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Parallel methods for the preparation and SAR exploration of *N*-ethyl-4-[(8-alkyl-8-aza-bicyclo[3.2.1]-oct-3-ylidene)-aryl-methyl]-benzamides, powerful mu and delta opioid agonists

Steven J. Coats,* Mark J. Schulz, John R. Carson, Ellen E. Codd, Dennis J. Hlasta, Philip M. Pitis, Dennis J. Stone, Jr., Sui-Po Zhang, Ray W. Colburn and Scott L. Dax

Drug Discovery, Johnson and Johnson Pharmaceutical Research and Development, L.L.C., Welsh and McKean Roads, PO Box 776, Spring House, PA 19477-0776, USA

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Abstract—Two parallel synthetic methods were developed to explore the structure–activity relationships (SAR) of a series of potent opioid agonists. This series of tropanylidene benzamides proved extremely tolerant of structural variation while maintaining excellent opioid activity. Evaluation of several representative compounds from this series in the mouse hot plate test revealed potent antinociceptive effects upon oral administration.

Morphine remains the analgesic of choice for severe pain but the accompanying constipation, addiction liability, respiratory depression, and other unwanted side effects drive the search for safer opioid agonists. The ability to modulate the unwanted effects of a mu opioid agonist by modifying its relative affinity for the delta receptor has been postulated[1] and later supported experimentally.[2]

We recently disclosed our delta/mu opioid agonist series of tropanylidenes typified by *N*-ethyl-4-[(8-phenethyl-8-aza-bicyclo[3.2.1]oct-3-ylidene)-phenyl-methyl]-benzamide (Fig. 1; **1**).[3] This compound was initially derived metabolically from the powerful delta agonist *N*,*N*-diethyl-4-[(8-phenethyl-8-aza-bicyclo[3.2.1]oct-3-ylidene)-phenyl-methyl]-benzamide **2**.

The unexpected discovery of potent mu opioid activity in a series that was predicted to display delta selectivity led us to explore analogs of **1** and hold constant this newly found ethyl amide mu address.[4] With the goal of identifying safer opioid agonists to treat severe pain, our efforts to develop both solution and solid-phase parallel synthetic approaches[5] to this series is disclosed herein, along with the evaluation of these compounds with respect to opioid affinity, relative affinity for the delta receptor, and their antinociceptive effects in rodent models.

We knew from our earlier work[3] that the 1*S*,5*R* configuration (as in Fig. 1) of **3** would, in general, display the strongest affinity for the mu and delta opioid receptors; however there are several cases where 1*R*,5*S* enantiomer produced a more potent compound or opposite delta/mu selectivity (Fig. 2). We had previously performed the initial opioid binding assay on the racemic mixture

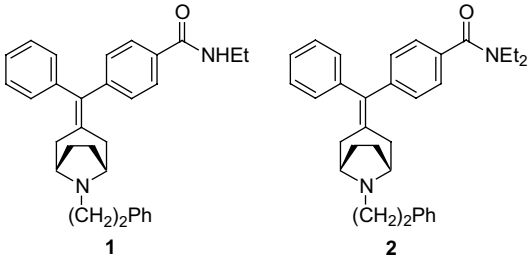

Figure 1. Initial tropanylidene opioid leads.

Keywords: Opioid; Delta; Mu; Parallel; Antinociception.
* Corresponding author. Tel.: +1 215 628 5266; fax: +1 215 628 3297; e-mail: scoats@prdus.jnj.com

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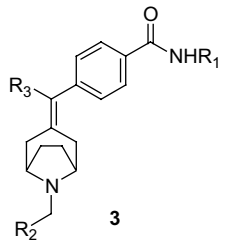


Figure 2. Generic tropanylidene scaffold.

of compounds of type **3** and found that the data correlated well with those obtained for the individual enantiomers. In consideration of these factors we chose to prepare the more readily available racemic compounds for initial in vitro screening while single enantiomers of selected compounds were prepared for subsequent profiling.

