Synthesis and Evaluation of Phorboid 20-Homovanillates: Discovery of a Class of Ligands Binding to the Vanilloid (Capsaicin) Receptor with Different Degrees of Cooperativity

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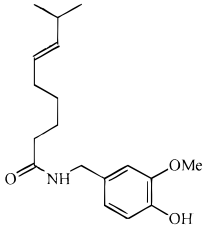
A number of phorboid 20-homovanillates were prepared by condensation of phorbol 12,13-diesters and 12-dehydrophorbol 13-esters with Mem-homovanillic acid followed by removal of the protecting group with $SnCl_4$ in THF. These compounds were evaluated for their ability to inhibit [^{3}H]resiniferatoxin (RTX) binding to rat spinal cord membranes. Compounds bearing a lipophilic ester group on ring C were considerably active, but a surprising tolerance of the vanilloid receptor toward the location and the orientation of this ester group was disclosed. Unexpectedly, these ligands could also diminish, to a variable degree, the positive cooperativity which characterizes RTX binding to the vanilloid receptor. Phorbol 12-phenylacetate 13-acetate 20-homovanillate (PPAHV, **6a**), a compound which abolished binding cooperativity, was further tested in a variety of *in vivo* assays used to characterize vanilloid-like activity. PPAHV showed only a marginal pungency and failed to induce a measurable hypothermia response at doses (up to 200 mg/kg) at which it effectively desensitized against neurogenic inflammation. These data suggest that the peculiar binding behavior of these ligands might be associated with a distinct spectrum of biological activity.

Introduction

Capsaicin (CPS, **1**), the pungent principle of hot pepper, excites and then desensitizes a subset of primary sensory neurons involved in nociception, neurogenic inflammation, thermoregulation, and a variety of local regulatory functions.[1,2] Upon binding to a specific receptor, CPS opens a ligand-gated channel permeable to both monovalent and divalent cations.[3] This leads to calcium influx and a subsequent release of inflammatory neuropeptides (e.g., tachykinins, calcitonin gene-related peptide) which mediate the afferent and efferent functions of CPS-sensitive neurons.[1−3] The remarkable selectivity of CPS for primary sensory neurons makes desensitization to CPS an attractive therapeutical approach to mitigate neurogenic pain and inflammation.[4−6] In fact, CPS-containing creams (e.g., Axsain, Zostrix) are already commercially available for these purposes, but irritance severely limits the pharmacological use of CPS. The synthesis of capsaicin-like compounds with an improved desensitization/irritation ratio or with a selective spinal antinociceptive activity has thus been pursued, with very limited success, for decades.[7−13] These efforts have been further stimulated by the discovery of a naturally occurring ultrapotent agonist (resiniferatoxin, RTX, **2**)[14,15] and by the synthesis of a competitive antagonist (capsazepine, **3**).[16]



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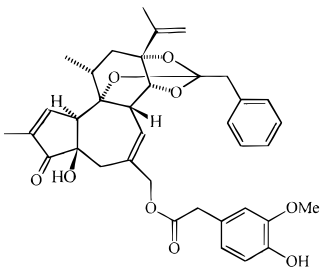

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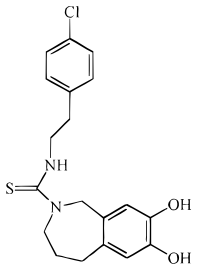

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RTX is structurally related to phorbol esters, but it neither acts as a tumor-promoter[17] nor binds to the PKC isoforms targeted by phorbol esters.[15] Though structurally and biogenetically unrelated in their carbon skeleton, RTX and CPS share a vanillyl (4-hydroxy-3-methoxybenzyl) moiety which is essential for their biological activity; thus the common membrane recognition site for these compounds was named the 'vanilloid' receptor.[18] Specific binding of [^{3}H]RTX provided the first direct proof for the existence of this receptor[19] and

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was then used to visualize CPS-sensitive neurons by an autoradiographic approach and to explore their pharmacology.[20]

As a general rule, vanilloid receptors bind CPS and RTX in a positive cooperative fashion.[21] The biological role for this binding behavior is unknown, but it might well serve to amplify the effects of endogenous ligand(s) produced in critically low concentrations.[22] Positive binding cooperativity thus points to the existence of endogenous vanilloids. Although RTX mimics CPS qualitatively, it shows striking differences in potency relative to CPS, ranging from several thousandfold higher potency to equipotency depending on the biological end points examined.[15,18] Moreover, RTX also has unique actions, which might contribute to its broad therapeutic range. For example, unlike capsaicin, RTX is able to desensitize certain end points (e.g., pulmonary J1 receptors) without any apparent prior excitation.[15,18] As yet, it is unclear to what extent these differences between RTX and CPS actions reflect receptor heterogeneity, but the observation that vanilloids show different structure−activity relations for receptor binding and stimulation of calcium uptake, respectively, suggests the existence of distinct receptor subclasses.[23,24]

Capsaicinoids are relatively simple from the structural point of view, and their structure−activity relationships have been explored in depth.[7−13] By contrast, limited information exists on the RTX pharmacophore.[15,23−25] The homovanillyl residue at C-20 and the lipophilic orthoester group on ring C are both necessary for the activity,[15] but the key elements of the terpenoid core are still ill defined, since RTX is not commercially available in synthetically useful amounts and is of difficult and limited accessibility from natural sources.

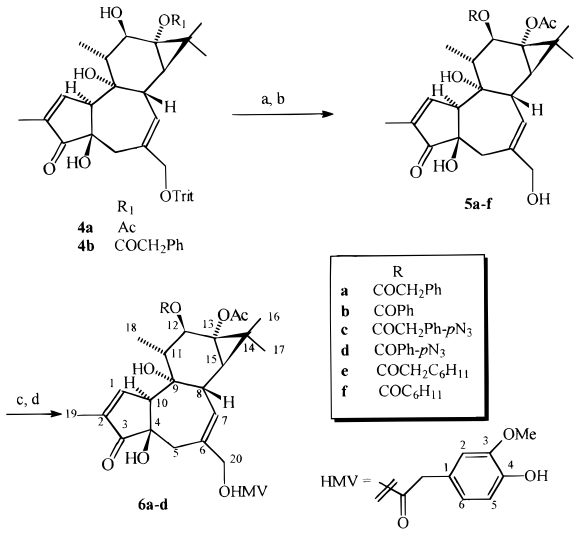
The therapeutical potential of RTX[26] has stimulated synthetic activity aimed at the total synthesis of the biologically active portion of the molecule and eventually of RTX itself.[27] In the context of a more modest but potentially rewarding project, we have investigated the possibility to obtain vanilloids structurally related to RTX using phorbol as starting material. This compound is related to the terpenoid core of RTX (resiniferonol), and the acylation of certain phorbol-related diterpenoids [12-deoxyphorbol 13-phenylacetate, 20-deoxy-20-aminophorbol 12,13-bis(benzoate)] with homovanillic acid has already yielded vanilloids with unique activity.[25,28] However, the possibility of using phorbol itself for the synthesis of vanilloids has so far been largely unaddressed, despite its availability from a commercial source (croton oil)[29] and the fact that its chemistry has been extensively investigated,[29] affording a large pool of related substrates (phorbobutanone, crotophorbolone, 4α-phorbol, neophorbol, phorboisobutanone)[29] to test the topology of the vanilloid binding site.

In spite of these potential advantages, the development of phorbol-based ligands has so far been hampered by the lack of an efficient synthetic method for the preparation of 20-homovanillyl esters of these functionalized tiglianes.[30] We report here an efficient protocol to solve this problem and the biological evaluation of a series of phorbol 20-homovanillates prepared in this way.

Chemistry

Using known phorbol chemistry,[29] the 12-acyl 13-acetyl diesters **5a**−**f** were prepared from phorbol 13-acetate 20-trityl ether (**4a**) (Scheme 1).[31] To investigate the introduction of the homovanillyl group, the primary allylic alcohol at C-20 was esterified with a series of protected [acetyl (Ac), triethylsilyl (Tes), methoxymethyl (Mom), (methoxyethoxy)methyl (Mem)] homovanillic (HMV) acids, and the deprotection was tested under standard conditions (pyrrolidine−water or $NaHCO_3$ for the acetyl group,[27] fluorides for the Tes group, H^+ or Lewis acids ($ZnBr_2$, $MgBr_2$) for the Mom, Mem, and Tes groups). Mixtures of products were obtained, resulting from competitive hydrolysis of the 12- and 13-esters, loss of the homovanillyl moiety, and/or degradation of the acid- and base-sensitive terpenoid core. After considerable experimentation, we eventually found that the Mem group could be removed in high yield with $SnCl_4$ in THF. In these conditions, the Mom group could not be cleaved so effectively, presumably because of poorer chelation. $SnCl_4$ is insoluble in THF, but, as the reaction proceeded, a homogeneous solution was obtained. The slow delivery of the oxyphilic species is important, since in CH_2Cl_2 the yield was lower and byproducts were observed. This protocol to introduce the homovanillyl moiety (esterification with Mem-HMV acid and deprotection with $SnCl_4$ in THF) was used throughout.

Scheme 1[a]

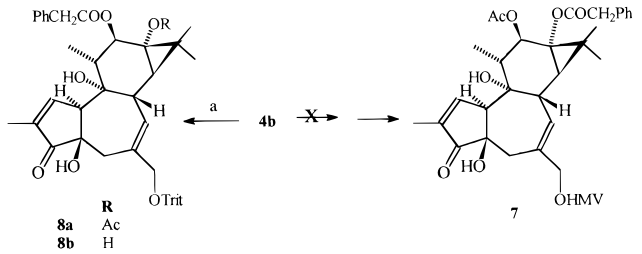


[a] (a) RCOOH (3 mol equiv), DCC, DMPA, CH_2Cl_2; (b) 0.01 N $HClO_4$, MeOH (60−80% from **4a**); (c) Mem-HMVA, DCC, DMAP, CH_2Cl_2 (80−95%); (d) $SnCl_4$, THF (70−85%).

Attempts to prepare analogues of the type **7**, with a reverse location of the 12,13-diester groups, failed, since acyl rearrangement took place in the acetylation of 13-acylphorbol 20-trityl ethers. Thus, treatment of 13-(phenylacetyl)phorbol 20-trityl ether (**4b**) with acetic acid−DCC gave phorbol 12-phenylacetate 13-acetate 20-trityl ether (**8a**) as the major reaction product, along with the 12-monoester **8b** (Scheme 2). **8a** was also the major reaction product when the acetylation was carried out with Ac_2O−pyridine or acetyl choride−triethylamine. The location of the ester group of **8a** was verified by NOESY spectroscopy and further confirmed by inspection of the long-range (HMBC) spectrum of the homovanillate **6a** obtained from this compound.

