Vanilloids. 1. Analogs of Capsaicin with Antinociceptive and Antiinflammatory Activity

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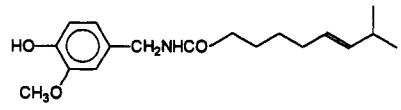
As part of a program to establish structure–activity relationships for vanilloids, analogs of the pungent principle capsaicin, the alkyl chain portion of the parent structure (and related compounds derived from homovanillic acid) was varied. In antinociceptive and antiinflammatory assays (rat and mouse hot plate and croton oil-inflamed mouse ear), compounds with widely varying alkyl chain structures were active. Short-chain compounds were active by systemic administration in the assays mentioned above but they retained the high pungency and acute toxicity characteristic of capsaicin. In contrast, the long chain *cis*-unsaturates, NE-19550 (vanillyloleamide) and NE-28345 (oleylhomovanillamide), were orally active, less pungent, and less acutely toxic than capsaicin. The potential of these compounds as antiinflammatory/analgesic agents is discussed in light of recent data on the mechanism of action of vanilloids on sensory nerve fibers.

Capsaicin, 1, is the primary pungent principle in peppers of the *Capsicum* family. Capsaicin and dihydrocapsaicin comprise over 90% of the capsaicinoids derived from crude oleoresin, with the remaining compounds being a mixture of higher and lower homologs with and without the double bond.[1] While most commonly used as a spice, hot peppers have been used for centuries for their medicinal effects, primarily for stomach disorders and as topical counter-irritants for relief of pain and inflammation.[2]



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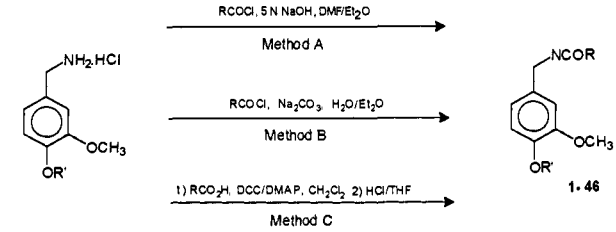
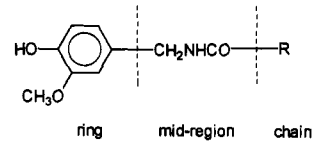
Pioneering work by a group of Hungarian workers, led by the late Nicholas Jancso, led to the discovery that the pain and inflammation produced by topical application of capsaicin could be reduced or eliminated by repeated application.[3] This capsaicin-induced desensitization inhibited the pain response caused by a variety of noxious chemical agents, while the response to mechanical stimuli remained unchanged. This selective response on chemonociceptors led Jancso and co-workers to suggest that the sensory nerve ending was the site of capsaicin's action. In adult animals, the effects of capsaicin were shown to be selective for a subset of sensory nerve fibers known as C-fiber polymodal nociceptors, which are small diameter unmyelinated fibers.[4] These fibers are responsive to noxious stimuli of thermal, mechanical, and chemical origin. These early observations stimulated a great deal of work over the last decade using capsaicin as a tool to probe the functioning of sensory neurons, much of which has been summarized in recent reviews.[5]

The effects of capsaicin on sensory fibers led us to examine the effect of capsaicin in animal models of analgesia. We found that adult mice and rats treated with vanilloids showed reversible antinociceptive responses in thermal antinociceptive assays (hot plate, tail-flick).[6] These data and similar observations of thermal antinociception following intrathecal[7] and systemic administration[8] suggested that capsaicin could have application as an analgesic agent.[9] Our goal at the outset was to investigate the effect of structural variants of capsaicin on nociception, inflammation, and acute toxicity with the objective of optimizing the therapeutic index, defined as the ratios of antinociceptive and antiinflammatory potencies to acute toxicity. Our approach was to make systematic changes to the capsaicin structure which we divided into three parts: the chain, the midregion, and the ring. This paper summarizes the results of changes in the chain.

Scheme I





Chemistry

The vanillylamine amides (Scheme I) were prepared by acylation of vanillylamine in DMF or THF with the appropriate acid chloride in ether or $CHCl_3$ (method A) or in a two-phase system using water and ether (method B). In one case, protected vanillylamine (4-ethylvinyl ether) was coupled to the desired carboxylic acid with *N,N'*-dicyclohexylcarbodiimide and catalytic 4-(dimethylamino)pyridine (method C). The homovanillic acid amides (Scheme II) were prepared by the reaction of 4-acetoxy-3-methoxyphenylacetic acid chloride (4-acetylhomovanillic acid chloride) with the appropriate amine (2 equiv, method D or 1 equiv of the desired amine and 1

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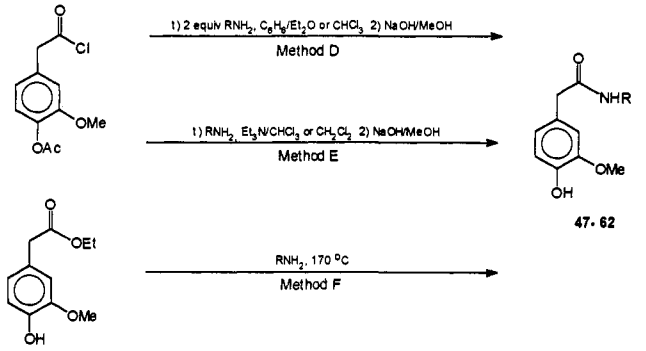
Scheme II



equiv of triethylamine, method E), followed by hydrolysis. In one case, neat ethyl (4-hydroxy-3-methoxyphenyl)-acetate (ethyl homovanillate) was reacted with the desired amine at elevated temperature (method F). The chemical data are summarized in Tables I and II. Several compounds are known, and their pungency has been studied.[10] The synthesis of the requisite acids and amines, when not commercially available, is given in the Experimental Section.

Results

Our primary screens for antinociception were the mouse and rat hot plate tests. Our hot plate procedure closely resembles the 55 °C hot plate test described by Eddy et al.[11] Variations of this test are commonly used to assess antinociceptive efficacy in rodents. In general, opiates but not nonsteroidal antiinflammatory agents (NSAID's), display efficacy in this model. With capsaicin, variations in the experimental protocol (species and strain of animal,[12] dose, time between dosing the testing, hot plate temperature, endpoint[13]) can affect the result.[14] In addition, the role that C-fibers play in thermal stimulation of glaborous skin of the rat paw is controversial.[15]

To evaluate antiinflammatory efficacy, we used the topical croton oil-inflamed mouse ear test. Croton oil contains phorbol esters, compounds known to induce neurogenic inflammation which is C-fiber mediated. We also determined the acute ip toxicity in mice in an attempt to find compounds devoid of the high acute toxicity of capsaicin.

1. **Antinociceptive Assays.** In the mouse hot plate test, a wide variety of amides were active but only after systemic administration (sc). For the vanillylamine amides (Table III), the saturated straight chain amides 1–10 show a progressive change in efficacy with increasing chain length, with the heptanoic through undecanoic acid-derived analogs 4–8 having the greatest efficacy. One member of this series, vanillylnonamide, 6 ("synthetic capsaicin"), is virtually identical with natural capsaicin in our assays and was routinely used as the positive control. Further increases in chain length, demonstrated with the dodecanoic and octadecanoic acid-derived analogs 9 and 10, eliminate activity. Lee et al.[16] have recently reported on the antinociceptive activity of a series of homologous vanilloids in the mouse phenylquinone writhing assay. In contrast to the present study, antinociceptive activity was observed for compounds over a broader range of chain lengths, including analog 10.

