ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/262054122

α-Methylated simplified resiniferatoxin (sRTX) thiourea analogues as potent and stereospecific TRPV1 antagonists

ARTICLE *in* BIOORGANIC & MEDICINAL CHEMISTRY LETTERS · APRIL 2014

Impact Factor: 2.42 · DOI: 10.1016/j.bmcl.2014.04.054 · Source: PubMed

CITATIONS
3

READS
34

12 AUTHORS, INCLUDING:

Tran Phuong-Thao
Seoul National University
7 PUBLICATIONS **13** CITATIONS
SEE PROFILE

Van-Hai Hoang
Seoul National University
7 PUBLICATIONS **31** CITATIONS
SEE PROFILE

Jihyae Ann
Seoul National University
13 PUBLICATIONS **25** CITATIONS
SEE PROFILE

Available from: Tran Phuong-Thao
Retrieved on: 13 January 2016

Bioorganic & Medicinal Chemistry Letters 24 (2014) 2685–2688

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

α-Methylated simplified resiniferatoxin (sRTX) thiourea analogues as potent and stereospecific TRPV1 antagonists

Ho Shin Kim [a], Mi-Kyoung Jin [a], Sang-Uk Kang [a], Ju-Ok Lim [a], Phuong-Thao Tran [a], Van-Hai Hoang [a], Jihyae Ann [a], Tae-Hwan Ha [a], Larry V. Pearce [b], Vladimir A. Pavlyukovets [b], Peter M. Blumberg [b], Jeewoo Lee [a,*]

[a] Laboratory of Medicinal Chemistry, Research Institute of Pharmaceutical Sciences, College of Pharmacy, Seoul National University, Seoul 151-742, Republic of Korea
[b] Laboratory of Cancer Biology and Genetics, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, MD 20892, USA

ARTICLE INFO

Article history:
Received 21 March 2014
Revised 10 April 2014
Accepted 12 April 2014
Available online 23 April 2014

Keywords:
Vanilloid receptor 1
TRPV1 antagonist
Capsaicin
Resiniferatoxin

ABSTRACT

A series of α-methylated analogues of the potent sRTX thiourea antagonists were investigated as rTRPV1 ligands in order to examine the effect of α-methylation on receptor activity. The SAR analysis indicated that activity was stereospecific with the (*R*)-configuration of the newly formed chiral center providing high binding affinity and potent antagonism while the configuration of the C-region was not significant.

© 2014 Elsevier Ltd. All rights reserved.

The vanilloid receptor TRPV1 has emerged as an exciting therapeutic target for a broad range on conditions, reflecting the fundamental role of the C-fiber sensory afferent neurons in which they are expressed.[1] While TRPV1 was initially defined as the site of action of naturally occurring agonists such as capsaicin (CAP) or the ultrapotent resiniferatoxin (RTX), a key advance was the finding that TRPV1 antagonism could be achieved with appropriate ligands, building on the developing insights in vanilloid structure activity relations.[2,3] These initial findings have fueled vigorous efforts in medicinal chemistry and in structural understanding of vanilloid–TRPV1 interactions.[4–12] Because of the great potency at TRPV1 of RTX compared to CAP, one approach we have taken has been to try to retain such enhanced potency of RTX upon structural simplification.

Structurally, both CAP and RTX have a vanilloid moiety (A-region) that is connected through either ester or amide linkages (B-region) to a long alkyl chain or diterpene moiety (C-region), respectively (Fig. 1).

The synthetic surrogates 1 and 2 were designed to mimic the principal pharmacophores of capsaicin and resiniferatoxin, respectively, and proved to be potent agonists in the rat and human TRPV1/CHO systems.[13] The 3-pivaloyloxy-2-benzylpropyl group, which represents the C-region of 2, was derived from the key

