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Synthesis and evaluation of novel peripherally restricted κ-opioid receptor agonists

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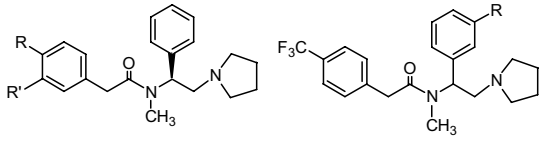
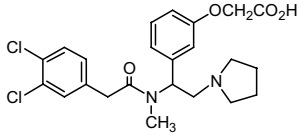
Abstract—A series of 3-substituted analogs (**3**) of the parent κ agonist, **1**, were prepared to limit access to the central nervous system. With the exception of compound **3j**, all other compounds bound to the human κ opioid receptor with high affinity (K_i = 0.31–9.5 nM) and were selective for κ over μ and δ opioid receptors. Compounds **3c**, **d**, and **3g–i** produced potent antinociceptive activity in the rat formalin assay (i.paw) and the mouse acetic acid-induced writhing assay (s.c.), with weak activity in the mouse platform sedation test. The peripheral restriction indices of **3c**, **d**, **3g**, and **3i** were improved 2- to 7-fold compared to the parent compound **1**, and these compounds were approximately 2- to 5-fold more potent than the peripheral κ agonist ICI 204448.

Although centrally active kappa (κ) opioid receptor agonists have analgesic properties, they have been of limited therapeutic use because of centrally mediated side effects such as sedation, dysphoria, and diuresis.[1–3] Recent evidence indicates that opioid antinociception can also be mediated by activation of opioid receptors located outside the central nervous system (CNS).[4,5] Our goal was to modify centrally active κ agonists to reduce their penetration into the CNS, thus minimizing or eliminating side effects. Previously, we reported 2-(4-trifluoromethylphenyl)-*N*-methyl-*N*-[1-phenyl-2-(1-pyrrolidinyl)ethyl]-acetamide (**1**)[6] as a highly potent agonist that was peripherally restricted compared to the centrally acting κ agonist ICI 199441 (**2**).[7] To further improve the peripheral restriction of **1**, we synthesized a series of analogs of general structure **3** with modifications on the central phenyl ring and evaluated them for in vitro binding affinity, in vivo antinociceptive activity, and peripheral restriction.

Modifications of the central phenyl ring at the 3-position were chosen since there are literature precedents[2,3] indicating that this position in the molecule can tolerate a wide variety of substituents without detrimental effects on κ opioid receptor affinity. The substituents at the 3-position were selected from a group, which may have ionizable potential at physiological pH, thus limiting the CNS penetration exemplified by ICI 204448, a reported opioid receptor agonist with reduced access to the CNS.[8]



1 (R = CF_3; R' = H)
2 (R = R' = Cl, ICI 199441)
3



ICI 204448

The 3-oxygenated analogs of the central phenyl ring of **1** were prepared as racemates as shown in Scheme 1. Synthesis of compounds (*R*,*S*)-**3**(**a**–**d**) required the diamine (*R*,*S*)-**6**, which was prepared according to the literature published by the ICI group.[9] Compound (*R*,*S*)-**3b** was

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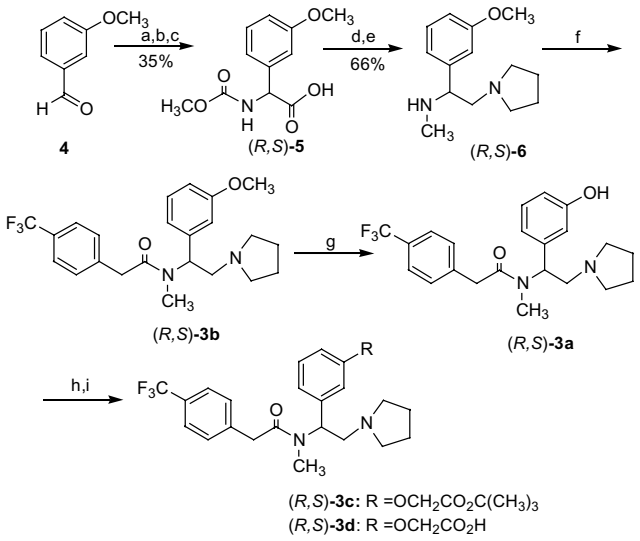


