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N-(3-Acyloxy-2-Benzylpropyl)-*N*′-Dihydroxytetrahydrobenzazepine and Tetrahydroisoquinoline Thiourea Analogues as Vanilloid Receptor Ligands

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Abstract—The vanilloid receptor represents a promising target for drug development. Building on our previous strategies which have generated potent agonists for VR1, we now describe a series of novel *N*-(3-acyloxy-2-benzylpropyl)-*N*′-dihydroxytetrahydrobenzazepine and tetrahydroisoquinoline thiourea analogues, several of which are potent VR1 antagonists. We report here the rationale for the design, the synthesis, and the in vitro characterization of activity in assays for [^{3}H]resiniferatoxin binding and ^{45}Ca influx using heterologously expressed rat VR1.

Introduction

The vanilloid receptor (VR) is a specific neuronal membrane recognition site for vanilloids like capsaicin and resiniferatoxin (RTX),[1] and its expression is a prominent feature of nociceptive primary afferents of the C-fiber 'pain' pathway.[2] Desensitization of the receptor caused by specific ligands has long been recognized as a promising therapeutic approach for the treatment of neuropathic pain and other pathological conditions involving C-fiber neurons.[3–6]

The functional vanilloid receptor subtype VR1, which is activated not only by capsaicin but also by noxious heat and low pH, has recently been cloned.[7] The VR1 protein is structurally a distant homologue of the transient release potential (TRP) family of store-operated calcium channels, comprising six transmembrane domains and three ankyrin repeats at the N-terminus.[8] VR1 is a nonselective cation channel with a preference for Ca^{++} and was shown, in studies with knockout mice, to be essential for response to vanilloids as well as a major mediator of thermal nociception.[2,9,10]

Among a number of VR agonists studied, only a few compounds are currently marketed (e.g., capsaicin) or undergoing clinical trial (e.g., resiniferatoxin, DA-5018).[11] The primary mechanistic rationale of vanilloids for treatment of pain is through C-fiber desensitization. The initial response of VR1 to vanilloids is to permit a large influx of Ca^{++} and C-fiber depolarization. Subsequently, depending on dose of vanilloid, time of exposure, and other factors, desensitization, potentially reflecting multiple mechanisms, develops. For capsaicin, the initial phase of acute stimulation, causing a severe burning sensation, represents the limiting toxicity. Apparently, pungency, reflecting the acute pain response, and the subsequent desensitization are not directly coupled. Thus, RTX is much more potent as a ligand at VR1 but is only modestly more pungent.[1] One direction for drug development, therefore, is design of analogues in which pungency and desensitization have been dissociated. An inherent advantage/problem in this approach is the potential long duration of desensitization. A single treatment with RTX, for example, can cause desensitization for multiple weeks.[1]

An alternative strategy, optimal when only a short duration of inhibition is desirable, is to block VR1 function either through a channel blocker specific for VR1 or else through a competitive receptor antagonist.[3,12] Currently, capsazepine is the only commercially

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available competitive antagonist for VR1, inhibiting biological responses of capsaicin and RTX in a competitive manner in a variety of bioassays.[13,14] Capsazepine has several drawbacks such as moderate potency, susceptibility to metabolic clearance, inhibitory activity on several channels other than VR1, and poor solubility. Nonetheless, the inhibition of nociceptive and hyperalgesic responses by capsazepine proves in principle that VR1 antagonists have an analgesic potential.[15]

Based on structure–activity relationships for stimulation of Ca^{2+} uptake by capsaicin derivatives, a model for ligand interaction at the receptor has been proposed in which three pharmacophoric elements, the so-called A, B and C regions, are crucial for activity.[16–18] Molecular modeling of capsazepine suggests that the twisted orientation of the A and C regions imposed by the benzazepine ring is responsible for its activity as an antagonist.[14]

The identification of RTX as a novel vanilloid with potency 3–4 orders of magnitude greater than that of capsaicin suggested exciting opportunities for exploitation through medicinal chemistry.[19] Structurally, RTX represents a typical vanilloid in the A and B regions, but with a tetracyclic diterpene in place of the typical C region substituents in capsaicin and its congeners. Over the past few years, we have explored simplified RTX analogues as VR1 ligands based on our hypothetical pharmacophore model.[20] Using this approach, we have recently reported ultrapotent VR agonists, **27** and **28**, with K_i values of 19 and 11 nM, respectively, in an [^{3}H]RTX binding assay on dorsal root ganglion neurons. These compounds are thus approximately 280 and 480 times more potent than capsaicin.[21] 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. In the present study, we have sought to combine features of the key pharmacophores of capsazepine and our simplified RTX analogues as a possible route to novel, potent antagonists (Fig. 1). We incorporated the benzazepine ring of capsazepine as an A region and the 3-acyloxy-2-benzylpropyl group as a C region. We reasoned that the benzazepine (or tetrahydroisoquinoline) template of the target compounds might induce antagonistic binding to the receptor conformationally and the 3-acyloxy-2-benzylpropyl group might confer tight binding as shown previously.

In this paper, we describe the syntheses of 3-acyloxy-2-benzyl-propyl thiourea analogues of tetrahydrobenzazepine and tetrahydroisoquinoline and characterize their in vitro receptor activities by calcium influx and binding affinity assays. The analysis was carried out using rat VR1 heterologously expressed in CHO cells in place of the more problematic dorsal root ganglion cultures used previously.

