Analogues of Capsaicin with Agonist Activity as Novel Analgesic Agents; Structure–Activity Studies. 3. The Hydrophobic Side-Chain "C-Region"

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Structural variants of the hydrophobic side chain ("C region") of the capsaicin molecule have been incorporated into a series of vanillylamides and vanillylthioureas. These compounds have been tested in an *in vitro* assay for agonism ($^{45}Ca^{2+}$ influx into dorsal root ganglia neurones), previously shown to be predictive of analgesic activity. The results of this study have established the requirement for a hydrophobic substituent of limited size (molar refractivity, MR, <55) in order to obtain high potency. Combination of the information gained here about the "C-region" of the capsaicin molecule with the studies described in the preceding two papers provides a rational basis for the design of compounds of increased potency.

Introduction

The potential of analogues of capsaicin (1) as novel analgesic agents has been discussed in the preceding papers of this series.

The data presented in this current paper completes the preliminary systematic structure–activity study of various regions of the capsaicin molecule. Herein we describe the consequences of structural variations of the hydrophobic side chain ("C-region", see Scheme I) on activity in *in vitro* bioassays which we have established are predictive of analgesic activity.

Chemistry

The majority of the C-region variants listed in Table I retain the structural features of capsaicin in the A- and B-regions; i.e. they are vanillylamides.

The common synthetic route to these molecules, namely the formation of the amide bond, was achieved by condensation of vanillylamines 3, either as the parent amine or with the phenolic OH group protected as the 2-ethoxyethoxy ether,[1] with an acylating agent derived from an alkyl, aralkyl, or aralkenyl carboxylic acid. The carboxylic acid moiety was activated either as an acid chloride (method a), mixed anhydride (method b), with DCCI (method c), or as the N-hydroxysuccinimide ester (method d). These various coupling methods (see Table I) gave rise to the miscellaneous set of target structures, 4, 6, 8, 10, and 12. In the cases where the phenolic OH group in the resulting amide was protected, treatment of the intermediate with dilute acid gave the free phenol in good yields. The details of the synthetic methods used are unexceptional and need no further description here. They are, however, given in the Experimental Section. Several of the target structures described above were subsequently transformed into other target compounds. Thus, treatment of 4b with TFA removed the Boc protecting group to give the amine 4c as the trifluoroacetate salt. The ester 4d was saponified to the acid 4e. Coupling of the amine 4h, using the mixed anhydride activation method, with 3,6,9-trioxadecanoic acid, gave the product 4n. Catalytic hydrogenation of the phenylethenyl compound 6b gave the saturated analogue 8b. Deprotection of the acetoxyphenyl derivative 8d using sodium bicarbonate gave the phenol 8e. The homovanillic acid amide

Scheme I

1
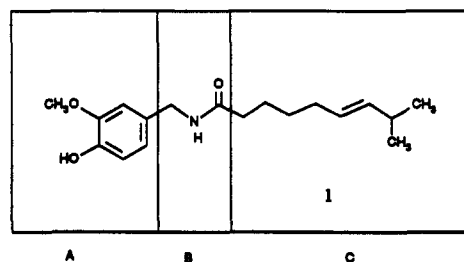
13 ("reverse" amide series) was prepared by the general method described in paper 2 of this series using 2-(*p*-chlorophenyl)ethylamine. The thioureas 15 were made by condensing the vanillylamines 3 with the appropriate isothiocyanate 14. The vanillylamines were used either as the parent amine, following the method of Buckwalter and Lahann[1] for the synthesis of 15f, or with the phenolic OH group protected as the ethoxyethyl ether,[1] as before. The isothiocyanates were commercially available except in the case of the target 15m, where the isothiocyanate was made by a literature method.[2] The primary thiourea 15a was made by treatment of the tritylthiourea 15p with dilute acid.

Biological Results and Discussion

The contributions of the aromatic (A) region and the amide bond (B) region to the analgesic activity of capsaicin analogues have been described in the preceding papers. This paper describes a similar investigation of the hydrophobic side-chain (C) region.

The data from the primary assay ($^{45}Ca^{2+}$ influx into dorsal root ganglia neurones in culture, induced by the capsaicin analogues) is presented in Table I. The close correspondence of activity in this assay compared with that from the guinea pig ileum assay is shown in Table II. The representative analgesia data presented reinforce the earlier derived conclusion that only potent capsaicin-like agonists are active in the mouse tail-flick assay. This data is included in Figure 1 of paper 1.

Several conclusions can be drawn from a consideration of the data presented in Table I.

Capsaicin (1), dihydrocapsaicin (2), and the octylamide 4a are approximately equipotent, suggesting that either

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Table I. Coupling Method and $^{45}Ca^{2+}$ Influx Activity for a Series of C-Region Analogues

	R_2	coupling[a] method	Ca^{2+} influx EC_{50} (μM)		R_2	coupling[a] method	Ca^{2+} influx EC_{50} (μM)
1	$(CH_2)_4(E)$-CH=CH CH$(CH_3)_2$		0.30 ± 0.04	4h	$(CH_2)_{10}NH_2$	c	NT[i]
2[b]	$(CH_2)_6CH(CH_3)_2$		0.19 ± 0.02	4i[c]	$(CH_2)_{16}CH_3$	a	>100
4a	$(CH_2)_7CH_3$	a	0.55 ± 0.08	4j[c]	$(CH_2)_7$-(E)-CH=CH$(CH_2)_7CH_3$	a	0.36 ± 0.07
4b	$(CH_2)_5$NH Boc	d	79.80 ± 10.70	4k[d]	$(CH_2)_7$-(Z)-CH=CH$(CH_2)_7CH_3$	a	0.17 ± 0.03
4c	$(CH_2)_5NH_3^+TFA^-$		>100	4l[e]	CH_2Br	a	>100
4d	$(CH_2)_{10}CO_2CH_3$	a	NT[i]	4m[e]	CH_2Cl	a	>100
4e	$(CH_2)_{10}CO_2H$		>100	4n	$-(CH_2)_{10}NHCOCH_2O$		>100
4f	$CH_2O(CH_2)_2OCH_3$	b	>100		$\quad\quad\quad | \atop CH_3[O(CH_2)_2]_2$		
4g	$CH_2O(CH_2)_2O(CH_2)_2OCH_3$	b	>100				

	alkene	X	coupling[a] method	Ca^{2+} influx EC_{50} (μM)		alkene	X	coupling[a] method	Ca^{2+} influx EC_{50} (μM)

	alkene	X	coupling[a] method	Ca^{2+} influx EC_{50} (μM)		alkene	X	coupling[a] method	Ca^{2+} influx EC_{50} (μM)
6a[e]	E	4-H	a	11.80 ± 1.90	6g	E	4-N$(CH_3)_2$	c	4.39 ± 0.67
6b	E	4-Cl	a	1.24 ± 0.11	6h	E	4-I	a	0.35 ± 0.05
6c	Z	4-Cl	a	50.10 ± 0.80	6i	E	4-NHCHO	b	1000
6d	E	4-NO$_2$	a	4.58 ± 0.29	6j	E	2,4-Cl$_2$	b	0.62 ± 0.25
6e	E	4-CN	a	26.50 ± 5.87	6k	E	3-Cl	a	2.58 ± 0.06
6f	E	4-Ph	a	0.24 ± 0.026^f					

	X	coupling method	Ca^{2+} influx EC_{50} (μM)		X	coupling method	Ca^{2+} influx EC_{50} (μM)

	X	coupling method	Ca^{2+} influx EC_{50} (μM)		X	coupling method	Ca^{2+} influx EC_{50} (μM)
8a[e]	H	a	45.26 ± 2.91	8d	4-OCOCH$_3$	b	NT[i]
8b	4-Cl		3.09 ± 0.07	8e	4-OH		>100
8c[e]	4-NO$_2$	a	21.0 ± 1.1	8f	4-N$_3$	d	4.20 ± 0.27

	structure	coupling[a] method	Ca^{2+} influx EC_{50} (μM)		structure	coupling[a] method	Ca^{2+} influx EC_{50} (μM)
10		a	7.77 ± 1.07	13			0.66 ± 0.07
12		a	4.10 ± 0.25				

	R_2	Ca^{2+} influx EC_{50} (μM)		R_2	Ca^{2+} influx EC_{50} (μM)

	R_2	Ca^{2+} influx EC_{50} (μM)		R_2	Ca^{2+} influx EC_{50} (μM)
15a	H	>100	15i	$(CH_2)_{11}CH_3$	0.16 ± 0.03
15b	$(CH_2)_3CH_3$	9.48 ± 1.05	15j	$(CH_2)_{15}CH_3$	>100
15c	$(CH_2)_4CH_3$	1.96 ± 0.51	15k	$(CH_2)_{17}CH_3$	8.1 ± 1.0
15d	$(CH_2)_5CH_3$	0.18 ± 0.02	15l	$(CH_2)_7$-(Z)-CH=CH$(CH_2)_7CH_3$	>100
15e	$(CH_2)_6CH_3$	0.29 ± 0.03	15m	adamantyl	1.08 ± 0.05
15f[h]	$(CH_2)_7CH_3$	0.06 ± 0.01	15n	CH(Ph)$_2$	0.27 ± 0.02
15g	$(CH_2)_8CH_3$	0.10 ± 0.01	15p	C(Ph)$_3$	>100
15h	$(CH_2)_9CH_3$	0.11 ± 0.01			

[a] See the text. [b] Reference 4. [c] Reference 5. [d] Reference 6. [e] Reference 3. [f] This value is an overestimate because of the limited aqueous solubility of 6f. [g] Reference 7. [h] Reference 1. [i] Not tested.

