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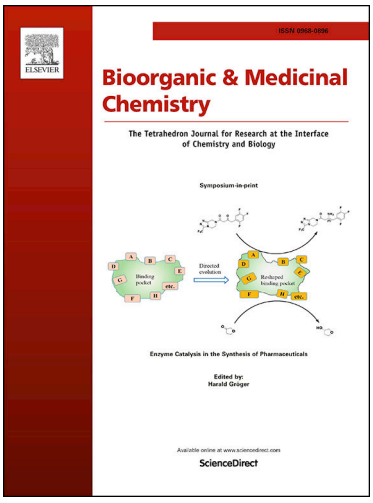

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Variations in the C-unit of bedaquiline provides analogues with improved biology and pharmacology

Hamish S. Sutherland[a], Amy S.T. Tong[a], Peter J. Choi[a], Adrian Blaser[a], Scott G. Franzblau[c], Christopher B. Cooper[d], Anna M. Upton[d], Manisha Lotlikar[d], William A. Denny[a,b,*], Brian D. Palmer[a,b,]

Affiliations [a]Auckland Cancer Society Research Centre, School of Medical Sciences, and [b]Maurice Wilkins Centre, University of Auckland, Private Bag 92019, Auckland 1142, New Zealand; [c]Institute for Tuberculosis Research, College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, Illinois 60612, USA; [d]Global Alliance for TB Drug Development, 40 Wall St, New York, New York 10005, USA

Keywords

CFU; colony-forming units; hERG, the alpha subunit of a K+ channel that contributes to the electrical activity of the heart; HPLC, high-peformance liquid chromatography; LDA, lithium diisopropylamide; LiTMP, lithium tetramethylpiperidide; LORA, low oxygen recovery assay; MABA, microplate alamar blue assay; MIC_{90}, minimum concentration for 90% inhibition; *M.tb*, *Mycobacterium tuberculosis*; TB, tuberculosis

Abstract

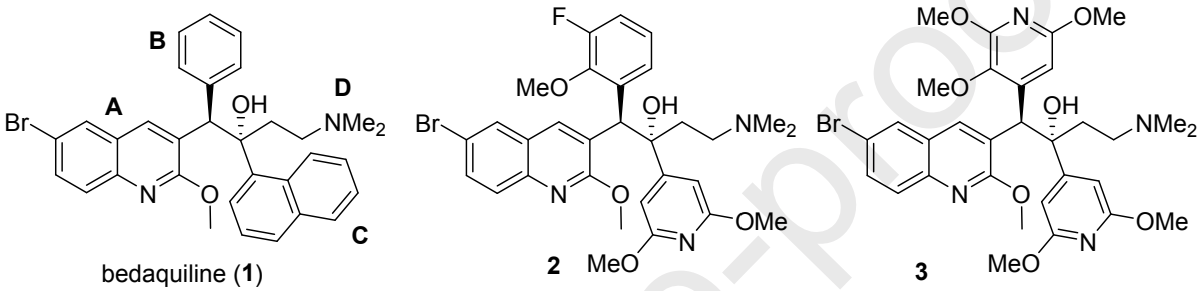
Analogues of the anti-tuberculosis drug bedaquiline, bearing a 3,5-dimethoxy-4-pyridyl C-unit, retain high anti-bacterial potency yet exert less inhibition of the hERG potassium channel, *in vitro*, than the parent compound. Two of these analogues (TBAJ-587 and TBAJ-876) are now in preclinical development. The present study further explores structure-activity relationships across a range of related 3,5-disubstituted-4-pyridyl C-unit bedaquiline analogues of greatly varying lipophilicity (clogP from 8.16 to 1.89). This broader class shows similar properties to the 3,5-dimethoxy-4-pyridyl series, being substantially more potent *in vitro* and equally active in an *in vivo* (mouse) model than bedaquiline, while retaining a lower cardiovascular risk profile through greatly attenuated hERG inhibition.

1. Introduction

The introduction of the mycobacterial ATP synthase inhibitor bedaquiline (**1**) for the treatment of multidrug-resistant tuberculosis (MDR-TB) is seen as a considerable step forward, although it has been noted[1] that the promising results to date have been based largely on culture conversion[2] rather than durable therapeutic response. One issue with bedaquiline is its long *in vivo* half-life (due significantly to its very high lipophilicity; clogP 7.25), which may increase the risk of acquired resistance.[3] In addition, it shows potent (IC_{50} 1.6 μM) inhibition of the hERG potassium channel, which can contribute to the risk of cardiac QTc prolongation.[4] Although this can be ameliorated by the co-administration of verapamil,[5] it is still a risk factor that must be considered in the formulation of multi-drug treatment regimens that include bedaquiline.[6]

With this in mind, we have recently reported[7,8] systematic studies towards the development of less lipophilic and less (potentially) cardiotoxic second-generation analogues of **1**. These studies have shown that, while there is a broad positive correlation between clogP and anti-mycobacterial potency, compounds such as **2** and **3**, with calculated clogP values two logs lower than **1**, can have equivalent or even superior biological activity. This is true both *in vitro* (MIC_{90} value against replicating *M.tb* strain H37Rv) and *in vivo* (reduction in colony-forming units in mice after daily treatment with 20 mg/kg for 12 days) (Figure 1).[7,8] We have also shown that C-unit pyridyl analogues[6] (and particularly C-unit 3,5-dialkoxy-4-pyridyl analogues[7]) have a greatly-reduced inhibitory activity towards the hERG cardiac potassium channel protein. Compounds **2** (TBAJ-587)[8] and **3** (TBAJ-876)[8] are now in preclinical development.[9-11]

Figure 1. Properties of bedaquiline (BDQ) and 2 advanced analogues



clogP	7.25	5.80	5.15
hERG IC_{50} (μM)	1.6	13	>30
M.tb MIC_{90} (μM)	0.03	0.006	0.004
$log_{10}CFU_{reduction}$	4.5–6.1	4.8	>5.5

In the present paper we follow up this work by exploring bedaquiline analogues with a wider range of 3,5-disubstituted 4-pyridyl C-units, further extending the structure-activity relationships for these variations for their effects on drug lipophilicity, anti-mycobacterial potency and, particularly, hERG channel inhibition.

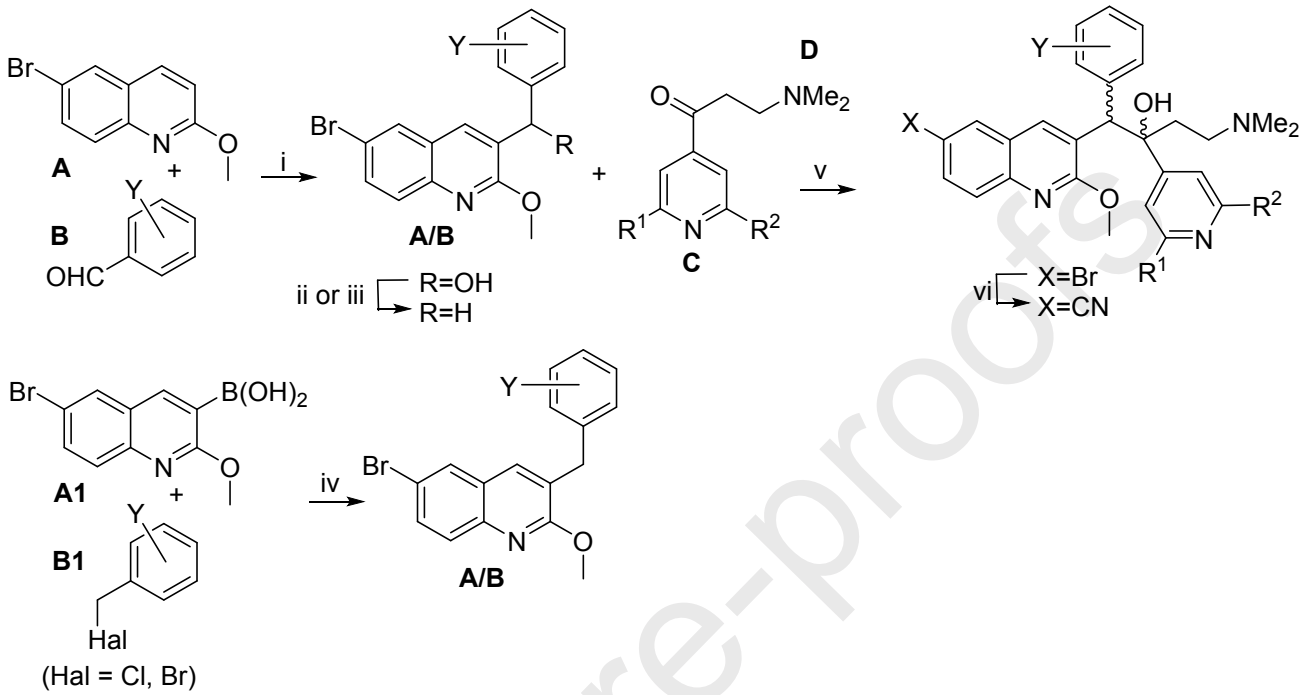
2. Results and Discussion

2.1 Chemistry

The new 3,5-disubstituted 4-pyridyl analogues were prepared by the general route outlined in Scheme 1. The A/B units were assembled as described previously,[7,8,12] from 6-bromo-2-methoxyquinoline (**A**) and the required aldehydes (**B**), or by palladium mediated coupling of a quinolone boronic acid (**A1**) and a (halomethyl)arene (**B1**). The C/D-units were prepared in high yields via the Weinreb amides, as shown in Scheme 2 and Table 1. Details of the preparation of the required acids (**I**) in Scheme 2 are given in Supplementary Information. The LDA mediated coupling of the benzylquinoline A/B-units and 1-(2,6-disubstituted pyridin-4-yl)-3-(dimethylamino)propan-1-one (Mannich base) C/D-units (Scheme 1) gave the compounds of Table 2, as a racemic mixture of four diastereomers. The desired *RS,SR* diastereomer (depicted) was then isolated by super-critical fluid HPLC at BioDuro LLC (Beijing). The 6-cyano compounds were prepared from the corresponding 6-bromo analogues by direct cyanation under previously reported conditions.[7,8] The C-unit SO_2Me and SO_2Et

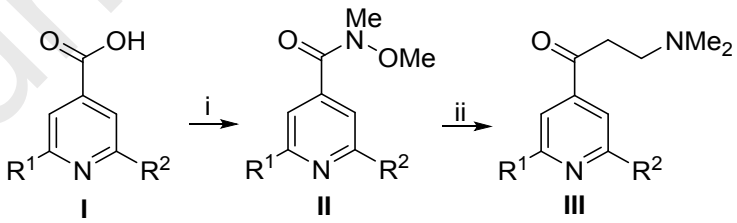
analogues **23** and **43** were prepared from the corresponding SMe and SEt compounds **21** and **42** respectively by oxidation with *N*-methylmorpholine *N*-oxide/OsO_4.

Scheme 1: Synthesis of 3,5-disubstituted 4-pyridyl analogues of 1



Reagents and conditions: (i) LiTMP, THF, -75 °C, 1.5 h then the appropriate aldehyde **B**, -75 °C, 4 h; (ii) Et_3SiH, TFA, DCM; (iii) MsCl, Et_3N, DMF, then $NaBH_4$; (iv) Cs_2CO_3, $Pd(PPh_3)_4$, PhMe/DMF, 110 °C (sealed tube), 5 h; (v) LDA, THF, -75 °C, 1.5 h then the appropriate ketone **C**/**D**, then HOAc; (vi) $Zn/Zn(CN)_2$, $Pd_2(dba)_3/P(o\text{-}tol)_3$, DMF, 50 °C, then separation of the diastereomers by SFC HPLC.

Scheme 2: Synthesis of pyridyl 3-(dimethylamino)propan-1-one Mannich bases



Reagents and conditions: (i) $(COCl)_2$, cat. DMF, DCM, then MeNH(OMe).HCl, pyridine; (ii) vinylMgBr, THF; then Me_2NH, water.

Table 1. New 3,5-disubstituted Mannich bases

R_1	R_2	Yields **I-II** / **II-III**
OMe	OEt	97 / 67

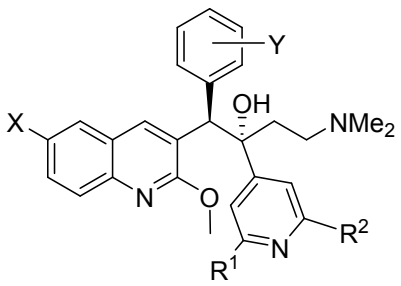
OMe	O^{i}Pr	97 / 99
OMe	O^{n}Pr	91 / 99
OMe	O^{c}Bu	85 / 99
OMe	OCF_2H	81 / 99
OMe	SMe	98 / 99
OMe	NMe_2	86 / 72
OEt	O^{i}Pr	97 / 99
OEt	O^{n}Pr	96 / 98
SMe	SMe	82 / 81
SEt	SEt	96 / 66
SEt	NMe_2	86 / 81

Li-mediated coupling of the benzylquinoline A/B-units and 1-(2,6-disubstituted pyridin-4-yl)-3-(dimethylamino)propan-1-one (Mannich base) C/D-units (Scheme 1) gave the compounds of Table 2, as a racemic mixture of four diastereomers. The desired *RS*,*SR* diastereomer (depicted) was then isolated by super-critical fluid HPLC at BioDuro LLC (Beijing).

2.2 Structure-activity relationships

In earlier work[7], we showed that while analogues of bedaquiline bearing a wide variety of methyl- and methoxy-substituted pyridyls as the C-unit were less lipophilic but equally potent *M.tb* inhibitors, there was little improvement in their hERG liability. In contrast, as noted above, analogues bearing symmetrical 3,5-dimethoxy- or 3,5-diethoxy-4-pyridyl C-units retained high potency against both replicating[13] and non-replicating[13,14] cultures of *M.tb*, together with marked improvements in hERG liability[8] (Fig. 1). In the present work, we explore this particular substitution pattern in more detail, with a wider variety of both symmetric and asymmetric 3,5-diethoxy-4-pyridyl C-units, to extend our understanding of how these changes affect both anti-tubercular activity and hERG liability (Table 2).

