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Novel *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines as μ opioid receptor antagonists with improved opioid receptor selectivity profiles

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Abstract—A series of *N*-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines, μ opioid receptor antagonists, analogs of alvimopan, were prepared using solid phase methodology. This study led to the identification of a highly selective μ opioid receptor antagonist, which interacts selectively with μ peripheral receptors.

Endogeneous and exogenous activation of opioid receptors in the gut contributes to the pathophysiology of both opioid-induced constipation and postoperative ileus, an impairment of gastrointestinal function that often delays hospital discharge for abdominal surgery patients. Initial efforts to reverse the gastrointestinal effects of opioids were made using the opioid antagonist naloxone, but its therapeutic utility was limited due to concurrent antagonism of opioid analgesia. An alternative strategy to prevent reversal of central opioid actions is to use opioid antagonists that have limited intestinal absorption and low oral bioavailability, and that act selectively in the gastrointestinal tract after oral administration to antagonize peripheral opioid receptors, thereby stimulating gut motility and secretion without compromising analgesia.[1] Alvimopan (**1**) is a peripherally-acting, *N*-substituted *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine μ opioid receptor antagonist.[2,3] The size and polarity of the *N*-substituent limit gastrointestinal absorption and prevent penetration across the blood-brain barrier. The opioid antagonist activity in the *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines has been shown to be a consequence of substitution at the 3-position of the piperidine ring.[4] The structure activity relationship (SAR) in this *trans*-3,4-dimethyl-4-(3-hydroxyphenyl)piperidine series has been focused largely on the substitution of the piperidine nitrogen. These studies demonstrated that both the binding potency and efficacy of the antagonists were directly related to the structure of the *N*-substituent.[5–7]

We now report the synthesis, opioid receptor binding properties and in vitro functional activity of a series of analogs of alvimopan. In particular, we have altered the *N*-substituent of alvimopan (formula I and II, compounds **2**–**28**) and examined the resultant effects on μ opioid receptor binding affinity and antagonist activity, as well as μ opioid receptor selectivity. Compounds **1**–**28** were tested for their affinities toward the cloned human μ, δ and κ opioid receptors as measured by their abilities to displace [^{3}H]-diprenorphine from its specific binding sites.[8] The μ antagonist potencies of these compounds were assessed by their abilities to inhibit loperamide-stimulated guanosine 5′-O-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) binding to membranes containing μ opioid receptors.[8] The antagonist potencies were expressed as IC_{50} values. No agonist activity was detectable for compounds **1**–**28** at concentrations up to 10 μM. The biological activity of the target compounds is summarized in Tables 1 and 2.

Keywords: Opioid; μ receptors; Solid phase synthesis.
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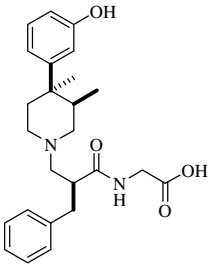
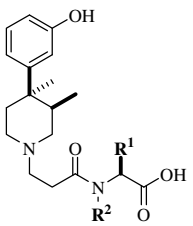
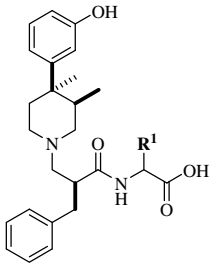


1 (alvimopan)