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

compd	Ca^{2+} influx	guinea pig ileum contraction	analgesia ED_{50} (μmol Kg^{-1})
	EC$_{50}$ (μM)		
capsaicin	0.30 ± 0.04	0.26 ± 0.06	15.0
4a	0.55 ± 0.08	0.40 ± 0.10	5.0
15b	9.48 ± 1.05	3.40 ± 1.45	>50.0
15c	1.96 ± 0.51	1.80 ± 0.53	>50.0
15d	0.18 ± 0.02	0.18 ± 0.05	12.5
15e	0.29 ± 0.03	0.10 ± 0.03	15.0
15f	0.06 ± 0.01	0.06 ± 0.01	3.2

Table III. $^{45}Ca^{2+}$ Influx Activity of a Series of 4-Chlorophenyl Substituted Derivatives

	structure^a	Ca^{2+} influx EC_{50} (μM)
6b	A-NHCO$\diagdown$R	1.24 ± 0.11
6c	A-NHCO$\diagdown$R	50.1 ± 0.1
8b	A-NHCO$\diagdown$R	3.09 ± 0.07
10	A-NHCO$\diagdown$R	7.77 ± 1.07
12	A-NHCO$\equiv$R	4.10 ± 0.25
13	A-CONH$\diagdown$R	0.66 ± 0.07

^{a} A = 3-methoxy-4-hydroxybenzyl, R = 4-chlorophenyl.

the *overall* size or the hydrophobicity (or both) are more important than the double bond and the branched side chain. For simplicity the octyl side chain has been used as the prototype throughout this work. The importance of size and hydrophobicity is also emphasized by the lack of activity shown by compounds with short side chains (4l, 4m, 15a, 15b), with long *polar* side chains (4f, 4g, 4n), with polar functional groups attached to the end of hydrophobic chains (4b, 4c, 4e), and with longer hydrophobic chains (4i, 15j, 15k). However two compounds possessing long aliphatic monounsaturated side chains (4j and 4k) are active in the *in vitro* and *in vivo* assays. We have no explanation for this apparent anomaly. One of these compounds, 4k (Olvanil) has been extensively investigated as an analgesic agent for local application.[8]

The results discussed above support the existence of a hydrophobic binding site of a limited size which can accommodate the C-region of the capsaicin molecule. In an attempt to delineate this site more clearly, we have investigated the activity of compounds containing substituted aromatic rings in the C-region. In contrast to indications from the early literature,[3] it is clear from the data shown in Table I that aromatic substituents can be accommodated in this putative binding site, although the mode of attachment to the "B-region" unit is critical. This is exemplified by compounds 6–13, shown in Table I. In particular, the subset of p-chlorophenyl-substituted compounds shown in Table III suggest that a constrained, linear, extended conformation of a two-carbon spacer unit is optimal (high potency of 6b and 12). Conformationally flexible analogues (8b and the bioequivalent "reverse" amide 13; see paper 2 of this series) can be accommodated well by this model, whereas the shorter chain length analogue 10 is accepted less well, and the *cis*-constrained isomer 6c is accepted poorly.

Comparison of the dehydro series exemplified by 6b in Table III with the saturated series (8b) shows that the former is more potent (cf 6a vs 8a, 6b vs 8b, and 6d vs 8c)
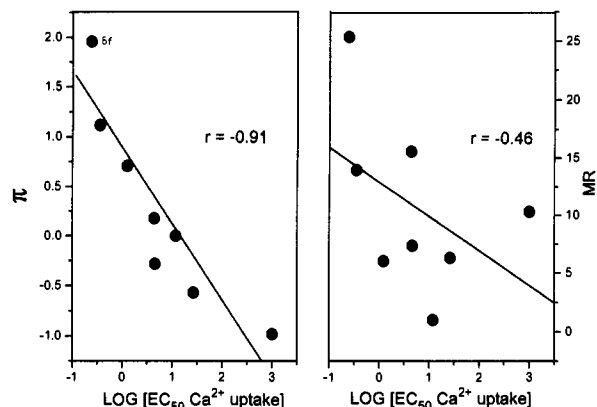
Figure 1. Graphs of data from Table IV: (1) hydrophobic substituent constant, π, values plotted against log EC_{50} values from the $^{45}Ca^{2+}$ influx assay and (2) molar refractivity (MR) values plotted against log EC_{50} values from the $^{45}Ca^{2+}$ influx assay.

Table IV. $^{45}Ca^{2+}$ Influx Activity, Hydrophobic Substituent Constants, and Molar Refractivity Values for a Series of N-(4-Hydroxy-3-methoxybenzyl)-(E)-3-(4-substituted phenyl)propenamides

compd	X	Ca^{2+} influx EC_{50} (μM)	π^a	MR^a
6a	H	11.8 ± 1.9	0	1.03
6b	Cl	1.24 ± 0.11	0.71	6.03
6d	NO$_2$	4.58 ± 0.29	−0.28	7.36
6e	CN	26.50 ± 5.87	−0.57	6.33
6f	C$_6$H$_5$	0.24 ± 0.03	1.96	25.36
6g	N(CH$_3$)$_2$	4.39 ± 0.67	0.18	15.55
6h	I	0.35 ± 0.05	1.12	13.94
6i	NHCHO	1000	−0.98	10.31

^{a} Reference 9.

and consequently a more extensive study has been carried out on the *trans*-alkene analogues 6a,b,d-k.

Variation of the para-substituent on the aromatic ring (in 6a, 6b, and 6d-i) shows that a very good correlation exists ($r = 0.91, P < 0.01$) between potency in the $^{45}Ca^{2+}$ uptake assay and the value of the hydrophobic substituent constant π^9 (Figure 1). The substituents were chosen in an attempt to remove any covariance between the hydrophobic parameter (π) and steric parameter (exemplified here by the molar refractivity MR[9]). It appears from the poor correlation of MR with agonist activity ($r = 0.46, P > 0.1$; see Table IV and Figure 1) that the steric bulk of these relatively small functional groups is not an important controlling factor *in this series*.

Compounds 6f (X = p-Ph) and 6h (X = p-I) were synthesized to test the hypothesis that increasing the hydrophobicity of the para-substituent would increase activity. While 6h falls well onto the correlation line, the p-phenyl-substituted compound 6f is less active than the hypothesis would predict (see Figure 1). However, this compound is poorly soluble in water and it is likely that the measured EC_{50} value is an overestimate, as the material at this nominal concentration is not completely in solution.

These data further support the existence of a hydrophobic binding site. More information about the *size* of this site has been obtained from the thiourea series (15). It has been established (from data in paper 2 of this series
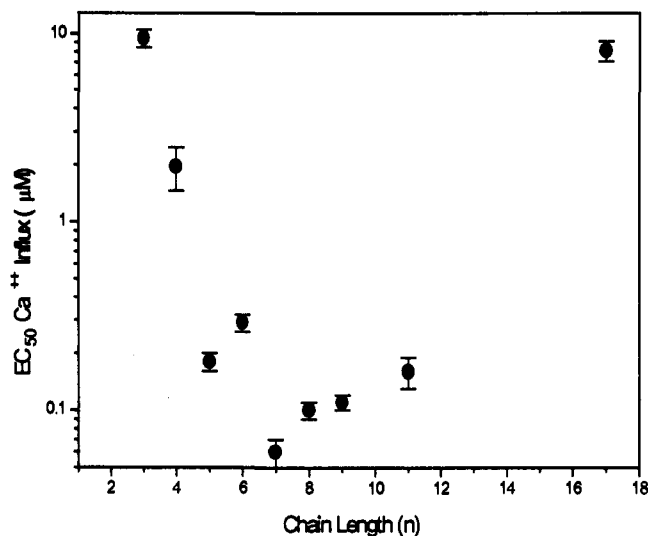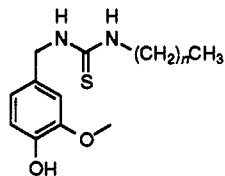
Figure 2. Graph of log EC_{50} values from the $^{45}Ca^{2+}$ influx assay against side-chain length (n) in a series of thioureas (15).

Figure 3. Graph of partition coefficients (log P) against HPLC retention times for a series of thioureas (15).

and in a forthcoming publication) that the thiourea moiety is the most potent B-region recognition function and we have used this series also to investigate the binding requirements in the C-region.

From the data provided in Table I and plotted in Figure 2 it is clear that potency increases with increasing chain length for the series 15a–k to a plateau at a chain length of 8–12 carbon atoms. Compounds with longer chain lengths are poorly active or inactive.

Because there are no convenient hydrophobic substituent parameters for these polymethylene alkyl side chains, we have attempted to use reverse-phase HPLC[10,11] to obtain a measure of the contribution of these alkyl substituents to the overall hydrophobicity of the molecule. By the use of the classical shake-flask technique,[12] the measurement of log P values of a subset of the thioureas (15b–g) has enabled calibration of the HPLC method for assessing hydrophobicity. From the data plotted in Figure 3 it appears that HPLC retention time is a good linear indicator of hydrophobicity (as measured by log P) of this series of molecules and that the hydrophobicity of the whole molecule increases in a linear manner with increasing side-chain length. At side-chain lengths of greater than C_9 the shake-flask method becomes impracticable because of very low aqueous solubility. However, the correlation between chain length and retention time remains linear (data not shown).

