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Lead identification of benzimidazolone and azabenzimidazolone arylsulfonamides as CC-chemokine receptor 4 (CCR4) antagonists

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ABSTRACT

A knowledge-based library of 2,3-dichlorophenylsulfonyl derivatives of commercially available aryl amines was synthesised and screened as human CCR4 antagonists, in order to identify a suitable hit for the start of a lead-optimisation programme. Hits were required to be more potent than an existing indazole series, have better physicochemical properties ($c\log P$ <3.5, chrom $\log D_{7.4}$ <5.3 and CLND solubility >116 μg/mL), and be stable to acid and light. The benzimidazol-2-one core was identified as a hit suitable for further investigation. Substitution at N1 with small alkyl groups was tolerated; however, these analogues were inactive in the whole blood assay (pA_2 <5). Azabenzimidazolone analogues were all found to be active, with compound **38** exhibiting whole blood activity of 6.1, low molecular weight (389) and chrom $\log D_{7.4}$ (2.4), high LE (0.43), and solubility (152 μg/mL). In addition, **38** had human serum albumin binding of around 93% and met all the criteria for progression to lead optimisation.

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 (Fig. 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**–**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.[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.[25] Herein we disclose the identification of a second lead series, the benzimidazol-2-one sulfonamides.

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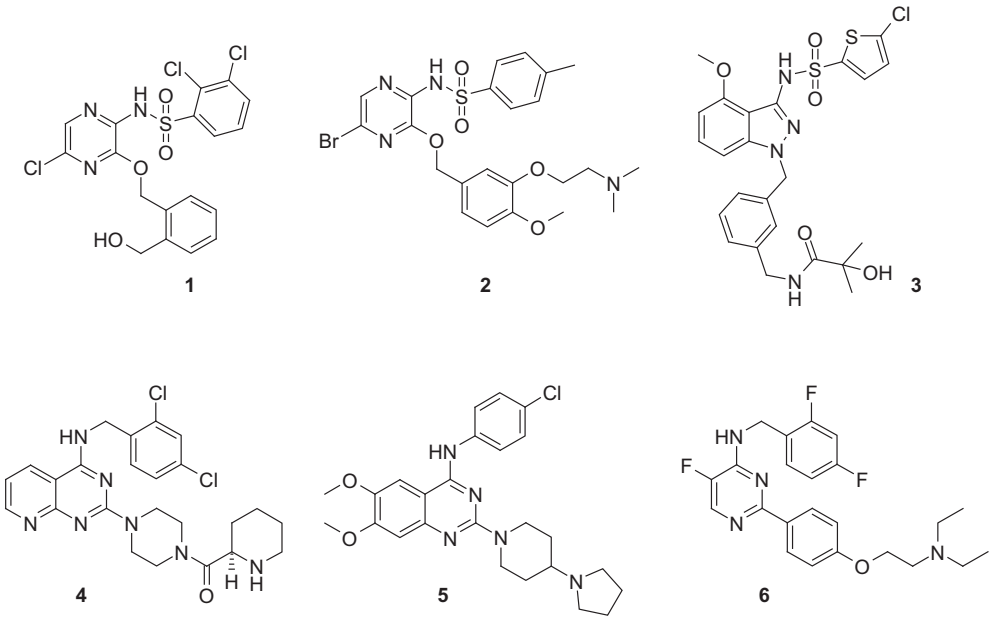


Figure 1. Structures for some recently published CCR4 antagonists.

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, *c*log*P* <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 pIC_{50} greater than that of the starting point of the indazole series (**7**) in the GTPγS assay, and also better physicochemical properties than **7** (*c*log*P* <3.5, chrom log$D_{7.4}$ <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 series[25] 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 (see Fig. 2).

