Bioorganic & Medicinal Chemistry Letters 22 (2012) 7326–7329

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design and biological evaluation of imidazo[1,2-*a*]pyridines as novel and potent ASK1 inhibitors

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ARTICLE INFO

Article history:
Received 20 August 2012
Revised 16 October 2012
Accepted 17 October 2012
Available online 23 October 2012

Keywords:
ASK1
Imidazo[1,2-*a*]pyridine
LLE

ABSTRACT

Imidazo[1,2-*a*]pyridine derivatives were designed, synthesized, and evaluated as inhibitors of the apoptosis signal-regulating kinase 1 (ASK1). These were based on a benzothiazole derivative that was discovered from high-throughput screening of our compound library. As a result, we identified potent, selective, and orally bioavailable ASK1 inhibitors for wide range of therapeutic targets.

Apoptosis signal-regulating kinase 1 (ASK1) was originally identified as a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family that activates both p38 MAP kinase and c-Jun N-terminal kinase (JNK) pathways.[1] ASK1 is stimulated by various cell stressors including cytotoxic cytokines, reactive oxygen species (ROS), and endoplasmic reticulum stress. Recent studies revealed that ASK1 contributes not only to the regulation of cell death, but also to cytokine responses, cell differentiation, and immune regulation. Therefore, ASK1 inhibitors are thought to have potential for the protection of cells from various stresses in wide-ranging pathological situations such as autoimmune disease, diabetes, cardiovascular disease, neurodegenerative disorders, and inflammatory disorders.[2,3] For example, ASK1 is activated by the tumor necrosis factor α, which impairs insulin action.[4] A high glucose level also induces activation of the ASK1 signal that mediates cellular senescence, which is assumed to lead to vascular aging in diabetic patients.[5] Thus, ASK1 is related to the pathological situation occurring in relation to diabetes. In addition, amyloid β induces neuronal cell death, which plays a central role in Alzheimer's disease through ROS-mediated ASK1 activation.[6] Furthermore, ASK1 knockout mice inhibited Angiotensin II-induced cardiac hypertrophy.[7] Therefore, inhibition of ASK1 could have great therapeutic potential in terms of the treatment of these diseases. However, there have so far been few reports on selective ASK1 inhibitors.[8–10]

To obtain useful compounds for exploring ASK1 inhibition, we focused our early efforts on the discovery of potent, selective, and orally bioavailable ASK1 inhibitors. We initially identified benzothiazole **1** (IC_{50}: 260 nM), with inhibitory activity against ASK1, by high-throughput screening of our in-house compound library (Fig. 1). On the basis of our speculation that compound **1** is as an ATP-competitive inhibitor, the essential interactions were assumed to be hydrogen bonds between the aminothiazole moiety and Val757 in the hinge region of ASK1.[11] We also speculated that the *tert*-butylphenyl moiety of compound **1** was directed to the solvent-exposed region, while the benzothiazole was located at the inner binding pocket (Fig. 2). On the other hand, compound **1** showed high lipophilicity (log*D*, measured at pH 7.4) and low ligand-lipophilicity efficiency (LLE, pIC_{50}–log*D*), which often raises the issue of undesirable physical properties and ADME (absorption, distribution, metabolism, and elimination) profiles and promotes interactions with other protein kinases and adverse biological activities.[12] To avoid these issues, our approach was aimed at the enhancement of the LLE and the reduction of the lipophilicity through the transformation of the benzothiazole core and the introduction of hydrophilic moieties on compound **1**. First, the 2-aminoimidazo[1,2-*a*]pyridine derivative was designed to reduce the lipophilicity of compound **1**. This scaffold was reported as a new class of ATP-competitive kinase inhibitors.[13,14] As a result, compound **3a** showed a reduction in lipophilicity (log*D*: 3.78). Interestingly, compound **3a** (IC_{50}: 22 nM) was 10 times more potent than compound **1**, which has the benzothiazole with the same substituents as **3a**. This improvement is presumably due to

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http://dx.doi.org/10.1016/j.bmcl.2012.10.084

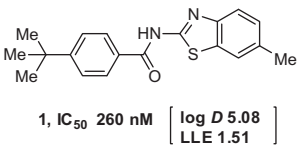


Figure 1. Benzothiazole derivative **1** (hit compound).

