Analogues of Capsaicin with Agonist Activity as Novel Analgesic Agents; Structure-Activity Studies. 1. The Aromatic "A-Region"

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A series of analogues of capsaicin, the pungent principle of chilli peppers, was synthesized and tested in assays for capsaicin-like agonism *in vitro*. The results of these assays were compared with activities in an acute nociceptive model and a correlation was observed which established that the results of these *in vitro* assays were predictive of analgesia. Using a modular approach the structure-activity profile of specific regions of capsaicin congeners was established using an *in vitro* assay measuring $^{45}Ca^{2+}$ uptake into neonatal rat dorsal root ganglia neurones. Substituted benzylnonanamides **2a-z** and *N*-octyl-substituted phenylacetamides **4a-v** were made to test the requirements for activity in the aromatic "A-region" of the molecule. Compounds with the natural substitution pattern (**2b** and **4c**) and the corresponding catechols (**2i** and **4g**) were the most potent, although the catechols were less potent *in vivo*. Other substitution patterns have reduced activity. These results have established stringent structural requirements for capsaicin-like activity in this part of the molecule.

Introduction

Current pain treatments rely heavily on two types of analgesic agents, the opioids and the nonsteroidal anti-inflammatory agents. In an attempt to develop new analgesics we have concentrated our efforts on a novel mechanistic approach aimed at the nociceptive afferent neurone. More specifically the objective has been to develop compounds which selectively modulate the responses of these sensory nerves. Capsaicin is one such prototype compound. Capsaicin is a pungent compound produced by chilli peppers and related plants of the Capsicum family.[1] The compound was isolated in 1876[2] and the structure was determined as *N*-(4-hydroxy-3-methoxybenzyl)-8-methylnon-6-enamide by Nelson in 1919.[3] The total synthesis was first described in 1955[4] and recent papers[5,6] have extended this route to make a variety of close analogues of capsaicin which also occur in pungent Capsicum extracts.

Capsaicin has a wide spectrum of biological actions[7,8] with effects on the cardiovascular and respiratory systems. It also causes the induction of pain on topical application to skin. This is followed by a period of desensitization, both to further applications of capsaicin and also to other noxious chemical and thermal and mechanical stimuli. Administration of capsaicin to neonatal rats results in permanent loss of the majority of unmyelinated sensory neurones. This neurotoxic effect is much decreased in the adult animal.[9]

The effects of capsaicin seem to be mediated by its actions on a subset of peripheral sensory neurones, the polymodal nociceptors,[10] and although the precise molecular basis for this cellular specificity is not understood, the available evidence suggests that capsaicin interacts with a specific membrane receptor/ion channel complex which is peculiar to this subset of sensory neurones.[11] These effects of capsaicin are cell-specific and dose-dependent. The limited amount of "structure-activity" information available from the literature also supports this hypothesis.

Early structure-activity work[12] established a correlation between pungency and structure in a series of capsaicin analogues and this was followed by the important studies of Szolcsanyi and Jancso-Gabor,[13,14] who, using the rat eye-wipe test, established a structure-activity profile for a limited series of synthetic capsaicin analogues and described a schematic representation of the hypothetical capsaicin receptor. More recently,[15] a series of capsaicin analogues were synthesized in an attempt to separate the antinociceptive properties of these compounds from their hypothermic effects. These efforts met with little success.

Recent work suggests that the plant toxin resiniferatoxin (RTX, see Scheme I) acts on sensory neurones by the same mechanism as capsaicin.[16,17] Binding sites for (^{3}H)RTX that also recognise capsaicin have been found on dorsal root ganglia,[18] and this ligand is now being used to characterize the receptor.[16]

The studies described here investigate the structure-activity profile for capsaicin analogues as agonists *in vitro* and as antinociceptive/analgesic agents. The effect of systematic structural variation of the parent molecule on *in vitro* activity established a coherent structure-activity profile for agonism. A subset of these analogues, which were diverse in both structure and activity, were tested in a model of antinociception *in vivo* and established a correlation between *agonist* and antinociceptive potencies. Subsequently we have used these *in vitro* assays as the biological indices of activity in our drug development program.

Concurrent with our investigations, a group at the Procter and Gamble Co. have undertaken similar studies based on antinociceptive activity *in vivo* and their findings, culminating in the development of Olvanil (see Scheme I) as a topical analgesic agent,[19] are broadly in line with our own.

Our approach has been to subdivide the capsaicin molecule into three regions, henceforth labeled A, B, and C (see Scheme I). Analogues were designed and synthesized by systematically modifying each of these regions in turn while holding the others constant. The compounds were evaluated *in vitro* as described below and this has enabled a comprehensive structure-activity picture of capsaicin agonists to be built up.

The details of our structure-activity investigations using

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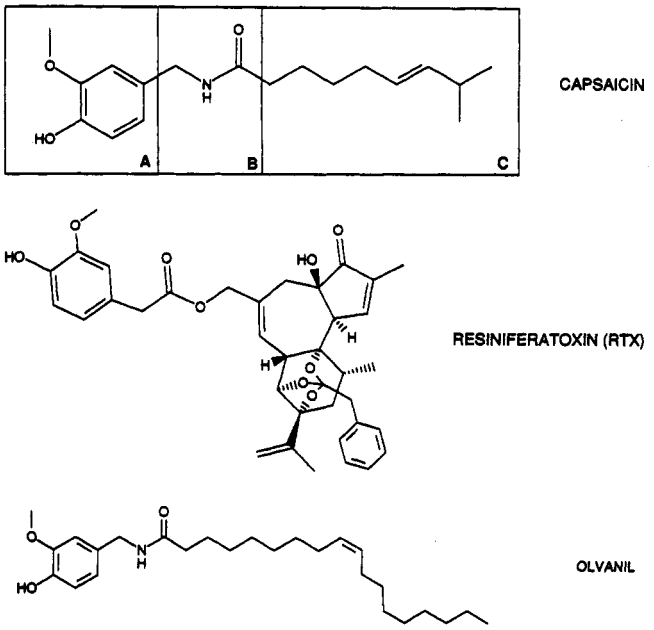
Scheme I



in vitro assays are given below and in the accompanying two papers. Our attempts to exploit this information to develop novel analgesics will be described in a forthcoming publication. This paper describes variations in the aromatic ring of the capsaicin molecule (the A-region).

Chemistry

For the investigation of the structural requirements of the aromatic portion of the capsaicin molecule (the A-region) the following design strategy has been used: (1) Simplification of the side chain (C-region) to octyl (see paper 3 in this series). (2) Synthesis of both amides **2**, and "reverse" amides **4**, is based on the observation of the equipotent biological effects of these two moieties (see also paper 2). This stratagem has been expedient in that it allowed access to a wider variety of commercially available starting materials and increased the flexibility of the synthetic routes.

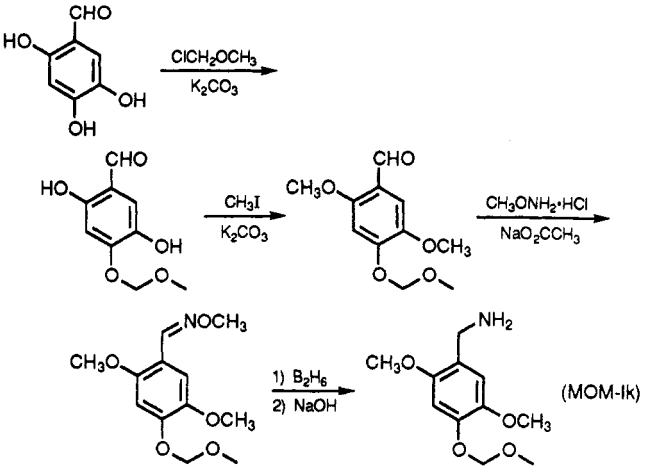
The amides **2** were made by coupling the appropriate amine **1**, with functional group protection if necessary, to an activated nonanoyl function, either an acid chloride (method a) or an *N*-hydroxysuccinimide ester (method b; see Table I).

The amine starting materials **1**, for the synthesis of the amides **2a–z**, shown in Table I, were obtained from commercial sources, synthesized by literature methods (see Experimental Section), or were made as outlined below. Amine **1j** was made from 3,5-dihydroxybenzonitrile by half-alkylation with dimethyl sulfate followed by Raney nickel catalyzed reduction at a pressure of 3 atm of H_2 gas.

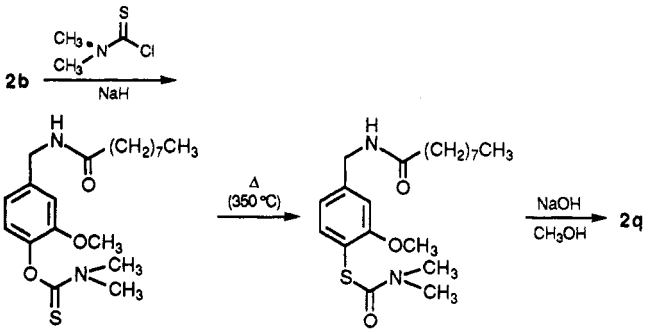
Amine **1k**, protected as the methoxymethyl (MOM) ether, was made by the route outlined in Scheme II. After coupling of protected **1k** with nonanoyl chloride, the deprotection of the MOM group was effected by HCl to give the tetrasubstituted target molecule **2k**.

Amine **1l** was made from the commercially available 2-methoxy-4-(benzyloxy)benzaldehyde by diborane reduction of the intermediate *O*-methylaldoxime. Similarly, amine **1o** was made from 2,3,4-tris(benzyloxy)benzaldehyde.[20] 3-Methoxy-4-nitrobenzylamine (**1r**) was prepared from the corresponding benzaldehyde[21] by reduction of the *O*-methylaldoxime, as before, using diborane. Amine

Scheme II



Scheme III



1v was made by medium-pressure reduction using Raney nickel of the known 2-methoxy-4-cyanopyridine.[22,23]

The target molecules **2m** and **2p** were made by deprotection of the amide precusors **2l** and **2o**, respectively. Thiophenol **2q** was made from **2b** by the route described in Scheme III. The crucial Newman–Kwart rearrangement[24,25] was achieved in moderate yield by pyrolysis at 350 °C. Catalytic hydrogenation of **2r** provided *N*-(4-amino-3-methoxybenzyl)nonanamide (**2s**). The pyridine *N*-oxide **2u** was made by oxidation of **2t**.

The "reverse" amides **4** were made by reacting an activated aryl acetate unit **3** with octylamine. In the three different coupling procedures used, the acid was activated either as an acid chloride (method c), with DCCI (method d) or as a mixed anhydride (method e; see Table II). The details of these coupling procedures are described in the Experimental Section.

The arylacetic acid precursors **3** were obtained from commercial sources with the exception of the benzodioxane **3s**[26] and the related tetrasubstituted compound **3u**. Tl^{III}-mediated oxidative rearrangement[27] of 4-(benzyloxy)-2,3-dimethoxyacetophenone[28] followed by saponification gave the acid **3u**. The acid **3q** was made from 4-aminophenylacetic acid using the Skraup procedure.[29]

4f was made from the commercially available 3,4-(methylenedioxy)phenylacetic acid. Appropriate deprotections of **4h**, **4s**, and **4u**, gave the target compounds **4i**, **4t**, and **4v**, respectively. Compounds **4k** and **4l** were made from the nitro compound **4j**.

