SUPPLEMENTARY INFORMATION

Screening a Protein Kinase Inhibitor Library against *Plasmodium falciparum*

Irene Hallyburton,[a] Raffaella Grimaldi,[a] Andrew Woodland,[a] Beatriz Baragaña,[a] Torsten Luksch,[a] Daniel Spinks,[a] Daniel James,[a] Didier Leroy,[b] David Waterson,[b] Alan H. Fairlamb,[a] Paul G. Wyatt,[a] Ian H. Gilbert,[a,]* Julie A. Frearson.[a]

a. Drug Discovery Unit, Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, Dundee, DD1 5EH, UK.

b. Medicines for Malaria Venture, 20, Route de Pré-Bois, 1215 Geneva 15, Switzerland

Tables and Figures

Name	Concentration, mM	Percentage of Inhibition
Mitoxantrone dihydrochloride	0.004	140 ± 2
Doxorubicin hydrochloride	0.003	137 ± 2
Daunorubicin hydrochloride	0.004	132 ± 1
Pyrvinium pamoate	0.001	107 ± 1
Demecarium bromide	0.003	104 ± 2
Chloroquine diphosphate	0.004	104 ± 1
Emetine dihydrochloride	0.004	103 ± 2
Cycloheximide	0.007	103.1 ± 0.1
Benzethonium chloride	0.004	102.5 ± 0.7
Anisomycin	0.008	102 ± 2
Cephaeline dihydrochloride heptahydrate	0.003	101 ± 3
Cinchonine	0.007	101 ± 5
Pyrimethamine	0.008	101 ± 2
Methotrexate	0.004	100.9 ± 0.7
Suloctidil	0.006	101 ± 1
(-)-Cinchonidine	0.007	100 ± 1
Pentamidine isethionate	0.003	100 ± 4
Quinacrine dihydrochloride dihydrate	0.004	100± 2
Quinidine hydrochloride monohydrate	0.005	100 ± 2
Artemisinin	0.007	99.5 ± 0.1
Primaquine diphosphate	0.004	100 ± 4
Hydroquinine hydrobromide hydrate	0.005	99 ± 2
Atovaquone	0.005	99 ± 2
Berberine chloride	0.005	99 ± 2
Propafenone hydrochloride	0.005	99 ± 1
Chlorhexidine	0.004	98 ± 1
Mefloquine hydrochloride	0.005	97.4 ± 0.4
Astemizole	0.004	97.3 ± 0.8
Puromycin dihydrochloride	0.004	96 ± 5
Verteporfin	0.003	95 ± 2
Doxazosin mesylate	0.004	84.0 ± 0.6
(d,l)-Tetrahydroberberine	0.006	83 ± 2
Cyclosporin A	0.002	81.4 ± 0.6
Thonzonium bromide	0.003	70 ± 1
Triamterene	0.008	69 ± 1
Bufexamac	0.009	69 ± 2
Paclitaxel	0.002	65 ± 2
Decamethonium bromide	0.005	64 ± 3
Mycophenolic acid	0.006	63 ± 3
Metergoline	0.005	56 ± 2
Bephenium hydroxynaphthoate	0.005	55.1 ± 0.5
Clobetasol propionate	0.004	50 ± 16
Proguanil hydrochloride	0.007	49 ± 4

Table S1. Inhibitors with known anti-malarial activity identified in the pilot screen (Prestwick Library). Antimalarial drugs are shown in bold.

