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In vitro and in vivo evaluation of *O*-alkyl derivatives of tramadol

Liming Shao,[a,*] Michael Hewitt,[a] Thomas P. Jerussi,[a] Frank Wu,[a] Scott Malcolm,[a] Paul Grover,[a] Kevin Fang,[a] Patrick Koch,[a] Chris Senanayake,[b] Nandkumar Bhongle,[a] Seth Ribe,[a] Roger Bakale[c] and Mark Currie[d]

[a]*Sepracor Inc., Medicinal Chemistry, 84 Waterford Drive, Marlborough, MA 01752, USA*
[b]*Boehringer-Ingleheim, 900 Ridgebury Road, Ridgefield, CT 06877-0368, USA*
[c]*Cephalon, 41 Moores Road, Frazer, PA 01955, USA*
[d]*Microbia, 320 Bent Street, Cambridge, MA 02141, USA*

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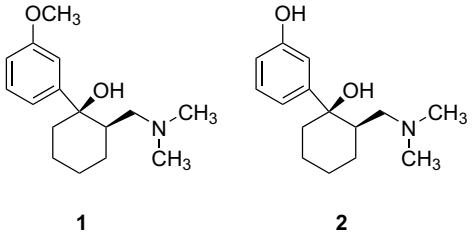
Abstract—Tramadol is a centrally acting opioid analgesic structurally related to codeine and morphine. *O*-Alkyl, *N*-desmethyl, and non-phenol containing derivatives of tramadol were synthesized to probe their effect on metabolic stability and both in vitro and in vivo potency.

(±)-*cis* Tramadol is used for the treatment of moderate to moderately severe pain.[1] Tramadol has a relatively short duration of analgesic effect due to extensive first pass metabolism and as such is dosed as frequently as 100 mg every 4–6 h.[2] The analgesic activity of tramadol is thought to be the result of a dual mechanism—the parent (**1**) acts as an inhibitor of norepinephrine and serotonin reuptake and the major *O*-desmethyl metabolite (**M1**) is a potent μ-opioid receptor agonist (Fig. 1).[3]

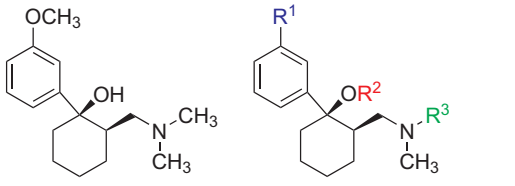
The opioid antagonist naloxone only partially inhibits the analgesic activity of tramadol in animal tests. Not unlike other opioids, tramadol causes a number of side effects including constipation, nausea, dizziness, and somnolence. A tramadol analog that had a longer half-life (and therefore required less-frequent dosing) and was devoid of opioid side effects would have therapeutic benefit. Herein we wish to disclose our efforts along these lines (See Fig. 2).

Our approach (Fig. 1) to the design of an 'improved' tramadol relied on traditional structural modifications and was based on the observation that *O*-methyl tramadol **3** (Scheme 1) possessed greater potency (ED_{50} = 0.7 mg/kg) than tramadol (ED_{50} = 11.3 mg/kg)



1 **2**

Figure 1. (±)-Tramadol **1** and metabolite (±)-M1, **2.**



(±)-Tramadol

R^1 = OCH_3, OH, CN, H
R^2 = alkyl, hydroxy-alkyl
R^3 = H, CH_3

Figure 2. Structural modifications to tramadol scaffold.

in the rat tail-flick model of pain (tail vein injection of compound), albeit with decreased bioavailability (F = 11% for O–Me **3** compared to F = 36% for tramadol, **1**, vida supra). The cause of the decreased

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* Corresponding author. Tel.: +1 508 357 7468; fax: +1 508 490 5454; e-mail: Liming.shao@sepracor.com