The araalkyl analogs 11–15 are surprisingly devoid of antinociceptive activity. These were chosen to approximate the hydrophobicity of the straight-chain analogs 4–7, which have considerable antinociceptive activity, based on the estimate that the phenyl group is approximately equivalent to a propyl group in hydrophobicity.[17]

Compounds 16–19 are all derived from substituted octadecanoic acids. Analog 16, derived from 9-methylstearic acid, shows antinociceptive activity while the 9-ketostearic acid-derived analog 17 does not. Aside from the obvious structural difference, there is a considerable difference in physical properties. For example, 16 is an oil while 17 is a high (>100 °C) melting solid. Since the physical properties of the analogs can determine bioavailability, some of the differences observed in *in vivo* testing may be related to differences in physical properties, as will be discussed in greater detail below. The *cis* and *trans* epoxides 18 and 19 have marginal activity in the hot plate test.

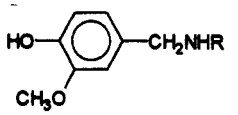
A systematic examination of the effect of unsaturation provided the most interesting results. *cis*-Capsaicin (20) is very efficacious, demonstrating that the stereochemistry of the double bond, which is *trans* in natural capsaicin, is not critical. The terminal olefin 21 shows considerably more efficacy than the terminal acetylene 22. When the unsaturation is internal, activity is observed over a range of chain lengths. The Δ9 *cis* compounds 23, 24, 26 and 30 (NE-19550), where the chain length increases from C_{12} to C_{18}, demonstrate this trend. Among the other analogs derived from isomeric octadecenoic acids, the Δ6 *cis* and Δ11 *cis* compounds 28 and 32 show biological activity similar to Δ9 *cis* compound NE-19550 (30), while the Δ2 *cis*, Δ6 *trans*, and Δ9 *trans* isomers 27, 29, and 31 show considerably less efficacy. Further increases in chain length eventually result in the loss of antinociceptive activity when the chain length increased to C_{24} (35). The geminally disubstituted olefinic acid-derived analog 37 and the acetylenic analog 38 illustrate other structural variants consistent with activity.

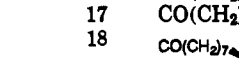
The analogs 39–45, derived from polyunsaturated fatty acids, all show considerable antinociceptive activity regardless of the stereochemistry of the double bonds. These were all potentially more susceptible to air oxidation on storage and offered no clear advantage over NE-19550 (30).

For the homovanillic acid amides (Table IV), similar structure–activity trends were seen in the mouse hot plate data. Considerable efficacy was observed for a range of compounds derived from short chain acyclic and cyclic fatty amines (47–50, 53) while further increases in chain length resulted in a loss of activity (51–52). Among the unsaturated analogs 54–61, good activity was seen for the two compounds tested, 54 and 58.

At this point, we discovered that, while no activity could be observed in the mouse after oral dosing due to poor bioavailability,[18c] many of the long-chain unsaturates displayed considerable activity via the oral route in the rat. As one of our long-term goals was to identify orally active compounds, the oral rat hot plate assay became our primary screen. All of the unsaturated homovanillic acid amides 54–61 showed good activity. Evaluation of the vanillylamine amides (Table III) verified that activity was observed only for unsaturates. While the terminally unsaturated compounds 21 and 22 and the acetylenic analogs 38 showed only marginal activity, the remaining unsaturates were considerably more active including the *trans* unsaturates, especially 31 and 41, which had no or low activity in the mouse hot plate assay.

Table I. Synthesis and Purification of Vanillylamine Amides



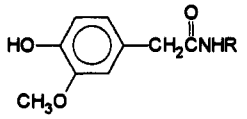
compd	R	synthetic method[a]	mp (°C)	yield (%)	purification method[b] cryst solv	purification method[b] chromat solv	purification method[b] distilled	anal.[c]
1	COC_8H_7	A	78–79	60		65% A	X	$C_{12}H_{17}NO_3$
2	COC_4H_9	A	54–55	55		60% A	X	$C_{13}H_{19}NO_3$
3	COC_8H_{11}	A	54–55	56			X	$C_{14}H_{21}NO_3$
4	COC_8H_{13}	A	58–59	59	Et_2O			$C_{18}H_{23}NO_3$
5	COC_7H_{15}	A	44–45	61			X	$C_{18}H_{25}NO_3$
6	COC_8H_{17}	A	57–58	59	Et_2O/pent			$C_{17}H_{27}NO_3$
7	COC_9H_{19}	A	61–63	69	benz/hex			$C_{18}H_{29}NO_3$
8	$COC_{18}H_{21}$	Pd/C/H_2 of 21[d]	59–61	63	benz/hex			$C_{18}H_{31}NO_3$
9	$COC_{11}H_{23}$	A	63–66	27	benz/hex			$C_{20}H_{33}NO_3$
10	$COC_{17}H_{35}$	A	90–91	48	benz/hex, EtOH/H_2O			$C_{26}H_{43}NO_3$
11	$CO(CH_2)_3Ph$	A	oil	48		40% A		$C_{18}H_{21}NO_3$
12	$CO(CH_2)_4Ph$	A	54–55	56		60% A		$C_{18}H_{23}NO_3$
13	$CO(CH_2)_5Ph$	A	54–56	55		60% A	X	$C_{20}H_{25}NO_3$
14	$CO(CH_2)_5Ph$	A	65–66	64	Et_2O/pent			$C_{21}H_{27}NO_3$
15	$COPh\text{-}p\text{-}C_6H_{11}$	A	112–114	71	tol/pent			$C_{20}H_{25}NO_3$
16	$CO(CH_2)_7CH(CH_3)C_9H_{19}$	A	oil	45		45% A		$C_{27}H_{47}NO_3$
17	$CO(CH_2)_7COC_9H_{19}$	A	105–106	40	MeOH			$C_{26}H_{43}NO_4$
18		*m*-CPBA on 30[e]	77–78	53	act/hex, MeOH/H_2O			$C_{26}H_{43}NO_4$
19		*m*-CPBA on 31[e]	71–72	58	act/hex, MeOH/H_2O			$C_{26}H_{43}NO_4$
20	*cis*-$CO(CH_2)_4CH{=}CHCHMe_2$	A	72–73	70	Et_2O/hex	60% A		$C_{18}H_{27}NO_3$
21	$COC_{10}H_{19}\Delta_{10}$	A	56–58	79		60% A		$C_{19}H_{29}NO_3$
22	$COC_{18}H_{17}\Delta_{10}$	A	81–82	63		60% A		$C_{18}H_{27}NO_3$
23	$COC_{11}H_{21}\Delta_{9,cis}$	A	oil	90		40% A		$C_{20}H_{31}NO_3$
24	$COC_{18}H_{25}\Delta_{9,cis}$	A	oil	78		50% A		$C_{22}H_{35}NO_3$
25	$COC_{18}H_{25}\Delta_{9,trans}$	B	56–57	80	EtOAc/hex			$C_{22}H_{55}NO_3$
26	$COC_{18}H_{29}\Delta_{9,cis}$	A	34–35	77		50% A		$C_{24}H_{29}NO_3$
27	$COC_{17}H_{33}\Delta_{2,cis}$	A	90–91	67		40% A		$C_{26}H_{43}NO_3$
28	$COC_{17}H_{33}\Delta_{6,cis}$	A	47–49	84	Et_2O/pent			$C_{26}H_{43}NO_3$
29	$COC_{17}H_{33}\Delta_{6,trans}$	A	75–76	67		50% A		$C_{26}H_{43}NO_3$
30	$COC_{17}H_{33}\Delta_{9,cis}$	A, B	43–44	85	Et_2O/pent			$C_{26}H_{43}NO_3$
31	$COC_{17}H_{33}\Delta_{9,trans}$	A	87–88	68	tol			$C_{26}H_{43}NO_3$
32	$COC_{17}H_{33}\Delta_{11,cis}$	A	43–45	80	pent	40% A		$C_{26}H_{43}NO_3$
33	$COC_{18}H_{35}\Delta_{10,cis}$	A	40–41	77		40% A		$C_{27}H_{45}NO_3$
34	$COC_{21}H_{41}\Delta_{13,cis}$	A	50–51	67		40% A		$C_{30}H_{51}NO_3$
35	$COC_{23}H_{45}\Delta_{15,cis}$	A	74–75	51	EtOAc/hex			$C_{32}H_{55}NO_3$
36	$COC_{17}H_{32}\Delta_{9,cis}$-12-OH	A	oil	42		60% A		$C_{26}H_{43}NO_4$
37	$CO(CH_2)_7C(C_8H_{19}){=}CH_2$	C	39–40	68		50% A		$C_{27}H_{45}NO_3$
38	$COC_{17}H_{31}\Delta_9$	A	63–64	54	tol/hex, benz/hex			$C_{26}H_{41}NO_3$
39	$COC_{17}H_{31}\Delta_{9,12,cis,cis}$	A	oil	88		50% A		$C_{26}H_{41}NO_3$
40	$COC_{17}H_{31}\Delta_{9,11,cis,trans}$ + $COC_{17}H_{31}\Delta_{9,12,cis,trans}$	A	oil	79		40% A		$C_{26}H_{41}NO_3$
41	$COC_{17}H_{31}\Delta_{9,12,trans,trans}$	A	53–54	88	Et_2O/pent			$C_{26}H_{41}NO_3$
42	$COC_{18}H_{33}\Delta_{10,13,trans,trans}$	A	64–65	80		45% A		$C_{27}H_{43}NO_3$
43	$COC_{19}H_{35}\Delta_{11,14,cis,cis}$	A	37–38	64		40% A	X	$C_{28}H_{48}NO_3$
44	$COC_{18}H_{35}\Delta_{11,14,trans,trans}$	A	61–62	68		45% A		$C_{28}H_{48}NO_3$
45	$COC_{17}H_{29}\Delta_{9,12,15,cis,cis,cis}$	A	oil	81		45% A		$C_{26}H_{39}NO_3$
46	$COC_{18}H_{31}\Delta_{5,8,11,14\ all\ cis}$	A	oil	34		40% A	X	$C_{26}H_{41}NO_3$

