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Discovery and SAR of 2-amino-5-(thioaryl)thiazoles as potent and selective Itk inhibitors

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Abstract—A series of structurally novel aminothiazole based small molecule inhibitors of Itk were prepared to elucidate their structure–activity relationships (SARs), selectivity, and cell activity in inhibiting IL-2 secretion in a Jurkat T-cell assay. Compound **3** is identified as a potent and selective Itk inhibitor which inhibits anti-TCR antibody induced IL-2 production in mice in vivo and was previously reported to reduce lung inflammation in a mouse model of ovalbumin induced allergy/asthma.

Itk (Interleukin-2-inducible T cell kinases), also known as Emt or Tsk, is expressed mainly in T, natural killer, and mast cells.[1] Itk is tyrosine phosphorylated and activated in response to cross linking of TCR, CD28 or CD2 and has been implicated in thymocyte development and activation of T cells through TCR and CD28 engagement.[2] Gene knockout studies have revealed that mice lacking Itk have fewer T cells, especially $CD4^+$ T cells, and mature T cells isolated from these mice exhibit defective TCR mediated responses such as calcium mobilization, IL-2 secretion, and proliferation.[3] In addition, Itk deficient mice are unable to establish functional Th2 cells resulting in their inability to clear parasitic infections dependent upon a Th2 response.[4] Recent studies have shown that Itk deficient mice have drastically reduced lung inflammation, eosinophil infiltration, and mucous production in response to OVA induced induction of allergic asthma.[5] These studies suggest that a selective Itk inhibitor should be useful as an immunosuppressive and/or anti-inflammatory agent and may be an attractive modulator of dysregulated allergic pathways mediated by Th2 cells.[6]

In an earlier report we described SAR optimization of the directed screening hit **1** that led to the discovery of **2** as a potent and selective Itk inhibitor.[7] In this communication, we outline SAR studies in a related and novel 2-amino-5-thioaryl-thiazole series that culminated in the identification of **3** as a highly potent and selective small molecule Itk inhibitor with excellent activity in reducing T cell proliferation in vitro and Il-2 production in mice in vivo (Fig. 1). As reported earlier,[8] compound **3** reduces lung inflammation in a mouse model of ovalbumin induced allergy/asthma.

The synthesis of compounds related to **3** is shown in Schemes 1–3. Scheme 1 outlines a general route to the carboxamide analogs **9**. Reaction of commercially available 2-amino-5-bromothiazole **4** with the appropriately substituted 3-carboxythiophenol **5** in the presence of sodium methoxide in methanol followed by addition of anhydrous hydrogen chloride in dioxane formed the thioether **6**. Protection of the amino group as a *tert*-butylcarbamate and subsequent base hydrolysis of the methyl ester afforded acid **7**. Coupling of acid **7** with the appropriate amines under standard conditions followed by BOC-deprotection with TFA formed amine **8** which was further coupled with an acid chloride in the presence of pyridine to form **9**.

A similar but more concise route was used for the synthesis of **3**. Reaction of 2-amino-5-bromothiazole **4**