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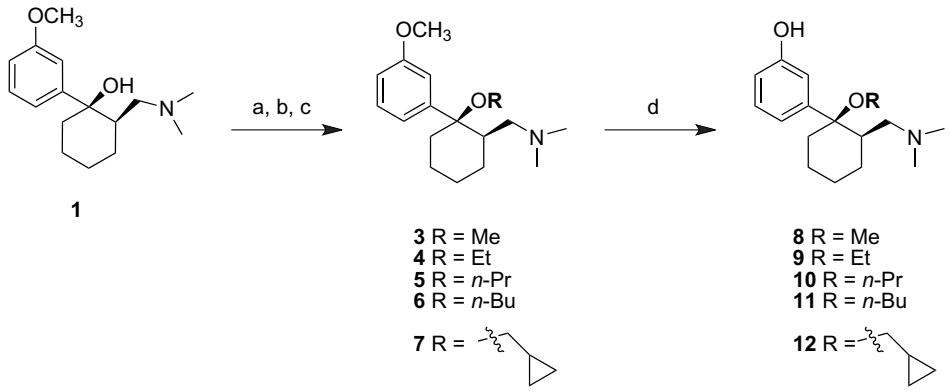
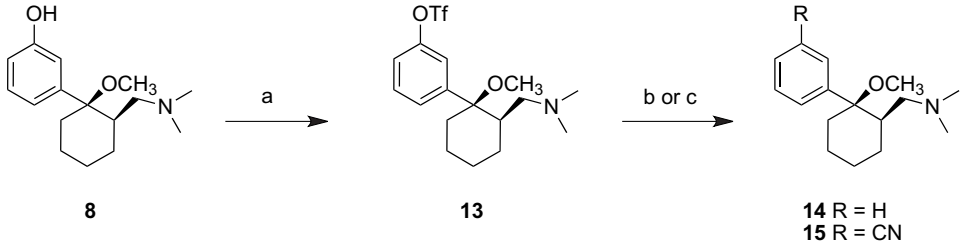


Scheme 1. Reagents and conditions: (a) BnBr, THF; (b) Alkyl halide, NaH, DMF; (c) Pd/C, MeOH, H_2; (d) $PHPh_2$, *n*-BuLi, THF, −78 °C.



Scheme 2. Reagents and conditions: (a) Tf_2O, TEA, CH_2Cl_2; (b) $Pd(OAc)_2$, HCO_2H, TEA, PPh_3; (c) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 90 °C.

bioavailability was thought to be rapid first-pass metabolism via *N*- or *O*-dealkylation and/or conjugation. *O*-alkyl, *N*-desmethyl, and non-phenol containing derivatives of tramadol were synthesized and tested in both in vitro potency and metabolic stability assays and in vivo in the rat tail flick latency assay.

Synthesis[4] of the alkylated tramadol analogs (Scheme 1) commenced with benzylation of racemic tramadol free base **1** to the quanternary ammonium salt, followed by reaction with NaH and an alkyl halide (Scheme 1).[5] Hydrogenolysis with catalytic palladium on carbon delivered ethers **3**–**7**, which could then be demethylated with *lithio* diphenylphosphine in THF to give phenols **8**–**12**.[6]

Cyano and unsubstituted phenyl derivatives were synthesized starting from phenol **8** (Scheme 2). Formation of aromatic triflate **13** was followed by either transfer hydrogenation to give unsubstituted aromatic compound **14** or Pd-catalyzed cyanation to give nitrile **15**.

Synthesis of some glycol derivatives was also explored as a route to tramadol derivatives with a lower log *P* and potentially greater solubility. Formation of the *N*-benzyl salt followed by alkylation of the tertiary hydroxyl group provided *O*-allyl bromide salt **16** (Scheme 3). Ozonolysis and reductive workup with $NaBH_4$ was followed by hydrogenolysis to give primary alcohol **17** without incident. *Lithio* diphenyl phosphine was used to expose the phenol and deliver highly polar derivative **18**. Hydroboration on the *N*-benzyl salt **16** did not, however, give any of desired 3-carbon glycol. This led us to investigate an alternative protecting group strategy for the dimethyl amine functionality (Scheme 4).