Chemistry

The thiourea VR1 ligands were conventionally synthesized from the condensation between amines of the A region and isothiocyanates of the C region. Isothiocyanates **1**–**8** were prepared from the corresponding

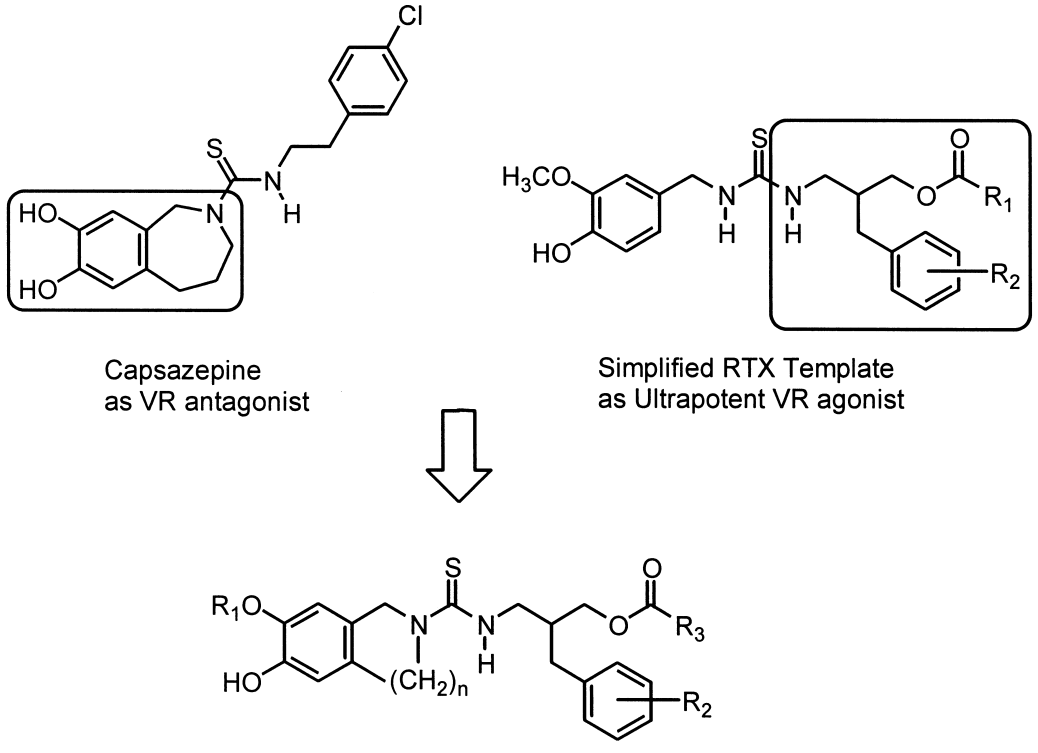


Figure 1.

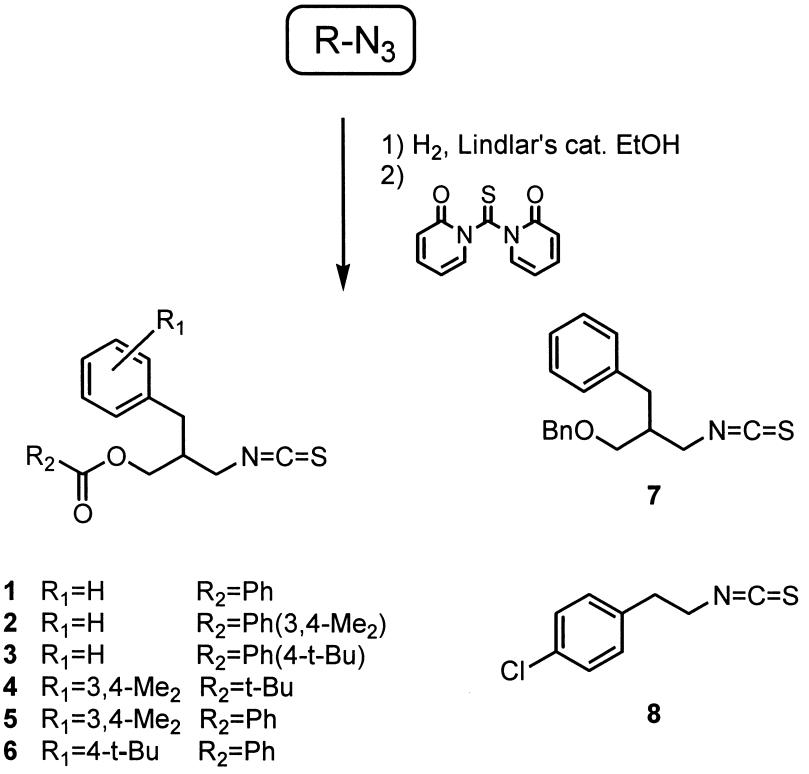
azides, whose syntheses were reported in a previous paper,[21] by catalytic hydrogenation with Lindler's catalyst followed by condensation with 1,1′-thiocarbonyldi-2(1H)-pyridone[22] (Scheme 1). Two tetrahydro-2-benzazepines, **9** and **10**, were efficiently prepared from isovanillin in seven steps by our procedure.[23] Catechols **11** and **12** were obtained from commercially available 6,7-dimethoxytetrahydroisoquinoline and 4-hydroxy-3-methoxybenzylamine, respectively, under refluxing 48% hydrobromic acid.[14] The condensation between isothiocyanates (**1**–**8**) and amines (**9**–**12**) proceeded smoothly in THF or DMF to afford thioureas; the reaction was carried out in the presence of base if amine salts were used.

