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Stilbenes as κ-Selective, Non-nitrogenous Opioid Receptor Antagonists

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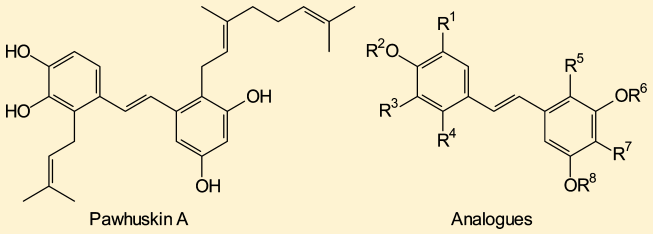
Supporting Information

ABSTRACT: The natural stilbene pawhuskin A has been shown to function as an opioid receptor antagonist, with preferential binding to the κ receptor. This finding encouraged assembly of a set of analogues to probe the importance of key structural features. Assays on these compounds determined that one (compound **29**) shows potent opioid receptor binding activity and significantly improved selectivity for the κ receptor. These studies begin to illuminate the structural features of these non-nitrogenous opioid receptor antagonists that are required for activity.



In 2004 Belofsky and co-workers reported a small set of prenylated stilbenes that they named the pawhuskins.[1] This family of compounds, exemplified by pawhuskins A (**1**) and C (**2**), was isolated from the common North American purple prairie clover (*Dalea purpurea*) collected near Pawhuska, Oklahoma. Extracts of this plant reportedly have been made into teas and used by Native American peoples as a prophylactic and for treatment of various ailments.[2] Belofsky's findings support this ethnomedical use, since the pawhuskins were shown to modulate opioid receptors by displacement of a nonselective radioactive antagonist in rat brain striatal tissue.[1] Pawhuskin A was the most potent member of the family, making it one of a small group of non-nitrogenous compounds with effects on the opiate receptor system. As part of an ongoing interest in natural prenylated stilbenes,[3,4] we undertook studies to elucidate the character and receptor subtype selectivity of opioid modulation by pawhuskins. This effort already has led to the synthesis of both pawhuskins A[5] and C,[6] and here we report the results of our further studies on this class of compounds.

Several non-nitrogenous opioid receptor modulators have been isolated from natural sources. The most studied compound is salvinorin A (**3**), a potent hallucinogen isolated from *Salvia divinorum*.[7] Salvinorin A has been shown to be a κ-opioid (KOP) receptor agonist, and KOP receptor ligands have become of interest with respect to studies of addiction and other disorders.[8] Two total syntheses of salvinorin A have been reported,[9,10] but modifications of the isolated natural product have driven more extensive structure–activity studies.[11−17] The non-nitrogenous compound dioflorin (**4**), a prenylated flavonoid, was isolated from the Brazilian vine *Dioclea grandiflora* through activity-guided fractionation[18−20] and shown to have analgesic activity.[21] More extensive efforts to categorize the opioid receptor binding of dioflorin have not yet been reported. Bioassays with a series of other natural flavonoids including catechin (**5**) and hesperetin (**6**) have been conducted and demonstrate that this scaffold may have considerable potential for development of opioid receptor ligands.[22] Other structural subtypes with opioid-binding activity are becoming more common,[23−25] including stilbenoids more reminiscent of the pawhuskins such as resveratrol (**7**)[26,27] and, more recently, chlorophorin (**8**).[28]

Salvinorin A (**3**) has been shown to be a functional agonist. Dioflorin (**4**) and other isolates of *Dioclea* display morphine-like analgesia that is inhibited by naloxone, a nonspecific opioid receptor antagonist, so they are presumably agonists as well.[20] While the flavan-3-ol catechin (**5**) had good activity as an antagonist at the KOP receptor (K_e = 320 nM), the flavanone hesperetin (**6**) had no activity at the μ, δ, or κ receptors.[22] The work of Sobolev and co-workers on peanut phytoalexins such as stilbene **8** determined the selectivity of these compounds against each opioid receptor, but these compounds have not yet been fully characterized using functional assays.[28] Here we report the opioid receptor binding affinity and selectivity of pawhuskin A using a functional assay based on [^{35}S]GTP-γ-S binding. We also report initial results of structure–activity relationship studies, which begin to illuminate the significance of the phenols and the prenyl group for activity.

RESULTS AND DISCUSSION

We began this exploration of pawhuskin opioid activity by probing the selectivity of pawhuskin A for human κ (KOP), μ (MOP), and δ (DOP) receptors. Even at a 10 μM

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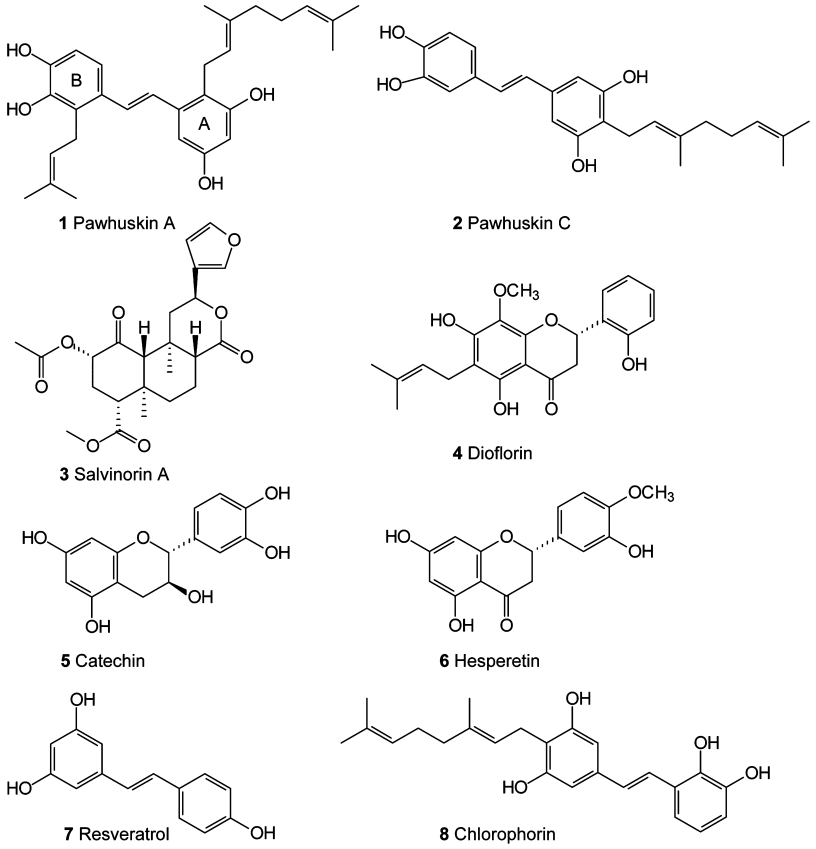

Figure 1. Structures of some non-nitrogenous opioid receptor modulators.

concentration, pawhuskin A was found to have no intrinsic agonist activity at these receptors. However, further testing showed antagonist activity at all three of the opioid receptor subtypes. Furthermore, pawhuskin A caused a rightward shift in the agonist concentration response curve, and its antagonism was surmountable, suggesting a competitive mode of antagonism (Figure 2).[29] Pawhuskin A is modestly selective for the κ receptor, with a K_e of 203 nM (δ/κ = 14.5, μ/κ = 2.9). Pawhuskin C (**2**) also displayed some antagonist activity at the KOP receptor, but was much less potent than compound **1**.