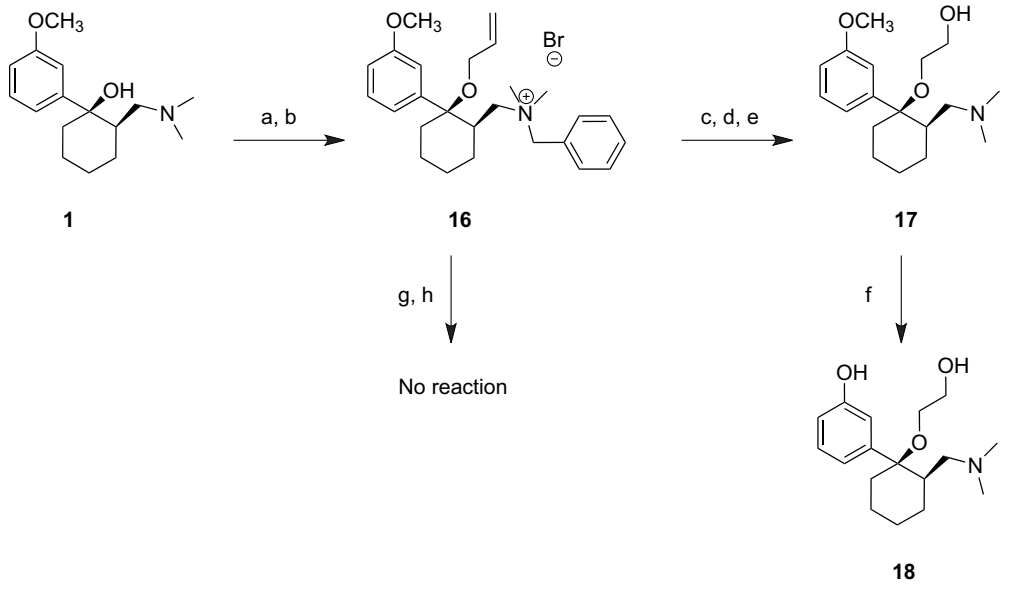
Methyl carbonate **19** allowed for reductive ozonolysis as well as hydroboration, followed by reductive workup. LAH reduction transformed the *N*-methyl methyl carbamate to the desired dimethyl amine of **17**. Hydroboration and oxidative workup on methyl carbamate **20** were successful and were followed by final LAH reduction to give dimethyl amine **21**.

Representative mono-methyl amines were synthesized as shown in Scheme 5. *O*-methyl tramadol **3** was mono-desmethylated with diethylazadicarboxylate (DEAD) followed by acidic hydrolysis to provide **22**; the phenol was exposed using *lithio*diphenylphospine to give **23**.

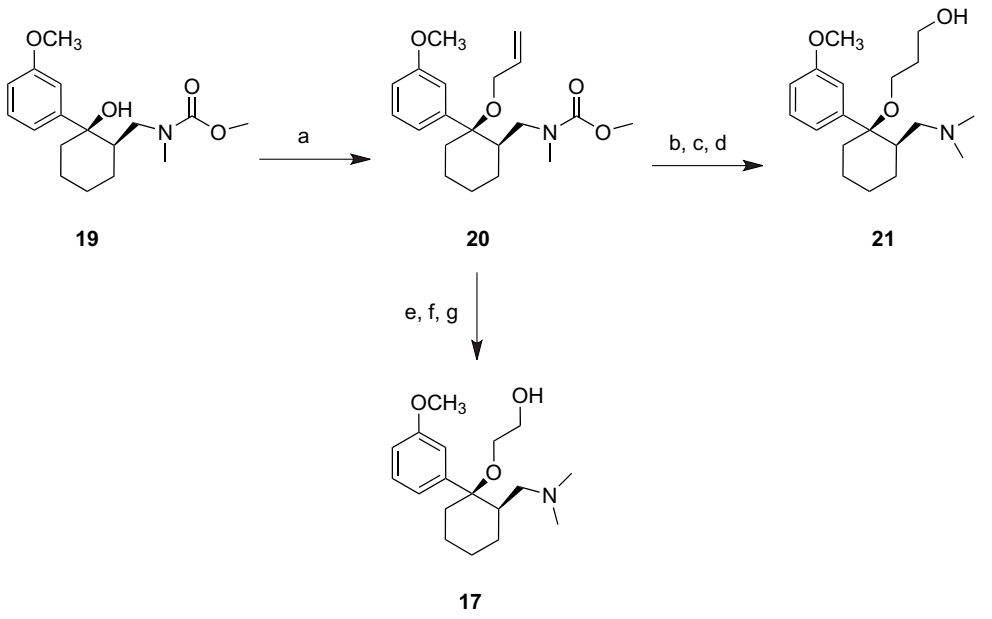
Several *O*-methylated chiral amines were also prepared as single isomers via resolution of tramadol with dibenzoyl tartaric acid[7] and chemical modification of the purified R, R- and S, S-tramadol isomers (Scheme 6). Of interest was dual action of 1-chloroethyl chloroformate: in CH_3CN (step a) and methanol (step b) to synthesize the methyl ether and in dichloroethane followed by acidic hydrolysis to mono-demethylate the dimethyl amine (step d and e).

The receptor binding affinity at the mu-opioid receptor (μ, IC_{50}) and functional monoamine uptake inhibition (5-HT and NE, IC_{50}) by tramadol and the alkylated tramadol analogs are shown in Table 1.