[a] Refer to Experimental Section for details. [b] Solvent abbreviations: A = % ethyl acetate in an ethyl acetate/hexane mixture; pen = pentane; benz = benzene; hex = hexanes; tol = toluene; act = acetone. [c] All compounds gave elemental analyses within 0.4% of theoretical values. [d] Hydrogenation over palladium on charcoal. [e] *m*-Chloroperbenzoic acid oxidation.

One trend evident in the oral rat hot plate data is that antinociceptive activity is correlated with both chain length and melting point. Since chain length and lipophilicity are highly correlated for these compounds, this relationship may be expressed in terms of either parameter. An important aspect of the chain length (or lipophilicity) effect on oral activity would appear to be the more rapid hepatic metabolism of the short-chain compounds, leading to lower bioavailability.[18] The net result is that many long-chain compounds are more potent than short-chain compounds. For long-chain compounds, the low-melting analogs are more potent than the higher melting compounds. The effect of melting point on potency may be related to the greater oil and water solubility of low-melting compounds relative to high melting ones.[19] If transport into or through membranes is important for vanilloid activity, one would expect that low-melting vanilloids would be the most potent. Figure 1 shows the relationship of melting point, log octanol/water partition coefficient (proportional, for the most part, to chain length), and activity in the rat hot plate test. Most of the active compounds are clustered in the upper right-hand corner of the graph and are characterized by log k_{oct} values greater than 6 (corresponding to alkyl chains C_{14} or longer) and melting points less than 65 °C.

The activity seen in the rat hot plate test after oral

Table II. Synthesis and Purification of Homovanillic Acid Amides



compd	R	synthetic method[a]	mp (°C)	yield (%)	purification method[b]			anal.[c]
					cryst solv	chromat solv	distilled	
47	C_8H_{13}	D	oil	76			X	$C_{18}H_{23}NO_3$
48	C_7H_{15}	D	50–51	45		60% A		$C_{18}H_{25}NO_3$
49	C_8H_{17}	D	63–64	63	Et_2O/pent			$C_{17}H_{27}NO_3$
50	C_9H_{19}	D	55–56	44	benz/hex	60% A		$C_{18}H_{29}NO_3$
51	$C_{10}H_{21}$	D	64–65	78	benz/hex	60% A		$C_{18}H_{31}NO_3$
52	$C_{12}H_{25}$	D	66–68	43	benz/hex			$C_{21}H_{35}NO_3$
53	c-C_8H_{15}	D	80–81	58	tol/hex	60% A		$C_{17}H_{25}NO_3$
54	$C_{10}H_{19}\Delta_9$	E	55–56	59	EtOAc/pent	50% A		$C_{19}H_{29}NO_3$
55	$C_{14}H_{27}\Delta_{9,cis}$	F	35–36	52		35% A		$C_{23}H_{37}NO_3$
56	$C_{18}H_{31}\Delta_{9,cis}$	F	oil	51		40% A		$C_{25}H_{41}NO_3$
57	$C_{17}H_{33}\Delta_{8,cis}$	E	41–43	42		3% i-PrOH/CH_2Cl_2		$C_{26}H_{43}NO_3$
58	$C_{18}H_{35}\Delta_{9,cis}$	E/F	42–43	28	EtOH/H_2O	40% A		$C_{27}H_{45}NO_3$
59	$C_{18}H_{35}\Delta_{11,cis}$	D	49–50	53		25% A		$C_{27}H_{45}NO_3$
60	$C_{18}H_{35}\Delta_{9,trans}$	F	64–65	45	hex	60% $CHCl_3$/pent		$C_{27}H_{45}NO_3$
61	$C_{18}H_{33}\Delta_{9,12,cis,cis}$	F	oil	39		60% $CHCl_3$/pent		$C_{27}H_{43}NO_3 \cdot 0.2CHCl_3$

[a] Refer to Experimental Section for details. [b] Solvent abbreviations: A = % ethyl acetate in an ethyl acetate/hexane mixture; pent = pentane; benz = benzene; hex = hexane; tol = toluene. [c] All compounds gave elemental analyses within 0.4% of theoretical values.

dosing is a composite of intrinsic activity, absorption, and metabolism. To get a better measure of intrinsic activity, selected vanilloids were dosed intravenously. The vanillylamine amide NE-19550 (**30**) and the related homovanillic acid amide NE-28345 (**58**) were administered iv in polyethylene glycol 200 at doses from 0.1 to 0.5 mg/kg. Both were found to have similar antinociceptive potency on the hot plate with a doubling dose[20] (approximately the dose required to double the pretest latency value) 30 min postdose of 0.24 mg/kg for analog **30** and 0.22 mg/kg for analog **58**. The similar potencies seen by the iv route contrasts with the 3-fold greater potency of analog **58** when given orally. The greater resistance of analog **58** toward metabolism likely accounts for the difference.[18b]

The correlation between hot plate activity for analogs **30** and **58** following iv and oral dosing suggests that the large database generated from oral dosing is relevant to intrinsic activity. While relative potencies change, the major differences between oral and iv dosing are as follows: (1) the 100-500-fold higher doses required for activity via the oral route and (2) the profound cardiovascular effects (labored breathing, increases in respiratory rate) and toxic side effects (tremor, convulsions, and occasional deaths) via the iv route.

