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Highly potent aminopyridines as Syk kinase inhibitors

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ABSTRACT

A novel class of potent Syk inhibitors has been developed from rational design. Highly potent aminopyridine derivatives bearing a 4-trifluoromethyl-2-pyridyl motif and represented by compound **13b** IC_{50}: 0.6 nM were identified. Substitution by a 2-pyrazinyl motif and SAR expansion in position 4 of the central core provided diverse potent non-cytotoxic Syk inhibitors showing nanomolar activity inhibiting human mast cell line LAD2 degranulation.

Spleen tyrosine kinase (Syk) is a cytosolic non-receptor protein tyrosine kinase essential for the signalling of several immunoreceptors including B-cell receptor, Fce and Fcg receptors. Upon receptor engagement by allergen, antigen or immune complex Syk mediates cellular responses (proliferation, differentiation, adhesion, cytokine production, mast cell degranulation) that are important for both allergic and antibody-mediated diseases.[1] Thus, pharmacological inhibition of Syk activity can modulate the inflammatory response in allergic and autoimmune diseases.[2] Furthermore, as Syk is positioned upstream in the cell signalling pathway, therapies with Syk inhibitors may be more advantageous relative to drugs that inhibit a single downstream event.

In addition to these adaptive immune response functions, recent studies have described new functions for Syk in innate immune response by coupling activation of lectins and integrins receptors to cellular responses.[3] Syk is currently considered an interesting biological target and the possibility to treat allergic, autoimmune diseases and malignancies has prompted the development of Syk inhibitors.[4]

To date, few compounds are into clinical development. Fostamatinib currently in phase II for rheumatoid arthritis, other autoimmune diseases and lymphoma has shown promising results.[5] Other molecules are in early phases of clinical studies, including PRT062607[6] for autoimmune diseases and R343 for asthma.[7] Recently R343 has shown activity in early and late asthmatic response in a phase I study in mild asthmatic patients by inhaled route.[8]

As part of ongoing efforts toward the discovery of novel Syk inhibitors,[9] and based on previous knowledge[10] we designed and identified compound **1** (Fig. 1) as an interesting starting point where the nitrogen atom (1) of the picoline group and the attached NH (2) could act as hinge binding motifs with the backbone of Ala451. We also hypothesized that and the presence of nitrogen atom 3 could help to keep the planarity between rings to allow the right positioning for substitution in the upper pyridine ring and in position 4.

We envisaged two areas of further optimization: exploration of different rings and substitutions in the upper pyridine area and systematic exploration of the picoline motif.

Initial SAR efforts were directed towards replacement and substitution of upper pyridyl motif to improve potency by capturing new interactions with the protein. In accordance with docking studies we envisaged that substitution in the *meta* position should allow to establish a salt bridge interaction with Asp512. Thus, a

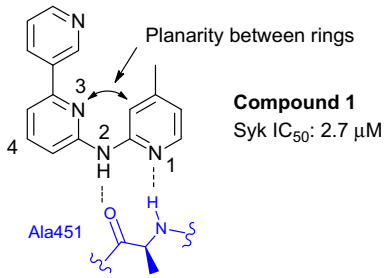

Figure 1. Knowledge based designed starting point.