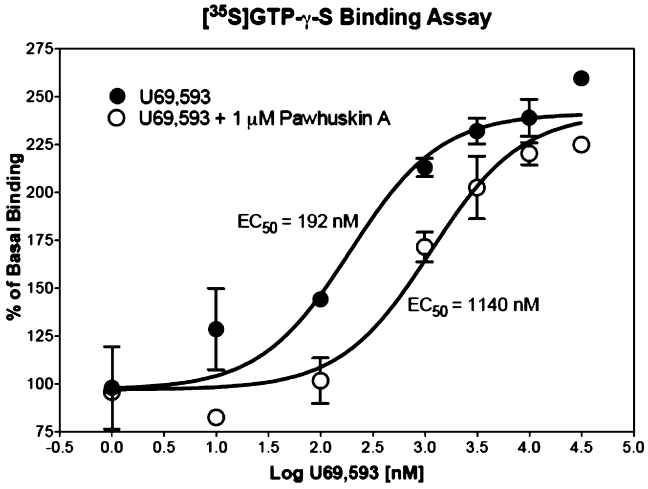

Figure 2. Representative graph of the antagonist activity of pawhuskin A in the KOP receptor affinity assay. Each data point represents the mean and SEM of duplicate samples.

While the natural product salvinorin A and many of its analogues are KOP receptor agonists, there are only limited examples of non-nitrogenous KOP receptor antagonists including some flavanoids.[22] Pawhuskin A rivals the potency of the flavonoids, although catechin (**5**) displayed higher selectivity versus the other opioid receptors (μ/κ > 31). However improved KOP receptor selectivity might be uncovered by a synthetic exploration involving the pawhuskin's stilbene scaffold. Synthetic efforts along these lines are encouraged by the recent interest in KOP receptor antagonists as potential treatments for stimulant abuse. Such agents might be of particular value as potential preventatives for relapse. While there has been some interest in using KOP agonists for treatment of substance abuse, compounds such as salvinorin A have been accompanied by serious side effects including potent hallucinogen activity. There is a significant relationship between relapse to stimulant abuse and stress.[30] Indeed encounters with stressors, and even images that induce stress, have been shown to induce craving in stimulant abusers.[31,32] The potent KOP receptor antagonist JDTic[33] has been shown to block stress-induced cocaine seeking behavior and also has demonstrated antidepressant-like activity.[34] This result was confirmed and expanded to show that pretreatment with the κ-opioid antagonist arodyn prevented stress-related induction of cocaine-conditioned place preference,[35] which further heightens interest in κ-selective antagonists.

Our approach to exploration of the structure–activity relationships of pawhuskin A analogues took advantage of a core strategy used in the synthesis of other natural stilbenes (Figure 3).[36−38] A disconnection of the central olefinic moiety (**9**) through a Horner–Wadsworth–Emmons transform allows

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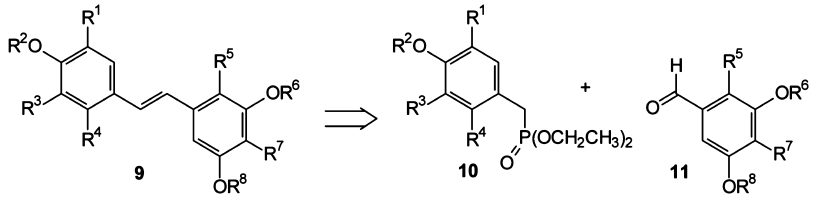
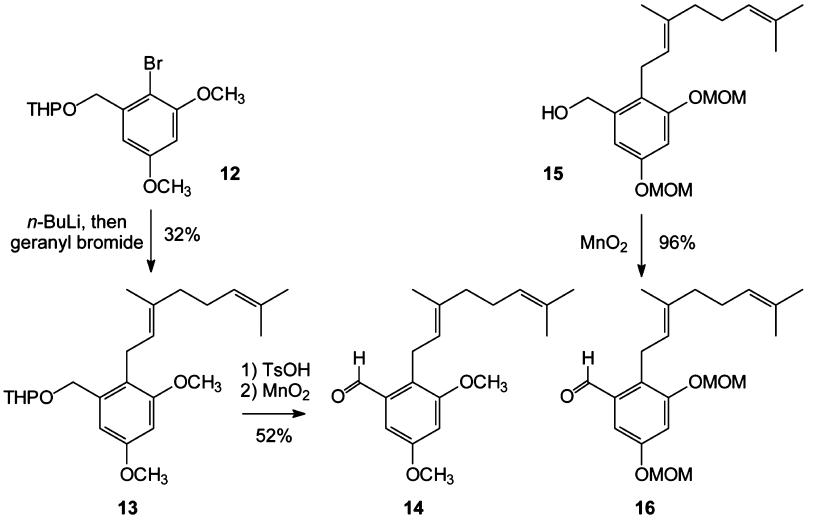


Figure 3. Synthetic strategy for pawhuskin analogues.

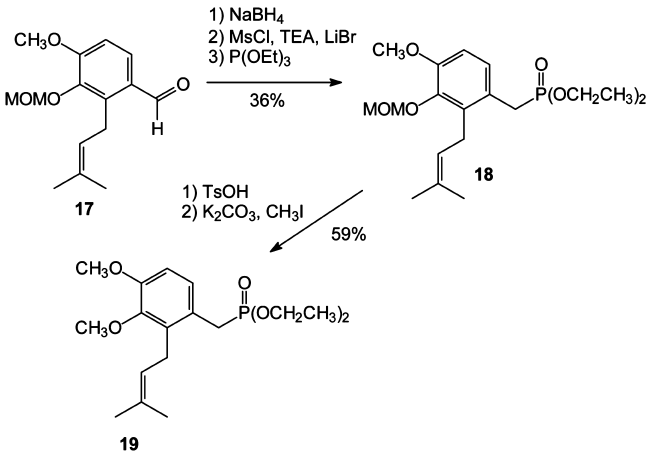
Scheme 1. Synthesis of the Aldehydes 14 and 16



choice of phosphonate coupling partners such as **10** and aldehydes such as **11**, although the reversed pairing is also viable.[39,40] This permits maximum convergence and provides for divergence through condensations of one aldehyde with several phosphonates or one phosphonate with several aldehydes.[41] To begin exploration of the pharmacophore of pawhuskin A and the essential binding motifs for κ-selective antagonist activity, we undertook syntheses aimed at preparation of a small set of analogues through this strategy. Phenolic H-bonding is important to the KOP receptor selectivity of the antagonist JDTic and other members of the phenylpiperidine class of opioid receptor modulators.[42] Furthermore, as in past studies of salvinorin A,[43] the lack of a readily ionizable group that would form salt bridges with an opioid receptor suggested that attention should be directed at the H-bonding groups of pawhuskin A. Thus, we chose to prepare various methylated analogues to assess the importance of H-bond donation from the various hydroxy groups without a significant change in electron donation.

To allow efficient preparation of several analogues, as well as synthesis of regiospecifically methylated materials, the per-methylated pawhuskin A analogue was pursued through preparation of both coupling partners aldehyde **14** (Scheme 1) and phosphonate **19** (Scheme 2) rather than methylation of the natural product. To access compound **14**, halogen metal exchange was carried out on the known bromide **12**.[44,45] Treatment of the lithiated arene with geranyl bromide afforded the THP ether **13**. Hydrolysis of the acetal protecting group and oxidation of the resulting benzylic alcohol with MnO_2 gave aldehyde **14** in satisfactory yield. The alcohol **15** is known from our synthesis of pawhuskin A,[5] and oxidation of that benzylic alcohol gave the methoxymethyl (MOM)-protected aldehyde **16**.

Scheme 2. Synthesis of Phosphonates 18 and 19



To prepare the complementary phosphonate **19**, the known aldehyde **17**[46] was reduced to the corresponding alcohol, and the alcohol was treated with mesyl chloride and Et_3N, then LiBr, and finally allowed to react with triethyl phosphite to obtain the phosphonate **18**. After hydrolysis of the MOM protecting group, standard reaction with MeI and base afforded the dimethylated phosphonate **19**.