Biological Results and Discussion

Previous work from these laboratories[8,17] has established that capsaicin induces an uptake of calcium ions into a subpopulation of dorsal root ganglia (DRG) neurones. This

effect is specific for some sensory afferent neurones and is dose-dependent (EC_{50} for capsaicin is 0.2 μM). Close structural analogues of capsaicin show similar agonist properties but with different potencies, and we have used this assay system as a convenient primary evaluation of the capsaicin-like agonist properties of the compounds presented in these papers. The results of testing the amides and reverse amides in this assay are given in Tables I and II, respectively.

Compounds which showed activity in this ion-flux assay were tested in other *in vitro* assays. In particular, capsaicin induces a dose-dependent contraction of guinea pig ileum and this provides a convenient measure of a capsaicin-like response in an intact tissue. There was a good correlation between the potencies of individual compounds in the two assays (see Table III and Figure 1).

Our studies showed that agonism *in vitro* is prerequisite for analgesic activity in these capsaicin analogues. Analgesic activity was evaluated using the tail flick latency to a noxious thermal stimulus in the mouse. The ED_{50} values of compounds in this analgesic assay are shown in Table III; compounds were considered "inactive" if no significant increase in tail flick latency was noted at doses of 50 μmol/kg. From the data shown in Figure 1 (which includes molecules having structural modifications in *all* parts of the capsaicin prototype) and from Table III it is clear that only potent ($EC_{50} < 1$ μM in $^{45}Ca^{2+}$ influx assay) capsaicin-like agonists *in vitro* show significant analgesic properties. Weak agonists, partial agonists, or antagonists showed no significant analgesic activity. These data show that potent agonism is a necessary, though not necessarily sufficient, prerequisite for analgesic activity.

In this discussion the assumption has been made that the amide and reverse amide moieties in compounds **2** and **4**, respectively, confer similar biological potencies on the two series and are thereby interchangeable functions for structure–activity comparisons. Several observations from the specific structure-activity results can be drawn. These are as follows.

(1) Compounds with the "parent" substitution 3-methoxy-4-hydroxybenzyl are most potent.

(2) Substitutions at positions 2, 5, and 6 on the aromatic ring lead, either singly or in any combination, to poorly active or inactive compounds (e.g. see **4v**, **4r**, and **2k**, respectively).

(3) Simple alkylation of the 4-OH reduces or removes activity (cf. **2b** with **2c** and **2h**, **4c** with **4f**, **4e** with **2e**, and **4g** with **4p**).

(4) Complete removal of the 4-OH substituent leads to loss of agonist activity. In contrast to other analogues which are inactive as agonists, the 3-methoxy compounds **2d** and **4a** act as weak *antagonists* of capsaicin-induced Ca^{2+} influx into DRG neurones.

(5) Variation of the 4-substituent (see **2q–s**) removes or decreases activity, but it is interesting that residual activity is retained with the nitro substituent.

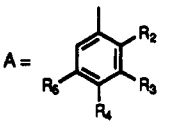
(6) Removal of the 3-methoxy group reduces activity (cf. **4e** with **4c**).

(7) Interchanging the phenol and methoxy substituents at positions 3 and 4 retains but decreases activity (cf. **4p** with **4c**).

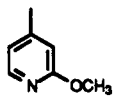
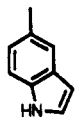
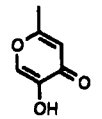
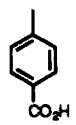
(8) Changing the substituent at the 3-position leads to a reduction or abolition of activity with the exception of the chloro compound **4m**, which, although potent, is a weak partial agonist (see Table IV). Attempts to correlate

Table I. $^{45}Ca^{2+}$ Influx Activity of Substituted Benzyloctylamides

A $CH_2NHCO(CH_2)_7CH_3$
2 a–z

A = 

	R_2	R_3	R_4	R_5	method[a]	Ca^{2+} influx EC_{50} (μM)
2a[b]	H	H	H	H	a	>100
2b	H	OCH_3	OH	H	a	0.55 ± 0.08
2c	H	OCH_3	OCH_3	H		6.41 ± 0.14
2d	H	OCH_3	H	H	a	>100
2e	H	H	OCH_3	H	a	>100
2f	OCH_3	H	H	H	a	>100
2g	H	OH	H	H		>100
2h[c]	H	–OCH_2O–		H	a	>100
2i[d]	H	OH	OH	H		0.63 ± 0.03
2j	H	OCH_3	H	OH	b	>100
2k	OCH_3	H	OH	OCH_3	a	>100
2l	OCH_3	H	OCH_2Ph	H	a	NT[e]
2m	OCH_3	H	OH	H		>100
2n	H	OCH_3	OCH_3	OCH_3	a	>100
2o	OCH_2Ph	OCH_2Ph	OCH_2Ph	H	a	NT[e]
2p	OH	OH	OH	H		7.64 ± 1.09
2q	H	OCH_3	SH	H		>100
2r	H	OCH_3	NO_2	H	a	7.91 ● 1.95
2s	H	OCH_3	NH_2	H		>10

	A	method[a]	Ca^{2+} influx EC_{50} (μM)
2t		a	28.0 ± 3.0
2u			>100
2v			31.5 ± 6.5
2w		a	>100
2x		b	>100
2y		b	>100
2z		b	>100

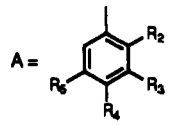
[a] See the text. [b] Reference 30. [c] Reference 31. [d] Reference 15. [e] Not tested.

the changes in physical properties consequent on these structural modifications with *in vitro* activity have been unsuccessful.

The pK_a of the phenol does not correlate with the alterations in the electronic properties (σ) of the adjacent 3-substituent (R, Table IV). Obfuscating steric effects no

Table II. $^{45}Ca^{2+}$ Influx Activity of Substituted *N*-Octylphenylacetamides

A $CH_2CONH(CH_2)_7CH_3$
4 a–v

A = 

	R_2	R_3	R_4	R_5	method[a]	Ca^{2+} influx EC_{50} (μM)
4a	H	OCH_3	H	H	c	>100
4b[b]	H	OCH_3	$OCOCH_3$	H	c	NT[d]
4c[c]	H	OCH_3	OH	H		0.30 ± 0.13
4d	H	H	$OCOCH_3$	H	c	NT[d]
4e[c]	H	H	OH	H		6.50 ● 0.50
4f	H	$-OCH_2O-$		H	c	>100
4g[c]	H	OH	OH	H		0.41 ● 0.03
4h	H	CH_3	OCH_3	H	c	>100
4i	H	CH_3	OH	H		>100
4j	H	NO_2	OH	H	d	1.04 ± 0.02
4k	H	NH_2	OH	H		>100
4l	H	$NHCOCH_3$	OH	H		>100
4m	H	Cl	OH	H	e	0.26 ± 0.13[e]
4n	H	OC_2H_5	OH	H	e	4.34 ± 0.29
4p	H	OH	OCH_3	H	e	1.82 ± 0.13
4q	H	$-CH=CH-CH=N-$		H	e	>100
4r	H	OCH_3	OH	OCH_3	e	>100
4s	$-OCH_2CH_2O-$		OCH_2Ph	H	e	NT[d]
4t	$-OCH_2CH_2O-$		OH	H		>100
4u	OCH_3	OCH_3	OCH_2Ph	H	e	NT[d]
4v	OCH_3	OCH_3	OH	H		>100

[a] See text. [b] Reference 32. [c] Reference 33. [d] Not tested. [e] Partial agonist: 20% efficacy relative to maximal response evoked by capsaicin.

Table III. Comparison of $^{45}Ca^{2+}$ Influx Agonist Activity, Guinea Pig Ileum Contraction, and Analgesia (Mouse Tail Flick Latency) for a Series of "A-Region" Analogues

	EC_{50} (μM)		
compd	Ca^{2+} influx	guinea pig ileum	analgesia ED_{50} (μmol kg^{-1})
capsaicin	0.30 ± 0.04	0.26 ± 0.06	15.00
2b	0.55 ± 0.08	0.40 ± 0.10	5.00
2c	6.41 ± 0.14	1.15 ± 0.15	>50.00
2e	>100.00	22.25 ± 6.55	>50.00
2i	0.63 ± 0.03	0.22 ● 0.03	20.00
4n	4.34 ± 0.29	4.96 ± 1.04	>50.00
4c	0.30 ± 0.13	0.33 ● 0.08	9.20
4e	6.50 ± 0.50	2.75 ± 0.50	>50.00
4g	0.41 ± 0.03	0.33 ± 0.40	~50.00
4j	1.04 ± 0.02	2.78 ± 0.66	>50.00
4m	0.26 ± 0.13[a]	0.68 ● 0.16	>50.00

[a] Partial agonist; 20% efficacy relative to the maximal response evoked by capsaicin.

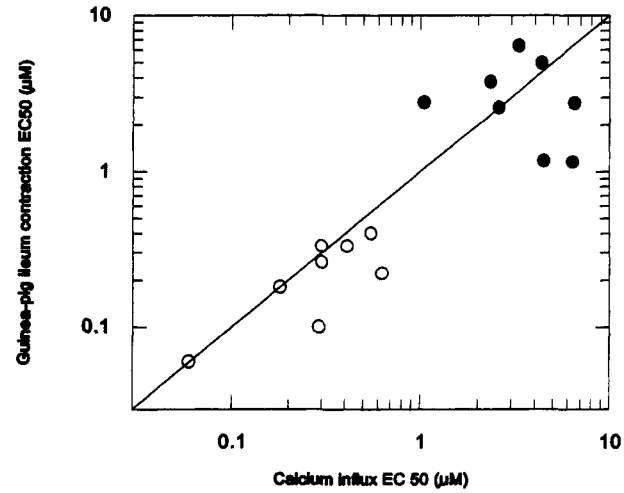


Figure 1. Graph of data from Table III: relationship between $^{45}Ca^{2+}$ influx agonist activity, guinea pig ileum contraction and analgesia (mouse tail flick latency). ○, ED_{50} <50 μmol/kg; ●, ED_{50} ≥ 50 μmol/kg.

doubt contribute to this. There is also no correlation of biological activity with pK_a of the phenol.

In contrast, the chemical shift of the H_6-proton (δ H_6) does appear to be a manifestation of the electronic effects (σ) of the 3-R substituent but does not correlate with biological activity (see Figure 2).

(9) It is interesting that the catechol analogues (**2i** and **4g**) are equipotent *in vitro* with compounds having the capsaicin substitution pattern (**2b** and **4c**) but that the analgesic activity is reduced (Table III). This may be due to faster metabolic inactivation of the catechols.

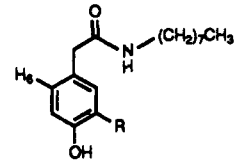
(10) In a further attempt to evaluate the function of the 4-hydroxy substituent, compounds **2x** and **4q** were synthesized and tested. Humber[34,35] has proposed that, under some circumstances, there is bioisosterism between a phenolic hydroxyl group and a pyrrole ring in the case where there is a hydrogen-bond-*donor* contribution to the biological "acceptor" molecule. As a model for cases where a phenol group acts as a hydrogen-bond *acceptor* he has also proposed the pyridine nitrogen atom. Consequently the indole **2x** and the quinoline **4q** were made to test the importance of the H-bond-donor and -acceptor properties of the phenol, respectively. Unfortunately both compounds are inactive, so the caveats that Humber[34] has invoked for failure of this idea generally, e.g. the extra steric constraints that these substituents impose, may also apply here.

(11) The more radical changes of the aromatic ring exemplified by **2t–w** led to loss of or diminution in potency.

(12) The carboxylic acid **2a**, previously reported in the patent literature[36] as an analgesic and anti-inflammatory agent, showed no significant activity in the calcium flux assay.

