Design and synthesis of novel non-peptidic KISS1R antagonists to restore drug sensitivity to triple-negative breast cancer patients

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Table of Contents

i. Acknowledgements

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ii. Abbreviations

ABC	ATP binding cassatte
ABCG2	ATP binding cassatte G2
AKT	Protein kinase B
ATP	Adenosine 5'-triphosphate
BCa	Breast cancer
BCRP	Breast cancer resistant protein
BL1	Basal-like 1
BL2	Basal-like 2
Bn	Benzyl
br	Broad
d	Doublet
DCC	*N*,*N'*-dicyclohexylmethanediimine
DCM	Dichloromethane
dd	Doublet of doublets
ddd	Doublet of doublet of doublets
DIPEA	Diisopropylethylamine
DMF	*N*,*N*, dimethylformamide
dt	Doublet of triplets
ECM	Extracellular matrix
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDG	Electron donating group
ER	Oestrogen receptor
ERK1/2	Extracellular signal-regulated kinases 1 and 2
GPCR	G-protein coupled receptor
HER1	Human epidermal growth factor receptor 1
HER2	Human epidermal growth factor receptor 2
HPG	Hypothalamic-pituitary-gonadal
Hz	Hertz
IC_{50}	Half maximal inhibitory concentration
IM	Immunomodulatory
IMS	Industrial methylated spirits
IR	Infra-red
J	Coupling constant
KISS1R	Kisspeptin 1 receptor
KP	Kisspeptin
M	Mesenchymal
***m*-CPBA**	3'-chloroperbenzoic acid
MMP	Matrix metalloproteinase
mp	Melting point
MSL	Mesenchymal stem-like
MT1	Metallothionein
mTOR	Mammalian target of rapamycin
MW	Microwave
NMR	Nuclear magnetic resonance
Nrf2	Nuclear factor-erythroid factor 2-related factor 2
P13K	Phosphoinositide 3-kinases
P-234	Peptide-234
PLC-β	Phospholipase C - β
PR	Progestogen receptor
RBF	Round bottom flask
rt	Room temperature
s	Singlet
t	Triplet
td	Triplet of doublets
THF	Tetrahydrofuran
TLC	Thin-layered chromatography
TMSCl	Trimethylsilyl chloride
TMSI	trimethylsilyl iodide
TNBC	Triple-negative breast cancer
tt	Triplet of triplets

iii. Abstract

Breast cancer (BCa) is the most common neoplasm among women and the leading cause of cancer related mortalities.[1] Globally, basal-like triple-negative breast cancer (TNBC) accounts for approximately 1 in 5 cases of all BCa malignancies but is disproportionally responsible for all BCa associated deaths.[1] The ability of cancer cells to become resistant to chemotherapy continues to be a major obstacle in treating TNBC.[2,3] KISS1R, a G-protein coupled receptor (GPCR), signals through a plethora of diverse molecular mechanisms that have the potential to regulate the processes navigating TNBC clinical outcomes. Heightened KISS1R signaling promotes chemotherapeutic desensitisation, due to the overexpression of the drug efflux transporter ABCG2 (BCRP).[4] Hence, there is an intense interest for the development of novel, synthetic agents, which may synergise with current BCa therapeutic options. Herein, novel compounds designed as small antagonists of KISS1R, with the outlook of restoring drug sensitivity to patients suffering from TNBC are reported. Methodologies for the synthesis of novel pyridine derivatives have been described. The KISS1R antagonistic efficacy for compounds **1** and **2** were evaluated in an outsourced cellular functional assay. Compared to **1**, compound **2** exhibited a greater antagonistic effect – thus, **2** presents a potential template for further analogue optimisation and development. Spectroscopic data including high-resolution mass spectra, IR, ^{1}H NMR, in addition to ^{13}C and 2D NMR constitute evidence for the chemical structures for all synthesised compounds. This paper also reports on the preliminary *in vitro* proliferation assay profile of the peptidic KISS1R antagonists, P-234, in MCF-7 and MDA-MB-231 cell lines. As observed, peptidic antagonists of KISS1R promote cell proliferation in TNBC model cell lines, contrary to PR^{+}/ER^{+} BCa cell lines.

1. Introduction

1.1. Fundamentals of triple-negative breast cancer

The global cancer statistics, for 2020, showed that there were approximately 2.2 million new cases of female breast cancer (BCa) and 684,996 associated deaths.[1] BCa is the most common neoplasm among women and the leading cause of cancer related mortalities.[1] The hypernym, BCa, describes a diverse heterogeneous tumour group with a highly variable treatment response. According to the St Gallen 2011 and 2013 guidelines, a proxy for molecular subtype classification, there are seven intrinsic molecular subgroups of BCa: 1) luminal A, 2) luminal B, 3) human epidermal growth factor receptor 2 (HER2) overexpressing, 4) basal-like (2 types – BL1 and BL2), 5) immunomodulatory (IM), 6) mesenchymal (M), 7) mesenchymal stem-like (MSL), and normal breast-like tumours.[5,6] In addition, based on the expression profiles of the progesterone receptor (PR), oestrogen receptor (ER), and HER2, BCa can be divided into three clinical subtypes: hormone receptor positive (ER^+, PR^+, PR^-, $HER2^-$), HER2-positive ($HER2^+$), and triple-negative (ER^-, PR^-, $HER2^-$).[7] The triple-negative phenotype shows a significant overlap (60-90%) with the basal-like molecular subtype of breast cancer.[3,8] As such, basal-like triple-negative breast cancer (TNBC) is a BCa subtype of particular interest. Clinically, basal-like TNBC is defined by its lack of expression of the ER, PR, and HER2 receptors which are used as essential indicators to determine the optimal therapeutic protocol.[9] As reviewed by Yadav *et al*, patients with hormone dependent positive BCa benefit from chemo-targeted and hormone therapy, whereas treatment for TNBCs are currently limited to surgery and conventional cytotoxic agents.[2] Basal-like TNBC is difficult to manage, with the disease characterised by poor prognosis due to limited treatment options and subsequent drug resistance.[10] Despite promising initial response rates to chemotherapies, including anthracyclines and taxanes-based regimens, patients with TNBC often develop chemoresistance.[8] In comparison to other BCa subtypes, TNBC tumours are frequently larger, less differentiated, and have a higher incidence of distant metastases, proliferation and recurrence.[11,12] Basal-like TNBC accounts for approximately 1 in 5 cases of BCa but is disproportionately responsible for all BCa associated deaths.[11] The ability of cancer cells to become resistant to chemotherapy continues to be a major obstacle in treating TNBC patients.

1.2. KP/KISS1R signaling network in triple-negative breast cancer

Kisspeptin receptor 1 (KISS1R; aka GPR54, OT7T175, AXOR12) is a $G\alpha_{q/11}$-coupled G-protein coupled receptor (GPCR) and a key regulator of the hypothalamic-pituitary-gonadal (HPG) axis. KISS1R is highly expressed in the brain, including the hypothalamus and pituitary gland as well as peripheral regions.[13] Kisspeptins (KPs), a product of the *KISS1* gene, are a group of peptide fragments that bind to and activate the KISS1R (Fig. 1).[14] The *KISS1* gene encodes a polypeptide consisting of 145 amino acids, known as the precursor peptide (KP-145).[13] KP-145 gives rise to a secretory protein of 126 amino acids that is proteolytically cleaved into smaller fragments: KP-54 (aka Metastin), KP-14, KP-13 and KP-10.[13] All peptide fragments bind to and activate the KISS1R with equal potency.[15] Each peptide fragment shares the same 10 terminal amino acid sequence, KP-10, the smallest fragment necessary for binding to and activating KISS1R.[15] The identification of the *KISS1* gene was initially championed for its anti-metastatic role.[16] *KISS1* activated KISS1R signaling has been shown to suppress cancer metastasis by inhibiting cancer cell migration and invasion. Downregulation of *KISS1* was clinically established with a worse prognosis among those diagnosed with melanoma,[16] colorectal,[17] prostate,[18] ovarian,[19] lung,[20] and bladder cancers.[21] Paradoxically, elevated KISS1R signaling appears to play a pro-metastatic role in some cancers such as breast and liver cancer.[22,23] Successive findings have likened the metastases of TNBC basal-like malignancies, and subsequent drug resistance, with the overexpression of *KISS1*.[4] As such, *KISS1* expression might be a useful predictive biomarker in medical outcomes.[16]

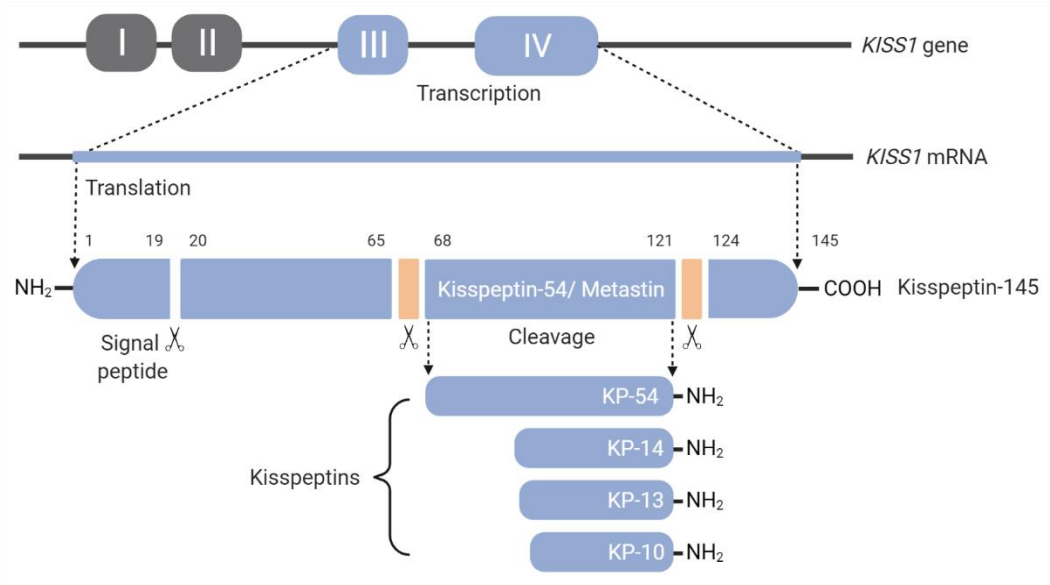


Figure 1. Kisspeptins (KPs) formation. The KISS1 gene is located in the long-arm of chromosome 1 (1q32-q41) and encodes a 145 amino-acid sequence (KP-145), which is subsequently cleaved into smaller C-terminal fragments: KP-54, KP-14, KP-13 and KP-10. All KP fragments possess biological activity and are endogenous ligands to the KISS1R. Figure created using BioRender.com.

KISS1R signals through a plethora of diverse molecular mechanisms that have the potential to regulate the processes navigating TNBCs clinical diagnoses. The underlying mechanisms by which KP/KISS1R regulates tumourigenesis in TNBC has been reviewed (Fig. 2). Goertzen *et al.* showed that G-protein dependent KISS1R promotes TNBC cell invasion by stimulating the secretion of ERK1/2.[24] It was demonstrated that β-arrestin2, not only desensitises G-protein signaling but also acts as a molecular scaffold and activates a series of signaling pathways such as ERK1/2.[25] KISS1R activation in metastatic TNBC cell lines increases the activity of cortactin, cofilin and MT1-MMP to stimulate invadopodia formation and extracellular matrix degradation through ERK1/2.[24] Conversely, depletion of KISS1R signaling inhibited metastatic TNBC cell migration, invasion, and malignant transformation.[24] Cvetković *et al*. reported that KISS1R activation, in metastatic TNBC MDA-MB-231 cells, leads to human epidermal growth factor receptor (HER1, EGFR) transactivation *via* IQGAP1 and β-arrestin2 mediated pathways.[26] The multi-domain scaffold protein, IQGAP1, is implicated in the P13K-AKT- mTOR (PAM), and KEAP1-Nrf2 pathways.[27] Thereafter, Nrf2 stimulates *BCRP* transcription, thus, inducing the ATP-binding cassette (ABC) transporter family.[28] Blake *et al.* demonstrated that KISS1R signaling promotes the expression of the drug efflux transporter, ABC-G member 2 (ABCG2, BCRP), in metastatic TNBC cell lines.[4] It was reported that BCRPs were highly expressed in SKBR3FlAG-KISS1R cells, compared to control cells, thus reducing the cellular accumulation of the chemotherapeutic doxorubicin.[4] The increased drug outflow proposes a role for KISS1R-dependent doxorubicin efflux as a mechanism for chemotherapeutic desensitisation.[28] Thereby, there is an intense interest for novel, synthetic agents, which may synergise with current chemotherapeutic options and, consequently, attenuate the signaling potential of KISS1R.

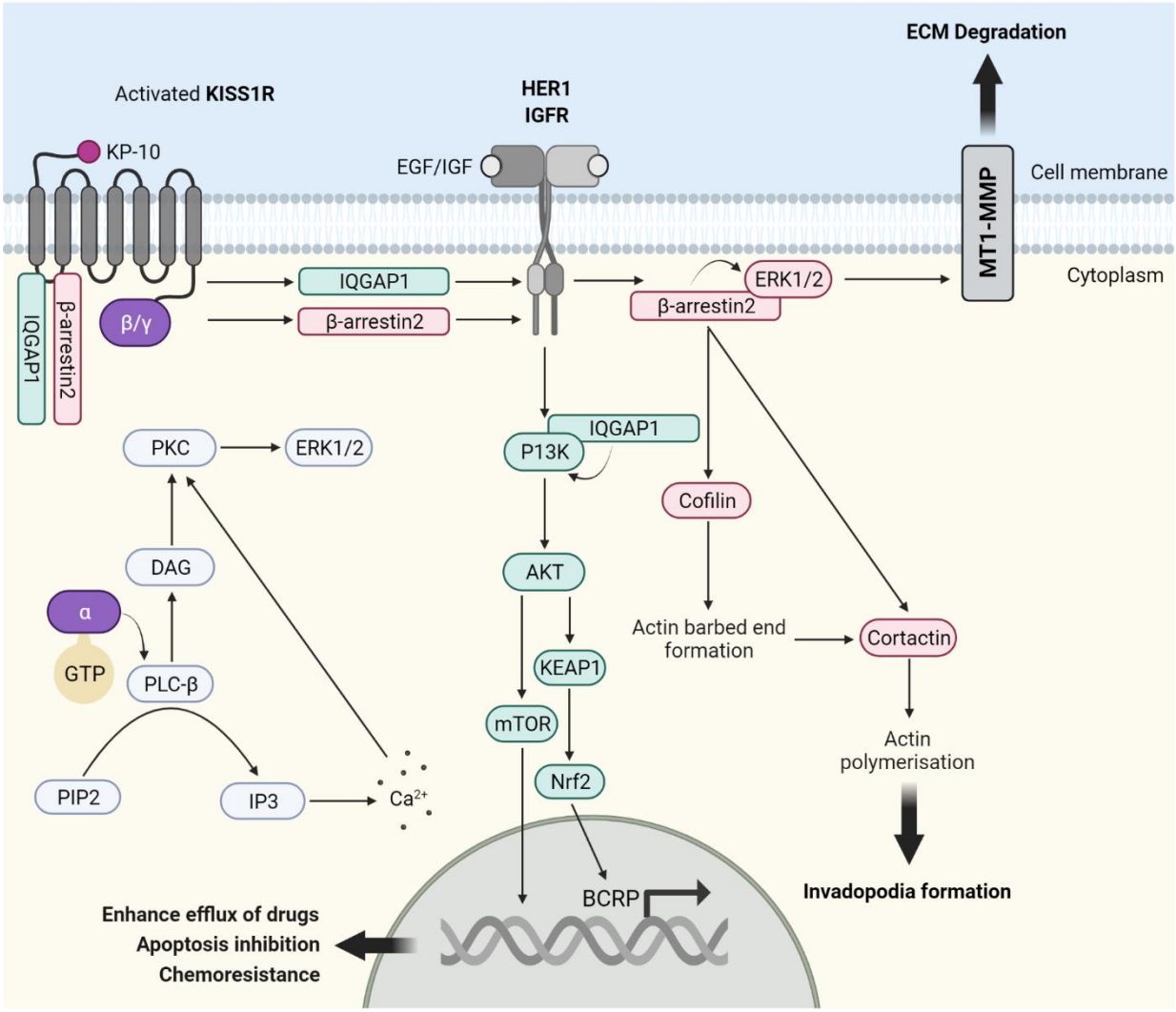


Figure 2. Schematic diagram of KISS1R signaling pathways activated upon KP-10 stimulation. KISS1R couples to the β/γ subunit, post KP-10 stimulation of the $G\alpha_{q/11}$-coupled receptor. The α subunit activates the primary effector phospholipase C - β (PLC-β) and extracellular signal-regulated kinases 1 and 2 (ERK1/2). KISS1R can also signal *via* a G-protein independent pathway to activate IQGAP1, β-arrestin2 and HER1. KISS1R signaling, *via* β-arrestin2, regulates extracellular matrix (ECM) degradation, and the phosphorylation levels of invadopodia proteins cofilin and cortactin. The KISS1R signaling mechanism *via* IQGAP1 regulates the P13K-AKT-mTOR (PAM), and KEAP1-Nrf2 pathways. Subsequent transcription of target gene, *BCRP*, enhance the efflux of chemotherapeutics *via* ABC transporters, including BCRP. Figure created using BioRender.com.

1.3. Inhibition of KISS1R expression

KISS1R stimulation is reliant on its cognate KPs. KP analogues have been synthesised based on the structure of human KP-10, as this is the smallest fragment needed to bind to and activate KISS1R.[14] The antagonistic efficacy of KP-10 analogues were tested in terms of their ability to inhibit KP-stimulated PLC-β mediated inositol phosphate and intracellular calcium release (Fig. 2).[29,30] Roseweir *et al*. established peptide-234 (P-234), a KP-10 analogue, as a potent KISS1R antagonist, inhibiting KP-stimulated calcium release by 89 %, with an IC_{50} of 1.0 nM.[30] Subsequently, P-234 has been used to block KP-10 stimulation in various *in vivo* and *in vitro* systems.[31] Blake *et al*. reported that P-234 increased drug sensitivity in metastatic TNBC MDA-MB-231 cells which express endogenous KISS1R.[4] Dissimilar to its peptide counterpart, which needs to be administered by injection, small molecule antagonists are more likely to be orally active and would therefore be more amendable to patient compliance in the clinic.[30]

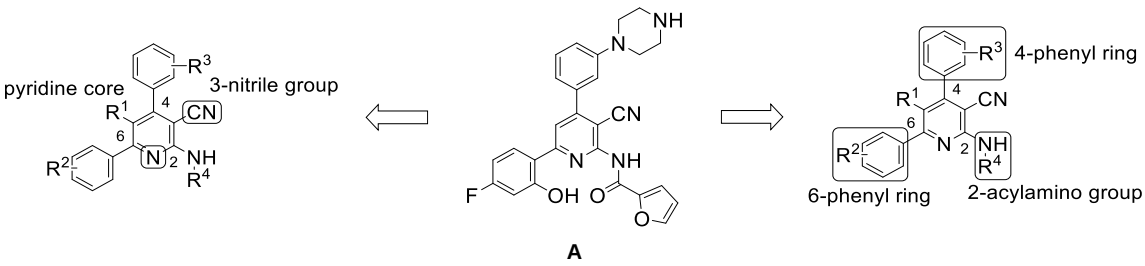


Figure 3. Structure of hit compound **A**, as established by Kobayashi *et al*., and synthetic strategies. The 2-acylamino-4,6-diphenylpyridine template will be utilised for the development of novel synthetic methodologies and derivatives. This research project is aimed at expanding upon the family of existing 2-acylamino-4,6-diphenylpyridines to generate novel compounds.

