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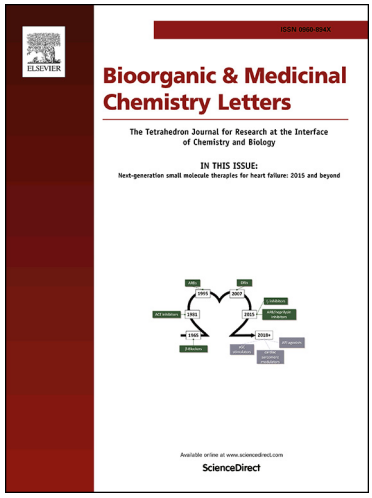

2-Methylacrylamide as a Bioisoster of Thiourea Group for 1,3-Dibenzylthioureido TRPV1 Receptor Antagonists

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2-Methylacrylamide as a Bioisoster of Thiourea Group for 1,3-Dibenzylthioureido TRPV1 Receptor Antagonists

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ABSTRACT

In order to replace thiourea group with the more drug-like moiety for 1,3-dibenzylthioureas having TRPV1 antagonist activity, we introduced a set of functional groups between the two aromatic rings based on bioisosteric replacement. The synthesized bioisosteres of 1,3-dibenzylthioureas were tested for their antagonist activities on TRPV1 by $^{45}Ca^{2+}$-influx assay using neonatal rat cultured spinal sensory neurons. Among the tested 14 kinds of bioisosters, 2-methylacrylamide group was the best candidate to replace thiourea group. Compound **7c**, 2-methylacrylamide analog of ATC-120, showed as potent as ATC-120 in its antagonist activity. In addition, 2-methylacrylamide analog **7e** having vinyl moiety showed the most potent activity with 0.022 μM of IC_{50} value, indicating that thiourea group of 1,3-dibenzylthioureas could be replaced to 2-methylacrylamide without loss of their potencies.

Key Words: 2-methylacrylamide, bioisoster, TRPV1, 1,3-dibenzylthioureas, antagonist, $^{45}Ca^{2+}$-influx assay

The transient receptor potential vanilloid-1 (TRPV1) is a ligand-gated nonselective cation channel with high Ca^{2+} permeability,[1] emerging as an attractive target for the treatment of chronic and inflammatory pain.[2] Capsaicin, resiniferatoxin,[3] and SDZ-249482[4] represent the most well-known agonists to date. However, due to their undesirable side effects such as pungency and/or hypothermia responses,[5] recent efforts have been focused on the discovery of novel antagonists.[6] We and co-workers discovered the potent antagonists (MK-056,[7a] SC-0030,[7b,c] and ATC-120[7d]) by changing phenolic hydroxyl group of SDZ-249482 to the corresponding methanesulfonylamido group. (Figure 1) Over the past few years, we have demonstrated that a series of 1,3-dibenzylthioureas having methanesulfonylamido group were potent TRPV1 antagonists active against multiple activators.[8] In these SAR studies, we have found that thiourea moiety of 1,3-dibenzylthioureas is very important pharmacophore for their high potencies. However, in view of drug-like properties, there is a need to develop the more drug-like moiety than is thiourea. Thus, we decide to investigate the new pharmacophoric alternatives to replace thiourea group of the 1,3-dibenzylthiourea series.

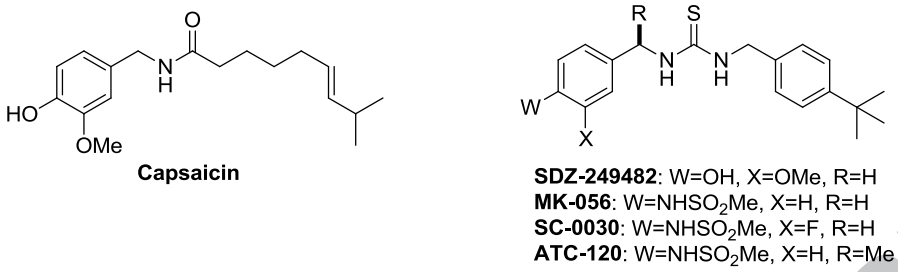
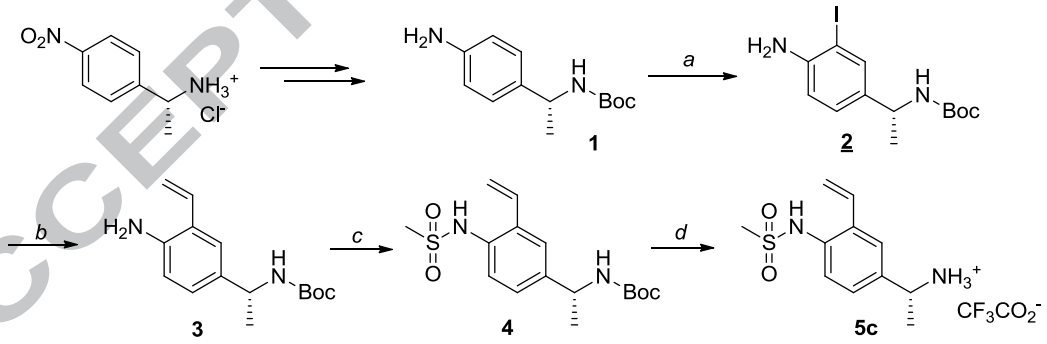


