Lead Identification of Benzimidazolone and Azabenzimidazolone Arylsulfonamides as CC-Chemokine Receptor 4 (CCR4) Antagonists.

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1. Introduction

T helper 2 (Th2) cytokines in inflamed tissues lead to eosinophilia, high levels of serum IgE and mast cell activation, all of which contribute to the pathogenesis of allergic diseases.\(^1\) Upon exposure to allergen, dendritic cells within tissue secrete macrophage-derived chemokine (MDC), and thymus activation-regulated chemokine (TARC) which can recruit Th2 cells from the circulation. The T cells can then migrate along this chemokine gradient to the dendritic cells. The latter migrate from the inflamed tissue to local lymph nodes where MDC and TARC may recruit further T cells. Elevated levels of TARC and MDC as well as accumulation of CCR4-positive cells were observed in lung biopsy samples from patients with atopic asthma following allergen challenge.\(^2\) CCR4 is also expressed by immune suppressive regulatory T cells,\(^3,4\) and a minor subset of Th17 cells.\(^5,6\) Hence CCR4 antagonists represent a novel therapeutic intervention in diseases where CCR4 is involved, such as asthma,\(^7\) lung disease,\(^3\) atopic dermatitis,\(^8\) allergic bronchopulmonary aspergillosis,\(^9\) cancer,\(^3,10\) inflammatory bowel disease,\(^4\) the mosquito-borne tropical diseases, such as Dengue fever,\(^11\) and allergic rhinitis.\(^12\) In addition, CCR4 antagonists were used as molecular adjuvants in vaccines.\(^13-16\) Finally, CCR4 monoclonal antibodies were recently explored for CCR4\(^+\) T cell leukaemia and one such antibody, Mogamulizumab, was approved in Japan for the treatment of relapsed or refractory adult T cell leukaemia/lymphoma.\(^17,18\) The approval of the humanised monoclonal antibody Mogamulizumab underlines the value of generating small molecule CCR4 antagonists in this area. The AstraZeneca pyrazine sulfonamide 1,\(^19\) the Ono sulfonamide 2,\(^20\) and the GlaxoSmithKline indazole 3\(^21,22\) are recently published sulfonamide CCR4 receptor antagonists (Figure 1). Sulfonamides 1 – 3 bind at an intracellular allosteric binding site, which is different from the extracellular binding site where lipophilic amine antagonists such as 4 to 6 bind. The two allosteric binding sites are distinct from each other and from the orthosteric binding site where TARC and MDC bind.\(^23\)
Indazole 3 was the first candidate to be progressed to human studies; however, the compound suffered from low solubility and weak potency.\textsuperscript{24} A back-up programme was therefore initiated to identify novel templates for lead optimisation studies. Our group has recently reported the identification of bicyclic heteroarenes as novel lead series of sulfonamide CCR4 antagonists.\textsuperscript{25} Herein we disclose the identification of a second lead series, the benzimidazol-2-one sulfonamides.

\textbf{Figure 1}. Structures for some recently published CCR4 antagonists.

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1.png}
\end{figure}

2. Chemistry

The initial hit came from a focused library of 2,3-dichlorobenzenesulfonamides designed on a knowledge driven approach. A virtual set of 1600 commercially available aryl or heteroaryl primary amines were enumerated as 2,3-dichlorobenzenesulfonamides, ensuring that the set contained novel starting points with desired physicochemical properties (MW <400, clogP
<4, polar surface area <100, H-bond acceptor <10, H-bond donor <5). A library of about 100 sulfonamides was synthesised and assayed in the GTPγS assay. Hits were required to have a pIC₅₀ greater than that of the starting point of the indazole series (7) in the GTPγS assay, and also better physicochemical properties than 7 (clogP <3.5, chrom logD₇.₄ <5.3 and CLND solubility >116 μg/mL). A subset of 14 of the most active compounds was tested for chemical stability in simulated gastric fluid and for photo-stability, and the unstable compounds were removed from the set to leave 10 hits. We have already reported for our indazole series,²² and more recently for the bicyclic heteroarene series²⁵ that introduction of a hydrogen bond acceptor (HBA), suitably orientated to hydrogen-bond to the sulfonamide NH, increased the potency of these compounds approximately ten-fold. A methoxy group was then introduced to each of the 10 stable hits of our sulfonamide library to act as an HBA for the sulfonamide NH. Following screening of these methoxy analogues in the GTPγS assay the benzimidazol-2-one sulfonamide 8 was identified as one of five hits suitable for further investigation. In this publication we disclose initial structure–activity relationships in this series, and the identification of a lead compound.

![Figure 2: Initial indazole (7) and benzimidazolone (8) hits](image)

Substitution with large benzyl groups was tolerated at the N1 position of the indazole series (for example compound 3).²¹,²² It was therefore important to find out whether substitution in the heterocyclic ring of the benzimidazolone scaffold was tolerated. Alkylation of 8 would
produce three potential products, which would require separation and extensive characterisation. It was also known from previous studies\textsuperscript{22} that alkylation of the sulfonamide nitrogen provided compounds devoid of any CCR4 activity. It was considered that treatment of 5-methoxy-6-nitro-benzimidazol-2-one \textit{9} with one equivalent of electrophile in the presence of a base might produce one major product regioselectively as the acidity of the nitrogen atoms of the imidazolone ring was substantially different. The calculated pK\textsubscript{a} values (ACD software, version 11) for N1 (\textit{para} to the nitro- group) was 10.9 and for N3 (\textit{para} to methoxy- group) was 17.1. Hence, treatment of \textit{9} with one equivalent of sodium hydride in DMF and one equivalent of electrophile was expected to give the N1-alkylation product as the major component. Indeed when iodomethane was used as the electrophile \textit{10a} was obtained in 25\% yield. The other mono-methyl product \textit{11a} was present in the reaction mixture only in trace amounts. The dimethyl product \textit{12a} was however also isolated in 14\% yield, which suggested that product \textit{10a} reacted further with iodomethane. The structure of the monomethyl regioisomer \textit{10a} was confirmed by NOE experiments. The HSQC spectrum confirmed the chemical shifts of the aromatic protons at the C4 (7.51 ppm) and C7-position (7.12 ppm). Irradiating the \textit{N}-methyl protons (3.34 ppm) and \textit{O}-methyl protons (3.94 ppm), resulted in both sets of protons giving an NOE to the proton at the C7-position (7.12 ppm), confirming the regiochemistry of the alkylation (Figure 3).

\begin{figure}[h]
\centering
\includegraphics[width=0.4\textwidth]{structure.png}
\caption{NOE confirmation of the structure of N1-methyl regioisomer \textit{10a}. The numbering of the N1-position in relation to the MeO- and nitro- groups shown above could change depending on the substituent used, so throughout the report, the N1-position as shown above is used as an arbitrary number for ease of discussion.}
\end{figure}
Treatment of the sodium salt of 9 with 2-bromoacetamide gave a 9:1 mixture of 10b and 11b (Scheme 1). Similarly treatment of 9 with iso-propyl bromide or with 3-iodo-oxetane gave 10c and 10d respectively. The nitro-group of 10a-d, 11b and 12a,b was reduced with hydrogen over 10% palladium on carbon to give the corresponding aromatic amines, which were sulfonylated with 2,3-dichlorobenzenesulfonyl chloride in pyridine to give 13a-d, 14b and 15a.

Scheme 1  Reagents & Conditions: (a) 17.5% HNO₃, 0 °C 1h, 20 °C, 3 h, 36%; (b) NaH, DMF, 20 °C then MeI (10a), or NH₂COCH₂Br (10b), or iso-PrBr (10c), or 3-iodo-oxetane (10d); (c) H₂, 10% Pd/C, EtOH, EtOAc, 18 h; (d) 2,3-dichlorobenzenesulfonyl chloride, pyridine, 20 °C

The general method shown in Scheme 1 was unsuccessful for the preparation of the N3-methylated regioisomer 11a, so an alternative route to explore the N3-substitution using allyl
protection was investigated (Scheme 2). The allyl group was chosen as a protecting group as it is stable to both acidic (nitric acid) and basic (sodium hydride) conditions, key requirements in the proposed synthetic route. Reaction of commercially available 2-fluoro-4-methoxy-1-nitrobenzene 16 with neat allylamine at room temperature afforded the nitroaniline 17 in 97% yield. The nitro group of 17 was reduced with iron and ammonium chloride to provide the phenylenediamine 18, which was then treated with CDI to give the methoxy benzimidazolone 19 in 45% yield. Nitration of 19 using fuming nitric acid and concentrated sulfuric acid or neat fuming nitric acid gave the undesired dinitro product 20. Investigation of various other concentrations of nitric acid and sulfuric acid, identified 4M nitric acid as the best reagent for the preparation of the desired mono nitro product, 21 (23% yield). A small amount of the dinitro product, 20 and starting material were also present in the reaction mixture. Alkylation of 21 with methyl iodide was facile providing the methylated benzimidazolone 22 in excellent yield. Removal of the allyl group proved to be problematic. Attempts to use palladium catalysed conditions, involving the formation of π-allyl complex, 26 and those based on isomerisation of the allylamine double bond using RuCl3 catalyst, followed by hydrolysis of the resulting enamine, were all found to be unsuccessful.27-29
Scheme 2. Reagents & Conditions: (a) allylamine, 20 °C, 1.5 h, 97%; (b) Fe, NH₄Cl, EtOH, H₂O, 100 °C, 22 h, 83%; (c) CDI, THF, 50 °C, 2 h, 45%; (d) 4M HNO₃, 0 – 20 °C, 4 h, 23%; (e) NaH, MeI, DMF, 0 – 20 °C, 2.5 h, 99%; (f) dichloro(2,6,10-dodecatriene-1,12-diyl)ruthenium(IV) (24), KIO₄, H₂O, 100 °C, 72 h, 39%; (g) H₂, 10% Pd/C, EtOH-EtOAc (1:1), 5 h, 99%; (h) 2,3-dichlorobenzensulfonyl chloride, pyridine, 20 °C, 1 h, 22%.

The method of Nebra,³⁰ utilising commercially available ruthenium catalyst 24, and stoichiometric potassium periodate in water gave 23 in 40% yield. Finally reduction of 23 using catalytic hydrogenation over 10% Pd/C catalyst, followed by reaction with 2,3-dichlorobenzensulfonyl chloride in pyridine gave the desired N3-methylated sulfonamide.
The N1-propyl analogue 13e was also obtained from this route by catalytic hydrogenation of 21 followed by sulfonylation.