Coupling of aldehyde **14** and phosphonate **19** via Horner–Wadsworth–Emmons condensation afforded the fully methylated pawhuskin A analogue **20** in good yield (Scheme 3). With this compound in hand, we employed our small library of readily available phosphonates of type **10** to synthesize additional pawhuskin analogues. Thus, coupling of aldehyde **14** with known phosphonate **21**[47] afforded the stilbene **22**, which upon deprotection gave stilbene **23**. Exhaustive

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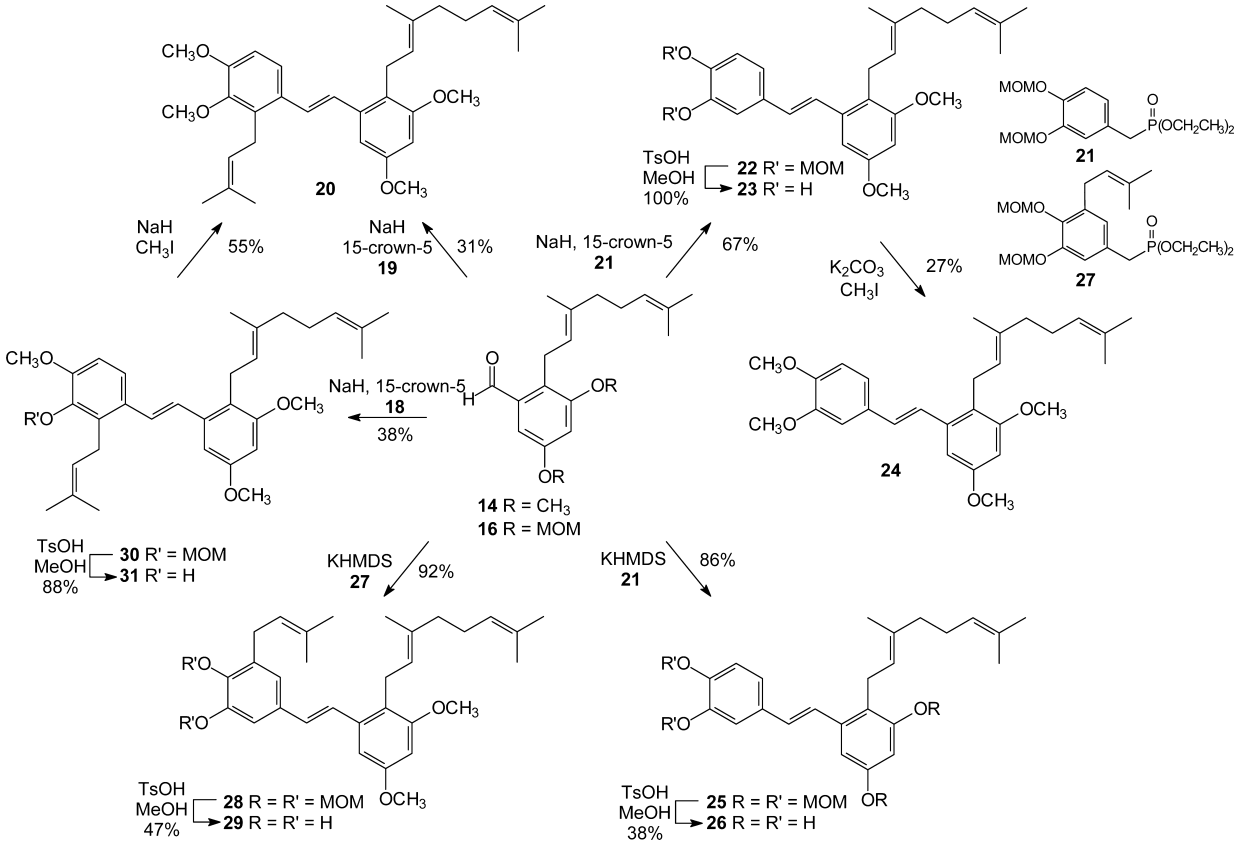
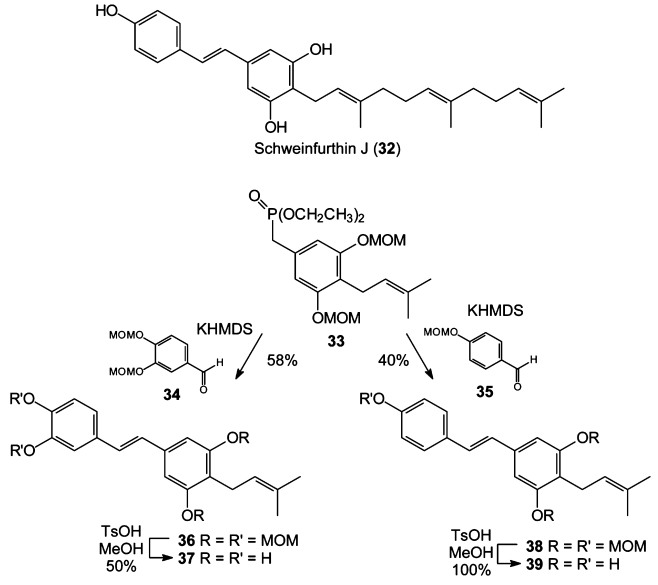
Scheme 3. Synthesis of Pawhuskin A Analogues 20, 23, 24, 26, 29, and 31



methylation of compound **23** then gave the permethylated analogue **24**. Condensation of compound **16** with phosphonate **21**,[38] followed by hydrolysis of the protecting groups in the resulting stilbene, gave the free phenolic pawhuskin A analogue **26**. To reposition the prenyl group so that it is isomeric to pawhuskin A (**1**), phosphonate **27** was prepared from the corresponding benzylic alcohol under standard conditions. That prenylated benzylic alcohol could be prepared from bromovanillin through reactions parallel to those reported in a schweinfurthin synthesis.[39] Condensation of phosphonate **27** with aldehyde **14** gave stilbene **28**, and hydrolysis of the MOM groups gave the dimethylated analogue **29**. Finally, condensation of aldehyde **14** with phosphonate **18** gave the selectively trimethylated pawhuskin A derivative **30**. Hydrolysis of the MOM acetal gave the specific phenol **31**. This compound was methylated to provide permethylated pawhuskin A (**20**) via a different route.

Because pawhuskin C (**2**) showed activity,[1] we tested several analogues of this chemotype. This set includes the natural product schweinfurthin J (**32**, Scheme 4), which was isolated from the African plant *Macaranga schweinfurthii*[48,49] and can be viewed as lacking one phenolic hydroxy group and bearing a farnesyl side chain in relation to pawhuskin C. We then used the known phosphonate **33**[50,51] to access the prenylated pawhuskin C analogue **37** and the natural product *trans*-arachidin-2 (**39**). Condensation of phosphonate **33** with the known aldehydes **34** and **35**[52] gave the protected stilbenes **36** and **38**, respectively. Hydrolysis of the four MOM acetals of compound **36** gave stilbene **37**. Use of the MOM protecting group for all the hydroxy groups of compound **38** allows for deprotection to stilbene **39** in a single step, which is more efficient than the previous synthesis.[53]

Scheme 4. Pawhuskin C Analogues



Of the various analogues studied for binding to opioid receptors, only the pawhuskin A analogue **29** and schweinfurthin J (**32**) demonstrated appreciable activity (Table 1). Schweinfurthin J with a 3 μM K_e for the MOP receptor and limited selectivity (δ/κ = 0.67, μ/κ = 0.33) is the only stilbene we have studied that shows selectivity for the μ-opioid receptor. Interestingly, schweinfurthin J is also closely related to chlorophorin (**8**), which was shown by Sobolev and co-workers to lower agonist binding to the κ and δ receptors to an equal

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Table 1. Apparent Affinity of Compounds Tested

	apparent affinity of competitive antagonists (K_e) in μM			selectivity	
compd	KOP	DOP	MOP	δ/κ	μ/κ
Paw A (**1**)	0.20	2.9	0.57	14.5	2.9
Paw C (**2**)	>10	>10	>10		
20	>10	>10	>10		
23	>10	>10	>10		
24	>10	>10	>10		
26	>10	>10	>10		
29	0.15	>10	>10	>67	>67
31	>10	>10	>10		
32	9	6	3	0.67	0.33
37	>10	>10	>10		
39	>10	>10	>10		

extent but to have no substantial effect on the binding of agonists to the μ-opioid receptor.[28]

Of greater interest is analogue **29**, which showed better binding affinity to the κ receptor than pawhuskin A and also demonstrated dramatically improved selectivity (δ/κ at least 4-fold larger and μ/κ at least 20-fold larger for compound **29** than for pawhuskin A). Indeed we could not find antagonist activity at the μ or δ receptors for compound **29** up to the highest concentrations typically tested (10 μM). This compound demonstrates that methylation of the malonate-derived hydroxy groups on the pawhuskin A scaffold does not abrogate the KOP receptor antagonist activity on this stilbene scaffold. Comparisons to compounds **20**, **23**, and **24** indicate that the presence and position of the prenyl substituent are important factors in binding to the KOP receptor. These results point to the importance of the shikimate-derived substructure and should allow further design with the aim of introducing more drug-like characteristics. Work on this strategy is currently under way.