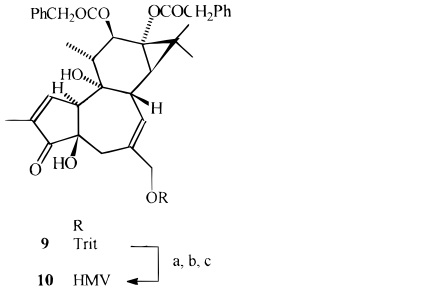
The 12,13-bis(phenylacetate) 20-homovanillate **10** was prepared from the corresponding 20-trityl ether (Scheme 3). The 12-dehydrophorbol homovanillates **12a,b** were prepared from the corresponding 13-esters by oxidation (PCC) and homovanyllation in the usual

Scheme 2[a]



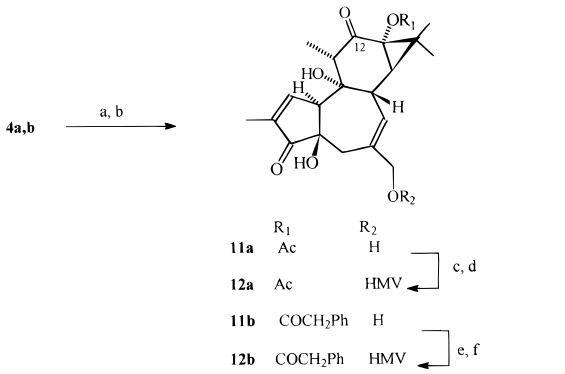
[a] (a) AcOH, DCC, CH_2Cl_2 (30% **8a**, 15% **8b**).

Scheme 3[a]



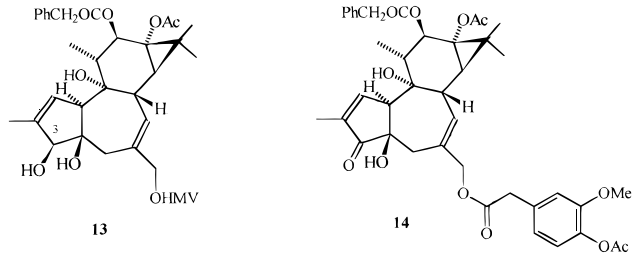
[a] (a) 0.01 N $HClO_4$, MeOH; (b) Mem-HMVA, DCC, DMAP (65% from **9**); (c) $SnCl_4$, THF (77%).

Scheme 4[a]



[a] (a) PCC, CH_2Cl_2; (b) 0.01 N $HClO_4$, MeOH (52% from **4a**, 48% from **4b**); (c) Mem-HMVA, DCC, DMAP (95%); (d) $SnCl_4$, THF (78%); (e) Mem-HMV, DCC, DMAP (75%); (f) $SnCl_4$, THF (82%).

way (Scheme 4), and the 3β-alcohol **13** was prepared from the borohydride reduction of the enone **6a** under Luche conditions.[32] The acetate **14** was prepared by esterification of **5a** with acetylhomovanillic acid.[33]



Biological Results

From the radioligand binding experiments listed in Table 1, it is evident that, with the exception of **6f**, **12a**, and **13**, all the ligands synthesized are more potent than capsaicin to compete for specific [^{3}H]RTX binding sites in rat spinal cord membranes, although none of them approached the very high affinity (20 pM) of RTX. Parameters of RTX binding to rat spinal cord membranes were analyzed in the presence of increasing

Table 1. Binding Affinity of Vanilloids to Vanilloid Receptors in Rat Spinal Cord Membranes and Their Effect on the Cooperativity of Resiniferatoxin Binding, as Quantitated by the Hill Coefficients[a]

compd	affinity (K_i, μM)	Hill coefficient
capsaicin (**1**)	2.0 ± 0.3	2.0 ± 0.2
RTX (**2**)	0.000 02	2.3 ± 0.1
capsazepine (**3**)	1.2 ± 0.1	2.4 ± 0.2
6a	0.6 ± 0.3	1.1 ± 0.2
6b	0.4 ± 0.2	1.7 ± 0.3
6c	0.2 ± 0.1	1.4 ± 0.3
6d	0.2 ± 0.2	1.5 ± 0.2
6e	0.4 ± 0.1	1.1 ± 0.1
6f	2.2 ± 0.1	1.0 ± 0.1
10	0.3 ± 0.1	1.3 ± 0.1
12a	>10	
12b	1.3 ± 0.1	0.9 ± 0.1
13	>10	
14	1.7 ± 0.7	1.0 ± 0.1

[a] Mean ± SEM; each experiment was performed at least three times.

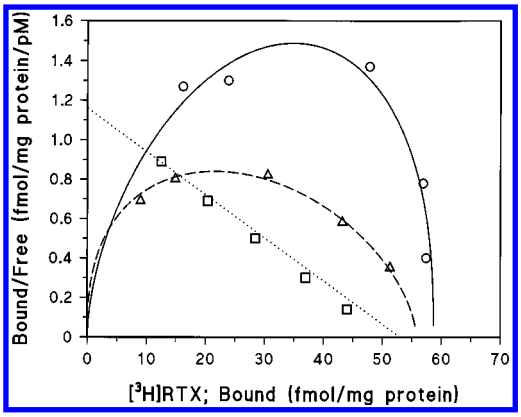


Figure 1. Scatchard plots of specific binding of [^{3}H]RTX to rat spinal cord membranes in the absence (○) or presence of 0.3 μM **6d** (△) or 1.0 μmM **6f** (□). Observe that the convexity of the Scatchard plot, indicative of positive cooperative binding, is diminished in the presence of **6d** and completely abolished (linear plot) in the presence of **6f**. The experiments shown are representative. For binding parameters (average ± SEM), see Table 1.

concentrations (6−400 pM) of [^{3}H]RTX and the concentration of phorboid 20-homovanillates which inhibited RTX binding by 50% in the competition experiments. It turned out that those ligands which competed for RTX binding sites also reduced the positive cooperativity characteristic of RTX binding to rat spinal cord membranes (Table 1, Figure 1). There appears to be little or no correlation between binding affinities and apparent Hill coefficients. The maximal density of binding sites (B_{max} values) was, however, not affected. **6a** also inhibited RTX binding to rat urinary bladder (K_i = 2.0 μM) membranes (a single experiment, not shown). **6a** and RTX showed the same rank order of affinity using rat, porcine, and human spinal cord preparations (rat > pig > human). Inhibitory constants (K_i) for RTX were 54 ± 6 pM in rat, 120 ± 15 pM in pig, and 450 ± 52 pM in human spinal cord, respectively (mean ± SEM, three determinations). **6a** inhibited [^{3}H]RTX binding to rat, pig, and human spinal cord membranes with K_i values of 0.6 ± 0.3, 2.1 ± 0.4, and 6.4 ± 1.2 μM, respectively (mean ± SEM, three measurements). As **6a** is a vanilloid receptor agonist in *in vitro* calcium influx experiments,[34] this ligand was further characterized in a variety of *in vivo* assays used to measure vanilloid-like activity. **6a** was approximately 100-fold less potent than capsaicin to provoke eye-wiping movements when

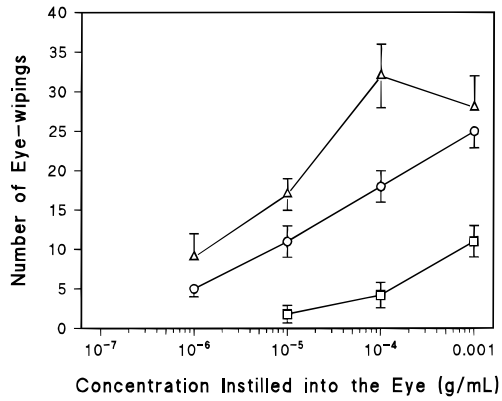


Figure 2. Protective eye-wiping movements with the forelegs in response to instillation of RTX (△), CPS (○), or **6a** (□) into the eyes of rats in the indicated concentrations (mean ± SD for eight animals).

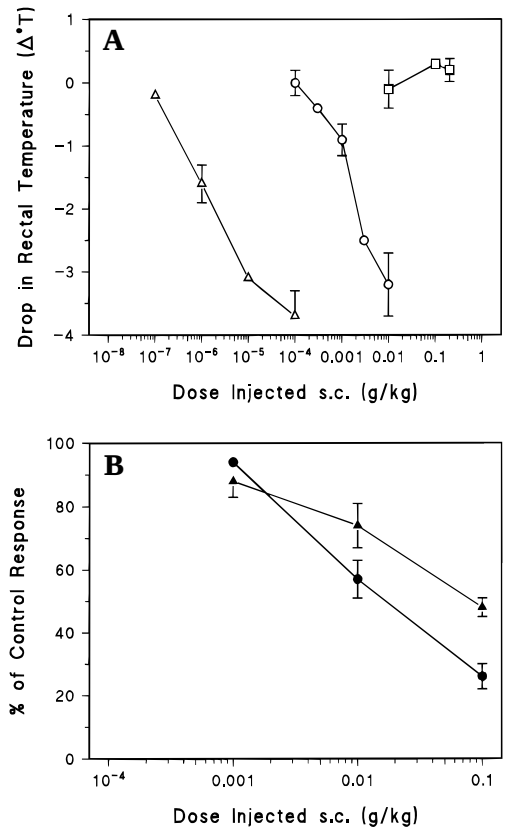


Figure 3. A: Hypothermia in rats by RTX (△) and CPS (○) and the lack of this response by **6a** (□), as determined 1 h after sc injection of the compounds at the indicated doses (mean ± SD for 10 animals). B: **6a**-treated animals from experiment A (distributed randomly into two groups) 24 h later showing a dose-dependent decrease in ear edema formation in response to topical RTX administration (●) as well as a concomitant loss of specific [^{3}H]RTX binding sites in spinal cord preparations (▲) (mean ± SD for five animals).

instilled into the eye of rats (Figure 2). At doses up to 10 mg/ear, **6a** failed to induce an erythema response (not shown). When given sc, **6a**, up to 200 mg/kg, did not cause any measurable hypothermia response (30 min, 1 h, 2 h postinjection) (Figure 3A), although at the examined doses it desensitized against RTX-induced ear edema formation (with an estimated ED_{50} of 10 mg/kg, determined 24 h after the administration of **6a**) and depleted vanilloid receptors from the spinal cord (Figure 3B). The subcellular mechanism(s) underlying *in vivo* vanilloid receptor loss following systemic vanilloid treatment is(are) essentially unknown, but this receptor loss is entirely due to a reduction of B_{max}.[35] In parallel experiments, both RTX (with an ED_{50} of 3 μg/kg) and capsaicin (with an ED_{50} of 3 mg/kg) induced a maximal (approximately 4 °C) drop in rectal temperature when measured 1 h after treatment (Figure 3A).

