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Potent Small Molecule Inhibitors of Spleen Tyrosine Kinase (Syk)

Justine Y. Q. Lai,[†] Paul J. Cox,* Rajesh Patel, Shazia Sadiq, David J. Aldous, Sukanthini Thurairatnam, Keith Smith, Darren Wheeler, Savita Jagpal, Sofia Parveen, Gary Fenton, Trevor K. P. Harrison, Clive McCarthy and Paul Bamborough

Aventis Pharmaceuticals, Route 202/206, Brigdewater, NJ 08807, USA

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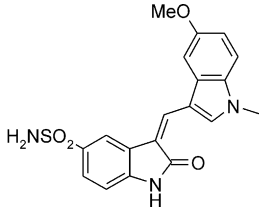
Abstract—A series of oxindoles demonstrating inhibition of the phosphorylation of biotinylated substrates of Syk and IgE/FcεRI triggered basophil cell degranulation has been identified. A study of the SAR around sulfonamide **31** ($IC_{50}=5$ nM, $EC_{50}=1400$ nM) is discussed. The modest cellular activity representative of the sulfonamide series was overcome when the Polar Surface Area was lowered to <110 Å^2, leading to the identification of amide **32** ($IC_{50}=145$ nM, $EC_{50}=100$ nM).

Introduction

Syk (Spleen Tyrosine Kinase), a double SH2 domain containing cytoplasmic protein, plays an important role in mediating inflammatory cell signalling. In mast cells and basophils, Syk plays a key role in IgE/FcεRI triggered cell degranulation,[1] resulting in the release of a variety of inflammatory mediators that orchestrate the allergic response. In macrophages, Fcγ-receptor mediated phagocytosis and mediator release is also dependent on the activation and phosphorylation of Syk.[2] Furthermore, Syk is involved in B-cell receptor signalling and activation,[3] as well as IL5 and GM-CSF mediated survival of eosinophils.[4] More recently, a critical role for Syk has been identified in Fc receptor mediated antigen presentation and induction of dendritic cell maturation.[5] Collectively, these data suggest that inhibitors of Syk kinase may be effective in inhibiting the function of a variety of cell populations involved in the inflammatory response.

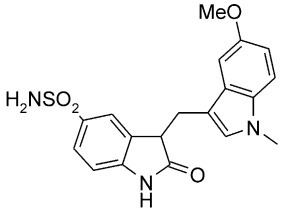
As such, the aim of our study was to identify orally available inhibitors of Syk in the belief that they may be of therapeutic use for the treatment of asthma and other inflammatory diseases. High-throughput screening led to the identification of oxindoles **1**–**3** (Table 1). Further optimisation led to the identification of **31** with $IC_{50}=5$ nM.

31
$IC_{50}=5$nM

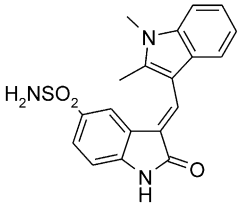


Results and Discussion

A study of the importance of the double bond revealed that saturation to give **4** led to loss in potency whilst the *Z*-analogue **5** was also inactive.



4
$IC_{50}=8100$ nM



5
$IC_{50}=$ inactive at 4 μM

*Corresponding author. E-mail: paul.cox@aventis.com
[†]Present address: Millennium Pharmaceuticals Research and Development Limited, Oncology Discovery Chemistry, Granta Park, Great Abington, Cambridge CB1 6ET, UK.

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Compounds **6** and **7** (Table 1) indicate that one sulfonamide NH is essential for potency. Increasing the bulk of the substituent at the sufonamide (**7**–**10**) led to a drop in potency. Flexible chains with acidic groups are less tolerated than carboxamide groups (**11**—**13**). Substitution of the indole nitrogen showed that, given the correct chain length, acids and polar groups such as alcohols and carboxamide groups are tolerated (**14**–**19**). However, introduction of the ester group led to a loss in potency (**20**–**23**).

Although potent inhibitors of Syk were identified in the primary screen, the compounds were all weakly active or inactive in the cellular assays, with the exception of **2** and **7**. It had been observed on a number of occasions that the compounds were very insoluble, and would precipitate out at low concentrations. Hence, it was speculated that the poor cellular activity was related to the solubility of the compounds. Attempts to improve the solubility were made by adding hydrophilic groups to the molecule. Since substitution off the indole NH tolerated amides (see **19**), hydrophilic amides were synthesised (**24**–**25**). They demonstrated a 10–50-fold loss in potency, but suggest that the solubility could be improved.

