Pergamon

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry Letters 13 (2003) 1415–1418

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Discovery and SAR of Novel [1,6]Naphthyridines as Potent Inhibitors of Spleen Tyrosine Kinase (SYK)

Charles L. Cywin,[a,*] Bao-Ping Zhao,[b] Daniel W. McNeil,[a] Matt Hrapchak,[a] Anthony S. Prokopowicz, III,[a] Daniel R. Goldberg,[a] Tina M. Morwick,[a] Amy Gao,[a] Scott Jakes,[a] Mohammed Kashem,[a] Ronald L. Magolda,[a] Richard M. Soll,[b] Mark R. Player,[b] Mark A. Bobko,[b] James Rinker,[b] Renee L. DesJarlais[b] and Michael P. Winters[b]

[a]*Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, Ridgefield, CT 06801-0368, USA*
[b]*3-Dimensional Pharmaceuticals, Inc., 665 Stockton Drive, Suite 104, Exton, PA 19341, USA*

Received 2 January 2003; accepted 11 February 2003

Abstract—The discovery of novel 5,7-disubstituted[1,6]naphthyridines as potent inhibitors of Spleen Tyrosine Kinase (SYK) is discussed. The SAR reveals the necessity for a 7-aryl group with preference towards *para* substitution and that this in combination with 5-aminoalkylamino substituents further improved the potency of the compounds. The initial SAR as well as a survey of the other positions is discussed in detail.

Introduction

Asthma and allergic disorders remain areas with unmet medical needs. Mast cells play an important part in the pathophysiology of these disorders by releasing pro-inflammatory mediators and cytokines. Activation of mast cells by antigen-mediated aggregation of FcεRI (the high-affinity receptor for IgE) results in a series of signaling events leading to the release of mediators, including histamine, proteases, leukotrienes and cytokines. These mediators play key roles in the etiology and symptoms of asthma and allergic disorders. A crucial downstream event in the signaling pathway following the activation of mast cells is activation of spleen tyrosine kinase (SYK).[1–3] SYK acts as a central initiator of the FcεRI-mediated signaling pathways and has been shown to be necessary for antigen-induced mediator release.[3,4] It has been shown that clustering of SYK chimera, introduced into a transmembrane protein in a mast cell line, was sufficient to stimulate the events leading to mediator release normally induced by clustering of FcεRI and that these events were dependent on the kinase activity of SYK.[4] A mast cell line lacking SYK was unable to signal without transfection of active SYK.[5] Additionally, inhibition of the antigen-stimulated phosphorylation of SYK by piceatannol (a non-selective SYK inhibitor) inhibited functional responses in mast cells, including mediator release.[6] These findings support the hypothesis that inhibiting SYK activity would attenuate FcεRI-mediated release of pro-inflammatory mediators and cytokines from mast cells. As part of our continuing efforts to identify new therapeutics directed at asthma and allergic disorders we sought to find novel inhibitors of SYK. Compound **1** and its analogue **2** were identified as a potentially interesting series for further SAR work which is the subject of this report (Fig. 1).

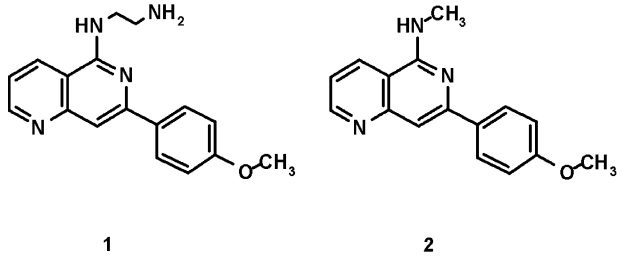
Results and Discussion

General chemistry

The compounds were synthesized as shown in Scheme 1. A 2-methylnicotinic acid derivative (**I**) is treated with lithium diisopropylamide (LDA) in THF at about −78 to 0 °C to form the dianion.[7] Subsequent addition of a nitrile (**II**) at −78 °C, and continuing the reaction at

*Corresponding author. Tel.: +1-203-791-6401; fax: +1-203-791-5868; e-mail: ccywin@rdg.boehringer-ingelheim.com

0960-894X/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0960-894X(03)00163-X



1 2

Figure 1.