Discussion

Phorbol 12-phenylacetate 13-acetate 20-homovanillate (PPAHV, **6a**) inhibited specific binding of [^{3}H]RTX to vanilloid receptors in rat spinal cord membranes with an affinity (K_i = 0.6 μM) higher than that of capsaicin (K_i = 2.0 μM) but lower than that of RTX (K_i = 20 pM) (Table 1). Replacement of the phenylacetyl moiety with related acids containing a lipophilic aromatic group [benzoic (**6b**), *p*-azidophenylacetic (**6c**), *p*-azidobenzoic (**6d**)] led to a modest increase of the affinity, as did replacement with a cyclohexylacetyl group (**6e**), whereas a cyclohexanecarboxylate group decreased the activity (**6f**). None of these compounds approached the affinity of RTX, but their activity is surprising, since all diterpenoid ligands of the vanilloid receptors reported to date have a lipophilic ester group on the opposite (α) face of ring C.[15,23−25,28]

Comparison of the 12-phenylacetyl 13-acetyl derivative **6a** and the 12,13-bis(phenylacetyl) derivative **10** showed that the presence of lipophilic ester groups on both faces of ring C increases the affinity. However, comparison of **6a**−**f** with compounds having an inverted location of the ester groups in the northern hemisphere (that is, 12-acetyl 13-acyl phorbol esters like **7**) could not be done, since acyl rearrangement took place in the acetylation of the 13-acyl derivatives, affording 12-acyl 13-acetyl esters as the major reaction products (Scheme 2).[36]

The importance of a lipophilic ester group on ring C is highlighted by comparison of the activity of the 12-dehydrophorbol ligands **12a,b**. Indeed, only the 13-phenylacetate **12b** was active, whereas the 13-acetate **12a** was devoided of activity. Reduction of the carbonyl group of **6a** gave the 3β-alcohol **13** and caused a dramatic loss of activity. This finding epitomizes the strict structural requirements necessary for vanilloid activity and suggests that the C-3 carbonyl is involved in receptor binding.

We have recently reported that **6a** can abolish positive cooperativity of binding by the vanilloid receptor.[34] As predicted by the modified Hill equation for positive binding cooperativity, an initial enhancement by nonradioactive RTX of specific [^{3}H]RTX binding was seen at low fractional receptor occupancies, preceding inhibition.[34] In parallel experiments, **6a** failed to enhance specific RTX binding,[34] in accordance with a noncooperative binding mechanism. Table 1 shows that this behavior is not unique of **6a**. Indeed, those phorboid 20-homovanillates which competed for specific RTX binding sites also reduced positive cooperativity of binding, suggesting that this binding behavior is a ligand-induced feature rather than an inherent property of vanilloid receptors. The molecular mechanism(s) responsible for positive cooperative binding is(are) essentially unknown. It is, however, generally accepted that positive cooperativity represents a self-regulatory process in which the binding of a ligand molecule to a member of a receptor oligomer increases the affinity of the other members of the same receptor oligomer for

additional molecules. Inasmuch, positive binding cooperativity reflects conformational changes in the receptor protein. Our present finding that phorboid 20-homovanillates bind to vanilloid receptors with dissimilar Hill coefficients is consistent with the view that different ligand−receptor complexes may have different molecular conformations,[37] resulting in different degrees of binding cooperativity.

The synthesis of the phorboid homovanillate ligands reported in Table 1 is straightforward and could be scaled to gram amounts (see the Experimental Section). We have thus been able to test **6a** in a variety of *in vivo* assays used to characterize vanilloid-like activity. **6a** showed only a marginal pungency (Figure 2) and failed to induce an ear erythema response. Furthermore, no hypothermic response (Figure 3A) could be observed in the rat at doses at which **6a** could effectively desensitize against neurogenic inflammation and deplete vanilloid receptor in the spinal cord (Figure 3B). The marginal activity of topical **6a** to induce acute ocular pain (as quantified by counting protective eye wipings) might simply be a pharmacokinetical artifact. For example, tinyatoxin, an RTX analogue lacking the methoxyl on the 20-ester group, is likewise inactive in the eye-wiping assay, although it is only 5−10-fold less potent than RTX to induce other biological responses, including hypothermia.[15,18] However, the failure of systematically administered **6a** to reduce body temperature more likely reflects receptor heterogeneity. 12-Deoxyphorbol 13-phenylacetate 20-homovanillate is also a very weak inducer of the hypothermia response,[28] suggesting that the vanilloid receptor mediating this response is particularly sensitive to changes in the diterpene moiety. This finding might be of great practical importance for drug development, since hypothermia is a clearly undesirable side effect of vanilloid treatment. The relationship between the peculiar spectrum of biological activity of **6a** and its noncooperative binding to vanilloid receptor remains to be established. A comparison of the biological activity of phorboid 20-homovanillates with different degrees of binding cooperativity is expected to reveal the biological relevance of this binding behavior.

Alkylation or acylation of the phenolic hydroxyl reduces or completely removes the activity of capsaicinoids.[11] Interestingly, phorboid ligands seem to tolerate better these chemical modifications. For example, acetyl RTX is comparable in potency to RTX in the mouse erythema assay,[38] whereas the homoveratryl analogue of RTX binds to rat spinal chord membranes with only 8-fold less affinity than does RTX.[39] In keeping with this, acetylation of the phenolic hydroxyl of **6a** resulted in a minimal drop in activity (**14**, Table 1). Removal of the homovanillyl moiety caused instead a complete loss of activity (K_i > 200 μM for **5a**).

The extraordinary potency of RTX is presumably the result of an ideal alignment between the terpenoid core, the lipophilic orthoester group on the α-face of ring C, and the C-20 homovanillate. The activity of the homovanillates **6a**−**f**, which lack a lipophilic residue on the α-face of ring C, and **10** and **12b**, where the 9,13,14-*o*-phenylacetate is replaced by a 13-phenylacetate, points to the possibility of alternative alignments. These, although less efficient in terms of affinity, might be useful to dissect receptor subclasses, ultimatively leading to more selective and therapeutically useful second-generation vanilloids.

Experimental Section

WARNING: 12,13-Diesters of phorbol are powerful tumor promoters and irritants.[29] Great care should thus be taken in the manipulation of 5a−f and related compounds with a free 20-hydroxyl, and all diterpene intermediates and final products should be treated as potentially dangerous compounds.

General Methods. Anhydrous conditions were achieved (when indicated) by flame-drying flasks and equipment. Reactions were monitored by TLC on Merck 60 F254 (0.25 mm) plates, which were visualized with 5% H_2SO_4 in EtOH and heating. Merck silica gel (70−230 mesh) was used for open-column chromatography. A Waters microporasil column (0.8 × 30 cm) was used for HPLC, with detection by a Waters differential refractometer 340. Isocratic solvent systems of composition stated in the text were used to assess the purity of the compounds. Melting points were obtained on a Büchi SMP-20 apparatus and are uncorrected. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded on a Bruker AC-300 spectrometer at 25 °C. The spectra were fully assigned using two-dimensional techniques [^2J and ^3J (HMBC) ^{1}H−^{13}C correlations].

Materials. [^{3}H]RTX (37 Ci/mmol) was synthesized by the Chemical Synthesis and Analysis Laboratory, NCI-FCRDC, Frederick, MD, and kindly donated by Dr. P. Blumberg (NCI, Bethesda, MD). Homovanillonitrile for the synthesis of Mem-homovanillic acid (Mem-HMVA) was purchased by Aldrich, and croton oil for the isolation of phorbol[29] was from Calbiochem-Novabiochem AG. Commercially available reagents and solvents were used without further purification. CH_2Cl_2 was dried by distillation from CaH_2 and THF by distillation from sodium benzophenone.

3-Methoxy-4-[(2-methoxyethoxy)methoxy]phenylacetic Acid (Mem-homovanillic acid). To a solution of homovanillonitrile (4.0 g, 24.5 mmol) in dry CH_2Cl_2 (15 mL) were added *N*-ethyldiisopropylamine (13.97 mL, 10.3 g, 81.2 mmol, 3.3 equiv) and Mem-chloride (9.28 mL, 13.2 g, 81.2 mmol, 3.3 equiv). The reaction mixture was stirred at room temperature for 48 h and then diluted with CH_2Cl_2 (ca. 40 mL), washed with dilute HCl (2 × 15 mL), saturated $NaHCO_3$ (2 × 15 mL), and brine, and dried (Na_2SO_4). Removal of the solvent left an oil, which was dissolved in 10 mL of EtOH; 15 mL of 6.6 N KOH was then added, and the solution was refluxed for 12 h under a nitrogen atmosphere. After cooling and dilution with water (ca. 50 mL), the solution was extracted wtih EtOAc (2 × 15 mL). The pH of the aqueous phase was adjusted to 3.0−3.5 with concentrated HCl, and the suspension was extracted with EtOAc (4 × 20 mL). The organic phase was washed with brine, dried (Na_2SO_4), and evaporated. The residue was crystallized from ether to give 4.08 g (62%) of a pale yellow powder: mp 59 °C; IR (KBr) 3400−2800 (br), 1701, 1514, 1261, 1217, 1101, 1001 cm^{-1}; MS (EI) *m/e* 270 (M^+) (20), 149 (70), 89 (100), 59 (90), 43 (35); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.80 (br s, 1H), 7.15 (br d, J = 7.8 Hz, 1H), 6.82 (br s, 1H), 6.80 (br d, J = 7.8 Hz, 1H), 5.30 (s, 2H), 3.86 (s, 2H), 3.85 (m, 3H), 3.59 (s, 2H), 3.57 (m, 2H), 3.38 (s, 3H). Anal. ($C_{13}H_{18}O_6$) C, H.