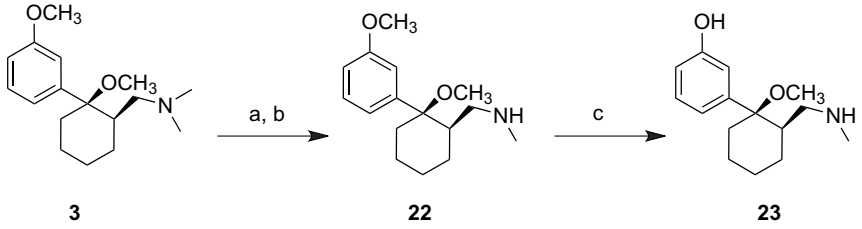
The increased μ potency of phenol derivatives compared to their OCH_3 counterparts was apparent across all of the *O*-alkylated derivatives shown in Table 1. The potency difference at μ was as large as a 161-fold change



Scheme 3. Reagents and conditions: (a) BnBr, THF; (b) Allyl bromide, NaH, DMF; (c) O_3, CH_2Cl_2/MeOH; (d) $NaBH_4$, CH_2Cl_2/MeOH; (e) Pd/C, MeOH, H_2; (f) $PHPh_2$, *n*-BuLi, THF, −78 °C; (g) 9-BBN, THF, DMSO; (h) H_2O_2, NaOH.

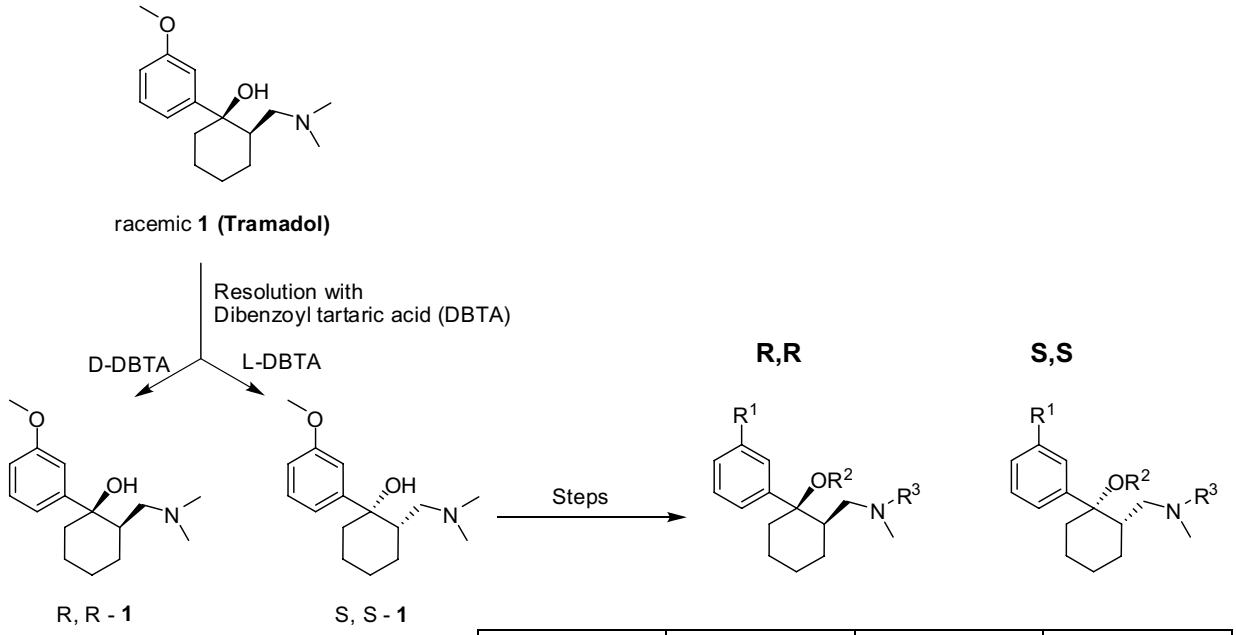


Scheme 4. Reagents: (a) Allyl bromide, NaH, DMF; (b) 9-BBN, THF, DMSO; (c) H_2O_2, NaOH; (d) LAH, THF; (e) O_3, CH_2Cl_2/MeOH; (f) $NaBH_4$, CH_2Cl_2/MeOH; (g) LAH, THF.



Scheme 5. Reagents and conditions: (a) DEAD; (b) EtOH, NH_4Cl; (c) $PHPh_2$, *n*-BuLi, THF, −78 °C.

for tramadol and the M1 metabolite (entries 1 and 2) and 82-fold for the *N*HCH_3derivatives (entries 3 and 6), but did not drop below a ~50-fold difference for any of the synthesized derivatives.