Table 2. Structural and *in vitro* data on 3,5-disubstituted pyridyl analogues of bedaquiline



	X	Y	R_1	R_2	Yld[a]	MIC_{90}		hERG[c]	clogP[d]
						(MABA)[b]	(LORA)[b]		
1	bedaquiline					0.03	0.06	1.6	7.25
2[e]	Br	2-F, 3-OMe	OMe	OMe		0.006	0.01	13	5.80
3[e]	Br	4-aza, 2,3,5-triOMe	OMe	OMe		0.004	0.006	>30	5.15
4	Br	2-F, 3-OMe	OMe	OEt	51	<0.01	<0.01	>30	6.33
5	Br	3-Me	OMe	OEt		<0.02	<0.02	>10	6.83
6	Br	4-aza, 2,3-diOMe	OMe	OEt	80	<0.02	<0.02	3.3	5.30
7	Br	2-F, 3-OMe	OMe	O^{i}Pr	43	<0.02	0.03	>30	6.64
8	Br	4-aza, 2,3-diOMe	OMe	O^{i}Pr	70	<0.01	<0.01	17	5.61
9	Br	3-Me	OMe	O^{i}Pr	18	0.01	0.01	>10	7.13
10	Br	3-Me	OMe	O^{n}Pr	22	0.01	0.02	>10	7.35
11	Br	4-aza, 2,3-diOMe	OMe	O^{n}Pr	45	0.01	0.06	7.8	5.83
12	Br	4-aza, 2,3-diOMe	OMe	O^{c}Bu	24	<0.004	0.01	>10	5.68
13	Br	2,3-O$(CH_2)_2$O-	OMe	OCF_2H	34	<0.01	<0.01	>10	6.17
14	CN	2,3-O$(CH_2)_2$O-	OMe	OCF_2H	73[f]	0.02	0.03	4.8	4.81
15	Br	2-F, 3-Me	OMe	OCF_2H	56	0.01	0.03	3% / 10[g]	6.88
16	CN	2-F, 3-Me	OMe	OCF_2H	58[f]	0.01	0.03	>30	5.53
17	Br	2-F, 3-OMe	OMe	OCF_2H	49	0.04	<0.02	>30	6.25
18	CN	2-F, 3-OMe	OMe	OCF_2H	49[f]	<0.02	<0.02	5.3	4.89
19	Br	4-aza, 2,3-diOMe	OMe	OCF_2H	60	<0.004	<0.004	>10	5.21
20	CN	4-aza, 2,3-diOMe	OMe	OCF_2H	68[f]	<0.02	0.04	7.0	3.86
21	Br	2-F, 3-OMe	OMe	SMe	46	<0.02	<0.02	>30	6.14
22	CN	2-F, 3-OMe	OMe	SMe	51[f]	0.02	0.04	>10	4.79
23	Br	2-F, 3-OMe	OMe	SO_2Me	87[h]	0.46	0.73	1.8	4.73
24	Br	4-aza, 2,3-diOMe	OMe	NMe_2	68	0.45	0.02	ND	5.08
25	CN	4-aza, 2,3-diOMe	OMe	NMe_2	84[f]	0.06	0.06	3.4	3.73
26	Br	4-aza, 2,3-diOMe	OEt	O^{i}Pr	56	<0.02	<0.02	>10	6.14
27	Br	4-aza, 3,5-diOMe	OEt	O^{i}Pr	59	<0.02	<0.02	>10	6.89
28	Br	2-F, 3-OMe	SMe	SMe	61	<0.02	<0.02	>10	6.46
29	CN	2-F, 3-OMe	SMe	SMe	88[f]	0.03	0.05	5.1	5.10
30	Br	4-aza, 2,3-diOMe	SMe	SMe	67	<0.02	<0.02	2.8	5.43
31	Br	2,3-O$(CH_2)_2$O-	SMe	SMe	38	<0.02	<0.02	>10	5.69
32	CN	2,3-O$(CH_2)_2$O-	SMe	SMe	67[f]	0.01	0.03	5.9	5.36
33	Br	2-F, 3-Me	SMe	SMe	63	<0.004	0.06	13% / 3[g]	7.10
34	CN	2-F, 3-Me	SMe	SMe	88[f]	0.02	0.17	>10	5.74
35	Br	H	SEt	SEt	55	0.02	0.05	>30	8.18
36	Br	4-aza, 2, 3-diOMe	SEt	SEt	53	0.04	0.21	9.2	7.30
37	Br	2-F, 3-OMe	SEt	SEt	70	<0.02	<0.02	>10	7.52
38	CN	2-F, 3-OMe	SEt	SEt	82[f]	0.10	0.20	>10	6.16
39	CN	2-F, 3-Me	SEt	SEt	87	<0.02	0.06	>10	8.16
40	Br	2,3-O$(CH_2)_2$O-	SEt	SEt	62	0.01	0.06	>10	6.39
41	CN	2,3-O$(CH_2)_2$O-	SEt	SEt	58[f]	<0.02	0.05	>10	5.36
42	CN	4-aza, 2,3-diOMe	SEt	SEt	79[f]	0.06	0.03	1.5	5.13
43	CN	4-aza, 2,3-diOMe	SO_2Et	SO_2Et	76[f,h]	4.4	5.0	[i]ND	1.89
44	Br	3-Me	SEt	NMe_2	43	0.14	0.12	23% / 3[g]	7.46
45	CN	3-Me	SEt	NMe_2	81[f,h]	0.12	0.17	51% / 3[g]	6.10
46	Br	4-aza, 2,3-diOMe	SEt	NMe_2	37	0.04	0.57	>10	5.93

Footnotes for Table 2

[a]Yields in the AB/CD coupling step to give bedaquiline analogues (as racemic mixtures). [b]MIC_{90} (μg/mL); minimum inhibitory concentration for 90% inhibition of growth of *M.tb* strain H37Rv, determined under aerobic, replicating (MABA) (ref. 13) or non-replicating (LORA) (ref. 14) conditions, determined at the Institute for Tuberculosis Research, University of Illinois at Chicago. [c]Inhibition of hERG (IC_{50} in μM); [d]clogP calculated by ChemDraw Ultra v12.0.2. (CambridgeSoft); [e]Data from ref. 7; [f]Yields for the Br/CN conversion; [g]hERG single concentration determination; % inhibition at the stated dose (μM); [h]Yield for oxidation of the corresponding SMe or SEt analogue with N-methylmorpholine N-oxide/OsO_4; [i]ND; not done due to poor MIC value.

Overall, while the analogues covered a wide range of lipophilicity, from 8.16 to 1.89 (calculated logP (clogP) values), there was relatively little variation in MIC with changing lipophilicity. All compounds except the very hydrophilic **43** retained high potency against *M. tb in vitro* (in many cases beyond the range of the assay) and mostly lower potency against hERG blockade compared to bedaquiline (Table 2).

Compounds **4**-**12** explored asymmetric 3,5-dialkoxy-4-pyridyl units. Replacing one methoxy substituent in **2** (TBAJ-587) with an ethoxy (**4**) or isopropoxy (**7**) had minimal effect on MIC values but showed an improvement in hERG IC_{50}, albeit with increased lipophilicity. Increasing the steric bulk around one alkoxy group while retaining the remainder of the molecular structure (compounds **6**, **8**, **11** and **12**) yielded little change in either MIC or hERG values were observed, suggesting some bulk tolerance in this position for both activities. The retention of MIC activity in the 3-ethoxy-5-isopropoxy-4-pyridyl derivative (**27**) (<0.02 μg/mL) provides further evidence for steric tolerance around the pyridyl group.

The mildly electron-withdrawing -OCF_2H group (compounds **13**-**20**) had no effect on MIC, and most of these compounds also showed low hERG inhibition. In contrast, the introduction of a strongly electron-withdrawing SO_2Me substituent in compound **23** resulted in significant loss of MIC potency and a hERG IC_{50} of 1.8 μM. Since compounds of similarly overall low lipophilicity (e.g., **14**, **18**, **22**) retained good anti-tubercular potency, the electronic effects of substituents appear important.

Compounds **21**-**23** looked at mixed OMe/SMe pyridyl units. The SMe group is moderately electron donating, but has the potential of metabolic oxidation to likely less effective -SO_2Me analogues. However, **21** and **22** retained good MIC and hERG values and in vivo activity (Table 3). Retention of potency was also observed when an OMe group was replaced with the strongly electron donating NMe_2 group in compounds **24** and **25**. However, **25** does not present an improvement over bedaquiline in hERG potency (IC_{50} 3.4 μM) compared to many of the other compounds.

Compounds **28**-**34** bear 3,5-diSMe-4-pyridyl C-units, while compounds **44**-**46** bear 2-SEt, 5-NMe_2-4-pyridyl C-units. Unsurprisingly, in light of the results for **21** and **22** (OMe/SMe

substituents), these compounds also retain good MIC potency (~0.02 μg/mL) and most have similar hERG properties (IC_{50}s 5 to >10 μM). Compounds **35-42** have analogous 3,5-diSEt-4-pyridyl C-units, and their results are broadly similar in terms of MIC (~0.02 μg/mL) and (except **42**) hERG (>10 μM) properties, although these compounds are significantly more lipophilic (clogP from 6-8). Chemical oxidation of **42** gave the extremely polar **43** (clogP 1.89), with very electron-withdrawing diSO_2Et substitution, which not surprisingly had significantly attenuated in vitro MIC values (5 μg/mL).

A substantial number of the compounds, representative of the sub-classes studied, were evaluated for a series of relevant pharmacological and *in vivo* antibacterial properties, in an endeavour to discern structure-activity relationships for these analogues. The data are shown in Table 3. The compounds were additionally tested for mammalian cell toxicity (with Vero green monkey-derived epithelial kidney cells),[14,15] where all compounds had IC_{50}s >10 μg/mL, except for compounds **28** (IC_{50} of 9.6 μg/mL), **37** (IC_{50} of 6.5 μg/mL), and **43** (not tested) (data not shown). In comparison, the value for **1** in repeat assays was between 4-16 μg/mL

Table 3. Additional biological data on selected analogues of Table 1

No	Microsomal clearance		Mouse PK parameters				Mouse Acute TB efficacy	
	H Cl_{int}	M Cl_{int}	IV Cl	Vz	AUC_{inf}	F	Log reduction in mouse lung CFU	
	μL/min/mg protein[a]		*ml/min /kg*[b]	*L/kg*[c]	*μg*h/ mL*[d]	*(%)*[e]	*Test compd*[f]	*Beda-quiline*[g]
1	3.0	7.3	7.0	22	17.4	56	--	4.5-6.1
2[g]	2.2	18	48	95	1.7	48	4.8	6.1
3[g]	2.1	22	13	31	5.6	44	>5.5	>5.5
4	4.1	14	19	51	4.0	46	4.1	4.6
5	4.4	7.8	12	45	9.4	65	0.7	5.5
6	3.8	7.6	9.5	28	12.7	70	5.4	5.6
7	3.4	6.0	8.9	34	9.0	43	4.1	4.6
8	6.4	6.7	6.8	19	5.6	22	2.6	4.6
11	8.2	6.5	6.9	44	10.9	50	5.0	5.5
12	2.5	4.6	11	33	8.4	56	5.2	4.9
14	1.6	15	27	41	3.2	51	5.1	4.6
16	0.6	5.9	13	38	8.6	66	>5.2	4.9
17	0.1	6.4	9.7	18	14.4	83	4.4	6.1
19	2.1	4.9	8.7	27	9.8	50	4.3	>5.5
20	2.5	11	24	40	3.0	43	5.2	>5.5
21	4.8	20	13	32	4.6	31	4.7	4.5[h]
22	16	36	42	54	1.1	28	>4.3	4.9
25	8.4	16	26	14	3.3	50	5.2	4.9
26	1.8	1.6	3.1	9.1	32.0	58	4.7	4.5[h]

27	1.1	2.3	2.7	20	33.0	58	4.5	>5.5
28	3.4	17	13	15	8.4	66	5.0	5.6
29	15	20	44	53	1.1	28	2.0	4.5[h]
30	8.8	10	18	64	3.9	41	4.1	5.6
34	9.2	13	22	79	2.4	31	3.9	4.9
35	2.3	17	11	30	4.0	24	1.8	4.6
37	4.9	12	3.6	14	11.9	25	4.2	5.6
38	17	27	29	93	1.4	24	1.7	5.0
39	9.1	33	9.4	41	4.0	22	2.9	5.6
40	9.5	12	3.4	9.4	5.5	11	2.9	5.6

Footnotes for Table 3

[a]Clearance of compound by human or mouse liver microsomes (μL/min/mg protein); [b]IV clearance in mice (μg*h/mL); [c]IV apparent volume of distribution during terminal phase, mouse (L/kg); [d]Oral exposure, area under the curve 0 to infinity, in mice (μg*h/mL); [e]Oral bioavailability in mice; [f]Log reduction in colony-forming units (CFU) compared to vehicle when dosed at 20 mg/kg daily in mice for 12 days, beginning 10 days after *M.tb* inoculation; [g]Results for bedaquiline (positive control) under the same conditions in the same assay. [h]Bedaquiline test concentration was 16.7 mg/kg

The data in Table 2 show that the two "lead" unit C 3,5-dimethoxy compounds **2** and **3**[9-11] do not show much improvement in the very slow human clearance of bedaquiline (**1**); However, compounds with pyridyl sulfur substituents do achieve this; e.g., **21** and **22** (SMe/OMe), **28-34** (SMe/SMe) and **35-40** (SEt/SEt). The last group have an average 3-fold higher H_{clint} value than **1**. In contrast, the mixed dialkoxy compounds **4-12** were no better than **1**, and the OCF_2H analogues **14-20** had even slower clearances than **1**.