General Procedure for the Synthesis of Phorboid 20-Homovanillates from Phorboid 20-Trityl Ethers: Synthesis of Phorbol 12-Phenylacetate 13-Acetate 20-Homovanillate (PPAHV, 6a). (a) To a solution of phorbol 13-acetate 20-trityl ether (**4a**)[30] (4.93 g, 7.6 mmol) in dry CH_2Cl_2 (110 mL) were added dicyclohexylcarbodimide (DCC; 4.70 g, 22.8 mmol, 3 equiv), phenylacetic acid (3.1 g, 22.8 mmol, 3 equiv), and 4-(dimethylamino)pyridine (DMAP; 50 mg). After stirring for 90 min at room temperature, the reaction mixture was diluted with ether (ca. 100 mL) and filtered to remove the precipitate of dicyclohexylurea (DCU). The filtrate was washed with brine (2 × 30 mL), dried (Na_2SO_4), and evaporated. The residue, still containing DCU, was dissolved in 0.01 N methanolic $HClO_4$ (200 mL). After stirring at room temperature for 50 min, the solution was diluted with water (ca. 400 mL), neutralized with solid NaOAc, and extracted with petroleum ether and then $CHCl_3$. The chloroform phase was washed with brine, dried (Na_2SO_4), and evaporated to give 4.6 g of a solid residue. Part of this (460 mg) was purified by

column chromatography (7 g of silica gel, hexane−EtOAc, 3:7, as eluant) to give 290 mg (73% from **4a**) of **5a** as a white powder: mp 104 °C; IR (KBr) 3300, 1730, 1460, 1320, 1240, 980 cm^{-1}; MS (CI, isobutane) *m/e* 525 ($M^+ + H$) (100); ^1H NMR (300 MHz, $CDCl_3$) δ 7.56 (br s, H-1), 7.29 (m, *Ph*CH_2CO), 5.64 (d, J = 5.4 Hz, H-7), 5.53 (br s, OH), 5.40 (d, J = 10.5 Hz, H-12), 4.02 (d, J = 13.3 Hz, H-20a), 3.96 (d, J = 13.3 Hz, H-20b), 3.66 (d, J = 14.7 Hz, Ph*CH*$_2$CO), 3.62 (d, J = 14.7 Hz, Ph*CH*$_2$CO), 3.20 (br s, H-8, H-10), 2.55 (br d, J = 19.5 Hz, H-5a), 2.46 (br d, J = 19.5 Hz, H-5b), 2.07 (s, Ac), 1.76 (d, J = 1.6 Hz, H-19), 1.08 (s, H-16), 1.01 (s, H-17), 0.84 (d, J = 6.4 Hz, H-18).

To avoid unnecessary manipulation of dangerous products,[29] the crude diesters were directly used for the esterification with Mem-homovanillic acid. When the procedure was tested on both purified and crude diesters (compounds **5a**,**b**), no detrimental decrease of the yield was observed.

(b) To a solution of crude phorbol 13-acetate 12-phenylacetate (**5a**) [2.70 g, corresponding to 1.70 g (3.24 mmol) of pure diester on the basis of the extrapolated yield] in 70 mL of dry CH_2Cl_2 were added Mem-homovanillic acid (1.74 g, 6.48 mmol, 2 equiv), DCC (1.33 g, 6.48 mmol, 2 equiv), and DMAP (50 mg). After stirring for 1 h at room temperature, the solution was diluted with ether (70 mL) and filtered. The filtrate was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (50 g of silica gel, hexane−EtOAc, 3:7, as eluant) to give 2.360 g (94%) of the 20-Mem-homovanillate as a white powder: mp 85 °C; IR (KBr) 3400, 1740, 1380, 1320, 1220, 1130, 990 cm^{-1}; MS (CI, isobutane) *m/e* 777 ($M^+ + H$) (100); ^1H NMR (300 MHz, $CDCl_3$) δ 7.55 (s, H-1), 7.29 (m, *Ph*CH_2CO), 7.11 (br d, J = 8.1 Hz, 6-HMV), 6.80 (br s, 2-HMV), 6.75 (d, J = 8.1 Hz, 5-HMV), 5.63 (br s, H-7), 5.48 (br s, OH), 5.39 (d, J = 10.4 Hz, H-12), 5.28 (s, 2H, Mem), 4.49 (d, J = 12.4 Hz, H-20a), 4.46 (d, J = 12.4 Hz, H-20b), 3.85 (s, HMV-OMe), 3.84 (m, 2H, Mem), 3.69 (d, J = 14.7 Hz, Ph*CH*$_2$CO), 3.65 (d, J = 14.7 Hz, Ph*CH*$_2$CO), 3.55 (m, 2H, Mem), 3.55 (br s, HMV-CH_2), 3.36 (s, Mem-OMe), 3.16 (br s, H-8, H-10), 2.40 (d, J = 19.0 Hz, H-5a), 2.36 (d, J = 19.0 Hz, H-5b), 2.09 (m, H-11), 2.07 (s, Ac), 1.76 (br s, H-19), 1.07 (s, H-16), 0.99 (s, H-17), 0.96 (d, J = 5.4 Hz, H-14), 0.83 (d, J = 6.4 Hz, H-18).

(c) To a solution of the Mem-homovanillate (1.33 g, 1.71 mmol) in dry THF (100 mL) was added $SnCl_4$ (1.00 mL, 2.23 g, 8.6 mmol, 5 equiv) dropwise. During the addition, a white precipitate was formed, which then dissolved as the reaction proceeded. After stirring for 90 min under a nitrogen atmosphere, the reaction mixture was worked up by the addition of saturated $NaHCO_3$ (ca. 25 mL) and extracted with EtOAc (2 × 50 mL). After washing with brine, drying (Na_2SO_4), and evaporation, the residue was purified by column chromatography (25 g of silica gel, hexane−EtOAc, 7:3, as eluant) to give 1.57 g (76%) of **6a** as a white powder: mp 75 °C; IR (KBr) 3420, 1740, 1390, 1275, 1150, 1040, 990 cm^{-1}; MS (CI, isobutane) *m/e* 689 ($M^+ + H$) (100); ^1H NMR (300 MHz, $CDCl_3$) δ 7.53 (s, H-1), 7.27 (m, *Ph*CH_2CO), 6.79 (br d, J = 8.0 Hz, 6-HMV), 6.77 (br s, 2-HMV), 6.72 (br d, J = 8.0 Hz, 5-HMV), 5.57 (br s, OH), 5.53 (br s, H-7), 5.43 (br s, OH), 5.38 (d, J = 10.6 Hz, H-12), 4.51 (br d, J = 12.8 Hz, H-20a), 4.42 (br d, J = 12.8 Hz, H-20b), 3.87 (OMe), 3.64 (d, J = 14.7 Hz, Ph*CH*$_2$CO), 3.62 (d, J = 14.7 Hz, Ph*CH*$_2$CO), 3.50 (br s, HMV-*CH*$_2$), 3.12 (br s, H-8, H-10), 2.41 (br d, J = 19.1 Hz, H-5a), 2.27 (br d, J = 19.1 Hz, H-5b), 2.07 (s, Ac), 2.07 (m, H-11), 1.76 (br s, H-19), 1.05 (s, H-16), 0.99 (s, H-17), 0.88 (d, J = 5.1 Hz, H-14), 0.81 (d, J = 6.2 Hz, H-18); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.5 (d, C-1), 135.3 (s, C-2), 208.1 (s, C-3), 73.5 (s, C-4), 38.7 (t, C-5), 132.7 (s, C-6), 131.9 (d, C-7), 39.2 (d, C-8), 78.0 (s, C-9), 56.1 (d, C-10), 42.8 (d, C-11), 77.4 (d, C-12), 65.4 (d, C-13), 36.0 (d, C-14), 25.8 (s, C-15), 23.5 (q, C-16), 16.3 (q, C-17), 14.2 (q, C-18), 10.0 (q, C-19), 68.7 (t, C-20), 171.4 (s, HMV *CO*), 41.0 (t, HMV-*CH*$_2$), 125.6 (s, 1-HMV), 111.7 (d, 2-HMV), 146.4 (s, 3-HMV), 144.7 (s, 4-HMV), 114.3 (d, 5-HMV), 122.1 (d, 6-HMV), 173.6 (s, PhCH_2*CO*), 41.7 (t, Ph*CH*$_2$CO), 134.0 (s, *i*-Ph), 129.0 (d, *o*-Ph), 128.5 (d, *m*-Ph), 127.0 (d, *p*-Ph), 171.2 (s, Ac), 21.0 (q, Ac), 56.0 (q, OMe). Anal. ($C_{39}H_{44}O_{11}$) C, H.

Phorbol 12-benzoate 13-acetate 20-homovanillate (6b): mp 75 °C; IR (KBr) 3390, 1720, 1375, 1270, 1150, 785, 710 cm^{-1}; MS (CI, isobutane) *m/e* 675 ($M^+ + H$) (100); ^1H NMR (300 MHz, $CDCl_3$) δ 8.03 (d, J = 7.6 Hz, *o*-Bz), 7.59 (br s, H-1), 7.58 (t, J = 7.6 Hz, *p*-Bz), 7.46 (t, J = 7.6 Hz, *m*-Bz), 6.79 (br d, J = 8.0 Hz, 6-HMV), 6.77 (br s, 2-HMV), 6.72 (br d, J = 8.0 Hz, 5-HMV), 5.66 (d, J = 9.9 Hz, H-12), 5.61 (br s, H-7), 5.56 (br s, OH), 4.54 (d, J = 12.4 Hz, H-20a), 4.47 (d, J = 12.4 Hz, H-20b), 3.91 (s, OMe), 3.55 (br s, HMV-*CH*$_2$), 3.28 (br s, H-8), 3.22 (br s, H-10), 2.48 (d, J = 19.0 Hz, H-5a), 2.33 (d, J = 19.0 Hz, H-5b), 2.25 (m, H-11), 2.15 (s, Ac), 1, 78 (br s, H-19), 1.35 (s, H-16), 1.20 (s, H-17), 1.01 (d, J = 5.1 Hz, H-14), 0.94 (d, J = 6.6 Hz, H-18). Anal. ($C_{38}H_{42}O_{11}$) C, H.

Phorbol 12-*p*-azidophenylacetate 13-acetate 20-homovanillate (6c): mp 80 °C; IR (KBr) 3400, 2120, 1750, 1375, 1260, 1140, 980, 790 cm^{-1}; ^1H NMR (300 Mhz, $CDCl_3$) δ 7.53 (br s, H-1), 7.24 (d, J = 8.4 Hz, Ph), 6.98 (d, J = 8.4 Hz, Ph), 6.79 (br d, J = 8.0 Hz, 6-HMV), 6.77 (s, 2-HMV), 6.71 (d, J = 8.0 Hz, 5-HMV), 5.52 (br s, H-7), 5.44 (s, OH), 5.38 (d, J = 10.6 Hz, H-12), 4.49 (d, J = 12.5 Hz, H-20a), 4.41 (d, J = 12.5 Hz, H-20b), 3.86 (s, OMe), 3.56 (d, J = 15.0 Hz, Ph*CH*$_2$CO), 3.52 (d, J = 15.0 Hz, Ph*CH*$_2$CO), 3.37 (br d, HMV-*CH*$_2$), 3.13 (br s, H-8, H-10), 2.39 (d, J = 19.1 Hz, H-5a), 2.28 (d, J = 19.1 Hz, H-5b), 2.09 (m, H-11), 2.08 (s, Ac), 1.76 (br s, H-19), 1.08 (s, H-16), 1.03 (s, H-17), 0.89 (d, J = 5.1 Hz, H-14), 0.80 (d, J = 6.6 Hz, H-18); HMRS (FAB^+) *m/e* calcd for ($M^+ + 1$) $C_{39}H_{44}N_3O_{11}$ 730.2976, found 730.2985; analytical HPLC (hexane−EtOAc, 1:1) t_R = 14.6 min (96% pure).

