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Phenolic Modification as an Approach to Improve the Pharmacology of the 3-Acyloxy-2-benzylpropyl Homovanillic Amides and Thioureas, a Promising Class of Vanilloid Receptor Agonists and Analgesics

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Abstract—In order to improve the analgesic activity and pharmacokinetics of thioureas **2** and **3,** which we previously developed as potent vanilloid receptor (VR) agonists, we prepared and characterized phenolic modifications of them and of their amide surrogates (**7**, **8**). The aminoethyl analogue of the amide template **13** was a potent analgesic with an $EC_{50} = 0.96\ \mu g/kg$ in the AA-induced writhing test and with better in vivo stability than the parent phenol.

Introduction

The vanilloid receptor (VR) is a nociceptor that responds to multiple pain-producing stimuli such as heat and protons and is activated by ligands such as capsaicin (CAP),[1,2] resiniferatoxin (RTX),[3] and natural aldehydes[4] such as scutigeral. The vanilloid receptor has been shown to be expressed on a subset of primary afferent neurons of the C-fiber 'pain' pathway. The activation of this receptor triggers cation influx and firing of action potentials, leading centrally to the perception of pain.[5] Desensitization of the receptor in response to specific ligands has long been considered to be a promising therapeutic approach for the treatment of neuropathic pain and other pathological conditions involving C-fiber neurons.[6,7]

Among the VR agonists studied, only a few compounds are currently marketed (e.g., capsaicin) or undergoing clinical trial (e.g., resiniferatoxin, DA-5018).[8] The identification of RTX as a novel vanilloid with a potency three to four orders of magnitude greater than that of capsaicin suggested exciting opportunities for exploitation through medicinal chemistry.[9–14] Structurally, RTX represents a typical vanilloid in the A and B regions (correspond to 4-hydroxy-3-methoxyphenyl and amide bond in CAP), but with a tricyclic diterpene in place of the typical C region substituents (hydrophobic side chain) found in capsaicin and its congeners.[2] Over the past few years, we have explored simplified RTX analogues as VR ligands based on our hypothetical pharmacophore model and have disclosed the first lead compound **1** (Fig. 1).[15] Using this approach, we recently reported ultrapotent VR agonists, thioureas **2** and **3**, with K_i values of 19 and 11 nM, respectively, in an [^{3}H]RTX binding assay on dorsal root ganglion neurons. These two compounds are thus approximately 280- and 480-fold more potent than capsaicin for receptor binding.[16] The particular feature of these compounds is a 3-acyloxy-2-benzylpropyl moiety as a C-region which we designed to mimic key putative pharmacophores of the diterpene moiety in RTX.

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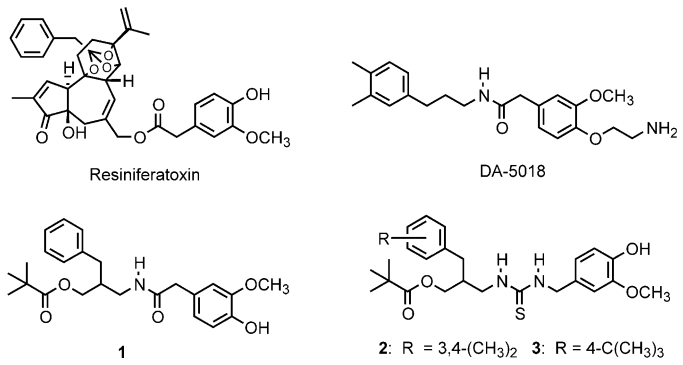


Figure 1.

One of the pharmacological problems for capsaicin analogues as analgesics is that the pharmacophoric 3-methoxy-4-hydroxyphenyl A-region is metabolically labile, mostly as the result of *O*-glucuronidation of the phenolic hydroxyl, leading to low bioavailability. Incorporation of an aminoethyl group into capsaicin analogues, for example DA-5018 and SDZ-249,482, was found to reduce their pungencies and improve their pharmacokinetic profiles by blocking metabolic clearance.[17] In the present study, we have sought to combine aminoethyl or acidic groups with our potent VR agonists in order to improve their pharmacokinetic profiles and analgesic activities.

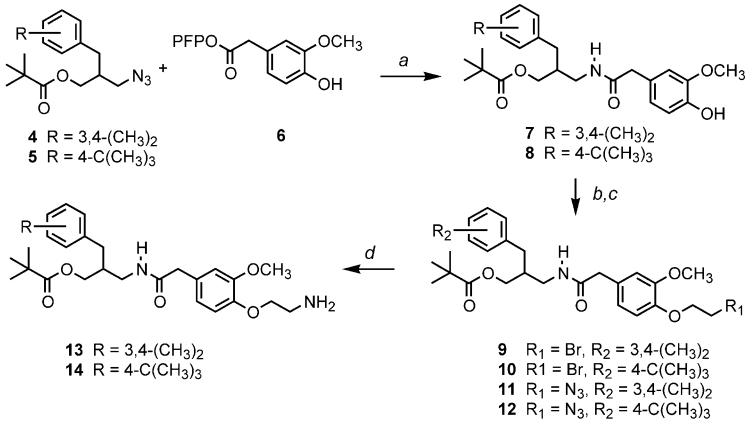
In this paper, we describe the syntheses, in vitro receptor activities, antinociceptive activities and in vivo stability of *O*-aminoethyl, acetate and succinate analogues of 3-acyloxy-2-benzylpropyl homovanillic thioureas and amides.

Chemistry

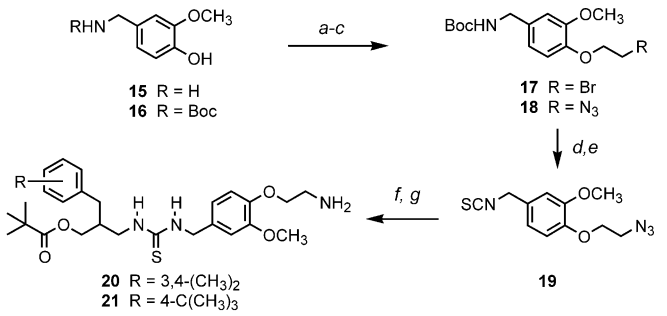
The syntheses of the amide templates (**7**, **8**) and their 2-aminoethy analogues (**13**, **14**) are described in Scheme 1. Azides (**4**, **5**), previously reported,[16] were reduced to amines by hydrogenation under Lindler's catalyst, which in situ reacted with the pentafluorophenyl ester of homovanillate (**6**) to afford amides **7** and **8**, respectively. Phenolic hydroxyls of **7** and **8** were alkylated with 1,2-dibromoethane and then their bromides were substituted by azide to give **11** and **12**, respectively. Reduction of the azides under catalytic hydrogenation finally produced the 2-aminoethyl analogues **13** and **14**.