Keywords: Amino-(thioaryl)thiazoles; Selective Itk inhibitor.
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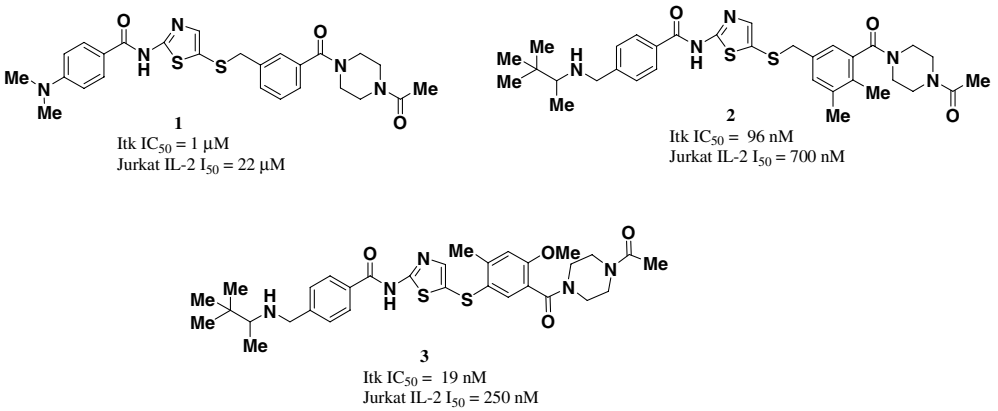
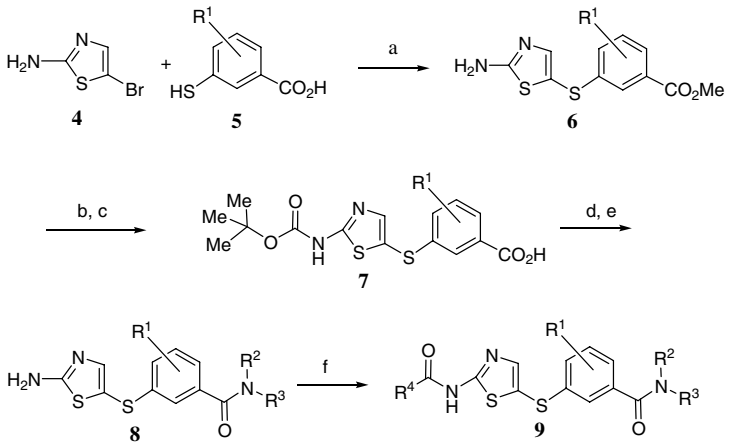
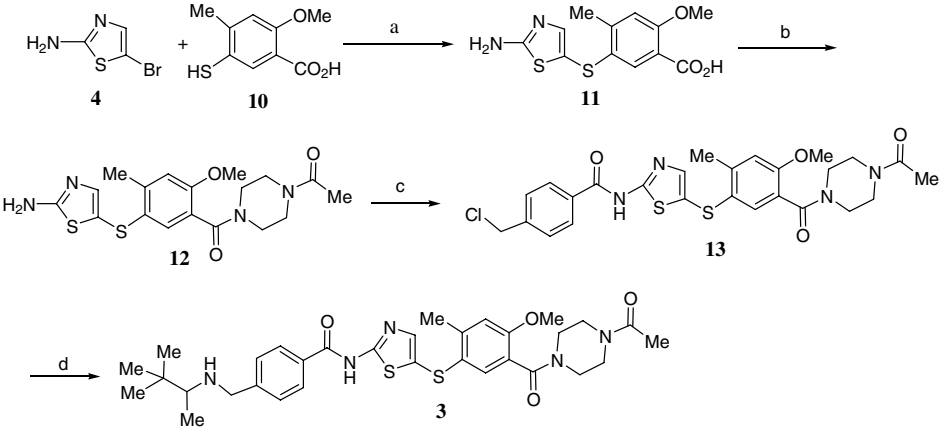


Figure 1. Itk inhibitory activity of aminothiazoles **1**–**3**.



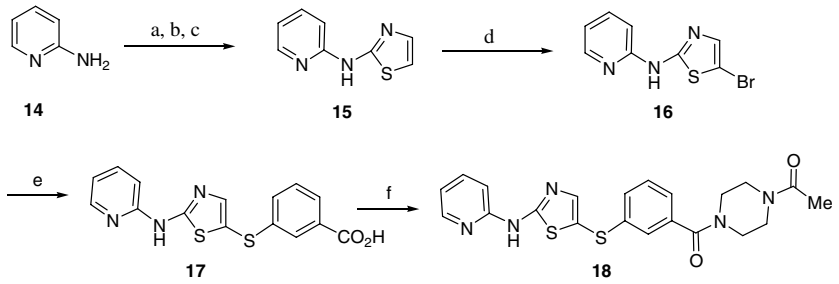
Scheme 1. Reagents: (a) i—NaOMe, MeOH; ii—4 M HCl, dioxane; (b) di-*tert*-butylcarbonate, DMAP, THF; (c) aq NaOH, MeOH; (d) EDAC, HOAt, *i*-Pr_2NEt, R^2R^3NH, THF; (e) TFA; (f) CH_2Cl_2, Py, R^4COCl.