Phorbol 12-*p*-azidobenzoate 13-acetate 20-homovanillate (6d): mp 98 °C; IR (KBr) 3400, 2140, 1720, 1380, 1275, 1180, 1135, 1100 cm^{-1}; MS (CI, isobutane) *m/e* 716 ($M^+ + H$) (100); ^1H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, J = 8.8 Hz, *o*-Bz), 7.59 (s, H-1), 7.08 (d, J = 8.8 Hz, *m*-PH), 6.83 (br d, J = 7.9 Hz, 6-HMV), 6.80 (br s, 2-HMV), 6.76 (br d, J = 7.0 Hz, 5-HMV), 5.64 (d, J = 10.2 Hz, H-12), 5.57 (br s, H-7), 5.53 (br s, OH), 4.54 (d, J = 12.5 Hz, H-20a), 4.47 (d, J = 12.5 Hz, H-20b), 3.90 (s, OMe), 3.40 (HMV-*CH*$_2$), 3.25 (br s, H-8), 3.21 (br s, H-10), 2.48 (d, J = 19.1 Hz, H-5a), 2.33 (d, J = 19.1 Hz, H-5b), 21.8 (m, H-11), 2.15 (s, Ac), 1.79 (br s, H-19), 1.34 (s, H-16), 1.20 (s, H-17), 1.00 (d, J = 5.2 Hz, H-14), 0.93 (d, J = 6.6 Hz, H-18); HMRS (FAB^+) *m/e* calcd for ($M^+ + 1$) $C_{38}H_{42}N_3O_{11}$ 716.2819, found 716.2810; analytical HPLC (hexane−EtOAc, 1:1) t_R = 13.8 min (97% pure).

Phorbol 12-cyclohexylacetate 13-acetate 20-homovanillate (6e): mp 70 °C; IR (KBr) 3416, 1728, 1516, 1450, 1377, 1253, 1149, 987 cm^{-1}; MS (CI, isobutane) *m/e* 695 ($M^+ + H$) (100); ^1H NMR (300 MHz, $CDCl_3$) δ 7.57 (br s, H-1), 6.82 (br d, J = 8.0 Hz, 6-HMV), 6.79 (br s, 2-HMV), 6.73 (br d, J = 8.0 Hz, 5-HMV), 5.57 (br s, H-7), 5.40 (d, J = 10.4 Hz, H-12), 4.49 (d, J = 12.5 Hz, H-20a), 4.45 (d, J = 12.5 Hz, H-20b), 3.88 (s, OMe), 3.70 (d, J = 14.5 Hz, HMV-C*H*$_2$), 3.57 (d, J = 14.5 Hz, HMV-*CH*$_2$), 3.15 (br s, H-8, H-10), 2.43 (d, J = 19.0 Hz, H-5a), 2.30 (d, J = 19.0 Hz, H-5b), 2.17 (br s, C_6H_{11}-*CH*$_2$CO), 2.09 (m, H-11), 2.09 (s, Ac), 1.78 (br s, H-19), 1.70−1.30 (m, *C*$_6$*H*$_{11}$-CH_2CO), 1.21 (s, H-16), 1.17 (s, H-17), 0.93 (d, J = 5.2 Hz, H-14), 0.83 (d, J = 6.6 Hz, H-18); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.7 (d, C-1), 135.2 (s, C-2), 208.5 (s, C-3), 73.5 (s, C-4), 38.7 (t, C-5), 132.8 (s, C-6), 132.0 (d, C-7), 39.2 (d, C-8), 78.0 (s, C-9), 56.0 (s, C-10), 42.7 (d, C-11), 76.2 (d, C-12), 65.5 (s, C-13), 35.9 (d, C-14), 25.4 (s, C-15), 23.6 (q, C-16), 16.7 (q, C-17), 14.3 (q, C-18), 10.0 (q, C-19), 68.8 (t, C-20), 171.0 (s, HMV-*CO*), 41.0 (t, HMV-*CH*$_2$), 152.6 (s, 1-HMV), 111.7 (d, 2-HMV), 144.2 (s, 3-HMV), 146.5 (s, 4-HMV), 114.3 (d, 5-HMV), 122.1 (d, 6-HMV), 56.0 (q, OMe), 172.9 (s, C_6H_{11}-CH_2*CO*), 42.4 (t, C_6H_{11}-*CH*$_2$CO), 35.2 (d, *C*$_6$*H*$_{11}$-CH_2CO), 32.9 (t, *C*$_6$*H*$_{11}$-CH_2-CO), 26.0 (t, *C*$_6$*H*$_{11}$-CH_2CO), 26.0 (t, *C*$_6$*H*$_{11}$-CH_2CO), 173.7 (s, Ac), 21.0 (q, Ac); HMRS (FAB^+) *m/e* calcd for ($M^+ + 1$) $C_{39}H_{51}O_{11}$ 695.3431, found 695.3437; analytical HPLC (hexane−EtOAc, 1:1) t_R = 15.2 min (99% pure).

Phorbol 12-cyclohexanecarboxylate 13-acetate 20-homovanillate (6f): mp 68 °C; IR (KBr) 3400, 1725, 1630, 1516, 1377, 1273, 1263, 1130, 1034 cm^{-1}; MS (CI, isobutane) *m/e* 681 ($M^+ + H$) (100); ^1H NMR (300 MHz, $CDCl_3$) δ 7.56 (s, H-1), 6.81 (d, J = 8.0 Hz, 6-HMV), 6.78 (br s, 2-HMV), 6.73 (br d, J = 8.0 Hz, 5-HMV), 5.56 (br s, H-7), 5.53 (br s, OH), 5.49 (br s, OH), 5.37 (d, J = 10.6 Hz, H-12), 4.49 (d, J = 12.7 Hz, H-20a), 4.45 (d, J = 12.7 Hz, H-20b), 3.88 (s, OMe), 3.73 (d, J = 15.0 Hz, HMV-*CH*$_2$), 3.53 (d, J = 15.0 Hz, HMV-*CH*$_2$), 3.15 (br s, H-8, H-10), 2.43 (d, J = 19.0 Hz, H-5a), 2.33 (m, *C*$_6$*H*$_{11}$-CO),

2.30 (d, J = 19 Hz, H-5b), 2.09 (s, Ac), 2.08 (m, H-11), 1.87 (m, C_6H_{11}-CO), 1.79 (br s, H-19), 1.70 (m, C_6H_{11}-CO), 1.45 (m, C_6H_{11}-CO), 1.30 (m, C_6H_{11}-CO), 1.21 (s, H-16), 1.17 (s, H-17), 0.93 (d, J = 5.4 Hz, H-14), 0.85 (d, J = 6.6 Hz, H-18); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.0 (d, *C*-1), 135.2 (s, C-2), 208.6 (s, C-3), 73.5 (s, C-4), 38.7 (t, C-5), 132.8 (s, C-6), 132.1 (d, C-7), 39.2 (d, C-8), 78.1 (s, C-9), 56.0 (d, C-10), 42.9 (d, C-11), 76.3 (d, C-12), 65.5 (s, C-13), 36.0 (d, C-14), 25.5 (s, C-15), 23.8 (q, C-16), 16.9 (q, C-17), 14.4 (q, C-18), 10.1 (q, C-19), 68.9 (t, C-20), 171.4 (s, HMV-*CO*), 41.1 (t, HMV-CH_2), 125.6 (s, 1-HMV), 111.7 (d, 2-HMV), 146.5 (s, 3-HMV), 144.7 (s, 4-HMV), 114.3 (d, 5-HMV), 122.2 (d, 6-HMV), 56.0 (q, OMe), 175.8 (s, $C_5H_{11}CO$), 43.3 (d, C_6H_{11}CO), 29.1 (t, $C_6H_{11}CO$), 28.9 (t, $C_6H_{11}CO$), 25.4 (t, C_6H_{11}-*CO*), 25.3 (t, C_6H_{11}CO), 25.8 (t, C_6H_{11}CO), 173.8 (s, Ac), 21.2 (q, Ac); HMRS (FAB$^+$) *m/e* calcd for (M$^+$ + 1) $C_{38}H_{49}O_{11}$ 681.3275, found 681.3288; analytical HPLC (hexane−EtOAc, 4:6) t_R = 12.9 min (98% pure).

Attempted Synthesis of Phorbol 12-Acetate 13-Phenylacetate 20-Trityl Ether. (a) To a solution of phorbol 20-trityl ether (1.0 g, 1.65 mmol) in dry CH_2Cl_2 (45 mL) were added DCC (340 mg, 1.65 mmol, 1 equiv), phenylacetic acid (225 mg, 1.65 mmol, 1 equiv), and DMAP (15 mg). After stirring for 30 min under a nitrogen atmosphere, the reaction mixture was worked up by dilution with ether (ca. 40 mL) and filtration. The filtrate was evaporated, and the residue was purified by column chromatography (hexane−EtOAc, 7:3) to give 415 mg (33%) of phorbol 13-phenylacetate 20-trityl ether (**4b**) as a powder: mp 95 °C; IR (KBr) 3430, 1709, 1448, 1269, 1159, 1030, 702 cm^{-1}; MS (CI, isobutane) *m/e* 725 (M$^+$ + H) (100); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (s, H-1), 7.44−7.20 (aromatics), 5.56 (br s, H-7), 3.92 (d, J = 10.0 Hz, H-12), 3.69 (br s, H-20a,b), 3.55 (br s, OH), 3.07 (br s, H-8), 3.02 (br s, H-10), 2.46 (br d, J = 19.1 Hz, H-5a), 2.38 (br d, J = 191.1 Hz, H-5b), 1.78 (br s, H-19), 1.23 (s, H-16), 1.09 (s, H-17), 1.04 (d, J = 6.2 Hz, H-18), 0.94 (d, J = 5.1 Hz, H-14).

