Bioorganic & Medicinal Chemistry Letters 22 (2012) 5485–5492

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

N-1-Alkyl-2-oxo-2-aryl amides as novel antagonists of the TRPA1 receptor

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ARTICLE INFO

Article history:
Received 12 June 2012
Revised 4 July 2012
Accepted 6 July 2012
Available online 14 July 2012

Keywords:
Ion channel
TRPA1
Antagonist

ABSTRACT

A series of potent antagonists of the ion channel transient receptor potential A1 (TRPA1) was developed by modifying lead structure **16** that was discovered by high-throughput screening. Based on lead compound **16**, a SAR was established, showing a narrow region at the nitro-aromatic R^1 moiety and at the warhead, while the R^2 side had a much wider scope including ureas and carbamates. Compound **16** inhibits Ca^{2+}-activated TRPA1 currents reversibly in whole cell patch clamp experiments, indicating that under in vivo conditions, it does not react covalently, despite its potentially electrophilic ketone.

TRPA1 is a nonselective cation channel,[1] which has gained high interest as a target for future analgesics during recent years.[2] A large number of agonists for TRPA1 have been reported,[3] many of them being of electrophilic nature such as acrolein (**1**),[4] allyl isothiocyanate (**2**),[5] allicin (**3**),[6] cinnamaldehyde (**4**),[4] ozone[7] or the highly potent CR (**5**) and other tear gas ingredients (Fig. 1).[8] It has been speculated that TRPA1 is a part of a warning system against noxious chemicals.[9]

But also reversible TRPA1 agonists have been found, such as Δ^9-tetrahydrocannabinol (**6**),[10] nifedipine (**7**),[11] the TRPM8 agonist menthol (**8**),[12] the TRPV1 agonist BCTC (**9**),[13] acetic acid (**10**),[14] or carbon dioxide.[15] Even simple ions like intracellular Ca^{2+} and Zn^{2+} have been reported to open the TRPA1 channel.[16]

In contrast, far fewer antagonists have been reported, many of them with a very limited scope (Fig 2). Most appear in patent applications such as de-aza caffeine-derived compound **11** (Hydra)[17] and similar compounds **12** (Glenmark)[18] tricyclic pyrimidones and –thiones (**13**, Janssen)[19] decalines (**14**, Merck)[20] and trichloro(sulfanyl)ethyl benzamides (**15**, Amgen).[21]

Our aim was to indentify novel TRPA1 antagonists and in order to avoid covalently binding activators we developed a fluorescent assay where Ca^{2+} was used as stimulating agonist of TRPA1. Ca^{2+} has been suggested to bind to the EF-hand motif in the intracellular N-terminus of the channel.[22,23] This assay was used in a HTS campaign and led to the finding of the potent singleton **16** (Fig. 3). This compound features two groups, a nitro, and an aromatic (electrophilic) ketone, which we sought to replace. The nitro group since it is known to impact several drug properties (e.g. solubility) and is known to carry toxicity risks, the latter also associated with ketone groups.[24]

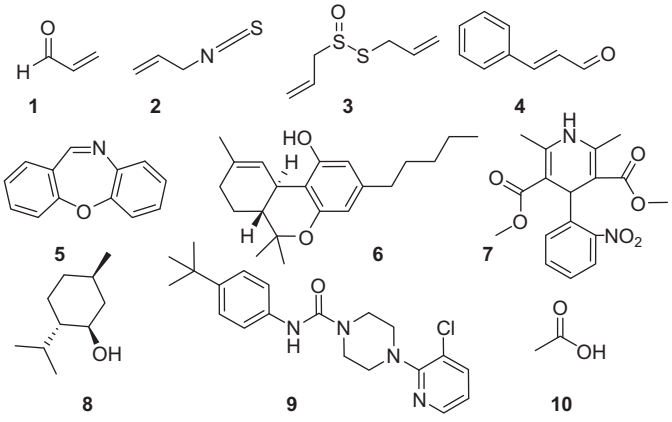

Figure 1. TRPA1 agonists.

