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Brief Article

14β-Arylpropiolylamino-17-cyclopropylmethyl-7,8-dihydronormorphinones and Related Opioids. Further Examples of Pseudoirreversible μ Opioid Receptor Antagonists

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14β-4′-Chlorocinnamoylaminodihydronormorphinone (**2a**), and analogues, are selective pseudoirreversible antagonists of the μ opioid receptor (MOR). The preparation of analogues with ethynic bonds, replacing the ethenic bond of **2a**, is described. The new ligands, in mouse antinociceptive assays, had pseudoirreversible MOR antagonist activity, which, in the case of **8b** was of longer duration than that of **2a**. The related codeinone (**9b**) had only antagonist activity in vivo, in contrast to **2a**'s codeinone equivalent **3a**, which had potent antinociceptive activity.

Introduction

The 14β-hydroxy-7,8-dihydronormorphinone derivatives naloxone (**1a**) and naltrexone (**1b**) were the prototypic μ opioid receptor (MOR[a]) antagonists, and both have been introduced into clinical practice. We have been interested in derivatives of the structurally equivalent 14β-amino-17-cyclopropylmethyl-7,8-dihydronormorphinone (**1c**) for some time, with particular attention to the 14β-cinnamoylamino derivatives (**2**, **3**)[1] and related side-chain analogues (**6**−**7**).[2] The most studied derivatives have been clocinnamox (C-CAM, **2a**)[11] and methcinnamox (M-CAM, **2b**)[10] and their codeinone precursors (**3a**, **3b**).[3] They are impressive MOR-selective pseudoirreversible antagonists, with only the codeinones (**3a**, **3b**) having any in vivo antinociceptive activity.[1] The present study was undertaken to determine the OR profile of the analogues of 14β-cinnamoylamino and 14β-cinnamylamino derivatives (**2**−**5**) in which the *trans*-ethenic bond in the cinnamoyl or cinnamyl group is replaced by an ethynic bond in the arylpropiolylamino derivatives (**8**, **9**) and arylpropargylamino derivatives (**10**, **11**). The ethynic bond in the new ligands places the key aromatic group further from C14 than in the cinnamoylamino and cinnamylamino ligands previously studied. The data collected in the present study show that the arylpropiolylamino morphinones (**8**) are pseudoirreversible MOR antagonists at least the equal of their cinnamoylamino analogues (Chart 1).

Synthesis

While phenylpropiolic acid is commercially available, *p*-chlorophenylpropiolic acid (**15**) and *p*-chlorophenylpropargyl bromide (**17**) were obtained by preparation from the appropriate cinnamic acid (Scheme 1).[4,5] Target compounds (**9**) were then accessed by acylation of *N*-cyclopropylmethyl-14β-aminodihydronorcodeinone (**18b**) (Scheme 2).[6,7] The equivalent morphinones (**8**) were accessed from codeinones (**9**) by 3-*O*-demethylation with boron tribromide. Direct alkylation of *N*-cyclopropylmethyl-14β-aminodihydronorcodeinone and *N*-cyclopropylmethyl-14β-aminodihydronorcodeinone using the arylpropargyl bromide (**17**) gave target compounds **10**, **11** (Scheme 2).

Results

Affinity for the individual types of opioid receptors (OR) was determined by displacement binding assays using membranes prepared from Chinese hamster ovary (CHO) cells expressing recombinant human opioid receptors. The selective radioligands used were [^{3}H]-DAMGO (MOR), [^{3}H]U-69593 (κ opioid receptor, KOR), and [^{3}H]Cl-DPDPE (δ opioid receptor, DOR).[8] All the new morphinones (**8a**, **8b**, **10**) had high affinity for all OR with no selectivity for any one (Table 1). The equivalent codeinones (**9a**, **9b**, **11**) had generally lower OR affinity, particularly at DOR and KOR. Overall, the affinities of the new series (**8**−**11**) were similar to the affinities displayed by the equivalent cinnamoylamino derivatives (**2**, **3**) and cinnamylamino derivatives (**4**, **5**).