Pharmacokinetic studies in male CD-1 mice used single oral gavage dosing at 10 mg/kg or single intravenous bolus injection at 2–3 mg/kg. Oral bioavailability was generally good; mostly in the 40-60% range typical of **1** and with little apparent correlation with lipophilicity. Again, compounds with sulfur-bearing pyridyl subunits seemed on average to have the lowest F values, maybe reflecting sulfur oxidative metabolism. Volumes of distribution varied widely, with no clear correlation to specific structural features. As demonstrated previously for **1**[16] and its analogues[8], human plasma protein binding was always >99.5%.

The compounds were evaluated for *in vivo* efficacy in female BALB/c mice (n = 7 per group), dosed by oral gavage at 20 mg/kg daily for 12 days, beginning 10 days after the aerosol inoculation of *M.tb* H37Rv, as previously described.[8] Compounds were formulated in 20% hydroxy-beta-cyclodextrin. Efficacy was determined by the reduction in colony-forming units (CFU) of *M. tb* bacteria recovered from lung homogenates from compound-treated mice, relative to the bacterial burden in mice administered with vehicle only, compared to the bacterial reduction shown by treatment with **1** as a positive control.

The average CFU for vehicle-only treated control groups was approximately 5.5-8 logs; thus a CFU reduction in the treated groups of >4.5 log units shows clearance of bacteria in the lungs of mice within the limit of detection (30 CFU/mouse) after 12 days of treatment. Ten of

the new compounds in Table 3 (**6**, **11**, **12**, **14**, **16**, **20**, **26**, **28**) achieved this high level of efficacy, with log CFU reduced to ~1.5 CFU, which reflecting essentially similar efficacy to **1**, given the assay-to-assay variation of these experiments.

3. Conclusions

Previous work[8] showed that analogues of **1** bearing 3,5-dimethoxy-4-pyridyl C-units in place of the naphthalene possessed good *in vitro* and *in vivo* anti-mycobacterial activity and greatly-reduced hERG potassium channel blockade (a potential liability of **1**) and identified two preclinical candidates (**2** and **3**). The present study broadens the scope of this SAR, showing that analogues with a wide variety of other 3,5-disubstituted-4-pyridyl units retain similarly-high antibacterial efficacy and similar or better *in vitro* hERG safety. Of the eight "best" new in vivo compounds (Table 3), five had a unit B 3,5-dialkoxy substitution pattern. The "best" unit C substitutions for in vivo activity were 3,5-diOalkyl (four), 3-OMe, 5-CF_2H (three) and 3,5-siSMwe (one). Effective suppression of hERG channel stimulation (IC_{50} >10 μM) was much less dependent on structure, with the great majority of the Table 3 compounds, bearing a cross-section of the unit C substitutions, meeting this criterion. As noted above, many of these compounds (e.g., **12**, **14**, **16**, **20**) also have much faster clearance rates than **1**, making them further potential backup candidates.

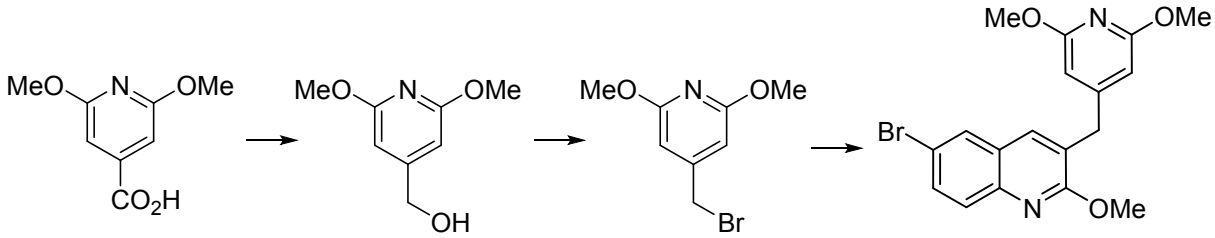
4. Experimental

4.1. Chemistry

Compounds were analysed for purity by reverse-phase HPLC (Alltima C18 5 μm column, 150 × 3.2 mm; Alltech Associated, Inc., Deerfield, IL) using an Agilent HP1100 equipped with a diode-array detector. Mobile phases were gradients of 80% CH_3CN/20% H_2O (v/v) in 45 mM NH_4HCO_2 at pH 3.5 and 0.5 mL/min. Purity was determined by monitoring at 330 ± 50 nm and was ≥95% for all final products. NMR spectra were obtained on a Bruker Avance 400 spectrometer at 400 MHz for ^1H. Low-resolution atmospheric pressure chemical ionization (APCI) mass spectra were measured for organic solutions on a ThermoFinnigan Surveyor MSQ mass spectrometer, connected to a Gilson autosampler. Melting points were determined on an Electrothermal 9100 melting point apparatus.

4.1.1. Scheme 1. New A/B units

*4.1.1.1. Scheme 1: 6-bromo-3-((2,6-dimethoxypyridin-4-yl)methyl)-2-methoxyquinoline (AB-7) for compound **27** of Table 1*



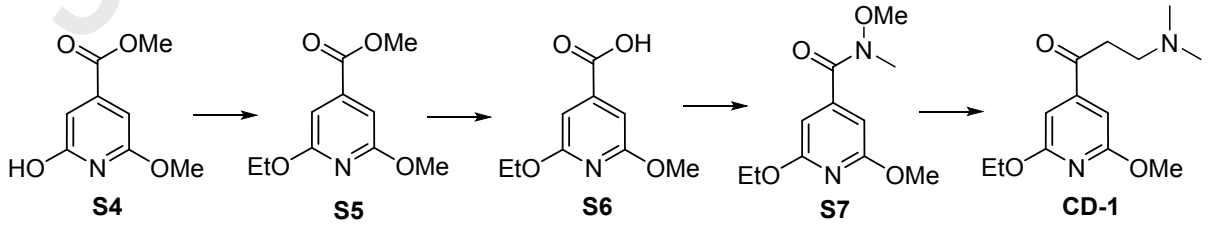
Borane-dimethylsulfide (4.60 mL, 48.5 mmol) and trimethylborate (5.58 mL, 49.1 mmol) were added to a solution of 2,6-dimethoxyisonicotinic acid (**S1**) (3.00 g, 16.4 mmol) in THF (100 mL, dist. Na) at 0 °C, the solution was then stirred at r.t. for 18 h, cooled to 0 °C, quenched with MeOH and evaporated. The residue was partitioned between EtOAc and water and the organic fraction was dried and evaporated. Column chromatography (2:1 hexanes:EtOAc) gave (2,6-dimethoxypyridin-4-yl)methanol (**S2**) (2.72 g, 98%). ^{1}H NMR ($CDCl_3$) δ 6.30 (s, 2H), 4.64 (d, J = 5.8 Hz, 2H), 3.91 (s, 6H), 1.75 (t, J = 6.1 Hz, 1H). Found: [M+H]=170.1.

A solution of **S2** (1.57 g, 9.30 mmol) in DCM (40 mL, anhydrous) at 0 °C was treated with triethylamine (2.60 mL, 18.6 mmol) then mesyl chloride (1.08 mL, 13.9 mmol), the mixture was stirred at 0 °C for 1 h then partitioned between DCM and water. The organic fraction was dried and evaporated and the residue was dissolved in acetone (50 mL). LiBr (4.04 g, 46.5 mmol) was added and the mixture was refluxed for 1 h then evaporated. The residue was partitioned between DCM and water and the organic fraction was dried and evaporated. Column chromatography (DCM) gave 4-(bromomethyl)-2,6-dimethoxypyridine (**S3**) (1.96 g, 91%). ^{1}H NMR ($CDCl_3$) δ 6.32 (s, 2H), 4.29 (s, 2H), 3.91 (s, 6H). Found: [M+H] = 232.0

A mixture of (6-bromo-2-methoxyquinolin-3-yl)boronic acid (2.20 g, 7.80 mmol), **S3** (1.93 g, 8.30 mmol), Cs_2CO_3 (5.10 g, 15.7 mmol) and $Pd(PPh_3)_4$ (0.45 g, 0.39 mmol) in DMF (10 mL) and toluene (20 mL) was purged with nitrogen, then heated to 80 °C for 4 h under nitrogen. The mixture was partitioned between EtOAc and water and the organic fraction was dried and evaporated. Column chromatography (3:1 hexanes:DCM) eluted non polar impurities, elution with DCM gave 6-bromo-3-((2,6-dimethoxypyridin-4-yl)methyl)-2-methoxyquinoline (**AB-7**) (1.95 g, 64%). ^{1}H NMR ($CDCl_3$) δ 7.78 (d, J = 2.1 Hz, 1H), 7.69 (d, J = 8.9 Hz, 1H), 7.63 (dd, J = 8.9, 2.2 Hz, 1H), 7.57 (s, 1H), 6.17 (s, 2H), 4.06 (s, 3H), 3.92 (s, 2H), 3.89 (s, 6H). Found: [M+H] = 389.1

4.1.2. New C/D units of Table 1

*4.1.2.1. 3-(Dimethylamino)-1-(2-ethoxy-6-methoxypyridin-4-yl)propan-1-one (**CD-1**)*



A suspension of 2,6-dihydroxyisonicotinic acid (10.00 g, 64.5 mmol) in MeOH (60 mL) was treated dropwise with H_2SO_4 (10 mL, 18.4 M, 184 mmol). The solution was refluxed for 72 h and then evaporated. The residue was treated with sat. aq. $NaHCO_3$ to pH 8 and extracted with

EtOAc (3 x 200 mL). The organic extracts were washed with sat. aq. $NaHCO_3$ and brine, then dried and evaporated to give methyl 2-hydroxy-6-methoxyisonicotinate (**S4**) (3.55 g, 30%). ^{1}H NMR (DMSO-d_6) δ 11.2 (bs, 1H), 6.61 (bs, 2H), 3.84 (s, 3H), 3.83 (s, 3H). Found: [M+H]=184.2.

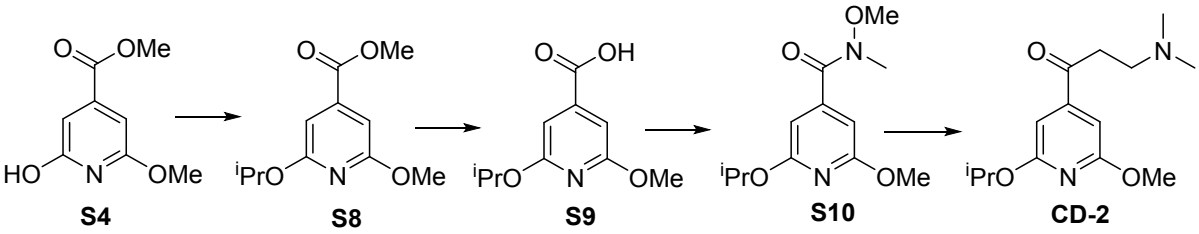
A solution of **S4** (6.96 g, 38.0 mmol) in DMF (100 mL, anhydrous) was treated with K_2CO_3 (6.57 g, 47.6 mmol) and then iodoethane (3.85 mL, 47.6 mmol). The mixture was stirred at r.t. for 24 h, partitioned between EtOAc and water and the aqueous layer was extracted with EtOAc. The organic fractions were washed with water, dried and evaporated, chromatography (DCM) gave methyl 2-ethoxy-6-methoxyisonicotinate (**S5**) (6.20 g, 77%). ^{1}H NMR ($CDCl_3$) δ 6.84 (s, 2H), 4.35 (q, J = 7.1 Hz, 2H), 3.93 (s, 3H), 3.91 (s, 3H), 1.40 (t, J = 7.1 Hz, 3H). Found: [M+H]=212.1.

A solution of LiOH (2.10 g, 87.7 mmol) in water (60 mL) was added to a solution of **S5** (6.20 g, 29.4 mmol) in MeOH (60 mL) and THF (60 mL), the solution was stirred at r.t. for 18 h and then evaporated. The residue was dissolved in water (150 mL) and acidified to pH 3 with 2 M HCl. The precipitate was filtered and dried to give 2-ethoxy-6-methoxyisonicotinic acid (**S6**) (5.61 g, 97%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 13.54 (bs, 1H), 6.73 (d, J = 1.0 Hz, 1H), 6.71 (d, J = 1.0 Hz, 1H), 4.32 (q, J = 7.0 Hz, 2H), 3.87 (s, 3H), 1.33 (t, J = 7.0 Hz, 3H). Found: [M+H]=198.2.

Oxalyl chloride (2.18 mL, 25.8 mmol) was added to a suspension of **S6** (4.23 g, 21.5 mmol) in DCM (100 mL, anhydrous) and DMF (0.3 mL) at r.t.. The mixture was stirred at r.t. for 1 h to give a colourless solution which was cooled to 0 °C. *N*,*O*-Dimethylhydroxylamine hydrochloride (2.51 g, 25.8 mmol) and pyridine (5.2 mL, 64.3 mmol) were added sequentially and the mixture was stirred at r.t. for 18 h, then partitioned between DCM and sat. aq. $NaHCO_3$. Column chromatography with 3:1 hexanes:EtOAc gave 2-ethoxy-*N*,6-dimethoxy-N-methylisonicotinamide (**S7**) (5.02 g, 97%). ^{1}H NMR ($CDCl_3$) δ 6.46 (s, 1H), 6.45 (s, 1H), 4.35 (q, J = 7.1 Hz, 2H), 3.92 (s, 3H), 3.59 (bs, 3H), 3.32 (s, 3H), 1.40 (t, J = 7.1 Hz, 3H). Found: [M+H]=241.1.