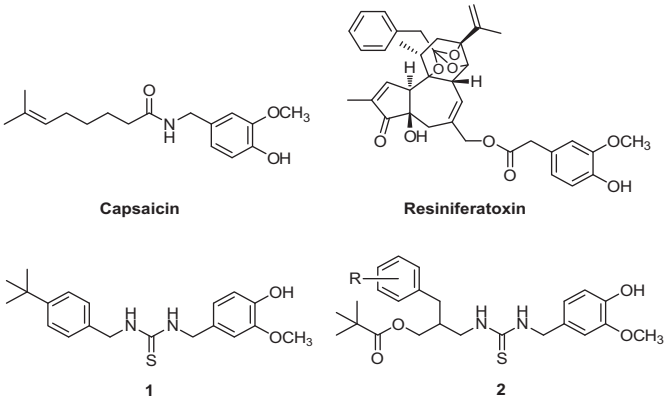


Figure 1. Natural and synthetic TRPV1 agonists.

pharmacophores of the diterpene in RTX in an effort to capture the markedly higher binding affinity to TRPV1 of RTX relative to that of capsaicin.

We have previously reported that isosteric replacement of the phenolic hydroxyl group in the A-region of the potent agonists 1 and 2 with the methylsulfonamido group provided the potent antagonists 3 and 5, respectively, which inhibited the activation by capsaicin of rat and human TRPV1 expressed in CHO cells

* Corresponding author. Tel.: +82 2 880 7846; fax: +82 2 888 0649.
E-mail address: jeewoo@snu.ac.kr (J. Lee).

http://dx.doi.org/10.1016/j.bmcl.2014.04.054
0960-894X/© 2014 Elsevier Ltd. All rights reserved.

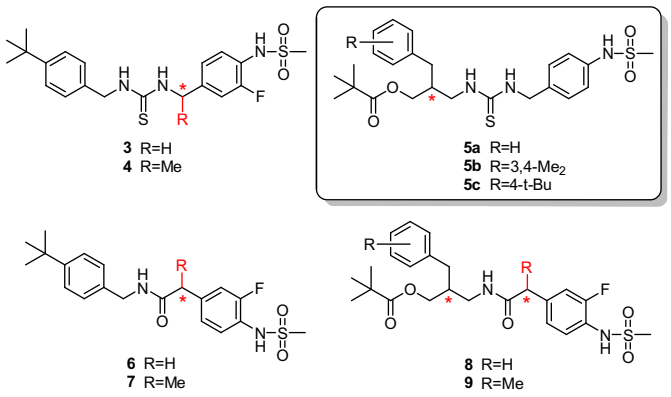
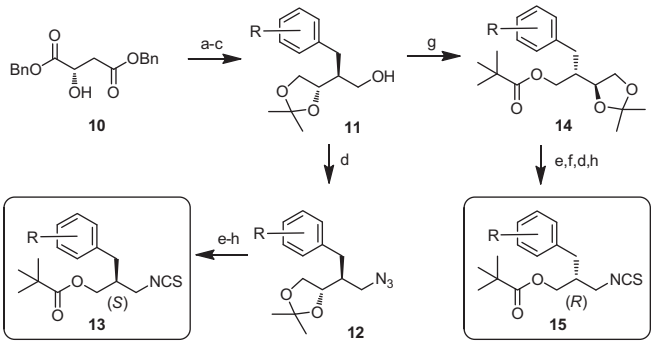


Figure 2. Lead TRPV1 antagonists.

(Fig. 2).[14,15] The SAR investigation of the B-region of 3 indicated that compound 4, the α-methyl substituted analogue of 3, showed similar potency to that of 3 but displayed enhanced binding to the receptor with stereospecificity for the (*R*)-configuration.[16]

Impressively, in the amide B-region surrogates **6** and **8**, α-methylation led to greater improvement in receptor potency and specificity (Fig. 2). Although compound **6** displayed moderate binding affinity and antagonism, its α-methylated analogue **7** showed an approximately 10-fold increase in receptor potency.[17] Of its two chiral analogues, the (*S*)-configuration showed approximately a further two-fold enhancement in receptor activity compared to the corresponding racemate, whereas the (*R*)-configuration provided weak antagonism. In addition, the α-methylation of the simplified RTX (sRTX) amide antagonist **8** provided compound **9** as a diastereomeric mixture, which also showed a dramatic increase in receptor potency compared to **8**.[18] The receptor activities of the four different isomers of **9** indicated that the (*S*)-configuration of the α-methyl group displayed high potency irrespective of the