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[22] that alkylation of the sulfonamide nitrogen provided compounds devoid of any CCR4 activity. It was considered that treatment of 5-methoxy-6-nitrobenzimidazol-2-one **9** with one equivalent of electrophile in the presence of a base might produce one major product

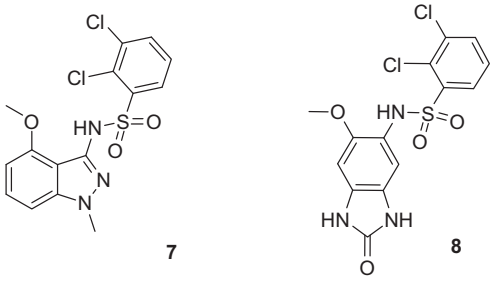


Figure 2. Initial indazole (**7**) and benzimidazolone (**8**) hits.

regioselectively as the acidity of the nitrogen atoms of the imidazolone ring was substantially different. The calculated pK_a values (ACD software, version 11) for N1 (*para* to the nitro-group) was 10.9 and for N3 (*para* to methoxy-group) was 17.1. Hence, treatment of **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 **10a** was obtained in 25% yield. The other mono-methyl product **11a** was present in the reaction mixture only in trace amounts. The dimethyl product **12a** was however also isolated in 14% yield, which suggested that product **10a** reacted further with iodomethane. The structure of the monomethyl regioisomer **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 *N*-methyl protons (3.34 ppm) and *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 (Fig. 3).

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**.

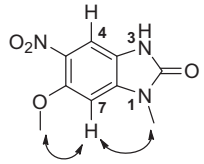


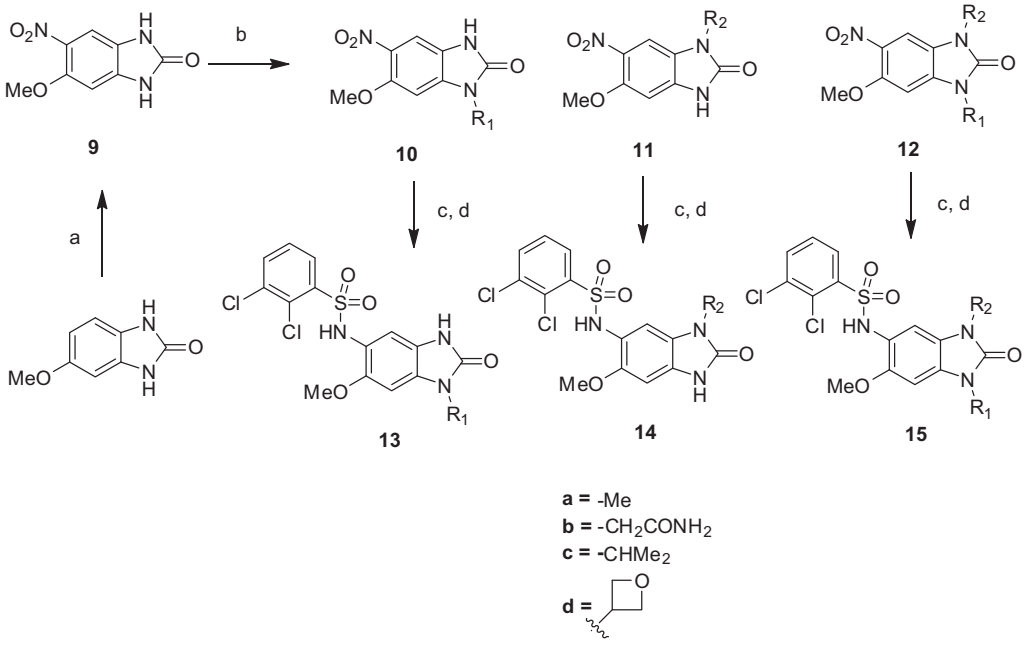
Figure 3. NOE confirmation of the structure of *N*1-methyl regioisomer **10a**. The numbering of the N1-position in relation to the MeO- and nitro-groups shown above could change depending on the substituent used, so throughout the report, the N1-position as shown above is used as an arbitrary number for ease of discussion.