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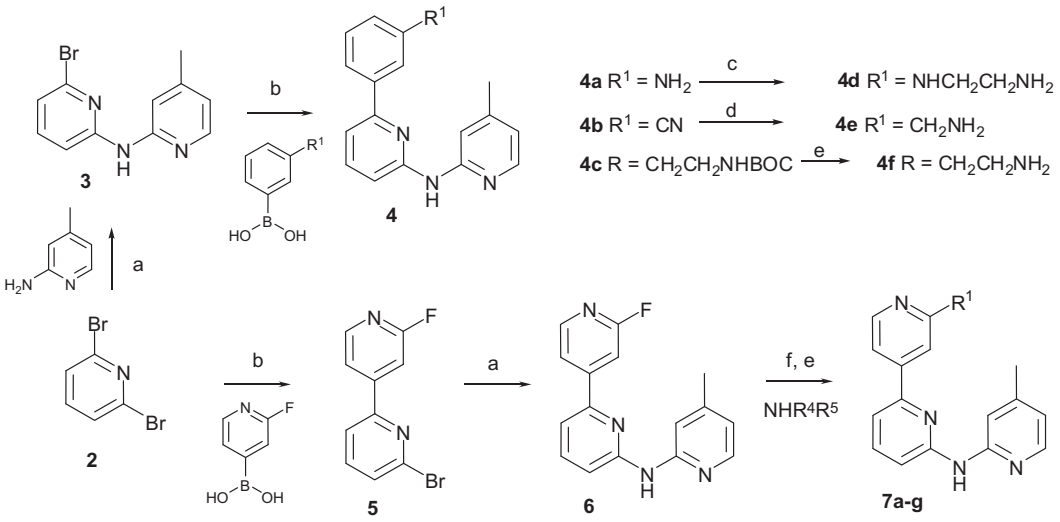
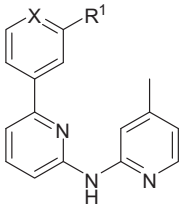
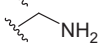
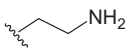
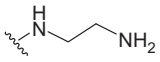
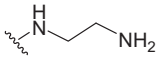
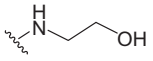
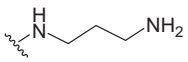
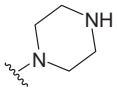
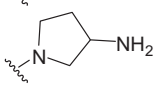
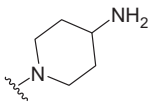
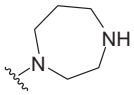


Scheme 1. Reagents and conditions: (a) *t*-BuOK, benzene, 80 °C, 67%; (b) $PdCl_2$dppf·DCM, Na_2CO_3, EtOH-toluene, 90 °C, 34–85%; (c) (i) $BOCNHCH_2CHO$, $NaBH_3CN$, MeOH-AcOH, r.t.; (ii) TFA, CH_2Cl_2, r.t. 40% two steps; (d) Pd/C, H_2, MeOH-aq. HCl, rt, 75%; (e) TFA, CH_2Cl_2, r.t. 65%; (f) K_2CO_3, DMSO, 100 °C, sealed tube, 54–84%.

Table 1
Syk inhibitory activity[11] of selected aminopyridine derivatives[a]

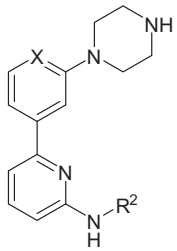


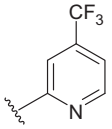
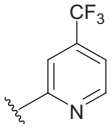
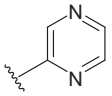
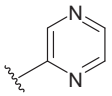
Compound	X	R^1	Syk IC_{50} (nM)
4a	CH		3242
4d	CH		1671
4e	CH		160
4f	CH		91
7a	N		200
7b	N		1872
7c	N		528
7d	N		14
7e	N		450
7f	N		114
7g	N		27

[a] IC_{50} values are an average of two or more determinations.

convergent synthesis was devised in order to rapidly access molecules containing different substituents at this position (Scheme 1).

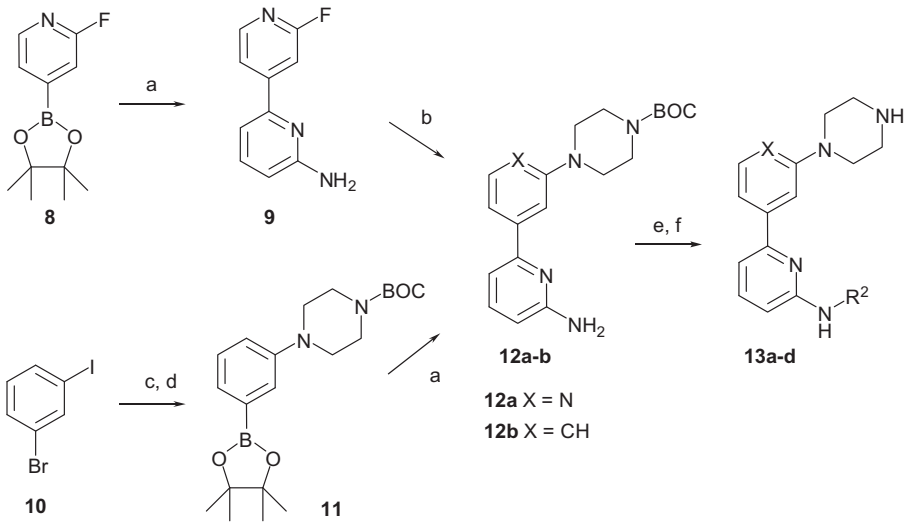
Table 2
Syk inhibitory activity of selected piperazine derivatives[a]



Compound	X	R^2	Syk IC_{50} (nM)
13a	N		1.8
13b	CH		0.6
13c	N		62
13d	CH		43

[a] IC_{50} values are an average of two or more determinations.

