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Oxime derivatives related to AP18: Agonists and antagonists of the TRPA1 receptor

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ABSTRACT

AP18 **1** was recently disclosed as an antagonist of the TRPA1 receptor by the research group of Patapoutian. However, no detailed structure–activity relationships around **1** have been disclosed. Thus, a small number of oximes related to AP18 were examined in order to characterize the determinants of TRPA1 activity. Congeners of AP18 were found to possess both agonist and antagonist activity, suggesting that AP18 may behave as a covalent antagonist of the TRPA1 ion-channel.

The ability to detect and respond to noxious stimuli is critical for survival and is usually interpreted as a painful experience. Nociceptors are the sensory neurons that detect noxious stimuli and transduce them to neural signaling. These cells typically express one or more Transient Receptor Potential (TRP) receptors: a functionally-diverse group of six-transmembrane domain ion-channels capable of responding to a variety of physical and chemical stimuli.[1] One such family member, Ankyrin-repeat Transient Receptor Potential 1 (TRPA1), is expressed in the peptidergic subset of sensory fibers and has been shown to respond to a variety of noxious stimuli, including environmental irritants, cold temperatures, and pungent plant-metabolites.[2,3] Despite differences in chemical functionality among small molecule irritants that agonize TRPA1, such as allyl isothiocyanate (mustard oil) **1**, iodoacetamide **2**, cinnamaldehyde **3** and diallyl disulfide **4**, they all activate the channel via a common mechanism. The agonists act as electrophiles, covalently modifying conserved cysteine residues present almost exclusively in the cytoplasmic, amino-terminal domain of the channel (Fig. 1).[2b,4] In the case of cinnamaldehyde (CA), the α,β unsaturated bond is attacked in a Michael fashion, and the resulting covalent addition leads to an opening of the ion-channel, an influx of calcium into the cell, and ultimately, a perception of a painful stimulus.

Endogenous mediators of inflammation and pain also interact with TRPA1. Prostaglandins, hydroxynonenal, and reactive oxygen species have all been shown to act as TRPA1 agonists, consistent with a role for TRPA1 as a mediator of inflammatory responses.[5] Various knock-down or knockout studies in rodents have demonstrated reduced nociceptive behavior in a number of preclinical pain models,[6] and attenuated airway inflammation in models of asthma.[7] In addition, genetic ablation experiments have shown that TRPA1 acts downstream of the pronociceptive agent bradykinin,[3d] further implicating TRPA1 in pain and inflammatory pathways. In vivo experiments using TRPA1 inhibitors have yielded similar results to genetic studies, indicating that inhibition of TRPA1 can produce analgesic and anti-inflammatory effects, and underscoring the potential of TRPA1 as a pharmacological target. At present, four classes of TRPA1 antagonist have been disclosed (Fig. 2). These are the oxime AP18 **5** from the research group of Patapoutian,[8] a series of trichlorosulfide antagonists from Amgen, for example, AMG7160 **6** (related in structure to the TRPA1 agonist, mustard gas **7**),[9] a diaminohexane derivative **8** (Patapoutian)[8a] and

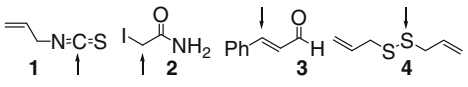

Figure 1. Representative covalent agonists of TRPA1 (site of reaction with cytoplasmic N-terminal cysteine residues denoted by arrow).

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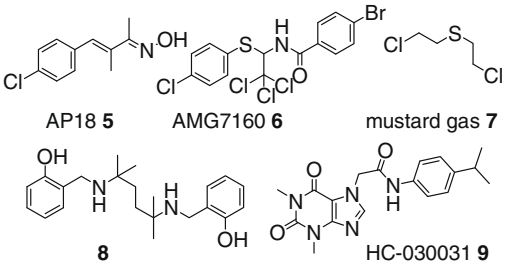
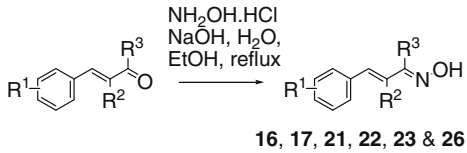


Figure 2. Literature antagonists of TRPA1.

