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Discovery of Narrow Spectrum Kinase Inhibitors: New Therapeutic Agents for the Treatment of COPD and Steroid-Resistant Asthma

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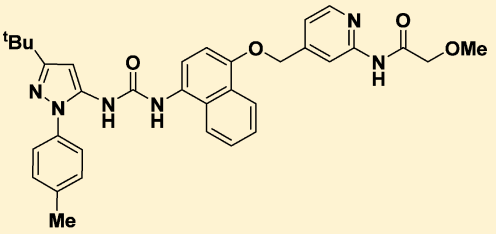
ABSTRACT: The discovery of a novel series of therapeutic agents that has been designed and optimized for treating chronic obstructive pulmonary disease is reported. The pharmacological strategy was based on the identification of compounds that inhibit a defined subset of kinase enzymes modulating inflammatory processes that would be effective against steroid refractory disease and exhibit a sustained duration of action after inhaled delivery.



Narrow Spectrum Kinase Inhibitor (NSKI) Profile

INTRODUCTION

Corticosteroids are widely used in the clinic for the treatment of pulmonary inflammatory disorders, including asthma and chronic obstructive pulmonary disease (COPD). However, their therapeutic benefit is frequently limited by a steroid-refractory phenotype.[1,2] This resistance to treatment can be an inherent feature of the condition or may be induced by external factors that provoke disease exacerbation, such as a viral infection,[3] or by long-term inhalation of noxious gases and particles, such as cigarette smoke or kitchen smoke.[4] Marked steroid resistance impedes the effective management of these maladies, and increasing the drug load in order to compensate for reduced efficacy is often associated with potentially severe side-effects, especially on chronic treatment.[5,6] The objective of the present study was to identify effective agents for the treatment of corticosteroid-resistant symptoms of COPD and asthma that would be suitable for chronic use.

COPD is a major and increasing global health problem with an enormous expenditure of indirect/direct health care costs.[7] COPD now affects over 10% of the world population over the age of 40,[8] and the burden of the disease is particularly high in developing countries. COPD is a complex disease combining inflammatory processes and structural changes to the affected tissues,[9] and long-acting bronchodilators (LAMA and LABA), not anti-inflammatory drugs, are currently the mainstay of COPD pharmacological treatment.[10] While the underlying inflammatory mechanisms are complex, alveolar macrophages are known to play a critical role in the pathophysiology of the disease by secreting proinflammatory proteins, including certain cytokines, chemokines, reactive oxygen species, and electrolytic enzymes.[11,12] Macrophages are markedly elevated in number in the lung and alveolar spaces of patients with COPD and are localized to sites of alveolar destruction.[12] The higher number of macrophages observed may result from increased recruitment of blood monocytes as well as prolonged survival in the lung.[13] To a lesser extent, the rise in macrophage levels may derive from increased proliferation in response to oxidative stress, such as exposure to tobacco smoke.[14] It is relevant to the disease symptomology that alveolar macrophages obtained from COPD patients are resistant to the anti-inflammatory effects of corticosteroids.[15] These differentiated cells recruit neutrophils from the bloodstream to the site of inflammation by the production of cytokines such as CXCL8 and TNFα.

Oral therapies targeting the p38 mitogen activated protein kinases (MAPK) have been proposed as an alternative treatment to corticosteroids owing to their ability to inhibit the production of proinflammatory cytokines.[16] The p38 MAP kinase family comprises four distinct protein isoforms, α, β, γ, and δ, each of which displays different patterns of tissue expression.[17] The p38 MAPK α and β isoforms are distributed widely in the body, being present in many cell types. While the α isoform is well-characterized as a mediator of inflammation, p38 MAPK δ and γ are less well studied. There is evidence to suggest that inhibition of p38 MAPK γ is the mechanism by which the β2-agonist formoterol reverses steroid resistance in asthma.[18] In addition,

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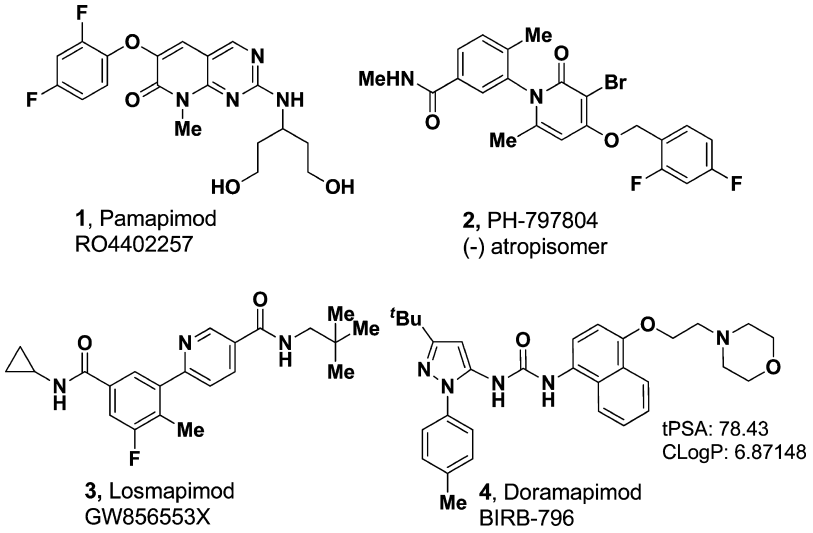

Figure 1. p38 MAPK inhibitors profiled in the clinic.

p38 MAPK γ expression is reported to be upregulated in peripheral blood mononuclear cells obtained from severe asthmatics.[18] This body of evidence has impelled numerous efforts to discover small molecule inhibitors of the p38 MAPK family, in particular the α isoform.

A recent example of a p38 MAPK α inhibitor entering the clinic is **1** (Pamapimod, RO4402257; Figure 1). It was previously reported to be efficacious in several in vivo models of inflammatory arthritis.[19] However, in a 12 week clinical study in patients with rheumatoid arthritis, the compound did not demonstrate significant efficacy either as a monotherapy or in combination with methotrexate.[20] Moreover, typical side effects associated with orally delivered p38 MAPK inhibitors were observed, including skin rash and elevated activity levels of liver enzymes.[21] Dose-limiting toxicology is the norm for p38 MAPK inhibitors, and it has been suggested that first-generation compounds, such as **1**, are much less selective agents than was first considered.[22] The latest generation of inhibitors, typified by **2** (PH-797804), is reported to be more selective and demonstrate little off-target kinase activity.[23] In a 6 week trial involving 230 patients with COPD, the oral administration of **2** was well-tolerated and demonstrated a statistical improvement in FEV1, while the usual skin rash was observed in only 7% of patients.[24] Another oral p38 MAPK inhibitor, **3** (Losmapimod, GW856553), was tested in a 12 week trial involving 302 patients with COPD. This compound was also well-tolerated and demonstrated a statistical reduction of plasma fibrinogen, although there was no significant improvement on lung function or sputum neutrophils.[25] However, the effect was not sustained in a 6 month study.[26] Selective p38 MAPK α inhibitors may not provide an effective therapeutic intervention in a chronic setting.

In contrast to **1**−**3**, urea **4** (Doramapimod, BIRB-796) from Boehringer Ingelheim is a pan active inhibitor of the four p38 MAPK isoforms as well as other non-MAP kinases.[27] This compound has undergone clinical assessment in healthy patients injected with LPS and was found to reduce the levels of induced TNFα and IL-1β release, along with other cytokines.[28] However, in patients with Crohn's disease, little efficacy was demonstrated and only a transient reduction in the inflammation marker, C-reactive protein (CRP), was observed.[29] These transient effects and disease resistance have been observed with other p38 MAP kinase inhibitors, such as VX702[30] and **1** Pamapimod,[20] in rheumatoid arthritis and could be explained by activation of both redundant and/or alternative kinase signaling pathways.[31]

For lung diseases, topical administration of p38 MAPK inhibitors is expected to significantly reduce their resulting systemic exposure, especially to the liver, and thereby minimize the likelihood of side effects.[32] This method of delivery is ideally suited to treating conditions like COPD in which the principal drug targets are epithelial cells and/or resident inflammatory cells such as macrophages. It remains a concern that for selective p38 MAPK agents redundancy in cell signaling cascades will result in disease resistance.[33] In addition, Smith and colleagues reported while p38 MAPK α inhibitors proved to be effective on LPS-stimulated IL-6 and GM-CSF release in monocytes they displayed limited maximal inhibition in macrophages and also no beneficial effects on LPS-stimulated CXCL8 release in macrophages.[34]

In addition to the p38 MAPK family, tyrosine kinases have been implicated in inflammatory disease. Of particular interest is hematopoietic kinase (HCK), which has been shown to modulate adhesion, granulocytosis, and cytokine production in neutrophils.[35] HCK is also reported to possess a preeminent role in LPS/toll like-receptor-4-induced TNF and IL-6 production in human primary macrophages and is upregulated in PBMCs from COPD patients.[36] This kinase belongs to the Src family, and its expression is limited to the hematopoietic cells, predominantly in neutrophils.[37] It was postulated that additional inhibition of relevant kinases, such as HCK, could enhance the efficacy of p38 inhibitors. This approach of inhibiting a subset of kinases is increasingly used in the oncology field and has provided compounds showing greater efficacy when compared to those that act on a single kinase target.[38,39]

The two main objectives for the project were to design molecules that, first, had p38α/γ activity and, second, had a longer duration of action than BIRB-796 when delivered topically to the lung. To the first end, we were assisted by the availability of crystallography data for our target enzymes with an array of ligands in the Protein Data Bank (PDB).[40] The design paradigm was to identify commonalities between the p38 isoforms that would also promote the DFG-out protein conformation observed for BIRB-796 (1kv2.pdb). A second design parameter was to focus on lower solubility derivatives that were anticipated

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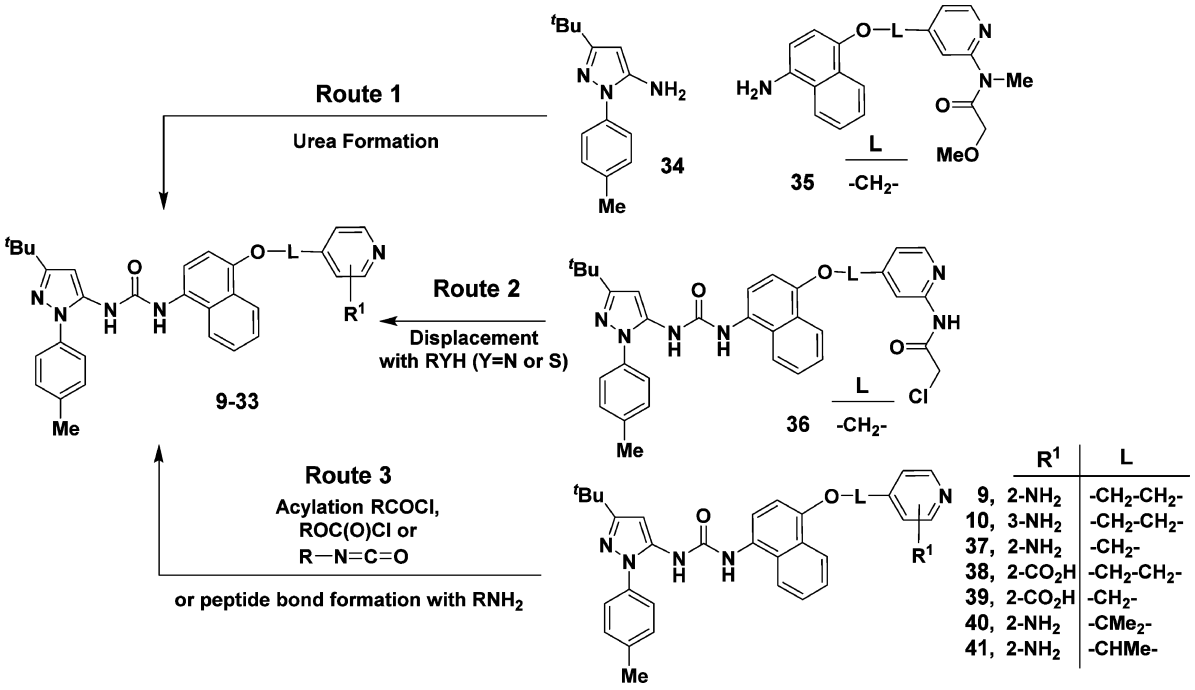
Scheme 1. General Synthetic Strategy



to promote slow dissolution in the airways, leading to persistent target occupancy that is not dependent on systemic PK.

This article describes the discovery of novel, small molecule agents that inhibit a selected set of therapeutically relevant kinases for the treatment of steroid-refractory pulmonary disorders such as COPD. In addition, the compounds possess pharmacokinetic properties that enable sustained activity in vivo.

CHEMISTRY

The compound examples described herein were prepared by one of three alternative processes as the final or penultimate step. Formation of the unsymmetrical central urea (Scheme 1, Route 1) was performed via activation of aminopyrazole **34**[41] by its transformation into the corresponding isocyanate or equivalent, followed by addition of the desired naphthylamine **35**. However, many analogues differed only in the nature of the substituent appended to the pyridine nucleus. In some instances, this structural divergence was readily incorporated by a displacement reaction on chloroacetamide intermediate **36** (Scheme 1, Route 2) with an appropriate nucleophile. In most cases, the compounds were obtained from common pyridine amines **9**, **10**, **37**, **40**, and **41** or carboxylic acid building blocks **38** and **39** by acylation or an amide coupling reaction, respectively (Scheme 1, Route 3).

Intermediates containing an ethylene linker (Scheme 1, L, $-CH_2CH_2-$) were obtained from the corresponding ethyl alcohol precursors (Scheme 2, **42**, **44**, and **45**). Boc-protected 2-aminopyridine **42** was derived from ethyl 2-(2-chloropyridin-4-yl)acetate by a Buchwald coupling[42] with *tert*-butylcarbamate followed by reduction of the ester to the alcohol, under standard conditions. The corresponding methyl ester **44** was accessed via hydrolysis of a protected picolinonitrile,[43] **43**, which, in turn, was obtained from commercial 2-(pyridin-4-yl)ethanol by silyl ether protection, followed by N-oxide formation and cyanide insertion. Treatment of 4-methyl-3-nitropyridine with base and *para*-formaldehyde provided aminopyridine ethanol **45** after nitro reduction.[44] Ether intermediates **46a–c** were generated either by an S_NAr displacement on 1-fluoro-4-nitronaphthalene or by a Mitsonobu coupling reaction on 4-nitronaphthalen-1-ol. Subsequent reduction of the nitro groups, with iron in acetic acid or by catalytic hydrogenation, gave the desired naphthylamines **47a–c**. The unsymmetrical ureas **9**/**10** and **38** were produced by prior activation of aminopyrazole **34** with either CDI or a phosgene equivalent followed by treatment with the requisite naphthylamine **47a–c** and, where necessary, deprotection of the product. In the case of 2-aminopyridine **9**, the Boc group was removed from intermediate **48** by exposure to TFA, whereas for pyridine carboxylic acid **38**, the acid was revealed by saponification of ester **49**. These advanced intermediates were transformed into final test substances by an N-acylation reaction with an acid chloride or isocyanate or amidation under standard peptide bond forming conditions.

Imidazolyl analogue **17** was readily accessible via an amination reaction on the known alkyl iodide **50**[45] with 2-nitroimidazole under basic conditions (Scheme 3). The resulting nitroarene **51** was converted into the test compound by reduction of the nitro group followed by acylation with acetyl chloride in the presence of DIPEA.