Kobayashi *et al*. adopted a combinatorial chemistry approach to identify compound **A** as the most potent antagonist of KISS1R-Metastin signaling within the HPG axis.[32,33] Although, compound **A** showed encouraging levels of KISS1R binding affinity (IC_{50} of 0.93 μM) *in vitro*, as well as some limited activity after dosing *in vivo*, compound **A** did not antagonise calcium release to the same extent as P-234.[32] These facts suggested that this class of compounds would be an appropriate source of leads for the discovery of a novel KISS1R antagonist, upon optimisation of the 2-acylamino-4,6-diphenylpyridine derivatives. The outlined synthetic strategies have been developed around the hit compound **A** with the aim of working towards achieving this objective. This is with the outlook of restoring drug sensitivity to patients suffering from basal-like TNBC (Fig. 3). Currently, there are no small molecule KISS1R inhibitors reported against basal-like TNBC chemotherapeutic desensitisation.

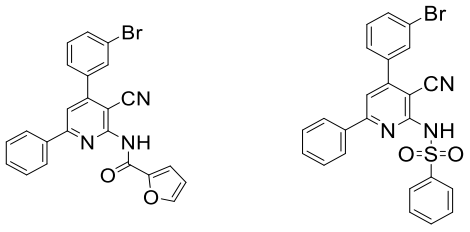


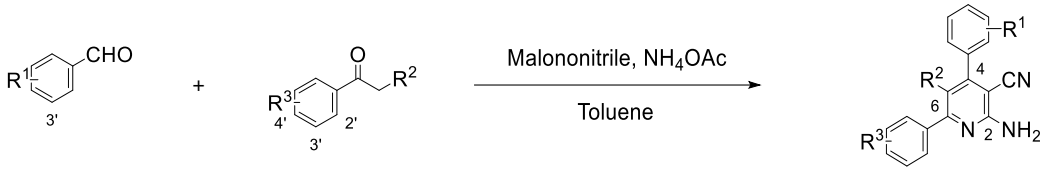
Figure 4. The novel derivatives, **1** and **2** were evaluated for their antagonist effect in an outsourced KP-10 stimulated, GPCR cellular functional assay (Eurofins, Cerep).[34]

Herein the synthesis of pyridyl derivatives bearing appropriate modification to the 4-phenyl ring, 6-phenyl ring, 3-cyano group, 2-acylamino group and pyridine core, as shown in Figure 3, are reported. The afforded novel derivatives, **1** and **2** (Fig. 4), were evaluated for their antagonist efficacy *via* a, KP-10 stimulated, GPCR cellular functional assay (see section 2.4). This report also proposes that P-234 may be employed, as a control, when testing for *in vitro* dependent variables, and compared to the afforded synthesised substrates. As such, a preliminary *in vitro* proliferation assay analysis of P-234, in MCF-7 and MDA-MB-231 cell lines was recorded (see section 2.5).

2. Results and Discussion

2.1. Synthesis of 2-acylamino-3-cyano-4,6-diphenylpyridine derivatives

The enclosed methodologies for the procurement of 2-acylamino-3-cyano-4,6-diphenylpyridine derivatives, as presented in Scheme 1, proceeded *via* a facile 4-component condensation reaction. Bearing the appropriate moiety on the corresponding aldehydes and ketones, a catalogue of pyridinium intermediates was synthesised. Compounds **8a**, **8d-f**, **8h-i**, and **8k** were prepared successfully through the treatment of ketone **2a-h** with the corresponding benzaldehyde, malononitrile, and ammonium acetate in toluene.



1a: R^1 = H
1b: R^1 = 3'-Br
1c: R^1 = 3'-F
1d: R^1 = 4'-N(Me)$_2$

2a: R^2 = H, R^3 = H
2b: R^2 = H, R^3 = 3'-NO$_2$
2c: R^2 = H, R^3 = 2'-NO$_2$
2d: R^2 = H, R^3 = 4'-OH
2e: R^2 = H, R^3 = 2'-OH
2f: R^2 = Br, R^3 = 4'-Cl
2g: R^2 = Br, R^3 = 2'-NO$_2$
2h: R^2 = H, R^3 = 4'-Br

8a: R^1 = H, R^2 = H, R^3 = H,
8b: R^1 = H, R^2 = H, R^3 = 3'-NO$_2$
8c: R^1 = H, R^2 = H, R^3 = 2'-NO$_2$
8d: R^1 = H, R^2 = H, R^3 = 4'-OH
8e: R^1 = H, R^2 = H, R^3 = 2'-OH
8f: R^1 = 3'-Br, R^2 = H, R^3 = H,
8g: R^1 = H, R^2 = Br, R^3 = 4'-NO$_2$
8h: R^1 = F, R^2 = H, R^3 = H
8i: R^1 = F, R^2 = H, R^3 = 2'-OH
8j: R^1 = 4'-N(Me)$_2$, R^2 = H, R^3 = 3'-Br,
8k: R^1 = OH. R^2 = H, R^3 = 3'-Br

Scheme 1. Synthesis of 2-acylamino-4,6-diphenylpyridines derivatives. Reagents and conditions: Aromatic aldehyde (**1a-d**), aromatic ketone (**2a-h**), malononitrile, NH_4OAc, toluene, reflux.

Initially, benzaldehyde (**1a**) and acetophenone (**2a**) were used as model reactants for the optimisation of the condensation reaction. According to previous literature, ethanol was found to be the optimum solvent for the one-pot cyclo-condensation reaction.[35,36] Conversely, upon using ethanol at their reflux temperatures, none of the desired product was observed. Employment of a protic, hygroscopic solvent, aka ethanol, in concurrence with water facilitates the ancillary hydrolysis of the imine intermediate (Fig. 5). The aforementioned mechanism is catalysed by the conjugate base of ammonium acetate, consequently returning the ketone starting reagent. In addition, no corresponding product was afforded when the reaction was carried out, thermally, in the absence of solvent or in the presence of MeCN.[36] Screening of several solvents revealed that toluene (anhydrous) was the most suitable option, as per Kobayashi *et al.*'s methodologies,[33] affording the desired product, **8a**, in 15 % yield.

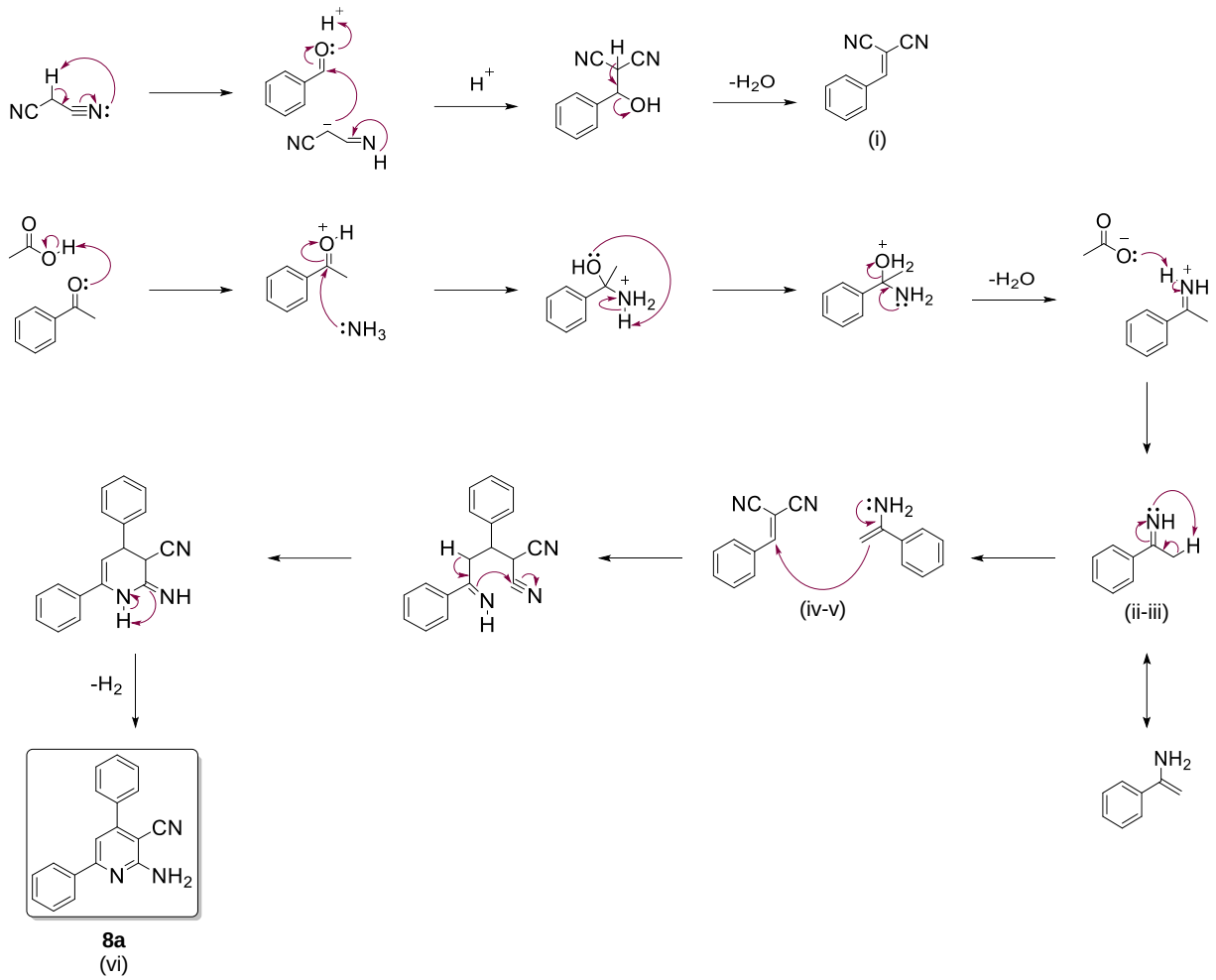
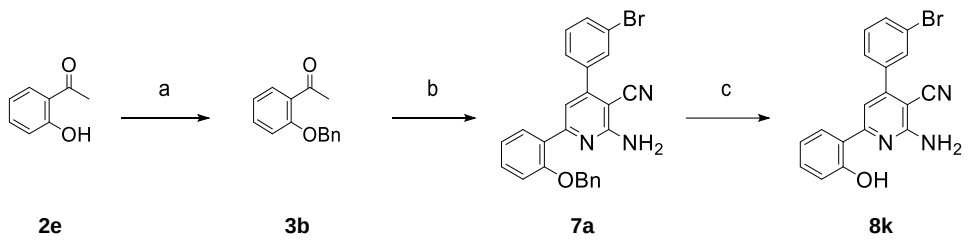


Figure 5. Possible mechanism of the one-pot formation of product **8a**. (i) 2-benzylidenemalononitrile formation: Knoevenagel condensation of malononitrile and benzaldehyde (**1a**); (ii) Enamine formation: Leuckart reductive amination of acetophenone (**2a**) and ammonium acetate; (iii) tautomerisation; (iv, v) cycloaddition, isomerisation; (vi) aromatisation.

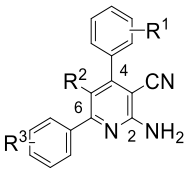
Having identified optimised conditions for the chemistry shown in Scheme 1, subsequent methodologies for the synthesis of pyridinium derivatives examined the scope and limitations of various aromatic aldehydes and ketones. Regarding modification of the 6-phenyl ring, the inclusion of electron withdrawing groups consisting of *meta*-nitro or *para*-nitro moieties (**8b** and **8c**) did not yield any product. To optimise the proton donor ability of the pyridinium scaffold, introduction of *para*-substituted or *ortho*-substituted hydroxy acetophenones gave trace amounts of product **8d** and **8e**. Kobayashi *et al*. identified that a 2'-postion hydroxy group on the 6-phenyl ring was essential for the affinity of KISS1R.[33] Thus, optimisation in the procurement of such an intermediate bearing a hydroxyl moiety on the 6-phenyl ring, as shown in Scheme 2, was proposed. Protection of the hydroxy moiety on **2e**, *via* the instalment of a benzyl (Bn) group, followed by the 4-component condensation reaction, incorporating **3b**, 3-bromobenzaldehyde, malononitrile and ammonium acetate afforded **7a** in 46 % yield. Subsequent ether cleavage of the benzyl protecting group, *via* the *in-situ* formation of TMSI, returned the hydroxy moiety for **8k** in 61 % yield.



Scheme 2. Optimised **s**ynthesis of 2-acylamino-4,6-diphenylpyridine (**8k**) with a hydroxyl group on the 6-phenyl ring. Reagents and conditions: (a) benzyl bromide, K_2CO_3, acetone, 60 °C; (b) 3-bromobenzaldehyde (**1b**), malononitrile, NH_4OAc, toluene, reflux; (c) TMSCl, NaI, MeCN, 120 °C.

Modification of the 4-phenyl ring, through the introduction of a halide substitute on the *meta*-position of an aromatic aldehyde afforded products **8f**, **8h**-**i** and **8k** in moderate yields. Halo-substituted aromatic aldehydes (3-bromobenzaldehyde **1b** and 3-flurobenzaldehyde **1c**) reacted readily with acetophenone, thus affording a *de facto* 'synthetic handle' on the pyridinium scaffold. The inclusion of a bromo or fluoro moiety on the *meta* position of the 4-phenyl ring (see compounds **8f** and **8h**) abetted subsequent coupling reactions, as shown in Scheme 7 (see section 2.2.). The synthetic potential of reacting 4'-dimethylaminobenzaldehyde (**1d**) with 4'-bromoacetopheonone (**2h**) was next examined, and *in-situ* analysis *via* ^{1}H-NMR highlighted partial formation of **8j**. Nonetheless, minimal product formation and comparable analyte elution time did not warrant any following scale-up synthesis. Thus, this report did not proceed with the synthesis of compound **8j**. While electron-deficient aromatic aldehydes (i.e., **1b** and **1c**) can react with malononitrile to generate 2-benzylidenemalononitrile intermediates (Fig. 5),[37] it was ascertained that electron-rich **1d** would require harsher reaction conditions. The scope of the synthesis of 2-acylamino-3-cyano-4,6-diphenylpyridine derivatives can be seen in Table 1.

Table 1. Scope of one-pot, 4-component condensation reaction of 2-acylamino-3-cyano-4,6-diphenylpyridine derivatives (Scheme 1 and Scheme 2).

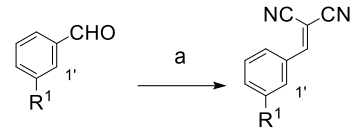


Compound	R^1	R^2	R^3	Scheme	Yield (%)[a]
7a	3'-Br	H	2'-OBn	2	46
8a	H	H	H	1	15
8b	H	H	3'-NO_2	1	0
8c	H	H	2'-NO_2	1	0
8d	H	H	4'-OH	1	6
8e	H	H	2'-OH	1	0.4
8f	3'-Br	H	H	1	10
8g	H	Br	4'-NO_2	1	0
8h	3'-F	H	H	1	26
8i	3'-F	H	2'-OH	1	4
8j	4'-N(Me)	H	4'-Br	1	0
8k	3'-Br	H	2'-OH	1, 2	6, 25[b]
9a	H	OH	4'-Cl	2	0

[a] Isolated yield and based on aromatic aldehyde (reagent **1a-d)**; Scheme 1.
[b] Overall yield, from the multistep, linear synthesis of **8k**, as shown in Scheme 6.

The facile Knoevenagel condensation of aromatic aldehydes with malononitrile, as shown in Scheme 3, afforded **4a** and **4b** in 92 % and 83 % yields respectively. Compounds **11a**-**b** were then obtained using a one-pot process, *via* a multicomponent domino coupling pathway, in combination with the appropriate benzylidenemalononitrile (Scheme 4).

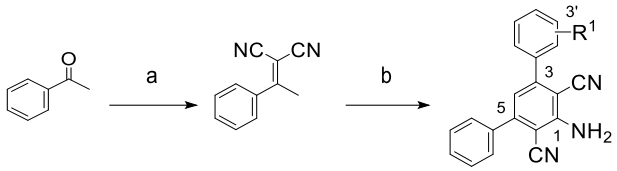
Scheme 5 depicts the attempted utilision of a pyridinium ylide intermediate as a means to obtain compound **21a**. Pyridinium salt **6a** was, however, successfully synthesised from pyridine and chloroacetonitrile in 54 % yield.[38]



2a: R^1 = H
2b: R^2 = Br

4a: R^1 = H
4b: R^2 = Br

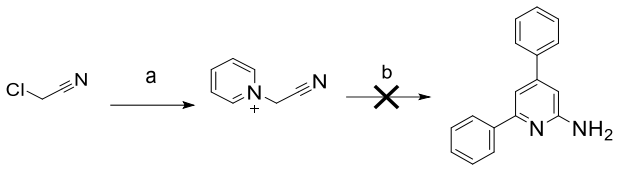
Scheme 3. Preparation of 2-benzylidenemalononitrile derivatives. Reagents and conditions: (a) malononitrile, piperidine, IMS, rt.



2a **5a**

11a: R^1 = H
11b: R^1 = Br

Scheme 4. Preparation of compound **11a-b**. Reagents and conditions: (a) malononitrile, glacial AcOH, NH_4OAc, toluene, reflux; (b) **4a** or **4b**, **5a**, piperidine, MeCN, reflux.



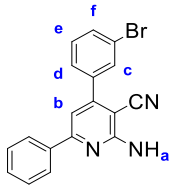
6a **21a**

Scheme 5. Preparation of 4,6-diphenylpyridin-2-amine **21a**. Reagents and conditions: (a) pyridine, MeCN, rt; (b) acetophenone, benzaldehyde, NH_4OAc, glacial AcOH, reflux.