Scheme 2. Reagents: (a) NaOMe, MeOH, 87%; (b) EDAC, HOAt, *i*-Pr_2NEt, *N*-acetylpiperazine, THF, 78%; (c) CH_2Cl_2, *i*-Pr_2NEt, 4-chloromethylbenzoyl chloride; (d) MeOH, 2-amino-3,3-dimethylbutane, 36% overall yield in two steps.

and 3-carboxy-4-methoxy-6-methylthiophenol **10** with methanolic sodium methoxide at 0 °C to rt formed acid **11** which was then treated with *N*-acetylpiperazine to form aminothiazole **12**. Subsequent reaction of **12** with 4-chloromethylbenzoyl chloride and diisopropylethyl amine at ambient temperature formed **13** which upon treatment with 2-amino-3,3-dimethylbutane in refluxing methanol afforded **3**. This synthetic route eliminated the protection/deprotection steps outlined in Scheme 1.

Preparation of 2-heteroarylamine analogs required a different approach as illustrated in Scheme 3 with the synthesis of 2-aminopyridine **18**. Reaction of 2-amino-pyridine **14** with benzoylisothiocyanate in acetone



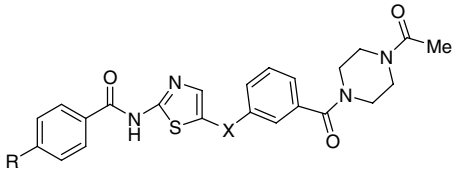
Scheme 3. Reagents: (a) acetone, benzoylisothiocyanate, 100%; (b) aq NaOH, 73%; (c) aq EtOH, chloroacetaldehyde, 79%; (d) bromine, AcOH, 86%; (e) 3-carboxythiophenol, NaOMe, MeOH, 86%; (f) EDAC, HOAt, *i*-Pr_2NEt, *N*-acetylpiperazine, THF, 100%.

followed by basic hydrolysis of the benzoyl protecting group afforded a thiourea intermediate[9] which was condensed with chloroacetaldehyde[10] in aq ethanol to form thiazole **15**. Bromination of **15** with bromine and acetic acid at 40 °C afforded 5-bromothiazole **16** which was then treated with 3-carboxythiophenol to form the acid **17**. Reaction of **17** with *N*-acetylpiperazine under standard conditions afforded 2-pyridylamino derivative **18**. This synthetic route is quite general and was used for the synthesis of other heteroaryl amino derivatives.

Aminothiazole **1** and its analogs were tested for their ability to inhibit the phosphorylation of an exogenous substrate (GST-fused SLP-76)[8] using a recombinant Itk kinase domain as source of the enzyme (IC_{50}, Tables 1–6).

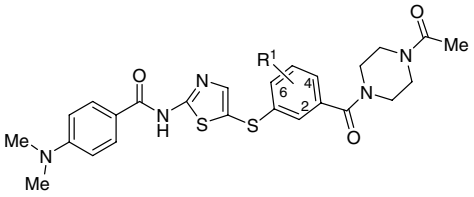
Table 1 outlines the SAR observed with the thiomethyl ether linkage modification of the initial screening hit **1**. Truncation of the thiomethyl linker by deletion of either the methylene group (**9a**) or the sulfur atom (**1a**) retains the biochemical potency of the parent **1**. In the 4-*tert*-butyl benzamide series the thiomethyl linker can be replaced with an extended ether (**1b**) or *N*-methylated amine (**1c**) without any loss of potency. The *N*-desmethyl analog (**1d**) corresponding to **1c** is at least an order of magnitude less potent. Based on its biochemical potency and selectivity over other kinases (data not shown) **9a** was selected for further optimization.

Table 1. SAR for thioether linker modification



Compound	X	R	Itk inhibition IC_{50} (μM)[8]
1	SCH_2	Me_2N	1.0
9a	S	Me_2N	0.97
1a	CH_2	Me_2N	0.82
1b	CH_2OCH_2	*t*-Bu	0.93
1c	$CH_2N(Me)\ CH_2$	*t*-Bu	2.19
1d	$CH_2NH\ CH_2$	*t*-Bu	22.34