Results and Discussion

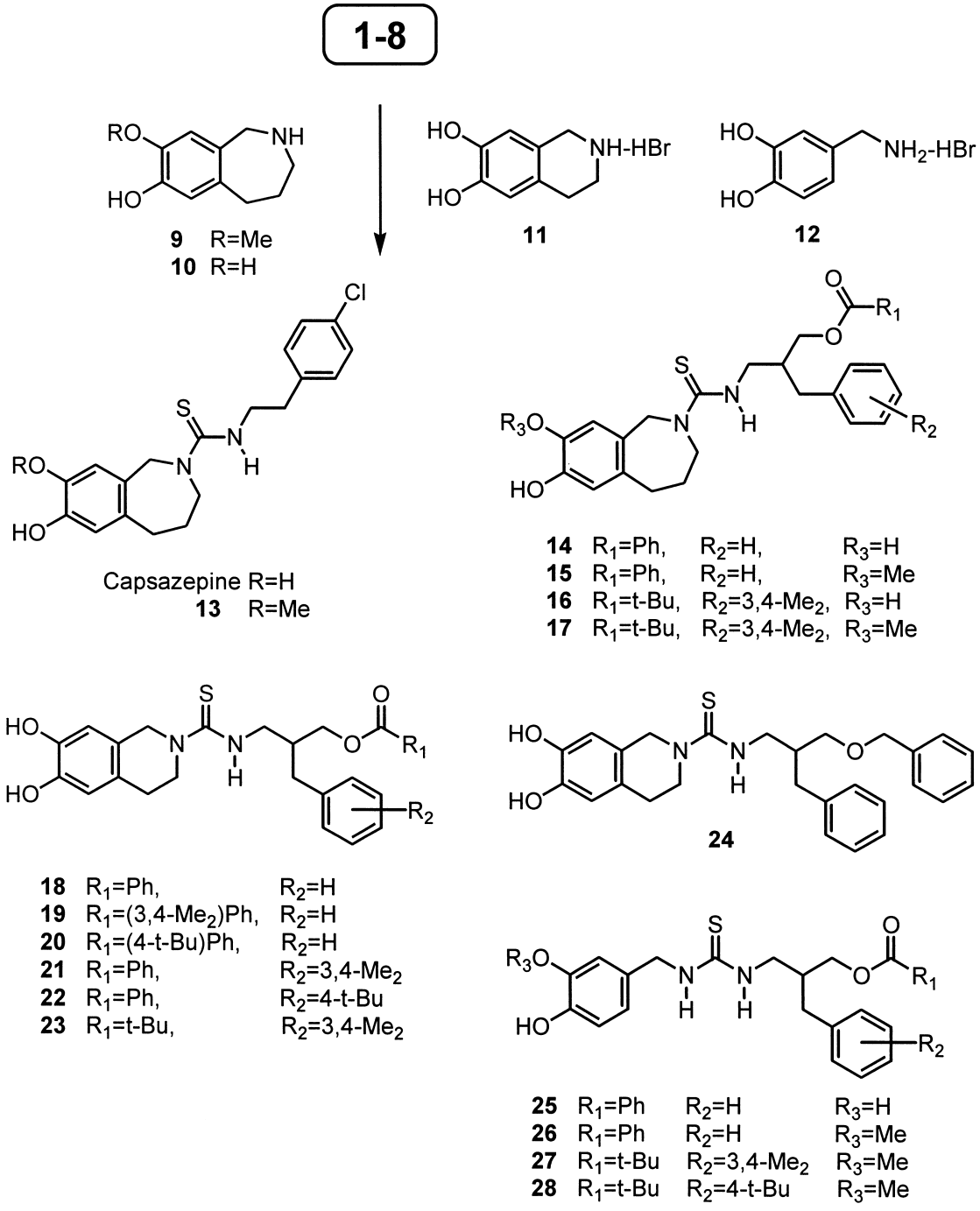
The activities of synthesized VR ligands as agonists and antagonists were assessed from assay of ^{45}Ca influx and their affinities for receptor binding were measured by competition of [^{3}H]RTX binding. Both assays were carried out using rat VR1 expressed in Chinese hamster ovary cells (CHO/VR1 cells) as previously described.[24] Compounds with measurable affinity for [^{3}H]RTX binding were first tested as possible agonists for ^{45}Ca uptake. Compounds without agonist activity were evaluated as possible antagonists. Antagonists were assayed in the presence of 50 nM capsaicin (K_D for ^{45}Ca uptake is 81 nM). K_i values generated from these experiments can be compared with that for capsazepine ($K_i = 0.56\,\mu M$), the one competitive antagonist of VR1. The results are presented in Table 1.

According to the calcium uptake assay, **13**, **14** and **18** among the tested VR ligands displayed antagonistic activity, with K_i values of 0.884, 0.966, and 0.194 μM, respectively. The antagonist **18** was ca. 3-fold more potent than capsazepine under these assay conditions. The binding assays of **13** ($K_i = 0.884\,\mu M$), **14** ($K_i = 0.223\,\mu M$) and **18** ($K_i = 0.81\,\mu M$) showed enhanced affinities compared to capsazepine ($K_i = 3.22\,\mu M$) of between 15- and 4-fold. It is interesting that while the 6,7-dihydroxytetrahydroisoquinoline surrogate of capsazepine was an agonist rather than an antagonist,[14] the 6,7-dihydroxytetrahydroisoquinoline analogue **18**, which is generated from the replacement of the chlorophenylethyl moiety in capsazepine by a 3-benzoyloxy-2-benzylpropyl group, is an antagonist. This suggests that the 3-benzoyloxy-2-benzylpropyl moiety in the C region might be responsible for its antagonistic activity.

The antagonistic activities of **14** and **18** were lost upon quite small structural modifications in the A and C regions. Within the A region, the corresponding monomethoxy analogues, **15** and **17**, were full agonists. In contrast, the 8-methoxy analogue of capsazepine **13**, retained its activity as an antagonist. This result indicated that a free catechol moiety may be important for antagonist activity in this series, but its importance depends on the specific structural context. In the C region, any modifications at the R_1 and R_2 groups (R_1 = pivaloyl, 3,4-dimethylphenyl or 4-*t*-butyl; R_2 = 3,4-dimethylphenyl or 4-*t*-butylphenyl) also switched both **14** and **18** into agonists (**16**–**17** and **19**–**23**), most of whose potencies were comparable to capsaicin in calcium influx and receptor binding assays. Removal of the



Scheme 1.