The information gained from this structure–activity study clearly defines the substitution of this part of the

Table IV. Comparison of *in Vitro* Biological Activities and Physicochemical Properties of 3-Substituted-4-hydroxy-*N*-octylphenylacetamides



compd	R	EC_{50} (μM) Ca^{2+} influx	EC_{50} (μM) guinea pig ileum	pK_a[a]	δ-H_6	σ_{para}[b]
4c	OCH_3	0.30 ± 0.13	0.33 ± 0.08	9.9	6.72	−0.27
4e	H	6.50 ± 0.50	2.75 ± 0.50	10.2	6.80	0
4g	OH	0.41 ● 0.03	0.33 ± 0.40	9.2	6.59	−0.37
4i	CH_3	>100	NT[d]	10.6	6.90	−0.17
4j	NO_2	1.04 ● 0.02	2.78 ± 0.66	7.7	7.54	0.78
4k	NH_2	>100	NT[d]	10.0	6.44	−0.66
4l	$NHCOCH_3$	>100	NT[d]	9.7	6.89	0.21
4m	Cl	0.26 ± 0.13[c]	0.68 ± 0.16	9.0	7.06	0.23
4n	OC_2H_5	4.34 ± 0.29	4.96 ± 1.04	10.4	6.70	−0.24

[a] pK_a measured spectrophotometrically using the automated flow system described: Clark, J.; Cunliffe, A. E. *Chem. Ind.* **1973**, 281. [b] σ_{para} values taken from: Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; J. Wiley and Sons: New York, 1979. [c] Partial agonist: 20% efficacy relative to the maximal response evoked by capsaicin. [d] Not tested.

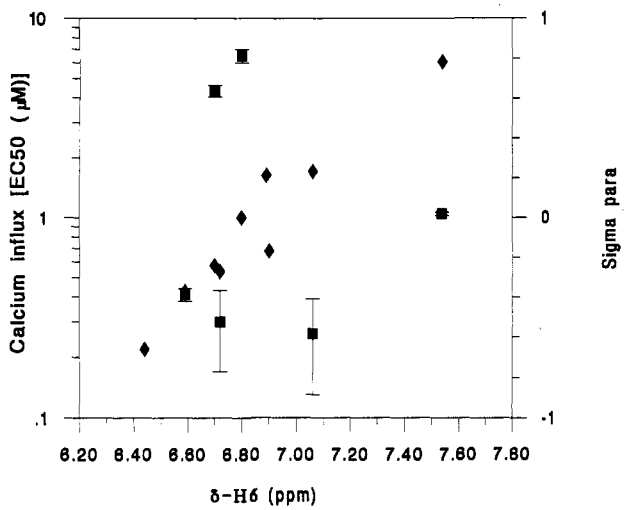


Figure 2. Graph of data from Table IV: relationship between the NMR chemical shift of the aromatic H_6 proton (δ H_6), the electronic substituent parameter, σ_{para}, and $^{45}Ca^{2+}$ Influx Activity. ■, Ca^{2+} influx; ◆, σ_{para}.

capsaicin molecule necessary for high potency in an *in vitro* assay, which we have established is predictive of analgesia. However, a precise understanding of the interaction of this moiety with the putative capsaicin receptor has not emerged. It appears that substitution on the aromatic ring very similar to the parent molecule is all that is tolerated for high potency. Seemingly, the 3 and 4 substituents are best considered as one structural/operational unit in that attempts to dissect these substituents individually has led to diminution or loss of activity. Our assumption that the interaction involves multiple hydrogen bonding has not been disproved but evidence for this proposition is still circumstantial.

However, the definition of a minimal structure for agonism, namely a 4-OH substituent with an adjacent substituent providing increased potency (3-OH or 3-OCH_3) forms a valuable piece of information in assembling a structure–activity profile for capsaicin analogues. This information, taken together with similar studies of other parts (B- and C-regions) of the capsaicin molecule (papers 2 and 3 of this series) form a basis to make compounds of increased potency.

Experimental Section

General Information. Melting points were determined using a Reichert hot-stage microscope and are uncorrected. Routine NMR spectra were recorded using a Hitachi Perkin-Elmer R12B machine. High-field spectra were recorded using Varian VX400 400-MHz (University College London Chemistry Department) and Bruker AM360 360-MHz (Sandoz, Basle, Switzerland) instruments. All spectra were recorded using tetramethylsilane (TMS) as an internal standard and chemical shifts are reported in parts per million (δ) downfield from TMS. Coupling constants are reported in hertz. A Perkin-Elmer 781 machine was used to record IR spectra. Elemental analyses were performed by the Analytical Department of University College London and were within 0.4% of the theoretical values unless otherwise indicated. Mass spectra were recorded by the Mass Spectrometry Department of University College London, using a VG 7070F/H spectrometer, and FAB spectra were recorded at Sandoz (Basle, Switzerland), using a VG 70-SE spectrometer. TLC was performed using Merck Kieselgel 60 F_{254} silica plates or Merck aluminum oxide 60 F_{254} plates, and components were visualized using UV light and iodine vapor. HPLC was performed using a Waters 600 system [μ-Bondapak C-18 column (RP_{18}), using CH_3CN/0.1% aqueous TFA gradients of the compositions stated in the text]. Compounds were purified by flash column chromatography[37] using Merck Kieselgel 60 (230–400 mesh) or Merck neutral aluminum oxide (70–230 mesh) unless otherwise indicated. Solvents were HPLC grade and were used without further purification. Solvents were dried according to the standard procedures.[38] Test compounds were homogeneous by TLC or HPLC unless otherwise stated. Chemical yields were not optimized.

***N*-(3,4-Dimethoxybenzyl)nonanamide (2c).** Compound **2b** (1.0 g, 3.4 mmol) was dissolved in acetone (15 mL) and K_2CO_3 (0.47 g, 3.4 mmol) was added. The suspension was stirred under N_2, methyl iodide (1.9 g, 13.7 mmol) was added, and the mixture refluxed for 18 h. Water (10 mL) was added, and the phases were separated. The organic phase was extracted with 1 N NaOH, washed with water, saturated NaCl, and then dried over Na_2SO_4. The solvent was removed *in vacuo*, leaving a white solid which was purified by flash column chromatography (silica, CH_2Cl_2/MeOH 50:1) to give a colorless crystalline solid: yield 0.35 g (37%); mp 92–94 °C; TLC (silica, CH_2Cl_2/MeOH 50:1) R_f 0.32; ^1H NMR ($CDCl_3$, 60 MHz) δ 0.9 (3H, t, alkyl CH_3), 1.3 (12H, env, alkyl CH_2), 2.2 (2H, t, $COCH_2CH_2$), 3.9 (6H, s, $Ar_{3,4}OCH_3$), 4.4 (2H, d, $ArCH_2NH$), 5.9 (1H, br s, amide NH), 6.9 (3H, m, ArH); MS *m/e* 307 (M^+). Anal. ($C_{18}H_{29}NO_3$) C, H, N.

***N*-(3-Methoxybenzyl)nonanamide (2d).** Compound **2d** was prepared by the condensation of 3-methoxybenzylamine with nonanoyl chloride, in the presence of triethylamine, by analogy with the preparation of **2a**.[30] Recrystallization from petroleum ether (bp 100–120 °C) afforded colorless needles: yield 69%; mp

47–48 °C; TLC (silica; CH_2Cl_2/MeOH 20:1) R_f 0.6; ^1H NMR ($CDCl_3$, 60 MHz) δ 0.9 (3H, t, alkyl CH_3), 1.25 (12H, env, alkyl CH_2), 2.15 (2H, t, $COCH_2CH_2$), 3.75 (3H, s, $ArOCH_3$), 4.35 (2H, δ, $ArCH_2NH$), 5.90 (1H, br s, amide NH), 6.8 (3H, m, $ArH_{2,5,6}$), 7.2 (1H, m, ArH_4); MS *m/e* 277 (M^+). Anal. ($C_{17}H_{27}NO_2$) C, H, N.

***N*-(4-Methoxybenzyl)nonanamide (2e).** Compound **2e** was prepared as described for **2d** from 4-methoxybenzylamine and was recrystallized from the same solvent to give colorless crystals: yield 69%; mp 97.5–98.5 °C; TLC (silica, CH_2Cl_2/MeOH 20:1) R_f 0.54; ^1H NMR ($CDCl_3$, 60 MHz) δ 0.9 (3H, t, alkyl CH_3), 1.25 (12H, env, alkyl CH_2), 2.15 (2H, t, $COCH_2CH_2$), 3.80 (3H, s, $ArOCH_3$), 4.35 (2H, d, $ArCH_2NH$), 6.0 (1H, br s, amide NH), 6.85 (2H, m, $ArH_{3,5}$), 7.2 (2H, m, $ArH_{2,6}$); MS *m/e* 277 (M^+). ($C_{17}H_{27}NO_2$) C, H, N.

***N*-(2-Methoxybenzyl)nonanamide (2f).** Compound **2f** was prepared as described for **2d** from 2-methoxybenzylamine and was recrystallized from the same solvent to give colorless crystals: yield 61%; mp 55–56 °C; TLC (silica, CH_2Cl_2/MeOH 20:1) R_f 0.63; ^1H NMR ($CDCl_3$, 60 MHz) δ 0.90 (3H, t, alkyl CH_3), 1.25 (12H, env, alkyl CH_2), 2.15 (2H, t, $COCH_2CH_2$), 3.85 (3H, s, $ArOCH_3$), 4.45 (2H, d, $ArCH_2NH$), 6.0 (1H, br s, amide NH), 7.0–7.30 (4H, m, ArH); MS *m/e* 277 (M^+). Anal. ($C_{17}H_{27}NO_2$) C, H, N.

***N*-(3-Hydroxybenzyl)nonanamide (2g).** Compound **2d** (0.3 g, 1.1 mmol) was dissolved in CH_2Cl_2 (10 mL) and stirred, on ice, under N_2. Boron trichloride (BCl_3, 1.4 mL of a 2.1 M solution in CH_2Cl_2, 2.9 mmol) was slowly added and the reaction mixture allowed to stir for 2 h. After this time there was little reaction evident by TLC and so additional BCl_3 (1 mL, 2.1 mmol) was added and the reaction stirred at room temperature for 18 h. After this time complete consumption of **2d** was indicated by TLC and so the reaction was stopped by the addition of MeOH (5 mL). The solvent was removed *in vacuo*, leaving an oil which was refluxed in MeOH (20 mL) for 20 min. The solvent was removed once more and the residue partitioned between CH_2Cl_2 and 1 N NaOH. The aqueous layer was acidified to pH 2 and extracted with CH_2Cl_2, which was washed with saturated NaCl and then dried over Na_2SO_4. Removal of the solvent *in vacuo* left a colorless solid, which was recrystallized from petroleum ether (bp 100–120 °C)/EtOAc to give colorless crystals: yield 0.06 g (21%); mp 63–64 °C; TLC (silica, $CHCl_3$/MeOH 25:1) R_f 0.14; ^1H NMR ($CDCl_3$, 60 MHz) δ 0.9 (3H, t, alkyl CH_3), 1.25 (12H, env, alkyl CH_2), 2.2 (2H, t, $COCH_2CH_2$), 4.35 (2H, d, $ArCH_2NH$), 6.28 (1H, br t, amide NH), 6.7–6.9 (4H, m, $ArH_{2,5,6}$ and ArOH), 7.05–7.3 (1H, m, ArH_4); MS *m/e* 263 (M^+). Anal. ($C_{16}H_{25}NO_2$) C, H, N.