Most of the lower homologues (Scheme 1, L, $-CH_2-$) were prepared using essentially the same synthetic strategies outlined above, starting from the appropriately substituted pyridinemethyl alcohols **52** and **53**, which were available from commercial sources (Scheme 4). The corresponding methoxy linked biaryls **54a–b** were obtained by a Mitsunobu coupling. N-Methyl methoxyacetamide **55b** was prepared by alkylation of acylated intermediate **55a** with methyl iodide in the presence of sodium hydride. The resulting nitronaphthylenes were converted into naphthylamines **35** and **56a–b** either by a dissolving metal reduction or by catalytic hydrogenation, and the products were subsequently exposed to a preactivated solution of 5-aminopyrazole **34**. In the case of tertiary amide **35**, this provided N-methylated analogue **31** directly. The protected intermediate obtained from the urea coupling step, ester **57**, was transformed into carboxylic acid **39** by basic hydrolysis. Final drug substances

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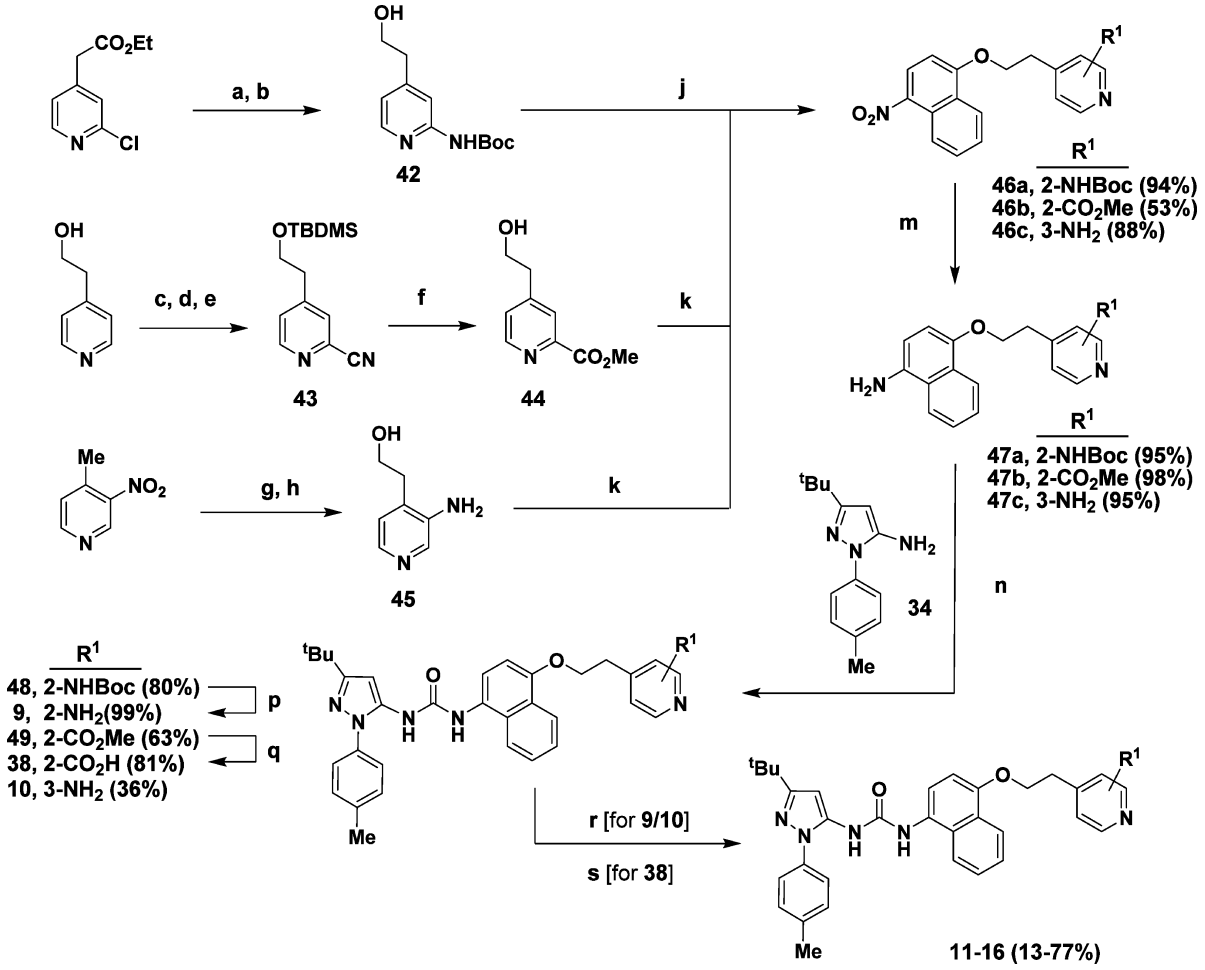
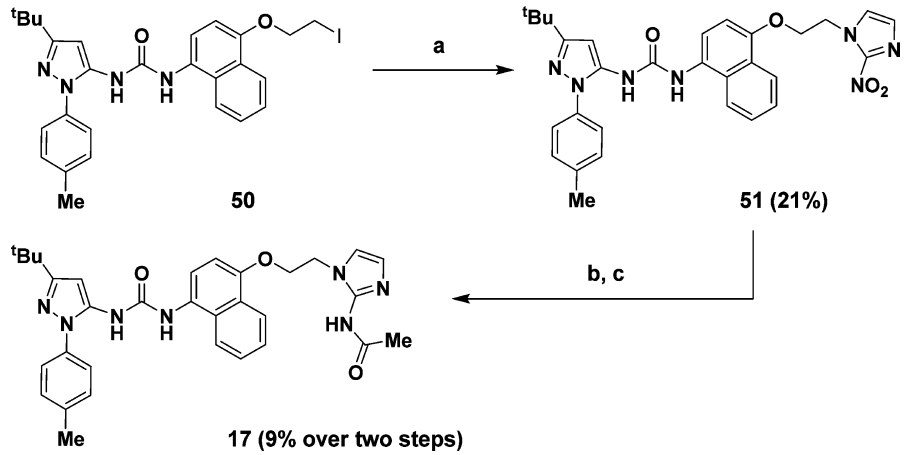
Scheme 2. Synthetic Route To Access the Ethylene Linker Intermediates[a]



[a]Reagents: (a) *t*-BuOCONH$_2$, Pd$_2$(dba)$_3$, Cs$_2$CO$_3$, Xantphos, THF, 65 °C; (b) DIBAL, −78 °C, THF; (c) TBDMSCl, imidazole, DCM, rt; (d) H$_2$O$_2$, MeReO$_3$, DCM, rt; (e) TMS-CN, Me$_2$NC(O)Cl, DCM, rt; (f) Na, MeOH, then aq. HCl, rt; (g) Triton B, paraformaldehyde, DMSO, rt; (h) H$_2$, Pd/C, MeOH; (j) NaH, 1-fluoro-4-nitronaphthalene, THF, 0 °C; (k) 4-nitronaphthalen-1-ol, DIAD, PPh$_3$, THF, −78 °C; (m) Fe, AcOH, 50 °C (**46a**) or H$_2$, Pt/C, rt (**46b** and **46c**); (n) CDI, DCM (**47a** and **47b**) or diphosgene, aq. NaHCO$_3$ (**47c**); (p) TFA, DCM; (q) aq. NaOH; (r) RCOCl, base, THF/DMF, rt; (s) RNH$_2$, HATU, DMF, rt.

Scheme 3. Synthesis of the Imidazolyl Analogue[a]



[a]Reagents: (a) 2-nitro-1*H*-imidazole, K$_2$CO$_3$, DMF, 50 °C; (b) Fe, AcOH, 50 °C; (c) MeCOCl, DIPEA, DCM, rt.

were generated from amine precursors **37** by acylation and sulfonylation and from acid **39** by amide couplings.

Additional 2-aminopyridine N-acetyl analogues were prepared by a process that is highly convergent and expedient for preparing this compound class (Scheme 5). Reaction of the parent amine **37** with chloroacetyl chloride provided chloroacetamide intermediate **36**. This reactive alkylating agent was isolated and then subjected to displacement reactions with

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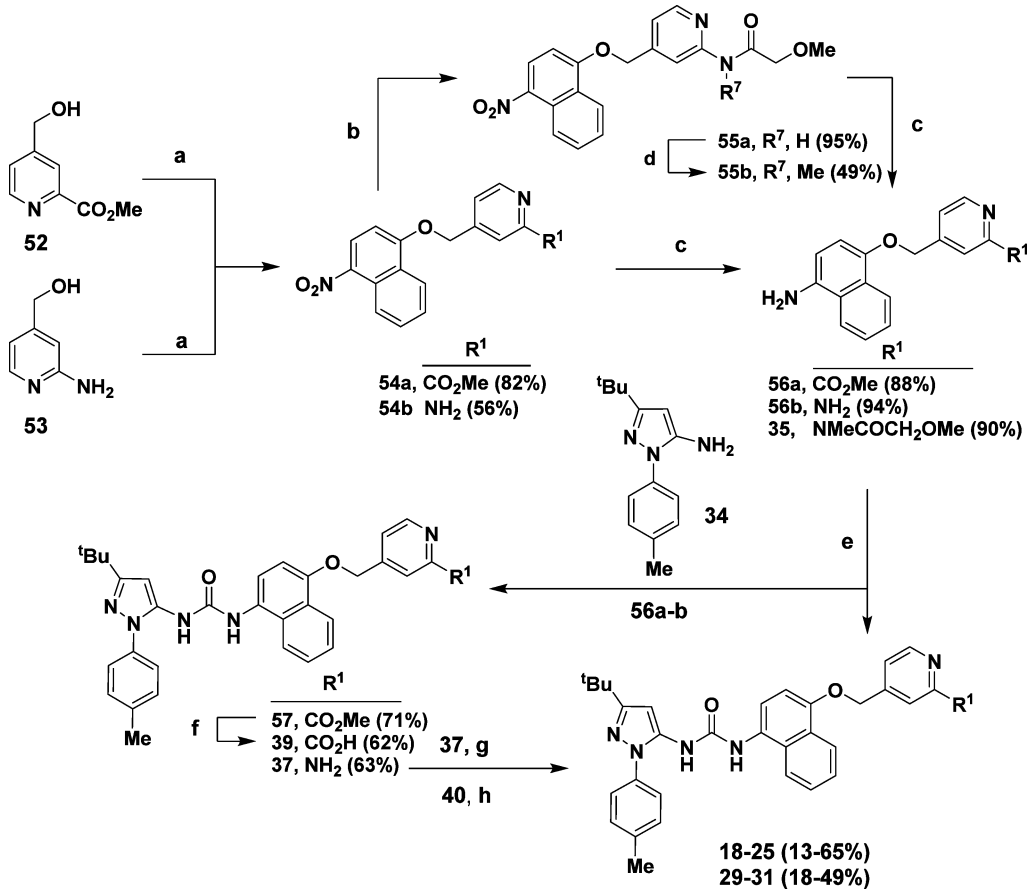
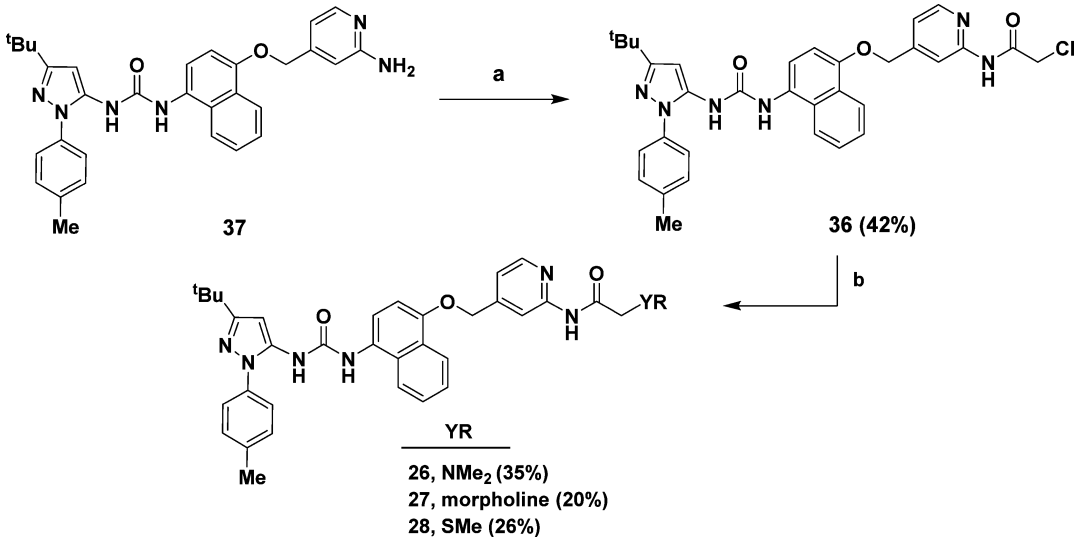
Scheme 4. Synthetic Route To Access the Methylene Linker Intermediates[a]



[a]Reagents: (a) 4-nitronaphthalen-1-ol, DIAD, PPh_3, −78 °C, THF; (b) $MeOCH_2COCl$, DIPEA, THF, rt; (c) Fe, AcOH, 50 °C or H_2, Pt/C, rt; (d) NaH, MeI, THF, 0 °C; (e) CDI, **34**, DCM; or diphosgene, $NaHCO_3$, DCM, 0 °C; (f) LiOH, THF/H_2O, rt; (g) RCOCl, DIPEA, THF/DMF, rt; or ROCOCl, DIPEA, THF/DMF, rt; or RSO_2Cl, DIPEA, THF/DMF, rt; or RNCO, DCM/DMF, rt; (h) HATU, RNH_2, DMF, rt.

Scheme 5. Synthesis of N-Acetyl Analogues[a]



[a]Reagents: (a) $ClCH_2COCl$, DCM, DIPEA, rt; (b) RYH, DIPEA, DCM/DMF, 40 °C (Y = N, S).

nucleophiles to provide, inter alia, aminoacetamide derivatives **26** and **27** and thioether **28**.

Analogues **32** and **33** were accessed with similar chemistry as that for compounds **18**−**25**; however, the side chain substitution (R^2 = H, Me) was introduced early in the synthesis. Treatment of commercial methyl 2-aminoisonicotinate with methyl lithium furnished both ketone **58** and tertiary alcohol **59a**. Standard reduction of **58** with $NaBH_4$ gave secondary alcohol **59b**.

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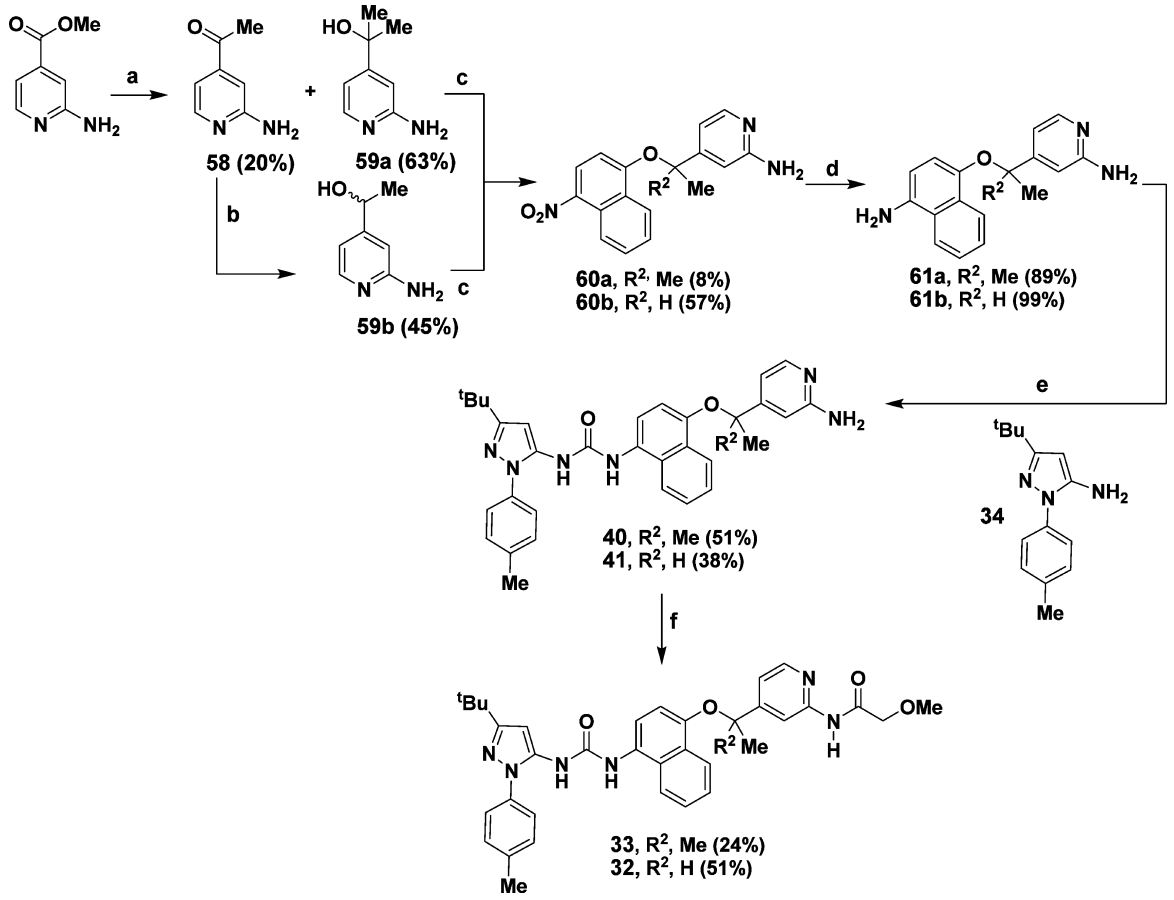
Scheme 6. Synthetic Route To Access the Side-Chain-Substituted Analogues[a]



[a]Reagents: (a) MeLi, THF, −78 °C; (b) $NaBH_4$, MeOH, 0 °C; (c) NaH, 1-fluoro-4-nitronaphthalene, DMF, 0 °C; (d) H_2, Pt/C, rt; (e) diphosgene, aq. $NaHCO_3$, 0 °C to rt; (f) $MeOCH_2COCl$, DIPEA, THF/DCM, 0 °C.

Elaboration of **59a−b** under standard chemistry gave **32** and **33** (Scheme 6).

