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The Discovery of RPR 200765A, a p38 MAP Kinase Inhibitor Displaying a Good Oral Anti-Arthritic Efficacy

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Abstract—RPR132331, a 2-(2-dioxanyl)imidazole, was identified as an inhibitor of tumour necrosis factor (TNF)α release from lipopolysaccharide (LPS)-stimulated human monocytes. An intensive programme of work exploring the biology, toxicity and physical chemistry of a novel series of inhibitors, derived from RPR132331, has led to the identification of RPR200765A, a development candidate for the treatment of rheumatoid arthritis (RA). RPR200765A is a potent and selective inhibitor of p38 MAP kinase (IC_{50} = 50 nM). It inhibits LPS-stimulated TNFα release both in vitro, from human monocytes (EC_{50} = 110 nM), and in vivo in Balb/c mice (ED_{50} = 6 mg/kg). At oral doses between 10 and 30 mg/kg/day it reduces the incidence and progression in the rat streptococcal cell wall (SCW) arthritis model when administered in either prophylactic or therapeutic dosing regimens. The compound, which is a mesylate salt and exists as a stable monohydrate, shows good oral bioavailabiltiy (F = 50% in the rat) and excellent chemical stability. The data from the SCW disease model suggests that RPR200765A could exhibit a profile of disease modifying activity in rheumatoid arthritis (RA) patients which is not observed with current drug therapies.

Introduction

Rheumatoid arthritis (RA) is a chronic debilitating disease, which is estimated to afflict more than 2 million people in the United States alone.[1] Although palliative treatments (non-steroidal anti-inflammatory drugs) are abundant, the current disease modifying anti-rheumatic drugs (DMARDS) are generally poorly tolerated and, as a result, treatment regimens are frequently dose restricted and insufficient to modify the insidious progression of the disease, which frequently leads to physical incapacitation and reduced life expectancy.

Tumour necrosis factor (TNF)α and interleukin (IL)-1β are pro-inflammatory cytokines implicated as causal agents in the onset and progression of the bone and joint destruction which characterises chronic rheumatoid arthritis.[2] The release of, and responses to, TNFα and IL-1β are regulated by a mitogen-activated protein (MAP) kinase known as p38.[3a,b] Indeed, selective inhibitors of this kinase, such as the prototypical SB203580,[3a] potently suppress the release of these pro-inflammatory cytokines from mononuclear phagocytes and block their actions in a variety of inflammatory cells. P38 is thus an important potential target for the discovery of novel, disease modifying anti-RA drugs. The relevance of p38 and other kinases to inflammatory diseases has been reviewed.[4]

A series of diphenylimidazoles, typified by RP66425, had been developed by Rhône Poulenc chemists as potent, stable and well absorbed ACAT (acyl-CoA: cholesterol *O*-acyl transferase) inhibitors.[5] However, unlike SB203580, no members of this series of compounds were found to inhibit release of TNFα from human monocytes following LPS stimulation. It was

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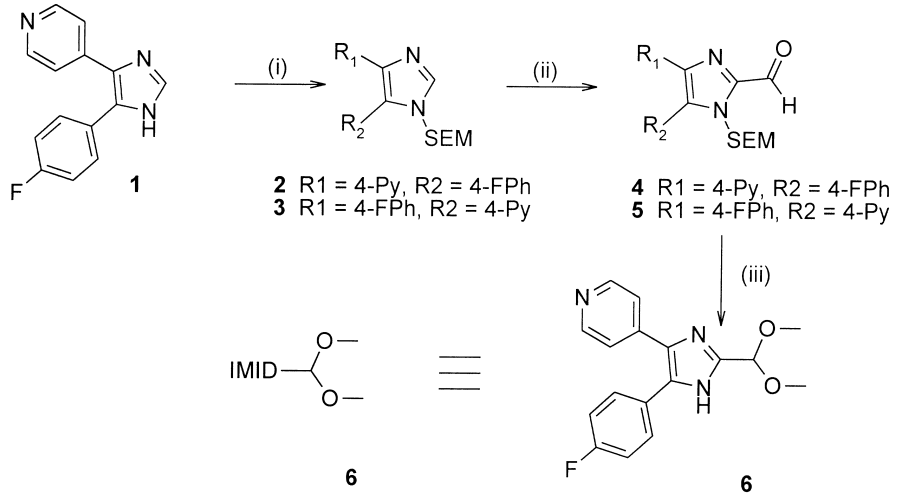
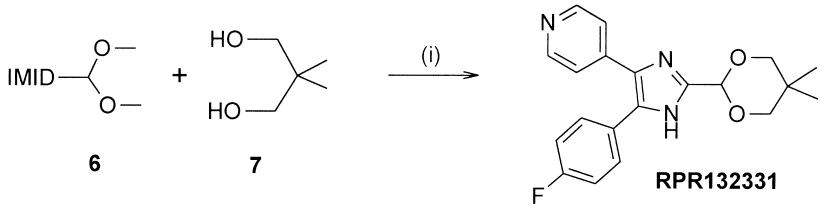
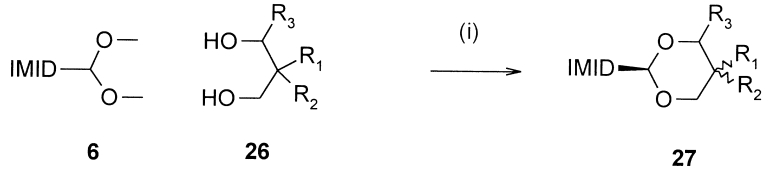


Scheme 1. Preparation of the dimethylacetal **6**. (i) $(CH_3)_3SiCH_2CH_2OCH_2Cl$, NaH, DMF; (ii) BuLi, *N*-formylmorpholine, THF; (iii) methanol, $HC(OCH_3)_3$, *p*-toluenesulphonic acid.



Scheme 2. Preparation of RPR132331. (i) 4-Toluenesulphonic acid, THF.



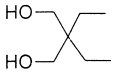
Scheme 3. General method A, library 1 (see Table 1 for the list of diols **26**). (i) General method A: 4-toluenesulphonic acid, CH_2Cl_2.

postulated that the existence of a 4-pyridyl group may be a requisite for such activity. RPR132331, a 4-pyridyl analogue of RP66425, was duly prepared (Scheme 2) and shown to have an EC_{50} in this assay of 800 nM. This paper describes the rapid optimisation of RPR132331 to provide the development candidate RPR200765A.

Several groups have reported p38 inhibitors with potent in vitro and in vivo activity. SB203580 itself has been shown to have many interesting activities in vivo,[6] but toxicological studies on rats have shown liver weight increases and significant elevation of hepatic P450 enzymes,[7] whilst in vitro studies have demonstrated inhibitory effects on human cytochrome P450 enzymes.[7] However, more recent analogues from the SB laboratories, SB220025[8] and SB226882,[9] which incorporate an aminopyrimidine moiety, display a markedly reduced cytochrome P450 inhibition profile.[10] Inhibitors have also been reported by other groups, for example, Merck (L167307),[11] R.W. Johnson (RWJ68354)[12] and Vertex (VK19911)[13] but toxicity of these compounds has not been disclosed. Two compounds have been reported to be in clinical phase I and II, respectively, for rheumatoid arthritis, SB242235[14a] (structure not disclosed) was shown to be orally potent[14b] although no toxicity issues were discussed and VX745[15a,b] was reported to be devoid of toxicity side effects arising from cytochrome P 450 modulation.[15c]

The mode of binding of this class of compound to p38 has been revealed through X-ray analysis of protein-inhibition complexes.[16,17] The inhibitors bind in the region otherwise occupied by ATP. The importance of the 4-pyridyl is clear as the pyridine nitrogen forms a key interaction with the main chain NH of Met109. The 4-fluorophenyl group sits in a hydrophobic pocket which includes the specificity residue Thr106[18,19] and the central imidazole ring forms interactions with the enzyme through water bridges. Substituents at position C2 of the imidazole bind in the ATP phosphate binding region whilst a piperidine ring at N1, as found in

Table 1. Diols (**26**) used with general method A, Scheme 6 for library 1

Diols (**26**)	*Cis*/*trans* isomers Isolated?
	NA
	No
	Yes
	NA
	NA
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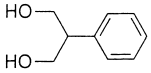
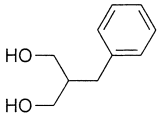
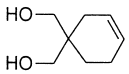
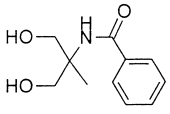
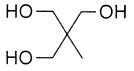
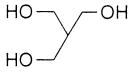
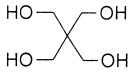
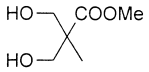
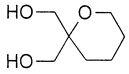
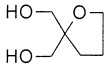
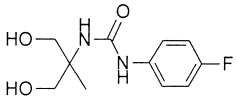
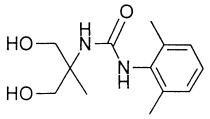
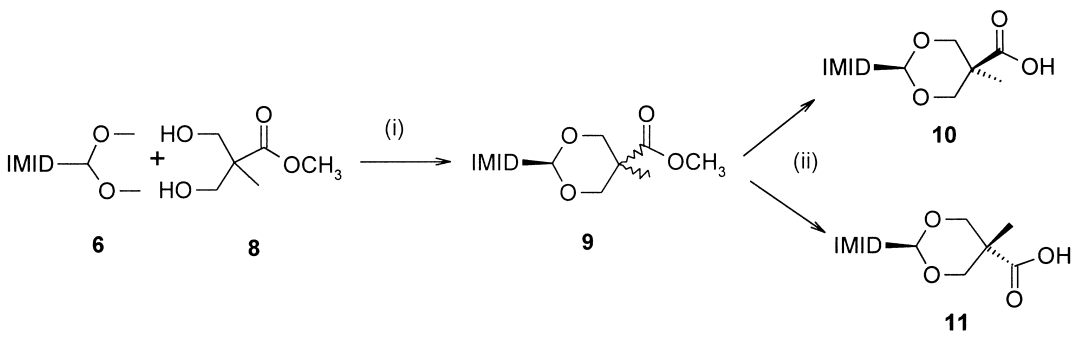
	No
	Yes
	No
	No
	Yes
	Yes
	NA
	Yes
	Yes
	Yes
	No
	Yes

VK19911,[17] appears to form a salt bridge with Asp168.