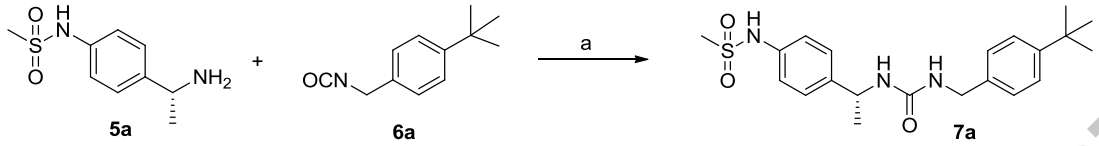
Figure 1. Structure of capsaicin and 1,3-dibenzylthioureas

A number of functional groups including urea, amide, acrylamide and glycolamide were chosen as bioisosteres of thiourea. ATC-120 was also chosen as reference compounds in order to clarify the effect of bioisosteric replacement. The target compounds were synthesized *via* the route outlined in Scheme 1-5. 4-Methanesulfonamido-α-methylbenzyamines **5a~c** were coupled with 4-*tert*-butylbenzenes **6a~e** having the requisite functional groups. (*S*)-4-Methanesulfonamido-α-methylbenzylamine (**5a**) and (*S*)-3-Fluoro-4-methanesulfonamido-α-methylbenzylamine (**5b**) were prepared according to the previously reported methods.[7d, 9] (*S*)-3-Vinyl-4-methanesulfonamido-α-methylbenzyamine (**5c)** was prepared *via* the route outlined in Scheme 1. Treatment of **1** with iodine monochloride produced **2** regioselectively in 47% yield. The iodide **2** was then converted the vinyl compound **3** using by Stille's coupling, followed by methanesulfonylation and deprotection to give the (*S*)-3-vinyl-4-methanesulfonamido-α-methylbenzyamine (**5c**).



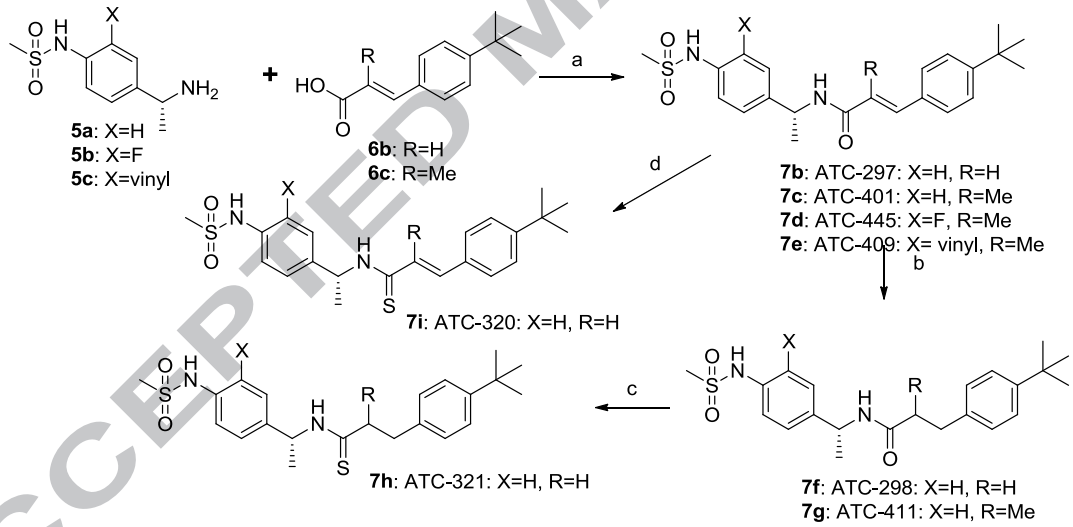
Scheme 1. Synthesis of chiral amine **5c**: a) ICl, CH_2Cl_2, 47%; b) $Bu_3SnCH{=}CH_2$, LiCl, $Pd(PPh_3)_4$, DMF, reflux, 72%; c) $(CH_3SO_2)_2O$, pyridine, CH_2Cl_2, 47%; d) CF_3CO_2H, CH_2Cl_2, 100%.