MMV Series	MMV02	MMV03		MMV04	MMV05	MMV06	MMV08	MMV09	MMV10	MMV11
Compound	A0002	A0012	A0003	A0004	A0005	A0006	A0008	A0009	A0010	A0011
MKK1	36	109	93	99	84	57	72	94	79	90
ERK1	97	92	101	84	91	108	113	97	92	108
ERK2	124	89	96	108	116	110	106	103	101	110
JNK1	93	101	103	97	112	110	104	96	101	105
JNK2	86	97	117	126	126	117	111	125	100	129
p38a MAPK	66	74	68	90	80	86	97	84	74	90
p38b MAPK	63	67	78	95	98	100	112	100	87	95
p38g MAPK	88	103	95	102	100	100	111	101	93	113
p38d MAPK	85	88	97	101	94	97	111	89	83	89
ERK8	86	78	90	88	104	57	74	90	97	55
RSK1	89	89	82	96	109	111	66	87	101	42
RSK2	66	77	114	107	97	116	104	113	87	48
PDK1	105	101	98	93	96	106	112	100	91	121
PKBa	37	102	98	97	83	117	110	93	99	71
PKBb	58	10	112	100	87	109	101	105	116	105
SGK1	83	88	88	98	113	100	89	87	84	86
S6K1	95	124	98	112	100	120	115	96	105	125
PKA	96	104	96	97	77	95	90	84	94	96
ROCK2	93	103	93	111	98	100	94	96	112	101
PRK2	90	101	94	101	92	98	106	93	92	76
PKCa	120	69	60	86	87	115	75	88	89	88
PKCZ	75	91	86	88	88	82	93	92	89	103
PKD1	56	128	110	100	101	112	96	100	95	102
MSK1	56	112	109	104	101	100	113	89	110	84
MNK1	44	90	93	96	98	113	85	103	93	76
MNK2	44	93	97	101	104	88	52	87	100	81
MAPKAP-K2	108	104	108	97	128	102	92	93	89	126
PRAK	98	89	86	101	89	104	78	94	100	107
CAMKKb	92	88	89	103	92	71	75	74	100	89
CAMK1	7	76	99	109	33	96	76	95	101	59
SmMLCK	22	72	219	95	39	106	31	92	112	73
PHK	98	105	97	118	122	110	68	106	112	73
CHK1	80	97	99	84	109	113	99	95	88	102
CHK2	61	64	92	94	93	48	72	94	82	107
GSK3b	92	97	106	96	58	100	102	105	95	120

CDK2-Cyclin A	97	89	89	96	103	95	99	96	96	86
PLK1 (Okadaic Acid)	108	87	89	108	115	109	102	84	99	120
Aurora B	72	76	89	105	109	57	81	85	95	23
AMPK	47	86	102	107	94	104	99	94	117	88
MARK3	98	105	104	111	110	107	114	103	101	110
BRSK2	88	73	87	100	95	100	109	97	79	138
MELK	97	105	102	86	79	90	114	108	106	77
CK1	105	121	90	97	100	121	40	100	110	107
CK2	103	97	101	104	99	97	93	91	116	109
DYRK1A	86	81	79	92	96	94	62	93	85	117
DYRK2	90	88	114	104	92	93	71	101	99	111
DYRK3	89	99	91	92	91	92	109	87	83	113
NEK2a	91	101	93	91	89	87	84	87	93	79
NEK6	101	94	100	88	103	109	103	106	91	108
IKKb	137	200	196	101	110	99	103	198	208	125
PIM1	94	103	102	93	113	104	100	101	105	97
PIM2	100	96	103	90	83	104	118	97	86	123
PIM3	89	62	35	22	76	90	88	97	78	82
SRPK1	88	81	97	106	102	124	95	88	96	116
MST2	97	92	96	103	85	92	108	92	98	92
EF2K	83	105	107	106	99	125	114	93	109	101
HIPK2	86	93	99	93	86	106	111	90	98	102
PAK4	45	76	94	105	77	53	77	85	99	57
PAK5	87	77	76	93	140	82	97	92	96	87
PAK6	100	109	111	111	115	100	109	89	105	117
Src	103	81	90	106	103	64	92	95	95	85
Lck	53	116	80	92	91	65	90	109	87	39
CSK	98	112	105	88	112	99	99	94	89	112
FGF-R1	103	58	75	128	100	97	115	100	91	73
IRR	65	70	77	80	86	90	67	90	56	99
EPH A2	96	96	91	108	96	107	98	115	94	61
MST4	77	86	83	105	69	90	106	93	90	88
SYK	108	84	85	102	132	114	99	95	91	103
YES1	84	83	61	101	95	45	76	80	81	53
IGF-1R	107	90	102	109	128	116	116	110	109	97
VEG-FR	91	53	98	97	99	81	77	100	99	87
BTK	85	87	86	100	92	77	100	90	80	51
IR-HIS	78	97	66	78	92	92	47	76	86	76
EPH-B3	88	81	71	108	97	102	95	78	76	103
TBK1 (du12569)	102	104	97	101	104	112	108	109	94	123
IKKe(14231)	88	98	88	98	98	92	103	103	95	101

Table S2 – Percentage activity of the panel of mammalian (mainly human) protein kinases when treated with hit compounds at a concentration of 10 μM.