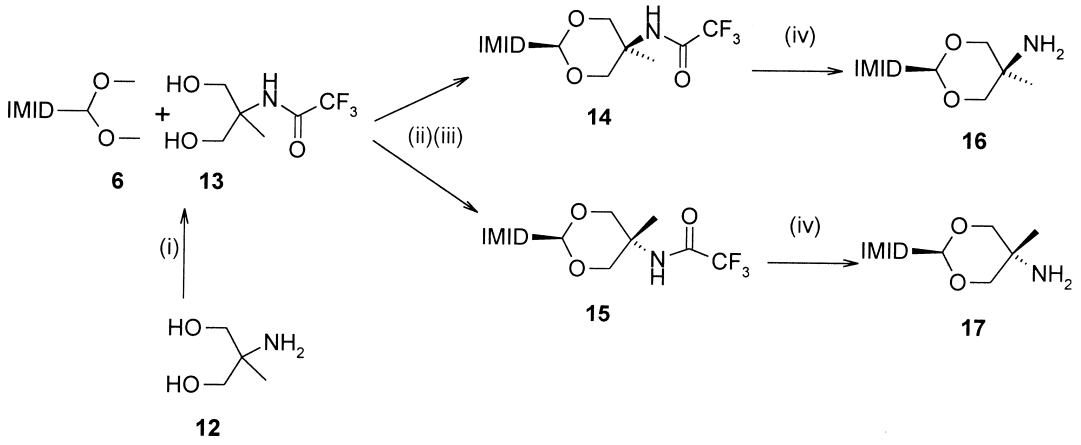
The passage of the p38 inhibitors into clinical development has been surprisingly slow and at the commencement of the work described here it appeared likely that toxicity was an issue with compounds of this type. Certainly at that time, toxicity in the rat had been reported for two compounds of similar structure to SB203580: SK&F105809 and SK&F-86002, with the first shown to be genotoxic and hepatocarcinogenic[20] and the second to induce hepatocellular hypertrophy.[21] Therefore, it was decided, before the optimisation of RPR132331 was initiated, to determine toxicity parameters that could be incorporated into the overall optimisation process. For this reason SB203580 was evaluated in models of mutagenicity and hepatotoxicity. In vitro, in the Chinese hamster ovary micronucleus assay,[22] it was found that SB203580 was indeed a powerful mutagen (see Table 8), whilst when dosed to rats at 100 and 200 mg/kg/day for 14 days the compound was found to produce a large increase in both liver weight and CYP 1A1 content (induction of CYP 1A1 could be associated with the development of hepatocarcinomas in rats).[23] In addition to these toxicity concerns it was known that the series of ACAT inhibitors, from which RPR132331 was derived, can produce adrenal toxicity which is demonstrable in the guinea pig.[24] Therefore, evaluation of mutagenicity, CYP 1A1 induction and guinea pig toxicity were all incorporated into the optimisation process, which also involved evaluation of cellular activity, in vivo activity, bioavailability, disease modifying activity, selectivity and physical chemical properties. The overall objective of this work was to identify a p38 inhibitor with in vivo disease modifying activities similar to SB203580, but without the toxicities associated with either SB203580 or ACAT inhibitors. Clearly the identification of p38 inhibitors, suitable for clinical evaluation, is a highly competitive area and, consequently, it was decided that the evaluation of RPR132331 should be completed within one year. To achieve this, a rigid project plan was designed, in which a programme of rapid parallel synthesis was allied to a rigid screening cascade. A four-month period was allotted in this plan for parallel synthesis and seven libraries, derived from seven reasonably accessible templates, were designed to make best use of this time.

Chemistry

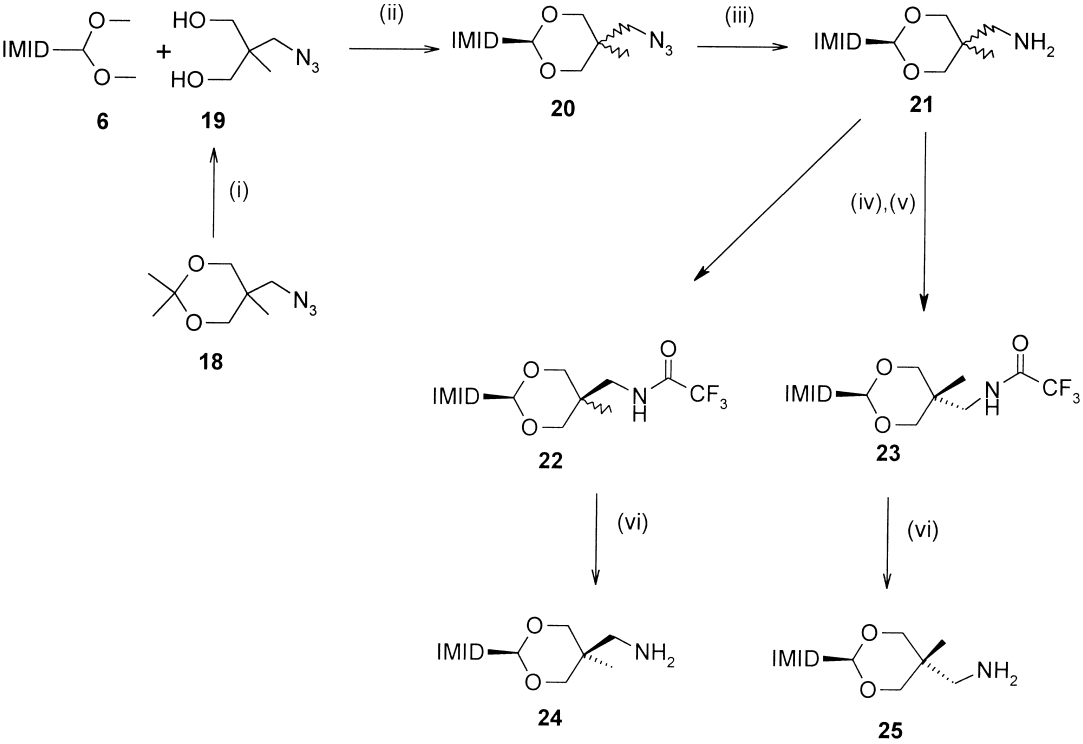
Preparation of the dimethyl acetal **6**, the key intermediate to all this work, from 4-(4-pyridyl)-5-(4-fluorophenyl)imidazole[25] **1** is shown in Scheme 1. Scheme 2 shows the preparation in one step of our lead compound, RPR132331 from the intermediate **6** with 2,2-dimethyl-1,3-propane diol **7**. Details of the seven small libraries are given in Schemes 3, 7 and 8. The library members were carefully selected to include a wide range of biologically acceptable groups capable of exploring H-bonding, charge, hydrophobic and aromatic interactions. Each of the seven libraries required the preparation of a template with an appropriate synthetic handle to allow rapid and simple compound preparation. In addition, a molecular weight restriction of 550 and a



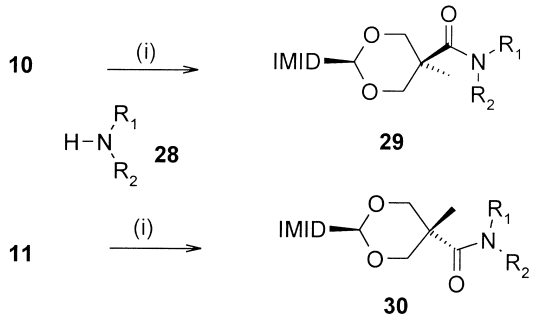
Scheme 4. Preparation of the two acids **10** and **11**. (i) 4-Toluenesulphonic acid, THF; (ii) NaOH, H_2O, methanol.



Scheme 5. Preparation of the two amines **16** and **17**. (i) K_2CO_3, $(CF_3CO)_2O$, DMF; (ii) 4-toluenesulphonic acid, THF; (iii) flash chromatography on silica; (iv) K_2CO_3, methanol, H_2O.



Scheme 6. Preparation of the two amines **24** and **25**. (i) HCl (conc), methanol; (ii) 4-toluenesulphonic acid, THF; (iii) $HCOONH_4$, methanol. Pd/C (iv) K_2CO_3, $(CF_3CO)_2O$, DMF; (v) preparative HPLC; (vi) K_2CO_3, methanol, H_2O.



Scheme 7. General method B for libraries 2 and 3. General method B: (i) EDCI, HOBT, *N*,*N*-diisopropylamine, DMF.

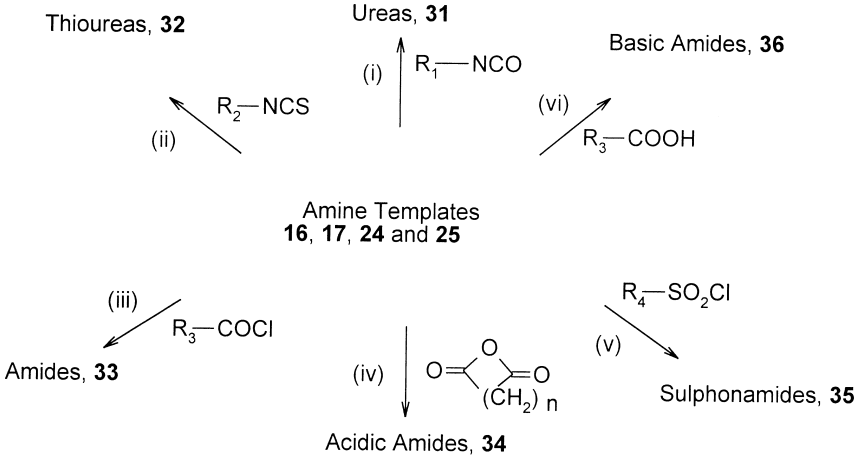
clogP restriction of **5** were imposed on the compounds in order to increase the probability for oral absorption. Compounds in the library 1, which were prepared in a similar manner to RPR132331 using commercially available 1,3-diols (Table 1 shows the list of diols), is depicted in Scheme 3. For this library (Scheme 3) a solution of **6** in dichloromethane, was dispensed into 18 flasks, treated with 2 equiv of diols **26** and allowed to stand for 3 days during which time efficient dioxane formation took place. Isolation and purification was carried out by preparative TLC with characterisation largely by MS. In some cases two isomers (*cis* and *trans* across the dioxane ring) were possible (when R1≠R2). Whenever possible, separation of these two isomers was achieved using preparative TLC, with assignment of structure according to NMR chemical shifts. This was possible as it has been shown previously[5] that the chemical shift of hydrogens on groups disposed axially at position 5 of the dioxane ring are shifted 0.5 ppm downfield relative to the corresponding equatorial isomer. The synthesis of the remaining six libraries required preparation of two isomeric acid templates **10** and **11** (Scheme 4), two isomeric amine templates **16** and **17** (Scheme 5) and the two isomeric extended amines **24** and **25** (Scheme 6). The two acids **10** and **11** (Scheme 4) were prepared in two steps from the ester diol **8**, following hydrolysis and separation of the isomers. The two amines **16** and **17** (Scheme 5) were prepared in three steps, starting from 2-amino-2-methyl-1,3-propanediol **12** which was protected as the trifluoroacetamide prior to dioxane formation, isomer separation and hydrolysis. The two amines **24** and **25** (Scheme 6) were made in five steps from the 2-azidomethyl-2-methyl-1,3-propanediol **19**, the intermediate amine **21** being trifluoroacetylated to facilitate separation of the isomers which were in turn hydrolysed.

Schemes 7 and 8 give details of the six libraries prepared from the acid and amine templates, respectively. Each of the library compounds was prepared from 50 mg of template with yields of between 10 and 100%.

Libraries 2 and 3 (Scheme 7) were prepared from acid templates **10** and **11**, respectively. For each template a solution in DMF was dispensed into 32 flasks and coupled with an appropriate amine using conventional amide coupling reagents (EDCI/HOBT). Isolation and purification of the amide products was achieved by simple aqueous work up with TLC to assess purity and MS to characterise products (the amines utilised are shown in Table 2).

Libraries 4, 5, 6 and 7 (Scheme 8) were prepared from templates **16**, **17**, **24** and **25**, respectively. These were dispensed, as THF solutions, into flasks and treated with an appropriate electrophile leading to the generation of amides, sulphonamides, thioureas and ureas. These were isolated by preparative TLC and characterised by MS (the electrophiles utilised are shown in Table 3). This approach enabled the preparation of more than 200 compounds over a three months period, of which 190 passed the acceptance criteria (Table 4).

