Bioorganic & Medicinal Chemistry Letters 18 (2008) 6231–6235

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery and SAR of novel 4-thiazolyl-2-phenylaminopyrimidines as potent inhibitors of spleen tyrosine kinase (SYK)

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ARTICLE INFO

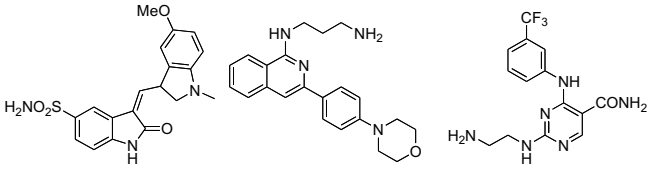
Article history:
Received 9 July 2008
Revised 25 September 2008
Accepted 29 September 2008
Available online 7 October 2008

Keywords:
SYK
Spleen tyrosine kinase
Asthma

ABSTRACT

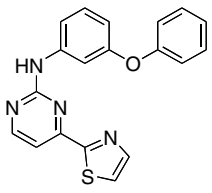
A series of SYK inhibitors based on the phenylamino pyrimidine thiazole lead **4** were prepared and evaluated for biological activity. Lead optimization provided compounds with nanomolar K_i's against SYK and potent inhibition in mast cell degranulation assays.

Asthma and allergic disorders remain an area of high interest for drug discovery. Drugs, such as bronchodilators or anti-inflammatory agents, are currently available to treat either acute or persistent symptoms of asthma. While these therapies provide effective and safe treatments for quick relief of asthma, many of these treatments for persistent symptoms have either side effects, low efficacy or show little efficacy in refractory patients.[1] Activation of Spleen tyrosine kinase (SYK) was shown to be the earliest detectable signaling response to cross-linking of the high affinity IgE receptor (FcεRI) cross-linking on mast cells, an event which subsequently leads to mast cell degranulation.[2–4] Such a critical role for SYK makes it a good target for therapeutic intervention in allergic diseases such as asthma.[5a,b] Interestingly, other roles have recently been reported for SYK as a tumor suppressor in breast cancer,[6] and a regulator of metastatic behavior in human melanoma cells[7a] and as an attractive target for B-cell malignancies.[7b] Over the last few years, SYK inhibition[4] has been an area of extensive research in asthma resulting in the disclosure of several potent SYK inhibitors of varied chemotypes. Lai et al.[8] and Cywin et al.[9] have reported on a novel series of oxindoles **1** and [1,6]naphthyridines **2** which were very potent SYK inhibitors, and Hisamichi et al.[10] published on a potent series with the disclosure of pyrimidine-5-carboxamides **3** based inhibitors.



1 IC_{50} = 5 nM **2** IC_{50} = 8 nM **3** IC_{50} = 41 nM

In our effort to discover potent and selective SYK inhibitors, we identified a small molecule lead from a HTS campaign, *N*-(3-phenoxyphenyl)-4-(thiazol-2-yl)pyrimidin-2-amine (**4**), shown below that demonstrated good affinity for SYK (K_i = 630 nM). The novel phenyl amino pyrimidine pharmacophore-based analog served as the starting point for further optimization.



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Based on this lead, a novel series of SYK inhibitors was prepared and evaluated (Tables 1–3). The syntheses of thiazole precursors **7a–c** and **9a,c** are described in Scheme 1. Lithiation of thiazoles[11] **5a–d** with *n*-butylithium in ether at −78 °C, followed by the addition of methoxymethy acetamide, afforded good yields of

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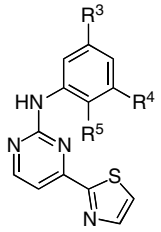
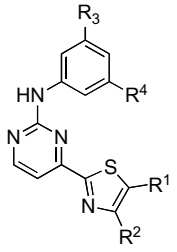
doi:10.1016/j.bmcl.2008.09.106

Table 1
Binding affinities and cellular activity from aniline variation



Compound	R^3	R^4	R^5	SYK K_i (μM)	CDK-2 K_i (μM)	ROCK K_i (μM)	SRC K_i (μM)	ZAP-70 K_i (μM)	Mast Cell Degran IC_{50} (μM)	Rat Microsome Stability. (% unchanged after 30 min.)
17	Me	Me	H	0.008	>5	>2.5	1.062	0.4191	0.55	24
18	OMe	CF3	H	0.028	>5	>2.5	1.833	2.145	0.49	54
34	OH	CF3	H	0.044	1.652	>2.5	0.991	0.754	0.41	24
19	Me	CF3	H	0.11	>5	>2.5	1.357	0.891	0.12	N.A.
35	$O(CH_2)_3OH$	CF3	H	0.026	>5	>2.5	0.422	0.677	0.09	68
20	OMe	OMe	H	0.014	>5	>2.5	0.862	0.318	0.07	12
21	Me	H	H	0.082	2.452	>.5	2.984	1.551	0.14	N.A.
22	Me	H	Me	1.817	>5	NA	0.153	NA	NA	N.A.