2.1.1. Spectroscopic analyses of 2-acylamino-3-cyano-4,6-diphenylpyridine derivatives 8f and 8k

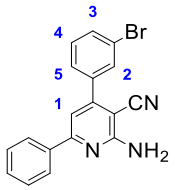
The ^{1}H-NMR spectrum of **8f** displayed a broad singlet at δ 5.43 ppm, in addition to an uncoupled singlet at δ 6.86 ppm. Both peaks are owed to the formation of the amino group and the lone hydrogen on the pyridine core. Formation of the nitrile group was underpinned by the presence of an IR absorbance peak at 2224 cm^{-1}. ^{1}H NMR multiplets in the δ 7.60-7.58 ppm and δ 7.53-7.52 ppm regions, and a series of doublets at δ 7.57 (J = 1.9 Hz), δ 7.51 (J = 2.0 Hz), and δ 7.50 (J = 2.1 Hz) ppm correspond to the five hydrogen environments on the 6-phenyl ring of **8f**. Hydrogen atom 'c' is split *via meta* positioned protons 'f' and 'd', affording the corresponding coupling constants: $^4J_{cf}$ = 1.8 Hz $^4J_{cd}$ = 1.8 Hz. Hydrogen 'e' is split by two non-equivalent, *ortho* positioned hydrogen-environments 'f' ($^3J_{cf}$ = 7.8 Hz) and 'd' ($^3J_{cd}$ = 8.0 Hz). The inductive effect of the halide moiety de-shields hydrogen 'f' and thus positions 'f' further downfield, relative to the hydrogen environment of 'd'. Complete evaluation of compound **8f**, including 2D-^{1}H, DEPTQ-HSQC NMR, are shown in Table 2 and Table 3.

Table 2. Deconstructing the ^{1}H NMR for the pyridine core and 4-phenyl ring of intermediate **8f** in $CDCl_3$-*d*.



Compound	H atom	^{1}H (δ) ppm	$^{x}J_{(H-H)}$	Multiplicity	Integration
8f	a	5.43	-	*br*	2
	b	6.86	-	*s*	1
	c	7.69	$^{4}J_{cf}$ = 1.8 Hz $^{4}J_{cd}$ = 1.8 Hz	*t*	1
	d	7.54	$^{3}J_{de}$ = 8.0 Hz, $^{4}J_{dc}$ = 2.0 Hz, $^{4}J_{df}$ = 1.0 Hz	*ddd*	1
	e	7.38	$^{3}J_{cf}$ = 7.8 Hz, $^{3}J_{cd}$ = 8.0 Hz	*t*	1
	f	7.62	$^{3}J_{fe}$ = 7.7 Hz, $^{4}J_{fc}$ = 1.8 Hz, $^{4}J_{fd}$ = 1.0 Hz	*ddd*	1

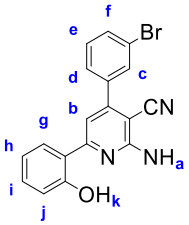
Table 3. Deconstruction of the 2D-^{1}H, DEPTQ-HSQC NMR data of **8f** in $CDCl_3$-*d*.



Compound	H atom	^{1}H (δ) ppm	C atom	^{13}C (δ) ppm
8f	b	6.86	1	120.0
	c	7.69	2	131.2
	d	7.54	5	127.1
	e	7.38	4	130.3
	f	7.62	3	132.7

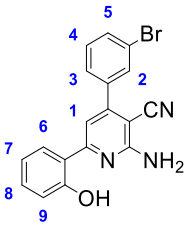
The IR spectrum of product **8k** displayed absorption bands at 3400, 3260, 2214, 1241 and 757 cm^{-1} which are characteristic of NH_2, OH, C≡N, C-N and C-Br bonds respectively. The ^{1}H-NMR spectrum of **8k** presented two broad singlets: hydrogens 'a' at δ 7.54 ppm and hydrogen 'k' at δ 13.37 ppm. Proton signal environments 'a' and 'k' were suppressed, upon the addition of D_2O, thereby displaying the triplet splitting pattern of hydrogen 'e' at δ 7.51 ppm. Hydrogen 'c' is split *via* protons 'd' and 'f', with coupling constants J = 1.7 Hz and J = 2.1 Hz respectively. The singlet at 7.45 ppm is indicative of the lone hydrogen environment 'd' on the pyridine core. Hydrogen 'g' is split *via* proton 'h' (^{3}J = 7.0 Hz) and 'i' (^{4}J = 1.2 Hz) affording a doublet of doublets. Likewise, hydrogen 'j' gives a doublet of doublets as this proton is split *via* proton 'i' (^{3}J = 8.3 Hz) and 'h' (^{4}J = 1.2 Hz). Complete evaluation of compound **8k**, including 2D-^{1}H, DEPTQ-HSQC NMR, are shown in Table 4 and Table 5.

Table 4. Deconstruction of the ^{1}H NMR spectra of **8k** in DMSO-d_6.



Compound	H atom	^{1}H (δ) ppm	$^{x}J_{(H-H)}$	Multiplicity	Integration
8k	a	7.54	-	*br*	2
	b	7.45	-	*s*	1
	c	7.89	$^{4}J_{cf}$ = 1.9 Hz $^{4}J_{cd}$ = 1.9 Hz	*t*	1
	d	7.69	$^{3}J_{de}$ = 7.8 Hz, $^{4}J_{dc}$ = 1.7 Hz, $^{4}J_{df}$ = 1.0 Hz	*ddd*	1
	e	7.51	$^{3}J_{cf}$ = 7.8 Hz, $^{3}J_{cd}$ = 8.0 Hz	*t*	1
	f	7.76	$^{3}J_{fe}$ = 8.1 Hz, $^{4}J_{fc}$ = 2.1 Hz, $^{4}J_{fd}$ = 1.1 Hz	*ddd*	1
	g	6.88	$^{3}J_{gh}$ = 7.0 Hz, $^{4}J_{gi}$ = 1.2 Hz	*dd*	1
	h	7.35	^{3}J = 8.5$_{hi}$ Hz, $^{3}J_{hg}$ = 7.1 Hz, $^{4}J_{hj}$ = 1.6 Hz	*ddd*	1
	i	8.10	$^{3}J_{ii}$ = 8.3 Hz, $^{4}J_{ig}$ = 1.2 Hz	*dd*	1
	j	6.91	$^{3}J_{ji}$ = 8.2 Hz, $^{4}J_{jh}$ = 1.6 Hz	*dd*	1
	k	13.37	-	*s*	1

Table 5. Deconstruction of the 2D-^{1}H, DEPTQ-HSQC NMR data of **8k** in DMSO-d_6.

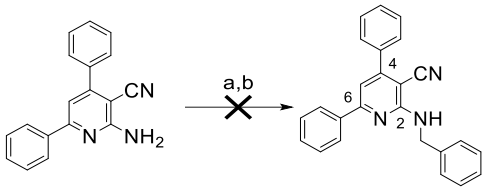


Compound	H atom	^{1}H (δ) ppm	C atom	^{13}C (δ) ppm
8k	b	7.45	1	108.6
	c	7.89	2	131.4
	d	7.69	3	128.1
	e	7.51	4	131.2
	f	7.76	5	133.0
	g	6.88	6	119.0
	h	7.35	7	133.0
	i	8.10	8	129.0
	j	6.91	9	118.6

2.2. Synthesis of 2-acylamino-3-cyano-4,6-diphenylpyridine and their amido and sulfonamido derivatives

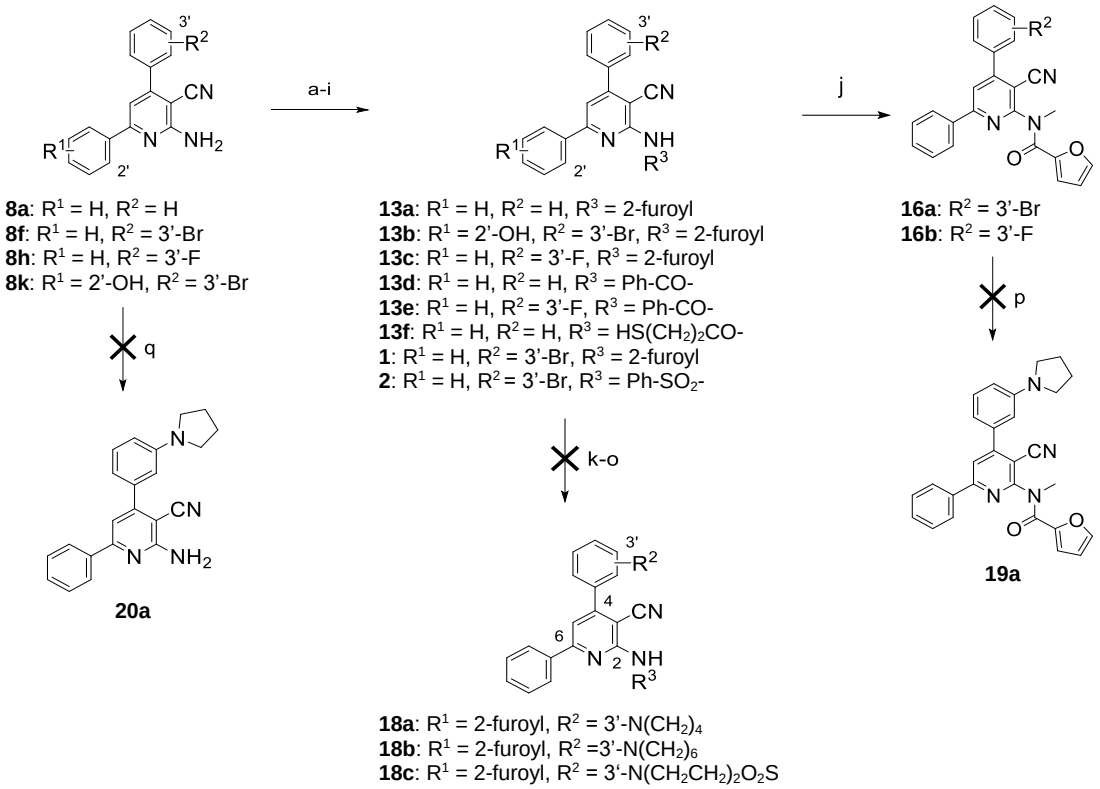
In the initial attempt to obtain *N*-(3-cyano-4,6-diphenylpyridin-2-yl)furan-2-carboxamide **13a**, an acylation between 2-amino-4,6-diphenylnicotinonitrile **8a**, with 2-furoyl chloride (**12a**) was performed. Kobayashi *et al*., adopted a combinatorial approach to identify the 2-furoyl moiety to be the most active antagonist of all tested 2-acylamino-3-cyano-4,6-diphenylpyridine derivatives.[32] The synthetic purpose of developing 2-furanylcarbonyl substituted 2-acylamino-3-cyano-4,6-diphenylpyridines was to investigate any corresponding structural-activity relationships with the novel chemical series of KISS1R antagonists. Table 6 lists the representative data obtained for the synthesis of the 2-acylamino pyridines, whereby different operatory conditions were selected and tested for the acylation reaction. The direct reaction of acyl chloride and amine is a common procedure for amide bond formation and, on first inspection, would appear to be a viable approach for the synthesis of **1**, **2** and compounds **13a-f**. As such, Kobayashi *et al*. facilitated their amide formation, through the modification of the 2-amino group (Fig. 3),[32,33] employing **12a** and pyridine. As observed, in Scheme 7, target amide **13a** was not produced when the reaction conditions proceeded *via* the use of triethylamine, DIPEA or pyridine (Table 6, entries 1-3). Presumably due to the 3-CN group and pyridine core, their close proximity and inductive effects upon the 2-amino group impair the amines nucleophilicity. To overcome some of these problems associated with the aforesaid approach, the possibility of generating the amidation method starting from the carboxylic acid was explored (Table 6, entries 4-9). Basavaprabhu *et al*.

described a facile, $FeCl_3$ catalysed, coupling of carboxylic acids and poorly nucleophilic anilines.[39] Adoption of their methodology (Table 6, entry 4) involved 1.0 equiv. of **8a**, glacial AcOH, $FeCl_3$ (20 mol%), and 2-furoic acid (1.2 equiv.) in toluene. However, as reported, employment of the catalyst, $FeCl_3$, did not abet the desired procedure, instead it returned the initial starting reagents. Moreover, the utilisation of carbodiimide coupling agents, EDC or DCC, were also ineffective. The difficulties observed with these couplings were linked to the electron deficient and sterically hindered 2-amino group, in combination with a weak electrophile. It was reasoned that a coupling reaction that minimised steric hinderance between the coupling partners would more likely succeed. As such, a one-pot HATU-mediated amide formation, as per Wren *et al.*,[40] was employed. The proposed aminium mediated formation of compound **13** proceeded as such: to a premixed solution of benzoic acid (**12c**, 1.1 equiv.) and HATU (1.1 equiv.) in DMF and pyridine, a solution of **8a** in DMF was added dropwise. Nonetheless, upon TLC analysis, after a reaction time of 24 h, no novel spots were observed. Although not presented in Table 6, the proposed reductive amination of **8a** (Scheme 6) further establishes the relatively inert nature of the 2-amino group.



8a **15a**

Scheme 6. Unsuccessful syntheses of 2-amino-6-(4-chlorophenyl)-5-hydroxy-4-phenylnicotinonitrile **15a**. Reagents and conditions: (a) Et_3N, DCM, rt; (b) benzaldehyde, $NaBH_4$, THF, rt.



8a: R^1 = H, R^2 = H
8f: R^1 = H, R^2 = 3'-Br
8h: R^1 = H, R^2 = 3'-F
8k: R^1 = 2'-OH, R^2 = 3'-Br

13a: R^1 = H, R^2 = H, R^3 = 2-furoyl
13b: R^1 = 2'-OH, R^2 = 3'-Br, R^3 = 2-furoyl
13c: R^1 = H, R^2 = 3'-F, R^3 = 2-furoyl
13d: R^1 = H, R^2 = H, R^3 = Ph-CO-
13e: R^1 = H, R^2 = 3'-F, R^3 = Ph-CO-
13f: R^1 = H, R^2 = H, R^3 = $HS(CH_2)_2CO$-
1: R^1 = H, R^2 = 3'-Br, R^3 = 2-furoyl
2: R^1 = H, R^2 = 3'-Br, R^3 = Ph-SO_2-

16a: R^2 = 3'-Br
16b: R^2 = 3'-F

20a

19a

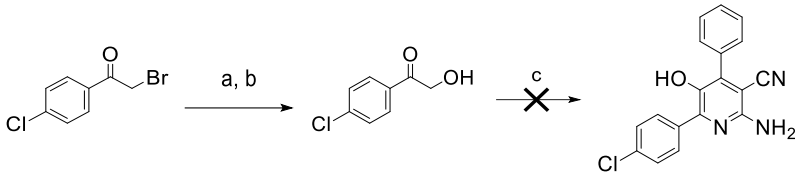
18a: R^1 = 2-furoyl, R^2 = 3'-$N(CH_2)_4$
18b: R^1 = 2-furoyl, R^2 =3'-$N(CH_2)_6$
18c: R^1 = 2-furoyl, R^2 = 3'-$N(CH_2CH_2)_2O_2S$

Scheme 7. Reagents and conditions for the synthesis of compounds **1-2** and compounds **13a-f** : (a) **8a**, 2-furoyl chloride, Et_3N, DCM, rt; (b) **8a**, 2-furoyl chloride, DIPEA, DCM, rt; (c) **8a**, 2-furoyl chloride, pyridine, rt; (d) **8a**, furoic acid, $FeCl_3$, glacial AcOH, toluene, reflux; (e) **8a**, furoic acid, EDC, pyridine, DMF, reflux; (f) **8a**, furoic acid, DCC, pyridine, DMF, reflux; (g) **8a**, thioglycolic acid, HATU, pyridine, DMF, reflux; (h) **8a** or **8h**, benzoic acid, HATU, pyridine, DMF, reflux; (i) compound **8**, 2-furoyl chloride or benzene sulfonyl chloride, pyridine, 120 °C, MW conditions. • Methylation of amide moiety, reagents and conditions: (j) MeI, KOtBu, THF, rt. • Reagents and conditions for the proposed synthesis of compound **18** with a cyclic tertiary amine on its 4-phenyl side chain and their amide derivatives: (k) **1**, pyrrolidine, $Pd_2(dba)_3$, *S*-BINAP, Cs_2CO_3, toluene, reflux; (l) **1**, hexamethyleneimine, $Pd(PPh_3)_4$, Na_2CO_3, KOtBu, toluene, reflux;

(m) **1**, thiomorpholine-1,1-dioxide, $Pd(OAc)_2$, KOtBu, 1,4-dioxane, reflux; (n) **1**, $Pd(OAc)_2$, *S*-BINAP, thiomorpholine-1,1-dioxide, Cs_2CO_3, toluene, reflux (o) **1**, thiomorpholine-1,1-dioxide, $Pd(OAc)_2$, Cs_2CO_3, toluene, reflux. • Reagents and conditions for the proposed synthesis of **19a** with a tertiary amine on its 4-phenyl side chain: (p) **16b**, pyrrolidine, BuLi, THF, 0 °C. • Reagents and conditions for the proposed synthesis of **20a** with a tertiary amine on its 4-phenyl side chain: (q) **8f**, K_3PO_4, CuI, pyrrolidine, ethylene glycol, propan-2-ol, rt.

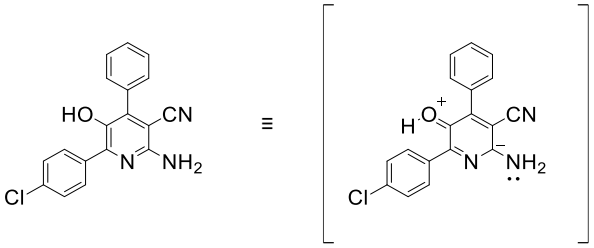
On the basis of the earlier results, a reasoned strategy, for the construction C-N coupled products, involved the instalment of electron-donating groups (EDG) on the pyridine core, prior to amidation. Inclusion of an EDG would increase the electron density of the pyridine core and inhibit any inductive effects *via* the 3-CN group. Moreover, the attachment of a pi-donor would increase the basicity on the 2-amino group, thus increasing its nucleophilicity (Fig. 6).

The displacement of the bromo moiety with an acetyl group on **2f** proceeded *via* sodium acetate in a water-MeOH solution. Subsequent hydrolysis of the corresponding carboxylic ester gave product **3a**. Next, acid catalysed hydrolysis of **2f** (using HCl) afforded **3a** in 29 % yield, as shown in Scheme 2. The unsuccessful, yet proposed, synthesis of **9a** proceeded *via* a facile 4-component condensation reaction employing **1a**, **3a**, malononitrile and ammonium acetate.