Scheme 1. Synthesis of chiral C-region. Reagents and conditions: (a) LHMDS, $ArCH_2Br$, HMPA, THF, 55–68%; (b) $LiAlH_4$, THF, 55–60%; (c) pTsOH, acetone, 76–82%; (d) PPh_3, DPPA, DEAD, THF, 88–90%; (e) H_5IO_6, ether, 98%; (f) $NaBH_4$, MeOH, 72–89%; (g) Me_3CCOCl, pyridine, 80–82%; (h) CS_2, PPh_3, THF, reflux, 80–90%.

chirality of the C-region. Modeling analysis suggested that the α-methyl in the propanamide B-region constituted an additional pharmacophore group which interacted with a small hydrophobic pocket on the receptor.[18]

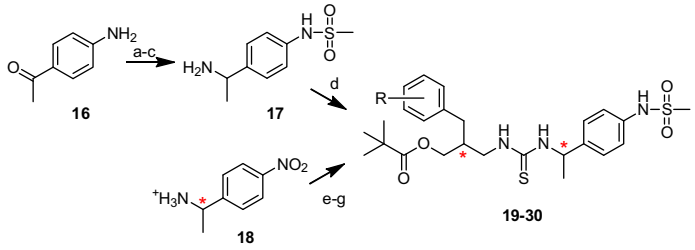
As part of our continuing effort to examine the effect of α-methylation on potent TRPV1 ligands, we investigated the α-methylated analogues of simplified RTX thiourea antagonist **5a–c** and its phenylalanol analogues. In this paper, we described the efficient syntheses of the racemic/chiral α-methylated thiourea derivatives, their receptor activities and SAR analysis.

The synthesis of the chiral C-region of the target compounds was accomplished by Seebach's diastereoselective alkylation of the α-alkoxide enolate as a key reaction (Scheme 1). Anti-selective alkylations of dibenzyl L-malate **10** with substituted benzyl bromides afforded 3*R*-benzyl-2*S*-hydroxysuccinates, which were reduced and then protected to provide the diverging intermediates **11**. For the synthesis of the (*S*)-isomeric isothiocyanate **13**, the hydroxyl group of **11** was converted into the corresponding azide **12** and then its acetonide was transformed into the 3-pivaloyloxy group in three steps. For the synthesis of the chiral (*R*)-isomeric isothiocyanate **15**, the hydroxyl group of **11** was pivaloylated to afford **14** and then its acetonide was transformed into the corresponding isothiocyanate in four steps. The racemic C-region was prepared as described in the previous report.[19]

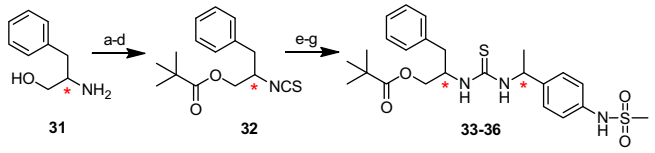
The syntheses of the stereo isomeric compounds **19–30** were generally accomplished by the coupling of the corresponding C-region isothiocyanates with A-region amines (Scheme 2). For the syntheses of the racemic α-methyl A-region analogues, commercially available 4-aminoacetophenone **16** was mesylated and then its methylketone was converted into the corresponding amine via the oxime to afford **17**, which was condensed with the C-region isothiocyanates to afford the α-methyl analogues **23**, **24**, **27** and **28**. For the syntheses of the chiral α-methyl A-region analogues, commercially available optical (*R* or *S*)-α-methyl-4-nitrobenzylamine **18** were condensed with the C-region isothiocyanates, respectively, and then the 4-nitro group was converted to the corresponding 4-methylsulfonamide to afford the chiral α-methyl analogues **19–22**, **25**, **26**, **29** and **30**. As references, the chiral isomers of **5a** was prepared from (4-methylsulfonylamino)benzylamine by the same method shown in Scheme 2.

For the syntheses of phenylalanol-type chiral C-region analogues, commercially available optical (*R* or *S*)-phenylalanol **31** was transformed into the corresponding isothiocyanate **32** in four steps, respectively, which was converted to the thioureas **33–36** by following the same methods described in Scheme 2 (Scheme 3).