Treatment of commercially available 2,6-dibromopyridine **2** with 2-aminopicoline followed by Suzuki-type coupling reaction with different phenylboronic acids afforded the corresponding aminopyridines **4a–c**. Functional group transformations provided final compounds **4d–f**. For a pyridine as the upper ring, treatment of (**2**) with 2-fluoro-4-pyridylboronic provided key intermediate (**5**). Pd catalyzed *N*-arylation with 2-aminopicoline followed by heating pyridyl fluoride with the appropriate amines provided compounds **7a–g** that were submitted (except for **7b**) to a deprotection of the BOC group with TFA.



Scheme 2. Reagents and conditions: (a) 2-amino-6-bromopyridine, $PdCl_2$dppf.DCM, Na_2CO_3, EtOH-toluene, 90 °C, 62–63%; (b) K_2CO_3, DMSO, 100 °C, sealed tube, 54–84%; (c) K*t*-BuO, $Pd_2(dba)_3$, BINAP, toluene, 100 °C, 53%; (d) bis(pinacolato)diboron, $PdCl_2$dppf, dppf, KOAc, 80 °C, 90%; (e) 2-bromo-4-(trifluoromethyl)pyridine (for **13a–b**) or 2-chloropyrazine (for **13c–d**), Cs_2CO_3, $Pd_2(dba)_3$, Xantphos, dioxane, 95 °C, 65–82%; (f) TFA, CH_2Cl_2, rt 83–98%.

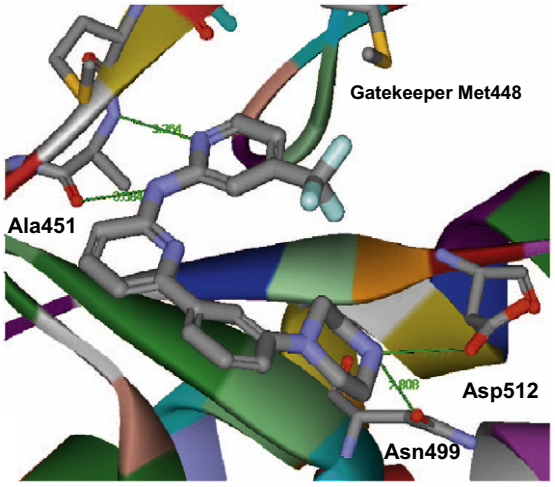
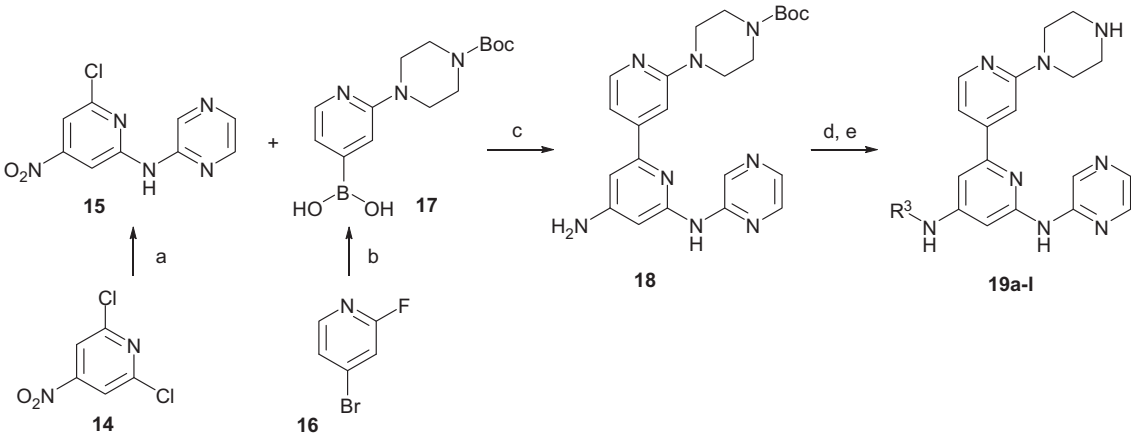


Figure 2. X-ray co-crystal structure of compound **13b** in the ATP binding site of Syk kinase.