During the course of the project we switched the agonist in the assay from Ca^{2+} to Zn^{2+} for reasons of assay stability and a possible in vitro/in vivo translation. The Ca^{2+} assay initially had stability

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http://dx.doi.org/10.1016/j.bmcl.2012.07.032

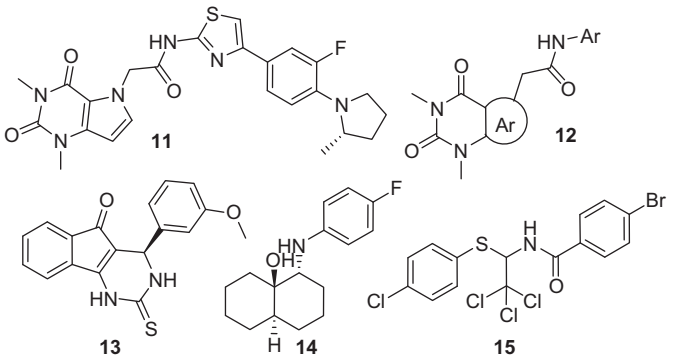


Figure 2. TRPA1 antagonists.

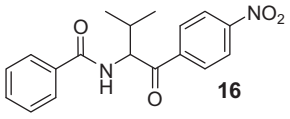


Figure 3. Lead compound.

issues, which later were recognized to depend on the sporadic addition of probenicid, an inhibitor for the anion-exchange protein in Chinese hamster ovar (CHO) cells. The root cause was (at that time the unknown fact) that probenicid is a TRPA1 agonist.[25,26] Once the issues due to probenicid in the Ca^{2+} assay had been identified and the results from the effected experiments had been discarded, the Zn^{2+} had been developed as well. Zn^{2+} is a TRPA1 agonist that has been suggested to interact with an intracellular C-terminal site in the channel.[27–29] Zn^{2+} has been shown to induce nocifensive behaviour via activation of TRPA1[27] and using an in vitro assay with Zn^{2+}as agonist may be of translational value as the relationship between in vitro IC_{50} values and effect of in vivo models would be clearer and easier to interpret. In the series described here no major differences in antagonistic potency (IC_{50}) were found when the agonist was changed. When comparing the assays the compound IC_{50} values correlated well and the ranking order of potency stayed the same (Supplementary data) and we therefore decided to treat the data as if it came from *one* assay regardless of the agonist used. This explains why some of the compounds in this study only have IC_{50} values in either the Zn^{2+} or the Ca^{2+} assay. However, for a structurally different series there was a clear difference between the assays and those observations will be published later on.[30] To be certain that the observed inhibition was not due to auto fluorescence caused by potential inherent spectrophotometric properties of test compounds, the activity on all compounds with pIC_{50} >5 were confirmed in a automated electrophysiology assay on IonWorks (Molecular Devices, data not shown).

Herein we report our SAR study effort divided into three major parts: Substitution modifications at positions R^1, R^2 and of the warhead (Fig. 4, Tables 1–3).

We initially prepared a series of substituted phenyl moieties in the R^1 region and the SAR is summarized in Table 1. Introduction of a halogen at the *para* position (compounds **18–20**), increased binding activity was found compared to the unsubstituted phenyl **17**, especially with chlorine (**19a**). The phenyl substitution SAR seemed rather narrow and by moving the chlorine to the meta position all activity was lost (**21**). Dichloride substitution though was accepted (**22**). Further efforts to replace the nitro group in the highly potent compound **16** resulted in decreased activity, for example, nitrile in **23**, or no activity at all when changing to sulfone group (**24**) or amino moieties as in **25–26**. By separating racemic **19a** we observed that only one isomer was active, **19b** versus **19c.** Early attempts to crystallise a single enantiomer failed, as well as efforts to synthesise compounds starting from enantiomerically pure valine, which led to inconclusive outcomes, most probably due to racemisation issues. On the other hand, the absolute configuration of a similar compound sharing the same warhead has been determined to be (*R*) via vibrational circular dicroism (VCD) experiments.[31]

