An Algorithm-Directed Two-Component Library Synthesized Via Solid-Phase Methodology Yielding Potent and Orally Bioavailable p38 MAP Kinase Inhibitors

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Previously we reported the identification of RPR200765A, a potent orally bioavailable pyridine−imidazole inhibitor of p38 mitogen-activated protein (MAP) kinase which suppressed paw swelling and joint pathology in streptococcal cell wall-induced arthritis. Herein, we report the use of solid-phase combinatorial organic synthesis for the parallel processing of a related pyrimidine−imidazole-based library with two points of structural variability. We report also that the application of a computer algorithm, the Monte Carlo Monomer Selection, maximized both the combinatorial synthetic efficiency and the bioavailability of the final compounds. In conjunction with the synthetic protocols, the polymer-supported quench technique was applied to the purification of the final compounds. Through rapid evaluation of the library using a p38 kinase assay and permeability assays, it was possible to identify a number of potent and orally bioavailable p38 MAP kinase inhibitors suitable for further biological investigation.

Introduction

Pro-inflammatory cytokines tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β) are implicated in the pathology of several chronic inflammatory diseases[1] including rheumatoid arthritis.[2] They are released from several inflammatory cells, particularly mononuclear phagocytes. The synthesis of both TNF-α and IL-1β is regulated by a kinase referred to as p38, which is a mitogen-activated protein (MAP) kinase belonging to the serine/threonine kinase superfamily.[3] It is activated by inflammatory stimuli such as lipopolysaccharide (LPS) as well as extracellular stresses including heat, UV light, and osmotic stress and is often referred to as a stress-activated protein kinase.[4] In its activated state, p38 phosphorylates intracellular protein substrates which regulate the biosynthesis of TNF-α and IL-1β post-transcriptionally. Previous studies have demonstrated that specific inhibitors of this kinase suppress production of these cytokines from mononuclear cells, making p38 a very attractive target for novel antiiinflammatory therapy. Thus, our aim was to identify small-molecule inhibitors of p38, for the treatment of inflammatory conditions resulting from excessive cytokine production.[5]

Our series of imidazoyl-based cyclic acetals as functional inhibitors of TNF-α originated from our previous knowledge that the 1,3-dioxane was stable when attached to the imidazole nucleus.[6] This knowledge when coupled to the postulate that the 4-pyridyl group attached to the imidazole nucleus may be a crucial entity for such biological activity led us to synthesize a large array of 4-pyridyl-5-(4-fluorophenyl)imidazoyl cyclic acetals.[7] Herein, we report our later efforts in the realization via solid-phase combinatorial organic synthesis (SPCOS) of a pyrimidinoimidazole-based library in which we made use of a computer algorithm in the library design to engender greater bioavailability to the series. Reaction transformations were monitored by single-bead FT-IR,[8] and a polymer-supported quench (PSQ) technique[9] following cleavage from the solid support allowed for rapid purification of the library. Thereafter, rapid biological evaluation identified a number of potent and orally bioavailable p38 MAP kinase inhibitors.

Chemistry

The investigations centered around 4-pyridyl-5-(4-fluorophenyl)imidazoyl cyclic acetals led to the identification of RPR200765A (**3**; Figure 1) as a novel p38 inhibitor and to its being accepted as a development candidate.[7] Our follow-up strategy for this initial work was to synthesize specifically (i) solution-based libraries[10] in which substituted pyrimidines, replacing the pyridine nucleus of **3**, were combined with the most favorable substituted dioxanes identified during the discovery of **3** and (ii) a larger library in which we sought variations in both the amide portion of **3** and the pyrimidine nucleus of the molecule simultaneously. This larger library is the subject of the work reported here and was prepared via SPCOS (Figure 1).

As already stated, we wished to introduce our diversity at the 2-position of the pyrimidine (**Nu**, R^3R^4N-) and the amide unit (**Am**, R^1R^2N-) of the dioxane. We envisaged that the former diversity **Nu** could be introduced through a nucleophilic cleavage from an appropriately activated resin while the latter **Am** could be introduced at a prior point on the resin-bound substrate. We hoped to maximize the bioavailability within this series through careful choice of complimentary substituents, a strategic decision which drove us to develop the Monte Carlo Monomer Selection (MCMS)

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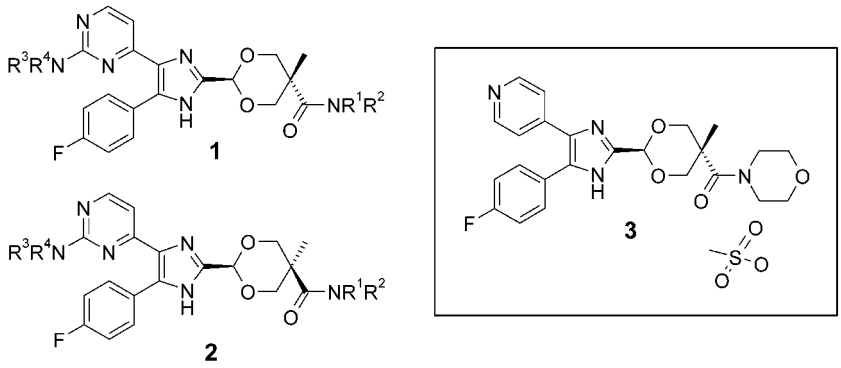


Figure 1. SPCOS-targeted compounds, *trans*-**1** and *cis*-**2** isomers; based on **3**, RPR200765A.

algorithm to direct these variations. It should be noted that these compounds can exist as *cis* or *trans* isomers, according to their relative stereochemistry about the dioxane ring. For this study we decided to work with both isomers, treating each as a separate library. All of the library design was carried out for the *trans* isomers **1** and then transferred to the *cis* isomer **2** library.

In this work we utilized two sets of monomers, both amines: the first set (**Am**, R^1R^2NH) for the amide-forming reaction, the second set (**Nu**, R^3R^4NH) for the nucleophilic displacement. MCMS applies a scoring function, controlled by a series of weights, to drive the monomer selection toward a solution which best satisfies a set of selection objectives. It is possible to define many different objectives for the function, but in this case the desire was to have a library of acceptable products (i.e., ones which calculation suggested would be absorbed from the intestine), which was as close to a 20 × 20 combinatorial solution as possible and which allowed each resin to be used exactly 20 times. This latter criterion was deemed essential on synthetic grounds as the preparation of the resin is difficult and time-consuming and to leave resin unused would be highly wasteful. The method of synthesis for these libraries did not necessitate a full combinatorial selection. However, the synthetic ease increased dramatically the closer the design was to a combinatorial one. Thus, the criteria with which we set out to constrain the library can be summarized in the following fashion.

(a) Synthetic Constraints. Am (amide variant) to be equal to 20 (i.e., ideally 20 resins to be prepared) and **Nu** (variation in the amine nucleophile) equal to or greater than 20 (i.e., use whatever nucleophiles necessary, but drive toward the ideal of a combinatorial solution) while selecting at least 400 compounds and using each amide resin at least 20 times.

(b) Absorption/Bioavailability Requirements. A polar surface area (PSA)[11] under 140 Å^2 and satisfaction of a modified rule-of-five in which compounds were acceptible only if cLogP < 6.2, MW < 550, number of H-bond donors < 5, and number of H-bond acceptors < 12. These modified values differ from those of the classical Lipinski rule-of-five[12] but were defined through previous experience with bioavailable compounds investigated during the identification of **3**.

The importance of MCMS in monomer selection has been reported elsewhere,[13] and here is illustrated by the simplified example in Figure 2.

Assume we require nine products originating from a virtual library of a possible 30 compounds (a 5 × 6 matrix). It can be seen from Figure 2, in which any shading indicates a possible selection according to the operators chosen (e.g., PSA or cLogP), that no 3 × 3 subsets exist; however, several 3 × 4 solutions exist. In solution 1, as indicated by the heavy shading within the left-hand figure, one **B** reagent (**B6**) is used only once and the **A** groups (**A1**, **A4**, and **A5**) are used between two and four times. However, a solution also exists in which each **A** is used three times (**A1**, **A3**, and **A4**) and each **B** (**B1**, **B3**, **B4**, and **B5**) at least twice, indicated in solution 2. Thus, this latter solution would then be preferred from a synthetic point of view, in which the reagents are used maximally, creating a good combinatorial solution. Hence, in a similar vein, the virtual library conceived for this work initially contained 1485 compounds (33 **Nu** × 45 **Am** matrix). However, following filtering by way of PSA and the rule-of-five, it was reduced to 770. Still, within this subset no 20 × 20 array could be achieved. A further complication to the ideal solution being realized was (i) the desire to include some known active monomers for both **Nu** and **Am** and (ii) the wish to combine each amide resin with 20 nucleophiles to use the precious resin efficiently (see Figure 3 for a list).

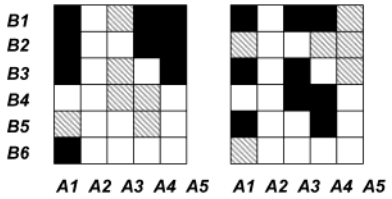


Figure 2. A virtual data set, a 5 × 6 matrix, with shading indicating that a monomer coselection (**A*X***, **B*X***) is possible under the chosen criteria. Darker shading indicates the chosen subsets, solutions 1 and 2, respectively (vide infra).