Since thiourea groups are labile to base-mediated alkylation conditions, we synthesized aminoethyl analogues (**20**, **21**) of the thiourea templates (**2**, **3**) by another route shown in Scheme 2. Commercially available 4-hydroxy-3-methoxybenzylamine hydrochloride (**15**) was readily converted into **18** by following the previous scheme. *N*-*tert*-Butoxycarbonylamine **18** was transformed into the corresponding isothiocyanate **19** by acidic deprotection followed by condensation with 1,1-thiocarbonyl di-2(1H)-pyridone.[18] Coupling of **19** with amines prepared from **4** and **5**, followed by PPh_3-mediated reduction produced 2-aminoethyl analogues of the thiourea templates (**20**, **21**), respectively.

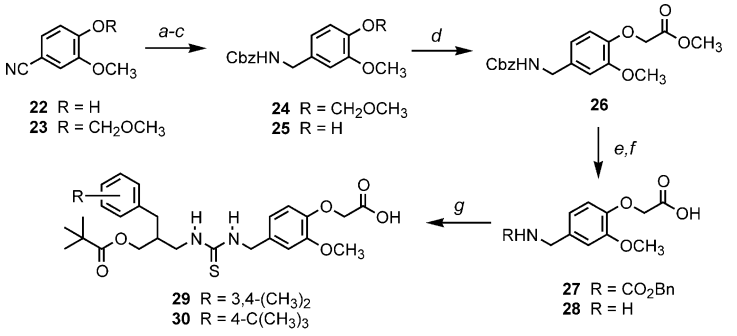
The syntheses of α-substituted acetic acid analogues (**29**, **30**) of the thiourea template as outlined in Scheme 3 were started from commercially available 4-hydroxy-3-methoxybenzonitrile (**22**). Nitrile **22** was converted into the corresponding carbobenzyloxyamino (Cbz) group to afford **25** in the conventional way in three steps. The phenol of **25** was alkylated with methyl bromoacetate to produce **26** which was then hydrolyzed and deprotected to amine **28**. Condensation of **28** with 2-(3,4-dimethyl(or 4-*tert*-butyl)benzyl)-3-pivaloyloxypropyl isothiocyanate, previously reported,[19] afforded the final compounds **29** and **30**, respectively.



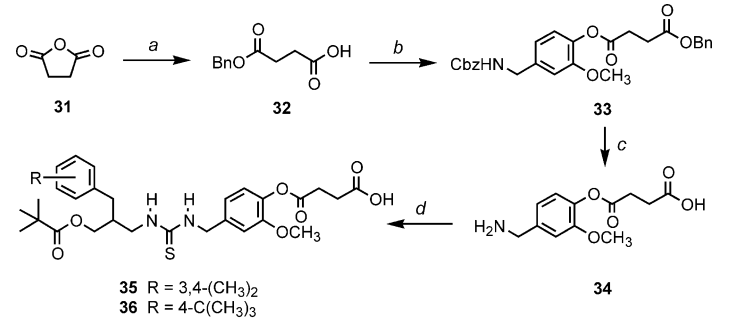
Scheme 1. Reagents: (a) (i) H_2, Lindler's cat, EtOH; (ii) **6**, EtOAc; (b) $BrCH_2CH_2Br$, KOH, Bu_4NOH; (c) NaN_3, DMF; (d) H_2, Pd/C, MeOH.



Scheme 2. Reagents: (a) Boc_2O, NEt_3, dioxane–H_2O; (b) $BrCH_2CH_2Br$, KOH, Bu_4NOH; (c) NaN_3, DMF; (d) CF_3CO_2H, CH_2Cl_2; (e) 1,1-thiocarbonyl di-2(1H)-pyridone, NEt_3, DMF; (f) (i) **4** (or **5**), H_2, 5% Pd/C, MeOH; (ii) CH_2Cl_2; (g) PPh_3, H_2O–THF.



Scheme 3. Reagents: (a) $ClCH_2OCH_3$, $NEt(i\text{-}Pr)_2$, CH_2Cl_2; (b) (i) $LiAlH_4$, THF; (ii) BnOCOCl, NEt_3, CH_2Cl_2; (c) CF_3CO_2H, CH_2Cl_2; (d) $BrCH_2CO_2CH_3$, K_2CO_3, acetone; (e) NaOH, THF–H_2O; (f) H_2, Pd/C MeOH; (g) 2-(3,4-dimethyl(or 4-*t*-butyl)benzyl)-3-pivaloyloxypropylisothiocyanate, NEt_3, DMF.



Scheme 4. Reagents: (a) BnOH, NaH, THF; (b) **25**, DCC, DMAP, CH_2Cl_2; (c) H_2, Pd/C, MeOH; (d) 2-(3,4-dimethyl(or 4-*t*-butyl)benzyl)-3-pivaloyloxypropylisothiocyanate, DMF.

The synthesis of succinate analogues (**35**, **36**) was shown in Scheme 4. Refluxing of succinic anhydride (**31**) with sodium salt of benzyl alcohol gave monobenzyl succinate **32** which was then esterified with phenol **25** to produce **33**. Hydrogenation of **33** followed by condensation with isothiocyanates used in Scheme 3 afforded **35** and **36**, respectively.

Results and Discussion

The activities of the synthesized vanilloid analogues as agonists were assessed in vitro using a ^{45}Ca influx assay and their antinociceptive activities were measured using the writhing test. The calcium influx assay was carried out using cultured rat dorsal root ganglion (DRG) neurons as previously described and the potencies of the compounds were expressed as the $EC_{50} \pm$ SEM.[17] Maximal response was compared to that induced by capsaicin at a concentration of 3 μM. The analgesic activities of the compounds were evaluated in the phenyl-*p*-benzoquinone (PBQ)-induced or acetic acid (AA)-induced writhing test in mice; the ED_{50} values indicate the concentrations required to cause a 50% reduction in the number of writhes.[16] Both the PBQ- and AA-induced writhing tests usually showed similar values, as indicated for ovanil and DA-5018, which were used as reference compounds. The in vitro and in vivo results are presented in Table 1.

