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Synthesis of *N*,*N*′,*N*″-Trisubstituted Thiourea Derivatives and Their Antagonist Effect on the Vanilloid Receptor

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Abstract—Twenty-seven *N*,*N*′,*N*″-trisubstituted thiourea derivatives were prepared. Among them, 1-[3-(4′-hydroxy-3′-methoxy-phenyl)-propyl]-1,3-diphenethyl-thiourea (**8**l, $IC_{50} = 0.32\,\mu M$), showed 2-fold higher antagonistic activity than that of capsazepine (**3**, $IC_{50} = 0.65\,\mu M$) against the vanilloid receptor in a $^{45}Ca^{2+}$-influx assay.

Capsaicin (**1**), the pungent component of chili pepper, opens a novel cation selective ion channel in the plasma membrane of peripheral sensory neurons via binding to the vanilloid receptor (VR1), which is a polymodal nociceptor.[1,2] Resiniferatoxin (RTX, **2**), isolated from the cactus-like succulent *Euphobia resinifera*, shows thousands-fold greater agonistic activity than that of capsaicin.[3] These agonists induce pain upon topical application in the early stage, which is followed by a period of desensitization.[4] The desensitization led to the consideration of the agonists as potential analgesic agents. Through extensive structure–activity relationship studies,[5,6] several potent synthetic agonists were introduced, such as SDZ-249-482,[5e] KR-25018.[6a] However, their initial irritation[7] makes them less appealing as orally active analgesics. Due to the unavoidable initial excitatory side effect of the agonists, competitive antagonists have been pursued as novel analgesic agents. Only a few antagonists have been reported so far. The first competitive antagonist, capsazepine (**3**, $IC_{50} = 0.65\,\mu M$), was prepared by introducing a saturated seven-membered rigid ring system, which maintains a virtually orthogonal conformation between each *N*-substituent in the thiourea structure.[8] 5-Iodo-RTX,[9] prepared from RTX by semi-synthesis, showed the most potent antagonistic activity (IC_{50}, 3.9 nM), but there is a limitation in the RTX supply from the natural source. Tetetrahydrobenzazepine and tetrahydroisoquinoline thiourea derivatives have been prepared as antagonists by the replacement of the *p*-chlorophenethyl group with 3-acyloxy-2-benzylpropyl groups.[6d] We also recently reported the five-membered pyrrolidine derivative (**4**, $IC_{50} = 3.0\,\mu M$) as a moderate antagonist by the modification of capsazepine.[10] As part of our program to develop new efficient analgesic agents, we continued the structure–activity relationship studies on **4** by introducing further modifications. In this communication, we report the synthesis of *N*,*N*′,*N*″-trisubstituted thioureas and their potent antagonistic effects against VR1.

Our previous studies revealed that the orthogonal conformation of capsazepine (**3**) is not as critical as the lengths of the *N*(2)-substituents, which could play more important roles in the antagonistic activity.[10] Based on these results, we designed the ring-opened thiourea **5**, with variation in the lengths of the two *N*(2)-substituents. Our rationale was that the increased flexibility

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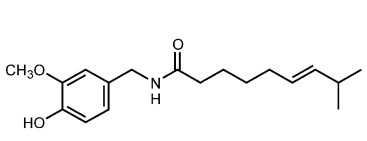
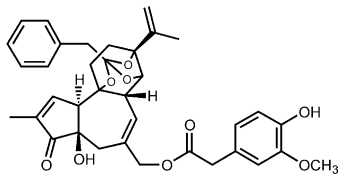


Capsaicin, **1**



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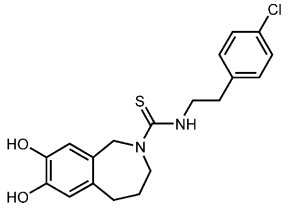
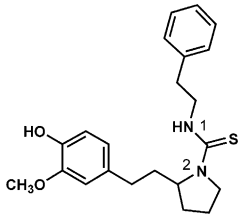
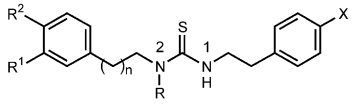