3-Hydroxy-5-methoxybenzonitrile. 3,5-Dihydroxybenzonitrile (2.0 g, 15 mmol) and K_2CO_3 were suspended in dry acetonitrile (100 mL) under N_2. Dimethyl sulfate (1.9 g, 15 mmol) was added and the mixture heated under reflux for 2 h. The solution was then cooled, filtered, and washed with acetonitrile and the solvent removed *in vacuo* to leave a white solid which was purified by flash column chromatography (silica, $CHCl_3$) to give white crystals: yield 1.7 g (76.0%); TLC (silica, CH_2Cl_2/MeOH 25:1) R_f 0.45; ^1H NMR (CD_3OD, 60 MHz) δ 3.72 (3H, s, $ArOCH_3$), 6.65–6.98 (3H, m, ArH); MS *m/e* 149 (M^+).

3-Hydroxy-5-methoxybenzylamine. 3-Hydroxy-5-methoxybenzonitrile (0.62 g, 4.0 mmol) was dissolved in MeOH which had been presaturated with NH_3 (50 mL). Raney nickel catalyst (1 g, wet) was added, the mixture placed in a Cook hydrogenator and agitated for 100 min under 3 atm H_2. The catalyst was removed by filtration through a bed of Celite and washed with MeOH. The combined filtrate and washings were evaporated *in vacuo* to leave a pale green powder which was used without purification: yield 0.62 g (100%); TLC (silica, CH_2Cl_2/MeOH 5:1) R_f 0.25; MS *m/e* 153 (M^+).

***N*-(3-Hydroxy-5-methoxybenzyl)nonanamide (2j).** A solution of nonanoic acid NHS ester[39] (0.55 g, 2.15 mmol) in dry EtOAc (10 mL) was stirred, on ice, under N_2. A solution of 3-hydroxy-5-methoxybenzylamine (0.33 g, 2.15 mmol) in EtOAc (5 mL) was slowly added and the reaction stirred at room temperature for 18 h. After this time, water (20 mL) was added, and the phases were separated. The organic phase was washed with 3 × 20 mL of water and then saturated NaCl and finally dried over Na_2SO_4. The solvent was removed *in vacuo* leaving an off-white solid which was purified by flash column chromatography (silica, $CHCl_3$/MeOH 50:1) and recrystallized from $CHCl_3$ to give white crystals: yield 36%; mp 93–95 °C; TLC (silica, CH_2Cl_2/MeOH 20:1) R_f 0.35; ^1H NMR ($CDCl_3$, 60 MHz) δ 0.95 (3H, m, alkyl CH_3), 1.34 (12H, m, alkyl CH_2), 2.2 (2H, t, $COCH_2CH_2$), 3.75 (3H, s, $ArOCH_3$), 4.39 (2H, d, $ArCH_2NH$), 6.4 (3H, m, ArH), 8.2 (1H, br t, amide NH), 9.4 (1H, br s, ArOH), MS *m/e* 293 (M^+). Anal. ($C_{17}H_{27}NO_3$) C, H, N.

2,5-Dihydroxy-4-(methoxymethoxy)benzaldehyde. This compound was prepared as described for 2-hydroxy-4-(methoxymethoxy)benzaldehyde, from 2,4,5-trihydroxybenzaldehyde and was purified by flash column chromatography (silica, cyclohexane/EtOAc 2:1): yield 94%; TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.3; ^1H NMR ($CDCl_3$, 60 MHz) δ 3.8 (3H, s, OCH_3), 5.3 (2H, br s, ArOH), 5.6 (2H, s, $ArOCH_2O$), 7.0 (1H, s, ArH), 7.4 (1H, s, ArH), 9.1 (1H, s, CHO).

2,5-Dimethoxy-4-(methoxymethoxy)benzaldehyde. This compound was prepared from 2,5-dihydroxy-4-(methoxymethoxy)benzaldehyde by treatment with dimethyl sulfate in the presence of K_2CO_3 as described for 2-methoxy-4-(methoxymethoxy)benzaldehyde (see supplementary material) from 2,5-dihydroxy-4-(methoxymethoxy)benzaldehyde and was purified by flash column chromatography (silica, cyclohexane/EtOAc 2:1): yield 66%; TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.8; ^1H NMR ($CDCl_3$, 60 MHz) δ 3.5 (3H, s, OCH_3), 3.85 (6H, s, $Ar_{2,5}OCH_3$), 5.3 (2H, s, $ArOCH_2O$), 6.8 (1H, s, ArH), 7.3 (1H, s, ArH), 10.8 (1H, s, CHO).

***O*-Methyl-2,5-dimethoxy-4-(methoxymethoxy)benzaldoxime.** This compound was prepared as described for *O*-methyl-2-methoxy-4-(methoxymethoxy)benzaldoxime, from 2,5-dimethoxy-4-(methoxymethoxy)benzaldehyde and was purified by flash column chromatography (silica, cyclohexane/EtOAc 3:1): yield 80%; TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.9; ^1H NMR ($CDCl_3$, 60 MHz) δ 3.5 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.9 (3H, s, OCH_3), 5.2 (2H, s, $ArOCH_2O$), 6.75 (1H, s, ArH), 7.3 (1H, s, ArH), 8.4 (1H, s, oxime CH).

2,5-Dimethoxy-4-(methoxymethoxy)benzylamine. *O*-Methyl-2,5-dimethoxy-4-(methoxymethoxy)benzaldoxime (0.5 g, 2.0 mmol) was dissolved in dry THF (50 mL) under N_2 and cooled in an ice bath. A 1 M Diborane solution in THF (2 mL, 2 mmol) was added and the mixture heated under reflux for 3 h, at which time TLC showed complete conversion of the oxime. The reaction mixture was cooled in an ice bath and 2 N NaOH (1 mL) added with stirring. The resulting mixture was refluxed for 18 h, cooled, diluted to 200 mL with water, and extracted with EtOAc (3 × 100 mL). The combined organic extracts were dried over $MgSO_4$ and filtered, and the solvent evaporated *in vacuo* to leave a pale yellow oil which was purified by flash column chromatography (silica, EtOAc/cyclohexane 3:2) to afford a white solid: yield 0.12 g (27%); ^1H NMR ($CDCl_3$, 60 MHz) δ 3.45 (3H, s, OCH_3), 3.75 (6H, s, $Ar_{2,5}OCH_3$), 5.1 (2H, s, OCH_2O), 6.75 (1H, s, ArH), 6.9 (1H, s, ArH).

***N*-[2,5-Dimethoxy-4-(methoxymethoxy)benzyl]nonanamide.** This compound was prepared as described for **2d**, from 2,5-dimethoxy-4-(methoxymethoxy)benzylamine, and was purified by flash column chromatography (silica, cyclohexane/EtOAc 2:1) to afford a white solid: yield 52%; TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.7; ^1H NMR ($CDCl_3$, 60 MHz) δ 0.85 (3H, m, alkyl CH_3), 1.25 (12H, m, alkyl CH_2), 2.2 (2H, m, $COCH_2CH_2$), 3.5 (2H, s, OCH_3), 3.8 (6H, s, $Ar_{2,5}OCH_3$), 4.9 (2H, d, $ArCH_2NH$), 5.25 (2H, s, OCH_2O), 6.85 (1H, s, ArH), 6.95 (1H, s, ArH).

***N*-(2,5-Dimethoxy-4-hydroxybenzyl)nonanamide (2k).** *N*-[2,5-Dimethoxy-4-(methoxymethoxy)benzyl]nonamide (0.071 g, 0.19 mmol) was dissolved in ethanol (2 mL) and the solution was saturated with HCl gas. The solvent was removed *in vacuo* to leave a brown oil which was purified by flash column chromatography (silica, cyclohexane/EtOAc 2:1) to give white crystals: yield 0.020 g (32%); mp 66–68 °C; TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.4; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3H, t, J = 6.9 Hz, alkyl CH_3), 1.26 (10H, m, alkyl CH_2), 1.6 (2H, m, $COCH_2CH_2$), 2.15 (2H, t, J = 7.6 Hz, $COCH_2CH_2$), 3.78 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 4.35 (2H, d, J = 5.9 Hz, $ArCH_2NH$), 5.65 (1H, br s, ArOH), 5.89 (1H, br s, amide NH), 6.56 (1H, s, ArH), 6.84 (1H, s, ArH); MS *m/e* 323 (M^+); HPLC RP_{18} (gradient 10–70% CH_3CN/0.1% aqueous TFA) >98% pure.

O-Methyl-2-methoxy-4-(benzyloxy)benzaldoxime. This compound was prepared as described for O-methyl-2,3,4-tris(benzyloxy)benzaldoxime, from 2-methoxy-4-(benzyloxy)benzaldehyde and was purified by flash column chromatography (silica, cyclohexane/EtOAc 3:1): yield 85%; TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.75; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 3.75 (3H, s, $NOCH_3$), 3.9 (3H, s, $ArOCH_3$), 5.05 (2H, s, OCH_2Ar), 6.5 (2H, m, ArH), 7.4 (5H, m, ArH), 7.75 (1H, m, ArH), 8.35 (1H, s, $ArCH{=}N$).

2-Methoxy-4-(benzyloxy)benzylamine Hydrochloride. This compound was prepared as described for 2,5-dimethoxy-4-(methoxymethoxy)benzylamine from O-methyl-2-methoxy-4-(benzyloxy)benzaldoxime. The crude amine was extracted with EtOAc, dried over Na_2SO_4, and evaporated to give a colorless oil. The oil was redissolved in diethyl ether and saturated with dry HCl gas, causing the hydrochloride to precipitate as white crystals, yield 72%. The hydrochloride was used without further purification.

N-[2-Methoxy-4-(benzyloxy)benzyl]nonanamide (2l). Compound **2l** was prepared as described for **2d** from 2-methoxy-4-(benzyloxy)benzylamine hydrochloride and was purified by flash column chromatography (silica, cyclohexane/EtOAc 3:1): yield 59%; TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.8; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 0.8 (3H, m, alkyl CH_3), 1.2 (12H, env, alkyl CH_2), 2.5 (2H, m, $COCH_2CH_2$), 3.7 (3H, s, $ArOCH_3$), 4.25 (2H, d, $ArCH_2NH$), 5.1 (2H, s, $ArOCH_2Ar$), 6.7 (2H, m, ArH), 7.45 (5H, m, ArH), 7.75 (1H, m, ArH).

N-(2-Methoxy-4-hydroxybenzyl)nonanamide (2m). Compound **2m** was prepared as described for **2p** from **2l** and was purified by flash column chromatography (silica, cyclohexane/EtOAc 2:1): yield 85%; mp 85 °C; TLC (silica cyclohexane/EtOAc 2:1) R_f 0.35; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86 (3H, t, J = 6.9 Hz, alkyl CH_3), 1.24 (10H, m, alkyl CH_3), 1.61 (2H, m, alkyl CH_2), 2.17 (2H, t, J = 7 Hz, $COCH_2$), 3.79 (3H, s, $ArOCH_3$), 4.34 (2H, d, J = 6 Hz, $ArCH_2NH$), 6.02 (1H, br t, amide NH), 6.35 (1H, m, ArH), 6.45 (1H, m, ArH), 7.26 (1H, m, ArH), 7.35 (1H, s, ArOH); MS m/e 293 (M^+). Anal. ($C_{17}H_{27}NO_3$) C, H, N.