Table 2
Substituted thiazoles: aniline mix/match



VRT number	R^1	R^2	R^3	R^4	SYK K_i (μM)	CDK-2 K_i (μM)	ROCK K_i (μM)	SRC K_i (μM)	ZAP70 K_i (μM)	Mast cell degran. IC_{50} (μM)	Rat microsome stability (% unchanged after 30 min)
23	Me	H	Me	Me	0.023	1.1	>2.5	0.348	0.41	0.2	15
24	H	Me	Me	Me	0.056	0.1	>2.5	2.03	1.03	1.55	5
25	CH_2OH	H	Me	Me	0.004	>2.5	>2.5	2	0.27	0.04	37
26	H	CH_2OH	Me	Me	0.01	2.4	>2.5	2.23	0.45	0.05	35
27	$(CH_2)_2OH$	Me	Me	Me	0.032	2.27	>2.5	3.5	0.56	0.07	65
28	$(CH_2)_2OH$	Me	Me	CF_3	0.022	>2.5	>2.5	3.5	3.5	0.18	80
29	H	CH_2OH	OMe	CF_3	0.033	>2.5	>2.5	3.5	1	0.18	N.A.
30	H	CH_2OH	OH	CF_3	0.05	1.5	>2.5	4	0.68	0.09	50
31	Me	H	OMe	CF_3	0.023	>2.5	>2.5	3.7	1.14	0.22	N.A.
32	H	CH_2OH	Me	CF_3	0.01	1.5	>2.5	1.8	0.75	0.07	64

the desired 2-acetyl thiazoles **6a–c**. Bromination of 4- and 5-methyl-2-acetyl thiazoles **6b,c** with NBS and a catalytic amount of AIBN provided, after treatment with potassium acetate in acetic acid, the desired 4- and 5-hydroxymethyl thiazoles intermediates **8a,b** in good overall yield. 2-Acethyl thiazoles **6a–d** and **8a,b** were treated with neat DMF–DMA at 80 °C to afford the key intermediate enaminones **7a–d** and the corresponding alcohols **9a,b** in moderate to high yield.

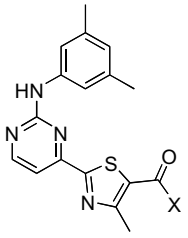
5-Carboethoxy-4-methyl-thiazole enaminone intermediate **12** was prepared according to Scheme 2. Diazotization of 2-amino thiazole **10** followed by treatment with hypophosphorous acid[12] afforded intermediate **11** in good yield. Deprotonation with lithium hexamethyldisilazide in the presence of methoxymethyl acetamide gave, after treatment with Bredereck's reagent,[13] enaminone **12**.

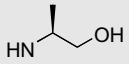
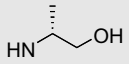
The phenylamino pyrimidines **17–33** were prepared according to Scheme 3. The enaminones **7, 9** and **12** were cyclized with guanidines **13–16** to provide pyrimidine analogs **17–33** in moderate to good yields.

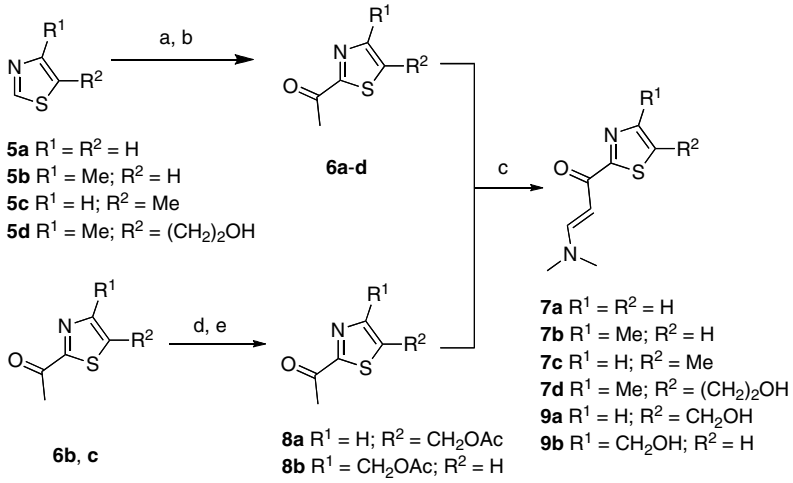
Pyrimidines **34–37** were prepared as depicted in Scheme 4. Demethylation of compound **18** with TMSI in quinoline at 180 °C proceeded smoothly to give intermediate phenol **34** in high yield. Alkylation with bromopropanol in the presence of potassium carbonate in DMF gave hydroxyalkyl ether **35**. Saponification of ester **33** with potassium hydroxide in methanol provided the corresponding intermediate acid, which was coupled with both enantiomers of alanol to give, after deprotection with TFA, the desired amides **36 and 37** in good yields.