Capsazepine, **3**



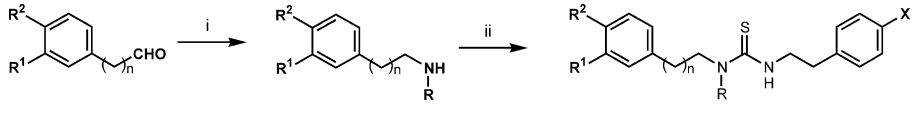
4



n = 0 - 3

5

of the ring-opened thiourea **5** might enhance the affinity for VR1 receptor. As shown in Scheme 1, a series of *N*,*N*′,*N*″-trisubstituted thiourea derivatives (**8a**–**a**′)[11] were prepared from **6** via reductive amination with the corresponding amines followed by reaction with the appropriate isothiocynates in 75–95% yield (two steps).[12] The biological activities of the prepared compounds were evaluated as both agonists and antagonists in the $^{45}Ca^{2+}$-influx assay, by using neonatal rat cultured spinal sensory neurons (Tables 1–3).[13] As shown in Table 1, most of the ring-opened thiourea derivatives showed comparable or higher antagonistic activities than **4**. The optimal length of the carbon chain between *N*(2) and the vanilloid (4′-methoxy-3′-hydroxyphenyl) ring was C_3 (C_1, **8a**, NE at 30 μM; C_2, **8b**, NE at 30 μM; C_3, **8l**, $IC_{50} = 0.32$ μM; C_4, **8o**, $IC_{50} = 6.1$ μM). Depending on the R group, quite wide variations in the activity were observed in the series of *N*(2)-(4′-hydroxy-3′-methoxyphenyl) propyl derivatives (**8c**–**n**). When the bulkiness of R was increased from H (**8c**, NE at 30 μM) to *n*-butyl (**8g**, $IC_{50} = 10$ μM), the activities changed from low-level agonists to modest antagonists, but the longer hydrocarbon chain (**8h**, $IC_{50} > 30$ μM) dramatically decreased the antagonistic activity. The α-branched alkyl or benzyl derivatives (**8i**, **8j**, **8n**) showed relatively higher antagonistic activities than those of the non-branched derivatives (**8e**, **8g**, **8k**), respectively. These findings suggest that the bulky R groups could assume more preferable molecular conformations for the antagonistic binding, but the size is limited for efficient binding. Interestingly, most R groups involving a phenyl ring provided quite high activities, implying that there might be an extra binding affinity, such as π–π stacking or a hydrophobic interaction, between the phenyl ring in R and the aromatic or hydrophobic amino acid residues in the receptor (**8k**–**n**). The best result was obtained with the phenethyl derivative, **8l** ($IC_{50} = 0.32$ μM), which had 10-fold higher potency as compared with the compound, **4** ($IC_{50} = 3.0$ μM). Table 2 clearly shows that the vanilloid group is very important for the antagonistic activity, in accordance with the previous results.[10] The electronic effects on the phenyl ring in each of the *N*(1) and *N*(2) phenethyl groups against the antagonistic activities are shown in Table 3 (**8u**–**a**′). There was no significant difference in the *N*(2)-phenethyl groups (**8x**–**8a**′) except for the *p*-fluoro derivative (**8w**); however introduction of the *p*-Cl (**8u**) and *p*-F (**8v**) groups on the phenyl ring of the *N*(1)-phenethyl group reduced their antagonistic activities.

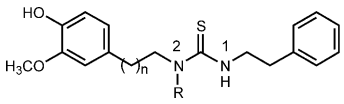


n = 0-3 n = 0-3 n = 0-3

6 **7** **8**

Scheme 1. Reactions and conditions: (i) RNH_2, 10% Pd/C, H_2, MeOH, rt; (ii) 4-X-$PhCH_2CH_2NCS$, CH_2Cl_2, rt.

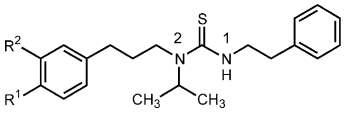
Table 1. $^{45}Ca^{2+}$-influx activity of the *N*,*N*′,*N*″-trisubstituted thiourea derivatives



No.	*n*	R	$^{45}Ca^{2+}$-influx activity (μM)[a]	
			Agonist (EC_{50})	Antagonist (IC_{50})
8a	0	$PhCH_2CH_2$	NE[b]	NE
8b	1	$PhCH_2CH_2$	NE	NE
8c	2	H	30 <[c]	NE
8d	2	CH_3	30 <	NE
8e	2	CH_3CH_2	NE	30 <
8f	2	$CH_3CH_2CH_2$	NE	12
8g	2	$CH_3(CH_2)_2CH_2$	NE	10
8h	2	$CH_3(CH_2)_6CH_2$	NE	30 <
8i	2	$(CH_3)_2CH$	NE	2.5
8j	2	C_6H_{11}	NE	3.0
8k	2	$PhCH_2$	NE	0.95
8l	2	$PhCH_2CH_2$	NE	0.32
8m	2	$PhCH_2CH_2CH_2$	NE	3.2
8n	2	$(Ph)_2CH$	NE	0.46
8o	3	$PhCH_2CH_2$	NE	6.1

[a]EC_{50} (the concentration of test compound necessary to produce 50% of the maximal response) and IC_{50} values (the concentration of test compound necessary to reduce the response to 0.5 μM capsaicin by 50%) were estimated with at least three replicates at each concentration. Each compound was tested in two independent experiments. Antagonist data were fitted with a sigmoidal function.
[b]NE, not effective at 30 μM.
[c]Only partial activity was observed at 30 μM.