A regioselective synthetic route for the preparation of N1-substituted benzimidazolones was developed and is outlined in Scheme 3. Reaction of 16 with a range of primary amines, such as N,N-dimethylaminoethylamine, 2-aminopropane-1,3-diol, and 3-amino-1-BOC-azetidine in the presence of diisopropylamine (DIPEA) in DMF at room temperature gave 25 in 83–93% yields. Reduction of the nitro group of 25 with catalytic hydrogenation over 10% Pd/C in ethanol gave 26 in 70–97% yields. In the case of 25f the hydroxyls were protected as TBDMS ethers before proceeding to the hydrogenation step. The aromatic diamines 26 were then cyclised with carbonyl diimidazole (CDI) in THF at 50 °C to give 27 in 69–83% yield. Benzimidazolones 27 were nitrated using 4M nitric acid to give the desired mono nitro products 10 in 33–53% yield, accompanied by a small amount of the di-nitration product. In the case of azetidine 10g the BOC protecting group was removed under the nitration conditions and it was re-installed using BOC₂O in THF in the presence of NaHCO₃. The nitro group of 10 was reduced by catalytic hydrogenation over 10% Pd/C to give 28 in high yields. The synthesis was completed by sulfonylation of 28 with 2,3-dichlorobenzenesulfonyl chloride in pyridine to give the desired sulfonamide 13 in moderate yields. In the case of BOC-13g the protecting group was removed using 4M HCl in dioxane. Finally, 13g was converted to 13h using one equivalent of acetic anhydride in pyridine.
Scheme 3. Reagents & Conditions: (a) RNH₂, DIPEA, DMF, 20 °C; (b) H₂, 10% Pd/C, EtOH; (c) CDI, THF, 50 °C; (d) 4M HNO₃, 0–20 °C; (e) 2,3-dichlorobenzenesulfonyl chloride, pyridine, 20 °C.

Introduction of nitrogen at the C4-position of the benzimidazolone series is outlined in Scheme 4. Commercially available 3-hydroxy pyridine 29 was reacted with bromine in aqueous sodium hydroxide to give the dibromide 30 according to the method of Clark and Deady. The latter was methylated with iodomethane in the presence of potassium carbonate in DMSO to give 31 in 80% yield. Nitration of 31 with conc. HNO₃ and conc. H₂SO₄ at 65 °C gave 32 in 28% yield. The two bromine atoms of 32 were readily displaced by aqueous ammonia using microwave irradiation using a modification of the Barraclough procedure to give diamine 33 in 78% yield. The latter was reacted with BOC₂O to give the bis-protected diamine 34 in 66% yield, followed by reduction of the nitro group using iron in acetic acid to provide amine 35 in 78% yield. This compound was then heated in pyridine in a microwave oven at 100 °C to give the cyclised aza-benzimidazolone 36 in 74% yield. Cleavage of the
BOC group with TFA gave 37 in 93%, and reaction of this product with 2,3-dichlorobenzenesulfonyl chloride in pyridine afforded the desired sulfonamide 38 in moderate yield (30%).

Scheme 4. Reagents & Conditions: (a) Br₂, 10% NaOH, 0 °C, 1 h, 20 °C, 4 h, 46%; (b) MeI, K₂CO₃, DMSO, reflux, 2 h, 80%; (c) conc. HNO₃, conc. H₂SO₄, 65 °C, 36 h, 28%; (d) aq. NH₃, 90 °C, 1 h, μwave, 78%; (e) BOC₂O, K₂CO₃, DMF, 20 °C, 18 h, 66%; (f) Fe, AcOH, 20 °C, 2 h, 78%; (g) pyridine, 100 °C, 1 h, μwave, 74%; (h) TFA, 20 °C, 30 min, 93%; (i) 2,3-dichlorobenzenesulfonyl chloride, pyridine, 20 °C, 2 h, 30%.

Commercially available 6-methoxypyridine-2,3-diamine (39) was used for the synthesis of the other regioisomeric azabenzimidazolone series (Scheme 5). Reaction of 39 with CDI was slow giving the benzimidazolone 40 in only 25% yield after 48 h. Nitration of 40, using triflic anhydride and fuming nitric acid gave the desired nitro-benzimidazolone 41, which
was reduced by catalytic hydrogenation over 10% Pd/C to give the amino benzimidazolone 42. Finally, reaction with 2,3-dichlorobenzenesulfonyl chloride afforded the target sulfonamide 43.

Scheme 5. Reagents & Conditions: (a) CDI, Et₃N, THF, 20 °C, 48 h, 31%; (b) Tf₂O, fuming HNO₃, 20 °C, 3 h, 47%; (c) H₂, 10% Pd/C, EtOH, 2 h, 76%; (d) 2,3-dichlorobenzenesulfonyl chloride, pyridine, 20 °C, 1 h, 48%.

The preparation of the pyrazine analogue is shown in Scheme 6. Reaction of commercially available 5-bromopyrazine-2,3-diamine (44) with CDI gave the diaza-benzimidazolone 45 in 85% yield. Nitration, using nitronium tetrafluorobororate gave the nitro compound 46 in 50% yield. The bromine of 46 was displaced by methoxide to give the methoxy diazabenzimidazolone 47 in 49% yield. Reduction of 47 using catalytic hydrogenation conditions over 10% Pd/C gave 48 (80% yield), which was then sulfonylated with 2,3-dichlorobenzenesulfonyl chloride in pyridine to afford the desired sulfonamide 49.
Scheme 6. Reagents & Conditions: (a) CDI, THF, 50 °C, 48 h, 85%; (b) NO_2BF_4, CH_3CN, 20 °C, 1 h, 50%; (c) NaOMe, MeOH, 20–50 °C, 20 h, 49%; (d) H_2, 10% Pd/C, EtOH–EtOAc (1:1), 1 h, 80%; (e) 2,3-dichlorobenzenesulfonyl chloride, pyridine, 20 °C, 1 h, 7%.

3. Results and Discussion

All compounds in Table 1 were tested as human CCR4 antagonists in vitro. Antagonist potency was determined by a [³⁵S]-GTPγS radioligand competition functional assay using recombinant CCR4-expressing CHO cell membranes adhered to WGA-coated Leadseeker SPA beads in assay buffer at pH 7.4. Another assay using human whole blood was used as a secondary screen to determine potency against the native receptor for the more potent compounds in the primary assay. The assay quantified cytoskeletal reorganization (formation of filamentous (F-) actin) which occurs in a variety of cells in response to chemoattractants and is a prelude to chemotaxis. This was achieved by staining the F-actin with a fluorescent derivative of phalloidin, which binds with high affinity and specificity to the interface between actin monomers in F-actin. The response was measured as an increase in the fluorescence intensity of the target cell population in a flow cytometer and was expressed as a pA₂. In this assay, human CD4⁺ CCR4⁺ lymphocytes were identified by staining with antibodies to CD4 and CCR4. Calculated partition coefficient (clogP),
chromatographic logD$_{7.4}$ (chrom logD at pH 7.4) and ChemiLuminescent Nitrogen Detection (CLND) kinetic solubility are included for all test compounds in this study. The high throughput CLND solubility assay involved addition of aqueous buffer to a test compound DMSO solution over a period of time until the compound precipitated. Ligand efficiency (LE) was obtained from the equation $LE = \frac{-1.36 \times pIC_{50}}{HAC}$, where HAC is the heavy atom count (number of non-hydrogen atoms present in the molecule), with a desirable figure of LE being $> 0.3$.

Compounds 2 and 4 were used as standards in the GTP$_{\gamma}$S assay and included in Table 1. The data generated for 7, the initial lead that lead to indazole candidate 3, are also included in Table 1 for comparison. The initial benzimidazolone hit 8 was slightly more potent than 7 ($pIC_{50} = 6.5$), with higher LE (0.38), and lower lipophilicity (chrom logD$_{7.4}$ 3.2), but with lower solubility. Substitution at the benzimidazolone N1-position with methyl or acetamide groups (13a and 13b) was tolerated, however, substitution at N3 (14a and 14b) reduced potency by 100-fold. The N1,N3-dimethyl analogue 15a was ten-fold less potent than 13a.

It was encouraging to observe that removal of a hydrogen bond donor (HBD) from the benzimidazolone core was tolerated. In general reducing the number of HBD’s in a compound increases permeability, although the solubility might be reduced.$^{34,35}$ The N1-position was further explored with small lipophilic, polar and weakly basic groups in an attempt to improve the potency, solubility and physicochemical property of the series.

Replacing the methyl group at the N1-position for n-propyl group (13c) reduced both the potency and the solubility. Replacing methyl for isopropyl group (13d) caused a marginal increase in potency, accompanied by increased lipophilicity (clogP = 4.4). The two basic analogues 13e and 13g, the diol 13f, and the amide 13h were made to increase the solubility of the molecules. However, despite the observed increases in solubilities, their potencies were lower than the previous compounds. The oxetane group was utilised to improve the
solubility and permeability of other scaffolds.\textsuperscript{36} The oxetanyl analogue 13i was equipotent to the methyl analogue 13a, and it was also less lipophilic, however, its solubility remained low. Furthermore, 13i was found to be unstable, and no further substitutions were investigated.

It was noted that all three sulfonamide CCR4 antagonist candidates 1, 2 and 3 contain a nitrogen heterocycle (pyrazine or indazole), and that the nitrogen atom of the heterocyclic ring was ortho- to the sulfonamide nitrogen. Similarly, a 10-fold increase of potency was observed in our lead generation work for a back-up series to compound 3 after the introduction of an ortho- nitrogen atom to phenylpyrazole to form pyridylpyrazole sulfonamides. Furthermore, the introduction of nitrogen into the phenyl ring of the benzimidazolone 8 was expected to enhance its solubility and physicochemical properties. This strategy was more successful than the removal of an HBD, or the introduction of solubilising groups. Thus, replacement of the C4-methine with nitrogen (compound 38) resulted in more than a log unit increase in GTP\(_{\gamma}\)S potency (pIC\(_{50}\) = 7.6), and a substantial increase in ligand efficiency (LE = 0.43). Furthermore, its lipophilicity was decreased (clogP = 3.6 and chrom logD\(_{7.4}\) = 2.4), and its solubility increased by more than three-fold compared to 8. Introducing nitrogen at C7 (compound 43) also increased potency in the GTP\(_{\gamma}\)S assay, but only by half a log unit while it increased its LE to 0.40. The lipophilicity was also decreased, but not by as much as in the case of 38. Its solubility was increased by three-fold to 126 \(\mu\)g/mL. Introducing two nitrogen atoms to the benzimidazolone phenyl ring provided pyrazine 49, which increased its pIC\(_{50}\) to 7.3, LE to 0.41, and its solubility by three-fold higher than 8.