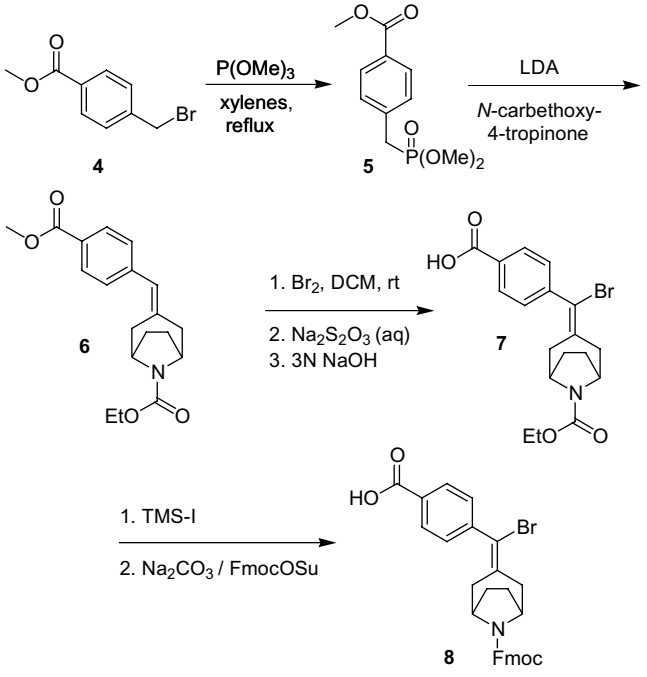
Our original route to **1** relied on a McMurray coupling as a key step in the synthetic sequence.[6,7] This linear route was quite efficient for the preparation of single compounds but lacked the features necessary for facile parallel analog construction. We therefore developed a new synthetic route,[8] which allowed us to easily generate variation of the amide R_1 group, the tropanylidene nitrogen R_2 substituent, and the olefinic R_3 group of racemic **3** (Fig. 2).

4-Bromomethyl methyl benzoate, **4** was allowed to react with trimethyl phosphite to provide the Horner–Emmons–Wittig precursor **5** (Scheme 1). The use of trimethyl phosphite in place of higher homologs allowed for a simple evaporative workup, which was quite useful on large scale. Olefin **6** was prepared in good yield from *N*-carbethoxy-4-tropinone in the presence of LDA.

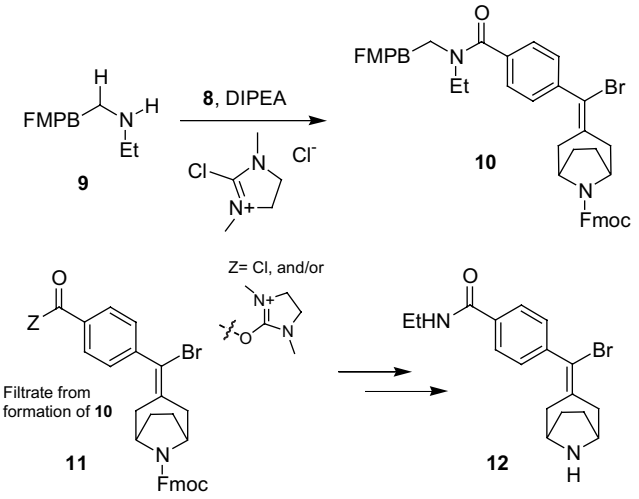
The one pot conversion of **6** to the carboxylic acid **7** involved: dibromination, consumption of excess bromine with sodium thiosulfate, and saponification of the methyl ester and elimination of HBr by sodium hydroxide. Removal of the ethyl carbamate was initially carried out on the crude intermediate dibromide by refluxing in ethanolic KOH. This was appealing in that three synthetic steps could be carried out in a single reaction pot. However we found the yields were improved by the use of a two-pot approach using NaOH to give an intermediate vinyl bromide carboxylic acid **7**, which was then treated with TMSI. Neutralization and protection with FmocOSu gave (21 g, 40%, seven steps) key intermediate **8** necessary for our solid phase analog program.

Our earlier work[3] had shown that the secondary carboxamido *N*-substituent could not deviate far in size from ethyl without sharply decreasing mu receptor affinity. Consequently we designed our parallel synthetic approach to hold this group constant as the ethyl amide. Accordingly, FMPB resin[9] was reacted with ethylamine under reductive amination conditions to give secondary amine resin **9** to which carboxylic acid **8** was coupled using 2-chloro-1,3-dimethylimidazolinium chloride (Scheme 2). After shaking for 24 h, the resin slurry of **10** was filtered and the resin was washed two times with dichloromethane. The combined filtrate containing excess **11** was allowed to react with ethylamine followed by Fmoc removal[10] to produce the ethyl amide **12**, which was used in our parallel solution phase approach described below.

