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Bioorganic & Medicinal Chemistry Letters 13 (2003) 197–200

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# Synthesis of 2-Substituted-Pyrrolidinethiourea Derivatives and Their Antagonist Effect on Vanilloid Receptor

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Received 29 August 2002; accepted 15 October 2002

**Abstract**—Four pyrrolidine derivatives were prepared by the formation of a 5-membered ring based on capsazepine. Among them, the two carbon extended derivatives, **4a** ($IC_{50}=55\,\mu M$) and **4b** ($IC_{50}=3\,\mu M$), both showed different levels of antagonist activity against the vanilloid receptor in a $^{45}Ca^{2+}$-influx assay.


Capsaicin (**1**), the pungent component of chili pepper, activates the vanilloid receptor (VR1) which is a polymodal nociceptor.[1] The activation opens a novel cation selective ion channel in the plasma membrane of peripheral sensory neurons.[2] Capsaicin induces pain upon topical application in the early stage, which is followed by a period of desensitization.[3] Since this agent became a target for the design of a novel class of analgesics, its structure and activity relationships have been studied by modifications of capsaicin.[4,5] However, the potent synthetic agonists (SDZ-249-482,[4e] KR-25018[5a]) could not be developed as orally active analgesics, because of their initial irritancy.[6] Since it has been impossible to remove the initial excitatory side effect in the agonist, a competitive antagonist has been pursued as a novel pharmacological agent for analgesics, rather than an agonist.

In 1994, the first competitive antagonist, capsazepine (**2**), was reported by introducing a saturated 7-membered rigid ring system.[7] The rationale for the major difference between the agonist and the antagonist is in the structural relationship between the catechol or vaniloid aromatic ring (A region) and the amide bond (B region). In the antagonist structure, the A and B regions are virtually orthogonal, in contrast to the agonist structure, in which they are approximately coplanar.[7] This conformational difference was supported by an X-ray crystallographic analysis as well as a molecular modeling technique. Recently, tetrahydrobenzazepine and tetrahydroisoquinoline thiourea derivatives have been prepared as antagonists by the replacement of the *p*-chlorophenethyl group with 3-acetoxy-2-benzylpropyl groups.[5d] As part of our program to find a new scaffold for a competitive antagonist against the capsaicin receptor, we modified capsazepine by introducing a 5-membered pyrrolidine ring (**3**, **4**), which has a similar conformation based on molecular modeling. In this communication, we report the synthesis of 2-substituted-pyrrolidinethiourea derivatives and their biological activities. The molecular modeling studies proposed the pyrrolidine derivatives (**3**) as target molecules with an orthogonal conformation between the A and B regions. The energy-minimized conformation of **3a** was very similar to that of capsazepine, as shown in Figure 1. Both structures showed the *E*,*E*-thiourea-π–π-stacking form (**3a**: 5.91 kcal/mol; **2**: 4.24 kcal/mol) rather than the *E*,*E*-thiourea-π–π-nonstacking form (**3a**: 8.86 kcal/mol; **2**: 6.68 kcal/mol) because of the stabilizing effect induced by the π–π stacking interaction of the two corresponding benzene moieties.[8]

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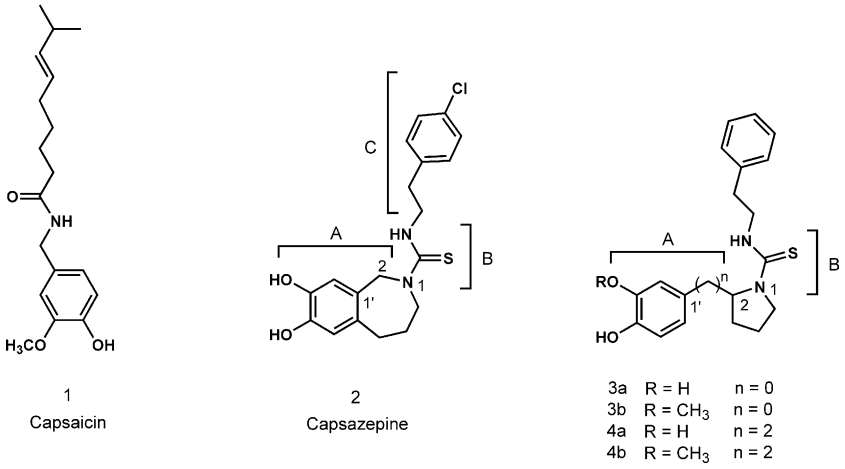
PII: S0960-894X(02)00887-9