Human whole blood F-actin polymerisation assay and physicochemical data were obtained for the most potent compounds of the benzimidazolone series, and the results are also summarised in Table 1. The parent benzimidazolone 8 was weakly active in this assay and
had a $pA_2$ value of 5.3. Although the N1 isopropyl analogue 13d was slightly more potent than 8 in the GTP$_{yS}$ assay, it was inactive in the whole blood assay ($pA_2 < 5$). The three aza-benzimidazolone analogues 38, 43 and 49 were all found to be active, with compound 38 exhibiting whole blood activity similar to that of the indazole candidate 3. The whole blood activity data for 38 ($pA_2 = 6.1$) is very encouraging as its molecular weight is lower than 3, (389 vs 549), it displays a higher LE of 0.43 (cf. 3 LE = 0.29), and it is more soluble than 3. It is also much less lipophilic than 3 (chrom logD$_{7.4} = 2.4$ vs 4.3). In addition, the azabenzimidazolones 38, 43 and 49 have human serum albumin binding around 93%, which is substantially lower than 3 (96.5%). This translates to twice the unbound fraction of 38 available for distribution to the desired tissues.

4. Conclusion

The aim of this work was to obtain human CCR4 antagonists which were more potent than the starting point of our indazole candidate, had better physicochemical properties (clogP < 3.5, chrom logD$_{7.4} < 5.3$ and CLND solubility > 116 $\mu$g/mL), and were stable to acid and light. A novel series of 2,3-dichlorophenylsulfonamide derivatives of benzimidazol-2-one were synthesised and examined as CCR4 antagonists. Substitution with small alkyl groups at N1 was tolerated; however, substitution at N3 or di-substitution at both N1 and N3 was not tolerated. Introduction of nitrogen in the benzimidazolone phenyl ring increased potency, ligand efficiency and solubility, whilst reducing the lipophilicity and human serum albumin binding. Aza-benzimidazolone 38 was equipotent with our indazole candidate 3 in the whole blood assay with a $pA_2$ of 6.1, making 38 a very attractive starting point for lead optimisation. The CLND solubility of 38 was 152 $\mu$g/mL, which is on the lower limit for an oral candidate. Introduction of small water-solubilising groups, either as substituents at N1,
or in the aromatic ring of the core would be expected to overcome this problem. Further investigation of this template will not be undertaken, however, due to closure of the project.

5. **Experimental Section**

Organic solutions were dried over anhydrous Na$_2$SO$_4$ or MgSO$_4$. TLC was performed on Merck 0.25 mm Kieselgel 60 F$_{254}$ plates. Products were visualised under UV light and/or by staining with aqueous KMnO$_4$ solution. LCMS analysis was conducted on either System A or System B. System A: Acquity UPLC BEH C18 column (2.1 mm × 50 mm i.d. 1.7 μm packing diameter) eluting with 0.1% formic acid in water (solvent A), and 0.1 % formic acid in acetonitrile (solvent B), using the following elution gradient 0.0 – 1.5 min 3 – 100 % B, 1.5 – 1.9 min 100% B, 1.9 – 2.0 min 3% B, at a flow rate of 1 mLmin$^{-1}$ at 40 °C. The UV detection was an averaged signal from wavelength of 210 nm to 350 nm, and mass spectra were recorded on a mass spectrometer using alternate-scan electrospray positive and negative mode ionization (ES+ve and ES-ve). System B: Acquity UPLC BEH C$_{18}$ column (50 mm × 2.1 mm ID, 1.7 μm packing diameter) eluting with 10 mM ammonium bicarbonate in water adjusted to pH 10 with ammonia solution (solvent A), and MeCN (solvent B) using the following elution gradient 0 – 1.5 min 1 – 97 % B, 1.5 – 1.9 min 97% B, 1.9 – 2.0 min 100% B at a flow rate of 1 mL min$^{-1}$ at 40 °C. Column chromatography was performed on Flashmaster II. Mass-directed auto-preparative HPLC (MDAP) was conducted on a Sunfire C18 column (150 mm × 30 mm i.d. 5 μm packing diameter) at ambient temperature eluting with 0.1 % formic acid in water (solvent A) and 0.1 % formic acid in acetonitrile (solvent B), using an appropriate elution gradient over 15 min at a flow rate of 40 mL min$^{-1}$ and detecting at 210–350 nm at room temperature. Mass spectra were recorded on Micromass ZMD mass spectrometer using electro spray positive and negative mode, alternate scans. $^1$H NMR spectra were recorded at 400 MHz, unless otherwise stated. The chemical shifts are
expressed in ppm relative to tetramethylsilane. High resolution positive ion mass spectra were acquired on a Micromass Q-Tof 2 hybrid quadrupole time-of-flight mass spectrometer. The purity of all compounds screened in the biological assays was examined by LCMS analysis and was found to be ≥ 95%, unless otherwise specified. All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals. The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.

5.1 2,3-Dichloro-N-(6-methoxy-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)benzenesulfonamide (8)  

A solution of 5-amino-6-methoxy-1,3-dihydro-2H-benzimidazol-2-one (55.6 mg, 0.31 mmol) and 2,3-dichlorobenzenesulfonyl chloride (76 mg, 0.31 mmol) in pyridine (0.5 mL) was stirred overnight. The reaction mixture was concentrated under a stream of nitrogen in a blow-down apparatus at 40 °C. The residue was purified by Mass-Directed Auto-Preparative HPLC (MDAP) to give 8 (65 mg, 54%) as a solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 10.55$ (s, 1H), 10.36 (s, 1H), 9.56 (s, 1H), 7.87 (dd, $J = 8.0, 2.0$ Hz, 1H), 7.70 (dd, $J = 8.0, 2.0$ Hz, 1H), 7.40 (t, $J = 8.0$ Hz, 1H), 6.75 (s, 1H), 6.44 (s, 1H), 3.33 (s, 3H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta = 155.5$, 149.6, 140.3, 134.0, 133.8, 129.7, 129.4, 129.2, 127.7, 122.6, 116.4, 109.4, 93.6, 55.5; HRMS (ESI) calcld. for C$_{14}$H$_{12}$Cl$_2$N$_3$O$_4$S (M+H)$^+$ 387.9920; found: 387.9927; LCMS (System A) RT = 0.85 min, 95%, ES+ve $m/z$ 388, 390, 392 (M+H)$^+$, 429, 431, 433 (M+H+MeCN)$^+$.

5.2 5-Methoxy-6-nitro-1H-benzo[d]imidazol-2(3H)-one (9)
A solution of 17.5% fuming nitric acid (12.5 mL, 48.9 mmol) was slowly added to 5-methoxy-1H-benzo[d]imidazol-2(3H)-one (0.25 g, 1.5 mmol) at 0 ºC, and the reaction was stirred for 1 h at 0 ºC, and at 20 ºC for 3 h. The reaction mixture was cooled to 0 ºC and neutralised with saturated aqueous NaHCO₃ solution. The product was extracted with EtOAc (2 × 50 mL), washed with water, dried, and concentrated to give 9 (113 mg, 36%) as a yellow solid: ¹H NMR (400 MHz, DMSO-d₆) δ = 11.21 (br s, 1H), 10.81 (br s, 1H), 7.47 (s, 1H), 6.80 (s, 1H), 3.89 (s, 3H); MS ES+ve m/z 210 (M+H)⁺, 251 (M+H+MeCN)⁺.

5.3 6-Methoxy-1-methyl-5-nitro-1H-benzo[d]imidazol-2(3H)-one (10a) and 5-methoxy-1,3-dimethyl-6-nitro-1H-benzo[d]imidazol-2(3H)-one (12a)

Sodium hydride (15 mg of a 60% w/w dispersion in mineral oil, 0.38 mmol) was added to a stirring solution of 9 (75 mg, 0.36 mmol) in anhydrous DMF (1 mL) at 0 ºC. Methyl iodide (0.02 mL, 0.36 mmol) was added and the reaction mixture was stirred at ambient temperature for 4 h. The reaction mixture was diluted with water (15 mL) and ethyl acetate (15 mL). The organic layer was separated, washed with water (10 mL), brine (10 mL), dried and evaporated under reduced pressure. The residue was taken up in DMSO (2 mL) and purified by MDAP. The appropriate fractions were combined and the solvent was evaporated under reduced pressure to give 10a (20 mg, 25%); ¹H NMR (400 MHz, DMSO-d₆) δ = 11.06 (br s, 1H), 7.51 (s, 1H), 7.12 (s, 1H), 3.94 (s, 3H), 3.34 (s, 3H); LCMS (System A) RT = 0.60 min, 95%, ES+ve m/z 265 (M+H+MeCN)⁺, ES-ve m/z 222 (M-H)⁻, and 12a (12 mg, 14%): ¹H NMR (400 MHz, DMSO-d₆) δ = 7.80 (s, 1H), 7.19 (s, 1H), 3.95 (s, 3H), 3.39 (s, 3H), 3.34 (s, 3H); ¹H NMR (400 MHz, MeOD-d₄) δ = 7.74 (s, 1H), 7.06 (s, 1H), 3.99 (s, 3H), 3.45 (s, 3H), 3.41 (s, 3H); LCMS (System A) RT = 0.69 min, 99%, ES+ve m/z 238 (M+H)⁺, 279 (M+H+MeCN)⁺.
5.4 2,3-Dichloro-N-(6-methoxy-1-methyl-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)benzenesulfonamide (13a)

A solution of 10a (20 mg, 0.09 mmol) in ethanol (2 mL) and ethyl acetate (2 mL) was hydrogenated over 10% palladium on carbon (5 mg). The catalyst was collected by filtration through celite, and washed with ethanol. The filtrate and washings were evaporated under reduced pressure and the residue was dissolved in anhydrous pyridine (0.6 mL). 2,3-Dichlorobenzenesulfonyl chloride (20 mg, 0.09 mmol) was added and stirred for 1 h. The reaction mixture was evaporated under reduced pressure and the residue was purified by MDAP. The solvent was evaporated under reduced pressure to afford 13a (9 mg, 25%) as a white solid: \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta = 10.57\) (s, 1H), 9.61 (br s, 1H), 7.86 (dd, \(J = 8.0, 1.5\) Hz, 1H), 7.69 (dd, \(J = 8.0, 1.5\) Hz, 1H), 7.39 (t, \(J = 8.0\) Hz, 1H), 6.80 (s, 1H), 6.72 (s, 1H), 3.39 (s, 3H), 3.21 (s, 3H); LCMS (System A) RT = 0.89 min, 95%, ES+ve \(m/z\) 402, 404 (M+H)+, 443, 445, 447 (M+H+MeCN)+.