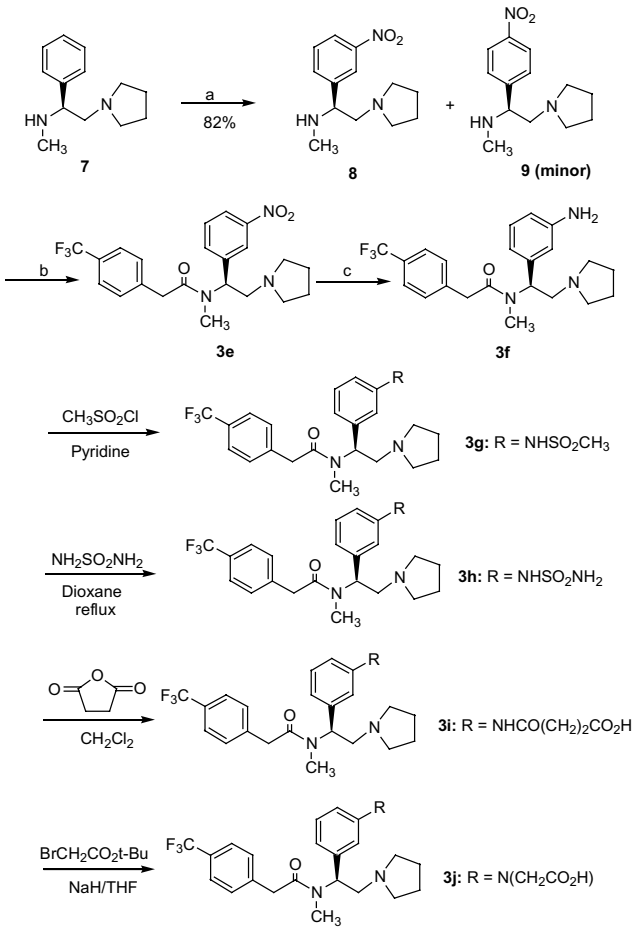
Scheme 1. Reagents and conditions: (a) NaCN, NH_4Cl; (b) HCl (6 N), refluxed; (c) methyl chloroformate, NaOH (1 N); (d) HOBt/DCC, pyrrolidine; (e) LAH, THF; (f) 4-trifluoromethylphenyl acetic acid, HOBt/DCC, CH_2Cl_2; (g) BBr_3, CH_2Cl_2, −70 °C; (h) *t*-butyl bromoacetate, K_2CO_3, DMF; (i) HCl (6 N), 95–100 °C.

prepared by coupling of the diamine with 4-trifluoromethylphenyl acetic acid in the presence of HOBt/DCC. Demethylation of methoxy ether (*R*,*S*)-**3b** was accomplished with BBr_3, which gave the phenol (*R*,*S*)-**3a**. Compounds (*R*,*S*)-**3c** and **d** were obtained by alkylation of (*R*,*S*)-**3a** with *t*-butyl bromoacetate followed by acid hydrolysis.

The corresponding 3-amino analogs (Scheme 2) were prepared by following the procedure described by Chang et al.[10] The nitration of diamine **7**[7] resulted in the 3-nitro compound (**8**) as a major product contaminated with 4-nitro compound (**9**). These compounds were not separated at this stage and were coupled with 4-trifluoromethylphenyl acetic acid to give a mixture of enantiomers and regioisomers. The desired isomer **3e** was purified by chromatography and recrystallization. Compound **3e** was then reduced with Raney-nickel/hydrazine hydrate, which resulted in compound **3f**. Reaction of **3f** with various electrophiles (methane sulfonyl chloride, sulfamide, succinic anhydride, and *t*-butyl bromoacetate) gave compounds **3g**–**j** (Table 1).

Receptor binding: Kappa (κ), mu (μ), and delta (δ) opioid receptor affinities were determined by displacement of bound [^{3}H]diprenorphine from membranes prepared from cells expressing cloned human opioid receptors. Details of the methodology have been previously reported in the literature.[11,12] Kappa selectivity over μ and δ opioid receptors was defined by the ratio of the K_i values.

Opioid receptor binding affinities of newly synthesized compounds are summarized in Table 1. With the exception of compound **3j**, all other compounds bound to the human κ opioid receptor with high affinity and good selectivity for κ compared to μ or δ opioid receptors. The K_i values for most of the compounds, (*R*,*S*)-**3a**,**b**,

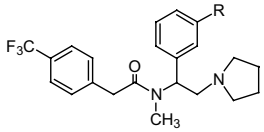


Scheme 2. Reagents and conditions: (a) HNO_3/H_2SO_4; (b) 4-trifluoromethylphenylacetic acid, DCC/HOBt; (c) Raney Ni, hydrazine, EtOH, 50 °C.

and **3e**–**h**, were in the subnanomolar range (K_i = 0.31–0.63 nM). In general, smaller attachments at the 3-position of the parent compound, either with ether or amino groups, resulted in compounds with high affinity for the κ opioid receptor. On the other hand, κ opioid receptor selectivity was reduced for compound (*R*,*S*)-**3a** and **3j** with respect to μ and δ opioid receptors. The zwitterionic characteristics of compounds containing carboxyl groups, (*R*,*S*)-**3d** and **3i**, also resulted in approximately one order of magnitude lower affinity for the κ opioid receptor, with K_i values of 3.7 and 9.5 nM, respectively. However, the dicarboxylic derivative, **3j**, exhibited a considerable reduction in κ opioid receptor binding affinity (K_i = 2300 nM). This reduction in affinity indicates the intolerance of the κ opioid receptor for compounds containing bis-ionizable groups in the central phenyl ring.