(b) To a solution of **4b** (134 mg, 0.18 mmol) in dry CH_2Cl_2 (3 mL) were added glacial acetic acid (20.6 μL, 21.8 mg, 0.36 mmol, 2 equiv), DCC (74.3 mg, 0.36 mL, 2 equiv), and DMAP (10 mg). After stirring for 3 h at room temperature, the reaction mixture was worked up by dilution with ether (ca. 3 mL) and filtered. The filtrate was evaporated and the residue purified by HPLC (hexane−EtOAc, 7:3) to give 42 mg (30%) of phorbol 12-phenylacetate 13-acetate (**8a**) (identified by comparison of the ^{1}H NMR and IR spectra with an authentic sample prepared from **4a**) and 20 mg (15%) of phorbol 12-phenylacetate 20-trityl ether (**8b**). **8a:** foam; IR (KBr) 3400, 1720, 1450, 1380, 1260, 980, 705 cm^{-1}; MS (CI, isobutane) *m/e* 767 (M$^+$ + H) (90); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (br s, H-1), 7.43−7.21 (aromatic, 20 H), 5.61 (br s, H-7), 5.42 (d, J = 11.5 Hz, H-12), 3.66 (d, J = 14.2 Hz, Ph-CH_2CO), 3.63 (d, J = 14.2 Hz, Ph-CH_2CO), 3.51 (br s, H-20a,b), 3.24 (br s, H-10), 3.11 (br s, H-8), 2.46 (d, J = 18.9 Hz, H-5a), 2.40 (d, J = 18.9 Hz, H-5b), 2.05 (s, Ac), 1.72 (br s, H-19), 1.10 (s, H-16), 1.03 (s, H-17), 0.84 (d, J = 6.4 Hz, H-18).

8b: powder; mp 96−98 °C; MS (CI, isobutane) *m/e* 707 (M$^+$ + H − H_2O) (100); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (br s, H-1), 7.43−7.21 (m, aromatics, 20 H), 5.58 (br d, J = 5.8 Hz, H-7), 5.45 (d, J = 10.3 Hz, H-12), 5.35 (s, OH), 3.65 (br s, H-20a,b), 3.22 (br s, H-8), 3.08 (br s, H-10), 2.48 (d, J = 19.0 Hz, H-5a), 2.40 (d, J = 19.0 Hz, H-5b), 2.06 (m, H-11), 1.76 (br s, H-19), 0.97 (s, H-16), 0.88 (s, H-17), 0.83 (d, J = 6.2 Hz, H-18).

Phorbol 12,13-Bis(phenylacetate) 20-Homovanillate (10). (a) To a solution of phorbol 20-trityl ether (210 mg, 0.34 mmol) in dry CH_2Cl_2 (10 mL) were added pyridine (117 μL, 109 mg, 1.36 mmol, 4 equiv) and phenylacetyl chloride (183 μL, 214 mg, 1.36 mmol, 4 equiv). After stirring for 1 h at room temperature, the reaction mixture was worked up by addition of water, and the organic phase was washed with dilute HCl, saturated $NaHCO_3$, and brine. After drying (Na_2SO_4) and removal of the solvent, the residue was purified by column chromatography (10 g of silica gel, hexane−EtOAc, 8:2, as eluant) to give 258 mg (90%) of phorbol 12,13-bis(phenylacetate) 20-trityl ether (**9**) as a powder: mp 54−56 °C; IR (KBr) 3416, 1713, 1497, 1267, 1151, 707 cm^{-1}; MS (CI, isobutane) *m/e* 843 (M$^+$ + H) (25); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.59 (br s, H-1), 7.43−7.25 (m, aromatics, 25 H), 5.58 (d, J = 5.4 Hz, H-7), 5.40 (d, J = 10.6 Hz, H-12), 5.36 (br s, OH), 5.26 (br s, OH), 3.66 (br m, 2 × Ph-CH_2CO), 3.49 (br s, H-20a, b), 3.22 (br s, H-10), 3.09 (br s, H-8), 2.41 (d, J = 19.0 Hz, H-5a), 2.37 (d, J = 19.0 Hz, H-5b), 2.06 (m, H-11), 1.77 (br s, H-19), 0.98 (s, H-16), 0.89 (s, H-17), 0.86 (d, J = 6.2 Hz, H-18).

(b) Conversion of **9** to **10** was carried out according to the general procedure employed for the synthesis of **6a**. **10** was obtained as a white powder: mp 64 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (br s, H-1), 7.35−7.25 (m, 2 × *Ph*-CH_2CO), 6.86 (d, J = 8.0 Hz, 6-HMV), 6.78 (br s, 2-HMV), 6.71 (br d, J = 8.0 Hz, 5-HMV), 5.52 (br d, J = 4.0 Hz, H-7), 5.44 (d, J = 10.4 Hz, H-12), 5.39 (s, OH), 4.48 (d, J = 12.8 Hz, H-20a), 4.41 (d, J = 12.8 Hz, H-20b), 3.89 (s, OMe), 3.65 (m, 2 × Ph-CH_2CO), 3.51 (s, HMV-CH_2), 3.11 (br s, H-8, H-10), 2.41 (d, J = 18.9 Hz, H-5a), 2.28 (d, J = 18.9 Hz, H-5b), 1.77 (br s, H-19), 0.94 (s, H-16), 0.87 (s, H-17), 0.83 (d, J = 6.1 Hz, H-18); HMRS (FAB$^+$) *m/e* calcd for (M$^+$ + 1) $C_{45}H_{49}O_{11}$ 765.3275, found 765.3282; analytical HPLC (hexane−EtOAc, 4:6) t_R = 10.2 min (99% pure).

12-Dehydrophorbol 13-Acetate 20-Homovanillate (12a). (a) To a solution of phorbol 13-acetate 20-trityl ether (**4a**) (1.06 g, 1.60.4 mmol) in dry CH_2Cl_2 (26 mL) were added pyridinium chlorochromate (PCC; 1.77 g, 8.2 mmol, 5 equiv) and powdered molecular sieves (4 Å, 1.37 g). After stirring at room temperature for 3 h, the reaction mixture was worked up by the addition of ether (ca. 60 mL) and filtration through Celite. After removal of the solvent, the residue was purified by column chromatography (hexane−EtOAc, 7:3) to give 703 mg (67%) of the 13-dehydro derivative as a white powder: mp 120−123 °C; IR (KBr) 3420, 1730, 1700, 1445, 1375, 1260, 760, 700 cm^{-1}; MS (CI, isobutane); *m/e* 647 (M$^+$ + H) (60); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (br s, H-1), 7.45−7.22 (aromatic, 15 H), 5.76 (br d, J = 5.0 Hz, H-7), 5.40 (br s, OH), 3.55 (br s, H-20a,b), 3.24 (br s, H-8, H-10), 2.94 (q, J = 6.4 Hz, H-11), 2.61 (d, J = 18.5 Hz, H-5a), 2.43 (d, J = 18.5 Hz, H-5b), 2.17 (s, Ac), 1.78 (br s, H-19), 1.49 (d, J = 4.9 Hz, H-14), 1.37 (s, H-16), 1.23 (s, H-17), 1.19 (d, J = 6.4 Hz, H-18).

(b) The conversion of 12-dehydrophorbol 13-acetate 20-trityl ether to **12a** was carried out according to the general procedure used for the synthesis of **6a**. **12a** was obtained as a white powder: mp 120 °C; IR (KBr) 3410, 1732, 1701, 1516, 1271, 1255, 1147 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (br s, H-1), 6.81 (br d, J = 8.0 Hz, 6-HMV), 6.78 (br s, 2-HMV), 6.46 (br d, J = 8.0 Hz, 5-HMV), 5.66 (br s, OH), 5.57 (br s, H-7), 5.43 (br s, OH), 4.54 (d, J = 12.7 Hz, H-20a), 4.46 (d, J = 12.7 Hz, H-20b), 3.87 (s, OMe), 3.53 (br s, HMV-CH_2), 3.13 (br s, H-10), 2.87 (q, J = 6.4 Hz, H-11), 2.87 (br s, H-8), 2.51 (br d, J = 19.0 Hz, H-5a), 2.34 (br d, J = 19.0 Hz, H-5b), 2.17 (s, Ac), 1.78 (br s, H-19), 1.32 (s, H-16), 1.18 (s, H-17), 1.15 (d, J = 6.4 Hz, H-18); HMRS (FAB$^+$) *m/e* calcd for (M$^+$ + 1) $C_{31}H_{37}O_{10}$ 569.2387, found 569.2374; analytical HPLC (hexane−EtOAc, 1:1) t_R = 14.1 min (98.5% pure).

12-Dehydrophorbol 13-Phenylacetate 20-Homovanillate (12b). Phorbol 13-phenylacetate 20-trityl ether (**4b**) was oxidized as described above for **4a**. The 12-dehydro derivative was obtained as a colorless foam: MS (CI, isobutane) *m/e* 723 (M$^+$ + H) (100); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (br s, H-1), 7.45−7.19 (aromatics, 20 H), 5.67 (br s, H-7), 5.34 (br s, OH), 3.76 (Ph-CH_2CO), 3.53 (br s, H-20a,b), 3.22 (br s, H-8, H-10), 2.96 (q, J = 6.4 Hz, H-11), 2.52 (d, J = 19.0 Hz, H-5a), 2.46 (d, J = 19.0 Hz, H-5b), 1.78 (br s, H-19), 1.31 (s, H-16), 1.28 (s, H-17), 1.16 (d, J = 6.4 Hz, H-18).

Conversion of **12b** was carried out according to the general procedure described for the synthesis of **6a**. **12b** was obtained as a powder: mp 120 °C; IR (KBr) 3410, 1730, 1698, 1516, 1273, 1234, 1151 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.49 (br s, H-1), 7.36−7.29 (*Ph*-CH_2CO), 6.85 (br d, J = 8.0 Hz, 6-HMV), 6.78 (br s, 2-HMV), 6.70 (br d, J = 8.0 Hz, 5-HMV), 5.64 (br s, OH), 5.55 (br s, H-7), 5.37 (br s, OH), 4.52 (d, J = 12.6 Hz, H-20a), 4.44 (d, J = 12.6 Hz, H-20b), 3.84 (s, OMe), 3.75 (br s, Ph-CH_2CO), 3.52 (s, HMV-CH_2), 3.12 (br s, H-10), 2.86 (q, J = 6.4 Hz, H-11), 2.86 (br s, H-8), 2.49 (d, J = 19.0 Hz, H-5a), 2.32 (d, J = 19.0 Hz, H-5b), 1.79 (br s, H-19), 1.20 (s, H-16), 1.17 (s, H-17), 1.15 (d, J = 6.4 Hz, H-18): HMRS (FAB$^+$) *m/e* calcd for (M$^+$ + 1) $C_{37}H_{41}O_{10}$ 645.2700, found 645.2709; analytical HPLC (hexane−EtOAc, 1:1) t_R = 9.6 min (99% pure).