5.5 2,3-Dichloro-N-(6-methoxy-1,3-dimethyl-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)benzenesulfonamide (15a)

Was prepared from 12a (30 mg, 0.13 mmol) according to the procedure described for the preparation of 13a. Obtained 15a as a white solid (20 mg, 38%): \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta = 9.67\) (br s, 1H), 7.87 (dd, \(J = 8.0, 1.5\) Hz, 1H), 7.70 (dd, \(J = 8.0, 1.5\) Hz, 1H), 7.38 (t, \(J = 8.0\) Hz, 1H), 7.01 (s, 1H), 6.77 (s, 1H), 3.35 (s, 3H), 3.27 (s, 3H), 3.26 (s, 3H); LCMS (System A) RT = 0.95 min, 93%, ES+ve \(m/z\) 416, 418,420 (M+H)+, 457, 459, 461 (M+H+MeCN)+.

5.6 2-(6-Methoxy-5-nitro-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)acetamide (10b) and 2-(5-methoxy-6-nitro-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)acetamide (11b)
were prepared from 9 (200 mg, 0.956 mmol) and 2-bromoacetamide (132 mg, 0.956 mmol) according to the procedure described for the preparation of 13a. A 9:1 mixture of 10b and 11b (90 mg, 32%) was obtained as a yellow solid: 1H NMR (400 MHz, DMSO-\textit{d}_6) major $\delta = 11.11$ (br s, 1H), 7.62 (br s, 1H), 7.52 (s, 1H), 7.22 (br s, 1H), 7.11 (s, 1H), 4.48 (s, 2H), 3.89 (s, 3H); MS ES+ve m/z 267 (M+H)$^+$.  

5.7 2-(5-(2,3-Dichlorophenylsulfonamido)-6-methoxy-2-oxo-2,3-dihydro-1H-benzo[\textit{d}]imidazol-1-yl)acetamide (13b) N19663-84-3 and 2-(6-(2,3-dichlorophenylsulfonamido)-5-methoxy-2-oxo-2,3-dihydro-1H-benzo[\textit{d}]imidazol-1-yl)acetamide (14b)  

were prepared from a mixture of 10b and 11b (9:1) (60 mg, 0.22 mmol) according to the procedure described for the preparation of 13a. Obtained 13b (18 mg, 18%) as a white solid: mp = 301–304 °C; 1H NMR (400 MHz, DMSO-\textit{d}_6) $\delta = 10.59$ (br s, 1H), 9.61 (br s, 1H), 7.87 (d, $J = 8.0$ Hz, 1H), 7.72 (dd, $J = 8.0$, 1.5 Hz, 1H), 7.52 (br s, 1H), 7.40 (t, $J = 8.0$ Hz, 1H), 7.11 (br s, 1H), 6.79 (s, 1H), 6.66 (s, 1H), 4.31 (s, 2H), 3.36 (s, 3H); 1H NMR (400 MHz, MeOD-\textit{d}_4) $\delta = 7.79$ – 7.66 (m, 2H), 7.27 (t, $J = 8.0$ Hz, 1H), 7.13 (s, 1H), 6.59 (s, 1H), 4.47 (s, 2H), 3.52 (s, 3H); HRMS (ESI) calcd. for C_{16}H_{15}Cl_2N_4O_5S (M+H)$^+$ 445.0140; found = 445.0152; LCMS (System A) RT = 0.80 min, 95%, ES+ve m/z 445, 447, 449 (M+H)$^+$. and 14b (4 mg, 4%) as a white solid: 1H NMR (400 MHz, MeOD-\textit{d}_4) $\delta = 7.86$ – 7.60 (m, 2H), 7.27 (t, $J = 8.0$ Hz, 1H), 7.04 (s, 1H), 6.59 (s, 1H), 4.48 (s, 2H), 3.51 (s, 3H); LCMS (System A) RT = 0.79 min, 95%, ES+ve m/z 445, 447, 449 (M+H)$^+$.  

5.8 1-Isopropyl-5-methoxy-6-nitro-1H-benzo[\textit{d}]imidazol-2(3H)-one (10c)
From 9 according to the procedure described for the preparation of 10a: $^1$H NMR (400 MHz, DMSO-$d_6$) δ = 11.10 (br s, 1H), 7.50 (s, 1H), 7.13 (s, 1H), 4.62 (spt, $J$ = 7.0 Hz, 1H), 3.95 (s, 3H), 1.47 (d, $J$ = 7.0 Hz, 6H); MS ES+ve m/z 252 (M+H)$^+$, 293 (M+H+MeCN)$^+$.

5.9 2,3-Dichloro-N-(1-isopropyl-6-methoxy-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)benzenesulfonamide (13c)

From 10c according to the procedure described for the preparation of 13a afforded 13c (13 mg, 70%) as a white solid: $^1$H NMR (DMSO-$d_6$, 600 MHz) δ 10.54 (s, 1H), 9.63 (br s., 1H), 7.92 – 7.82 (m, 1H), 7.73 (dd, $J$=8.1, 1.5 Hz, 1H), 7.41 (t, $J$=8.1 Hz, 1H), 6.78 (s, 1H), 6.76 (s, 1H), 4.48 (spt, $J$=6.9 Hz, 1H), 3.41 (s, 3H), 1.39 (d, $J$=6.8 Hz, 6H); LCMS (System A) RT = 1.00 min, 100%, ES+ve m/z 430, 432, 434 (M+H)$^+$.

5.10 2,3-Dichloro-N-(6-methoxy-1-(oxetan-3-yl)-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)benzenesulfonamide (13d)

as a white solid: $^1$H NMR (400 MHz, DMSO-$d_6$) δ = 10.70 (br s, 1H), 9.69 (br s, 1H), 7.87 (d, $J$ = 8.0 Hz, 1H), 7.73 (dd, $J$ = 8.0, 1.5 Hz, 1H), 7.41 (t, $J$ = 8.0 Hz, 1H), 6.96 (s, 1H), 6.84 (s, 1H), 5.46 – 5.33 (m, 1H), 5.05 (t, $J$ = 6.5 Hz, 2H), 4.86 (m, 2H), 3.44 (s, 3H); LCMS (System B) RT = 0.93 min, 100%, ES+ve m/z 444, 446, 448 (M+H)$^+$.

5.11 N-Allyl-5-methoxy-2-nitroaniline (17)

Allylamine (2.19 mL, 29.2 mmol) was added to 2-fluoro-4-methoxy-1-nitrobenzene (16) (2.5 g, 15 mmol) and the reaction was stirred at ambient temperature for 1.5 h. The reaction mixture was concentrated under reduced pressure to remove excess allylamine. The residue was dissolved in ethyl acetate (300 mL) and washed with HCl (1M, 150 mL), followed by saturated aqueous solution of sodium bicarbonate (100 mL), water (50 mL), dried, and
evaporated under reduced pressure to afford 17 (2.94 g, 97%) as a yellow solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 8.47$ (t, $J = 5.0$ Hz, 1H), 8.04 (d, $J = 9.0$ Hz, 1H), 6.34 – 6.28 (m, 2H), 5.99 – 5.88 (m, 1H), 5.27 (dd, $J = 17.0$, 1.5 Hz, 1H), 5.20 (dd, $J = 10.3$, 1.5 Hz, 1H), 4.08 – 4.03 (m, 2H), 3.83 (s, 3H); MS ES+ve m/z 209 (M+H)$^+$, 250 (M+H+MeCN)$^+$.  

5.12 N1-allyl-5-methoxybenzene-1,2-diamine (18)

Iron powder (3.5 g, 325 mesh, 62 mmol) was added to a solution of 17 (2.6 g, 12 mmol) in ethanol (70 mL) and water (35 mL). Ammonium chloride (1.8 g, 33 mmol) was added in one portion, and the reaction mixture was refluxed for 24 h. Ethanol was removed under reduced pressure, and the residue was partitioned between ethyl acetate (500 mL) and saturated aqueous sodium bicarbonate solution (500 mL). The organic layer was separated and the aqueous layer was extracted with more ethyl acetate ($2 \times 300$ mL). The combined organic extracts were washed with brine ($2 \times 200$ mL), dried, and concentrated under reduced pressure to afford 18 (1.84 g, 83%) as a dark purple oil: LCMS (System A) RT = 0.50 min, 80%, ES+ve m/z 179 (M+H)$^+$  A portion (40 mg) of the product was purified by MDAP to give 18 (22 mg) as an orange oil: $^1$H NMR (400 MHz, CDCl$_3$) $\delta = 8.16$ (br s, 1H), 6.72 (d, $J = 7.0$ Hz, 1H), 6.32 – 6.16 (m, 2H), 6.05 – 5.92 (m, 1H), 5.47 – 5.24 (m, 5H), 5.19 (d, $J = 10.0$ Hz, 1H), 3.76 (s, 3H); LCMS (System A) RT = 0.50 min, 91%, ES+ve m/z 179 (M+H)$^+$.  

5.13 1-Allyl-6-methoxy-1H-benzo[d]imidazol-2(3H)-one (19)

CDI (110 mg, 0.68 mmol) was added to a stirring solution of 18 (110 mg, 0.62 mmol) in THF (2 mL). The reaction mixture was heated to 50 °C under nitrogen for 2 h, concentrated, and purified by MDAP to give 19 (57 mg, 45%) as a pale purple solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 10.63$ (br s, 1H), 6.87 (d, $J = 8.5$ Hz, 1H), 6.67 (d, $J = 2.5$ Hz, 1H), 6.56 (dd, $J
= 8.5, 2.5 Hz, 1H), 5.92 – 5.81 (m, 1H), 5.15 (dd, J = 10.0, 1.5 Hz, 1H), 5.09 (dd, J = 17.0, 1.5 Hz, 1H), 4.38 (m, 2H), 3.72 (s, 3H); MS ES+ve m/z 205 (M+H)+, 246 (M+H+MeCN)+.

5.14 1-Allyl-6-methoxy-5-nitro-1H-benzo[d]imidazol-2(3H)-one (21)

Nitric acid (4M, 66.5 mL) was cooled to 0 ºC and added slowly to 19 (1.9 g, 9.3 mmol). The reaction mixture was stirred for 1 h at 0 ºC and then allowed to warm to ambient temperature and stirred for 3 h. Some of the starting material dropped out of solution and formed a gum. Glacial acetic acid (5 mL) was added and the reaction was stirred at ambient temperature for 2 h. The reaction mixture was poured onto 30 mL of iced-water and was neutralised with saturated sodium bicarbonate solution. The product was extracted with ethyl acetate (2 × 300 mL), and the combined organic extracts were washed with brine (100 mL), dried and evaporated under reduced pressure. The residue was purified by chromatography on silica (100 g cartridge) eluting with a gradient of 0-100% ethyl acetate–cyclohexane over 60 min. The appropriate fractions were combined and evaporated under reduced pressure to afford 21 (0.53 g, 23%) as a brown solid: 1H NMR (400 MHz, DMSO-d6) δ = 11.17 (br s, 1H), 7.54 (s, 1H), 7.06 (s, 1H), 6.27 – 5.62 (m, 1H), 5.41 – 4.85 (m, 2H), 4.49 (m, 2H), 3.91 (s, 3H); MS ES+ve m/z 250 (M+H)+, 291 (M+H+MeCN)+.