From the data reported above (Figure 1) on the substituted phenylpropenamides 6 we have established that there is a correlation of activity with hydrophobicity (π) but not with molecular size (MR). If the assumption is made that the propenamides 6 and the aliphatic thioureas 15 bind at the same site (C-region) it appears that the molecular size of the side-chain substituent is not a controlling factor on activity up to a certain size (i.e., not unreasonably, the binding site has a finite size). This is illustrated by the data presented in Figure 2, where the potency of the thioureas increases with increasing chain
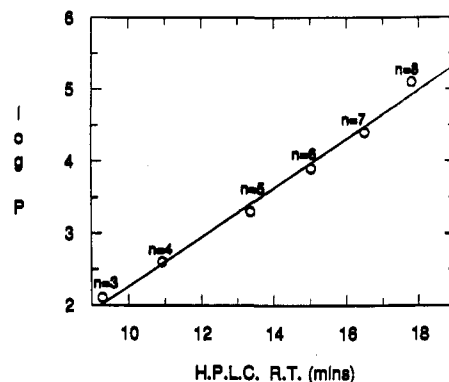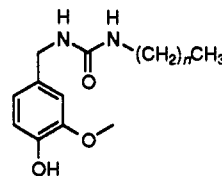
length up to approximately 12 carbon atoms but then decreases with even longer (n = 15, 17) side chains. The fact that the activities of the compounds which are smaller than this size appear to asymptote to this limit may simply reflect the increasing difficulty of fitting a substituent approaching the size limit. A more meaningful estimate of the "size" of this hydrophobic binding site can be presented in terms of molar refractivity, MR. The dodecyl side chain has a MR of 57.[9] In this context it is of interest that the relatively potent benzhydrylthiourea 15n, which has a nominal aliphatic polymethylene "chain length" of 6.5 from its HPLC retention time (from Figure 3), fits well onto the curve in Figure 2. The molar refractivity of the benzhydryl substituent is 54, i.e., just below the size limit for the binding site. From these considerations it is possible to make an estimate of the properties of a substituent which should bind optimally to the C-region binding site. It is expected that small, hydrophobic substituents will confer high potency.

Exploitation of these findings will be the subject of a publication in preparation which attempts to combine the information gained from these systematic studies of the three (A, B, C) regions of the prototype capsaicin molecule, in order to produce compounds which exhibit higher *in vitro* potencies. It is anticipated that this approach will lead to novel analgesic agents.

Experimental Section

formed using Merck Kieselgel 60 F$_{254}$ silica plates or Merck aluminium 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$_3$CN/0.1% aqueous TFA gradients of compositions stated in the text). Compounds were purified by flash column chromatography[13] using Merck Kieselgel 60 (230–400 mesh) or Merck neutral aluminium 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.[14] Test compounds were homogeneous by TLC or HPLC unless otherwise stated. Chemical yields were not optimized.

N-(4-Hydroxy-3-methoxybenzyl)-6-(tert-butoxycarboxamido)hexanamide (4b). Boc-ϵ-Aminocaproic acid N-hydroxysuccinimide ester (1 g, 3.05 mmol) was dissolved in dry DMF (5 mL) and stirred on ice, under N$_2$.

4-(2-ethoxyethoxy)-3-methoxybenzylamine[1] (0.7 g, 3.1 mmol), as a solution in DMF (3 mL), was slowly added. The reaction was stirred overnight before the removal of solvent *in vacuo*. The residue was redissolved in CH$_2$Cl$_2$ and washed with H$_2$O. The organic layer was evaporated *in vacuo* to leave a colorless oil, which was redissolved in THF (10 mL). 2 N HCl (1 mL) was added to the stirred solution. After 10 min of stirring, the solvent was again removed *in vacuo* and the residue partitioned between CH$_2$Cl and water. The organic layer was washed with saturated NaCl and dried over Na$_2$SO$_4$. Removal of the solvent *in vacuo* gave a colorless solid which was recrystallized from petroleum ether (bp 100–120 °C)/EtOAc to give white crystals (0.8 g, 72%); mp 52–56 °C; TLC (silica, CH$_2$Cl$_2$/MeOH 5:1) R_f 0.7; ^{1}H NMR [CDCl$_3$, 60 MHz] δ 1–1.6 (15H, env, alkyl CH$_2$ and BOC CH$_3$), 2.2 (2H, t, COCH$_2$CH$_2$), 3.1 (2H, d of t, CH$_2$CH$_2$NH), 3.85 (3H, s, ArOCH$_3$), 4.35 (2H, d, ArCH$_2$NH), 4.6 (1H, br s, NH), 5.9 (1H, br s NH), 6.8 (3H, m, ArH); MS *m/e* 366 (M$^+$). Anal. (C$_{19}$H$_{30}$N$_2$O$_5$) C, H, N.

N-(4-Hydroxy-3-methoxybenzyl)-6-aminohexanamide Trifluoroacetate (4c). N-(4-Hydroxy-3-methoxybenzyl)-6-(tert-butoxycarboxamido)hexanamide (0.55 g, 1.5 mmol) was dissolved in distilled trifluoroacetic acid (1 mL) and stirred at room temperature for 1 h. After this time, the TFA was removed *in vacuo* to leave a colorless oil which was left under high vacuum overnight to remove traces of TFA (0.38 g, 98%): TLC (silica, CH$_2$Cl$_2$/MeOH 5:1) R_f 0.13 (ninhydrin); ^{1}H NMR (D$_2$O, 60 MHz) δ 1–1.7 (6H, env, alkyl CH$_2$), 2.1 (2H, t, COCH$_2$), 2.8 (2H, t, CH$_2$CH$_2$ND$_3$$^+$), 3.6 (3H, s, ArOCH$_3$), 4.03 (2H, s, ArCH$_2$NDCO), 4.95 (1H, s, ArOH), 6.7 (3H, m, ArH); MS *m/e* 266 (M$^+$); HRMS *m/e* calcd for C$_{14}$H$_{22}$N$_2$O$_3$ 266.1631, found 266.1608; HPLC RP$_{18}$ (gradient 10–50% MeCN/0.1% aqueous TFA) 98% pure.

N-(4-Hydroxy-3-methoxybenzyl)-11-carbomethoxyundecanamide. This compound was prepared by the method described for 8a[7] (see supplementary material). 11-carbomethoxyundecanoyl chloride (100% unpurified) and N-[4-(2-ethoxyethoxy)-3-methoxybenzyl]-11-carbomethoxyundecanamide were intermediates. The product from the final deprotection reaction was an oil which crystallized on standing (58% yield): TLC (silica, CH$_2$Cl$_2$/MeOH 10:1) R_f 0.33; ^{1}H NMR (CDCl$_3$, 60 MHz) δ 1.2 (16H, env, alkyl CH$_2$), 2.2 (4H, 2 × overlapping t, CH$_2$CH$_2$CONH, CH$_2$CH$_2$COOCH$_3$), 3.7 (3H, s, ester OCH$_3$), 3.9 (3H, s, ArOCH$_3$), 4.2 (2H, d, ArCH$_2$NH), 5.3 (1H, br s, ArOH), 5.4 (1H, br t, NH), 6.8 (3H, m, ArH).

N-(4-Hydroxy-3-methoxybenzyl)-11-carboxyundecanamide (4e). N-(4-Hydroxy-3-methoxybenzyl)-11-carbomethoxyundecanamide (40 mg, 0.11 mmol) was dissolved in MeOH (2 mL) and to this was added 5N NaOH (0.5 mL, 2.5 mmol). The solution was stirred at room temperature for 2 h, after which time no starting material was present by TLC. The reaction mixture was diluted with CH$_2$Cl$_2$ (20 mL) and water (20 mL). The aqueous layer was separated and acidified to approximately pH 1, precipitating a white solid. The solid was collected by filtration and dried *in vacuo* to give colorless crystals (30 mg, 78%): mp 103–105 °C; TLC (silica, CH$_2$Cl$_2$/MeOH 10:1) R_f 0.09; ^{1}H NMR (CDCl$_3$, 60 MHz) δ 1.3 (16 H, env, alkyl CH$_2$), 2.25, 2.35 (4H, 2 × overlapping t, CH$_2$CH$_2$CONH, CH$_2$CH$_2$COOH), 3.9 (3H, s, ArOCH$_3$), 4.35 (2H, d, ArCH$_2$NH), 6.0 (1H, br t, NH), 6.7–7.0 (3H, m, ArH); MS *m/e* 365 (M$^+$). Anal. (C$_{20}$H$_{31}$NO$_5$·0.3H$_2$O) C, H, N.