In vitro OR functional activity of the new ligands was determined in assays in which stimulation of [^{35}S]GTPγS binding is measured for individual recombinant human OR transfected into CHO cells.[8,9] The morphinones (**8a**, **8b**, **10**) were potent antagonists for all three OR, except that **8a** showed low efficacy agonism at KOR (Table 1). The equivalent codeinones (**9a**, **9b**, **11**) had MOR antagonist activity of lower potency than their morphinone equivalents (**8a**, **8b**, **10**). The only codeinone with both KOR and DOR antagonist activity was the arylpropargyl derivative (**11**), but potency was low in each case so that **11** profiled as a MOR antagonist of some selectivity. The arylpropiolylamino codeinones (**9a**, **9b**) were KOR partial agonists; **9a** also had DOR partial agonist activity, whereas **9b** was a DOR antagonist (Table 1).

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[a] Abbreviations: OR, opioid receptors; C-CAM, 14β-4′-chlorocinnamoylaminodihydronormorphinone; M-CAM, 14β-4′-methylcinnamoylaminodihydronormorphinone; norBNI, nor-binaltorphimine; TW, tail withdrawal; AS, abdominal stretch.

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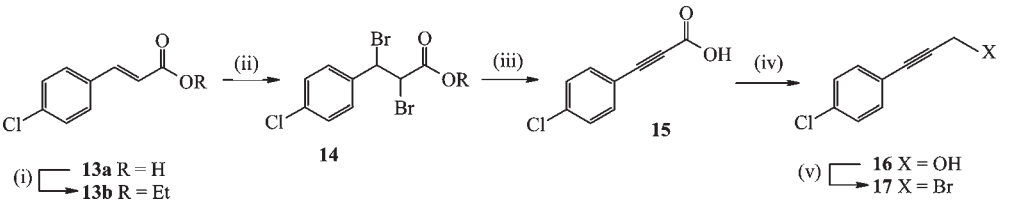
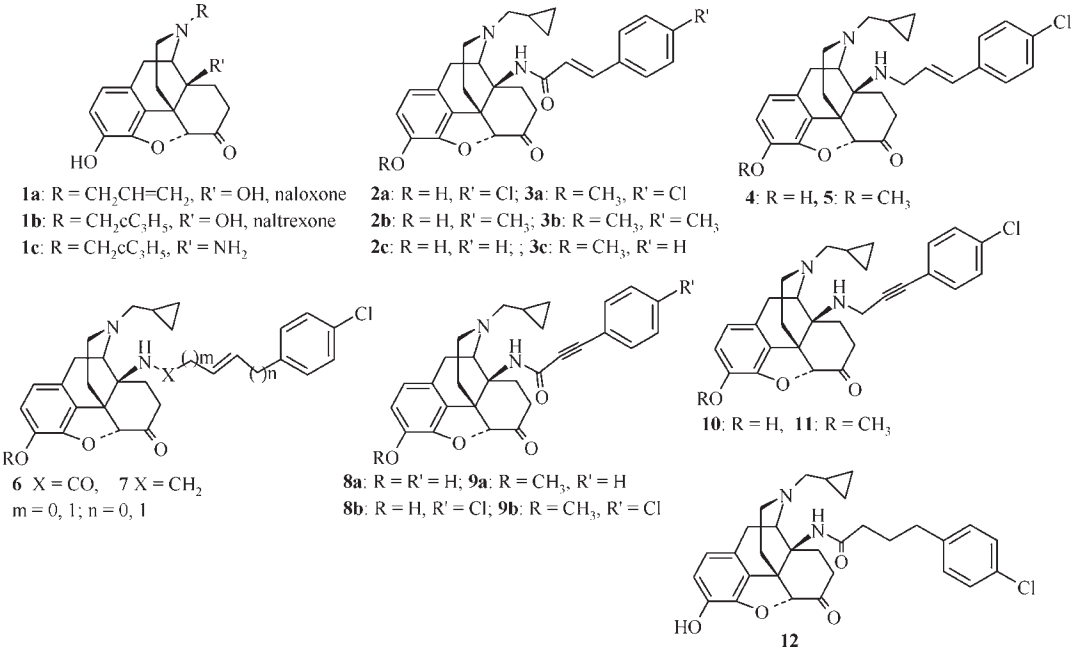
Scheme 1[a]