Scheme 2.

carbonyl group in antagonist **18** to afford **24** also converted its activity from an antagonist to an agonist. Apparently, the interaction of the 3-benzoyloxy-2-benzylpropyl group shared commonly in **14** and **18** with the receptor seems to be very specific for conferring their antagonistic activity. The acyclic surrogate **25** of the two antagonists **14** and **18**, which represents a conformationally flexible analogue, was also prepared and found to be a full agonist with an $EC_{50} = 0.165\,\mu M$ in the calcium influx assay and a $K_i = 0.253\,\mu M$ in binding affinity. This agonist was of equal or greater potency than the other tetrahydrobenzazepine and tetrahydroisoquinoline surrogates in both assays, but was 8-fold less potent in the binding assay than the corresponding 7-methoxy analogue **26**, for which the $K_i = 0.031\,\mu M$ was reported previously.[21] Enhanced agonistic activity associated with conformational flexibility was also evident in the conversion of the benzazepine **17** ($K_i = 1.42\,\mu M$) into the acyclic analogue **27** ($K_i = 0.0033\,\mu M$), resulting in a dramatic increase in binding affinity of 430-fold. The SAR in this series revealed that, whereas the antagonistic activity at VR1 requires appropriate conformational restriction of the 3,4-dihydrohydroxybenzyl amine, agonistic activity was enhanced when the flexibility was increased and when the 3-hydroxy group of catechol was methylated. Of

Table 1. In vitro assays of synthesized compounds toward VR1 receptor

Compounds	^{45}Ca uptake		[^{3}H]RTX binding	Relative potency
	Agonist (ED_{50}, μM)	Antagonist (K_i, μM)	(K_i, μM)	Ca^{++} uptake versus Binding
Capsaicin	0.081 (±0.011)		1.7[a]	0.02[a]
	0.038[a]			
		0.14[a]	6.6[a]	
Capsazepine		0.56 (±0.12)	3.22 (±0.48)	
13	NE[c]	0.884 (±0.152)	0.865 (±0.051)	
14	NE	0.966 (±0.118)	0.223 (±0.052)	
15	1.15 (±0.42)		0.59 (±0.18)	1.9
16	5.01 (±0.74)		1.156 (±0.047)	4.3
17	0.57 (±0.29)		1.42 (±0.049)	0.4
18	NE	0.196 (±0.05)	0.81 (±0.065)	
19	0.36 (±0.11)		0.40 (±0.068)	0.9
20	0.80 (±0.32)		0.52 (±0.15)	1.5
21	0.187 (±0.033)		0.627 (±0.074)	0.3
22	0.21 (±0.076)		0.233 (±0.003)	0.9
23	0.36 (±0.24)		7.1 (±2.4)	0.05
24	0.12 (±0.040)		0.267 (±0.059)	0.45
25	0.165 (±0.032)		0.253 (±0.063)	0.65
26	NT[d]		0.031 (±0.009)[b]	
27	0.0033 (±0.0015)		0.0067 (±0.0009)	0.49
28	0.0031 (±0.0002)		0.0032 (±0.0001)	0.97

[a]Literature value in ref 24.
[b]Literature value in ref 21.
[c]Not effective.
[d]Not tested.

course, constraint in the conformation optimized for agonist activity might yield yet greater potency, as suggested by the K_i of 0.00001 μM for RTX, an analogue with a rigid C region but still with flexibility between the A and B regions (Scheme 2).[24]

It has been noted that whereas capsaicinoids seem to be consistently less potent for inhibiting RTX binding than for inducing $^{45}Ca^{2+}$-uptake, RTX analogues are reciprocally more potent for inhibiting RTX binding (usually calcium uptake/binding affinity $= EC_{50}/K_i < 1$ for capsaicinoids and > 1 for resiniferanoids).[1,25] The relative potencies for calcium uptake and binding affinity of the compounds in the series described are shown in Table 1. Most are between 0.5 and 1. These values reveal that these ligands bind to VR1 in CHO cells and induce calcium uptake by them with similar affinities as do the RTX amide and thiourea analogues, with ratios of 1 and 1.2, respectively, for assay in cultured DRG neurons.[26]

Conclusion

Thirteen thiourea compounds having a dihydroxy-tetrahydrobenzazepine or dihydroxy tetrahydroisoquinoline template for the A region and a 3-acyloxy-2-benzyl-propyl moiety for the C region were synthesized as VR1 ligands and their in vitro receptor activities were measured by ^{45}Ca influx and [^{3}H]RTX binding assays. Among them, **14** and **18** were found to be potent VR antagonists. Compared to capsazepine, the only competitive VR1 antagonist described so far, **14** and **18** were 0.6- and 3-fold as potent when assayed as antagonists for calcium influx, and they were 15- and 4-fold as potent in binding affinity. The SAR study of this series indicated that both conformational constraint at the A region and the 3-benzoyloxy-2-benzylpropyl group at the C region were crucial for the antagonistic activities of **14** and **18** and their binding affinities to the receptor; their acyclic analogues and any structurally modified analogues in the C region were full agonists with an activity comparable to capsaicin. Our results suggest novel opportunities for the design of VR1 antagonists, a promising therapeutic objective.

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. Infrared spectra were recorded on a Perkin-Elmer 1710 Series FTIR. Mass spectra were recorded on a VG Trio-2 GC–MS. Elemental analyses were performed with an EA 1110 Automatic Elemental Analyzer, CE Instruments.

General procedure for the synthesis of isothiocyanates (1–8)

A suspension of Lindler's catalyst (0.05 g) in a solution of azide (0.5 mmol) in ethanol (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 1,1′-thiocarbonyldi-2(1H)-pyridone (0.5 mmol). After stirring for 16 h at room temperature, the reaction mixture was concentrated in

vacuo. The residue was purified by flash column chromatography over silica gel with EtOAc/hexane (10:1) as eluant to give the corresponding isothiocyanate.

2-Benzyl-3-isothiocyanatopropyl benzenecarboxylate (1). 90% yield; a colorless oil; ^{1}H NMR ($CDCl_3$) δ 8.04 (d, 2H), 7.60 (t, 1H), 7.47 (t, 2H) 7.2–7.35 (m, 5H, phenyl), 4.43 (dd, 1H, J = 4.8 and 11.4 Hz, $BzOCH_2$), 4.28 (dd, 1H, $BzOCH_2$), 3.63 (ddd of AB, 2H, CH_2NCS), 2.82 (dd of AB, 2H, CH_2Ph), 2.47 (m, 1H, CH).