N-(4-Hydroxy-3-methoxybenzyl)-3,6-dioxaheptanamide (4f). 3,6-Dioxaheptanoic acid (2 g, 14.9 mmol) was dissolved in dry DMF (20 mL), N-methylmorpholine (1.5 g, 14.9 mmol) was added, and the mixture was stirred, under N$_2$ at –15 °C. Isobutyl chloroformate (2.2 g, 16.1 mmol) was slowly added such that the temperature did not exceed –10 °C. The mixture was stirred for 5 min before the slow addition of 4-(2-ethoxyethoxy)-3-methoxybenzylamine[1] (3.35 g, 14.9 mmol) in solution in DMF (5 mL). The reaction was stirred at room temperature overnight before removal of the solvent *in vacuo*. The residue was redissolved in THF (100 mL), 1 N HCl (5 mL) was added, and the reaction was stirred for 5 min. The solvent was removed *in vacuo* and the residue was purified by flash column chromatography (silica, EtOAc) to give a colorless oil on removal of the solvent (1.9 g, 47%): TLC (silica, CH$_2$Cl$_2$/MeOH 25:1) R_f 0.14; ^{1}H NMR (CDCl$_3$, 60 MHz) δ 3.25 (3H, s, CH$_2$CH$_2$OCH$_3$), 3.48–3.8 (4H, m, OCH$_2$CH$_2$O), 3.82 (3H, s, ArOCH$_3$) 4.05 (2H, s, COCH$_2$O), 4.4 (2H, d, ArCH$_2$NH), 6.8–6.85 (3H, m, ArH), 7.05 (1H, br s, ArOH), 7.5 (1H, br t, amide NH); MS *m/e* 269 (M$^+$); HRMS, *m/e* calcd for C$_{13}$H$_{19}$NO$_5$ 269.1263, found 269.1279; HPLC RP$_{18}$ (gradient 10–50% MeCN/0.1% aqueous TFA) >99% pure.

N-(4-Hydroxy-3-methoxybenzyl)-3,6-9-trioxadecanamide (4g). This compound was prepared by the method described for 4f and was purified by flash column chromatography (silica, CH$_2$Cl$_2$/MeOH 35:1) to give a colorless oil on removal of the solvent *in vacuo*: 54% yield; TLC (silica, CH$_2$Cl$_2$/MeOH 25:1) R_f 0.17; ^{1}H NMR (CDCl$_3$, 60 MHz) δ 3.3 (3H, s, CH$_2$CH$_2$OCH$_3$), 3.5–3.75 (8H, m, 2 × OCH$_2$CH$_2$O), 3.86 (3H, s, ArOCH$_3$), 4.05 (2H, s, COCH$_2$O), 4.4 (2H, d, ArCH$_2$NH), 5.95 (1H, br s, ArOH), 6.9–7.0 (3H, m, ArH), 7.3 (1H, br t, amide NH); MS *m/e* 313 (M$^+$); HRMS, *m/e* calcd for C$_{15}$H$_{23}$NO$_6$ 313.1525, found 313.1515; HPLC RP$_{18}$ (gradient 10–50% MeCN/0.1% aqueous TFA) >99% pure.

N-[4-(2-ethoxyethoxy)-3-methoxybenzyl]-11-(tert-butoxycarboxamido)undecanamide. 11-(tert-butoxycarboxamido)undecanoic acid[15] (4 g, 13 mmol) and 4-(2-ethoxyethoxy)-3-methoxybenzylamine (3 g, 13 mmol) were dissolved in dry CH$_2$Cl$_2$ (50 mL), and the solution was stirred on ice, under N$_2$. DCCI (2.95 g, 14.3 mmol), in CH$_2$Cl$_2$ (10 mL), was added and the mixture stirred overnight. After this time the precipitate was removed by filtration and the solvent was removed *in vacuo*. The residual oil was redissolved in diethyl ether and the precipitate again removed by filtration. The ether solution was cooled to 0 °C, causing a white compound to crystallize. The crystals were collected by filtration and air-dried: yield 5.1 g (75%); TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.19; ^{1}H-NMR (CDCl$_3$, 60 MHz) δ 1.15 (3H, t, OCH$_2$CH$_3$), 1.3 (16H, env, alkyl CH$_2$), 1.4 (3H, d, CH$_3$CH(OC$_2$H$_5$)) overlapping 1.45 (9H, s, Boc 3 × CH$_3$), 2.2 (2H, t, CH$_2$CH$_2$CO), 3.2 (2H, d of t, CH$_2$CH$_2$NH), 3.7 (2H, q, OCH$_2$CH$_3$), 3.9 (3H, s, ArOCH$_3$), 4.4 (2H, d, ArCH$_2$NH), 4.5 (1H, br s, NH), 5.5 (1H, q, CH$_3$CH(OR)OC$_2$H$_5$), 6.0 (1H, br s, NH), 6.9 (3H, m, ArH).

N-(4-Hydroxy-3-methoxybenzyl)-11-aminoundecanamide Trifluoroacetate (4h). N-[4-(2-ethoxyethoxy)-3-methoxybenzyl]-11-(tert-butoxycarboxamido)undecanamide (1 g, 2 mmol) was dissolved in dry CH$_2$Cl$_2$ (10 mL) and stirred on ice, under N$_2$. TFA (5 mL, 66 mmol) was added and the mixture was stirred for 30 min, after which time no starting material remained by TLC. The solvent was removed *in vacuo*, leaving a colorless oil which was purified by flash column chromatography (silica, CH$_2$Cl$_2$/MeOH 10:1, then MeOH). The pure fractions were evaporated *in vacuo* to give a colorless oil: 0.85 g (97%); TLC (silica, CH$_2$Cl$_2$/MeOH 5:1, ninhydrin) R_f 0.11.

N-(4-Hydroxy-3-methoxybenzyl)-11-(3,6,9-trioxadecanamido)undecanamide (4n). 3,6,9-Trioxadecanoic acid (0.4 g, 2.2 mmol) was dissolved in dry DMF (10 mL) and N-methylmorpholine (0.22 g, 2.2 mmol) was added. The solution was stirred, under N$_2$, at –15 °C while isobutyl chloroformate (0.3 g, 2.4 mmol) in DMF was slowly added. After 5 min of stirring, 4h (1 g, 2.2 mmol) was added, in DMF (1 mL), together with N-methylmorpholine (0.25 g, 2.5 mmol). The reaction mixture was stirred overnight before removal of the solvent *in vacuo*. The crude product was purified by flash column chromatography (silica, CH$_2$Cl$_2$/MeOH 25:1) to give a colorless oil: yield 0.3 g (27%); TLC (silica, CH$_2$Cl$_2$/MeOH 10:1) R_f 0.42; ^{1}H NMR (CDCl$_3$, 400 MHz) δ 1.25 (12H, env, alkyl CH$_2$), 1.5 (2H, m, COCH$_2$CH$_2$), 1.65 (2H, m, NHCH$_2$CH$_2$), 2.2 (2H, t, COCH$_2$CH$_2$), 3.28 (2H, d

of t, CH$_2$CH$_2$NH), 3.39 (3H, s, OCH$_3$), 3.57–3.66 (8H, m, 2 ×
OCH$_2$CH$_2$O), 3.87 (3H, s, ArOCH$_3$), 3.98 (2H, s, COCH$_2$O), 4.36
(2H, d, ArCH$_2$NH), 5.31 (1H, s, ArOH), 5.86 (1H, br t, amide
NH), 6.75 (1H, d of d, J = 2 Hz, J' = 8 Hz, ArH$_6$), 6.80 (1H, d,
J = 2 Hz, ArH$_2$), 6.86 (1H, d, J' = 8 Hz, ArH$_5$), 7.05 (1H, br t,
amide NH); MS *m/e* 496 (M⁺); HRMS *m/e* calcd for C$_{26}$H$_{44}$N$_2$O$_7$
496.3150, found 496.3207; HPLC RP$_{18}$ (gradient 10–50% MeCN/
0.1% aqueous TFA) >95% pure.

**N-(4-Hydroxy-3-methoxybenzyl)-(E)-3-(4-chlorophenyl)-
propenamide (6b).** This compound was prepared from 4-chlo-
rocinnamic acid in three steps by a method similar to that
described for 8a[7] (see supplementary material). 4-Chlorocin-
namoyl chloride (98% without purification) and N-[4-(2-ethox-
yethoxy)-3-methoxybenzyl]-(E)-3-(4-chlorophenyl)propena-
mide (33% after recrystallization from EtOAc) were intermediates.
The crude product from the final deprotection step was recrys-
tallized from EtOAc to yield fine, colorless needles (400 mg,
97%): mp 151–153 °C; TLC (silica, CHCl$_3$/MeOH 5:1) R$_f$ 0.58;
^{1}H NMR (DMSO-d$_6$, 60 MHz) δ 3.75 (3H, s, ArOCH$_3$), 4.3 (2H,
d, CH$_2$NH), 6.6 (1H, d, J = 15 Hz, *trans* COCH=CH), 6.7–6.9
(3H, m, vanillyl ArH), 7.45 (1H, d, J = 15 Hz, *trans* COCH=CH),
7.5–7.6 (4H, m, 4-Cl-phenyl ArH), 8.2–8.6 (1H, broad t, NH), 8.8
(1H, s, ArOH); MS *m/e* 317 (M⁺). Anal. (C$_{17}$H$_{16}$NO$_3$Cl·H$_2$O) C,
H, N, Cl.