RESULTS AND DISCUSSION

Highly potent drugs are usually required for inhaled administration owing to the small doses that can be delivered conveniently, typically no more than a few hundred micrograms as a single dose. A long duration of action is also highly desirable to maintain therapeutic benefit, for example, during the night and to allow once daily dosing. In addition to these characteristics, low oral bioavailability (<5%) is an important advantageous property to minimize systemic availability and side effects. Slow dissolution and absorption through the lungs have also been implicated as desirable properties, and these attributes have recently been referred to as forming part of an "inhalation by design" strategy.[46] In view of the modest metabolizing potential of lung epithelium and the route administration, functional groups and molecular properties that might be considered detrimental for the development of an oral medicine were not necessarily excluded from consideration.[47,48] Indeed, increasing the molecular complexity by the introduction of additional polar groups and hydrogen-bond donors was anticipated to modulate both solubility and permeability positively for our inhaled program.

For this purpose, U937 cells were treated with phorbol myristate acetate (PMA) to evoke their differentiation into macrophages-like cells. Stimulation of the resulting cells (d-U937 cells) with lipopolysaccharide (LPS) induces the production of proinflammatory cytokines including TNFα and CXCL8. The potency of test compounds in this system was evaluated by the determination of both IC_{50} values as well as their maximum inhibitory effect (E_{max}). This latter property was considered to be an important criterion that resulted from the inhibition of multiple targets and was therefore used to select compounds that displayed the kinase profile described above.

Upon evaluation of potential alternative hinge binders in the pyrazole urea series in comparison to BIRB-796 (**4**), **5**−**8** (Table 1)[27] demonstrated that p38 inhibition could be maintained. 4-Pyridyl analogue **8** was superior to its regioisomeric analogues **6** and **7** and to imidazole **5**, as the former maintained significant potency toward the γ isozyme and introduced reasonable potency against HCK. Additionally, compound **8** demonstrated moderate activity in d-U937 cells.

We next focused on further modification of compound **8**; with the aim of promoting interactions with the peptide backbone of the kinase hinge, a number of heteroaryl amine, amide, and urea analogues were prepared (Table 2).

From public X-ray structures of BIRB-796 with p38 α (1KV2.pdb) and atrial natriuretic peptide (ANP) with p38 γ (1CM8.pdb), we identified a difference in the presentation of hydrogen-bonding residues to the ligand from the hinge region of the protein: BIRB-796 being monodentate in p38 α and ANP being bidentate in p38 γ. Inspection of other p38 α structures (e.g., 3FLN.pdb) showed that, when it is presented with a ligand with bidentate hydrogen-bonding capacity, p38 α is able to

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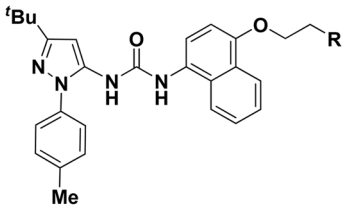
Table 1



Compound	R	IC_{50} values (nM) at isolated enzymes			IC_{50} values (nM) and /or [E_{max} %]	
		p38 α	p38 γ	HCK	TNFα	CXCL8
4 [BIRB-796]		20	102	>19000	15 [69]	[56]
5		22	2050	4937	[45]	[28]
6		167	2950	>19000	[16]	[3]
7		12	616	>19000	[3]	[-1]
8		17	213	901	[51]	[45]

undergo a rotation of the hinge region to accommodate the extra hydrogen bond. This suggested that a bidentate hinge binder would be tolerated by p38 α and would yield stronger binding to p38 γ. It was also postulated that the firmer anchor of the bidentate hinge binding in p38 γ may help to promote the trapping of a DFG-out protein conformation, which was suspected to be required for a longer duration of action. An unintended consequence of this change to a bidentate ligand was to promote the inhibition of other kinases such as HCK, which was found to be beneficial to the overall cellular profile of the compounds (greater E_{max} for the inhibition of TNFα in U937 cells).

Simple amines **9** and **10** maintained moderate activity toward the kinases of interest and displayed a promising increase in cellular potency. In addition, it was encouraging to see that the pyridine nucleus could be functionalized with such a synthetically useful and adaptable amino group. The corresponding acetamide derivatives **11** and **12** demonstrated similar p38 MAPK profiles as those of the amine precursors as well as a modest increase in activity at HCK. Of particular interest at this stage was the ability of ortho-substituted analogue **11** to inhibit the stimulated release of TNFα from d-U937 cells with an IC_{50} value of <20 nM and with a high maximal activity.

A consistent SAR trend in favor of the ortho-isomer was evident for the two methoxyacetamido-substituted pyridines, **13** and **14**. Although activity against the γ isoform of p38 MAPK remained weak, ortho-analogue **14** displayed an impressive cellular profile, especially against TNFα release, for which the IC_{50} value was single-digit nanomolar. This compound was also the first from this chemotype to potently inhibit the release of CXCL8 from the differentiated, macrophage-like U937 cells.

In contrast, "reverse" amides **15** and **16** retained activity against p38 MAPK α, failing to demonstrate any activity at the γ isoform or at HCK together with little activity at the cellular level. This data suggested that the disposition of the amidic carbonyl is of critical importance in determining the pharmacology of this compound series. Imidazole analogue **17** was also largely selective for the α isoform of p38 MAPK. Its cellular profile was comparable with that of 2-aminopyridine **9**, and consequently this chemotype was discounted from further study.

The in vitro biology of the compounds, summarized in Table 2, together with that of additional analogues (data not shown) revealed that 2-acylaminopyridine was a favored fragment that promoted inhibitory activity against the triad of isolated enzymes as well as potency in cellular systems. The data also indicated that p38 MAPK inhibitors with additional HCK kinase activity demonstrate an anti-inflammatory biological phenotype in a disease-relevant cell line.

To assess the degree of CYP inhibition inherent in this series, 4-pyridyl derivative **8** was compared, against a standard isoform panel, with the corresponding 2- and 3-N-acylamino derivatives, **11** and **13**, respectively (Table 3). The former compound exhibited significant inhibitory activity, particularly against the enzyme CYP 3A4. This is most likely to arise from a direct interaction with the pyridyl nitrogen as a similar profile is evident for meta-substituted analogue **13**. In contrast, 2-acetamido derivative **11** displayed hardly any activity at either the CYP 2D6 or 3A4 isoform. The superior profile shown by **11** likely derives from the presence of the ortho substituent in the pyridine nucleus, which presumably disrupts the undesired interaction with the basic nitrogen.

Despite the encouraging efficacy results for acylaminopyridine **11**, a significant increase in potency in d-U937 cells was deemed to be necessary for such compounds to meet their intended product profile as inhaled medicines. We next studied shortening of the three-atom spacer between the naphthyl and pyridine groups (Table 4). Consistent with the early findings for these chemotypes, pyridine carboxamides **18** and **19** remained selective potent inhibitors of the p38 MAPK α isozyme while being, at best, weak inhibitors of p38 MAPK γ and HCK, and they did not reach maximum efficacy in cells. In contrast, the

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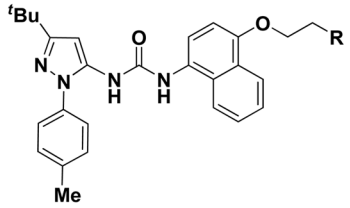
Table 2



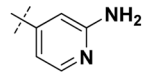
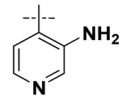
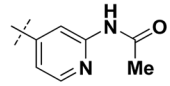
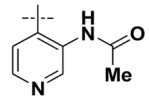
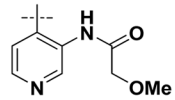
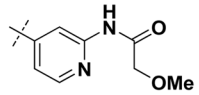
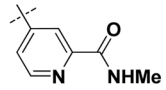
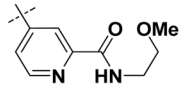
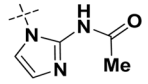
Compound	R	IC$_{50}$ values (nM) at isolated enzymes: p38 α	p38 γ	HCK	IC$_{50}$ values (nM) and /or [E$_{max}$ %]: TNFα	CXCL8
9		60	1504	829	31 [74]	32 [71]
10		11	591	625	110 [60]	[46]
11		11	972	82	17 [83]	816 [66]
12		8	634	482	[42]	[46]
13		3	354	1117	[42]	[49]
14		31	647	164	4 [80]	37 [76]
15		33	>17000	>17000	[34]	[47]
16		25	>16000	>16000	[33]	[39]
17		6	>17000	3221	37 [78]	88 [60]

Table 3

Compound	Inhibition (%) of CYP 450 enzyme isoforms at 30 μM: 2C19	2D6	3A4	2C9
BIRB-796	35	11	−23	13
8	38	44	67	31
11	25	3	6	23
13	25	73	64	37

profiles of a series of functionalized 2-aminopyridines was markedly enhanced by the reduction in the chain length to a two-atom linker. For example, carbamate derivative **20** is a potent, dual p38 MAPK α/HCK inhibitor, and this is reflected by its superior potency in cells. Sulfonamide **21** has more modest but comparable activity at the γ isoform and HCK. Notable is the direct comparison of the in vitro profile of acetamide **22** and its higher homologue, compound **11** (Table 2). This reveals that the methoxy-linked compounds can possess good to excellent activity against the triad of isolated enzymes examined in this study and potently inhibit the inflammatory mediators TNFα and CXCL8 in differentiated macrophage-type cells. Equally gratifying was the observation that the maximal inhibitory values reached in these latter assay systems were ≥90%, which is a highly desirable property, postulated to be the result of simultaneous inhibition of the α and γ protein isoforms of p38 MAPK.

The markedly superior pharmacological profile of N-sulfonyl and N-acyl 2-aminopyridines **21**−**23** provided the incentive to explore this particular compound class in more detail (Table 5). Results obtained for benzamide **24** and phenylacetamide **25** were both surprising and disappointing. Both compounds displayed in vitro profiles comparable to those of earlier structures, namely, inhibitory activity strongly in favor of the p38 MAPK α enzyme. While high potencies are preserved in the cellular system, the

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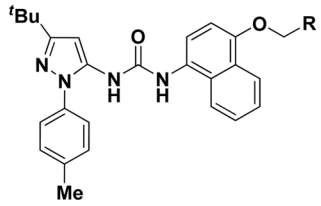
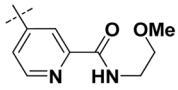
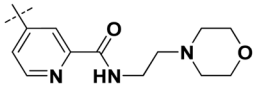
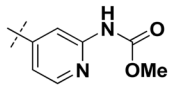
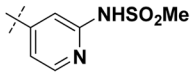
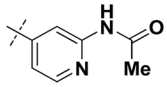
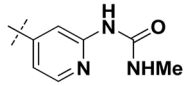
Table 4



Compound	R	IC$_{50}$ values (nM) at isolated enzymes: p38 α	p38 γ	HCK	IC$_{50}$ values (nM) and [E$_{max}$ %]: TNFα	CXCL8
18		123	>16000	>16000	23 [77]	30 [64]
19		6	>15000	>15000	5 [79]	14 [63]
20		13	>17000	26	4 [82]	10 [76]
21		18	303	2141	4 [77]	41 [65]
22		2	195	51	4 [93]	12 [101]
23		6	169	26	5 [90]	9 [90]

corresponding E_{max} values are less than or equal to 80% for these two compounds.

Additional compound analogues revealed that a diversity of functional modification was well-tolerated and could be exploited to refine the biological phenotype. Tertiary amines **26** and **27** exhibited characteristics similar to those of earlier, favored analogues (**21**−**23**, Table 4) and offer the opportunity to modulate global physicochemical parameters, including the possibility of salt formation and selection. Incorporation of a thioether, **28**, or a sulfone, **29**, was also tolerated as both exhibited the desired narrow spectrum kinase inhibitor (NSKI) activity and showed excellent potencies and efficacies in cells. These findings confirm that a broad range of functionality can be introduced at the β carbon of the acetamido group while maintaining the desired biological profile. Methoxyacetamide analogue **30** was found to be a highly active NSKI and to potently inhibit the release of inflammatory mediators in d-U937 cells. The poor activity of N-methylated analogue **31** supports the hypothesis that the N-acyl 2-aminopyridine pharmacophore operates in a donor/acceptor binding mode. Perturbation of this critical interaction has a negative impact on p38 MAPK γ and HCK kinase inhibition. This disruption often leads to a loss of the NSKI phenotypic profile and reduction in cellular efficacy.

As reducing the linker length had such a profound enhancing effect on the pharmacological profile of N-acyl 2-aminopyridine series, additional modifications were investigated that would either perturb its conformation or shorten it further. Increasing steric constraints from monomethyl **32** (as a racemate) to gem-dimethyl analogue **33** gave a general decrease in isolated enzyme and cellular profile (Table 6). Further reduction to an ether or methane linker lead to a significant change in pharmacological profile, which is beyond the scope of this article.[49]

In view of its promising in vitro biology, methoxyacetamide **30** was selected for further profiling in an in vivo time course experiment to assess the compound's duration of action against BIRB-796 (Figure 2). The test compound was dosed (at 0.2 mg mL^{-1}) by intratracheal (it) administration to mice (N = 8). Following this event and after increasing intervals of time (the preincubation time) starting with a delay of 2 h, which was incrementally increased up to 18 hours, animals were challenged with an endotoxin (LPS) to evoke an inflammatory response in the lungs. An additional 8 h after the insult, each animal was sacrificed and bronchoalveolar lavage fluid (BALF) was collected. The number of neutrophils per milliliter of fluid was counted to determine the effectiveness of the potential drug.

The decrease in the number of neutrophils at t = 2 h is an indication of an agent's anti-inflammatory activity. The persistence of the observed effect as the delay (preincubation time) before administration of the challenge is increased indicates the compound's duration of action. BIRB-796 and compound **30** initially show comparable anti-inflammatory effects in murine lung, but the therapeutic effect of BIRB-796 rapidly diminishes and has all but disappeared at 8 h post dose. By contrast, compound **30** retained a significant proportion of its initial activity

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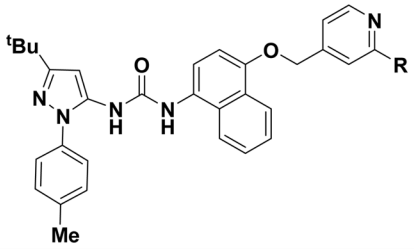
Table 5



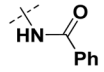
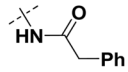
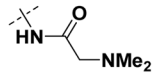
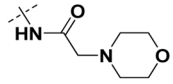
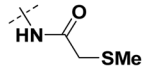
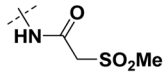
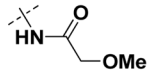
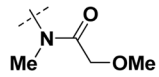
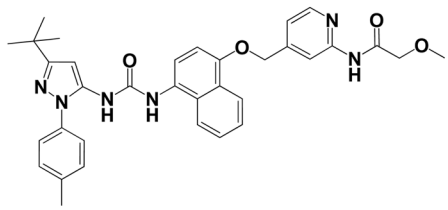
Compound	NHCOR	IC50 values (nM) at isolated enzymes: p38 α	p38 γ	HCK	IC50 values (nM) and [Emax %]: TNFα	CXCL8
24		24	8359	13291	5 [82]	9 [80]
25		9	378	692	2 [83]	3 [74]
26		1	461	197	5 [78]	37 [94]
27		2	352	187	5 [79]	5 [87]
28		2	148	208	1 [95]	2 [103]
29		8	55	110	2 [90]	15 [92]
30		5	40	52	4 [88]	7 [88]
31		27	2438	8673	6 [72]	8 [68]

Table 6



Compound	R	IC50 values (nM) at isolated enzymes: p38 α	p38 γ	HCK	IC50 values (nM) and [Emax %]: TNFα	CXCL8
32	H	52	902	807	286 [60]	9 [75]
33	Me	38	1941	8552	[35]	1581 [51]

out to the 12 hour time point, indicating a $t_{1/2}$ in the region of 14 h and its likely suitability for once daily dosing in the clinic.

It is interesting to compare the physiochemical properties of BIRB-796 and compound **30**. While they have similar lipophilicity (cLog P 6.87 vs 6.66), BIRB-796 has a more strongly basic center, which would be anticipated to lead to better solubility. Lung permeability may also be reduced by the additional H-bond present in compound **30** and its higher polar surface

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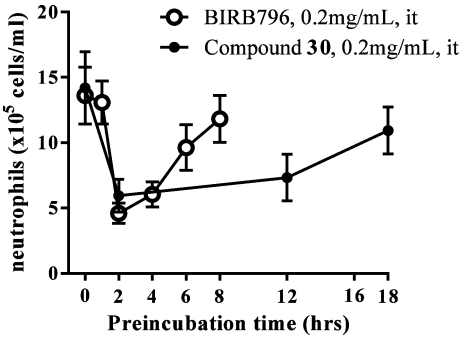

Figure 2. Comparison of the effects of treatment with BIRB-796 or compound **30** on LPS-induced airway neutrophilia in mice. it = intratracheal.

area (tPSA 78.43 vs 116.6).[46] We postulate that these properties contribute to the excellent in vivo duration of action of compound **30**.