The syntheses of the two compounds RPR200765A (identified from library 3) and RPR201227A (identified from library 5), which were required for more detailed evaluation, were investigated in some detail and are shown in Scheme 9 and 10 respectively. Both compounds were required as the *trans* isomers. The synthesis



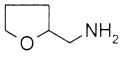
Scheme 8. General methods C–G for libraries 4, 5, 6 and 7. General method C: (i) THF, rt. General method D: (ii) THF, reflux. General method E: (iii) triethylamine, THF, rt. General method F: (iv) triethylamine, THF, reflux. General method G: (v) triethylamine, THF, reflux. General method H: (vi) EDCI, HOBT, *N*,*N*-diisopropylamine, DMF.

of RPR200765A was more favourable than RPR201227A as the required *trans* intermediate **11** was obtained as the equivalent product (*cis*:*trans*—1:1) whilst for RPR201227A the *trans* intermediate **17** was the minor product (*cis*:*trans*—3:1).

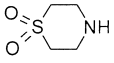
Biology

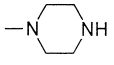
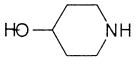
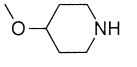
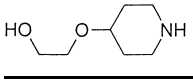
The biological screening strategy is shown in Figure 2. Potency cut-offs were set to ensure compounds were at least equivalent to SB203580. Samples prepared using library techniques were used for all stages of the project prior to drug disposition studies. In this way the investigation of the series was able to progress very rapidly through the early stages of the biological assessment. The number of compounds eliminated at early stages was high, as can be seen from Table 4.

Table 2. Amines (**28**, Scheme 7)

List of amines
NH_3
$MeNH_2$
$EtNH_2$
nPrNH$_2$
cPrNH$_2$
cPentNH$_2$
cHexNH$_2$
(nPr)$_2$NH
(Me)$_2$NH
HOCH$_2$CH$_2$NH$_2$
HOCH$_2$CH$_2$CH$_2$NH$_2$
CH$_3$OCH$_2$CH$_2$NH$_2$
CH$_3$OCH$_2$CH$_2$CH$_2$NH$_2$

2-PyCH$_2$NH$_2$
3-PyCH$_2$NH$_2$
4-PyCH$_2$NH$_2$
2-FurylCH$_2$NH$_2$
PhCH$_2$CH$_2$NH$_2$
2-PyCH$_2$CH$_2$NH$_2$
3-IndolylCH$_2$CH$_2$NH$_2$
(Me)$_2$NCH$_2$CH$_2$CH$_2$NH$_2$
(Me)$_2$NCH$_2$CH$_2$NH$_2$





The 12 library samples showing in vivo activity < 30 mg/kg are shown in Table 5 with the corresponding in vitro, and CYP1A1 induction data. These 12 compounds can be seen to come from six of the seven libraries. However, four of the 12 are the original amine templates for libraries 4, 5, 6 and 7 which were included in the respective library. Of the four compounds identified with an acceptable induction profile, two were templates (**16** [Lib4_1] and **17** [Lib5_1]) whilst the other two were true library products: Lib3_28 and Lib5_14 (Table 5). The two library compounds were resynthesised as RPR200765A and RPR201227A, respectively (both as mesylate salts). For each of the four, oral and intravenous drug disposition studies were carried out in the rat. Disappointingly the two templates **16** and **17** were not found to produce significant blood levels. This came as a surprise as good in vivo activity had been seen, following oral dosing, in the mouse. There would appear to be a species difference between rat and mouse in the absorption of these primary amines. The project plan utilised the rat as the species for both disease model evaluation and toxicity and consequently **16** and **17** were abandoned. However, when converted to the mesylate salt both RPR200765A and RPR201227A showed excellent bioavailability with high blood levels. The pharmacokinetic parameters for the two compounds dosed orally at 10 mg/kg or iv at 1 mg/kg are compared in Table 6.

Interestingly RPR201227A showed very high plasma concentrations resulting in a very low volume of distribution which may reflect high plasma protein binding. Both compounds were considered suitable for further evaluation.

Comparison of RPR201227A and RPR200765A

The two selected compounds were compared in vitro and in vivo with each other. Inhibitory activity on various kinases was first performed with results shown in Table 7.

Both compounds show p38 kinase inhibition at a level similar to SB203580 (IC_{50} for SB203580 = 56 nM) and both show good specificity for p38 over other kinases. The low micromolar potency against the src familly kinase, lck has been noted previously with other potent p38 inhibitors.[15b]

A full ED_{50} for mouse LPS-stimulated TNFα release was performed for the two compounds and SB203580. Results are presented in Figure 3 where it can be seen that the compounds have potencies very similar to SB203580.

RPR200765A and RPR201227A were both effective in the SCW arthritis model in the Lewis rat using a prophylactic

Table 3. Electrophiles used for preparation of libraries 4, 5, 6 and 7 (Scheme 8)

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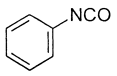
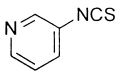
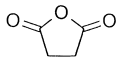
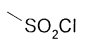
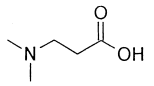
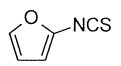
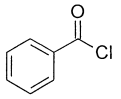
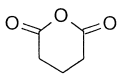
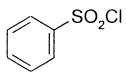
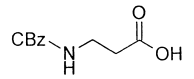
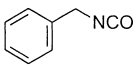
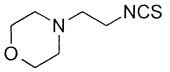
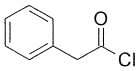
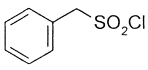
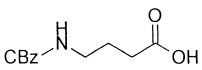
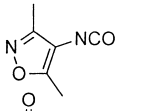
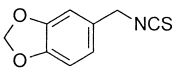
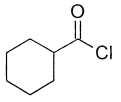
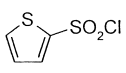
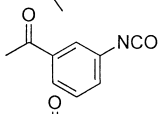
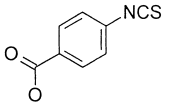
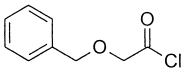
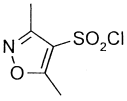
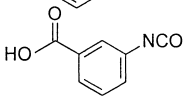
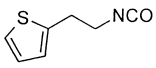
					
					
					
					
					
					
					

Table 4. Numbers of library compound accepted at early stages of the project

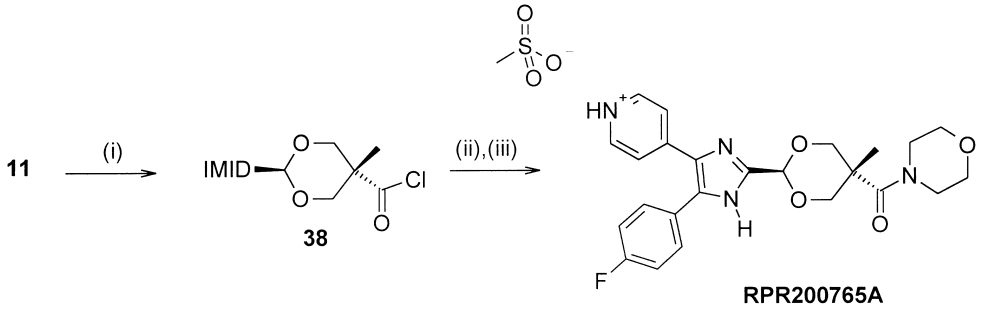
	Acceptance criteria	Compounds accepted
Library synthesis	TLC single spot/M.S. correct	190
Cellular assay	$ED_{50} < 200$ nM	24
In vivo activity	$ED_{50} < 30$ mg/kg	12
CYP 1A1 induction	No induction	4

oral dosing regimen. Activities were again comparable to SB203580 with all compounds showing ED_{50}s on paw swelling of 10–30 mg/kg/day (Fig. 4) and statistically significant dose dependent effects on histological parameters of 3–30 mg/kg/day (decrease in pannus area, increase in area of intact joint surface and decrease synovial hyperplasia and decrease in bone resorption cavities, data not shown).

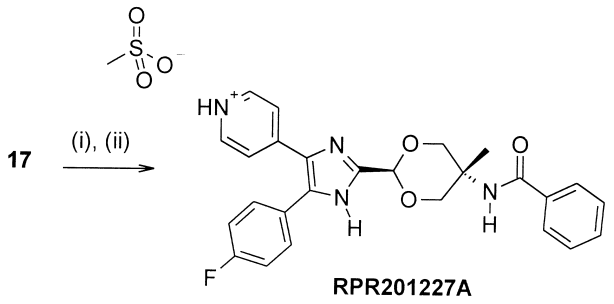
Drug safety

Table 8 summarises the toxicity of the two compounds RPR200765A and RPR201227A compared on Ames test, micronucleus test, CYP 1A1 induction in vitro and in vivo and in the guinea pig adrenotoxicity study.

Unlike SB203580, neither RPR201227A nor RPR200765A were able to induce chromosome damage or induce CYP 1A1. RPR200765A was a very weak inhibitor of ACAT activity and was also found to be free of adrenotoxicity in the guinea pig at doses up to 800 mg/kg/day. However, RPR201227A was a moderately active inhibitor of ACAT and, more importantly, was found to be highly toxic to guinea pigs (100 mg/kg/day) requiring the study to be halted at an early stage. The severity of the toxicity was such that it was decided to abandon RPR201227A at this stage (see Table 8).



Scheme 9. Preparation of RPR200765A. (i) $SOCl_2$, rt; (ii) morpholine, CH_2Cl_2; (iii) CH_3SO_3H, THF, rt.



Scheme 10. Preparation of RPR201227A. (i) Benzoic acid, HATU, DIPEA, DMF; (ii) CH_3SO_3H, THF, rt.

Two further biological studies were carried out with RPR200765A. Firstly, efficacy was evaluated in the SCW arthritis model using a therapeutic oral dosing regimen and dosing alongside SB203580 for comparative purposes. This study showed that, as with prophylactic dosing, RPR200765A and SB203580 produced significant effects on paw swelling and histological parameters at 10–30 mg/kg/day on both joint swelling and histological parameters (Fig. 5). Finally, RPR200765A and SB203580 were examined for potential to inhibit CYP enzymes. As can be seen from the results in Table 9, weak inhibition of CYP 1A2 ($IC_{50} = 4.2\,\mu M$) by RPR200765A was observed but this was more than 80 times the IC_{50} seen for p38 inhibition.