Keeping the biaryl amine motif fixed, we proceeded to examine the effects of various substituents in R^1 in Syk inhibitory activity (Table 1).[11] Increasing the chain length resulted in a rapid increase in potency with an optimal chain length of 4 atoms (see **4f** and **7a** vs **4e** and **7c**). Replacement of the free amine in the chain by a hydroxyl group results in a drop of activity (**7a** vs **7b**) probably because the ammonium motif is involved in a salt bridge interaction with Asp514 of the Syk protein. Interestingly the presence of a cyclic amine substituent in R^1 such as piperazine or homopiperazine (**7d** and **7g**) provided the best Syk inhibitory activities while nitrogen extrusion as in the aminopyrrolidine and aminopiperidine derivatives **7e** and **7f** resulted less active.

We then explored replacement of the picoline motif by 4-trifluoromethyl-2-pyridyl and 2-pyrazinyl derivatives keeping the piperazine motif fixed. As observed in Table 2, outstanding potencies were observed with trifluoromethyl analogues **13a** and **13b** whereas similar potencies were observed for **13c** and **13d** with a 4.4-fold drop in potency when **13c** is compared to the picoline analogue **7d**.



Scheme 3. Reagents and conditions: (a) 2-aminopyrazine, Cs_2CO_3, $Pd_2(dba)_3$, Xantphos, dioxane, 95 °C, 63%; (b) (i) *tert*-butyl piperazine-1-carboxylate, K_2CO_3, DMSO, 100 °C, sealed tube, 78%; (ii) bis(pinacolato)diboron, $PdCl_2$dppf, dppf, KOAc, dioxane, 80 °C, 100%; (c) (i) $PdCl_2$dppf·DCM, Na_2CO_3, EtOH-toluene, 90 °C, 42%; (ii) Fe, aq. NH_4Cl/EtOH, 70 °C, 45%; (d) for **19b–c**: R^4CHO, $NaBH_3CN$, MeOH-AcOH, r.t., 54–85%; for **19d–j**: R^4COOH, EDC·HCl, Et_3N, HOBt, CAN, rt or R^4COOH, HATU, DIEA, DMF, or R^4COCl, DIPEA, DCM, −78 °C, 60–100%; for **19k–l**: (i) MeOOCR^4COOH, EDC·HCl, Et_3N, HOBt, r.t., 56–74%; (ii) LiOH, THF/H_2O, rt 33–75% (e) TFA, CH_2Cl_2, rt 60–98%.

Piperazinyl derivatives **13a–d** were prepared by the synthetic route described in Scheme 2. Suzuki coupling of boronate **8** with 2-amino-6-bromopyridine provided intermediate **9** that by substitution with a conveniently protected piperazine led to intermediate **12a**. Commercially available 1-bromo-3-iodobenzene (**10**) was converted to boronate **11** which upon Suzuki coupling with 2-amino-6-bomopyridine provided intermediate **12b**. Palladium catalyzed *N*-arylation of **12a-b** with the corresponding 2-haloheterocycles followed by removal of the BOC protecting group yielded compounds **13a–d**.

X-ray structure of compound **13b** bound to Syk (Fig. 2) confirmed our postulated binding mode.[12] The pyridine N1 atom and 2-amino group form bidentate hydrogen bonds with the backbone of the hinge residue Ala451. These position the trifluoromethyl moiety of the inhibitor in the hydrophobic area close to the gatekeeper residue Met448. Probably this hydrophobic interaction contributes to the high potency observed. In addition hydrogen bonding and salt bridge interactions are observed between the ionized nitrogen of the piperidine and Asn499 and Asp512 'respectively'.

Analysis of the crystal structure of **13b** suggested that large substituents in position 4 of the central pyridine ring would be accommodated, and allow exploration of additional interaction sites or modulation of physicochemical properties since it should be pointing towards the solvent exposed area.