CONCLUSIONS

This study has shown that the natural stilbene pawhuskin A is a competitive antagonist with selectivity for the KOP receptor. We also have shown that improved selectivity for the KOP versus the DOP and MOP receptors is possible within the constraints of the stilbene structure, which encourages further efforts to improve these molecules via synthesis. The isomeric pawhuskin A analogue **29** exhibited greater affinity and selectivity for the KOP receptor than pawhuskin A itself, indicating that the shikimate-derived ring is a key for κ-opioid receptor binding. Compound **29** shows significantly greater selectivity for the KOP than PF-04455242, which was advanced to phase 1 clinical trials for alcohol dependency, albeit with significantly lower potency (~150 nM vs 3 nM).[54,55]

Thus far, none of the new compounds reported here have shown any agonist activity. While the study of KOP agonists for treatment of pain and addiction has been moving forward, their potential may be limited by side effects such as hallucinations and dysphoria. This makes the discovery of additional classes of KOP antagonists appealing. Therefore, this stilbene scaffold may present new opportunities for the discovery of compounds with utility in the treatment of addiction and depression.

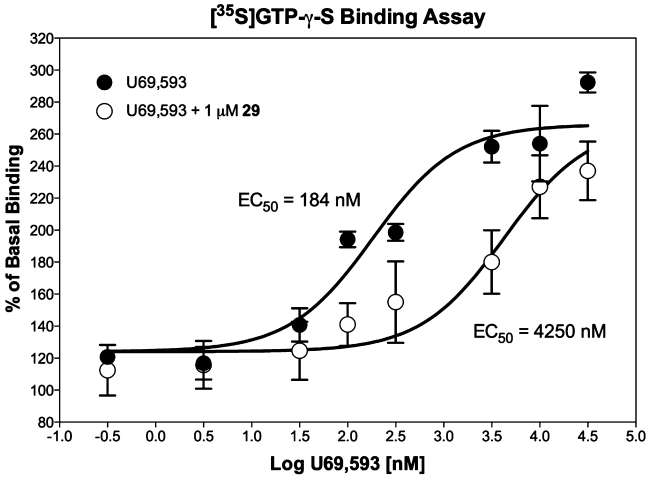

Figure 4. Representative graph of the antagonist activity of compound **29** in the KOP receptor affinity assay. Each data point represents the mean and SEM of duplicate samples.

EXPERIMENTAL SECTION

General Experimental Procedures. Both THF and Et_2O were freshly distilled from Na/benzophenone. Both CH_2Cl_2 and Et_3N were distilled from CaH_2 prior to use. Solutions of *n*-BuLi were purchased from a commercial source and titrated with diphenylacetic acid prior to use. All other reagents and solvents were purchased from commercial sources and used without further purification. All reactions in nonaqueous solvents were conducted in flame-dried glassware under a positive pressure of Ar and with magnetic stirring. NMR spectra were obtained at 300−500 MHz for ^{1}H and 75−125 MHz for ^{13}C with $CDCl_3$ or CD_3OD as solvent and $(CH_3)_4Si$ (^{1}H, 0.00 ppm) or $CDCl_3$ (^{13}C, 77.0 ppm or 49.0 ppm) as internal standards unless otherwise noted. The ^{31}P chemical shifts were reported in ppm relative to 85% H_3PO_4 (external standard). High-resolution mass spectra were obtained at the University of Iowa Mass Spectrometry Facility. Silica gel (60 Å, 0.040−0.063 mm) was used for flash chromatography.

2-Geranyl-3,5-dimethoxytetrahydropyranylbenzyl Alcohol (13). To a stirred solution of TMEDA (0.58 mL, 3.9 mmol) and *n*-BuLi (2.47 M solution in hexanes, 1.5 mL, 3.6 mmol) in Et_2O (20 mL) at −10 °C was added the bromide **12** (979 mg, 3.0 mmol) dissolved in Et_2O (4 mL). After 45 min of stirring, CuI (742 mg, 3.9 mmol) was added and then geranyl bromide (840 mg, 3.9 mmol) was added slowly over 8 min to the reaction. After the mixture was stirred overnight, the reaction was quenched by addition of saturated aqueous NH_4Cl. The resulting mixture was extracted with EtOAc, and the combined organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Final purification by flash column chromatography (4% EtOAc in hexanes) afforded compound **13** (364 mg, 32%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.61 (d, J = 2.4 Hz, 1H), 6.42 (d, J = 2.5 Hz, 1H), 5.07−5.04 (m, 2H), 4.74 (d, J = 12.2 Hz, 1H), 4.70 (t, J = 3.7 Hz, 1H), 4.48 (d, J = 12.1 Hz, 1H), 3.94−3.90 (m, 1H), 3.80 (s, 3H), 3.79 (s, 3H), 3.37−3.29 (m, 2H), 2.06−1.80 (m, 5H), 1.80−1.54 (m, 5H), 1.75 (s, 3H), 1.65 (s, 3H), 1.57 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.6, 158.4, 138.0, 134.5, 131.2, 124.4, 123.3, 121.3, 105.0, 98.0 (2C), 66.9, 62.2, 55.7, 55.3, 39.8, 30.7, 26.8, 25.7, 25.5, 24.1, 19.5, 17.7, 16.1; HRMS (ESI) m/z calcd for $C_{24}H_{36}O_4Na$ $(M + Na)^+$ 411.2511, found 411.2495.

2-Geranyl-3,5-dimethoxybenzaldehyde (14). To a solution of the THP acetal **13** (364 mg, 0.9 mmol) in MeOH (8 mL) at room temperature was added TsOH (356 mg, 1.9 mmol). The solution was stirred for 2.5 h and quenched by addition of $NaHCO_3$. The mixture was extracted with EtOAc, and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford the benzylic alcohol as a yellow oil. This material was used in further reactions without additional purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.59 (d, J = 2.4 Hz, 1H), 6.43 (d, J = 2.2 Hz, 1H), 5.09−5.02 (m, 2H) 4.64 (d, J = 3.9 Hz, 2H), 3.81 (s, 3H), 3.80 (s, 3H), 3.35 (d, J = 6.8 Hz, 2H), 2.10−1.94 (m, 4H), 1.76 (s, 3H), 1.65 (s, 3H), 1.57 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.7, 158.3, 140.6, 135.0, 131.4, 124.1, 123.5, 120.3, 104.0, 97.9, 63.3, 55.6, 55.3, 39.6,

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26.6, 25.6, 23.7, 17.6, 16.1; HRMS (EI) m/z calcd for $C_{19}H_{28}O_3$ $(M)^+$ 304.2038, found 304.2044.