N-(3,4,5-Trimethoxybenzyl)nonanamide (2n). Compound **2n** was prepared as described for **2d** from 3,4,5-trimethoxybenzylamine and recrystallized from the same solvent to give white crystals: yield 72%; mp 86 °C; TLC (silica, CH_2Cl_2/MeOH 25:1) R_f 0.5; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 0.8 (3H, m, alkyl CH_3), 1.25 (12H, m, alkyl CH_2), 2.1 (2H, m, $COCH_2CH_2$), 3.78 (9H, s, $ArOCH_3$), 4.25 (2H, d, $ArCH_2NH$), 6.5 (2H, m, ArH); MS m/e 337 (M^+). Anal. ($C_{19}H_{31}NO_4$) C, H, N.

O-Methyl-2,3,4-tris(benzyloxy)benzaldoxime. 2,3,4-Tris(benzyloxy)benzaldehyde[20] (5.0 g, 12 mmol), methoxylamine hydrochloride (2.0 g, 25 mmol) and sodium acetate (1.9 g, 25 mmol) were suspended in MeOH (30 mL) and heated under reflux for 3 h. The reaction mixture was cooled, the solvent removed *in vacuo*, and the residue suspended in water (100 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were dried over Na_2SO_4 and filtered, and the solvent was removed *in vacuo* to leave a yellow oil. The residual oil was purified by flash column chromatography (silica, cyclohexane/EtOAc 3:1) to give pale yellow crystals: yield 4.8 g (88%); ^{1}H NMR ($CDCl_3$, 60 MHz) δ 3.9 (3H, s, OCH_3), 5.05 (6H, s, $Ar_{2,3,4}$-OCH_2Ar), 6.65–7.65 (17H, m, ArH), 8.3 (1H, s, oxime CH).

2,3,4-Tris(benzyloxy)benzylamine Hydrochloride. 2,3,4-Tris(benzyloxy)benzylamine hydrochloride was prepared as described for 2,5-dimethoxy-4-(methoxymethoxy)benzylamine from O-methyl-2,3,4-tris(benzyloxy)benzaldoxime. The crude amine was extracted with EtOAc, dried over Na_2SO_4, and evaporated to leave a pale yellow oil. The residual oil was dissolved in Et_2O and acidified with HCl gas, and the resulting crystals were filtered off and air-dried: yield 65%; MS m/e 425 (M^+).

N-[2,3,4-Tris(benzyloxy)benzyl]nonanamide (2o). Compound **2o** was prepared as described for **2d** from 2,3,4-tris(benzyloxy)benzylamine hydrochloride and was purified by flash column chromatography (silica, cyclohexane/EtOAc 3:1) to give a white solid: yield 46%; TLC (silica, cyclohexane/EtOAc 3:1) R_f 0.25; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 0.95 (3H, m, alkyl CH_3), 1.35 (12H, m, alkyl CH_2), 2.15 (2H, m, $COCH_2CH_2$), 4.32 (2H, d, $ArCH_2$-NH), 5.15 (6H, m, $Ar_{2,3,4}OCH_2Ar$), 5.7 (1H, br s, amide NH), 6.8–7.5 (2H, m, ArH); MS m/e 565 (M^+).

N-(2,3,4-Trihydroxybenzyl)nonanamide (2p). N-[2,3,4-Tris(benzyloxy)benzyl]nonanamide (0.40 g, 0.7 mmol) in MeOH (1 mL) was added to a suspension of 5% Pd/C (0.04 g) in MeOH (3 mL) under N_2. The reaction vessel was evacuated, an atmosphere of H_2 was introduced, and the resulting mixture was stirred at room temperature for 1 h. The H_2 atmosphere was replaced with N_2 and the catalyst was filtered off through a bed of Celite and washed with MeOH. The combined filtrate and washings were evaporated *in vacuo* to leave a black oil, which was purified by flash column chromatography (silica, cyclohexane/EtOAc 1:1) to afford a colorless oil: yield 0.11 g (67%); TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.2; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3H, t, J = 7 Hz, alkyl CH_3), 1.25 (10H, m, alkyl CH_2), 1.59 (2H, m, $COCH_2CH_2$), 2.21 (2H, t, J = 7.5 Hz, $COCH_2CH_2$), 4.24 (2H, d, J = 6.6 Hz, $ArCH_2NH$), 5.44 (1H, s, ArOH), 5.95 (1H, s, ArOH), 6.38 (1H, br t, amide NH), 6.44 (1H, d, J = 8.4 Hz, ArH), 6.51 (1H, d, J = 8.4 Hz, ArH), 10.09 (1H, s, Ar_2OH); MS m/e 295 (M^+), HRMS m/e calcd for $C_{16}H_{25}NO_4$ 295.1783, found 295.1769; HPLC RP_{18} (CH_3CN/0.1% aqueous TFA, 10–70%) >98% pure.

N-[4-[[Dimethyl(thiocarbamoyl)]oxy]-3-methoxybenzyl]-nonanamide. NaH (3.6 g, 150 mmol) was suspended in dry DMF (50 mL) and stirred on ice, under N_2. A solution of **2b** (20 g, 68 mmol) in dry DMF (100 mL) was slowly added and the mixture stirred until gas evolution ceased. A solution of dimethylthiocarbamoyl chloride (11.23 g, 91 mmol) in dry DMF (20 mL) was then added. After addition was complete the ice bath was removed and the temperature was gradually raised to 80 °C and maintained at this temperature for 45 min, after which time no **2b** remained by TLC. The cooled reaction mixture was then diluted with water (2 L) and then extracted with EtOAc/petroleum ether (bp 60–80 °C) 2:1 (5 × 200 mL). The combined organic extracts were washed with 1 N NaOH (200 mL), water (200 mL), and saturated NaCl (100 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* to leave a yellow oil which was purified by flash column chromatography (silica, CH_2Cl_2/MeOH 50:1). An oil was obtained, after removal of solvent *in vacuo*, which crystallized on standing to give colorless crystals: yield 9.6 g (37%); TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.25; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 0.89 (3H, t, alkyl CH_3), 1.30 (12H, env, alkyl CH_2), 2.22 (2H, t, $COCH_2CH_2$), 2.35 (3H, s, NCH_3), 2.45 (3H, s, NCH_3), 3.80 (3H, s, $ArOCH_3$), 4.42 (2H, d, $ArCH_2NH$), 5.92 (1H, br t, amide NH), 6.92 (3H, m, ArH); MS m/e 381 (M^+).

N-[4-[(Dimethylcarbamoyl)thio]-3-methoxybenzyl]nonanamide. N-[4-[[Dimethyl(thiocarbamoyl)]oxy]-3-methoxybenzyl]nonanamide (5 g, 13 mmol) was heated until molten (Bunsen flame) under a stream of N_2. The stirred melt was carefully heated until an exothermic reaction occurred which caused the melt to boil in the absence of heating. The reaction persisted for a further 2 min, after which time the bubbling stopped. No starting material remained at this time by TLC. The crude product was purified by flash column chromatography (silica, EtOAc/cyclohexane 1:1) to give a colorless oil which crystallized on standing: yield 2.5 g (50%); TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.08; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 0.9 (3H, t, alkyl CH_3), 1.25 (12H, env, alkyl CH_2), 2.2 (2H, t, $COCH_2CH_2$), 3.05 (6H, s, $N(CH_3)_2$), 3.84 (3H, s, $ArOCH_3$), 4.42 (2H, d, $ArCH_2NH$), 5.57 (1H, br s, amide NH), 6.90–7.28 (3H, m, ArH).

N-(3-Methoxy-4-mercaptobenzyl)nonanamide (2q). N-[4-[(Dimethylcarbamoyl)thio]-3-methoxybenzyl]nonanamide (1.00 g, 2.55 mmol) was dissolved in MeOH (50 mL) and 5 N NaOH (5 mL, 25 mmol) was added. The mixture was stirred and refluxed for 2 h, after which time no starting material remained by TLC. The MeOH was removed *in vacuo* and the resulting solution was acidified to pH 1 with concentrated HCl and then extracted with EtOAc. The organic phase was washed with saturated NaCl and dried over Na_2SO_4. Removal of the solvent *in vacuo* gave an oil which crystallized on standing. The solid was recrystallized from cyclohexane/diethyl ether to give colorless crystals: yield 0.78 g (96%); mp 60–63 °C; TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.3; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 0.9 (3H, t, alkyl CH_3), 1.30 (10H, env, alkyl CH_2), 1.70 (2H, m, $COCH_2CH_2$), 2.15 (2H, t, $COCH_2$-CH_2), 3.80 (1H, s, ArSH), 3.90 (3H, s, $ArOCH_3$), 4.35 (2H, d, $ArCH_2NH$), 5.95 (1H, br s, amide NH), 6.70–6.90 (2H, m, $ArH_{2,6}$), 7.22 (1H, d, ArH_5); MS m/e 309 (M^+). Anal. ($C_{17}H_{27}NO_2S$) C, H, N.

O-Methyl-3-methoxy-4-nitrobenzaldoxime. This com-

pound was prepared as described for *O*-methyl-2,3,4-tris(benzyloxy)benzaldoxime from 4-nitro-3-methoxybenzaldehyde[21] and the crude product was recrystallized from ethanol to give pale yellow crystals: yield 52%; TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.54; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 3.98 (3H, s, oxime OCH_3), 4.0 (3H, s, $ArOCH_3$), 7.15 (1H, d of d, ArH_6), 7.47 (1H, d, J = 2 Hz, ArH_2), 7.9 (1H, d, J = 8 Hz, ArH_5), 8.04 (1H, s, aldoxime H).

3-Methoxy-4-nitrobenzylamine. This compound was prepared as described for 2,5-dimethoxy-4-(methoxymethoxy)-benzylamine from *O*-methyl-3-methoxy-4-nitrobenzaldoxime to give a brown oil which was used without purification: yield 70%; TLC [silica, CH_2Cl_2/MeOH/AcOH (32% aqueous) 120:90:5] R_f 0.55 (pink stain with ninhydrin); ^{1}H NMR ($CDCl_3$, 60 MHz) δ 1.82 (2H, br s, amine NH_2), 3.7–3.9 (5H, s and d, $ArOCH_3$, $ArCH_2NH_2$), 6.85 (1H, d of d, ArH_6), 6.96 (1H, d, J = 2 Hz, ArH_2), 7.7 (1H, d, J = 8 Hz, ArH_5).

***N*-(3-Methoxy-4-nitrobenzyl)nonanamide (2r).** Compound **2r** was prepared as described for **2d** from 3-methoxy-4-nitrobenzylamine and the crude product was purified by flash column chromatography (silica, cyclohexane/EtOAc 1:1) to give a pale yellow crystalline solid: yield 57%; mp 86–88 °C; TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.25; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 0.85 (3H, t, alkyl CH_3), 1.18 (12H, env, alkyl CH_2), 2.17 (2H, t, $COCH_2CH_2$), 3.81 (3H, s, $ArOCH_3$), 4.35 (2H, d, $ArCH_2NH$), 6.1 (1H, br s, amide NH), 6.82 (1H, d of d, J = 8 Hz, J' = 2 Hz, ArH_6), 6.92 (1H, d, J' = 2 Hz, ArH_2), 7.7 (1H, d, J = 8 Hz, ArH_5); MS m/e 322 (M^+). Anal. ($C_{17}H_{26}N_2O_4$) C, H, N.