a series of purine acetamides, exemplified by HC-030031 **9**, from Hydra Biosciences.[10] While the exact mechanism of action for these antagonists is not known, it is possible that compounds such as **5** and **6** are capable of covalently modifying TRPA1, but are unable to trigger a conformational change that would result in channel activation. In this Letter we explore the structure–activity relationship of simple analogues related to AP18 **5**, in an effort to characterize the determinants of agonist and antagonist activities against human TRPA1. Our results may suggest that AP18 behaves as a covalent modifier of TRPA1. Additionally, we detail 3-methyl-4-phenylbut-3-en-2-one oxime **11**, a congener of AP18, as a new antagonist of the TRPA1 receptor.[11]

In order to undertake a preliminary examination of the structure–activity relationships (SAR) around AP18, a number of related oximes were purchased from commercial sources. Additionally, further oximes were prepared using the chemistry detailed in Scheme 1. Thus, an appropriate cinnamaldehyde or benzylideneacetone starting material was reacted with hydroxylamine hydrochloride, in a mixture of aqueous NaOH and EtOH, to afford the desired oxime product.[12] For some reactions, both oxime geometrical isomers could be isolated; the (*E*)-isomer was presumed to predominate for such reactions.[12b,c] In total, 19 oximes were purchased, or synthesized, for evaluation as modulators of TRPA1 (Fig. 3).[13]

Compounds were tested for an ability to agonize the TRPA1 ion-channel by assessing the influx of ^{45}Ca into TRPA1-expressing cells upon exposure to test compounds. Cinnamaldehyde (CA) was used as a positive control in each experiment. The results from our investigations are shown in Table 1.[13] Some notable features in the structure–activity relationships (SAR) were apparent. As expected AP18 **5**, displays no agonist activity at the TRPA1 receptor. Likewise, when the *para*-chloro group of AP18 was moved to the *ortho*-position (compound **10**), or was replaced with a hydrogen atom (compound **11**), or nitro group (compound **12**), no agonist activity was observed (entries 2–5). However, when the chloro group of AP18 was replaced with a methoxy substituent, to give compound **13**, weak agonist activity at TRPA1 was noted (entry 6). This agonist activity was even more pronounced when



Scheme 1. Preparation of oximes from aldehyde or ketone precursors.

the *para*-chloro group of AP18 was replaced with a *meta*-methyl substituent; the resulting compound (**14**) had comparable agonist activity to CA (entry 7). Deleting one, or more methyl groups in AP18 also had a marked effect upon TRPA1 agonist activity. For example, deletion of the C_3 methyl group in AP18 resulted in the emergence of TRPA1 agonist behavior (**15**, entry 8). Deletion of both methyl groups in AP18 gave rise to a particularly potent agonist, which had similar relative efficacy to CA, but was substantially more potent in terms of its EC_{50} (compound **16**; entry 9). Significantly, compound **17**, a geometrical isomer of **16**, was less potent in its agonist behavior at TRPA1 (compare entries 9 and 10).

It is also interesting to note the SAR for compound **11** and its demethylated congeners. For instance, selective deletion of a methyl group in compound **11** gave rise to **18** and **19**, which both showed weak agonist activity at TRPA1 (entries 11 and 12). In contrast to the behavior observed with congeners of AP18 above, deletion of both methyl groups in **11** resulted in a compound (**20**) with no agonist activity (entry 13; compare entries 9 and 13).

Table 1
TRPA1 agonist activity of AP18 and related oximes

Entry	Compound	Agonist	Relative efficacy to CA[a]	EC_{50}[a] (μM)
1	CA **3**	Yes	1.0	51.4 (±5.8)
2	**5**	No	0	nd
3	**10**	No	0	nd
4	**11**	No	0	nd
5	**12**	No	0	nd
6	**13**	Yes	0.23 (*n* = 1)	46.1 (*n* = 1)
7	**14**	Yes	0.84 (±0.03)	14.4 (±3.3)
8	**15**	Yes	0.66 (±0.07)	27.6 (±1.2)
9	**16**	Yes	0.85 (±0.12)	9.4 (±0.6)
10	**17**	Yes	0.46 (*n* = 1)	nd
11	**18**	Yes	0.18 (±0.08)	69.4 (±10.1)
12	**19**	Yes	0.27 (±0.02)	96.6 (±3.2)
13	**20**	No	0	nd
14	**21**	Yes	0.85 (±0.01)	63.1 (±19.4)
15	**22**	Yes	0.21 (±0.15)	nd
16	**23**	Yes	0.13 (±0.03)	nd
17	**24**	Yes	0.87 (±0.07)	15.9 (±9.6)
18	**25**	Yes	0.54 (±0.16)	11.5 (±2.0)
19	**26**	Yes	0.15 (*n* = 1)	nd
20	**27**	Yes	<0.1	nd

[a] Values are means of two or three experiments (standard error is given in brackets). nd = not determined.