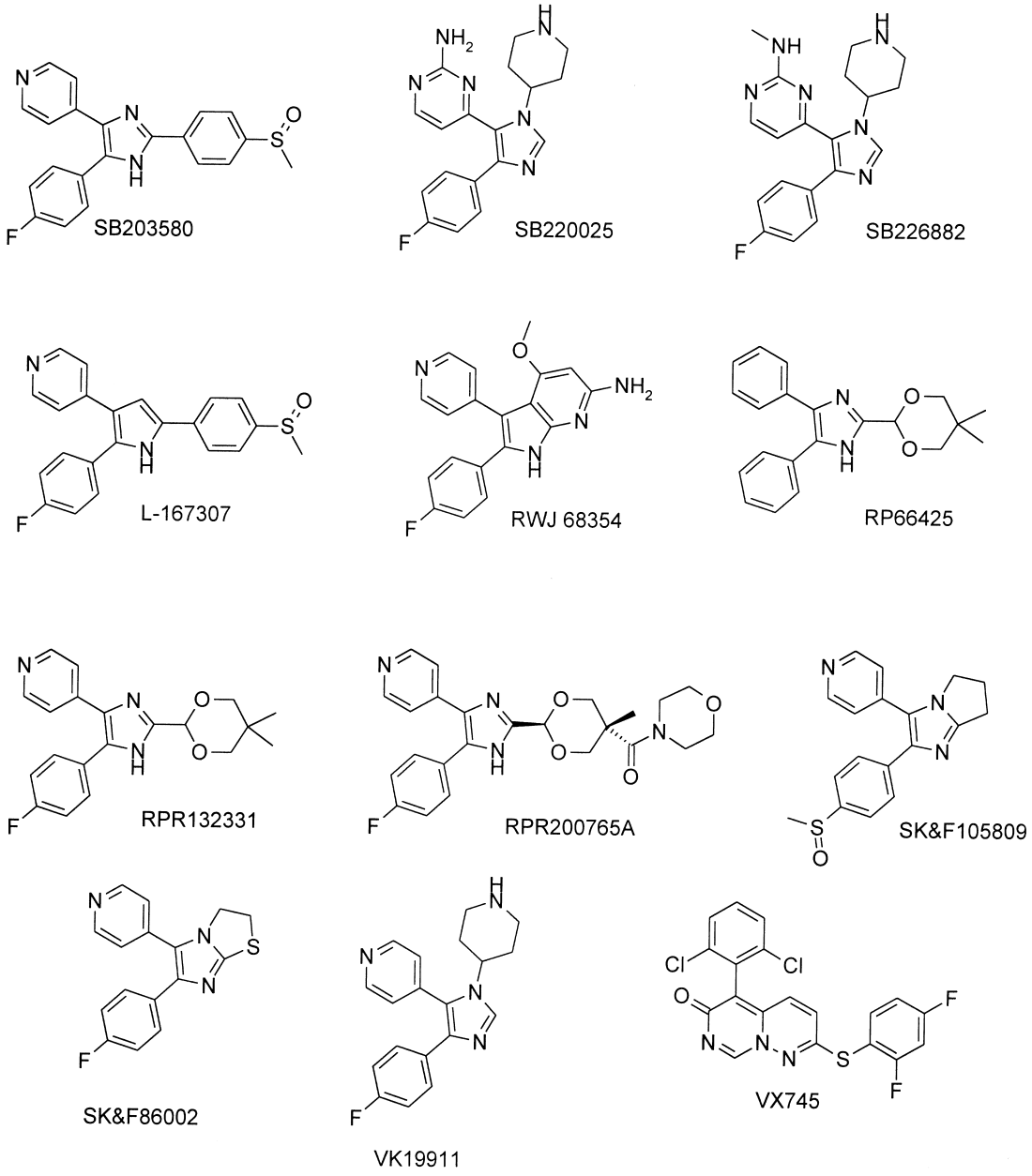


Figure 1. Structures of compounds relevant to this work.

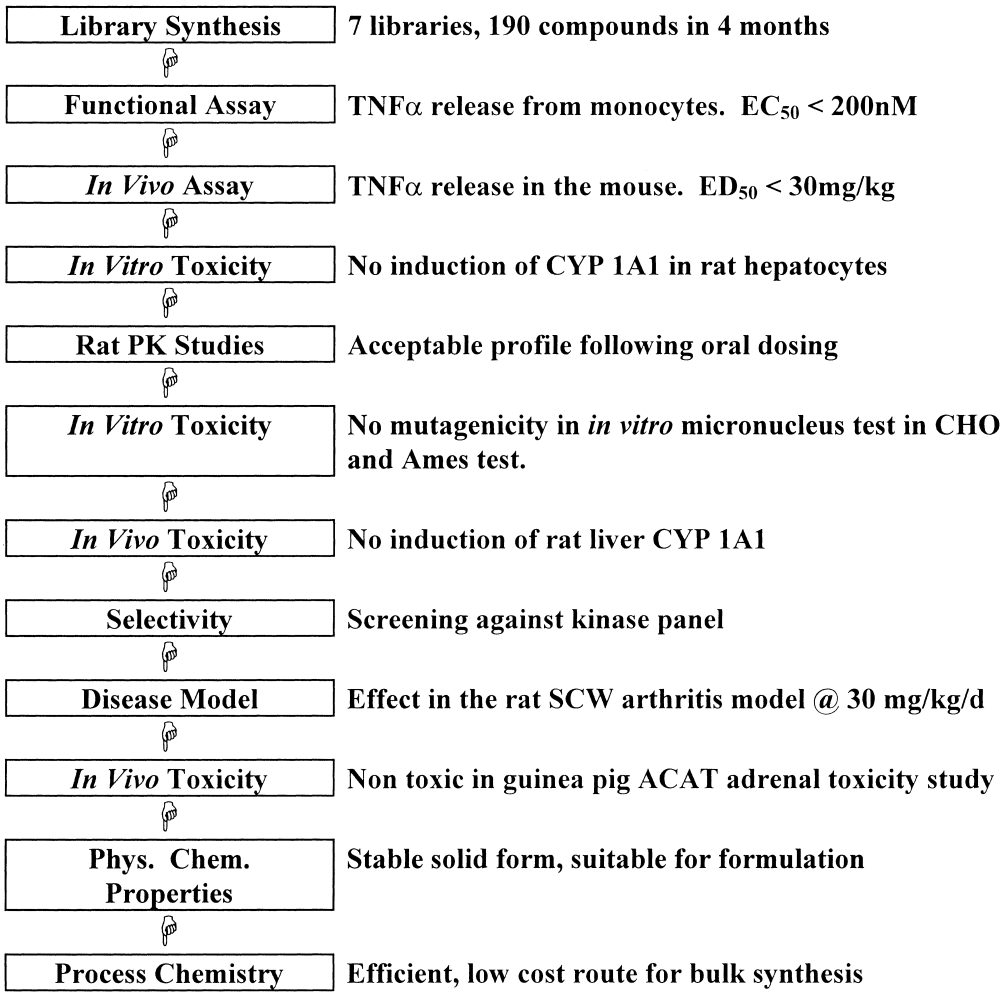


Figure 2. Screening strategy adopted for optimisation of RPR132331. The stages are carried out sequentially and the acceptance criteria are quoted at each stage.

RPR200765A—Chemical and physical properties

RPR200765A was highly stable in acidic, basic and oxidative environments. At an early stage it had been prepared as a mesylate salt to assist solubility and bioavailability. The increase in solubility in distilled water was great (free base 13 mg/L, mesylate 14 g/L). This salt formed a stable, crystalline hydrate and only one morphological form was found. The pharmaceutical properties of the mesylate salt were excellent.

A very efficient process route, was developed for bulk preparation of the compound.[26]

Conclusions

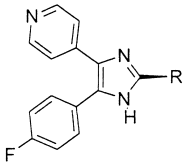
RPR132331 was identified as a novel, weak inhibitor of TNFα release from human monocytes. From this initial lead, parallel synthesis and biological filtering techniques were employed in the rapid identification of a development candidate: RPR200765A. The goal was to complete the project within one year and towards this end a great saving in chemical effort was achieved by avoiding full resynthesis and characterisation of compounds until firm candidates had been identified. Considerable effort was applied to the definition of the project objectives. This enabled a rational screening strategy to be devised and allowed key toxicity screens to be applied at a very early stage (Fig. 2). The excellent oral bioavailability and disease modifying activity of RPR200765A (both prophylactically and therapeutically), the absence of toxicity indicators (CYP 1A1 induction, mutagenicity and guinea pig adrenotoxicity) combined with excellent physical properties enabled RPR200765A to be proposed and accepted as a development candidate for the potential treatment of rheumatoid arthritis.

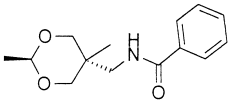
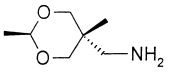
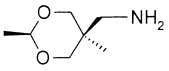
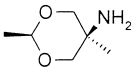
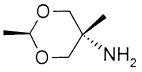
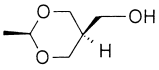
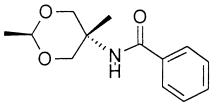
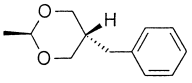
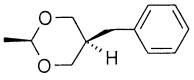
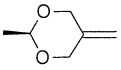
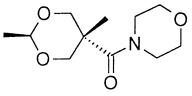
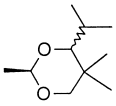
Experimental

Chemistry

Analytical data were recorded for the compounds described below using the following general procedures. Proton NMR spectra were recorded on a Varian VXR 400; chemical shifts were recorded in ppm (δ) from an internal tetramethylsilane standard in deuterochloroform

Table 5. Library samples showing activity in the mouse LPS stimulated TNFa release assay



R	Library code	EC_{50} (nM)[a]	ED_{50} (mg/kg)[b]	CYP 1A1 induction[c]
SB203580		146 (±53)	10–30	Inducer
	Lib7_13	84 (±13)	< 30	Inducer
	Lib7_1	38 (±8)	10–30	Inducer
	Lib6_1	33 (±11)	10–33	Inducer
	Lib4_1	55 (±20)	10–30	Non-inducer
	Lib5_1	180 (±33)	< 30	Non-inducer
	Lib1_12a	200 (±80)	10–30	Inducer
	Lib5_14	110 (±24)	10–30	Non-inducer
	Lib1_8b	191 (±42)	10–30	Inducer
	Lib1_8a	145 (±48)	< 10	Inducer
	Lib1_6	117 (±13)	< 30	Inducer
	Lib3_28	110 (±11)	< 10	Non-inducer
	Lib1_5	115 (±21)	< 30	Inducer

[a]Rapid cellular screening was first carried out using two concentrations only (100 and 300 nM, $n = 4$). Full IC_{50} determinations were then carried out for compounds showing more than 50% inhibition at 300 nM. Full protocol can be found in the Experimental.
[b]Rapid screening was carried out in this assay using doses of 10 and 30 mg/kg only ($n = 8$). The criteria for acceptance was < 30 mg/kg. SB203580 commonly produced results between 10 and 30 mg/kg with such a protocol.
[c]See Experimental for protocols.

Table 6. Pharmacokinetic parameters for RPR200765A and RPR201227A in the rat

Compound	Oral dosing, 10 mg/kg				iv Dosing, 1 mg/kg			
	Cp_{Max} (ng.mL^{-1})	AUC (h.ng.mL^{-1})	T_{max} (h)	$T_{1/2}$ (h)	VD (L.kg^{-1})	Cl (L.kg^{-1}.h^{-1})	$T_{1/2}$ (h)	F (%)
RPR200765A	5154	11,111	0.25–0.5	2.8	0.7	1	1.6	50
RPR201227A	50134	480,463	1–2	6.2	0.1	0.013	5.6	64

Table 7. IC_{50}s (μM) for different kinases (see Experimental for assay conditions)[a]

Kinase	RPR200765A	RPR201227A
p38	0.050	0.044
ERK	>100	~100
ZAP70	>40	>40
SYK	>40	>40
Lck	1.6	0.4

[a]IC_{50} for SB203580 = 0.056 nM.