***N*-(4-Amino-3-methoxybenzyl)nonanamide (2s).** A portion (150 mg) of 5% Pd/C was suspended in MeOH (4 mL) and stirred in an atmosphere of hydrogen for 5 min. A solution of **2r** (3.5 g, 10.9 mmol) was added and stirring continued for 3 h. The hydrogen atmosphere was evacuated and the catalyst was removed by filtration through Celite. The solvent was removed *in vacuo* to leave a tan solid which was purified by flash column chromatography (silica, cyclohexane/EtOAc 1:1) to give a pale yellow crystalline solid: yield 2.1 g (66%); mp 64–65 °C; TLC (silica, cyclohexane/EtOAc 1:2) R_f 0.3 (pink stain with ninhydrin); ^{1}H NMR ($CDCl_3$, 60 MHz) δ 0.9 (3H, t, alkyl CH_3), 1.3 (12H, env, alkyl CH_2), 2.2 (2H, t, $COCH_2CH_2$), 3.84 (3H, s, $ArOCH_3$), 3.5–3.9 (2H, br s, amine NH_2), 4.32 (2H, d, $ArCH_2NH$), 5.75 (1H, br s, amide NH), 6.6–6.8 (3H, m, ArH); MS m/e 292 (M^+). Anal. ($C_{17}H_{27}N_2O_2$) C, H, N.

***N*-(4-Pyridylmethyl)nonanamide (2t).** This compound was prepared as described for **2d**, purified by flash column chromatography (silica, CH_2Cl_2/MeOH 25:1), and then recrystallized from petroleum ether (bp 100–120 °C)/diethyl ether to give colorless crystals: 52% yield; mp 37–39 °C; TLC (silica, CH_2Cl_2/MeOH 10:1) R_f 0.35; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 0.9 (3H, t, alkyl CH_3), 1.30 (12H, env, alkyl CH_2), 2.25 (2H, t, $COCH_2CH_2$), 4.45 (2H, d, $ArCH_2NH$), 6.35 (1H, br s, amide NH), 7.15 (2H, d of d, pyridine $ArH_{3,5}$), 8.5 (2H, d of d, pyridine $ArH_{2,6}$); MS m/e 248 (M^+). Anal. ($C_{13}H_{24}N_2O \cdot 0.5H_2O$) C, H, N.

4-[(Nonanoylamino)methyl]pyridine 1-Oxide (2u). Compound **2t** (6.0 g, 22.7 mmol) was dissolved in glacial acetic acid (60 mL) and stirred at 5 °C during the addition of 30% H_2O_2 (3.36 mL, 28.8 mmol). The mixture was stirred at 70 °C for 18 h before removal of the solvent *in vacuo*, leaving a pale yellow oil. The oil was dissolved in CH_2Cl_2 (100 mL), washed with saturated $NaHCO_3$ solution and then saturated NaCl, and finally dried over Na_2SO_4. Removal of the solvent *in vacuo* gave a colorless solid which was recrystallized from petroleum ether (bp 100–120 °C)/EtOAc to give colorless crystals: yield 4.2 g (65%); mp 72–74 °C; TLC (silica, CH_2Cl_2/MeOH 1:1) R_f 0.52; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 0.85 (3H, t, alkyl CH_3), 1.2–1.6 (12H, env, alkyl CH_3), 2.28 (2H, t, $COCH_2CH_2$), 4.1 (2H, d, $ArCH_2NH$), 6.7 (1H, br t, amide NH), 7.2 (2H, d of d, pyridine $ArH_{3,5}$), 8.06 (2H, d of d, pyridine $ArH_{2,6}$); MS m/e 264 (M^+). Anal. ($C_{15}H_{24}N_2O_2$) C, H, N.

4-(2-Methoxypyridyl)methylamine Hydrochloride. 4-Cyano-2-methoxypyridine[22,23] (3.0 g, 22.4 mmol) was dissolved in MeOH saturated with dry NH_3 (80 mL) and placed in a Cook hydrogenator. Raney nickel (2.0 g) was added and 5 atm of H_2 was introduced and the mixture was shaken for 75 min before removal of the H_2 atmosphere and purging with N_2. The catalyst was removed by filtration through Celite, and the filtrate was acidified to pH 2 with methanolic HCl. The addition of diethyl ether (100 mL) caused the precipitation of a colorless solid which was collected by filtration and washed several times with diethyl ether to yield 3.1 g (79%).

***N*-(2-Methoxy-4-pyridylmethyl)nonanamide (2v).** 4-(2-Methoxypyridyl)methylamine hydrochloride (1.0 g, 5.7 mmol) was dissolved in DMF (15 mL), 5 N NaOH (2.9 mL, 14.3 mmol) was added, and the solution was stirred under N_2, on ice. Nonanoyl chloride (1.13 g, 6.4 mmol), in DMF (5 mL), was added and the reaction mixture stirred for 18 h. The solvent was removed *in vacuo* and the residue redissolved in CH_2Cl_2, washed with H_2O and saturated NaCl, and dried over Na_2SO_4. The crude product was purified by flash column chromatography (silica, cyclohexane/EtOAc 1:1) and then recrystallized from diisopropyl ether/petroleum ether (bp 100–120 °C) to give colorless crystals: yield 0.62 g (41%); mp 52–55 °C; TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.2; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 0.9 (3H, t, alkyl CH_3), 1.3 (12H, env, alkyl CH_2), 2.2 (2H, t, $COCH_2CH_2$), 3.92 (3H, s, pyridine $ArOCH_3$), 4.35 (2H, d, $ArCH_2NH$), 6.3 (1H, br t, amide NH), 6.6 (1H, d, J = 1.6 Hz, pyridine ArH_2), 6.75 (1H, d of d, J = 1.6 Hz, J' = 5 Hz, pyridine ArH_6), 8.1 (1H, d, J' = 5 Hz, pyridine ArH_5); MS m/e 278 (M^+). Anal. ($C_{16}H_{26}N_2O_2$) C, H, N.

3-[(Nonanoylamino)methyl]pyridine (2w). Compound **2w** was prepared as described for **2d** from 3-aminomethylpyridine and was purified by flash column chromatography (silica, cyclohexane/EtOAc 3:1) to give white crystals: yield 49%; mp 71–73 °C; TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.55; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 0.9 (3H, m, alkyl CH_3), 1.28 (12H, m, alkyl CH_2), 2.2 (2H, m, $COCH_2CH_2$), 4.45 (2H, d, $ArCH_2NH$), 6.85 (1H, br t, amide NH), 7.2–7.75 (2H, m, pyridine $ArH_{5,6}$), 8.55 (2H, m, pyridine $ArH_{2,4}$); MS m/e 284 (M^+). Anal. ($C_{15}H_{24}N_2O$) C, H, N.

***N*-(5-Indolylmethyl)nonanamide (2x).** Compound **2x** was prepared as described for **2j** from 5-(methylamino)indole.[40] The product was recrystallized from petroleum ether (bp 100–120 °C)/EtOAc to give colorless crystals: yield 42%; mp 68–70 °C; TLC (silica, CH_2Cl_2/MeOH 25:1) R_f 0.53; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 0.87 (3H, t, alkyl CH_3), 1.25 (10H, env, alkyl CH_2), 1.55–1.75 (2H, m, $COCH_2CH_2$), 2.18 (2H, t, $COCH_2CH_2$), 4.49 (2H, d, $ArCH_2NH$), 5.85 (1H, br t, amide NH), 5.5 (1H, m, indole ArH_3), 7.0–7.4 (3H, m, indole $ArH_{4,6,7}$), 7.53 (1H, m, indole ArH_2), 8.76 (1H, br s, indole NH); MS m/e 286 (M^+). Anal. ($C_{18}H_{26}N_2O$) C, H, N.

2-[(Nonanoylamino)methyl]-5-hydroxypyran-4-one (2y). Compound **2y** was prepared as described for **2x**, from kojic amine dihydrobromide[41] (with the addition of 2.2 equiv of Et_3N to the suspension of the amine salt prior to acylation), purified by flash column chromatography (silica, CH_2Cl_2/MeOH 25:1), and recrystallized from chloroform: yield 45%; mp 142 °C; TLC (silica, CH_2Cl_2/MeOH 5:1) R_f 0.45; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 0.8 (3H, m, alkyl CH_3), 1.2 (12H, m, alkyl CH_2), 2.05 (2H, m, $COCH_2CH_2$), 4.05 (2H, d, $ArCH_2NH$), 6.15 (1H, s, ArH), 7.95 (1H, s, ArH), 9.0 (1H, s, ArOH); MS m/e 281 (M^+). Anal. ($C_{15}H_{23}NO_4 \cdot 0.4H_2O$) C, H, N.

***N*-Octyl-3-methoxyphenylacetamide (4a).** 3-Methoxyphenylacetic acid (2 g, 12 mmol) was suspended in distilled thionyl chloride and the mixture was refluxed for 1 h. Thionyl chloride was removed *in vacuo* to leave a yellow oil, which was redissolved in benzene. The solvent was removed *in vacuo* and this procedure was repeated. The resulting acid chloride, a yellow oil, was used in the next step without purification.

A solution of octylamine (1.7 g, 13 mmol) and Et_3N (1.3 g, 13 mmol) in EtOAc (100 mL) was stirred, on ice, under N_2. A solution of the acid chloride (2.2 g, 12 mmol) in EtOAc (10 mL) was added dropwise and the mixture allowed to stir for 12 h before the addition of water (100 mL) and the separation of the phases. The organic phase was washed with 1 N HCl (50 mL), water (50 mL), and the saturated NaCl before drying over Na_2SO_4. The solvent was removed *in vacuo* leaving a yellow oil which was crystallized from petroleum ether (bp 100–120 °C)/diethyl ether to give cream platelets: yield 1.6 g (47%); mp 45–47 °C; TLC (silica, $CHCl_3$/MeOH 25:1) R_f 0.5; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 0.9 (3H, t, alkyl CH_3), 1.25 (12H, env, alkyl CH_2), 3.2 (2H, d of t, $NHCH_2CH_2$), 3.55 (2H, s, $ArCH_2CO$), 3.8 (3H, s, $ArOCH_3$), 5.55 (1H, br s, amide NH), 6.75–6.9 (3H, m, $ArH_{2,5,6}$), 7.2 (1H, m, ArH_4); MS m/e 277 (M^+). Anal. ($C_{17}H_{27}NO_2$) C, H, N.

N-Octyl-3,4-(methylenedioxy)phenylacetamide (4f). Compound **4f** was prepared by a method similar to that of **4a**, from 3,4-(methylenedioxy)phenylacetic acid and was recrystallized from diethyl ether/petroleum ether (bp 100–120 °C): yield 64%; mp 90–95 °C; TLC (silica, CH_2Cl_2/MeOH 20:1) R_f 0.55; ^1H NMR ($CDCl_3$, 60 MHz) δ 0.9 (3H, t, alkyl CH_3), 1.25 (12H, env, alkyl CH_2), 3.2 (2H, d of t, $NHCH_2CH_2$), 3.48 (2H, s, $ArCH_2CO$), 5.6 (1H, br s, amide NH), 5.95 (2H, s, methylenedioxy CH_2), 6.76 (3H, m, ArH); MS *m/e* 291 (M^+). Anal. ($C_{17}H_{25}NO_3$) C, H, N.

N-Octyl-4-methoxy-3-methylphenylacetamide (4h). Compound **4h** was prepared by a method similar to that of **4a**, from 4-methoxy-3-methylphenylacetic acid and was purified by flash column chromatography (silica, cyclohexane/EtOAc 2:1) to give an off-white solid: yield 74%; TLC (silica, cyclohexane/EtOAc 2:1) R_f 0.14; ^1H NMR ($CDCl_3$, 60 MHz) δ 0.9 (3H, t, alkyl CH_3), 1.3 (12H, env, alkyl CH_2), 2.2 (3H, s, $ArCH_3$), 3.2 (2H, d of t, $NHCH_2CH_2$), 3.45 (2H, s, $ArCH_2CO$), 3.8 (3H, s, $ArOCH_3$), 5.5 (1H, br s, amide NH), 6.7–7.1 (3H, m, ArH).

