOH, DCE, r.t.; (d) (Boc)₂O, DMAP, DIPEA, DCM, reflux; (e) NBS, benzoyl peroxide, CCl₄, reflux; (f) boronic acid or pinacol ester, Pd(dppf)Cl₂, K₂CO₃, acetone, water, 100°C w/microwave irradiation; (g) secondary amine, K₂CO₃, DMF, r.t.

Scheme 2. Synthesis of intermediates 4d-e.^a

^a Reagents and conditions: (a) 3-bromopropionyl chloride, K₂CO₃, DCM, r.t.; (b) NaOtBu, DMF, r.t.; (c) TfOH, DCE, r.t.; (d) (Boc)₂O, DMAP, DIPEA, DCM, reflux.

Scheme 3. Final steps in synthesis of 10a-o^a

^a Reagents and conditions: (h) (*R*)-(+)-2-methyl-2-propanesulfinamide, Ti(OEt)₄, THF, reflux; (i) NaBH₄, THF; (j) conc. HCl, 1,4-dioxane, r.t.; (k) Boc-L-Dmt, PyBOP, DIPEA, HOBt-Cl, DMF, r.t.; (l) TFA, DCM, r.t.; (m) (Ac)₂O, pyridine.

Table 1Opioid receptor binding affinities and clogP values for analogues 10a-o.^a

Binding, Ki (nM)			clogP ^c
Compound	R1	R2	MOR	DOR	KOR	
(Fig 1a)		H	0.22±0.02 ^b	9.4±0.8 ^b	68±2 ^b	3.7
10a		H	0.66±0.08	17±4	66±8	2.2
10b		H	0.3±0.1	120±29	29±9	2.7
10c		H	0.15±0.02	61±9	3.6±0.7	3.3
10d		H	0.6±0.1	140±67	170±32	1.4

Binding, Ki (nM)			clogP ^c
Compound	R1	R2	MOR	DOR	KOR	
10e		H	3.1±0.6	50±14	450±14	0.75
10f		H	0.8±0.2	18±6	20±3	2.9
10g		H	2.1±0.6	23±5	120±21	3.6
10h		H	0.10±0.02	1.5±0.2	16±4	3.6
10i		H	0.12±0.01	4.3±0.8	21±2	3.7
10j		H	0.15±0.08	15±5	2±1	3.1
10k		H	0.03±0.01	3.1±0.2	2.2±0.4	3.6

Binding, Ki (nM)			clogP ^c
Compound	R1	R2	MOR	DOR	KOR	
10l		H	0.15±0.01	4.8±0.9	37±8	4.1
10m		Acetyl	0.19±0.1	0.89±0.2	0.78±0.1	3.2
10n		Acetyl	0.32±0.09	2.6±0.2	7±3	2.7
10o		Acetyl	0.8±0.2	2±1	15±6	3.7

^a Binding affinities (K_i) were obtained by competitive displacement of [³H]diprenorphine in membrane preparations expressing either MOR, DOR or KOR. All values are mean ± SEM of three separate assays performed in duplicate.

^b Data from Ref. 17

^c Calculated using ChemBioDraw Ultra version 14.0

Table 2Opioid receptor efficacy for analogues 10a-o.^a

Compound	EC ₅₀ (nM)			% stimulation		
	MOR	DOR	KOR	MOR	DOR	KOR
(Fig 1a)	1.6±0.3 ^b	110±6 ^b	540±72 ^b	81±2 ^b	16±2 ^b	22±2 ^b
10a	93±20	dns	dns	37±7	dns	dns
10b	9±1	dns	dns	73±8	dns	dns
10c	25±11	dns	dns	52±2	dns	dns
10d	60±2	dns	dns	82±2	dns	dns
10e	dns	dns	dns	dns	dns	dns
10f	72±24	dns	> 1000	18±2	dns	> 40
10g	23±13	dns	dns	34±6	dns	dns
10h	2.2±0.9	dns	dns	84±6	dns	dns
10i	14±3	dns	dns	36±3	dns	dns
10j	3±1	dns	15±9	96±4	dns	14±2
10k	0.4±0.1	dns	90±65	105±6	dns	25±4
10l	2.0±0.5	dns	600±400	56±2	dns	14±1
10m	6±2	dns	160±36	91±8	dns	46±5
10n	0.9±0.4	dns	400±130	118±5	dns	32±1
10o	40±20	dns	> 2000	72±3	dns	> 20

^a Efficacy data were obtained using agonist induced stimulation of [³⁵S]GTPγS binding in membrane preparations expressing either MOR, DOR or KOR. Potency is represented as EC₅₀ (nM) and efficacy as percent maximal stimulation relative to standard agonist DAMGO (MOR), DPDPE (DOR), or U69,593 (KOR) at 10 μM. All values are expressed as the mean ± SEM of three separate assays performed in duplicate. dns: does not stimulate.

^b Data from Ref. 17