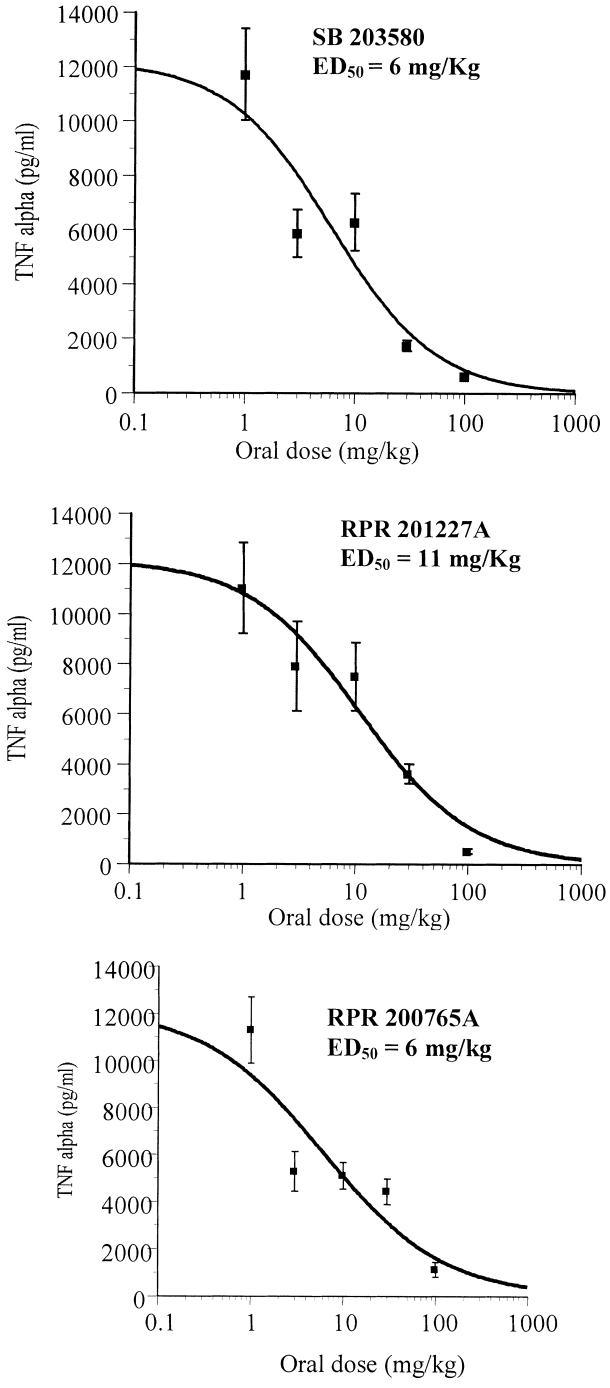


Figure 3. Effect of SB203580, RPR200765A and RPR201227A on LPS (0.1 mg/kg) induced TNF alpha release in male Balb/c mice.

or deuterodimethyl sulphoxide as specified below. Coupling constants (*J*) were recorded in Hz. Mass spectra (MS) were recorded on a VG 7070E/250 spectrometer using chemical ionisation with NH_3 as the carrier gas. Microanalyses were performed on a Carlo-Erba 1106 microanalyser. Where analyses are indicated by the symbols of the elements, results obtained were within 0.4% of the theoretical values. Melting points were taken on an Electrothermal melting point apparatus and are uncorrected. Reagents were purchased from commercial sources. Anhydrous magnesium sulphate was used routinely to dry the combined organic layers from extractions. Solvent was routinely removed in vacuo using a rotary evaporator.

Commonly used abbreviations: THF (tetrahydrofuran), DMF (dimethylformamide), DMSO (dimethyl sulphoxide), TLC (thin layer chromatography).

In many cases crude products were used, without further purification, as intermediates in subsequent reactions. In such cases characterisation of the crude product was carried through examination of the proton NMR and identification of an appropriate molecular ion in the MS. For these syntheses the overall yields reported were based on the original starting compound.

All library compounds were single spot by TLC and characterised by MS and, in cases where there existed the possibility of two isomers (*cis* and *trans*), the products were prepared for screening as 10 mM solutions in deuterodimethyl sulphoxide and the relative orientation assigned by ^1H NMR prior to biological examination.

4-[2-Dimethoxymethyl-5-(4-fluoro-phenyl)-1*H*-imidazol-4-yl]-pyridine 6. (Scheme 1). A stirred solution of 5-(4-fluoro-phenyl)-4-(4-pyridyl)-imidazole[25] (450 mg, 1.9 mmol, **1**) in dry DMF (5 mL) was treated portionwise with sodium hydride (90 mg, 60% dispersion in mineral oil). The mixture was stirred at room temperature until the evolution of hydrogen stopped, then treated dropwise with 2-(trimethylsilyl)ethoxymethyl chloride (340 mg, 2 mmol). The mixture was then stirred at room temperature for 1.5 h, the reaction mixture was treated

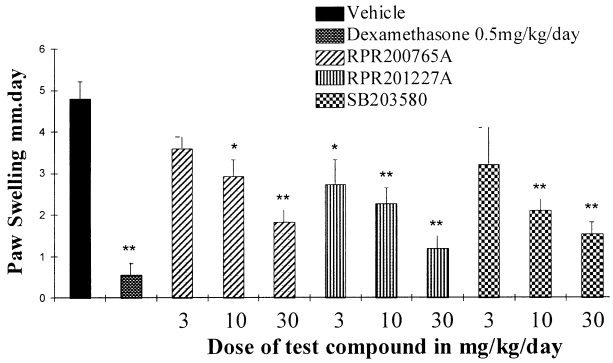


Figure 4. SCW arthritis model. Effects of SB203580, RPR200765A and RPR201227A on paw swelling following a prophylactic dosing regimen (po, bid.). The paw swelling is measured twice daily and the result is given on the *y*-axis as mm per day. Mean±S.E.M. $*P < 0.05$, $**P < 0.01$, ANOVA with post hoc Dunnett's test compared to vehicle-treated animals.

Table 8. Summary of toxicological parameters determined

Compound	Ames	Micronucleus	CYP1A1 (in vitro)	CYP1A1 (in vivo)	ACAT IC_{50} μM	Guinea pig adrenotox.
SB203580	Negative up to 2500 μg/plate[a]	Positive at 50 μg/mL and above	+	+	40	ND
RPR200765A	Negative up to 2500 μg/plate[a]	Negative up to 25 g/mL[a]	–	–	62	–
RPR201227A	Negative up to 1000 μg/plate[a]	Negative up to 75 μg/mL[a]	–	–	2.5	[b]

[a]Lowest insoluble concentration.
[b]Study abandoned due to animal death.

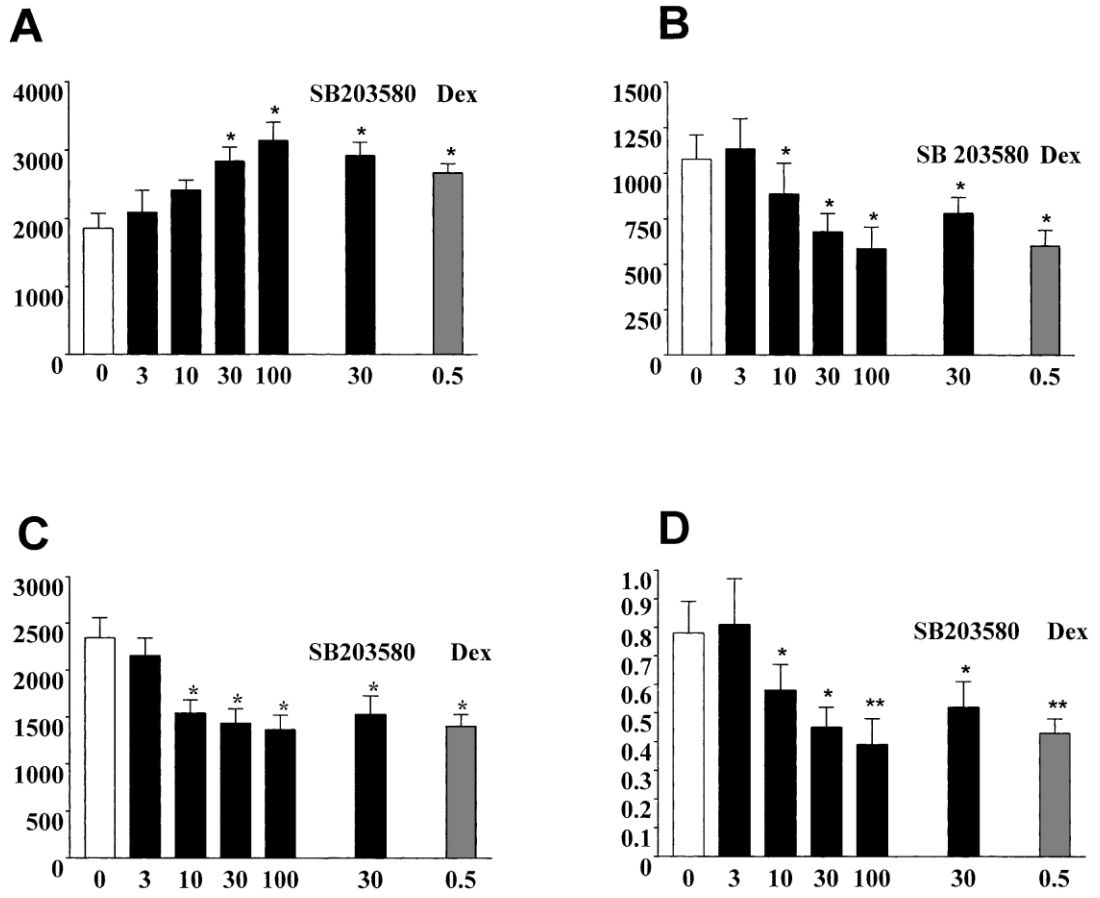


Figure 5. Effects of RPR200765A administered in a therapeutic regimen on histological parameters. RPR200765A was dosed at 3, 10, 30 and 100 mg/kg/day (po, bid.), SB203580 used as a comparison at 30 mg/kg/day (po, bid.) and the standard dexamethasone (Dex) at 0.5 mg/kg/day. The histology was assessed in joints obtained at the study termination (day 28) and quantified in terms of joint area (panel A), pannus (panel B), synovial hyperplasia (panel C) and resorption index (panel D) by histomorphometric analysis. Results represent means ± S.E.M. $*P<0.05$, $**P<0.01$, $***P<0.001$ compared to relevant vehicle control.

with water (0.2 mL) and evaporated. The residual oil was then partitioned between ethyl acetate (5 mL) and water (5 mL) and the aqueous phase was extracted twice further with ethyl acetate. The combined organic phases were washed with brine, dried and evaporated. The residual oil was subjected to flash chromatography on silica gel, eluting with a mixture of methanol:dichloromethane (98:2, v/v), to give a mixture of the silyl derivatives 4-[5-(4-fluoro-phenyl)-[1 or 3]-[(2-(trimethylsilyl)ethoxy)methyl]-1*H*-imidazol-4-yl]-pyridine (0.529 g, 1.4 mmol, **2** and **3**) as a yellow oil. This intermediate was dissolved in dry THF (5 mL) and treated with a solution of *n*-butyllithium in hexane (0.7 mL, 2.1 M) to give a dark green solution which was stirred at −78 °C for 15 min before treating with a solution of *N*-formylmorpholine (160 mg, 1.4 mmol) in THF (10 mL). After stirring for 15 min at −78 °C, the mixture was allowed to warm to room temperature and stirred for a further 1 h. The reaction mixture was quenched with water (10 mL) and extracted with ethyl acetate (3×30 mL). The combined extracts were dried and evaporated. The residual oil was subjected to flash chromatography on silica gel, eluting with ethyl acetate, to give a mixture of