These multiple objectives were converted into weightings for MCMS which, after a few minutes on an SG R10000 processor, provided an optimized solution which utilized 21 **Am** groups and 24 **Nu** groups. Within this selection 89% of the compounds, or 449 of 504 possible, satisfied the library design criteria. Other programs have been reported for deriving optimized monomer selections: PLUMS,[14] VOLGA,[15] GALOPED,[16] and MFA.[17] Invariably these techniques are designed to produce a full combinatorial solution. MCMS differs in that it is applicable to situations where a near-combinatorial solution is acceptable.

From a chemical strategy viewpoint the retrosynthetic analysis of the library mandated that key intermediates were prepared in solution (vide infra), attached to the

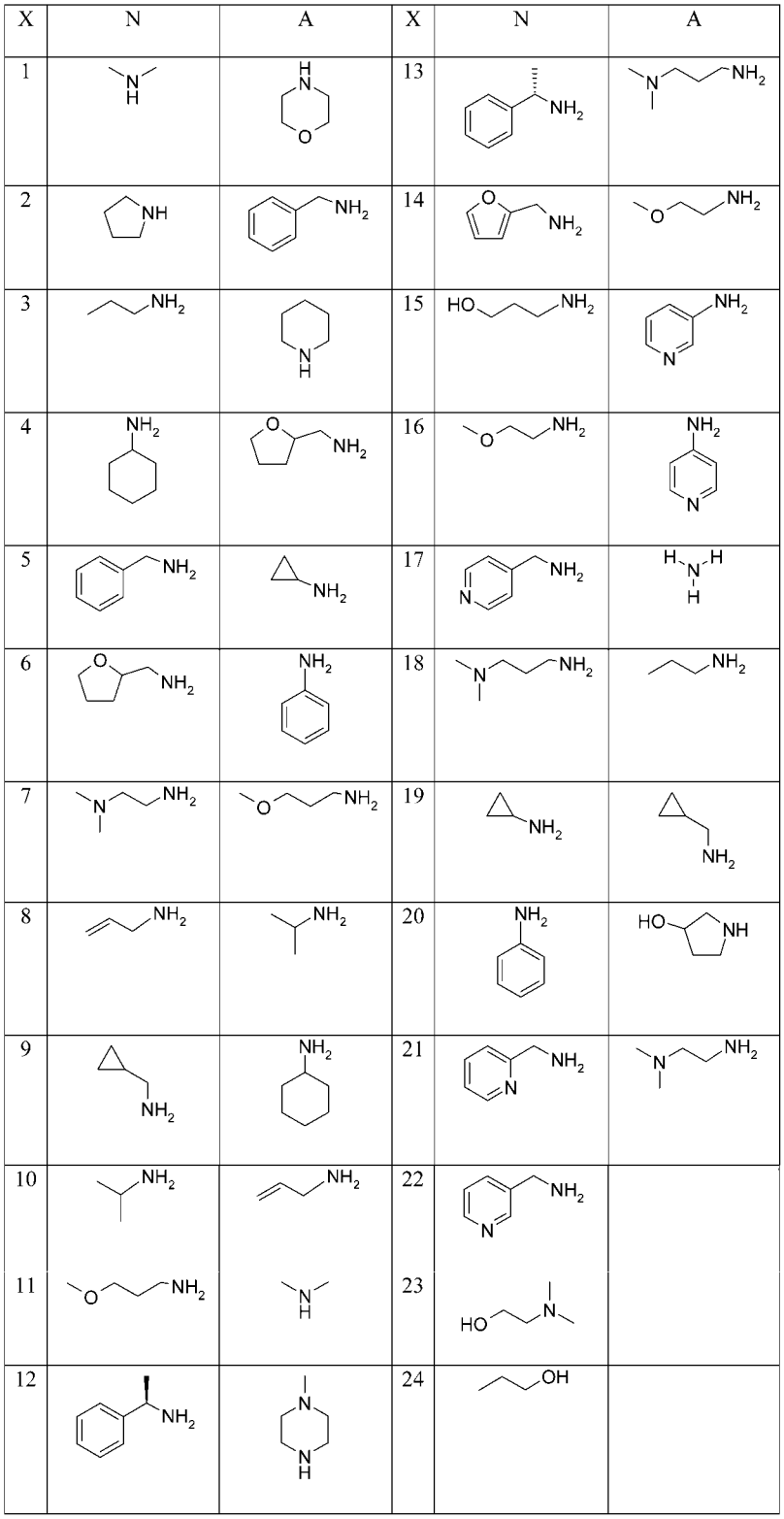
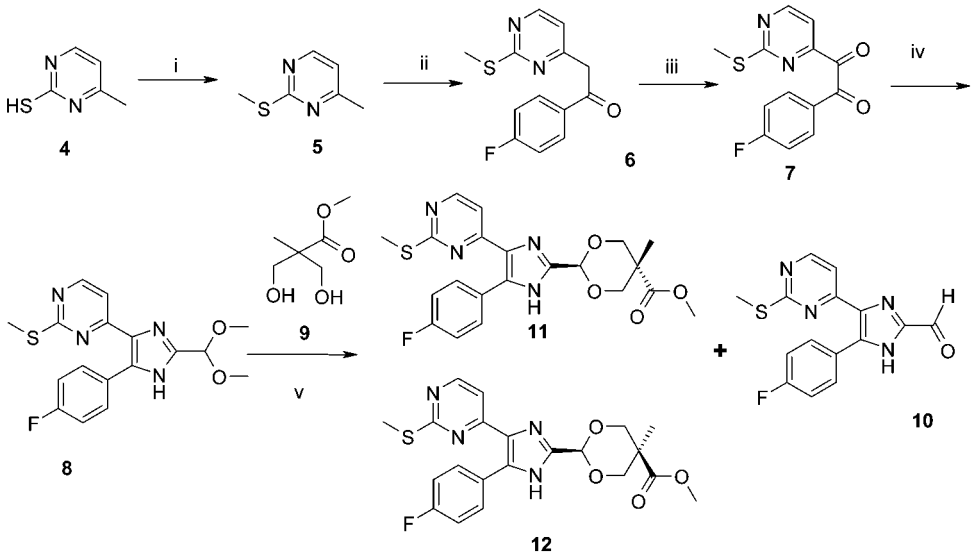


Figure 3. Targeted structure with the nucleophile (**N*X***) and amide (**A*X***) monomers as defined by the MCMS algorithm. **A1** (morpholine) was only used as the amide component for the *cis*-dioxane series and at the expense of **A18** (propylamine).[10]

polymer, and subsequently functionalized. This desire when coupled with a requirement to design a single-compound−single-well methodology meant that we were to follow a parallel-processing route. An advantage of this procedure was seen in the analysis of the library, as each well would be expected to return a single product of defined identity. Thus, mass spectral analysis of the crude products would confirm the identity of each material, and HPLC analysis could be carried out for product purity. Thus, we used parallel LC−MS in conjunction with ELS detection to analyze reaction efficiency, product identity, and homogeneity for all of the reaction wells. A limiting factor within our series and hence chemistry strategy was a thermodynamic mixture of dioxanes being formed during a transacetalation procedure. This both could not be avoided and

Scheme 1[a]



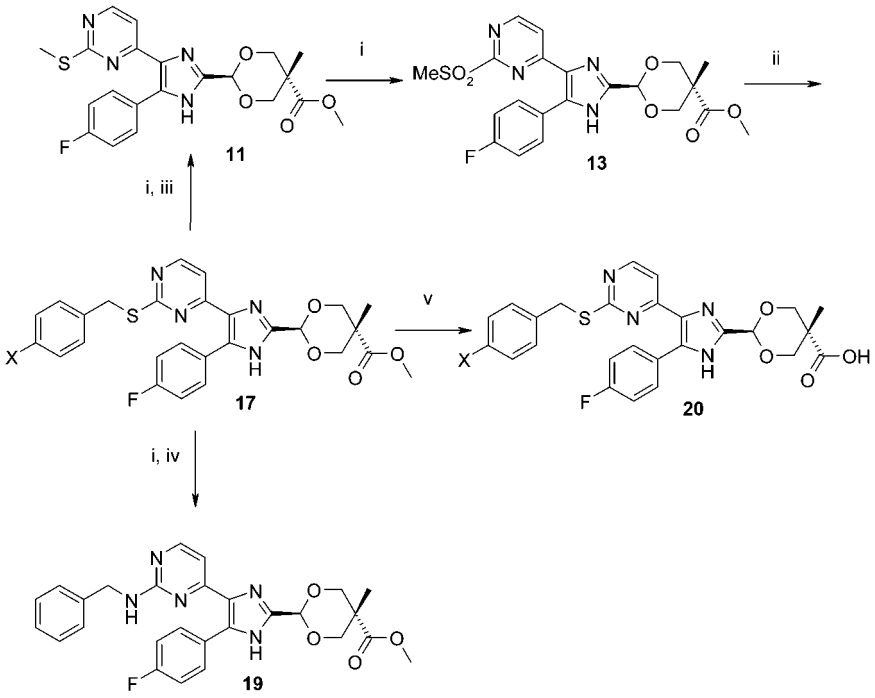
[a] Reagents: (i) NaOH, MeI, EtOH; (ii) NaHMDS, THF, 4-F(C_6H_4)CO_2Me; (iii) 48% HBr, DMSO; (iv) NH_4OAc, glyoxal dimethyl acetal, TBME, MeOH; (v) DMF, TsOH, $CH(OMe)_3$, **9**.