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 4 M 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 $RuCl_3$ catalyst, followed by hydrolysis of the resulting enamine, were all found to be unsuccessful.[27–29]

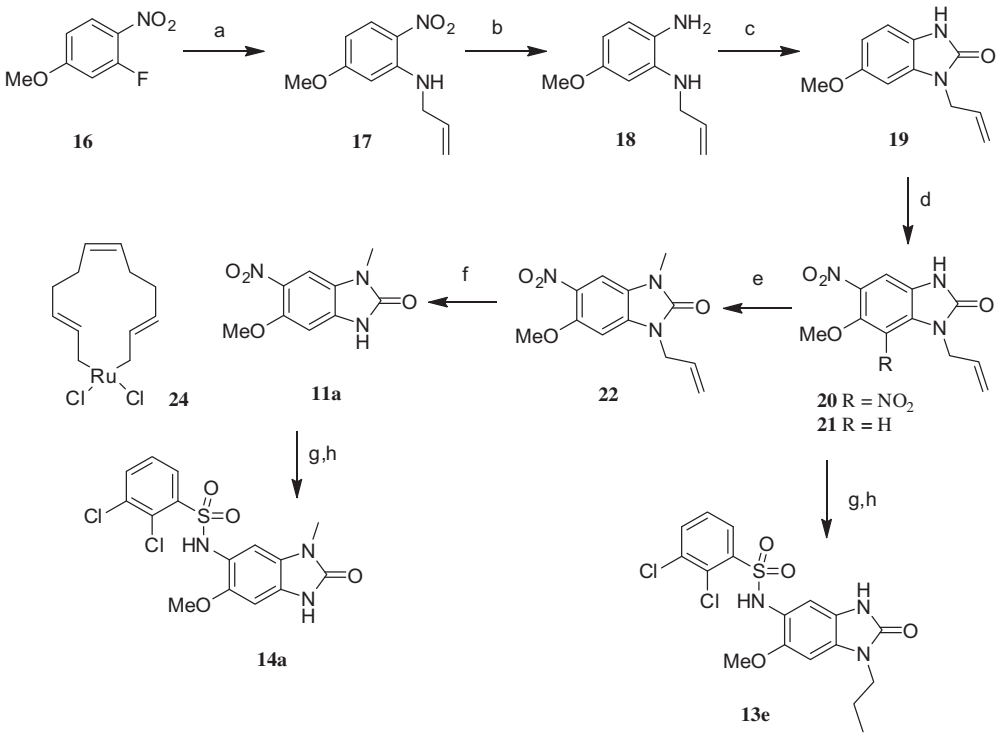
The method of Nebra,[30] utilising commercially available ruthenium catalyst **24**, and stoichiometric potassium periodate in water gave **23** in 40% yield. Finally reduction of **23** using catalytic hydrogenation over 10% Pd/C catalyst, followed by reaction with 2,3-dichlorobenzenesulfonyl chloride in pyridine gave the desired N3-methylated sulfonamide **14a** in 7% yield. The *N*1-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 4 M 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 BOC_2O in THF in the presence of $NaHCO_3$. 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 4 M HCl in dioxane. Finally, **13g** was converted to **13h** using one equivalent of acetic anhydride in pyridine.

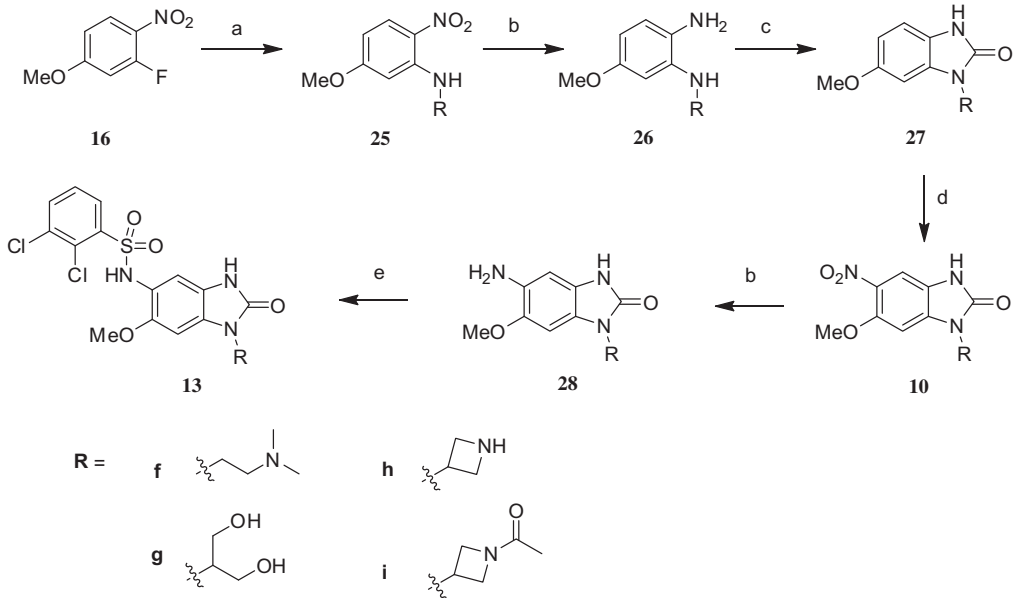
Introduction of nitrogen at the C4-position of the benzimidazolone series is outlined in Scheme 4. Commercially available 3-hydroxy pyridine **29** was reacted with bromine in aqueous sodium hydroxide to give the dibromide **30** according to the method of Clark and Deady.[31] The latter was methylated with iodomethane in the presence of potassium carbonate in DMSO to give **31** in 80% yield. Nitration of **31** with concd HNO_3 and concd