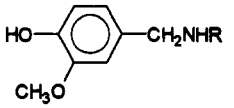
2. Antiinflammatory Assay. We selected the topical croton oil-induced inflamed mouse ear test as our primary antiinflammatory screen. The inflammation induced by this agent is known to have a strong neurogenic component which is C-fiber mediated and should be subject to inhibition by vanilloids. Inhibition of inflammation was seen over a broad range of saturated chain lengths for both the vanillylamine amides (**2–9**) and the homovanillic acid amides (**48–53**) with the exception of the shortest chain member of each series (**1** and **47**). Otherwise, the only compounds with low activity were octadecanoic and *p*-pentylbenzoic analogs **10** and **15**. Both these latter compounds had considerably higher melting points than the other compounds of this group that were tested. With regard to the effect of melting point, it is interesting to compare the inactive *p*-pentylbenzamide analog **15** (mp 112–114 °C) with the isomeric 6-phenylhexanoyl analog **13** (mp 54–56 °C) which is active in this assay. Broad activity was also observed for the unsaturates in both series of compounds. Among the unsaturated vanillylamine amides, which were more extensively tested than the homovanillic acid amides, the lowest activity was seen for the *trans* monounsaturated analogs **29** and **31** which also were among the highest melting. As observed for antinociceptive activity in the rat hot plate assay, low melting compounds gave the greatest antiinflammatory activity in the croton oil-induced inflamed mouse ear test. Figure 2 demonstrates this trend for a series of C_{18}–C_{20} vanillylamine amides.

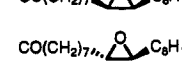
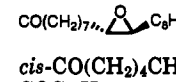
Acute Toxicity. The short-chain saturated vanilloids displayed low acute toxicity by the oral route due to poor absorption and rapid metabolism. However, when given ip considerable acute toxicity was observed. As one long-term goal was to discover vanilloids with good oral bioavailability and improved safety, we sought to minimize this potential toxicity. The LD_{50} for synthetic capsaicin **6** was 8 mg/kg. However, for those compounds with chain length $\geq C_{16}$ and with internal olefinic unsaturation, the LD_{50}'s were ≥200 mg/kg for a ≥25-fold decrease in acute toxicity. Among the compounds tested, the only exceptions are the *trans* monounsaturates **29** and **31** which appear to be slightly more toxic.

The SAR trends discussed above suggest that problems of acute toxicity and the lack of antinociceptive activity by the oral route characteristic of synthetic capsaicin, **6**, can be partly overcome with analogs derived from unsaturated long-chain acids and amines. For example, compounds with C_{18} chains, the vanillylamine amide **30** (NE-19550) and the homovanillic acid **58** (NE-29345), display good antinociceptive (both sc in the mouse hot plate assay and orally in the rat hot plate assay) and antiinflammatory activity with a ≥25-fold reduction in acute toxicity.

The pharmacologies of NE-19550 (**30**) and NE-28345 (**58**) have been compared with each other and with benchmark analgesic (codeine) and antiinflammatory (indomethacin, hydrocortisone) agents in a variety of assays (Table V).[21] The antinociceptive assays show that NE-28345 is generally more potent than NE-19550, as mentioned above. In the rat hot plate and mouse abdominal constriction assays, NE-28345 has potencies

Table III. In Vivo Biological Activity of Vanillylamine Amides

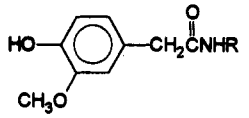


compd	R	mouse hot plate test (sc)[a] (0.68 mmol) latency (s, mean ± SD)	response	rat hot plate test (oral)[b] (300 mg/kg) max % analg	inflamed mouse ear test (top.)[c] (1% EtOH) % inhibition (mean ± SD)	toxicity (ip)[d] dose (mg/kg): % lethality
1	COC_3H_7	10.8 ± 6.1	4/8		3.2 ± 20.3	100:40
2	COC_4H_9	9.6 ± 3.8	2/8		24.2 ± 31.7	100:60
3	COC_5H_{11}	16.8 ± 17.9	4/8		33.6 ± 19.0	50:60
4	COC_6H_{13}	29.8 ± 20.0	6/8		33.7 ± 39.4	50:85
5	COC_7H_{15}	>51.3 ±13.8[e]	6/6		45.8 ± 13.9	50:90
6	COC_8H_{17}	>31.2 ± 18.8	8/8	17	72.3 ± 24.3	8:50
7	COC_9H_{19}	>43.0 ± 15.8	5/5		71.4 ± 10.5	50:80
8	$COC_{10}H_{21}$	47.7 ± 19.3	7/8		29.6 ± 39.6	50:80
9	$COC_{11}H_{23}$	9.9 ± 0.7	0/8		48.1 ± 16.4	50:100; 15:25
10	$COC_{17}H_{35}$	5.9 ± 1.4	0/8	3	11.3 ± 31.9	50:0
11	$CO(CH_2)_3Ph$	5.6 ± 0.4	NR[f]		40.9 ± 30.0	50:75
12	$CO(CH_2)_4Ph$	12.2 ± 3.6	NR		56.4 ± 20.9	50:90
13	$CO(CH_2)_5Ph$	6.2 ± 1.2	NR		57.6 ± 32.7	50:70
14	$CO(CH_2)_6Ph$	6.8 ± 0.3	NR	1	46.2 ± 28.7	50:60
15	$COPh\text{-}p\text{-}C_5H_{11}$	6.9 ± 0.3	NR		9.3 ± 24.1	50:60
16	$CO(CH_2)_7CH(CH_3)C_8H_{19}$	26.9 ± 17.7	4/8		88.3 ± 15.6	100:40
17	$CO(CH_2)_7COC_8H_{19}$	11.2 ± 7.1	NR			
18		13.5 ± 3.7	NR	5	54.5 ± 31.6	200:40
19		10.7 ± 2.9	NR		26.0 ± 19.6	200:60
20	$cis\text{-}CO(CH_2)_4CH{=}CHCHMe_2$	>39.9 ± 20.3[g]	6/7	0	79.5 ± 10.0	25:50
21	$COC_{10}H_{19}\Delta_{10}$	55.5 ± 12.8	NR	8	65.7 ± 14.9	33:50
22	$COC_{10}H_{17}\Delta_{10}$	17.1 ± 2.7	NR	15	36.4 ± 33.4	50:90
23	$COC_{11}H_{21}\Delta_{9,cis}$	>60	NR		76.2 ± 10.3	100:50
24	$COC_{13}H_{25}\Delta_{9,cis}$	>50.6 ± 13.6	8/8	23	88.9 ± 13.8	200:60
25	$COC_{13}H_{25}\Delta_{9,trans}$			22		
26	$COC_{15}H_{29}\Delta_{9,cis}$	>48.8 ± 15.5	7/8		79.3 ± 27.5	200:10
27	$COC_{17}H_{33}\Delta_{2,cis}$	7.5 ± 2.3	0/8			
28	$COC_{17}H_{33}\Delta_{6,cis}$	46.1 ± 5.8	NR	37	68.4 ± 16.9	200:30
29	$COC_{17}H_{33}\Delta_{6,trans}$	11.5 ± 4.4	2/8	27	26.7 ± 18.5	200:60
30	$COC_{17}H_{33}\Delta_{9,cis}$	>38.5 ± 20.7	7/8	69[h]	81.7 ± 18.9	400:30
31	$COC_{17}H_{33}\Delta_{9,trans}$	7.1 ± 1.9	0/8	37	10.9 ± 29.4	200:60
32	$COC_{17}H_{33}\Delta_{11,cis}$	>39.9 ± 20.8	8/8		60.0 ± 21.3	200:30
33	$COC_{18}H_{35}\Delta_{10,cis}$	35.7 ± 12.0	NR	43	76.9 ± 8.7	200:40
34	$COC_{21}H_{41}\Delta_{13,cis}$	10.8 ± 3.9; 46.3 ± 2.3	NR		29.0 ± 13.1	200:0
35	$COC_{23}H_{45}\Delta_{15,cis}$	6.1 ± 2.3	0/8		48.0 ± 11.8	200:0
36	$COC_{17}H_{32}\Delta_{9,cis}\text{-}12\text{-}OH$	12.0 ± 1.0	NR		53.0 ± 13.7	
37	$CO(CH_2)_7C(C_9H_{19}){=}CH_2$	>22.8 ± 17.6	2/8	67	96.2 ± 10.2	200:60
38	$COC_{17}H_{31}\Delta_9$	>18.6 ± 17.4	2/8	15	50.3 ± 17.0	200:70
39	$COC_{17}H_{31}\Delta_{9,12,cis,cis}$	>47.6 ± 18.4	7/8		93.7 ± 6.7	400:40
40	$COC_{17}H_{31}\Delta_{9,11,cis,trans}$ + $COC_{17}H_{31}\Delta_{9,12,cis,trans}$	>36.6 ± 23.5	6/8	34	66.8 ± 21.3	200:0
41	$COC_{17}H_{31}\Delta_{9,12,trans,trans}$	17.6 ± 8.3	2/8	57	61.6 ± 21.8	400:30
42	$COC_{18}H_{33}\Delta_{10,13,trans,trans}$	39.2 ± 1.6	NR			
43	$COC_{18}H_{35}\Delta_{11,14,cis,cis}$	>60; 19.3 ± 2.0	NR	50	82.1 ± 17.3	200:50
44	$COC_{18}H_{35}\Delta_{11,14,trans,trans}$	28.3 ± 0.8	NR			
45	$COC_{17}H_{29}\Delta_{9,12,15,cis,cis,cis}$	22.3 ± 16.5; >60	NR	37	78.5 ± 31.9	200:10
46	$COC_{19}H_{31}\Delta_{5,8,11,14\ all\ cis}$	8.1 ± 0.7	NR	51		200:40