had to be carried out in solution with the components being separated chromatographically and then utilized as the core monomers in our library. Our synthetic route[18] to the imidazole C-2 dimethyl acetal **8** installed both the acetal and imidazole C-2 carbons in a single step, using glyoxal dimethyl acetal and ammonium acetate together with the requisite benzyl **7**, which itself originated from methyl 4-fluorobenzoate and 2-mercapto-4-methylpyrimidine (**4**). This procedure was found to be preferable to the alternative strategy in which the acetal was installed through a lithiation protocol utilizing an SEM-protected imidazole (Scheme 1).[7]

Thus, through a four-step route from commercial starting material **4**, we were able to synthesize the parent dimethyl acetal **8** in good yield. A simple acid-catalyzed transetherification under thermodynamic control yielded the desired 1,3-dioxanes as a mixture (3:2 *cis*-**12**/*trans*-**11**). The reaction mixture also contained varying amounts of aldehyde **10**; however, all of the components were separable by flash chromatography, and further the dioxanes could be interconverted through reequilibration when necessary. Following oxidation to their respective sulfonyl derivatives **13** and **14**, both the *cis* and *trans* isomers were attached to a Merrifield thiol resin (**16**).[19] The two resulting fictionalized resins **17** and **18** were then ready for parallel manipulation and library generation.[20] It has been well documented[21] that the sulfonyl group in the 2-position of the pyrimidine is more reactive than the corresponding chloro derivative; both of these are still far more reactive than the corresponding thioether. Hence, the intrinsic advantage of this strategy allowed us to selectively activate the 2-position and displace the substituent with an alternate thiol, thereby inactivating the substrate to nucleophilic cleavage until we wished to cleave our products from the resin, which would again be through an oxidation−nucleophilic displacement protocol.[22] However, this "traceless" technology was not without a problem as simple reaction analysis was not possible by direct cleavage from the resin. Analysis of the resin using magic-angle spinning ^{1}H NMR spectroscopy[23] yielded a spectrum in which the peaks were still broadened and hence not predictive in either reaction quantification or analysis, and thus we looked at alternate means to both follow and characterize our transformations. Single-bead FT-IR microspectroscopy[8] proved to be an excellent qualitative and quantitative measure for the transformations carried out. To determine the loading of the resin, oxidation of substrate **17** using a 5-fold excess of *m*CPBA[21,24] and thereafter displacement of the bound substrate from the now activated sulfonyl resin with sodium thiomethoxide regenerated our 2-thiomethyl derivative **11**. We determined the loading of the substrate from the recovered material to be 0.75 mmol of substrate/g of resin, while FT-IR analysis of the spent resin showed that complete cleavage had occurred. We also conducted this exercise using benzylamine as the nucleophile, yielding **19**, both indicating that cleavage was possible from the resin using an amine and further determining the loading obtained from the thiomethoxide experiment (see Scheme 2).

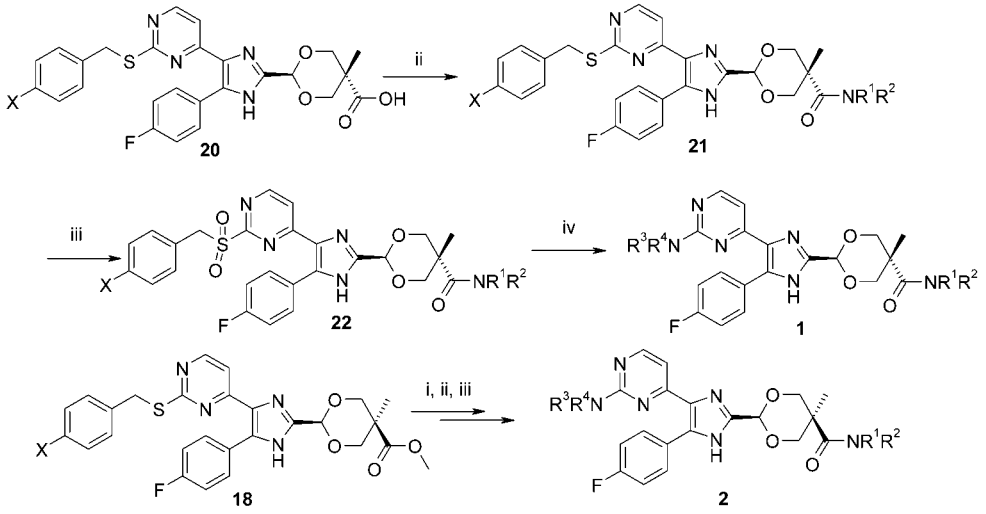
Thus, with resin loading and synthetic viability in hand we could continue on our pathway. Hydrolysis of the ester **17** was achieved using sodium hydroxide in THF, giving the carboxylic acid **20** on the solid support, the first point at which we wished to introduce the diversity into our route. Thus, division and subsequent reaction of this acid under optimized conditions was carried out. The amide formation yielding **21** proceeded well either through formation of the acid chloride, oxalyl chloride, and catalytic DMF or by using excess HATU. Interestingly EDCI proved to be of limited value in our hands especially for nonnucleophilic amines, which gave slow and incomplete reactions even over extended reaction times. Oxidation to the sulfonylpyrimidine resin **22** could be carried out using *m*CPBA; however, there were certain chemoselectivity problems that this reagent would present in a number of cases in our library. Thus, to avoid oxidation of alkenes and pyridines under these latter conditions, we turned to OXONE as the preferred oxidant for our libraries.[25] Once the resin was cleanly oxidized, cleavage was best

Scheme 2[a]



[a] Reagents: (i) *m*CPBA, CH_2Cl_2; (ii) Merrifield thiol resin **16**, DMF, NaH; (iii) DMF, NaSMe; (iv) $PhCH_2NH_2$, DME; (v) NaOH, THF.

Scheme 3[a]



[a] Reagents: (i) NaOH, H_2O, methanol; (ii) CH_2Cl_2, $(COCl)_2$, catalytic DMF, amines **A1**−**A21**; (iii) *m*CPBA, CH_2Cl_2, or OXONE, THF, MeOH; (iv) NMP, amines **N1**−**N22**, 80 °C; then CH_2Cl_2, isocyanate resin.

achieved using an excess of nucleophile at an elevated temperature. With DMF as solvent, we observed various quantities of the 2-dimethylamino compound as an impurity. Alternative inert solvents for this reaction were dimethoxyethane (DME) and *N*-methylpyrrolidinone (NMP). However, under the automated conditions we sought for cleavage of the library from the solid support, NMP was preferred as it did not develop any vapor pressure at 80 °C, the chosen reaction temperature. The conditions used for the nucleophilic cleavage were a 10-fold excess of nucleophile in NMP for 16 h at 80 °C (Scheme 3).

Following cleavage from the resin, the crude compounds were subjected to a PSQ, involving an isocyanate resin. Analysis of the members of the library was carried out using a parallel LC−MS in conjunction with ELS detection, allowing us to determine reaction efficiency and purity. The cleavage procedure from the solid support yielded a 570-member library;[26] analysis of these showed that 85% of the compounds were of greater than 85% purity, with 90% of these being of 100% purity (Figure 4).

Biological Results and Discussion

Dissolution of the cleaved products into 10 mM solutions in DMF allowed rapid biological evaluation of the library.[7,10] Following parallel analyses of the compounds in a primary functional assay, the CaCo-2 monolayer assay, and in an in vitro metabolic stability study, an assessment of the compound bioavailability was carried out in a combi-PK cassette-dosing experiment. Our target, through the algorithm-based library

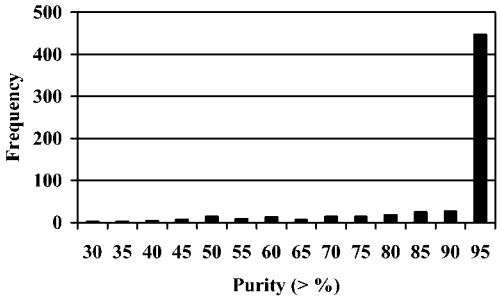


Figure 4. Overall purity profile as delineated by parallel LC–MS and ELS.

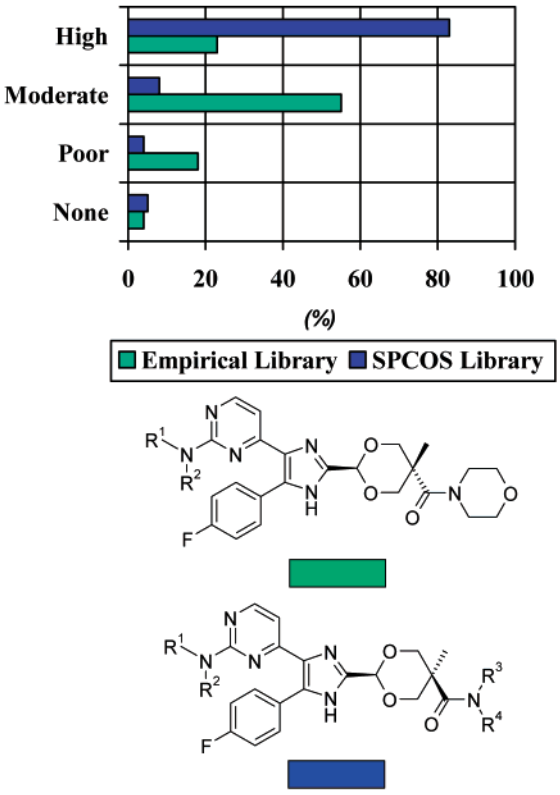


Figure 5. Comparison of experimental CaCo-2 absorption data for the empirically designed library and the algorithm-driven SPCOS library, the latter designed to have high absorption properties.

design, was to maximize the bioavailability within the series. It is not easy to demonstrate the success of the approach without constructing an identical library with randomly selected monomers. However, at least within the in vitro setting of the CaCo-2 monolayer assay, which is a model for intestinal absorption, the designed objective appears to have been born out with 80% of the compounds exhibiting a high absorption (>20%).[27] To provide some sort of benchmark, these results were compared with those of a previously prepared library of structurally related p38 inhibitors. These had been designed empirically with only a very simple constraint on molecular weight (under MW 550). The overall profile of the algorithm-driven library with regard to the CaCo-2 assay is compared to that of the manually chosen library in Figure 5.