We next turned our attention to the impact on in vivo potency of the R^2 substituents. As summarized in Table 2, SAR at this side of the warhead seem to be broader compared to the R^1 region. Benzonitrile (**23**) was used as the default R^1 analogue due to its good solubility of 150 μM.[32] Both electron withdrawing and donating groups were tolerated on the phenyl ring. Chlorine in the *para* position (**27**) showed a significant increase in activity compared to the unsubstituted phenyl ring (**23**) and the electron-rich methoxy group (**28**). Disubstituted phenyl rings with di-chloro (**29**), or Cl/F (**30**, **31**) showed activities similar to the plain phenyl (**23**). Interestingly, introduction of CF_3 in the 5-position in combination with Cl in the 2-position showed high activity (**32**). Removing the chloro substituent at the 2-position furnished a 10-fold activity drop (**33**). Introducing heteroaromatic groups at the R^2 position tolerated as well, especially with a bulky alkyl group as the ortho-substituent (**34**–**37**). Even a long, non-functionalized alkyl group as the R^2 moiety was found active (**38**). Results from the introduction of an amino or ether linker at the R^2 position, which generated ureas and carbamates, are disclosed in Table 3. Secondary ureas showed no or low activity, whereas N-alkylation increased potency significantly (**39–44**). The corresponding carbamate analogues showed similar activities (**45** and **46**). We also discovered that by introducing a fluoro atom at the meta position in the R^1 region the activity was increased (**47**). Making the R^1 region less lipophilic by use of a pyridine (**48**) led to loss of activity. However, no general correlation between lipophilicity and potency in the Zn^{2+} assay was observed, when the 31 compounds active in the zinc assay were examined.[33]

As shown in Table 4, the linker region had a very tight SAR. Lipophilic bulky *iso*-propyl-(**19a**) and *tert*-butyl groups (**49**), as the alkyl part were tolerated. The cyclopropyl group as in **50** was acceptable, whereas, surprisingly, the unbranched *n*-propyl (**51**) had no in vitro activity at all. The attempt to remove potential racemisation issues of the chiral sp^3 carbon by introducing an additional methyl group resulted in reduced potency (**52**).[34] Rigidification of the *tert*-butyl group to bicyclic **53** was unfavourable as was making the symmetric cyclobutyl compound **54**. Removing the central alkyl chain resulted in inactive **55**. Combining high potency fragments from R^1, R^2 and the alkyl chain generated active

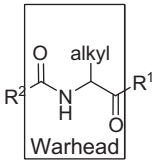
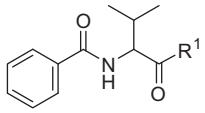
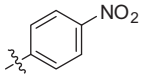
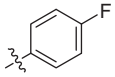
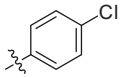
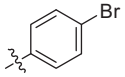
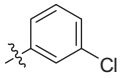
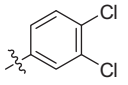
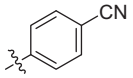
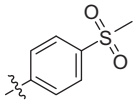
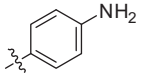
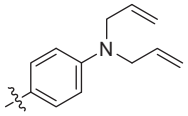


Figure 4. Warhead.

Table 1
SAR at the R^1 region



Compound[a]	R^1	pIC_{50} Zn^{2+},[b]	pIC_{50}, Ca^{2+},[b]
16		6.6	6.5
17		<4.5[c]	NV
18		5.1	5.3
19		**19a**, 5.9, racemate **19b**, 6.4, isomer 1 **19c**, NV, isomer 2	5.6 6.0 NV
20		5.9	5.1
21		<4.5[c]	NV
22		NR	5.4
23		NR	5.4
24		NV	NV
25		NV	NV
26		<4.5[c]	NV

[a] All compounds were racemates except **19b–c**.
[b] NV = no value (no observed effect), NR = not run.
[c] pIC_{50} <4.5 = 30–50% inhibition in highest concentration.

substance **56** with a good solubility profile (80 μM).[32] The nitro bioisostere benzooxodiazole **57** was active. Analogue **58** with the original R^1 moiety was highly active, although with an unfavourable solubility of less than 1 μM.[32]