Compounds **17–32** and **34–37** were tested for SYK inhibition and selectivity against a small panel of other key kinases. Furthermore, their ability to inhibit signaling activity through the high affinity IgE receptor (FcεRI) was evaluated in the mast cell degranulation assay, a more physiologically relevant endpoint for asthma. Screening numerous aniline aryl substitutions led us to the 3,5-disubstituted patterns as being optimal for SYK inhibition. As an example, 3,5-dimethyl aniline based compound **17** was a good starting point with a potent SYK K_i of 8 nM and an IC_{50} of

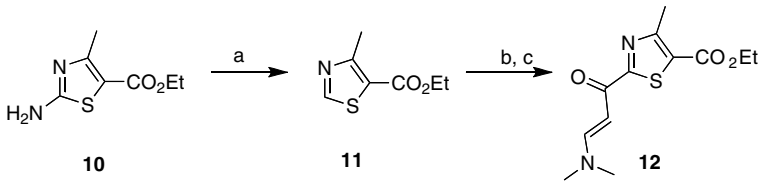
Table 3
Addition of chiral motifs



Compound	X	SYK K_i (μM)	CDK-2 K_i (μM)	ROCK K_i (μM)	SRC K_i (μM)	ZAP70 K_i (μM)	Mast cell degran. IC_{50} (μM)
36		0.009	>3.33	2.3	>3.33	0.24	0.07
37		0.21	>3.33	>2.5	>3.33	1.5	4.2



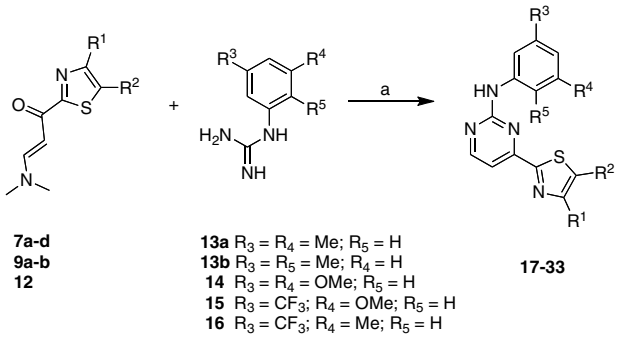
Scheme 1. Reagents and conditions: (a) *n*-BuLi, −78 °C, ether, 15 min; (b) AcN(OMe)Me, THF, −78 °C to rt (70–85%); (c) DMF–DMA, 85 °C, 16 h (60–90%); (d) NBS, CCl_4 70 °C, 16 h (73%); (e) KOAc, HOAc, rt, 16 h (67%).



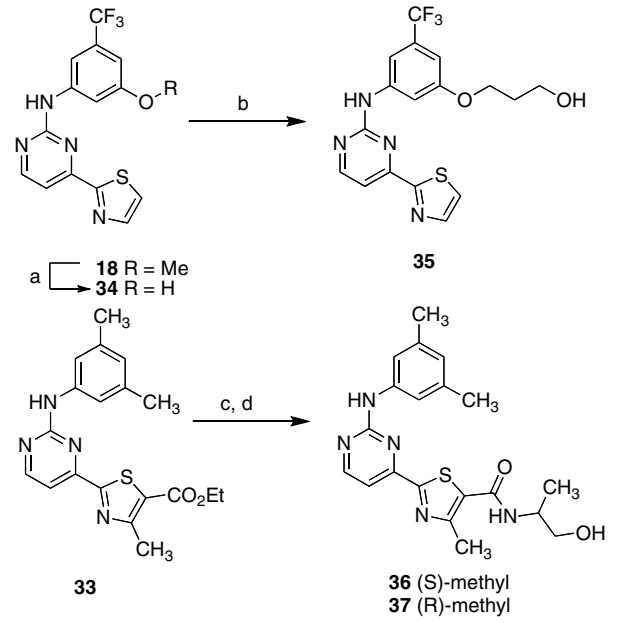
Scheme 2. Reagents and conditions: (a) $NaNO_2$, H_2O, −5 °C then H_3PO_2, −5 °C then NaOH, rt (70%); (b) LiHMDS, THF, −78 °C, 30 min then AcN(OMe)Me, THF, −78 °C to rt (61%); (c) Bredereck's reagent (74%).