Vinylmagnesium bromide (16.6 mL, 1 M, 16.6 mmol) was added to a solution of **S7** (2.00 g, 8.30 mmol) in THF (100 mL, dist. Na) at 0 °C, the brown solution was warmed to r.t. for 1 h then dimethylamine in THF (2M, 16.6 mL, 33.2 mmol) and water (25 mL) were added. The solution was stirred at r.t. for 1 h, then partitioned between EtOAc and water. The solution was dried and evaporated, column chromatography of the residue (95:5 DCM:MeOH) gave **3-**(dimethylamino)-1-(2-ethoxy-6-methoxypyridin-4-yl)propan-1-one (**CD-1**) (1.40 g, 67%). ^{1}H NMR ($CDCl_3$) δ 6.73 (s, 1H), 6.72 (s, 1H), 4.37 (q, J = 7.0 Hz, 2H), 3.93 (s, 3H), 3.06 (t, J = 7.4 Hz, 2H), 2.72 (t, J = 7.4 Hz, 2H), 2.27 (s, 6H), 1.41 (t, J = 7.0 Hz, 3H). Found: [M+H]=253.2.

*4.1.2.2. 3-(Dimethylamino)-1-(2-isopropoxy-6-methoxypyridin-4-yl)propan-1-one (**CD-2**)*



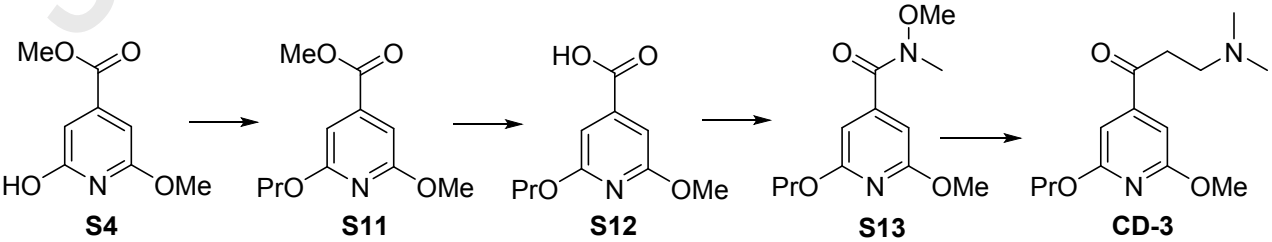
A solution of **S4** (5.04 g, 27.5 mmol) in DMF (100 mL, anhydrous) was treated with K_2CO_3 (4.75 g, 34.4 mmol) and then 2-iodopropane (3.43 mL, 34.4 mmol). The mixture was stirred at r.t. for 24 h, more 2-iodopropane (3.43 mL, 34.3 mmol) was added and the mixture was stirred for a further 72 h, then partitioned between EtOAc and water and the aqueous layer was extracted further with EtOAc. The organic fractions were washed with water, dried and evaporated. Chromatography (DCM) gave methyl 2-isopropoxy-6-methoxyisonicotinate (**S8**) (6.21 g, 100%). ^{1}H NMR ($CDCl_3$) δ 6.81 (s, 2H), 5.24 (sp, J = 6.2 Hz, 1H), 3.92 (s, 3H), 3.90 (s, 3H), 1.36 (d, J = 6.2 Hz, 6H). Found: [M+H]=226.2.

A solution of LiOH (1.98 g, 82.7 mmol) in water (60 mL) was added to a solution of **S8** (6.20 g, 27.5 mmol) in MeOH (60mL) and THF (60 mL) and the solution was stirred at r.t. for 18 h and then evaporated. The residue was dissolved in water (150 mL) and acidified to pH 3 with 2 M HCl. The precipitate was filtered and dried to give 2-isopropoxy-6-methoxyisonicotinic acid (**S9**) (5.33 g, 92%) as a white solid. ^{1}H NMR (DMSO-d_6) δ 13.50 (bs, 1H), 6.70 (d, J = 1.0 Hz, 1H), 6.66 (d, J = 1.0 Hz, 1H), 5.20 (sp, J = 6.2 Hz, 1H), 3.86 (s, 3H), 1.31 (d, J = 6.2 Hz, 6H). Found: [M+H]=212.1.

Oxalyl chloride (1.75 mL, 20.7 mmol) was added to **S9** (3.65 g, 17.3 mmol) in DCM (100 mL, anhydrous) and DMF (0.3 mL) at r.t.. The mixture was stirred at r.t. for 1 h to give a colourless solution which was cooled to 0 °C. *N*,*O*-Dimethylhydroxylamine hydrochloride (2.03 g, 20.8 mmol) and pyridine (4.2 mL, 51.9 mmol) were added sequentially and the mixture was stirred at r.t. for 18 h, then partitioned between EtOAc and water. Column chromatography with 3:1 hexanes:EtOAc gave 2-isopropoxy-*N*,6-dimethoxy-*N*-methylisonicotinamide (**S10**) (4.58 g, 100%) which was used directly. ^{1}H NMR ($CDCl_3$) δ 6.42 (s, 1H), 6.41 (s, 1H), 5.24 (sp, J = 6.2 Hz, 1H), 3.90 (s, 3H), 3.60 (bs, 3H), 3.32 (bs, 3H), 1.35 (d, J = 6.2 Hz, 6H). Found: [M+H]=255.2.

Vinylmagnesium bromide (36 mL, 1 M, 36 mmol) was added to a solution of **S10** (4.54 g, 17.9 mmol) in THF (200 mL, dist. Na) at 0 °C, the brown solution was warmed to r.t. for 1 h, then dimethylamine in THF (36 mL, 2 M, 72 mmol) and water (30 mL) were added. The solution was stirred at r.t. for 1 h and then partitioned between EtOAc and water. Column chromatography using a gradient of 97.5:2.5 DCM:MeOH to 95:5 DCM:MeOH gave 3-(dimethylamino)-1-(2-isopropoxy-6-methoxypyridin-4-yl)propan-1-one (**CD-2**) (3.58 g, 75%). ^{1}H NMR ($CDCl_3$) δ 6.70 (d, J = 1.0 Hz, 1H), 6.69 (d, J = 1.0 Hz, 1H), 5.25 (sp, J = 6.2 Hz, 1H), 3.92 (s, 3H), 3.05 (t, J = 7.4 Hz, 2H), 2.72 (t, J = 7.4 Hz, 2H), 2.26 (s, 6H), 1.36 (d, J = 6.2 Hz, 6H). Found: [M+H]=267.2.

*4.1.2.2. 3-(Dimethylamino)-1-(2-methoxy-6-propoxypyridin-4-yl)propan-1-one (**CD-3**)*



A solution of **S4** (6.00 g, 32.8 mmol) in DMF (100 mL, anhydrous) was treated with K_2CO_3 (6.80 g, 49.2 mmol) and then 1-iodopropane (4.8 mL, 49.2 mmol). The mixture was stirred at r.t. for 48 h, partitioned between EtOAc and water and the aqueous layer was extracted with

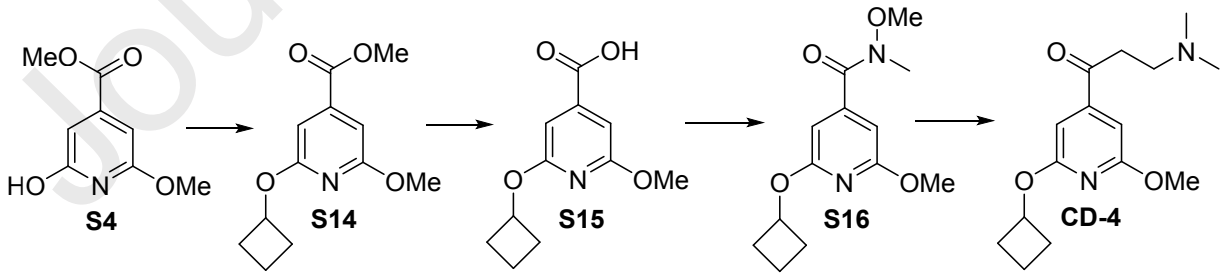
EtOAc. The organic fractions were washed with water, dried and evaporated. Column chromatography (DCM) gave methyl 2-methoxy-6-propoxyisonicotinate (**S11**) (6.59 g, 89%). ^{1}H NMR ($CDCl_3$) δ 6.85 (d, J = 1.0 Hz, 1H), 6.83 (d, J = 1.0 Hz, 1H), 4.24 (t, J = 6.7 Hz, 2H), 3.93 (s, 3H) , 3.91 (s, 3H), 1.80 (qt, J = 7.4, 6.7 Hz, 2H), 1.02 (t, J = 7.4 Hz, 3H). Found: [M+H]=226.1.

A solution of LiOH (2.04 g, 85.2 mmol) in water (60 mL) was added to a solution of **S11** (6.40 g, 28.4 mmol) in THF (60 mL) and MeOH (60 mL), the solution was stirred at r.t. for 18 h and then evaporated. The residue was dissolved in water (200 mL) and acidified to pH 3 with 2 M HCl. The precipitate was filtered and dried to give 2-methoxy-6-propoxyisonicotinic acid (**S12**) (5.39 g, 90%). ^{1}H NMR (DMSO-d_6) δ 13.53 (bs, 1H), 6.73 (d, J = 1.0 Hz, 1H), 6.72 (d, J = 1.0 Hz, 1H), 4.23 (t, J = 6.6 Hz, 2H), 3.87 (s, 3H), 1.73 (qt, J = 7.4, 6.6 Hz, 2H), 0.96 (t, J = 7.4 Hz, 3H). Found: [M+H]=212.1.

Oxalyl chloride (2.76 mL, 32.6 mmol) was added to **S12** (5.74 g, 27.2 mmol) in DCM (100 mL, anhydrous) and DMF (0.4 mL, 5.2 mmol) at r.t.. The mixture was stirred at r.t. for 1 h to give a colourless solution which was cooled to 0 °C. *N*,*O*-Dimethylhydroxylamine hydrochloride (3.18 g, 32.6 mmol) and pyridine (6.6 mL, 81.6 mmol) were added sequentially and the mixture was stirred at r.t. for 18 h, then partitioned between DCM and water. Column chromatography on alumina with DCM gave *N*,2-dimethoxy-*N*-methyl-6-propoxyisonicotinamide (**S13**) (6.29 g, 91%). ^{1}H NMR ($CDCl_3$) δ 6.42 (s, 1H), 6.41 (s, 1H), 5.24 (sp, J = 6.2 Hz, 1H), 3.90 (s, 3H), 3.59 (bs, 3H), 3.32 (s, 3H), 1.35 (d, J = 6.2 Hz, 6H). Found: [M+H]=255.1.

Vinylmagnesium bromide (43 mL, 1 M in THF, 43 mmol) was added to a solution of **S13** (5.78 g, 21.7 mmol) in THF (100 mL, dist. Na) at 0 °C, the brown solution was stirred at 0 °C for 1 h and then dimethylamine (43 mL, 2 M in THF, 86 mmol) and water (40 mL) were added. The solution was stirred at r.t. for 1 h then partitioned between EtOAc and water. The solution was dried and evaporated to give 3-(dimethylamino)-1-(2-methoxy-6-propoxypyridin-4-yl)propan-1-one (**CD-3**) (5.75 g, 100%). ^{1}H NMR ($CDCl_3$) δ 6.73 (d, J = 1.1 Hz, 1H), 6.72 (d, J = 1.1 Hz, 1H), 4.26 (t, J = 6.7 Hz, 2H), 3.93 (s, 3H), 3.06 (t, J = 7.4 Hz, 2H), 2.72 (J = 7.4 Hz, 2H), 2.27 (s, 6H), 1.80 (qt, J = 7.4, 6.7 Hz, 2H), 1.03 (t, J = 7.4 Hz, 3H). Found: [M+H]=267.2.

*4.1.2.3. 1-(2-Cyclobutoxy-6-methoxypyridin-4-yl)-3-(dimethylamino)propan-1-one (**CD-4**)*



A solution of **S4** (3.00 g, 16.4 mmol) in DMF (50 mL, anhydrous) was treated with K_2CO_3 (4.52 g, 32.7 mmol) and then bromocyclobutane (2.00 mL, 25.0 mmol). The mixture was stirred at r.t. for 48 h, partitioned between EtOAc and water and the aqueous layer was extracted with EtOAc. The organic fractions were washed with water, dried and evaporated. Column chromatography (DCM) gave methyl 2-cyclobutoxy-6-methoxyisonicotinate (**S14**) (2.21 g, 57%). ^{1}H NMR ($CDCl_3$) δ 6.84 (d, J = 1.0 Hz, 1H), 6.79 (d, J = 1.0 Hz, 1H), 5.08

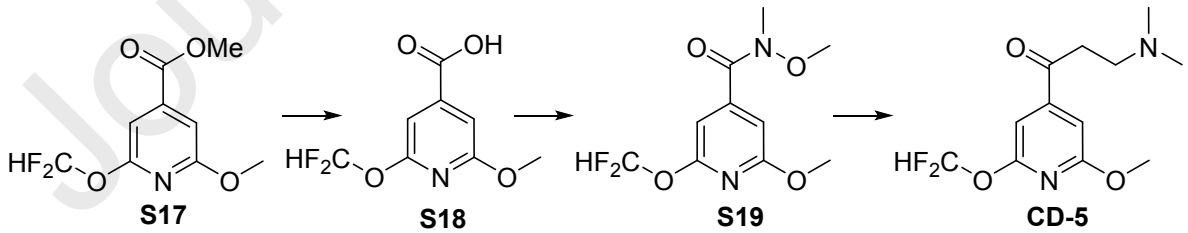
(pd, J = 7.4, 0.8 Hz, 1H), 3.91 (s, 3H), 3.90 (s, 3H), 2.42-2.52 (m, 2H), 2.12-2.24 (m, 2H), 1.80-1.90 (m, 1H), 1.62-1.75 (m, 1H). Found: [M+H]=238.2.