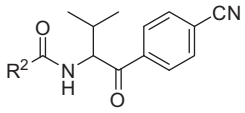
Introduction of a methylene spacer in the linker region had a negative impact in compounds tested (Fig 5 and 59–61). As pointed out initially, we wanted to replace the ketone functionality, due to potential toxicity liabilities. Generating isosteres by removing the carbonyl (**62**), reducing the ketone (**63**), ring formation to the R^1 region to benzotriazole **64**, or sulfonamide **65** all led to inactive compounds. Methylation of the nitrogen showed that an N–H as donor is crucial for the activity (**66**). Efforts to move the H-donor function to the aromatic R^2 moiety (**67**), or to change the amide to sulfonamide **68**, as well as reversing the amide as in **69**, also generated only compounds which lacked activity.

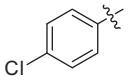
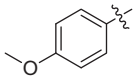
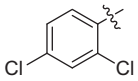
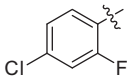
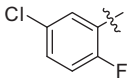
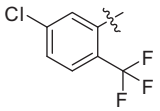
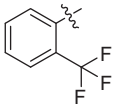
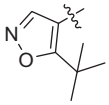
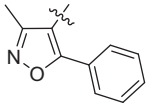
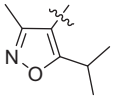
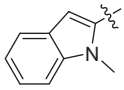
Whole cell patch clamp experiments were performed to confirm whether the ketone containing original HTS hit **16** acted on TRPA1 in a reversible manner. Application of 10 μM compound **16** resulted in a rapid and complete inhibition of Ca^{2+} activated TRPA1 currents. Washout of **16** led to full recovery of the TRPA1 currents within seconds.[35] The reversibility of inhibition suggests that the mechanism of TRPA1 inhibition by **16** does not involve the irreversible formation of covalent bonds with the ion channel protein.

With regards to the general in vivo safety of ketones in drugs, there seems to be a lack of general studies and no generic standard assays are available. Generally, a ketone moiety could be expected to react with amines (lysine or N-termini), thiols (cysteine) or alcohols (serine). Although covalent bonds could be formed, the hemiacetal-type products from serine or cysteine would be instable. For example, a hemiacetal from a ketone and Porcine Pancreatic Elastase has been reported,[36] but could only be observed in the enzyme itself. The reaction of a ketone with a lysine moiety would result in an imine, which also can be a reversible reaction. Mutation studies suggest that the described compounds most likely bind in the pore region of TRPA1.[37] As there are no cysteine or lysine residues in that particular region, covalent binding to TRPA1 can be considered to be unlikely. This would be furthermore in accordance to the results of the patch clamp experiments (vide supra).

In addition, the general reactivity of 15 compounds was determined in a human microsome gluthathione (GSH) trapping assay.[38] Of those tested, nine compounds did not show any adducts

Table 2
SAR at the R^2 region



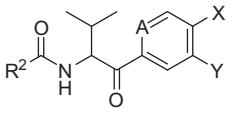
Compound	R^2	pIC_{50} Zn^{2+}	pIC_{50} Ca^{2+}
23		NR	5.4
27		NR	6.4
28		5.2	4.9
29		5.6	5.3
30		5.4	5.5
31		5.7	NV
32		6.5	6.4
33		5.3	5.5
34		6.3	6.0
35		5.4	5.1
36		5.7	5.8
37		5.8	5.4
38		6.0	5.7

(among them compound **16**), 7 showed GSH-adducts on levels between 17% and 40% compared to the standard clozapine and only 1 compound formed GSH-adducts in high rate: **41** was found 6 times more reactive as clozapine. However, there was no correlation between TRPA1 inhibition and adduct formation. Although this assay typically is used to determine the risk of reactive metabolite formation, it can be used as an indicator for reactive group containing compounds, as would be the case to see whether or not the ketone moiety would react with either a free amino or free thiol group (in glutathione) and thus useful as an indicator for potential disadvantageous covalent binding to proteins in e.g. the liver or other organs in vivo. The present warhead does not seem to show this type of reactive behaviour.