5.15 1-Allyl-6-methoxy-3-methyl-5-nitro-1H-benzo[d]imidazol-2(3H)-one (22)

Sodium hydride (18 mg of a 60% w/w dispersion in mineral oil, 0.46 mmol) was added to a stirring solution of 21 (105 mg, 0.42 mmol) in anhydrous DMF (1 mL) at 0 ºC. The mixture was stirred for 20 min, then methyl iodide (0.03 mL, 0.46 mmol) was added and the reaction mixture was allowed to warm to ambient temperature over 2 h. The reaction mixture was quenched with water (20 mL) and the product was extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were washed with water (20 mL), brine (2 × 20 mL), dried
and evaporated under reduced pressure to afford 22 (110 mg, 99%) as an orange solid: $^1H$
NMR (400 MHz, DMSO-$d_6$) $\delta = 7.83$ (s, 1H), 7.13 (s, 1H), 5.97 – 5.86 (m, 1H), 5.18 (dd, $J = 10.5$, 1.5 Hz, 1H), 5.13 (dd, $J = 18.5$, 1.5 Hz, 1H), 4.54 (m, 2H), 3.92 (s, 3H), 3.36 (s, 3H); MS ES+ve $m/z$ 264 (M+H)$^+$, 305 (M+H+MeCN)$^+$. 

5.16 5-Methoxy-1-methyl-6-nitro-1H-benzo[d]imidazol-2(3H)-one (11a)
Compound 22 (30 mg, 0.11 mmol), dichloro(2,6,10-dodecatriene-1,12-diyl)ruthenium(IV) (24) (2.2 mg, 6.8 µmol) and potassium periodate (52 mg, 0.23 mmol) were suspended in water (1 mL) and stirred in a sealed vessel at 100 °C for 72 h. Ethyl acetate (10 mL) was added and the organic layer was separated, washed with brine (10 mL), dried and evaporated under reduced pressure. The residue was purified by MDAP to give 11a (10 mg, 39%) as a yellow solid: $^1H$ NMR (400 MHz, MeOD-$d_4$) $\delta = 7.70$ (s, 1H), 6.93 (s, 1H), 3.94 (s, 3H), 3.38 (s, 3H), the NH was not observed due to exchange; MS ES+ve $m/z$ 224 (M+H)$^+$, 265 (M+H+MeCN)$^+$. 

5.17 2,3-Dichloro-N-(6-methoxy-3-methyl-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)benzenesulfonamide (14a)
From 11a according to the procedure described for the preparation of 13a afforded 14a (4 mg, 22%) as a white solid: $^1H$ NMR (400 MHz, CDCl$_3$) $\delta = 9.03$ (br, 1H), 7.82 (dd, $J = 8.0$, 1.5 Hz, 1H), 7.61 (dd, $J = 8.0$, 1.5 Hz, 1H), 7.47 (br s, 1H), 7.20 (t, $J = 8.0$ Hz, 1H), 7.18 (s, 1H), 6.52 (s, 1H), 3.61 (s, 3H), 3.38 (s, 3H); LCMS (System A) RT = 0.88 min, 96%, ES+ve $m/z$ 443, 445, 447 (M+H+MeCN)$^+$. 

5.18 2,3-Dichloro-N-(6-methoxy-2-oxo-1-propyl-2,3-dihydro-1H-benzo[d]imidazol-5-yl)benzenesulfonamide (13e)
From 21 according to the procedure described for the preparation of 13a afforded 13e (5 mg, 7%) as a beige coloured solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 10.55$ (br s, 1H), 9.60 (br, 1H), 7.87 (dd, $J = 8.0$, 1.5 Hz, 1H), 7.72 (dd, $J = 8.0$, 1.5 Hz, 1H), 7.40 (t, $J = 8.0$ Hz, 1H), 6.79 (s, 1H), 6.74 (s, 1H), 3.67 (t, $J = 7.0$ Hz, 2H), 3.40 (s, 3H), 1.60 (sxt, $J = 7.0$ Hz, 2H), 0.83 (t, $J = 7.0$ Hz, 3H); LCMS (System A) RT = 1.00 min, 100%, ES+ve $m/z$ 430, 432, 434 (M+H)$^+$; 471, 473, 475 (M+H+MeCN)$^+$.

5.19 $N_1$-(5-Methoxy-2-nitrophenyl)-$N_2$,$N_2$-dimethylethane-1,2-diamine (25f)

$N_1$,$N_1$-Dimethylaminoethylamine (0.96 mL, 8.8 mmol) was added to a solution of 16 (1.0 g, 5.8 mmol), in anhydrous DMF (2 mL). DIPEA (2.0 mL, 12 mmol) was added and the reaction mixture was stirred under nitrogen at ambient temperature for 3 h. The reaction mixture was diluted with ethyl acetate (50 mL) and the organic layer was washed with water (2 $\times$ 50 mL), brine (50 mL), dried and evaporated under reduced pressure to afford 25f (1.45 g, 93%) as a yellow solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 8.50$ (br s, 1H), 8.02 (d, $J = 9.5$ Hz, 1H), 6.35 – 6.28 (m, 2H), 3.87 (s, 3H), 3.41 – 3.36 (m, 2H), 2.55 (t, $J = 6.0$ Hz, 2H), 2.22 (s, 6H); MS ES+ve $m/z$ 240 (M+H)$^+$.

5.20 2-((5-Methoxy-2-nitrophenyl)amino)propane-1,3-diol (25g)

Was similarly prepared to give 25g (1.3 g, 83%) as a yellow solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 8.57$ (d, $J = 8.0$ Hz, 1H), 8.02 (d, $J = 9.5$ Hz, 1H), 6.46 (d, $J = 2.5$ Hz, 1H), 6.30 (dd, $J = 9.5$, 2.5 Hz, 1H), 4.95 (t, $J = 5.5$ Hz, 2H), 3.86 (s, 3H), 3.77 – 3.68 (m, 1H), 3.67 – 3.48 (m, 4H); MS ES+ve $m/z$ 243 (M+H)$^+$.

5.21 N-(5-Methoxy-2-nitrophenyl)-2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecan-6-amine (25g-bis-TBMS ether)
TBDMS-Cl (2.3 g, 15 mmol) and imidazole (1.0 g, 15 mmol) was added to a solution of 25g (1.3 g, 6.8 mmol) in anhydrous DMF (15 mL) and the reaction mixture was stirred under nitrogen at ambient temperature for 3 h. The reaction mixture was partitioned between water (50 mL) and ethyl acetate (3 × 50 mL). The organic extracts were washed with brine (50 mL), dried, and evaporated under reduced pressure to afford 25g-bis-TBMS ether (3.38 g, 95%) as a yellow oil: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 8.60$ (d, $J = 8.5$ Hz, 1H), 8.02 (d, $J = 9.5$ Hz, 1H), 6.44 (d, $J = 2.5$ Hz, 1H), 6.30 (dd, $J = 9.5, 2.5$ Hz, 1H), 4.06 – 3.93 (m, 1H), 3.85 (s, 3H), 3.83 – 3.77 (m, 2H), 3.77 – 3.62 (m, 2H), 0.87 (s, 18H), 0.04 (s, 6H), 0.03 (s, 6H); MS ES+ve $m/z$ 471 (M+H)$^+$. 

5.22 tert-Butyl 3-((5-methoxy-2-nitrophenyl)amino)azetidine-1-carboxylate (N-BOC-25h)

Was prepared according to the procedure described for the preparation of 25f to give 25h (819 mg, 84%) as a yellow gum: $^1$H NMR (400 MHz, CDCl$_3$) $\delta = 8.37$ (d, $J = 4.5$ Hz, 1H), 8.16 (d, $J = 9.5$ Hz, 1H), 6.29 (dd, $J = 9.5, 2.5$ Hz, 1H), 5.83 (d, $J = 2.5$ Hz, 1H), 4.38 – 4.24 (m, 3H), 3.89 – 3.82 (m, 2H), 3.83 (s, 3H), 1.43 (s, 9H); MS ES+ve $m/z$ 647 (2M+H)$^+$. 

5.23 N1-(2-(Dimethylamino)ethyl)-5-methoxybenzene-1,2-diamine (26f)

A solution of 25f (1.45 g, 6.06 mmol) in ethanol (100 mL) was hydrogenated over 10% palladium on carbon (0.65 g) for 18 h. The catalyst was removed by filtration through a pad of celite, washed with ethanol (150 mL) and concentrated under reduced pressure to give 26f (1.15 g, 91%) as a dark purple oil: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 6.51$ (d, $J = 8.0$ Hz, 1H), 6.08 (d, $J = 2.5$ Hz, 1H), 6.03 (dd, $J = 8.0, 2.5$ Hz, 1H), 4.46 (m, 1H), 3.97 (br s, 2H), 3.64 (s, 3H), 3.11 – 3.04 (m, 2H), 2.54 – 2.45 (m, 2H), 2.21 (s, 6H).
5.24 5-Methoxy-N1-(2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecan-6-yl)benzene-1,2-diamine (26g-bis-TBMS ether)

(2.53 g, 70%) as a dark purple oil: \(^1\)H NMR (400 MHz, DMSO-\textit{d}_6) \(\delta = 6.58\) (d, \(J = 8.0\) Hz, 1H), 6.16 (d, \(J = 2.5\) Hz, 1H), 6.08 (dd, \(J = 8.5, 2.5\) Hz, 1H), 4.25 (d, \(J = 8.5\) Hz, 1H), 3.92 (s, 2H), 3.77 - 3.67 (m, 4H), 3.66 (s, 3H), 3.47 – 3.40 (m, 1H), 0.93 (s, 18H), 0.09 (s, 12H); MS ES+ve m/z 441 (M+H)

5.25 tert-Butyl 3-((2-amino-5-methoxyphenyl)amino)azetidine-1-carboxylate (N-BOC-26h)

as a dark solid (723 mg, 97%); \(^1\)H NMR (400 MHz, DMSO-\textit{d}_6) \(\delta = 9.13\) (br s, 2H), 7.05 (d, \(J = 8.5\) Hz, 1H), 6.30 (dd, \(J = 8.5, 2.5\) Hz, 1H), 6.20 (br s, 1H), 6.02 (d, \(J = 2.5\) Hz, 1H), 4.30 – 4.12 (m, 3H), 3.74 – 3.64 (m, 2H), 3.71 (s, 3H), 1.39 (s, 9H); MS ES+ve m/z 294 (M+H)