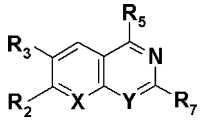
ambient temperature until completion provides the [1,6]naphthyridin-5-ol (**III**), which may also exist as the tautomeric 6*H*-[1,6]naphthyridin-5-one (**IIIa**).[8] The [1,6]naphthyridin-5-ol is then treated with $POCl_3$, with or without base (i.e., *N,N*-diethylaniline) at 100–135 °C, to give the 5-chloro-[1,6]naphthyridine (**IV**). This is then reacted with an excess of the desired amine (R′R″NH) at 100–140 °C, in a sealed reaction vessel, to provide the 5-amino substituted compounds (**V**) shown in the tables.[9] Alternatively, the 5-substituted ethers were available by reacting **IV** with the desired alcohol (R′OH), in the presence of sodium hydride in DMF at room temperature to give ethers **VI**.

Structure–activity relationships

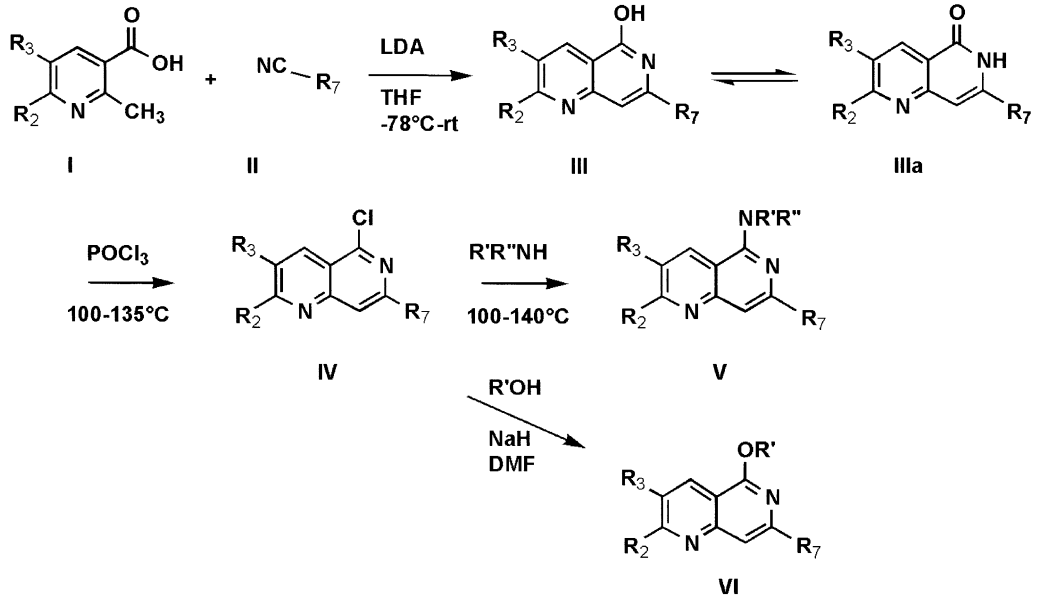
A rapid survey of the molecule was undertaken to determine the best path for further SAR studies (Table 1).[10] Compounds **1**–**5** show that the [1,6]naphthyridine core system is essential for maintaining potency. The 5-amino substituent appeared to directly correlate with activity (compounds **1**, **2**, **6**, **7**) and appeared to be a good site for further exploration. Most notably, the requirement for a substituted amine and the dependence of activity on chain length was observed. The 7-aryl moiety also appeared essential. Replacement by *t*-butyl (**8**) and other alkyl substituents resulted in loss of activity. While variation of the 7-aryl group could be beneficial (**9**), substitution at the 2-position, as well as the 4-, and 8-positions (data not shown), resulted in compounds with generally reduced potency. While the 3-position tolerated small substituents like bromo (**13**) and methyl (data not shown), larger substituents like phenyl were considerably less potent. Based on the initial SAR we focused on determining the SAR of the 5-amino and 7-aryl substituents more thoroughly.