2-Benzyl-3-isothiocyanatopropyl 3,4-dimethylbenzenecarboxylate (2). 82% yield; a colorless oil; ^{1}H NMR ($CDCl_3$) δ 7.78 (m, 2H), 7.2–7.35 (m, 6H), 4.40 (dd, 1H, $OCOCH_2$), 4.34 (dd, 1H, $OCOCH_2$), 3.67 (ddd of AB, 2H, CH_2NCS), 2.7–2.9 (m, 2H, CH_2Ph), 2.46 (m, 1H, CH), 1.35 (s, 9H, $C(CH_3)_3$).

2-Benzyl-3-isothiocyanatopropyl 4-*tert*-butylbenzenecarboxylate (3). 73% yield; a colorless oil; ^{1}H NMR ($CDCl_3$) δ 7.99 (d, 2 H), 7.50 (d, 2H) 7.2–7.35 (m, 5 H, phenyl), 4.43 (dd,1 H, $BzOCH_2$), 4.24 (dd, 1H, $BzOCH_2$), 3.62 (ddd of AB, 2H, CH_2NCS), 2.7–2.9 (m, 2H, CH_2Ph), 2.46 (m, 1H, CH), 1.35 (s, 9H, $C(CH_3)_3$).

2-(3,4-Dimethylbenzyl)-3-isothiocyanatopropyl pivalate (4). 94% yield; a colorless oil; ^{1}H NMR ($CDCl_3$) δ 6.8–7.3 (m, 3H), 4.04 (m, 2H, $OCOCH_2$), 3.36 (m, 2H, CH_2NCS), 2.63 (m, 2H, CH_2Ph), 2.2–2.3 (m, 7H, 2× CH_3 and CH), 1.23 (s, 9H, $C(CH_3)_3$).

2-(3,4-Dimethylbenzyl)-3-isothiocyanatopropyl benzenecarboxylate (5). 58% yield; a colorless oil; ^{1}H NMR ($CDCl_3$) δ 8.05 (d, 2H), 7.61 (t, 1H), 7.57 (t, 2H) 6.9–7.1 (m, 3 H), 4.42 (dd,1 H, $BzOCH_2$), 4.28 (dd, 1H, $BzOCH_2$), 3.64 (ddd of AB, 2H, CH_2NCS), 2.85 (m, 2H, CH_2Ph), 2.4–2.5 (m, 7H, 2× CH_3 and CH).

2-(4-*tert*-Butylbenzyl)-3-isothiocyanatopropyl benzenecarboxylate (6). 94% yield; a colorless oil; ^{1}H NMR ($CDCl_3$) δ 8.05 (d, 2H), 7.61 (t, 1H), 7.57 (t, 2H), 7.33 (d, 2H) 7.15 (d, 2H), 4.44 (dd, 1H, $BzOCH_2$), 4.27 (dd, 1H, $BzOCH_2$), 3.64 (ddd of AB, 2H, CH_2NCS), 2.7–2.9 (m, 2H, CH_2Ph), 2.45 (m, 1H, CH), 1.31 (s, 9H, $C(CH_3)_3$).

1-[(2-Benzyl-3-isothiocyanatopropoxy)methyl]benzene (7). 51% yield; a colorless oil; ^{1}H NMR ($CDCl_3$) δ 7.1–7.4 (m, 10H, phenyl), 4.50 (s, 2H, OCH_2Ph), 3.3–3.6 (m, 4H, CH_2N_3 and CH_2O), 2.6–2.7 (m, 2H, CH_2Ph), 2.24 (m, 1H, CH).

1-Chloro-4-(2-isothiocyanatoethyl)-benzene (8). 95% yield; a colorless oil; ^{1}H NMR ($CDCl_3$) δ 7.33 (d, 2H, Ph), 7.16 (d, 2H, Ph), 3.71 (t, 2H, CH_2Ph), 2.95 (t, 2H, CH_2NCS).

General procedure for the synthesis of thioureas

Method A. A mixture of isocyanate (0.5 mmol) and 7-hydroxy-8-methoxy-2,3,4,5-tetrahydro-1H-2-benzazepine (0.5 mmol) in THF (5 mL) was stirred for 18 h at room temperature. The reaction mixture was concentrated in vacuo and the residue was purified by flash column chromatography over silica gel with EtOAc/hexane (1:1) as eluant to give the thiourea.

***N*-(4-Chlorophenethyl)-7-hydroxy-8-methoxy-1,3,4,5-tetrahydro-2H-2-benzazepine-2-carbothioamide (13).** 90% yield; a white solid; mp 58 °C; ^{1}H NMR ($CDCl_3$) δ 7.23 (d, 2H, Ph), 6.96 (d, 2 H, Ph), 6.75 (s, 1H, ArH), 6.53 (s, 1H, ArH), 5.59 (s, 1H, ArOH), 5.37 (t, 1H, NH), 4.60 (s, 2H, $ArCH_2N$), 4.08 (m, 2H, $PhCH_2CH_2NH$), 3.88 (dd, 2H, $ArCH_2CH_2CH_2N$), 3.73 (s, 3H, $ArOCH_3$), 2.8–2.9 (m, 4H, $ArCH_2CH_2CH_2N$ and CH_2Ph), 1.81 (m, 2H, $ArCH_2CH_2CH_2N$); IR (KBr) 3396, 1591, 1489, 1288, 759 cm^{-1}; Anal. calcd for $C_{20}H_{23}ClN_2O_2S$: C, 61.45; H, 5.93; N, 7.17; S, 8.20. Found: C, 61.65; H, 5.95; N, 7.15; S, 8.17.