2f **3a** **9a**

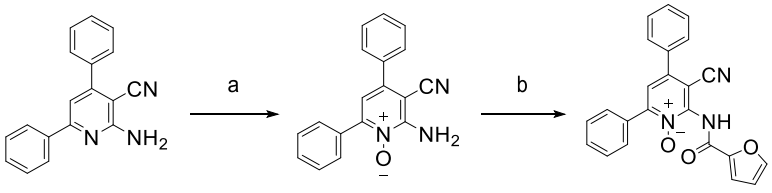
Scheme 8. Introduction of hydroxyl moiety to afford **3a** and, synthesis of 2-amino-6-(4-chlorophenyl)-5-hydroxy-4-phenylnicotinonitrile (**9a**). Reagents and conditions: (a) water-MeOH, sodium acetate, reflux; (b) HCl, MeOH, reflux; (c) benzaldehyde, malononitrile, NH_4OAc, toluene, reflux.



9a

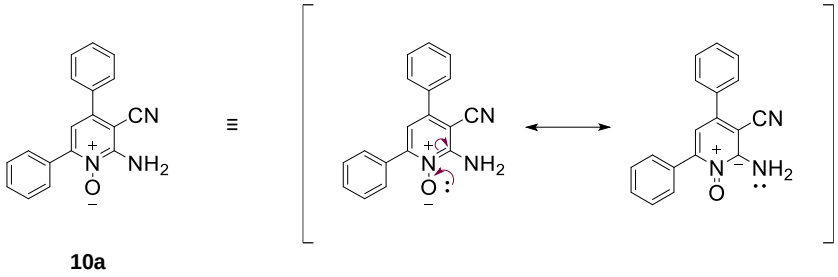
Figure 6. Increasing of electron density on the pyridine core *via* the resonance donating effect of the 5-hydroxy moiety.

By the introduction of *m*-CPBA, as shown in Scheme 9, it was feasible to mediate the formation of the *N*-oxide, whereby the oxide group exerts a strong electron-donating effect on the pyridine core (Fig. 7). When **8a** (0.42 mmol) was reacted with 0.84 mmol of *m*-CPBA the pyridinium oxide, **10a**, was obtained in 94 % yield. As shown in entry 10 (Table 6), the mild acylation of the 2-amino group (using 1.0 equiv. of 2-furoyl chloride) gave product **14a** in 10 % yield. The results indicate that the *N*-oxide, 2-amino-3-cyano-4,6-diphenylpyridine-1-oxide **10a**, could abet the mechanism for the instalment of the 2-furoyl moiety, consistent with Londregan *et al.*.[46]



8a **10a** **14a**

Scheme 9. Synthesis of 2-amino-3-cyano-4,6-diphenylpyridine 1-oxide (**10a**) and its amide derivative **14a**. Reagents and conditions: (a) *m*-CPBA, DCM, -15 °C; (b) 2-furoyl chloride, Et_3N, rt.



10a

Figure 7. Increasing of electron density on the pyridine core *via* the resonance donating effect of the *N*-oxide.

Microwave (MW) irradiation has also been widely used in amide formation, due to a reduction in reaction times and its good yields in an *N*-acylation application.[47] In this work it has been demonstrated that MW irradiation upon compounds **8**, in pyridine, mediated the coupling with **12a** to give products **13a-c** and proposed KISS1R antagonist **1** in moderate to excellent yields (Table 6, entries 11–14). Prompted by these results and having identified optimised MW mediated conditions, it was found that aryl-sulfonyl chlorides are suitable substrates for the corresponding coupling reaction. The MW irradiation of **8f** and benzene-sulfonyl chloride (**12e**) proceeded efficiently and the proposed KISS1R antagonist, compound **2**, was obtained in excellent yield (entry 15, 84 %).

Table 6. Optimisation of reaction conditions for the acylation of compound **8** with compound **10**. Reaction conditions for the coupling of compound **10a** and **14a**.

Entry	Reagent 1	Reagent 2	Coupling Agent	Solvent	Base	Product	Yield (%)
1	**8a**	2-Furoyl chloride	-	DCM	Et_3N	**13a**	0
2	**8a**	2-Furoyl chloride	-	DCM	DIPEA	**13a**	0
3	**8a**	2-Furoyl chloride	-	Pyridine	-	**13a**	0 [b]
4	**8a**	2-Furoic acid	$FeCl_3$	Toluene/ AcOH	-	**13a**	0
5	**8a**	2-Furoic acid	EDC	DMF/ Pyridine	-	**13a**	0
6	**8a**	2-Furoic acid	DCC	DMF/ Pyridine	-	**13a**	0
7	**8h**	Benzoic acid	HATU	DMF/ Pyridine		**13e**	0
8	**8a**	Benzoic acid	HATU	DMF/ Pyridine	-	**13d**	0
9	**8a**	Thioglycolic acid	HATU	DMF/ Pyridine	-	**13f**	0
10	**10a**	**14a**	-	DCM	Et_3N	**14a** [a]	10
11	**8a**	2-Furoyl chloride	-	Pyridine	-	**13a** [a]	71 [c]
12	**8f**	2-Furoyl chloride	-	Pyridine	-	**1** [a]	46 [c]
13	**8h**	2-Furoyl chloride	-	Pyridine	-	**13c** [a]	69 [c]
14	**8k**	2-Furoyl chloride	-	Pyridine	-	**13b** [a]	27 [c]
15	**8f**	Benzene-sulfonyl chloride	-	Pyridine	-	**2** [a]	84 [c]

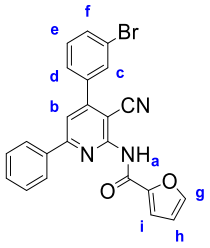
[a] Novel compounds: No reference to **13a-c**, **1**, **2,** and **14a** found in Scifinder-n (as of 28th April 2022).[45]
[b] Room temperature for 48 h.
[c] MW mediated reaction, 120 °C for 12 h.

2.2.1 Spectroscopic analyses of 2-acylamino-3-cyano-4,6-diphenylpyridine and their amido and sulfonamido derivatives

The reaction of **8f** and **12a** led to the formation of compound **1**. The IR spectrum of **1** displayed absorption bands at 3340, 2220, 1673, and 690 cm^{-1} which are characteristic of N-H, C≡N, C=O and C-Br bonds. The ^{1}H NMR spectrum of **1** showed a singlet at δ 7.73 ppm for hydrogen 'b' on the pyridine core (Table 7). In addition, there is a broad singlet at δ 8.86 ppm which is associated with the N-H functionality. The furoyl moiety bears three different nuclei (in terms of magnetic nuclei) coupling together- an *ihg* system. Within this system there are three different coupling constants: $^3J_{hi}$ = 3.6 Hz, $^3J_{hg}$ = 1.8, $^4J_{ig}$ = 0.9 Hz, so that, for instance, the 'h' resonance is split into a doublet of spacing J_{hi} and then each line of the doublet is further split into a doublet by J_{hg}. Thus, characteristically, there are three different chemical shifts (doublet of doublets) per nuclus environment: δ 6.65 ppm ('h'), δ 7.38 ppm ('i'), and δ 7.66 ppm ('g'). The same splitting pattern is observed on the furoyl moiety of **16a** (Table 9). Complete evaluation of compound **1**, including 2D-^{1}H, DEPTQ-HSQC NMR, are shown in Table 7

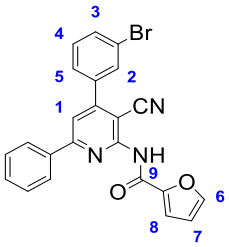
and Table 8. Moreover, evaluation of product **2** and **16a** ^{1}H NMR spectra for compounds **2** and **16a** are shown in Tables 9 and 10 respectively.

Table 7. Deconstructing the ^{1}H NMR spectrum of **1** in CD_2Cl_2-d_2.



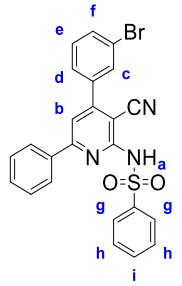
Compound	H atom	^{1}H (δ) ppm	$^{x}J_{(H-H)}$	Multiplicity	Integration
	a	8.86	-	*br*	1
	b	7.73	-	*s*	1
	c	7.83	$^{4}J_{cf}$ = 1.8 Hz $^{4}J_{cd}$ = 1.8 Hz	*t*	1
	d	7.67	-	*m*	1
1	e	7.47	$^{3}J_{cf}$ = 7.9 Hz, $^{3}J_{cd}$ = 7.9 Hz	*t*	1
	f	7.71	$^{3}J_{fe}$ = 8.0 Hz, $^{4}J_{fc}$ = 1.9 Hz, $^{4}J_{fd}$ = 0.9 Hz	*ddd*	1
	g	7.66	$^{3}J_{gh}$ = 1.8 Hz, $^{4}J_{gi}$ = 0.8 Hz	*dd*	1
	h	6.65	$^{3}J_{hi}$ = 3.6 Hz, $^{4}J_{hg}$ = 1.8	*dd*	1
	i	7.38	$^{3}J_{ih}$ = 3.6 Hz, $^{4}J_{ig}$ = 0.9	*dd*	1

Table 8. Deconstructing the 2D-^{1}H, DEPTQ-HSQC NMR data for **1** in $CDCl_3$-*d*.



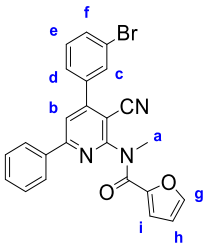
Compound	H atom	^{1}H (δ) ppm	C atom	^{13}C (δ) ppm
	b	7.73	1	120.0
	c	7.83	2	131.2
	d	7.67	5	127.1
	e	7.47	4	130.3
1	f	7.71	3	132.7
	g	7.66	6	145.5
	h	6.65	7	113.3
	i	7.38	8	116.9
	-	-	9	160.5

Table 9. Deconstructing the ^{1}H NMR spectrum of **2** in DMSO-d_6.



Compound	H atom	^{1}H (δ) ppm	$^{x}J_{(H-H)}$	Multiplicity	Integration
2	a	11.87	-	*br*	1
	b	7.86	-	*s*	1
	c	7.93	$^{4}J_{cf}$ = 1.9 Hz $^{4}J_{cd}$ = 1.9 Hz	*t*	1
	d	7.70	$^{3}J_{de}$ = 7.7 Hz, $^{4}J_{dc}$ = 1.4 Hz	dt	1
	e	7.55	$^{3}J_{cf}$ = 7.9 Hz, $^{3}J_{cd}$ = 7.9 Hz	*t*	1
	f	7.78	$^{3}J_{fe}$ = 8.0 Hz, $^{4}J_{fc}$ = 1.8 Hz, $^{4}J_{fd}$ = 0.9 Hz	*ddd*	1
	g	7.84	$^{3}J_{gh}$ = 8.0 Hz, $^{4}J_{gi}$ = 1.4 Hz Hz	*dd*	2
	h i	7.46	-	*m*	3

Table 10. Deconstructing the ^{1}H NMR spectrum of **16b** in $CDCl_3$-*d*.



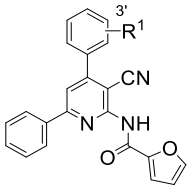
Compound	H atom	^{1}H (δ) ppm	$^{x}J_{(H-H)}$	Multiplicity	Integration
16b	a	3.70	-	*s*	3
	b	7.76	-	*s*	1
	c	7.74	$^{4}J_{cf}$ = 1.9 Hz $^{4}J_{cd}$ = 1.9 Hz	*t*	1
	d	7.56	$^{3}J_{de}$ = 7.8 Hz	*d*	1
	e	7.74	$^{3}J_{cf}$ = 7.9 Hz, $^{3}J_{cd}$ = 7.9 Hz	*t*	1
	f	7.68	$^{3}J_{fe}$ = 7.9 Hz, $^{4}J_{fc}$ = 2.0 Hz	*dd*	1
	g	7.24	$^{3}J_{gh}$ = 1.9 Hz	*d*	1
	h	6.41	$^{3}J_{hi}$ = 3.6 Hz, $^{4}J_{ig}$ = 1.7	*dd*	1
	i	7.02	$^{3}J_{ih}$ = 3.6 Hz	*d*	1

2.3. Proposed *N*-arylation methodologies for the instalment of cyclic amine moiety

A wide variety of palladium-catalysed C-N bond forming methodologies are available through catalysed reactions by Pd(0) compounds.[43,44] The initially proposed catalytic reactions were conducted by heating a solution of **1**, amine, Pd(0) catalyst, and base in either toluene or 1,4-dioxane, as shown in Table 11. In the initial attempt to obtain a cyclic amine on the 4-phenyl ring of **1**, 2 mol% $Pd_2(dba)_3$ and Cs_2CO_3 were employed for the arylation of the weakly acidic pyrrolidine (**17a**; entry 1). To further elucidate the generality of this practical approach, the use of a number of Pd(0) compounds and cyclic amines were investigated in the amination reaction (Scheme 7, Table 11). It was reasoned that the failure of reaction in entries 1, 4 and 5 were due to the relatively low pK_a values of caesium and sodium carbonates, thus making it harder to deprotonate the Pd-coordinated amine during the catalytic cycle. As per Lin *et al.*,[48] it was proposed that the cross-coupling of a fluoroarene with **17a** may be facilitated *via* an organolithium species. It was reasoned that due to base-labile functional groups being present on **13c**, a stronger base would perform worse. As such, protection of the amide nitrogen *via* 1.5 equiv. of MeI gave both products, **16a** and **16b**, in 95 % yield. Unfortunately, contrary to Lin's work,[48] the direct substitution of pyrrolidine on compound **16** in the presence of a strong base, *n*BuLi, did not afford **19a –** instead the reaction returned starting materials. Next, this study moved on to investigate the use of C-N coupling conditions previously described by Buchwald *et al.*.[41,42] For

example, the proposed reaction of **17a** with **8f** was carried out in the presence of 0.19 mmol of CuI, ethylene glycol and 2.0 equiv. of K_3PO_4 in iso-propyl alcohol. However, such a reaction course did not prove compatible for the *N*-arylation of compound **8f**. It should be stressed amination of the 4-phenyl ring is important for the antagonistic efficacy of the pyridine-based scaffolds.[32,33] On the basis of these results, further optimisation and substrate scope is required.

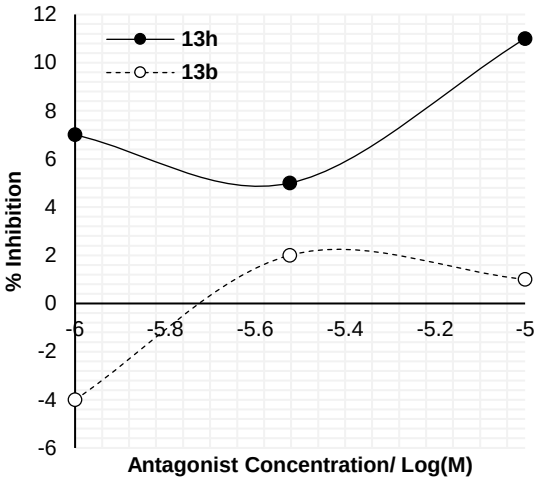
Table 11. Scheme 7: The proposed *N*-arylation methodologies for the instalment of a cyclic amine moiety (R^1) on the 4-phenyl ring of compound **1**.

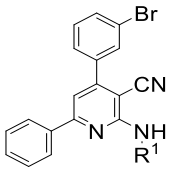


Entry	Catalyst	Base	Solvent	R^1	Duration / h	Compound	Yield (%)
1	2 mol% $Pd_2(dba)_3$, 3 mol% *S*-BINAP	Cs_2CO_3	Toluene		48	**18a**	0
2	2 mol% $Pd(PPh_3)_4$	KOtBu, Na_2CO_3	Toluene		24	**18b**	0
3	5 mol% $Pd(OAc)_2$	KOtBu	1,4-Dioxane		24	**18c**	0
4	2 mol% $Pd(OAc)_2$, 3 mol% *S*-BINAP	Cs_2CO_3	Toluene		48	**18c**	0
5	5 mol% $Pd(OAc)_2$	Cs_2CO_3	Toluene		24	**18c**	0

2.4. Antagonist activity of compounds 1 and 2 at the KISS1R

Kobayashi *et al.* established small molecular non-peptide-based antagonists containing a 2-acylamino-3-cyano-4,6-diphenylpyridine scaffold, identifying the 2-furoyl group to be the most active antagonist of all derivatives tested.[32] Presented herein are the antagonistic activities of **2**, bearing a sulfone substitution on the 2-amino position of the 2-acylamino-3-cyano-4,6-diphenylpyridine scaffold, evaluated side-by-side with the 2-furoyl pyridinium derivative **1**. To evaluate the KISS1R antagonistic activities of **1** and **2**, a cellular Ca^{2+} mobilisation assay was carried out at the human KiSS1/KISS1R receptor expressed in rat basophil leukemia cells. Compounds **1** and **2** were assessed at 1.0 µM, 3.2 µM and 10 µM, with their results expressed as a percent inhibition of the control response to Metastin (45-54) at 10 nM (Eurofins Cerep).[34] The results of the antagonistic assay are shown in Figure 8. Introduction of the bulkier benzene sulfone moiety (**2**) on the 2-amino group of the pyridine scaffold enhanced calcium inhibition, relative to the 2-furoyl moiety. At 10 µM, **2** displayed weak-moderate antagonist activity with an 11.2 % Ca^{2+} inhibition, whereas **1** did not adequately suppress Ca^{2+} influx - with an inhibition of 0.6 % at 10 µM. The maximum level of Ca^{2+} inhibition for **1** was 2.2 %, as shown at 3.2 µM. Interestingly, **1** induced Ca^{2} influx at 1.0 µM, whereby **2** subdued Ca^{2+} mobilisation by 7.5 % at the same concentration.





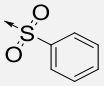
Compound	R^1	Max. % Ca^{2+} inhibition
1		2.2
2		11.1

Figure 8. Outsourced study, Eurofins Cerep.[34] Evaluation of the antagonistic activity of compound **1** and **2** at the human KiSS1/KISS1R receptor, determined by measuring their effect on agonist induced cytosolic Ca^{2+} ion mobilisation.

Although only modestly active, **2** bore a strong preference to KISS1R antagonism, relative to **1**. While further studies are required, the data outlined in this paper purports that substrates bearing a sulfonamido functional group could feasibly be an alternative to existing amide bearing small pyridine-based antagonists.