Table 9. IC_{50} (μM) for inhibition of various human CYP enzymes

Compound	CYP 3A4	CYP 2D6	CYP 1A2	CYP 2C9
RPR200765	20	> 50	4.2	12
SB203580	4.4	28	2.6	0.44

formylated products 4-[5-(4-fluoro-phenyl)-2-formyl-[1 or 3]-[(2-(trimethylsilyl)ethoxy)methyl]-1*H*-imidazol-4-yl]-pyridine (525 mg, 1.3 mmol, **4** and **5**) as a yellow oil. This intermediate was dissolved in methanol (10 mL) and treated with trimethylorthoformate (5 mL) then with 4-toluenesulphonic acid (0.39 g). The reaction mixture was refluxed for 5 h, cooled to room temperature and evaporated under reduced pressure. The residue was partitioned between ethyl acetate (30 mL) and saturated aqueous sodium bicarbonate solution (30 mL). The aqueous phase was extracted four times with ethyl acetate (20 mL). The combined organic phases were washed with water (15 mL), brine (15 mL), dried over magnesium sulphate and evaporated. The residual oil was subjected to flash chromatography on silica gel eluting with a mixture of dichloromethane:methanol (8:1, v/v) to give **6** (0.344 g, 58% overall) as a white solid having mp 186–189 °C. ^{1}H NMR (DMSO-d_6) 8.35 (d, J = 6 Hz, 2H), 7.43 (d, J = 6 Hz, 2H), 7.36 (dd, J = 8 Hz and J = 4 Hz, 2H), 7.19 (t, J = 8 Hz, 2H), 5.39 (s, 1H), 3.34 (s, 6H). Anal. ($C_{17}H_{16}FN_3O_2$) C, H, N.

4-[2-(5,5-Dimethyl-[1,3]dioxan-2-yl)-5-(4-fluoro-phenyl)-1*H*-imidazol-4-yl]-pyridine RPR132331. (Scheme 2). A stirred solution of 4-[2-dimethoxymethyl-5-(4-fluoro-phenyl)-1*H*-imidazol-4(5)-yl]-pyridine (5.0 g, 15.9 mmol, **6**) and 2,2-dimethyl-1,3-propanediol (3.33 g, 31.8 mmol, **7**) and 4-toluenesulphonic acid (6.8 g, 39.7 mmol) in dry THF (200 mL) was heated at reflux for 6 h. The mixture was allowed to cool to room temperature then partitioned between ethyl acetate (200 mL) and saturated sodium bicarbonate solution (200 mL). The organic phase was washed with water (100 mL), brine (100 mL), then dried and evaporated to give RPR132331 (3.36 g, 61%) as a cream solid having mp 248–249 °C (d). ^{1}H NMR (DMSO-d_6) 8.72 (d, J = 8 Hz, 2H), 7.95 (d, J = 8 Hz, 2H), 7.62 (dd, J = 8 Hz and J = 4 Hz, 2H), 7.40 (t, J = 8 Hz, 2H), 5.68 (s, 1H), 3.72 (s, 4H), 1.25 (s, 3H, CH_3-ax), 0.79 (s, 3H, CH_3-eq). Anal. ($C_{20}H_{20}FN_3O_2 \cdot 0.5H_2O$) C, H, N.

General method A. Preparation of compounds in library 1. (Scheme 3). A solution of 4-[2-dimethoxymethyl-5-(4-fluoro-phenyl)-1*H*-imidazol-4-yl]-pyridine **6** (50 mg, 0.16 mmol) and 4-toluenesulphonic acid (70 mg, 0.4 mmol) in dichloromethane (5 mL) was treated with an appropriately substituted 1,3-propanediol (2 equiv, **26**, Table 1) and allowed to stand for 3 days. Each reaction mixture was then washed with saturated sodium bicarbonate solution (5 mL), dried and evaporated. The crude residues were then subjected to preparative thick layer chromatography on silica gel eluting with mixtures of dichloromethane/methanol to provide the library product (**27**) which was prepared, for biological evaluation, as a 10 mM solution in DMSO.

***c*-2-[5-(4-Fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl]-5-methyl-[1,3]dioxane-*r*-5-carboxylic acid 10 and *t*-2-[5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl]-5-methyl-[1,3]dioxane-*r*-5-carboxylic acid 11.** (Scheme 4). A stirred solution of 4-[2-dimethoxymethyl-5-(4-fluoro-phenyl)-1*H*-imidazol-4(5)-yl]-pyridine (370 mg, 1.2 mmol, **6**), methyl 2,2-bis(hydroxymethyl)propionate (380 mg, 2.4 mmol, **8**) and 4-toluenesulphonic acid (520 mg, 3 mmol) in dry THF (20 mL) was heated at reflux for 6 h. The mixture was allowed to cool to room temperature then partitioned between ethyl acetate (20 mL) and saturated sodium bicarbonate solution (20 mL). The organic phase was washed twice with water (2×10 mL), brine (10 mL), dried and evaporated. The residue was subjected to chromatography on silica gel eluting with a mixture of dichloromethane:methanol (9:1, v/v) to provide *c*/*t*-2-[5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl]-5-methyl-[1,3]dioxane-*r*-5-carboxylic acid methyl esters (*cis*- and *trans*-isomers) as a cream solid (340 mg, 0.86 mmol, **9**). This mixture of intermediate esters was dissolved in methanol (15 mL), treated with aqueous sodium hydroxide solution (1.71 mL, 1 N) and heated at reflux for 7 h. The reaction mixture was cooled and evaporated. The residual cream powder was dissolved in methanol (10 mL) and the solution acidified to pH 5–6 by addition of glacial acetic acid. The resulting white precipitate was filtered and washed with pentane to give the *trans* isomer **11** (0.11 g, 24% overall) as a white solid having mp 265–267 °C (d). ^{1}H NMR (DMSO-d_6) 8.50 (m, 2H), 7.53–7.28 (m, 6H), 5.51 (s, 1H), 4.08 (d, J = 10 Hz, 2H), 3.90 (d, J = 10 Hz, 2H), 1.48 (s, 3H, CH_3-ax). Anal. ($C_{20}H_{18}FN_3O_4$) C, H, N. The filtrate plus washings were separated by flash chromatography on silica gel eluting with a mixture of dichloromethane: methanol (9:1, v/v) to give the *cis* isomer **10** (0.14 g, 30% overall) as a white solid having mp 290 °C (d). ^{1}H NMR (DMSO-d_6) 8.44 (m, 2H), 7.46 (dd, J = 8 Hz and J = 4 Hz, 2H), 7.36 (d, J = 8 Hz, 2H), 7.30–7.21 (m, 2H), 5.66 (s, 1H), 4.47 (d, J = 12 Hz, 2H), 3.71 (d, J = 12 Hz, 2H), 0.94 (s, 3H, CH_3-eq). Anal. ($C_{20}H_{18}FN_3O_4 \cdot CH_3OH$) C, H, N.

***c*-5-Methyl-2-{5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl}-[1,3]dioxan-*r*-5-ylamine *cis*-isomer 16 and *t*-5-methyl-2-{5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl}-[1,3]dioxan-*r*-5-ylamine *trans*-isomer 17.** (Scheme 5). A stirred solution of 2-amino-2-methyl-1,3-propanediol (7.5 g, 71.4 mmol, **12**) in dry DMF (150 mL), at room temperature, was treated with potassium carbonate (11.2 g). After stirring at room temperature for 15 min the mixture was treated dropwise with trifluoroacetic anhydride (11.5 mL) and stirred for a further 18 h. The reaction mixture was evaporated and the residue was subjected to flash chromatography on silica gel eluting with a mixture of dichloromethane:methanol (95:5, v/v) to give 2-methyl-2-trifluoroacetamido-1,3-propanediol (7.38 g, 36.7 mmol, **13**). This intermediate was dissolved in dry THF (200 mL) and treated with 4-[2-dimethoxymethyl-5-(4-fluoro-phenyl)-1*H*-imidazol-4-yl]-pyridine (5.75 g, 18.3 mmol, **6**) and 4-toluenesulphonic acid (8.03 g, 46.7 mmol) and the mixture heated at reflux temperature for 6 h. The mixture was cooled, treated with saturated sodium bicarbonate solution (200 mL), and extracted with ethyl acetate (200 mL). The organic phase was washed with water (2×100 mL), brine (100 mL), dried and evaporated. The residue was subjected to chromatography on silica gel eluting with a mixture of dichloromethane:methanol (95:5, v/v) to give *c*-2,2,2-trifluoro-*N*-[5-methyl-2-{5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl}-[1,3]dioxan-*r*-5-yl]-acetamide **14**

(*cis*-isomer, 4.3 g, 9.5 mmol) and *t*-2,2,2-trifluoro-*N*-[5-methyl-2-{5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl}-[1,3]di-oxan-*r*-5-yl]-acetamide **15** (*trans*-isomer, 1.62 g, 3.6 mmol) both as light brown gums. Both intermediates were dissolved separately in methanol (*cis* isomer: 300 mL; *trans* isomer: 100 mL) and treated with potassium carbonate (*cis* isomer: 2.6 g, 19 mmol; *trans* isomer: 1 g, 7.2 mmol) and water (*cis* isomer: 7.5 mL; *trans* isomer: 2.5 mL). The mixtures were heated for 8 h, evaporated, and residual water removed by azeotroping with toluene. Each residue was then subjected to flash chromatography on silica gel eluting with a mixture of dichloromethane:methanol (4:1,v/v) to provide the two title products as white solids: the *cis*-product **16** (2.4 g, 37.7% overall) having mp 194 °C (d). ^{1}H NMR (DMSO-d_6) 8.47 (m, 2H), 7.51 (dd, $J=8$ Hz and $J=4$ Hz, 2H), 7.39 (d, $J=6$ Hz, 2H), 7.31 (m, 2H), 5.64 (s, 1H), 3.83 (d, $J=12$ Hz, 2H), 3.68 (d, $J=12$ Hz, 2H), 0.90 (s, 3H, CH_3-eq). Anal. ($C_{20}H_{18}FN_3O_4 \cdot 0.25H_2O$) C, H, N and the *trans*-product **17** (0.93 g, 14.2% overall) having mp 254 °C (d). ^{1}H NMR (DMSO-d_6) 8.36 (m, 2H), 7.43 (m, 2H), 7.32 (d, $J=6$ Hz, 2H), 7.36 (m, 2H), 5.47 (s, 1H), 3.63 (d, $J=10$ Hz, 2H), 3.54 (d, $J=10$ Hz, 2H), 1.26 (s, 3H, CH_3-ax). Anal. ($C_{20}H_{18}FN_3O_4 \cdot 0.25H_2O$) C, H, N.