The majority of the compounds have been prepared by the route described in Scheme 1, where protected amino acid **49–0** was converted to Weinreb-amide **49–1**, which was reacted with a Grignard-reagent to form the ketone functionality in **49–2**. The amine was then de-protected (**49–3**) and reacted with an acid chloride, alternatively with an acid and an appropriate coupling reagent, to form the desired product **49**. The alternative route described in Scheme 2 made use of an intermediate 4-isopropyl-2-phenyloxazol-5(4*H*)-one (**19a–1**), which was treated with an acid chloride and subsequently hydrolyzed to form the desired product **19a**.[39]

In summary, a series of potent TRPA1 inhibitors were developed by modifying lead structure **16**. Based on the lead compound, SAR

Table 3
Ureas and carbamates



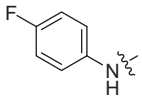
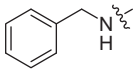
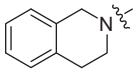
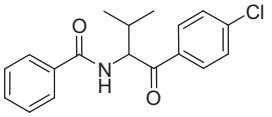
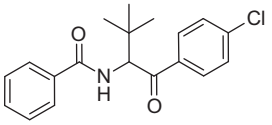
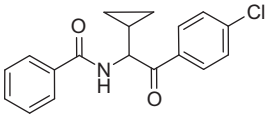
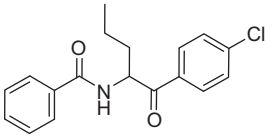
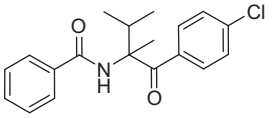
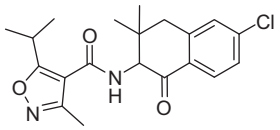
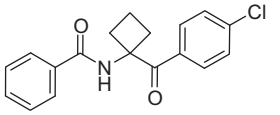
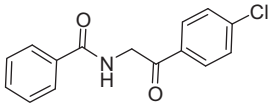
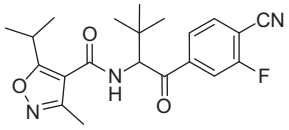
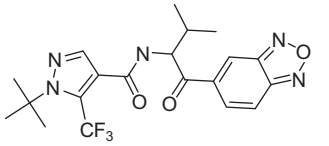
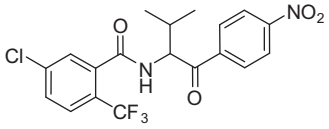
Compound	R^2	X/Y/A	pIC_{50} Zn^{2+}	pIC_{50} Ca^{2+}
39		CN/H/C	NV	NV
40		CN/H/C	NV	NV
41		CN/H/C	5.3	5.3
42		CN/H/C	NR	5.4
43		Cl/H/C	6.2	6.6
44		Cl/H/C	5.6	5.7
45		Cl/H/C	6.1	6.0
46		CN/H/C	5.6	5.2
47		CN/F/C	5.7	5.9
48		Cl/H/N	4.8	5.3

Table 4
SAR at the warhead

Compound	Structure	pIC_{50} Zn^{2+}[a]	pIC_{50} Ca^{2+}[a]
19a		5.9	5.6
49		6.4	6.0
50		5.7	5.7
51		NV	NV
52		NV	5.5

(*continued on next page*)

Table 4 (*continued*)

Compound	Structure	pIC_{50} Zn^{2+}[a]	pIC_{50} Ca^{2+}[a]
53		NV	NR
54		NV	NV
55		NV	NV
56		6.7	NR
57		6.4	NR
58		8.0	NR

[a] NV = no value (no observed effect), NR = not run.