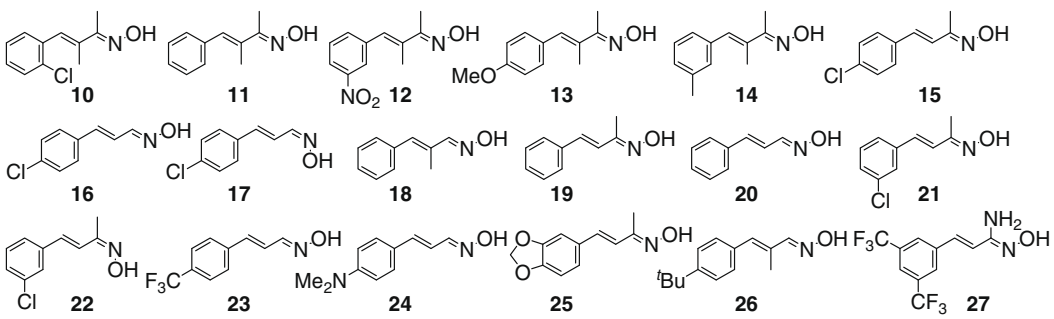


Figure 3. Compounds tested for activity at TRPA1.

Table 2
TRPA1 antagonist activity of oximes related to AP18

Entry	Compound	Antagonist	IC_{50}[a] (μM)
1	**5**	Yes	2.8 (±0.3)
2	**11**	Yes	2.7 (±0.4)
3	**15**	No	nd
4	**18**	No	nd
5	**20**	No	nd
6	**25**	No	nd
7	**26**	No	nd
8	**27**	No	nd

[a] Values are means of two experiments (standard error is given in brackets). nd = not determined.

Another set of oximes (**21**–**27**) were also tested for agonist activity at TRPA1. All of these compounds exhibited agonist behavior, although the magnitude of the effect was variable (entries 14–20). Interestingly, as was seen in the case of compound **16** and its isomer **17** mentioned above, the stereochemistry of the oxime group seemed to play a role in the magnitude of agonist response. Thus, the agonist activity of compound **21** differed significantly to that of its geometrical isomer **22** (compare entries 14 and 15; note the similarities to entries 9 and 10).[14]

A select number of non-agonist oxime analogues, or those with a low relative efficacy, were tested for an ability to antagonise the effect of CA to TRPA1-expressing cells. The results are shown in Table 2.[13] As can be seen, only AP18 **5** and compound **11** were found to act as antagonists. Thus, the results in Tables 1 and 2 indicate that a number of compounds appeared to be neither agonists of TRPA1 nor antagonists of its activation by CA (e.g., compounds **15**, **18**, **20**, **25**–**27**).

Finally, the pA2 values for the antagonists AP18 **5** and **11** were determined using CA as an agonist (Fig. 4). The results indicated that AP18 has a pA2 value of 1.26 ± 0.15 μM, while compound **11** has a pA2 of 1.91 ± 0.09 μM.[15]