Supplementary information: **Medicinal Chemistry Evaluation**

MMV05

MMV05 is a 3,6-disubstituted 1H-pyrazolo[3,4-b]pyridine (Supplementary Figure 1). Only one compound A0005 was identified in the initial screening campaign, this compound could not be repurchased and was already under development by MMV so no further work was conducted on this series. The hit compound A0005 is 10 nM *vs*. *P. falciparum* and showed a 100 fold selectivity window over MRC5 cells. A0005 was tested vs. the 76 kinases in the DSTT panel, inhibiting only CAMK1 and SmMLCK by 67% and 61% respectively at 10 µM. The presence of a large number of metabolically labile groups would be of concern if this series was to be progressed. Table 3 in main body of the paper contains a summary of A0005 and its physical properties as generated by Stardrop.

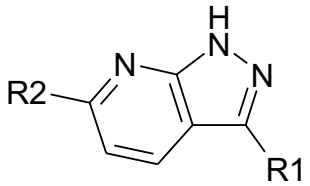


Figure S1 – MMV05 Scaffold

MMV06

MMV06 is a 2,6-disubstituted pyrimidine (Supplementary Figure 2). Only one example of MMV06 was identified in the initial screening campaign, A0006. A0006 is very active against *P. falciparum* and >350 fold selective over MRC5 cells. A0006 was tested in the DSTT kinase panel inhibiting CHK2 and YES1 by 52% and 55% respectively at 10 µM. A0006 is no longer commercially available so could not be reconfirmed and since MMV06 was under development by MMV, no further work was conducted on this series. Table 3 (main body of the paper) contains a summary of A0006 and its physical properties as generated by Stardrop.

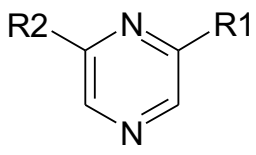


Figure S2 – MMV06 Scaffold

MMV08

MMV08 consists of a 3,8-disubstituted imidazo[1,2-a]pyrazine core (Supplementary Figure 3). Only one example of MMV08 was identified in the primary screen. The hit A0008 has a low nanomolar EC_{50} against MRC5 cells but is over 20 fold less active against *P. falciparum*. Interestingly the MRC5 toxicity is not reflected in the kinase panel results, with only SmMLCK, CK1 and IR-HIS inhibited by between 50-70% at 10 µM. The MMV08 hit could not be repurchased and as MMV was already developing the series we did not progress it into hit expansion. Table 3 (main body of the paper) contains a summary of A0008 and its physical properties as generated by Stardrop.

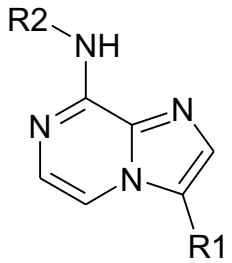


Figure S3 – MMV08 Scaffold

MMV09

MMV09 is a 1,4'-disubstituted 1-(thiazol-2-yl)ethanol (Supplementary Figure 4). Only one sub-micromolar example was identified in the primary screen. The hit A0009 was repurchased and retesting the compound confirmed the data obtained from the screening stocks. A0009 was tested in the kinase panel but did not hit any of the 76 kinases, A0009 is also a >75 fold selective inhibitor of *P. falciparum* over MRC5 cell growth. MMV was already developing analogues of MMV09 so no further work was conducted on this series. Table 3 (main body of the paper) contains a summary of A0009 and its physical properties as generated by Stardrop.

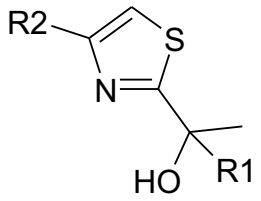


Figure S4 – MMV09 Scaffold

MMV11

MMV11 is a 3,6-disubstituted imidazo[1,2-b]pyridazine (Supplementary Figure 5). Only one sub-micromolar example of this series was identified in the primary screen. A0011 was tested in the kinase panel inhibiting RSK1, RSK2, Aurora B and Lck by between 50-80% at 10 µM. A0011 is only a 10 fold selective inhibitor of *P. falciparum* over MRC5 cell growth. The hit A0011 could not be repurchased and as this series was under development by MMV, no further work was conducted. Table 3 (main body of the paper) contains a summary of A0011 and its physical properties as generated by Stardrop.