Scheme 1. Reagents and conditions: (a) 17.5% HNO_3, 0 °C 1 h, 20 °C, 3 h, 36%; (b) NaH, DMF, 20 °C then MeI (**10a**), or NH_2COCH_2Br (**10b**), or *iso*-PrBr (**10c**), or 3-iodo-oxetane (**10d**); (c) H_2, 10% Pd/C, EtOH, EtOAc, 18 h; (d) 2,3-dichlorobenzenesulfonyl chloride, pyridine, 20 °C.



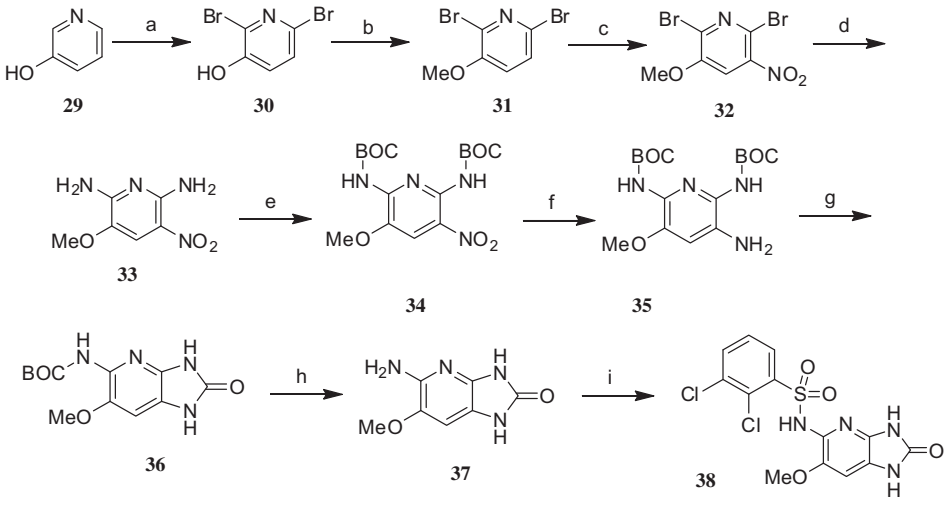
Scheme 2. Reagents and conditions: (a) allylamine, 20 °C, 1.5 h, 97%; (b) Fe, NH_4Cl, EtOH, H_2O, 100 °C, 22 h, 83%; (c) CDI, THF, 50 °C, 2 h, 45%; (d) 4 M HNO_3, 0–20 °C, 4 h, 23%; (e) NaH, MeI, DMF, 0–20 °C, 2.5 h, 99%; (f) dichloro(2,6,10-dodecatriene-1,12-diyl)ruthenium(IV) (**24**), KIO_4, H_2O, 100 °C, 72 h, 39%; (g) H_2, 10% Pd/C, EtOH–EtOAc (1:1), 5 h, 99%; (h) 2,3-dichlorobenzenesulfonyl chloride, pyridine, 20 °C, 1 h, 22%.