In the calcium influx assay, the parent phenolic analogues (thioureas: **2**, **3**; amides: **7**, **8**) demonstrated potent agonistic activities with EC_{50} values of 0.058, 0.361, 0.162, 0.182 μM, which were 7.5, 1.2, 2.7 and 2.4-fold (**2**, **3**, **7** and **8**) more potent than capsaicin (EC_{50} = 0.435 μM), respectively. Their analgesic activities in

the PBQ-induced writhing test improved much more than in the calcium influx assay. Amides **7** and **8** with ED_{50} values of 12 and 3.5 μg/kg were 25- and 86-fold more potent than capsaicin (ED_{50} = 300 μg/kg) in this assay for analgesia, as previously reported in a series of thiourea analogues (**2**, **3**).[16]

Incorporation of the 2-aminoethyl group into the phenols led to a series of compounds (**13**, **14**, **20** and **21**) in which agonistic and analgesic activities were comparable or a little reduced compared to the parent compounds, with the exception of **13**. Compound **13** possessed potent analgesic activity (ED_{50} = 0.96 μg/kg) in the AA-induced writhing assay, being ca. 18- and 2.4-fold more potent than olvanil and DA-5018. Although the in vitro activity of **13** was less than that of the parent compound **7**, presumably its enhanced in vivo stability more than compensated. In a series of capsaicin analogues, it has been noted previously that only 2-aminoethyl analogues among blocking groups of the phenolic OH group retained the antinociceptive properties of the parent compounds, showed reduced irritancy, and, by avoiding rapid phenolic glucuronidation in vivo, displayed better pharmacokinetic profiles.[17] This conclusion appears to hold likewise for the present series.

In order to examine the in vivo stability of the two potent analgesics **2** and **13**, the compounds were injected intravenously into the rat and then measured in plasma collected at regular intervals (Fig. 2). Whereas the phenolic compound **2** showed a very short half life with $t_{1/2}$ = 3 min and was completely cleared in ca. 10 min, the aminoethyl analogue **13** showed enhanced stability with $t_{1/2}$ = 30 min, indicating that the aminoethyl surrogate was more metabolically stable than the parent phenol. The higher analgesic potency of **13** compared to the parent compound presumably reflects its better pharmacokinetics.

Unlike the aminoethyl analogues, both the α-substituted acetic acid (**29**, **30**) and succinate analogues (**35**, **36**) possessed dramatically reduced agonistic and analgesic activities even though previous report described these analogues of synthetic capsanoid showed a little better antinociceptive effect than parent one.[20] These findings indicate that a hydrogen bonding acceptor such as carboxylic acid is not an appropriate substituent, unlike the amino group as a hydrogen bonding donor in the phenolic modification for improving VR mediated calcium influx and analgesic activity, and are consistent with those of capsainoid analogues previously reported[17] in

Table 1. Calcium influx assay and writhing test

	^{45}Ca Influx (EC_{50} = μM)	PBQ-induced writhing test (ED_{50} = μg/kg)	AA-induced writhing test (ED_{50} = μg/kg)
Amides			
7	0.162 (±0.33)	12	
8	0.182 (±0.45)	3.5	
13	1.57 (±0.23)		0.960
14	0.878 (±0.17)		8.12
Thioureas			
2	0.058 (±0.008)	0.5[16]	
3	0.361 (±0.054)	1.0[16]	
20	0.083 (±0.012)		14.0
21	0.377 (±0.067)		48.2
29	7.77 (±0.80)		431
30	12.3 (±1.58)		365
35	7.04 (±1.3)		53.1
36	7.00 (±1.2)		60
Capsaicin	0.435	300	
RTX		0.01	
Olvanil		30	17.4
DA-5018	0.234	3	2.27
Indomethacinn		400	
Aspirin		3500	
Morphine		1000	

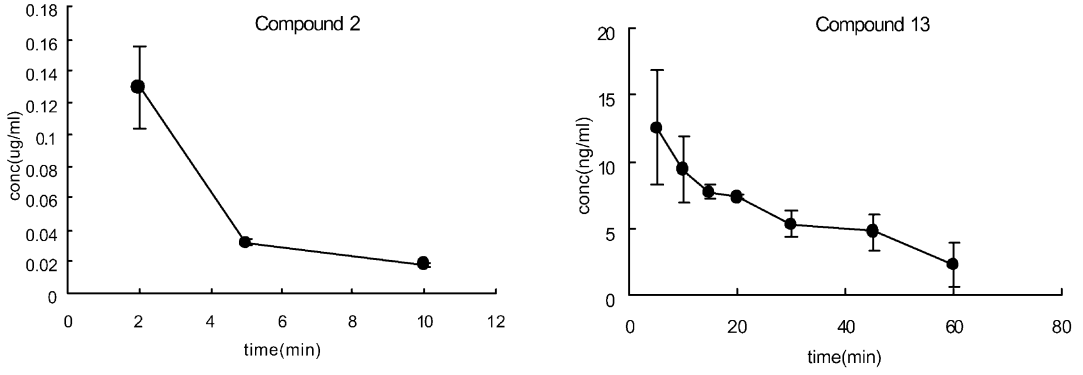


Figure 2. In vivo stability of compounds **2** and **13**.

which substitution of basic nitrogen acting as a hydrogen bonding acceptor for free amine reduced its in vitro and in vivo activities.

In conclusion, we investigated amide surrogate and phenolic modifications of our previously discovered potent vanilloid receptor agonists **2** and **3** and found the aminoethyl analogue of amide template **13** to be a potent analgesic due to improved pharmacokinetics. Compound **13** was selected as a candidate for further development of novel analgesics.

Experimental

All chemical reagents were commercially available. Melting points were determined on a Melting Point Büchi B-540 apparatus and are uncorrected. Silica gel column chromatography was performed on silica gel 60, 230–400 mesh, Merck. Proton NMR spectra were recorded on a JEOL JNM-LA 300 at 300 MHz. Chemical shifts are reported in ppm units with Me_4Si as a reference standard. Mass spectra were recorded on a VG Trio-2 GC–MS. Elemental analyses were performed with an EA 1110 Automatic Elemental Analyzer, CE Instruments.

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-2-[4-hydroxy-3-methoxyphenyl]acetamide (7) and *N*-[2-(4-*tert*-butylbenzyl)-3-(pivaloyloxy)propyl]-2-[4-hydroxy-3-methoxyphenyl]acetamide (8).** A suspension of **4** (or **5**) (2.0 mmol) and 5% palladium on carbon (100 mg) in MeOH (10 mL) was hydrogenated under a balloon of hydrogen for 2 h. The reaction mixture was filtered and the filtrate was concentrated *in* vacuo. The residue was dissolved in CH_2Cl_2 (10 mL) and treated with homovanillic pentafluorophenyl ester (**6**) (696 mg, 2 mmol) prepared from homovanillic acid and pentafluorophenol by DCC coupling. After being stirred overnight at room temperature, the reaction mixture was concentrated in vacuo and the residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (1:2) as eluant to afford **7** (or **8**).