When comparing the κ opioid receptor affinity of newly synthesized analogs with the parent compound **1**, it was evident that the present compounds were at least 5-fold less potent in the κ opioid receptor binding assay, with varying degrees of selectivity for μ and δ opioid receptors. Nonetheless, compounds (*R*,*S*)-**3a**, **b**, and **3e**–**h** were more potent than ICI 204448.[8] Three other compounds, (*R*,*S*)-**3c**, **d**, and **3i**, had affinities for the κ

Table 1. In vitro binding to cloned human opioid receptors



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Compound	R	Yield (%)[a]	K_i (nM) for κ[b][^{3}H]-diprenorphine	Selectivity for κ over μ (fold)	Selectivity for κ over δ (fold)
1	H	—	0.058 (0.046–0.073)	> 1000	> 1000
	ICI 204448	—	2.69 (1.58–5.01)	162	69
(*R*,*S*)-**3a**	OH	28	0.44 (0.28–0.67)	68	230
(*R*,*S*)-**3b**	OCH_3	65	0.53 (0.3–0.93)	870	> 1000
(*R*,*S*)-**3c**	$OCH_2CO_2C(CH_3)_3$	63	2.9 (1.7–4.8)	330	79
(*R*,*S*)-**3d**	OCH_2CO_2H	62	3.7 (2.5–5.6)	350	190
(*S*)-**3e**	NO_2	94	0.63 (0.34–1.2)	920	> 1000
(*S*)-**3f**	NH_2	48	0.31 (0.22–0.44)	110	130
(*S*)-**3g**	$NHSO_2CH_3$	45	0.36 (0.25–0.5)	420	> 1000
(*S*)-**3h**	$NHSO_2NH_2$	39	0.48 (0.39–0.59)	310	> 1000
(*S*)-**3i**	$NHCO(CH_2)_2CO_2H$	74	9.5 (8.4–11.0)	57	11
(*S*)-**3j**	$N(CH_2CO_2H)_2$	54	2300 (1700.0–3100.0)	> 43	> 43

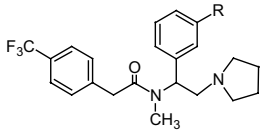
[a] The compounds were isolated as hydrochloride or methane sulfonic acid salts and characterized by MS, NMR, mp, and elemental analyses.
[b] Values are geometric means of three to ten experiments and 95% confidence intervals are given in parentheses.

opioid receptor that were comparable to ICI 204448 (Table 1). In a direct comparison of ICI 204448 with compound (*R*,*S*)-**3d**, the latter compound had comparable κ opioid receptor affinity, with improved selectivity for μ and δ opioid receptors.

Formalin-induced flinching:[13] The formalin test was used as an initial in vivo screen for the newly synthesized compounds. Due to the potential toxicity of compounds **3e** (nitro) and **3f** (aniline), they were excluded from the initial screen and the data for the rest of the compounds are presented in Table 2. Generally, 3-oxygen substituted compounds (*R*,*S*)-**3a**–**d** showed analgesic activity in this test comparable to compound **1** and the 3-nitrogen substituted compounds (**3g**–**i**) were slightly less active. The compounds that inhibited flinching by approximately 70% or higher at 300 μg given i.paw (Table 2), with no observable CNS or minimum side effects, were advanced for further evaluation in the mouse acetic acid-induced writhing test. Compound **3a** showed significant sedation in the formalin test; an attribute of centrally active κ opioid agonists and no additional testing was warranted.

Acetic acid-induced writhing:[14] All the tested compounds exhibited potent (ED_{50} = 0.15–1.7 mg/kg s.c., Table 2) analgesic activity in the acetic acid-induced writhing test in mice. Compound (*R*,*S*)-**3a** was not tested due to observed side effects in the rat formalin test. The ED_{50} values of the *t*-butyl ester (*R*,*S*)-**3c** and the corresponding acid (*R*,*S*)-**3d** were essentially equal to ICI 204448 in this assay. The aniline substituted compounds (**3g**, **h**, and **i**) were slightly more active than the corresponding ethers with the exception of compound (*R*,*S*)-**3b**,