The shift of compounds into the higher absorption category is striking. Thus, through the use of the algorithm we appear to have significantly improved the distribution of the library toward highly permeable compounds when compared to the similar library in which an empirical approach was taken.

It is also worth noting that this increased absorption profile was not at the expense of in vitro potency. Within the designed library, 85% of the compounds surpassed the activity of the reference compound RPR200765A for inhibition of p38 kinase activity while for the empirically designed library only 60% incidence of increased potency was achieved.

Following the triage of library compounds through the outlined assays and decision points, we selected 56 compounds for evaluation in rat pharmacokinetic studies, with compounds evaluated as mixtures of 14 in a combi-PK protocol at 1 mg/kg. With the benefit of these results we were able to identify 10 compounds worthy of in vivo efficacy studies. These compounds were relatively structurally diverse and potent and also exhibited acceptable systemic exposure in the combi-PK studies (Cp max >25 ng/mL), data that were then confirmed through a single-compound PK study to determine relative absorption ranking. The following data (Table 1) show the structural diversity, potency both against the isolated enzyme and functionally, and also the preliminary rat PK data for these compounds. It is of great interest to see that the compounds which progressed were obtained from both *cis* and *trans* libraries and utilized six different **Nu**'s and six different **Am**'s. This clearly demonstrates the strength of the lead optimization library. Traditional medicinal chemistry, in which one variable is changed at a time, could not have produced such a large number of relatively diverse, progressable compounds in such a short period of time.

Analysis of the results (Table 1) shows the overall trend that the *cis* isomers (entries **A**–**C**) are less potent in vitro than the *trans* isomers (entries **D**–**J**) although similar in vivo potency is achieved.

In the p38 assay, compounds showing 10-fold improved potencies over **3** have been identified (comparing entries **D**–**J** with entry **3**). In the in vitro whole cell assay, compounds with greater than 10-fold improved potencies compared to **3** have been synthesized (comparing entries **D**, **E**, **G**, **I**, **J**, and **3**), and compound **D** shows a 300-fold improvement in potency over **3**. Overall, four compounds—entries **D**, **E**, **G**, **I**, and **J**—exhibited improved enzyme and whole cell potencies compared to **3**, the other two (entries **B** and **C**) being comparable. Furthermore, in the in vivo LPS-induced TNF-α release assay in the rat, compounds having 10-fold improved ED_{50} compared to RPR200765A have been identified (compare entries **B** and **F** with entry **3**), and the other compounds (except entry **E**) have ED_{50} values comparable to that of **3**.

The pharmacokinetic parameters for most of the compounds are comparable to or better than those obtained with the free base of RPR200765. Entries **A**–**C** and **F**–**J** showed higher Cp(max) values when compared with entry **K**, and entries **D**–**J** show better AUCs than entry **K**. Compounds **I** and **J** have PK parameters comparable to that of the bioavailable, optimized mesylate salt RPR200765A (entry **3**).

The 10 compounds identified have different hydrophobic and hydrophilic side chains attached to the pyrimidine ring and also different small alkyls and alkyl ether amides linked to the dioxane ring. Secondary

Table 1. In Vitro, Functional, in Vivo, and Pharmacokinetic Parameters of the Compounds Following Oral Dosing in the Rat at 1 mg/kg

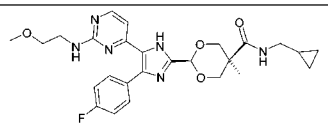
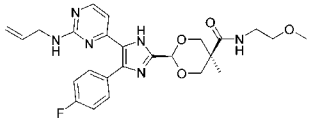
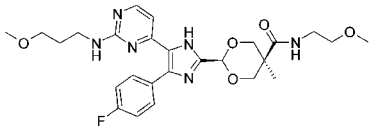
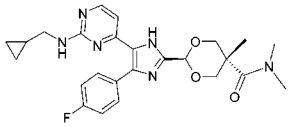
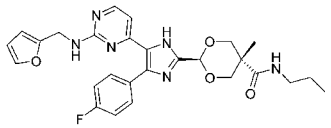
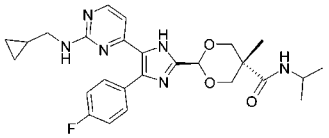
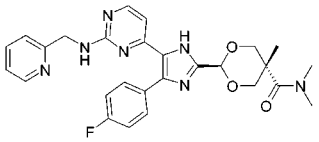
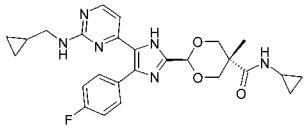
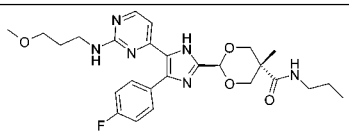
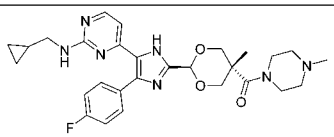
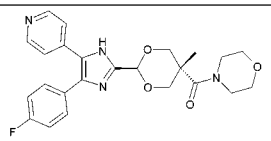
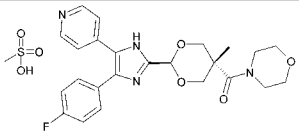
Compound {N*X*, A*X*} Entry	**RPR #**	P38 IC_{50} (nM)	THP-1 cell TNFα IC_{50} (nM)	TNF-α ED_{50} ($mgkg^{-1}$)	Cp max (ng/ml; rat)	AUC (ng/ml.hr; rat)	Structure
2{16, 19} **A**	*235455*	47	-	3	62	121	
2{8, 14} **B**	*235315*	62	73	0.2	95	169	
2{11, 14} **C**	*235316*	127	71	5	89	138	
1{9, 11} **D**	*237897*	6	0.2	9	29	276	
1{14, 18} **E**	*239938*	6	3	-	39	257	
1{9, 8} **F**	*239497*	12	-	0.7	141	412	
1{21, 11} **G**	*238778*	4	9	8	110	529	
1{9, 5} **H**	*239457*	5	-	5	91	288	
1{11, 18} **I**	*239939*	6	3.5	6	270	738	
1{9,12}	*238677*	4	2	10	221	541	

Table 1 (Continued)

J							
RPR200765	-	-	-	-	60	247	
K							
RPR200765A	-	110	60	3-10	277	628	
3							

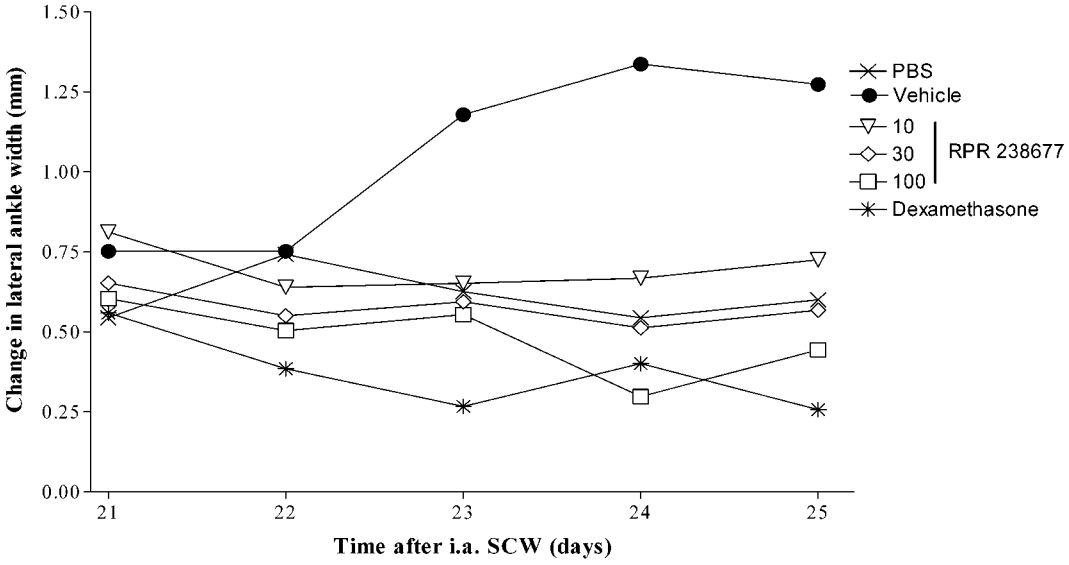


Figure 6. Change in lateral ankle width (mean, injected − uninjected) from sensitized Lewis rats over the 5 days following intravenous challenge with SCW (100 mg) or phosphate-buffered saline (PBS) on day 21. Vehicle RPR238677 (10, 30, and 100 (mg/kg)/day) or dexamethasone (0.3 (mg/kg)/day) was given orally (as a split dose, b.i.d.) for 5 days from 1 day prior to intravenous challenge with SCW. $n = 10$ rats/group except for PBS where $n = 5$ rats/group and vehicle-treated where $n = 15$ rats/group.