The binding affinities and potencies as agonists/antagonists of the synthesized TRPV1 ligands were assessed in vitro by a binding competition assay with [^{3}H]RTX and by a functional $^{45}Ca^{2+}$ uptake assay using rat TRPV1 heterologously expressed in Chinese hamster ovary (CHO) cells, as previously described.[3] The results are summarized in Tables 1 and 2, together with the potencies of **5a–c** as references.



Scheme 2. Synthesis of 3-pivaloyl-2-benzyl-propyl C-region analogues. Reagents and conditions: (a) CH_3SO_2Cl, pyridine, 95%; (b) NH_2OH–HCl, pyridine, 85–88%; (c) H_2, Pd–C, HCl, MeOH, 96–98%; (d) RNCS, CH_2Cl_2 or DMF, 82–93%; (e) RNCS, NEt_3, CH_2Cl_2, 82–84%; (f) H_2, Pd–C, MeOH, 96–98%; (g) CH_3SO_2Cl, pyridine, 92–94%.

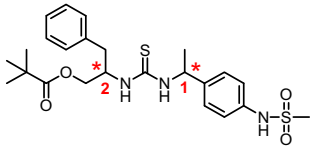


Scheme 3. Synthesis of 2-pivaloyl-1-benzyl-ethyl C-region analogues. Reagents and conditions: (a) Boc_2O, CH_2Cl_2, rt, 94%; (b) Me_3CCOCl, NEt_3, CH_2Cl_2, 0 °C, 88%; (c) CF_3CO_2H, CH_2Cl_2, 0 °C; (d) thiocarbonyldiimidazole, NEt_3, DMF, 50 °C, 82%; (e) RNCS, NEt_3, CH_2Cl_2, 82–84%; (f) H_2, Pd–C, MeOH, 96–98%; (g) CH_3SO_2Cl, pyridine, 92–94%.

First, we examined the effect of α-methylation on **5a** with R = H. In the two chiral isomers of **5a**, its (*R*)-configuration showed slightly better antagonism than that of (*S*)-configuration. Incorporation of α-methyl group into **5a** provided the four different stereo-isomers **19**–**22** (C_1: α-methyl, C_2: C-region chiral centers), respectively. The result indicated that the α-methylation produced the stereospecific antagonism and the C_1-configuration in the two chiral centers was critical for the receptor activity in which (*R*)-configuration of C_1 (**19**, **20**) showed much better binding affinity and antagonism than those of (*S*)-configuration of C_1 (**21**, **22**) irrespective of C_2 chirality. Compounds **19** and **20** showed approximately a 10-fold increase in binding affinity and slight or no enhancement in antagonism compared to (*R*)-**5a** and (*S*)-**5a**, respectively. The preference for the *R*-configuration of C_1 in receptor activity was also examined in the thiourea antagonist **4** with a 4-*t*-butylbenzyl C-region.[16] In the presence of the same C_1 chirality, the receptor activity upon changing the C_2-configuration gave mixed results.

We next investigated the α-methylated analogues of **5b** and **5c** with R = 3,4-Me_2 and 4-*t*-Bu, respectively, which were found to be the most potent antagonists in this series.[14] Since the *R*-configuration of C_1 in the series of the α-methylated **5a** proved to be the active one, we examined only the (*R*)-configuration of C_1 of the α-methylated **5b** and **5c**. As examined above, α-methylation of **5b** and **5c** also provided stereospecific antagonism in which the (*R*)-isomers **24** and **28** in the α-methyl analogues of **5b** and **5c** showed 5- and 6-fold increases in binding affinity and 10- and 4-fold increases in antagonism, compared to **5b** and **5c**, respectively. The C_2-configuration in **24** and **28** also appeared not to be significant for receptor activity (**25** vs **26**, **29** vs **30**). We previously demonstrated a similar SAR pattern in the amide B-region surrogate of **27**–**30** in which the receptor activity resided only in the (*S*)-configuration of C_1 while the C_2-configuration was not critical for receptor activity.[18] Nevertheless, among this series, the (*R*,*S*)-configuration of C_1 and C_2 seemed to be the optimal configuration in which compounds **26** and **30** showed high binding affinities with K_i = 2.12 nM and 6.75 nM, respectively.