Since the above pharmacodynamic screening method is sensitive to corticosteroid, compound **30** was next evaluated in an animal model of steroid-refractory inflammation.[50] Mice exposed repeatedly, over 11 days, to cigarette smoke (CS) develop pulmonary inflammation that is inert to treatment with fluticasone propionate (FP) (Figure 3). The two test substances were administered to mice intranasally at 40 μg mL^{-1} (35 μL per mouse, $N = 6$) twice daily for 3 days following their exposure to cigarette smoke. The ineffectiveness of FP, when given at 50 μg mL^{-1}, to reduce neutrophil influx into the lungs of the animals attests to the steroid insensitivity of the stimulated inflammatory response. It is particularly relevant that BIRB-796 demonstrated little or no effect on neutrophil accumulation when dosed intranasally at 40 μg mL^{-1}. This result confirms that the compound is ineffective at controlling inflammatory processes that are untreatable with corticosteroids. However, when administered at the same dose, **30** brought about a dramatic reduction in the number of neutrophils recruited into mouse lung. These results support the hypothesis that a NSKI such as **30** is able to inhibit corticosteroid-refractory inflammation by exerting inhibitory activity on a predefined panel of therapeutically relevant kinases.

Additional attributes of the drug substance **30** enhance its suitability as a prospective inhaled medicine. Separate studies (data not shown) reveal that the compound has an oral bioavailability of less than 5% and displays high plasma protein binding of >99%. When used at therapeutically relevant doses, these properties should ensure that systemic exposure will be very low, reducing the risk of side effects. Relevant physical properties of the compound were also investigated and are suitable for dry powder inhaled formulation, the intended method of use in the clinic. Compound **30** is currently undergoing clinical trials.

CONCLUSIONS

A series of functionalized 2-aminopyridines has been derived that potently inhibit both p38 and SRC family kinases. It has been demonstrated that these kinase inhibitors, such as acetamide **22**, urea **23**, thioether **28**, and sulfonyl derivative **29**, potently reduce the stimulated release of proinflammatory mediators from disease-relevant cells, with high maximal efficacy. As an example of this chemotype, methoxy acetamide **30** has been identified to demonstrate a long and advantageous duration of action in an in vivo pharmacodynamic model. The same compound is also highly effective at reversing cigarette smoke-induced inflammation in mice, showing that it is effective against a model of the disease state that is normally resistant to treatment with corticosteroids. It has been established that a diverse range of substituents, appended to a common pharmacophore, is compatible with the desired pharmacological profile. This permits the selection of compounds having physicochemical properties that are suitable for various pharmaceutical formulations including those used for dry powder inhalation.

EXPERIMENTAL SECTION

Enzyme Inhibition Assays. The enzyme inhibitory activities of compounds disclosed herein were determined by FRET using synthetic peptides labeled with both donor and acceptor fluorophores (Z-LYTE, Invitrogen Ltd., Paisley, UK). Inhibitory activities against the p38 MAPK α isoform (MAPK14: Invitrogen) were evaluated indirectly by determining the level of activation/phosphorylation of the downstream molecule, MAPKAP-K2. The p38 MAPK α protein (80 ng mL^{-1}, 2.5 μL in 2× kinase buffer provided in the kit) was mixed with the test compound (2.5 μL of either 4.000, 0.400, 0.040, or 0.004 μg mL^{-1} in 4% DMSO in H_2O) for 2 h at rt. The mix solution in 1× kinase buffer (2.5 μL) of the p38 α inactive target MAPKAP-K2 (Invitrogen, 600 ng mL^{-1}) and FRET peptide (8.0 μM; a phosphorylation target for MAPKAP-K2) was then added, and the kinase reaction was initiated by adding ATP (40 μM, 2.5 μL in 1× kinase buffer). The mixture was incubated for 1 h at rt. Development reagent (protease diluted 1:100 in development buffer, 5.0 μL) was added 1 h prior to detection in a fluorescence microplate reader (Varioskan Flash, ThermoFisher Scientific).

The inhibitory activities against p38 MAPK γ (MAPK12: Invitrogen) and HCK were evaluated in a similar fashion to that described above. The enzyme (800 ng mL^{-1}, 2.5 μL in 2× kinase buffer) was incubated with the test compound (2.5 μL at either 4.000, 0.400, 0.040, or 0.004 μg mL^{-1} in 4% DMSO in H_2O) for 2 h at rt. The FRET peptides

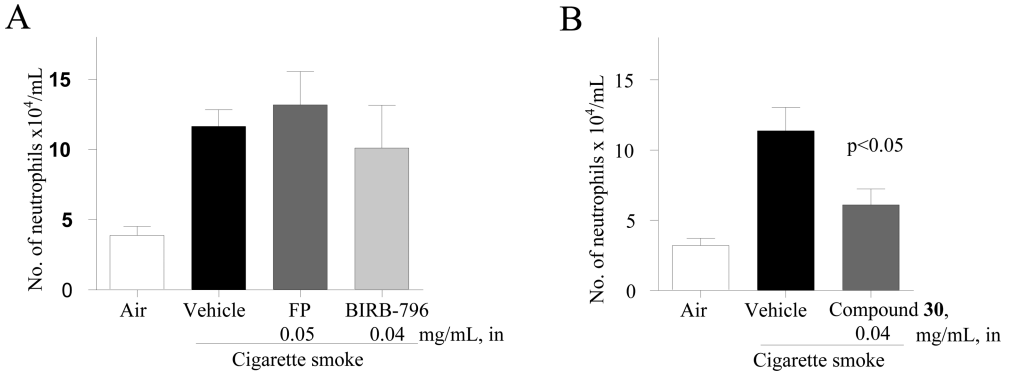

Figure 3. Effect of treatment with either FP (A), BIRB-796 (A), or **30** (B) on tobacco smoke-induced airway neutrophilia in mice. in = intranasal.

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(8.0 μM, 2.5 μL in 1× kinase buffer) and the appropriate ATP solution (2.5 μL, 400 μM for p38 MAPK γ or 2.5 μL, 60 μM for HCK in 1× kinase buffer) were then added to the enzyme/compound mixtures and incubated for 1 h.

In all cases, the site-specific protease cleaves nonphosphorylated peptide only and eliminates the FRET signal. Phosphorylation levels of each reaction were calculated using the ratio of coumarin emission (donor) over fluorescein emission (acceptor), for which high ratios indicate low levels of phosphorylation and low ratios indicate high levels of phosphorylation. The percentage inhibition of each reaction was calculated relative to noninhibited control, compounds were tested in quadruplicate at each concentration, and the 50% inhibitory concentration (IC_{50} value) was then calculated from the concentration−response curve.

LPS-Induced TNFα/CXCL8 Release in d-U937 Cells. U937 cells, a human monocytic cell line, suspended in 50 mL of 10% FBS RPMI-1640 media were incubated with 5.0 μL of PMA stock solution (1.0 mg mL^{-1} in neat DMSO), resulting in a final PMA concentration of 100 ng mL^{-1}, for 48 to 72 h and differentiated into macrophage-type cells. The resulting cells were incubated in 5% FBS RPMI-1640 media. The test compounds were prepared as 2.0 mg mL^{-1} neat DMSO solution. The stock solutions were serially diluted in DMSO to be 200-fold desirable final concentrations. This allows the final DMSO concentration to be constant across the plate at 0.5%. The cells were then preincubated with the test compound in triplicate prepared above for 2 h and stimulated with 50 μL of LPS (1 mg mL^{-1} in PBS) (from *E. coli*: O111:B4, Sigma) for 4 h. Thus, the final concentration of LPS was 0.1 μg mL^{-1}. The supernatant was collected for determination of TNFα and CXCL8 concentrations by sandwich ELISA (DuoSet, R&D systems). The inhibition of TNFα and CXCL8 production was calculated at each concentration of test compound by comparison with vehicle control. The 50% inhibitory concentration (IC_{50}) was determined from the resultant concentration−response curve.

LPS-Induced Neutrophil Accumulation in Mice. Nonfasted Balb/c mice were dosed by the intratracheal route with either 20 μL of vehicle (10% DMSO in isotonic saline) or the test substance at the indicated times (within the range 2−18 h) before stimulation of the inflammatory response by application of an LPS challenge. At $t = 0$, mice were placed into an exposure chamber and exposed to LPS (7.0 mL, 0.5 mg mL^{-1} solution in PBS for 30 min). After a further 8 h, the animals were anesthetized, their tracheas were cannulated, and bronchoalveolar lavage fluid (BALF) was extracted by infusing and then withdrawing from their lungs 1.0 mL of PBS via the tracheal catheter. Total and differential white cell counts in the BALF samples were measured using a Neubaur hemocytometer. Cytospin smears of the BALF samples were prepared by centrifugation at 200 rpm for 5 min at rt and stained using a DiffQuik stain system (Dade Behring). Cells were counted using oil immersion microscopy. Data for the number of neutrophils in BAL are shown as mean ± SEM (standard error of the mean). The percentage inhibition of neutrophil accumulation was calculated for each treatment relative to vehicle treatment. This study was conducted at Pneumolabs UK Ltd. (London, UK).

Cigarette Smoke-Induced Inflammatory Accumulation in Mice. A/J mice (males, 5 weeks old) were exposed to cigarette smoke (4% cigarette smoke, diluted with compressed air) for 30 min per day for 11 days using a tobacco smoke inhalation experiment system for small animals (model SIS-CS; Sibata Scientific Technology, Tokyo, Japan). Test substances were given intranasally (35 μL of solution in 50% DMSO in isotonic saline) and therapeutically twice daily for 3 days after the final cigarette smoke exposure. At 96 h after the last tobacco smoke exposure (meaning 12 h after the last dosing), animals were anesthetized, the trachea was cannulated, and BALF was collected as previously reported.[51] The procedure for this involved infusing at one-third of 20 mL/kg of PBS, leaving it in the airway for 10 s, and withdrawing infused PBS gradually at room temperature. This step was repeated three times in total. The BALF was then centrifuged (1200 rpm for 10 min at 4 °C), the supernatant was drawn off, and the resulting cell pellet was resuspended in 0.2% NaCl to induce hemolysis of erythrocytes. After isotonization with the same volume of 1.6% NaCl, the total BAL cells were counted. The percent population of alveolar macrophages and neutrophils was determined by FACS analysis (EPICS ALTRA II, Beckman Coulter, Inc., Fullerton, CA, USA) using anti-mouse MOMA2 antibody (macrophage) or anti-mouse 7/4 antibody (neutrophil), and the cell number was calculated with % population and total BAL cell number. This study was conducted at the Department of Physiology and Anatomy, Nihon University School of Pharmacy (Chiba, Japan).

Chemistry. All starting materials and solvents were obtained either from commercial sources or prepared according to the literature citation. Unless otherwise stated, all reactions were stirred magnetically. Organic solutions were routinely dried over anhydrous magnesium sulfate and filtered. Hydrogenations were performed on a ThalesNano H-cube flow reactor under the conditions stated. In the case of aniline products, these were analyzed by LC-MS and used directly without isolation.

Column chromatography was performed on prepacked silica cartridges (230−400 mesh, 40−63 μm) using the amount indicated. SCX resin was purchased from Supelco and treated with 1 M hydrochloric acid prior to use. Unless stated otherwise, the reaction mixture to be purified was first diluted with MeOH and then made acidic with a few drops of AcOH. The resulting solution was loaded directly onto a column packed with the SCX resin, which was washed with MeOH. The desired material was then eluted with 0.7 M NH_3 in MeOH. All ^1H NMR spectra were acquired on a Bruker Avance III spectrometer at 400 MHz using residual undeuterated solvent as reference and, unless specified otherwise, were run in DMSO-d_6. Test compounds were all >95% pure, as determined by HPLC and ^1H NMR except where indicated otherwise.

Preparative Reverse-Phase High-Performance Liquid Chromatography. Agilent Scalar column C18, 5 μm (21.2 × 50 mm), flow rate 28 mL/min, eluting with a H_2O−MeCN gradient containing 0.1% v/v formic acid over 10 min using UV detection at 215 and 254 nm. Gradient information: 0.0−0.5 min, 95% H_2O−5% MeCN; 0.5−7.0 min, ramped from 95% H_2O−5% MeCN to 5% H_2O−95% MeCN; 7.0−7.9 min, held at 5% H_2O−95% MeCN; 7.9−8.0 min, returned to 95% H_2O−5% MeCN; 8.0−10.0 min, held at 95% H_2O−5% MeCN.

Analytical Reverse-Phase High-Performance Liquid Chromatography-Mass Spectrometry. Agilent Scalar column C18, 5 μm (4.6 × 50 mm), or Waters XBridge C18, 5 μm (4.6 × 50 mm), flow rate 2.5 mL/min, eluting with a H_2O−MeCN gradient containing 0.1% v/v formic acid over 7 min employing UV detection at 215 and 254 nm. Gradient information: 0.0−0.1 min, 95% H_2O−5% MeCN; 0.1−5.0 min, ramped from 95% H_2O−5% MeCN to 5% H_2O−95% MeCN; 5.0−5.5 min, held at 5% H_2O−95% MeCN; 5.5−5.6 min, held at 5% H_2O−95% MeCN, flow rate increased to 3.5 mL/min; 5.6−6.6 min, held at 5% H_2O−95% MeCN, flow rate 3.5 mL/min; 6.6−6.75 min, returned to 95% H_2O−5% MeCN, flow rate 3.5 mL/min; 6.75−6.9 min, held at 95% H_2O−5% MeCN, flow rate 3.5 mL/min; 6.9−7.0 min, held at 95% H_2O−5% MeCN, flow rate reduced to 2.5 mL/min.

***tert*-Butyl 4-(2-Hydroxyethyl)pyridin-2-ylcarbamate (42).** To a solution of ethyl 2-(2-(*tert*-butoxycarbonylamino)pyridin-4-yl)-acetate[41] (10.0 g, 35.7 mmol) under nitrogen in THF (100 mL) at −78 °C was added a solution of DIBAL (1 M in THF, 71 mL, 71 mmol) over 1 h. The reaction mixture was stirred at −78 to −60 °C for 40 min and was then warmed to −15 °C over 1 h. The solution was recooled to −78 °C and was treated with additional DIBAL (1 M in THF, 36 mL, 36 mmol). The mixture was allowed to warm to −40 °C and was stirred for 1 h. Water (10 mL) was added cautiously to quench the reaction followed by $MgSO_4$ (20 g), and the solids were removed by filtration. The filtrate was concentrated to dryness in vacuo, and the residue was purified by flash column chromatography (SiO_2, 330 g, EtOAc in isohexanes, 65%, isocratic elution) to give the title compound (**42**) as a yellow solid (6.00 g, 64%). LC-MS: m/z 239 $(M + H)^+$ (ES^+). ^1H NMR: δ 1.47 (9H, s), 2.70 (2H, t), 3.62 (2H, m), 4.68 (1H, t), 6.88 (1H, dd), 7.67 (1H, m), 8.10 (1H, d), 9.61 (1H, s).

***tert*-Butyl 4-(2-(4-Nitronaphthalen-1-yloxy)ethyl)pyridin-2-ylcarbamate (46a).** To a solution of *tert*-butyl 4-(2-hydroxyethyl)-pyridin-2-ylcarbamate (**42**) (6.00 g, 25.2 mmol) in THF (70 mL) was added sodium hydride (2.52 g, 60 wt % dispersion in mineral oil, 63.0 mmol) at 0 °C. The resulting bright yellow suspension was stirred

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for 20 min at 0 °C, and then 1-fluoro-4-nitronaphthalene (4.81 g, 25.2 mmol) was added in a single portion. The reaction mixture was warmed to rt for 2 h, and then water (100 mL) and EtOAc (100 mL) were added. An insoluble solid formed between the layers and was collected by filtration. The organic phase was separated and washed with saturated aq. $NaHCO_3$ (100 mL) and brine (100 mL) and then dried and evaporated in vacuo. The residue was combined with the collected solid and triturated with MeOH (50 mL) to afford the title compound (**46a**) as a yellow solid (11.9 g, 94%). LC-MS: m/z 410 $(M + H)^+$ (ES^+). ^1H NMR: δ 3.20 (2H, t), 4.55 (2H, t), 7.08 (1H, d), 7.17 (1H, d), 7.63 (1H, m), 7.82 (1H, m), 7.91 (1H, bs), 8.16 (1H, dd), 8.32 (1H, m), 8.44 (1H, d), 8.58 (1H, dt), 9.68 (1H, bs).