amines can be introduced in the right-hand side to make a tertiary amide without loss in activity (entries **D**, **G**, and **J**), but only primary amines, linked to the pyrimidine ring on the left-hand side, led to active compounds. There is no obvious structure−activity relationship (SAR) that can be discerned from these results, demonstrating that they could not have been obtained as rapidly following a traditional sequential approach. This MCMS approach was used to maximize the different in vitro, in vivo, and PK parameters of the compounds at the same time, avoiding a more traditional, sequential SAR approach. Compound **J** (RPR238677) was selected for further evaluation on the basis of its good PK profile and the possibility of preparing a salt formulation. Compound **J** was further evaluated in vivo in an SCW (streptococcal cell wall) induced arthritis model in the Lewis rat. It suppressed paw swelling with an ED_{50} of 10−30 mg/kg (Figure 6), Furthermore, administration of RPR238677 was associated with marked preservation of bone anatomy and a reduction in erosion areas. This was particularly apparent at the 30 and 100 mg/kg dose levels, but there was some evidence of conservation of joint anatomy at 10 mg/kg. Periosteal inflammation was less apparent in the 30 and 100 mg/kg dose groups (Figure 7).

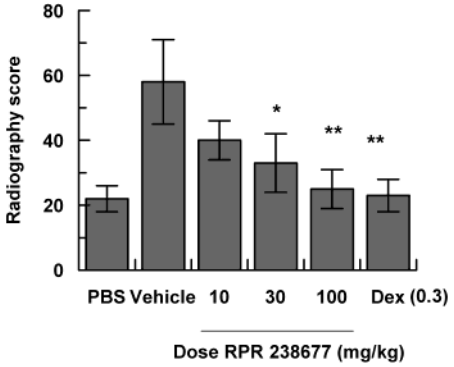


Figure 7. Anatomical assessment of radiographs. Mean ± SEM. One asterisk indicates $p < 0.05$ and two asterisks indicate $p < 0.01$ compared to vehicle control. One-way ANOVA with correction for Dunnett's multiple *t*-test.

Conclusion

As part of a program to identify a backup compound for the p38 inhibitor **3**, an algorithmically guided SPCOS approach has been used to generate a library of more than 570 compounds varying structurally in a combinatorial fashion at two positions. The algorithm was designed to produce a library which was constructed in a synthetically efficient manner and contained a high proportion of compounds with good oral PK parameters. From this library 10 relatively structurally diverse compounds were identified which possessed an improved in vitro potency over RPR200765A and good Cp max values and AUCs in the rat. The identification of these compounds would not have been possible without the use of both a solid-phase synthesis coupled with the Monte Carlo computer algorithm MCMS. The synthesis made use of a traceless linker amenable to nucleophilic displacement for the introduction of diversity within the pyrimidine nucleus, and purification of the cleaved products of the library thereafter was achieved through the utilization of a quench resin.

This technique has enabled the rapid identification of RPR238677 (F = 20%), a potential drug candidate.

Experimental Section

Chemical Methods. All reagents were received and used without further purification. All spectral data were recorded on a Varian VXR 400 and are reported as chemical shifts in parts per million (ppm) from an internal standard, tetramethylsilane (δ_H 0.00) in deuteriochloroform (δ_H 7.27, δ_C 77.0) or deuteriodimethyl sulfoxide (δ_H 2.49, δ_C 39.4), with coupling constants (J) given in hertz. Mass spectra were recorded on a VG 7070/250 spectrometer, and microanalyses were performed on a Carlo-Erba 1106 microanalyzer. Melting points are uncorrected and were obtained using an electrothermal apparatus. Chromatography was carried out using silica gel eluting with the indicated solvent systems.

4-Methyl-2-thiomethylpyrimidine (5). To a solution of 2-mercapto-4-methylpyrimidine hydrochloride (**4**) in ethanol and 1.0 M sodium hydroxide (2 equiv) was added methyl iodide (1 equiv). The reaction was stirred for 16 h, evaporated to half-volume, and extracted with ethyl acetate. The combined organic phases were washed with water and brine, dried (sodium sulfate), and then concentrated to yield **5** (90%) as an oil: ^{1}H NMR ($CDCl_3$) 2.45 (s, 3H), 2.56 (s, 3H), 6.82 (d, J = 5.1 Hz, 1H), 8.36 (d, J = 5.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) 14.0, 24.0, 115.9, 156.6, 167.3, 172.2; MS (CI) m/z 141 $[MH]^+$.

2-(2-Thiomethylpyrimidin-4-yl)-1-(4-fluorophenyl)ethanone (6). To a cooled solution (0 °C) of sodium bis(trimethylsilyl)amide (2.2 equiv) in THF was added a solution of **5**, and the resulting solution was stirred for 30 min. A solution of methyl 4-fluorobenzoate was then added dropwise and stirred while attaining ambient temperature over 3 h. The reaction was quenched by addition of ammonium chloride and extracted into ethyl acetate, and the combined organic phases were washed with brine, dried (sodium sulfate), and concentrated in vacuo to yield, after trituration with hexanes, a solid, **6** (99%): mp 88−90 °C; ^{1}H NMR ($CDCl_3$) (60% enol) 2.61 (s, 3H), 5.91 (s, 1H), 6.64 (d, J = 5.3 Hz, 2H), 7.15 (m, 2H), 7.83 (m, 2H), 8.31 (d, J = 5.3 Hz, 1H); (40% ketone) 2.52 (s, 3H), 4.35 (s, 2H), 6.98 (d, J = 5.0 Hz, 1H), 7.15 (m, 2H), 8.07 (m, 2H), 8.46 (d, J = 5.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) 14.1, 47.7, 93.1, 115.6 (d, J = 21.9 Hz), 112.7, 115.9 (d, J = 21.9 Hz), 116.5, 128.0 (d, J = 9.0 Hz), 131.5 (d, J = 8.3 Hz), 156.3, 157.2, 163.1 (d, J = 251.3 Hz), 164.4, 165.2 (d, J = 255.8 Hz), 167.1, 169.7, 172.9, 193.7; MS (CI) m/z 263 $[MH]^+$. Anal. ($C_{13}H_{11}N_2SOF$) C, H, N.

2-Thiomethylpyrimidin-4-yl-4-fluorophenylbenzil (7). To a solution of **6** in DMSO at 55 °C was added dropwise 48% aqueous HBr (3 equiv). The reaction mixture was stirred for 3 h at that temperature and then cooled to ambient temperature. The mixture was poured into ice/water, a solution of sodium acetate then added dropwise, and subsequently the pH then adjusted to 7 using solid sodium bicarbonate. The solid was filtered off, washed with water, and dried, yielding **7** (82%): mp 73−75 °C; ^{1}H NMR ($CDCl_3$) 2.32 (s, 3H), 7.22 (m, 2H), 7.95 (m, 2H), 8.84 (d, J = 4.8 Hz, 1H); ^{13}C NMR ($CDCl_3$) 6.5 (d, J = 21.9 Hz), 13.9, 112.7, 117.64 (d, J = 4.8 Hz), 129.3, 132.2 (d, J = 9.8), 157.0, 159.5, 166.7 (d, J = 258.9 Hz), 174.1, 192.9, 193.5; MS (CI) m/z 277 $[MH]^+$. Anal. ($C_{13}H_9N_2SO_2F$) C, H, N.

2-Dimethoxymethyl-4-(4-fluorophenyl)-5-(2-methylsulfanylpyrimidin-4-yl)-1*H*-imidazole (8). To a solution of **7** and glyoxal dimethyl acetal (1.2 equiv) in *tert*-butyl methyl ether was added a solution of ammonium acetate (2.5 equiv) in methanol. The resulting solution was stirred at ambient temperature for 4 h. The reaction mixture was partitioned between ethyl acetate and water, and the combined organic phases were washed with brine, dried (sodium sulfate), and concentrated in vacuo to yield an oil. Addition of diethyl ether induced slow crystallization of a solid, **8** (86%): mp 149−151 °C; ^{1}H NMR (DMSO-d_6) 2.04 (s, 3H), 3.38 (s, 6H), 5.50 (s, 1H), 7.26 (app t, J = 8.8 Hz, 2H), 7.60 (m, 3H), 8.50 (d, J = 5.2 Hz, 1H), 12.95 (s, 1H); ^{13}C (DMSO-d_6) 12.6, 53.4, 98.2, 112.3, 114.6 (d, J = 21.9 Hz), 126.9, 132.0 (d, J = 8.3 Hz), 132.5, 145.2, 160.7, 161.9 (d, J = 250.0 Hz), 170.4; MS (CI) m/z 361 $[NH]^+$. Anal. ($C_{17}H_{17}N_4SO_2F$) C, H, N.