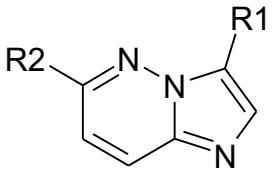
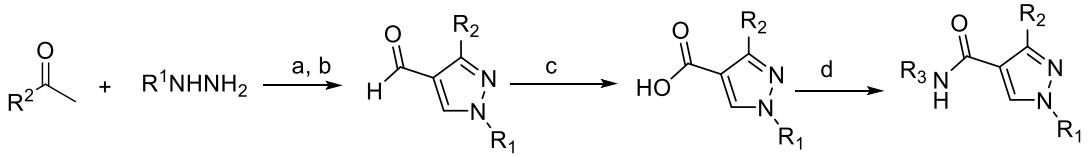


Figure S5 – MMV11 Scaffold

Chemistry Experimental

Chemistry. *General***.** Solvents and reagents were purchased from commercial suppliers and used without further purification. Dry solvents were purchased in sure sealed bottles stored over molecular sieves. Normal phase TLCs were carried out on pre-coated silica plates (Kieselgel 60 F_{254}, BDH) with visualisation *via* UV light (UV254/365 nm) and/or ninhydrin solution. Flash chromatography was performed using Combiflash Companion Rf (Teledyne ISCO) and prepacked RediSep silica gel columns purchased from Teledyne ISCO. Preparative HPLC separations were performed with a Gilson HPLC (321 pumps, 819 injection module, 215 liquid handler/injector) connected to a Gilson 155 UV/vis detector. HPLC chromatographic separations were conducted using Waters XBridge C18 columns, 19 x 100 mm, 5 um particle size; using 0.1% ammonia in water or 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) as mobile phase. ^{1}H NMR spectra were recorded on a Bruker Avance DPX 500 spectrometer (^{1}H at 500.1 MHz). Chemical shifts (δ) are expressed in ppm recorded using the residual solvent as the internal reference in all cases. Signal splitting patterns are described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broadened (br), or a combination thereof. Coupling constants (*J*) are quoted to the nearest 0.1 Hz. Low resolution electrospray (ES) mass spectra were recorded on a Bruker Daltonics MicroTof mass spectrometer, run in positive mode. LC-MS analysis and chromatographic separation were conducted with a Brucker Daltonics MicrOTOf mass spectrometer or an Agilent Technologies 1200 series HPLC connected to an Agilent Technologies 6130 quadrupole LC/MS, where both instruments were connected to an Agilent diode array detector. The column used was a Waters XBridge column (50 mm × 2.1 mm, 3.5 µm particle size,) and the compounds were eluted with a gradient of 5 to 95% acetonitrile/water +0.1% Ammonia. All final compounds showed chemical purity ≥ 95% as determined by the UV chromatogram (190-450nm) obtained by LC-MS analysis. Unless otherwise stated herein reactions have not been optimized. Compounds **A0012, A0036, A0037** and **A0038** were purchased from Enamine and tested without further purification after purity was determined to be ≥ 95% by integration of the UV chromatogram (190-450nm) obtained by LC-MS analysis.



Conditions: (a) AcOH, EtOH, 90°C, 1h 30min; (b) $POCl_3$, DMF, reflux, overnight; (c) $KMnO_4$, KOH, dioxane/water 4/1; (d) Amine, EDC, HOBt, DIPEA, DMF

Supplementary Figure 6. General synthetic route.

General procedure A: Synthesis of pyrazole-4-aldehydes. To a solution of the methyl ketone (1 eq) and the corresponding hydrazine (1 eq) in ethanol (1 mL/mmol), acetic acid (1 mL/ 25 mmol) was added dropwise at room temperature. Reaction mixture was heated at 90°C for 1h and 30 min. Solvents were removed under reduced pressured and residue partitioned between NaHCO3 saturated aqueous solution (50 mL) and DCM (250 mL). The organic layer was dried over $MgSO_4$ and solvents were removed under reduced pressured. The residue was dissolved in DMF (7 mL) and added to a previously prepared mixture of $POCl_3$ (2 eq) and DMF (2 eq) dropwise in an ice bath. The mixture was stirred at room temperature for 30 min and then refluxed overnight. Reaction was quenched over ice water (50 mL) followed by 2.5 M NaOH (15 mL) and extracted with DCM (250 mL). The organic layer was dried over $MgSO_4$ and solvents were removed under reduced pressured to obtain the desire product that was used without further purification.

General procedure B: Oxidation of pyrazole-4-aldehydes. To a solution of the pyrazole-4-carbaldehyde (1 eq) in a mixture of dioxane/water 4/1 (5 mL/mmol), a solution of KOH (1.5 eq) in water (8 mL) and $KMnO_4$ (1.5 eq) were added. The reaction mixture was stirred overnight at room temperature. The reaction was quenched with a mixture of hydrogen peroxide/water 1/1 (10 mL) and filtered through Celite. Solvents were removed and pH was adjusted to pH 3 with 3N HCl. Precipitate was filtered and washed with water. The product was dried under vacuum and used without further purification.