Scheme 3. Reagents and conditions: (a) RNH_2, DIPEA, DMF, 20 °C; (b) H_2, 10% Pd/C, EtOH; (c) CDI, THF, 50 °C; (d) 4 M HNO_3, 0–20 °C; (e) 2,3-dichlorobenzenesulfonyl chloride, pyridine, 20 °C.

H_2SO_4 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.[32] The latter was reacted with BOC_2O 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 azabenzimidazolone **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%).

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



Scheme 4. Reagents and conditions: (a) Br_2, 10% NaOH, 0 °C, 1 h, 20 °C, 4 h, 46%; (b) MeI, K_2CO_3, DMSO, reflux, 2 h, 80%; (c) concd HNO_3, concd H_2SO_4, 65 °C, 36 h, 28%; (d) aq NH_3, 90 °C, 1 h, μwave, 78%; (e) BOC_2O, K_2CO_3, 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%.

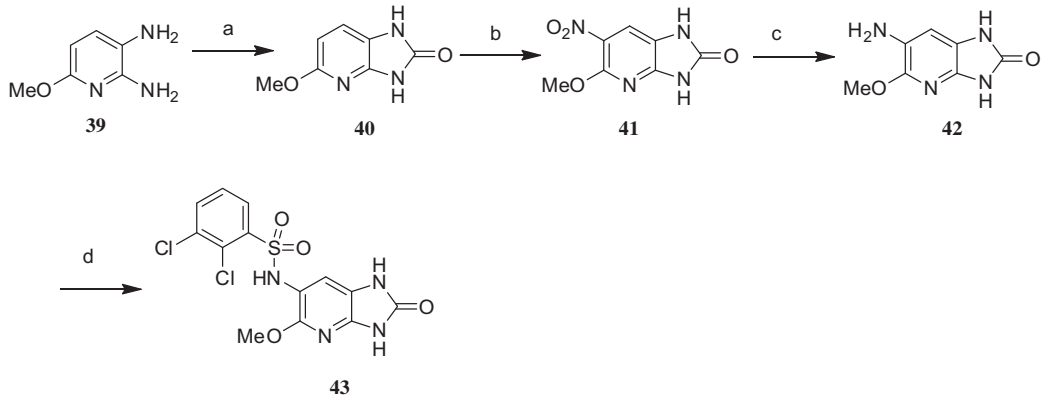
of **40**, using triflic anhydride and fuming nitric acid gave the desired nitro-benzimidazolone **41**, which was reduced by catalytic hydrogenation over 10% Pd/C to give the amino benzimidazolone **42**. Finally, reaction with 2,3-dichlorobenzenesulfonyl chloride afforded the target sulfonamide **43**.

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 diazabenzimidazolone **45** in 85% yield. Nitration, using nitronium tetrafluoroborate 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**.

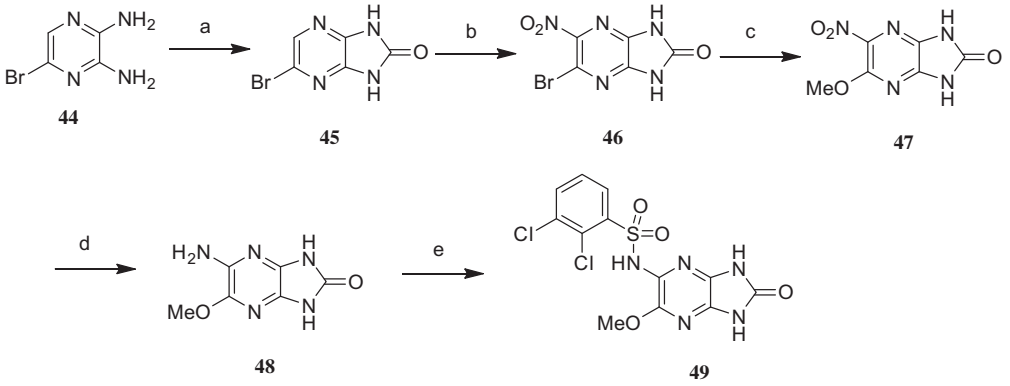
3. Results and discussion

All compounds in Table 1 were tested as human CCR4 antagonists in vitro. Antagonist potency was determined by a [^{35}S]-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.[33] 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.[22] 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 pA_2. In this assay, human $CD4^+$ $CCR4^+$ lymphocytes were identified by staining with antibodies to CD4 and CCR4. Calculated partition coefficient (*c*log*P*), chromatographic log$D_{7.4}$ (chrom log*D* 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 = $-1.36 \times pIC_{50}$/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 GTPγS 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