**N-(4-Hydroxy-3-methoxybenzyl)-(Z)-3-(4-chlorophenyl)-
propenamide (6c).** A solution of 4-chlorocinnamic acid (400
mg) in MeOH (25 mL) was irradiated for 24 h with UV light (λ
= 254 nm). Removal of the solvent *in vacuo* yielded a white
crystalline material consisting of a mixture of Z and E isomers
in a 1:3 ratio (by NMR). The mixed (Z)- and (E)-cinnamic acids
(0.39 g, 2.14 mmol) were dissolved in dry DMF (15 mL) and
cooled to –15 °C. N-Methylmorpholine (0.22 g, 2.1 mmol) was
added, followed by the slow addition of isobutyl chloroformate
(0.32 g, 2.3 mmol), the temperature being kept below –10 °C. The
mixture was stirred for 10 min, after which time 4-(2-ethoxy-
ethoxy)-3-methoxybenzylamine (0.48 g, 2.1 mmol) in dry DMF
(1 mL) was slowly added and the mixture stirred for 2 h. Removal
of the solvent *in vacuo* left a yellow oil, which was dissolved in
EtOAc and washed with 1 M Na$_2$CO$_3$ and then dried over MgSO$_4$.
The solvent was removed *in vacuo* to leave a pale yellow oil (0.8
g, 2 mmol) which was dissolved in THF (20 mL) and cooled on
ice. 1 N HCl (3 mL) was then added and the mixture stirred
under N$_2$ for 1 h, after which time the solution was neutralized
with 1 N NaOH and the solvent removed *in vacuo*. The crude
product was purified by flash column chromatography (silica,
cyclohexane/EtOAc 2:3) to give a colorless oil: yield 150 mg (22%);
TLC (silica, cyclohexane/EtOAc 2:3) R$_f$ 0.26; ^{1}H NMR (CDCl$_3$,
400 MHz) δ 3.83 (3H, s, ArOCH$_3$), 4.34 (2H, d, CH$_2$NH), 5.62–5.8
(2H, br s, NH, ArOH), 6.0 (1H, d, J = 12 Hz, *cis* COCH=CH),
6.65–6.72 (3H, m, vanillyl ArH), 6.83 (1H, d, J = 12 Hz, *cis*
COCH=CH), 7.2–7.4 (4H, m, 4-Cl-Ph ArH); MS *m/e* 317 (M⁺);
HRMS, *m/e* calcd for C$_{17}$H$_{16}$NO$_3$Cl 317.0819, found 317.0835;
HPLC RP$_{18}$ (gradient 10–50% MeCN/0.1% aqueous TFA) >99%
pure.

**N-(4-Hydroxy-3-methoxybenzyl)-(E)-3-(4-nitrophenyl)-
propenamide (6d).** This compound was prepared from 4-ni-
trocinnamic acid in three steps by a method similar to that
described for 8a[7] (see supplementary material). 4-Nitrocin-
namoyl chloride (83%) and N-[4-(2-ethoxyethoxy)-3-methoxy-
benzyl]-(E)-3-(4-nitrophenyl)propenamide (69% after recrys-
tallization from EtOAc) were intermediates. The crude product
from the subsequent deprotection step was crystallized from
MeOH/petroleum ether (bp 100–120 °C) to give yellow crystals,
90% yield: mp 170–173 °C; TLC (CHCl$_3$/MeOH 5:1) R$_f$ 0.55; ^{1}H
NMR (DMSO-d$_6$, 60 MHz) δ 3.8 (3H, s, ArOCH$_3$), 4.35 (2H, d,
CH$_2$NH), 6.8–7.0 (3H, m, vanillyl ArH), 6.9 (1H, d, J = 15 Hz,
trans CH=CHPh), 7.65 (1H, d, J = 15 Hz, *trans* CH=CHPh),
7.8–8.4 (4H, m, ArH), 8.7 (1H, br t, NH), 8.9 (1H, br s, ArOH);
MS *m/e* 328 (M⁺). Anal. (C$_{17}$H$_{18}$N$_2$O$_5$) C, H, N.

**N-(4-Hydroxy-3-methoxybenzyl)-(E)-3-(4-cyanophenyl)-
propenamide (6e).** This compound was prepared from 4-cy-
anocinnamic acid by a method similar to that described for 8a[7]
(see supplementary material): yield 76% overall; mp 179–179
°C; TLC (silica, cyclohexane/EtOAc 1:1) R$_f$ 0.08; ^{1}H NMR
[DMSO-d$_6$, 60 MHz] δ 3.83 (3H, s, ArOCH$_3$), 4.35 (2H, d, J = 6
Hz, CH$_2$NH), 6.8–6.98 (3H, m, vanillyl ArH) overlapping (1H, d,

J = 15 Hz, *trans* CH=CHPh) 7.65 (1H, d, J = 15 Hz, *trans*
CH=CHPh), 7.8 (4H, s, ArH), 8.1 (1H, br s, amide NH); FAB
MS *m/e* 309 (M + 1⁺). Anal. (C$_{18}$H$_{16}$N$_2$O$_3$) C, H, N.

**N-(4-Hydroxy-3-methoxybenzyl)-(E)-3-(4-phenylphenyl)-
propenamide (6f).** This compound was prepared by the method
described for 8a[7] (see supplementary material). 4-Phenylcin-
namoyl chloride (98% unpurified) and N-[4-(2-ethoxyethoxy)-
3-methoxybenzyl]-(E)-3-(4-phenylphenyl)propenamide (94%)
were intermediates. The final deprotection step gave a white
solid which was recrystallized from MeOH to give colorless crystals
(43% yield): mp 200–203 °C; TLC (cyclohexane/EtOAc 1:1) R$_f$
0.2; ^{1}H NMR (DMSO-d$_6$, 60 MHz) δ 3.8 (3H, s, ArOCH$_3$), 4.35
(2H, d, J = 6 Hz, CH$_2$NH), 6.8 (1H, d, J = 15 Hz, *trans*
CH=CHPh), 6.8–7.8 (12H, m, ArH), 7.65 (1H, d, J = 15 Hz,
trans CH=CHPh), 8.5 (1H, t, J = 6 Hz, NH), 8.85 (1H, s, ArOH);
MS *m/e* 359 (M⁺). Anal. (C$_{23}$H$_{21}$NO$_3$) C, H, N.

**N-(4-Hydroxy-3-methoxybenzyl)-(E)-3-[4-(dimethylami-
no)phenyl]propenamide (6g).** A solution of DCCI (3.55 g, 17
mmol) in CH$_2$Cl$_2$ (20 mL) was slowly added to an ice-cooled
mixture of 4-(2-ethoxyethoxy)-3-methoxybenzylamine[1] (3.53 g,
16 mmol) and 4-(dimethylamino)cinnamic acid (3.0 g, 16 mmol)
in CH$_2$Cl$_2$ (20 mL) and the mixture stirred overnight under N$_2$.
The resulting precipitate of DCU was filtered off and washed
with acetone and the filtrate evaporated *in vacuo* to yield a yellow
solid (3.2 g, 47%). The solid (2 g, 5 mmol), in THF (10 mL), was
deprotected without further purification by the addition of 1 N
HCl (5 mL) with stirring on ice for 2 h. The solvent was removed
in vacuo to yield a yellow solid which was dried and recrystallized
from CHCl$_3$/petroleum ether (bp 80–100 °C) to give pale yellow
crystals (0.3 g, 5% overall): mp 168–171 °C; TLC (silica,
cyclohexane/EtOAc 1:2) R$_f$ 0.3; ^{1}H NMR (CDCl$_3$, 60 MHz) δ 3.0
(6H, s, N(CH$_3$)$_2$), 3.85 (3H, ArOCH$_3$), 4.45 (2H, d, ArCH$_2$NH),
5.2 (1H, br s, ArOH), 5.95 (1H, d, J = 15 Hz, COCH=CHAr), 6.45
(1H, d, J = 15 Hz, CH=CHAr), 6.55–7.75 (7H, m, ArH), 7.3 (1H,
br t, CH$_2$NH); MS *m/e* 326 (M⁺). Anal. (C$_{19}$H$_{22}$N$_2$O$_3$) C, H, N.

**N-(4-Hydroxy-3-methoxybenzyl)-(E)-3-(4-iodophenyl)-
propenamide (6h).** This compound was prepared by the method
described for 8a[7] (see supplementary material). 4-Iodocinnamoyl
chloride (100% unpurified) and N-[4-(2-ethoxyethoxy)-3-meth-
oxybenzyl]-(E)-3-(4-iodophenyl)propenamide (95%) were in-
termediates. The final deprotection step gave an off-white solid
which was recrystallized from EtOAc to give colorless crystals
(90% yield): mp 171–173 °C; TLC (silica, CHCl$_3$/MeOH 5:1) R$_f$
0.57; ^{1}H NMR (DMSO-d$_6$, 60 MHz) δ 3.75 (3H, s, ArOCH$_3$), 4.3
(2H, d, J = 6 Hz, CH$_2$NH), 6.7 (1H, d, J = 15 Hz, *trans*
CH=CHPh), 6.75–6.95 (3H, m, ArH), 7.35–7.75 (4H, m, Ar(I)-
H), 7.45 (1H, d, J = 15 Hz, *trans* CH=CHPh), 8.45 (1H, br t,
NH), 8.8 (1H, s, ArOH); MS *m/e* 409 (M⁺). Anal. (C$_{17}$H$_{16}$NO$_3$)
C, H, N.