General procedure C: Synthesis of amides. To a solution of the pyrazoloze-4-carboxylic acid (1 eq) in DMF (5 mL), the corresponding amine (1.2 eq), diisopropylethylamine (1.2 eq), EDC (2 eq) and HOBt (2 eq) were added at room temperature. The reaction mixture was stirred overnight at room temperature. The reaction was partitioned between $NaHCO_3$ saturated aqueous solution (10 mL)

and DCM (2 x 25 mL). The combined organic layers were dried over $MgSO_4$ and solvents were removed under reduced pressure. The product was purified by column chromatography.

1-Phenyl-3-(thiophen-2-yl)-1*H*-pyrazole-4-carboxylic acid. Prepared following general procedures A and B starting from 1 2-acetylthiophene (1.7 mL, 16 mmol) and phenyl hydrazine (1.6 mL, 16 mmol) to obtain 1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazole-4-carboxylic acid as a brown solid (1.3 g, 30% yield). ^{1}H NMR (500 MHz, DMSO) δ 9.08 (s, 1H), 8.17 (d, J=2.7 Hz, 1H), 7.96 (d, J=7.7 Hz, 2H), 7.62 - 7.59 (m, 1H), 7.55 (dd, J=8.0, 8.0 Hz, 2H), 7.40 (dd, J=7.4, 7.4 Hz, 1H), 7.17 (dd, J=3.8, 5.0 Hz, 1H). LC-MS (ESI) *m/z* 271 $[M + H]^+$.

***N*-(1H-benzo[d]imidazol-2-yl)-1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazole-4-carboxamide (A0034).** Prepared following general procedure C starting from 1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazole-4-carboxylic acid (200 mg, 0.7 mmol) and purified by column chromatography using a silica cartridge (12 g) and DCM (A) and 10% MeOH in DCM (B) as eluent to obtain *N*-(1H-benzo[d]imidazol-2-yl)-1-phenyl-3-(thiophen-2-yl)-1*H*-pyrazole-4-carboxamide as a white solid (13 mg, 5% yield). ^{1}H NMR (500 MHz, DMSO) δ 9.32 (s, 1H), 8.19 (d, J=3.0 Hz, 1H), 7.90 (d, J=7.9 Hz, 2H), 7.66 - 7.59 (m, 3H), 7.49 (dd, J=3.2, 5.4 Hz, 2H), 7.47 - 7.41 (m, 2H), 7.20 - 7.11 (m, 4H). LC-MS (ESI) *m/z* 386 ($[M + H]^+$.

3-Isopropyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde. Prepared following general procedure A starting from 3-methyl-2-butanone (2.2 mL, 20 mmol) and phenyl hydrazine (1 eq) to obtain 3-isopropyl-1-phenyl-1H-pyrazole-4-carbaldehyde as a brown solid (0.4 g, 10% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.05 (s, 1H), 8.37 (s, 1H), 7.75 - 7.72 (m, 2H), 7.52 - 7.48 (m, 2H), 7.37 (t, J=7.4 Hz, 1H), 3.56 - 3.47 (m, 1H), 1.43 - 1.42 (m, 6H). LC-MS (ESI) *m/z* 215 $[M + H]^+$.

3-Isopropyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid. Prepared following general procedure B starting from 3-isopropyl-1-phenyl-1H-pyrazole-4-carbaldehyde (0.4 g, 2 mmol) to obtain 3-isopropyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid as a brown solid (0.4 g, 84% yield). ^{1}H NMR (500 MHz, DMSO) δ 12.44 - 12.44 (m, 1H), 8.87 (s, 1H), 7.89 (d, J=7.6 Hz, 2H), 7.53 - 7.49 (m, 2H), 7.34 (t, J=7.4 Hz, 1H), 3.58 - 3.51 (m, 1H), 1.29 (d, J=6.9 Hz, 6H). LC-MS (ESI) *m/z* 231 $[M + H]^+$.