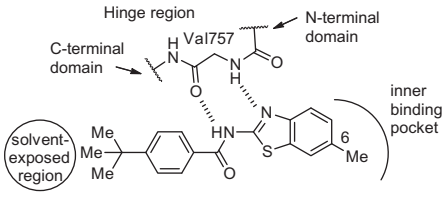
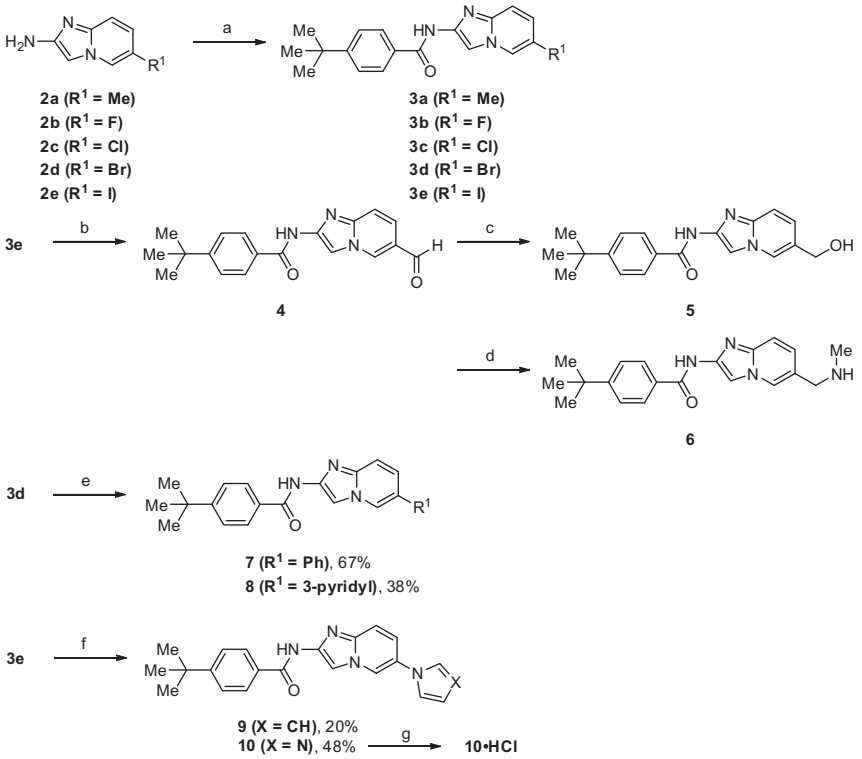


Figure 2. Binding mode of compound **1** in ASK1.

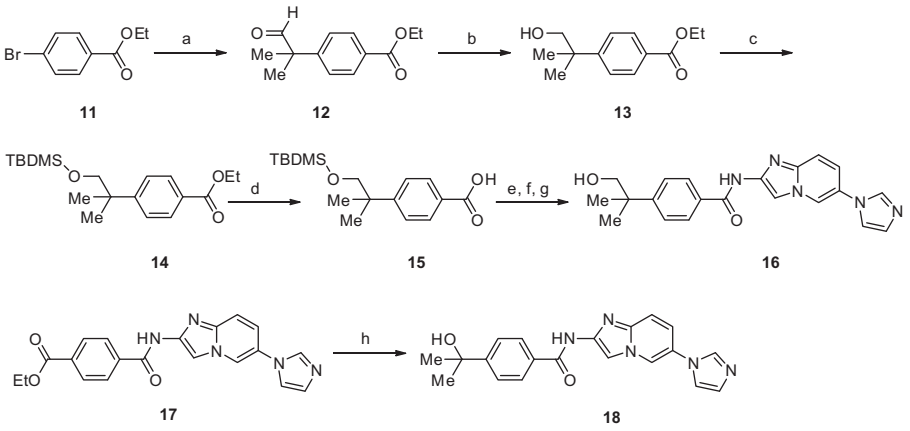
the increase of the pK_a (calc) value on the nitrogen at the 1-position compared to the benzothiazole (**1**: 5.43 versus **3a**: 10.07), enhancing the electron-donating ability. The LLE value also increased dramatically from 1.51 to 3.88, suggesting that the core structure has the potential for further optimization as a proficient ASK1 inhibitor.

2-Aminoimidazo[1,2-*a*]pyridines[15] with several kinds of substituents at the 6-position (**3**, **5–10**) and on the *tert*-butyl moiety (**16** and **18**) were prepared for the investigation of the structure–activity relationship (SAR) (Scheme 1). Aldehyde **4**, leading to compounds **5** and **6**, was prepared from aryl iodide **3e** and *i*-PrMgCl by halogen–metal exchange followed by the addition of DMF as an electrophile.[16] The reaction of aryl iodide **3e** and pyrrole in the presence of a catalytic amount of CuI gave the N–H arylated compound **9**. Imidazole was also used as a coupling substrate to give compound **10**. The arylation of isobutyraldehyde with aryl bromide **11** in the presence of $Pd(OAc)_2$-P(*t*-Bu)$_3$ using Cs_2CO_3 gave the α-arylated aldehyde **12**, which led to the propanol derivative **16** (Scheme 2).[17] Treatment of ester **17**, which was synthesized in a same manner as the procedure in compound **10**, with MeMgBr gave *tert*-alcohol **18**.