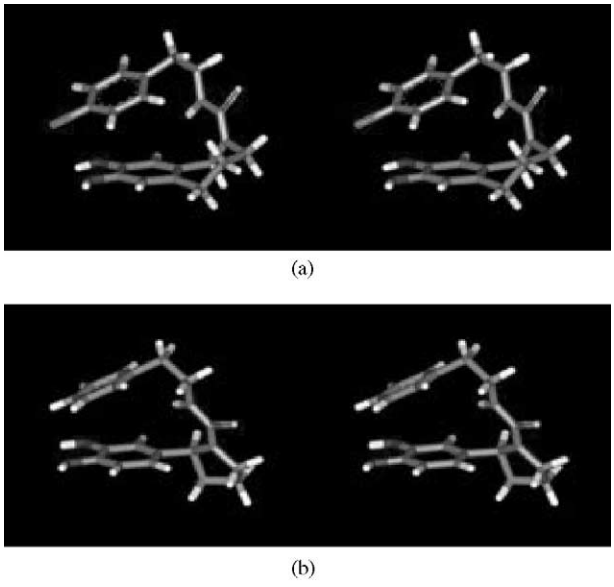
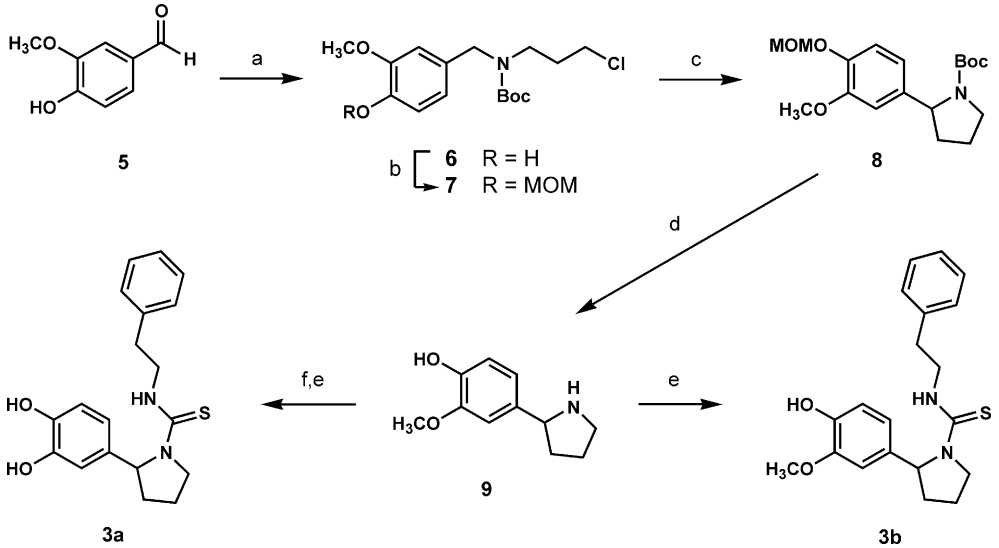



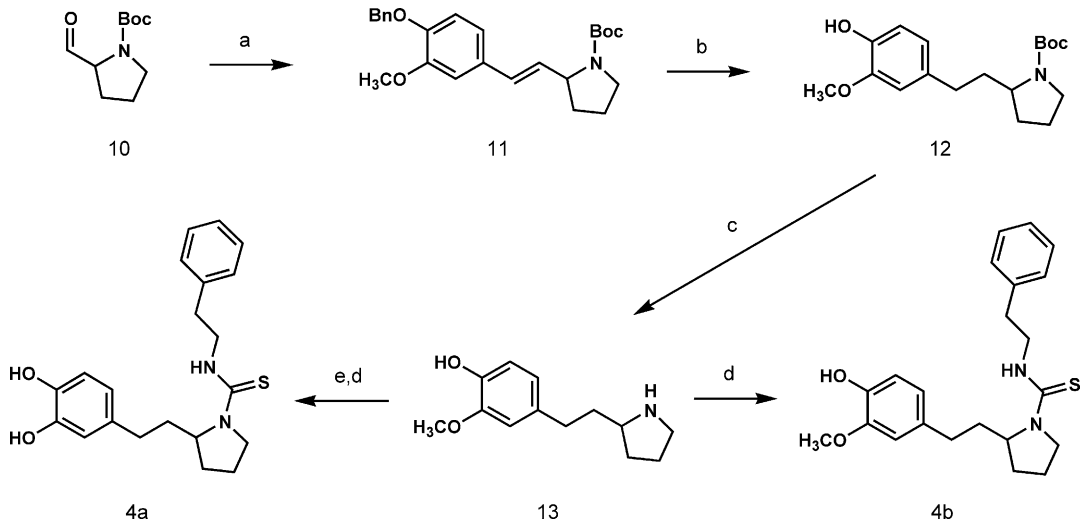

**Figure 1.** Stereopair modeling representation of the preferred three-dimensional conformation. (a) Energy-minimized conformation of **2**; (b) energy-minimized conformation of **3a**.