Table 2. In vivo profile of κ-opioid agonists



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Compound[a]	R	Formalin flinching %A @ 300 μg, i.paw	Writhing ED_{50} (mg/kg), s.c.	Platform sedation ED_{50} (mg/kg), s.c.	Peripheral restriction index
1	H	92	0.005	0.13	25
	ICI 204448	—	1.6	52	32.5
(*R*,*S*)-**3a**	OH	99	ND	ND	—
(*R*,*S*)-**3b**	OCH_3	99	0.15	ND	—
(*R*,*S*)-**3c**	$OCH_2CO_2C(CH_3)_3$	84	1.7	140	81
(*R*,*S*)-**3d**	OCH_2CO_2H	93	1.7	> 300	> 180
(*S*)-**3g**	$NHSO_2CH_3$	77	0.56	89	160
(*S*)-**3h**	$NHSO_2NH_2$	73	0.74	13	18
(*S*)-**3i**	$NHCO(CH_2)_2CO_2H$	73	0.87	47	54

[a] Compounds were administered in 20% aqueous creamaphor solution.

which was the most active of the 3-substituted compounds. Even though all of the new compounds showed potent analgesic activity in the writhing test, the potency of these compounds was substantially lower than the parent compound **1** (ED_{50} = 0.005 mg/kg). The greater potency of the parent compound in the mouse writhing assay may be in fact due to contributions from central κ opioid receptors as well as peripheral receptors.

Platform sedation:[15] The peripheral selectivity indices were determined only for compounds with minimum observable side effects in the mouse writhing test. Thus, compounds (*R*,*S*)-**3a** and **b** were not tested in the platform sedation assay in mice. The peripheral index was calculated for the rest of the compounds in Table 2. The parent compound **1** was very potent in the mouse platform sedation assay with an ED_{50} of 0.13 mg/kg (s.c.). Its peripheral restriction index was 25, due to its very potent activity in the mouse writhing test. All other substituted compounds, with the exception of compound **3h**, had a peripheral restriction index approximately 2- to 7-fold higher than the parent compound **1**, and in some cases 2- to 5-fold better than the known peripheral compound ICI 204448 (Table 2). Two compounds, (*R*,*S*)-**3d** and **3g**, were the most promising leads, with peripheral indices of >180 and 160, respectively. The high peripheral indices of these two compounds could be attributed to the ionizability characteristics at physiological pH of the substituents at the 3-position of (*R*,*S*)-**3d** ($CH_2CO_2O^-$) and **3g** ($^-NSO_2CH_3$), thus limiting access to the CNS. The compounds are being further evaluated in a number of assays for development as potential antinociceptive agents.

All new compounds with the exception of **3j** potently bound to the κ receptor in vitro, with K_i values in the nanomolar range. The new 3-substituted compounds, except for (*R*,*S*)-**3a**, **3i**, and **3j**, were over 100-fold selective for κ over μ. The selectivity for κ over δ was slightly higher than that for κ over μ except for compounds (*R*,*S*)-**3c**, **3d**, **3i**, and **3j**. By substituting in the central phenyl ring of parent compound **1** with ether or amino containing groups, the degree of peripheral restriction of these κ agonists was improved approximately 2- to 7-fold. The carboxymethyl ether group (compound, (*R*,*S*)-**3d**) and methanesulfonamido group (compound, **3g**) offered the most improvement for the peripheral restriction index and represented at least a 5-fold improvement over ICI 204448.

Overall, 3-substituted compounds were less potent in vitro and in vivo compared to the parent compound **1**. However, the objective of this work was achieved in finding at least two compounds with an improvement in the peripheral index compared to compound **1**, as a measure of limiting access to the CNS.

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$$\frac{(\text{Mean formalin response} - \text{Mean saline response}) - \text{Individual response}}{\text{Mean formalin response} - \text{Mean saline response}} \times 100$$

The mean formalin response was the mean number of flinches in rats treated with vehicle prior to formalin injection. The mean saline response was the mean number of flinches in rats treated with 50 μL of saline. The data are presented in Table 2 and compared with compound **1** and ICI 204448.
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expressed as the percent inhibition of acetic acid-induced writhing, when compared to the mean number of writhes observed in vehicle-treated mice.

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Sedation was measured by the latency of a mouse to completely step off a slightly raised platform ($11 \times 8 \times 3$ cm). Prior to treatment, mice were tested once for baseline latencies. Mice with baseline latencies >15 s were not used. Mice were injected s.c. (back of the neck) with either vehicle or drug and 20 min later tested for the latency to step off the platform. A 30 s cut-off was utilized. The percent sedation was calculated by the following formula:

$$\frac{\text{Test latency} - \text{Baseline latency}}{(30\text{ s} - \text{Baseline latency})} \times 100$$

Peripheral restriction index = platform sedation ED_{50}/writhing ED_{50}.