Among the halogen-substituted compounds (**3b–d**), the Cl derivative **3c** (IC_{50}: 16 nM) showed potent inhibitory activity (Table 1). Introduction of a hydroxymethyl moiety (**5**) for the reduction of log *D* led to a decrease in inhibitory activity, while the LLE value was comparable to that of compound **3a**, suggesting that the introduction of a polar moiety was tolerable in terms of LLE. To explore the potential of aromatic substituents, we synthesized and evaluated several compounds. The phenyl compound **7** (IC_{50}: 82 nM) had a reduced LLE value (2.26). On the other hand, the 3-pyridyl derivative **8** (IC_{50}: 21 nM) with polar substituents showed not only potent inhibitory activity, but also an efficient LLE value (4.01). The 1-imidazolyl derivative **10** (LLE: 4.69) showed a higher LLE value than the 1-pyrrolyl derivative **9** (LLE: 3.28). These results suggested that the nitrogen atom at the 3-position of the pyridyl and imidazolyl moieties might be involved in an interaction with ASK1. This hypothesis was confirmed when the co-crystal structure of compound **10** with ASK1 revealed direct interaction between the imidazole nitrogen and the side chain of Lys709 (Fig. 3).[18] In addition, several other interactions were observed, including the water-mediated interaction between the amide carbonyl of the compound and Ser761, as shown in Figure 3 (dotted lines). As we expected, two hydrogen bonds interactions between the imidazo[1,2-*a*]pyridine and Val757 were detected in the hinge region. The protein–ligand complex also suggested that

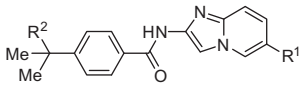


Scheme 1. Synthesis of 6-substituted imidazo[1,2-*a*]pyridine derivatives. Reagents and conditions: (a) 4-*t*-butylbenzoyl chloride, NEt_3, THF, room temp, 10–69%; (b) *i*-PrMgCl, DMF, THF; (c) $LiBH_4$, THF, room temp, 18% (2 steps); (d) $MeNH_2$, $NaBH(OAc)_3$, AcOH, CH_2Cl_2–DMF, room temp, 25% (2 steps); (e) $ArB(OH)_2$, Na_2CO_3, $Pd(PPh_3)_4$, DME-H_2O, 100 °C; (f) imidazole, K_2CO_3, CuI, DMF, reflux, 43%; (g) HCl in ethyl acetate, room temp, 60%.



Scheme 2. Synthesis of compounds **16**, **18**. Reagents and conditions: (a) isobutyraldehyde, Cs_2CO_3, $Pd(OAc)_2$–$P(t\text{-}Bu)_3$, dioxane, reflux; (b) $NaBH_4$, THF, room temp, 44% (2 steps); (c) TBDMSOTf, 2,6-lutidine, CH_2Cl_2, room temp, quant; (d) KOH (aq), MeOH-THF, room temp, 82%; (e) $(COCl)_2$, DMF, CH_2Cl_2 and then **3e**, NEt_3, THF, room temp, 35%; (f) imidazole, K_2CO_3, CuI, DMF, reflux, 54%; (g) TBAF, THF, room temp, 74%; (h) MeMgBr, THF, reflux, 41%.

Table 1
In vitro activities of imidazo[1,2-*a*]pyridine derivatives



Compound	R^1	R^2	IC_{50} (nM)[a]	log*D*[b]	LLE[c]
3a	Me	Me	22	3.78	3.88
3b	F	Me	34	3.55	3.92
3c	Cl	Me	16	4.04	3.76
3d	Br	Me	21	4.17	5.51
5	CH_2OH	Me	129	2.83	4.09
6	CH_2NHMe	Me	870	2.40	3.66
7	1-Phenyl	Me	82	4.83	2.26
8	3-Pyridyl	Me	21	3.60	4.01
9	1-Pyrrolyl	Me	30	4.24	3.28
10	1-imidazolyl	Me	14	3.16	4.69
16	1-imidazolyl	$HOCH_2$	32	1.62	5.87
18	1-imidazolyl	HO	70	1.27	5.88