I

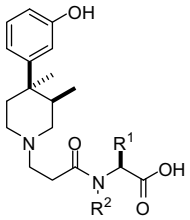


II

As shown in Table 1, replacement of the benzyl group of **1** with a hydrogen atom (compound **2**) resulted in an almost complete loss of μ binding. This result supports the existence of an important lipophilic binding region for the μ receptor in the proximity of the piperidine nitrogen-binding site.[7] Based on this information, we have introduced various lipophilic substituents at the Cα position of the glycyl moiety of **2** (compounds **3**–**6**) and examined the resultant effects on μ opioid receptor binding affinity. The synthesis of compounds **3**–**6** is outlined in Scheme 1. Deprotection of Wang resin bound Fmoc protected α-amino acids **31** with piperidine/DMF followed by coupling with the carboxylic acid **30** (obtained by basic hydrolysis of the methyl ester **29**[9]), and subsequent cleavage of the resin using trifluoroacetic acid provided the derivatives **3**–**6**, further purified by HPLC. As shown in Table 1, introduction of a benzyl group at the Cα position of the glycyl moiety of **2** (compound **3**) resulted in a marked increase in μ opioid receptor binding. Hence, compound **3**, which bound to the μ receptor with a K_i of 36 nM, also displayed potent μ in vitro antagonist activity (IC_{50} = 7.8 nM). Replacement of the benzyl group of **3** with a cyclohexylmethyl moiety (compound **4**) resulted in a slight increase in μ binding, but also led to a decreased selectivity for μ versus δ receptors. Furthermore, introduction of a biphenylmethyl or phenethyl moieties (compounds **5** and **6**, respectively) in place of the benzyl group of **3** did not result in an improvement of the binding affinity toward the μ opioid receptor. We then prepared analogs of **3**, incorporating at the R^2 position (see formula I), various lipophilic moieties (compounds **7**–**15**). The synthesis of compounds **7**–**15** is showed in Scheme 1. Removal of the Fmoc protecting group of **31a**, followed by reductive aminations using previously reported strategy[10] provided the resin-bound secondary amine intermediates **33**. The *N*-phenyl derivative **34** was obtained from **31a** according to the method described by Combs and collaborators.[11] The secondary amine derivatives **35** were obtained from **31a** using a solid-phase variant of the Fukuyama–Mitsunobu process.[12–14] Despite many attempts, conducted using a wide range of coupling reagents and reaction conditions, the coupling of the carboxylic acid **30** with resin-bound amines **33**–**35** failed. As an alternative strategy, coupling of resins **33**–**35** with acryloyl chloride in the presence of triethylamine provided the resin-bound acrylamide derivatives **34** which reacted with (+)-4(*R*)-(3-hydroxyphenyl)-3(*R*),4-dimethyl-1-piperidine (**37**[9]) to provide the corresponding resin-bound conjugate addition products. Cleavage of the resulting resins using trifluoroacetic acid provided derivatives **7**–**15**, further purified by HPLC. As shown in Table 1, the best compound in this series (compound **15**) displayed good μ binding affinity, potent μ antagonist activity (IC_{50} = 6.8 nM), comparable to the μ antagonist activity of naloxone (IC_{50} = 7.3 nM), but a low selectivity profile for μ versus δ and κ receptors.

The second part of the study was to investigate the SAR at the Cα position (R^1 substituent of formula II) of the glycyl moiety of **1**. The target compounds **16**–**27** were prepared according to Scheme 2. Deprotection of Wang resin bound Fmoc protected α-amino acids **31** with piperidine/DMF followed by coupling with the carboxylic acid **38**,[9] and subsequent cleavage of the resin using trifluoroacetic acid provided the derivatives **16**–**27**, further purified by HPLC. As shown in Table 2, introduction of a methyl group at the R^1 position (compound **16**) was well tolerated. Compound **16** and its diastereoisomer analog **17** had similar binding affinity at the μ opioid receptor. Introduction of lipophilic moieties of various size and flexibility at the R^1 position provided ligands (compounds **18**–**23**) with good μ binding affinity and antagonist activity. However, these modifications to the structure of **1** also led to an increase of the affinity of the ligands toward the δ opioid receptor. For example, compound **21** binds with equipotent affinity to μ and δ opioid receptors (K_i of 2.0 and 3.2 nM, respectively). In the functional assay, compound **21** was found to be a potent μ and δ[15] opioid receptor antagonist (IC_{50} values of 6.2 and 7.8 nM, respectively). Interestingly, introduction at the R^1 position, of alkyl chains containing polar substituents, had an important effect on opioid receptor selectivity. Indeed, the carboxymethylene and carboxyethylene derivatives (compounds **24** and **25**, respectively) exhibited good μ binding affinity and μ antagonist activity while displaying greater selectivity versus the κ receptors (>1000-fold) when compared to **1** (210-fold). Furthermore, introduction of an aminopropylene or aminobutylene moieties at the R^1 position provided ligands with μ subnanomolar binding affinity, potent μ antagonist activity and excellent opioid receptor selectivity. In particular, compound **27** with binding