We undertook an analysis of the substituent tolerance at the *ortho*, *meta* and *para* positions of the 7-phenyl group (Table 2). It was quickly discerned that *ortho* substitution was not tolerated (**16**–**18**). Small *meta* substituents generally had little effect on potency (**19**–**22**) while larger aryl substituents (**23**–**24**) led to less potent compounds. The *para* position proved to be the most fruitful avenue of exploration with a larger variety of substituents being tolerated. The *para*-position appears to have both a steric requirement and an electronic component. Small electron withdrawing substituents

Table 1. Initial SAR Survey

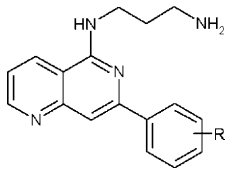


Compd	X	Y	R_2	R_3	R_5	R_7	SYK IC_{50} (μM)
1	N	C	H	H	$NH(CH_2)_2NH_2$	4-MeOPh	1.5
2	N	C	H	H	$NHCH_3$	4-MeOPh	0.49
3	N	N	H	H	$NHCH_3$	4-MeOPh	>30 μg/mL
4	C	C	H	H	$NHCH_3$	4-MeOPh	>30 μg/mL
5	C	N	H	H	$NHCH_3$	4-MeOPh	40
6	N	C	H	H	NH_2	4-MeOPh	17
7	N	C	H	H	$NH(CH_2)_3NH_2$	4-MeOPh	0.14
8	N	C	H	H	$NH(CH_2)_3NH_2$	*t*-Bu	>30 μg/mL
9	N	C	H	H	$NH(CH_2)_3NH_2$	2-Thienyl	0.44
10	N	C	H	H	$NH(CH_2)_2NH_2$	4-Me_2NPh	0.19
11	N	C	OH	H	$NH(CH_2)_3NH_2$	4-MeOPh	>30 μg/mL
12	N	C	CF_3	H	$NH(CH_2)_3NH_2$	4-MeOPh	>30 μg/mL
13	N	C	H	Br	$NH(CH_2)_3NH_2$	4-MeOPh	0.12
14	N	C	H	Ph	$NH(CH_2)_3NH_2$	4-MeOPh	3.6



Scheme 1. General synthetic method.

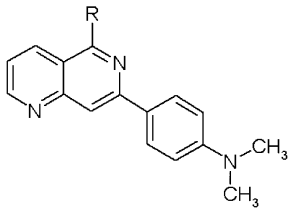
Table 2. 7-aryl SAR



Compd	R	SYK IC_{50} (μM)
15	H	0.41
16	*o*-Br	25
17	*o*-OCH_3	>30 μg/mL
18	*o*-SCH_3	>30 μg/mL
19	*m*-OCH_3	0.50
20	*m*-F	0.85
21	*m*-Cl	0.26
22	*m*-OCF_3	0.83
23	*m*-(thien-3-yl)[a]	1.5
24	*m*-(tolu-4-yl)[a]	3.8
7	*p*-OCH_3	0.14
25	*p*-F	1.1
26	*p*-Cl	1.2
27	*p*-(thien-3-yl)[a]	1.6
28	*p*-(tolu-4-yl)[a]	0.90
29	*p*-$N(CH_3)_2$	0.034
30	*p*-$N(CH_2CH_3)_2$	0.020
31	*p*-(*N*-morpholinyl)	0.008
32	*p*-$(N(CH_3)(CH_2)_2N(CH_3)_2)$	0.071
33	*p*-$(N(CH_3)(CH_2)_3N(CH_3)_2)$	0.42
34	*p*-Br	0.26
35	*p*-CH_3	0.063
36	*p*-SCH_3	0.034
37	*p*-$CH(CH_3)_2$	0.090

[a]Prepared via the corresponding bromides via Suzuki couplings.

Table 3. 5-Position SAR



Compd	R	SYK IC_{50} (μM)
38	$NHCH_3$	0.23
39	$N(CH_3)_2$	0.42
10	$NH(CH_2)_2NH_2$	0.19
29	$NH(CH_2)_3NH_2$	0.034
40	$NH(CH_2)_4NH_2$	0.019
41	$NH(CH_2)_5NH_2$	0.21
42	$O(CH_2)_3OH$	0.20
43	$O(CH_2)_4NH_2$	0.16
44	$NH(CH_2)_4OH$	0.20
45	$N(CH_3)(CH_2)_3NH_2$	0.072
46	$NH(CH_2)_3NHCH_3$	0.078
47	$N(CH_3)(CH_2)_3NHCH_3$	0.89
48	$NH(CH_2)_3N(CH_3)_2$	1.7
49	$N(CH_3)(CH_2)_3N(CH_3)_2$	8.6
50	$NHCH_2CH(OH)CH_2NH_2$	0.043
51	$NHCH_2C(CH_3)_2CH_2NH_2$	0.31
52	$NHCH_2$*c*-Hex	5.9
53	3-Pyridylmethylamino	1.3
54	3-Pyridylmethylamino	0.50
55	(3-Aminomethylbenzylamino)	0.33
56	(2-Aminobenzylamino)	0.85
57	(4-Aminobenzylamino)	13