Table 2. SAR for thioaryl linker modification

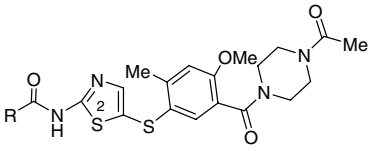


Compound	R^1	Itk inhibition IC_{50} (μM)[8]
9a	H	0.97
9b	2-Me	>25
9c	4-Me	0.29
9d	6-Me	0.94
9e	4-Cl	0.6
9f	4-NH_2	0.09
9g	4-OH	0.03
9h	4-OMe	0.034
9i	4-NHAc	0.25
9j	5-NH_2	1.13
9k	5-CF_3	19.24
9l	4,5-di-Me	0.21
9m	5,6-di-Me	3.9
9n	4,6-di-Me	0.06
9o	4-OH,6-Me	0.012
9p	4-OMe,6-Me	0.018

A profound increase in biochemical potency was observed upon introduction of substituents on the central thiophenyl ring (Table 2). A methyl group scan revealed the importance of such substitution at the C4- or C6-position of the phenyl ring (**9d**–**e**). A wide variety of small substituents (**9e**–**i**) is tolerated at the C4-position. In general electron donating groups led to more potent analogs (**9f**–**h**). A similar trend is observed with respect to substitution at the C5-position of the phenyl ring (**9j**–**k**). Finally disubstitution in the phenyl ring led to some of the more potent analogs. 4-Hydroxy-6-methyl and 4-methoxy-6-methyl analogs **9o** and **9p** are identified as the two most active compounds in this series.

To optimize the potency further, we next turned to the modification of the C2′-benzamide moiety of compound **9p** (Table 3). Replacement of the *N*,*N*-dimethyl group on the benzamide with an ether (**9q**–**r**) has little impact on the biochemical and cell potency of these analogs.

Table 3. SAR for C_2-amide modification



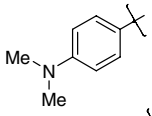
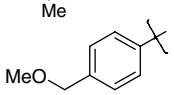
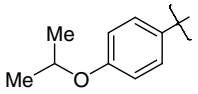
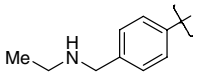
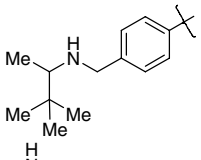
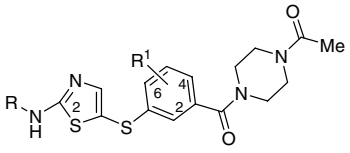
Compound	R	Itk inhibition IC_{50} (μM)[8]	Jurkat Il-2 inhibition IC_{50} (μM)[8]
9p		0.018	0.28
9q		0.02	0.42
9r		0.047	0.36
9s		0.029	3.4
3		0.015	0.25
9t		0.014	0.25
9u		0.009	0.69

Table 4. SAR for C_2 amine modification



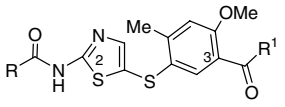
Compound	R	R^1	Itk inhibition IC_{50} (μM)[8]	Jurkat IL-2 inhibition IC_{50} (μM)[8]
18a	Ph	H	34.2	ND[a]
18	2-Pyridyl	H	0.69	24
18b	3-Pyridyl	H	>50	ND[a]
18c	2-Pyrimidinyl	H	46.3	ND[a]
18d	2-Pyrazinyl	H	6.06	ND[a]
18e	2-Thiazolyl	H	7.07	ND[a]
18e	2-Pyridyl	4-OH	0.03	2.18
18f	6-Br-2-pyridyl	4-OH	0.007	0.37
18g	4,6-di-Me-2-pyridyl	4-OH	0.007	0.46
18h	4,6-di-Me-2-pyridyl	4-OMe, 6-Me	0.006	0.23

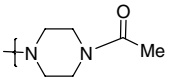
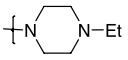
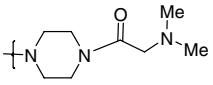
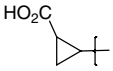
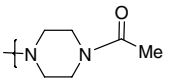
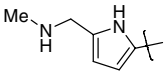
[a] ND, not determined.

Furthermore, the benzylamine analog **9s** is an order of magnitude less potent than the parent **9p** in the cell based assay despite being biochemically equipotent. Introduction of a large hydrophobic group on the benzyl amine led to analog **3** as a highly potent Itk inhibitor both biochemically and in the cell. The benzamide can be replaced with a pyrrolo amide (**9t**) or cyclopropyl amide (**9u**) with no loss of potency in vitro. However, the cyclopropyl amide **9u** is roughly 2-fold less potent in the Jurkat cell based assay.