c-C-[5-Methyl-2-(5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl)-[1,3]dioxan-*r*-5-yl]-methylamine *cis*-isomer 24 and *t*-C-[5-methyl-2-(5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl)-[1,3]dioxan-*r*-5-yl]-methylamine *trans*-isomer 25. (Scheme 6). 5-Azidomethyl-2-dimethyl-5-methyl-1,3-dioxane[27] (1.48 g, 8 mmol, **18**) was dissolved in dry THF (10 mL), treated with concentrated hydrochloric acid (0.3 mL) and water (0.8 mL) before heating at reflux temperature for 1 h. The mixture was then cooled and evaporated to give 2-azidomethyl-2-methyl-1,3-propanediol (1.3 g, 8.9 mmol, **19**) as a colourless oil. This intermediate was dissolved in dry THF (50 mL) and treated with 4-[2-dimethoxymethyl-5-(4-fluoro-phenyl)-1*H*-imidazol-4-yl]-pyridine (1.4 g, 4.4 mmol, **6**) and 4-toluenesulphonic acid (1.9 g, 11.3 mmol) and the mixture heated at reflux temperature for 6 h. The mixture was cooled, treated with saturated sodium bicarbonate solution (50 mL) and extracted with ethyl acetate (50 mL). The organic phase was washed with water (2×25 mL), brine (25 mL), dried and evaporated. The residue was subjected to flash chromatography on silica gel eluting with a mixture of dichloromethane: methanol (9:1 v/v) to give 4-[2-(*c*/*t*-5-azidomethyl-5-methyl-[1,3]dioxan-*r*-2-yl)-5-(4-fluoro-phenyl)-1*H*-imidazol-4-yl]-pyridine, *cis*- and *trans*-isomers **20** (1.56 g, 3.9 mmol) as a white solid. This intermediate mixture of isomers was dissolved in methanol (100 mL), treated with ammonium formate (1 g) and 10% palladium on activated carbon (0.15 g). The mixture was stirred for 3.5 h, filtered through diatomaceous earth and evaporated. The residual orange solid was subjected to flash chromatography on silica gel eluting with a mixture of dichloromethane:pentane:methanol:concentrated ammonia (55:25:18:2 v/v/v/v) to give a mixture of *C*-[5-methyl-2-{5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl}-[1,3]dioxan-5-yl]-methylamine, *cis*- and *trans*-isomers **21** (0.980 g, 2.7 mmol) as a yellow solid. This mixture of isomers was suspended in dichloromethane (50 mL) and treated with triethylamine (1 mL) and trifluoroacetic anhydride (1 mL). After stirring at room temperature for 4.5 h the reaction mixture was partitioned between ethyl acetate (50 mL) and water (50 mL). The organic phase was washed with water (50 mL), brine (50 mL), dried and evaporated. The residue was subjected to preparative HPLC, using methanol:water 40:60 v/v to 5:95 v/v over 20 min to give *c*-2,2,2-trifluoro-*N*-[5-methyl-2-{5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl}-[1,3]dioxan-*r*-5-ylmethyl]-acetamide, *cis*-isomer **22** (750 mg, 1.6 mmol, $R_T=12$ min) and *t*-2,2,2-trifluoro-*N*-[5-methyl-2-{5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl}-[1,3]dioxan-*r*-5-ylmethyl]-acetamide, *trans*-isomer **23** (300 mg, 0.64 mmol, $R_T=11$ min). Both intermediates were dissolved separately in methanol (*cis* isomer: 100 mL; *trans* isomer: 50 mL) and treated with potassium carbonate (*cis* isomer: 1.11 g, 8 mmol; *trans* isomer: 440 mg, 3.2 mmol) and water (*cis* isomer: 2.5 mL; *trans* isomer: 1.2 mL). The mixtures were heated for 8 h, evaporated and residual water removed by azeotroping with toluene. Each residue was subjected to flash chromatography on silica gel eluting with a mixture of dichloromethane:pentane:methanol:concentrated ammonia (55:25:18:2, v/v/v/v) to provide the two title products as white solids: the *cis*-product **24** (370 mg, 23% overall) having mp 203–205 °C. ^{1}H NMR (DMSO-d_6) 8.45 (d, $J=6$ Hz, 2H), 7.46 (dd, $J=8$ Hz and $J=4$ Hz, 2H), 7.36 (d, $J=6$ Hz, 2H), 7.28 (t, $J=8$ Hz, 2H), 5.58 (s, 1H), 3.93 (d, $J=12$ Hz, 2H), 3.60 (d, $J=12$ Hz, 2H), 2.88 (s, 2H), 0.65 (s, 3H, CH_3-eq). Anal. ($C_{20}H_{18}FN_3O_4$) C, H, N and the *trans*-product **25** (150 mg, 9% overall) having mp 158—165 °C. ^{1}H NMR (DMSO-d_6) 8.44 (d, $J=6$ Hz, 2H), 7.48 (dd, $J=8$ Hz and $J=4$ Hz, 2H), 7.37 (d, $J=6$ Hz, 2H), 7.27 (t, $J=8$ Hz, 2H), 5.53 (s, 1H), 3.77 (d, $J=12$ Hz, 2H), 3.72 (d, $J=12$ Hz, 2H), 2.65 (s, 2H), 1.21 (s, 3H, CH_3-ax). Anal. ($C_{20}H_{18}FN_3O_4$) C, H, N.

General method B. Preparation of compounds in libraries 2 and 3. (Scheme 7). A solution of *cis* or *trans*-2-[5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl]-5-methyl-[1,3]dioxane-5-carboxylic acid (*cis*- or *trans*-isomer) **10** or **11** (50 mg, 0.13 mmol), an appropriate primary or secondary amine (1.1 equiv, **28** Table 2), 1-(3-dimethyl-amino-propyl)-3-ethylcarbodiimide (1.1 equiv), 1-hydroxybenzotriazol hydrate (1.1 equiv) and *N*,*N*-diisopropylethylamine (3 equiv) in dry DMF (5 mL) was stirred at room temperature for 18 h. For each reaction the mixture was evaporated and the residue partitioned between ethyl acetate and water. The organic phase was washed with brine and evaporated to give the required library compound **29** or **30** which was prepared, for biological evaluation, as a 10 mM solution in DMSO.

General method C. Preparation of ureas in libraries 4, 5, 6 and 7. (Scheme 8). A solution of 5-methyl-2-{5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl}-[1,3]dioxan-5-ylamine (*cis*- or *trans*-isomer) **16** or **17** (50 mg, 0.13 mmol) or *C*-[5-methyl-2-(5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl)-[1,3]dioxan-5-yl]-methylamine (*cis*- or *trans*-isomer) **24** or **25** (50 mg, 0.13 mmol) and

an appropriately substituted isocyanate (1 equiv, Table 3) in dry THF was stirred at room temperature for 30 min. For each reaction the mixture was evaporated and the residue subjected to preparative thick layer chromatography on silica gel, eluting with a mixture of dichloromethane:methanol (9:1 v/v), and the required library compound **31** prepared, for biological evaluation, as a 10 mM solution in DMSO.

General method D. Preparation of thioureas in libraries 4, 5, 6 and 7. (Scheme 8). A solution of 5-methyl-2-{5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl}-[1,3]dioxan-5-ylamine (*cis*- or *trans*-isomer) **16** or **17** (50 mg, 0.13 mmol) or *C*-[5-methyl-2-(5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl)-[1,3]dioxan-5-yl]-methylamine **24** or **25** (*cis*- or *trans*-isomer) (50 mg, 0.13 mmol) and an appropriately substituted isothiocyanate (1 equiv, Table 3) in dry THF (5 mL) was heated at reflux for 18 h. For each reaction the mixture was evaporated and the residue subjected to preparative thick layer chromatography on silica gel, eluting with a mixture of dichloromethane:methanol (9:1 v/v), and the required library compound **32** isolated and prepared, for biological evaluation, as a 10 mM solution in DMSO.

General method E. Preparation of neutral amides in libraries 4, 5, 6 and 7. (Scheme 8). A solution of 5-methyl-2-{5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl}-[1,3]dioxan-5-ylamine (*cis*- or *trans*-isomer) **16** or **17** (50 mg, 0.13 mmol) or *C*-[5-methyl-2-(5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl)-[1,3]dioxan-5-yl]-methylamine **24** or **25** (*cis*- or *trans*-isomer) (50 mg, 0.13 mmol) and triethylamine (1 equiv) in dry THF (5 mL) was treated with an appropriately substituted acid chloride (1 equiv, Table 3) and the mixture stirred for 18 h at room temperature. For each reaction the mixture was evaporated and the residue subjected to preparative thick layer chromatography on silica gel, eluting with a mixture of dichloromethane:methanol (9:1 v/v), and the required library compound **33** isolated and prepared, for biological evaluation, as a 10 mM solution in DMSO.

General method F. Preparation of acidic amides in libraries 4, 5, 6 and 7. (Scheme 8). A solution of 5-methyl-2-{5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl}- [1,3]dioxan-5-ylamine (*cis*- or *trans*-isomer) **16** or **17** (50 mg, 0.13 mmol) or *C*-[5-methyl-2-(5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl)-[1,3]dioxan-5-yl]-methylamine (*cis*- or *trans*-isomer) **24** or **25** (50 mg, 0.13 mmol) and triethylamine (1.2 equiv) in dry THF (5 mL) was treated with an appropriately substituted cyclic acid anhydride (1 equiv, Table 3) and the mixture heated at reflux temperature for 8 h. For each reaction the mixture was evaporated and the residue subjected to preparative thick layer chromatography on silica gel, eluting with a mixture of dichloromethane:methanol (9:1 v/v), and the required library compound **34** isolated and prepared, for biological evaluation, as a 10 mM solution in DMSO.

General method G. Preparation of sulphonamides in libraries 4, 5, 6 and 7. (Scheme 8). A solution of 5-methyl-2-{5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl}-[1,3]dioxan-5-ylamine (*cis*- or *trans*-isomer) **16** or **17** (50 mg, 0.13 mmol) or *C*-[5-methyl-2-(5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl)-[1,3]dioxan-5-yl]-methylamine (*cis*- or *trans*-isomer) **24** or **25** (50 mg, 0.13 mmol) and triethylamine (1 equiv) in dry THF (5 mL) was treated with an appropriately substituted sulphonyl chloride (1 equiv, Table 3) and the mixture heated at reflux temperature for 8 h. The reaction mixture was evaporated and the residue subjected to preparative thick layer chromatography on silica gel, eluting with a mixture of dichloromethane:methanol (9:1 v/v). The required library compound **35** was isolated and prepared, for biological evaluation, as a 10 mM solution in dimethylsulphoxide.

General method H. Preparation of basic amides in libraries 4, 5, 6 and 7. (Scheme 8). A solution of 5-methyl-2-{5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl}-[1,3]dioxan-5-ylamine (*cis*- or *trans*-isomer) **16** or **17** (50 mg, 0.13 mmol) or *C*-[5-methyl-2-(5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl)-[1,3]dioxan-5-yl]-methylamine (*cis*- or *trans*-isomer) **24** or **25** (50 mg, 0.13 mmol) an appropriate carboxylic acid (1.1 equiv, Table 3), 1-(3-dimethylamino-propyl)-3-ethylcarbodiimide (1.1 equivalents), 1-hydroxybenzotriazol hydrate (1.1 equiv) and *N*,*N*-diisopropylethylamine (3 equiv) in dry DMF (5 mL) was stirred at room temperature for 18 h. For each reaction the mixture was evaporated and the residue partitioned between ethyl acetate and sodium bicarbonate solution. The organic phase was dried, evaporated and the residue subjected to preparative thick layer chromatography on silica gel, eluting with dichloromethane:methanol, to provide the required library compound **36** which was prepared, for biological evaluation, as a 10 mM solution in DMSO.