Methyl Bis(2,2-hydroxymethyl)propanoate (9). To a solution of bis(2,2-hydroxymethyl)propionic acid in methanol at 0 °C was added thionyl chloride. The resulting solution was heated at reflux for 16 h and evaporated and the residue distilled (97−99 °C, 0.1 mmHg):

^{1}H NMR (DMSO-d_6) 1.04 (s, 3H), 3.40 (d, J = 10.4 Hz, 2H), 3.50 (d, J = 10.4 Hz, 2H), 3.57 (s, 3H), 4.66 (br s, 2H); ^{13}C NMR (DMSO-d_6) 16.8, 50.2, 51.2, 63.8, 175.2; MS (CI) m/z 149 $[MH]^+$.

***cis*- and *trans*-2-(5-Methyl-5-carboxymethyl-1,3-dioxan-2-yl)-4-(4-fluorophenyl)-5-(2-methylsulfanylpyrimidin-4-yl)-1*H*-imidazole (11 and 12).** To a solution of **8** in DMF were added **9** (1.6 equiv), trimethylorthoformate (2.5 equiv), and 4-toluenesulfonic acid (2.5 equiv). The reaction mixture was heated at 80 °C for 4 h before cooling. The reaction mixture was partitioned between ethyl acetate and sodium bicarbonate and extracted three times, and the combined organic phases were washed with brine and dried (sodium sulfate). Following flash chromatography on silica gel eluting with ethyl acetate the following were obtained, in order of elution: the hydrolysis product **10**, **11**, and **12**.

Data for 2-Carboxyaldehyde-4-(4-fluorophenyl)-5-(2-methylsulfanylpyrimidin-4-yl)-1*H*-imidazole (10): yield 16%; mp 187−188 °C; ^{1}H NMR (DMSO-d_6) 2.06 (s, 3H), 7.31 (m, 2H), 7.68 (m, 3H), 8.60 (m, 1H), 9.75 (s, 1H), 14.20 (s, 1H); ^{13}C NMR (DMSO-d_6) 12.6, 112.7, 114.9 (d, J = 21.1 Hz), 126.0, 132.2 (d, J = 8.3 Hz), 136.4, 145.0, 157.9, 160.0, 162.4 (d, J = 245.2 Hz), 171.0, 181.5; MS (CI) m/z 315 $[MH]^+$. Anal. ($C_{15}H_{11}N_4SOF$) C, H, N.

Data for *trans* Isomer 11: yield 24%; mp 199−200 °C; ^{1}H NMR (DMSO-d_6) 1.54 (s, 3H), 2.02 (s, 3H), 3.67 (s, 3H), 3.96 (d, J = 11.1 Hz, 2H), 4.16 (d, J = 11.1 Hz, 2H), 5.68 (s, 1H), 7.27 (m, 2H), 7.58 (m, 3H), 8.50 (m, 1H), 13.15 (s, 1H); ^{13}C NMR (DMSO-d_6) 12.6, 19.0, 51.9, 71.1, 95.5, 112.2, 114.6 (d, J = 21.9 Hz), 127.0, 132.1 (d, J = 8.3 Hz), 132.4, 132.8, 144.3, 157.4160.6, 162.0 (d, J = 245.2 Hz), 170.4, 173.1; MS (CI) m/z 445 $[MH]^+$. Anal. ($C_{21}H_{21}N_4SO_4F$) C, H, N.

Data for *cis* Isomer 12: yield 36%; mp 161−163 °C; ^{1}H NMR (DMSO-d_6) 0.98 (s, 3H), 2.00 (s, 3H), 3.72 (s, 3H), 3.79 (d, J = 11.1 Hz, 2H), 4.47 (d, J = 11.1 Hz, 2H), 5.71 (s, 1H), 7.25 (m, 2H), 7.56 (m, 3H), 8.49 (m, 1H), 13.05 (s, 1H); ^{13}C NMR (DMSO-d_6) 12.5, 17.5, 52.2, 72.1, 95.7, 112.2, 114.6 (d, J = 21.9 Hz), 127.0, 132.2 (d, J = 9.0 Hz), 132.5, 132.7, 144.2, 157.3, 160.6, 162.2 (d, J = 245.0 Hz), 170.4, 174.0; MS (CI) m/z 445 $[MH]^+$. Anal. ($C_{21}H_{21}N_4SO_4F$) C, H, N.

***trans*-2-(5-Methyl-5-carboxymethyl-1,3-dioxan-2-yl)-4-(4-fluorophenyl)-5-(2 -methylsulfonylpyrimidin-4-yl)-1*H*-imidazole (13)**: To a solution of **11** in dichloromethane and methanol (9:1 v/v) was added *m*CPBA (2.2 equiv), and the mixture was stirred for 16 h. The reaction mixture was diluted with diethyl ether, washed with sodium bisulfite, sodium bicarbonate, and brine, dried (sodium sulfate), and concentrated in vacuo to yield a foam, **13** (85%): ^{1}H NMR (DMSO-d_6) 1.54 (s, 3H), 2.97 (s, 3H), 3.67 (s, 3H), 4.02 (d, J = 11.1 Hz, 2H), 4.18 (d, J = 11.1 Hz, 2H), 5.71 (s, 1H), 7.28 (m, 2H), 7.69 (m, 2H), 8.10 (br s, 1H), 8.92 (m, 1H), 13.3 (s, 1H); ^{13}C NMR (DMSO-d_6) 19.0, 38.2, 51.9, 71.1, 95.4, 114.8 (d, J = 21.9 Hz), 119.0, 126.2, 131.3, 132.1 (d, J = 8.3 Hz), 145.0, 158.8, 161.4, 162.1 (d, J = 246.0 Hz), 165.0, 173.1; MS (CI) m/z 477 $[MH]^+$. Anal. ($C_{21}H_{21}N_4SO_6F$) C, H, N.

***cis*-2-(5-Methyl-5-carboxymethyl-1,3-dioxan-2-yl)-4-(4-fluorophenyl)-5-(2- methylsulfonylpyrimidin-4-yl)-1*H*-imidazole (14).** In a similar fashion using the *cis* isomer **12**, we obtained **14** as a foam (82%): ^{1}H NMR (DMSO-d_6) 0.99 (s, 3H), 2.96 (s, 3H), 3.72 (s, 3H), 3.82 (d, J = 11.2 Hz, 2H), 4.50 (d, J = 11.2 Hz, 2H), 5.74 (s, 1H), 7.26 (m, 2H), 7.65 (m, 2H), 8.10 (br s, 1H), 8.92 (d, J = 5.2 Hz, 1H), 13.2 (s, 1H); ^{13}C NMR (DMSO-d_6) 17.5, 38.2, 42.0, 52.2, 72.1, 95.5, 114.7 (d, J = 21.9 Hz), 119.0, 126.2, 131.5, 132.2 (d, J = 8.3 Hz), 134.4, 144.9, 158.7, 161.4, 162.1 (d, J = 246.0 Hz), 165.0, 174.0; MS (CI) m/z 477 $[MH]^+$. Anal. ($C_{21}H_{21}N_4SO_6F$) C, H, N.

Preparation and Characterization of Resin-Bound Substrates. FT-IR analysis on a number of beads from each reaction well was performed, ensuring both homogeneity and identity (ν, cm^{-1}, intensity).

A suspension of Merrifield resin (20 g, 1.1 mmol g^{-1}) in DMF was treated with potassium thioacetate (5 equiv). After 24 h at ambient temperature the resulting resin **15** was filtered, then washed with DMF, THF, water, THF, and finally dichloromethane, and then dried under vacuum at 60 °C: IR 3024s, 2920s, 1691s, 1599m, 1493s, 1453s, 1130m, 758s, 700s.

A suspension of the resin **15** in THF was treated with lithium borohydride. After 24 h the resin was washed with THF, 1 N HCl, THF (7:3), water, THF, methanol, and finally dichloromethane and then dried under vacuum at 60 °C, yielding **16**: IR 3026s, 2924s, 1599w, 1493s, 1452s, 757s, 700s.

A suspension of the thiol resin **16** in DMF was treated with sodium hydride (60% dispersion in mineral oil) and agitated for 15 min. It was then treated with a DMF solution of **13** or **14** (2 equiv) and subsequently heated for 20 h. Filtration, washing with THF, methanol, and finally dichloromethane, and then drying under vacuum at 60 °C yielded **17** or **18**, respectively: IR 3021m, 2920m, 1732m, 1569m, 1452s, 1091m, 835m, 757s, 699s.

Resin Loading Determination. To a suspension of the resin **17** (100 mg) in dichloromethane and methanol (10:1) was added *m*CPBA (3 equiv). After 48 h of agitation the resin was filtered off, washed with dichloromethane, and dried under vacuum at 60 °C. The resin was split into two equal portions and treated with (1) sodium thiomethoxide (3 mmol) in THF at ambient temperature and (2) benzylamine (3 mmol) in DME at reflux. Isolation of the products by preparative TLC eluting with ethyl acetate yielded, respectively, **11**, 16.5 mg, indicating 0.75 mmol of substrate/g of resin and ***trans*-2-(5-methyl-5-carboxymethyl-1,3-dioxan-2-yl)-4-(4-fluorophenyl)-5-(2-*N*-benzylaminopyrimidin-4-yl)-1*H*-imidazole (19)** as a foam (15.2 mg, 80% based on previous loading determination): ^{13}C NMR (DMSO-d_6) 19.1, 40.3, 43.5, 51.9, 71.2, 95.7, 114.3 (d, J = 21.9 Hz), 119.0, 126.2, 131.5, 132.2 (d, J = 8.3 Hz), 134.4, 140.6, 143.8, 158.0, 161.4, 162.1 (d, J = 246.0 Hz), 165.0, 173.2; MS (CI) m/z 504 $[MH]^+$. Anal. ($C_{27}H_{26}N_5O_4F$ $0.4H_2O$) C, H, N.