A solution of LiOH (0.71 g, 29.6 mmol) in water (20 mL) was added to a solution of **S14** (2.20 g, 9.29 mmol) in MeOH (20mL) and THF (20 mL); the solution was stirred at r.t. for 18 h and then evaporated. The residue was dissolved in water (80 mL) and acidified to pH 3 with 2 M HCl. The precipitate was filtered and dried to give 2-cyclobutoxy-6-methoxyisonicotinic acid (**S15**) (2.02 g, 97%). ^{1}H NMR (DMSO-d_6) δ 13.56 (bs, 1H), 6.74 (d, J = 1.0 Hz, 1H), 6.67 (d, J = 1.0 Hz, 1H), 5.07 (pd, J = 7.1, 0.7 Hz, 1H), 3.85 (s, 3H), 2.37-2.46 (m, 2H), 2.14-2.22 (m, 2H), 1.74-1.83 (m, 1H), 1.59-1.72 (m, 1H). Found: [M+H]=224.2.

Oxalyl chloride (0.45 mL, 5.32 mmol) was added to a suspension of **173*S15** (1.00 g, 4.48 mmol) in DCM (50 mL, anhydrous) and DMF (0.2 mL) at r.t.. The mixture was stirred at r.t. for 1 h to give a colourless solution which was cooled to 0 °C. *N*,*O*-Dimethylhydroxylamine hydrochloride (0.52 g, 5.33 mmol) and pyridine (1.09 mL, 13.5 mmol) were added sequentially and the mixture was stirred at r.t. for 18 h, then partitioned between DCM and sat. aq. $NaHCO_3$. Column chromatography on alumina with DCM gave 2-cyclobutoxy-*N*,6-dimethoxy-*N*-methylisonicotinamide (**S16**) (1.01 g, 85%). ^{1}H NMR ($CDCl_3$) δ 6.46 (s, 1H), 6.40 (d, J = 0.6 Hz, 1H), 5.08 (pd, J = 7.4, 0.9 Hz, 1H), 3.90 (s, 3H), 3.58 (bs, 3H), 3.32 (s, 3H), 2.41-2.51 (m, 2H), 2.12-2.23 (m, 2H), 1.80-1.90 (m, 1H), 1.62-1.74 (m, 1H). Found: [M+H]=267.2.

Vinylmagnesium bromide (8.0 mL, 1 M, 8.0 mmol) was added to a solution of **S16** (1.02 g, 3.84 mmol) in THF (50 mL, dist. Na) at 0 °C, the brown solution was warmed to r.t. for 1 h then dimethylamine in THF (8.0 mL, 2M, 16.0 mmol) and water (10 mL) were added. The solution was stirred at r.t. for 1 h, and then partitioned between EtOAc and water. The solution was dried and evaporated, column chromatography (95:5 DCM:MeOH) gave 1-(2-cyclobutoxy-6-methoxypyridin-4-yl)-3-(dimethylamino)propan-1-one (**CD-4**) (1.05 g, 97%). ^{1}H NMR ($CDCl_3$) δ 6.73 (d, J = 1.1 Hz, 1H), 6.68 (d, J = 1.2 Hz, 1H), 5.10 (pd, J = 7.4, 1.1 Hz, 1H), 3.91 (s, 3H), 3.05 (t, J = 7.0 Hz, 2H), 2.72 (t, J =7.0 Hz, 2H), 2.42-2.50 (m, 2H), 2.27 (s, 6H), 2.13-2.23 (m, 2H), 1.80-1.90 (m, 1H), 1.62-1.74 (m, 1H). Found: [M+H]=279.2.

*4.1.2.4. 1-(2-(Difluoromethoxy)-6-methoxypyridin-4-yl)-3-(dimethylamino)propan-1-one (**CD-5**)*



To a solution of **S4** (3.00 g, 16.4 mmol) in DMF (40 mL) was added sodium chlorodifluoroacetate (7.50 g, 49.2 mmol) and K_2CO_3 (2.73 g, 21.3 mmol). The reaction mixture was stirred at 80 °C for 72 h, then washed with water (50 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to obtain a yellow residue. Purification by flash column chromatography using hexanes:EtOAc (1:1) gave ethyl 2-(difluoromethoxy)-6-methoxyisonicotinate (**S17**) as a white solid (2.02 g, 52%). ^{1}H NMR

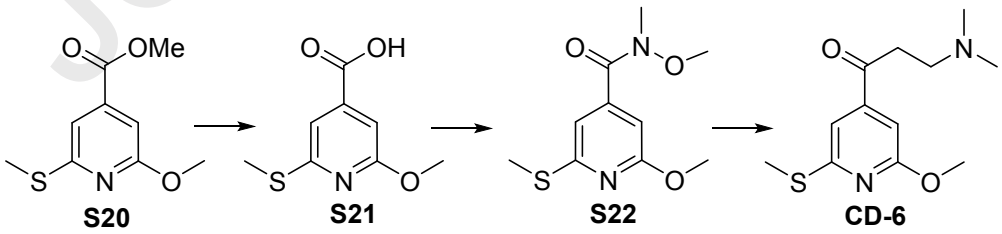
($CDCl_3$) δ 7.40 (t, J = 73.0, 1H), 7.10 (d, J = 1.0 Hz, 1H), 7.00 (d, J = 1.0 Hz, 1H), 3.94 (s, 3H), 3.93 (s, 3H). Found: [M+H]=234.5.

To a solution of **S17** (2.02 g, 8.66 mmol) in MeOH:THF:H_2O (60 mL, 1:1:1) was added lithium hydroxide (0.62 g, 26.0 mmol) and the reaction mixture was stirred at r.t. for 72 h. The solvent was removed under reduced pressure, water (50 mL) was added and the mixture was washed with EtOAc (50 mL) which was discarded. 2M HCl (50 mL) was added to the aqueous layer, which was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to obtain 2-(difluoromethoxy)-6-methoxyisonicotinic acid (**S18**) as a white solid (1.90 g, 99%). ^{1}H NMR (DMSO-d_6) δ 13.85 (bs, 1H), 7.76 (t, J = 72.5 Hz, 1H), 7.03 (d, J = 0.96 Hz, 1H), 6.95 (d, J = 0.92 Hz, 1H), 3.91 (s, 3H). Found: [M+H]=220.6.

To a solution of **S18** (1.70 g, 8.53 mmol), hydroxybenzotriazole (1.29 g, 9.54 mmol), *N,O*-dimethylhydroxylamine hydrochloride (1.27 g, 13.0 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.48 g, 9.54 mmol) in DCM (50 mL) was added DMF (4.83 mL, 34.7 mmol). The reaction mixture was stirred at r.t for 24 h. The reaction mixture was washed with water (100 mL) and extracted with DCM (3 x 50 mL). The organic phase was dried with Na_2SO_4 and concentrated to give a yellow residue. Purification by flash column chromatography using hexanes:EtOAc (1:1) gave 2-(difluoromethoxy)-*N*,6-dimethoxy-*N*-methylisonicotinamide (**S19**) as a yellow oil (1.85 g, 81%). ^{1}H NMR ($CDCl_3$) δ 7.41 (t, J = 73.1 Hz, 1H), 6.73 (d, J = 0.68 Hz, 1H), 6.65 (d, J = 0.64 Hz, 1H), 3.92 (s, 3H), 3.58 (s, 3H), 3.34 (s, 3H). Found: [M+H]=263.5.

To a solution of **S19** (1.85 g, 7.06 mmol) in THF (100 mL) at 0 °C was added vinylmagnesium bromide (1M solution in THF, 14.8 mL, 14.8 mmol) and the solution stirred at 0 °C for 3 h. Dimethylamine (2M solution in THF, 14.8 mL, 29.7 mmol) was added followed by water (40 mL). After 30 minutes stirring at r.t., the reaction mixture was concentrated under reduced pressure to obtain a brownish residue. This was extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give 1-(2-(difluoromethoxy)-6-methoxypyridin-4-yl)-3-(dimethylamino)propan-1-one (**CD-5**) as a brown oil (1.92 g, 99%). ^{1}H NMR ($CDCl_3$) δ 7.40 (t, J = 73.0 Hz, 1H), 6.97 (d, J = 1.1 Hz, 1H), 6.89 (d, J = 1.1 Hz, 1H), 3.95 (s, 3H), 3.07 (t, J = 7.0 Hz, 2H), 2.73 (t, J = 7.3 Hz, 2H), 2.27 (s, 6H). Found: [M+H]= 275.6.

*4.1.2.5. 3-(Dimethylamino)-1-(2-(dimethylamino)-6-methoxypyridin-4-yl)propan-1-one (**CD-6**)*



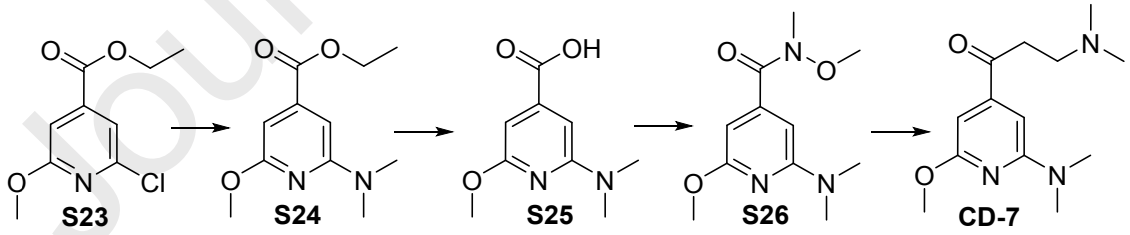
To a mixture of methyl 2-methoxy-6-(methylthio)isonicotinate (**S20**) (WO 2010/036632) (2.07 g, 9.71 mmol) in MeOH:THF:H_2O (60 mL, 1:1:1) was added lithium hydroxide (0.697 g, 29.1 mmol). The reaction mixture was stirred at r.t. for 24 h. The solvent was removed under reduced pressure, water (50 mL) was added and the mixture was washed with EtOAc (50 mL)

which was discarded. 2M HCl (50 mL) was added to the aqueous layer, which was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to obtain 2-methoxy-6-(methylthio)isonicotinic acid (**S21**) as a white solid (1.70 g, 88%). ^{1}H NMR (DMSO-d_6) δ 13.67 (s, 1H), 7.22 (d, J = 0.92 Hz, 1H), 6.86 (d, J = 0.92 Hz, 1H), 3.90 (s, 3H), 2.55 (s, 3H). Found: [M+H]=200.5.

To a solution of **S21** (1.70 g, 8.53 mmol) in DCM (200 mL) was added DMF (0.13 mL), followed by dropwise addition of oxalyl chloride (0.88 mL, 10.2 mmol). The mixture was stirred at r.t. for 2 h. The reaction mixture was cooled to 0 °C and *N,O*-dimethylhydroxylamine hydrochloride (0.915, 9.38 mmol) followed by pyridine (2.27 mL, 28.2 mmol) were added and resulting mixture was stirred at r.t. for 18 h. The mixture was poured onto sat. $NaHCO_3$ (150 mL) and extracted with DCM (150 mL) and EtOAc (100 mL). The combined organic phase was dried with Na_2SO_4 and concentrated to give a yellow residue. Purification by flash column chromatography using hexanes:EtOAc (4:1) gave *N*,2-dimethoxy-*N*-methyl-6-(methylthio)isonicotinamide (**S22**) as yellow oil (2.07 g, 99%). ^{1}H NMR ($CDCl_3$) δ 6.93 (d, J = 0.72 Hz, 1H), 6.59 (s, 1H), 3.97 (s, 3H), 3.57 (s, 3H), 3.32 (s, 3H), 2.57 (s, 3H). Found: [M+H]=243.5.

To a solution of **S22** (2.07 g, 8.54 mmol) in THF (100 mL) at 0 °C was added vinylmagnesium bromide (1M solution in THF, 17.9 mL, 17.9 mmol) and the solution was stirred at 0 °C for 3 h. Dimethylamine (2M solution in THF, 17.9 mL, 35.9 mmol) was added followed by water (40 mL). After 30 minutes stirring at r.t. the reaction mixture was concentrated under reduced pressure to obtain a brownish residue. This was extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give 3-(dimethylamino)-1-(2-methoxy-6-(methylthio)pyridin-4-yl)propan-1-one (**CD-6**) as a brown oil (2.17 g, 99%). ^{1}H NMR ($CDCl_3$) δ 7.19 (d, J = 1.2 Hz, 1H), 6.82 (d, J = 1.1 Hz, 1H), 3.98 (s, 3H), 3.04 (t, J = 7.0 Hz, 2H), 2.72 (t, J = 7.3 Hz, 2H), 2.58 (s, 3H), 2.27 (s, 6H). Found: [M+H]=255.6.

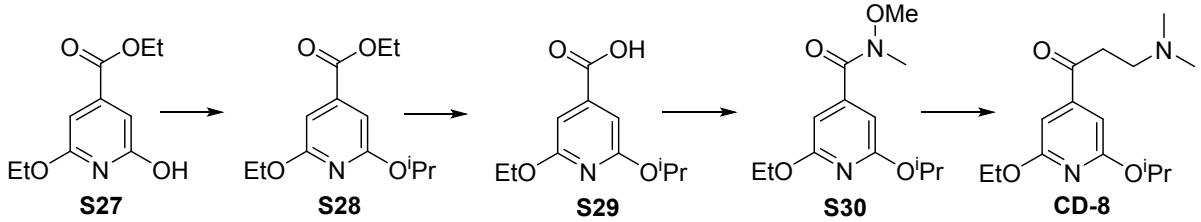
*4.1.2.6. 3-(Dimethylamino)-1-(2-(dimethylamino)-6-methoxypyridin-4-yl)propan-1-one (**CD-7**)*



To a glass tube was charged ethyl 2-chloro-6-methoxyisonicotinate (**S23**) (WO 2009/024905) (4.01 g, 18.60 mmol), diphenylphosphino-1,1'-binaphthol (1.85 g, 2.976 mmol) and cesium carbonate (8.49 g, 26.10 mmol) under continuous nitrogen flow. Anhydrous toluene (72 mL) was added. The mixture was purged with nitrogen for 5 min. Palladium acetate (0.33 g, 1.488 mmol) was added, the mixture was purged again with nitrogen. Dimethylamine in tetrahydrofuran (2M, 11.2 mL, 22.3 mmol) was added and the mixture was sealed in the tube and heated at 80 °C for 19.5 hours. The mixture was filtered through Celite, washing with EtOAc. The filtrate was concentrated in vacuo to yield the crude product as a dark red liquid.