To a stirred solution of the benzylic alcohol (285 mg, 0.9 mmol, assuming 100% conversion in the previous step) in CH_2Cl_2 (15 mL) was added activated MnO_2 (815 mg, 9.4 mmol). The mixture was stirred overnight and subsequently was filtered and concentrated *in vacuo*. Final purification by flash column chromatography (12% EtOAc in hexanes) afforded aldehyde **14** (146 mg, 52% from **13**) as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.3 (s, 1H), 6.98 (d, J = 2.2 Hz, 1H), 6.68 (d, J = 1.9 Hz, 1H), 5.13−5.07 (m, 1H), 5.05−5.00 (m, 1H), 3.82 (s, 6H), 3.70 (d, J = 6.5 Hz, 2H), 2.24−1.90 (m, 4H), 1.76 (s, 3H), 1.64 (s, 3H), 1.56 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.8, 158.8, 158.6, 135.2, 134.9, 131.4, 127.3, 124.0, 123.4, 104.8, 101.9, 55.8, 55.5, 39.5, 26.5, 25.6, 22.5, 17.6, 16.2; HRMS (ESI) m/z calcd for $C_{19}H_{26}O_3Na$ $(M + Na)^+$ 325.1780, found 325.1783.

2-Geranyl-3,5-bis(methoxymethoxy)benzaldehyde (16). Activated MnO_2 (644 mg, 7.1 equiv) was added to a solution of alcohol **15**[5] (267 mg, 0.7 mmol) in CH_2Cl_2 (15 mL) at room temperature, and the mixture was stirred overnight. The mixture was filtered, and the filtrate was concentrated *in vacuo*. Final purification by flash column chromatography (50% EtOAc in hexanes) afforded aldehyde **16** (256 mg, 96%) as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.26 (s, 1H), 7.21 (d, J = 2.7 Hz, 1H), 7.04 (d, J = 2.2 Hz, 1H), 5.21 (s, 2H), 5.19 (s, 2H), 5.14−5.10 (m, 1H), 5.05−5.00 (m, 1H), 3.73 (d, J = 6.5 Hz, 2H), 3.48 (s, 6H), 2.08−1.93 (m, 4H), 1.86 (s, 3H), 1.73 (s, 3H), 1.65 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 191.6, 156.3, 156.2, 135.3 (2C), 131.5, 128.1, 124.1, 123.2, 109.3, 108.7, 94.7, 94.5, 56.2, 56.1, 39.6, 26.6, 25.6, 22.9, 17.6, 16.3; HRMS (ESI) m/z calcd for $C_{21}H_{30}O_5Na$ $(M + Na)^+$ 385.1991, found 385.1983.

Diethyl{[4-methoxy-3-(methoxymethoxy)-2-(prenyl)-phenyl]methyl} Phosphonate (18). To a stirred solution of aldehyde **17** (1.16 g, 4.5 mmol) in MeOH (10 mL) at 0 °C was added $NaBH_4$ (282 mg, 7.5 mmol) as a single aliquot. This solution was stirred for 30 min, and then H_2O (50 mL) was added and the resulting solution was extracted with EtOAc. After concentration *in vacuo*, the resulting alcohol (1.07 g, 90%) was dissolved in THF (10 mL) and treated with Et_3N (0.80 mL, 5.7 mmol) followed by methanesulfonyl chloride (0.31 mL, 4.01 mmol). After 15 min, LiBr (391 mg, 0.45 mmol) was added in THF (15 mL). The resulting solution was stirred for an additional 45 min and quenched by addition of H_2O. This mixture was extracted with EtOAc, and then the combined organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The resulting yellow oil was dissolved in triethyl phosphite (5 mL) and heated at reflux for 5 days. Removal of excess triethyl phosphite *in vacuo* gave a yellow oil. Purification by flash column chromatography (100% EtOAc) afforded phosphonate **18** (622 mg, 40%) as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.97−6.93 (m, 1H), 6.67−6.63 (m, 1H), 4.95 (br s, 3H), 3.92−3.86 (m, 4H), 3.70 (s, 5H), 3.56 (s, 3H), 3.01 (d, J_{HP} = 21 Hz, 2H), 1.70 (s, 3H), 1.58 (s, 3H), 1.16−1.10 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.8 (d, J_{CP} = 3.6 Hz), 143.7 (d, J_{CP} = 3.7 Hz), 134.8 (d, J_{CP} = 6.7 Hz), 131.3, 126.3 (d, J_{CP} = 5.2 Hz), 122.7 (d, J_{CP} = 9.3 Hz), 122.5, 109.4 (d, J_{CP} = 3.9 Hz), 98.6, 61.6 (d, J_{CP} = 7.1 Hz, 2C), 57.1, 55.2, 29.5 (d, J_{CP} = 139 Hz), 25.3, 25.2, 17.6, 16.0 (d, J_{CP} = 5.8 Hz, 2C); ^{31}P NMR (122 MHz, $CDCl_3$) 27.2; HRMS (EI) m/z calcd for $C_{19}H_{31}O_6P$ $(M)^+$ 386.1858, found 386.1857.

Diethyl{[3,4-dimethoxy-2-(prenyl)phenyl]methyl} Phosphonate (19). To a stirred solution of MOM ether **18** (103 mg, 0.3 mmol) in EtOH (2.5 mL) was added TsOH (152 mg, 0.8 mmol). The solution was stirred overnight, quenched by addition of saturated aqueous NH_4Cl, and extracted with EtOAc. The combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated *in vacuo* to afford the phenol as a yellow oil. To a stirred solution of the phenol (88 mg, 0.3 mmol) in acetone (6 mL) were added K_2CO_3 (242 mg, 1.8 mmol) and MeI (0.1 mL, 1.6 mmol). After the mixture was heated to reflux and stirred overnight, it was quenched by addition of H_2O, and the mixture was extracted with EtOAc. The organic extracts were washed with 2 M NaOH, dried ($MgSO_4$), and concentrated *in vacuo*. Without additional purification, methyl ether **19** (56 mg, 59%, 2 steps) was obtained as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.96 (dd, J = 8.7 Hz, J_{HP} = 3.2 Hz, 1H), 6.68 (d, J = 8.8 Hz, 1H), 4.96 (t, J = 6.5 Hz, 1H), 3.99−3.86 (m, 4H), 3.79 (s, 3H), 3.70 (s, 3H), 3.42 (d, J = 6.5 Hz, 2H), 3.04 (d, J_{HP} = 21 Hz, 2H), 1.72 (s, 3H), 1.61 (s, 3H), 1.18 (td, J = 7.5 Hz, J_{HP} = 3.7 Hz, 6H).

2-Geranyl-3,5,3′,4′-tetramethoxy-2′-prenyl-(*E*)-stilbene (20). To a stirred suspension of NaH (60% dispersion in mineral oil, washed with hexanes, 33 mg, 0.8 mmol) in THF (2.5 mL) were added phosphonate **19** (56 mg, 0.2 mmol), aldehyde **14** (39 mg, 0.1 mmol), and 15-crown-5 (3 drops). The mixture was stirred for 2 h and quenched by addition of saturated aqueous NH_4Cl. The resulting mixture was extracted with EtOAc, and the combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. Final purification by flash column chromatography (10% EtOAc in hexanes) afforded stilbene **20** (20 mg, 31%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (d, J = 8.7 Hz, 1H), 7.12 (s, 2H), 6.80 (d, J = 8.8 Hz, 1H), 6.71 (d, J = 2.5 Hz, 1H), 6.21 (d, J = 2.5 Hz, 1H), 5.16−5.11 (m, 2H), 5.07−5.04 (m, 1H), 3.88 (s, 3H), 3.84 (s, 3H), 3.81 (s, 3H), 3.81 (s, 3H), 3.52−3.50 (m, 2H), 3.42 (d, J = 6.7 Hz, 2H), 2.07−2.03 (m, 2H), 1.98−1.92 (m, 2H), 1.81 (s, 3H), 1.78 (s, 3H), 1.67 (s, 3H), 1.62 (s, 3H), 1.55 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.5, 158.4, 152.3, 146.9, 138.4, 134.4, 134.0, 131.4, 131.2, 130.4, 128.0, 127.0, 124.4, 123.5, 123.3, 121.6, 121.1, 110.2, 101.6, 97.9, 60.7, 55.7 (2C), 55.3, 39.7, 26.8, 25.7, 25.6, 25.5, 24.3, 18.1, 17.6, 16.3; HRMS (EI) m/z calcd for $C_{33}H_{44}O_4$ $(M)^+$ 504.3240, found 504.3237.