(3*R*)-Dihydrophorbol 12-Phenylacetate 13-Acetate 20-Homovanillate (13). To a solution of **6a** (100 mg, 0.14 mmol) in MeOH (5 mL) were added 54 mg of $CeCl_3 \cdot 7H_2O$ (0.14 mmol, 1 equiv) and $NaBH_4$ (10 mg). After 5 min, the reaction mixture was worked up by dilution with saturated NH_4Cl and extracted with $CHCl_3$. The organic phase was washed with brine, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography (4 g of silica gel, hexane−EtOAc, 6:4, as eluant) to give 63 mg (63%) of **13** as a colorless foam: IR (KBr) 3420, 1730, 1380, 1275, 1150, 1040, 990 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.30 (m, *Ph*-CH_2CO), 6.81 (br d, J = 8.0 Hz, 6-HMV), 6.78 (br s, 2-HMV), 6.77 (br d, J = 8.0 Hz, 5-HMV), 5.75 (br s, *H*-1), 5.57 (br s, H-7), 5.56 (br s, OH), 5.34 (d, J = 10.2 Hz, H-12), 4.48 (d, J = 12.2 Hz, H-20a), 4.45 (d, J = 12.2 Hz, H-20b), 3.88 (s, OMe), 3.65 (br s, Ph-CH_2CO), 3.54 (s, HMV-CH_2), 2.67 (br s, H-8), 3.12 (m, H-10), 2.66 (d, J = 19.3 Hz, H-5a), 2.38 (d, J = 19.3 Hz, H-5b), 2.07 (s, Ac), 1.67 (br s, H-19), 1.09 (s, H-16), 1.01 (s, H-17), 0.89 (d, J = 6.4 Hz, H-18); HMRS (FAB^+) *m/e* calcd for ($M^+ + 1$) $C_{39}H_{47}O_{11}$ 691.3118, found 691.3125; analytical HPLC (hexane−EtOAc, 1:1) t_R = 12.9 min (96% pure). The stereochemistry of the 3-hydroxyl was assigned by analogy with the results reported for other phorbol esters.[40]

Phorbol 12-Phenylacetate 13-Acetate 20-Acetylhomovanillate (14). To a solution of phorbol 12-phenylacetate 13-acetate (**5a**) (100 mg, 0.19 mmol) in dry CH_2Cl_2 (5 mL) were added acetylhomovanillic acid[33] (0.38 mmol, 2 equiv) and DCC (79 mg, 0.38 mmol, 2 equiv). After stirring for 1 h at room temperature, the reaction mixture was worked up by dilution with ether (10 mL) and filtered. The filtrate was evaporated and the residue purified by column chromatography (5 g of silica gel, hexane−EtOAc, 6:4, as eluant) to give 125 mg (90%) of **14**: mp 92−95 °C; IR (KBr) 3412, 1767, 1735, 1263, 1198, 1152, 984 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.53 (br s, H-1), 7.30 (m, *Ph*-CH_2CO), 6.97 (br d, J = 8.0 Hz, 6-HMV), 6.90 (br s, 2-HMV), 6.84 (br d, J = 8.0 Hz, 5-HMV), 5.61 (br s, H-7), 5.46 (br s, OH), 5.40 (d, J = 10.5 Hz, H-12), 4.52 (d, J = 12.4 Hz, H-20a), 4.45 (d, J = 12.4 Hz, H-20b), 3.84 (s, OMe), 3.65 (d, J = 14.9 Hz, Ph-CH_2CO), 3.62 (d, J = 14.9 Hz, Ph-CH_2CO), 3.59 (s, HMV-CH_2), 3.14 (br s, H-8, H-10), 2.36 (d, J = 19.0 Hz, H-5a), 2.30 (s, Ac), 2.28 (d, J = 19.0 Hz, H-5b), 2.08 (m, H-11), 2.08 (s, Ac), 1.77 (br s, H-19), 1.08 (s, H-16), 0.99 (s, H-17), 0.96 (d, J = 5.0 Hz, H-14), 0.88 (d, J = 6.4 Hz, H-18); HMRS (FAB^+) *m/e* calcd for ($M^+ + 1$) $C_{41}H_{47}O_{12}$ 731.3067, found 731.3077; analytical HPLC (hexane−EtOAc, 7:3) t_R = 9.9 min (99% pure).

Biological Evaluation. Radioligand Binding Studies. Binding assays using rat spinal cord, rat urinary bladder, porcine dorsal horn, and human dorsal horn (obtained *post-mortem*) membranes were performed as described previously.[20,21] Briefly, tissues were disrupted with the aid of a Polytron tissue homogenizer in ice-cold buffer A (pH 7.4), containing (in mM) NaCl, 5.8; KCl, 5; $CaCl_2$, 0.75; $MgCl_2$, 2; sucrose, 137; and HEPES, 10. Tissue homogenates were first centrifuged for 10 min at 1000*g* (4 °C), and then the resulting supernatants were further centrifuged for 30 min at 35000*g* (4 °C). The pellets from the second centrifugation resuspended in buffer A were aliquoted and then kept at −80 °C until assayed. For competition studies, 50 μg protein aliquots of rat spinal cord membranes were incubated in triplicate with 20 pM [^{3}H]RTX, the appropriate K_d from the saturation experiments, in 500 μL of buffer A containing 0.25 mg/mL bovine serum albumin (Cohn fraction V) in the absence or presence of 100 nM nonradioactive RTX. In this way, non-specific binding could be determined. Competing ligands (dissolved in ethanol and then diluted in buffer A containing 10 mg/mL bovine serum albumin) were added using 1:3 dilutions; the final concentration of the organic solvents in the assay mixture never exceeded 0.1% (v/v) and did not have any measurable effect on specific RTX binding. For the determination of RTX binding curves in the presence of competing ligands, membranes were incubated with increasing concentrations (6−400 pM) of [^{3}H]RTX in the absence or presence of the concentration of the test compounds which inhibited RTX binding by 50% in the competition experiments. The binding reaction was initiated by transferring the assay tubes into a shaking water bath (37 °C) and then terminated following a 60 min incubation by cooling the assay mixtures on ice. Nonspecific binding was then reduced by adding 100 μg of bovine α_1-acid glycoprotein (AGP; Sigma, St. Louis, MO) to each tube.[41] AGP works by sequestering free [^{3}H]RTX which is in equilibrium with nonspecifically bound [^{3}H]RTX.[41] Since the off-rate of receptor-bound RTX is unmeasurably slow at 0 °C, addition of AGP does not compromise specific RTX binding.[39] Bound and free [^{3}H]RTX were separated by pelleting the membranes in a Beckman 12 microfuge (maximal velocity, 15 min) and then quantitated by scintillation counting. Binding data were analyzed either by the collection of computer programs of PcPherson, collectively referred to as KELL (Biosoft, Cambridge, U.K.), or by a computer program which fits the allosteric Hill equation to the measured values (FitP, Biosoft).[21]

Determination of *in Vivo* Vanilloid-like Activity in the Rat. Acute pain-producing potency of capsaicin and **6a** was determined in the eye-wiping assay of Jancso and co-workers:[42] Increasing concentrations (from 10^{-6} to 10^{-3} g/mL) of the compounds in 10% ethanol/10% Tween 80/80% physiological saline were instilled into the eyes of rats (female Sprague−Dawley rats weighing approximately 200 g), and the number of protective wiping movements with the forelegs was counted.

In other experiments, capsaicin and **6a** (up to 10 mg/ear) dissolved in acetone were applied on the inner surface of rat ear; animals were kept under surveillance for a period of 6 h to detect erythema (ear reddening).

To examine the acute hypothermic action of vanilloids, rectal temperature of rats habituated to room temperature (20 °C) was determined using a small animal temperature probe (introduced into a depth of 5 cm) as described by Szikszay et al.,[43] both before and 60 min after vanilloid treatment. For vanilloid treatment, compounds dissolved in ethanol were injected sc into the scruff of the neck of the animals in a volume of 100 μL under light ether anesthesia in the following dose ranges: RTX, from 10^{-7} to 10^{-4} g/kg; capsaicin, from 10^{-4} to 10^{-2} g/kg; PPAHV (**6a**) from 1 to 200 mg/kg. After the measurement of hypothermic effect had been completed, animals injected with 1, 10, and 100 mg/kg PPAHV, respectively, were randomly distributed into two groups (five animals each): In the first group, animals were challenged the day after with 5 μg/ear RTX applied topically on the inner surface of the rat ear; animals were killed by cervical dislocation 30 min later, and a 0.5 cm diameter ear plug was removed and weighed to quantify edema formation.[44] Rats belonging to the second group were sacrificed under CO_2 anesthesia 24 h after treatment, the spinal cord was removed, and membranes were prepared for [^{3}H]RTX binding experiments in order to determine vanilloid receptor loss.[45] Whenever possible, animal experimentation was carried out under light ether anesthesia to avoid any unncessary discomfort to the rats. All protocols were approved by the institutional ethics committee.

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References

(1) Buck, S. H.; Burks, T. F. The Neuropharmacology of Capsaicin: a Review of Some Recent Observations. *Pharmacol. Rev.* **1986**, *38*, 179−226.
(2) Holzer, P. Capsaicin: Cellular targets, Mechanisms of Action, and Selectivity for the Thin Sensory Neurons. *Pharmacol. Rev.* **1991**, *43*, 144−201.
(3) Bevan, S.; Docherty, R. J. Cellular Mechanism of Action of Capsaicin. In *Capsaicin in the Study of Pain*; Wood, J. N., Ed.; Academic Press: London, 1993; pp 27−44.
(4) Rumsfield, J. A.; West, D. P. Topical Capsaicin in Dermatological and Peripherical Pain Disorders. *DICP Ann. Pharmacother.* **1991**, *25*, 381−387.
(5) Szolcsanyi, J. Perspectives of Capsaicin-type Agents in Pain Therapy and Research. In *Contemporary Issues in Chronic Pain Management*; Parris, W. C. V., Ed.; Kluwer Academic: Boston, 1991; pp 97−124.