2-Benzyl-3-{[(7-hydroxy-8-methoxy-1,3,4,5-tetrahydro-2H-2-benzazepin-2-yl)carbothioyl]amino}propyl benzoate (15). 87% yield; a white solid; mp 58 °C; ^{1}H NMR ($CDCl_3$) δ 8.06 (d, 2H, Bz), 7.64 (t, 1H, Bz), 7.49 (t, 2H, Bz), 7.2–7.34 (m, 5H, Ph), 7.12 (s, 1H, Ar), 6.73 (s, 1H, Ar), 6.43 (t, 1H, NH), 5.50 (s, 1H, OH), 4.73 (s, 2 H, $ArCH_2NH$), 4.60 (dd, 1H, CH_2OBz), 3.97 (dd, 2 H, CH_2OBz and CH_2NHCSN), 3.81 (s, 3H, $ArOCH_3$), 3.23 (m, 1H, CH_2CH_2N), 2.88 (m, 4 H, $ArCH_2CH_2$ and CH_2Ph), 2.50 (m, 1H, CH),1.88 (m, 2H, $ArCH_2CH_2$); IR (KBr) 3408, 1514, 1276, 1100 cm^{-1}; Anal. calcd for $C_{29}H_{32}N_2O_4S$: C, 69.02; H, 6.39; N, 5.55; S, 6.35. Found: C, 69.27; H, 6.41; N, 5.53; S, 6.33.

2-Benzyl-3-{[(7-hydroxy-8-methoxy-1,3,4,5-tetrahydro-2H-2-benzazepin-2-yl)carbothioyl]amino}propyl pivalate (17). 81% yield; a white solid; mp 60 °C; ^{1}H NMR ($CDCl_3$) δ 6.9–7.2 (m, 4H, Ph and ArH), 6.73 (s, 1H, Ar), 6.43 (m, 1H, NH), 5.50 (s, 1H, OH), 4.68 (s, 2H, $ArCH_2NH$), 4.60 (ddd, 2H, CH_2OCO), 3.99 (m, 1H, CH_2NHCSN), 3.84 (s, 3H, $ArOCH_3$), 3.72 (m, 1H, CH_2CH_2N), 2.83 (m, 2 H, $ArCH_2CH_2$), 2.23 (m, 7H, 2×CH_3 and CH), 1.86 (m, 2 H, $ArCH_2CH_2$), 1.30 (s, 9H, $C(CH_3)_3$); IR (KBr) 3408, 1713, 1515, 1287, 1164 cm^{-1}; Anal. calcd for $C_{29}H_{40}N_2O_4S$: C, 67.94; H, 7.86; N, 5.46; S, 6.25. Found: C, 68.15; H, 7.88; N, 5.48; S, 6.27.

Method B. A solution of amine salt (1.0 mmol) and triethylamine (1.5 mmol) in DMF (1 mL) was stirred for 1 h and treated with isocyanate (1.2 mmol). After stirring for 20 h at room temperature, the reaction mixture was diluted with H_2O and extracted with EtOAc several times. The combined organic layer was 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/hexane (2:1) as eluant to give the thiourea.

2-Benzyl-3-{[(7,8-dihydroxy-1,3,4,5-tetrahydro-2H-2-benzazepin-2-yl)carbothioyl]amino}propyl benzoate (14). 41% yield; a white solid; mp 123 °C; ^{1}H NMR ($CDCl_3$) δ 8.10 (d, 2H, Bz), 7.66 (t, 1H, Bz), 7.52 (t, 2H, Bz), 7.2–7.3 (m, 5H, Ph), 6.89 (s, 1H, Ar), 6.73 (s, 1H, Ar), 6.01 (bs, 1H, NH), 5.50 (bs, 1H, OH), 4.50 (m, 3H, $ArCH_2NH$ and CH_2OBz), 4.03 (dd, 2H, CH_2OBz and CH_2NHCSN), 3.32 (m, 1H, CH_2CH_2N), 2.85 (m, 4H, $ArCH_2CH_2$ and CH_2Ph), 2.49 (m, 1H, CH), 1.85 (m,

2H, $ArCH_2C\underline{H}_2$). IR (KBr) 3407, 1539, 1277 cm^{-1}; Anal. calcd for $C_{28}H_{30}N_2O_4S$: C, 68.55; H, 6.16; N, 5.71; S, 6.54. Found: C, 68.72; H, 6.18; N, 5.69; S, 6.52.

2-(3,4-Dimethylbenzyl)-3-{[(7,8-dihydroxy-1,3,4,5-tetrahydro-2H-2-benzazepin-2-yl)carbothioyl]amino}propyl pivalate (16). 35% yield; a yellow solid; mp 76 °C; ^1H NMR ($CDCl_3$) δ 6.9–7.2 (m, 3H, Ph), 6.86 (s, 1H, Ar), 6.73 (s, 1H, Ar), 5.94 (bs, 1H, NH), 5.88 (bs, 1H, NH), 4.45 (m, 2H, $ArCH_2NH$), 4.12 (dd, 1H, CH_2OCO), 3.8–4.0 (m, 2H, CH_2OCO and CH_2NHCSN), 3.22 (m, 1H, CH_2NHCSN), 2.83 (d, 2H, $ArC\underline{H}_2CH_2$), 2.6 (m, 2H, $CHC\underline{H}_2Ph$), 2.23 (m, 7H, 2× $C\underline{H}_3$ and CH), 1.81 (m, 2H, $ArCH_2C\underline{H}_2$), 1.30 (s, 9H, $C(CH_3)_3$); IR (KBr) 3401, 1537, 1293 cm^{-1}; Anal. calcd for $C_{28}H_{38}N_2O_4S$: C, 67.44; H, 7.68; N, 5.62; S, 6.43. Found: C, 67.66; H, 7.70; N, 5.60; S, 6.42.