2-Geranyl-3,5-dimethoxy-3′,4′-bis(methoxymethoxy)-(*E*)-stilbene (22). To a stirred suspension of NaH (60% dispersion in mineral oil, washed with hexanes, 28 mg, 0.7 mmol) in THF (2.5 mL) were added aldehyde **14** (35 mg, 0.1 mmol), phosphonate **21**[38] (49 mg, 0.1 mmol), and 15-crown-5 (3 drops). The mixture was stirred for 2 h and quenched with saturated aqueous NH_4Cl. The resultant mixture was extracted with EtOAc, and the combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. Final purification of the residue by flash column chromatography (10% EtOAc in hexanes) gave stilbene **22** (38 mg, 67%) as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (d, J = 1.6 Hz, 1H), 7.21 (d, J = 16.1 Hz, 1H), 7.15 (d, J = 8.4 Hz, 1H), 7.10 (dd, J = 8.5 Hz, 1.7 Hz, 1H), 6.89 (d, J = 16.2 Hz, 1H), 6.72 (d, J = 2.2 Hz, 1H), 6.42 (d, J = 2.1 Hz, 1H), 5.28 (s, 2H), 5.26 (s, 2H), 5.14−5.03 (m, 2H), 3.85 (s, 3H), 3.81 (s, 3H), 3.54 (s, 3H), 3.53 (s, 3H), 3.43 (d, J = 6.7 Hz, 2H), 2.08−1.95 (m, 4H), 1.81 (s, 3H), 1.61 (s, 3H), 1.54 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.5, 158.3, 147.3, 146.8 137.8, 134.3, 132.4, 131.2, 129.7, 125.8, 124.3, 123.5, 121.1, 120.8, 116.5, 114.6, 101.2, 98.0, 95.4, 95.3, 56.2 (2C), 55.6, 55.3, 39.7, 26.7, 25.6, 24.3, 17.6, 16.2; HRMS (ESI) m/z calcd for $C_{30}H_{41}O_6$ $(M + H)^+$ 497.2903, found 497.2918.

2-Geranyl-3,5-dimethoxy-3′,4′-dihydroxy-(*E*)-stilbene (23). To a solution of bis(methoxymethyl) ether **22** (18 mg, 0.04 mmol) in MeOH (2 mL) was added TsOH (29 mg, 0.15 mmol). After the solution was stirred overnight, the reaction was quenched by addition of saturated aqueous $NaHCO_3$. The resultant mixture was extracted with EtOAc, and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Final purification of a portion (25%) of the residual oil by preparative TLC (25% EtOAc in hexanes) afforded the stilbene **23** (5 mg, 100% by NMR) as a yellow oil; the remaining material (75%) was moved forward without additional purification. For compound **23**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20−6.82 (m, 5H), 6.71 (m, 1H), 6.42 (m, 1H), 5.19−4.98 (m, 2H), 3.86 (s, 3H), 3.81 (s, 3H), 3.42 (d, J = 5.9 Hz, 2H), 2.07−1.97 (m, 4H), 1.80 (s, 3H), 1.62 (s, 3H), 1.55 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.5, 158.4, 143.8, 143.5, 137.9, 134.5, 131.3, 129.9, 125.1, 124.3, 123.5, 121.1, 119.9, 115.5, 113.1, 110.9, 101.4, 97.9, 55.7, 55.4, 39.7, 26.7, 25.6, 24.3, 17.7, 16.3; HRMS (ESI) m/z calcd for $C_{26}H_{33}O_4$ $(M + H)^+$ 409.2379, found 409.2374.

2-Geranyl-3,5,3′,4′-tetramethoxy-(*E*)-stilbene (24). To a stirred solution of stilbene **23** in acetone (3 mL) was added K_2CO_3 (35 mg, 0.25 mmol) followed by MeI (38 μL, 0.61 mmol). The mixture was stirred for 2 days, and the reaction was quenched with H_2O. The resulting mixture was extracted with EtOAc, and the combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. Final purification by flash column

chromatography (gradient of hexanes to 40% EtOAc in hexanes) provided stilbene **24** (4 mg, 27%) as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27−7.18 (m, 1H), 7.06−7.04 (m, 2H), 6.94−6.85 (m, 2H), 6.75 (d, J = 2.7 Hz, 1H), 6.43 (d, J = 2.4 Hz, 1H), 5.14 (t, J = 7.5 Hz, 1H), 5.06 (t, J = 7.5 Hz, 1H), 3.94 (s, 3H), 3.91 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H), 3.46−3.44 (m, 2H), 2.05−1.95 (m, 4H), 1.82 (s, 3H), 1.62 (s, 3H), 1.55 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.9, 149.1, 138.0, 134.2, 131.3, 130.8, 130.1, 125.2, 124.2, 123.4, 121.1, 119.9, 111..3, 108.8, 107.1, 105.8, 101.4, 97.9, 56.0, 55.8, 55.7, 55.4, 39.7, 26.8, 25.6, 24.4, 17.6, 16.3; HRMS (EI) m/z calcd for $C_{28}H_{36}O_4$ $(M)^+$ 436.2614, found 436.2606.

2-Geranyl-3,5,3′,4′-tetrakis(methoxymethoxy)-(*E*)-stilbene (25). To a solution of potassium hexamethyldisilazane (KHMDS) (0.5 M solution in toluene, 2.3 mL, 1.16 mmol) in THF (1.5 mL) were added phosphonate **21** (46 mg, 0.13 mmol) and aldehyde **16** (35 mg, 0.10 mmol). After the solution was stirred for 4 h, the reaction was quenched by addition of NH_4Cl. The resulting mixture was extracted with EtOAc, washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. Final purification by flash column chromatography (7% EtOAc in hexanes) provided stilbene **25** (46 mg, 86%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31−7.11 (m, 4H), 6.96 (s, 1H), 6.88 (d, J = 15.7 Hz, 1H), 6.74 (s, 1H), 5.27−5.05 (m, 10H), 3.54−3.48 (m, 14H), 2.04−1.97 (m, 4H), 1.82 (s, 3H), 1.61 (s, 3H), 1.54 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.1, 155.8, 147.4, 146.9, 138.2, 134.5, 132.4, 131.3, 130.1, 125.5, 124.2, 123.3, 122.8, 120.9, 116.7, 114.8, 106.2, 102.9, 95.5, 95.4, 94.6 (2C), 56.2 (2C), 56.0 (2C), 39.7, 26.7, 25.6, 24.7, 17.6, 16.2; HRMS (EI) m/z calcd for $C_{32}H_{44}O_8$ $(M)^+$ 556.3036, found 556.3056.