550 nM. Changing the position of the two methyl groups on the aniline moiety from 3,5- to 2,5-, as in compound **22**, resulted in lower activity against SYK activity with a K_i of 1820 nM. A first round of optimization explored variations of aniline 3,5-substitutions (Table 1). Removal of one of the methyl groups in compound **17**, as in compound **21**, led to a 10-fold decrease in SYK affinity. The replacement of a methyl by a trifluoromethyl group such as compound **19** also resulted in a 12-fold decrease in activity against SYK. 3-Methoxy-5-trifluoromethyl aniline based analogs **18** and the de-methylated methyl ether counterpart **34** also retained activity in enzyme assay for SYK with respect to lead compound **17**, the latter being more selective against ZAP70. The most notable improvement in cellular activity was observed for the 3,5-dimethoxy aniline and 3-hydroxy propyloxy-5-trifluoromethyl analogs **20** and **35** with IC_{50}'s of 70 and 90 nM, respectively. Unfortunately, a reduced selectivity against Zap70 was observed for both compounds. All of the other 3,5-analogs were very selective against CDK-2 and ROCK, and moderately selective against SRC and ZAP70.

The poor correlation between IC_{50} and K_i for compounds **17** and its des-methyl analog **21** was attributed to the very poor solubility



Scheme 3. Reagents and conditions: (a) K_2CO_3, DMF, 85 °C, 16 h (40–80%).



Scheme 4. Reagents and conditions: (a) TMSI, quinoline, 180 °C (80%); (b) $Br(CH_2)_3OH$, K_2CO_3, DMF, 68 °C (75%); (c) KOH, MeOH, H_2O, 60 °C, 5 h; (d) EDC, HOBT, DIEA, DCM, $H_2NCH(Me)CH_2OH$ (70–73%).

of the latter. This hypothesis was further supported by the correlation from methyl-thiazole regioisomers **23** and **24**. Both compounds had high affinity for SYK while only compound **23** elicited moderate cellular activity which was explained by the better solubility of **23** (100 μM) compared to **24** (<2.5 μM). The most important increase in cellular activity was obtained via the functionalization of the 2-thiazole moiety, which resulted in compounds with cellular potency below 100 nM (Table 2). The 5-hydroxymethyl thiazolyl phenylaminopyrimidine **25** showed a 2-fold increase in SYK potency, and more importantly, a 14-fold increase in cellular activity over unsubstituted thiazole **17**. The 4-regioisomer **26** showed comparable activity, and both compounds exhibited weak residual activity against ZAP70. The 4-hydroxyethyl analog **27** was also active in cells, albeit with weaker inhibition of SYK (K_i of 32 nM). Selective SYK inhibitors for the potential treatment of asthma should be devoid of activity against kinases involved in cellular proliferation, such as CDK-2, or the immune response, such as SRC and ZAP70. Since the most potent cell active compounds were also associated with sub-micro molar activity for ZAP70, further selectivity optimization was needed. Replacement of the 3,5-dimethyl substituents for a 3-trifluoro methyl-5-methyl or methoxy groups as for compounds **28, 29, 31** and **32** led to an improved selectivity against Zap70 while maintaining moderate to good cellular potency.

Based on the high homology observed among kinases near the hinge-binding region, it was also hypothesized that the addition of chiral motifs may interact with amino acid residues distal to that region and may result in an improvement in kinase selectivity. Results are shown in Table 3. (*S*)-Alanol based compound **36** exhibited good SYK activity with a K_i of 9 nM and an IC_{50} of 70 nM. However, compound **36** showed no improvement over **25** in selectivity against Zap70. The (*R*)-enantiomer **37** was 20-fold less active than **36** for SYK and again with no improvement observed for Zap70 selectivity. Compounds **17, 23** and **24** only exhibited poor rat liver microsome stability after 30 min incubation with only 24%, 15% and 5% remaining, respectively. Variation of substitutions on either the phenyl or thiazole ring (Tables 1 and 2) led to some improvements in metabolic stability. The replacement of the benzylic alcohol of **25** by an alkanol side chain, shown in compound **27**, led to an improvement of metabolic stability (from 37% to 65%). Furthermore, the substitution of a methyl group by a trifluoromethyl group on the phenyl ring, as for compound **28** improved stability to 80%.