In some cases the reaction was performed with an amino acid protected as a benzyloxycarbonyl derivative. The products from these reactions were subsequently treated with palladium, 5% activated on carbon and stirred at room temperature under a hydrogen atmosphere for 8 h, filtering through a pad of diatomaceous earth, evaporated and the residue subjected to preparative thick layer chromatography on silica gel, eluting with dichloromethane:methanol (7:3 v/v) to provide the required primary amine product which was prepared, for biological evaluation, as a 10 mM solution in DMSO.

{*t*-2-[4-(4-Fluoro-phenyl)-5-pyridin-4-yl-1*H*-imidazol-2-yl]-5-methyl-[1,3]dioxan-*r*-5-yl}-morpholin-4-yl-methanone methane sulphonic acid salt, *trans*-isomer, RPR200765A. (Scheme 9). *t*-2-[5-(4-Fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl]-*r*-5-methyl-[1,3]dioxane-5-carboxylic acid, *trans*-isomer **11** (7.70 g, 20 mmol) was added portionwise to stirred thionyl chloride (50 mL). The reaction mixture was stirred at room temperature for 1.25 h, evaporated and the residue azeotroped with dry toluene to yield the crude acid chloride **38** which was dissolved in dry dichloromethane (150 mL) and treated with morpholine (30 mL). The mixture was stirred at room temperature for 2.5 h, evaporated and the residue partitioned between ethyl acetate (250 mL) and saturated sodium bicarbonate

(200 mL). The insoluble product at the interface was filtered off and washed with water (20 mL), methanol (10 mL), diethyl ether (20 mL) to give the free base of the title compound (7.43 g, 16.9 mmol) which was dissolved in hot THF (500 mL) and treated with a solution of methane sulphonic acid (1.578 g) in THF. After standing at room temperature for 18 h the reaction mixture was filtered and the solid recrystallised from acetonitrile with hot filtration through Celite to give RPR200765A (6.00 g, 53% overall) as a pale yellow crystalline solid having mp 242–246 °C (d). ^{1}H NMR (DMSO-d_6) 8.65 (d, J = 8 Hz, 2H), 7.86 (d, J = 8 Hz, 2H), 7.60 (dd, J = 8 Hz and J = 4 Hz, 2H), 7.40 (t, J = 8 Hz, 2H), 5.69 (s, 1H), 4.12 (s, 4H), 3.59–3.50 (m, 8H), 2.31 (s, 3H, CH_3SO_3), 1.58 (s, 3H, CH_3-ax). Anal. ($C_{24}H_{25}FN_4O_4 \cdot CH_3SO_3H$) C, H, N.

***t*-*N*-{2-[4-(4-Fluoro-phenyl)-5-pyridin-4-yl-1*H*-imidazol-2-yl]-5-methyl-(1,3)dioxan-*r*-5-yl}-benzamide, methane sulphonic acid salt, *trans*-isomer RPR201227A.** (Scheme 10). A stirred suspension of 5-methyl-2-{5-(4-fluoro-phenyl)-4-pyridin-4-yl-1*H*-imidazol-2-yl}-[1,3]dioxan-5-ylamine, *trans* isomer **17** (1.76 g, 5 mmol), benzoic acid (0.67 g, 5.5 mmol) and diisopropylethylamine (1.39 mL) in dry DMF (50 mL) was treated with {0-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate} (1.88 g, HATU) under a nitrogen atmosphere. The mixture was stirred at room temperature for 2 h and evaporated to dryness. The residue was partitioned between ethyl acetate (70 mL) and saturated sodium bicarbonate (50 mL). The organic phase was washed twice with water (50 mL), then with brine (30 mL) and then evaporated. The residue was subjected to flash chromatography on silica gel eluting with ethyl acetate to give the free base of the title product (0.85 g, 1.85 mmol). This was dissolved in THF (100 mL) and treated with methane sulphonic acid (0.178 g, 1.85 mmol). The reaction mixture was agitated for 5 min then evaporated to dryness and dried under high vacuum. The residual solid was recrystallised from ethyl acetate containing a minimum volume of acetonitrile to give RPR201227A (900 mg, 32% overall) as a yellow crystalline solid having mp 165–169 °C. ^{1}H NMR (DMSO-d_6) 8.65 (d, J = 8 Hz, 2H), 8.03 (s, 1H), 7.87–7.80 (m, 4H), 7.63–7.51 (m, 3H), 7.47 (t, J = 8 Hz, 2H), 7.39 (t, J = 8 Hz, 2H), 5.77 (s, 1H), 4.31 (d, J = 10 Hz, 2H), 4.12 (d, J = 10 Hz, 2H), 2.31 (s, 3H, CH_3SO_3), 1.71 (s, 3H, CH_3-ax). Anal. ($C_{26}H_{23}FN_4O_3 \cdot CH_3SO_3H$) C, H, N.

Biological Methods

Kinases assay

The p38 enzyme assay is carried out at room temperature for 1 h, using 40 ng/well of the mouse enzyme. The substrate, 50 μg/mL ATF-2 is coated onto 96-well plates, the assay is carried out in 25 mM Hepes buffer, pH 7.7 containing 25 mM magnesium chloride, 2 mM dithiothreitol, 1 mM sodium orthovanadate and 100 μM ATP. Phosphorylated ATF-2 is quantitated using a phospho-specific ATF-2 primary antibody (rabbit anti-human) followed by a europium-labelled secondary antibody (sheep anti-rabbit IgG) with addition of the DELFIA enhancement solution resulting in fluorescence. ERK was measured using a [^{33}P] ATP filtration assay format using for substrates myelin basic protein. ZAP-70, Syk and Lck kinase activities were measured using the homogeneous time-resolved fluorescence methodology (HTRF) with the catalytic domains of each of the tyrosine kinases, biotinylated, specific peptide substrates, streptavidin-linked APC and europium cryptate-conjugated anti-phosphotyrosine antibody. Results represent means ± S.E.M. (n = 3).

Monocyte TNFα release assay. Adherent human monocytes (100,000 cells/well) were incubated with LPS (10 ng/mL) in the absence and presence of compound for 18 h. Individual experiments were carried out in quadruplicate samples. TNFα was measured by sandwich ELISA and IC_{50} values calculated for the activity of individual compounds. IC_{50} values shown from repeat experiments are means±S.E.M. (n = 3). means ± S.E.M. (n = 3).

Mouse TNFα release assay. Compound was administered orally to balb/c mice 30 min prior to LPS (0.1 mg/kg ip) challenge. Serum TNFα levels were determined 90 min after LPS insult. Results represent means ± S.E.M. (n = 3).

Hepatocyte CYP induction assay. Primary hepatocytes were isolated from Sprague–Dawley rats, cultured in 6-well plates and treated with vehicle (0.25% DMSO) or compound (30, 50 and 100 μM) for a period of 3 days. At the end of the incubation, cells were harvested, homogenised and the post-nuclear fractions isolated. Content of CYP isozymes (1A1, 1A2, 2B1 and 2B2) were evaluated by Western blot using isozyme specific antibodies.

Rat drug distribution studies. Three rats were dosed orally (10 mg/kg) with a suspension of compound in methylcellulose or iv (1 mg/kg) with a solution of compound in *N*-methylpyrrolidone. Blood samples were taken at intervals over 24 h and analysed for compound. Averaged results are reported with ± S.E.M. (n = 3).

Rat CYP induction assay. Compound was dosed orally to three male rats at 100 and 200 mg/kg/day for 5 days. Animals were sacrificed and liver CYP (1A1, 1A2, 2B1, 2B2, 3A1 and 2E1) levels assessed by Western blot analysis.

Rat streptococcal cell wall (SCW) induced arthritis model. This model was performed using Lewis Rats in either a prophylactic or therapeutic regimen. On day 0 an intra-articular injection of 10 μg of SCW 100P fraction (in 10 μL) was made into the tibiotarsal joint of one hind limb of anaesthetised female rat. Any animals which had not developed a swollen ankle 2 days later were excluded from the study. Challenge with iv SCW was made on day 21 and compounds given on day 20–24 (po, bid.) for the prophylactic studies and 21–24 for the therapeutic studies. Paw swelling was calculated by summation of daily measurement of the paw medial and lateral malleoli width post challenge with iv SCW until

termination of study. Radiography of tibio-tarsal joints was undertaken using a Phillips Practix X-ray unit. Histopathology was performed on formalin-fixed tissues, processed and stained with Alcian Blue. Histomorphometry of histological structures was performed by image analysis using an Apple Quadra 700 computer attached to an Olympus BX50 microscope using the Image-Grabber™ program and analysed by Optilab™ software (both ME Electronics Ltd, Reading, UK). Structures examined were: unaffected joint area (area of histologically normal tibio-tarsal tissues); synovial hyperplasia (the intrusion of activated synovial villi into the articular space); pannus (total pannus area within the sub-chondral bone); bone resorption index (the number of activated cavities divided by the total cavity number). Compound effects on paw swelling indices were compared to vehicle groups using ANOVA and Dunnetts test ($n=3$). Radiographic and histological indices were analysed using the Mann–Whitney U test. Groups of 10 rats were used for each dosing protocol. Mean ± S.E.M. * $P<0.05$, ** $P<0.01$, ANOVA with post hoc Dunnett's test compared to vehicle-treated animals.

Human CYP inhibition assay. Rapid inhibition assays were undertaken to determine the inhibitory potency (IC_{50}) of the p38 compounds using Gentest recombinant human CYPs. Probe substrates were incubated with the expressed CYPs in the presence or absence of test compound (0.4, 2, 10 and 50 μM). Gentest protocols were followed except for a reduction in the incubation for CYP2C9 assays (30 min).[28]

Guinea pig adrenotoxicity assay. Three guinea pigs were dosed orally with compound at 100, 300 and 800 mg/kg/day for 4 days. Animals were sacrificed and adrenal glands examined histologically for anomalies.

Bacterial reverse mutation test (Ames test)

The potential of the compounds to induce point mutations was evaluated in three *Salmonella typhimurium* strains TA98, TA100 and TA102, both with and without metabolic activation by liver homogenate from rats pretreated with Aroclor (S9 mix), using plate incorporation method.[29] The compound was considered positive when it induces at least a 2-fold increase in the number of revertant colonies.

In vitro micronucleus test in Chinese hamster ovary (CHO) cells

The potential to induce chromosome damage was evaluated by measuring the increase in the number of micronucleated cells. CHO-WBL cells were exposed for 3 h to the compound in the presence or in the absence of metabolic activation by liver homogenate from rats pretreated with Aroclor (S9 mix). After treatment, cells were grown for 20 h with 2 μg/mL cytochalasine B in order to inhibit cytoplasm division without inhibiting nucleus division. At the end of this 20-h period, cells were grown for 1.5 h and then fixed and stained. The compound was considered positive if it induces at least a 2-fold increase in the incidence of micronucleated cells.

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