**N-(4-hydroxy-3-methoxybenzyl)-(E)-3-(4-formamidophe-
nyl)propenamide (6i).** This compound was prepared as des-
cribed for 4f and the crude product from the final deprotection
reaction was purified by recrystallization from MeOH/H$_2$O to
give a cream-colored, crystalline product (0.1 g, 8%): mp 180 °C
dec; TLC (CHCl$_3$/MeOH 5:1) R$_f$ 0.46; ^{1}H NMR (DMSO-d$_6$, 400
MHz) δ 3.75 (3H, s, ArOCH$_3$), 4.28 (2H, d, ArCH$_2$NHCO), 6.6
(1H, d, J = 16 Hz, *trans* CO-CH=CH-Ph), 6.7–6.9 (3H, m, vanillyl-
ArH), 7.2–7.7 (4H, m, ArH), 7.4 (1H, d, J = 16 Hz, *trans* CO-
CH=CH-Ph), 8.3 (1H, d, ArNHCHO), 8.45 (1H, t, CONHCH$_2$),
8.85 (1H, s, ArOH), 10.35 (1H, br s, ArNHCHO); MS *m/e* 326
(M⁺). Anal. (C$_{18}$H$_{18}$N$_2$O$_4$) C, H, N.

**N-(4-Hydroxy-3-methoxybenzyl)-(E)-3-(2,4-dichlorophe-
nyl)propenamide (6j).** This compound was prepared as des-
cribed for 4f and the crude product from the final deprotection
reaction recrystallized from EtOAc/petroleum ether to give
colorless crystals (45%): mp 163–166 °C; TLC (cyclohexane/
EtOAc 1:1) R$_f$ 0.18; ^{1}H NMR (DMSO-d$_6$, 60 MHz) δ 3.85 (3H, s,
ArOCH$_3$), 4.4 (2H, d, ArCH$_2$NHCO), 6.75–7.9 (6H, m, ArH), 6.85
(1H, d, J = 16.8 Hz, *trans* CO-CH=CH-Ph), 7.85 (1H, d, J = 16.8
Hz, *trans* CO-CH=CH-Ph), 8.7 (1H, t, NH), 8.95 (1H, s, ArOH);
MS *m/e* 351 (M⁺). Anal. (C$_{17}$H$_{15}$NO$_3$Cl$_2$) C, H, N.

**N-(4-Hydroxy-3-methoxybenzyl)-(E)-3-(3-chlorophenyl)-
propenamide (6k).** This compound was prepared as described
for 8a[7] (see supplementary material) and the crude product from
the deprotection step was recrystallized from EtOAc/petroleum
ether (100–120 °C) to give colorless crystals (82%); mp 171–173

°C; TLC (cyclohexane/EtOAc 1:1) R_f 0.23; ^{1}H NMR (DMSO-d_6, 60 MHz) δ 3.8 (3H, s, ArOCH₃), 4.35 (2H, d, CH_2NH), 6.8 (1H, d, J = 16 Hz, *trans* COCH=CHPh) 6.8–7.0 (3H, m, vanillyl ArH), 7.35–7.75 (3H, m, ArH), 7.55 (1H, d, J = 16 Hz, *trans* COCH=CHPh), 8.55 (1H, t, NH), 8.9 (1H, s, ArOH); MS *m/e* 317 (M⁺). Anal. (C₁₇H₁₆NO₃Cl) C, H, N.

N-(4-Hydroxy-3-methoxybenzyl)-3-(4-chlorophenyl)propanamide (8b). *N*-(4-hydroxy-3-methoxybenzyl)-(*E*)-3-(4-chlorophenyl)propenamide (0.1 g, 0.3 mmol) was dissolved in dry MeOH (10 mL) and added to a suspension of 10% Pd/C (10 mg) in dry MeOH (10 mL), under a H₂ atmosphere, and stirred overnight at room temperature. The catalyst was removed by filtration and the solvent removed *in vacuo* to leave a clear oil which was recrystallized from EtOAc/petroleum ether and dried to give colorless needles: yield 0.065 g (68%); mp 97–99 °C; TLC (CHCl₃/MeOH 5:1) R_f 0.58; ^{1}H NMR (CDCl₃, 60 MHz) δ 2.5 (2H, t, COCH_2CH₂), 2.95 (2H, t, COCH₂CH_2Ar), 3.82 (3H, s, ArOCH₃), 4.28 (2H, d, ArCH_2NH), 5.6–6.3 (2H, broad, ArOH, NH), 6.65–6.8 (3H, m, vanillyl ArH), 7.1–7.3 (4H, m, 4-Cl phenyl ArH); MS *m/e* 319 (M⁺). Anal. (C₁₇H₁₆NO₃Cl) C, H, N.

N-(3-Methoxy-4-hydroxybenzyl)-3-(4-acetoxyphenyl)propanamide. 4-Acetoxyphenylpropanoic acid and 4-(2-ethoxyethoxy)-3-methoxybenzylamine[1] were condensed using the isobutyl chloroformate method (38% yield) and deprotected using 1 N HCl in THF (98%) as described for **4f**: TLC (alumina, CH₂Cl₂/MeOH 5:1) R_f 0.72; ^{1}H NMR (CDCl₃, 60 MHz) δ 2.25 (3H, s, COCH₃), 2.48 (2H, t, COCH_2CH₂), 2.98 (2H, t, CH₂CH_2Ar), 3.8 (3H, s, ArOCH₃), 4.3 (2H, d, CH_2NH), 5.75–6.05 (1H, br s, NH), 6.65–7.3 (7H, m, ArH).

N-(3-Methoxy-4-hydroxybenzyl)-3-(4-hydroxyphenyl)-propionamide (8e). *N*-(3-Methoxy-4-hydroxybenzyl)-3-(4-acetoxyphenyl)propionamide (2.6 g, 7.6 mmol) was dissolved in MeOH (100 mL) and added to a suspension of NaHCO₃ (6.5 g, 77 mmol) in 50% MeOH/H₂O (30 mL) and the reaction stirred under N₂ overnight. 1 N HCl was added until no more frothing occurred and the product came out of solution as a white precipitate. This was collected by filtration, and the filtrate and reaction mixture were extracted with CH₂Cl₂, the phases were separated, and the organic phase was washed with 1 N HCl and dried over MgSO₄. The solvent was removed *in vacuo*, and the precipitate and the extracts were combined and recrystallized from EtOAc/petroleum ether (bp 100–120 °C) to give colorless crystals (2.1 g, 92%): mp 125–127 °C; TLC (alumina, CH₂Cl₂/MeOH 5:1) R_f 0.58; ^{1}H NMR (DMSO-d_6, 60 MHz) δ 2.45 (2H, t, COCH_2CH₂) 2.62 (2H, t, COCH₂CH_2), 3.72 (3H, s, ArOCH₃), 4.15 (2H, d, CH_2NH), 6.55–7.15 (7H, m, ArH), 8.15 (1H, broad t, NH), 8.95 (2H, broad, 2 × ArOH); MS *m/e* 301 (M⁺). Anal. (C₁₇H₁₉NO₄) C, H, N.

3-(4-Azidophenyl)propanoic Acid. 3-(4-Aminophenyl)propanoic acid (3 g, 18 mmol) was dissolved in 6 N H₂SO₄ (18 mL) and stirred on ice. A solution of sodium nitrite (1.24 g, 18 mmol) in water (10 mL) was slowly added, the temperature not being allowed to exceed 5 °C. A solution of sodium azide (1.6 g, 24 mmol) in water (10 mL) was then added, dropwise. The frothy suspension gradually precipitated a cream-colored solid which was collected by filtration and washed with cold water and then diethyl ether. The solid was dried *in vacuo* to give a cream solid (2.9 g, 86%); IR (Nujol) ν 2600–2900 (br, CO₂H), 2100 (m, N₃), 1700 (s, CO) cm⁻¹.

4-Azidophenylpropanoic Acid N-Hydroxysuccinimide Ester. 3-(4-Azidophenyl)propanoic acid (2 g, 10.5 mmol) was dissolved in dry CH₂Cl₂ (100 mL), and N-hydroxysuccinimide (1.2 g, 10.5 mmol) was added and the solution was stirred, on ice, under N₂. DCCI (2.3 g, 11.5 mmol) in CH₂Cl₂ (30 mL) was added and the solution stirred overnight. After this time the precipitate which had formed was removed by filtration and the solvent was removed *in vacuo*. The residue was dissolved in acetone and filtered again, the solvent was removed, and the residue was recrystallized from petroleum ether (bp 80–100 °C)/EtOAc to give beige crystals (2.2 g, 72%): TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.4 (streaks); ^{1}H NMR (CDCl₃, 60 MHz) δ 2.83 (4H, s, NHS ester 2 × CH₂), 2.85 (2H, t, COCH_2CH₂) 2.95 (2H, t, COCH₂-CH_2), 7.1 (4H, m, ArH).

N-(3-Methoxy-4-hydroxybenzyl)-3-(4-azidophenyl)-propanamide(8f). 3-(4-Azidophenyl)propanoic acid N-hydroxysuccinimide ester (0.5 g, 1.7 mmol) was dissolved in dry DMF

and stirred at room temperature, under N₂. 4-(2-Ethoxyethoxy)-3-methoxybenzylamine, in DMF (1 mL), was added and the reaction left stirring overnight. After this time water (100 mL) was added, the solution was extracted with CH₂Cl₂ (3 × 50 mL), and the combined organic extracts were dried over Na₂SO₄. The solvent was removed *in vacuo* to leave an oil which was deprotected without purification by dissolving the oil in THF (10 mL) and treatment with 1 N HCl (10 mL). The reaction mixture was stirred for 10 min before removal of the solvent *in vacuo* to leave a cream solid which was recrystallized from petroleum ether (bp 100–120 °C)/dioxane to give cream-colored needles (0.15 g, 25%): mp 105–110 °C; TLC (silica, cyclohexane/EtOAc 1:3) R_f 0.18 ^{1}H NMR (CDCl₃, 60 MHz) δ 2.55 (2H, t, COCH_2CH₂), 2.95 (2H, t, CH₂CH_2Ar), 3.85 (3H, s, ArOCH₃), 4.35 (2H, d, CH_2NH), 5.6 (2H, br s, ArOH, NH), 6.7–7.1 (7H, m, ArH); MS *m/e* 326 (M⁺). Anal. (C₁₇H₁₈N₄O₃) C, H, N.