In order to better understand and improve selectivity for our inhibitor series, the X-ray crystal structure[14] for compound **36** bound to the kinase domain of SYK was solved. As Fig. 1 shows, residue A451, in the hinge region of SYK, acts as an H-bond donor for the pyrimidine ring nitrogen and as an H-bond acceptor for the –NH– group of compound **36**. Based on this (Fig. 1), it was suggested that the planarity of the aniline ring with respect to the pyrimidine core might be important for its activity. This may explain the dramatic decrease in activity for compound **22** which bears an *ortho*-methyl thereby forcing the aniline out of the plane.

The environment of the 3,5-dimethyl phenyl moiety is primarily hydrophobic, being approximately bounded by residues L377, V385, K386, and K387 above and residues G454, P455, and K458 below. As a consequence, removal of one of the *meta*-methyl groups (compound **17**–**21**) from this series results in an approximately 10-fold loss in activity. The orientation of the 3,5-dimethylphenyl pocket relative to the hinge-binding elements, forces the pyrimidine-NH-phenyl unit to remain almost completely planar. Moving a methyl group from the 3- to the 2-position (compound **17**–**22**) stabilizes a non-planar arrangement of this unit resulting in SYK inhibition that is approximately 200-fold reduced. Interestingly, the same change results in SRC inhibition that is about 10-fold better. This is likely resulting from the smaller serine residue (S345) for SRC compared to (Pro 455 in SYK; shown in red) providing additional space for the phenyl ring to twist out of the plane of the pyrimidine.

The X-ray crystal structure for the complex of (*S*)-alanol **36** in the vicinity of salt-bridge residue K402 is shown in Fig. 2. Here we ob-

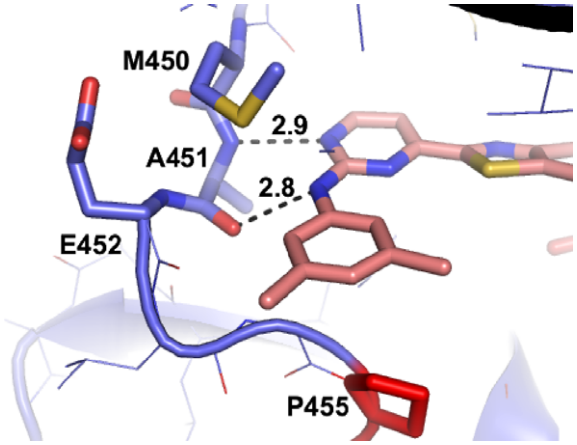


Figure 1.

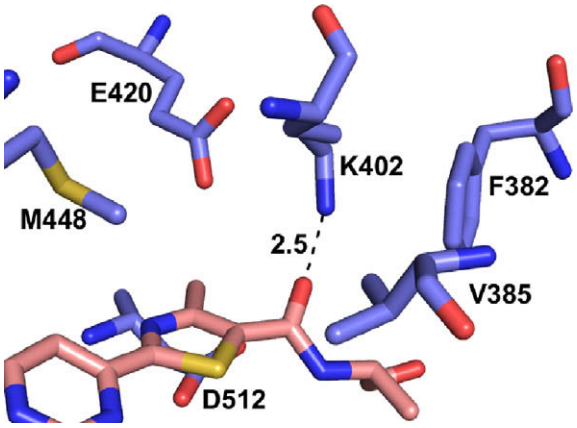


Figure 2.

served the amide carbonyl oxygen accepting an H-bond from the side chain of K402. Interestingly, alanol based enantiomer **37** was 20-fold less active than compound **36**. One explanation for this discrepancy may be that a small rotation of the NCCO torsion of **36** could place the terminal OH group within H-bonding distance of the catalytic aspartic acid residue (D512). Our crystallographic analysis suggests that the CH_2OH terminal group is conformationally mobile.

Selectivity against Zap70 in our most potent cell active compounds as well as metabolic stability is being addressed. More substitution exploration at the 3,5-position of the aniline ring is also currently underway to improve the solubility of this scaffold. Further optimization with branched amide capped aminoacids analogs to achieve both an improvement in SYK biological activity and greater selectivity will be subsequently reported.

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