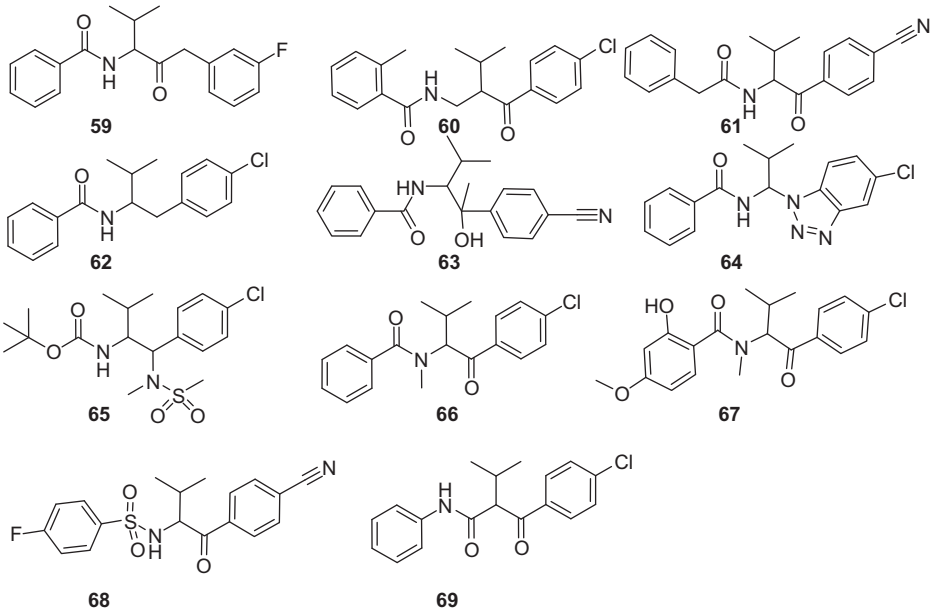
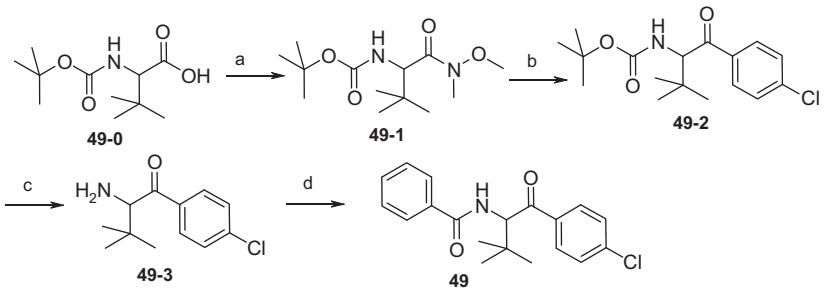
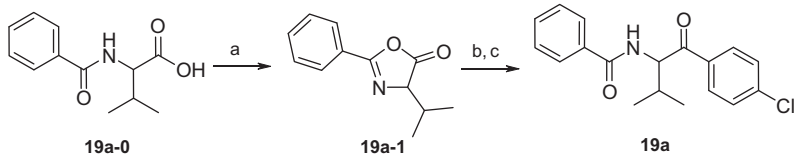


Figure 5. Warhead changes, leading to inactive compounds.

studies have been focused on modifications at the R^1- and R^2-position, as well as the warhead. Despite these efforts, nitro-containing compounds remained among the most potent, with low solubility severely limiting in vivo testing potential. Two different control studies could conclude that the presence of an aromatic ketone in the inhibitor has no negative influence: (1) Patch clamp studies showed that **16** does not form unwanted covalent bonds to the channel protein, and (2) GSH-trapping experiments showed little or no reactivity in the assay for many compounds, among them the original hit **16**.



Scheme 1. Reagents and conditions: (a) *N1*-((ethylimino)methylene)-*N3,N3*-dimethylpropane-1,3-diamine hydrochloride, *N,O*-dimethylhydroxylamine hydrochloride, *N*-ethyl-*N*-isopropylpropan-2-amine, DCM, rt, 48 h; (b) (4-chlorophenyl)magnesium bromide, THF, rt, 16 h; (c) 5–6 N HCl in 2-propanol, MeOH, rt, 16 h; (d) benzoyl chloride, pyridine, 0–25 °C, 16 h.



Scheme 2. Reagents and conditions: (a) ethyl chlorocarbonate, triethylamine, THF, 0–25 °C 1 h; (b) 4-chlorobenzoyl chloride, $MgCl_2$, triethylamine, THF, 0–25 °C, 30 min; (c) 6 M HCl, 0–50 °C, 30 min.

Acknowledgments

The authors would like to thank Erwan Arzel, Robert Pendrill, Jonas Johansson, Anna Laurell, Sandra Lindberg, Jens Nolsöe and the chemists at Naeja for the synthesis of several of the analogues.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.07.032.

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