***tert*-Butyl 4-(2-(4-Aminonaphthalen-1-yloxy)ethyl)pyridin-2-ylcarbamate (47a).** A suspension of *tert*-butyl 4-(2-(4-nitronaphthalen-1-yloxy)ethyl)pyridin-2-ylcarbamate (**46a**) (5.20 g, 12.7 mmol) and iron mesh (4.30 g, 76.0 mmol) in a mixture of AcOH and EtOH (1:2 v/v, 120 mL) was placed in a preheated oil bath at 60 °C and stirred rapidly for 2 h. The mixture was cooled to rt, poured carefully onto saturated aq. $NaHCO_3$ (1 L), and extracted with EtOAc (2 × 500 mL). The combined organic extracts were washed with saturated aq. $NaHCO_3$ (1 L), water (1 L), and brine (1 L) and then dried and evaporated in vacuo to furnish the title compound (**47a**) as a yellow oil (5.00 g, 95%). LC-MS: m/z 380 $(M + H)^+$ (ES^+). ^1H NMR: δ 1.27 (9H, s), 2.92 (2H, t), 4.05 (2H, t), 4.97 (2H, bs), 6.40 (1H, d), 6.57 (1H, d), 6.86 (1H, dd), 7.18 (2H, overlapping m), 7.69 (1H, bs), 7.81 (2H, overlapping m), 7.96 (1H, d), 9.47 (1H, bs).

4-(2-((4-Nitronaphthalen-1-yl)oxy)ethyl)pyridin-3-amine (46c). To a solution of 4-nitronaphthol (0.95 g, 5.0 mmol), PPh_3 (1.97 g, 7.50 mmol), and 2-(3-aminopyridin-4-yl)ethanol[43] (**45**) (1.04 g, 7.50 mmol) in THF (20 mL) at −15 °C was added DIAD (590 μL, 3.75 mmol) dropwise. The mixture was warmed to rt for 1 h and was then evaporated in vacuo. The residue was taken up in MeOH, evaporated onto silica (20 g), and purified by flash column chromatography (SiO_2, 80 g, EtOAc in isohexane, 50−100%, gradient elution, then 5% MeOH in EtOAc, isocratic elution) to give the title compound (**46c**) as a yellow solid (1.40 g, 88%). LC-MS: m/z 310 $(M + H)^+$ (ES^+). ^1H NMR: δ 3.14 (2H, t), 4.50 (2H, t), 5.31 (2H, bs), 7.10 (1H, d), 7.15 (1H, d), 7.68 (1H, m), 7.72 (1H, d), 7.81 (1H, m), 7.96 (1H, s), 8.32 (1H, dd), 4.43 (1H, d), 8.57 (1H, d).

4-(2-((4-Aminonaphthalen-1-yl)oxy)ethyl)pyridin-3-amine (47c). A solution of 4-(2-((4-nitronaphthalen-1-yl)oxy)ethyl)pyridin-3-amine (**46c**) (700 mg, 2.26 mmol) in a mixture of MeOH (50 mL), EtOAc (25 mL), and DCM (25 mL) was passed through a ThalesNano H-cube (10% Pt/C, 30 mm, CatCart, 1.0 mL min^{-1}, at 40 °C in full hydrogen mode). The volatiles were evaporated in vacuo to give the title compound (**47c**) as a brown solid (612 mg, 95%). LC-MS: m/z 280 $(M + H)^+$ (ES^+). ^1H NMR: δ 3.02 (2H, t), 3.17 (2H, d), 4.21 (2H, t), 5.23 (2H, bs), 6.58 (1H, d), 6.75 (1H, d), 7.08 (1H, d), 7.38−7.41 (2H, overlapping m), 7.72 (1H, d), 7.95 (1H, s), 7.98−8.02 (2H, overlapping m).

Methyl 4-(2-((4-Nitronaphthalen-1-yl)oxy)ethyl)picolinate (46b). To a solution of methyl 4-(2-hydroxyethyl)picolinate[42] (**44**) (1.04 g, 5.74 mmol) in THF (10 mL) at 0 °C were added 4-nitronaphthalen-1-ol (0.72 g, 3.80 mmol), PPh_3 (1.31 g, 4.97 mmol), and DIAD (0.93 mL, 4.80 mmol). The reaction was warmed to rt for 18 h. The reaction mixture was diluted with EtOAc (100 mL), washed with saturated aq. $NaHCO_3$ (2 × 50 mL), and then dried and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, 40 g, EtOAc in isohexane, 10−100%, gradient elution) followed by trituration from MeOH to give the title compound (**46b**) as a yellow solid (0.71 g, 53%). LC-MS: m/z 353 $(M + H)^+$ (ES^+). ^1H NMR δ 3.38 (2H, t), 3.88 (3H, s), 4.65 (2H, t), 7.21 (1H, d), 7.67 (1H, td), 7.71 (1H, dd), 7.83 (1H, m), 8.16 (1H, s), 8.25 (1H, d), 8.45 (1H, d), 8.58 (1H, d), 8.67 (1H, dd).

Methyl 4-(2-((4-Aminonaphthalen-1-yl)oxy)ethyl)picolinate (47b). A solution of methyl 4-(2-((4-nitronaphthalen-1-yl)oxy)ethyl)-picolinate (**46b**) (1.2 g, 3.4 mmol) in a mixture of MeOH (60 mL) and DCM (20 mL) was passed through a ThalesNano H-cube (10% Pt/C 30 mm CatCart, 1.0 mL min^{-1}, at rt, in full hydrogen mode). Evaporation of the volatiles in vacuo gave the title compound (**57b**) as a brown oil (1.10 g, 98%). LC-MS: m/z 323 $(M + H)^+$ (ES^+).

4-(2-((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)-naphthalen-1-yl)oxy)ethyl)picolinic Acid (38). To a solution of CDI (0.85 g, 5.2 mmol) in DCM (1.0 mL) was added a solution of 3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-amine[41] (**34**) (1.20 g, 5.21 mmol) in DCM (2.0 mL) dropwise over 1 h. After an additional 2 h, a solution of methyl 4-(2-((4-aminonaphthalen-1-yl)oxy)ethyl)picolinate (**47b**) (1.12 g, 3.47 mmol) in DCM (3.0 mL) was added over 5 min. After an additional 2 h, the mixture was diluted with MeOH and was evaporated directly onto silica gel. The mixture was purified by flash column chromatography (SiO_2, 80 g, EtOAc in isohexane, 30−100%, gradient elution) to give methyl 4-(2-((4-(3-(3-(*tert*-butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)naphthalen-1-yl)oxy)ethyl)picolinate (**49**) as a chalky solid (1.28 g, 63%). LC-MS: m/z 578 $(M + H)^+$ (ES^+). ^1H NMR δ 1.25 (9H, s), 2.37 (3H, s), 3.30 (2H, t), 3.85 (3H, s), 4.43 (2H, t), 6.33 (1H, s), 6.97 (1H, d), 7.30−7.35 (2H, overlapping m), 7.40−7.45 (2H, overlapping m), 7.47 (1H, m), 7.55 (1H, m), 7.60 (1H, d), 7.70 (1H, dd), 7.87 (1H, d), 8.05 (1H, dd), 8.15 (1H, dd), 8.55 (1H, bs), 8.63 (1H, dd), 8.75 (1H, bs).

To a suspension of methyl ester (550 mg, 0.95 mmol), prepared above, in THF (10 mL) was added an aqueous solution of lithium hydroxide (1M, 1.90 mL, 1.90 mmol). After 15 min, AcOH (1.0 mL) was added and the reaction mixture was extracted into DCM. The combined organic extracts were washed with water and brine and then evaporated in vacuo to give the title compound (**38**) as a white solid (440 mg, 81%). LC-MS: m/z 564 $(M + H)^+$ (ES^+). ^1H NMR: δ 1.25 (9H, s), 2.30 (3H, s), 3.25 (2H, t), 4.37 (2H, t), 6.32 (1H, s), 6.93 (1H, d), 7.28−7.36 (3H, overlapping m), 7.38−7.48 (4H, overlapping m), 7.56 (1H, d), 7.87 (1H, d), 8.01 (1H, s), 8.03 (1H, d), 8.45 (1H, d), 9.17−9.23 (2H, overlapping m).

1-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-3-(4-(2-(2-nitro-1*H*-imidazol-1-yl)ethoxy)naphthalen-1-yl)urea (51). A mixture of 1-(3-(*tert*-butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-3-(4-(2-iodoethoxy)-naphthalen-1-yl)urea[45] (**50**) (200 mg, 0.35 mmol), 2-nitroimidazole (44 mg, 0.39 mmol), and K_2CO_3 (146 mg, 1.06 mmol) in DMF (2.0 mL) was heated at 50 °C for 3 h. The resulting mixture was cooled to rt, poured onto water, and extracted with EtOAc. The combined organic extracts were dried and evaporated in vacuo. The residue was purified by flash column chromatography (SiO_2, 12 g, EtOAc in isohexane, 10−80%, gradient elution) to give the title compound (**51**) as a yellow solid (41 mg, 21%). LC-MS: m/z 554 $(M + H)^+$ (ES^+). ^1H NMR: δ 1.26 (9H, s), 2.37 (3H, s), 4.52 (2H, t), 4.96 (2H, t), 6.33 (1H, s), 6.93 (1H, d), 7.21 (1H, d), 7.32 (2H, m), 7.42 (2H, m), 7.46−7.57 (2H, overlapping m), 7.60 (1H, dd), 7.86−7.90 (2H, overlapping m), 8.07 (1H, dd), 8.55 (1H, bs), 8.75 (1H, bs).

Methyl 4-(((4-Nitronaphthalen-1-yl)oxy)methyl)picolinate (54a). To a mixture of methyl 4-(hydroxymethyl)picolinate (**52**) (374 mg, 2.06 mmol), 4-nitronaphthalen-1-ol (389 mg, 2.06 mmol), and PPh_3 (648 mg, 2.47 mmol) in THF (15 mL) at −50 °C was added DIAD (490 μL, 2.47 mmol) dropwise over 10 min. The resulting mixture was warmed to rt for 1.5 h, and then the volatiles were evaporated in vacuo. The residue was triturated with MeOH (8.0 mL) to give the title compound (**54a**) as a yellow solid (0.60 g, 82%). LC-MS: m/z 339 $(M + H)^+$ (ES^+). ^1H NMR: δ 3.89 (3H, s), 5.65 (2H, s), 7.21 (1H, d), 7.75 (1H, m), 7.83−7.88 (2H, overlapping m), 8.22 (1H, d), 8.44−8.48 (2H, overlapping m), 8.59 (1H, d), 8.77 (1H, d).

Methyl 4-(((4-Aminonaphthalen-1-yl)oxy)methyl)picolinate (56a). A solution of methyl 4-(((4-nitronaphthalen-1-yl)oxy)methyl)-picolinate (**54a**) (796 mg, 2.35 mmol) in a mixture of MeOH (150 mL) and DCM (150 mL) was passed through a ThalesNano H-cube (10% Pt/C, 30 mm, CatCart, 1.0 mL min^{-1}, at rt in full hydrogen mode). The volatiles were evaporated in vacuo to give the title compound (**56a**) as a purple solid (710 mg, 88%, 90% purity). LC-MS: m/z 309 $(M + H)^+$ (ES^+).

Methyl 4-(((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-ureido)naphthalen-1-yl)oxy)methyl)picolinate (57). A solution of 3-(*tert*-butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-amine[41] (**34**) (713 mg, 3.11 mmol) in DCM (3.0 mL) was added dropwise over 1 h to a suspension of CDI (504 mg, 3.11 mmol) in DCM (3.0 mL). The mixture was kept at rt

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for 2 h and was then treated dropwise with a solution of methyl 4-(((4-aminonaphthalen-1-yl)oxy)methyl)picolinate (**56a**) (366 mg, 1.41 mmol) in DCM (4.0 mL). After 30 min at rt, MeOH (20 mL) was added to the reaction mixture, which gave a precipitate. The mixture was reduced in volume to ca. 20 mL by evaporation in vacuo, and the precipitate was collected by filtration and washed with MeOH and diethyl ether to give the title compound (**57**) as a beige solid (0.92 g, 71%, 91% purity). LC-MS: m/z 564 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.27 (9H, s), 2.39 (3H, s), 3.89 (3H, s), 5.47 (2H, s), 6.35 (1H, s), 7.01 (1H, d), 7.35 (2H, m), 7.43 (2H, m), 7.56−7.64 (3H, overlapping m), 7.81 (1H, dd), 7.93 (1H, m), 8.20 (1H, m), 8.31 (1H, m), 8.58 (1H, bs), 8.75 (1H, d), 8.79 (1H, bs).

4-(((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)-naphthalen-1-yl)oxy)methyl)picolinic Acid (39). A suspension of methyl 4-(((4-(3-(3-(*tert*-butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)-naphthalen-1-yl)oxy)methyl)picolinate (**57**) (100 mg, 0.16 mmol) in a mixture of THF (3.0 mL) and water (2.0 mL) was treated with lithium hydroxide (17 mg, 0.71 mmol), and the mixture was maintained at rt for 3 h. Acetic acid (3.0 mL) was added, and the mixture was purified by SCX capture and release. The resultant solid was triturated with diethyl ether to afford the title compound (**39**) as a white solid (55 mg, 62%). LC-MS: m/z 550 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.27 (9H, s), 2.35 (3H, s), 5.40 (3H, s), 6.31 (1H, s), 6.99 (1H, d), 7.31 (2H, m), 7.45 (2H, m), 7.53−7.60 (3H, overlapping m), 7.95 (1H, m), 8.05 (1H, s), 8.27 (1H, m), 8.55 (1H, d), 9.06 (1H, bs), 9.18 (1H, bs).

2-Amino-4-((4-nitronaphthalen-1-yloxy)methyl)pyridine (54b). To a solution of 4-nitronaphthol (5.17 g, 27.3 mmol), PPh_3 (10.8 g, 41.0 mmol), and 2-aminopyridine-4-methanol (**53**) (5.09 g, 41.0 mmol) in THF (50 mL) at −15 °C was added DIAD (8.07 mL, 41.0 mmol) dropwise. The reaction mixture was warmed to rt for 18 h and was then evaporated in vacuo. The residue was triturated with EtOAc (150 mL), and the solids were collected by filtration and washed with EtOAc (100 mL). A second trituration with MeOH (100 mL) gave the title compound (**54b**) (4.54 g, 56%) as a yellow solid. LC-MS: m/z 296 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 5.35 (2H, s), 6.00 (2H, bs), 6.60 (2H, overlapping m), 7.15 (1H, d), 7.72 (1H, m), 7.85 (1H, m), 7.97 (1H, dd), 8.42 (2H, d), 8.60 (1H, d).

2-Amino-4-((4-aminonaphthalen-1-yloxy)methyl)pyridine (56b). A solution of 2-amino-4-((4-nitronaphthalen-1-yloxy)methyl)-pyridine (**54b**) (4.50 g, 15.24 mmol) in a mixture of MeOH (200 mL) and AcOH (200 mL) was passed through a ThalesNano H-cube (2.0 mL min^{-1}, 40 °C, 55 mm, 10% Pt/C Cat-Cart, full hydrogen mode). The volatiles were removed in vacuo, and the crude product was subjected to SCX capture and release to give the title compound (**56b**) as a purple solid (3.82 g, 94%). LC-MS: m/z 266 $(M + H)^+$ (ES^+).

1-(4-((2-Aminopyridin-4-yl)methoxy)naphthalen-1-yl)-3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)urea (37). To a solution of CDI (4.18 g, 25.8 mmol) in DCM (15 mL) was added a solution of 3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-amine[41] (**34**) (5.91 g, 25.8 mmol) in DCM (15 mL) dropwise over 40 min. The resulting solution was stirred at rt for 1 h and was then added dropwise to a solution of 2-amino-4-((4-aminonaphthalen-1-yloxy)methyl)pyridine (**56b**) (3.80 g, 12.9 mmol) in DCM (25 mL). The mixture was maintained at rt for 18 h. The volatiles were removed in vacuo, and the crude product was purified by flash column chromatography (SiO_2, 120 g, MeOH in EtOAc, 0−6%, gradient elution) to give the title compound (**37**) as an off-white solid (4.27 g, 63%). LC-MS: m/z 521 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.24 (9H, s), 2.38 (3H, s), 5.17 (2H, s), 5.95 (2H, bs), 6.35 (1H, s), 6.58−6.63 (2H, overlapping), 6.97 (1H, d), 7.35 (2H, m), 7.43 (2H, m), 7.54−7.63 (3H, overlapping m), 7.88−7.93 (2H, overlapping m), 8.30 (1H, dd), 8.57 (1H, bs), 8.77 (1H, bs).