5.26 1-(2-(Dimethylamino)ethyl)-6-methoxy-1H-benzod[d]imidazol-2(3H)-one (27f)

A mixture of 26f (1.15 g, 5.49 mmol) and CDI (0.89 g, 5.49 mmol) in anhydrous THF (20 mL) was heated to 50 °C for 2 h. The mixture was concentrated under reduced pressure, and the residue was taken up in ethyl acetate (40 mL). The organic layer was washed with sodium bicarbonate solution (40 mL), brine (40 mL), dried, and evaporated under reduced pressure to afford 27f (1.26 g, 83%) as a dark purple solid: \(^1\)H NMR (400 MHz, DMSO-\textit{d}_6) \(\delta = 10.54\) (br s, 1H), 6.85 (d, \(J = 8.0\) Hz, 1H), 6.76 (d, \(J = 2.5\) Hz, 1H), 6.55 (dd, \(J = 8.0, 2.5\) Hz, 1H), 3.84 (t, \(J = 6.5\) Hz, 2H), 3.74 (s, 3H), 2.53 – 2.47 (m, 2H), 2.18 (s, 6H); MS ES+ve m/z 236 (M+H)

5.27 6-Methoxy-1-(2,2,3,3,9,9,10,10-octamethyl-4,8-dioxa-3,9-disilaundecan-6-yl)-1H-benzod[d]imidazol-2(3H)-one (27g-bis-TBMS ether)
(2.04 g, 69%) as a dark brown solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 10.56$ (br s, 1H), 6.81 (d, $J = 8.5$ Hz, 1H), 6.79 (d, $J = 2.5$ Hz, 1H), 6.53 (dd, $J = 8.5$, 2.5 Hz, 1H), 4.40 – 4.31 (m, 1H), 4.06 (dd, $J = 10.5$, 8 Hz, 2H), 3.97 (dd, $J = 10.5$, 6 Hz, 2H), 3.71 (s, 3H), 0.75 (s, 18H), -0.04 (s, 6H), -0.11 (s, 6H); MS ES+ve m/z 467 (M+H)$^+$. 

5.28 tert-Butyl 3-(5-methoxy-2-oxo-1H-benzo[d]imidazol-3(2H)-yl)azetidine-1-carboxylate (N-BOC-27f)

(620 mg, 79%) as a beige coloured solid: $^1$H NMR (400 MHz, CDCl$_3$) $\delta = 8.61$ (br s, 1H), 6.99 (d, $J = 8.5$ Hz, 1H), 6.98 (d, $J = 2.5$ Hz, 1H), 6.69 (dd, $J = 8.5$, 2.5 Hz, 1H), 5.34 – 5.24 (m, 1H), 4.52 – 4.43 (m, 2H), 4.44 – 4.35 (m, 2H), 3.83 (s, 3H), 1.51 (s, 9H); MS ES+ve m/z 320 (M+H)$^+$, 639 (2M+H)$^+$. 

5.29 1-(2-(Dimethylamino)ethyl)-6-methoxy-5-nitro-1H-benzo[d]imidazol-2(3H)-one (10f)

A suspension of 27f (1.26 g, 4.55 mmol) in nitric acid (4M, 60 mL) was stirred at ambient temperature for 22 h. The reaction mixture was poured over ice (50 mL), neutralised with solid sodium bicarbonate, and extracted with ethyl acetate (2 × 150 mL). The combined organic extracts were then washed with brine (100 mL), dried, and evaporated under reduced pressure to afford 10f (750 mg, 53%) as a yellow solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 11.05$ (s, 1H), 7.51 (s, 1H), 7.15 (s, 1H), 4.00 – 3.88 (m, 2H), 3.94 (s, 3H), 2.55 (t, $J = 6.5$ Hz, 2H), 2.19 (s, 6H); MS ES+ve m/z 281(M+H)$^+$. 

5.30 1-(1,3-Dihydroxypropan-2-yl)-6-methoxy-5-nitro-1H-benzo[d]imidazol-2(3H)-one (10g)
(40 mg, 33%) as a yellow solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 11.03$ (br s, 1H), 7.49 (s, 1H), 7.14 (s, 1H), 4.87 (t, $J = 5.5$ Hz, 2H), 4.42 – 4.34 (m, 1H), 3.95 – 3.88 (m, 2H), 3.91 (s, 3H), 3.81 – 3.74 (m, 2H); MS ES+ve m/z 284 (M+H)$^+$.  

5.31 tert-Butyl 3-(6-methoxy-5-nitro-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)azetidine-1-carboxylate (N-BOC-10h)

4M Nitric acid (30 mL) was added to N-BOC-27h (620 mg, 1.94 mmol) and the suspension was shaken at ambient temperature until the solid dissolved. After a couple of minutes a fine powder started to precipitate, and the mixture was stirred for 4 h. The product was very soluble in water and was thus converted to the N-BOC protected analogue by treating the solution with solid sodium bicarbonate to pH 8, and then adding THF (20 mL) and Boc$_2$O (0.76 mL, 3.27 mmol). The mixture was stirred at ambient temperature for 48 h, and then extracted with ethyl acetate (3 × 50 mL). The combined organic extracts were washed with HCl (2M, 40 mL), sodium bicarbonate solution (40 mL), brine (40 mL), dried, and evaporated under reduced pressure. The residue was purified by column chromatography on silica (100 g cartridge) eluting with 0–50% ethyl acetate–cyclohexane for 30 min. The solvent was evaporated under reduced pressure to afford N-BOC-10h (368 mg, 52%) as a dark yellow solid: $^1$H NMR (400 MHz, CDCl$_3$) $\delta = 9.01$ (s, 1H), 7.77 (s, 1H), 7.14 (s, 1H), 5.28 (m, 1H), 4.54 – 4.36 (m, 4H), 3.99 (s, 3H), 1.51 (s, 9H); MS ES+ve m/z 365 (M+H)$^+$.  

5.32 5-Amino-1-(2-(dimethylamino)ethyl)-6-methoxy-1H-benzo[d]imidazol-2(3H)-one (28f)

A solution of 10f (0.75 g, 2.4 mmol) in a mixture of ethanol (50 mL) and ethyl acetate (50 mL) was hydrogenated over 10% palladium on carbon (0.77 g) for 18 h. The catalyst was collected by filtration through a pad of celite, washed with a mixture of ethanol and ethyl
acetate (1:1; 100 mL), and then the filtrate was concentrated under reduced pressure to afford 28f (0.63 g, 94%) as a brown oil: ¹H NMR (400 MHz, DMSO-d₆) δ = 10.21 (s, 1H), 6.70 (s, 1H), 6.38 (s, 1H), 4.40 (br s, 2H), 3.77 (t, J = 6.5 Hz, 2H), 3.75 (s, 3H), 2.48 (t, J = 6.5 Hz, 2H), 2.18 (s, 6H).

5.33 5-Amino-1-(1,3-dihydroxypropan-2-yl)-6-methoxy-1H-benzo[d]imidazol-2(3H)-one (28g)

From 10g (40 mg, 0.14 mmol) obtained 28g (28 mg, 78%) as a dark purple oil: ¹H NMR (400 MHz, DMSO-d₆) δ = 10.24 (s, 1H), 6.74 (s, 1H), 6.37 (s, 1H), 4.80 (br t, J = 5.5 Hz, 2H), 4.34 (br s, 2H), 4.19 (quin, t, J = 6.5 Hz, 1H), 3.87 – 3.74 (m, 4H), 3.73 (s, 3H).

5.34 tert-Butyl 3-(5-amino-6-methoxy-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)azetidine-1-carboxylate (N-BOC-28h)

From N-BOC-10h (367 mg, 1.00 mmol) obtained N-BOC-28h (321 mg, 95%) as a light brown solid: ¹H NMR (400 MHz, DMSO-d₆) δ = 10.94 (s, 1H), 10.0 – 9.30 (br, 2H), 7.10 (s, 2H), 5.23 – 5.14 (m, 1H), 4.49 – 4.33 (m, 2H), 4.23 (t, J = 9 Hz, 2H), 3.87 (s, 3H), 1.43 (s, 9H); MS ES+ve m/z 335 (M+H)⁺, 376 (M+H+MeCN)⁺.

5.35 2,3-Dichloro-N-(1-(2-(dimethylamino)ethyl)-6-methoxy-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)benzenesulfonamide (13f)

From 28f (100 mg, 0.40 mmol) and 2,3-dichlorobenzenesulfonyl chloride (108 mg, 0.44 mmol) according to the procedure described for the preparation of 13a afforded 13f (67 mg, 36%) as a beige coloured solid: ¹H NMR (400 MHz, DMSO-d₆) δ = 10.56 (s, 1H), 9.60 (br, 1H), 7.88 (dd, J = 8.0, 1.5 Hz, 1H), 7.72 (dd, J = 8.0, 1.5 Hz, 1H), 7.40 (t, J = 8.0 Hz, 1H), 6.78 (s, 1H), 6.74 (s, 1H), 3.80 (t, J = 6.5 Hz, 2H), 3.40 (s, 3H), 2.46 (t, J = 6.5 Hz, 2H), 2.15
(s, 6H); $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ = 154.6, 150.0, 140.7, 134.2, 134.0, 130.0, 129.9, 129.6, 128.0, 121.3, 117.3, 109.5, 93.8, 56.7, 56.0, 45.4, 38.3.

HRMS (ESI) calcd. for C$_{18}$H$_{21}$Cl$_2$N$_4$O$_4$S (M+H)$^+$ 459.0661; found = 459.0654; LCMS (System A) RT = 0.69 min, 90%, ES+ve m/z 459, 461, 463 (M+H)$^+$.

5.36 2,3-Dichloro-N-(1-(1,3-dihydroxypropan-2-yl)-6-methoxy-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)benzenesulfonamide (13g)

(21 mg, 48%) as a white solid; $^1$H NMR (400 MHz, DMSO-$d_6$) δ = 10.55 (s, 1H), 9.59 (br s, 1H), 7.88 (dd, $J$ = 8.0, 1.5 Hz, 1H), 7.73 (dd, $J$ = 8.0, 1.5 Hz, 1H), 7.41 (t, $J$ = 8.0 Hz, 1H), 6.76 (s, 2H), 4.77 (t, $J$ = 5.5 Hz, 2H), 4.26 – 4.17 (m, 1H), 3.86 – 3.77 (m, 2H), 3.76 – 3.67 (m, 2H), 3.38 (s, 3H). HRMS (ESI) calcd. for C$_{17}$H$_{18}$Cl$_2$N$_3$O$_6$S (M+H)$^+$ 462.0293; found 462.0295; LCMS (System A) RT = 0.77 min, 100%, ES+ve m/z 462, 464, 466 (M+H)$^+$.