3-Isopropyl-1-phenyl-*N*-(pyridin-3-yl)-1*H*-pyrazole-4-carboxamide (A0039). Prepared following general procedure C starting from 3-isopropyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid (158 mg, 0.7

mmol) and purified by column chromatography using a silica cartridge (12 g) and DCM (A) and 10% NH_3-MeOH in DCM (B) as eluent to obtain 3-isopropyl-1-phenyl-N-(pyridin-3-yl)-1*H*-pyrazole-4-carboxamide as a white solid (149 mg, 70% yield). ^{1}H NMR (500 MHz, DMSO) δ 10.07 (s, 1H), 9.07 (s, 1H), 8.86 (d, J=2.2 Hz, 1H), 8.30 (dd, J=1.4, 4.7 Hz, 1H), 8.17 - 8.14 (m, 1H), 7.83 (d, J=7.6 Hz, 2H), 7.59 - 7.55 (m, 2H), 7.41 - 7.36 (m, 2H), 3.65 - 3.56 (m, 1H), 1.32 (d, J=6.9 Hz, 6H). LC-MS (ESI) *m/z* 307 $[M + H]^+$.

3-Methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde. Prepared following general procedure A starting from acetone (1.5 mL, 20 mmol) and phenyl hydrazine (1 eq) to obtain 3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde as a brown solid (1.3 g, 35 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.00 (s, 1H), 8.35 (s, 1H), 7.71 - 7.68 (m, 2H), 7.51 - 7.47 (m, 2H), 7.37 (dd, J=7.4, 7.4 Hz, 1H), 2.59 (s, 3H). LC-MS (ESI) *m/z* 187 $[M + H]^+$.

3-Methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid. Prepared following general procedure B starting from 3-methyl-1-phenyl-1H-pyrazole-4-carbaldehyde (1.4 g, 7.2 mmol) to obtain 3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid as a brown solid (0.8 g, 56% yield). ^{1}H NMR (500 MHz, CDCl3) δ 8.45 (s, 1H), 7.72 (d, J=7.4 Hz, 2H), 7.52 - 7.49 (m, 2H), 7.37 (t, J=7.4 Hz, 1H), 2.62 (s, 3H). LC-MS (ESI) *m/z* 203 $[M + H]^+$.

3-Methyl-1-phenyl-*N*-(pyridin-3-yl)-1*H*-pyrazole-4-carboxamide (A0040). Prepared following general procedure C starting from 3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid (139 mg, 0.7 mmol) and purified by column chromatography using a silica cartridge (12 g) and DCM (A) and 10% NH_3-MeOH in DCM (B) as eluent to obtain 3-methyl-1-phenyl-*N*-(pyridin-3-yl)-1*H*-pyrazole-4-carboxamide as a white solid (78 mg, 40% yield). ^{1}H NMR (500 MHz, MeOD) δ 10.86 (s, 1H), 9.90 (s, 1H), 9.68 (d, J=2.4 Hz, 1H), 9.11 (dd, J=1.3, 4.7 Hz, 1H), 8.96 (A, J=1.5, 2.6, 8.3 Hz, 1H), 8.63 (d, J=7.6 Hz, 2H), 8.40 - 8.36 (m, 2H), 8.23 - 8.18 (m, 2H), 3.31 (s, 3H). LC-MS (ESI) *m/z* 279 $[M + H]^+$.

4-(4-Formyl-3-(pyridin-3-yl)-1*H*-pyrazol-1-yl)benzonitrile. Prepared following general procedure A starting from 2-acetylpyridine (1.8 mL, 16 mmol) and 4-cyanophenyl hydrazine hydrochloride (1 eq) to obtain 4-(4-formyl-3-(pyridin-3-yl)-1*H*-pyrazol-1-yl)benzonitrile as an off-white solid (2.4 g, 55 % yield). ^{1}H NMR (500 MHz, DMSO) δ 10.02 (s, 1H), 9.59 (s, 1H), 9.12 (d, J=1.4 Hz, 1H), 8.70 (dd, J=1.7,

4.8 Hz, 1H), 8.37 - 8.33 (m, 1H), 8.24 (d, J=8.8 Hz, 2H), 8.10 (d, J=8.8 Hz, 2H), 7.57 (dd, J=5.2, 7.6 Hz, 1H). LC-MS (ESI) *m/z* 275 $[M + H]^+$.

1-(4-Cyanophenyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carboxylic acid. Prepared following general procedure B starting from 4-(4-formyl-3-(pyridin-3-yl)-1*H*-pyrazol-1-yl)benzonitrile (2.4 g, 8.7 mmol) to obtain 1-(4-cyanophenyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carboxylic acid as an off-white solid (2.1 g, 85% yield). ^{1}H NMR (500 MHz, DMSO) δ 9.39 (s, 1H), 9.35 (s, 1H), 8.91 (d, J=4.4 Hz, 1H), 8.86 (d, J=8.2 Hz, 1H), 8.25 (d, J=9.0 Hz, 2H), 8.07 - 8.00 (m, 3H).LC-MS (ESI) *m/z* 291 $[M + H]^+$.