N-Octyl-4-hydroxy-3-methylphenylacetamide (4i). Compound **4h** (2.0 g, 6.9 mmol) was dissolved in CH_2Cl_2 (100 mL) and stirred on ice, under N_2. Boron tribromide (BBr_3, 4 mL, 42 mmol) was slowly added and the reaction stirred for 2 h. After this time no **4h** remained by TLC (silica cyclohexane/EtOAc 1:1), so water (100 mL) was added, and the phases were separated. The organic phase was washed with saturated NaCl and dried over Na_2SO_4. The solvent was removed *in vacuo*, leaving a pale yellow oil which crystallized on standing. The solid was recrystallized from petroleum ether (bp 100–120 °C)/EtOAc to give pale cream crystals: yield 1.4 g (73%); mp 56–58 °C; TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.32; ^1H NMR ($CDCl_3$, 60 MHz) δ 0.9 (3H, t, alkyl CH_3), 1.3 (12H, env, alkyl CH_2), 2.25 (3H, s, $ArCH_3$), 3.2 (2H, d of t, $NHCH_2CH_2$), 3.5 (2H, s, $ArCH_2CO$), 5.6 (2H, br s, amide NH, ArOH), 6.8–7.0 (3H, m, ArH); MS *m/e* 277 (M^+). Anal. ($C_{17}H_{27}NO_2$) C, H, N.

N-Octyl-4-hydroxy-3-nitrophenylacetamide (4j). 4-Hydroxy-3-nitrophenylacetic acid (4.80 g, 24 mmol) was dissolved in dry EtOAc (100 mL) and *N*-methylmorpholine (2.46 g, 24 mmol) was added. The mixture was stirred at −15 °C, under N_2, during the addition of isobutyl chloroformate (3.61 g, 26 mmol) at a slow enough rate to ensure that the temperature did not rise above −10 °C. The mixture was allowed to stir for 5 min after complete addition of the chloroformate before the dropwise addition of octylamine (3.10 g, 0.024 mmol). The mixture was stirred for 12 h at room temperature before the addition of 1 N HCl (100 mL) and the separation of the phases. The organic phase was washed with water and then saturated NaCl and dried over Na_2SO_4. The solvent was removed *in vacuo*, leaving a yellow oil which was purified by flash column chromatography (silica, cyclohexane/EtOAc 1:1) and then crystallization from EtOAc to give tan crystals: yield 2.10 g (28%); mp 80–83 °C; TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.3; ^1H NMR ($CDCl_3$, 360 MHz) δ 0.86 (3H, t, alkyl CH_3), 1.24 (10H, env, alkyl CH_2), 1.48 (2H, m, $NHCH_2CH_2$), 3.23 (2H, d of t, $NHCH_2CH_2$), 3.50 (2H, s, $ArCH_2CO$), 5.45 (1H, br s, amide NH), 7.14 (1H, d, J = 8.8 Hz, ArH_5), 7.54 (1H, d of d, J = 8.8 Hz, J' = 2.2 Hz, ArH_6), 8.0 (1H, d, J' = 2.2 Hz, ArH_2), 10.52 (1H, s, ArOH); MS *m/e* 308 (M^+). Anal. ($C_{16}H_{24}N_2O_4$) C, H, N.

N-Octyl-3-amino-4-hydroxyphenylacetamide (4k). A 50-mg portion of 10% Pd/C was stirred in MeOH (0.5 mL) under a H_2 atmosphere for 5 min before the addition of **4j** (0.5 g, 1.6 mmol) dissolved in MeOH (3 mL). The reaction mixture was stirred for 80 min, after which time no **4j** remained by TLC (CH_2Cl_2/MeOH 5:1). The H_2 atmosphere was removed and replaced with N_2, and the catalyst was removed by filtration and washed with MeOH. The solvent was removed *in vacuo* and the residue was recrystallized from EtOAc to give beige crystals: yield 0.2 g (44%); mp 122–135 °C; TLC (silica, CH_2Cl_2/MeOH 5:1) R_f 0.57; ^1H NMR ($CDCl_3$, 360 MHz) δ 0.88 (3H, t, alkyl CH_3), 1.24 (10H, env, alkyl CH_2), 1.42 (2H, m, $NHCH_2CH_2$), 2.46 (2H, br s, NH_2), 3.14 (2H, d of t, $NHCH_2CH_2$), 3.41 (2H, s, $ArCH_2CO$), 3.85 (1H, br s, ArOH), 5.66 (1H, br s, amide NH), 6.44 (1H, d of d, J = 8 Hz, J' = 2 Hz, ArH_6), 6.56 (1H, d, J' = 2 Hz, ArH_2), 6.76 (1H, d, J = 8 Hz, ArH_5); MS *m/e* 278 (M^+). Anal. ($C_{16}H_{26}N_2O_2 \cdot 0.25H_2O$) C, H, N.

N-Octyl-4-acetoxy-3-nitrophenylacetamide. Compound **4j** (1.5 g, 4.9 mmol) was stirred, under N_2, in EtOAc (50 mL), and triethylamine (0.5 g, 5 mmol) was added. Acetyl chloride (0.38 g, 4.9 mmol) in EtOAc (1 mL) was added dropwise and the reaction mixture stirred for 12 h before the addition of water (100 mL) and the separation of the phases. The organic phase was washed with 1 N HCl, 1 N $NaHCO_3$, water, and saturated NaCl and then dried over Na_2SO_4. The solvent was removed *in vacuo* to leave pale yellow crystals: yield 1.47 g (86%); TLC (alumina, CH_2Cl_2/MeOH 25:1) R_f 0.75; ^1H NMR ($CDCl_3$, 60 MHz) δ 0.9 (3H, t, alkyl CH_3), 1.25 (12H, env, alkyl CH_2), 2.35 (3H, s, ArOAc), 3.30 (2H, d of t, $NHCH_2CH_2$), 3.55 (2H, s, $ArCH_2CO$), 6.1 (1H, br s, amide NH), 7.2 (1H, d, J = 8.6 Hz, ArH_5), 7.62 (1H, d of d, J = 8.6 Hz, J' = 2 Hz, ArH_6), 8.02 (1H, d, J' = 2 Hz, ArH_2).

N-Octyl-3-(acetylamino)-4-hydroxyphenylacetamide (4l). Compound **4l** was prepared by hydrogenation of *N*-octyl-4-acetoxy-3-nitrophenylacetamide, by a similar method to **4k**, and subsequent rearrangement. Purification was effected by flash column chromatography (silica, CH_2Cl_2/MeOH 10:1): yield 41%; mp 163–165 °C; TLC (silica, CH_2Cl_2/MeOH) R_f 0.2; ^1H NMR ($CDCl_3$, 360 MHz) δ 0.89 (3H, t, alkyl CH_3), 1.25 (10H, env, alkyl CH_2), 1.42 (2H, m, $NHCH_2CH_2$), 2.22 (3H, s, NAc), 3.16 (2H, d of t, $NHCH_2CH_2$), 3.44 (2H, s, $ArCH_2CO$), 5.79 (1H, br t, CH_2NHCO), 6.9 (2H, m, $ArH_{5,6}$), 7.44 (1H, s, ArH_2), 8.92 (1H, br s, ArNHAc), 9.42 (1H, s, ArOH); MS *m/e* 320 (M^+). Anal. ($C_{18}H_{28}N_2O_3$) C, H, N.

N-Octyl-3-ethoxy-4-hydroxyphenylacetamide (4n). Compound **4n** was prepared as described for **4j** from 3-ethoxy-4-hydroxyphenylacetic acid and purified by flash column chromatography (silica, cyclohexane/EtOAc 1:1) and recrystallization from petroleum ether (bp 100–120 °C)/diethyl ether: yield 35%; mp 50–52 °C; TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.21; ^1H NMR ($CDCl_3$, 360 MHz) δ 0.88 (3H, t, alkyl CH_3), 1.22 (10H, env, alkyl CH_2), 1.41 (2H, m, $NHCH_2CH_2$), 1.45 (3H, t, ethoxy CH_3), 3.18 (2H, d of t, $NHCH_2CH_2$), 3.48 (2H, s, $ArCH_2CO$), 4.11 (2H, q, ethoxy CH_2), 5.38 (1H, br s, amide NH), 5.69 (1H, s, ArOH), 6.70 (1H, d of d, J = 8 Hz, J' = 2 Hz, ArH_6), 6.74 (1H, d, J' = 2 Hz, ArH_2), 6.9 (1H, d, J = 8 Hz, ArH_5); MS *m/e* 307 (M^+). Anal. ($C_{18}H_{29}NO_3$) C, H, N.

N-Octyl-3-hydroxy-4-methoxyphenylacetamide (4p). Compound **4p** was prepared as described for **4j** from 3-hydroxy-4-methoxyphenylacetic acid and purified by flash column chromatography (silica, CH_2Cl_2/MeOH 25:1): yield 68%; mp 72–74 °C; TLC (silica, CH_2Cl_2/MeOH 10:1) R_f 0.7; ^1H NMR ($CDCl_3$, 60 MHz) δ 0.9 (3H, t, alkyl CH_3), 1.25 (12H, env, alkyl CH_2), 3.2 (2H, m, $CONHCH_2CH_2$), 3.5 (2H, s, $ArCH_2CO$), 3.95 (3H, s, $ArOCH_3$), 5.4 (1H, s, ArOH), 5.8 (1H, br s, amide NH), 6.8 (3 H, m, ArH); MS *m/e* 293 (M^+). Anal. ($C_{17}H_{27}NO_3$) C, H, N.

N-Octyl-6-quinolylacetamide (4q). Compound **4q** was prepared as described for **4j** from 6-quinolineacetic acid[29] and purified by flash column chromatography (silica, CH_2Cl_2/MeOH 25:1) and recrystallization from petroleum ether (bp 100–120 °C)/EtOAc: yield 8%; mp 98–100 °C; TLC (CH_2Cl_2/MeOH 5:1) R_f 0.62; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.86 (3H, t, alkyl CH_3), 1.21 (10H, env, alkyl CH_2), 1.25 (2H, m, $NHCH_2CH_2$), 3.23 (2H, d of t, $NHCH_2CH_2$), 3.75 (2H, s, Ar{6-quinoline}CH_2CO), 5.50 (1H, br t, amide NH), 7.42 (1H, d of d, J = 8.4 Hz, J' = 4.2 Hz, ArH_3), 7.61 (1H, d of d, J = 8.4 Hz, J'' = 1.7 Hz, ArH_4), 7.72 (1H, d, J''' = 1.8 Hz, ArH_5), 8.11 (2H, m, $ArH_{7,8}$), 8.91 (1H, d of d, J' = 4.2 Hz, J'' = 1.7 Hz, ArH_2); MS *m/e* 298 (M^+). Anal. ($C_{19}H_{26}N_2O \cdot 0.3H_2O$) C, H, N.

N-Octyl-3,5-dimethoxy-4-hydroxyphenylacetamide (4r). Compound **4r** was prepared as described for **4j** from 3,5-dimethoxy-4-hydroxyphenylacetic acid and purified by flash column chromatography (silica, cyclohexane/EtOAc 1:2) and recrystallization from petroleum ether (bp 100–120 °C)/EtOAc: yield 25% (first crop); mp 75–77 °C; TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.15; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.87 (3H, t, alkyl CH_3), 1.24 (10H, env, alkyl CH_2), 1.42 (2H, m, $NHCH_2CH_2$), 3.19 (2H, d of t, $NHCH_2CH_2$), 3.48 (2H, s, $ArCH_2CO$), 3.88 (6H, s, $ArOCH_3$), 5.42 (1H, br s, amide NH), 5.54 (1H, s, ArOH), 6.47 (2H, s, $ArH_{2,6}$); MS *m/e* 323 (M^+). Anal. ($C_{18}H_{29}NO_4$) C, H, N.