Table 1. Opioid receptor (μ, κ and δ) binding data and in vitro antagonist activity (μ) of *N*-substituted-*trans*-3,4-dimethyl-4-(3-hydroxyphenyl) piperidines



Compound	R^1	R^2	K_i (μ) (nM)[a] or %inh. at 10 μM[c]	IC_{50} (μ) (nM)[b]	K_i (κ) (nM)[a] or %inh. at 10 μM[c]	K_i (δ) (nM)[a] or %inh. at 10 μM[c]	κ/μ[d]	δ/μ[e]
Naloxone			3.7	7.3	9.2	33	2.5	9
1 (Alvimopan)			0.47	1.7	100	12	210	30
2	–H	–H	20%	nd[f]	4%	5%	nd[f]	nd[f]
3	–CH_2–Ph	–H	36	7.8	39%	990	>300	27
4	–CH_2–cyclohexyl	–H	20	5.5	44%	170	>500	9
5	–CH_2–biphenyl	–H	80	28	1100	160	13	2
6	–$(CH_2)_2$–Ph	–H	58	10	960	360	16	6
7	–CH_2–Ph	–CH_3	66	14	35%	620	>150	9
8	–CH_2–Ph	–CH_2CH_3	19	110	44%	510	>500	26
9	–CH_2–Ph	–$CH_2CH(CH_3)_2$	28	43	2200	29	78	1
10	–CH_2–Ph	–$CH_2CH(CH_2)_2$	22	16	2200	100	100	5
11	–CH_2–Ph	–Ph	32	70	1400	67	43	2
12	–CH_2–Ph	–CH_2–Ph	23	25	550	39	24	2
13	–CH_2–Ph	–$(CH_2)_2$–Ph	66	63	1200	63	18	1
14	–CH_2–Ph	–CH_2–biphenyl	74	36	230	52	3	1
15	–CH_2–Ph	–CH_2–(2-Cl-Ph)	15	6.8	460	24	30	2

[a] The potencies of the compounds were determined by testing the ability of a range of concentrations of each compound to inhibit the binding of the non-selective opioid antagonist, [^{3}H]diprenorphine, to cloned human μ, κ, and δ opioid receptors, expressed in separate cell lines.[8] K_i values are geometric means computed from at least three separate determinations.

[b] The potencies of the antagonists were assessed by their abilities to inhibit agonist (loperamide) stimulated [^{35}S]GTPγS binding to membranes containing the cloned human μ opioid receptor.

[c] % Inhibition of [^{3}H]diprenorphine binding to the cloned human μ, κ, and δ opioid receptors using a concentration of the competitor of 10 μM.

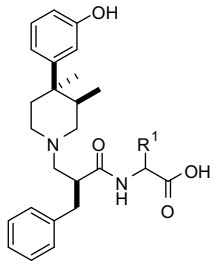
[d] K_i (κ)/K_i (μ).

[e] K_i (δ)/K_i (μ).

[f] nd, not determined.

K_i values of 0.50, 1500 and 580 nM at μ, δ and κ opioid receptors, respectively, is, to our knowledge, one of the most selective μ opioid receptor antagonist yet reported. The benzyl moiety of **27** was thought to have an important effect on μ opioid binding and μ opioid antagonist activity. The binding data (Table 2) for compound **28** (Scheme 1), which differs from **27** by the absence of this benzyl group, supported this hypothesis.