Table 2
In vitro rTRPV1 activities for 2-pivaloyloxy-1-benzylethyl C-region analogues



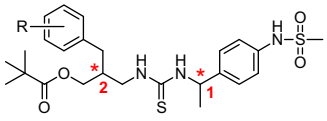
	C1	C2	K_i (nM)	EC_{50} (nM)	K_i (nM)
33	*R*	*R*	420 (±32)	NE	193 (±95)
34	*R*	*S*	272 (±28)	NE	290 (±66)
35	*S*	*R*	NE	NE	WE[a]
36	*S*	*S*	NE	NE	WE[a]

[a] Only fractional antagonism: 35, 47%; 36, 4%.

We also investigated the α-methylated thiourea analogues with a 2-pivaloyloxy-1-benzylethyl C-region (Table 2). Similar to the above, the receptor activity in this series resided only in the (*R*)-isomers of C_1, **33** and **34**, while the C_2-configuration did not have a significant effect on activity.

In summary, we have investigated a series of α-methylated analogues of the potent sRTX thiourea antagonists **5a**–**c**. The SAR analysis indicated that they showed stereospecific antagonism dependent on the α-methyl configuration, with the (*R*)-configuration proving to be the active one as had been found for the previous thiourea antagonists **3** and **4**. In addition, the C_2-configuration in the C-region appeared not to be significant for activity. Overall, we concluded that α-methylation at the benzylic position of 4-methylsulfonamidophenyl antagonists provided stereospecific and potent antagonists. Whereas the amide B-region analogues preferred the (*S*)-configuration for receptor activity, the thiourea B-region ones favored the (*R*)-configuration.

Table 1
In vitro rTRPV1 activities for 3-pivaloyloxy-2-benzylpropyl C-region analogues



	R	Chirality (C_1)	(C_2)	K_i (nM)	EC_{50} (nM)	$K_{i(CAP)}$ (nM)
(*R*)-**5a**	H		*R*	252 (±77)	NE	64.3 (±9.8)
(*S*)-**5a**	H		*S*	236 (±4.0)	NE	80.6 (±6.4)
19	H	*R*	*R*	22.7 (±4.4)	NE	44.5 (±9.9)
20	H	*R*	*S*	19.4 (±1.9)	NE	89 (±20)
21	H	*S*	*R*	1140 (±160)	NE	3500 (±300)
22	H	*S*	*S*	710 (±240)	NE	133 (±24)
5b				29.3	WE	67
23	3,4-Me_2			37.2 (±5.9)	NE	25.9 (±9.1)
24	3,4-Me_2	*R*		6.1 (±2.3)	NE	6.9 (±1.4)
25	3,4-Me_2	*R*	*R*	15.2 (±3.4)	NE	7.1 (±1.3)
26	3,4-Me_2	*R*	*S*	2.12 (±0.73)	NE	13.2 (±5.6)
5c				64	WE	86
27	4-*t*-Bu			42.7 (±5.6)	NE	28.7 (±8.7)
28	4-*t*-Bu	*R*		10.0 (±1.3)	NE	23.9 (±6.1)
29	4-*t*-Bu	*R*	*R*	17.9 (±1.2)	NE	53 (±11)
30	4-*t*-Bu	*R*	*S*	6.75 (±0.99)	NE	30.6 (±7.3)

Acknowledgments

This research was supported by Grants from the National Research Foundation of Korea (NRF) (R11-2007-107-02001-0), and by the Intramural Research Program of NIH, Center for Cancer Research, NCI (Project Z1A BC 005270).