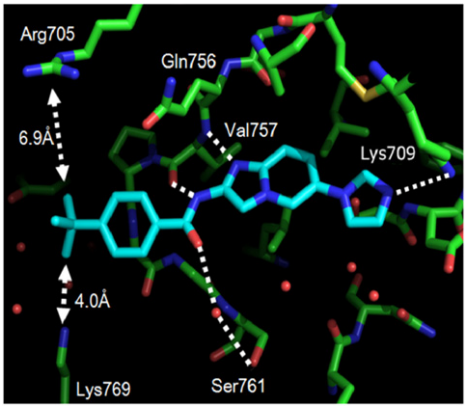
[a] All values are averages of $n = 2$.
[b] The log*D* values were determined at pH 7.4.
[c] The LLE values were determined by pIC_{50}–log*D*.

the nitrogen atoms of the Lys769 and Arg705 side chains were present near the *t*-butyl group in the outer pocket. Compounds **16** and **18**, with a hydroxyl group at the terminal lipophilic moiety, were designed to occupy the hydrophilic space. Consequently, these compounds showed very high LLE values and tolerable IC_{50} values, demonstrating the potential of additional interactions.

Table 2
Selectivity profile of compound **10·HCl** for representative kinases

Kinase	IC_{50} (μM)	Kinase	IC_{50} (μM)
ASK2	0.51	JNK1	>10
MEKK1	>10	p38α	>10
TAK1	>10	gsk3β	>10
IKKβ	>10	PKCθ	>10
ERK1	>10	B-raf	>10

Further in vitro profiles were evaluated using the 1-imidazoly derivatives. For preliminary kinase selectivity among representative kinases, compound **10·HCl** was found to be a selective inhibitor, except for ASK2 (Table 2).[19] As a cell assay of ASK1 inhibitors, the downstream phosphorylation of JNK and p38 was examined using compound **10**. Compound **10** was found to inhibit streptozotocin (STZ)-induced JNK in INS-1 pancreatic β cells from 0.3 μM (Fig. 4).[20] Phosphorylation of p38 was also inhibited in a dose-dependent manner. In addition, pharmacokinetic profiles in rats were tested for the representative compounds. Good oral bioavailability was detected for compounds **10·HCl** and **16** compared to that of the benzothiazole derivative **1** (Table 3). These findings



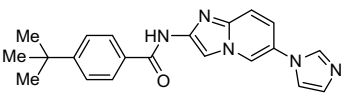


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Figure 3. Co-crystal structure of ASK1 and compound **10**.

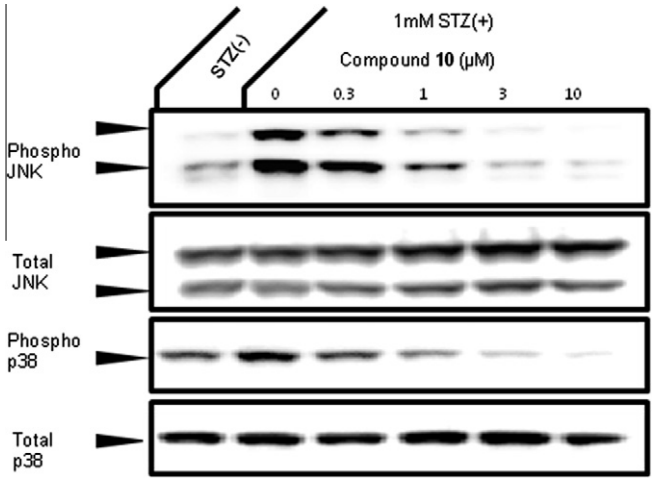

Figure 4. Inhibiton of STZ-induced MAPK phosphorylation in INS-1 cells.

Table 3
Pharmacokinetic parameters of compounds[a]

compound	C_{max} (ng/mL)	T_{max} (h)	$AUC_{po,0-8h}$ (ng.h/mL)	*F* (%)
1	—	—	<10	—
10·HCl	285.1	1.67	275.4	41.1
16	101.3	1.00	407.4	41.0

Average of three rats. *F* means bioavailability.
[a] Rat cassette doing at 0.1 mg/kg, iv and 1 mg/kg, po.

indicated that the lowered lipophilicity might contribute to the improvement of the PK profiles.

In summary, we have identified potent ASK1 inhibitors with high LLE values. This discovery could serve to provide promising and useful compounds for elucidating potential ASK1 inhibitors for medicinal applications; this will be addressed in future studies.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.10.084.

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18. The crystal structure of compound **10** bound to ASK1 has been deposited in the Protein Data Bank with deposition code 3VW6.
19. Compound **10 HCl** was also tested for its inhibitory activities toward a panel of 195 kinases (one dose assay at 1 μM). The inhibitory activities towards 187 kinases were less than 50%.
20. See protocol given in supplementary data.