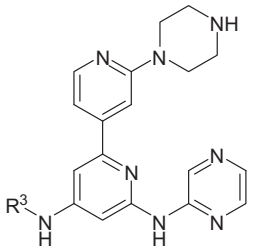
Unfortunately, most potent analogues identified (**7d**, **13a–b**) suffered from cytotoxicity in CHO cells in the range of 1–5 μM level. Although the cause of the high cytotoxicity of this compounds is unclear, we hypothesized that a decrease in lipophilicity (c log *P* of **7d**, **13a–b**: 3.7, 4 and 4.6) could be beneficial to identify less cytotoxic compounds. Indeed, pyrazine substituted analogues **13c–d** (c log *P*: 1.9 and 2.5) did show cytotoxicity values in CHO cells greater than 15 microM. We decided to go further by exploring SAR in position 4 keeping the pyrazine motif in the hinge binding area as well as the piperazinyl substituted pyridine in the upper region.

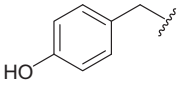
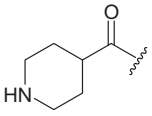
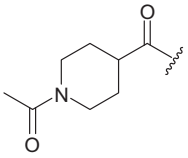
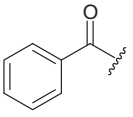
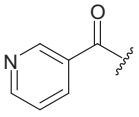
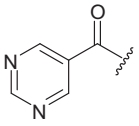
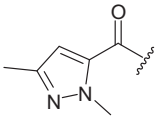
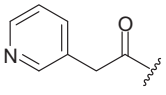
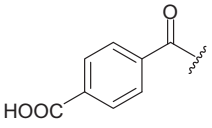
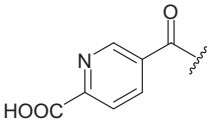
Diaminopyridine derivatives **19a–l** were prepared following the synthetic route described in Scheme 3. Key intermediate **18** was prepared from Suzuki coupling of chloropyridine **15** and boronic acid **17**. Direct removal of BOC group yields compound **19a** whereas reductive amination or standard coupling conditions for amide formation with intermediate **18** followed by BOC removal provided compounds **19b–l**.

As can be observed from Table 3, the placement of a primary amine at position 4 (compound **19a**) resulted in more than 10-fold increase in enzymatic potency compared to analogue **13c**. With single digit nanomolar potency achieved we began to profile compounds in a degranulation assay in the human mast cell line LAD2 cells.[13] Methyl or benzyl substitution in R^3 (**19b–c**) did not result in significant impact on Syk enzymatic potency compared to **19a** with a slight drop in cellular potency for **19b** and 5-fold decrease for **19c** 'respectively'. When amides in R^3 were explored, 4-pyperidinyl analogues **19d–e** produced comparable potency to **19a** although a dramatic drop of activity in LAD2 was observed. The presence of an ionisable group in **19d** may be the reason for the low activity whereas **19e** kept some cellular activity. Aryl, heteroaryl and heteroarylmethyl amides (**19f–j**) were also well tolerated with single digit nanomolar activities inhibiting Syk (except for **19f**) and LAD2 potency in a range of IC_{50} = 122–362 nM. Zwitterionic compounds **19k–l** were also examined, keeping both enzymatic and cellular potencies. Compounds **19a–l** did not show cytotoxicity in CHO cells (IC_{50} >20 μM).

Compound **19a** was tested in a kinase selectivity panel,[15] being the kinases most potently inhibited (>90%) Fgr, Flt3, Lyn and Yes. Considering the role of Lyn in the signaling of FcεRI receptor[16] it can not be excluded some contribution of off-target activities to the cell activity observed.

Table 3
Inhibition of Syk activity and LAD2 cells degranulation[14] of 4,6-diaminopyridine derivatives[a,b]



Compound	R^3	Syk IC_{50} (nM)	LAD2 IC_{50} (nM)
19a	H	4	118
19b	Me	4	188
19c		8	585
19d		4	14%@1 μM
19e		5	1000
19f		28	362
19g		6	122
19h		9	331
19i		7	169
19j		4	352
19k		8	140
19l		10	204

[a,b] IC_{50} and percentage of inhibition values are an average of two or more determinations.

