Bioorganic & Medicinal Chemistry Letters 28 (2018) 2153–2158

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

N-aryl-piperidine-4-carboxamides as a novel class of potent inhibitors of MALT1 proteolytic activity

Achim Schlapbach *, Laszlo Revesz, Carole Pissot Soldermann, Thomas Zoller, Catherine H. Régnier, Frédéric Bornancin, Thomas Radimerski, Jutta Blank, Ansgar Schuffenhauer, Martin Renatus, Paulus Erbel, Samu Melkko, Richard Heng, Oliver Simic, Ralf Endres, Markus Wartmann, Jean Quancard

Novartis Institutes for BioMedical Research, CH-4002 Basel, Switzerland

ARTICLE INFO

Article history:
Received 20 February 2018
Revised 16 April 2018
Accepted 8 May 2018
Available online 9 May 2018

Keywords:
MALT1
Paracaspase
Protease inhibitors
Autoimmune disease
B-cell lymphoma

ABSTRACT

Starting from a weak screening hit, potent and selective inhibitors of the MALT1 protease function were elaborated. Advanced compounds displayed high potency in biochemical and cellular assays. Compounds showed activity in a mechanistic Jurkat T cell activation assay as well as in the B-cell lymphoma line OCI-Ly3, which suggests potential use of MALT1 inhibitors in the treatment of autoimmune diseases as well as B-cell lymphomas with a dysregulated NF-κB pathway. Initially, rat pharmacokinetic properties of this compound series were dominated by very high clearance which could be linked to amide cleavage. Using a rat hepatocyte assay a good *in vitro-in vivo* correlation could be established which led to the identification of compounds with improved PK properties.

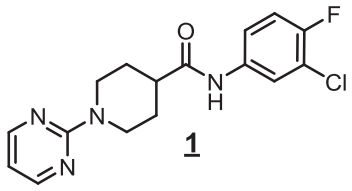
The NF-κB pathway is of chief importance in immunity and cancer biology.[1] Attempts to target this pathway by small molecule inhibitors have had little success so far, essentially because NF-κB is required for tissue homeostasis, in particular in the liver.[2] As a consequence, full blockade of this pathway turned out to be associated with toxicities which prevented therapeutic use of this concept.[2] Mucosa-associated lymphoid tissue lymphoma translocation protein 1 (MALT1) plays a key role in NF-κB pathway activation, in particular in T and B lymphocytes.[3,4] Upon antigen receptor stimulation, MALT1 is recruited to CARD11 (CARMA1) together with BCL10 to form the 'CBM' complex that triggers inhibitor of kappa-B kinase (IKK) activation. Akin to the structurally related caspases, MALT1 (aka paracaspase 1) possesses an original cysteine protease domain. However, in contrast to caspases, MALT1 cleaves substrates at arginine residues.[5] X-ray crystal structures of the MALT1 catalytic domain show a dimeric organization in a classical caspase-like fold.[6] Several studies using mouse models of MALT1 deficiencies have shown that MALT1 plays a role in autoimmune diseases.[7] Furthermore, first MALT1 inhibitors and their activity *in vitro* and in animal models have been disclosed.[8] Collectively, this provides evidence to support MALT1 as an attractive novel protease target for development of small molecule inhibitors with the potential to dampen NF-κB signaling in B-cell lymphomas and autoimmune diseases.[9]

Following the discovery of MALT1's protease function and its key role in lymphocyte signaling[3] a high throughput screen was initiated to search for compounds which inhibit the cleavage of an artificial MALT1 substrate.[10] In addition to several known, mostly irreversible, cysteine protease inhibitors, compound **1** devoid of any classical covalent warhead functionality was discovered (Fig. 1). We observed that **1** not only inhibited the MALT1 protease function *in vitro* with an IC_{50} of 1.6 μM, but that it also displayed activity in a cellular reporter gene assay (RGA), measuring NF-κB activation in a HEK293 reporter cell line stably transfected with the fusion protein cIAP2-MALT1.[10,11] Furthermore, compound **1** inhibited the cleavage of one of the MALT1 substrates, BCL10 in the OCI-Ly3 cell line which is characterized by constitutive CBM assembly, resulting in high spontaneous MALT1 activity.[12,13] Based on these results an optimization campaign was initiated.