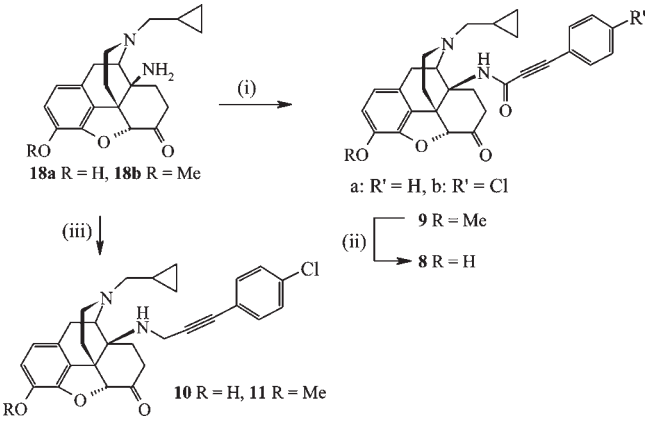


[a] (i) EtOH, c·H_2SO_4, reflux, 4 h, 80%; (ii) Br_2, DCM, rt, overnight, 70%; (iii) KOH, EtOH, reflux, 6 h, 40%; (iv) DIBAL, Et_2O, −78 °C to rt, overnight, 64%; (v) PPh_3, imidazole, Br_2, DCM, rt, 1.5 h, 79%.

Chart 1. 14-Substituted 7,8-Dihydromorphinones and Codeinones



Scheme 2[a]



[a] (i) R′$C_6H_4CCCOCl$, NEt_3, DCM, rt, overnight, 27−71%; (ii) BBr_3, DCM, −30 °C to rt, 0.5 h, 72−75%; (iii) $ClC_6H_4CCCH_2Br$, K_2CO_3, DMF, 90 °C, 3 h, 63−74%.

The arylpropiolylamino derivatives (**8a**, **8b**, **9a**, **9b**) were evaluated in vivo in mouse antinociceptive assays using thermal (50 °C water tail withdrawal; TW) and chemical (acetic acid induced stretching; AS) nociceptors.[10] Only the phenylpropiolylamino codeinone (**9a**) had any antinociceptive activity in these assays. In TW, it was a partial agonist with peak effect (at 3.2 mg/kg), reaching about two-thirds of the maximum possible (Figure 1). In AS, **9a** at a dose of 0.32 mg/kg totally inhibited the stretching response. This effect was reversed by the selective MOR antagonist methcinnamox (M-CAM, **2b**) but not by naltrindole (DOR) and partially by norBNI (KOR) (Table 2).

All of the arylpropiolylamino ligands were able to antagonize the antinociceptive effects of morphine in TW (Table 3). When a high dose of the test ligand (32 mg/kg) was administered 30 min before morphine, **8a**, **8b**, and **9b** all flattened the morphine dose−response curve and were shown to retain antagonist activity beyond 48, 144, and 24 h, respectively. **9a**, which had agonist activity in TW, was only tested at 24 h and beyond; it was active as an MOR antagonist at 24 h (Table 3).

The most impressive in vivo morphine antagonist among the new ligands was thus the *p*-chlorophenylpropiolylamino morphinone (**8b**). Its OR antagonist selectivity was investigated in AS against ED_{100} doses of the selective agonists morphine (MOR), bremazocine (KOR), and BW373U86 (DOR). At a dose of 3.2 mg/kg of **8b**, the inhibitory effect of each of the selective agonists was fully reversed; 1 mg/kg **8b** was also substantially effective, with the MOR and DOR activity showing superiority over KOR activity (Figure 2). With 24 h pretreatment, 3.2 mg/kg **8b** still showed substantial OR antagonist activity; this activity was significantly MOR selective (Figure 3).

Table 1. Binding Affinities (K_i) of New Compounds to Opioid Receptors and Antagonist Activity (K_e) in the [^{35}S]GTPγS Binding Assay

