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Tricyclic 3,4-dihydropyrimidine-2-thione derivatives as potent TRPA1 antagonists

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ABSTRACT

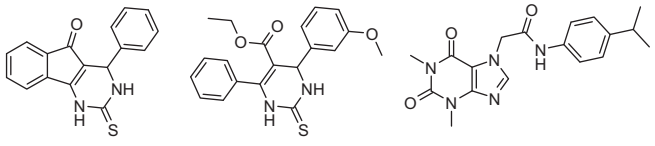
The transient receptor potential A1 (TRPA1) channel has been implicated in a number of inflammatory and nociceptive processes, and antagonists of the TRPA1 receptor could offer a potential treatment for conditions such as inflammatory or neuropathic pain, airway disorders, and itch. In a high throughput screen aimed at the identification of TRPA1 antagonists, 4-phenyl-2-thioxo-1,2,3,4-tetrahydro-indeno[1,2-*d*]pyrimidin-5-one (**1**) was identified as a potent TRPA1 receptor antagonist. A series of analogous tricyclic 3,4-dihydropyrimidine-2-thiones has been prepared via the multi-component Biginelli reaction and subsequent derivatization. This has led to TRPA1 antagonists with potencies around 10 nM for both rat and human derived TRPA1 receptors. The activity was shown to reside exclusively in the 4*R*-enantiomers.

The transient receptor potential A1 (TRPA1, formerly named ANKTM1) channel is a polynodal nociceptor of various noxious stimuli including pungent chemicals such as allylisothiocyanate present in mustard oil, thiosulfinates present in garlic, cinnamaldehyde, and tear gases.[1] Many structurally diverse compounds have been described as TRPA1 activators.[2] The majority of these are electrophilic agents, which most likely activate the TRPA1 channel via a covalent interaction with the many cysteine residues present in the TRPA1 channel.[1] In this way, the TRPA1 channel can be regarded as a sensor to chemical irritants, which is illustrated by the identification of the TRPA1 channel as the biological target for many of the currently used tear gasses.[3] Antagonists of the TRPA1 receptor could find possible therapeutic use as antinociceptive or anti-inflammatory agents,[4] or be used as protective agents against irritant chemicals and tear gases. Recently, the TRPA1 has also been linked to play a role in airway disorders[5] and itch.[6] To determine the full therapeutic potential of TRPA1 channel modulation, there is a need for potent TRPA1 antagonists. Compared to the diversity of available agonists, relatively few classes of TRPA1 antagonists have been reported up to now.[2] Structural variation of some of the electrophilic series has resulted in compounds displaying antagonism towards TRPA1.[7] The distinction between activation or inhibition of the TRPA1 channel has been shown to be dependent on small structural variations. Several groups have reported species differences for TRPA1 agonism/antagonism, which could complicate extrapolation of results obtained from animal models to humans.[8,9] A clear goal for our program has therefore been to identify compounds displaying clear antagonism for both the rat and human TRPA1 channel.

In a high throughput screen aimed at the identification of TRPA1 antagonists, 4-phenyl-2-thioxo-1,2,3,4-tetrahydro-indeno[1,2-*d*]pyrimidin-5-one (**1**) was identified as a TRPA1 receptor antagonist (Fig. 1). With a potency in our assay exceeding the previously described antagonists **2** and **3** (HC-030031),[10] we embarked on the exploration of this tricyclic class of dihydropyrimidones (DHPMs). This paper describes the optimization efforts and initial SAR findings.

A number of commercially available thiourea analogs of **1**, exemplifying various ring disconnections or simplifications of **1**, were tested for rat and human TRPA1 antagonism and all found to be inactive, illustrating the requirement for the tricyclic DHPM for optimal activity (**4**–**11**, Fig. 2).

Also the corresponding urea analog of **1**, compound **12**, did not display TRPA1 antagonism at concentrations up to 10 μM. (Fig. 3). Chiral separation of **1** into its enantiomers via SFC and subsequent



1
hTRPA1 IC_{50} 1.8 μM
rTRPA1 IC_{50} 1.9 μM

2
hTRPA1 IC_{50} >10 μM
rTRPA1 IC_{50} 9.5 μM

3
hTRPA1 IC_{50} >10 μM
rTRPA1 IC_{50} 5.6 μM

Figure 1. Structure and activity of HTS hit and known TRPA1 antagonists.