2.5. P-234 cell proliferation assay

In this study the activity of P-234 on the proliferation of MCF-7 (Fig. 9) and MDA-MB-231 (Fig. 10) cell lines were examined *via* Incucyte and WST-1 cell-imagining assays. According to the Incucyte assay result, supernatants containing P-234 in the MCF-7 cell lines markedly reduced proliferation in a dose and time dependent manner. In sharp contrast to MCF-7 (PR^+/ER^+), anti-proliferation activity for P-234 was observed in MDA-MB-231 cell lines (TNBC, $PR^-/ ER^-/ HER2^-$, model). With reference to the mechanism outlined in Figure 2, it was reasoned that P-234 would inhibit cell-proliferation in MDA-MB-231 cell lines, by blocking endogenous *KISS1*/KISS1R expression and/or succumb to auxiliary cytotoxic effects. Data on the effect of P-234 on breast cancer cell proliferation are limited. However, Zarnani *et al*. evaluated the potential effects of placenta KPs (KISS1R agonists) on the proliferation of MCF-7 and MDA-MB-231 breast cancer cells.[49] Interestingly, Zarnani's results demonstrated that KPs reduced proliferation of breast cancer cells,[49] hypothesising that KISS1R antagonist may promote proliferation. As observed, synthetic antagonists of KISS1R promote cell proliferation in TNBC model cell

lines, contrary to PR^+/ER^+ BCa cell lines. Such reverse effects may be due, in part, by the differential responsiveness and relative stability of P-234 in MCF-7 and MDA-MB-231 cell lines.

Next, this study reported on the WST-1 proliferation study of MCF-7 and MDA-MB-231 cell lines over a period of 72 h at 0 nM, 10 nM, 100 nM and 1000 nM. The data on the modulatory effect of P-234 on cell proliferation in MCF-7 showed no association between P-234 inhibition and cancer cell growth (Fig. 9). Figure 10 demonstrates the anti-proliferation effect of P-234, in MDA-MB-231, with the greatest rate of growth at 1000 nM after 24 h.

An outlook of this study would be to employ P-234, as a control, when testing for *in vitro* dependent variables, and compared to the afforded synthesised substrates. Going forward, due to the heterogeneity and the complexity of TNBC tumourigenesis, it is important to confirm and to validate results in other cell line systems and cultures (i.e. BT459, 3D culture systems) - this is with the outlook of reporting a comprehensive *in vitro* based analyses.

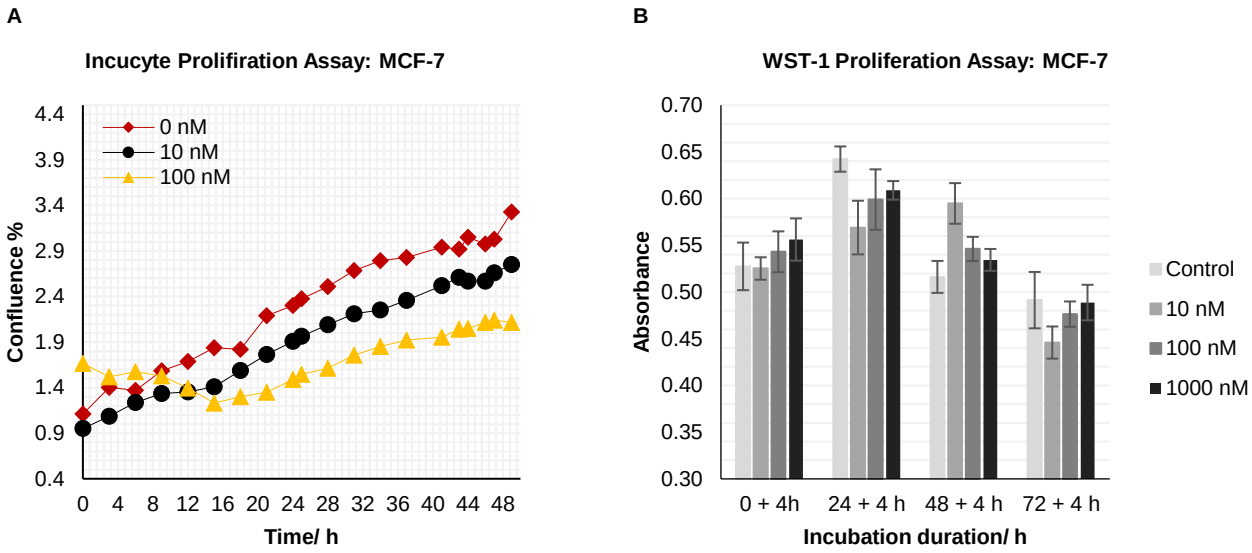


Figure 9. The effect of P-234 on the proliferation of MCF-7 breast cancer cells. A) Incuctye, cell imagining proliferation assay for P-234 assessed at concentrations of 0 nM, 10 nM and 100 nM, over a 48 h period. B) WST-1 proliferation assay for P-234 assessed at concentrations of 0 nM, 10 nM, 100 nM and 1000 nM, over a 72 h period. The additional 4 h accounts for the incubation period upon the addition of WST-1.

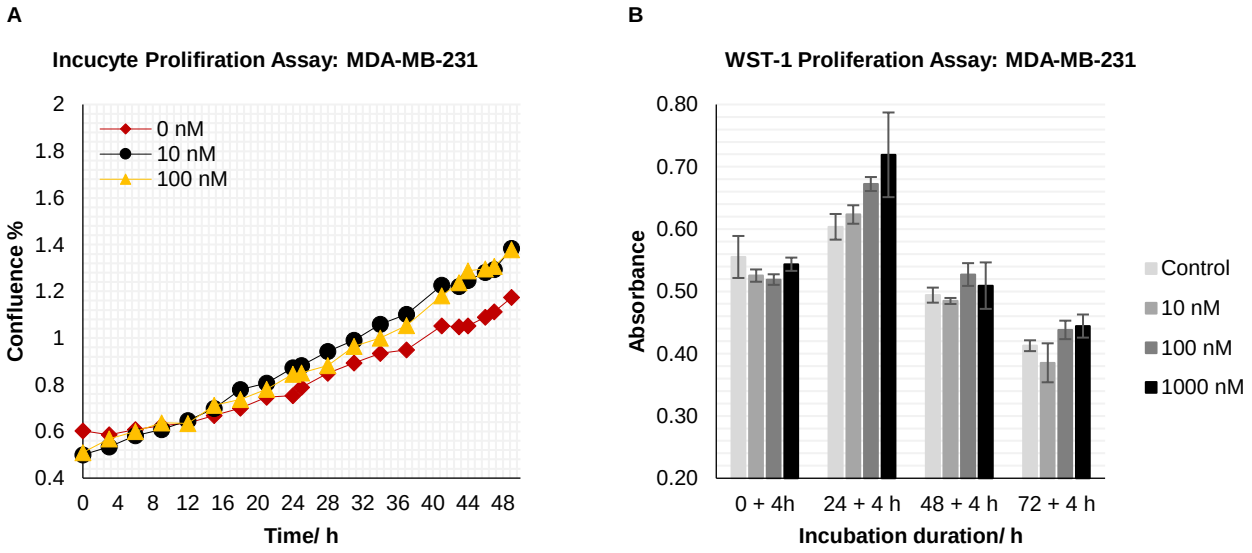


Figure 10. The effect of P-234 on the proliferation of MDA-MB-231 breast cancer cells. A) Incuctye, cell imagining proliferation assay for P-234 assessed at concentrations of 0 nM, 10 nM and 100 nM, over a 48 h period. B) WST-1 proliferation assay for P-234 assessed at concentrations of 0 nM, 10 nM, 100 nM and 1000 nM, over a 72 h period. The additional 4 h accounts for the incubation period upon the addition of WST-1.

3. Conclusion and Outlook

Characterised by poor survival rates, high instance of distance metastases, and limited therapeutic options, TNBC is the most aggressive form of BCa.[50] Thus, there is a particular medical interest to elucidate the molecular drivers behind TNBC chemoresistance.[4] KISS1R signals through a plethora of diverse molecular mechanisms that have the potential to regulate the processes navigating TNBC clinical outcomes.[4]

In an attempt to find novel KISS1R antagonists, this paper has reported on the synthesis of novel 2-acylamino-4,6-diphenylpyridine derivatives, whereby the antagonistic effects of both **1** and **2** were evaluated *in vitro*. The synthetic protocols outlined in the paper have the capacity to expand upon the existing chemical series of small pyridine KISS1R antagonists. Spectral and analytical data of the newly synthesised compounds were all in good agreement with the proposed chemical structures. Compounds **8** were accessed *via* a four-component condensation reaction. It is noteworthy that hydrogen donor functionality is necessary for KISS1R-substrate binding. Thus, Scheme 2 describes a methodology in procuring a hydroxy moiety on the 6-phenyl ring (Fig 11, region 2). In addition, a set of substrates were mediated *via* MW conditions to afford C-N and CN-S coupled products (Scheme 7). However, the optimum specification for amination, on the 4-phenyl ring was not established. Interestingly, a benzene-sulfone group was found to be an appropriate substitute of the 2-furoyl moiety, when evaluated upon its capacity to inhibit Ca^{2+} mobilisation. The structural-activity optimisation, for compound **2**, would see the instalment of a cyclic amine moiety (i.e., piperazine) on region '1' and a hydroxy group on region '2' (Fig. 11).

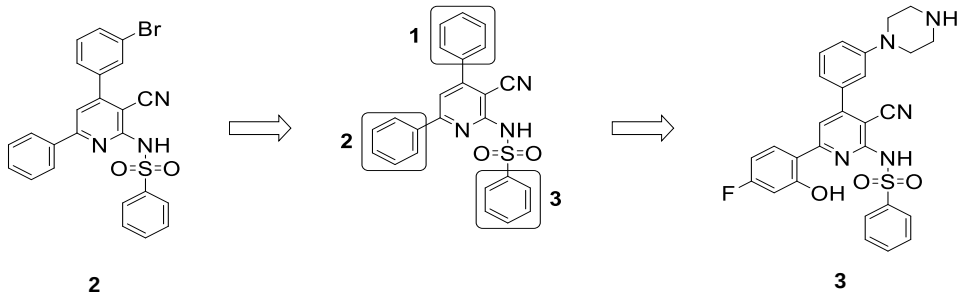


Figure 11. i) Compound **2** and structural regions for exploration. ii) Compound **3**, adapted from compound **A** (Fig. 3) could be a viable pyridine scaffold for future studies.

Following the appraisal of compound **2**, which showed apparent antagonistic activity in a cellular functional assay, further proof-of-concept studies are warranted. Going forward, the principal objective should be to evaluate the viability of KISS1R antagonism, as a therapeutic option in TNBC. Initially, more highly selective and potent KISS1R inhibitors should be developed to obtain better tolerability and anticancer efficacy. Second, optimised combination regimens, with existing chemotherapeutics (i.e., doxorubicin), need to be investigated for the best synergic effect. Third, additional studies should be conducted to identify reliable biomarkers, to predict antagonistic response and to minimise any adverse effects. Moreover, KISS1R inhibitor resistance should also be investigated.

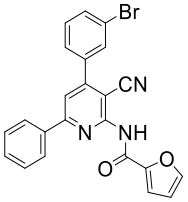
4. Experimental

The hotplate magnetic stirrer used was supplied by Camlab (MS-H280-Pro). TLC analyses were performed using aluminium backed silica gel coated plate ($60F_{254}$) and developed using a UVGL-58 Handheld UV Lamp (254-365 nm). Microwave mediated reactions were operated using a Biotage® Intiator 2.5. Column chromatography was performed using Davisil® chromatography grade silica (pore size 60 angstrom, particle size 35-70 micron). Melting points were determined on a Gallenkamp (electronic) apparatus. IR spectra were recorded on a Nicolet iS5 spectrometer, with the Thermo ScientificTM iD7 ATR-diamond accessory included. The following abbreviations are used for IR absorbance bands: *w* (weak), *m* (medium), *s* (strong), *br* (broadened). Nuclear magnetic resonance (NMR) spectra were recorded on an Bruker NMR ADVANCE II instrument operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. NMR spectra were obtained as $CDCl_3$ – *d*, CD_2Cl_2–d_2, DMSO-d_6 or acetone-d_6 solutions (reported in ppm), using $CDCl_3$-*d* (^{1}H: 7.25 ppm; ^{13}C: 77.16 ppm), CD_2Cl_2–d_2 (^{1}H: 5.32 ppm; ^{13}C: 54.00 ppm), DMSO-d_6 (^{1}H: 2.50 ppm; ^{13}C: 39.52 ppm) or acetone-d_6 (^{1}H: 2.05 ppm; ^{13}C: 206.26 ppm) as the corresponding reference standard. The following abbreviations are used for peak multiplicities: *s* (singlet), *d* (doublet), *t* (triplet), *m* (multiplet), *br* (broadened), *dd* (doublet of doublets), *ddd* (doublet of doublet of doublets), *dt* (doublet of triplets), *td* (triplet of doublets) and *tt* (triplet of triplets). Coupling constants (*J*) are reported in Hertz (Hz). Mass spectra were recorded on a Bruker Daltonics micrOTOF and measured in *m/z*. Calculated *m/z* data is founded on each element's monoisotopic mass.

4.1. Materials

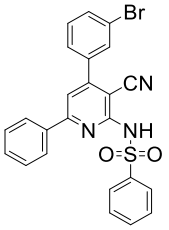
2-Furoic acid, (98 %), 2'-hydroxyacetophenone (99 %), 3-bromobenzaldehyde (97%), *N*,*N*, dimethylformamide (DMF, anhydrous), tetrahydrofuran (THF; 99.85%, extra dry), and trimethylsilyl chloride (TMSCl) were purchased from Alfa Aesar (Heysham, UK). Acetonitrile (MeCN), benzenesulfonyl chloride (99 %), calcium chloride ($CaCl_2$), conc. hydrochloric acid (HCl), dichloromethane (99.8%, extra dry over molecular sieve, stabilized), ethyl acetate (EtOAc), ethylene glycol, glacial acetic acid (AcOH), hexane, industrial methylated spirit (IMS), magnesium sulfate ($MgSO_4$), methanol (MeOH), potassium carbonate (K_2CO_3), potassium hydroxide (KOH), propan-2-ol, sodium acetate, sodium borohydride ($NaBH_4$), sodium hydrogen carbonate ($NaHCO_3$) and toluene (anhydrous) were purchased from Fisher Scientific (Loughborough, UK). 1,4-Dioxane, 2-furoyl chloride, acetone-d_6 (>99.8 %), acetophenone, ammonium acetate, chloroform-*d* ($CDCl_3$-*d*; >99.8 %), diisopropylethylamine (DIPEA), dimethylsulphoxide-d_6 (DMSO-d_6; >99.8%), HATU, hexamethyleneimine, malononitrile, methylene dichloride ($CDCl_2$-d_2; >99.8 %), $Pd(OAc)_2$, potassium tert-butoxide (KOtBu), thiomorpholine 1,1-dioxide, triethylamine, tris(dibenzylideneacetone)dipalladium (0) ($Pd_2(dba)_3$), and tetrakis(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$) were purchased from Flurochem (Hadfield, UK). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), 2-bromo-4'-chloroacetopheonone, 2-bromo-4'-nitroacetopheonone, 2'-hydroxyacetopheonone, 2'-nitroacetopheonone, 3-chloroperbenzoic acid (≤ 77 %), 3'-flurobenzaldehyde, 3'-nitroacetophenone, 4'-bromoacetopheonone, 4'-hydroxyacetopheonone, benzaldehyde, benzoic acid, butyllithium (2.5 M in hexane), chloroacetonitrile (99%), copper (I) iodide (CuI), dimethylaminobenzaldehyde, iodomethane, iron (III) chloride ($FeCl_3$), *N*,*N'*-dicyclohexylmethanediimine (DCC), piperidine, pyridine, pyrrolidine, *S*-BINAP, sodium iodide (NaI), thioglycolic acid, tripotassium phosphate were obtained from Merk (Gillingham (Dorset), UK).

4.1.1. *N*-(4-(3-bromophenyl)-3-cyano-6-phenylpyridin-2-yl)furan-2-carboxamide (1)



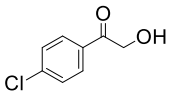
N-[3-cyano-4-(3-bromophenyl)-6-phenyl-2-pyridyl]furan-2-carboxamide **1** was prepared from 2-amino-4-(3-bromophenyl)-6-phenyl-pyridine-3-carbonitrile **8f** (100 mg, 0.29 mmol, 1.0 equiv.) and 2-furoyl chloride **12a** (74 mg, 0.43 mmol, 1.5 equiv.) in a manner similar to that described for **13a** (*Procedure E)* as a white solid in 46 % (58.6 mg) yield. mp. 214 -215 °C. ν_{max} (ATR)/ cm^{-1}: 3340*m* (N-H), 2220*w* (C≡N), 1673*s* (2° amide, C=O), 1591*m* (N-H), 690*s* (C-Br). ^{1}H NMR (400 MHz, CD_2Cl_2-d_2): δ_H 8.86 (*s*, 1H, NH), 8.15 – 8.08 (*m*, 2 arom. H), 7.83 (*t*, *J* = 1.8 Hz, 1 arom. H), 7.73 (*s*, 1 arom. H), 7.71 (*ddd*, *J* = 8.0, 1.9, 0.9 Hz, 1 arom. H), 7.69 – 7.66 (*m*, 1 arom H), 7.66 (*dd*, *J* = 1.8, 0.8 Hz, 1H, CH), 7.55 – 7.50 (*m*, 3 arom. H), 7.47 (*t*, *J* = 7.9 Hz, 1 arom. H), 7.38 (*dd*, *J* = 3.6, 0.9 Hz, 1H, CH), 6.65 (*dd*, *J* = 3.6, 1.8 Hz, 1H, CH). ^{13C} NMR (100 MHz, CD_2Cl_2-d_2): δ_C 160.5, 155.37, 153.8, 153.0, 146.7, 145.5, 138.3, 137.2, 133.5, 131.5, 130.8, 130.5, 129.0, 128.1, 127.4, 127.3, 122.0, 117.6, 116.9, 115.2, 113.3, 101.7. MS (ESI, MeOH): ($C_{23}H_{14}BrN_3O_2$) $[M+H]^+$ found: 444.0783, calcd.: 444.0269.