Steps	R, R Compound	Substitution	S, S Compound
a, b, c, d, e	**24**	$R^1 = OCH_3$, $R^2 = CH_3$, $R^3 = H$	**25**
a, b, c, d, e, f	**26**	$R^1 = OH$, $R^2 = CH_3$, $R^3 = H$	**27**
a, b, c, f, g, h	**28**	$R^1 = H$, $R^2 = CH_3$, $R^3 = CH_3$	**29**

Scheme 6. Reagents and conditions: (a) 1-Chloroethyl chloroformate, CH_3CN; (b) MeOH; (c) EtOAc/hexane recrystallization; (d) 1-chloroethyl chloroformate, dichloroethane; (e) NH_4Cl (aq); (f) $PHPh_2$, *n*-BuLi, THF, −78 °C; (g) Tf_2O, TEA, CH_2Cl_2; (h) $Pd(OAc)_2$, HCO_2H, TEA, PPh_3.

It was also apparent that the phenol moiety was required for μ affinity as shown by the 84-fold potency drop in the unsubstituted phenyl derivative (entry 9) compared to its phenol counterpart (entry 14). In addition, other *meta* substitution on the phenyl ring (i.e., CN) was deleterious with respect to μ potency (compare entries 12–9), and also had a negative effect on potency at SERT and NET (compare entries 12 and 9 or 13).

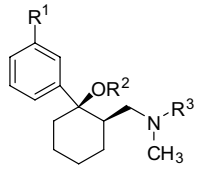
There was not a large difference between the racemic compounds and single enantiomers in the *N*HCH$_3$ anisole and phenol series (entries 3–5 and 6–8). For the unsubstituted phenyl dimethyl amine series (entries 9–11) most of the activity seemed to reside in one enantiomer, compound **28**.

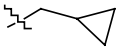
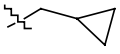
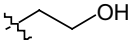
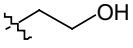
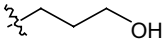
The other trend that was readily apparent in Table 1 was the divergent SAR in the *O*-alkylated compounds with respect to μ and SERT: while changing from *O*CH3 to *O*H on the aromatic ring increased μ potency dramatically, it decreased potency against SERT. For all of the *O*-alkylated derivatives the trend held up, including the -*O*Et derivatives (eightfold decrease in SERT and 75-fold increase in μ, entries 15 and 16), –*O*Pr (111-fold on SERT and 42-fold on μ, entries 17 and 18), –*O*Bu (24-fold for SERT and 66-fold for μ, entries 19 and 20) and *O*-cyclopropyl (146-fold for SERT and 61-fold for μ, entries 21 and 22).

The metabolic stability[8] of a range of tertiary alcohol tramadol derivative were evaluated in human and rat liver microsomes as shown in Table 2. Tramadol, (entry 1) included as a reference, showed moderate stability in rat liver microsomes (RLM) and excellent stability in human liver microsomes (HLM). Several trends were apparent in the tertiary alcohol *O*CH$_3$ series from our limited metabolic SAR study: (a) the phenol derivatives were much more stable in RLM than their *O*CH$_3$ counterparts (compare entry 2 to entry 3 and entry 7 to entry 8); (b) unsubstituted phenyl was more stable than *O*CH$_3$ in RLM (compare entry 3 to entry 4); (c) aromatic nitrile substitution was comparable from a stability standpoint to aromatic *O*H (entries 5 and 2) and (d) changing tertiary amine to secondary amine did not have a big impact on metabolic stability (compare entry 3 to entry 6). It was also apparent from entries 8 and 9 that *O*-alkylation beyond CH$_3$ was quite deleterious from a metabolic viewpoint. *O*-butyl (entry 8) and *O*-cyclopropyl (entry 9) derivatives were almost completely consumed in the RLM assay and substantially consumed in the HLM assay.

The *O*-methyl analogs were further evaluated in vivo in the rat tail-flick assay (Table 3).[9] Compared to tramadol, analog **3** was significantly more potent (ED_{50} of 0.7 mg/kg for **3** vs 11.3 mg/kg for tramadol **1**) but had a shorter duration of effect (20 min for **2** vs 69 min for tramadol). The

Table 1. μ-Opioid binding and 5-HT and NE reuptake inhibition of (±)-*cis* Tramadol and *O*-alkylated derivatives