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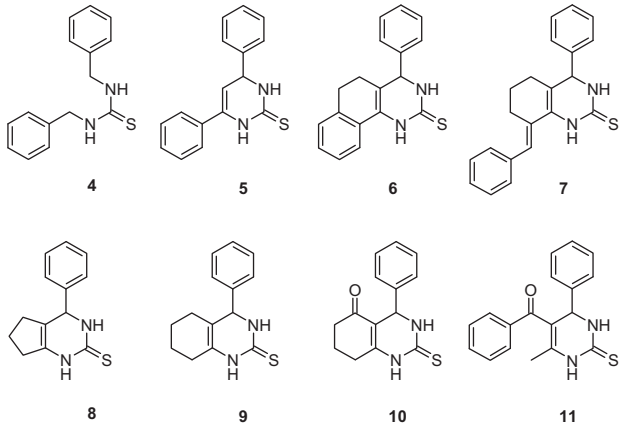
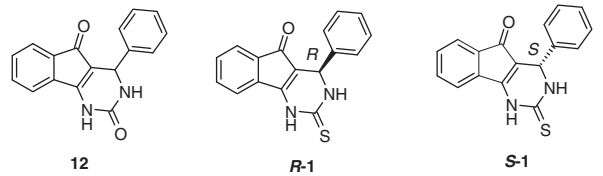


Figure 2. Inactive thiourea analogs of **1**. hTRPA1 IC50 all >10 μM.



12	*R*-1	*S*-1
hTRPA1 IC_{50} >10 μM	hTRPA1 IC_{50} 0.77 μM	hTRPA1 IC_{50} 8.1 μM
rTRPA1 IC_{50} >10 μM	rTRPA1 IC_{50} 0.31 μM	rTRPA1 IC_{50} >10 μM

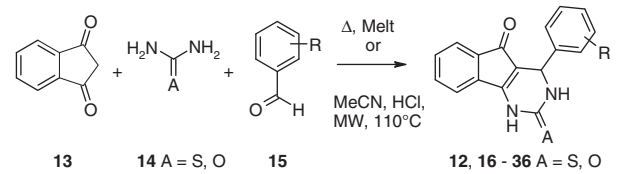
Figure 3. TRPA1 activity of urea analog and enantiomers of **1**.

testing, proved *R*-**1** to be the eutomer (absolute configuration determined via VCD). The clear difference in activity between the two enantiomers gave us the confidence that **1** was not a false positive hit, and a series of analogs was prepared.

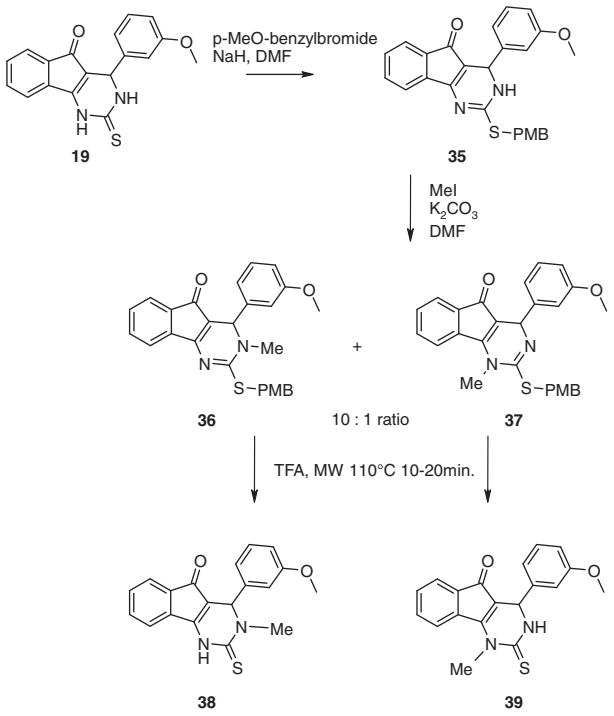
The synthesis of **1** and analogous tricyclic DHPMs is most easily achieved via a Biginelli three component reaction between indane-1,3-dione **13**, urea or thiourea **14** and benzaldehyde **15** (Scheme 1)[11,12] In general, when using acetonitrile as a solvent, the desired products precipitated from the reaction mixture and were easily isolated by filtration.