4.1.2. *N*-(4-(3-bromophenyl)-3-cyano-6-phenylpyridin-2-yl)benzenesulfonamide (2)



N-(4-(3-bromophenyl)-3-cyano-6-phenylpyridin-2-yl)benzenesulfonamide **2** was prepared from 2-amino-4-(3-bromophenyl)-6-phenyl-pyridine-3-carbonitrile **8f** (100 mg, 0.29 mmol, 1.0 equiv.) and benzene sulfonyl chloride **12e** (74 mg, 0.43 mmol, 1.5 equiv.) in a manner similar to that described for **13a** (*Procedure E)* as a white solid in 84.4 % (119.7 mg) yield. mp. 279 °C. ν_{max} (ATR)/ cm^{-1}: 3407*br*, 2219*m* (C≡N), 2158*m*, 1584*m*, 1538*m*, 1452*s*, 1328*s* (S=O), 1164*m*, 1164*s*, 1023*s*, 1001*s*, 744*s* (C-Br) . ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 11.87 (*s*, 1H, NH), 8.09 – 8.06 (*m*, 2 arom. H), 7.93 (*t*, *J* = 1.9 Hz, 1 arom. H), 7.86 (*s*, 1 arom. H), 7.84 (*dd*, *J* = 8.0, 1.4 Hz, 2 arom. H), 7.78 (*ddd*, *J* = 8.0, 1.8, 0.9 Hz, 1 arom. H), 7.70 (*dt*, *J* = 7.7, 1.4 Hz, 1 arom. H), 7.68 – 7.61 (*m*, 3 arom. H), 7.55 (*t*, *J* = 7.9 Hz, 1 arom. H), 7.51 – 7.42 (*m*, 3 arom. H). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 157.8, 154.1, 153.1, 141.6, 138.0, 136.26, 132.8, 130.9, 130.8, 129.1, 132.8, 128.7, 127.9, 127.7, 126.9, 122.0, 116.0, 115.2, 95.9. MS (ESI, MeOH): ($C_{24}H_{16}BrN_3O_2S$) $[M+H]^+$ found: 489.0147, calcd.: 489.0128.

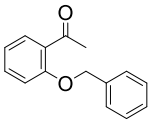
4.1.3. 1-(4-Chlorophenyl)-2-hydroxy-ethanone (3a)



Sodium acetate (30.0 mmol, 2.46 g, 3.0 equiv.) was added to a solution of 2-bromo-4-chloroacetophenone **2f** (2.34 g, 10.0 mmol, 1.0 equiv.) in a MeOH-water mixture (1:3, 100 mL) and magnetically stirred at room temperature for 24 h. After the solvent was evaporated, under reduced pressure, the crude residue was partitioned between water (100 mL) and EtOAc (100 mL). The solution was washed with 2 x 50 mL of NaCl (aq.), dried over $MgSO_4$ and concentrated under reduced pressure. MeOH (20 mL) and HCl (5 M, 20 mL) were added to the crude residue and the mixture was heated at 60 °C for 18 h. The product was concentrated, diluted with water (200 mL), and extracted with 3 x 100 mL of EtOAc. The combined organic

extracts were dried over $MgSO_4$ and concentrated under vacuum. The residue was purified by silica-gel column chromatography (gradient elution, EtOAc/hexane = 1:9 to 1:1) to give 1-(4-chlorophenyl)-2-hydroxy-ethanone **3a** in 29 % (487 mg) as a white solid. mp 141 °C (Lit.: 125-127 °C).[51] ν_{max} (ATR)/ cm^{-1}: 3375*br* (O-H), 1678*s* (C=O), 1591*s*, 1575*s*, 1492*m* (alkane, C-H), 1410*s*, 1280*m*, 1230*s*, 1111*s*, 1091*s*, 1033*m*, 974*s*, 824*s*, 805*s* (Ph-Cl), 605*s*. ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ_H 7.87 (*d*, *J* = 8.8 Hz, 2 arom. H), 7.49 (*d*, *J* = 8.8 Hz, 2 arom. H), 4.85 (*s*, 2H, CH_2), 3.44 (*s*, 1H, OH).

4.1.4. 1-(2-(Benzyloxy)phenyl)ethan-1-one (3b)

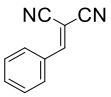


Within an oven dried, nitrogen purged, 250 mL one-necked round bottom flask, benzyl bromide (5.64 g, 33 mmol, 1.1 equiv.) and K_2CO_3 (4.56 g, 33 mmol, 1.1 equiv.) were added to a solution of 2-hydroxyacetopheone **2e** (4.08 g, 30 mmol, 1.0 equiv.) in 45 mL of acetone. The reaction mixture was stirred vigorously and heated to 60 °C for 24 h. Upon completion of the reaction, as shown *via* TLC analysis, the solvent was removed under reduced pressure. The crude residue was diluted with EtOAc (250 mL), washed with brine (4 x 100 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica-gel column chromatography (EtOAc/hexane = 7:3) to give 1-(2-(benzyloxy)phenyl)ethan-1-one **3b** as transparent, colourless crystals in 87 % (5.94 g) yield. mp 34 °C (Lit.: 40 °C).[52] ν_{max} (ATR)/ cm^{-1}: 2929*br*, 1734*w*, 1670*s* (C=O), 1595*s*, 1292*s* (Ph-O), 1234*s*, 753*s*. ^{1}H NMR (400 MHz, Acetone-d_6): δ_H 7.52 (*dd*, *J* = 7.7, 1.8 Hz, 1 arom. H), 7.44 – 7.40 (*m*, 2 arom. H), 7.37 (*ddd*, *J* = 8.4, 7.3, 1.8 Hz, 1 arom. H), 7.32 – 7.27 (*m*, 2 arom. H), 7.24 (*dt*, *J* = 4.7, 1.9 Hz, 1 arom. H), 7.11 (*d*, *J* = 8.4 Hz, 1 arom. H), 6.89 (*td*, *J* = 7.6, 0.9 Hz, 1 arom. H), 5.14 (*s*, 2H, CH_2), 2.39 (*s*, 3H, CH_3). MS (ESI, MeOH): ($C_{15}H_{14}O_2$) $[M+Na]^+$ found: 249.0835, calcd.: 249.0892.

4.1.5. General procedure for the synthesis of 4a and 4b

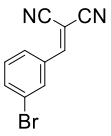
Aromatic aldehyde **1a** or **1b** (18 mmol, 1.2 equiv.), malononitrile (0.99 g, 15 mmol, 1.0 equiv.), piperidine (15 µL, 0.15 mmol, 0.01 equiv.) and 15mL of IMS were magnetically stirred at room temperature for 10 mins. Aldehyde **1a** and **1b** was washed with sat. $NaHCO_3$ (aq.) and distilled over $CaCl_2$, prior to use. Piperidine was distilled over KOH, prior to use. The white precipitate formed was filtered, washed with chilled IMS, and dried under vacuum.

4.1.6. 2-Benzylidenemalononitrile (4a)



2-Benzylidenemalononitrile **4a** was prepared from benzaldehyde **1a** (1.91 g, 18 mmol, 1.2 equiv.), in accordance with the general procedure, as a white solid in 93 % **(**2.31 g) yield. mp 102 °C (Lit.: 101 °C).[53] ν_{max} (ATR)/ cm^{-1}: 3033*m*, 2223*m* (C≡N), 1589*m* (C=C), 1568*m*, 1492*m*, 1450*s*, 1317*w*, 1298*w*, 1217*w*, 1055*w*, 970*s*. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.55 (s, 1 H), 7.98 – 7.93 (*m*, 2 arom. H), 7.73 – 7.67 (*m*, 1 arom. H), 7.65 – 7.60 (*m*, 2 arom. H). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 161.6, 134.4, 131.3; 130.5, 129.5, 114.2, 113.2, 81.62.

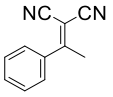
4.1.7. 2-(3-Bromobenzylidene)malononitrile (4b)



2-(3-Bromobenzylidene)malononitrile **4b** was prepared from 3-bromobenzaldehyde **1b** (3.33 g, 18 mmol, 1.2 equiv.), in accordance with the general procedure, as a white solid in 82 % (2.84 g) yield. mp. 112 °C (Lit.: 110 °C).[54] ν_{max} (ATR)/ cm^{-1}:

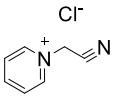
3303*br*, 2220*w* (C≡N), 1674*s*, 1591*s*, 1508*s*, 1409*m*, 1375*s*, 1286*s*, 1012*br*, 872*s*, 798*m* (Ph-Br), 763*s* (C-H). ^{1}H NMR (400 MHz, Acetone-d_6): δ_H 8.35 (*s*, 1H, CH), 8.17 (*t*, *J* = 1.9 Hz, 1 arom. H), 8.04 (*ddd*, *J* = 7.9, 1.8, 0.8 Hz, 1 arom. H), 7.88 (*ddd*, *J* = 8.1, 2.0, 1.0 Hz, 1 arom. H), 7.61 (*t*, *J* = 8.0 Hz, 1 arom. H). ^{13}C NMR (100 MHz, Acetone-d_6): δ_H 159.6, 136.6, 133.6, 133.1, 131.4, 129.0, 122.7, 113.5, 112.6, 84.3.

4.1.8. 2-(1-Phenylethylidene)malononitrile (5a)



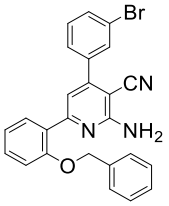
To a 100 mL round bottom flask was added acetophenone **2a** (3.61 g, 0.03 mmol, 1.0 equiv.), malononitrile (3.30 g, 0.05 mmol, 1.7 equiv.), ammonium acetate (1.16 g, 0.09 mmol, 0.5 equiv.) and glacial AcOH (2.87 mL) in 40 mL of toluene. The reaction solution was refluxed for 12 h. After cooling of the reaction mixture, the contents of the RBF were decanted onto an ice-bath and neutralised with saturated $NaHCO_3$ (aq.). The solution was washed with EtOAc (3 x 100 mL) and the combined organic extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was recrystalised from IMS, collected by vacuum filtration, and washed with chilled IMS to give 2-(1-phenylethylidene)malononitrile **4a** as a white solid in 28 % (1.31 g) yield. mp. 106 °C (Lit.: 99 °C).[55] ν_{max} (ATR)/ cm^{-1}: 2928*w* (alkene, C-H), 2227*s* (C≡N), 1585*s*, 1565, 1492*m*, 1442*m* (methyl, C-H), 1376*m*, 1306*m*, 1190*m*, 1051*m*, 769*s*, 709*s*. ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ_H 7.57 – 7.47 (*m*, 5 arom. H), 2.63 (*s*, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$-*d*): δ_C 175.6, 135.9, 132.3, 129.2, 127.4, 127.4, 112.9, 84.7, 24.3.

4.1.9. 1-(Cyanomethyl)pyridin-1-ium (6a)



To an oven dried, nitrogen purged, 100 mL two-necked round bottom flask, chloroacetonitrile (453 mg, 6.0 mmol, 1.2 equiv.) was added to a solution of pyridine (396 mg, 5.0 mmol, 1.0 equiv.) in acetonitrile (15 mL). The mixture was magnetically stirred at room temperature for 96 h. Acetonitrile was distilled over $CaCl_2$ prior to use. The white precipitate formed was filtered, washed with acetone, and dried under vacuum to afford the pure 1-(cyanomethyl)pyridin-1-ium chloride salt **6a** as a while solid in 54 % (419 mg) yield. mp. 174 °C (Lit.: 178 °C).[56] ν_{max} (ATR)/ cm^{-1}: 3045*s*, 3030*s*, 2865*s*, 2731*w*, 2622*w*, 2255*w* (C≡N), 1629*m* (aromatic, C-H), 1498*s*, 1484*s*, 1411*m*, 1387*m*, 1314*m* (C-N), 1214*s*, 1186*s*, 1053*m*, 1025*m*, 946*m*. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 9.35 (*d*, *J* = 5.9 Hz, 2 arom. H), 8.75 (*t*, *J* = 7.8 Hz, 1 arom. H), 8.31 – 8.24 (*m*, 2 arom. H), 6.30 (*s*, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 145.6, 128.7, 114.4, 47.5.

4.1.10. 2-Amino-6-(2-(benzyloxy)phenyl)-4-(3-bromophenyl)nicotinonitrile (7a)

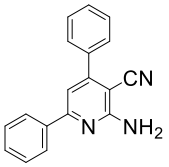


Within a 100 mL one-necked round bottom flask 1-(2-(benzyloxy)phenyl)ethan-1-one **3b** (1.13 g, 5.0 mmol, 1.0 equiv.), malononitrile (0.33 g, 5.0 mmol, 1.0 equiv.), ammonium acetate (0.52 g, 15.0 mmol, 1.5 equiv.) were added to a solution of bromobenzaldehyde **1b** (0.93 g, 5.0 mmol, 1.0 equiv.) in 25 mL of toluene. The reaction mixture was refluxed and stirred vigorously for 24 h. 3-Bromobenzaldehyde **1b** was washed with saturated $NaHCO_3$ (aq.) and distilled over $CaCl_2$, prior to use. The solvent was removed under reduced pressure and the afforded crude residue was dissolved in 75 mL of DCM. The solution was washed thrice with 25 mL of saturated $NaHCO_3$ (aq.), dried over $MgSO_4$, and concentrated under reduced pressure. The obtained product was purified *via* silica gel column chromatography (EtOAc/hexane = 3:7) and recrystalised from EtOH to give 2-amino-6-(2-(benzyloxy)phenyl)-4-(3-bromophenyl)nicotinonitrile **7a** as a white solid in 46 % (0.47 g) yield. mp. 200 °C. ν_{max} (ATR)/ cm^{-1}: 3474*s*, 3296*m* (2° amine, N-H), 3162*br*, 2923*m*, 2203*w* (C≡N), 1636*s*, 1600*w* (aromatic, C-H), 1389*s*, 1363*s*, 1289*s*, 1244*s* (Ph-O), 724*s*, 713*s*, 690*s*. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 7.88 – 7.81 (*m*, 1 arom. H), 7.71 (*d*, *J* = 9.1 Hz, 2 arom. H), 7.49 – 7.38 (*m*, 4 arom. H), 7.35 – 7.24 (*m*, 6 arom. H), 7.10 (*t*, *J* = 7.5 Hz, 1 arom. H), 7.00 (*s*, 2H, NH_2), 5.16 (*s*, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 160.7, 157.9, 156.5, 151.8, 139.3, 136.6, 132.2, 131.2, 130.9, 130.6, 128.4, 128.0, 127.9, 127.2, 127.1, 121.9, 120.8, 116.8, 114.0, 113.3, 85.9, 70.1. MS (ESI, MeOH): ($C_{25}H_{18}BrN_3O$) $[M+H]^+$ found: 456.1045, calcd.: 456.0712.

4.1.11. General procedure for the synthesis of 8a-j

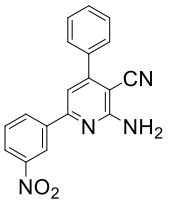
Within a 100 mL one-necked round bottom flask. Aromatic aldehyde **1** (10.0 mmol, 1.0 equiv.), aromatic ketone **2** (10.0 mmol, 1.0 equiv.), malononitrile (0.66 g, 10.0 mmol, 1.0 equiv.), ammonium acetate (1.16 g, 15.0 mmol, 1.5 equiv.) and 25 mL of toluene were refluxed and stirred for 24 h. Compound **1** was washed with saturated $NaHCO_3$ (aq.) and distilled over $CaCl_2$, prior to use. The solvent was removed under reduced pressure and the afforded crude residue was dissolved in 200 mL of EtOAc. The solution was washed thrice with 75 mL of saturated $NaHCO_3$ (aq.), dried over $MgSO_4$ and concentrated under reduced pressure. The obtained product was purified *via* silica gel column chromatography (EtOAc/hexane = 3:7) and recrystallised from an equal part solution of DCM and MeOH.

4.1.12. 2-Amino-4,6-diphenylnicotinonitrile (8a)



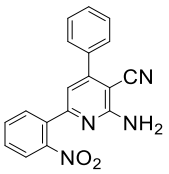
2-Amino-4,6-diphenyl-pyridine-3-carbonitrile **8a** was prepared from benzaldehyde **1a** (1.06 g, 10.0 mmol, 1.0 equiv.) and acetophenone **2a** (1.20 g, 10.0 mmol, 1.0 equiv.), in accordance with the general procedure, as a white solid in 15 % (410 mg) yield. mp 175 °C (Lit.: 178 °C).[57] ν_{max} (ATR)/ cm^{-1}: 3463*m* (1° amine, N-H), 3300*m*, 3175*br* (O-H), 2205*m* (C≡N), 1633*s* (N-H), 1584*s*, 1571*s*, 1544*s*, 1494*s*, 1451*s*, 1370*m*, 1258*m* (C-N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ_H 8.17 – 8.10 (*m*, 2 arom. H), 7.72 – 7.65 (*m*, 2 arom. H), 7.60 – 7.53 (*m*, 3 arom. H), 7.53 – 7.45 (*m*, 3 arom. H), 7.28 (*s*, 1 arom. H), 7.00 (*s*, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 160.9, 158.7, 154.9, 137.0, 130.1, 129.6, 128.8, 128.7, 128.4, 127.3, 117.1, 109.3, 86.7. MS (ESI, MeOH): ($C_{18}H_{13}N_3$) $[M+Na]^+$ found: 294.1128, calcd.: 294.1007.

4.1.13. 2-Amino-6-(3-nitrophenyl)-4-phenylnicotinonitrile (8b)



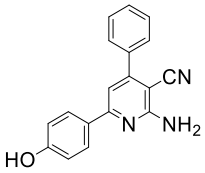
An attempt to prepare 2-amino-6-(3-nitrophenyl)-4-phenylnicotinonitrile **8b** from benzaldehyde **1a** and 3'-nitroacetophenone **2b**, in accordance with the general procedure, was made. Starting materials returned.

4.1.14. 2-Amino-6-(2-nitrophenyl)-4-phenylnicotinonitrile (8c)



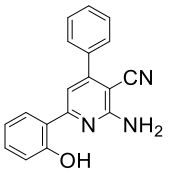
An attempt to prepare 2-amino-6-(2-nitrophenyl)-4-phenylnicotinonitrile **8c** was prepared from benzaldehyde **1a** and 2'-nitroacetophenone **2c**, in accordance with the general procedure, was made. Starting materials returned.