***N*-(1H-Benzo[d]imidazol-2-yl)-1-(4-cyanophenyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carboxamide (A0041).** Prepared following general procedure C starting from 1-(4-cyanophenyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carboxylic acid (199 mg, 0.7 mmol) and purified by column chromatography using a silica cartridge (4 g) and DCM (A) and 20% NH_3-MeOH in DCM (B) as eluent to obtain *N*-(1H-benzo[d]imidazol-2-yl)-1-(4-cyanophenyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carboxamide as a white solid (60 mg, 21% yield). ^{1}H NMR (500 MHz, DMSO) δ 9.49 (s, 1H), 9.05 (d, J=2.2 Hz, 1H), 8.65 (dd, J=1.7, 4.8 Hz, 1H), 8.29-8.26 (m, 1H), 8.15 (d, J=8.9 Hz, 2H), 8.11 (d, J=8.9 Hz, 2H), 7.54-7.51 (m, 1H), 7.48-7.44 (m, 2H), 7.14-7.11 (m, 2H). LC-MS (ESI) *m/z* 406 $[M + H]^+$.

1-(4-Fluorophenyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carbaldehyde. Prepared following general procedure A starting from 2-acetylpyridine (1.8 mL, 16 mmol) and 4-fluorophenyl hydrazine hydrochloride (1 eq) to obtain 1-(4-fluorophenyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carbaldehyde as an off-white solid (1.3 g, 36 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.08 (s, 1H), 9.13 (d, J=1.4 Hz, 1H), 8.74 (dd, J=1.7, 4.9 Hz, 1H), 8.54 (s, 1H), 8.26 - 8.23 (m, 1H), 7.82 - 7.79 (m, 2H), 7.47 (dd, J=4.9, 7.1 Hz, 1H), 7.29 - 7.24 (m, 2H). LC-MS (ESI) *m/z* 268 $[M + H]^+$.

1-(4-Fluorophenyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carboxylic acid. Prepared following general procedure B starting from 1-(4-fluorophenyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carbaldehyde (1.3 g, 4.9 mmol) to obtain 1-(4-fluorophenyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carboxylic acid as an off-white solid (1.3 g, 96% yield). ^{1}H NMR (500 MHz, DMSO) δ 9.26 (s, 1H), 9.19 (s, 1H), 8.84 (d, J=1.6 Hz, 1H), 8.73 (d, J=8.0 Hz, 1H), 8.08 - 8.04 (m, 2H), 7.92 (dd, J=5.4, 7.8 Hz, 1H), 7.42 (dd, J=8.8, 8.8 Hz, 2H). LC-MS (ESI) *m/z* 284 $[M + H]^+$.

***N*-(1H-Benzo[*d*]imidazol-2-yl)-1-(4-fluorophenyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carboxamide (A0042).** Prepared following general procedure C starting from 1-(4-fluorophenyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carboxylic acid (195mg, 0.7 mmol) and purified by column chromatography using a silica cartridge (4 g) and DCM (A) and 20% NH_3-MeOH in DCM (B) as eluent to obtain *N*-(1H-benzo[*d*]imidazol-2-yl)-1-(4-fluorophenyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carboxamide as an off-white solid (18 mg, 7% yield). ^{1}H NMR (500 MHz, MeOD) δ 9.03 (d, J=1.4 Hz, 1H), 8.87 (s, 1H), 8.48 (d, J=3.6 Hz, 1H), 8.33 (d, J=8.0 Hz, 1H), 7.84 - 7.80 (m, 2H), 7.47 - 7.42 (m, 3H), 7.25 (dd, J=8.7, 8.7 Hz, 2H), 7.19 (dd, J=3.1, 5.9 Hz, 2H). LC-MS (ESI) *m/z* 399 $[M + H]^+$.

1-(*tert*-Butyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carbaldehyde. Prepared following general procedure A starting from 2-acetylpyridine (1.8 mL, 16 mmol) and *tert*-butyl hydrazine hydrochloride (1 eq) to obtain 1-(*tert*-butyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carbaldehyde as an off-white solid (1.9 g, 49 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.97 (s, 1H), 9.06 (d, J=1.4 Hz, 1H), 8.68 (dd, J=1.3, 4.8 Hz, 1H), 8.20 - 8.18 (m, 2H), 7.42 (dd, J=5.0, 7.7 Hz, 1H), 1.69 (s, 9H). LC-MS (ESI) *m/z* 230 $[M + H]^+$.