7: 96% yield, colorless oil; ^{1}H NMR ($CDCl_3$) δ 6.65–7.05 (m, 6H, Ar), 5.74 (t, 1H, NH), 4.00 (m, 1H, CH_2OCO), 3.88 (s, 3H, OCH_3), 3.80 (m, 1H, CH_2OCO), 3.46 (s, 2H, $COCH_2Ar$), 3.30 (m, 1H, CH_2NH), 3.08 (m, 1H, CH_2NH), 2.59 (dd, 1H, $J=7.3$, 12 Hz, CH_2Ph), 2.48 (d, 1H, $J=7.3$ Hz, CH_2Ph), 2.0–2.3 (m, 7H, 2 × CH_3 and CH), 1.20 (s, 9H, $CO(CH_3)_3$); MS *m*/*e* 441 (M^+). Anal. calcd for $C_{26}H_{35}NO_5$: C, 70.72; H, 7.99; N, 3.17. Found: C, 70.99; H, 8.01; N, 3.15.

8: 94% yield, colorless oil; ^{1}H NMR ($CDCl_3$) δ 7.28 (d, 2H, $J=8.3$ Hz, Ar), 7.02 (d,2H, $J=8.3$ Hz), 6.88 (d, 1H, $J=7.8$ Hz), 6.77 (d, 1H, $J=2.8$ Hz), 6.72 (dd, 1H, $J=2.8$, 7.8 Hz), 5.73 (t, 1H, NH), 4.00 (dd, 1H, CH_2OCO), 3.89 (s, 3H, OCH_3), 3.82 (dd, 1H, CH_2OCO), 3.46 (s, 2H, $COCH_2Ar$), 3.32 (m, 1H, CH_2NH), 3.08 (m, 1H, CH_2NH), 2.53 (d, 2H, $J=7.8$ Hz, CH_2Ph), 2.10 (m, 1H, CH), 1.30 (s, 9H, $C(CH_3)_3$), 1.20 (s, 9H, $CO(CH_3)_3$); MS *m*/*e* 469 (M^+). Anal. calcd for $C_{28}H_{39}NO_5$: C, 71.61; H, 8.37; N, 2.98. Found: C, 71.84; H, 8.39; N, 2.97.

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-2-[4-(2-bromoethoxy)-3-methoxyphenyl]acetamide (9) and *N*-[2-(4-*tert*-butylbenzyl)-3-(pivaloyloxy)propyl]-2-[4-(2-bromoethoxy)-3-methoxyphenyl]acetamide (10).** A mixture of **6** (or **7**) (1 mmol), 40% KOH (2 mL), tetrabutylammonium hydroxide (40% in H_2O, 0.2 mL) and 1,2-dibromoethane (1.5 mL) was heated at 50 °C for 2 h. The reaction mixture was diluted with CH_2Cl_2, washed with H_2O and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (1:1) as eluant to afford **9** (or **10**).

9: 78% yield, yellow oil; ^{1}H NMR ($CDCl_3$) δ 6.7–7.05 (m, 6H, Ar), 5.76 (t, 1H, NH), 4.32 (t, 2H, $J=6.6$ Hz, OCH_2CH_2Br), 4.00 (m, 1H, CH_2OCO), 3.86 (s, 3H, OCH_3), 3.80 (m, 1H, CH_2OCO), 3.64 (t, 2H, $J=6.6$ Hz, OCH_2CH_2Br), 3.47 (s, 2H, $COCH_2Ar$), 3.30 (m, 1H, CH_2NH), 3.10 (m, 1H, CH_2NH), 2.60 (dd, 1H, $J=7.3$, 12.0 Hz, CH_2Ph), 2.49 (d, 1H, $J=7.3$ Hz, CH_2Ph), 2.0–2.3 (m, 7H, 2 × CH_3 and CH), 1.20 (s, 9H, $CO(CH_3)_3$).

10: 88% yield, yellow oil; ^{1}H NMR ($CDCl_3$) δ 7.28 (d, 2H, $J=8.3$ Hz, Ar), 7.02 (d, 2H, $J=8.3$ Hz), 6.88 (d, 1H, $J=7.8$ Hz), 6.80 (d, 1H, $J=2.8$ Hz), 6.74 (dd, 1H, $J=2.8$, 7.8 Hz), 5.80 (t, 1H, NH), 4.32 (t, 2H, $J=5.4$ Hz, OCH_2CH_2Br), 4.02 (dd, 1H, $J=4.6$, 11.2 Hz, CH_2OCO), 3.89 (s, 3H, OCH_3), 3.83 (dd, 1H, $J=5.2$, 11.2 Hz, CH_2OCO), 3.64 (t, 2H, $J=5.4$ Hz, OCH_2CH_2Br), 3.49 (s, 2H, $COCH_2Ar$), 3.32 (m, 1H, CH_2NH), 3.10 (m, 1H, CH_2NH), 2.56 (d, 2H, $J=7.6$ Hz, CH_2Ph), 2.12 (m, 1H, CH), 1.30 (s, 9H, $C(CH_3)_3$), 1.20 (s, 9H, $CO(CH_3)_3$).

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-2-[4-(2-azidoethoxy)-3-methoxyphenyl]acetamide (11) and *N*-[2-(4-*tert*-butylbenzyl)-3-(pivaloyloxy)propyl]-2-[4-(2-azidoethoxy)-3-methoxyphenyl]acetamide (12).** A mixture of **9** (or **10**) (1 mmol) and sodium azide (195 mg, 3 mmol) in DMF (2 mL) was heated at 100 °C for 8 h. The reaction mixture was diluted with H_2O and extracted with EtOAc several times. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (1:1) as eluant to afford **11** (or **12**).

11: 95% yield, colorless oil; ^{1}H NMR ($CDCl_3$) δ 6.7–7.05 (m, 6H, Ar), 5.76 (t, 1H, NH), 4.17 (t, 2H, $J=5.3$ Hz, $OCH_2CH_2N_3$), 4.00 (m, 1H, CH_2OCO), 3.86 (s, 3H, OCH_3), 3.80 (m, 1H, CH_2OCO), 3.62 (t, 2H, $J=5.3$ Hz, $OCH_2CH_2N_3$), 3.48 (s, 2H, $COCH_2Ar$), 3.30 (m, 1H, CH_2NH), 3.10 (m, 1H, CH_2NH), 2.60 (dd, 1H, $J=7.3$, 12.0 Hz, CH_2Ph), 2.49 (d, 1H, $J=7.3$ Hz, CH_2Ph), 2.0–2.3 (m, 7H, 2 × CH_3 and CH), 1.20 (s, 9H, $CO(CH_3)_3$).