Entry	Compound	R_1	R_2	R_3	μ IC_{50} (nM)	5-HT IC_{50} (nM)	NE IC_{50} (nM)
1	**1** (tramadol)	OCH_3	H	CH_3	7,600	1493	3861
2	**2, M1**	OH	H	CH_3	47	>10,000	>10,000
3	**22**	OCH_3	CH_3	H	>10,000	2686	3873
4	**24**	OCH_3	CH_3	H	>10,000	3172	>10,000
5	**25**	OCH_3	CH_3	H	>10,000	3378	898
6	**23**	OH	CH_3	H	122	6922	2250
7	**26**	OH	CH_3	H	48	5263	>10,000
8	**27**	OH	CH_3	H	84	1052	8999
9	**14**	H	CH_3	CH_3	4750	2534	3789
10	**28**	H	CH_3	CH_3	2590	2955	1518
11	**29**	H	CH_3	CH_3	>10,000	3880	1565
12	**15**	CN	CH_3	CH_3	>10,000	6236	4495
13	**3**	OCH_3	CH_3	CH_3	7870	927	161
14	**8**	OH	CH_3	CH_3	56	10,000	525
15	**4**	OCH_3	Et	CH_3	>10,000	181	1010
16	**9**	OH	Et	CH_3	130	1424	3776
17	**5**	OCH_3	Pr	CH_3	7900	25	3867
18	**10**	OH	Pr	CH_3	190	2798	6248
19	**6**	OCH_3	*n*-Bu	CH_3	6490	110	6688
20	**11**	OH	*n*-Bu	CH_3	97	2744	>10,000
21	**7**	OCH_3		CH_3	7670	25	3776
22	**12**	OH		CH_3	130	3667	>10,000
23	**17**	OCH_3		CH_3	>10,000	>10,000	>10,000
24	**18**	OH		CH_3	190	1503	5948
25	**21**	OCH_3		CH_3	8000	1493	3861

*IC_{50} is the concentration required to inhibit 50% binding to μ-opioid receptors or reuptake of 5-hydroxytryptamine (5-HT) and norepinephrine (NE).

shorter duration of action was probably due to more rapid metabolism, as shown by % remaining in the RLM in vitro assay (58% remaining for tramadol **1** vs 17% remaining for methoxy ether **3**) and was reflected in decreased bioavailability in rats ($F = 36\%$ for tramadol **1** and 11% for **3**). The cause for the increased potency of **3** (compared to tramadol **1**) may have been due to its profile at SERT and NET, which were markedly improved compared to tramadol (see Table 1); both compounds had metabolites with potent μ activity.

Racemic compound **22** and its enantiomer **25** also showed improved potency compared to tramadol in the in vivo assay, albeit with a decreased duration of effect (evaluated for enantiomer **25** only). The cause for the decreased duration of effect was likely more rapid metabolism when compared to tramadol. The lack of activity of highly hydrophilic compound **23** was unexpected given its strong affinity for the μ opioid receptor (122 nM) and may have been due to poor brain exposure.

Unsubstituted phenyl derivative enantiomer **29** also showed improved potency compared to tramadol (3.3 mg/kg for **29** vs 11.3 mg/kg for tramadol **1**), although the duration of effect was reduced (13 min for **29** vs 69 min for **1**). The efficacy of compound **29**

Table 2. In vitro rat and human microsomal stability assay for alkyl tramadol compounds[6]

Entry	Compound	% Remaining after 60 min			
		RLM	Rat S9	HLM	Human S9
1	Tramadol, **1**	58	56	96	95
2	**8**	88	74	104	98
3	**3**	17	12	82	85
4	**14**	56	61	96	101
5	**15**	69	83	89	94
6	**22**	31	26	77	76
7	**23**	86	82	103	108
8	**6**	3	4	9	10
9	**7**	3	6	29	35

Table 3. In vivo results for tramadol (1) and *O*-methyl tramadol analogs

Compound	ED_{50} (mg/kg)	Duration (min)	*F* (%, rat)
(±) −**1** (tramadol)	11.3	69	36
(±) −**3**	0.7	20	11
(±) −**22****	2.9		
25	3.1	27	
24	Toxicity*		
(±) −**14**	Toxicity*		
28	Toxicity*		
29	3.3	13	
(±) −**23**	No analgesia at subtoxic doses		

Toxicity*—compound demonstrated some analgesic activity, but toxicity (twitching, convulsions and death) was observed before the dose could be pushed high enough to observe maximum analgesia.

** Duration was not determined for **22** due to a lack of available compound.

in the tail flick assay was surprising given its lack of a strong μ opioid pharmacology, and may have been due to its weak affinity for the serotonin and norepinephrine transporters, the result of formation of a potent oxidative phenol metabolite in vivo, or to unknown off-target activity.