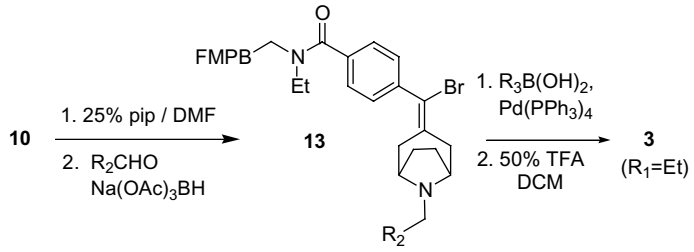
With versatile resin **10** in hand we began to prepare sets of 96 compounds utilizing the chemistry sequence outlined in Scheme 3. Removal of the Fmoc group was followed by reductive amination with a variety of aldehydes to give the resin bound vinyl bromides **13**.



Scheme 1. Preparation of a versatile tropanylidene.



Scheme 2. Loading **8** onto solid support.



Scheme 3. Solid phase parallel preparation of delta/mu opioid agonists.

A subsequent Suzuki reaction with a broad range of boronic acids was followed by cleavage from support to give functionalized tropanylidenes **3**. We were also successful with acylations and sulfonylations at the tropanylidene nitrogen as well as Stille reactions with the vinyl bromide.

From this approach we were able to rapidly obtain 243 compounds, which were all purified by reversed-phase HPLC, analyzed by LC/MS,[11] and assayed in vitro against the mu and delta opioid receptors. The techniques used for the determination of delta and mu opioid receptor binding have been previously described.[12] Functional activity[7] was measured by stimulation of [^{35}S]GTPγS binding in a manner similar to that previously employed to study bradykinin receptors.[13] With select compounds, in vivo activity was assessed using the mouse 48 °C hot plate test,[14] a stringent antinociceptive model.

We were quite surprised to find that 180 (74%) compounds had a K_i of less than 50 nM for either the delta or mu receptors; more amazingly 102 (42%) compounds had a K_i of less than 10 nM for either the delta or mu. Table 1 shows some selected compounds along with their delta and mu binding affinity.

We were encouraged to find that the tropanylidene ethyl benzamide series displayed a significantly improved delta opioid binding affinity relative to morphine. As shown in Table 1, substituted phenyl groups such as 3,4-methylenedioxy (**14**), 3-acetylamino (**19** and **22**), and 3-acetyl (**20**) were all well tolerated in the olefinic R_3 position of **3** for both the mu and delta binding. Basic groups such as 2-pyridyl (**15**) and 2-pyrazinyl (**16**) behaved similarly in the binding assays. While only 2-furanyl (**17**) is shown, we found that a broad range of other small heterocycles could effectively replace the olefinic R_3 substituent of **3** such as 3-furanyl, 2-thiophenyl, 3-thiophenyl, and 4-isoxazolyl.

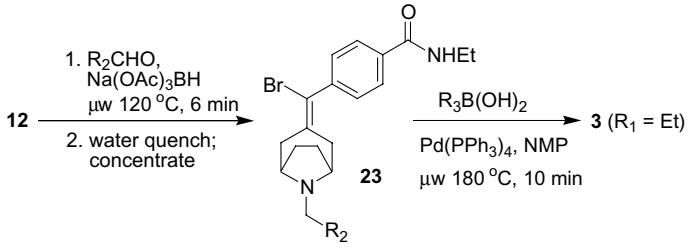
The R_2 substituent of **3** was also tolerant of small heterocyclic groups such as thienyl (**14**–**16**) and 4-imidazolo (**19**). Basic groups in the R_2 position generally led to a significant loss in opioid binding; unless matched with an optimal R_3 as in the case of 3-pyridyl (**21**) and 2-pyridyl (**22**). Groups containing acid functionality attached at R_2, as in the case of **18**, always led to a significant loss in activity. As was found in related delta opioid agonists[15] smaller groups at R_2 were optimal while larger groups (not shown) trended toward a loss of opioid binding affinity.

In our support of therapeutic team collaborations, we have come to increasing rely on the development of parallel solution phase strategies, which allow multiple sequential reactions to occur in a single reaction vessel with little or no workup involved between synthetic steps. Such sequences are, in many cases, more rapidly developed relative to their solid phase counterparts. This approach is well suited for the parallel preparation of sets of compounds in a discovery SAR program.