(6) Campbell, E.; Bevan, S.; Dray, A. Clinical Applications of Capsaicin and its Analogues. In *Capsaicin in the Study of Pain*; Wood, J. N., Ed.; Academic Press: London, 1993; pp 255−272.
(7) Szolcsanyi, J.; Jancso-Gabor, A. Sensory Effects of Capsaicin Congeners. II. Importance of Chemical Structure and Pungency in Desensitizing Activity of Capsaicin-type Compounds. *Drug Res.* **1976**, *26*, 33−37.
(8) Hayes, A. G.; Oxford, A.; Reynolds, M.; Shingler, A. H.; Skingle, M.; Smith, C.; Tyers, M. B. The Effect of a Series of Capsaicin Analogs on Nociception and Body Temperature in the Rat. *Life Sci.* **1984**, *34*, 1241−1248.
(9) Brand, L.; Berman, E.; Schwen, R.; Loomans, M.; Janusz, J.; Bohne, R.; Maddin, C.; Gardner, J.; LaHann, T.; Farmer, R.; Jones, L.; Chiabrando, C.; Fanelli, R. NE-19550: A Novel, Orally Active Anti-inflammatory Analgesic. *Drug Exp. Clin. Res.* **1987**, *13*, 259−265.
(10) Janusz, J. M.; Buckwalter, B. L.; Young, P. A.; LaHann, T. R.; Farmer, R. W.; Kasting, G. B.; Loomans, M. E.; Kerchkaert, G. A.; Maddin, C. S.; Berman, E. F.; Bohne, R. L.; Cupps, T. L.; Milstein, J. R. Vanilloids. 1. Analogs of Capsaicin with Antinociceptive and Antiinflammatory Activity. *J. Med. Chem.* **1993**, *36*, 2595−2604.
(11) Walpole, C. S. J.; Wrigglesworth, R.; Bevan, S.; Campbell, E. A.; Dray, A.; James, I. F.; Perkins, M. N.; Reid, D. J.; Winter, J. Analogues of Capsaicin with Agonist Activity as Novel Analgesic Agents; Structure-activity Studies. 1. The Aromatic "A-Region". *J. Med. Chem.* **1993**, *36*, 2362−2372.
(12) Walpole, C. S. J.; Wrigglesworth, R.; Bevan, S.; Campbell, E. A.; Dray, A.; James, I. F.; Masdin, K. J.; Perkins, M. N.; Winter, J. Analogues of Capsaicin with Agonist Activity as Novel Analgesic Agents; Structure-activity Studies 2. The Amide Bond "B-Region". *J. Med. Chem.* **1993**, *36*, 2373−2380.
(13) Walpole, C. S. J.; Wriggleworth, R.; Bevan, S.; Campbell, E. A.; Dray, A.; James, I. F.; Masdin, K. J.; Perkins, M. N.; Winter, J. Analogues of Capsaicin with Agonist Activity asNovel Analgesic Agents; Structure-Activity Studies. 3. The Hydrophobic Side-chain "C-Region". *J. Med. Chem.* **1993**, *36*, 2381−2389.
(14) Szallasi, A.; Blumberg, P. M. Resiniferatoxin, a Phorbol-Related Diterpene Acts as an Ultrapotent Analog of Capsaicin, the Irritant Constituent in Red Pepper. *Neuroscience* **1989**, *30*, 515−520.
(15) Blumberg, P. M.; Szallasi, A.; Acs, G. Resiniferatoxin, an Ultrapotent Capsaicin Analogue. In *Capsaicin in the Study of Pain*; Wood, J. N., Ed.; Academic Press: London, 1993; pp 45−62.
(16) Bevan, S.; Hothi, S.; Hughes, G.; James, I. F.; Rang, H. P.; Shah, K.; Walpole, C. S. J.; Yeats, J. C. Capsazepine: a Competitive Antagonist of the Sensory Neurone Excitant Capsaicin. *Br. J. Pharmacol.* **1992**, *107*, 544−552.
(17) ZurHausen, H.; Bornkamm, G. W.; Schmidt, R. J.; Hecker, E. Tumor Initiators and Promoters in the Induction of Epstein-Barr Virus. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 782−785.
(18) Szallasi, A.; Blumberg, P. M. Resiniferatoxin and its Analogs Provide Novel Insights into the Pharmacology of the Vanilloid (Capsaicin) Receptor. *Life Sci.* **1990**, *47*, 1399−1408.
(19) Szallasi, A.; Blumberg, P. M. Specific Binding of Resiniferatoxin, an Ultrapotent Capsaicin Ansalog, by Dorsal Root Ganglion Membranes. *Brain Res.* **1990**, *524*, 106−111.
(20) Szallasi, A. Autoradiographic Visualization and Pharmacological Characterization of Vanilloid (Capsaicin) Receptors in Several Species, Including Man. *Acta Physiol. Scand.* **1995**, *155* (Suppl. 629), 1−68.
(21) Szallasi, A.; Lewin, N. E.; Blumberg, P. M. Vanilloid (Capsaicin) Receptor in the Rat: Positive Cooperativity of Resiniferatoxin Binding and its Modulation by Reduction and Oxidation. *J. Pharmacol. Exp. Ther.* **1993**, *266*, 678−683.
(22) Maderspach, K.; Fajszi, C. β-Adrenergic Receptors of Brain Cells. Membrane Integrity Implies Apparent Positive Cooperativity and Higher Affinity. *Biochim. Biophys. Acta* **1982**, *692*, 469−478.
(23) Acs, G.; Lee, J.; Marquez, V.; Blumberg, P. M. Vanilloid Receptors: Distinct Structure-activity Relations for Simulation of ^{45}Ca Uptake and for High Affinity Binding in Cultured Rat Dorsal Root Ganglion Neurons and Dorsal Root Ganglion Membranes. *Mol. Brain Res.* **1996**, *35*, 173−185.
(24) Lee, J.; Acs, G.; Blumberg, P. M.; Marquez, V. E. Thiourea Analogues of Resiniferatoxin as Ligands for the Vanilloid Receptor. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1331−1334.
(25) Acs, G.; Lee, J.; Marquez, V. E.; Wang, S.; Milne, G. W.; Du, L.; Lewin, N. E.; Blumberg, P. M. Resiniferatoxin amide and Analogues as Ligands for Protein Kinase C and Vanilloid Receptors and Determination of Their Biological Activities as Vanilloids. *J. Neurochem.* **1995**, *65*, 301−308.
(26) Szallasi, A.; Blumberg, P. M. Mechanism and Therapeutic Potential of Vanilloids (Capsaicin-like Molecules). *Adv. Pharmacol.* **1993**, *24*, 123−155.
(27) Bloomfield, G. C.; Wrigglesworth, R.; Ritchie, T. J. Synthesis of 2,9,10-Trioxatricyclo[4.3.1.0^{3,8}]decane Analogues of Resiniferatoxin. *J. Chem. Soc., Perkin Trans. 1* **1992**, 1229−1236.
(28) Szallasi, A.; Sharkey, N. A.; Blumberg, P. M. Structure-activity Analysis of Resiniferatoxin Analogs. *Phytother. Res.* **1989**, *3*, 253−257.
(29) Hecker, E.; Schmidt, R. Phorbolesters, the Irritant Cocarcinogens of Croton Tiglium L. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Eds.; Springer: Wien, 1974; Vol. 31, pp 377−467.
(30) Blumberg, P. M. Class of Compounds Having a Variable Spectrum of Activities for Capsaicin-Like Responses, Composition and use Thereof. U.S. Patent 5,021,450, 1991.
(31) Sorg, B.; Fürstenberger, G.; Berry, D. L.; Hecker, E.; Marks, F. Preparation of Retinoic Acid Esters of Phorbol Derivatives. *J. Lipid Res.* **1982**, *23*, 443−447.
(32) Luche, J.-L.; Gemal, A. L. Lanthanides in Organic Synthesis. 6. The Reduction of α-Enones by Borohydride in the Presence of Lanthanoid Chlorides: Synthetic and Mechanistic Aspects. *J. Am. Chem. Soc.* **1981**, *103*, 5454−5459.
(33) Fisher, H. E.; Hibbert, H. Studies on Lignin and Related Compounds. LXXXIII. Synthesis of 3-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-2-propanone. *J. Am. Chem. Soc.* **1947**, *69*, 1208−1210.
(34) Szallasi, A.; Acs, G.; Cravotto, G.; Blumberg, P. M.; Lundberg, J. M.; Appendino, G. A Novel Agonist, PPAHV, Abolishes Positive Cooperativity of Binding by the Vanilloid Receptor. *Eur. J. Pharmacol.* **1996**, *299*, 221−228.
(35) (a) Goso, C.; Piovacari, G.; Szallasi, A. Resiniferatoxin-Induced Loss of Vanilloid Receptors is Reversible in the Urinary Bladder but not in the Spinal Cord of the Rat. *Neurosci. Lett.* **1993**, *162*, 197−200. (b) Szallasi, A.; Nilsson, S.; Farkas-Szallasi, T.; Blumberg, P. M.; Hökfelt, T.; Lundberg, J. M. Vanilloid (Capsaicin) Receptors in the Rat: Distribution in the Brain, Regional Differences in the Spinal Cord, Axonal Transport to the Periphery, and Depletion by Systemic Vanilloid Treatment. *Brain Res.* **1995**, *703*, 175−183.
(36) In all natural 12,13-phorbol diesters where one of the acyl residues is acetic acid and the other an aromatic ester group, the acetate is always bound to the C-13 oxygen (Evans, F. J.; Taylor, S. E. Pro-Inflammatory, Tumor-Promoting and Anti-Tumor Diterpenes of the Plant Families Euphorbiaceae and Thymelaeaceae. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Eds.; Springer: Wien, 1983; Vol. 44, pp 1−99), suggesting an inherent bias for this arrangement. The alternative arrangement (12-acetyl, 13-acyl) is known with linear aliphatic acids.[29]
(37) Koshland, D. E.; Nemethy, G.; Filmer, D. Comparison of Experimental Binding Data and Theoretical Models in Protein Containing Subunits. *Biochemistry* (*Washington, DC*) **1966**, *5*, 365−385.
(38) Schmidt, R. J.; Evans, F. J. Investigation into the Skin-irritant Properties of Resiniferonol Ortho Esters. *Inflammation* **1979**, *3*, 173−280.
(39) James, I. F.; Walpole, C. S. J.; Szallasi, A. Unpublished observations.
(40) Wender, P. A.; Irie, K.; Miller, B. L. Synthesis and Binding of Photoaffinity Ligand Candidates for Protein Kinase C. *J. Org. Chem.* **1993**, *58*, 4179−4181.
(41) Szallasi, A.; Lewin, N. E.; Blumberg, P. M. Identification of α1-Acid Glycoprotein (Orsomucoid) as a Major Vanilloid-binding Protein in Serum. *J. Pharmacol. Exp. Ther.* **1992**, *262*, 883−888.
(42) Jancso, N.; Jancso-Gabor, A.; Takats, J. Pain and Inflammation Induced by Nicotine, Acetylcholine and Structurally-related Compounds and Their Prevention by Desensitizing Agents. *Acta Physiol. Acad. Sci. Hung.* **1961**, *19*, 113−131.
(43) Szikszay, M.; Obal, F., Jr.; Obal, F. Dose-response Relationship in the Thermoregulatory Effects of Capsaicin. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1982**, *320*, 97−100.
(44) Szallasi, A.; Szolcsanyi, J.; Szallasi, Z.; Blumberg, P. M. Inhibition of [^{3}H]Resiniferatoxin Binding to Rat Dorsal Root Ganglion Membranes as a Novel Approach in Evaluating Compounds with Capsaicin-like Activity. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1991**, *344*, 551−556.
(45) Szallasi, A.; Blumberg, P. M. Vanilloid Receptor Loss in Rat Sensory Ganglia Associated with Long Term Densitization to Resiniferatoxin. *Neurosci. Lett.* **1992**, *136*, 51−54.

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