At first, we made urea analog **7a** as a thiourea bioisoster of ATC-120, as shown in Scheme 2. (*S*)-4-Methanesulfonamido-α-methylbenzylamine **5a** was treated with 4-*tert*-butylbenzylisocyanate **6a** under basic condition followed by deprotection to give the urea analog **7a** in 17% yield.



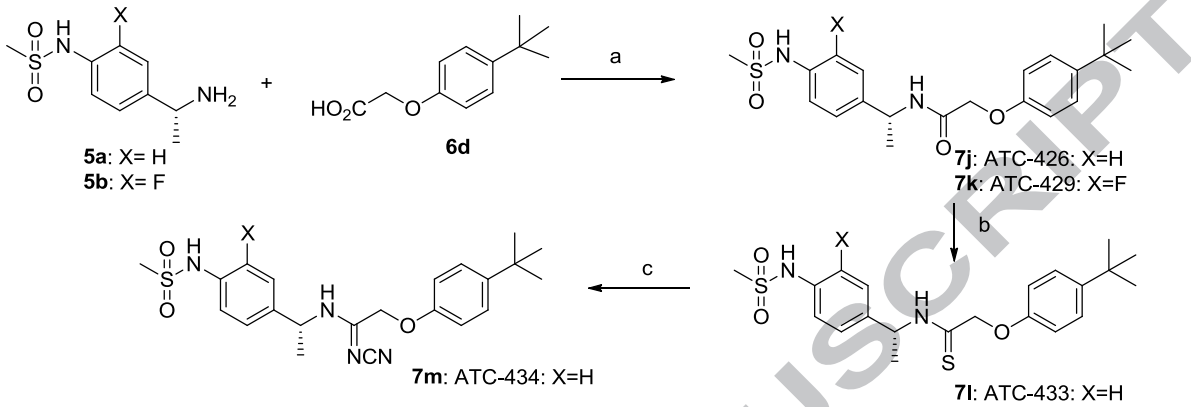
Scheme 2. Synthesis of urea **7a**: a) TEA, CH_2Cl_2, then CF_3CO_2H, 17 %.

Next, we focused on the design and synthesis of amide analogs due to their drug-like properties. Amides, acrylamides, thioamides, and thioacrylamides were designed and prepared *via* the route outlined in Scheme 3. (*S*)-4-Methanesulfonamido-α-methylbenzylamines (**5a~c**) were treated with (*E*)-3-[4-(*tert*-butyl)phenyl]acrylic acid (**6b**) or (*E*)-3-[4-(*tert*-butyl)phenyl]-2-methylacrylic acid (**6c**) with an aid of coupling agent DEPC under basic condition in DMF to produce the corresponding (methyl)acrylamides **7b~e** in 55~94% yields. Double bond reduction of (methyl)acrylamides **7b~c** by hydrogenolysis gave the (methyl)amides **7f~g** in good yields. Treatment of **7b** or **7f** with Lawesson's reagent gave the corresponding thio(acryl)amide **7h** or **7i** respectively.