For the solution phase preparation of functionalized tropanylidenes **3** we simply dispensed a dichloroethane (DCE) solution of **12** to a set of microwave tubes, added the aldehydes in DCE, added a DMF solution of sodium triacetoxy borohydride, and subjected the mixture

Table 1. Some representative tropanylidenes **3** (R_1 = Et) from solid phase

Compd	R_2	R_3	K_i mu (nM)	K_i delta (nM)	mu/delta
Morphine			1.8	90	0.020
1	Benzyl	Phenyl	72	0.24	300
2	Benzyl	Phenyl (R_1 = diethyl amide)	0.26	47	0.0055
14	3-Thienyl	3,4-Methylenedioxyphenyl	1.3	3.1	0.42
15	2-Thienyl	2-Pyridyl	6.0	3.8	1.6
16	3-Thienyl	2-Pyrazinyl	2.5	17	0.15
17	2-Methyl-propene	2-Furanyl	6.3	5.6	1.1
18	3-Carboxy phenyl	Phenyl	480	280	1.7
19	4-Imidazolo	3-Acetylamino phenyl	9.4	19	0.50
20	2-Methyl-propene	3-Acetylphenyl	1.9	1.8	1.1
21	3-Pyridyl	Phenyl	62	71	0.87
22	2-Pyridyl	3-Acetylamino phenyl	3.6	28	0.13



Scheme 4. Solution phase parallel preparation of delta/mu opioid agonists.

to microwave irradiation for 6 min at 120 °C (Scheme 4). Simply quenching the reductive amination reaction with water and subsequent concentration allowed us to directly perform a microwave assisted Suzuki reaction on crude **23**. This critical finding allowed us to rapidly prepare analogs of type **3** more quickly and efficiently than the above solid phase approach.

From this parallel solution phase approach[16] we rapidly obtained a total of 192 compounds, which were all purified by reversed-phase HPLC, analyzed by LC/MS, and assayed in vitro against the mu and delta opioid receptors. Of these, 123 (64%) compounds had a K_i of less than 10 nM for either the delta or mu receptors. An additional advantage of the parallel solution phase approach was that it allowed us to rapidly scale up selected compounds for further in vivo studies using the same solution phase sequence.

In our exploration of the R_2 position of **3** we found that 3-furanyl provided the broadest in vitro opioid activity of all substituents investigated. Table 2 shows

Table 2. Tropanylidene **3** (R_1 = Et, R_2 = 3-furanyl); a highly potent series

Compd	R_3	K_i mu (nM)	K_i delta (nM)	mu/delta
24	3-Amino-5-carboxy phenyl	9100	56	160
25	5-(1*H*-Tetrazole)	83	210	0.40
26	Cyano	1.0	0.30	3.3
27	3-Nitro-5-carboxy phenyl	12	0.94	13
28	2-Pyrazinyl	9.9	3.4	2.9
29	3-Carboxy phenyl	28	3.5	8.0
30	4-Carboxy phenyl	12	5.3	2.3
31	4-(3-Phenylpropionic acid)	3.0	0.78	3.9
32	5-Pyrimidinyl	6.9	2.3	3.0
33	4-Sulfamoyl phenyl	50	16	3.1
34	4-Methanesulfonyl phenyl	3.1	2.0	1.6
35	4-*N*,*N*-Dimethyl benzamide	6.7	4.6	1.5
36	2-Pyridyl	45	10	4.5
37	4-Pyridyl	2.2	6.0	0.37
38	H	110	93	1.2
39	3-Pyridyl	1.2	0.40	3.0
40	4-(3,5-Dimethyl-isoxazol-4-yl)	1.8	1.2	1.5
41	4-Benzamide	1.3	3.9	0.33
42	3-(3-Amino) phenyl	2.6	2.0	1.3
43	3-Hydroxy-3-pyridyl	4.1	0.52	7.9
44	3-(1*H*-Tetrazol-5-yl) phenyl	1500	0.73	2100
45	Br	1.9	0.80	2.4
46	3-Methoxy-3-pyridyl	1.9	0.46	4.1
47	3-Aminomethyl phenyl	71	20	3.6
48	3-Hydroxymethyl phenyl	0.81	0.15	5.4
49	3-Acetylamino phenyl	1.2	0.50	2.4
50	4-Acetylamino phenyl	0.80	0.34	2.4
51	4-Hydroxy-3-methoxy phenyl	0.59	0.30	2.0
52	4-Hydroxymethyl phenyl	19	2.7	7.0
53	3-(3-Cyano) phenyl	3.3	0.60	5.5
54	3-Acetyl phenyl	1.3	0.40	3.3
55	4-Acetyl phenyl	1.7	0.33	5.2
56	3,4-Methylenedioxyphenyl	1.1	1.1	1.0
57	4-Nitro phenyl	1.5	0.23	6.5
58	3-Quinolyl	4.0	20	0.20
59	3-Fluoro phenyl	3.7	0.40	9.3
60	4-Fluoro phenyl	1.7	1.4	1.2
61	4-(*N*,*N*-dimethylamino) phenyl	51	8.7	5.9
62	2,6-Dimethyl phenyl	1.4	1.3	1.1