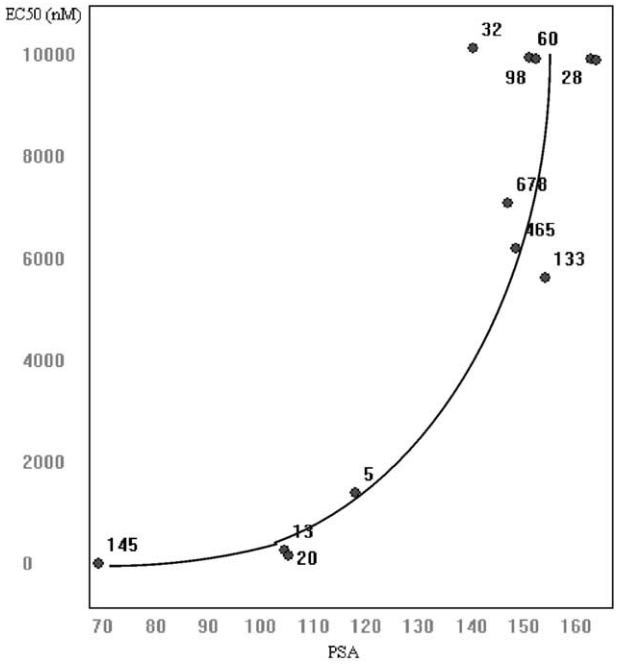
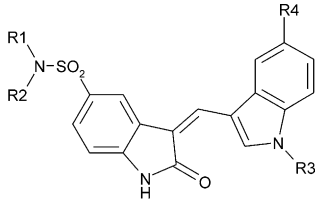
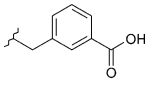
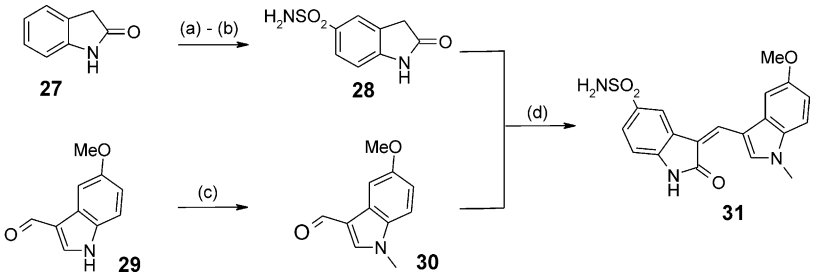


Figure 1. Correlation between EC_{50} and PSA. The labels show IC_{50} (nM) values.

Table 1. Comparison of the physicochemical properties and inhibitory activities of oxindoles in kinase and whole cell based assays

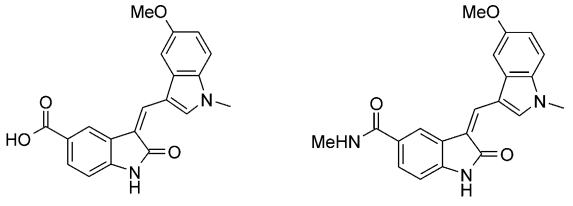


Compd	R1	R2	R3	R4	IC_{50} (nM)	EC_{50} (nM)	Solubility (mg/L)	PSA
1	H	H	H	H	128			121
2	H	H	Me	H	14	313		108
3	H	H	H	OMe	28	>10,000		162
31	H	H	Me	OMe	5	1400	<0.1	117
6	Me	Me	Me	OMe	Inactive @ 80 μM	>10,000	<0.1	94
7	H	Me	Me	OMe	20	110	<0.1	105
8	H	Et	Me	OMe	100			104
9	H	^{i}Bu	Me	OMe	937			101
10	H	*p*-MeOPhCH_2	Me	OMe	20% @ 4 μM			116
11	H	CH_2COOH	Me	OMe	658	1940	30	148
12	H	$CH_2CH_2C(=O)NH_2$	Me	OMe	60	>10,000	8	151
13	H	$CH_2C(=O)NH_2$	Me	OMe	98	>10,000	1	152
14	H	H	CH_2COOH piperidine salt	OMe	850		70	163
15	H	H	$(CH_2)_3COOH$	OMe	14			163
16	H	H	$(CH_2)_4COOH$	OMe	11	20,700	<0.1	163
17	H	H		OMe	27			149
18	H	H	$(CH_2)_3OH$	OMe	32	>10,000	<0.1	142
19	H	H	$(CH_2)_3CONH_2$	OMe	12	>10,000	5	164
20	H	H	$CH_2COO^{t}Bu$	OMe	1000			140
21	H	H	$(CH_2)_3COOEt$	OMe	204			147
22	H	H	$(CH_2)_4COOEt$	OMe	678	6960	<0.1	147
23	H	H	$(CH_2)_5COOEt$	OMe	465	6300	<0.1	147
24	H	H	$(CH_2)_3CO$-morpholino	H	133	5600	<0.1	153
25	H	H	$(CH_2)_3CO$-piperidine	H	616	>30,000	10	156