For SAR studies the molecule was derivatized in three major parts, the piperidine substituents, the core piperidine moiety and the amide substituent. Substituted benzenes and 6-ring heterocycles were tolerated as piperidine substituents (Table 1). Aliphatic *N*-substituents or a benzyl group led to inactive molecules (compounds **4** and **5**). The pyrimidine as it is present in the hit structure could be replaced by a phenyl-, a pyridyl- or pyridazyl-ring (compounds **2**, **3** and **10**). Substitution in *meta*-position led to reduced

* Corresponding author.
E-mail address: achim.schlapbach@novartis.com (A. Schlapbach).

https://doi.org/10.1016/j.bmcl.2018.05.017
0960-894X/



1

MALT1 biochemical: 1.6 μM
MALT1 cellular (RGA): 3.1 μM
BCL10 cleavage: 5.4 μM
Solubility pH 6.8: 21 μM
cLogP: 2.3

Fig. 1. Chemical structure and properties of HTS hit compound **1.**

potency (compound **6**); however a significant increase in potency was achieved by adding *ortho*-substituents, in particular lipophilic substituents like chlorine (compound **8**). Interestingly, the same increase in potency could be achieved by addition of an ortho amino group (compound **9**). With the exception of compound 8 which appears to be unexpectedly potent in our biochemical assay, biochemical activity generally translated well into cellular potency as measured in a NF-κB reporter gene assay, or more directly, by measuring inhibition of cleavage of the MALT1 substrate BCL10.

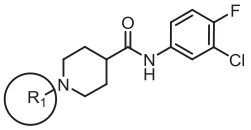
An aromatic group was required as amide substituent, as compound **11** containing a cyclohexyl substituent was devoid of any activity while the unsubstituted phenyl derivative **12** retained potency (Table 2). Lipophilic substituents, e.g. a chlorine in the *meta*-position increased potency tremendously (compound **13**). The *para*-position was very tolerant of modifications and several substitutions could lead to further potency increase. As such a para fluoro- or methoxy-group yielded potent inhibitors (**8** and **14**, respectively) and a triazole (1,2,3 or 1,2,4) was found to be the most beneficial *para*-substituent (compounds **15**–**18**). The triazole substituent could be further substituted, e.g. by an additional amide as in **18**. Ortho-substituents were not tolerated (not shown), and the phenyl ring could be replaced by a 3-pyridyl-substituent without major impact on potency, e.g. **16** vs. **17**.

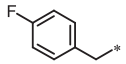
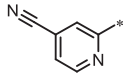
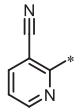
With potent MALT1 inhibitors at hand we turned our attention to analyzing their cellular effects. To assess the effects on T cell activation, selected compounds were tested using an IL-2 reporter gene assay in Jurkat T cells after stimulation with PMA/anti-CD28 monoclonal antibody (mAb).[10] In line with the compounds' effect on the protease function of MALT1, the IL-2 reporter gene was inhibited in the 50–100 nM range (Table 3).

In addition, compounds inhibited OCI-Ly3 cell proliferation with IC_{50} values in the low micromolar range, whereas proliferation of BJAB cells, which do not display constitutive MALT1/NF-kB activity was not affected, indicating that the anti-proliferative effect is linked to MALT1 inhibition[14] (Table 3).

Off-target activity of compound **17** was assessed in an extended selectivity panel. Apart from weak activity (8 μM) on Cox-2, no significant activity was found in a panel of 64 receptors, transporters and enzymes. In particular, only very high micromolar activity was noted in a panel of cysteine proteases.[15]

Table 1
SAR around the piperidine substituent.



	R_1	MALT1 biochemical IC_{50} (μM)	MALT1 cellular NF-κB RGA IC_{50} (μM)	MALT1 cellular BCL10 cleavage EC_{50} (μM)
1		1.62	3.1	5.4
2		0.82	3.2	9.2
3		1.90	n.d.	n.d.
4		>100	n.d.	n.d.
5		>100	n.d.	n.d.
6		9.0	n.d.	n.d.
7		0.42	2.7	2.1
8		0.028	1.35	1.5
9		0.10	0.42	0.54
10		0.10	0.29	0.40

For detailed assay descriptions see [10,13]. The IC_{50} values are averages of at least 2 separate determinations.