4.1.15. 2-Amino-6-(4-hydroxyphenyl)-4-phenylnicotinonitrile (8d)



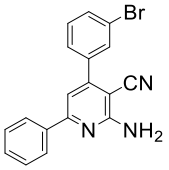
2-Amino-6-(4-hydroxyphenyl)-4-phenyl-pyridine-3-carbonitrile **8d** was prepared from benzaldehyde **1a** (1.06 g, 10.0 mmol, 1.0 equiv.) and 4'-hydroxyacetophenone **2d** (1.36 g, 10.0 mmol, 1.0 equiv.), in accordance with the general procedure, as a yellow solid in 6 % (185 mg) yield. mp. 234 °C (Lit.: 233 - 235 °C).[58] ν_{max} (ATR)/ cm^{-1}: 3393*m* (1° amine, N-H), 3316*m*, 3145*br* (O-H), 2211*m*, 1648*m* (aromatic, C-H) 1374*m*, 1355*m* (C-N). ^{1}H NMR (400 MHz, DMSO): δ_H 9.95 (*s*, 1H, OH), 8.02 – 7.97 (*m*, 2 arom. H), 7.67 – 7.61 (*m*, 2 arom. H), 7.57 – 7.51 (*m*, 3 arom. H), 7.15 (*s*, 1 arom. H), 6.87 (*s*, 2 H, NH_2), 6.86 – 6.82 (*m*, 2 arom. H). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 160.9, 159.6, 158.7, 154.6, 137.3, 129.6, 129.0, 128.8, 128.4, 128.39, 128.35, 117.4, 115.5, 108.2, 85.3. MS (ESI, MeOH): ($C_{18}H_{13}N_3O$) $[M+H]^+$ found: 288.1199, calcd.: 288.1059.

4.1.16. 2-Amino-6-(2-hydroxyphenyl)-4-phenylnicotinonitrile (8e)



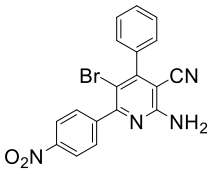
2-Amino-6-(2-hydroxyphenyl)-4-phenylnicotinonitrile **8e** was prepared from benzaldehyde **1a** (1.06 g, 10.0 mmol, 1.0 equiv.) and 2'-hydroxyacetophenone **2e** (1.36 g, 10.0 mmol, 1.0 equiv.), in accordance with the general procedure, as an orange solid in 0.4 % (9.8 mg) yield. mp 239 °C (Lit.: 240 °C).[59] ν_{max} (ATR)/ cm^{-1}: 3372*m* (1° amine, N-H), 3291*m*, 3171*br* (O-H), 2210*m* (C≡N), 1593*s*, 1568*s*, 1386*m*, 1356*m* (C-N). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.13 – 8.12 (*d*, *J* = 4.0 Hz, 2 arom. H), 7.69 – 7.67 (*d*, *J* = 8.0 Hz, 2 arom. H), 7.56 - 7.48 (*m*, 6 arom. H), 7.28 (*s*, 1 arom. H), 7.01 (*s*, 2H, NH_2). MS (ESI, MeOH): ($C_{18}H_{13}N_3O$) $[M+H]^+$ found: 288.1199, calcd.: 288.1059.

4.1.17. 2-Amino-4-(3-bromophenyl)-6-phenylnicotinonitrile (8f)



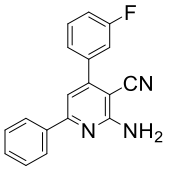
2-Amino-4-(3-bromophenyl)-6-phenyl-pyridine-3-carbonitrile **8f** was prepared from 3-bromobenzaldehyde **1b** (1.85 g, 10.0 mmol, 1.0 equiv.) and acetophenone **2a** (1.20 g, 10.0 mmol, 1.0 equiv.), in accordance with the general procedure, as a yellow solid in 10 % yield (359 mg). mp 220 °C (Lit.: 184 °C).[60] ν_{max} (ATR)/ cm^{-1}: 3330*m* (1° amine, N-H), 2981*br* (N-H), 2224*m* (C≡N), 1268*s* (C-N), 684*s* (C-Br). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.69 (*t*, *J* = 1.8 Hz, 1 arom. H), 7.62 (*ddd*, *J* = 8.0, 2.0, 1.0 Hz, 1 arom. H), 7.60 – 7.58 (*m*, 1 arom. H), 7.57 (*d*, *J* = 1.9 Hz, 1 arom. H), 7.54 (*ddd*, *J* = 7.7, 1.8, 1.0 Hz, 1 arom. H), 7.53 – 7.52 (*m*, 1 arom. H), 7.51 (*d*, *J* = 2.0 Hz, 1 arom. H), 7.50 (*d*, *J* = 2.1 Hz, 1 arom. H), 7.38 (*t*, *J* = 7.9 Hz, 1 arom. H), 6.86 (s, 1 arom. H), 5.43 (*s*, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 153.3, 150.5, 148.4, 139.4, 137.3, 132.8, 131.4, 130.5, 130.0, 129.1, 128.5, 127.2, 123.1, 120.0, 115.9, 115.7, 95.6, 94.9. MS (ESI, MeOH): ($C_{18}H_{13}BrN_3$) $[M+H]^+$ found: 350.0390, calcd.: 350.0293

4.1.18. 2-Amino-5-bromo-6-(4-nitrophenyl)-4-phenylnicotinonitrile (8g)



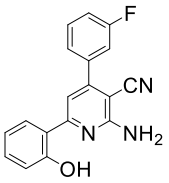
An attempt to prepare 2-amino-5-bromo-6-(4-nitrophenyl)-4-phenylnicotinonitrile **8g** was prepared from benzaldehyde **1a** and 2-bromo-4'-nitroacetopheonone **2g**, in accordance with the general procedure, were made. Starting materials were returned.

4.1.19. 2-Amino-4-(3-fluorophenyl)-6-phenylnicotinonitrile (8h)



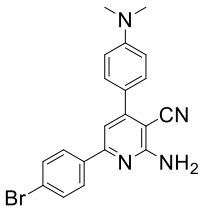
2-Amino-4-(3-fluorophenyl)-6-phenyl-pyridine-3-carbonitrile **8h** was prepared from 3-fluorobenzaldehyde **1c** (1.24 g, 10.0 mmol, 1.0 equiv.) and acetophenone **2a** (1.20 g, 10.0 mmol, 1.0 equiv.), in accordance with the general procedure, as an orange solid in 26 % (740 mg) yield. mp 262-264 °C (Lit.: 261 °C).[61] ν_{max} (ATR)/ cm^{-1}: 3464*m* (1° amine, N-H), 3301*m*, 3175*br*, 2204*s* (C≡N), 1632*m* (aromatic, C-H), 1585*s*, 1574*s*, 1370*m*, 1248*s* (C-N). ^{1}H-NMR (400 MHz, DMSO-d_6): δ_H 8.20 – 8.09 (*m*, 2 arom. H), 7.63 – 7.46 (*m*, 6 arom. H), 7.40 (*d*, *J* = 7.3 Hz, 1 arom. H), 7.31 (*s*, 1 arom. H), 7.07 (*s*, 2H, NH_2). ^{13}C NMR (100 MHz, DMSO-d_6): 163.3, 160.8, 158.9, 153.44, 153.42, 139.23, 139.20, 137.5, 131.2, 130.2, 128.7, 127.7, 125.1, 116.82, 116.80, 115.9, 109.3, 86.5. MS (ESI, MeOH): ($C_{18}H_{12}FN_3$) $[M+H]^+$ found: 290.1173, calcd.: 290.1094.

4.1.20. 2-Amino-4-(3-fluorophenyl)-6-(2-hydroxyphenyl)nicotinonitrile (8i)



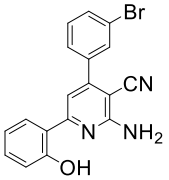
2-Amino-4-(3-fluorophenyl)-6-(2-hydroxyphenyl)nictotinonitrile **8i** was prepared from 3-fluorobenzaldehyde **1c** (1.24 g, 10.0 mmol, 1.0 equiv.) and 2-hydroxyacetophenone **2e** (1.36 g, 10.0 mmol, 1.0 equiv.), in accordance with the general procedure, as an orange solid in 4 % (11 mg) yield. mp 181 °C (Lit.: 190-192 °C).[59] ν_{max} (ATR)/ cm^{-1}: 3403*m* (1° amine, N-H), 3338*m*, 3227*m*, 2973*br* (O-H), 2219 (C≡N), 1656*m* (aromatic, C-H), 1576*s*, 1562*s*, 1238*m* (C-N), 1054*s* (Ph-F). ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 13.31 (*s*, 1H, OH), 8.03 (*dd*, *J* = 8.1, 1.7 Hz, 1 arom. H), 7.62 – 7.57 (*m*, 2 arom. H), 7.55 (*s*, 2H, NH_2), 7.47 – 7.40 (*m*, 3 arom. H), 7.38 – 7.31 (*m*, 1 arom. H), 6.93 (*dd*, *J* = 8.3, 1.2 Hz, 1 arom. H), 6.88 (*t*, *J* = 7.4 Hz, 1 arom. H). ^{13}C NMR (100 MHz, DMSO-d_6): 159.6, 159.3, 158.4, 150.1, 132.6, 131.9, 131.0, 128.3, 125.5, 124.9, 119.0, 118.14, 118.13, 118.1, 116.2, 115.9, 116.0, 109.1, 87.6. MS (ESI, MeOH): ($C_{18}H_{12}FN_3O$) $[M+H]^+$ found: 306.1199, calcd.: 306.1043.

4.1.21. 2-Amino-6-(4-bromophenyl)-4-(4-(dimethylamino)phenyl)nicotinonitrile (8j)



2-Amino-6-(4-bromophenyl)-4-(4-(dimethylamino)phenyl)nicotinonitrile **8j** was prepared from dimethylaminobenzaldehyde **1d** and 4'-bromoacetopheonone **2h**, in accordance with the general procedure., as a dark-purple solid. Crude product returned.

4.1.22. 2-Amino-4-(3-bromophenyl)-6-(2-hydroxyphenyl)nicotinonitrile (8k)

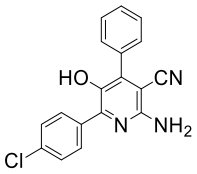


Procedure A: 2-Amino-4-(3-bromophenyl)-6-(2-hydroxyphenyl)nicotinonitrile **8k** was prepared from 3-bromobenzaldehyde **1b** (1.85 g, 10.0 mmol, 1.0 equiv.) and 2-hydroxyacetopheone **2e** (1.36 g, 10.0 mmol, 1.0 equiv.), in accordance with the general procedure, as a yellow-orange solid in 5.6 % (17 mg) yield. Analytical data for *Procedure A* (**8k**) is in accordance with the data presented in *Procedure B*.

Procedure B: To an oven dried, nitrogen purged, 100 mL two-necked round bottom flask, trimethylsilyl chloride was added dropwise to a solution of 2-amino-6-(2-(benzyloxy)phenyl)-4-(3-bromophenyl)nicotinonitrile **7a** (411 mg, 0.90 mmol, 1.0 equiv.) and NaI (338 mg, 2.25 mmol, 2.5 equiv.) in 20 mL of acetonitrile. Acetonitrile was distilled over $CaCl_2$ prior to use. The mixture was heated to 120 °C and stirred until the reaction was complete (72 h). The reaction mixture was quenched with distilled water and washed with EtOAc. The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The product was recrystalised from IMS to afford 2-amino-4-(3-bromophenyl)-6-(2-hydroxyphenyl)nicotinonitrile **8k**, as a white solid, in 61 % (201 mg) yield. mp. 250 °C. ν_{max} (ATR)/ cm^{-1}: 3400*m* (1° amine, N-H), 3336*m*, 3260*br* (O-H), 2214*s* (C≡N), 1653*m* (aromatic, C-H), 1576*s*, 1545*s*, 1274*m*, 1241*m* (C-N), 757*s* (Ph-Br). ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 13.37 (*s*, 1H, OH), 8.10 (*dd*, *J* = 8.2, 1.6 Hz, 1 arom. H), 7.89 (*t*, *J* = 1.9 Hz, 1 arom. H), 7.76

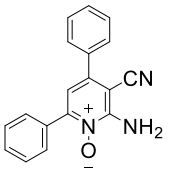
(*ddd*, *J* = 8.0, 2.1, 1.0 Hz, 1 arom. H), 7.69 (*ddd*, *J* = 7.8, 1.7, 1.0 Hz, 1 arom. H), 7.54 (*s*, 2H, NH_2), 7.51 (*t*, *J* = 8.0, 7.8 Hz, 2 arom. H), 7.45 (*s*, 1 arom. H), 7.35 (*ddd*, *J* = 8.5, 7.1, 1.6 Hz, 1 arom. H), 6.91 (*dd*, *J* = 8.3, 1.2 Hz, 1 arom. H), 6.88 (*dd*, *J* = 7.0, 1.2 Hz, 1 arom. H). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 159.7, 159.3, 158.9, 153.7, 139.4, 132.6, 132.5, 131.1, 130.8, 128.5, 127.6, 121.9, 118.9, 118.2, 118.1, 116.8, 108.2, 86.1. MS (ESI, MeOH): ($C_{18}H_{12}BrN_3O$) $[M+H]^+$ found: 366.0417, calcd.: 366.0164.

4.1.23. 2-Amino-6-(4-chlorophenyl)-5-hydroxy-4-phenylnicotinonitrile (9a)



An attempt to prepare 2-amino-6-(4-chlorophenyl)-5-hydroxy-4-phenylnicotinonitrile **9a** proceeded in accordance with the following methodology: to a 25 mL one-necked round bottom flask, 1-(4-chlorophenyl)-2-hydroxy-ethanone **3a** (74 mg, 0.44 mmol, 1.0 equiv.), benzaldehyde **1a** (46 mg, 0.44 mmol, 1.0 equiv.), malononitrile (28.7 mg, 0.44 mg, 1.0 equiv.), and ammonium acetate (50.3 mg, 0.65 mmol, 1.5 equiv.) were added to a 10 mL solution of toluene. The reaction mixture was refluxed and stirred for 24 h. Starting materials were returned.

4.1.24. 2-Amino-3-cyano-4,6-diphenylpyridine 1-oxide (10a)

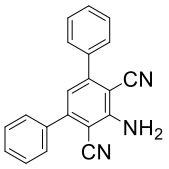


2-Amino-4,6-diphenyl-pyridine-3-carbonitrile **8a** (113 mg, 0.42 mmol, 1.0 equiv.) was dissolved in DCM (4 mL) and cooled (1:4 mass ratio of sodium chloride to ice) to -15 °C. A solution of *m*-CPBA (144 mg, 0.84 mmol, 2.0 equiv.) in 6 mL of DCM was added dropwise. The mixture was magnetically stirred for 3 h, with the temperature maintained at -15 °C, before being allowed to warm to room temperature for an additional reaction time of 24 h. The reaction mixture was diluted *via* 25 mL of DCM, washed with saturated $NaHCO_3$ (aq.; 6 x 50 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (EtOAc/hexane = 3:7) to give 2-amino-3-cyano-4,6-diphenylpyridine 1-oxide **10a** as a white solid in 94 % (54 mg) yield. mp. 230 °C. ν_{max} (ATR)/ cm^{-1}: 3681*w*, 2981*br*, 2844*br*, 2221*w* (C≡N), 1683*s*, 1615*m*, 1574*s* (N-O), 1497*w*, 1416*s*, 1301*s*, 1261*s*, 1206*s*, 1056*s*, 1033*s*, 1014*s*. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.02 – 7.98 (*m*, 2 arom. H), 7.97 (*br*, 2H, NH_2), 7.63 – 7.49 (*m*, 7 arom. H), 7.40 – 7.34 (*m*, 1 arom. H), 7.02 (*s*, 1 arom. H). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 166.5, 153.9, 149.2, 141.6, 136.3, 133.8, 133.4, 133.2, 131.1, 129.9, 129.3, 129.2, 128.9, 128.4, 128.4. MS (ESI, MeOH): ($C_{18}H_{13}N_3O$) $[M+H]^+$ found: 288.1202, calcd.: 288.1059.

4.1.25. General procedure for the synthesis of 11a-b

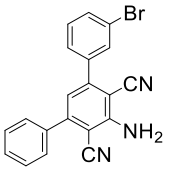
To a 50 mL one-necked round bottom flask, compound **5** (1.0 equiv.) was added to a solution of compound **4** (1.0 equiv.) and piperidine (1.0 equiv.) in 5 mL of acetonitrile. Piperidine was distilled over KOH, prior to use. The reaction mixture was refluxed and stirred for 24 h. The reaction mixture was concentrated under reduced pressure and the crude residue was partitioned between distilled water (25 mL) and EtOAc (25 mL). The organic layer was washed with brine (25 mL), dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was recrystalised from IMS, collected by vacuum filtration, and washed with chilled IMS.

4.1.26. 5'-Amino-[1,1':3',1''-terphenyl]-4',6'-dicarbonitrile (11a)



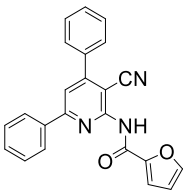
5'-Amino-[1,1':3',1''-terphenyl]-4',6'-dicarbonitrile **11a** was prepared from 2-benzylidenemalononitrile **4a** (68 mg, 0.44 mmol, 1.0 equiv), 2-(1-phenylethylidene)malononitrile **5a** (74 mg, 0.44 mmol, 1.0 equiv.) and piperidine (44 µL, 0.44 mmol, 1.0 equiv.), in accordance with the general procedure, as a white solid in 42 % **(**54.5 mg) yield. mp 243 °C (Lit.: 225 °C).[62] ν_{max} (ATR)/ cm^{-1}: 3477*m*, 3372*br* (N-H), 2214*s* (C≡N), 1630*s*, 1583*s*, 1570*s*, 1572*s*, 1284*s*, 763*s*, 693*s*. ^{1}H NMR (400 MHz, $CDCl_3$-*d*): δ_H 7.66 – 7.63 (*m*, 4 arom. H), 7.55 - 7.51 (*m*, 6 arom. H), 6.81 (*br*, 2H, NH_2), 6.80 (*s*, 1 arom. H). ^{13}C NMR (100 MHz, $CDCl_3$-*d*): δ_C 153.2, 150.1, 137.4, 129.7, 128.9, 128.4, 120.10, 116.0, 94.9. MS (ESI, MeOH): ($C_{18}H_{13}N_3$) $[M+H]^+$ found: 272.1249, calcd.: 272.1188

4.1.27. 5'-Amino-3-bromo-[1,1':3',1''-terphenyl]-4',6'-dicarbonitrile (11b)



5'-Amino-3-bromo-[1,1':3',1''-terphenyl]-4',6'-dicarbonitrile **11b** was prepared from 2-(3-bromobenzylidene)malononitrile **4b** (204 mg, 0.88 mmol, 1.0 equiv.), 2-(1-phenylethylidene)malononitrile **5a** (148 mg, 0.88 mmol, 1.0 equiv.) and piperidine (86 µL, 0.88 mmol, 1.0 equiv.), in accordance with the general procedure, as a white solid in 55 % (180 mg) yield. mp 207 °C. ν_{max} (ATR)/ cm^{-1}: 3460*s*, 3342*br* (N-H), 3237*s*, 2213*s* (C≡N), 1637*s*, 1576*s*, 1541*s*, 790*s*, 771*s* (Ph-Br), 660*s*. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.69 (*d*, *J* = 2.2 Hz, 1 arom. H), 7.66 – 7.45 (*m*, 7 arom. H), 7.38 (*t*, *J* = 7.9 Hz, 1 arom. H), 6.86 (*s*, 1 arom. H), 5.45 (*s*, 2H, NH_2). ^{13}C NMR (100 MHz, $CDCl_3$-*d*): δ_C 153.3, 150.5, 148.4, 139.4, 137.3, 132.8, 131.4, 130.5, 130.0, 129.1, 128.5, 127.2, 123.1, 120.0, 115.9, 115.7, 95.6, 94.9. MS (ESI, MeOH): ($C_{20}H_{12}BrN_3$) $[M+H]^+$ found: 374.0455, calcd.: 374.0215.