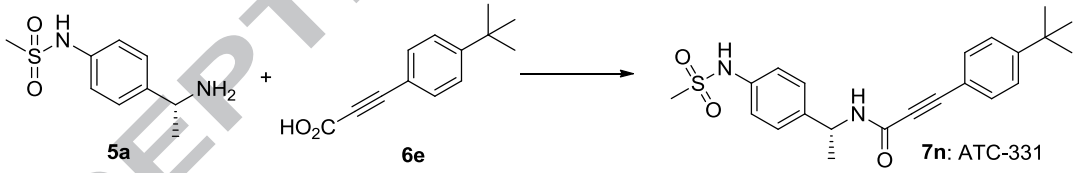
Scheme 3. Synthesis of amides and thioamides: a) DEPC, TEA, DMF, 55~94 %; b) H_2, Pd/C, quant.; c) Lawesson's reagent, toluene, reflux, 87%; d) Lawesson's reagent, toluene, reflux, 87 %.

We also designed the glycolamides **7j,k** and its analogs **7l** and **7m** as a bioisoster of thiourea ATC-120. Synthese of **7j~m** are outlined in Scheme 4. (*S*)-4-Methanesulfonamido-α-methylbenzylamines (**5a~b**) were treated with 2-[4-(*tert*-butyl)phenoxy]acetic acid (**6d**) with an aid of coupling agent DEPC under basic condition in DMF to produce the corresponding glycolamides **7j,k**. Treatment of **7j** with Lawesson's reagent gave the corresponding thioglycolamide **7l** in 88% yield. By reacting with cyanamide and $HgCl_2$, thioglycolamide **7l** could be converted to the corresponding *N*-cyanoacetimidamide **7m** in 98% yield.



Scheme 4. Synthesis of glycolamides and its analogs: a) DEPC, TEA, DMF, 77~88 %; b) Lawesson's reagent, toluene, reflux, 88 %; c) $HgCl_2$, H_2NCN, TEA, DMF, 98%.

Finally, we designed propiolamide as a bioisoster of thiourea of ATC-120. (*S*)-4-Methanesulfonamido-α-methylbenzylamine (**5a**) was treated with 2-[4-(*tert*-butyl)phenyl] propiolic acid (**6e**) with an aid of coupling agent DEPC under basic condition in DMF to produce the corresponding propiolamide **7n**, as shown in Scheme 5.

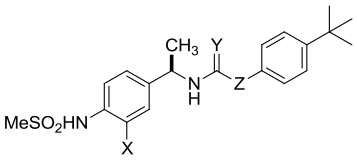


Scheme 5. Synthesis of propiolamide: a) DEPC, TEA, DMF, 61 %.

The prepared bioisosters for ATC-120 were tested for their antagonist activities on TRPV1 by $^{45}Ca^{2+}$ -influx assay using neonatal rat cultured spinal sensory neurons.[10] The results are summarized in Table 1. ATC-120 was used as reference compound. As is anticipated, urea analog **7a** showed 13-fold decrease in antagonist activity compared to thiourea analog ATC-120. Amide analogs **7f**, methyl-branched amide **7g**, and thioamide **7h** were less potent than thiourea analog ATC-120, but more active than urea analog **7a**. When an oxygen atom is introduced to β-position in place of CH_2 into the amide **7f**, the resulting glycolamide **7j** showed drastic increase of antagonistic potency with an IC_{50} of 0.096 μM, but still less potent (1/2 fold) as compared to thiourea ATC-120. Both *N*-cyanoacetimidamide **7m** and sulfur analogs **7l** of glycolamide **7j** exhibited 3 to 7 fold less potent antagonistic potency compared to glycolamide **7j**. Thus, we explored the modification of the aromatic ring attached to methansulfonamide group. When we

replaced the hydrogen atom with fluoride atom at X position of **7j** (Table 1), the antagonist activity increased up to 0.071 μM of IC_{50} value. However, we could not find out the better compounds than glycolamide **7j** from the modification study. Thus, we turned our attention to acrylamide analogs aiming that introduction of double bond could restrict the rotation around both amide bond and *tert*-butylated phenyl ring, thereby increasing the % population of bioactive conformation. *trans*-Acrylamide **7b** showed 0.16 μM of IC_{50}, 1.5 fold more potent than saturated amide **7f**, indicating that *trans*-conformation might be closer to the bioactive conformation. Next, we introduced triple bond between amide and 4-*tert*-butylphenyl ring, providing propiolamide **7n**, proved better antagonist with IC_{50} value of 0.1 μM. However, there is no space to modify around triple bond on propiolamide **7n**, we needed to explore the acrylamide further. Methyl-branching at α-position of acrylamide **7b**, providing **7c**, has an impact on the improvement in activity with IC_{50} value of 0.046 μM. It means that antagonistic potency increased approximately 4-fold compared to **7b**, comparable to that of thiourea analog ATC-120. Encouraged with the result, we explored the modification of the aromatic ring attached to methansulfonamide group. Substitution at X-position of **7c** with fluorine atom, providing **7d**, resulted in equipotent activity with parent compound **7c**. The best result obtained by introducing vinyl group at X-position of **7c** to provide compound **7e** with IC_{50} value of 0.022 μM, representing 2-fold increase in antagonistic potency compared to thiourea ATC-120. It is also notable that all thioamides including thioacrylamides and thioglycolamides studied here showed very weak antagonistic activities.