	K_i/nM[a]			K_e/nM[d]		
	MOR	DOR	KOR	MOR	DOR	KOR
8a	1.31 ± 0.27	1.60 ± 0.08	1.12 ± 0.06	0.37 ± 0.07	0.38 ± 0.03	agonist[f]
8b	3.75 ± 0.15	5.41 ± 1.2	2.73 ± 0.36	0.52 ± 0.08	0.73 ± 0.04	0.29 ± 0.03
10	0.97 ± 0.12	3.26 ± 0.32	2.89 ± 0.53	0.25 ± 0.02	1.43 ± 0.18	2.50 ± 0.09
9a	1.46 ± 0.31	28.3 ± 2.0	7.86 ± 1.8	4.60 ± 0.61	agonist[e]	agonist[g]
9b	7.43 ± 2.7	65.2 ± 5.6	13.7 ± 1.0	1.16 ± 0.06	29.5 ± 1.4	agonist[h]
11	9.68 ± 2.0	136 ± 17	74.4 ± 9.4	4.24 ± 0.43	152 ± 27	106 ± 12
2a[b]	2.98 ± 0.22	2.69 ± 0.23	1.41 ± 0.52	0.53 ± 0.13	0.19 ± 0.02	0.10 ± 0.006
3a[b]	4.78 ± 0.58	4.79 ± 0.73	16.4 ± 2.5	0.97 ± 0.15	7.16 ± 0.57	9.81 ± 0.88
4[c]	0.32 ± 0.03	0.63 ± 0.08	0.91 ± 0.12	NT	NT	NT
5[c]	0.70 ± 0.10	44.5 ± 4.6	53.6 ± 0.95	NT	NT	NT

[a] K_i/nM vs [^{3}H]DAMGO (MOR), [^{3}H]U69593 (KOR) and [^{3}H]DPDPE (DOR). Data are the mean of two experiments, each carried out in triplicate. [b] Data from Nieland et al.[1] [c] Data from Rennison et al.[2] [d] K_e/nM vs the standard agonists DAMGO (MOR), U69593 (KOR) and DPDPE (DOR). [e] Agonist activity, EC_{50} 75.1 ± 16.9 nM, 47% stimulation relative to DPDPE. [f] Agonist activity, EC_{50} 0.30 ± 0.09 nM, 21% stimulation relative to U69593. [g] Agonist activity, EC_{50} 3.18 ± 0.73 nM, 77% stimulation relative to U69593. [h] Agonist activity, EC_{50} 20.0 ± 2.78 nM, 25% stimulation relative to U69593. NT: not tested. Data supplied by the NIDA Addiction Treatment Discovery Program.

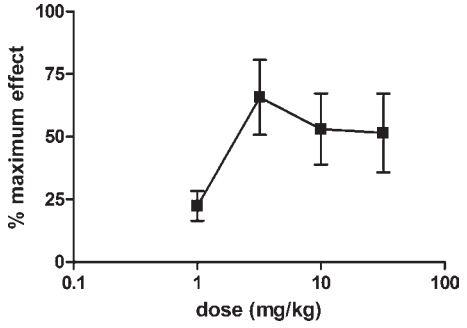


Figure 1. Antinociceptive activity of **9a** in the mouse warm water tail withdrawal assay.

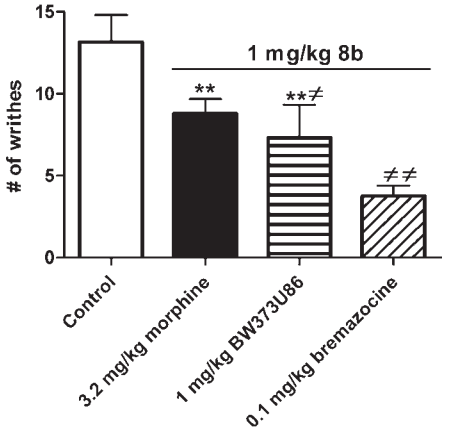


Figure 2. Antagonist activity of **8b** in the mouse abdominal stretch (AS) assay vs fully effective doses of standard opioid receptor agonists. ** $p < 0.01$ compared to morphine (MOR) or BW373-U86 (DOR) alone. ≠ $p < 0.05$, ≠≠ $p < 0.01$ compared to control (**8b** alone), which was not different from vehicle (saline). One way ANOVA with Dunnett's posthoc test.

Table 2. Agonist Selectivity of **9a** (0.32 mg/kg, sc) in the Mouse AS Assay

treatment	no. of stretches
control	10.0 ± 1.3
9a	0[a]
9a + MCAM (1.8 mg/kg, 1 h pretreatment)	12.0 ± 1.5
9a + naltrindole (10 mg/kg, 15 min pretreatment)	0[a]
9a + norBNI (32 mg/kg, 24 h pretreatment)	5.0 ± 2.0[a]

[a] Significantly different from controls.