***N*-(4-((4-(3-(3-*tert*-Butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)ureido)-naphthalen-1-yloxy)methyl) pyridin-2-yl)-2-chloroacetamide (36).** To a solution of 1-(4-((2-aminopyridin-4-yl)methoxy)naphthalen-1-yl)-3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)urea (**37**) (2.00 g, 3.84 mmol) in a mixture of DCM (40 mL) and DMF (8.0 mL) were added DIPEA (1.37 mL, 7.68 mmol) and chloroacetyl chloride (0.61 mL, 7.70 mmol). The reaction mixture was maintained at rt for 1 h and was then treated with a second aliquot of chloracetyl chloride (100 μL, 1.25 mmol). After 1 h at rt, the resulting mixture was partitioned between DCM (40 mL) and saturated aq. $NaHCO_3$ (40 mL). The organic phase was separated and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, 80 g, MeOH in DCM, 0−10%, gradient elution). The crude product so obtained was triturated with a mixture of diethyl ether (20 mL) and isohexane (20 mL) to afford the title compound (**36**) as a light purple solid (1.07 g, 42%). LC-MS: m/z 597, 599 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.25 (9H, s), 2.38 (3H, s), 4.35 (2H, s), 5.38 (2H, s), 6.35 (1H, s), 7.00 (1H, d), 7.29 (1H, dd), 7.35 (2H, m), 7.43 (2H, m), 7.55−7.63 (3H, overlapping m), 7.92 (1H, m), 8.28−8.34 (2H, overlapping m), 8.36 (1H, dd), 8.57 (1H, bs), 8.78 (1H, bs), 10.85 (1H, bs).

2-Methoxy-*N*-(4-(((4-nitronaphthalen-1-yl)oxy)methyl)-pyridin-2-yl)acetamide (55a). To a suspension of 2-amino-4-((4-nitronaphthalen-1-yloxy)methyl)pyridine (**54b**) (20.0 g, 68.0 mmol) in a mixture of DCM (100 mL) and THF (150 mL) at −5 °C was added DIPEA (17.7 mL, 102 mmol) followed by methoxyacetyl chloride (8.1 mL, 88 mmol) dropwise over 10 min. After 10 min, the reaction mixture was warmed to rt for an additional 30 min and was then treated with methanolic NH_3 (7 M, 30 mL). After 1 h, the volatiles were removed by evaporation in vacuo, and the residue was triturated with water (500 mL). The solids were collected by filtration and washed with water (300 mL) and diethyl ether (500 mL) to give the title compound (**55a**) as a yellow solid (24.0 g, 95%). LC-MS: m/z 368 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 3.37 (3H, s), 4.07 (2H, s), 5.57 (2H, s), 7.21 (1H, s), 7.30 (2H, dd), 7.75 (1H, m), 7.85 (1H, m), 8.32 (1H, s), 8.36 (1H, d), 8.44−8.47 (2H, overlapping m), 8.59 (1H, d), 10.10 (1H, bs).

2-Methoxy-*N*-methyl-*N*-(4-(((4-nitronaphthalen-1-yl)oxy)-methyl)pyridin-2-yl)acetamide (55b). To a solution of 2-methoxy-*N*-(4-(((4-nitronaphthalen-1-yl)oxy)methyl)pyridin-2-yl)acetamide (**55a**) (300 mg, 0.82 mmol) in THF at 0 °C was added sodium hydride (74 mg, 60 wt % dispersion in mineral oil, 1.20 mmol). After 30 min at rt, the mixture was treated with iodomethane (76 μL, 1.22 mmol), kept at rt for 1 h, and then heated at 50 °C. An additional aliquot of iodomethane (380 μL, 6.10 mmol) was added. After 1 h at 50 °C, the reaction mixture was cooled to rt and was poured into water. The mixture was extracted with EtOAc. The organic layer was separated and evaporated in vacuo. The residue was purified by flash column chromatography (SiO_2, 40 g, EtOAc in isohexane, 0−100%, gradient elution then MeOH in EtOAc, 0−10%, gradient elution) to furnish the title compound (**55b**) as a yellow solid (155 mg, 49%). LC-MS: m/z 382 $(M + H)^+$ (ES^+). ^{1}H NMR ($CDCl_3$) δ: 3.40 (3H, s), 3.45 (3H, s), 4.24 (2H, s), 5.40 (2H, s), 6.85 (2H, d), 7.32 (1H, dd), 7.58 (1H, bs), 7.67 (1H, m), 7.80 (1H, m), 8.37 (1H, d), 8.46 (1H, dq), 8.51 (1H, dd), 8.78 (1H, d).

***N*-(4-(((4-Aminonaphthalen-1-yl)oxy)methyl)pyridin-2-yl)-2-methoxy-*N*-methylacetamide (35).** The title compound was obtained in a similar manner as that for **56b** to provide **37** as a purple solid (130 mg, 90%). LC-MS: m/z 352 $(M + H)^+$ (ES^+).

1-(2-Aminopyridin-4-yl)ethanone (58). To a stirred solution of methyl-2-aminopyridine-4-carboxylate (1.00 g, 6.57 mmol) in THF (100 mL) at −78 °C under nitrogen was added methyllithium (1.6 M in diethyl ether, 16.4 mL, 26.3 mmol) over 10 min. After a further 30 min at −78 °C, the viscous reaction mixture was warmed to 0 °C. After a further 3 h, the reaction was quenched at 0 °C by the cautious addition of isopropanol (8.0 mL). The mixture was warmed to rt. Brine (200 mL) and EtOAc (150 mL) were added, and the layers were separated. The aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic extracts were dried, and the solvents were removed in vacuo. The crude residue was purified by column chromatography (SiO_2, 80 g, MeOH in EtOAc, 0−8%, gradient elution) to give 1-(2-aminopyridin-4-yl)ethanone (**58**) as a yellow powder (176 mg, 20%). LC-MS: m/z 137 $(M + H)^+$ (ES^+). ^{1}H NMR (MeOH-d_4): δ 2.54 (3H, s), 7.01 (1H, dd), 7.03 (1H, m), 8.03 (1H, dd).

2-(2-Aminopyridin-4-yl)propan-2-ol (59a). To a stirred solution of methyl-2-aminopyridine-4-carboxylate (2.00 g, 13.0 mmol) in THF (200 mL) at −78 °C under nitrogen was added methyllithium (1.6 M in diethyl ether, 33 mL, 53.0 mmol) over 10 min. After 30 min, the viscous reaction mixture was warmed to 0 °C for 3.5 h and was quenched at 0 °C by the cautious addition of isopropanol (15 mL). The mixture was warmed to rt, and brine (400 mL) and EtOAc (300 mL) were added. The aqueous layer was separated and extracted with EtOAc (3 × 200 mL).

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The combined organic extracts were evaporated in vacuo. The crude residue was purified by flash column chromatography (SiO_2, 120 g, MeOH in EtOAc, 0−10%, gradient elution) to afford the title compound (**59a**) (1.27 g, 63%) as a yellow amorphous solid. ^{1}H NMR (MeOH-d_4): δ 1.47 (6H, s), 6.68−6.71 (2H, overlapping m), 7.80−7.81 (1H, dd).

4-(2-(4-Nitronaphthalen-1-yloxy)propan-2-yl)pyridin-2-amine (60a). To a stirred solution of 2-(2-aminopyridin-4-yl)propan-2-ol (**59a**) (1.55 g, 10.0 mmol) in DMF (30 mL) under nitrogen at 0 °C was added sodium hydride (60 wt %, 0.61 g, 15.0 mmol). The resulting mixture was stirred at 0 °C for 5 min. A solution of 1-fluoro-4-nitronaphthalene (1.95 g, 10.0 mmol) in DMF (30 mL) was added dropwise, and the resulting dark-red mixture was stirred at 0 °C for a further 5 min and then at rt for 2 h. The reaction mixture was quenched by the addition of a saturated aq. NH_4Cl solution (10 mL). Water (150 mL) and EtOAc (150 mL) were added, and the aqueous layer was separated and extracted with EtOAc (3 × 100 mL). The combined organic extracts were washed with brine, dried, and evaporated in vacuo. The crude residue was purified by column chromatography (SiO_2, 80 g, EtOAc in isohexane, 0−60%, gradient elution) to afford the title compound (**60a**) (282 mg, 8%) as a red oil. LC-MS m/z 324 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.52 (6H, s), 5.92 (2H, br s), 6.45 (1H, s), 6.55 (1H, dd), 6.93 (1H, d), 7.75 (1H, m), 7.84−7.90 (2H, overlapping m), 8.35 (1H, d), 8.49 (1H, dd), 8.58 (1H, d).

4-(2-(4-Aminonaphthalen-1-yloxy)propan-2-yl)pyridin-2-amine (61a). A solution of 4-(2-(4-nitronaphthalen-1-yloxy)propan-2-yl)pyridin-2-amine (**60a**) (282 mg, 0.87 mmol) in MeOH (45 mL) was passed through a ThalesNano H-cube (1.0 mL min^{-1}, 30 °C, 55 mm 10% Pt/C Cat-Cart, full hydrogen mode). The reaction mixture was evaporated in vacuo to afford the title compound (**61a**) (253 mg, 89%) as a brown foam. LC-MS m/z 294 $(M + H)^+$ (ES^+).

1-(4-(2-(2-Aminopyridin-4-yl)propan-2-yloxy)naphthalen-1-yl)-3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)urea (40). A solution of 3-(*tert*-butyl)-5-isocyanato-1-(*p*-tolyl)-1*H*-pyrazole (see compound **10**) (447 mg, 1.75 mmol) in THF (15 mL) was added to a solution of 4-(2-(4-aminonaphthalen-1-yloxy)propan-2-yl)pyridin-2-amine (**61a**) (253 mg, 0.86 mmol) in THF (2.0 mL). DIPEA (451 μL, 2.59 mmol) was added, and the reaction mixture was stirred at rt for 2 h. Water (30 mL) and EtOAc (20 mL) were added, and the aqueous layer was separated and extracted with EtOAc (3 × 15 mL). The combined organic layers were washed with brine (40 mL) and evaporated in vacuo. The residue was purified by flash column chromatography (SiO_2, 40 g, MeOH in DCM, 0−10%, gradient elution) to afford the title compound (**40**) (249 mg, 51%) as a purple amorphous solid. LC-MS: m/z 549 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.27 (9H, s), 1.37 (6H, s), 2.38 (3H, s), 6.37 (1H, s), 6.97 (1H, dd), 7.36 (2H, m), 7.45 (2H, m), 7.47−7.55 (2H, overlapping m), 7.72 (2H, m), 7.92 (1H, d), 7.97 (1H, dd), 8.12 (1H, dd), 8.66−8.71 (2H, overlapping m), 8.93 (1H, bs).

1-(2-Aminopyridin-4-yl)ethanol (59b). To a mixture of 1-(2-aminopyridin-4-yl)ethanone (**58**) (168 mg, 1.23 mmol) in MeOH (10 mL) under nitrogen at 0 °C was added sodium borohydride (47 mg, 1.23 mmol). The resulting reaction mixture was stirred at rt for 2 h, and then the solvents were removed in vacuo. The residue was taken up into EtOAc (25 mL) and washed with a saturated aq. $NaHCO_3$ solution (30 mL), and the layers were separated. The aqueous layer was extracted with EtOAc (2 × 20 mL). The combined organic extracts were washed with brine (30 mL) and dried, and the solvents were removed in vacuo to give 1-(2-aminopyridin-4-yl)ethanol (**59b**) (77 mg, 45%) as a yellow oil. LC-MS: m/z 139 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.23 (3H, d), 4.50 (1H, m), 5.13 (1H, d), 5.75 (2H, br s), 6.40−6.43 (2H, overlapping m), 7.78 (1H, d).

4-(1-(4-Nitronaphthalen-1-yloxy)ethyl)pyridin-2-amine (60b). To a stirred solution of 1-(2-aminopyridin-4-yl)ethanol (**59b**) (73 mg, 0.53 mmol) in DMF (1.5 mL) under nitrogen at 0 °C was added sodium hydride (32 mg, 0.79 mmol, 60 wt % in mineral oil). The resulting mixture was stirred at 0 °C for 40 min before a solution of 1-fluoro-4-nitronaphthalene (101 mg, 0.53 mmol) in DMF (1.5 mL) was added dropwise. The resulting dark-red mixture was stirred at 0 °C for a further 5 min and warmed to rt. After a further 40 min, the reaction was quenched by the addition of a saturated aq. NH_4Cl solution (1.0 mL). Water (20 mL) and EtOAc (20 mL) were added, and the aqueous layer was separated and extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with brine and dried, and the solvents were removed in vacuo. The crude material was purified by column chromatography (SiO_2, 12 g, EtOAc in isohexane, 0−80%, gradient elution) to give 4-(1-(4-nitronaphthalen-1-yloxy)ethyl)-pyridin-2-amine (**60b**) (94.6 mg, 57%) as an orange gum. LC-MS: m/z 310 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.69 (3H, d), 5.75 (1H, q), 5.93 (2H, bs), 6.45 (1H, s), 6.55 (1H, dd), 6.93 (1H, d), 7.75 (1H, m), 7.84−7.89 (2H, overlapping m), 8.35 (1H, d), 8.50 (1H, dd), 8.58 (1H, d).

4-(1-(4-Aminonaphthalen-1-yloxy)ethyl)pyridin-2-amine (61b). 4-(1-(4-Nitronaphthalen-1-yloxy)ethyl)pyridin-2-amine (**60b**) (91 mg, 0.29 mmol) in MeOH (15 mL) and AcOH (3.0 mL) was passed through a ThalesNano H-cube (1.0 mL min^{-1}, 30 °C, 55 mm 10% Pt/C Cat-Cart, full hydrogen mode). The volatiles were removed in vacuo, and the residue was subjected to SCX capture and release to give 4-(1-(4-aminonaphthalen-1-yloxy)ethyl)pyridin-2-amine (**61b**) (81 mg, 99%) as a purple oil. LC-MS: m/z 280 $(M + H)^+$ (ES^+).

1-(4-(1-(2-Aminopyridin-4-yl)ethoxy)naphthalen-1-yl)-3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)urea (41). The title compound was prepared in a similar manner as that for **40** from 4-(1-(4-aminonaphthalen-1-yloxy)ethyl)pyridin-2-amine (**61b**) to give **41** as a beige powder (63 mg, 38%). LC-MS: m/z 535 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.24 (9H, s), 1.63 (3H, d), 2.37 (3H, s), 5.50 (1H, q), 5.80 (2H, bs), 6.31 (1H, s), 6.48 (1H, s), 6.55 (1H, dd), 6.75 (1H, d), 7.34 (2H, m), 7.42 (2H, m), 7.47 (1H, d), 7.55 (1H, m), 7.82 (1H, dd), 7.87 (1H, m), 8.32 (1H, m), 8.60 (1H, bs), 8.76 (1H, bs).

1-(4-(2-(2-Aminopyridin-4-yl)ethoxy)naphthalen-1-yl)-3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)urea (9). To a suspension of CDI (3.00 g, 18.2 mmol) in DCM (15 mL) was added a solution of 3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-amine[41] (**34**) (4.17 g, 18.2 mmol) in DCM (40 mL) over 1.5 h. After 2 h at rt, a solution of *tert*-butyl 4-(2-(4-aminonaphthalen-1-yloxy)ethyl)pyridin-2-ylcarbamate (**48a**) (3.00 g, 7.91 mmol) in DCM (15 mL) was added. After 18 h, the solution was diluted with MeOH (10 mL), evaporated onto silica gel (30 g), and subjected to column chromatography (SiO_2, 330 g, EtOAc in isohexane, 30−100%, gradient elution then MeOH in EtOAc, 0−6%, gradient elution) to give *tert*-butyl (4-(2-((4-(3-(3-(*tert*-butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)naphthalen-1-yl)oxy)ethyl)pyridin-2-yl)carbamate as a beige solid (4.2 g, 80%).

A portion of the above material (1.35 g, 2.20 mmol) was suspended in DCM (10 mL) and was treated with TFA (10 mL). The reaction mixture was kept at rt for 2 h and was then evaporated in vacuo. The residue was taken up in EtOAc (50 mL), washed with saturated aq. $NaHCO_3$ (50 mL) and brine (50 mL), and then dried and evaporated in vacuo to give the title compound (**9**) as a pale pink solid (1.20 g, 80% over two steps). LC-MS: m/z 535 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.26 (9H, s), 2.37 (3H, s), 3.00 (2H, t), 4.32 (2H, t), 5.80 (2H, s), 6.34 (1H, s), 6.45 (1H, s), 6.52 (1H, dd), 6.95 (1H, d), 7.32−7.36 (2H, overlapping m), 7.40−7.45 (2H, overlapping m), 7.50−7.55 (2H, overlapping m), 7.60 (1H, d), 7.81 (1H, dd), 7.87 (1H, m), 8.11 (1H, dd), 8.54 (1H, s), 8.74 (1H, s).

1-(4-(2-(3-Aminopyridin-4-yl)ethoxy)naphthalen-1-yl)-3-(3-(*tert*-butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)urea (10). To a solution of 3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-amine[41] (**34**) (1.00 g, 4.36 mmol) in DCM (90 mL) was added a saturated aq. $NaHCO_3$ solution (60 mL). The heterogeneous mixture was stirred vigorously at 0 °C, treated with diphosgene (2.10 mL, 17.4 mmol) in a single portion, and then warmed to rt for 1 h. The organic layer was separated, dried, and evaporated in vacuo to give a brown oil, which was triturated from isohexane (5.0 mL) and filtered. The filtrate was concentrated in vacuo to give 3-*tert*-butyl-5-isocyanato-1-*p*-tolyl-1*H*-pyrazole as a light brown oil (1.00 g, 3.92 mmol, 90%). LC-MS: m/z 288 (in MeOH) $(M + H+MeOH)^+$ (ES^+).