Alkylation of the tertiary alcohol of tramadol dramatically increased the potency of the novel tramadol analogs in the in vitro SERT reuptake inhibition assay and provided novel phenol metabolites with potent μ opioid binding properties. Several of these analogs proved to be superior to tramadol in the in vitro metabolic stability assay (greater % remaining) or the in vivo rat tail flick latency assay (lower ED_{50}). In particular, *N* and *O*-desmethylation led to a highly potent compound, **23**, which had both in vitro stability superior to tramadol in the in vitro RLM assay and potent affinity (122 nM) for the μ opioid receptor. This did not, however, translate to any in vivo efficacy in the rat tail flick assay, presumably due to poor CNS exposure. Our efforts to 'improve' tramadol led to some derivatives with superior in vivo potency in the rat tail flick assay but inferior pharmarcokinetics, or to compounds with superior in vitro stability and potency but a lack of activity in the in vivo tail flick assay. In all cases, the analogs synthesized could not measure up to the superior pharmacological properties present in the combination of racemic tramadol and its rapidly formed M1 metabolite.

References and notes

1. (a) Raffa, R. B.; Friderichs, E.; Reimann, W.; Shank, R. P.; Good, E. E.; Vaught, J. L. *J. Pharmacol. Exp. Ther.* **1992**, *260*, 275; (b) Gibson, T. P. *Am. J. Med.* **1996**, *101*(Suppl 1A), 47S; (c) Radbruch, L.; Grond, S.; Lehmann, K. *Drug Safety* **1996**, *15*, 8; (d) Raffa, R. B. *Am. J. Med.* **1996**, *101*(Suppl 1A), 41S; (e) Garrido, M.; Valle, M.; Campanero, M. A.; Calvo, R.; Troconiz, I. F. *J. Pharmacol. Exp. Ther.* **2000**, *295*, 352; (f) Subrahmanyam, V.; Renwick, A. B.; Walters, D. G.; Young, P. J.; Price, R. J.; Tonelli, A. P.; Lake, B. G. *Drug Metab. Dispos.* **2001**, *29*, 1146; (g) Wu, W. N.; McKown, L. A.; Liao, S. *Xenobiotica* **2002**, *32*, 411; (h) Leppert, W.; Luczak, J. *Support Care Cancer* **2005**, *13*, 5; (i) Duhmke, R. M.; Cornblath, D. D.; Hollingshead, J. R. *Cochrane Database Syst. Rev.* **2004**, *2*, CD003726; (j) Grond, S.; Sablotzki, A. *Clin. Pharmacokinet.* **2004**, *43*, 879; (k) Klotz, U. *Arzneim.-Forsch.* **2003**, *53*, 681.
2. PDR Electronic Library, Thomson Scientific, http://www.thomsonhc.com/pdrel/librarian, reference for Ultram© tablets (Ortho-McNeil).
3. Raffa, R. B.; Nayak, R. K.; Liao, S.; Minn, F. L. *Rev. Contemporary Pharmacother.* **1995**, *6*, 485.
4. All new compounds were characterized by ^{1}H NMR, HPLC and LCMS or GC-MS. In some cases ^{13}C or DEPT spectra were also recorded. Unless otherwise indicated, new compounds were analytically pure (>98% pure by HPLC) and the collected spectra was consistent with the assigned structure.
5. Synthesis of **4**: Tramadol *N*-benzyl salt (264 mg, 0.61 mmol) and NaH (60% dispersion in mineral oil, 37 mg, 0.92 mmol) were suspended in anhydrous DMF (6 mL) and cooled to 0 °C. Iodoethane (74 μL, 0.92 mmol) was added and the suspension was allowed to stir and warm to rt. After 16 h, MeOH (3 mL) was added and the solution was concentrated in vacuo and used directly in the next reaction. Crude ethyl tramadol *N*-benzyl salt (0.61 mmol) and 10% wt. Pd/C (150 mg) were suspended in 10:1 MeOH/H_2O (5.5 mL) in a 30 mL hydrogenation bomb. A stir bar was added, and the vessel was purged 3 times with H_2 then charged to 60 psi. After 16 h the suspension was diluted with MeOH (3 mL) and filtered through celite. The celite was washed with MeOH and CH_2Cl_2 and the combined washes concentrated to an orange oil. Purification by silica gel column chromatography on an Isco combiflash system utilized a 35 g column and a gradient of 0 → 20% MeOH in