N-alkylated derivatives could not be obtained directly via a Biginelli reaction using N-alkylated urea or thiourea, nor via an Atwal modification of the Biginelli reaction.[13] Direct alkylation of **19** predominantly resulted in S-alkylation. This was used to our advantage by initial S-alkylation of **19** to the *p*-methoxybenzyl (PMB) protected **35**, followed by N-methylation to give a mixture of **36** and **37**. After separation of the two isomers, subsequent removal of the *p*-methoxybenzyl group gave **38** and **39** (Scheme 2).

All products were tested on both the human and rat TRPA1 channel (Table 1).[14] In general, a comparable activity was observed for the human and rat channel, with for most compounds a slightly higher potency for rat TRPA1. At all concentrations tested, the compounds behaved as true antagonists, in competition with the applied agonists.[15] This is unlike many electrophilic ligands, which can display functional antagonism via desensitization of the channel, depending on their concentration.[16] Introduction of various substituents on the aryl substituent of **1** had a pronounced effect on the potency of the tricyclic DHPMs. Ortho- and para-substituted

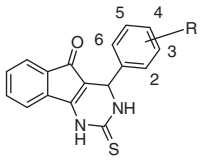


Scheme 1. Synthesis of target products via the Biginelli reaction.



Scheme 2. Synthesis of N-alkylated derivatives.

Table 1
Effect of aryl substitution on human and rat TRPA1 antagonism



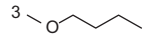
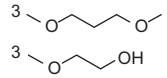
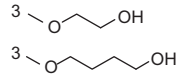
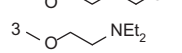
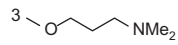
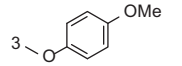
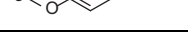
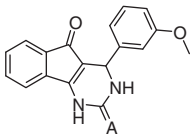
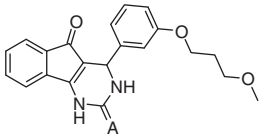
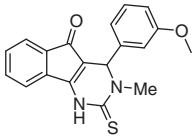
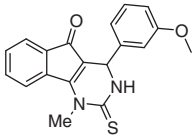
Compound	R	hTRPA1 IC_{50} (μM)	rTRPA1 IC_{50} (μM)
1	H	1.8	1.9
16	2-Fluoro	2.07	3.39
17	3-Fluoro	1.1	0.39
18	2-Methoxy	>10	9.4
19	3-Methoxy	0.13	0.02
20	4-Methoxy	3.1	3.5
21	3-Bromo	0.5	0.071
22	3-Hydroxy	0.64	0.23
23	3-Hydroxymethylene	0.71	0.044
24	3-Carboxylic acid	>10	>10
25	2,3-Dimethoxy	>10	2.7
26	2,5-Dimethoxy	>10	>10
27	3,5-Dimethoxy	2.78	0.80
28		0.31	0.07
29		0.05	0.011
30		0.21	0.047
31		0.07	0.010
32		>10	8.9
33		>10	>10
34		1.5	0.16

Table 2
Effect of stereochemistry, (thio)urea, and N-alkylation on human and rat TRPA1 antagonism

Structure	Compound	A	Stereochemistry	hTRPA1 IC_{50} (μM)	rTRPA1 IC_{50} (μM)
	**19**	S	*RS*	0.13	0.020
	40	S	*R*	0.075	0.012
	41	S	*S*	>10	>10
	42	O	*RS*	3.9	4.7
	**29**	S	*RS*	0.050	0.011
	43	S	*R*	0.013	0.004
	44	S	*S*	>10	>10
	45	O	*RS*	1.7	0.5
	**38**	S	*RS*	>10	0.98
	**39**	S	*RS*	0.085	0.003

compounds **16**, **18**, and **20** led to a drop in activity compared to the unsubstituted **1**. In contrast, meta-substitution, such as in **17**, **19**, and **21**–**23** increased the potency relative to **1**. Especially meta-methoxy substituted **19** displayed a more than 10-fold enhanced potency, and additional analogs around **19** were investigated.