Table 3. Antagonist Activity in the Mouse Tail-Withdrawal (50 °C) Test

	pretreatment	morphine ED_{50} mg/kg, ip	shift
control		9.3 (7.3−11.8)	
9a, 32 mg/kg	24 h	38.3 (12.2−119)[a]	4.1
	48 h	17.1 (10.7−27.2)	1.8
9b, 32 mg/kg	30 min	≫100[a,b]	*d*
	24 h	118.5 (99.4−141.2)[a]	12.7
	4 days	25.0 (8.2−76.1)	2.7
8a, 32 mg/kg	2.5 h	≫100[a,b]	*d*
	48 h	65.1 (28.1−150)[a]	7
8b, 32 mg/kg	30 min	≫100[a,b]	*d*
	3 day	≫100[a,c]	*d*
	4 day	112.9 (43.6−292)[a]	12.1
	6 day	40.8 (28.1 − 59.3)[a]	4.4

[a] Significantly different from control. [b] 0% effect, 100 mg/kg. [c] 20% effect, 100 mg/kg. [d] Shift was too large to determine.

Discussion

The data obtained from the evaluation of the new ligands synthesized in the present study conform to the pattern of structure−activity relationships established for the analogous 14-cinnamoylamino derivatives.[1] That is, the arylpropiolylamino morphinones (**8a**, **8b**) behave as pseudoirreversible MOR antagonists of long duration and that *p*-chloro substitution in the side chain aromatic ring eliminates agonist activity, enhances MOR antagonist activity, and increases the duration of the latter effect. Comparison with the equivalent cinnamoylaminomorphinones (**2a**, **2c**) reveals substantial similarity between **2a** and **8b** in binding and in vitro assays (Table 1). Comparison of the binding of the 14-propiolylamino codeinones (**9**) with the corresponding morphinones (**8**) shows little difference in MOR binding affinity but greater difference in KOR and particularly DOR binding. Similar effects were seen in comparison of the 14-cinnamoylamino morphinones (**2**) with codeinones (**3**). This confirms that for MOR binding the lipophilic C14 side chain is the dominant structural motif, whereas for KOR and DOR binding, the C3 phenolic group is also required. In vivo it appears that the arylpropiolylamino derivatives (**8a**, **8b**) have somewhat longer duration of morphine antagonist activity than the

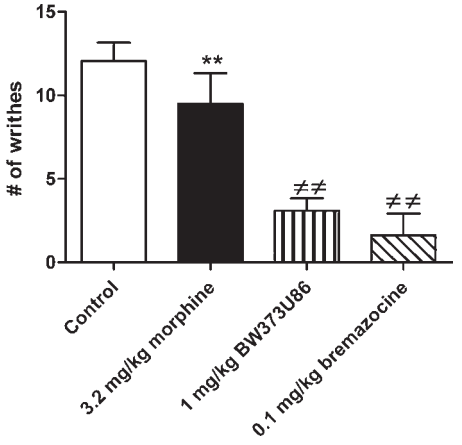


Figure 3. Antagonist selectivity of **8b** (3.2 mg/kg) against ED_{100} doses of the selective agonists morphine (MOR), BW373U86 (DOR) and bremazocine (KOR) in the mouse abdominal stretch (AS) assay after 24 h pretreatment. ** $p < 0.01$ compared to morphine. ≠≠ $p < 0.01$ compared to control (**8b** alone), which was not different from vehicle (saline). One way ANOVA with Dunnett's posthoc test.

cinnamoylamino equivalents (**2a**, **2c**).[1] When the morphine dose−response curve was determined 4 days after administration of a 32 mg/kg dose of the test antagonist, **2a** shifted the dose−response curve about 5-fold to the right,[11] whereas in the present study, the same dose of **8b** in the same protocol resulted in a 12-fold shift (Table 3).

There is perhaps greater difference in in vivo activity between the arylpropiolylamino codeinones (**9a**, **9b**) and the corresponding cinnamoylamino codeinones (**3a**, **3c**). Whereas **9b** had no antinociceptive activity in TW or AS, **3a**, though having no agonist activity in TW, has potent antinociceptive activity in AS, being fully effective at 0.2 mg/kg.[1,12,13] Similarly the MOR partial agonism shown in vivo by **3c** is of higher efficacy than that of **9a** in the present study.[1] Thus it appears that the conformationally linear side chains of the arylpropiolylamino derivatives (**8**, **9**) are associated with lower MOR efficacy and more profound MOR antagonism than the equivalent cinnamoylamino derivatives (**2**, **3**). This suggests that for antagonism, the optimum position for the lipophilic aryl moiety is further from C14 than is achieved in the cinnamoyl derivatives. Rennison et al.[2] showed that the 14β-phenylbutylamidomorphinone (**12**), with a longer spacer between C14 and the aryl group, was more effective than C-CAM in flattening the dose−response curve of DAMGO in a [^{35}S]GTPγS binding assay.