2-Benzyl-3-{[(6,7-dihydroxy-3,4-dihydro-2(1H)-isoquinolinyl)carbothioyl]amino}propyl benzoate (18). 62% yield; a yellow solid; mp 53 °C; ^1H NMR ($CDCl_3$) δ 8.06 (d, 2H, Bz), 7.60 (t, 1H, Bz), 7.47 (t, 2H, Bz), 7.2–7.35 (m, 5H, Ph), 6.88 (bs, 1H, ArOH), 6.67 (s, 1H, Ar), 6.66 (s, 1H, ArH), 6.53 (bs, 1H, ArOH), 6.36 (t, 1H, NH), 4.69 ($ArCH_2N$), 4.57 (dd of AB, 1H, CH_2OBz), 4.15 (m, 2H, CH_2OBz and $C\underline{H}_2NHCSN$), 3.78 (t, 2H, $CH_2C\underline{H}_2N$), 3.50 (m, 1H, $C\underline{H}_2NHCSN$), 2.7-2.83 (m, 4H, $C\underline{H}_2CH_2NCSNH$ and CH_2Ph), 2.60 (m, 1 H, CH); IR (KBr) 3375, 1712, 1524, 1275, 1101 cm^{-1}; Anal. calcd for $C_{27}H_{28}N_2O_4S$: C, 68.04; H, 5.92; N, 5.88; S, 6.73. Found: C, 68.29; H, 5.94; N, 5.86; S, 6.71.

2-Benzyl-3-{[(6,7-dihydroxy-3,4-dihydro-2(1H)-isoquinolinyl)carbothioyl]amino}propyl 3,4-dimethylbenzoate (19). 92% yield; a yellow solid; mp 45 °C; ^1H NMR ($CDCl_3$) δ 7.80 (m, 2H, Bz), 7.2–7.35 (m, 6H, Ph), 6.88 (m, 1H, ArOH), 6.65 (s, 1H, Ar), 6.63 (s, 1H, ArH), 6.43 (t, 1H, NH), 4.75 (d, 2H, $ArCH_2N$), 4.57 (dd of AB, 1H, CH_2OCO), 4.0–4.16 (m, 2H, CH_2OCO and $C\underline{H}_2NHCSN$), 3.78 (t, 2H, $CH_2C\underline{H}_2N$), 3.45 (m, 1H, $C\underline{H}_2NHCSN$), 2.7–2.81 (m, 4H, $C\underline{H}_2CH_2NCSNH$ and CH_2Ph), 2.57 (m, 1 H, CH), 2.32 (m, 6H, 2× CH_3); IR (KBr) 3389, 1696, 1524, 1267 cm^{-1}; Anal. calcd for $C_{29}H_{32}N_2O_4S$: C, 69.02; H, 6.39; N, 5.55; S, 6.35. Found: C, 69.27; H, 6.41; N, 5.54; S, 6.42.

2-Benzyl-3-{[(6,7-dihydroxy-3,4-dihydro-2(1H)-isoquinolinyl)carbothioyl]amino}propyl 4-*tert*-butylbenzoate (20). 85% yield; a yellow solid; mp 66 °C; ^1H NMR ($CDCl_3$) δ 8.06 (d, 2H, Bz), 7.60 (t, 1H, Bz), 7.47 (t, 2H, Bz), 7.2–7.35 (m, 5H, Ph), 6.65 (s, 2H, Ar), 6.38 (t, 1H, NH), 4.80 (d, 2H, $ArCH_2N$), 4.58 (dd of AB, 1H, CH_2OBz), 4.11 (m, 2H, CH_2OBz and $C\underline{H}_2NHCSN$), 3.74 (t, 2H, $CH_2C\underline{H}_2N$), 3.50 (m, 1H, $C\underline{H}_2NHCSN$), 2.6–2.8 (m, 4H, $C\underline{H}_2CH_2NCSNH$ and CH_2Ph), 2.57 (m, 1H, CH),1.29 (s, 9H, $C(CH_3)_3$); IR (KBr) 3375, 1699, 1522, 1277 cm^{-1}; Anal. calcd for $C_{31}H_{36}N_2O_4S$: C, 69.90; H, 6.81; N, 5.26; S, 6.02. Found: C, 70.09; H, 6.83; N, 5.24; S, 6.00.

2-(3,4-Dimethylbenzyl)-3-{[(6,7-dihydroxy-3,4-dihydro-2(1H)-isoquinolinyl)carbothioyl]amino}propyl benzoate (21). 66% yield; a yellow solid; mp 62 °C; ^1H NMR ($CDCl_3$) δ 8.08 (m, 2H, Bz), 7.4–7.6 (m, 3H, Bz), 6.9–7.1 (m, 5H, Ph), 6.67 (d, 2H, Ar), 6.32 (t, 1H, NH), 4.69 (s, 2H, $ArCH_2N$), 4.57 (dd of AB, 1H, CH_2OBz), 4.0–4.15 (m, 2H, CH_2OBz and $C\underline{H}_2NHCSN$), 3.74 (t, 2H, $CH_2C\underline{H}_2N$), 3.53 (m, 1H, $C\underline{H}_2NHCSN$), 2.7–2.8 (m, 4H, $C\underline{H}_2CH_2NCSNH$ and CH_2Ph), 2.57 (m, 1H, CH), 2.21 (m, 6H, 2× CH_3); IR (KBr) 3391, 1523, 1276, 1101 cm^{-1}; Anal. calcd for $C_{29}H_{32}N_2O_4S$: C, 69.02; H, 6.39; N, 5.55; S, 6.35. Found: C, 69.25; H, 6.42; N, 5.53; S, 6.33.