Scheme 5. Reagents and conditions: (a) CDI, Et_3N, THF, 20 °C, 48 h, 31%; (b) Tf_2O, fuming HNO_3, 20 °C, 3 h, 47%; (c) H_2, 10% Pd/C, EtOH, 2 h, 76%; (d) 2,3-dichlorobenzenesulfonyl chloride, pyridine, 20 °C, 1 h, 48%.



Scheme 6. Reagents and 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%.

Table 1
In vitro data pIC_{50} for humanCCR4 GTPγS binding assay, calculated log*P*, ligand efficiency, measured chrom log*D* at pH 7.4 and CLND solubility

Compd	GTPγS pIC_{50} ± SEM (*n*)	*c*Log*P*	LE	Chrom log*D*	CLND Solub. (μg/mL)	hWB pA_2
2	8.41 ± 0.02 (147)	4.4	0.34	2.9	193	6.6 ± 0.1 (24)
4	8.26 ± 0.01 (150)	4.9	0.33	2.9	145	7.3 ± 0.3 (4)
7	6.22 ± 0.03 (2)	3.5	0.35	5.3	116	<5.0 (6)
8	6.5 ± 0.1 (4)	3.1	0.38	3.2	32	5.3 (4)
13a	6.7 ± 0.1 (4)	3.5	0.36	3.8	12	<5
14a	4.7 ± 0.2 (2)	3.5	0.26	3.7	66	ND
15a	5.7 ± 0.1 (4)	3.3	0.30	4.4	58	ND
13b	6.34 ± 0.03 (3)	2.3	0.31	2.5	11	ND
14b	<4.5 (3)	2.3		2.4	167	ND
13c	6.0 ± 0.2 (3)	4.6	0.30	4.7	3	ND
13d	6.95 ± 0.06 (3)	4.4	0.35	4.6		<5
13e	5.4 ± 0.2 (2)	3.7	0.25	3.2	180	ND
13f	5.7 ± 0.3 (2)	2.5	0.27	2.6	176	ND
13g	5.8 ± 0.3 (2)	3.0	0.28	2.1	82	ND
13h	6.4 ± 0.1 (2)	2.6	0.28	3.0	194	ND
13i	6.7 ± 0.2 (5)	3.0	0.33	3.7	8	<5
38	7.6 ± 0.1 (2)	3.6	0.43	2.4	152	6.1
43	7.1 ± 0.2 (3)	4.0	0.40	3.2	126	5.6
49	7.3 ± 0.1 (2)	3.5	0.41	ND	130	5.2

ND: not determined.

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 log$D_{7.4}$ 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 *N*1, *N*3-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 *iso*propyl group (**13d**) caused a marginal increase in potency, accompanied by increased lipophilicity (*c*log*P* = 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.[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 GTPγS potency (pIC_{50} = 7.6), and a substantial increase in ligand efficiency (LE = 0.43). Furthermore, its lipophilicity was decreased ($c\log P$ = 3.6 and chrom $\log D_{7.4}$ = 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 GTPγS 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 μg/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 *N*1 isopropyl analogue **13d** was slightly more potent than **8** in the GTPγS assay, it was inactive in the whole blood assay (pA_2 <5). The three azabenzimidazolone 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 $\log D_{7.4}$ = 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 ($c\log P$ <3.5, chrom $\log D_{7.4}$ <5.3 and CLND solubility >116 μg/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. Azabenzimidazolone **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 μg/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_2SO_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 mL min^{-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-1*H*-benzo[*d*]imidazol-5-yl)benzenesulfonamide (8)