CH_2Cl_2 over 20 min. To prepare the free base, the pure product was redissolved in EtOAc (50 mL), washed $3 \times 20\%$ aqueous NaOH, dried (Na_2SO_4), filtered and concentrated to yield **4** (92 mg, 52%) as a white solid. ^1H NMR (400 MHz, $CDCl_3$) 7.23 (t, 1 H), 6.88–6.86 (m, 2H), 6.76–6.74 (m, 1H), 3.80 (s, 3H), 3.42–3.38 (m, 1H), 3.30–3.26 (m, 1H), 2.32 (dd, $J = 9.9$, 12.1 Hz, 1H), 2.05–2.02 (m, 1H), 1.92 (s, 6H), 1.89–1.79 (3H), 1.69 (dd, $J = 2.2$, 12.1 Hz, 1H), 1.63–1.34 (m, 5H), 1.24 (t, $J = 6.96$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 159.9, 144.8, 129.9, 118.5, 112.7, 111.9, 79.4, 60.6, 57.1, 55.4, 44.2, 43.9, 32.5, 28.6, 24.9, 21.3, 15.3.

6. Synthesis of **9**: A dry 2-neck flask equipped with a reflux condenser was charged with anhydrous THF (16 mL) and diphenyl phosphine (1.26 mL, 7.24 mmol). The clear solution was cooled to −10 °C and *n*-BuLi (2.5 M solution in hexane, 3.5 mL, 8.69 mmol) was added dropwise. The resultant bright orange solution was stirred for 30 min at −10 °C, then **4** (470 mg, 1.61 mmol) in anhydrous THF (16 mL) was added dropwise. The orange solution was warmed to 0 °C and stirred for 1 h, then placed in an oil bath and heated to reflux for 16 h. After cooling to rt, the reaction was quenched with 2 N aqueous HCl (40 mL) and washed with EtOAc (3×30 mL). The organic washes were discarded. The aqueous phase was brought up to pH 12 with sat'd aqueous K_2CO_3, washed with EtOAc (3×30 mL), and the combined organic extracts were dried (Na_2SO_4), filtered and concentrated. Purification by silica gel column chromatography with 90/9/1 CH_2Cl_2/MeOH/conc NH_4OH yielded **9** (177 mg, 40%) as an off white solid. ^1H NMR (400 MHz, $CDCl_3$) 7.16 (t, 1H), 6.70 (d, $J = 7.7$ Hz, 1H), 6.72 (s, 1H), 6.60 (dd, $J = 1.5$, 7.7 Hz, 1H), 3.41–3.28 (m, 2H), 2.36 (dd, $J = 9.2$, 12.8 Hz, 1H), 2.14–2.02 (m, 2H), 1.96 (s, 6H), 1.90–1.30 (m, 8H), 1.23 (at, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 157.0, 146.4, 129.0, 118.1, 114.4, 114.1, 80.2, 60.2, 56.8, 45.9, 45.3, 33.4, 28.0, 26.2, 22.1, 15.6.
7. Flick, K.; Frankus, E. U.S. Patent 3,652,589, 1972.
8. Microsomal stability assay was done using pooled human and rat liver microsomes (0.3 mg/mL protein). Compound was incubated for 60 min at pH 7.4 and 37 °C at a concentration of 1 μm. % remaining of parent compound was determined by HPLC-MS/MS. For a more detailed description see Kuhnz, W.; Gieschen, H. *Drug Metab. Dispos.* **1998**, *26*, 1120.
9. Male Sprague–Dawley rats (150–200 g) were tested for analgesia with tramadol analogs: Animals ($N = 4$–6/dose/compound) were administered test compound (0.1–30 mg/kg) in saline (vehicle) via tail vein injection in a volume of 0.2 mL over approximately 10 s. Five minutes later, the rat was placed into a Plexiglas restrainer with its tail positioned under a focused radiant (light) heat source. The light was activated with a timer, and when the animal flicked its tail a photocell was uncovered, stopping the timer. Tail-flick (TF) latencies were recorded to 0.1 s and a maxiμm latency of 10 s was used as a cut-off in order to prevent tissue damage. Analgesic response, expressed as percent of maxiμm possible effect (%MPE), was used to generate a dose-response curve according to the calculation: $\%MPE = \frac{\text{Mean TF Latency of Vehicle Group} - \text{Drug TF Latency} \times 100}{(10 - \text{Mean TF Latency of Vehicle Group})}$ ED_{50} and A_{99} values of all compounds were determined by linear regression analysis and the A_{99} value was used for assessment of the relative durations of analgesic activity. Analgesia (%MPE) in the TF assay was assessed at 5 to 10-min intervals until it fell below 20%.