like fluoro and chloro (**25**–**26**) were detrimental to activity however small electron donating groups like methyl (**35**) and methoxy (**7**) were beneficial. The *p*-dimethylamino (**29**) is preferred to the isosteric *p*-isopropyl (**37**) group indicating that the nitrogen, as well as the oxygen and sulfur atom of **7** and **36**, might be acting additionally as hydrogen bond acceptors. Further exploration of the *p*-amino substituent showed that larger substitution can be beneficial in conjunction with an additional polar functionality. For example, the *p*-morpholino compound **31** shows an IC_{50} less than 10 nM. There also appears to be a size restriction as shown by **32** and **33**.

We next explored SAR at the 5-position. Since we had already determined that an unsubstituted amine (**6**) was weakly active, the next step was to determine the effects of substitution (Table 3). Compound **39** showed that disubstitution on nitrogen was permitted albeit with slight loss in activity. 5-Aminoalkylamines of 3 to 4 carbons in length were optimal. A clear preference for propylenediamine and butylenediamine is shown by comparing **10**, **29**, **40**, and **41**. Examining substitution of the diamino group showed that both amines could be replaced individually or together by oxygen (**42**–**44**) with approximately a log loss in potency. Monomethylation of the propylenediamine at either nitrogen was tolerated (**45**–**46**). However, either di- or trimethylation resulted in a ~10–100-fold loss in activity (**47**–**49**). Substitution of the central carbon of the propylenediamine was also tolerated (**50**–**51**). Other groups such as cyclohexylmethylamino (**52**) proved detrimental. The key to activity proved to be the proper positioning of the second basic amine group. Compare compounds **53** and **55** to **57** (which can be considered ring constrained versions of **41**). The loss of activity in **57** is likely a result of both positioning as well as the basicity of the distal amine.

Conclusions

It has been shown that suitable 5,7-disubstituted-[1,6]naphthyridines are potent inhibitors of the tyrosine kinase SYK. The SAR has shown that a 7-aryl and preferably *para*-substituted aryl group as well as 5-alkyldiamines have synergistic effects on activity. The potencies ranged from high micromolar to low nanomolar. These compounds are of further interest and additional SAR and biological evaluations will be the subject of future reports.

Acknowledgements

The authors would like to thank Joachim Heider who originally synthesized **1** and **2**.

References and Notes

1. Turner, M.; Schweighoffer, E.; Colucci, F.; Di Santo, J. P.; Tybulewicz, V. L. *Immunology Today* **2000**, *21*, 148.
2. Siraganian, R. P.; Zhang, J.; Suzuki, K.; Sada, K. *Molecular Immunology* **2001**, *38*, 1229.