N-Octyl-4-(benzyloxy)-2,3-(ethylenedioxy)phenylacetamide (4s). Compound **4s** was prepared as described for **4j** from 2,3-(ethylenedioxy)-4-(benzyloxy)phenylacetic acid[26] and was used without further purification: 62% yield; TLC (silica, CH_2Cl_2/MeOH 10:1) R_f 0.8; ^1H NMR ($CDCl_3$, 60 MHz) δ 0.85 (3H, t, alkyl CH_3), 1.35 (10H, env, alkyl CH_2), 1.42 (2H, m,

$CONHCH_2CH_2$), 3.25 (2H, m, $CONHCH_2CH_2$), 3.4 (2H, s, $ArCH_2CO$), 4.30 (4H, s, OCH_2CH_2O), 5.10 (2H, s, $ArCH_2OAr$), 6.6 (2H, m, $ArH_{5,6}$), 7.4 (5H, m, benzyl ArH); MS *m/e* 411 (M^+).

***N*-Octyl-2,3-(ethylenedioxy)-4-hydroxyphenylacetamide (4t).** Compound **4s** was deprotected as described for **2p** from **2o** and was purified by flash column chromatography (silica, CH_2Cl_2/MeOH 25:1): 61.4%; yield; mp 88 °C; TLC (silica, CH_2Cl_2/MeOH 10:1) R_f 0.6; ^1H NMR ($CDCl_3$, 360 MHz) δ 0.87 (3H, t, alkyl CH_3), 1.25 (10H, env, alkyl CH_2), 1.42 (2H, m, $CONHCH_2CH_2$), 3.18 (2H, d of t, $CONHCH_2CH_2$), 3.44 (2H, s, $ArCH_2CO$), 4.31 (4H, s, OCH_2CH_2O), 5.46 (1H, br s, ArOH), 5.55 (1H, br t, amide NH), 6.52 (1H, d, J = 8 Hz, ArH_5), 6.67 (1H, d, J = 8 Hz, ArH_6); MS *m/e* 321 (M^+). Anal. ($C_{18}H_{27}NO_4$) C, H, N.

Methyl 4-(Benzyloxy)-2,3-dimethoxyphenylacetate. 4-(Benzyloxy)-2,3-dimethoxyacetophenone[28] (2.0 g, 6.9 mmol) was added to a stirred solution of thallium(III) nitrate (3.1 g, 6.9 mmol) in MeOH (20 mL) containing perchloric acid (4 mL) and the mixture was stirred for 3 h at room temperature. The precipitate that formed was removed by filtration and the filtrate was diluted with water (40 mL) and washed with $CHCl_3$ (3 × 25 mL). The combined extracts were dried over $MgSO_4$ and filtered, and the solvent was removed *in vacuo*, to leave a brown oil which became an off-white solid on trituration with diethyl ether. The solid was used without further purification: yield 1.6 g (73%); TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.75; ^1H NMR ($CDCl_3$, 60 MHz) δ 3.6 (2H, s, $ArCH_2CO_2CH_3$), 3.7 (3H, s, CO_2CH_3), 3.8 (6H, s, $ArOCH_3$), 5.1 (2H, s, $ArCH_2OAr$), 6.75 (2H, m, $ArH_{5,6}$), 7.4 (5H, m, benzyl ArH); MS *m/e* 316 (M^+).

4-(Benzyloxy)-2,3-dimethoxyphenylacetic Acid. Methyl 2,3-dimethoxy-4-(benzyloxy)phenylacetate (1.5 g, 4.7 mmol) was suspended in MeOH (75 mL) and a solution of NaOH (0.47 g, 120 mmol) in water (15 mL) was added. The mixture was refluxed for 5 h before being cooled in an ice bath and acidified with concentrated HCl until a heavy white precipitate formed. The precipitate, which was collected by filtration, washed with water, and air-dried, was used without further purification: yield 1.0 g (69%); TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.15; ^1H NMR ($CDCl_3$, 60 MHz) δ 3.6 (2H, s, $ArCH_2CO_2H$), 3.85 (6H, s, $ArOCH_3$), 5.1 (2H, s, $ArCH_2OAr$), 6.75 (2H, m, $ArH_{5,6}$), 7.4 (5H, m, benzyl ArH), 9.85 (1H, br s, $ArCO_2H$); MS *m/e* 302 (M^+).

***N*-Octyl-4-(benzyloxy)-2,3-dimethoxyphenylacetamide (4u).** Compound **4u** was prepared as described for **4j** from 4-(benzyloxy)-2,3-dimethoxyphenylacetic acid and purified by flash column chromatography (silica, $CHCl_3$): 62% yield; TLC (silica, CH_2Cl_2/MeOH 25:1) R_f 0.7; ^1H NMR ($CDCl_3$, 60 MHz) δ 0.9 (3H, t, alkyl CH_3), 1.25 (12H, env, alkyl CH_2), 3.2 (2H, m, $CONHCH_2CH_2$), 3.5 (2H, s, $ArCH_2CO$), 3.9 (6H, s, $ArOCH_3$), 5.1 (2H, s, $ArCH_2OAr$), 6.85 (2H, m, $ArH_{5,6}$), 7.45 (5H, m, benzyl ArH); MS *m/e* 413 (M^+).

***N*-Octyl-2,3-dimethoxy-4-hydroxyphenylacetamide (4v).** Compound **4v** was prepared by deprotection of **4u** as described for **2p** from **2o** and was purified by flash column chromatography (silica, $CHCl_3$): yield 24%; mp 71–73 °C; TLC (silica, CH_2Cl_2/MeOH 25:1) R_f 0.3; ^1H NMR ($CDCl_3$, 60 MHz) δ 0.9 (3H, t, alkyl CH_3), 1.3 (12H, env, alkyl CH_2), 3.25 (2H, m, $CONHCH_2CH_2$), 3.55 (2H, s, $ArCH_2CO$), 3.95 (6H, s, $ArOCH_3$), 5.8 (1H, br s, amide NH), 6.5 (1H, s, ArOH), 6.85 (2H, m, $ArH_{5,6}$); MS *m/e* 323 (M^+). Anal. ($C_{18}H_{29}NO_4$) C, H, N.

Biology. *In Vitro* Assay. $^{45}Ca^{2+}$ Uptake. The uptake and accumulation of $^{45}Ca^{2+}$ by capsaicin analogues was studied in neonatal rat cultured spinal sensory neurones by the method described in detail by Wood et al.[8] In brief, spinal (dorsal root) ganglia were dissected aseptically from newborn rats and incubated sequentially at 37 °C for 30 min with collagenase (Boehringer Mannheim) followed by 30 min in 2.5 mg/mL trypsin (Worthington), both enzymes made up in Ham's F-14 medium. The ganglia were then washed in medium supplemented with 10% horse serum and the cells dissociated by trituration through a Pasteur pipet. The cells were collected by centrifugation and resuspended in Ham's F-14 medium with 10% horse serum plus 1 μg/mL nerve growth factor. The neuronal preparation was plated onto poly(D-ornithine) Terasaki plates (Flow Laboratories) at a density of 1000 neurones/well. Cultures were incubated at 37 °C in a humidified incubator gassed with 3% CO_2 in air. After the cells had adhered, 10^{-4} M cytosine arabinoside, a mitotic inhibitor, was added to the culture for 48 h to kill the dividing non-neuronal cells.

$^{45}Ca^{2+}$ uptake assays were made on 3–7-day-old cultures. The Terasaki plates were washed four times with calcium-free Hank's balanced salt solution (BSS) buffered with 10 mM HEPES (pH 7.4). Excess medium was drained from the plate and then 10 μL of remaining medium removed from the individual wells. Medium (10 μL) containing the test concentration of compound plus 10 μCi/mL $^{45}Ca^{2+}$ (Amersham) was added to each well. All media contained 1% dimethyl sulfoxide (DMSO) to keep the compounds in solution. The neurones were incubated at room temperature for 10 min, then the Terasaki plates were washed six times in BSS and dried in an oven, and 10 μL of 0.3% sodium dodecyl sulfate was added to each well to dissolve the cells and extract the $^{45}Ca^{2+}$. The contents of each well were transferred to scintillation vials and counted in 1 mL of Beckman CP scintillation fluid. In all experiments one group of replicates were treated with medium alone to estimate the background uptake.

EC_{50} values (the concentration of drug necessary to produce 50% of the maximal response) were estimated with at least six replicates at each concentration. Each compound was tested in two or more independent experiments. Data were fitted with a sigmoidal function of the following form:

$$\text{total uptake} = a/(1 + (EC_{50}/\text{conc})^b) + c$$

where a = the maximum evoked uptake, b = the slope factor, c = the background uptake in the absence of compound. Results are given as mean ± SEM.

***In Vitro* Assay. Guinea Pig Ileum.** Male Dunkin–Hartley guinea pigs were killed and segments of ileum suspended in 8-mL organ baths in Tyrode's solution (37 °C) gassed continuously with 95% O_2/5% CO_2. The tissue was attached to isometric force transducers and allowed to equilibrate for 1 h under a tension of 0.5 g. Compounds were applied at intervals of 1 h using an increasing dose regimen with frequent washings between applications. The amplitude of contraction in response to drug application was measured and calculated as a percentage of the maximal response to capsaicin. From this data an EC_{50} value (i.e. the concentration of drug required to produce a contraction equivalent to 50% of the capsaicin response) was determined. The mean ± SEM EC_{50} value for test compounds was calculated from a minimum of four tissues.

***In Vivo* Antinociceptive Assay. Mouse Tail Flick.** Female mice (CD-1, Charles River, weight 20 g) were maintained in a controlled lighting environment (12 h on/12 h off) and fasted overnight prior to testing. On the day of testing animals were placed in a Perspex restrainer and a section of the tail marked 3.5 cm from the tip. Animals were then removed from the restrainer and either drug or vehicle (10 animals per group) was injected, subcutaneously, into the back of the neck. One hour postinjection of drug or vehicle, tail flick latency was determined with a tail flick analgesia meter (Muromachi Kikai Co. Ltd., Japan; cuttoff latency 30 s). Animals were immobilized in the restrainer and the previously marked area of the tail was placed over the focused light beam, which served as the noxious stimulus, and latency to tail flick was recorded.

The mean ± SEM latency in the drug-treated group was compared to the mean ± SEM latency in the vehicle-treated group using the Mann Whitney U test or Dunnett's multiple comparison, where appropriate. Where appropriate, the percentage analgesia at each dose was calculated using the following equation:

$$\frac{\text{mean latency (drug-treated)} - \text{mean latency (vehicle-treated)}}{\text{cutoff latency (30 s)} - \text{mean latency (vehicle-treated)}}$$

and from this data an ED_{50} was determined as the dose required to produce 50% analgesia. Compounds were considered to have interesting antinociceptive properties if they produced a significant increase in tail flick latency ($p < 0.05$, Student's T-test) at doses less than or equal to 50 μmol/kg. Morphine sulfate administered by the subcutaneous route (ED_{50} = 11.9 μmol/kg^{-1} [8 mg kg^{-1}]) was used as a standard reference compound. NSAIDs were inactive in this test.

Supplementary Material Available: Experimental protocols for the synthesis of compounds previously described in the literature where the methods described herein may differ from the published procedure (9 pages). Ordering information is given on any current masthead page.

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