Scheme 1. (a) HSO_3Cl, 60 °C, 2 h; (b) concd NH_3, rt, 20 h; (c) NaH, MeI, DMF, rt, 20 h; (d) piperidine, EtOH, 70 °C, 30 min.

A calculation method for determining the physicochemical properties[6] demonstrated that the compounds obeyed Lipinski's rules.[7] However, in the list of compounds tested in the cellular assay, apart from **31**, **2** and **7**, the polar surface area (PSA)[6] of the oxindoles were in excess of the recommended maximum value of 140 $Å^2$. Hence compounds with lower PSA were sought. As the sulfonamide group contributed to ca. 40 $Å^2$ to the PSA value, other functional groups were examined. It was observed that although replacement with an acid group[8] **26** led to a loss in potency, the compound demonstrated moderate cellular activity. The methylamide group **32** led to a 2-fold increase in potency in the kinase assay. Despite a reduced solubility compared to **26**, good cellular activity (EC_{50} = 100 nM) was observed.



26 **32**

IC_{50}	273 nM	145 nM
EC_{50}	3500 nM	100 nM
Solubility	5.6 mg/L	0.5 mg/L
PSA	81 $Å^2$	70 $Å^2$

These preliminary results suggested that when the PSA is <110 $Å^2$, good cellular penetration is achieved. However, poor cellular activity would be predicted if the PSA is >110 $Å^2$ (Fig. 1).

Chemistry

The synthesis of the oxindoles, as exemplified by **31**, is outlined in Scheme 1. Commercially available oxindole **27** was treated portion-wise with an excess of chlorosulfonic acid at 0 °C over 1 h and then stirred at 60 °C for 2 h. The precipitate obtained from quenching in ice was subjected to overnight treatment of concd NH_3 at rt to furnish sulfonamide **28**. Alkylation of the indole **29** was carried out using NaH in DMF and MeI. The condensation[9] of sulfonamide **28** with aldehyde **30** was carried out under refluxing conditions in the presence of catalytic piperidine,[10] where the desired product precipitated out of solution.

Biological Data

Binding assay

Syk kinase activity was measured using a homogeneous time-resolved fluorescence assay, essentially as described by Park et al.[11] *N*-Flag tagged catalytic domain of human SYK (Ala340-Asn635) was expressed in the yeast *Klyveromyces lactis* and purified using standard procedures. Kinase assays were performed at 2×Km for ATP using the biotinylated substrate, Biot-$(BA)_3$-DEEEYEIPP-NH_2 (capped). Phosphorylated products were detected, upon termination of reactions with EDTA and the addition of streptavidin-labelled allophycocyanin (streptavidin-XL665; Cis bio) and europium cryptate labelled anti-phosphotyrosine antibody (PY20; Cis bio) as fluorescence acceptor and donor respectively (above ref). Upon excitation at 337 nm, a long-lived, 665 nm energy transfer signal, measured on the Packard Discovery, correlates with the amount of phosphorylated peptide formed.

Functional assay

Syk kinase inhibitors were evaluated in an in vitro model of IgE/FcεRI triggered basophil cell degranulation, modified from that previously described by Beaven et al.[12] Briefly, rat-basophilic cells (RBL-2H3) were incubated overnight with IgE anti-DNP (1 μg/mL) and 5′-hydroxytryptamine binoxylate (5-HT; 0.5 μCi/mL). Cells were then washed and incubated for 30 min with various concentrations of compound, prior to the addition of antigen (DNP; 100 ng/mL) for a further 30 min. Degranulation was assessed by measuring the amount of 5-HT release in the supernatant, using a liquid scintillation counter.

Conclusion

This study demonstrated that oxindoles are potent inhibitors of Syk. Sulfonamide **31** inhibited Syk kinase activity with IC_{50} = 5 nM, EC_{50} = 1400 nM. Its modest cellular activity is representative of the sulfonamide series where the PSA is >140 $Å^2$. When the PSA was lowered to <110 $Å^2$, as exemplified with carboxamide **32**, good cellular activity (EC_{50} = 100 nM) was achieved.

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