Combination of a meta-methoxy substituent with additional methoxy substituents as in **25**–**27** led to a loss in activity. Elongation of the methoxy group proved to be tolerated better, with **29** and **31** being the most potent compounds identified. Both acidic and basic substituents were not allowed, as illustrated by **24** and **32**–**33**, respectively.

For two of the most interesting compounds, **19** and **29**, the racemates were separated into the enantiomers, and the corresponding dihydropyrimidones **42** and **45** were prepared, respectively. The results are shown in Table 2. In line with the data on the enantiomers of **1**, only the dextrarotary enantiomers **40** and **43** proved to be active. The absolute configuration of **40** was again determined to be 4*R* via VCD. In contrast to **12**, dihydropyrimidones **42** and **45** both had a measurable potency below 10 μM but were about two orders of magnitudes lower in activity than their thio-analogs **19** and **29**, respectively. A similar, positive influence of the meta substitution pattern was observed, with methoxypropoxy substituted **45** being more potent than methoxy derivative **42**.

The influence of alkylation of the thio-dihydropyrimidine nitrogens was briefly investigated via N-methylated compounds **38** and **39**. Methylation at N3 as in **38** led to a loss in potency compared to **19**, which was more pronounced for the human TRPA1 channel than for the rat TRPA1 channel. N1-methylated **39** displayed a similar potency as **19** in the human TRPA1 channel, and was one of the most potent antagonists to the rat TRPA1 channel.

The blockade of the TRPA1 channel by the antagonists was confirmed in electrophysiological experiments on both the human and rat TRPA1 channel for **40** and **41**.[3a] The eutomer **40** gave a 100% blockade of ionic currents with an EC_{50} of 0.01 μM of the human TRPA1 channel, and a 90% blockade with EC_{50} of 0.22 μM of the rat TRPA1 channel. The distomer **41** did not show any blockade of the rat channel and 40–70% blockade with an EC_{50} of 12 μM of the human channel.

The high potency of this class of TRPA1 antagonists for both the human and rat TRPA1 channel makes it a potentially attractive series to investigate the therapeutic use of TRPA1 antagonists in animal models. In general, however, this class of compounds displays a poor druglike profile, such as very low solubility and low metabolic stability. In addition, the thiopyrimidone class of compounds is intensely colored, ranging from yellow to dark red. The potential toxicity of thioureas is another concern.[17] Further optimization will be required to arrive at compounds which could be used as tool compound for in vivo studies. The tolerance of polar and flexible meta-phenyl substituents such as in **29** and **31**, and the acceptance of N1 substituents as in **39**, could offer initial handles for improvement of the druglike properties.

Acknowledgment

We thank Maarten te Molder for the synthesis of several of the analogs.

Supplementary data

Supplementary data (The details of the in vitro human and rat TRPA1 assays and the synthesis and analytical data of **19**, **29**, **40**, **41**, **43** and **44** are described) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.12.068.

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14. Ca^{2+} fluorometric measurements on hTRPA1 and rTRPA1 inducible HEK293 cells. All data shown for the hTRPA1 receptor were derived using the potent, human specific hTRPA1 agonist 5*H*-dibenzo[*b*,*e*]azepine-10-carboxylic acid methyl ester (see Gijsen, H. J. M.; Berthelot, D.; Zaja, M.; Brône, B.; Geuens, I.; Mercken, M. *J. Med. Chem.* **2010**, *53*, 7011). Comparable results were obtained when benzylisothiocyanate (BITC) was used as agonist, which is a less volatile alternative to allylisothiocyanate. Data shown for the rTRPA1 assay, were generated using BITC as agonist. For detailed procedures see supplementary material.
15. To investigate the possibility of covalent binding of the 3,4-dihydropyrimidine-2-thiones to the TRPA1 channel, **29** was incubated with benzylmercaptane in DMSO for several days as described in Ref. [3a] No reaction was observed, Although this does not completely rule out reversible covalent binding to the many cysteine residues in the TRPA1 channel, it indicates that this class of compounds does not easily form covalent bonds with thiols.
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