5.37 tert-Butyl 3-(5-(2,3-dichlorophenylsulfonamido)-6-methoxy-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-1-yl)azetidine-1-carboxylate (N-BOC-13h)

From N-BOC-28h (321 mg, 0.96 mmol) and 2,3-dichlorobenzenesulfonyl chloride (286 mg, 1.17 mmol) according to the procedure described for the preparation of 13a. (300 mg, 57%) as a colourless oil: $^1$H NMR (400 MHz, CDCl$_3$) δ = 9.99 (br, 1H), 7.77 (d, $J$ = 8.0 Hz, 1H), 7.58 (s, 1H), 7.56 (d, $J$ = 8.0 Hz, 1H), 7.17 (t, $J$ = 8.0 Hz, 1H), 6.77 (s, 1H), 5.25 (s, 1H), 5.23 – 5.16 (m, 1H), 4.39 – 4.28 (m, 4H), 3.60 (s, 3H), 1.43 (s, 9H); MS ES+ve m/z 543, 545, 547 (M+H)$^+$.

5.38 N-(1-(Azetidin-3-yl)-6-methoxy-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)-2,3-dichlorobenzenesulfonamide hydrochloride (13h)
4M HCl solution in dioxane (4 mL, 16 mmol) was added to a solution of \textit{N-BOC-13h} (300 mg, 0.552 mmol) in chloroform (1 mL), and the mixture was stirred at ambient temperature for 1.5 h. The white precipitate was collected by filtration to afford \textit{13h} (125 mg, 51%) as an off-white solid: \textsuperscript{1}H NMR (400 MHz, CD\textsubscript{3}OD) \(\delta = 7.72\) (br t, \(J = 8\) Hz, 2H), 7.26 (t, \(J = 8.0\) Hz, 1H), 7.14 (s, 1H), 6.75 (s, 1H), 5.31 – 5.22 (m, 1H), 4.85 – 4.79 (m, 2H), 4.45 (br t, \(J = 9\) Hz, 2H), 3.65 (s, 3H); LCMS (System A) RT = 0.68 min, 91%, ES+ve \textit{m/z} 443, 445, 447 (M+H).  

5.39 \textit{N-(1-(1-Acety lazetidin-3-yl)-6-methoxy-2-oxo-2,3-dihydro-1H-benzo[d]imidazol-5-yl)-2,3-dichlorobenzenesulfonamide (13i)}  
Acetic anhydride (50 µL, 0.53 mmol) was added to a solution of \textit{13h} (19 mg, 0.04 mmol) in pyridine (0.5 mL). The mixture was stirred at ambient temperature for 3 h; LCMS analysis showed that di-acetylation had occurred. MeOH (7.5 mL) and K\textsubscript{2}CO\textsubscript{3} (60 mg, 0.43 mmol) were added to the mixture, and after stirring for 24 h, 2M aq. HCl solution was added to pH 1. The mixture was extracted using ethyl acetate (20 mL) and the organic layer was washed with brine (20 mL), dried and concentrated under reduced pressure. The solid was dissolved in DMSO (1 mL), and purified by MDAP. Appropriate fractions were evaporated under reduced pressure to afford \textit{13i} (5 mg, 25%) as a white solid: \textsuperscript{1}H NMR (400 MHz, CD\textsubscript{3}OD) \(\delta = 7.73\) (m, 2H), 7.28 (t, \(J = 8.0\) Hz, 1H), 7.14 (s, 1H), 6.71 (s, 1H), 5.22 – 5.14 (m, 1H), 4.75 – 4.71 (m, 1H), 4.57 (t, \(J = 9.3\) Hz, 1H), 4.53 – 4.47 (m, 1H), 4.34 (t, \(J = 9.5\) Hz, 1H), 3.54 (s, 3H), 1.93 (s, 3H); LCMS (System A) RT = 0.82 min, 98%, ES+ve \textit{m/z} 485, 487, 489 (M+H).  

5.40 \textit{2,6-Dibromopyridin-3-ol (30)}
An ice-cold solution of bromine (16.2 mL, 315 mmol) in sodium hydroxide (2.5 M, 320 mL, 800 mmol) was added dropwise to a stirred solution of pyridin-3-ol (10.0 g, 105 mmol) in sodium hydroxide (2.5 M, 110 mL, 275 mmol). The solution was stirred at 0 °C for 1 h and then at room temp for 4 h. A small amount of precipitate that formed was removed by filtration. The filtrate was cooled, and concentrated hydrochloric acid was added until the pH reached 1. The solid was collected by filtration, washed with water, dried, and recrystallised from carbon tetrachloride to give 30 (12.1 g, 46%) as a beige coloured solid: \textsuperscript{1}H NMR (400 MHz, DMSO-d\textsubscript{6}) δ = 11.14 (br s, 1H), 7.47 (d, J = 8.0 Hz, 1H), 7.26 (d, J = 8.0 Hz, 1H); MS ES-ve m/z 250, 252, 254 (M-H\textsuperscript{-}).

5.41 2,6-Dibromo-3-methoxypyridine (31)

A suspension of 2,6-dibromopyridin-3-ol (12 g, 47 mmol), potassium carbonate (6.0 g, 43 mmol), methyl iodide (10.1 mL, 162 mmol) and DMSO (20 mL) was refluxed for 2 h. The reaction mixture was poured into water (60 mL) and was warmed gently at 50 °C with stirring for 30 min. The reaction mixture was cooled to room temperature and the precipitated solid was collected by filtration, and dried under vacuum. The residue was crystallised from cyclohexane to give 31 (10.1 g, 80%) as a pale orange solid: \textsuperscript{1}H NMR (400 MHz, DMSO-d\textsubscript{6}) δ = 7.66 (d, J = 8.0 Hz, 1H), 7.52 (d, J = 8.0 Hz, 1H), 3.90 (s, 3H); MS ES+ve m/z 266, 268, 270 (M+H\textsuperscript{+}).

5.42 2,6-Dibromo-3-methoxy-5-nitropyridine (32)

Fuming nitric acid (16M, 5 mL, 80 mmol) was added to a solution of 31 (2.0 g, 7.5 mmol) in sulfuric acid (18M, 8 mL, 144 mmol) at 0 °C, and then the mixture was heated at 65 °C for 18 h. Additional conc. nitric acid (2.5 mL, 40 mmol) was added and the reaction mixture was stirred at 65 °C for a further 18 h. The reaction mixture was cooled and cautiously
neutralised with solid sodium carbonate. The resulting mixture was extracted with ethyl acetate (3 × 50 mL), and the combined organic extracts were dried and concentrated under reduced pressure. The residue was purified by chromatography on a silica cartridge (100 g) eluting with a gradient of 0 – 25% ethyl acetate-cyclohexane over 60 min. The appropriate fractions were combined and evaporated under reduced pressure to give 32 (0.69 g, 28%) as a white solid: $^1$H NMR (400 MHz, DMSO-$d_6$) δ = 8.24 (s, 1H), 4.00 (s, 3H); LCMS (System A) RT=1.05 min, 98%, no mass ions observed in either +ve or –ve mode.

5.43 3-Methoxy-5-nitropyridine-2,6-diamine (33)

A solution of 32 (0.5 g, 1.6 mmol) in aqueous ammonia (15M, 12 mL, 180 mmol) was heated at 90 °C for 1 h in a microwave oven. The reaction mixture was cooled, the yellow solid was collected by filtration, washed with a small amount of water, and dried under vacuum to afford 33 (230 mg, 78%) as a yellow solid: $^1$H NMR (400 MHz, DMSO-$d_6$, 393 K) δ = 7.48 (s, 1H), 7.35 (br, 2H), 6.81 (br, 2H), 3.80 (s, 3H); MS ES+ve m/z 185 (M+H)$^+$.  

5.44 Di-tert-butyl (3-methoxy-5-nitropyridine-2,6-diyl)dicarbamate (34)

Di-tert-butyl dicarbonate (0.76 mL, 3.3 mmol) and K$_2$CO$_3$ (560 mg, 4.10 mmol) were added to a solution of 33 (150 mg, 0.82 mmol) in DMF (4 mL). The reaction mixture was stirred at ambient temperature for 18 h, and then diluted with water (20 mL) and ethyl acetate (40 mL). The organic layer was separated, washed with water (2 × 10 mL), brine (10 mL), dried, and concentrated under reduced pressure. The residue was purified by chromatography on a silica cartridge (50 g) eluting with a gradient of 0 – 50% ethyl acetate-cyclohexane over 40 min. The appropriate fractions were combined and evaporated under reduced pressure to give 34 (210 mg, 66%) as a yellow solid: $^1$H NMR (400 MHz, DMSO-$d_6$) δ = 9.86 (s, 1H),
9.22 (s, 1H), 7.91 (s, 1H), 3.89 (s, 3H), 1.45 (s, 9H), 1.40 (s, 9H); MS ES+ve m/z 385 (M+H).  

5.45 Di-tert-butyl (3-amino-5-methoxypyrind-2,6-diyl)dicarbamate (35)

Iron powder (290 mg, 5.2 mmol) was added to a stirring solution of 34 (200 mg, 0.52 mmol) in glacial acetic acid (10 mL). The reaction mixture was stirred at ambient temperature for 2 h, and then concentrated under reduced pressure. The residue was partitioned between sodium bicarbonate solution (10 mL) and ethyl acetate (20 mL), the aqueous was extracted with further portion of ethyl acetate (20 mL), and the organic extracts were washed with brine (20 mL), dried, and concentrated under reduced pressure to give 35 (170 mg, 78%) as a yellow oil: $^1$H NMR (400 MHz, DMSO-$d_6$) δ = 8.58 (s, 1H), 8.23 (s, 1H), 6.81 (s, 1H), 4.81 (s, 2H), 3.70 (s, 3H), 1.43 (s, 9H), 1.39 (s, 9H); MS ES+ve m/z 355 (M+H).  

5.46 tert-Butyl (6-methoxy-2-oxo-2,3-dihydro-$1H$-imidazo[4,5-$b$]pyridin-5-yl)carbamate (36)

A solution of 35 (150 mg, 0.42 mmol) in dry pyridine (1.5 mL) was heated a microwave oven for 1 h at 100 ºC. The mixture was concentrated under reduced pressure to give 36 (110 mg, 74%) as a brown solid: $^1$H NMR (400 MHz, DMSO-$d_6$) δ = 11.01 (br s, 1H), 10.71 (br s, 1H), 8.44 (s, 1H), 7.04 (s, 1H), 3.75 (s, 3H), 1.41 (s, 9H); MS ES+ve m/z 281 (M+H).  