12: 92% yield, colorless oil; ^{1}H NMR ($CDCl_3$) δ 7.28 (d, 2H, $J=8.3$ Hz, Ar), 7.03 (d, 2H, $J=8.3$ Hz), 6.88 (d, 1H,

$J = 7.8$ Hz), 6.80 (d, 1H, $J = 2.8$ Hz), 6.74 (dd, 1H, $J = 2.8$, 7.8 Hz), 5.76 (t, 1H, NH), 4.17 (t, 2H, $J = 5.2$ Hz, $OCH_2CH_2N_3$), 4.01 (dd, 1H, $J = 4.5$, 11.2 Hz, CH_2OCO), 3.86 (s, 3H, OCH_3), 3.80 (dd, 1H, $J = 5.0$, 11.2 Hz, CH_2OCO), 3.63 (t, 2H, $J = 5.2$ Hz, $OCH_2CH_2N_3$), 3.48 (s, 2H, $COCH_2Ar$), 3.32 (m, 1H, CH_2NH), 3.06 (m, 1H, CH_2NH), 2.54 (d, 2H, $J = 7.6$ Hz, CH_2Ph), 2.10 (m, 1H, CH), 1.30 (s, 9H, $C(CH_3)_3$), 1.20 (s, 9H, $CO(CH_3)_3$).

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-2-[4-(2-aminoethoxy)-3-methoxyphenyl]acetamide (13) and *N*-[2-(4-*tert*-butylbenzyl)-3-(pivaloyloxy)propyl]-2-[4-(2-aminoethoxy)-3-methoxyphenyl]acetamide (14).** A suspension of **11** (or **12**) (1 mmol) and 5% palladium on carbon (50 mg) in MeOH (5 mL) was hydrogenated under a balloon of hydrogen for 1 h. The reaction mixture was filtrated and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with CH_2Cl_2/MeOH (10:1) as eluant to afford **13** (or **14**).

13: 83% yield, white solid, mp 38 °C; ^{1}H NMR ($CDCl_3$) δ 6.7–7.05 (m, 6H, Ar), 5.78 (t, 1H, NH), 4.07 (t, 2H, $J = 4.9$ Hz, $OCH_2CH_2NH_2$), 4.00 (m, 1H, CH_2OCO), 3.85 (s, 3H, OCH_3), 3.80 (m, 1H, CH_2OCO), 3.47 (s, 2H, $COCH_2Ar$), 3.30 (m, 1H, CH_2NH), 3.15 (t, 2H, $J = 4.9$ Hz, $OCH_2CH_2NH_2$), 3.10 (m, 1H, CH_2NH), 2.60 (dd, 1H, $J = 7.5$, 12 Hz, CH_2Ph), 2.50 (d, 1H, $J = 7.5$ Hz, CH_2Ph), 2.15–2.3 (m, 6H, 2 × CH_3), 2.08 (m, 1H, CH), 1.21 (s, 9H, $CO(CH_3)_3$); MS *m*/*e* 484 (M^+). Anal. calcd for $C_{28}H_{40}N_2O_5$: C, 69.39; H, 8.32; N, 5.78. Found: C, 69.61; H, 8.36; N, 5.76.

14: 89% yield, white solid, mp 36 °C; ^{1}H NMR ($CDCl_3$) δ 7.28 (d, 2H, $J = 8.3$ Hz, Ar), 7.03 (d, 2H, $J = 8.3$ Hz), 6.88 (d, 1H, $J = 7.8$ Hz), 6.80 (d, 1H, $J = 2.8$ Hz), 6.74 (dd, 1H, $J = 2.8$, 7.8 Hz), 5.84 (t, 1H, NH), 4.07 (t, 2H, $J = 4.9$ Hz, $OCH_2CH_2NH_2$), 4.02 (dd, 1H, $J = 4.5$, 11.2 Hz, CH_2OCO), 3.85 (s, 3H, OCH_3), 3.82 (dd, 1H, $J = 5.2$, 11.2 Hz, CH_2OCO), 3.47 (s, 2H, $COCH_2Ar$), 3.32 (m, 1H, CH_2NH), 3.15 (t, 2H, $J = 4.9$ Hz, $OCH_2CH_2NH_2$), 3.08 (m, 1H, CH_2NH), 2.53 (d, 2H, $J = 7.6$ Hz, CH_2Ph), 2.12 (m, 1H, CH), 1.29 (s, 9H, $C(CH_3)_3$), 1.20 (s, 9H, $CO(CH_3)_3$); MS *m*/*e* 512 (M^+). Anal. calcd for $C_{30}H_{44}N_2O_5$: C, 70.28; H, 8.65; N, 5.46. Found: C, 70.50; H, 8.68; N, 5.45.

***tert*-Butyl *N*-(4-hydroxy-3-methoxybenzyl)carbamate (16).** A solution of 4-hydroxy-3-methoxy benzylamine hydrochloride (**15,** 500 mg, 2.64 mmol) and triethylamine (54 mg, 5.28 mmol) in H_2O (10 mL) was treated dropwise with a solution of di-*tert*-butyl dicarbonate (1.15 g, 5.28 mmol) in dioxane (2 mL) for 20 min. After being stirred for 24 h at room temperature, the reaction mixture was extracted with CH_2Cl_2 several times. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (1:1) as eluant to give **16** as an oil (663 mg, 99%). ^{1}H NMR ($CDCl_3$) δ 6.7–7.0 (m, 3H, Ar), 5.65 (s, 1H, OH), 4.79 (bs, 1H, NH), 4.23 (d, 2H, $J = 5.6$ Hz, CH_2NHBoc) 3.87 (s, 3H, OCH_3), 1.43 (s, 9H, $C(CH_3)_3$).

***tert*-Butyl *N*-[4-(2-bromoethoxy)-3-methoxybenzyl]carbamate (17).** The compound was obtained from **16** by following the method for **9** in 84% yield. ^{1}H NMR ($CDCl_3$) δ 6.75–6.90 (m, 3H, Ar), 4.79 (bs, 1H, NH), 4.31 (t, 2H, $J = 6.6$ Hz, OCH_2CH_2Br), 4.24 (d, 2H, $J = 5.8$ Hz, CH_2NHBoc), 3.86 (s, 3H, OCH_3), 3.64 (t, 2H, $J = 5.8$ Hz, OCH_2CH_2Br), 1.46 (s, 9H, $C(CH_3)_3$).