A solution of 3-*tert*-butyl-5-isocyanato-1-*p*-tolyl-1*H*-pyrazole (530 mg, 2.10 mmol) in THF (2.0 mL) was added to a solution of 4-(2-(4-aminonaphthalen-1-yloxy)ethyl)pyridin-3-amine (**47c**) (580 mg, 2.10 mmol) and DIPEA (1.1 mL, 6.20 mmol) in a mixture of THF (10 mL) and MeCN (1.0 mL). The reaction mixture was stirred at rt for 18 h, poured into brine (25 mL), and extracted with EtOAc (2 × 25 mL). The combined organics were dried, and the volatiles were removed in vacuo. The residue was taken up in MeOH, evaporated onto Hyflo Super Cel

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(10 g), and purified by reverse-phase flash column chromatography (C18 reverse-phase silica gel, 40 g, MeCN in H_2O, 0−100%, gradient elution) to afford the title compound (**10**) as an off-white solid (410 mg, 36%). LC-MS: m/z 535 $(M + H)^+$ (ES^+). ^1H NMR: δ 1.26 (9H, s), 2.38 (3H, s), 3.06 (2H, t), 4.34 (2H, t), 5.25 (2H, bs), 6.34 (1H, s), 6.95 (1H, d), 7.07 (1H, d), 7.35 (2H, m), 7.42 (2H, m), 7.45−7.57 (2H, overlapping m), 7.60 (1H, d), 7.71 (1H, dd), 7.87 (2H, d), 7.95 (1H, s), 8.15 (1H, dd), 8.55 (1H, bs), 8.75 (1H, bs).

***N*-(4-(2-((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-ureido)naphthalen-1-yl)oxy)ethyl)pyridin-2-yl)acetamide (11).** The title compound was prepared using the process described to make **14** by replacing methoxyacetyl chloride with acetyl chloride to give **11** as a white solid (41 mg, 77%). LC-MS: m/z 577 $(M + H)^+$ (ES^+). ^1H NMR: δ 1.26 (9H, s), 2.08 (3H, m), 2.38 (3H, s), 3.20 (2H, t), 4.37 (2H, t), 6.34 (1H, s), 6.95 (1H, d), 7.15 (1H, dd), 7.35 (2H, m), 7.40−7.50 (3H, overlapping m), 7.55 (1H, m), 7.60 (1H, m), 7.90 (1H, d), 8.15−8.23 (3H, overlapping m), 8.57 (1H, s), 8.77 (1H, bs), 10.40 (1H, s).

***N*-(4-(2-((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-ureido)naphthalen-1-yl)oxy)ethyl)pyridin-3-yl)acetamide (12).** The title compound was prepared in the same manner as that for **14** from 1-(4-(2-(3-aminopyridin-4-yl)ethoxy)naphthalen-1-yl)-3-(3-(*tert*-butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)urea (**10**) as the amine substrate by reaction with acetyl chloride to provide **12** as a white solid (20 mg, 46%). LC-MS: m/z 577 $(M + H)^+$ (ES^+). ^1H NMR: δ 1.24 (9H, s), 2.10 (3H, s), 2.38 (3H, s), 3.20 (2H, t), 4.38 (2H, t), 6.34 (1H, s), 6.95 (1H, d), 7.35 (2H, m), 7.42 (2H, m), 7.45−7.50 (2H, overlapping m), 7.55 (1H, m), 7.61 (1H, d), 7.87 (1H, d), 8.07 (1H, dd), 8.31 (1H, d), 8.52−8.56 (2H, overlapping m), 8.75 (1H, bs).

***N*-(4-(2-((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-ureido)naphthalen-1-yl)oxy)ethyl) pyridin-3-yl)-2-methoxyacetamide (13).** The title compound was prepared in the same manner as that for **12** from **10** by reaction with methoxyacetyl chloride to provide **13** as a pale pink solid (45 mg, 77%). LC-MS: m/z 607 $(M + H)^+$ (ES^+). ^1H NMR: δ 1.25 (9H, s), 2.38 (3H, s), 3.16 (3H, s), 4.07 (2H, s), 4.37 (2H, t), 6.34 (1H, s), 6.95 (2H, s), 7.33 (2H, m), 7.43 (2H, m), 7.46−7.55 (3H, overlapping m), 7.60 (1H, d), 7.88 (1H, d), 8.07 (1H, dd), 8.35 (1H, d), 8.51 (1H, s), 8.55 (1H, bs), 8.75 (1H, bs), 9.65 (1H, bs).

***N*-(4-(2-(4-(3-(3-*tert*-Butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)ureido)-naphthalen-1-yloxy)ethyl)pyridin-2-yl)-2-methoxyacetamide (14).** To a suspension of 1-(4-(2-(2-aminopyridin-4-yl)ethoxy)-naphthalen-1-yl)-3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)urea (**9**) (35 mg, 0.07 mmol) in DCM (0.5 mL) were added DIPEA (23 μL, 0.13 mmol) and methoxyacetyl chloride (7.0 μL, 0.07 mmol). The mixture was maintained at rt for 2 h and was then diluted with saturated aq. $NaHCO_3$ (1.5 mL). The organic layer was collected and evaporated in vacuo, and the residue was subjected to SCX capture and release. The resulting crude product was purified by preparative RP HPLC to give the title compound (**14**) as a white solid (5 mg, 13%). LC-MS: m/z 607 $(M + H)^+$ (ES^+). ^1H NMR: δ 1.26 (9H, s), 2.37 (3H, s), 3.20 (2H, t), 3.37 (3H, s), 4.06 (2H, s), 4.38 (2H, t), 6.33 (1H, s), 6.95 (1H, d), 7.19 (1H, dd), 7.33 (2H, m), 7.42−7.47 (3H, overlapping m), 7.54 (1H, m), 7.59 (1H, d), 7.87 (1H, d), 8.12 (1H, d), 8.18 (1H, bs), 8.23 (1H, d), 8.67 (1H, s), 8.84 (1H, s), 9.89 (1H, s).

4-(2-((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)-naphthalen-1-yl)oxy)ethyl)-*N*-methylpicolinamide (15). To a mixture of 4-(2-((4-(3-(3-(*tert*-butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)-naphthalen-1-yl)oxy)ethyl)picolinic acid (**38**) (100 mg, 0.18 mmol), HOBt (36 mg, 0.27 mmol), and EDC (51 mg, 0.27 mmol) in THF was added methylamine (2 M in THF, 890 μL, 1.8 mmol). The reaction was maintained at 45 °C for 2 h and then at rt for 18 h. The resulting mixture was diluted with saturated aq. $NaHCO_3$ and extracted with DCM. The organic extracts were evaporated in vacuo, and the residue was purified by flash column chromatography (SiO_2, 12 g, MeOH in DCM, 0−5%, gradient elution) to provide the title compound (**16**) (24 mg, 24%). LC-MS: m/z 577 $(M + H)^+$ (ES^+). ^1H NMR: δ 1.25 (9H, s), 2.37 (3H, s), 2.80 (3H, d), 3.30 (2H, obscured by DOH), 4.41 (2H, t), 6.32 (1H, s), 6.97 (1H, d), 7.32 (2H, m), 7.42 (2H, m), 7.46 (1H, m), 7.54 (1H, m), 7.60 (2H, d), 7.63 (1H, dd), 7.87 (1H, d), 8.05 (1H, dd), 8.10 (1H, d), 8.53−8.57 (2H, overlapping m), 8.70−8.76 (2H, overlapping m).

4-(2-((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)-naphthalen-1-yl)oxy)ethyl)-*N*-(2-methoxyethyl)picolinamide (16). The title compound was prepared by a similar procedure as that used for **15** by substituting methylamine with 2-methoxyethanamine to give **16** (58 mg, 53%). LC-MS: m/z 621 $(M + H)^+$ (ES^+). ^1H NMR: δ 1.25 (9H, s), 2.38 (3H, s), 3.25 (3H, s), 3.30 (2H, obscured by DOH), 3.45−3.48 (4H, overlapping m), 4.42 (2H, t), 6.33 (1H, s), 6.97 (1H, d), 7.33 (2H, m), 7.42 (2H, m), 7.45 (1H, m), 7.53 (1H, m), 7.60 (1H, d), 7.65 (1H, dd), 7.86 (1H, d), 8.05 (1H, dd), 8.12 (1H, d), 8.52−8.58 (2H, overlapping m), 8.65 (1H, m), 8.75 (1H, bs).

***N*-(1-(2-((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-ureido)naphthalen-1-yl)oxy)ethyl)-1H-imidazol-2-yl)-acetamide (17).** To a suspension of 1-(3-(*tert*-butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-3-(4-(2-(2-nitro-1*H*-imidazol-1-yl)ethoxy)naphthalen-1-yl)urea (**51**) (80 mg, 0.15 mmol) in a mixture of EtOH and AcOH (2:1v/v, 3.0 mL) was added iron mesh (48 mg, 0.87 mmol). The mixture was heated at 60 °C for 1 h, cooled to rt, and adjusted to pH 9 by the addition of a saturated aq. Na_2CO_3 solution, which resulted in the formation of a thick suspension. The volatiles were evaporated in vacuo, and the residue was subjected to SCX capture and release to afford a crude sample of 1-(4-(2-(2-amino-1*H*-imidazol-1-yl)ethoxy)naphthalen-1-yl)-3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)urea (80 mg). A portion of this material (53 mg, ∼0.10 mmol) in DCM (2.0 mL) was treated with DIPEA (35 μL, 0.20 mmol) and acetyl chloride (8 μL, 0.11 mmol). After 18 h at rt, the mixture was diluted with a saturated aq. $NaHCO_3$ solution (1.0 mL) and the layers were separated. The organic layer was evaporated in vacuo, and the residue was purified by flash column chromatography (SiO_2, 12 g, MeOH in EtOAc, 0−5%, gradient elution). The material so obtained was triturated from a mixture of diethyl ether and isohexane and was then subjected to SCX capture and release to give the title compound (**17**) as pale yellow solid (7 mg, 9%, 87% purity). LC-MS: m/z 566 $(M + H)^+$ (ES^+). ^1H NMR: δ 1.24 (9H, s), 2.05 (3H, s), 2.37 (3H, s), 4.25 (2H, m), 4.35 (2H, t), 6.35 (1H, s), 6.79 (1H, s), 6.90 (1H, d), 7.29−7.39 (3H, overlapping m), 7.42 (2H, m), 7.50−7.60 (2H, overlapping m), 7.61 (1H, d), 7.88 (1H, d), 8.15 (1H, d), 8.55 (1H, bs), 8.76 (1H, bs), 10.05 (1H, bs).

4-(((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)-naphthalen-1-yl)oxy)methyl)-*N*-(2-methoxyethyl)picolinamide (18). A mixture of 4-(((4-(3-(3-(*tert*-butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)naphthalen-1-yl)oxy) ethyl)picolinic acid (**39**) (35 mg, 0.06 mmol), 2-methoxyethylamine (6.0 μL, 0.07 mmol), DMAP (7.8 mg, 0.06 mmol), and EDC·HCl (16 mg, 0.08 mmol) in a mixture of DCM (1.0 mL) and DMF (1.0 mL) was kept at rt for 72 h. The neat reaction mixture was purified by flash column chromatography (SiO_2, 12 g, MeOH in DCM, 0−20%, gradient elution). The crude product so obtained was purified further by SCX capture and release to afford the title compound (**18**) as a light brown solid (7 mg, 17%). LC-MS: m/z 607 $(M + H)^+$ (ES^+). ^1H NMR: δ 1.25 (9H, s), 2.37 (3H, s), 3.27 (3H, s), 3.47 (4H, overlapping m), 5.48 (2H, s), 6.35 (1H, s), 7.00 (1H, s), 7.35 (2H, m), 7.43 (2H, m), 7.57−7.63 (3H, overlapping m), 7.75 (1H, dd), 7.92 (1H, m), 8.18 (1H, d), 8.31 (1H, m), 8.58 (1H, bs), 8.67 (1H, d), 8.72 (1H, m), 8.78 (1H, bs).

4-(((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)-naphthalen-1-yl)oxy)methyl)-*N*-(2-morpholinoethyl)-picolinamide (19). The title compound was prepared in the same manner as that described for **18** by replacing 2-methoxyethanamine with 2-morpholinoethanamine to furnish **19** as an off-white solid (10 mg, 16%). LC-MS: m/z 662 $(M + H)^+$ (ES^+). ^1H NMR: δ 1.27 (9H, s), 2.35−2.45 (7H, overlapping s), 2.45 (2H, obscured by DOH), 3.45 (2H, t), 3.55 (4H, t), 5.44 (2H, s), 6.35 (1H, s), 7.00 (1H, d), 7.35 (2H, m), 7.43 (2H, m), 7.58−7.62 (3H, overlapping, m), 7.75 (1H, dd), 7.93 (1H, m), 8.17 (1H, d), 8.31 (1H, m), 8.58 (1H, bs), 8.67 (1H, dd), 8.74 (1H, t), 8.79 (1H, bs).

Methyl (4-(((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-ureido)naphthalen-1-yl)oxy)ethyl)pyridin-2-yl)carbamate (20). To a solution of 1-(4-((2-aminopyridin-4-yl)methoxy)naphthalen-1-yl)-3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)urea (**37**) (40 mg, 0.08 mmol) in a mixture of DCM (0.9 mL) and DMF (0.1 mL) were added DIPEA (15 μL, 0.09 mmol) and methyl chloroformate (6.5 μL, 0.09 mmol). After 1 h, an additional aliquot of methyl chloroformate (6.5 μL, 0.09 mmol) was added. The mixture was maintained at rt for 1 h and was

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then evaporated in vacuo. The residue was triturated with methanol (2.0 mL). The resulting solid was collected by filtration and washed with MeOH (1.0 mL) and diethyl ether (2.0 mL) to give the title compound (**20**) as a beige powder (15 mg, 33%). LC-MS: m/z 579 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.25 (9H, s), 2.38 (3H, s), 3.67 (3H, s), 5.35 (2H, s), 6.35 (1H, s), 6.99 (1H, d), 7.19 (1H, dd), 7.35 (2H, m), 7.43 (2H, m), 7.56–7.63 (2H, overlapping m), 7.92 (1H, m), 8.05 (1H, s), 8.26–8.32 (2H, overlapping m), 8.58 (1H, bs), 8.79 (1H, bs), 10.25 (1H, bs).

***N*-(4-(((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)naphthalen-1-yl)oxy)methyl)yridin-2-yl)-methanesulfonamide (21).** To a solution of 1-(4-((2-aminopyridin-4-yl)methoxy)naphthalen-1-yl)-3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)urea (**39**) (100 mg, 0.19 mmol) in pyridine (1.0 mL) was added methanesulfonyl chloride (59 μL, 0.76 mmol), and the resulting yellow solution was kept at rt for 72 h. The mixture was treated with 1% NH_3 in MeOH (2.0 mL), and after 30 min, it was preabsorbed onto silica gel and purified by flash column chromatography (SiO_2, 12 g, MeOH in DCM, 0–5%, gradient elution) to furnish the title compound (**23**) as a light orange solid (49 mg, 50%). LC-MS: m/z 599 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.25 (9H, s), 2.39 (3H, s), 3.25 (3H, s), 5.32 (2H, s), 6.35 (1H, s), 7.00 (1H, d), 7.10 (1H, m), 7.21 (1H, m), 7.35 (2H, m), 7.43 (2H, m), 7.55–7.63 (3H, overlapping m), 7.92 (1H, dd), 8.21 (1H, m), 8.31 (1H, dd), 8.57 (1H, bs), 8.79 (1H, bs), 10.80 (1H, bs).

***N*-(4-(((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)naphthalen-1-yl)oxy)methyl)yridin-2-yl)acetamide (22).** To a solution of 1-(4-((2-aminopyridin-4-yl)methoxy)naphthalen-1-yl)-3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)urea (**37**) (40 mg, 0.08 mmol) and DIPEA (15 μL, 0.09 mmol) in a mixture of DCM (1.0 mL) and DMF (0.1 mL) was added acetyl chloride (6 μL, 0.08 mmol). After 40 min at rt, additional aliquots of acetyl chloride (4 μL, 0.05 mmol) and DIPEA (10 μL, 0.06 mmol) were added. The resulting mixture was kept at rt for an additional 30 min, treated with acetic acid (0.5 mL), and subjected to SCX capture and release. The crude material so obtained was triturated with DCM to afford the title compound (**22**) as a pale orange solid (30 mg, 69%). LC-MS: m/z 563 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.26 (9H, s), 2.09 (3H, s), 2.35 (3H, s), 5.35 (2H, s), 6.35 (1H, s), 7.00 (1H, d), 7.22 (1H, m), 7.35 (2H, m), 7.42 (2H, m), 7.55–7.63 (3H, overlapping m), 7.92 (1H, dd), 8.28–8.33 (2H, overlapping m), 8.57 (1H, bs), 8.78 (1H, bs), 10.54 (1H, bs).