A suspension of the resin **17** in THF was treated with 1 N NaOH and stirred for 8 h at 70 °C. The resin was filtered off, washed with 1 N HCl/THF (1:1), then THF, DMF, methanol, and finally dichloromethane, and then dried under vacuum at 60 °C, yielding **20**: IR 3060w, 3025m, 2923m, 2852w, 1720w, 1601m, 1571s, 1493s, 1452s, 1335s, 1235m, 1199m, 1183m, 1097s, 838m, 760s, 704s.

Table 2. FT-IR Frequencies for the Resin Bond Derivatives, before and after Oxidation with *m*CPBA

	amine for **Am**	ν(RSMe) (cm^{-1})	ν(RSO$_2$Me) (cm^{-1})
A1[a]	morpholine	1636	1635
A2	benzylamine	1666	1658
A3	piperidine	1626	1625
A4	tetrahydrofurfurylamine	1666	1655
A5	cyclopropylamine	1663	1645
A6	aniline	1674	1672
A7	3-methoxypropylamine	1663	1650
A8	isopropylamine	1666	1645
A9	cyclohexylamine	1666	1646
A10	allylamine	1669	1664
A11	dimethylamine	1630	1630
A12	1-methylpiperazine	1629	1637
A13	1,1-dimethylpropylidenediamine	1660	1652
A14	2-methoxyethylamine	1662	1657
A15	3-aminopyridine	1687	1689
A16	4-aminopyridine	1695	1711
A17	ammonia	1674	1665
A18[a]	propylamine	1653	1650
A19	cyclopropylmethylamine	1650	1645
A20	3-hydroxypyrrolidine	1630	1629
A21	1,1-dimethylethylenediamine	1660	1657

[a] **A1** (morpholine) was only used as the amide component for the *cis*-dioxane series and used at the expense of **A18** (propylamine).

A suspension of the resin **20** in dichloromethane was treated with a catalytic amount of DMF followed by oxalyl chloride. The addition of oxalyl chloride was repeated twice more before the resin was washed with dichloromethane. The resin was then treated with an appropriate amine **Am** (**A1**−**A21**), the reaction mixture was agitated for 30 min before being filtered, washed with THF, methanol, and finally dichloromethane, and then dried under vacuum at 60 °C. Alternatively, this procedure could be carried out using **20**, HATU, and triethylamine in DMF with agitation for 24 h: the derivatized resins were subsequently characterized by FT-IR. The carbonyl (C═O) stretching frequencies are given in Table 2.

To a suspension of the resin **21** (**A1**−**A21**) in dichloromethane and methanol (10:1) was added *m*CPBA (3 equiv). After 48 h of agitation the resin was filtered off, washed with dichloromethane, and dried under vacuum at 60 °C: carbonyl (C═O) stretching frequencies as characterization for **22** are given in the table. Alternatively, to a suspension of the resin **21** in methanol, THF, and water (1:1:0.1) was added OXONE. After 24 h of agitation the resin was filtered off, washed with methanol, THF, and dichloromethane, and dried under vacuum at 60 °C, yielding **22**.

Thus, the amines **N1**−**N22** were utilized in the nucleophilic displacements from the activated 2-sulfonylpyrimidine resins **22**. The reaction block (96-well; ACT) was set up with resin (30 mg) in NMP (1 mL) per well; thereafter was added the appropriate amine (10 equiv), and the block was heated to 80 °C for 16 h. Each well was flushed with NMP, and the solvents were then removed in vacuo. Addition of dichloromethane and isocyanate resin (3 equiv with respect to amine) and further agitation for 16 h followed by filtration and evaporation of the solvent in vacuo allowed the isolation of the library products. The yield of the resin-cleaved products ranged from 1% to 75%, with between 0.1 and 8.4 mg being isolated with analysis of the library being carried out by parallel LC−MS and ELS.

Data for the resynthesized compounds are as follows.

Data for *cis*-2-{4-(4-Fluorophenyl)-5-[2-(2-methoxyethylamino)pyrimidin-4-yl]-1*H*-imidazol-2-yl}-5-methyl-[1,3]-dioxane-5-carboxylic Acid Cyclopropylmethylamide (2{16, 19}, RPR235455): mp 168−170 °C; MS (HRMS) m/z calcd for $C_{26}H_{32}N_6O_4F$ (M + H) 511.2469, found 511.2447; ^{1}H NMR (DMSO-d_6) 0.23 (m, 2H), 0.40 (m, 2H), 0.95−1.10 (m, 1H), 0.98 (s, 3H), 3,17 (t, J = 6 Hz, 2H), 3.30 (s, 3H), 3.37 (m, 2H), 3.42 (m, 2H), 3,81 (d, J = 12 Hz, 2H), 4.42 (d, J = 12 Hz, 2H), 5.75 (s, 1H), 6.05 (m, 1H), 6.91 (br d, J = 5 Hz, 1H), 7.17 (t, J = 9 Hz, 2H), 7.31 (m, 1H), 7.68 (dd, J = 9 and 7 Hz, 2H),

8.19 (d, J = 5 Hz, 1H), 11.20−12.40 (m, 1H). Anal. ($C_{26}H_{31}FN_6O$) C, H, N.

Data for *cis*-2-[5-(2-Allylaminopyrimidin-4-yl)-4-(4-fluorophenyl)-1*H*-imidazol-2-yl]-5-methyl[1,3]dioxane-5-carboxylic Acid (2-Methoxyethyl)amide (2{8, 14}, RPR235315): mp 188−190 °C; MS (HRMS) *m/z* calcd for $C_{25}H_{30}N_6O_4F$ (M + H) 497.2313, found 497.2345; ^{1}H NMR (DMSO-d_6) 1.00 (s, 3H), 3.24 (s, 3H), 3,42 (m, 2H), 3.49 (t, J = 6 Hz, 2H), 3.70−4.00 (m, 2H), 3.82 (d, J = 12 Hz, 2H), 4.40 (d, J = 12 Hz, 2H), 5.02 (br d, J = 10 Hz, 1H), 5.13 (br d, J = 16 Hz, 1H), 5.75 (s, 1H), 5.75−6.00 (m, 1H), 6.00−6.50 (m, 2H), 7.17 (t, J = 9 Hz, 2H), 7.33 (m, 1H), 7.68 (dd, J = 9 and 6.5 Hz, 2H), 8.21 (d, J = 5 Hz, 1H), 11.50−12.50 (m, 1H). Anal. ($C_{25}H_{29}FN_6O_4$) C, H, N.

Data for *cis*-2-{4-(4-Fluorophenyl)-5-[2-(3-methoxypropylamino)pyrimidin-4-yl]-1*H*-imidazol-2-yl}-5-methyl-[1,3]dioxane-5-carboxylic Acid (2-Methoxyethyl)amide (2{11, 14}, RPR235316): mp 159−161 °C; MS (HRMS) *m/z* calcd for $C_{26}H_{34}N_6O_5F$ (M + H) 529.2575, found 529.2598; ^{1}H NMR (DMSO-d_6) 0.98 (s, 3H), 1.69 (m, 2H), 3.19 (m, 2H), 3.22 (s, 3H), 3.25 (s, 3H), 3,36 (m, 2H), 3.40 (t, J = 5.5 Hz, 2H), 3.47 (t, J = 6 Hz, 2H), 3.80 (d, J = 12 Hz, 2H), 4.40 (d, J = 12 Hz, 2H), 5.73 (s, 1H), 6.32 (m, 1H), 6.91 (m, 1H), 7.19 (t, J = 9 Hz, 2H), 7.44 (m, 1H), 7.69 (dd, J = 9 and 6.5 Hz, 2H), 8.19 (d, J = 5 Hz, 1H), 11.50−12.50 (br m, 1H). Anal. ($C_{26}H_{33}FN_6O_5$) C, H, N.

Data for *trans*-2-[5-[2-(Cyclopropylmethylamino)pyrimidin-4-yl]-4-(4-fluorophenyl)-1*H*-imidazol-2-yl]-5-methyl-[1,3]dioxane-5-carboxylic Acid Dimethylamide (1{9, 11}, RPR237897): mp 196−198 °C; MS (HRMS) *m/z* calcd for $C_{25}H_{30}N_6O_3F$ (M + H) 481.2363, found 481.2347; ^{1}H NMR (DMSO-d_6) 0.17 (m, 2H), 0.40 (m, 2H), 0.97 (m, 1H), 1.65 (s, 3H), 3.00 (s, 6H), 3.06 (m, 2H), 4.13 (d, J = 12 Hz, 2H), 4.20 (d, J = 12 Hz, 2H), 5.61 (s, 1H), 6.16 (m, 1H), 6.93 (m, 1H), 7.17 (t, J = 9 Hz, 2H), 7.68 (dd, J = 9 and 6.5 Hz, 2H), 8.20 (d, J = 5 Hz, 1H), 11.50−12.50 (br m, 1H). Anal. ($C_{27}H_{29}FN_6O_4\cdot 3H_2O$) C, H, N.