3. Taylor, J. A.; Karas, J. L.; Ram, M. K.; Green, O. M.; Seidel-Dugan, C. *Mol. Cell Biol.* **1995**, *15*, 4149.
4. Rivera, V. M.; Brugge, J. S. *Mol. Cell. Biol.* **1995**, *15*, 1582.
5. Zhang, J.; Berenstein, E. H.; Evans, R. L.; Siraganian, R. P. *J. Exp. Med.* **1996**, *184*, 71.
6. Oliver, J. M.; Burg, D. L.; Wilson, B. S.; McLaughlin, J. L.; Geahlen, R. L. *J. Biol. Chem.* **1994**, *269*, 29697.
7. For a recent report of analogous chemistry to produce isoquinol-1-ones see: Brun, E. M.; Gil, S.; Mestres, R.; Parra, M. *Synthesis* **2000**, 273.
8. Alternatively the method of Ames, D. E.; Dodds, W. D. *J. Chem. Soc., Perkin Trans. 1,* 705, could be employed for the synthesis of **III 1972**.
9. A typical experimental (preparation of **29**). LDA (2 M (THF), 12.5 mL, 25 mmol) was added to 2-methylnicotinic acid (1.37 g, 10 mmol) in THF (20 mL) at −78 °C. The mixture was stirred at −78 °C for 30 min then slowly warmed to 0 °C over 1.5 h. The mixture was cooled to −78 °C and a solution of 4-(dimethylamino)benzonitrile (2.19 g, 15 mmol) in THF (10 mL) was added. The mixture was warmed to rt and stirred for 16 h. H_2O (20 mL) was added and the THF was removed in vacuo. EtOAc (10 mL) was added and the solution was allowed to stand for 2 h. The precipitate was collected by filtration to afford the 7-(4-dimethylaminophenyl)-[1,6]naphthyridin-5-ol (1.2 g). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.56 (b, 1H), 8.86 (dd, 1H), 8.45 (dd, 1H), 7.72 (dd, 2H), 7.39 (dd, 1H), 6.81 (m, 3H), 2.99 (s, 6H), MS (*m*/*e*) calcd for $C_{16}H_{15}N_3O$ 265, found 266 (M + H). A mixture of naphthyridinol (1.2 g, 5 mmol), $POCl_3$ (10 mL) and *N*,*N*-diethylaniline (0.15 g, 1.0 mmol) were stirred at 110 °C for 16 h then cooled to rt. Excess $POCl_3$ was evaporated and the residue neutralized with aq Na_2CO_3 and extracted with CH_2Cl_2. The residue from the organic layer was purified by flash chromatography (hexanes/EtOAc) to afford 5-chloro-7-(4-dimethylaminophenyl)-[1,6]naphthyridine (1.1 g). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.03 (dd, 1H), 8.54 (d, 1H), 8.13 (s, 1H), 8.08 (d, 2H), 7.46 (dd, 1H), 6.81 (d, 2H), 3.05 (s, 6H). A mixture of the chloride (10 mg, 0.035 mmol) and 1,3-diaminopropane (200 μL, 2.4 mmol) was stirred at 100 °C for 5 h. After cooling to rt, H_2O was added and the mixture was extracted with CH_2Cl_2 (2×3 mL). Purification by preparative TLC afforded **29** (8.5 mg). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.85 (dd, 1H), 8.10 (d, 2H), 8.07 (d, 1H), 7.50 (s, 1H), 7.21 (dd, 1H), 6.81 (d, 2H), 3.87 (t, 2H), 3.02 (s, 6H), 2.97 (t, 2H), 1.90 (quint, 2H); MS (*m*/*e*) calcd for $C_{19}H_{23}N_5$ 321, found 322 (M + H).
10. The kinase activity was measured using DELFIA (Dissociation Enhanced Lanthanide Fluoroimmunoassay). The kinase assay was performed in buffer (50 mM HEPES, pH 7.0, 25 mM $MgCl_2$, 5 mM $MnCl_2$, 50 mM KCl, 100 μM Na_3VO_4, 0.2% BSA, 0.01% CHAPS). Test samples initially dissolved in DMSO at 5 mg/mL, were pre-diluted for dose response with the assay buffer in 96-well microtiter plates and a 25 μL aliquots of these diluted samples were added to a neutravidin coated 96-well white plate (PIERCE). A 25 μL volume of diluted enzyme (0.6 ng/mL final conc.) and a 50 μL volume of a mixture of substrates containing 200 nM ATP and 3.6 ng/μL PGTYR-biotin (CIS Biointernational) in the assay buffer was sequentially added and the assay plates were incubated for 30 min at RT. Following incubation, the plates were washed three times with 300 μL wash buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.05% Tween 20, 0.2% BSA). A 100 μL aliquot of Eu-labeled anti-phosphotyrosine (Eu^{3+}-PT66, Wallac CR04-100) diluted in DELFIA Assay Buffer (1 nM final conc.) was added to each well and incubated for 30 min at rt. The plates were washed four times with 300 μL of wash buffer and 100 μL of DELFIA Enhancement Solution (Wallac) was added to each well. After 10 min or longer, time-resolved fluorescence was measured on the LJL's Analyst (excitation at 360 nm, emission at 620 nm, EU 400 Dichroic Mirror) after a delay time of 250 μs.