Compounds **3a** and **3b** were prepared in 5 and 6 steps, respectively, from vanillin, as shown in Scheme 1. Reductive amination of vanillin with 3-chloropropylamine, using 10% Pd/C under atmospheric $H_2$, followed by sequential *N*-butyloxycarbonyl (Boc) protection and *O*-methoxymethyl (MOM) protection, gave **7**. The pyrrolidine ring system (**8**) was constructed by intramolecular cyclization of **7** under basic conditions.[9] The deprotection of the Boc and MOM groups with trifluoroacetic acid gave **9**, which was coupled with phenethylisothiocyanate to provide **3b**. Compound **3a** also could be prepared by demethylation of **9**, using c-HBr and the same coupling reaction as above.

The biological activities of **3a** and **3b** were evaluated as both agonists and antagonists in the $^{45}Ca^{2+}$-influx assay by using the neonantal rat cultured spinal sensory neurons.[10] Unexpectedly, none of derivatives showed both agonist and antagonist activity, in spite of their similar conformations to capsazepine based on the molecular modeling (Table 1). A possible explanation may come from the unfavorable rotation of C(1′)–C(2) of **3**, which may affect the binding interaction of the catechol or

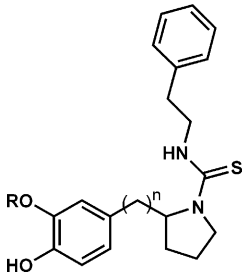



**Scheme 1.** Reaction and conditions: (a) 3-chloropropylamine, 10% Pd/C, $H_2$, MeOH, rt; $Boc_2O$, $CH_2Cl_2$, TEA, rt, 95%, (b) MOMCl, $K_2CO_3$, DMF, 50 °C, 93%, (c) *n*-BuLi, THF, −78–0 °C, 84%, (d) TFA, $CH_2Cl_2$, rt, 95%, (e) phenethylisothiocyanate, $CH_2Cl_2$, rt, 85%, (f) c-HBr, 100 °C, 57%.




**Scheme 2.** Reaction and conditions: (a) 4′-benzyoxy-3′-methoxybenzyltriphenylphosphoniumbromide, THF, *n*-BuLi, 0 °C, 85%, (b) 10% Pd/C, $H_2$, $CH_3OH$, rt, 95%, (c) TFA, $CH_2Cl_2$, rt, 95%, (d) phenethylisothiocynate, $CH_2Cl_2$, rt, 82%, (e) c-HBr, 100 °C, 67%.

**Table 1.** $^{45}Ca^{2+}$-influx activity of the pyrrolidine derivatives




| No. | R | n | $^{45}Ca^{2+}$ influx activity (μM)[a] | |
|---|---|---|---|---|
| | | | Agonist ($EC_{50}$) | Antagonist ($IC_{50}$) |
| **3a** | H | 0 | >100 | >100 |
| **3b** | $CH_3$ | 0 | >100 | >100 |
| **4a** | H | 2 | >100 | 55 |
| **4b** | $CH_3$ | 2 | >100 | 3 |
| **2** | — | — | >100 | 0.6 |

[a]$EC_{50}$ (the concentration of derivatives necessary to produce 50% of the maximal response) and $IC_{50}$ values (the concentration of derivatives 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 sigmodial function.

vanilloid moiety of **3** with the receptor. We extended two carbons between C(1′)–C(2) of **3**, which could give sufficient flexibility. The preparation of **4a** and **4b** is shown in Scheme 2. Wittig coupling with *N*-Boc-2-formypyrrolidine **10** and 3′-methoxy-4′-benzyloxybenzyltriphenylphosphoniumbromide, followed by hydrogenation, gave **12**. The deprotection of the Boc group with trifluoroacetic acid, followed by coupling with phenethylisothiocyanate, provided **4b**. Demethylation of **13** with c-HBr followed by treatment with phenethylisothiocyanate gave **4a**. As shown in Table 1, the $^{45}Ca^{2+}$-influx assay revealed that both **4a** and **4b** show antagonist activity and the vanilloid derivative, **4b** ($IC_{50} = 3\,\mu M$), is more active than the catechol derivative, **4a** ($IC_{50} = 55\,\mu M$). Interestingly, the difference in potency between the vanilloid derivative (**4b**) and the catechol derivative (**4a**) is congruent with the reported general tendency in agonists, but opposite to that of capsazepine and its vanilloid derivative.[11] These cumulative findings may suggest that the binding site of the vanilloid or catechol moiety in **4** may same as that of the general agonists, such as capsaicin.