A solution of 5-amino-6-methoxy-1,3-dihydro-2*H*-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) δ = 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) δ = 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) calcd for $C_{14}H_{12}Cl_2N_3O_4S$ $(M+H)^+$ 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-1*H*-benzo[*d*]imidazol-2(3*H*)-one (9)

A solution of 17.5% fuming nitric acid (12.5 mL, 48.9 mmol) was slowly added to 5-methoxy-1*H*-benzo[*d*]imidazol-2(3*H*)-one (0.25 g, 1.5 mmol) at 0 °C, and the reaction was stirred for 1 h at 0 °C, and at 20 °C for 3 h. The reaction mixture was cooled to 0 °C and neutralised with saturated aqueous $NaHCO_3$ 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: ^{1}H NMR (400 MHz, DMSO-d_6) 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-1*H*-benzo[*d*]imidazol-2(3*H*)-one (10a) and 5-methoxy-1,3-dimethyl-6-nitro-1*H*-benzo[*d*]imidazol-2(3*H*)-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 °C. 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%); ^{1}H NMR (400 MHz, DMSO-d_6) 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%): ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.80 (s, 1H), 7.19 (s, 1H), 3.95 (s, 3H), 3.39 (s, 3H), 3.34 (s, 3H); ^{1}H NMR (400 MHz, MeOD-d_4) 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-1*H*-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) δ = 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-1*H*-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) δ = 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-1*H*-benzo[*d*]imidazol-1-yl)acetamide (10b) and 2-(5-methoxy-6-nitro-2-oxo-2,3-dihydro-1*H*-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: ^{1}H NMR (400 MHz, DMSO-d_6) major δ = 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-1*H*-benzo[*d*]imidazol-1-yl)acetamide (13b) N19663-84-3 and 2-(6-(2,3-dichlorophenylsulfonamido)-5-methoxy-2-oxo-2,3-dihydro-1*H*-benzo[*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; ^{1}H NMR (400 MHz, DMSO-d_6) δ = 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); ^{1}H NMR (400 MHz, MeOD-d_4) δ = 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: ^{1}H NMR (400 MHz, MeOD-d_4) δ = 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-1*H*-benzo[d]imidazol-2(3*H*)-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-1*H*-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-1*H*-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 (1 M, 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) δ = 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. *N*1-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 × 300 mL). The combined organic extracts were washed with brine (2 × 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, $CDCl_3$) δ = 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-1*H*-benzo[*d*]imidazol-2(3*H*)-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) δ = 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-1*H*-benzo[*d*]imidazol-2(3*H*)-one (21)

Nitric acid (4 M, 66.5 mL) was cooled to 0 °C and added slowly to **19** (1.9 g, 9.3 mmol). The reaction mixture was stirred for 1 h at 0 °C 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: ^{1}H NMR (400 MHz, DMSO-d_6) 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-1*H*-benzo[*d*]imidazol-2(3*H*)-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 °C. 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: ^{1}H NMR (400 MHz, DMSO-d_6) δ = 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-1*H*-benzo[*d*]imidazol-2(3*H*)-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: ^{1}H NMR (400 MHz, MeOD-d_4) δ = 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-1*H*-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: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 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-1*H*-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) δ = 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 × 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) δ = 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) δ = 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) δ = 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, $CDCl_3$) δ = 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 (2 M+H)$^+$.

5.23. *N*1-(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) δ = 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-*N*1-(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-d_6) δ = 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-d_6) δ = 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-1*H*-benzo[*d*] imidazol-2(3*H*)-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-d_6) δ = 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)-1*H*-benzo[*d*]imidazol-2(3*H*)-one (27g-bis-TBMS ether)

(2.04 g, 69%) as a dark brown solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ = 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-1*H*-benzo[*d*]imidazol-3(2*H*)-yl)azetidine-1-carboxylate (*N*-BOC-27h)

(620 mg, 79%) as a beige coloured solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 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 (2 M+H)$^+$.