Flash column chromatography of the crude product using 3-10% Et_2O in hexanes provided ethyl 2-(dimethylamino)-6-methoxyisonicotinate (**S24**) as a yellow crystalline solid. Yield = 3.36 g, 80%. ^{1}H NMR ($CDCl_3$) δ 6.61 (1H, d, J = 0.96 Hz), 6.52 (1H, d, J = 0.88 Hz), 4.35 (2H, q, J = 7.1 Hz), 3.90 (3H, s), 3.1 (6H, s), 1.38 (3H, t, J = 7.2 Hz). Found: [M+H]=225.5.
To a solution of **S24** (6.77 g, 30.2 mmol) in EtOH (150 mL) was added at r.t. NaOH (30.2 mL, 60.4 mmol). The mixture was stirred at r.t. for 2 hours and then concentrated in vacuo to a light yellow solution, which was further diluted in water. The aqueous mixture was acidified to pH ~2 with 2M HCl, when bright yellow solids precipitated out. These were collected by filtration, washed with water and dried under ambient conditions to yield 2-(dimethylamino)-6-methoxyisonicotinic acid (**S25**) as a bright yellow powder. Yield = 5.60 g, 95%. ^{1}H NMR (DMSO-d_6) δ 13.25 (1H, br s), 6.54 (1H, d, J = 0.9 Hz), 6.33 (1H, d, J = 0.8 Hz), 3.82 (3H, s), 3.04 (6H, s).
To a suspension of **S25** (0.75 g, 3.81 mmol) in anhydrous DMF (22 mL) was added triethylamine (1.6 mL, 11.4 mmol), the mixture was cooled to 2 °C. Ethyl chloroformate (0.62 mL, 4.19 mmol) was added dropwise and fumes were evolved. The mixture was stirred from 2 °C to r.t. for 2.5 hours and then treated with *N*,*O*-dimethylhydroxylamine hydrochloride (0.557 g, 5.71 mmol) at 2 °C under nitrogen. The mixture was stirred to r.t. overnight. The mixture was diluted in water, and the aqueous mixture was extracted with EtOAc (4x) and the organic extract was washed with water, brine, dried ($MgSO_4$) and concentrated to afford the crude product. This was purified by repeated flash chromatography eluting with mixtures of 4:1 then 2:1 hexanes/EtOAc as eluent, yielding 2-(dimethylamino)-*N*,6-dimethoxy-*N*-methylisonicotinamide (**S26**) as a yellow oil. Yield = 0.70 g, 77%. ^{1}H NMR ($CDCl_3$) δ 6.17 (1H, s), 6.11 (1H, s), 3.89 (3H, s), 3.62 (3H, br s), 3.31 (3H, s), 3.08 (6H, s).
To a solution of **S26** (2.53 g, 10.60 mmol) in freshly distilled THF (96 mL) was added at 2 °C under nitrogen vinylmagnesium bromide in THF (1N, 21.1 mL, 21.10 mmol) dropwise. The mixture was stirred at 2 °C for 20 min, then at r.t. for 75 min. Dimethylamine in THF (2N, 21.1 mL, 42.20 mmol) was added, followed by water (40 mL). The mixture was stirred at r.t. for 1 hour. The aqueous mixture was partitioned between water and EtOAc and the organic phase was separated and the aqueous phase was extracted with EtOAc (2x). The organic extract was washed with brine, dried (Na_2SO_4) and concentrated in vacuo to give the crude product as a yellow-brown oil. Flash chromatography of the product using 2-8% MeOH in DCM as eluent gave 3-(dimethylamino)-1-(2-(dimethylamino)-6-methoxypyridin-4-yl)propan-1-one (**CD-7**) as a yellow oil which crystallised at -20 °C. Yield = 1.91 g, 72%. ^{1}H NMR ($CDCl_3$) δ 6.47 (1H, d, J = 1.0 Hz), 6.39 (1H, d, J = 1.0 Hz), 3.91 (3H, s), 3.10 (6H, s), 3.06 (2H, t, J = 7.0 Hz), 2.72 (2H, t, J = 7.1 Hz), 2.27 (6H, s).

4*.1.2.7. 3-(Dimethylamino)-1-(2-ethoxy-6-isopropoxypyridin-4-yl)propan-1-one (**CD-8**)*



A suspension of 2,6-dihydroxyisonicotinic acid (40.00 g, 258 mmol) in EtOH (300 mL) was treated dropwise with H_2SO_4 (40 mL, 18.4 M, 752 mmol). The solution was refluxed for 72 h then evaporated; the residue was treated with sat. aq. $NaHCO_3$ to pH 8 and then extracted with EtOAc (3 x 500 mL). The organic extracts were washed with sat. aq. $NaHCO_3$, brine, then dried and evaporated to give ethyl 2-ethoxy-6-hydroxyisonicotinate (**S27**) (10.86 g, 20%). ^{1}H NMR (DMSO-d_6) δ 11.15 (bs, 1H), 6.59 (d, J = 1.0 Hz, 1H), 6.57 (bs, 1H), 4.29 (q, J = 7.1 Hz, 2H), 4.25 (q, J = 7.1 Hz, 2H), 1.298 (t, J = 7.1 Hz, 3H), 1.296 (t, J = 7.1 Hz, 3H). Found: [M+H]=212.2.

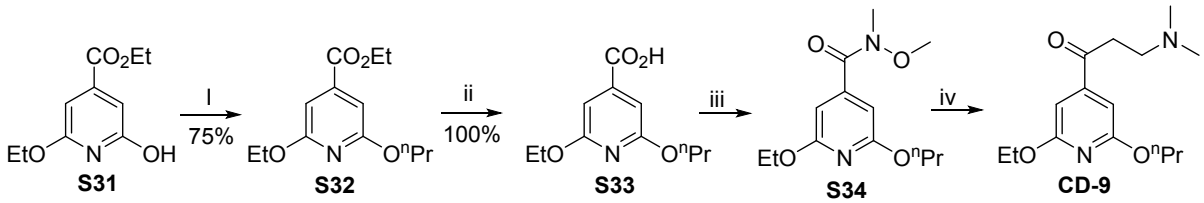
A solution of **S27** (10.82 g, 51.2 mmol) in DMF (125 mL, anhydrous) was treated with K_2CO_3 (8.65 g, 62.5 mmol) and then 2-iodopropane (6.4 mL, 64 mmol). The mixture was stirred at r.t. for 48 h, K_2CO_3 (8.65 g, 62.5 mmol) and 2-iodopropane (6.4 mL, 64 mmol) were added and the mixture was stirred for a further 24 h, partitioned between EtOAc and water and the aqueous layer was extracted with EtOAc. The organic fractions were washed with water, dried and evaporated. Chromatography (DCM) gave ethyl 2-ethoxy-6-isopropoxyisonicotinate (**S28**) (11.56 g, 89%). ^{1}H NMR (DMSO-d_6) δ 6.81 (s, 2H), 5.23 (sp, J = 6.2 Hz, 1H), 4.20-4.38 (m, 4H), 1.35-1.42 (m, 12H). Found: [M+H]=254.1.

A solution of LiOH (3.25 g, 136 mmol) in water (60 mL) was added to a solution of **S28** (11.42 g, 45.1 mmol) in THF (60 mL) and MeOH (60 mL), the solution was stirred at r.t. for 18 h and then evaporated. The residue was dissolved in water (150 mL) and acidified to pH 3 with 2 M HCl. The precipitate was filtered and dried to give 2-ethoxy-6-isopropoxyisonicotinic acid (**S29**) (10.03 g, 99%). ^{1}H NMR (DMSO-d_6) δ 13.48 (bs, 1H), 6.66 (d, J = 0.9 Hz, 1H), 6.64 (d, J = 0.9 Hz, 1H), 5.17 (sp, J = 6.2 Hz, 1H), 4.29 (q, J = 7.0 Hz, 2H), 1.32 (t, J = 7.0 Hz, 3H), 1.30 (d, J = 6.2 Hz, 6H). Found: [M+H]=226.1.

Oxalyl chloride (3.13 mL, 37 mmol) was added to **S29** (6.95 g, 30.8 mmol) in DCM (100 mL, anhydrous) and DMF (5.2 mmol) at r.t.. The mixture was stirred at r.t. for 1 h to give a colourless solution which was cooled to 0 °C. *N*,*O*-Dimethylhydroxylamine hydrochloride (3.61 g, 37.0 mmol) and pyridine (7.5 mL, 92.7 mmol) were added sequentially and the mixture was stirred at r.t. for 18 h, then partitioned between EtOAc and water. The organic fractions were washed with water, dried and evaporated. Column chromatography with 95:5 DCM:EtOAc gave 2-ethoxy-6-isopropoxy-*N*-methoxy-*N*-methylisonicotinamide (**S30**) (7.98 g, 97%). ^{1}H NMR ($CDCl_3$) δ 6.40 (s, 1H), 6.39 (s, 1H), 5.22 (sp, J = 6.2 Hz, 1H), 4.33 (q, J = 7.1 Hz, 2H), 3.60 (bs, 3H), 3.31 (s, 3H), 1.39 (t, J = 6.2 Hz, 3H), 1.34 (d, J = 7.1 Hz, 6H). Found: [M+H]=269.2.

Vinylmagnesium bromide (40 mL, 1 M in THF, 40 mmol) was added to a solution of **S30** (5.33 g, 19.9 mmol) in THF (100 mL, dist. Na) at 0 °C, the brown solution was stirred at 0 °C for 1 h and then dimethylamine (40 mL, 2 M in THF, 80 mmol) and water (40 mL) were added. The solution was stirred at r.t. for 1 h then partitioned between EtOAc and water. The solution was dried and evaporated, to give 3-(dimethylamino)-1-(2-ethoxy-6-isopropoxypyridin-4-yl)propan-1-one (**CD-8**) (5.57 g, 100%) as a brown oil. ^{1}H NMR ($CDCl_3$) δ 6.68 (d, J = 1.0 Hz, 1H), 6.67 (d, J = 1.0 Hz, 1H), 5.22 (sp, J = 6.2 Hz, 1H), 4.33 (q, J = 7.0 Hz, 2H), 3.05 (t, J = 7.5 Hz, 2H), 2.72 (t, J = 7.5 Hz, 2H), 2.26 (s, 6H), 1.40 (t, J = 6.2 Hz, 3H), 1.36 (d, J = 7.0 Hz, 6H). Found: [M+H]=281.7.

*4.1.2.9. 3-(Dimethylamino)-1-(2-ethoxy-6-propoxypyridin-4-yl)propan-1-one (**CD-9**)*



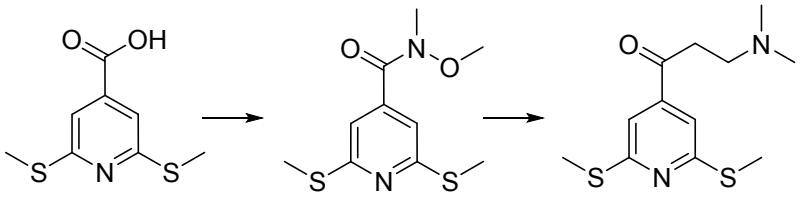
A mixture of ethyl 2-ethoxy-6-hydroxyisonicotinate (**S31**) (10.0 g, 47.3 mmol) and potassium carbonate (5.80 g, 59.2 mmol) in DMF (100 mL) was treated with 1-iodopropane (8.17 mL, 59.4 mmol) and then stirred at room temperature for 72 h. The mixture was partitioned with EtOAc and water. The organic fractions were washed with water, dried and evaporated. Column chromatography on silica with DCM gave ethyl 2-ethoxy-6-propoxyisonicotinate (**S32**) (8.98 g, 75%). ^{1}H NMR ($CDCl_3$) δ 6.84 (d, J = 1.1 Hz, 1H), 6.83 (d, J = 1.1 Hz, 1H), 4.36 (q, J = 7.2 Hz, 2H), 4.34 (q, J = 7.1 Hz, 2H), 4.23 (t, J = 6.7 Hz, 2H), 1.80 (qt, J = 7.4, 6.8 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H), 1.37 (t, J = 7.1 Hz, 3H), 1.02 (t, J = 7.4 Hz, 3H). Found: [M+H]=269.2.

A solution of lithium hydroxide (2.53, 106.0 mmol) in water (60 mL) was added to a solution of **S32** (35.4 mmol) in methanol (60mL) and THF (60 mL), the solution was stirred at room temperature for 18 h and then evaporated. The residue was dissolved in water (150 mL) and acidified with 2 M HCl to pH 3. The precipitate was filtered, dissolved in EtOAc, dried and evaporated to give 2-ethoxy-6-propoxyisonicotinic acid (**S33**) as a white solid (7.96 g, 100%). ^{1}H NMR (DMSO-d_6) δ 13.52 (bs, 1H), 6.70 (d, J = 1.0 Hz, 1H), 6.69 (d, J = 1.0 Hz, 1H), 4.30 (q, J = 7.0 Hz, 2H), 4.20 (t, J = 6.6 Hz, 2H), 1.78 (qt, J = 7.4, 6.8 Hz, 2H), 1.32 (t, J = 7.0 Hz, 3H), 0.95 (t, J = 7.4 Hz, 3H). Found: [M+H]= 226.1.