In conclusion, four pyrrolidine derivatives[12] were prepared by the formation of a 5-membered ring based on capsazepine. Even though the energy minimized molecular conformations of **3** are very similar to that of capsazepine, they show no biological activities. However, the two carbons extended derivatives, **4a** and **4b**, both show antagonist effects at different levels. This result suggests that the orthogonal conformation may not so critical for the antagonist activity but the variation in the length of the ligand could play more important role for the design of new scaffold as potent antagonist. The studies on the vanilloid type acyclic antagonist are currently being investigated.

## Acknowledgements

This research was supported by a grant from Amore Pacific Co. Ltd., Korea, and a grant of the Korea Health 21 R+D Project, Ministry of Health and Welfare, Republic of Korea: O2-PJ2-PG4-PT01-0014.

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8. The calculations were done using the program SYBYL 6.5 from Tripos Software Inc., St. Louis, MO, USA.

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10. The uptake and accumulation of $^{45}Ca^{2+}$ by the pyrrolidine derivatives was studied in neonatal rat cultured spinal sensory neurons by the method described in detailed by the reference, **3b**.

11. See the reference 7 and 5d *N*-(3,4-Dihydroxybenzyl)-*N*′-[(4-chlorophenyl)ethyl]thiourea ($EC_{50}$, 0.10 μM), *N*-(4-Hydroxy-3-methoxybenzyl)-*N*′-[(4-chlorophenyl)ethyl]thiourea ($EC_{50}$, 0.06 μM); Capsazepine ($IC_{50} = 0.56$ μM), the vanilloid derivative of Capsazepine ($IC_{50} = 0.97$ μM).

12. All compounds gave satisfactory spectroscopic data consistent with the proposed structures. Data for **3a**: mp 197 °C. $^1$H NMR ($CDCl_3$, 300 MHz), δ 6.98 (m, 8H), 4.60 (m, 1H), 3,99 (m, 2H), 3.85 (m, 2H), 2.72 (m, 2H), 2.40 (m, 1H), 1.87 (m, 3H). IR (KBr) 3384, 2924, 1644, 1618 $cm^{-1}$. MS (EI) *m*/*e*, 342 [$M^+$]. Anal. calcd for $C_{19}H_{22}N_2O_2S$: C, 66.64; H, 6.48; N, 8.18. Found: C, 66.59; H, 6.42; N, 8.09. **3b**: mp 148 °C. $^1$H NMR($CDCl_3$, 300 MHz), δ 7.06 (m, 5H), 6.65 (m, 3H), 4.61 (s, 1H), 4.01 (d, 2H, $J = 7.14$ Hz), 3.81 (m, 2H), 3.82 (s, 3H), 2.72 (m, 2H), 2.38 (m, 1H), 1.90 (m, 3H). IR (KBr) 3390, 2938, 1602, 1516 $cm^{-1}$. MS (EI) *m*/*e*, 356 [$M^+$]. Anal. calcd for $C_{20}H_{24}N_2O_2S$: C, 67.38; H, 6.79; N, 7.86. Found: C, 66.75; H, 6.70; N, 7.65. **4a**: mp 158 °C. $^1$H NMR ($CDCl_3$, 300 MHz) δ 7.12 (m, 5H), 6.56 (m, 2H), 6.39 (d, 1H, $J = 8.04$ Hz), 3.67 (m, 3H), 3.58 (m, 1H), 3.21 (t, 1H, $J = 1.58$ Hz), 2.76 (m, 2H), 2.36 (t, 2H, $J = 7.32$ Hz), 1.87 (m, 6H), 1.50 (m, 1H). IR (KBr) 3399, 2920, 1604, 1531, 1455 $cm^{-1}$. MS (EI) *m*/*e*, 370 [$M^+$]. Anal. calcd for $C_{21}H_{26}N_2O_2S$: C, 68.08; H, 7.07; N, 7.56. Found: C, 67.89; H, 7.17; N, 7.05. **4b**: mp 121 °C. $^1$H NMR ($CDCl_3$, 300 MHz) 7.18 (m, 5H), 6.75 (d, 1H, $J = 8.07$ Hz), 6.64 (s, 1H), 6.50 (d, 1H, $J = 7.86$ Hz), 3.97 (m, 7H), 3.52, (m, 1H), 2.81 (d, 2H, $J = 3.84$ Hz), 2.48 (m, 2H), 1.93 (m, 6H). IR (KBr) 3397, 2920, 1516, 1456, 1384 $cm^{-1}$. MS (EI) m/e, 384. Anal. calcd for $C_{22}H_{28}N_2O_2S$: C, 68.72; H, 7.34; N, 7.29. Found: C, 68.48; H, 7.40; N, 7.05.