We also investigated the effect of replacing the C2′-carboxamide with an aryl/heteroaryl amine (Table 4). Replacement with aniline (**18a**) resulted in substantial

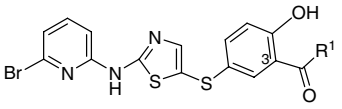
Table 5. SAR for C_2 and C_3 carboxamide modification

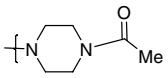
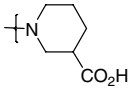
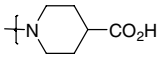
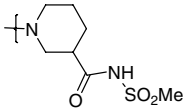


Compound	R	R^1	Itk inhibition IC_{50} (μM)[8]	Jurkat Il-2 inhibition IC_{50} (μM)[8]	MLM rate[a]
9u			0.009	0.69	High
9v			0.26	1.0	High
9w			0.019	0.21	Moderate
9x			0.32	25.32	Low
9y			0.007	0.26	Moderate

[a] MLM (mouse liver microsome) rate (nmol/min/mg of protein): high >0.3; moderate 0.1–0.29; low <0.1.

Table 6. SAR for C_3 carboxamide modification



Compound	R^1	Itk inhibition IC_{50} (μM)[8]	Jurkat Il-2 inhibition IC_{50} (μM)[8]	MLM rate[a]
18f		0.007	0.37	High
18j		0.034	>50	Low
18k		0.015	>50	Low
18l	N(Me)CH2CO_2H	0.37	>10	Low
18m		0.083	>10	Low

[a] MLM (mouse liver microsome) rate (nmol/min/mg of protein): high >0.3; moderate 0.1–0.29; low <0.1.

loss of potency. Heteroaryl amine substitution revealed that only the 2-pyridyl amine analog **18** retained the biochemical potency, while the isomeric pyridine derivative **18b** or other heteroaryl amines (**18c**–**e**) were significantly less potent. In the 2-pyridyl series an increase in potency was achieved by introduction of substituent in the central thiophenyl ring (**18e**). Substitution on the pyridine ring further increased both biochemical and cell potency. Compounds **18f**–**h** were identified as some of the most potent analogs in this series.

Our SAR studies so far identified several extremely potent Itk inhibitors (**3**, **9p**, **9t**, and **18f**–**h**) in this novel 2-amino-5-arylthio thiazole series. However, in vitro metabolism studies with both mouse and human liver enzyme homogenates revealed that this class of analogs

in general suffered from a rapid rate (>0.3 nmol/min/mg of protein) of degradation. To address this issue, we investigated the effect of incorporating an acidic or basic functionality in the molecule that could reduce its affinity for the cytochrome P450 isozymes. Table 5 outlines these results in the C2′-carboxamide series. For example, replacement of the *N*-acetylpiperazine group in the cyclopropyl amide analog **9u** with basic sidechains (**9v–w**) or introduction of a methylaminomethyl functionality in the pyrroloamide analog (**9y**) had little impact on the metabolic degradation rates of these compounds. The metabolically stable carboxylic acid analog **9x** was significantly less potent against the enzyme, and more importantly, in the cellular assay.

A similar trend was observed with the 2-pyridylamino analogs (Table 6). Several metabolically stable analogs were identified through the introduction of an acid (**18j–l**) or an acid surrogate group (**18m**). Some of these analogs (**18j–k**) retained the intrinsic biochemical potency of the parent compound **18f** but lacked any cellular activity in the Jurkat Il-2 inhibition assay. To summarize, the rapid rate of metabolic degradation of this class of compounds could not be addressed without subsequent loss of cellular potency.

Based on its potent enzyme and cellular activity, compound **3** was characterized for enzyme selectivity in vitro. As shown in Table 7, analog **3** exhibited exquisite selectivity over other kinases. It was at least 200-fold selective over Tec family kinases and over 55-fold selective against other kinases tested. In addition **3** showed ATP competitive kinetics[8].