Data for *trans*-2-(5-(4-Fluorophenyl)-4-{2-[(furan-2-ylmethyl)amino]pyrimidin-4-yl}-1*H*-imidazol-2-yl)-5-methyl-[1,3]dioxane-5-carboxylic Acid Propylamide (1{14, 18}, RPR239938): mp 158−160 °C; MS (HRMS) *m/z* calcd for $C_{27}H_{30}N_6O_4F$ (M + H) 521.2313, found 521.2310; ^{1}H NMR (DMSO-d_6) 0.91 (t, J = 7.5 Hz, 3H), 1.45−1.60 (m, 2H), 1.52 (s, 3H), 3.13 (m, 2H), 4.04 (d, J = 12 Hz, 2H), 4.12 (d, J = 12 Hz, 2H), 4.39 (m, 2H), 5.64 (s, 1H), 6.15 (m, 1H), 6.30−6.70 (br m, 1H), 6.34 (br s, 1H), 6.80−7.30 (br m, 1H), 7.13 (t, J = 9 Hz, 2H), 7.46 (br s, 1H), 7.69 (dd, J = 9 and 6.5 Hz, 2H), 8.23 (d, J = 5 Hz, 1H). Anal. ($C_{27}H_{29}FN_6O_4\cdot 2H_2O$) C, H, N.

Data for *trans*-2-[5-[2-(Cyclopropylmethylamino)pyrimidin-4-yl]-4-(4-fluorophenyl)-1*H*-imidazol-2-yl]-5-methyl-[1,3]dioxane-5-carboxylic Acid Isopropylamide (1{9, 8}, RPR239497): mp 138−140 °C; MS (HRMS) *m/z* calcd for $C_{26}H_{32}N_6O_3F$ (M + H) 495.2520, found 495.2531; ^{1}H NMR (DMSO-d_6) 0.15 (m, 2H), 0.40 (m, 2H), 0.97 (m, 1H), 1.16 (d, J = 7 Hz, 6H), 1.51 (s, 3H), 3.05 (m, 2H), 3.96 (m, 1H), 4.04 (d, J = 12 Hz, 2H), 4.09 (d, J = 12 Hz, 2H), 5.63 (s, 1H), 6.14 (m, 1H), 6.87 (m, 1H), 6.92 (m, 1H), 7.17 (t, J = 9 Hz, 2H), 7.69 (dd, J = 9 and 6.5 Hz, 2H), 8.19 (d, J = 5 Hz, 1H), 11.00−12.50 (br m, 1H). Anal. ($C_{26}H_{31}FN_6O_3\cdot 2.5H_2O$) C, H, N.

Data for *trans*-2-(4-(4-Fluorophenyl)-5-{2-[(pyridin-2-ylmethyl)amino]pyrimidin-4-yl}-1*H*-imidazol-2-yl)-5-methyl-[1,3]dioxane-5-carboxylic Acid Dimethylamide (1{21, 11}, RPR238778): mp 120−122 °C; MS (HRMS) *m/z* calcd for $C_{27}H_{29}N_7O_3F$ (M + H), 518.2316, found 518.2291; ^{1}H NMR (DMSO-d_6) 1.63 (s, 3H), 3.00 (s, 6 H), 4.13 (d, J = 12 Hz, 2H), 4.20 (d, J = 12 Hz, 2H), 4.51 (m, 2H), 5.62 (s, 1H), 6.71 (m, 1H), 6.97 (m, 1H), 7.10 (t, J = 9 Hz, 2H), 7.15−7.30 (m, 2H), 7.60−7.75 (m, 3H), 8.24 (d, J = 5 Hz, 1H), 8.54 (br d, J = 4.5 Hz, 1H), 11.70−12.40 (br m, 1H). Anal. ($C_{27}H_{28}FN_7O_3\cdot 0.5H_2O$) C, H, N.

Data for *trans*-2-[5-[2-(Cyclopropylmethylamino)pyrimidin-4-yl]-4-(4-fluorophenyl)-1*H*-imidazol-2-yl]-5-methyl-[1,3]dioxane-5-carboxylic Acid Cyclopropylamide (1{9, 5}, RPR239457): mp 186−189 °C; MS (HRMS) *m/z* calcd for $C_{26}H_{30}N_6O_3F$ (M + H) 493.2363, found 493.2401; ^{1}H NMR (DMSO-d_6) 0.12 (m, 2H), 0.37 (m, 2H), 0.50 (m, 2H), 0.66 (m, 2H), 0.93 (m, 1H), 1.50 (s, 3H), 2.69 (m, 1H), 3.00 (m, 2H), 4.00 (d, J = 12 Hz, 2H), 4.08 (d, J = 12 Hz, 2H), 5.61 (s, 1H), 6.31 (m, 1H), 6.94 (m, 1H), 7.19 (t, J = 9 Hz, 2H), 7.31 (br s, 1H), 7.67 (dd, J = 9 and 6.5 Hz, 2H), 8.20 (d, J = 5 Hz, 1H), 12.00−12.60 (br m, 1H). Anal. ($C_{26}H_{29}FN_6O_3\cdot H_2O$) C, H, N.

Data for *trans*-2-{4-(4-Fluorophenyl)-5-[2-(3-methoxypropylamino)pyrimidin-4-yl]-1*H*-imidazol-2-yl}-5-methyl-[1,3]dioxane-5-carboxylic Acid Propylamide (1{11, 18}, RPR239939): mp 176−177 °C; MS (HRMS) *m/z* calcd for $C_{26}H_{33}N_6O_4F$ (M + H) 513.2626, found 513.2623; ^{1}H NMR (DMSO-d_6) 0.90 (t, J = 7.5 Hz, 3H), 1.45−1.60 (m, 2H), 1.53 (s, 3H), 1.69 (m, 2H), 3.11 (m, 2H), 3.19 (broad m, 2H), 3.27 (s, 3H), 3.36 (t, J = 6.5 Hz, 2H), 4.03 (d, J = 12 Hz, 2H), 4.11 (d, J = 12 Hz, 2H), 5.63 (s, 1H), 6.30 (m, 1H), 6.94 (m, 1H), 7.19 (t, J = 9 Hz, 2H), 7.30 (m, 1H), 7.71 (dd, J = 9 and 6.5 Hz, 2H), 8.20 (d, J = 5 Hz, 1H), 12.00−12.50 (br m, 1H). Anal. ($C_{26}H_{33}FN_6O_4$) C, H, N.

Data for *trans*-2-{4-(4-Fluorophenyl)-5-[2-(3-cyclopropylmethyl)pyrimidin-4-yl]-1*H*-imidazol-2-yl}-5-methyl-[1,3]dioxane-5-carboxylic Acid 4-Methylpiperazinamide (1{9, 12}, RPR238677): mp 229−230 °C; ^{1}H NMR (DMSO-d_6) 0.15 (m, 2H), 0.41 (m, 2H), 0.98 (m, 1H), 1.63 (s, 3H), 2.25 (s, 3H), 2.38 (t, J = 5 Hz, 4H), 3.06 (m, 2H), 3.55 (t, J = 5 Hz, 4H), 4.15 (m, 4H), 5.63 (s, 1H), 6.13 (br m, 1H), 6.93 (br m, 1H), 7.17 (t, J = 9 Hz, 2H), 7.69 (dd, J = 9 and 5.5 Hz, 2H), 8.19 (d, J = 5.5 Hz, 1H), 11.50−12.50 (br m, 1H); MS (CI) *m/z* 536 $[MH]^+$. Anal. ($C_{28}H_{34}FN_7O_3$) C, H, N.

Biological Methods. Kinase 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-labeled 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 used for the substrate myelin basic protein. ZAP-70, Syk, and Lck kinase activities were measured using the homogeneous time-resolved fluorescence (HTRF) methodology 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 ± SEM (n = 3).

Human Monocytic Cell Line (THP-1) TNFα Release Assay. THP-1 cells were seeded into 96-well plates at 1 × 10^6-cells/mL (200 μL/well) in medium containing FCS (1%) and incubated overnight. Following pretreatment with compounds for 1 h, the cells were incubated with LPS (20 μg/mL) for a further 24 h. TNFα release was measured in the supernatants by sandwich ELISA. IC_{50} values shown from repeat experiments are means (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 (n = 3).

Rat Drug Distribution Studies. Three rats were dosed orally (1 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 analyzed for compound. Averaged results are reported (n = 3).

Rat SCW-Induced Arthritis Model. This model was performed using Lewis rats in either a prophylactic or therapeutic regimen with 10 animals per group. 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 anaesthetized 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 were given on days 20−24 (po, b.i.d.) 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 postchallenge with iv SCW until termination of the study. Radiography of tibio−tarsal joints was undertaken using a Phillips Practix X-ray unit. The radiographs were scored using a system which sums lesion scores for each of the bones—which are divided into quadrants.

The results represent means ± SEM. One asterisk indicates $p < 0.05$, and two asterisks indicate $p < 0.01$, ANOVA with post hoc Dunnett's test compared to vehicle-treated animals.

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(26) The difference between this and the targeted 800 compounds was a venting phenomenon within the automated reaction chamber, in which a high local vapor pressure forced the contents of a well to waste. Hence, upon completion of the reaction the well contained dry resin and no solvent, reagent, or product.

(27) This classification into high (>20%), medium (2−20%), and low (<2%) is used by the group in which this assay was carried out for all programs and compounds and thus is a fair judgment of the library with regard to the assay.

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