Oxalyl chloride (1.51 mL, 17.8 mmol) was added to **S33** (3.35 g, 14.9 mmol) in DCM (100 mL, anhydrous) and DMF (0.4 mL, 5.2 mmol) at r.t.. The mixture was stirred at r.t. for 1 h to give a colourless solution which was cooled to 0 °C. *N*,*O*-Dimethylhydroxylamine hydrochloride (1.74 g, 17.8 mmol) and pyridine (3.61 mL, 44.6 mmol) were added sequentially and the mixture was stirred at r.t. for 18 h, then partitioned between EtOAc and water. The organic fractions were washed with water, dried and evaporated. Column chromatography with DCM on alumina gave 2-ethoxy-*N*-methoxy-*N*-methyl-6-propoxyisonicotinamide (**S34**) (3.82 g, 96%). ^{1}H NMR ($CDCl_3$) δ 6.44 (s, 1H), 6.42 (s, 1H), 4.33 (q, J = 7.1 Hz, 2H), 4.22 (t, J = 6.7 Hz, 2H), 3.59 (bs, 3H), 3.32 (s, 3H), 1.79 (qt, J = 7.4, 6.8 Hz, 2H), 1.39 (t, J = 7.1 Hz, 3H), 1.01 (t, J = 7.4 Hz, 3H). Found: [M+H]=254.2.

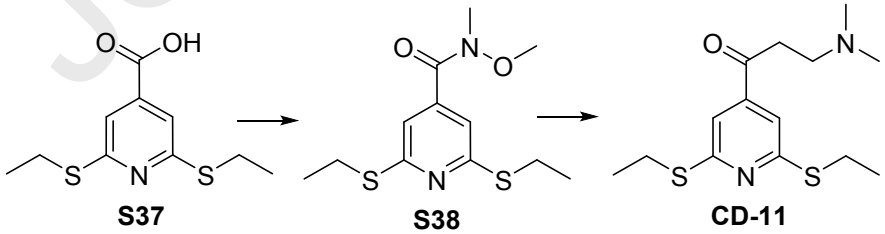
Vinylmagnesium bromide (28.0 mL, 1 M in THF, 28.0 mmol) was added to a solution of **S34** (3.78 g, 14.0 mmol) in THF (150 mL, dist. Na) at 0 °C, the brown solution was stirred at 0 °C for 1 h and then dimethylamine (28.0 mL, 2 M in THF, 56.0 mmol) and water (30 mL) were added. The solution was stirred at r.t. for 1 h then partitioned between EtOAc and water. The solution was dried and evaporated, to give 3-(dimethylamino)-1-(2-ethoxy-6-propoxypyridin-4-yl)propan-1-one (**CD-9**) (3.86 g, 98%) as a brown oil. ^{1}H NMR ($CDCl_3$) δ 6.72 (d, J = 1.1 Hz, 1H), 6.70 (d, J = 1.1 Hz, 1H), 4.34 (q, J = 7.1 Hz, 2H), 4.24 (t, J = 6.7 Hz, 2H), 3.05 (q, J = 7.0 Hz, 2H), 2.71 (q, J = 7.0 Hz, 2H), 2.27 (s, 6H), 1.80 (qt, J = 7.4, 6.8 Hz, 2H), 1.40 (t, J = 7.1 Hz, 3H), 1.02 (t, J = 7.4 Hz, 3H). Found: [M+H]=281.7.

*4.1.2.10. 1-(2,6-Bis(methylthio)pyridin-4-yl)-3-(dimethylamino)propan-1-one (**CD-10**)*



To a solution of 2,6-dichloroisonicotinic acid (4.00 g, 20.8 mmol) in DMF (40 mL) at 0 °C was added sodium thiomethoxide (4.38 g, 65.5 mmol). The reaction mixture was stirred at 150 °C for 18 h. Water (40 mL) was added to the resultant solution and the pH was adjusted to ~3 using 2M HCl solution. The aqueous solution was extracted with EtOAc (3 x 40 mL), dried with $MgSO_4$, filtered and concentrated under reduced pressure to give 2,6-bis(methylthio)isonicotinic acid (**S35**) as an orange solid, which was recrystallized from methanol (4.01 g, 90%). ^{1}H NMR ($CDCl_3$) δ 7.44 (s, 2H), 2.61 (s, 6H). Found: [M+H]=216.5.
To a solution of **S35** (5.03 g, 23.4 mmol) in DCM (200 mL) was added DMF (0.362 mL), followed by dropwise addition of oxalyl chloride (2.41 mL, 28.0 mmol). The mixture was stirred at r.t. for 2 h, cooled to 0 °C and *N,O*-dimethylhydroxylamine hydrochloride (2.51 g, 25.7 mmol) followed by pyridine (6.22 mL, 77.1 mmol) were added and resulting mixture was stirred at r.t. for 18 h. The mixture was poured onto sat $NaHCO_3$ (150 mL), extracted with DCM (150 mL) and EtOAc (100 mL). The organic phase was dried with Na_2SO_4 and concentrated to give *N*-methoxy-*N*-methyl-2,6-bis(methylthio)isonicotinamide (**S36**) as yellow oil, which was used without further purification for the next step (4.96 g, 82%). ^{1}H NMR ($CDCl_3$) δ 7.04 (s, 2H), 3.56 (s, 3H), 3.33 (s, 3H), 2.60 (s, 6H). Found: [M+H]=259.5.
To a solution of **S36** (4.96 g, 19.2 mmol) in THF (90 mL) at 0 °C was added vinylmagnesium bromide (1M solution in THF, 40.3 mL, 40.3 mmol) and the solution was stirred at 0 °C for 1 h. Dimethylamine (2M solution in THF, 40.3 mL, 80.6 mmol) was added followed by water (60 mL). After 30 minutes stirring at r.t., the reaction mixture was concentrated under reduced pressure to obtain a brownish residue. This was extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give 1-(2,6-bis(methylthio)pyridin-4-yl)-3-(dimethylamino)propan-1-one (**CD-10**) as a brown oil (4.87 g, 94%). ^{1}H NMR ($CDCl_3$) δ 7.26 (s, 2H), 3.04 (t, J = 7.0 Hz, 2H), 2.70 (t, J = 7.2 Hz, 2H), 2.61 (s, 6H), 2.26 (s, 6H). Found: [M+H]=271.6.

*4.1.2.11. 1-(2,6-Bis(ethylthio)pyridin-4-yl)-3-(dimethylamino)propan-1-one (**CD-11**)*



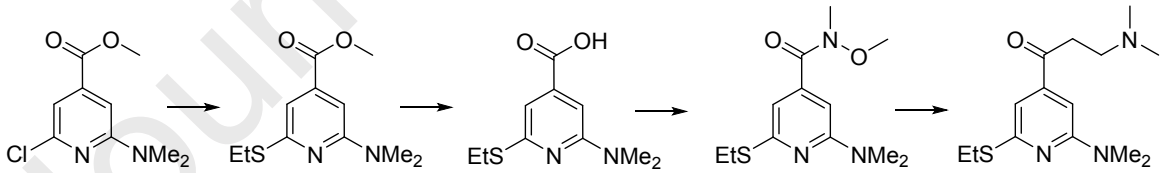
To a suspension of sodium hydride (1.82 g, 45.5 mmol) in DMF (20 mL) at 0 °C was added ethanethiol (3.29 mL, 45.5 mmol) dropwise. The milky foamy solution was stirred at 0 °C for 10 min 2,6-dichloroisonicotinic acid (3.17 g, 13.0 mmol) in DMF (5 mL) was added dropwise to the solution. The mixture was warmed to 50 °C and stirred for 18 h. Water (40 mL) was

added to the resultant solution and the pH was adjusted to ~3 using 2M HCl solution. The aqueous solution was extracted with EtOAc (3 x 30 mL), dried with $MgSO_4$, filtered and the solvent was evaporated to give 2,6-bis(ethylthio)isonicotinic acid **(S37**) as a yellow solid which was used for the next step without further purification (2.82 g, 89%). ^{1}H NMR ($CDCl_3$) δ 7.40 (s, 2H), 3.19 (q, J = 7.3 Hz, 4H), 1.39 (t, J = 7.3 Hz, 6H). Found: [M+H]=244.5.

To a solution of **S37** (3.24 g, 13.3 mmol) in DCM (150 mL) was added DMF (0.206 mL), followed by dropwise addition of oxalyl chloride (1.37 mL, 16.0 mmol). The mixture was stirred at r.t. for 2 h, then cooled to 0 °C and *N,O*-dimethylhydroxylamine hydrochloride (1.43 g, 14.6 mmol) followed by pyridine (3.55 mL, 43.9 mmol) were added and resulting mixture was stirred at r.t. for 18 h. The mixture was poured onto sat. $NaHCO_3$ (150 mL), extracted with DCM (150 mL) and EtOAc (100 mL). The combined organic phase was dried with Na_2SO_4 and concentrated to give a yellow residue. Purification by flash column chromatography using hexanes:EtOAc (1:1) gave 2,6-bis(ethylthio)-*N*-methoxy-*N*-methylisonicotinamide (**S38**) as colourless oil (3.65 g, 96%). ^{1}H NMR ($CDCl_3$) δ 7.02 (s, 2H), 3.56 (s, 3H), 3.32 (s, 3H), 3.19 (q, J = 7.3 Hz, 4H), 1.37 (t, J = 7.3 Hz, 6H). Found: [M+H]=287.5.

To a solution of **S38** (3.65 g, 12.7 mmol) in THF (150 mL) at 0 °C was added vinylmagnesium bromide (1M solution in THF, 31.5 mL, 31.5 mmol) which was then stirred at 0 °C for 4 h. Dimethylamine (2M solution in THF, 31.5 mL, 63.0 mmol) was added followed by water (60 mL). After 30 minutes stirring at r.t., the reaction mixture was concentrated under reduced pressure to obtain a brownish residue. This was extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a brown oil. Purification by flash column chromatography using EtOAc:MeOH (9:1) gave 1-(2,6-bis(ethylthio)pyridin-4-yl)-3-(dimethylamino)propan-1-one (**CD-11**) as a yellow oil (2.51 g, 66%). ^{1}H NMR ($CDCl_3$) δ 7.24 (s, 2H), 3.20 (q, J = 7.4 Hz, 4H), 3.03 (t, J = 7.1 Hz, 2H), 2.71 (t, J = 7.2 Hz, 2H), 2.26 (s, 6H), 1.38 (t, J = 7.4 Hz, 6H). Found: [M+H]=299.6.

*4.1.2.12. 3-(Dimethylamino)-1-(2-(dimethylamino)-6-(ethylthio)pyridin-4-yl)propan-1-one (**CD-12**)*



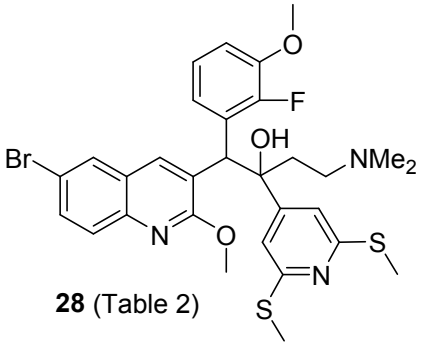
To a solution of methyl 2-chloro-6-(dimethylamino)isonicotinate (**S39**) (2.44 g, 11.4 mmol), rac-bis(diphenylphosphino)-1,1'-binaphthal (0.71 g, 1.14 mmol) and cesium carbonate (4.43 g, 13.6 mmol) under continuous nitrogen flow. Anhydrous toluene (30 mL) was added. The mixture was purged with nitrogen 5 minutes. Palladium acetate (0.26 g, 1.16 mmol) was added, the mixture was added purged again with nitrogen. Ethanethiol (1.0 mL, 13.6 mmol) was added and the mixture was sealed in the tube and heated at 150 °C for 22 hours. The mixture was filtered through celite, washed with ethyl acetate. The filtrate was concentrated in the fume hood by purging with air. Purification by flash column chromatography using diethyl ether:hexane (5:95) gave methyl 2-(dimethylamino)-6-(ethylthio)isonicotinate (**S40**) as a yellow crystalline solid (2.42 g, 88%). ^{1}H NMR ($CDCl_3$) δ 6.96 (d, J = 1.0 Hz, 1H), 6.73 (d, J

= 1.0 Hz, 1H), 3.89 (s, 3H), 3.16 (q, J = 7.3 Hz, 2H), 3.11 (s, 6H), 1.38 (t, J = 7.3 Hz, 3H). Found: [M+H]=241.2.

To a solution of **S40** (1.50 g, 6.25 mmol) in tetrahydrofuran (30 mL) was added at room temperature a solution of lithium hydroxide (0.45 g, 18.8) in water (15 mL). The reaction mixture was stirred at room temperautre overnight. Volatiles were removed in vacuo; the slurry was treated with 2M hydrochloric acid until pH reached 4. The yellow solids were collected by filtration, washed with water and dried to give 2-(dimethylamino)-6-(ethylthio)isonicotinic acid (**S41**) as a bright yellow solid (1.39 g, 98%). ^{1}H NMR ($CDCl_3$) δ 13.36 (s, 1H), 6.79 (d, J = 0.8 Hz, 1H), 6.69 (d, J = 0.8 Hz, 1H), 3.11 (q, J = 7.2 Hz, 2H), 3.07 (s, 6H), 1.30 (t, J = 7.2 Hz, 3H). Found: [M+H]=227.2.

To a solution of **S41** (2.21 g, 9.77 mmol) in anhydrous DMF (60 mL) was added triethylamine (4.1 ml, 29.3 mmol). The reaction mixture was cooled to 2 °C, ethyl chloroformate (1.6 ml, 10.7 mmol) was added dropwise. The mixture was stirred at ~4 °C for 1.5 h and was added *N,O*-dimethylhydroxylamine hydrochloride (1.43 g, 14.7 mmol). The mixture was stirred at room temperature for 18 h. The mixture was diluted in water, and the aqueous mixture was extracted with EtOAc (3 X 200 ml). The organic extract was washed with water, brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a crude product. Purification by flash column chromatography using hexane:EtOAc (2:1) gave 2-(dimethylamino)-6-(ethylthio)-*N*-methoxy-*N*-methylisonicotinamide (**S42**) as a yellow oil (4.09 g, 86%). ^{1}H NMR ($CDCl_3$) δ 6.57 (s, 1H), 6.32 (s, 1H), 3.60 (s, 3H), 3.31 (s, 3H), 3.14 (q, J = 7.2 Hz, 2H), 3.09 (s, 6H), 1.38 (t, J = 7.2 Hz, 3H). Found: [M+H]=270.2.