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

A suspension of **27f** (1.26 g, 4.55 mmol) in nitric acid (4 M, 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) δ = 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-1*H*-benzo[*d*]imidazol-2(3*H*)-one (10g)

(40 mg, 33%) as a yellow solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ = 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-1*H*-benzo[*d*]imidazol-1-yl)azetidine-1-carboxylate (*N*-BOC-10h)

4 M 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_2O (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 (2 M,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, $CDCl_3$) δ = 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-1*H*-benzo[*d*]imidazol-2(3*H*)-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: ^{1}H NMR (400 MHz, DMSO-d_6) 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-1*H*-benzo[*d*]imidazol-2(3*H*)-one (28g)

From **10g** (40 mg, 0.14 mmol) obtained **28g** (28 mg, 78%) as a dark purple oil: ^{1}H NMR (400 MHz, DMSO-d_6) 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 (quint, *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-1*H*-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: ^{1}H NMR (400 MHz, DMSO-d_6) 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-1*H*-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: ^{1}H NMR (400 MHz, DMSO-d_6) 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 $C_{18}H_{21}Cl_2N_4O_4S$ $(M+H)^+$ 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-1*H*-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 $C_{17}H_{18}Cl_2N_3O_6S$ $(M+H)^+$ 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-1*H*-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-1*H*-benzo[*d*]imidazol-5-yl)-2,3-dichlorobenzenesulfonamide hydrochloride (13h)

4 M HCl solution in dioxane (4 mL, 16 mmol) was added to a solution of ***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 **13h** (125 mg, 51%) as an off-white solid: ^{1}H NMR (400 MHz, CD_3OD) δ = 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 *m*/*z* 443, 445, 447 $(M+H)^+$.

5.39. *N*-(1-(1-Acetylazetidin-3-yl)-6-methoxy-2-oxo-2,3-dihydro-1*H*-benzo[*d*]imidazol-5-yl)-2,3-dichlorobenzenesulfonamide (13i)

Acetic anhydride (50 μL, 0.53 mmol) was added to a solution of **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 diacetylation had occurred. MeOH (7.5 mL) and K_2CO_3 (60 mg, 0.43 mmol) were added to the mixture, and after stirring for 24 h, 2 M 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 **13i** (5 mg, 25%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD) δ = 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 *m*/*z* 485, 487, 489 $(M+H)^+$.

5.40. 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: ^{1}H NMR (400 MHz, DMSO-d_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 $(M-H)^-$.

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: ^{1}H NMR (400 MHz, DMSO-d_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 $(M+H)^+$.

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

Fuming nitric acid (16 M, 5 mL, 80 mmol) was added to a solution of **31** (2.0 g, 7.5 mmol) in sulfuric acid (18 M, 8 mL, 144 mmol) at 0 °C, and then the mixture was heated at 65 °C for 18 h. Additional concd 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 (15 M, 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_2CO_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-methoxypyridine-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-1*H*-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 °C. 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-1*H*-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) δ = 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-1*H*-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) δ = 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-1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-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) δ = 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-1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-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: ^{1}H NMR (400 MHz, DMSO-d_6) δ = 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-1*H*-imidazo[4,5-*b*]pyridin-2(3*H*)-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: ^{1}H NMR (400 MHz, DMSO-d_6) δ = 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-1*H*-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: ^{1}H NMR (400 MHz, DMSO-d_6) δ = 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) δ = 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 $C_{13}H_{11}Cl_2N_4O_4S$ = 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-1*H*-imidazo[4,5-*b*]pyrazin-2(3*H*)-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 °C for 22 h. More CDI (0.60 g, 3.7 mmol) was added and the reaction was stirred at 50 °C 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: ^{1}H NMR (400 MHz, DMSO-d_6) 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-1*H*-imidazo[4,5-*b*]pyrazin-2(3*H*)-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: ^{1}H NMR (400 MHz, DMSO-d_6) δ = 12.67 (br s, 1H), 12.50 (br s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) 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-1*H*-imidazo[4,5-*b*]pyrazin-2(3*H*)-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 °C 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) δ = 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-1*H*-imidazo[4,5-*b*]pyrazin-2(3*H*)-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) δ = 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-1*H*-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_3OD) δ = 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 $(M+H)^+$.

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