[a] Mouse hot plate test, subcutaneous dosing, $n = 8$; 0.68 mM = 200 mg/kg for synthetic capsaicin (**6**); response = ratio of the number of responders (defined as animals which displayed latency times > the vehicle control mean latency plus 2 standard deviations) to the number of animals tested. [b] Rat hot plate test, oral dosing, $n = 8$; max % analgesia defined as [(postdose latency time−predose latency time)/(60−predose latency time)]100 at the time of maximal latency, generally 3 or 5 h postdose. [c] Croton oil-inflamed mouse ear test, topical dosing, $n = 10$. [d] Acute toxicity after a single intraperitoneal dose; % lethality = no. of deaths/no. of animals (n = 8–10). [e] Dose = 75 mg/kg. [f] Not recorded. [g] Dose = 40 mg/kg. [h] In a modified version of this assay, where jumping was taken as an additional endpoint, the magnitude of the antinociceptive effect was substantially reduced. The potency was also reduced with an increase in the doubling dose[19] (approximately the dose required to double the pretest latency time) from 74 mg/kg to 132 mg/kg. For a possible explanation, see ref 13.

similar to codeine. In antiinflammatory testing, the potency was less than indomethacin in the carrageenan-inflamed rat paw and arachidonic acid-inflamed ear assays whereas in the croton oil-inflamed ear assay, the potency was at least equivalent to hydrocortisone. Because both these vanilloids displayed some characteristics of both opioid agonists and peripherally acting NSAID's their pharmacology was investigated further. The activity of NE-19550 in the hot plate assays was not blocked by a high dose (10 mg/kg) of the opioid antagonist naloxone. Pretreatment of rats with antiinflammatory doses of NE-19550 failed to suppress prostaglandin or thromboxane synthesis *in vitro* in whole blood or tissues (lung, brain) while indomethacin reduced prostanoid levels in each case. These results suggest that the pharmacology of NE-19550 is not opiate or NSAID-like. Further testing showed that

Table IV. In Vivo Biological Activity of Homovanillic Acid Amides



compd	R	mouse hot plate test (sc)[a] (0.68 mmol) latency (s, mean ± SD)	response	rat hot plate test (oral)[b] (300 mg/kg) max % analg	inflamed mouse ear test (top.)[c] (1% EtOH) % inhibition (mean ± SD)	toxicity (ip)[d] dose (mg/kg): % lethality
47	C_8H_{13}	40.7 ± 5.4	NR[e]		-5.8 ± 5.6	15:71
48	C_7H_{15}	41.1 ± 5.5	NR		69.4 ± 33.9	15:100
49	C_8H_{17}	58.7 ± 1.7	NR	7	89.1 ± 15.7	4:50
		>60				
		32.3 ± 0.9				
50	C_9H_{19}	22.3 ± 1.9	NR		80.7 ± 23.0	15:86
51	$C_{10}H_{21}$	5.3 ± 0.2	NR		58.1 ± 29.4	15:100
52	$C_{12}H_{25}$	5.0 ± 0.1	NR		55.3 ± 20.7	50:0
53	c-C_8H_{15}	34.2 ± 15.9	6/6	4	34.7 ± 23.6	200:80
54	$C_{10}H_{10}\Delta_9$	32.4 ± 24.1	5/8	29	98.9 ± 11.0	100:90
55	$C_{14}H_{27}\Delta_{9,cis}$			74		
56	$C_{18}H_{31}\Delta_{9,cis}$			88		
57	$C_{17}H_{33}\Delta_{8,cis}$			66		
58	$C_{18}H_{35}\Delta_{9,cis}$	27.9 ± 14.7	7/8	74[f]	95.5 ± 10.8	200:50
59	$C_{18}H_{35}\Delta_{11,cis}$			62[g]		
60	$C_{18}H_{35}\Delta_{9,trans}$			95		
61	$C_{18}H_{33}\Delta_{9,12,cis,cis}$			89		

[a] Mouse hot plate test, subcutaneous dosing, $n = 8$; 0.68 mM = 200 mg/kg for synthetic capsaicin (6); response = ratio of the number of responders (defined as animals which displayed latency times > the vehicle control mean latency plus 2 standard deviations) to the number of animals tested. [b] Rat hot plate test, oral dosing, $n = 8$; max % analgesia defined as [(postdose latency time−predose latency time)/(60−predose latency time)]100 at the time of maximal latency, generally 3 or 5 h postdose. [c] Croton oil-inflamed mouse ear test, topical dosing, $n = 10$. [d] Acute toxicity after a single intraperitoneal dose; % lethality = no. of deaths/no. of animals ($n = 7$–10). [e] Not recorded. [f] In a modified version of this assay, where jumping was taken as an additional endpoint, the magnitude of the antinociceptive effect was slightly reduced. The potency was also reduced with an increase in the doubling dose[19] (approximately the dose required to double the pretest latency time) from 23 mg/kg to 33 mg/kg. For a possible explanation, see ref 13. [g] Dose = 150 mg/kg.