Table 1. $^{45}Ca^{2+}$ -Influx activity of the bioisosters of 1,3-dibenzylthioureido $TRPV_1$ receptor antagonist



Compound	X	Y	Z	$^{45}Ca^{2+}$ influx activity (μM)[a]	
				Agonist (EC_{50})	Antagonist(IC_{50})
ATC-120	H	S	-$NHCH_2$-	> 100	0.05
7a	H	O	-$NHCH_2$-	> 100	0.68
7f	H	O	-CH_2CH_2-	> 100	0.24
7g	H	O	-$CHMeCH_2$- (racemic)	> 100	0.27
7h	H	S	-CH_2CH_2-	> 100	0.30
7j	H	O	-CH_2O-	> 100	0.096
7l	H	S	-CH_2O-	> 100	0.68
7m	H	NCN	-CH_2O-	> 100	0.21

7k	F	O	$-CH_2O-$	> 100	0.071
7b	H	O	-CH=CH- (*trans*)	> 100	0.16
7i	H	S	-CH=CH- (*trans*)	> 100	5.0
7n	H	O	-C≡C-	> 100	0.10
7c	H	O	-C(Me)=CH- (*trans*)	> 100	0.046
7d	F	O	-C(Me)=CH- (*trans*)	> 100	0.041
7e	H_2C=CH	O	-C(Me)=CH- (*trans*)	> 100	0.022

[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 to 0.5 μM capsaicin by 50%) were estimated with at least 3 replicates at each concentration. Each compound was tested in two independent experiments. Antagonist data were fitted with a sigmoid function.

In summary, we have designed and synthesized a series of bioisosters of 1,3-dibenzylthiourea $TRPV_1$ antagonist ATC-120, focusing on the replacement of thiourea functionality to improve drug-likeness. Among the tested 14 kinds of bioisosters, 2-methylacrylamide group was the best candidate to replace thiourea group. Compound **7c**, 2-methylacrylamide analog of ATC-120, showed as potent as ATC-120 in its antagonist activity. In addition, 2-methylacrylamide analog **7e** showed the most potent activity with 0.022 μM of IC_{50} value, indicating that the less druggable thiourea group of 1,3-dibenzylthioureas could be replaced to the more drug-like 2-methylacrylamide group without loss of their potencies. This bioisosteric replacement might enable us to jump into the new chemical space of $TRPV_1$ related antagonists.

Acknowledgements

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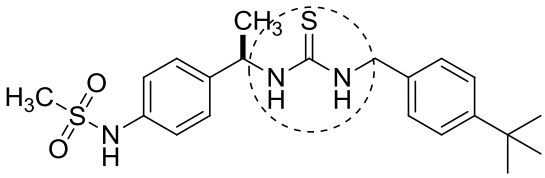
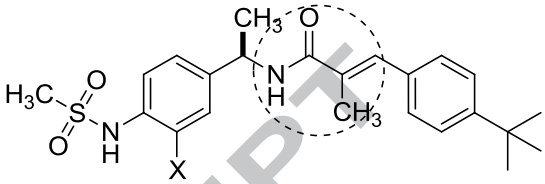


ATC-120: IC_{50} = 0.05 μM
$TRPV_1$ antagonist

Improve
drug-likeness



X=H: IC_{50} = 0.046 μM
X= vinyl: IC_{50} = 0.022 μM

Highlights

- 1,3-Dibenzylthioureas having methanesulfonylamide are potent TRPV1 antagonists.
- Thiourea moiety of 1,3-dibenzylthioureas could be replaced to 2-methylacrylamide.
- This replacement enable us to design the more druggable $TRPV_1$ related antagonists.