In the present study, there was pronounced similarity in OR binding and in vitro profiles between the *p*-chlorophenylpropiolylamino derivatives (**8b**, **9b**) and the *p*-chlorophenylpropargylamino derivatives (**10**, **11**). In this respect, the SAR followed that established by Rennison et al.[2] for a range of 14-acylamino- (**6**) and 14-alkylaminomorphinones and codeinones (**7**).

Conclusions

The 14β-arylpropiolylamino morphinones and codeinones of the present study provide further examples of pseudoirreversible MOR antagonists comparable to the previously reported clocinnamox and methcinnamox. The SAR of the new series is very similar to that of the equivalent cinnamoylamino series, but there are trends to lower MOR efficacy and more profound antagonism.

Experimental Section

Reagents and solvents were purchased from Aldrich or Lancaster and used as received. NMR Spectra: Jeol Lambda-270-MHz instrument: ^{1}H at 270 MHz, with TMS as an internal standard. Only representative examples of the synthesis are shown. Oxalate salts were formed prior to pharmacological evaluation. Tested compounds had purity ≥95%.

***N*-Cyclopropylmethyl-14β-[phenylpropioloylamino]-7,8-dihydronorcodeinone (9a).** Oxalyl chloride (8.8 equiv) and phenylpropiolic acid (1.1 equiv) in anhydrous toluene were heated at reflux for 1 h. The solvent was removed, the residue dissolved in anhyd CH_2Cl_2, added dropwise to a solution of **18b** (1 equiv) and triethylamine (1.1 equiv) in anhyd CH_2Cl_2, and stirred at rt overnight. The solvent was removed and the crude residue purified by column chromatography to yield a white solid (71%); R_f (CH_2Cl_2:MeOH, 50:1) 0.26. ^{1}H NMR ($CDCl_3$) 0.21 (2H, m), 0.60 (2H, m), 0.89 (1H, m), 2.32−2.52 (2H, m), 3.10 (1H, d), 3.88 (3H, s), 4.95 (1H, s), 6.63 (1H, d), 6.74 (1H, d), 7.30 (1H), 7.35−7.61 (5H, m).

***N*-Cyclopropylmethyl-14β-[phenylpropioloylamino]-7,8-dihydronormorphinone (8a).** To the codeinone (**9a**) in anhyd CH_2Cl_2 at −30 °C under N_2, was added BBr_3 (6 equiv, 1 M in CH_2Cl_2) slowly. The reaction was allowed to reach rt over 1 h before adding a 1:1 mixture of ice:ammonia (concd). The organic phase was isolated, the aqueous layer washed (×3) with $CHCl_3$:MeOH (3:1), the combined organic fractions washed with brine, dried, and evaporated to dryness. Column chromatography gave **8a** as a white solid (72%); R_f (CH_2Cl_2: MeOH, 20:1) 0.47. ^{1}H NMR (DMSO) 0.25 (2H, m), 0.62 (2H, m), 0.92 (1H, m), 3.10 (1H, d), 5.01 (1H, s), 6.61 (1H, d), 6.80 (1H, d), 7.38 (5H, m).

***N*-Cyclopropylmethyl-14β-[3′-(4″-chlorophenyl)-propargylamino]-7,8-dihydronormorphinone (10). 18a** was treated with 3-(4′-chlorophenyl)propargyl bromide (**17**, 1.1 equiv) in the presence of potassium carbonate (5 equiv) in dimethylformamide at 90 °C for 12 h. The solvent was removed and the crude residue was purified by column chromatography to afford **10** as a white solid (63%); R_f 0.42 (CH_2Cl_2:MeOH, 20:1). ^{1}H NMR ($CDCl_3$) δ 0.18 (2H, m), 0.54 (2H, m), 0.89 (1H, m), 3.06 (1H, d), 3.57 (2H, s), 4.70 (1H, s), 6.60 (1H, d), 6.71 (1H, d), 7.29 (2H, d), 7.37 (2H, d).

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Supporting Information Available: Full experimental details, including biological assay methods and microanalysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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