1-(*tert*-Butyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carboxylic acid. Prepared following general procedure B starting from 1-(*tert*-butyl)-3-(pyridin-3-yl)-1H-pyrazole-4-carbaldehyde (1.9 g, 8.1 mmol) to obtain 1-(*tert*-butyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carboxylic acid as an off-white solid (1.2 g, 60% yield). ^{1}H NMR (500 MHz, DMSO) δ 12.46 - 12.45 (m, 1H), 8.91 (d, J=1.4 Hz, 1H), 8.56 (dd, J=1.4, 4.7 Hz, 1H), 8.39 (s, 1H), 8.15 - 8.11 (m, 1H), 7.44 (dd, J=5.0, 7.6 Hz, 1H), 1.59 (s, 9H). LC-MS (ESI) *m/z* 246 $[M + H]^+$.

***N*-(1H-Benzo[*d*]imidazol-2-yl)-1-(*tert*-butyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carboxamide (A0043).** Prepared following general procedure C starting from 1-(tert-butyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carboxylic acid (169 mg, 0.7 mmol) and purified by column chromatography using a silica cartridge (4 g) and DCM (A) and 20% NH_3-MeOH in DCM (B) as eluent to obtain *N*-(1H-benzo[*d*]imidazol-2-yl)-1-(tert-butyl)-3-(pyridin-3-yl)-1*H*-pyrazole-4-carboxamide as a white solid (37 mg, 15% yield. ^{1}H NMR (500 MHz, MeOD) δ 8.96 (d, J=1.6 Hz, 1H), 8.54 (s, 1H), 8.51 (dd, J=1.6, 4.9 Hz, 1H), 8.28 - 8.24 (m, 1H), 7.46 (dd, J=4.9, 7.9 Hz, 1H), 7.41 - 7.38 (m, 2H), 7.18 - 7.16 (m, 2H), 1.64 (s, 9H). LC-MS (ESI) *m/z* 361 $[M + H]^+$.

1-Methyl-3-(thiophen-2-yl)-1*H*-pyrazole-4-carbaldehyde. Prepared following general procedure A starting from 2-acetylthiophene (1.7 mL, 16 mmol) and methyl hydrazine (1 eq) to obtain 1-methyl-3-(thiophen-2-yl)-1*H*-pyrazole-4-carbaldehyde as a brown oil (2.4 g, 79% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.02 (s, 1H), 7.95 (s, 1H), 7.78 (td, J=1.0, 3.6 Hz, 1H), 7.37 (td, J=1.0, 5.1 Hz, 1H), 7.13-7.11 (m, 1H), 3.96 (s, 3H). LC-MS (ESI) *m/z* 193 $[M + H]^+$.

1-Methyl-3-(thiophen-2-yl)-1*H*-pyrazole-4-carboxylic acid. Prepared following general procedure B starting from 1-methyl-3-(thiophen-2-yl)-1*H*-pyrazole-4-carbaldehyde (2.4 g, 13 mmol) to obtain 1-methyl-3-(thiophen-2-yl)-1*H*-pyrazole-4-carboxylic acid as a yellow solid (1.2 g, 48% yield). ^{1}H NMR (500 MHz, DMSO) δ 12.43 - 12.43 (m, 1H), 8.30 (s, 1H), 8.08 (dd, J=1.1, 3.6 Hz, 1H), 7.51 (dd, J=1.1, 5.2 Hz, 1H), 7.10 (dd, J=3.7, 5.1 Hz, 1H), 3.88 (s, 3H). LC-MS (ESI) *m/z* 209 $[M + H]^+$.

1-Methyl-*N*-(pyridin-3-yl)-3-(thiophen-2-yl)-1*H*-pyrazole-4-carboxamide (A0044). Prepared following general procedure C starting from 1-methyl-3-(thiophen-2-yl)-1*H*-pyrazole-4-carboxylic acid (143 mg, 0.7 mmol) and purified by column chromatography using a silica cartridge (12 g) and DCM (A) and 10% MeOH in DCM (B) as eluent to obtain as a white solid (43 mg, xx% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.36 (d, J=1.6 Hz, 1H), 8.30 (d, J=4.3 Hz, 1H), 8.13 - 8.08 (m, 2H), 7.98 (s, 1H), 7.52 - 7.48 (m, 2H), 7.18 (dd, J=3.6, 5.0 Hz, 1H), 3.92 (s, 3H). LC-MS (ESI) *m/z* 285 $[M + H]^+$.