To a solution of **S42** (3.95 g, 14.7 mmol) in THF (140 mL) at 0 °C was added vinylmagnesium bromide (1M solution in THF, 29.3 mL, 29.3 mmol) which was then stirred at 0 °C for 1.5 h. Dimethylamine (2M solution in THF, 29.3 mL, 29.3 mmol) was added followed by water (50 mL). After 60 minutes stirring at r.t., the reaction mixture was concentrated under reduced pressure to obtain a brownish residue. This was extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with brine (100 mL), dried over Na_2SO_4, filtered and concentrated under reduced pressure to give a brown oil. Purification by flash column chromatography using EtOAc:MeOH (9:1) gave 3-(dimethylamino)-1-(2-(dimethylamino)-6-(ethylthio)pyridin-4-yl)propan-1-one (**CD-12**) as a yellow oil (3.35 g, 81%). ^{1}H NMR ($CDCl_3$) δ 6.83 (d, J = 1.2 Hz, 1H), 6.59 (d, J = 1.2 Hz, 1H), 3.17 (q, J = 7.2 Hz, 2H), 3.12 (s, 6H), 3.06 (t, J = 7.4 Hz, 2H), 2.72 (t, J = 7.6 Hz, 2H), 2.28 (s, 6H), 1.39 (t, J = 7.2 Hz, 6H). Found: [M+H]=282.2.



28 (Table 2)

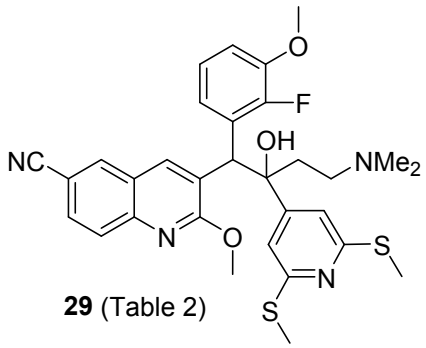
4.1.3. Example of coupling reaction for the bromo compouds of Table 2: 2-(2,6-bis(methylthio)pyridin-4-yl)-1-(6-bromo-2-methoxyquinolin-3-yl)-4-(dimethylamino)-1-(2-

*fluoro-3-methoxyphenyl)butan-2-ol (compound **28** of Table 2)***.** n-BuLi (4.79 mL of a 2N solution in cyclohexane, 9.57 mmol) was added at -40 °C under dry nitrogen to a solution of dry diisopropylamine (1.46 mL, 9.57 mmol) in dry THF (6 mL) and the solution was stirred at this temperature for 10 min, then cooled to -78 °C. A solution of 6-bromo-3-(2-fluoro-3-methoxybenzyl)-2-methoxyquinoline (**AB-10**) (3.00 g, 7.97 mmol) in dry THF (10 mL) was added dropwise and the mixture was stirred at -78 °C for 90 min, to give a dark, wine-red coloured solution. A solution of 1-(2,6-bis(methylthio)pyridin-4-yl)-3-(dimethylamino)propan-1-one (**CD-10**) (2.16 g, 7.97 mmol) in dry THF (7 mL) was added and the reaction mixture was stirred at this temperature for 5 h. Water (40 mL) was added and the mixture was extracted with EtOAc (2 X 50 mL). The combined organic extract was washed with sat. aq. $NaHCO_3$ solution, and brine, then dried with Na_2SO_4 and the solvent removed under reduced pressure. The residue was purified by flash column chromatography. Elution with hexanes:EtOAc (1:1) afforded isomer A of 28(1.77 g, 34%) followed by isomer B of **28** (1.38 g, 27%) as pale yellow foamy solids.

Isomer A. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.45 (s, 1H), 8.34 (br s, 1H), 7.83 (d, J = 2.2 Hz, 1H), 7.68 (d, J = 8.9 Hz, 1H), 7.62 (dd, J = 8.9, 2.2 Hz, 1H), 7.58-7.53 (m, 1H), 7.05 (br s, 2H), 6.85 (dt, J = 8.1, 1.5 Hz, 1H), 6.63 (dt, J = 8.1, 1.5 Hz, 1H), 5.35 (s, 1H), 4.15 (s, 3H), 3.71 (s, 3H), 2.55 (s, 6H), 2.28-2.20 (m, 1H), 2.04-1.99 (m, 1H), 2.01 (s, 6H), 1.98-1.91 (m, 1H), 1.71-1.66 (m, 1H). Found: [M+H]= 646.2.

Isomer B. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.58 (s, 1H), 8.31 (br s, 1H), 7.79 (d, J = 1.6 Hz, 1H), 7.56-7.50 (m, 2H), 7.28 (dd, J = 7.9, 1.5 Hz, 1H), 7.09 (br s, 2H), 6.95 (dt, J = 8.1, 1.4 Hz, 1H), 6.82 (dt, J = 8.1, 1.4 Hz, 1H), 5.30 (s, 1H), 3.91 (s, 3H) 3.91 (s, 3H), 2.49 (s, 6H), 2.29-2.20 (m, 1H), 2.06-2.01 (m, 1H), 2.04 (s, 6H), 1.99-1.93 (m, 1H), 1.65-1.60 (m, 1H). Found: [M+H]= 646.2.

The mixture was resolved into its four optical isomers using preparative supercritical fluid HPLC at BioDuro LLC (Beijing). The data in Table 1 are for the most active *R*,*S*-diastereomers. The other 6-bromo compounds in Table I were prepared and purified similarly.



29 (Table 2)

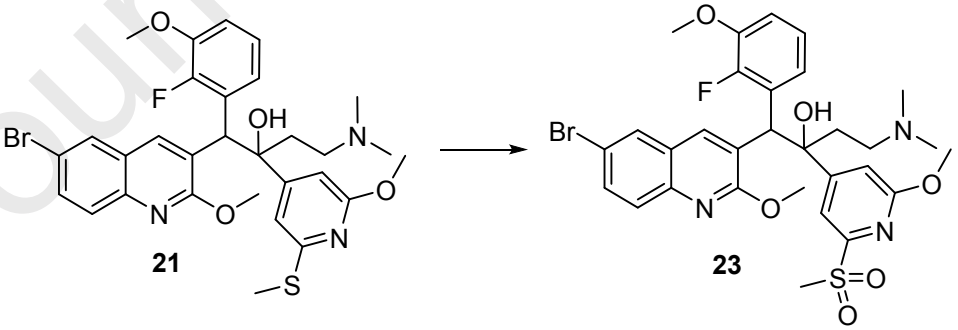
*4.1.4. Example of cyanation reaction: 3-(2-(2,6-bis(methylthio)pyridin-4-yl)-4-(dimethylamino)-1-(2-fluoro-3-methoxyphenyl)-2-hydroxybutyl)-2-methoxyquinoline-6-carbonitrile (compound **29** of Table 2).* A solution of Isomer A of **28** (Table 2) (1.06 g, 1.64 mmol) in DMF (10 mL, anhydrous) was purged with nitrogen and heated to 55 °C for 10 min Tri(o-tolyl)phosphine (0.10 g, 0.328 mmol), zinc dust (0.011 g, 0.164 mmol) and tris(dibenzylideneacetone)dipalladium(0) (0.150 g, 0.164 mmol) were then added, and the

reaction was again purged with nitrogen and heated for another 10 min at 55 °C. Zinc cyanide (0.135 g, 1.15 mmol) was then added and the reaction mixture was heated to 65 °C for 5 hours. The reaction was diluted with water and extracted with EtOAc (3 X 50 mL). The organic layer was washed with brine, dried with Na_2SO_4 and evaporated. Column chromatography with 1:1 hexane/EtOAc afforded Isomer A of **29** (0.48 g, 50%) as pale yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.57 (s, 1H), 8.40 (br s, 1H), 8.08 (d, J = 1.8 Hz, 1H), 7.85 (d, J = 8.6 Hz, 1H), 7.71 (dd, J = 8.6, 1.9 Hz, 1H), 7.58-7.52 (m, 1H), 7.05 (br s, 2H), 6.87 (dt, J = 8.1, 1.5 Hz, 1H), 6.65 (dt, J = 8.1, 1.5 Hz, 1H), 5.35 (s, 1H), 4.19 (s, 3H), 3.71 (s, 3H), 2.55 (s, 6H), 2.28-2.21 (m, 1H), 2.06-2.01 (m, 1H), 2.01 (s, 6H), 1.95-1.87 (m, 1H), 1.72-1.66 (m, 1H). Found: [M+H]= 593.2.

A solution of Isomer B of **28** (Table 2) (1.06 g, 1.64 mmol) in DMF (10 mL, anhydrous) was purged with nitrogen and heated to 55 °C for 10 min Tri(o-tolyl)phosphine (0.10 g, 0.328 mmol), zinc dust (0.011 g, 0.164 mmol) and tris(dibenzylideneacetone)dipalladium(0) (0.150 g, 0.164 mmol) were then added, and the reaction was again purged with nitrogen and heated for another 10 min at 55 °C. Zinc cyanide (0.135 g, 1.15 mmol) was then added and the reaction mixture was heated to 65 °C for 5 hours. The reaction was diluted with water and extracted with EtOAc (3 X 50 mL). The organic layer was washed with brine, dried with Na_2SO_4 and evaporated. Column chromatography with 1:1 hexane/EtOAc afforded Isomer B of **29** (0.59 g, 77%) as white solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.72 (s, 1H), 8.40 (br s, 1H), 8.02 (d, J = 1.7 Hz, 1H), 7.69 (d, J = 8.6 Hz, 1H), 7.63 (dd, J = 8.6, 1.8 Hz, 1H), 7.31-7.28 (m, 1H), 7.09 (br s, 2H), 6.98 (dt, J = 8.2, 1.4 Hz, 1H), 6.84 (dt, J = 8.1, 1.4 Hz, 1H), 5.28 (s, 1H), 3.94 (s, 3H) 3.91 (s, 3H), 2.49 (s, 6H), 2.31-2.21 (m, 1H), 2.07-2.04 (m, 1H), 2.03 (s, 6H), 2.02-1.93 (m, 1H), 1.65-1.60 (m, 1H). Found: [M+H]= 593.2.

The mixture was resolved into its four optical isomers using preparative supercritical fluid HPLC at BioDuro LLC (Beijing). The data in Table 1 are for the most active *R*,*S*-diastereomers. The other 6-cyano compounds in Table I were prepared and purified similarly.

*4.1.5. 1-(6-Bromo-2-methoxyquinolin-3-yl)-4-(dimethylamino)-1-(2-fluoro-3-methoxyphenyl)-2-(2-methoxy-6-(methylsulfonyl)pyridin-4-yl)butan-2-ol (compound **23** of Table 2)*



A solution of **21** (1.20 g, 1.90 mmol) in THF:water:acetone (2:1:1, 100 ml) was treated with *N*-methylmorpholine *N*-oxide (0.669 g, 5.71 mmol) followed by osmium tetroxide (1.81 g, 0.285 mmol) dropwise. The reaction was stirred for 27 h at room temperature. Reaction was washed with Na_2SO_3 (sat) solution, extracted with EtOAc (x 3), dried with Na_2SO_4, filtered and the solvent was evaporated to give a yellow oil. Purification by flash column chromatography with silica using hexanes:EtOAc (1:4) gave **23** as a white solid (1.10 g, 87%). ^{1}H NMR ($CDCl_3$) δ 8.63-8.56 (m, 3H), 7.88 (d, J = 2.0 Hz, 1H), 7.87-7.81 (m, 3H), 7.68 (d, J

= 8.9 Hz, 1H), 7.62 (dd, J = 8.8, 2.2 Hz, 1H), 7.56-7.48 (m, 3H), 7.40-7.33 (m, 1H), 7.16 (s, 1H), 7.02-6.96 (m, 1H), 6.88-6.82 (m, 2H), 6.66-6.60 (m, 1H), 5.39 (s, 1H), 5.35 (s, 1H), 4.13 (s, 3H), 3.95 (s, 3H), 3.91 (s, 6H), 3.90 (s, 3H), 3.68 (s, 3H), 3.13 (s, 3H), 3.07 (s, 3H), 2.22-2.13 (m, 2H), 2.13-2.05 (m, 4H), 2.04 (s, 6H), 2.02 (s, 6H), 1.82-1.69 (m, 2H). Found: [M+H]=662.2.

Acknowledgments

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Appendix A. Supplementary data

Supplementary date to this article can be found at *****. These data include MOL files and 1H NMR spectra of the new molecules of Table 1.

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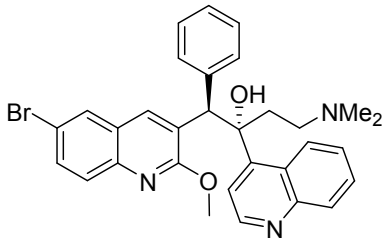
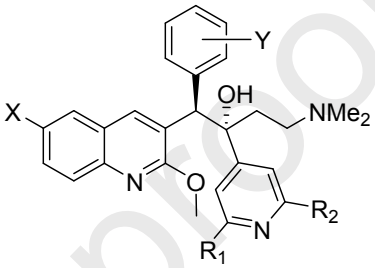
Supplementary data
Supplementary data (MOL files for the compound of Tables 2 and 5) associated with this article can be found in the online version

Graphical abstract





Property	Bedaquiline	Table 1 compounds
clogP	7.25	3.73 – 8.18
MIC	0.08	<0.004 – 0.46
hERG	1.6	1.5 - >30
HCl_{int}	3.0	0.1 – 17
AUC	17.4	1.1 – 33
F (%)	56	11 - 83

DARQ 6

Highlights

- bedaquiline analogues with 3,5-disubstituted-4-pyridyl C-units
- high potency and attenuated hERG inhibition cf bedaquiline
- 4-6.5 log units of bacterial clearance from lung in mouse model
- oral bioavailability generally 40-60%