In conclusion, a novel class of Syk inhibitors has been discovered from a structure-based design. Subnanomolar compound **13b** was co-crystalized bound to Syk enzyme. This allowed the identification of an area for SAR expansion that led to highly potent

and diverse non-cytotoxic derivatives showing good activity in LAD2 degranulation assay.

Acknowledgements

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References and notes

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11. *Syk kinase inhibition assay*: recombinant human full-length Syk was supplied by Millipore. The activity of Syk kinase was assessed using a radioactive filtration kinase assay. The kinase assay was performed in buffer (50 mM Tris–HCl (pH 7.5), 0.1 mM EGTA, 4 mM $Mg(CH_3COO)_2$, 0.1 mM Na_3VO_4, 0.1% (v/v) β-mercaptoethanol, 0.133 mg/ml BSA(bovine serum albumin). A 10 μL of substrate poly-GT (100 μg/mL final) and 4 μl of Syk (1 nM final) were added over 1.5 μl of various concentrations of compound or DMSO vehicle (5% final). Then, the reaction was initiated by the addition of 14.5 μL of 0.36 μCi [γ-^{33}P] ATP (10 mCi/ml, PerkinElmer) and 15 μM of ATP. The reaction mixture took place in a half-area NBS plate (Corning). After 40 min at room temperature, 15 μL of reaction mixture was transferred to a filter plate (Millipore) previously pre-wetted with a solution of 75 mM phosphoric acid. The filter plate was washed three times with 200 μL phosphoric acid per wash on a vacuum manifold (Millipore cat#/MSVMHTS00). Finally, 30 μL Optiphase™ Supermix (PerkinElmer) was added to each test well and allowed to incubate for at least 1 h before counting. Radioactivity counting was performed in a Wallac MicroBeta®, and the tyrosine phosphorylation activity by Syk was calculated.
12. The coordinates of **13b** bound to Syk have been deposited in the Protein Databank PDB ID code 4F4P.
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14. *LAD2 cells degranulation assay*: the human mast cell line LAD2, established at the National Institutes of Health (NIH) was provided by Dr. Arnold Kirshenbaum through a biological materials license agreement. Cells were sensitized in its normal growth media (Complete StemPro-34 SFM, containing StemPro-34 nutrient supplement; 2 mM glutamine and 100 ng/ml SCF) by adding 100 ng/ml of biotin-labeled IgE overnight (37 °, 5% CO_2) in a humidified atmosphere (90%). Sensitized LAD2 cells were washed with release buffer (137 mM NaCl, 2.7 mM KCl, 0.4 mM NaH_2PO_4, 5.6 mM glucose, 1.8 mM $CaCl_2$, 1.3 mM $MgSO_4$, 0.025% BSA) and adjusted to a density of 0.2×10^6/ml. Cells were transferred to the assay plate (10,000 cells/well) and pre-incubated with drugs for 30 min, and then activated with 125 ng/ml of streptavidin for 30 min. After the activation, supernatants were removed and transferred to another well for β-hexosaminidase determination. Cells were lyzed in 2 cycles of freeze-thaw and they were incubated for 90 min at 37 °C with 1 mM β-hexosaminidase substrate (*p*-nitrophenyl-*N*-acetyl-D-glucosamide, in citric buffer, pH 4.5). Reaction was stopped with 0.4 M glycine solution (pH 10.0) and absorbance was read at 405 nm.
15. *Kinases with < 50% inhibition at 1 μM*: Alk(h), Aurora-A(h), Axl(h), CaMKIIδ(h), CDK1/cyclinB(h), CHK1(h), CK1δ(h), FAK(h), FGFR3(h), Flt1(h), Fms(h), Fyn(h), GSK3β(h), IKKβ(h), JNK3(h), Met(h), MKK6(h), PDK1(h), Pim-1(h), PKCβII(h), PKCγ(h), PKCε(h), PRAK(h), Pyk2(h), ROCK-II(h), Rsk4(h),TAK1(h), TrkA(h), ZAP-70(h); kinases with >50% inhibition at 1 μM: BTK(h), cSRC(h), Fgr(h), Flt3(h), GSK3α(h), Hck(h), Itk(h), Lck(h), Lyn(h), Ret(h), Yes(h).
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