Based on potency and selectivity, **3** was analyzed further in cells. Compound **3** inhibited anti-CD3 antibody induced IL-2 secretion both in human Jurkat T-cells ($IC_{50} = 0.25 \pm 0.11$ μM), PBMCs ($IC_{50} = 0.39 \pm 0.16$ μM) and in murine EL4 cells ($IC_{50} = 0.07 \pm 0.04$ μM) and splenocytes ($IC_{50} = 0.38 \pm 0.18$ μM). The murine EL4 cell line appears to be the most sensitive to Itk inhibition.

Table 7. Enzyme selectivity of compound **3**

Enzyme	IC_{50} (μM)[8] (selectivity ratio)[a]
Itk	0.019 (1)
Txk	11 (550)
Tec	17 (850)
Btk	4.1 (200)
Bmax	>50 (>2500)
Lck	2.4 (120)
Fyn	1.1 (55)
Syk	>50 (>2500)
ZAP-70	>50 (>2500)
IR	1.1 (55)
EGFR	>50 (>2500)
Cdk2	29 (1450)
ERK-1	>50 (>2500)
PKA	>50 (>2500)
PKC	24 (1200)
Akt1	>50 (>2500)
IKKβ	>50 (>2500)
GSK-3β	36 (1800)

[a] Selectivity ratio, IC_{50}/IC_{50} (Itk).

Prior to assessing activity in vivo, the pharmacokinetic profile of **3** was determined in mouse (Table 8). Single iv and oral doses of **3** were administered. Concentrations of **3** were determined in plasma using an LC/MS assay. Plasma concentrations declined with a mean elimination half-life of 1.4 h upon oral dosing. Analog **3** suffered from a high rate of clearance compared to the mouse hepatic blood flow. The oral bioavailability of **3** based on the dose-normalized AUCs after oral and iv dosing was 6.4% in mice.

Compound **3** was tested for its ability to inhibit IL-2 production in vivo in mice following intravenous injection of anti-CD3 antibody (Fig. 2). A 50% inhibition of serum IL-2 production was observed at a dose of 50 mg/kg, sc, irrespective of the amount of the antibody used. In a separate experiment, the serum concentration of **3** was determined to be 1.89 ± 0.34 μM ($n = 5$), 1.75 h after administration at 50 mg/kg, sc. In addition we reported earlier[8] that compound **3** significantly reduced lung inflammation at a dose of 25 mg/kg, sc in a mouse model of ovalbumin induced allergy/asthma.

In summary, starting from a screening lead **1** with modest biochemical and cell potency we identified a novel series of potent and selective Itk inhibitors as exemplified by **3** through systematic SAR optimization. The problem of the metabolic instability of this series could not be solved without concomitant loss of cell potency. However, we demonstrated the potency of compound **3** in an in vivo mouse model. To our knowledge, **3** is the first example of a potent and selective small molecule Itk inhibitor to show efficacy in a mouse model of lung inflammation. This analog

Table 8. Single dose pharmacokinetic parameters of compound **3** in mice

iv route		Oral route	
Dose	10 mg/kg	Dose	20 mg/kg
Half-life (h)	1.3	Half-life (h)	1.4
Clearance (mL/min/kg)	116	C_{max} (μM)	0.069
MRT (h)	1	MRT (h)	3.1
T_{max} (h)	0.05	T_{max} (h)	1
V_{ss} (L/kg)	7	F (%)	6.4

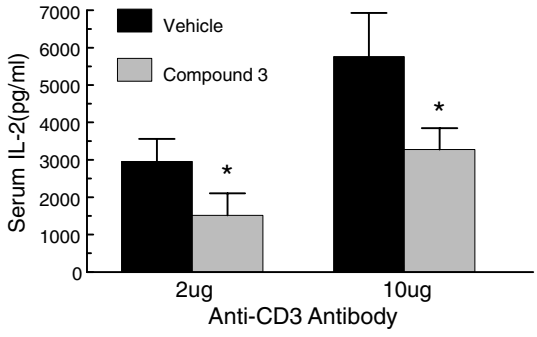


Figure 2. Inhibition of anti-CD3 antibody induced serum IL-2 production in mice in vivo by 50 mg/kg of compound **3**. $^{*}p < 0.05$ (Student's t test).

should serve as a useful tool in investigating the role of Itk in T cell signaling.

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