Table 2. $^{45}Ca^{2+}$-influx activity of the *N*,*N*′,*N*″-trisubstituted thiourea derivatives

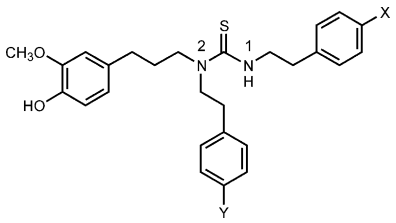


No.	R^1	R^2	$^{45}Ca^{2+}$-influx activity (μM)[a]	
			Agonist (EC_{50})	Antagonist (IC_{50})
8i	OH	OCH_3	NE[b]	2.5
8p	OH	OH	NE	9.3
8q	OCH_3	OCH_3	NE	10.5
8r	OH	H	NE	30 <[c]
8s	H	OCH_3	NE	NE
8t	H	H	NE	NE

[a]EC_{50} and IC_{50} values were estimated by the same method described in Table 1.
[b]NE, not effective at 30 μM.
[c]Only partial activity was observed at 30 μM.

In conclusion, we prepared 27 *N*,*N*′,*N*″-trisubstituted thiourea derivatives by the modification of **4**. The $^{45}Ca^{2+}$-influx assay provided that the 1-[3-(4′-hydroxy-3′-methoxy-phenyl)-propyl]-1,3-diphenethyl-thiourea, **8l** showed the highest activity among the prepared compounds, which is 2-fold higher than that of capsazepine. The cumulative findings from the structure–activity relationship studies, reveal that the acyclic *N*,*N*′,*N*″-trisubstituted thiourea would be a useful scaffold for potential analgesics. Further analyses of the structure–activity relationship are now under way, including replacement of the phenyl ring with other aromatic or heterocyclic ring systems.

Table 3. $^{45}Ca^{2+}$-influx activity of the *N*,*N*′,*N*″-trisubstituted thiourea derivatives



No.	X	Y	$^{45}Ca^{2+}$-influx activity (μM)[a]	
			Agonist (EC_{50})	Antagonist (IC_{50})
8l	H	H	NE[b]	0.32
8u	F	H	NE	5.1
8v	Cl	H	NE	2.4
8w	H	F	NE	1.2
8x	H	Cl	NE	0.55
8y	H	CH_3	NE	0.95
8z	H	OCH_3	NE	0.72
8a′	H	NO_2	NE	0.50

[a]EC_{50} and IC_{50} values were estimated by the same method described in Table 1.
[b]NE, not effective at 30 μM.

Acknowledgements

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11. All compounds gave satisfactory spectroscopic data consistent with the proposed structures.

12. Representative procedure for the synthesis of **8l**: A methanolic mixture (3 mL) of 3-(3′-methoxy-4′-hydroxyphenyl) propionaldehyde (100 mg, 0.55 mmol), phenethylamine (67 mg, 0.55 mmol), and a catalytic amount of 10%-Pd/C was stirred under an atmosphere of H_2 at room temperature (3 h). The methanol solvent was removed in vacuo. Without further purification, the residue was adapted to the following coupling reaction. To a methylenechloride solution (3 mL) of the residue was added phenethylisothiocynate (98 mg, 0.60 mmol) at 0 °C, and the reaction solution was stirred at room temperature (3 h). The solvent was removed in vacuo and the residue was purified by column chromatography (SiO_2, EtOAc/*n*-hexane = 1:3) to afford **8l** as white solid (216 mg, 87%); mp 107.7 °C. ^{1}H NMR ($CDCl_3$, 300 MHz), δ 7.17 (m, 10H), 6.79 (m, 1H), 6.55 (m, 2H), 5.50 (s, 1H), 5.05 (s, 1H), 3.81 (m, 5H), 3.68 (t, 2H, J = 7.43 Hz), 3.30 (t, 2H, J = 7.55 Hz), 2.80 (m, 4H), 2.43 (t, 2H, J = 7.43 Hz), 1.76 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz), δ 180.82, 146.48, 143.94, 138.85, 138.54, 132.58, 128.76, 128.71, 128.69, 128.62, 126.59, 126.53, 120.66, 114.34, 110.88, 55.89, 53.20, 50.10, 46.64, 34.96, 33.73, 32.41, 28.50. IR (KBr) 3397, 2934, 1604, 1618, 1518, 1452 cm^{-1}. MS (EI) *m*/*e*, 448 [M^+]. Anal. calcd for $C_{27}H_{32}N_2O_2S$: C, 72.29; H, 7.19; N, 6.24. Found: C, 72.05; H, 7.32; N, 6.15.

13. The uptake and the accumulation of $^{45}Ca^{2+}$ by the *N*,*N*′,*N*″-trisubstituted thiourea derivatives were studied in neonatal rat cultured spinal sensory neurons according to the described method in detail in ref 4b.