2-(4-*tert*-butylbenzyl-3-{[(6,7-dihydroxy-3,4-dihydro-2(1H)-isoquinolinyl)carbothioyl]amino}propyl benzoate (22). 94% yield; a yellow solid; mp 59 °C; ^1H NMR ($CDCl_3$) δ 8.05 (d, 2H, Bz), 7.60 (t, 1H, Bz), 7.48 (t, 2H, Bz), 7.2–7.35 (m, 5H, Ph), 6.88 (bs, 1H, ArOH), 6.67 (s, 2H, Ar), 6.39 (t, 1H, NH), 4.75 (d, 2H, $ArCH_2N$), 4.57 (dd of AB, 1H, CH_2OBz), 4.11 (ddd, 2H, CH_2OBz and $C\underline{H}_2NHCSN$), 3.74 (t, 2H, $CH_2C\underline{H}_2N$), 3.48 (m, 1H, $C\underline{H}_2NHCSN$), 2.7–2.79 (m, 4H, $C\underline{H}_2CH_2NCSNH$ and CH_2Ph), 2.57 (m, 1H, CH), 1.29 (s, 9H, $C(CH_3)_3$); IR (KBr) 3390, 1523, 1275, 1100 cm^{-1}; Anal. calcd for $C_{31}H_{36}N_2O_4S$: C, 69.90; H, 6.81; N, 5.26; S, 6.02. Found: C, 70.12; H, 6.83; N, 5.24; S, 6.00.

2-(3,4-Dimethylbenzyl)-3-{[(6,7-dihydroxy-3,4-dihydro-2(1H)-isoquinolinyl)carbothioyl]amino} propyl pivalate (23). 61% yield; a yellow solid; mp 78 °C; ^1H NMR ($CDCl_3$) δ 6.9–7.5 (m, 3H, Ph), 6.63 (d, 2H, ArH), 6.29 (t, 1H, NH), 4.67 (s, 2H, $ArCH_2N$), 4.32 (dd of AB, 1H, CH_2OCO), 3.93 (m, 2H, CH_2OCO and $C\underline{H}_2NHCSN$), 3.67 (m, 2H, $CH_2C\underline{H}_2N$), 3.41 (m, 1H, $C\underline{H}_2NHCSN$), 2.55–2.7 (m, 4H, $C\underline{H}_2CH_2NCSNH$ and CH_2Ph), 2.41 (m, 1H, CH), 2.20 (m, 6H, 2× CH_3), 1.26 (s, 9H, $C(CH_3)_3$). IR (KBr) 3390, 1697, 1523, 1279 cm^{-1}; Anal. calcd for $C_{27}H_{36}N_2O_4S$: C, 66.91; H, 7.49; N, 5.78; S, 6.62. Found: C, 67.14; H, 7.46; N, 5.76; S, 6.60.

***N*-(2-Benzyl-3-benzyloxypropyl)-6,7-dihydroxy-3,4-dihydro-2(1H)-isoquinoline carbothioamide (24).** 80% yield; a yellow solid; mp 45 °C; ^1H NMR ($CDCl_3$) δ 7.17–7.33 (m, 10H, 2× Ph), 6.88 (m, 2H, ArOH), 6.56 (s, 1H, Ar), 6.42 (s, 1H, Ar), 4.54 (s, 2H, $PhCH_2O$), 4.41 (s, 2H, OCH_2CH), 3.97 (m, 1H, $C\underline{H}_2NCS$), 3.78 (m, 2H, $ArC\underline{H}_2CH_2$), 3.65 (t, 2H, $C\underline{H}_2C\underline{H}_2N$), 2.67 (t, 3H, CH_2Ph), 2.37 (m, 3 H, $C\underline{H}_2CH_2\underline{N}CS$ and CH); IR (KBr) 3349, 1521, 1372, 1278 cm^{-1}; Anal. calcd for $C_{27}H_{30}N_2O_3S$: C, 70.10; H, 6.54; N, 6.06; S, 6.93. Found: C, 70.31; H, 6.56; N, 6.04; S, 6.91.

2-Benzyl-3-({[(3,4-dihydroxybenzyl)amino]carbothionyl}-amino)propyl benzoate (25). 81% yield; a yellow solid; mp 30 °C; ^1H NMR ($CDCl_3$) δ 8.02 (d, 2H, Ar), 7.60 (t, 1H, Ar), 7.47 (t, 2H, Ar), 7.1–7.3 (m, 5H, Ph), 6.84 (m, 2H, Ar), 6.67 (m, 1H, Ar), 6.36 (m, 2H, NH), 4.36 (m, 2H, $ArCH_2N$ and CH_2OCO), 4.15 (m, 2H, CH_2OCO and $C\underline{H}_2NHCSN$), 3.48 (m, 1H, $C\underline{H}_2NHCSN$), 2.69 (m, 2H, $C\underline{H}_2Ph$), 2.43 (m, 1H, CH); IR (KBr) 3363, 1602, 1556, 1280, 1113 cm^{-1}; Anal. calcd for $C_{25}H_{26}N_2O_4S$: C, 66.64; H, 5.82; N, 6.22; S, 7.12. Found: C, 66.83; H, 5.84; N, 6.20; S, 7.10.

Protocol of ^{45}Ca uptake by Tet-off VR1 expressing CHO cells

Cells (CHO-VR1 Tet-off cells)[22] were plated in 24-well plates to yield a cell density 20–40% of confluent. The next day, the medium was changed to remove the tetracycline and induce VR1 expression. Experiments were done approximately 36–40 h after induction. For assay of ^{45}Ca uptake, cells were incubated for 10 min at 37 °C in a total volume of 500 :L of serum free DMEM (containing 1.8 mM $CaCl_2$) in the presence of 0.25 mg/mL BSA (Sigma), 1 :Ci/mL ^{45}Ca (5–30 Ci/g from ICN, CA), and increasing concentrations of the compound to be tested. Immediately after the incubation, extracellular ^{45}Ca was removed by washing the cells three times with cold DPBS (containing 1.8 mM $CaCl_2$). Then 400 :L RIPA buffer (50 mM Tris pH 7.4; 150 mM NaCl; 1% Triton X-100; 0.1% SDS; 1% sodium deoxycholate) was added to each well in order to lyse the cells. Plates were shaken slowly for 20 min; then 300 :L of cell lysate was transferred from each well into a scintillation vial and radioactivity was determined by scintillation counting. For each data point in each experiment, four wells were assayed. Data from these experiments was analyzed by computer fit to the Hill equation. At least three separate experiments were carried out for each compound.

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References and Notes

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