Table 3. Selected compounds prepared as single enantiomers

Compd	R_2	R_3	Stereochemistry	K_i mu (nM)	K_i delta (nM)
63	2-Pyridyl	4-*N*,*N*-Dimethyl benzamide	1*S*,5*R*	14	0.20
64			1*R*,5*S*	100	0.50
65	2-Methyl-propene	3-Acetyl phenyl	1*S*,5*R*	2.8	1.5
66			1*R*,5*S*	680	28
67	3-Thiophenyl	4-Hydroxy methyl phenyl	1*S*,5*R*	17	2.0
68			1*R*,5*S*	80	17
69	2-Methyl-propene	3-Carboxy phenyl	1*S*,5*R*	58	2.2
70			1*R*,5*S*	24	30
71	3-Thiophenyl	3-(1*H*-Tetrazol-5-yl) phenyl	1*S*,5*R*	12	0.70
72			1*R*,5*S*	3100	31
73	3-Furanyl	Cyano	1*S*,5*R*	2.6	0.90
74			1*R*,5*S*	2.3	1.1
75	3-Furanyl	Br	1*S*,5*R*	0.20	0.30
76			1*R*,5*S*	39	29

all of the compounds we prepared where the R_2 of tropanylidene **3** is 3-furanyl and the R_1 is again held constant as ethyl. The compounds are listed in order of increasing Clog *P*, which ranges from 1.5 to 6. The variety of substituents tolerated at R_3 is quite impressive as we found that acidic, basic, neutral, large, and small groups all result in high affinity compounds. To our knowledge this level of broad substitution, which retains single digit nanomolar binding, is unprecedented in the opioid area.

A number of homochiral analogs of **3** were prepared by one of two routes, which we have previously described.[3] As can be seen in Table 3 the most active isomer in every case is the 1*S*,5*R* enantiomer; however the 1*R*,5*S* enantiomer in many cases also displays potent opioid binding and in some cases the selectivity for the opioid receptors is reversed.

Many compounds underwent functional testing and almost all showed agonist activity. A subset was tested orally at 150 μmol/kg in the mouse 48 °C hot plate test. We found that compounds **15**, **17**, **20**, and **73** provided robust antinociception and most induced Straub tail, a behavior often associated with mu opioid agonist activity. The 3- and 4-carboxy phenyl tropanylidenes **29** and **30** provided little or no analgesic effects in the same testing paradigm most likely due to poor oral absorption or brain penetration. We observed no instances of convulsions[17] or deaths with these compounds.

In summary, we have developed an efficient solid and solution phase approach for SAR exploration in the tropanylidene series of opioid agonists. In a collaboration that lasted a little over one year, 435 compounds were prepared for in vitro screening and over 60 compounds were resynthesized on larger scale to support further testing. We discovered that significant polarity could be introduced into the tropanylidene scaffold while maintaining excellent binding affinity and in many cases oral in vivo efficacy. This scaffold has proven to be an excellent opioid template as 225 unique compounds displayed potent binding affinity with K_i values of less than 10 nM for either the delta or mu opioid receptors.

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16. Representative procedure: To a solution of 4-[(8-azabicyclo[3.2.1]oct-3-ylidene)-bromo-methyl]-*N*-ethyl-benzamide, **12** (10 mg, 0.029 mmol) and the appropriate aldehyde (0.087 mmol) in dichloroethane (0.5 mL) was added sodium triacetoxyborohydride (12 mg, 0.058 mmol) in dimethylformamide (100 μL) and acetic acid (5 μL). The reaction mixture was irradiated (μw) at 120 °C for 6 min. After quenching with water, the mixture was concentrated in vacuo. To the resulting residue in *N*-methyl pyrrolidinone (0.3 mL) was added potassium carbonate (12 mg, 0.087 mmol) in water (100 μL), the appropriate boronic acid (0.087 mmol), and tetrakis(triphenylphosphine)palladium (1.5 mg, 0.001 mmol) in *N*-methyl pyrrolidinone (100 μL). The reaction mixture was irradiated (μw) at 180 °C for 10 min. After quenching with water, the mixture was absorbed onto diatomaceous earth and eluted with 5% methanol/ethyl acetate. The eluate was concentrated to a residue and purified by reversed-phase chromatography to furnish the product as a trifluoroacetate salt.
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