Table 2. Opioid receptor (μ, κ and δ) binding data and in vitro antagonist activity (μ) of alvimopan analogs

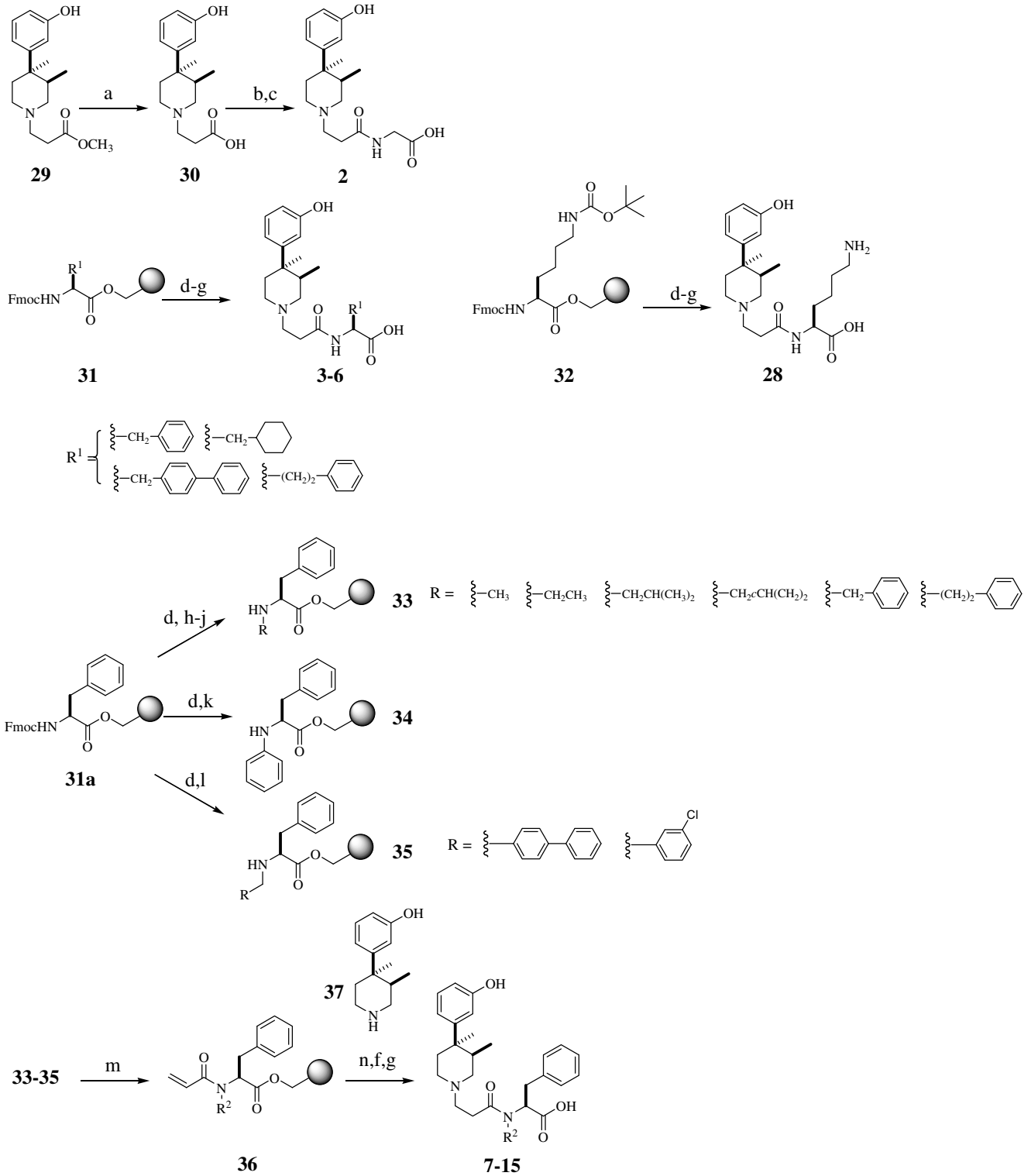


Compound	R^1	K_i (μ) (nM)[a] or %inh. at 10 μM[c]	IC_{50} (μ)[b] (nM)	K_i (κ) (nM)[a] or %inh. at 10 μM[c]	K_i (δ) (nM)[a] or %inh. at 10 μM[c]	κ/μ[d]	δ/μ[e]
16	(*S*)-CH_3	0.63	1.2	67	22	110	36
17	(*R*)-CH_3	2.9	3.0	910	74	310	26
18	(*S*)-CH_2-Ph	1.7	1.5	250	15	150	9
19	(*S*)-CH_2-biphenyl	1.9	21	160	8.3	84	4
20	(*S*)-CH_2-1-naphthyl	2.7	7.9	83	7.0	31	3
21	(*S*)-CH_2-2-naphthyl	2.0	6.2	240	3.2	120	2
22	(*S*)-CH_2-cyclohexyl	11	5.3	720	15	65	1
23	(*S*)-$(CH_2)_2$-Ph	4.7	9.9	510	10	110	2
24	(*S*)-CH_2CO_2H	7.3	3.8	27%	500	1400	68
25	(*S*)-$(CH_2)_2$-CO_2H	11	13	26%	360	>1000	
26	(*S*)-$(CH_2)_3$-NH_2	0.41	1.4	200	510	490	1200
27	(*S*)-$(CH_2)_4$-NH_2	0.50	1.2	580	1500	1200	3000
28[d]		45%	nd[e]	3%	11%	nd[e]	nd[e]

[a] See Table 1, footnote a.
[b] See Table 1, footnote b.
[c] See Table 1, footnotes c and e.
[d] See Table 1, footnote d and see structure in Scheme 1.
[e] nd, not determined.

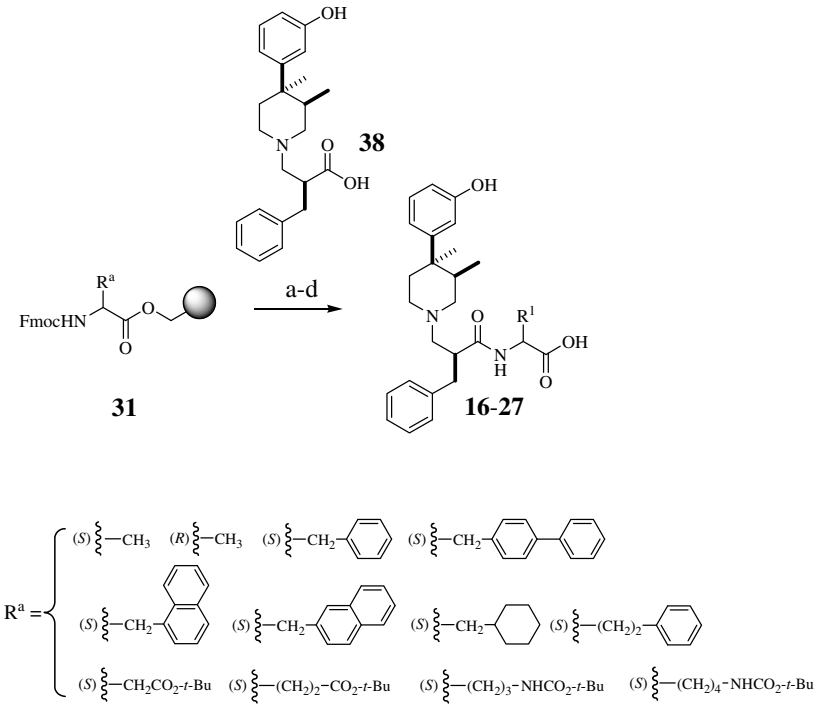