***tert*-Butyl *N*-[4-(2-azidoethoxy)-3-methoxybenzyl]carbamate (18).** The compound was obtained from **17** by following the method for **11** in 98% yield. ^{1}H NMR ($CDCl_3$) δ 6.75–6.90 (m, 3H, Ar), 4.79 (bs, 1H, NH), 4.24 (d, 2H, $J = 5.8$ Hz, CH_2NHBoc), 4.17 (t, 2 H, $J = 4.9$ Hz, $OCH_2CH_2N_3$), 3.86 (s, 3H, OCH_3), 3.62 (t, 2H, $J = 4.9$ Hz, $OCH_2CH_2N_3$), 1.46 (s, 9H, $C(CH_3)_3$).

4-(2-Azidoethoxy)-3-methoxybenzylisothiocyanate (19). A solution of **18** (322 mg, 1 mmol) in CH_2Cl_2 (3 mL) was treated with trifluoroacetic acid (0.9 mL) stirred at room temperature for 1 h. The reaction mixture was concentrated in vacuo by the aid of toluene to afford the corresponding amine salt as a brown solid.

The amine salt was dissolved in DMF (3 mL), treated with triethylamine (110 mg, 1.1 mmol) and stirred for 1 h. The mixture was treated with 1,1-thiocarbonyl di-2(1H)-pyridone (244 mg, 1.1 mmol) and stirred for 3 h at room temperature. The mixture was diluted with H_2O and extracted with diethyl ether several times. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (1:2) as eluant to afford **19** as an oil (270 mg, 74%). ^{1}H NMR ($CDCl_3$) δ 6.8–6.95 (m, 3H, Ar), 4.65 (s, 2H, CH_2NCS), 4.18 (t, 2H, $J = 5.1$ Hz, $OCH_2CH_2N_3$), 3.89 (s, 3H, OCH_3), 3.64 (t, 2H, $J = 5.1$ Hz, $OCH_2CH_2N_3$).

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-*N′*-[4-(2-aminoethoxy)-3-methoxybenzyl]thiourea (20) and *N*-[2-(4-*tert*-butylbenzyl)-3-(pivaloyloxy)propyl]-*N′*-[4-(2-aminoethoxy)-3-methoxybenzyl]thiourea (21).** A suspension of **4** (or **5**) (0.6 mmol) and 5% palladium on carbon (25 mg) in MeOH (5 mL) was hydrogenated under a balloon of hydrogen for 2 h. The reaction mixture was filtered and the filtrate was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (5 mL) and treated with isothiocyanate **19** (132 mg, 0.5 mmol). After being stirred for 15 h at room temperature, the reaction mixture was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (1:10) as eluant to afford thiourea. The above compound (0.3 mmol) was dissolved in THF (3 mL) was treated with triphenylphosphine (157 mg, 0.6 mmol) and H_2O (11 mg, 0.6 mmol), and stirred for 24 h at room temperature. The reaction mixture was concentrated in vacuo and the residue was purified by flash column chromatography over silica gel with CH_2Cl_2/MeOH (10:1) as eluant to afford **20** (or **21**).

20: 48% yield, sticky semisolid; ^{1}H NMR ($CDCl_3$) δ 6.75–7.05 (m, 6H, Ar), 6.47 (bs, 2H, NH), 4.43 (bs, 2H, $NHCH_2Ar$), 4.13 (m, 1H, CH_2OCO), 3.99 (t, 2H,

$J = 5.4$ Hz, $OCH_2CH_2NH_2$), 3.82 (s, 3H, OCH_3), 3.7–3.8 (m, 2H, CH_2OCO and $CHCH_2NH$), 3.31 (m, 1H, $CHCH_2NH$), 3.07 (t, 2H, $J = 5.4$ Hz, $OCH_2CH_2NH_2$), 2.45–2.7 (m, 2H, CH_2Ph), 2.15–2.3 (m, 7H, 2 × CH_3 and CH), 1.22 (s, 9H, $C(CH_3)_3$); MS *m*/*e* 515 (M^+). Anal. calcd for $C_{28}H_{41}N_3O_4S$: C, 65.21; H, 8.01; N, 8.15; S, 6.22. Found: C, 65.46; H, 8.04; N, 8.13; S, 6.19.

21: 54% yield, sticky semisolid; ^{1}H NMR ($CDCl_3$) δ 7.30 (d, 2H, $J = 8.3$ Hz), 7.10 (d, 2H, $J = 8.3$ Hz), 6.75–6.9 (m, 3H, Ar), 6.40 (bs, 2H, NH), 4.43 (bs, 2H, $NHCH_2Ar$), 4.12 (m, 1H, CH_2OCO), 3.98 (t, 2H, $J = 5.4$ Hz, $OCH_2CH_2NH_2$), 3.82 (s, 3H, OCH_3), 3.7–3.8 (m, 2H, CH_2OCO and $CHCH_2NH$), 3.30 (m, 1H, $CHCH_2NH$), 3.06 (t, 2H, $J = 5.4$ Hz, $OCH_2CH_2NH_2$), 2.45–2.7 (m, 2H, CH_2Ph), 2.20 (m, 1H, CH), 1.28 (s, 9H, $C(CH_3)_3$), 1.22 (s, 9H, $C(CH_3)_3$); MS *m*/*e* 543 (M^+). Anal. calcd for $C_{30}H_{45}N_3O_4S$: C, 66.26; H, 8.34; N, 7.73; S, 5.90. Found: C, 66.50; H, 8.33; N, 7.70; S, 5.88.

Benzyl *N*-(4-hydroxy-3-methoxybenzyl)carbamate (25). A solution of 4-hydroxy-3-methoxy-benzonitrile (**22**, 10 g, 67 mmol) and diisopropylethylamine (17.52 mL, 100.5 mmol) in CH_2Cl_2 (100 mL) was treated with chloromethylmethylether (6.11 mL, 80.4 mmol) and stirred for 3 h at room temperature. The mixture was diluted with CH_2Cl_2, washed with H_2O, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel using EtOAc/hexanes (1:2) as eluant to afford **23** (11.9 g, 92%). ^{1}H NMR ($CDCl_3$) δ 7.05–7.3 (m, 3H, Ar), 5.29 (s, 2H, $ArOCH_2$), 3.91 (s, 3H, $ArOCH_3$), 3.51 (s, 3H, CH_2OCH_3).