N-(4-Hydroxy-3-methoxybenzyl)-4-chlorophenylacetamide (10). This compound was prepared as described for **8a**[7] (see supplementary material) and the crude product from the deprotection step was recrystallized from EtOAc/petroleum ether (bp 100–120 C) to give colorless crystals (10% overall): mp 128–130 °C; TLC (CHCl₃/MeOH 5:1) R_f 0.58; ^{1}H NMR (CDCl₃, 60 MHz) δ 3.55 (2H, s, COCH_2Ar), 3.8 (3H, s, ArOCH₃), 4.3 (2H, d, CH_2NH), 5.7–6.2 (2H, br s, NH, ArOH), 6.7–6.8 (3H, m, vanillyl ArH), 7.25 (4H, m, ArH); MS *m/e* 305 (M⁺). Anal. (C₁₆H₁₆NO₃Cl) C, H. N.

N-(4-Hydroxy-3-methoxybenzyl)-2-(4-chlorophenyl)ethynamide (12). This compound was prepared as described for **8a**[7] (see supplementary material) and the crude product from the deprotection step was purified by flash column chromatography (silica, cyclohexane/EtOAc 1:1) and recrystallized twice from EtOAc/petroleum ether (100–120 °C) to give colorless crystals (11% overall yield): mp 117–119 °C; TLC (silica, cyclohexane/EtOAc 1:1) R_f 0.3; ^{1}H NMR (CDCl₃, 60 MHz) δ 3.85 (3H, s, ArOCH₃), 4.45 (2H, d, CH_2NH), 6.0 (1H, br s, ArOH), 6.75 (1H, br t, NH), 6.85–7.0 (3H, m, vanillyl ArH), 7.2–7.6 (4H, m, ArH); MS *m/e* 315 (M⁺). Anal. (C₁₇H₁₄NO₃Cl) C, H, N.

N-[2-(4-Chlorophenyl)ethyl]-4-hydroxy-3-methoxyphenylacetamide (13). Acetylhomovanilloyl chloride was prepared from acetylhomovanillic acid and thionyl chloride as described for **8a**[7] (see supplementary material) and was obtained as a yellow oil (99% yield), which was dissolved in EtOAc and added to an ice-cooled solution of 2-(4-chlorophenyl)ethylamine (0.7 g, 4.5 mmol) and triethylamine (4.8 mmol) in EtOAc, with stirring. The mixture was stirred overnight under N₂ before addition of water and separation of the phases. The organic layer was washed with water, 0.2 N HCl, and saturated NaCl and dried over MgSO₄ before removal of the solvent *in vacuo*. A pale orange oil was obtained which was used without further purification. The compound (1.4 g, 4 mmol) was dissolved in MeOH (5 mL) and deprotected by stirring overnight with a suspension of K₂CO₃ (6 g, 40 mmol) in 50% MeOH/H₂O (25 mL). 2 N HCl was added until frothing no longer occurred, CHCl₃ was added and the phases were separated. The organic phase was washed with water and saturated NaCl and dried over Na₂SO₄. Removal of the solvent *in vacuo* and crystallization of the resulting oil from EtOAc/petroleum ether (bp 80–100 °C) yielded buff-colored crystals (0.35 g, 27%): mp 124–127 °C; TLC (EtOAc) R_f 0.3; ^{1}H NMR (CDCl₃, 60 MHz) δ 2.65 (2H, t, J = 6 Hz, CH₂CH_2Ph), 3.3 (2H, t, J = 6 Hz, CH_2CH₂Ph), 3.4 (2H, s CH_2CO), 3.72 (3H, s, ArOCH₃), 5.9 (1H, br s, ArOH), 6.2 (1H, br s, NH), 6.65–7.3 (7H, m, ArH); MS *m/e* 319 (M⁺). Anal. (C₁₇H₁₈NO₃Cl) C, H, N.

N-(4-Hydroxy-3-methoxybenzyl)thiourea (15a). 4-[(2-Ethoxy)ethoxy]-3-methoxybenzylamine[1] (1.5 g, 6.6 mmol) was stirred, on ice, in EtOAc during the addition of trityl isothiocyanate (2 g, 6.6 mmol) in EtOAc. The solution was stirred at room temperature for 18 h, after which time no starting materials remained by TLC, which showed one product spot (silica, CH₂Cl₂/MeOH, R_f 0.8). The solvent was removed *in vacuo* and the reaction mixture was redissolved in THF (50 mL). 1 N HCl (10 mL) was added to the stirred solution, on ice, and the mixture allowed to stir for 30 min before the removal of the solvents *in vacuo*. The cream-colored residue was recrystallized from EtOAc/MeOH to give white crystals (1.15, 81%): mp 138–142 °C; TLC (silica, CH₂Cl₂/MeOH 25:1) R_f 0.12; ^{1}H NMR (CD₃OD, 60 MHz)

δ 3.85 (3H, s, ArOCH₃), 4.55 (2H, s, ArCH₂NH), 6.8–6.9 (3H, m, ArH); MS m/e 212 (M⁺). Anal. (C₉H₁₂N₂O₂S·0.5H₂O) C, H, N.

N-(4-Hydroxy-3-methoxybenzyl)-N'-butylthiourea (15b). This compound was prepared as described for 15f¹ (see supplementary material) and was recrystallized from diisopropyl ether/ EtOAc to give white crystals: 21% yield; mp 87–89 °C; TLC (silica, CH₂Cl₂/MeOH 20:1) R_f 0.39; ¹H NMR (CDCl₃, 60 MHz) δ 0.9 (3H, t, alkyl CH₃), 1.25 (4H, env, alkyl CH₂), 3.35 (2H, d of t, NHCH₂CH₂), 3.86 (3H, s, ArOCH₃), 4.54 (2H, d, ArCH₂NH), 5.70–6.2 (3H, br s, ArOH, 2 × thiourea NH), 6.85 (3H, m, ArH); MS m/e 268 (M⁺). Anal. (C₁₃H₂₀N₂O₂S) C, H, N.

N-(4-Hydroxy-3-methoxybenzyl)-N'-pentylthiourea (15c). This compound was prepared as described for 15f¹ and was purified by flash column chromatography (silica, cyclohexane/ EtOAc 2:1) and then recrystallized from petroleum ether (bp 100–120 °C)/diisopropyl ether to give white crystals: 20% yield; mp 85–87 °C; TLC (silica, CH₂Cl₂/MeOH 20:1) R_f 0.40; ¹H NMR (CDCl₃, 60 MHz) δ 0.88 (3H, t, alkyl CH₃), 0.95–1.25 (6H, env, alkyl CH₂), 3.33 (2H, d of t, NHCH₂CH₂), 3.9 (3H, s, ArOCH₃), 4.55 (2H, d, ArCH₂NH), 5.60–6.6 (3H, br s, ArOH, 2 × thiourea NH), 6.88 (3H, m, ArH); MS m/e 282 (M⁺). Anal. (C₁₄H₂₂N₂O₂S) C, H, N.

N-(4-Hydroxy-3-methoxybenzyl)-N'-hexylthiourea (15d). This compound was prepared as described for 15f¹ (see supplementary material) and was recrystallized from petroleum ether (bp 100–120 °C)/diisopropyl ether to give white crystals: 52% yield; mp 87–89 °C; TLC (silica, CH₂Cl₂/MeOH 20:1) R_f 0.41; ¹H NMR (CDCl₃, 60 MHz) δ 0.9 (3H, t, alkyl CH₃), 1.24 (8H, env, alkyl CH₂), 3.33 (2H, d of t, NHCH₂CH₂), 3.90 (3H, s, ArOCH₃), 4.54 (2H, d, ArCH₂NH), 5.70–6.2 (3H, br s, ArOH, 2 × thiourea NH), 6.85 (3H, m, ArH); MS m/e 296 (M⁺). Anal. (C₁₅H₂₄N₂O₂S) C, H, N.

N-(4-Hydroxy-3-methoxybenzyl)-N'-heptylthiourea (15e). This compound was prepared as described for 15f¹ (see supplementary material) and was purified by flash column chromatography (silica, cyclohexane/EtOAc 2:1) and then recrystallized from petroleum ether (bp 100–120 °C)/diisopropyl ether to give white crystals: 3% yield; mp 92–94 °C; TLC (silica, CH₂Cl₂/ MeOH 20:1) R_f 0.42; ¹H NMR (CDCl₃, 60 MHz) δ 0.88 (3H, t, alkyl CH₃), 1.25 (10H, env, alkyl CH₂), 3.36 (2H, d of t, NHCH₂CH₂), 3.88 (3H, s, ArOCH₃), 4.55 (2H, d, ArCH₂NH), 5.5–6.3 (3H, br s, ArOH, 2 × thiourea NH), 6.88 (3H, m, ArH); MS m/e 310 (M⁺). Anal. (C₁₆H₂₆N₂O₂S·0.15H₂O) C, H, N.