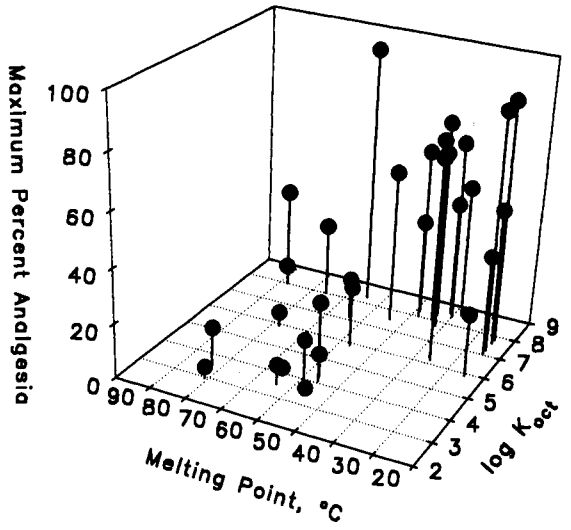


Figure 1. Oral activity of vanilloids in the rat hot plate test (z axis), plotted versus the melting point and octanol/water partition coefficient of each compound. Compounds that were oils at room temperature are plotted with a melting point of 25 °C.

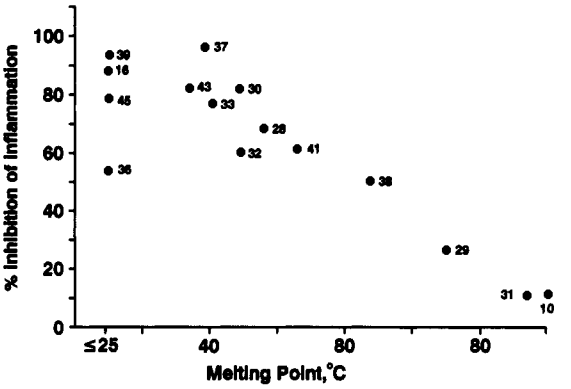


Figure 2. Antiinflammatory activity of C_{18}–C_{20} vanillylamine amides in the croton oil-induced inflamed mouse ear test, plotted versus the melting point of the compound. The number by each point is the compound identification number in Tables I and III.

a variety of neuronal antagonists (adrenergic, serotonergic, dopaminergic, cholinergic, GABA-ergic, histaminergic) did not inhibit NE-19550 antinociception on the hot plate.

Despite the lack of effect of the well-known antagonists listed above, there is evidence that vanilloid effects are receptor mediated. Early circumstantial evidence was based on the cellular specificity of capsaicin. *In vivo*, capsaicin selectively excites C-fibers without affecting other sensory neurons. In neonatal rats, capsaicin treatment leads to a selective loss of C-fibers as well as some A-δ fibers.[22] On the basis of the structural requirements established for the pungency of a variety of vanilloids, Szolcsanyi and Jancso-Gabor proposed a hypothetical model for the capsaicin receptor.[23]

Recent experiments have provided direct evidence for a capsaicin receptor which is coupled to a nonselective cation channel in the cell membrane of sensory neurones. The evidence includes the following: capsaicin-evoked uptake of Ca and Na ions by a variety of cells in vitro,[24] receptor labeling with a photoaffinity probe,[25] binding studies with resiniferatoxin, a hybrid of the capsaicin-like aromatic group and a phorbol-related diterpene, which possesses ultrapotent capsaicin-like activity,[26,27] and the existence of a competitive antagonist, capsazepine.[28–30]

With this new evidence that vanilloid biological effects are receptor-mediated and involve opening of an ion channel, Szolcsanyi has categorized the biological response to capsaicin into four stages which are dependent on concentration, dose, and exposure time:[5c] (1) excitation,

Table V. Comparison of NE-19550 (30) and NE-28345 (58) in a Variety of Antinociceptive and Antiinflammatory Assays

assay	route	NE-19550	NE-28345	benchmark compd
	Antinociceptive Assays (ED_{50} (mg/kg))			
rat hot plate	po	171	47	63 (codeine)
	sc	8	2	
	iv	0.5	0.3	
rat tail flick	po	321	318	12 (codeine)
mouse hot plate	sc	245	~300	59 (codeine)
mouse abdominal constriction	po	170	16	80 (codeine)
	sc	18		
	Antiinflammatory Assays			
carrageenan-inflamed rat paw	po (ED_{35})	195	85	1.4 (indomethacin)
croton oil-inflamed ear	1% topical	81% inhib	84% inhib	63% (hydrocortisone)
arachidonic acid-inflamed ear	1% topical	16% inhib	28% inhib	50% (indomethacin)
	Other Assays			
naloxone[a] reversal of antinociception	po	no	no	
blepharospasm[b]	1% intraocular	yes	yes	
thermoregulatory deficit[c]	po	no	no	
	sc	yes	yes	
acute lethality (LD_{50})	ip	350	>100; <1000	7 (capsaicin)

[a] Dose = 10 mg/kg, sc. [b] Eye wipe response (slow response, see text). [c] Difference in rectal temperature change versus vehicle control in response to 37.8 °C heat stress at doses up to 100 mg/kg.

(2) sensory neurone blockade, (3) long-term selective neurotoxic impairment, and (4) irreversible cell destruction. For the development of a useful vanilloid drug, one would want to avoid the neurotoxicity inherent in stages 3 and 4. The reversible desensitization, or stage 2 effect, is therefore the target for drug development. The antinociception observed on the hot plate for the compounds in Tables III and IV is reversible in hours to days which suggests that irreversible neurotoxicity is not involved. However, significant losses of unmyelinated axons in the rat ureter were found by electron microscopy 7 days after a 300 mg/kg oral dose of antinociceptive vanilloids, whereas no loss was observed after dosing an inactive analog. Using the method of Coggeshall,[31] the results (mean ± SE), expressed as a percent of the number of bundles of unmylenated axons observed in vehicle-treated rats, are: NE-19550, 36 ± 4% (n = 15); NE-28345, 44 ± 10% (n = 5); and for the inactive analog **10**, 104 ± 10% (n = 5).

One would also like to eliminate or minimize the stage I effects which, for example, cause a burning sensation that limits the utility of topical capsaicin for a variety of cutaneous disorders where there is evidence for a therapeutic effect (e.g., psoriasis, postherpetic neuralgia, and others).[32] There is some evidence that the stage I excitatory effect of NE-19550 is diminished relative to capsaicin based on the failure of NE-19550 to activate peripheral nerve fibers in a neonatal rat tail/spinal cord preparation *in vitro*.[33] Given that NE-19550 and capsaicin have similar antinociceptive potency *in vivo* according to our own work (Table V) and independent literature reports in the mouse (tail flick, acetic acid-induced writhing, phenylquinone-induced writhing,[12] yeast-induced inflammation) and rat (paw pressure),[34] these results suggest that NE-19550 may induce analgesia with little initial activation of nociceptors. However, an *in vivo* assessment of stage 1 effects using the blepharospasm response (eye blinks or wipes) upon instillation of dilute solutions into the eye of a rat noted considerable irritancy for NE-19550 when the observation time was extended from 60 s to 30 min.[35] No vanilloid analog was found to be desensitizing without first being irritating. This suggests that the two processes may be linked. The initial excitation would represent opening of a cation channel and depolarization of the C-fiber. The subsequent influx of ions produces changes that render the nerve incapable of response to additional noxious stimuli. These data suggest that the differences in bioavailability may account for the apparent change in irritancy of long-chain vanilloids. These compounds are much less water-soluble than capsaicin and would consequently be expected to diffuse less efficiently across biological barriers.