In the functional assay, compound **27** was also a potent inhibitor of loperamide-stimulated [^{35}S]GTPγS binding with an IC_{50} value of 1.2 nM, comparable to the μ in vitro antagonist activity of alvimopan (IC_{50} = 1.7 nM) and superior to the μ in vitro antagonist activity of naloxone (IC_{50} = 7.3 nM). In additional studies, compound **27** (0.1 and 1 mg/kg, s.c.) was tested for its ability to antagonize the gastrointestinal (GI) antitransit effect of morphine (3 mg/kg s.c.) in mice.[16a] Compound **27**, administered one hour prior to assessment of gastrointestinal transit (GIT), antagonized the antitransit effect of morphine by 64 and 82% at 0.1 and 1 mg/kg s.c., respectively. For comparison, compound **1** (0.1 mg/kg, s.c.), administered one hour prior to assessment of GIT, antagonized the GI antitransit effect of morphine by 69%. These data suggest that compounds **1** and **27** have similar potencies at the one hour time point after subcutaneous administration. Compound **27** was also active orally in the GIT assay at doses as low as 0.3 mg/kg p.o. Furthermore, compound **27** did not precipitate central opioid abstinence at 10 mg/kg s.c. in mice implanted with morphine pellets for three days, demonstrating that **27** is effectively excluded from the CNS.[16b]