A solution of **23** (9.66 g, 50 mmol) in THF (50 mL) was added dropwise to a stirred suspension of lithiumaluminum hydride (7.6 g, 200 mmol) in THF (150 mL). After being refluxed for 4 h, the reaction mixture was cooled in ice bath and quenched carefully with 5 N NaOH. Precipitated aluminum salts were removed by filtration and the filtrate was evaporated. The residue was partitioned with ether and H_2O. The ether layer was washed with H_2O and brine, dried over $MgSO_4$ and concentrated in vacuo to give amine. The amine was dissolved in CH_2Cl_2 (50 mL) and treated with triethylamine (14 mL, 100 mmol) and benzylchloro formate (10.7 mL, 75 mmol). After being stirred for 2 h at room temperature, the reaction mixture was diluted with CH_2Cl_2, washed with H_2O and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (1:2) as eluant to give **24** (8.28 g, 50%). ^{1}H NMR ($CDCl_3$) δ 7.3–7.35 (m, 5H, phenyl), 7.09 (d, 1H, $J = 8.1$ Hz), 6.80 (m, 2H), 5.21 (s, 2H, $ArOCH_2$), 5.14 (s, 2H, OCH_2Ph), 4.32 (d, 2H, $J = 6.1$ Hz, $NHCH_2Ar$), 3.85 (s, 3H, $ArOCH_3$), 3.51 (s, 3H, CH_2OCH_3).

A cooled solution of **24** (6.63 g, 20 mmol) in CH_2Cl_2 (20 mL) in ice bath was treated with trifluoroacetic acid (2 mL) slowly and stirred for 10 min at room temperature. After cooling in ice bath, the reaction mixture was neutralized with saturated $NaHCO_3$ solution, diluted with H_2O and extracted with CH_2Cl_2 several times. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (2:1) as eluant to give **25** (5.46 g, 95%). ^{1}H NMR ($CDCl_3$) δ 7.3–7.35 (m, 5H, phenyl), 6.86 (d, 1H, $J = 7.8$ Hz), 6.76 (m, 2H), 5.14 (s, 2H, OCH_2Ph), 4.30 (d, 2H, $J = 5.8$ Hz, $NHCH_2Ar$), 3.86 (s, 3H, OCH_3).

2-{[4-(Benzyloxycarbonylamino)methyl-2-methoxy]phenoxy}acetic acid (27). A solution of **25** (287 mg, 1 mmol) in acetone (20 mL) was treated with K_2CO_3 (552 mg, 4 mmol) and methyl bromoacetate (0.14 mL, 1.5 mmol) and refluxed for 3 h. After cooling, the reaction mixture was filtered and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (3:2) as eluant to afford **26** (323 mg, 90%). ^{1}H NMR ($CDCl_3$) δ 7.25–7.35 (m, 5H, phenyl), 6.75–6.85 (m, 3H, Ar), 5.11 (s, 2H, OCH_2Ph), 4.65 (s, 2H, OCH_2CO_2Me), 4.28 (d, 2H, $J = 5.8$ Hz, $NHCH_2Ar$), 3.81 (s, 3H, OCH_3), 3.76 (s, 3H, CO_2CH_3).

A solution of **26** (323 mg, 0.9 mmol) in THF (1 mL) was treated with 15% NaOH solution (1 mL) and stirred for 30 min at room temperature. The reaction mixture was neutralized with acetic acid, diluted with H_2O and extracted with CH_2Cl_2 several times. The combined organic layers were washed with H_2O, dried over $MgSO_4$ and concentrated in vacuo to afford **27** (276 mg, 89%). ^{1}H NMR (DMSO-d_6) δ 7.72 (t, 1H, NH), 7.25–7.4 (m, 5 H, phenyl), 6.65–6.85 (m, 3H, Ar), 5.02 (s, 2H, OCH_2Ph), 4.16 (s, 2H, OCH_2CO_2H), 4.09 (d, 2H, $J = 6.1$ Hz, $NHCH_2Ar$), 3.70 (s, 3H, OCH_3).

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-*N′*-[4-carboxymethoxy-3-methoxybenzyl]thiourea (29) and *N*-[2-(4-*tert*-butylbenzyl)-3-(pivaloyloxy)propyl]-*N′*-[4-carboxymethoxy-3-methoxybenzyl]thiourea (30).** A suspension of **27** (276 mg, 0.8 mmol) and 5% palladium on carbon (30 mg) in MeOH (5 mL) was hydrogenated under a balloon of hydrogen for 1 h. The reaction mixture was filtrated and the filtrate was concentrated to afford amine **28**. The amine was dissolved in DMF (1 mL) was treated with triethylamine (0.14 mL, 1.0 mmol) and stirred for 30 min. The solution was treated with a solution of 2-(3,4-dimethyl (or 4-*tert*-butyl)benzyl)-3-pivaloyloxypropyl isothiocyanate (0.8 mmol).[19] After stirring for 24 h at room temperature, the reaction mixture was diluted with H_2O and extracted with EtOAc several times. The combined organic layers were washed H_2O and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with CH_2Cl_2/MeOH/AcOH (100:10:0.5) as eluant to afford **29** (or **30**).

29: 37% yield, white solid, mp 50 °C; ^{1}H NMR ($CDCl_3$) δ 6.75–7.05 (m, 6H, Ar), 6.54 (bs, 2H, NH), 4.51 (bs, 2H, $NHCH_2Ar$), 4.46 (s, 2H, OCH_2CO_2H), 4.17 (m,

1H, CH_2OCO), 3.7–3.85 (m, 5H, OCH_3, CH_2OCO and CH_2NHCS), 3.22 (m, 1H, CH_2NHCS), 2.5–2.7 (m, 2H, CH_2Ar), 2.2–2.4 (m, 7H, 2 × CH_3 and CH), 1.23 (d, 9H, $C(CH_3)_3$); MS *m/e* 530 (M^+). Anal. calcd for $C_{28}H_{38}N_2O_6S$: C, 63.37; H, 7.22; N, 5.28; S, 6.04. Found: C, 63.61; H, 7.24; N, 5.25; S, 6.02.

30: 52% yield, white solid, mp 48 °C; ^1H NMR ($CDCl_3$) δ 7.28 (d, 2H, $J = 8.3$ Hz), 7.09 (d, 2H, $J = 8.3$ Hz), 6.65–6.9 (m, 3H, Ar), 6.54 (bs, 2H, NH), 4.58 (bs, 2H, $NHCH_2Ar$), 4.46 (s, 2H, OCH_2CO_2H), 4.11 (dd, 1H, $J = 4.2$, 11.5 Hz, CH_2OCO), 3.6–3.9 (m, 5H, OCH_3, CH_2OCO and $CHCH_2NH$), 3.26 (m, 1H, $CHCH_2NHCS$), 2.64 (dd, 1H, $J = 6.3$, 13.5 Hz, CH_2Ar), 2.53 (dd, 1H, $J = 8.3$, 13.5 Hz, CH_2Ar), 2.31 (m, 1H, CH), 1.29 (s, 9H, $C(CH_3)_3$), 1.21 (s, 9H, $C(CH_3)_3$); MS *m/e* 558 (M^+). Anal. calcd for $C_{30}H_{42}N_2O_6S$: C, 64.49; H, 7.58; N, 5.01; S, 5.74. Found: C, 64.73; H, 7.61; N, 5.00; S, 5.73.