In summary, capsaicin analogs (vanilloids) with variations on the alkyl chain part of the molecule have been prepared. These compounds are amides of vanillylamine (as is the parent capsaicin) or amides of homovanillic acid in which the orientation of the amide is reversed. *In vivo* tests for antinociceptive (mouse and rat hot plate assay) and antiinflammatory (croton oil-inflamed mouse ear assay) effects demonstrate that the incorporation of long unsaturated chains can significantly change the profile of biological activity compared to capsaicin. While compounds with a large range of alkyl chain lengths show reversible antinociceptive and antiinflammatory effects after systemic administration, vanilloids with long unsaturated chains show several potential advantages. The oleic acid and oleylamine derived compounds, NE-19550 and NE-28345, for example, are orally active, have reduced pungency, and are less acutely toxic. The antinociceptive and antiinflammatory effects may be due to selective sensory neurone blockade. More work needs to be done to substantiate this mechanism of action and to determine if the excitatory and neurotoxic side effects of vanilloids can be minimized or eliminated.

Future papers will address the antinociceptive and antiinflammatory effects of vanilloid analogs with changes in the midregion and ring.

Experimental Section

Melting points were obtained with a Thomas-Hoover melting point apparatus or hot-stage microscope and are uncorrected. Infrared spectra were obtained with a Perkin-Elmer Model 298 infrared spectrophotometer. ^{1}H NMR spectra were obtained with either a Varian T-60 or a General Electric QE-300 spectrometer. Mass spectra were obtained on a Hewlett-Packard 5985B GC/MS system. TLC analyses were obtained in the indicated solvent systems with Analtech Uniplates, 2.5 × 10 cm, silica gel GF; the plates were visualized by dipping them in 5%

phosphomolybdic acid in 2-propanol or 95:5 ethanol/sulfuric acid and by heating them on a hot plate. Dry THF and dry ether were obtained by distillation from sodium/benzophenone under argon immediately before use. Octanol/water partition coefficients were estimated using the MEDCHEM CLOGP program[36] plus a correction based on HPLC retention times of selected compounds versus known standards.[37]

The acids and acid chlorides needed for the synthesis of the vanillylamine amides in Table I were obtained as follows: The saturated acyclic acids and acid chlorides were obtained from Aldrich Chemical Co. The ω-phenylalkanoic acids were obtained from Fairfield Chemical Co. *p*-Pentylbenzoyl chloride was from Kodak Chemicals. All the unsaturated fatty acids, with the exceptions noted below, were high purity materials from Nu Chek Prep. 9-Ketostearic acid (for the synthesis of analog **17**) was prepared from octylmalonic acid and 8-carbomethoxyoctanoyl chloride (from azelaic acid monomethyl ester) via the method of Bowman and Fordham[38] followed by hydrolysis (KOH in EtOH/H_2O). Methyl 9-ketostearate was olefinated with methylenetriphenylphosphorane[39] and then hydrolyzed, as mentinoed above, to give 9-methylenestearic acid for the preparation of analog **37**. The olefinated ester was reduced with palladium on charcoal and hydrolyzed to give the methyl-branched acid for the synthesis of analog **16**. The epoxide analogs **18** and **19** were prepared by treating the corresponding olefins (analogs **30** and **31**) with *m*-chloroperbenzoic acid. *cis*-8-Methyl-6-nonenoic acid for the preparation of *cis*-capsaicin (analog **20**) was prepared by the reaction of 3-methyl-1-butyne with 5-bromopentanoic acid according to the method of Ames and Covell[40] followed by Lindlar reduction to the *cis*-olefin. This same sequence of steps was used to prepare *cis*-9 dodecanoic acid (from 1-butyne (10 equiv) and 8-bromooctanoic acid) for the preparation of analog **23**. *cis*-2-Octadecenoic acid for the preparation of analog **27** was made by reacting 1-heptadecyne (Farchan) with EtMgI and bubbling CO_2 through the resulting magnesium salt.[41] Catalytic reduction ($Pd/BaSO_4$/quinoline in MeOH) gave the *cis*-acid. 9-Octadecynoic acid (stearolic acid) for the preparation of analog **38** was prepared by the method of Butterfield and Dutton[42a] and was purified by recrystallization from petroleum ether.[42b] Analog **36** was derived from 12-hydroxy-*cis*-9-octadecenoic acid (ricinoleic acid, Nu Chek Prep) which was esterified (MeOH/HCl), silylated (TBDMSCl/imidazole/DMF), hydrolyzed (5 N NaOH/MeOH), and coupled as indicated in Table I. The silyl protecting group was removed (HOAc/THF/H_2O) to give the final product.

The amines used to prepare the analogs in Table II were obtained as follows: The saturated alkylamines were obtained from Aldrich. 1-Amino-9-decene, the precursor to analog **54**, was prepared from 9-decen-1-ol by conversion to the azide (2-fluoro-*N*-methylpyridinium tosylate/Et_3N then NaN_3/HMPA), followed by reduction with LAH.[43] *cis*-9-Tetradecylamine needed for the preparation of analog **55** was made from *cis*-9-methyltetradecenoate (methyl myristoleate, Nu Chek Prep) by conversion to the amide (NH_3/MeOH) followed by LAH reduction. *cis*-9-Hexadecylamine for the preparation of analog **56** was made from *cis*-9-hexadecen-1-ol by conversion to the mesylate (MsCl/pyridine) and then the azide (NaN_3/benzene/H_2O/Aliquot 336) and finally reduction ($SnCl_2$/MeOH) to the amine. Analog **57** was prepared from *cis*-8-heptadecenylamine which was prepared from *cis*-9-octadecenoic acid (oleic acid) by a variant of the Curtius reaction.[44] The amines required for the synthesis of analogs **58**, **60**, and **61** were prepared from the corresponding mesylates (Nu Chek Prep) via the Gabriel synthesis.[45] In each case the unsaturated amines were accompanied by 4–11% of the corresponding saturated amine. Pure oleylamine could be prepared by conversion of oleyl mesylate to the iodide (3.2 equiv of NaI, acetone, reflux) followed by reaction with sodium trifluoroacetamide according to the method of Harland et al.[46] *cis*-11-Octadecenylamine for the preparation of analog **59** was made from dodecyn-1-ol (mixture of isomers, Wiley) by the following procedures: (1) isomerization of the triple bond to produce 11-dodecyn-1-ol (with sodium rather than potassium 3-aminopropylamide, 55 °C, 150 min),[47] (2) alkylation with hexyl bromide (2 equiv of LDA in HMPA, RT, 2 h), (3) reduction ($Pd/CaCO_3$/quinoline), (4) conversion to the mesylate (MsCl, Et_3N, hexane, 0 °C, 2 h), and (5) conversion to the amine via the Gabriel synthesis referenced above.

Vanillylamine Amide Syntheses. Method A. The carboxylic acid was dissolved in neat oxalyl chloride (2–10 equiv) and was stirred for 3 h or until gas evolution ceased. The excess oxalyl chloride was evaporated. Volatile acid chlorides could be distilled, but generally the acid chlorides were used without further purification.