1-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-3-(4-((2-(3-methylureido)pyridin-4-yl)ethoxy)naphthalen-1-yl)urea (23). To a solution of 1-(4-((2-aminopyridin-4-yl)methoxy)naphthalen-1-yl)-3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)urea (**37**) (70 mg, 0.13 mmol) in pyridine (1.5 mL) was added methyl isocyanate (14 μL, 0.24 mmol). The reaction mixture was kept at rt for 72 h. The reaction mixture was evaporated in vacuo, and the residue was triturated with DCM (3.0 mL) to afford the title compound (**23**) as an off-white powder (36 mg, 45%). LC-MS: m/z 578 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.27 (9H, s), 2.39 (3H, s), 2.74 (3H, d), 5.30 (2H, s), 6.36 (1H, s), 6.99 (1H, d), 7.05 (1H, d), 7.35, (2H, m), 7.44 (2H, m), 7.54–7.64 (4H, overlapping m), 7.93 (1H, d), 8.19 (1H, d), 8.23 (1H, bs), 8.35 (1H, d), 8.58 (1H, bs), 8.79 (1H, bs), 9.36 (1H, bs).

***N*-(4-(((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)naphthalen-1-yl)oxy)methyl)yridin-2-yl)benzamide (24).** The title compound was prepared by the procedure described for **22** using benzoyl chloride in place of acetyl chloride to give **24** as a beige powder (19 mg, 39%). LC-MS: m/z 625 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.25 (9H, s), 2.39 (3H, s), 5.41 (2H, s), 6.35 (1H, s), 7.04 (1H, d), 7.31–7.37 (3H, overlapping m), 7.42 (2H, m), 7.50 (2H, m), 7.56–7.64 (4H, overlapping m), 7.93 (1H, m), 8.02 (2H, m), 8.32 (1H, m), 8.40–8.45 (2H, overlapping m), 8.58 (1H, bs), 8.79 (1H, bs), 10.90 (1H, bs).

***N*-(4-(((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)naphthalen-1-yl)oxy)methyl)yridin-2-yl)-2-phenylacetamide (25).** The title compound was obtained in a similar manner as that for **22** using phenylacetyl chloride in place of acetyl chloride to give **25** as a beige powder (7 mg, 13%). LC-MS: m/z 639 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.25 (9H, s), 2.37 (3H, s), 4.72 (2H, s), 5.34 (2H, s), 6.34 (1H, s), 6.97 (1H, d), 7.20–7.26 (2H, overlapping m), 7.27–7.37 (6H, overlapping m), 7.53–7.62 (3H, overlapping m), 7.92 (1H, m), 8.25–8.29 (2H, overlapping m), 8.33 (1H, dd), 8.57 (1H, bs), 8.78 (1H, bs), 10.77 (1H, bs).

***N*-(4-((4-(3-(3-*tert*-Butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)ureido)-naphthalen-1-yloxy)methyl)yridin-2-yl)-2-(dimethylamino)-acetamide (26).** To a solution of *N*-(4-((4-(3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)ureido)naphthalen-1-yloxy) ethyl) pyridin-2-yl)-2-chloroacetamide (**36**) (50 mg, 0.08 mmol) in a mixture of DCM (1.0 mL), DMF (0.1 mL), and DIPEA (17 μL, 0.10 mmol) was added a solution of dimethylamine (2.0 M in THF, 41 μL, 0.08 mmol). The reaction mixture was heated at 40 °C for 12 h. The resulting mixture was purified by flash column chromatography (12 g SiO_2, MeOH in DCM, 0–10%, gradient elution). The product obtained was triturated with a mixture of diethyl ether, DCM, and isohexane (2:1:2, 5.0 mL) to afford the title compound (**26**) as an orange solid (18 mg, 35%). LC-MS: m/z 607 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.27 (9H, s), 2.31 (6H, s), 2.39 (3H, s), 3.14 (2H, s), 5.39 (2H, s), 6.35 (1H, s), 7.01 (1H, d), 7.29 (1H, d), 7.35 (2H, d), 7.44 (2H, d), 7.65–7.55 (3H, overlapping m), 7.94 (1H, m), 8.38–8.28 (3H, overlapping m), 8.59 (1H, bs), 8.79 (1H, bs), 9.93 (1H, bs).

***N*-(4-((4-(3-(3-*tert*-Butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)ureido)-naphthalen-1-yloxy)methyl)yridin-2-yl)-2-morpholinoacetamide (27).** To a solution of *N*-(4-((4-(3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)ureido)naphthalen-1-yloxy)ethyl)pyridin-2-yl)-2-chloroacetamide (**36**) (50 mg, 0.08 mmol) in a mixture of DCM (1.0 mL), DMF (0.1 mL), and DIPEA (21.9 μL, 0.13 mmol) was added morpholine (11.0 μL, 0.13 mmol). The reaction mixture was kept at rt for 3 h, heated at 40 °C for 12 h, and then treated with a second aliquot of morpholine (11.0 μL, 0.13 mmol). After an additional 5 h at 40 °C, the crude reaction mixture was purified by flash column chromatography (12 g, SiO_2, MeOH in DCM, 0–10%, gradient elution). The impure product obtained was triturated with MeOH (5.0 mL), and the solid was collected by filtration to provide the title compound (**27**) as a light yellow solid (11 mg, 20%). LC-MS: m/z 648 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.27 (9H, s), 2.39 (3H, s), 2.54 (4H, m), 3.20 (2H, s), 3.63 (4H, m), 5.39 (2H, s), 6.35 (1H, s), 7.01 (1H, d), 7.28 (1H, d), 7.35 (2H, d), 7.43 (2H, d), 7.56–7.62 (3H, overlapping m), 7.92 (1H, d), 8.29–8.37 (3H, overlapping m), 8.58 (1H, bs), 8.90 (1H, bs), 10.01 (1H, bs).

***N*-(4-((4-(3-(3-*tert*-Butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)ureido)-naphthalen-1-yloxy)methyl)yridin-2-yl)-2-(methylthio)-acetamide (28).** To a stirred suspension of sodium thiomethoxide (35 mg, 0.50 mmol) in MeOH (5.0 mL) at rt was added portionwise *N*-(4-((4-(3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)ureido)naphthalen-1-yloxy)methyl)pyridin-2-yl)-2-chloroacetamide (**36**) (100 mg, 0.17 mmol). After 1 h at rt, the resulting mixture was evaporated in vacuo and the residue was partitioned between brine (20 mL) and DCM (30 mL). The organic layer was separated and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, 12 g, EtOAc in isohexane, 10–100%, gradient elution) to give the title compound (**28**) as a light yellow solid (28 mg, 26%). LC-MS: m/z 610 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.27 (9H, s), 2.16 (3H, s), 2.39 (3H, s), 3.53 (2H, s), 5.37 (2H, s), 6.35 (1H, s), 7.01 (1H, d), 7.26 (1H, dd), 7.35 (2H, m), 7.44 (2H, m), 7.55–7.64 (3H, m), 7.92 (1H, m), 8.30–8.35 (3H, overlapping m), 8.58 (1H, s), 8.78 (1H, s), 10.60 (1H, s).

***N*-(4-(((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)ureido)naphthalen-1-yl)oxy)methyl)yridin-2-yl)-2-(methylsulfonyl)acetamide (29).** To a suspension of methanesulfonylacetic acid (40 mg, 0.29 mmol) and oxalyl chloride (29 μL, 0.34 mmol) in DCM (1.0 mL) was added DMF (1 drop). The reaction mixture was stirred at rt for 1 h. The resulting solution was added dropwise to a mixture of 1-(4-((2-aminopyridin-4-yl)methoxy)naphthalen-1-yl)-3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)urea (**37**) (50 mg, 0.10 mmol) and DIPEA (167 μL, 1.00 mmol) in DCM/DMF (10:1 v/v, 1.1 mL). After 18 h, the reaction mixture was treated with 1% NH_3 in MeOH (2.0 mL) and was then evaporated in vacuo. The residue was subjected to SCX capture and release to afford the title compound (**29**) as a pale pink solid (11 mg, 18%). LC-MS: m/z 641 $(M + H)^+$ (ES^+). ^{1}H NMR: δ 1.27 (9H, s), 2.39 (3H, s), 3.17 (3H, s), 4.44 (2H, s), 5.40 (2H, s), 6.35 (1H, s), 7.02 (1H, d), 7.33 (1H, dd), 7.36 (2H, m), 7.44 (2H, m), 7.56–7.64 (3H, overlapping m), 7.93 (1H, m), 8.30–8.33 (2H, overlapping m), 8.39 (1H, dd), 8.59 (1H, bs), 8.79 (1H, bs), 10.98 (1H, bs).

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***N*-(4-((4-(3-(3-*tert*-Butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)ureido)-naphthalen-1-yloxy)methyl)yridin-2-yl)-2-methoxyacetamide (30).** To a solution of 1-(4-((2-aminopyridin-4-yl)methoxy)-naphthalen-1-yl)-3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)urea (**37**) (526 mg, 0.96 mmol) and DIPEA (184 μL, 1.06 mmol) in a mixture of DCM and DMF (10:1, 11 mL) was added methoxyacetyl chloride (92 μL, 1.01 mmol). After 1 h at rt, additional aliquots of DIPEA (184 μL, 1.06 mmol) and methoxyacetyl chloride (92 μL, 1.01 mmol) were added sequentially. Stirring was continued for 1 h. A solution of 1% NH_3 in MeOH (40 mL) was added, and after 15 min, the mixture was evaporated in vacuo. The residue was purified by flash column chromatography (SiO_2, 40 g, MeOH in DCM, 0−6%, gradient elution) to furnish the title compound (**30**) as a white solid (286 mg, 49%). LC-MS: *m*/*z* 593 $(M + H)^+$ (ES^+). ^1H NMR: δ 1.27 (9H, s), 2.39 (3H, s), 3.32 (3H, s), 4.08 (2H, s), 5.39 (2H, s), 6.36 (1H, s), 7.03 (1H, d), 7.28 (1H, dd), 7.36 (2H, m), 7.44 (2H, m), 7.56−7.64 (3H, overlapping m), 7.93 (1H, m), 8.30−8.35 (3H, overlapping m), 8.58 (1H, bs), 8.79 (1H, bs), 10.02 (1H, bs).

***N*-(4-(((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-ureido)naphthalen-1-yl)oxy)methyl) yridin-2-yl)-2-methoxy-*N*-methylacetamide (31).** The title compound was prepared in a similar manner as that for **37** from *N*-(4-(((4-aminonaphthalen-1-yl)oxy)-methyl)pyridin-2-yl)-2-methoxy-*N*-methylacetamide (**35**) to give **31** as a light beige solid (60 mg, 27%). LC-MS: *m*/*z* 607 $(M + H)^+$ (ES^+). ^1H NMR: δ 1.27 (9H, s), 2.38 (3H, s), 3.20 (3H, s), 3.29 (3H, s), 4.13 (2H, s), 5.39 (2H, s), 6.34 (1H, s), 7.02 (1H, d), 7.35 (2H, m), 7.41−7.45 (3H, overlapping m), 7.55−7.65 (3H, overlapping m), 7.70 (1H, s), 7.92 (1H, m), 8.31 (1H, m), 8.46 (1H, d), 8.57 (1H, bs), 8.78 (1H, bs).

***N*-(4-(1-((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-ureido)naphthalen-1-yl)oxy)ethyl)pyridin-2-yl)-2-methoxyacetamide (32).** The title compound (**32**) was prepared in a similar manner as that for **30** from 1-(4-(1-(2-aminopyridin-4-yl)ethoxy)-naphthalen-1-yl)-3-(3-(*tert*-butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)urea (**41**) as a beige solid (24 mg, 51%). LC-MS *m*/*z* 607 $(M + H)^+$ (ES^+). ^1H NMR: δ 1.26 (9H, s), 1.67 (3H, d), 2.38 (3H, s), 4.03 (2H, s), 5.75 (1H, q), 6.32 (1H, s), 6.81 (1H, d), 7.22 (1H, dd), 7.35 (2H, m), 7.42 (2H, m), 7.48 (1H, s), 7.59 (2H, m), 7.88 (1H, m), 8.24 (1H, bs), 8.27 (1H, d), 8.36 (1H, m), 8.59 (1H, bs), 8.76 (1H, bs), 9.99 (1H, bs).

***N*-(4-(2-((4-(3-(3-(*tert*-Butyl)-1-(*p*-tolyl)-1*H*-pyrazol-5-yl)-ureido)naphthalen-1-yl)oxy)propan-2-yl)pyridin-2-yl)-2-methoxyacetamide (33).** The title compound (**33**) was prepared in a similar manner as that for **30** from 1-(4-(2-(2-aminopyridin-4-yl)propan-2-yloxy)naphthalen-1-yl)-3-(3-*tert*-butyl-1-*p*-tolyl-1*H*-pyrazol-5-yl)urea (**42**) as a white solid (18 mg, 24%). LC-MS *m*/*z* 621 $(M + H)^+$ (ES^+). ^1H NMR: δ 1.28 (9H, s), 1.39 (6H, s), 2.39 (3H, s), 3.22 (3H, s), 3.80 (1H, d), 4.16 (1H, d), 5.27 (1H, s), 6.41 (1H, s), 7.26 (1H, dd), 7.37 (2H, m), 7.46 (2H, m), 7.56 (1H, d), 7.60 (2H, overlapping m), 7.76 (1H, br s), 7.95 (1H, m), 8.01 (1H, d), 8.09 (1H, m), 8.22 (1H, d), 8.94 (1H, bs), 9.28 (1H, bs).

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Notes

The authors declare the following competing financial interest(s): Stuart T. Onions, Richard J. Brown, Marie Colucci, Kevin Joly, Andrew Novak, Anjna Rani, Alun Smith, David M. Taddei, and Jonathan G. Williams were full-time employees of Sygnature Discovery Limited at the time this work was completed. Kazuhiro Ito, Catherine E. Charron, P. John Murray, Garth Rapeport, and Peter Strong were full-time employees of RespiVert Ltd at the time this work was completed.

DEDICATION

This paper is dedicated to the memory of Fritz Frickel.

ABBREVIATIONS USED

AcOH, glacial acetic acid; BALF, bronchoalveolar lavage fluid; b, broad; CatCart, catalytic cartridge; CDI, 1,1-carbonyl-diimidazole; COPD, chronic obstructive pulmonary disease; CS, cigarette smoke; Cs_2CO_3, cesium carbonate; CXCL8, interleukin 8; CYP, cytochrome P450; d-, differentiated; DFG, aspartic acid−phenylalanine−glycine; DIAD, diisopropylazadicarboxylate; DIPEA, *N*,*N*-diisopropylethylamine; EDC, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide; EtOAc, ethyl acetate; EtOH, ethanol; (ES^+), electrospray ionization, positive mode; FACS, fluorescence-activated cell sorting; FBS, fetal bovine sample; FEV1, forced expiratory volume in 1 s; FP, fluticasone propionate; FRET, fluorescence resonance energy transfer; GM-CSF, granulocyte-macrophage colony-stimulating factor; H_2O_2, hydrogen peroxide; HCl, hydrochloric acid; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; HCK, hematopoietic kinase; HOBt, 1-hydroxybenzotriazole; IBD, inflammatory bowel disease; K_2CO_3, potassium carbonate; IL, interleukin; IT, intratracheal; LiOH, lithium hydroxide; MAP, mitogen activated protein; MAPKAP-K2, mitogen activated protein kinase-activated protein kinase 2; MeCN, acetonitrile; MeI, methyl iodide; MeLi, methyllithium; MeOH, methanol; $(M + H)^+$, protonated molecule; min, minute(s); MOMA2, monocyte/macrophage marker antibody; *m*/*z*, mass-to-charge ratio; $MgSO_4$, magnesium sulfate; $NaBH_4$, sodium borohydride; NaCl, sodium chloride; $NaHCO_3$, sodium bicarbonate; NaOH, sodium hydroxide; NH_3, ammonia; NH_4Cl, ammonium chloride; NSKI, narrow spectrum kinase inhibitor; PBMC, peripheral blood mononucleated cell; $Pd_2(dba)_3$, tris(dibenzylideneacetone)dipalladium(0); PMA, phorbol myristate acetate; PPh_3, triphenylphosphine; RA, rheumatoid arthritis; RP, reverse phase; rt, room temperature; SCX, strong cation exchange (resin); SiO_2, silica; S_NAr, nucleophilic aromatic substitution; TBDMSCl, *tert*-butyldimethylsilyl chloride; TMS-CN, trimethylsilyl cyanide; Xantphos, 4,5-bis-(diphenylphosphino)-9,9-dimethylxanthene

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