N-(4-Hydroxy-3-methoxybenzyl)-N'-nonylthiourea (15g). This compound was prepared by the method described for 15f¹ (see supplementary material) and was recrystallized from petroleum ether (bp 80–100 °C)/diisopropyl ether to give white crystals: 54% yield; mp 85–89 °C; TLC (silica, CH₂Cl₂/MeOH 20:1) 0.44; ¹H NMR (CDCl₃, 60 MHz) δ 0.9 (3H, t, alkyl CH₃), 1.25 (14H, env, alkyl CH₂), 3.35 (2H, m, NHCH₂CH₂), 3.87 (3H, s, ArOCH₃), 4.55 (2H, d, ArCH₂NH), 5.7 (1H, s, ArOH), 5.9 (1H, br s, thiourea NH), 6.1 (1H, br s, thiourea NH), 6.85 (3H, m, ArH); MS m/e 338 (M⁺). Anal. (C₁₈H₃₀N₂O₂S) C, H, N.

N-(4-Hydroxy-3-methoxybenzyl)-N'-decylthiourea (15h). This compound was prepared as described for 15f¹ (see supplementary material) and was recrystallized from petroleum ether (bp 80–100 °C)/diethyl ether to give white crystals: 27% yield; mp 81–82 °C; TLC (silica, CH₂Cl₂/MeOH 20:1) R_f 0.45; ¹H NMR (CDCl₃, 60 MHz) δ 0.9 (3H, t, alkyl CH₃), 1.25 (16H, env, alkyl CH₂), 3.35 (2H, d of t, NHCH₂CH₂), 3.90 (3H, s, ArOCH₃), 4.55 (2H, d, ArCH₂NH), 5.70 (1H, s, ArOH), 5.90 (1H, br s, thiourea NH), 6.20 (1H, br s, thiourea NH), 6.85 (3H, m, ArH); MS m/e 352 (M⁺). Anal. (C₁₉H₃₂N₂O₂S) C, H, N.

N-(4-Hydroxy-3-methoxybenzyl)-N'-dodecylthiourea (15i). This compound was prepared as described for 15f¹ (see supplementary material) and was recrystallized from petroleum ether (bp 80–100 °C) to give white crystals: 48% yield; mp 86–88 °C; TLC (silica, CH₂Cl₂/MeOH 20:1) R_f 0.47; ¹H NMR (CDCl₃, 60 MHz) δ 0.88 (3H, t, alkyl CH₃), 1.25 (20H, env, alkyl CH₂), 3.35 (2H, m, NHCH₂CH₂), 3.85 (3H, s, ArOCH₃), 4.50 (2H, d, ArCH₂NH), 5.5–6.2 (3H, br s, ArOH, 2 × thiourea NH), 6.81 (3H, m, ArH); MS m/e 380 (M⁺). Anal. (C₂₁H₃₆N₂O₂S) C, H, N.

N-(4-Hydroxy-3-methoxybenzyl)-N'-hexadecylthiourea (15j). This compound was prepared as described for 15f¹ (see supplementary material) and was recrystallized from petroleum ether (bp 100–120 °C)/diisopropyl ether to give white crystals:

54% yield; mp 97.5–98.5 C; TLC (silica, CH₂Cl₂/MeOH 20:1) R_f 0.50; ¹H NMR (CDCl₃, 60 MHz) δ 0.9 (3H, t, alkyl CH₃), 1.25 (28H, env, alkyl CH₂), 3.4 (2H, d of t, NHCH₂CH₂), 3.9 (3H, s, ArOCH₃), 4.57 (2H, d, ArCH₂NH), 5.7–6.3 (3H, br s, ArOH, 2 × thiourea NH), 6.88 (3H, m, ArH); MS m/e 436 (M⁺). Anal. (C₂₅H₄₄N₂O₂S) C, H, N.

N-(4-Hydroxy-3-methoxybenzyl)-N'-octadecylthiourea (15k). This compound was prepared as described for 15f¹ (see supplementary material) and was recrystallized from petroleum ether (bp 100–120 °C)/diisopropyl ether to give white crystals: 12% yield; mp 98–99 °C; TLC (silica, CH₂Cl₂/MeOH 20:1) R_f 0.52; ¹H NMR (CDCl₃, 60 MHz) δ 0.9 (3H, t, alkyl CH₃), 1.25 (32H, env, alkyl CH₂), 3.33 (2H, d of t, NHCH₂CH₂), 3.88 (3H, s, ArOCH₃), 4.54 (2H, d, ArCH₂NH), 5.60–6.3 (3H, br s, ArOH, 2 × thiourea NH), 6.85 (3H, m, ArH); MS m/e 464 (M⁺). Anal. (C₂₇H₄₈N₂O₂S) C, H, N.

N-(4-Hydroxy-3-methoxybenzyl)-N'-oleylthiourea (15l). This compound was prepared by the method described for 15f¹ from oleyl isothiocyanate² and was purified by flash column chromatography (silica, CH₂Cl₂/MeOH 100:1). Recrystallization from petroleum ether (bp 100–120 °C) gave a white crystalline solid: yield (45% yield); mp 94–97 °C; TLC (silica, CH₂Cl₂/MeOH 25:1) R_f 0.56; ¹H NMR (CDCl₃, 400 MHz) δ 0.88 (3H, t, alkyl CH₃), 1.3 (22H, env, alkyl CH₂), 1.55 (2H, m, NHCH₂CH₂CH₂), 2.0 (4H, m, CH₂CH₂CH=CHCH₂CH₂), 3.38 (2H, br s, NHCH₂CH₂), 3.7 (3H, s, ArOCH₃), 4.5 (2H, br s, ArCH₂NH), 5.33 (2H, m, CH₂CH=CHCH₂), 6.0 (1H, thiourea NH), 6.15 (1H, br s, thiourea NH), 6.62–6.8 (3H, m, ArH); MS m/e 462 (M⁺); HPLC RP₁₈ (gradient 10–70% CH₃CN/0.1% aqueous TFA) >95% pure.

N-(4-Hydroxy-3-methoxybenzyl)-N'-adamantylthiourea (15m). This compound was prepared as described for 15f¹ (see supplementary material) and was purified by flash column chromatography (silica, CH₂Cl₂) and then recrystallized from petroleum ether (bp 80–100 °C)/diethyl ether to give white crystals: 49% yield; mp 140–144 °C; TLC (silica, CH₂Cl₂/MeOH 25:1) R_f 0.6; ¹H NMR (CDCl₃, 60 MHz) δ 1.7–2.05 (15H, m, adamantyl CH₂ and CH) 3.9 (3H, s, ArOCH₃), 4.7 (2H, d, ArCH₂NH), 5.7–6.1 (3H, br s, 2 × thiourea NH, ArOH), 6.9 (3H, m, ArH); MS m/e 364 (M⁺). Anal. (C₁₈H₂₆N₂O₂S) C, H, N.

N-(4-Hydroxy-3-methoxybenzyl)-N'-benzhydrylthiourea (15n). This compound was prepared by the same method as that described for 15a and the residue after removing the THF/1 N HCl *in vacuo* was recrystallized from petroleum ether (bp 100–120 °C)/EtOAc to give white crystals: yield 91%; mp 62–64 °C; TLC (silica, CH₂Cl₂/MeOH) R_f 0.59; ¹H NMR (CDCl₃, 400 MHz) δ 3.78 (3H, s, ArOCH₃)₁ 4.52 (2H, d, ArCH₂NH), 5.62 (1H, br s, ArOH), 5.95 (1H, br s, thiourea NH), 6.06 (1H, br s, thiourea NH), 6.46 (1H, br d, NHCHAr₂), 6.56 (1H, d of d, J = 2 Hz, ArH₆), 6.67 (1H, d, J = 2 Hz, ArH₂), 6.78 (1H, d, J' = 8 Hz, ArH₅), 7.15–7.38 (10H, m, benzhydryl ArH); MS m/e 378 (M⁺). Anal. (C₂₂H₂₂N₂O₂S·0.25H₂O) C, H, N.

N-(4-Hydroxy-3-methoxybenzyl)-N'-tritylthiourea (15p). This compound was prepared as described for 15f¹ (see supplementary material) and was purified by flash column chromatography (silica, CH₂Cl₂) and then recrystallized from petroleum ether (bp 80–100 °C) to give white crystals: 26% yield; mp 72–77 °C; TLC (silica, CH₂Cl₂/MeOH 25:1) R_f 0.7; ¹H NMR (CDCl₃, 60 MHz) δ 3.75 (3H, s, ArOCH₃), 4.42 (2H, d, ArCH₂NH), 5.35 (1H, br s, thiourea NH), 6.15 (1H, d of d, J = 2 Hz, J' = 8 Hz, ArH₆), 6.45 (1H, d, J = 2 Hz, ArH₂), 6.68 (1H, d, J' = 8 Hz, ArH₅), 7.3 (15H, s, trityl ArH), 7.3–7.5 (2H, br s, ArOH, thiourea NH); MS m/e 454 (M⁺). Anal. (C₂₈H₂₆N₂O₂S) C, H, N.

Biology. The *in vitro* and *in vivo* assays used in this paper are described in paper 1 of this series.

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