References and notes

1. Szallasi, A.; Blumberg, P. M. *Pharmacol. Rev.* **1999**, *51*, 159.
2. Bevan, S.; Hothi, S.; Hughes, G.; James, I. F.; Rang, H. P.; Shah, K.; Walpole, C. S.; Yeats, J. C. *Br. J. Pharmacol.* **1992**, *107*, 544.
3. Wang, Y.; Szabo, T.; Welter, J. D.; Toth, A.; Tran, R.; Lee, J.; Kang, S. U.; Suh, Y. G.; Blumberg, P. M.; Lee, J. *Mol. Pharmacol.* **2002**, *62*, 947 (erratum in *Mol. Pharmacol.* **2003**, *63*, 958).
4. Kym, P. R.; Kort, M. E.; Hutchins, C. W. *Biochem. Pharmacol.* **2009**, *78*, 211.
5. Wong, G. Y.; Gavva, N. R. *Brain Res. Rev.* **2009**, *60*, 267.
6. Gunthorpe, M. J.; Chizh, B. A. *Drug Discovery Today* **2009**, *14*, 56.
7. Lazar, J.; Gharat, L.; Khairathkar-Joshi, N.; Blumberg, P. M.; Szallasi, A. *Exp. Opin. Drug Disc.* **2009**, *4*, 159.
8. Voight, E. A.; Kort, M. E. *Exp. Opin. Ther. Pat.* **2010**, *20*, 1.
9. Szolcsányi, J.; Sándor, Z. *Trend Pharmacol. Sci.* **2012**, *33*, 646.
10. Szallasi, A.; Sheta, M. *Exp. Opin. Investig. Drug* **2012**, *21*, 1351.
11. Lee, J. H.; Lee, Y.; Ryu, H.; Kang, D. W.; Lee, J.; Lazar, J.; Pearce, L. V.; Pavlyukovets, V. A.; Blumberg, P. M.; Choi, S. *J. Comput. Aided Mol. Des.* **2011**, *25*, 317.
12. Liao, M.; Cao, E.; Julius, D.; Cheng, Y. *Nature* **2013**, *504*, 107.
13. Kim, M. S.; Ki, Y.; Ahn, S. Y.; Yoon, S.; Kim, S.-E.; Park, H.-G.; Sun, W.; Son, K.; Cui, M.; Choi, S.; Pearce, L. V.; Esch, T. E.; DeAndrea-Lazarus, I. A.; Blumberg, P. M.; Lee, J. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 382. and references therein.
14. Lee, J.; Lee, J.; Kang, M.; Shin, M.-Y.; Kim, J.-M.; Kang, S.-U.; Lim, J.-O.; Choi, H.-K.; Suh, Y.-G.; Park, H.-G.; Oh, U.; Kim, H.-D.; Park, Y.-H.; Ha, H.-J.; Kim, Y.-H.; Toth, A.; Wang, Y.; Tran, R.; Pearce, L. V.; Lundberg, D. J.; Blumberg, P. M. *J. Med. Chem.* **2003**, *46*, 3116.
15. Lee, J.; Kang, S.-U.; Lim, J.-O.; Choi, H.-K.; Jin, M.-K.; Toth, A.; Pearce, L. V.; Tran, R.; Wang, Y.; Szabo, T.; Blumberg, P. M. *Bioorg. Med. Chem.* **2004**, *12*, 371.
16. Chung, J.-U.; Kim, S. Y.; Lim, J.-O.; Choi, H.-K.; Kang, S.-U.; Yoon, H.-S.; Ryu, H.; Kang, D. W.; Lee, J.; Kang, B.; Choi, S.; Toth, A.; Pearce, L. V.; Pavlyukovets, V. A.; Lundberg, D. J.; Blumberg, P. M. *Bioorg. Med. Chem.* **2007**, *15*, 6043.
17. Kim, Y. S.; Kil, M.-J.; Kang, S.-U.; Ryu, H.; Kim, M. S.; Cho, Y.; Bhondwe, R. S.; Thorat, S. A.; Sun, W.; Liu, K.; Lee, J. H.; Choi, S.; Pearce, L. V.; Pavlyukovets, V. A.; Morgan, M. A.; Tran, R.; Lazar, J.; Blumberg, P. M.; Lee, J. *Bioorg. Med. Chem.* **2012**, *20*, 215.
18. Ryu, H.; Jin, M.-K.; Kang, S.-U.; Kim, S. Y.; Kang, D. W.; Lee, J.; Pearce, L. V.; Pavlyukovets, V. A.; Morgan, M. A.; Tran, R.; Toth, A.; Lundberg, D. J.; Blumberg, P. M. *J. Med. Chem.* **2008**, *51*, 57.
19. Lee, J.; Lee, J.; Kim, J.; Kim, S. Y.; Chun, M. W.; Cho, H.; Hwang, S. W.; Oh, U.; Park, Y. H.; Marquez, V. E.; Beheshti, M.; Szabo, T.; Blumberg, P. M. *Bioorg. Med. Chem.* **2001**, *9*, 19.