The results presented above are significant and may yield insights into the action of AP18 **5** at TRPA1. It is assumed that the activities observed for all oxime agonists presented in Table 1 result from a similar mechanism of action to that observed with CA. That is, the oxime agonists are assumed to act as Michael acceptors, reacting with cysteine residues located in the N-terminal domain of the TRPA1 receptor. This covalent modification results in a conformational change, allowing opening of the ion-channel and an influx of calcium into the cell. For those compounds with reduced efficacy compared to CA, it is not apparent whether this property is due to an inability to react with every cysteine that is modified by CA, a less efficacious transduction of the reaction with cysteines to channel activation, or a difference in 'on–off' rate (i.e., a difference compared to CA in the reversibility of the reaction with cysteines). Oximes **15**–**17**, which are all demethylated analogues of AP18, are agonists of the TRPA1 receptor. Given the structural homology between AP18 and **15**–**17**, it is conceivable that AP18 could be acting as a covalent modifier of the TRPA1 receptor. Under this hypothesis, the presence of the additional methyl group(s) in AP18 prevent the receptor from adopting an active conformation (open ion-channel) despite binding and cysteine modification.[16] In order to gain experimental support for such a model, it would be necessary to characterize fragments of the N-terminal domain of TRPA1 upon exposure to AP18 and **15–17**. The presence of covalently modified cysteine residues can be ascertained from mass-spectrometry measurements. Similar approaches have been used previously to demonstrate that noxious chemicals bind in a covalent manner,[4] and could illuminate the covalent or non-covalent mechanism by which AP18 and the oxime agonists and antagonists described in this Letter modulate TRPA1 activity.

In conclusion, this Letter describes the agonist and antagonist behavior of a set of oximes related to the literature TRPA1 antagonist, AP18. The results indicated that demethylated versions of AP18 behaved as agonists at TRPA1, and may suggest that AP18 is a covalent modifier of the TRPA1 receptor. In addition, our studies revealed that a related AP18 derivative, 3-methyl-4-phenylbut-3-en-2-one oxime **11**, also acted as a TRPA1 antagonist.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.113.

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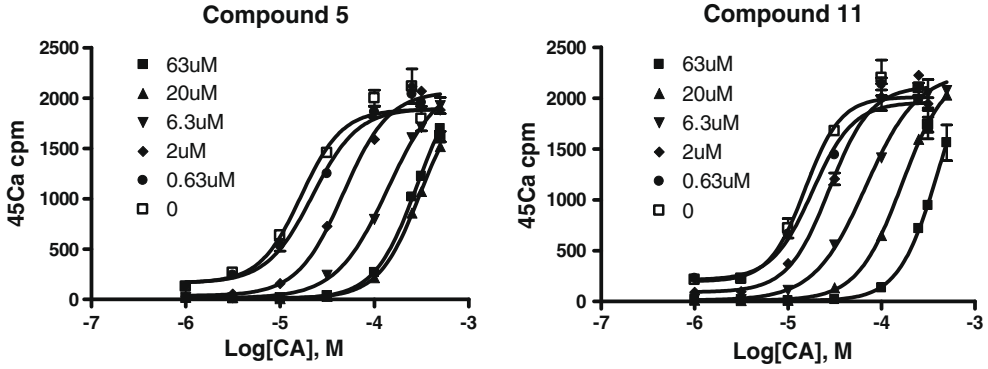

Figure 4. pA2 determination of AP18 **5** and related oxime antagonist **11**.

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11. After the submission of this Letter a series of related oxime derivatives from Abbott Laboratories were also disclosed as antagonists of the TRPA1 receptor. See Perner, R. J.; Kort, M. E.; Didomenico, S.; Chen, J.; Vasudevan, A. WO 2009089083; *Chem. Abstr.* **2009**, *151*, 148153.
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13. See Supplementary data for experimental procedures and a representative copy of the agonist and antagonist dose–response graphs for each compound described in this Letter.
14. A representative selection of substituted cinnamaldehyde and benzylideneacetone derivatives related to the oximes described in Table 1 were also tested for their ability to act as TRPA1 ligands. In each case, we found that the compounds functioned as TRPA1 agonists.
15. See Ref. 8b for the ability of AP18 **5** to act as an antagonist of TRPA1 when cinnamaldehyde, iodoacetamide, mustard oil (allyl isothiocyanate) and noxious cold were used as agonists. Confirmation of the antagonist properties of AP18 using electrophysiology experiments are also described in Ref. 8b.
16. A reviewer of this manuscript proposed that compounds **5** and **11** could also act as antagonists by 'binding' in the same pocket as cinnamaldehyde. However, the presence of additional methyl substitutions prevented reaction with the cysteine residues of TRPA1. We think this hypothesis to be unlikely, since literature suggests that chemical modulation of TRPA1 is dictated by the reactivity of a molecule, rather than its shape (see Refs. 1 and 4 for further information).