5.47 5-Amino-6-methoxy-$1H$-imidazo[4,5-$b$]pyridin-2(3$H$)-one (37)

A mixture of 36 (50 mg, 0.18 mmol) and TFA (0.25 mL) was stirred at ambient temperature for 30 min. The mixture was concentrated under reduced pressure, the residue was dissolved in methanol and dichloromethane (1:1, 1 mL), and passed down an aminopropyl ion-exchange cartridge (500 mg), eluting with methanol and dichloromethane (1:1, 10 mL). The
fractions were concentrated under reduced pressure to give 37 (30 mg, 93%) as a brown solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 10.56$ (br s, 1H), 10.12 (s, 1H), 6.82 (s, 1H), 5.12 (s, 2H), 3.73 (s, 3H); MS ES+ve $m/z$ 181 (M+H)$^+$.  

5.48 2,3-Dichloro-$N$-(6-methoxy-2-oxo-2,3-dihydro-1H-imidazo[4,5-b]pyridin-5-yl)benzenesulfonamide (38)  
From 37 (30 mg, 0.17 mmol) and 2,3-dichlorobenzenesulfonyl chloride (45 mg, 0.18 mmol) gave 38 (20 mg, 29%) as a white solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 11.00$ (s, 1H), 10.77 (s, 1H), 10.21 (s, 1H), 7.94 (dd, $J = 8.0$, 1.3 Hz, 1H), 7.90 (dd, $J = 8.0$, 1.3 Hz, 1H), 7.51 (t, $J = 8.0$ Hz, 1H), 7.02 (s, 1H), 3.58 (s, 3H); LCMS (System A) RT = 0.76 min, 100%, ES+ve $m/z$ 389, 391, 393 (M+H)$^+$.  

5.49 5-Methoxy-1H-imidazo[4,5-b]pyridin-2(3H)-one (40)  
CDI (1.25 g, 7.73 mmol) was added to a solution of 6-methoxypyridine-2,3-diamine (39) (1.0 g, 7.0 mmol) and triethylamine (2.0 mL, 14 mmol) in THF (13 mL), and the reaction was stirred for 2 h at ambient temperature under nitrogen. The reaction was evaporated under reduced pressure to remove THF, and the residue was triturated with ethyl acetate. The solid was collected by filtration, and further purified by chromatography on a silica cartridge (100 g) eluting with a gradient of 0 – 25% methanol-dichloromethane over 40 min. Appropriate fractions were evaporated under reduced pressure to give 40 (360 mg, 31%) as a white solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta = 11.18$ (br s, 1H), 10.54 (br s, 1H), 7.21 (d, $J = 8.5$ Hz, 1H), 6.35 (d, $J = 8.5$ Hz, 1H), 3.77 (s, 3H); MS ES+ve $m/z$ 166 (M+H)$^+$.  

5.50 5-Methoxy-6-nitro-1H-imidazo[4,5-b]pyridin-2(3H)-one (41)  
Compound 40 (266 mg, 1.61 mmol) was slowly added to trifluoroacetic anhydride (5.5 mL,
40 mmol) at 0 °C, and the reaction was stirred at this temperature for 1 h. Fuming nitric acid (0.14 mL of a 70 wt% aqueous solution, 1.63 mmol) was added dropwise and the reaction was stirred at 0 °C for a further 2 h. The reaction mixture was then slowly added to a solution of sodium metabisulfite (304 mg, 1.6 mmol) in water (14 mL) at 0 °C, and the precipitate formed was collected by filtration to give 41 (160 mg, 47%) as a bright yellow solid: \(^1H\) NMR (400 MHz, DMSO-\(d_6\)) \(\delta = 12.09\) (br s, 1H), 11.06 (br s, 1H), 7.85 (s, 1H), 3.98 (s, 3H); MS ES+ve \(m/z\) 211 (M+H)\(^+\); 228 (M+NH\(_4\))\(^+\).

5.51 6-Amino-5-methoxy-1H-imidazo[4,5-b]pyridin-2(3H)-one (42)

A solution of 41 (130 mg, 0.62 mmol) in ethanol (2 mL) was hydrogenated over 10% palladium on carbon (66 mg) for 2 h. The catalyst was removed by filtration through celite, washed with ethanol (30 mL), and the filtrate was concentrated under reduced pressure to give 42 (42 mg, 38%) as a yellow solid: \(^1H\) NMR (400 MHz, DMSO-\(d_6\)) \(\delta = 10.59\) (br s, 1H), 10.15 (s, 1H), 6.69 (s, 1H), 4.38 (s, 2H), 3.80 (s, 3H).

5.52 2,3-Dichloro-N-(5-methoxy-2-oxo-2,3-dihydro-1H-imidazo[4,5-b]pyridin-6-yl)benzenesulfonamide (43)

From 42 (23 mg, 0.13 mmol) and 2,3-dichlorobenzenesulfonyl chloride (35 mg, 0.14 mmol) gave 43 (24 mg, 48%) as a white solid: \(^1H\) NMR (400 MHz, DMSO-\(d_6\)) \(\delta = 11.23\) (br s, 1H), 10.52 (s, 1H), 9.90 (br s, 1H), 7.88 (dd, \(J = 8.0, 1.5\) Hz, 1H), 7.71 (dd, \(J = 8.0, 1.5\) Hz, 1H), 7.41 (t, \(J = 8.0\) Hz, 1H), 7.08 (s, 1H), 3.39 (s, 3H); \(^{13}C\) NMR (101 MHz, DMSO-\(d_6\)) \(\delta = 178.8, 154.4, 154.0, 140.4, 140.3, 134.1, 133.8, 129.7, 129.4, 127.8, 118.3, 117.1, 53.0\); Anal. Calcd. for C\(_{13}\)H\(_{11}\)Cl\(_2\)N\(_4\)O\(_4\)S = 388.9878. Found = 388.9881 (M+H)\(^+\); LCMS (System A) RT = 0.83 min, 100%, ES+ve \(m/z\) 389, 391, 393 (M+H)\(^+\).
5.53 5-Bromo-1H-imidazo[4,5-b]pyrazin-2(3H)-one (45)

A mixture of 5-bromopyrazine-2,3-diamine (44) (0.70 g, 3.7 mmol) and CDI (1.2 g, 7.4 mmol) in anhydrous THF (20 mL) was heated at 50 ºC for 22 h. More CDI (0.60 g, 3.7 mmol) was added and the reaction was stirred at 50 ºC for a further 24 h. The reaction mixture was concentrated under reduced pressure, and the residue was purified by chromatography on a silica cartridge (100 g), eluting with a gradient of 0 – 100% ethyl acetate-dichloromethane over 40 min. The appropriate fractions were combined and evaporated under reduced pressure to give 45 (0.68 g, 85%) as a white solid: ¹H NMR (400 MHz, DMSO-d₆) δ = 11.95 (br s, 2H), 7.97 (s, 1H); MS ES+ve m/z 215, 217 (M+H⁺).

5.54 5-Bromo-6-nitro-1H-imidazo[4,5-b]pyrazin-2(3H)-one (46)

Nitronium tetrafluoroborate (1.05 g, 7.91 mmol) was added in portions over 10 min to a suspension of 45 (0.68 g, 3.16 mmol) in MeCN (10 mL) and the mixture was stirred at ambient temperature for 1 h. The reaction mixture was poured over ice-water (40 mL), and the precipitate formed was collected by filtration, washed with water, and dried under reduced pressure to give 46 (0.41 g, 50%) as a yellow solid: ¹H NMR (400 MHz, DMSO-d₆) δ = 12.67 (br s, 1H), 12.50 (br s, 1H); ¹³C NMR (101 MHz, DMSO-d₆) 154.4, 146.2, 143.1, 138.1, 120.0; MS ES-ve m/z 258, 260 (M-H⁻).

5.55 5-Methoxy-6-nitro-1H-imidazo[4,5-b]pyrazin-2(3H)-one (47)

Sodium methoxide solution in methanol (25% w/v, 0.4 mL, 100 mg, 1.8 mmol) was added in portions to a solution of 46 (150 mg, 0.58 mmol) dissolved in MeOH (1 mL). The reaction was stirred at ambient temperature for 18 h and was then heated to 50 ºC and stirred at this temperature for a further 24 h. Water was added (1 mL) and the reaction mixture was diluted with DMSO (4 mL). The precipitated solid was removed by filtration. The filtrate was
diluted with more DMSO (2 mL), and purified by MDAP. The appropriate fractions were combined and the solvent was evaporated under reduced pressure to give 47 (60 mg, 49%) as a yellow solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ = 12.5 (br, 1H), 12.0 (br, 1H), 4.00 (s, 3H); MS ES-ve $m/z$ 210 (M-H$^-$).

5.56 5-Amino-6-methoxy-1H-imidazo[4,5-b]pyrazin-2(3H)-one (48)

A solution of 47 (60 mg, 0.28 mmol) in ethanol (2 mL) and ethyl acetate (2 mL) was hydrogenated over 10% palladium on carbon (91 mg) for 1 h. The catalyst was removed by filtration through celite, washed with a mixture of ethanol and ethyl acetate (1:1, 30 mL), and evaporated under reduced pressure to give 48 (41 mg, 80%) as a grey solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ = 11.9 (br s, 1H), 8.98 (s, 1H), 7.27 (s, 1H), 6.46 (s, 1H), 3.73 (s, 3H); MS ES+ve $m/z$ 182 (M+H$^+$).

5.57 2,3-Dichloro-N-(6-methoxy-2-oxo-2,3-dihydro-1H-imidazo[4,5-b]pyrazin-5-yl)benzenesulfonamide (49)

From 48 (41 mg, 0.23 mmol) and 2,3-dichlorobenzenesulfonyl chloride (70 mg, 0.28 mmol) to give 49 (8 mg, 7%) as a pale orange solid: $^1$H NMR (400 MHz, CD$_3$OD) $\delta$ = 8.06 (dd, $J$ = 8.0, 1.5 Hz, 1H), 7.77 (dd, $J$ = 8.0, 1.5 Hz, 1H), 7.44 (t, $J$ = 8.0 Hz, 1H), 3.83 (s, 3H); LCMS (System A) RT = 0.78 min, 88%; ES+ve $m/z$ 390, 392, 394 (M+H$^+$).

Acknowledgements. We thank Bill J. Leavens for collecting the HRMS data, and the Screening and Compound Profiling Department at GlaxoSmithKline for generating the human CCR4 GTP$\gamma$S data.
6. References


Table 1. In vitro data pIC$_{50}$ for humanCCR4 GTP$_{\gamma}$S binding assay, calculated logP, Ligand Efficiency, measured chrom logD at pH 7.4 and CLND solubility.

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