2-Geranyl-3,5,3′,4′-tetrahydroxy-(*E*)-stilbene (26). To a solution of stilbene **25** (23 mg, 0.04 mmol) in MeOH (4 mL) was added TsOH (63 mg, 0.33 mmol). The solution was stirred for 24 h, and the reaction was quenched by addition of $NaHCO_3$. The resulting mixture was extracted with EtOAc, dried ($MgSO_4$), and concentrated *in vacuo*. Final purification by preparative TLC (30% EtOAc in hexanes) afforded stilbene **26** (6 mg, 38%) as a yellow oil: ^{1}H NMR (500 MHz, CD_3OD) δ 6.96 (d, J = 16 Hz, 1H), 6.85 (d, J = 2.3 Hz, 1H), 6.71 (d, J = 2 Hz, 1H), 6.70 (d, J = 1.7 Hz, 1H), 6.65 (d, J = 15.8 Hz, 1H), 6.63 (d, J = 8.3 Hz, 1H), 6.44 (d, J = 2.1 Hz, 1H), 6.13 (d, J = 2.3 Hz, 1H), 5.01−5.00 (m, 1H), 4.95−4.92 (m, 1H), 3.27 (d, J = 6.6 Hz, 2H), 1.97−1.92 (m, 2H), 1.88−1.85 (m, 2H), 1.70 (s, 3H), 1.47 (s, 3H), 1.42 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 157.0, 156.7, 146.5, 146.4, 139.8, 134.5, 132.1, 131.6, 130.7, 125.8, 125.4 (2C), 120.0, 119.1, 116.4, 114.0, 104.3, 102.6, 40.8, 27.8, 25.8, 25.1, 17.7, 16.5; HRMS (EI) m/z calcd for $C_{24}H_{28}O_4$ $(M)^+$ 380.1988, found 380.2014.

2-Geranyl-3,5-dimethoxy-3′-prenyl-4′,5′-bis-(methoxymethoxy)-(*E*)-stilbene (28). To a stirred solution of aldehyde **14** (21 mg, 0.07 mmol) and phosphonate **27** (51 mg, 0.12 mmol) in THF (1.4 mL) at 0 °C was added KHMDS (0.5 M in toluene, 0.69 mL, 0.35 mmol). The solution was stirred for 22 h at rt, and the reaction was quenched with NH_4Cl. The resultant mixture was extracted with EtOAc, and the combined organic extracts were washed with brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Final purification by preparative TLC (30% EtOAc in hexanes) gave stilbene **28** (36 mg, 92%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.20 (d, J = 16.0 Hz, 1H), 7.14 (d, J = 1.8 Hz, 1H), 6.98 (d, J = 2.1 Hz, 1H), 6.87 (d, J = 15.7 Hz, 1H), 6.72 (d, J = 2.4 Hz, 1H), 6.41 (d, J = 2.6 Hz, 1H), 5.34−5.31 (m, 1H), 5.22 (s, 2H), 5.12 (s, 2H), 5.06−5.03 (m, 1H), 3.85 (s, 3H), 3.81 (s, 3H), 3.61 (s, 3H), 3.52 (s, 3H), 3.44−3.41 (m, 4H), 2.06−2.02 (m, 2H), 1.98−1.95 (m, 2H), 1.81 (s, 3H), 1.76 (s, 3H), 1.74 (s, 3H), 1.60 (s, 3H), 1.53 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.5, 158.4, 150.0, 144.4, 137.9, 136.1, 134.2, 133.9, 132.7, 131.2, 130.1, 126.3, 124.3, 123.5, 122.6, 121.5, 121.2, 112.1, 101.4, 99.1, 98.1, 95.2, 57.5, 56.2, 55.7, 55.4, 39.7, 28.6, 26.7, 25.8, 25.6, 24.4, 17.9, 17.6, 16.2; HRMS (ESI) m/z calcd for $C_{35}H_{49}O_6$ $(M + H)^+$ 565.3529, found 565.3524.

2-Geranyl-3,5-dimethoxy-3′-prenyl-4′,5′-dihydroxy-(*E*)-stilbene (29). To a solution of bis-MOM acetal **28** (36 mg, 0.06 mmol) in MeOH (6.4 mL) was added TsOH (49 mg, 0.26 mmol). After the solution was stirred for 19.5 h at rt, additional TsOH (25 mg, 0.13 mmol) was added due to incomplete conversion to product. After the solution was stirred for an additional 22.5 h, the reaction was quenched by addition of $NaHCO_3$. The resultant mixture was extracted with EtOAc, and the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Final purification by preparative TLC (35% EtOAc in hexanes) provided stilbene **29** (14 mg, 47%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (d, J = 15.6 Hz, 1H), 6.94 (s, 1H), 6.84−6.80 (m, 2H), 6.71 (d, J = 2.1 Hz, 1H), 6.40 (d, J = 2.0 Hz, 1H), 5.45 (br s, 1H), 5.34 (t, J = 6.1 Hz, 1H), 5.11 (t, J = 6.7 Hz, 1H), 5.06−5.04 (m, 1H), 3.84 (s, 3H), 3.80 (s, 3H), 3.42 (d, J = 6.5 Hz, 2H), 3.37 (d, J = 7.1 Hz, 2H), 2.07−2.03 (m, 2H), 1.98−1.95 (m, 2H), 1.81−1.79 (m, 9H), 1.61 (s, 3H), 1.54 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 158.5, 158.4, 144.2, 141.9, 138.0, 135.3, 134.4, 131.3, 130.6, 130.1, 127.3, 125.2, 124.3, 123.6, 121.6, 121.1, 120.5, 110.9, 101.5, 97.9, 55.7, 55.4, 39.7, 29.9, 26.8, 25.8, 25.6, 24.4, 17.9, 17.6, 16.3; HRMS (ESI) m/z calcd for $C_{31}H_{41}O_4$ $(M)^+$ 477.3005, found 477.2994.

2-Geranyl-3,5-dimethoxy-2′-prenyl-3′-(methoxymethoxy)-4′-methoxy-(*E*)-stilbene (30). To a stirred solution of aldehyde **14** (27 mg, 0.1 mmol) and **18** (51 mg, 0.1 mmol) in THF (1.5 mL) at room temperature were added NaH (60% dispersion in mineral oil, 22 mg, 0.6 mmol) and 15-crown-5 (2 drops). After the mixture was stirred overnight, the reaction was quenched by addition of NH_4Cl. The resulting mixture was extracted with EtOAc, and then the combined organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Final purification by flash column chromatography (10% EtOAc in hexanes) provided **30** (18 mg) in 38% yield as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (d, J = 8.7 Hz, 1H), 7.11 (m, 2H), 6.80 (d, J = 8.6 Hz, 1H), 6.70 (d, J = 2.3 Hz, 1H), 6.41 (d, J = 2.5 Hz, 1H), 5.16−5.03 (m, 3H), 5.09 (s, 2H), 3.86 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.60 (s, 3H), 3.57 (d, J = 6.6 Hz, 2H), 3.42 (d, J = 6.7 Hz, 2H), 2.05−1.96 (m, 4H), 1.80 (s, 3H), 1.78 (s, 3H), 1.68 (s, 3H), 1.62 (s, 3H), 1.55 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.8, 158.7, 152.1, 144.1, 138.7, 134.8, 134.5, 131.6 (2C), 130.9, 128.4, 127.3, 124.7, 123.8, 123.6, 122.2, 121.4, 110.4, 101.8, 99.4, 98.2, 58.0, 56.1, 56.0, 55.6, 40.1, 30.0, 27.1, 26.1, 26.0 (2C), 24.7, 18.5, 18.0, 16.6; HRMS (EI) m/z calcd for $C_{34}H_{46}O_5$ $(M)^+$ 534.3345, found 534.3330.