4-{4-[(Benzyloxycarbonylamino)methyl]-2-methoxyphenoxy}-4-oxobutanoic acid (33). A solution of benzyl alcohol (0.52 mL, 5 mmol) in THF (20 mL) was treated with NaH (60%, 0.2 g, 5 mmol) and succinic anhydride (**31**, 0.502 g, 5 mmol). After being refluxed for 4 h, the reaction mixture was diluted with H_2O and extracted with EtOAc several times. The combined organic layers were washed with H_2O and brine, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (1:1) as eluant to give **32** (0.866 g, 57%). ^1H NMR ($CDCl_3$) δ 7.25–7.40 (m, 5H, phenyl), 5.15 (s, 2H, $PhCH_2$), 2.70 (m, 4H, $COCH_2CH_2CO$).

A solution of **32** (0.866 g, 2.85 mmol) in CH_2Cl_2 (10 mL) was treated with **25** (2.85 mmol), dicyclohexylcarbodiimide (4.2 mmol) and 4-dimethylaminopyridine (0.42 mmol) successively and stirred for 24 h at room temperature. The reaction mixture was diluted with diethyl ether, precipitated solid was filtered and the filtrate was concentrated in vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexanes (2:1) as eluant to give **33** as a white solid (1.15 g, 85%); mp 64 °C; ^1H NMR ($CDCl_3$) δ 7.25–7.40 (m, 10H, 2 × phenyl), 6.75–6.95 (m, 3H, Ar), 5.15 (s, 2H, CH_2Ph), 5.14 (s, 2H, CH_2Ph), 4.34 (d, 2H, $J = 5.8$ Hz, CH_2NH), 3.75 (s, 3H, $ArOCH_3$), 2.93 (t, 2H, $J = 6.8$ Hz, $COCH_2CH_2CO$), 2.79 (t, 2H, $J = 6.8$ Hz, $COCH_2CH_2CO$).

***N*-[2-(3,4-Dimethylbenzyl)-3-(pivaloyloxy)propyl]-*N′*-{4-[(3-carboxypropanoyl)oxy]-3-methoxybenzyl}thiourea (35) and *N*-[2-(4-*tert*-butylbenzyl)-3-(pivaloyloxy)propyl]-*N′*-{4-[(3-carboxypropanoyl)oxy]-3-methoxybenzyl}-thiourea (36).** The compounds were obtained from **33** by following the method for **29** (or **30**) via **34**.

35: 24% yield, white solid, mp 48 °C; ^1H NMR ($CDCl_3$) δ 6.75–7.05 (m, 6H, Ar), 6.54 (bs, 2H, NH), 4.51 (bs, 2H, $NHCH_2Ar$), 4.17 (m, 1H, CH_2OCO), 3.7–3.85 (m, 5H, OCH_3, CH_2OCO and CH_2NHCS), 3.22 (m, 1H, CH_2NHCS), 2.4–2.5 (m, 4H, CH_2Ar and $COCH_2CH_2CO$), 2.34 (t, 2H, $J = 6.8$ Hz, $COCH_2CH_2CO$), 2.2–2.4 (m, 7H, 2 × CH_3 and CH), 1.23 (d, 9H, $C(CH_3)_3$); MS *m/e* 441 (M^+); MS *m/e* 572 (M^+). Anal. calcd for $C_{30}H_{40}N_2O_7S$: C, 62.91; H, 7.04; N, 4.89; S, 5.60. Found: C, 63.16; H, 7.06; N, 4.87; S, 5.58.

36: 21% yield, white solid, mp 46 °C; ^1H NMR ($CDCl_3$) δ 7.30 (d, 2H, $J = 8.3$ Hz), 7.06 (d, 2H, $J = 8.3$ Hz), 6.7–6.9 (m, 3H, Ar), 6.26 (bs, 2H, NH), 4.36 (d, 2H, $J = 4.1$ Hz, $NHCH_2Ar$), 4.11 (dd, 1H, $J = 4.4$, 11.4 Hz, CH_2OCO), 3.6–3.9 (m, 5H, OCH_3, CH_2OCO and $CHCH_2NH$), 3.27 (m, 1H, $CHCH_2NHCS$), 2.4–2.5 (m, 4H, CH_2Ar and $COCH_2CH_2CO$), 2.35 (t, 2H, $J = 6.6$ Hz, $COCH_2CH_2CO$), 2.31 (m, 1H, CH), 1.29 (s, 9H, $C(CH_3)_3$), 1.21 (s, 9H, $C(CH_3)_3$); MS *m/e* 600 (M^+). Anal. calcd for $C_{32}H_{44}N_2O_7S$: C, 63.98; H, 7.38; N, 4.66; S, 5.34. Found: C, 64.22; H, 7.41; N, 4.65; S, 5.32.

Characterization of pharmacokinetics for 2 and 13

Pharmacokinetics of **2** and **13** were studied in rats. Male Sprague–Dawley rats (body weight 250–300 g) were anesthetized by ether, and the femoral artery and vein were catheterized using polyethylene tubing (PE-50). After a recovery period of approximately 2 h, **2** and **13** were intravenously injected (dose 0.5 mg/kg) via the venous catheter. Arterial blood samples were collected various times up to 1 h and analyzed for **2** and **13**. Two hundred μL of acetonitrile was added to 100 μL of plasma for deproteination. The resulting mixture was centrifuged, an aliquot (270 μL) of the supernatant collected and evaporated under vacuum. The residue was reconstituted by LC mobile phase (see below) and an aliquot (20 μL) was injected on to an LC–MS system (Finnigan LCQ Deca). The mobile phase was consisted of acetonitrile and water (6:4 in volume), and the flow rate was 0.5 mL/min. Selected ions monitored for **2** and **13** were 495.2 and 485.2, respectively. The peaks of the drug were adequately separated from other peaks (i.e., retention times, **2**: 7.5 min, **13**: 5.3 min). The detection limit for this assay was 10 ng/mL for **2** and 1 ng/mL for **13**.

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