In summary, SAR studies at the *N*-substituent position of the μ opioid receptor antagonist alvimopan led to the identification of several ligands displaying



Scheme 1. Reagents and conditions: (a) NaOH, H_2O/THF, 25 °C; (b) TsO^- $H_3N^+CHCH_2CO_2$-*i*-Bu, *i*-Pr_2EtN, HATU, DMF, 25 °C; (c) LiOH, H_2O/THF, 25 °C; (d) piperidine/DMF, 25 °C; (e) 30, *i*-Pr_2EtN, HATU, CH_2Cl_2/DMF, 25 °C; (f) TFA/CH_2Cl_2, 25 °C; (g) HPLC purification; (h) (2,4-NO_2)$PhSO_2Cl$, 2,6-lutidine, THF/CH_2Cl_2, 25 °C; (i) ROH, DIAD, $P(C_6H_5)_3$, THF, 25 °C; (j) *n*-$BuNH_2$, DMF, 25 °C; (k) $C_6H_5B(OH)_2$, $Cu(OAc)_2$, Et_3N, 4 Å powder molecular sieves, THF, 25 °C; (l) 1—RCHO, $HC(OCH_3)_3$, 25 °C, 2—$NaBH_3CN$, AcOH, 25 °C; (m) CH_2=CHCOCl, *i*-Pr_2EtN, CH_2Cl_2, 25 °C; (n) 37, CH_3OH/THF, 2 cycles, 25 °C.

good affinity toward the μ opioid receptor and potent μ in vitro antagonist activity.[17] From this study, we identified compound **27**, a lysine analog of alvimopan, which is one of the most selective μ opioid receptor ligand yet reported. Compound **27** represents an important new pharmacological probe to further characterize peripheral versus central μ opioid mediated effects.



Scheme 2. Reagents and conditions: (a) piperidine/DMF, 25 °C; (b) **38**, *i*-Pr_2EtN, HATU, CH_2Cl_2/DMF, 25 °C; (c) TFA/CH_2Cl_2, 25 °C; (d) HPLC purification.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.01.106.

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16. In vivo test methods: (a) Gastrointestinal Transit (GIT) assay: Male Swiss-Webster mice (20–25 g) were fasted overnight prior to the experiment. Mice were treated with vehicle (10% DMSO:20% cremophor EL:70% saline), or test compound (administered using the subcutaneous route). Twenty-five minutes after administration of vehicle or tested compound, the mice were treated with morphine (3 mg/kg s.c.), and 10 min later received 0.3 mL of a charcoal meal consisting of charcoal:flour:water (1:2:8, w/w/v). Gastrointestinal transit (GIT) was measured 25 min after the administration of the charcoal meal. GIT was determined by removing the entire length of the small intestine and measuring how far the leading edge of the charcoal meal traveled in the small intestine; % GIT was determined by the following formula: % GIT = [distance to charcoal leading edge (cm)/length of small intestine (cm)] × 100. (b) Assessment of physical dependence: male Swiss-Webster mice (30–35 g) were implanted with a placebo or a 75 mg morphine base pellet (Murty Pharmaceuticals, Inc., Lexington, KY). Three days after the pellet implantation, mice were treated with vehicle (10% DMSO:20% Cremophor EL:70% Saline) or test compound (administered using the subcutaneous route at the

dose of 10 mg/kg). Immediately after the treatment, the mice were placed in individual plastic observation cylinders (26 cm high × 22 cm diameter) and the number of jumps and other abstinence behaviors were recorded for a 10 min period. One hour after the treatment, the incidence of diarrhea was assessed as absent or present for each mouse and a post treatment body weight was obtained.

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