2-Geranyl-3,5-dimethoxy-2′-prenyl-3′-hydroxy-4′-methoxy-(*E*)-stilbene (31). To a stirred solution of MOM ether **30** (27 mg, 0.1 mmol) in MeOH (2.5 mL) was added TsOH (40 mg, 0.2 mmol). The solution was stirred overnight, and the reaction was quenched by addition of saturated aqueous $NaHCO_3$. The resulting mixture was extracted with EtOAc, and the organic extracts were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Stilbene **31** (22 mg, 88%) was obtained as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.14 (m, 2H), 7.10 (m, 1H), 6.76 (m, 1H), 6.73 (d, J = 2.4 Hz, 1H), 6.42 (d, J = 2.2 Hz, 1H), 5.21−5.04 (m, 3H), 3.90 (s, 3H), 3.84 (s, 3H), 3.81 (s, 3H), 3.52 (d, J = 6.5 Hz, 2H), 3.43 (d, J = 6.4 Hz, 2H), 2.09−1.96 (m, 4H), 1.82 (s, 3H), 1.78 (s, 3H), 1.68 (s, 3H), 1.62 (s, 3H), 1.55 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.9, 158.1, 146.2, 143.6, 138.7, 134.7, 131.9, 131.5, 131.0, 128.5, 127.5, 126.1, 124.7, 123.9, 123.0, 121.5, 117.5, 108.8, 101.9, 98.3, 56.0, 55.7, 55.3, 39.8, 26.8, 25.7, 25.6, 25.1, 24.4, 18.1, 17.6, 16.3; HRMS (EI) m/z calcd for $C_{32}H_{42}O_4$ $(M + H)^+$ 490.3083, found 490.3087.

2-Geranyl-3,5,3′,4′-tetramethoxy-2′-prenyl-(*E*)-stilbene (20). To a solution of stilbene **31** (11 mg, 0.02 mmol) in THF (3 mL) were added NaH (60% dispersion in mineral oil, 6 mg, 0.2 mmol) and MeI (2 drops). The mixture was stirred for 5 h, and the reaction was quenched by addition of H_2O. The resultant mixture was extracted with EtOAc, and the combined organic extracts were washed with 2 M NaOH, dried ($MgSO_4$), and concentrated *in vacuo*. Stilbene **20** (6 mg, 55%) was obtained as a yellow oil, with ^{1}H NMR data that were identical to the data given above.

4-Prenyl-3,5,3′,4′-tetrakis(methoxymethoxy)-(*E*)-stilbene (36). To a solution of KHMDS (0.5 M solution in toluene, 2.12 mL, 1.06 mmol) in THF (1.5 mL) were added phosphonate **23**[S1] (46 mg, 0.11 mmol) and aldehyde **34**[S2] (20 mg, 0.09 mmol). After the solution was stirred for 2 h, the reaction was quenched by addition of NH_4Cl. The resulting mixture was extracted with EtOAc, washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. Final purification by flash

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column chromatography provided stilbene **36** (25 mg, 58%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (d, J = 1.9 Hz, 1H), 7.14−7.09 (m, 2H), 6.98−6.89 (m, 4H), 5.29 (s, 2H), 5.25−5.21 (m, 7H), 3.56 (s, 3H), 3.53 (s, 3H), 3.50 (s, 6H), 3.39 (d, J = 7.2 Hz, 2H), 1.79 (s, 3H), 1.66 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.8 (2C), 147.4, 146.8, 136.4, 132.1, 131.0, 127.7 (2C), 122.7, 121.0, 119.7, 116.6, 114.3, 106.0 (2C), 95.4 (2C), 94.5 (2C), 56.2 (2C), 56.0 (2C), 25.7, 22.7, 17.7; HRMS (EI) m/z calcd for $C_{27}H_{36}O_8$ $(M)^+$ 488.2410, found 488.2416.

4-Prenyl-3,5,3′,4′-tetrahydroxy-(*E*)-stilbene (37). After TsOH (40 mg, 0.21 mmol) was added to a solution of stilbene **36** (13 mg, 0.03 mmol) in MeOH (2.5 mL), the solution was stirred for 24 h. The reaction was quenched by addition of $NaHCO_3$, and the resulting mixture was extracted with EtOAc, dried ($MgSO_4$), and concentrated *in vacuo*. Final purification by preparative TLC (30% EtOAc in hexanes) gave stilbene **37** (4 mg, 50%) as a yellow oil. The ^{1}H and ^{13}C NMR spectra matched published data.[56,57]

4-Prenyl-3,5,4′-tris(methoxymethoxy)-(*E*)-stilbene (38). To a solution of KHMDS (0.5 M solution in toluene, 4.62 mL, 2.31 mmol) in THF (1.5 mL) were added phosphonate **33** (99 mg, 0.24 mmol) and aldehyde **35**[52] (32 mg, 0.19 mmol). After the solution was stirred for 3 h, the reaction was quenched by addition of NH_4Cl. The resulting mixture was extracted with EtOAc, washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. Final purification of the residue by flash column chromatography (4% EtOAc in hexanes) provided stilbene **38** (33 mg, 40%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (t, J = 2.7 Hz, 1H), 7.42 (t, J = 1.9 Hz, 1H), 7.03−7.01 (m, 2H), 6.98−6.95 (m, 1H), 6.92−6.90 (m, 1H), 6.92 (s, 2H), 5.24−5.19 (m, 5H), 5.19 (s, 2H), 3.50 (s, 6H), 3.49 (s, 3H), 3.39 (d, J = 7.2 Hz, 2H), 1.79 (s, 3H), 1.66 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.8, 155.8 (2C), 136.6, 131.3, 131.0, 127.7, 127.6 (2C), 127.2, 122.7, 119.6, 116.4 (2C), 106.0 (2C), 94.5 (2C), 94.4, 56.0 (3C), 25.8, 22.8, 17.8; HRMS (EI) m/z calcd for $C_{25}H_{32}O_6$ $(M)^+$ 428.2199, found 428.2191.

***trans*-Arachidin-2 (39).** To a solution of compound **38** (17 mg, 0.04 mmol) in MeOH (5 mL) was added TsOH (46 mg, 0.24 mmol). After the solution was stirred for 24 h, the reaction was quenched with $NaHCO_3$. The resulting mixture was extracted with EtOAc, dried ($MgSO_4$), and concentrated *in vacuo*. Final purification by flash column chromatography (12% EtOAc in hexanes) provided stilbene **39** (12 mg, 100%) as a yellow oil. Both the ^{1}H and ^{13}C data matched those of the known compound.[56,57]

Biological Assays. All compounds were initially screened for intrinsic and antagonist activity at 10 μM in the [^{35}S]GTP-γ-S binding assay at the human κ and the μ and δ opioid receptors overexpressed in CHO cells. These cell lines were kindly provided by Dr. Liu-Chen (Temple University, κ) and Dr. Larry Toll (SRI International, μ and δ). Compounds were identified as antagonist characterized for functional antagonism (K_e) and selectivity by measuring the ability of the test compounds to inhibit stimulated [^{35}S]GTP-γ-S binding produced by one of the selective agonists DAMGO (μ), DPDPE (δ), or U69,593 (κ). Agonist concentration−response curves were run in the presence or absence of a single concentration of test compound.

Briefly, the test compounds were assayed in duplicate in 1.4 mL polypropylene tubes in a 96-well format. CHO membrane homogenates (20−40 μg protein) were incubated with a positive control or the test compound, 0.1 nM [^{35}S]GTP-γ-S, and 1 μM GDP in 50 mM HEPES buffer (pH 7.4) at room temperature for 1 h, after which bound radioligand was separated from free radioligand via rapid vacuum filtration over GF-B filters with a Brandel Scientific (Gaithersburg, MD, USA) 96-well harvester. Bound radioactivity is determined using a TopCount 12-detector instrument (Packard Instruments) using standard scintillation counting techniques. Bound radioactivity is normalized to samples containing vehicle (basal binding). A four-parameter logistic function was fit to these data to calculate the EC_{50} and E_{max} values using Prism (v. 6; Graph Pad Software, San Diego, CA, USA). The K_e values were calculated using the formula K_e = [L]/DR − 1, where [L] is the concentration of test compound, and DR is the ratio of agonist EC_{50} value in the presence or absence of test compound.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supplementary data including the ^{1}H and ^{13}C NMR spectra for the key intermediates and final products in this article are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

We dedicate this contribution to our valued colleague Paul J. Klausmeyer, who isolated and characterized schweinfurthin J and who was killed in an automobile accident on January 22, 2013.

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