Vanillylamine hydrochloride was suspended in DMF (or THF), and 5 N NaOH (2 equiv) was added. The mixture was stirred for 30 min and cooled to 0 °C, and the acid chloride (1–1.2 equiv) was added dropwise in ether (or $CHCl_3$). The reaction was allowed to warm to room temperature and was stirred for 3–24 h. The reaction was poured into water (about 10× the volume of DMF; when THF was used as solvent, it was evaporated and ether was added). The layers were separated, and the aqueous layer was extracted with ether or chloroform. The combined extracts were washed with 1 N HCl, saturated $NaHCO_3$, water, and brine and then dried over $MgSO_4$. The solvent was removed in vacuo. The crude product was purified by silica gel flash chromatography and/or crystallization as indicated in Table I. Note that the workup can be modified to remove nonacidic impurities by extraction of the product from the organic phase into 2.5 N NaOH. Separation of the layers and acidification of the aqueous layer with HCl followed by ether extraction leads to recovery of product. This procedure is suitable only for the short-chain compounds. Longer chain compounds (e.g., oleyl) give emulsions.

Method B. The acid chloride was prepared as described in A. Vanillylamine hydrochloride was suspended in water (25 mL/10 mmol), and Na_2CO_3 (1–2 equiv) was added. The mixture was stirred for 10 min and cooled to 0 °C, and an equivalent volume of ether was added. The acid chloride (1 equiv) in ether was added dropwise, and the reaction was stirred for 3 h as it warmed to room temperature. The layers were separated, and the workup was continued as described for A.

Method C. Protected vanillylamine (4-ethylvinyl ether, 1 equiv) and the desired carboxylic acid (1.05 equiv) were combined in CH_2Cl_2 and cooled to 0 °C. *N,N'*-Dicyclohexylcarbodiimide (1.05 equiv) was added in a small amount of CH_2Cl_2 followed by addition of 4-(dimethylamino)pyridine (0.1 equiv). The stirred reaction mixture was allowed to warm to room temperature over 1 h. The precipitate was filtered and the filtrate washed with 1 N HCl, saturated $NaHCO_3$, H_2O, and brine and then dried over $MgSO_4$. The solvent was removed in vacuo. Hydrolysis of the protecting group (0.8 equiv of 1 N HCl, THF, rt, 45 min) gave the final product.

Homovanillic Acid Amide Syntheses. Method D. 4-Acetoxy-3-methoxyphenylacetic acid and $SOCl_2$ (3–12 equiv) were refluxed until gas evolution ceased. The excess $SOCl_2$ was evaporated. Benzene was added and evaporated to help remove the last traces of $SOCl_2$. The resulting acid chloride was dissolved in benzene (~20 mL/10 mmol) and cooled to 0 °C, and the amine (2.0 equiv) in ether or chloroform (approximately half the volume of benzene used) was added dropwise. The stirred reaction was allowed to warm to room temperature over 3 h. The precipitate was filtered and rinsed with ether. The filtrate was washed with H_2O and brine and then dried over $MgSO_4$. The solvent was removed in vacuo to give the protected amide. This was dissolved in MeOH (~25 mL/10 mmol), and 5 N NaOH (1.2–2.0 equiv) was added. The mixture was stirred for 3 h at room temperature, and the solvent was evaporated. The residue was dissolved in 1 N NaOH, extracted with ether, acidified while cooling with HCl, and extracted three times with ether. The combined extracts were washed with H_2O and brine and then dried over $MgSO_4$. The solvent was removed in vacuo to give crude product.

Method E. Similar to method D but done in $CHCl_3$ or CH_2Cl_2 with 1 equiv of amine and 1 equiv of Et_3N. When complete, the reaction was poured into water and the layers were separated. The organic layer was washed with 1 N HCl, and the workup and hydrolysis were continued as described for method D. The ether extraction of the NaOH solution was either omitted or done with gentle shaking to avoid emulsion formation.

Method F. The desired amine (1 equiv) and ethyl homovanillate (1–1.2 equiv) were combined and heated to 170 °C allowing the EtOH produced to distill off. After 3 h, a slight vacuum was applied to remove any remaining EtOH. The reaction was cooled, diluted and ether, washed with H_2O, 1 N HCl, $NaHCO_3$, and

brine, and then dried over $MgSO_4$. The solvent was removed in vacuo to give crude product.

4-Acetoxy-3-methoxyphenylacetic acid. Homovanillic acid (30 g, 0.17 mol), acetic anhydride (75 mL, 0.79 mol) and 2 drops of concentrated H_2SO_4 were combined and stirred at room temperature overnight. The reaction was poured into 1.8 L of H_2O and stirred for 3 h. The precipitate was filtered and the filtrate volume reduced to about 400 mL and additional precipitate collected. These were combined and crystallized from hot H_2O, filtered, and dried to give 29.4 g (79%) of product, mp 138–139 °C (lit.[48] mp 139–140 °C).

Biological Evaluations. Hot Plate Tests. Sprague–Dawley rats, 100–200 g, were double housed and acclimated for at least 4 days before initiation of the study. Rats were fasted for 18 h predose, and food was returned 5 h postdose. For thermal antinociception studies, a hotplate was used. The apparatus consisted of an insulated water-filled box with a copper surface maintained at 55 ± 0.5 °C by a circulating water bath. A bottomless glass container 10 cm high by 19 cm in diameter with a 7.5 cm diameter opening in the top was used to restrict the rat to a defined area of the hot plate. The animal was placed through the opening and a watchglass was placed over the opening to prevent the rat from escaping. Latency times were measured with a stopwatch. The endpoint was taken when the hindpaw reached the mouth (touch or lick). A 60 s limit was imposed on the latency time to prevent tissue damage. The rats were prescreened on the hotplate, and those with latencies of <12 s were used. In some cases when an insufficient number of animals met the prescreening criterion, the remaining animals were retested and those with the lowest latency times (all <30 s) were used. This latency time was recorded as the pretest time (0 h). Rats were randomized into groups of 8 and were dosed orally with vehicle control (sesame oil) or vanilloid in sesame oil (1 mL/200 g body weight). Latency times were measured at 1.5, 3, 5 h, and, when antinociception persisted, at 24 h postdose. Percent analgesia was calculated as follows: $[(LT(t) - LT(0))/(60 - LT(0))]100$ where $LT(t)$ is the latency time at time t and $LT(0)$ is the pretest latency time (typically 8 s). The mouse hot plate test was done similarly with male CF-1 mice, 8 animals/group, dosed sc with drug in 0.2 mL of ethanol/Tween-80/saline (10/10/80). The data are expressed as mean latency time (seconds) ± standard deviation (pretest latency ~5 s).

Inflamed Mouse Ear Test. Male Cox ICR mice (25–35 g), 10 animals/group, were treated topically on the left ear with 25 μL of a 1% solution of the test compound in ethanol. Approximately 17 h later, they were treated identically. Control animals were treated identically with vehicle only. One hour later, both ears were treated with 25 μL of 2% croton oil. Four hours later, the animals were sacrificed, the ears removed, and 5 mm punch biopsies taken from each ear and weighed to the nearest 0.1 mg on a Cahn electrobalance. Treatment effects were expressed as percent inhibition of swelling in the drug-treated versus the control group. This was calculated according to the formula: $[(C - T_i)/C]100$, where C is the average difference between the left and right ear weights of the control group and T_i is the difference between the left and right ear weights for a drug-treated animals. Individual values of % inhibition were averaged to obtain the reported result.

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Supplementary Material Available: Spectroscopic data for all compounds and details of the log P calculations (7 pages). Ordering information is given on any current masthead page.

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