4.1.28. N-(3-cyano-4,6-diphenylpyridin-2-yl)furan-2-carboxamide (13a)



Procedure A: Within a 25 mL, oven-dried, nitrogen purged, one-necked round bottom flask 2-amino-4,6-diphenyl-pyridine-3-carbonitrile **8a** (135 mg, 0.50 mmol, 1.0 equiv.) was added to an ice-cooled solution of triethylamine (102 mg, 1.10 mmol, 2.2 equiv.) in 10 mL of dry DCM. Triethylamine was distilled over KOH, prior to use. To the reaction mixture, 2-furoyl chloride **12a** (130 mg, 1.0 mmol, 2.0 equiv.) was added dropwise. The reaction solution was stirred at room temperature for 6 h and its progress monitored *via* TLC (EtOAc/ hexane = 3:7). Upon TLC diagnosis, it was observed that no further spots had emerged. A further 0.5 equiv. of triethylamine and 2-furoyl chloride were added at the 6 h point. The mixture was stirred at room temperature for a further 24 h. Starting materials were returned.

Procedure B: Within a 25 mL, oven-dried, nitrogen purged, one-necked round bottom flask 2-amino-4,6-diphenyl-pyridine-3-carbonitrile **8a** (65 mg, 0.24 mmol, 1.0 equiv.) was added to an ice-cooled solution of *N*,*N*-diisopropylethylamine (83 mg, 0.64 mmol, 2.5 equiv.) in 4 mL of dry DCM. To the reaction, a solution of 2-furoyl chloride **12a** (34 mg, 0.26 mmol,

1.1 equiv.) in 2 mL of DCM, was added dropwise. The reaction mixture was stirred at room temperature for 48 h. The reaction was monitored *via* TLC (EtOAc/ hexane = 3:7) and, upon diagnosis, it was noted that no further spots had emerged. Starting materials were returned.

Procedure C: Within a 25 mL, oven-dried, nitrogen purged, one-necked round bottom flask 2-amino-4,6-diphenyl-pyridine-3-carbonitrile **8a** (65 mg, 0.24 mmol, 1.0 equiv.) was added to a solution of 2-furoyl chloride **12a** (34 mg, 0.26 mmol, 1.1 equiv.) in 4 mL of pyridine. The reaction mixture was stirred at room temperature for 48 h. The reaction was monitored *via* TLC (EtOAc/ hexane = 3:7) and, upon diagnosis, it was observed that no further spots had emerged. Starting materials were returned.

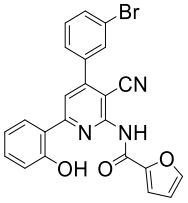
Procedure D: Within a 50 mL, oven-dried, nitrogen purged, two-necked round bottom flask, a solution of 2-amino-4,6-diphenyl-pyridine-3-carbonitrile **8a** (109 mg, 0.4 mmol, 1.0 equiv.) in 5 mL of toluene, was added to a 50 °C pre-heated mixture of 15 µL of glacial AcOH, $FeCl_3$ (20 mol%), and 2-furoic acid (56 mg, 0.5 mmol, 1.2 equiv.) in 10 mL of toluene. The reaction mixture was heated to 90 °C and stirred for 48 h. The reaction was monitored *via* TLC (EtOAc/ hexane = 3:7) and, upon diagnosis, it was observed that no further spots had emerged. Starting materials were returned.

Procedure E: Within a 25 mL, nitrogen purged, one-necked round bottom flask, 2-amino-4,6-diphenylnicotinonitrile **8a** (85 mg, 0.31 mmol, 1.0 equiv.), furoic acid **12a** (39 mg, 0.35 mmol, 1.1 equiv.), EDC (54 mg, 0.35 mmol, 1.1 equiv.), and pyridine (125 mg, 1.55 mmol, 5.0 equiv.) were dissolved in DMF and stirred for 24 h. Starting materials were returned.

Procedure F: Within a 25 mL, nitrogen purged, one-necked round bottom flask, 2-amino-4,6-diphenylnicotinonitrile **8a** (85 mg, 0.31 mmol, 1.0 equiv.), furoic acid **12a** (39 mg, 0.35 mmol, 1.1 equiv.), DCC (72.2 mg, 0.35 mmol, 1.1 equiv.), and pyridine (125 mg, 1.55 mmol, 5.0 equiv.) were dissolved in DMF and stirred for 24 h. Starting materials were returned.

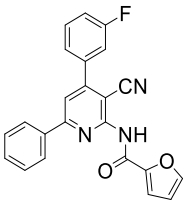
Procedure G: To a 5 mL microwave pressure vial, 2-amino-4,6-diphenyl-pyridine-3-carbonitrile **8a** (35 mg, 0.13 mmol, 1 equiv.) was added to a solution of 2-furoyl chloride **12a** (25 mg, 0.19 mmol, 1.5 equiv.) 1.5 mL of pyridine. The reaction vial was capped before being subjected to microwave mediated conditions (12 h, 110 °C). After cooling, the solvent was removed under high vacuum. The crude residue was partitioned between saturated $NaHCO_3$ (aq.; 30 mL) and DCM (50 mL), washed with distilled water, dried over $MgSO_4$, and concentrated under reduced pressure. The afforded product was purified by silica gel column chromatography (gradient elution EtOAc/hexane = 1:9 to 1:1) before being recrystallised from MeOH, collected by vacuum filtration, and washed with chilled MeOH to afford *N*-(3-cyano-4,6-diphenylpyridin-2-yl)furan-2-carboxamide **13a**, as a white solid in 71 % (35.4 mg) yield. mp 220 °C. ν_{max} (ATR)/ cm^{-1}: 3290*m* (N-H), 2921*br* (N-H), 2223*m* (C≡N), 1670*s* (2° amide, C=O), 1590*s* (N-H), 1489*s*, 1452*s*, 1170*s*, 1135*s* (C-O). ^{1}H NMR (400 MHz, CD_2Cl_2-d_2): 8.88 (*s*, 1H, NH), 8.16 – 8.08 (*m*, 2 arom. H), 7.76 (*s*, 1 arom. H), 7.74 – 7.71 (*m*, 1 arom. H), 7.71 (*d*, *J* = 2.1 Hz, 1 arom. H), 7.67 – 7.63 (*m*, 1H, CH), 7.59 (*d*, *J* = 1.7 Hz, 1H), 7.58 (*d*, *J* = 2.3 Hz, 2H), 7.53 (*d*, *J* = 1.9 Hz, 1H), 7.51 (*d*, *J* = 2.0 Hz, 2H), 7.38 (*dd*, *J* = 3.6, 0.9 Hz, 1H, CH), 6.65 (*dd*, *J* = 3.6, 1.8 Hz, 1H, CH). ^{13}C NMR (100 MHz, CD_2Cl_2-d_2): δ_C 159.8, 156.4, 156.0, 152.8, 147.4, 146.0, 137.5, 136.9, 131.4, 131.2, 130.7, 129.6, 129.5, 129.3, 129.2, 128.0, 120.7, 119.5, 117.9, 117.4, 116.2, 113.4, 100.8. MS (ESI, MeOH): ($C_{23}H_{15}N_3O_2$) $[M+H]^+$ found: 366.1519, calcd.: 366.1164.

4.1.29. *N*-(4-(3-bromophenyl)-3-cyano-6-(2-hydroxyphenyl)pyridin-2-yl)furan-2-carboxamide (13b)



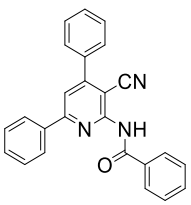
N-(4-(3-bromophenyl)-3-cyano-6-(2-hydroxyphenyl)pyridin-2-yl)furan-2-carboxamide **13b** was prepared from 2-amino-4-(3-bromophenyl)-6-(2-hydroxyphenyl)nicotinonitrile **8k** (64 mg, 0.17 mmol, 1.0 equiv.) and 2-furoyl chloride **12a** (45 mg, 0.35 mmol, 2.0 equiv.) in a manner similar to that described for **13a** (*Procedure E)* as a white solid in 27 % (21.0 mg) yield. mp. 218 °C. ν_{max} (ATR)/ cm^{-1}: 3288*br*, 2924*m*, 2220*m* (C≡N), 1674*s* (2° amide, C=O), 1589, 1566, 1510, 1284, 1171, 1074 (amine, C-N), 1012, 762*s* (Ph-Br). ^{1}H NMR (400 MHz, CD_2Cl_2-d_2): δ_H 7.91 (*dd*, *J* = 7.7, 1.8 Hz, 1 arom. H), 7.77 – 7.72 (*m*, 1 arom. H), 7.67 (*dt*, *J* = 7.9, 1.6 Hz, 1 arom. H), 7.60 (*d*, *J* = 1.7 Hz, 1H, CH), 7.58 (*dd*, *J* = 7.8, 1.8 Hz, 1 arom. H), 7.49 (*dd*, *J* = 7.6, 1.3 Hz, 1 arom. H), 7.46 (*dt*, *J* = 7.7, 1.6 Hz, 1 arom. H), 7.41 (*d*, *J* = 7.7 Hz, 1 arom. H), 7.39 (*dd*, *J* = 3.5, 0.9 Hz, 1H, CH), 7.34 (*dd*, *J* = 8.0, 1.3 Hz, 1 arom. H), 7.17 (*s*, 1 arom. H), 6.67 (*dd*, *J* = 3.6, 1.8 Hz, 1H, CH), 5.37 (*s*, 1H, OH). ^{13}C NMR (100 MHz, CD_2Cl_2-d_2): δ_C 161.1, 158.8, 154.1, 148.9, 144.9, 139.9, 133.8, 132.9, 132.0, 131.96, 131.92, 131.5, 128.0, 127.8, 124.7, 123.7, 120.9, 117.4, 115.3, 113.5. MS (ESI, MeOH): ($C_{23}H_{14}BrN_3O_3$) $[M+H]^+$ found: 460.0774, calcd.: 460.0219.

4.1.30. *N*-(3-cyano-4-(3-fluorophenyl)-6-phenylpyridin-2-yl)furan-2-carboxamide (13c)



N-(3-cyano-4-(3-fluorophenyl)-6-phenylpyridin-2-yl)furan-2-carboxamide **13c** was prepared from 2-amino-4-(3-fluorophenyl)-6-phenylnicotinonitrile **8h** (50 mg, 0.17 mmol, 1.0 equiv.) and 2-furoyl chloride **12a** (34 mg, 0.26 mmol, 1.5 equiv.) in a manner similar to that described for **13a** (*Procedure E)* as a white solid in 69 % (45.6 mg) yield. mp. 215 °C. ν_{max} (ATR)/ cm^{-1}: 3335*m* (N-H), 2221*w* (C≡N), 1674*s* (2° amide, C=O), 1589*m* (N-H), 1170*m* (Ph-F), 1074*m* (amine, C-N). ^{1}H NMR (400 MHz, CD_2Cl_2-d_2): δ_H 8.86 (*s*, 1H, NH), 8.23 – 8.03 (*m*, 2 arom. H), 7.75 (*s*, 1 arom. H), 7.66 (*d*, *J* = 1.7 Hz, 1H, CH), 7.61 – 7.56 (*m*, 1 arom. H), 7.53 (*m*, *J* = 6.2, 4.0 Hz, 4 arom. H), 7.42 (*dt*, *J* = 9.4, 2.1 Hz, 1 arom. H), 7.38 (*d*, *J* = 3.5 Hz, 1H, CH), 7.29 (*tdd*, *J* = 8.3, 2.6, 1.2 Hz, 1 arom. H), 6.66 (*dd*, *J* = 3.5, 1.8 Hz, 1H, CH). ^{13}C NMR (100 MHz, CD_2Cl_2-d_2): δ_C 160.0, 155.9, 154.7, 152.8, 147.3, 146.1, 138.8; 137.3, 133.6, 132.1, 131.4, 131.1, 129.6, 128.1, 128.0, 123.4, 117.7, 117.5, 115.8, 113.4, 100.7. MS (ESI, MeOH): ($C_{23}H_{14}FN_3O_2$) $[M+H]^+$ found: 384.1496, calcd.: 384.1070.

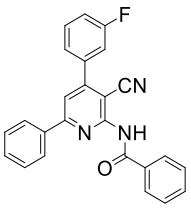
4.1.31. *N*-(3-cyano-4,6-diphenylpyridin-2-yl)benzamide (13d)



An attempt to prepare *N*-(3-cyano-4,6-diphenylpyridin-2-yl)benzamide **13d** proceeded in accordance with the following methodology: within a 25 mL, nitrogen purged, one-necked round bottom flask, 2-amino-4,6-diphenylnicotinonitrile **8a** (150

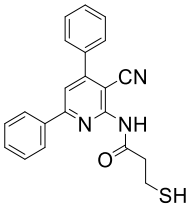
mg, 0.55 mmol, 1.0 equiv.), benzoic acid **12b** (0.61 mmol, 74 mg, 1.1 equiv.), HATU (0.61 mmol, 231 mg, 1.1 equiv.), and pyridine (218 mg, 2.77 mmol, 5.0 equiv.) were dissolved in DMF and stirred for 24 h. Starting materials were returned.

4.1.32. *N*-(3-cyano-4-(3-fluorophenyl)-6-phenylpyridin-2-yl)benzamide (13e)



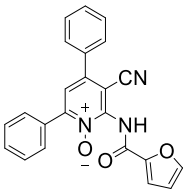
An attempt to prepare ***N***-(3-cyano-4-(3-fluorophenyl)-6-phenylpyridin-2-yl)benzamide **13e** proceeded in accordance with the following methodology: within a 25 mL, nitrogen purged, one-necked round bottom flask, 2-amino-4-(3-fluorophenyl)-6-phenylnicotinonitrile **8h** (159 mg, 0.55 mmol, 1.0 equiv.), benzoic acid **12b** (0.61 mmol, 74 mg, 1.1 equiv.), HATU (0.61 mmol, 231 mg, 1.1 equiv.), and pyridine (218 mg, 2.77 mmol, 5.0 equiv.) were dissolved in DMF and stirred for 24 h. Starting materials were returned.

4.1.33. *N*-(3-cyano-4,6-diphenylpyridin-2-yl)-2-mercaptoacetamide (13f)



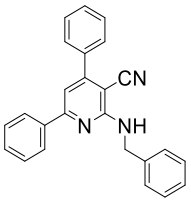
An attempt to prepare *N*-(3-cyano-4,6-diphenylpyridin-2-yl)-2-mercaptoacetamide **13f** proceeded in accordance with the following methodology: within a 25 mL, nitrogen purged, one-necked round bottom flask, 2-amino-4,6-diphenylnicotinonitrile **8a** (85 mg, 0.31 mmol, 1.0 equiv.), thioglycolic acid **12c** (0.35 mmol, 33 mg, 1.1 equiv.), HATU (0.35 mmol, 131 mg, 1.1 equiv.), and pyridine (125 mg, 1.57 mmol, 5.0 equiv.) were dissolved in DMF and stirred for 24 h. Starting materials returned.

4.1.34. 3-Cyano-2-(furan-2-carboxamido)-4,6-diphenylpyridine 1-oxide (14a)



To an oven dried, nitrogen purged, ice-cooled 25 mL one-necked round bottom flask, 2-furoyl chloride **12a** (55 mg, 0.42 mmol, 1.0 equiv.) and triethylamine (63 mg, 0.63 mmol, 1.5 equiv.) were added to a solution of 2-amino-3-cyano-4,6-diphenylpyridine-1-oxide **10a** (120 mg, 0.40 mmol, 1.0 equiv.) in 5 mL of DCM. The solution was warmed to room temperature and magnetically stirred for 48 h. After the solvent had been evaporated, under reduced pressure, the crude residue was diluted with EtOAc, washed with brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography (gradient elution, EtOAc/hexane = 1:9 to 1:1) to afford crude 3-cyano-2-(furan-2-carboxamido)-4,6-diphenylpyridine 1-oxide **14a,** as a white solid, in 10 % (16 mg) yield. mp. 215 - 220 °C. ^{1}H NMR (400 MHz, $CDCl_3$-*d*) δ_H 8.08 (*t*, *J* = 1.8, 2H), 7.99 (*dt*, *J* = 7.8, 1.3, 2H), 7.89 – 7.85 (*m*, 1H), 7.74 – 7.70 (*m*, 1H), 7.64 – 7.56 (*m*, 3H), 7.54 (*tt*, *J* = 4.3, 2.6, 3H), 7.44 (*d*, *J* = 0.6, 1H). ^{13}C NMR (100 MHz, $CDCl_3$-*d*): δ_C 170.4, 155.1, 150.9, 144.1, 134.7, 133.8, 131.0, 131.0, 130.4, 130.2, 129.8, 129.4, 129.2, 128.8, 128.6, 128.3, 121.9, 118.4, 113.0. MS (ESI, MeOH): ($C_{23}H_{15}N_3O_3$) $[M+H]^+$ found: 382.1447, calcd.: 382.1192.

4.1.35. 2-(Benzylamino)-4,6-diphenylnicotinonitrile (15a)



An attempt to prepare 2-(benzylamino)-4,6-diphenylnicotinonitrile **18c** proceeded in accordance with the following methodologies:

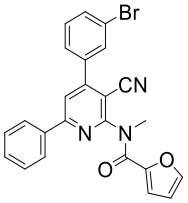
Procedure A: Within a 25 mL oven-dried, nitrogen purged, one-necked round bottom flask, benzyl bromide (32 mg, 0.18, 1.0 equiv.) was added to a solution of 2-amino-4,6-diphenyl-pyridine-3-carbonitrile **13a** (50 mg, 0.18 mmol, 1.0 equiv.) and triethylamine (28 mg, 0.28 mmol, 1.5 equiv.) in 4 mL of dry DCM. The reaction was stirred at room temperature for 24 h. The reaction was monitored *via* TLC (EtOAc/ hexane = 3:7) and, upon diagnosis, it was observed that no further spots had emerged. Starting materials were returned.

Procedure B: Within a 25 mL oven-dried, nitrogen purged, one-necked round bottom flask, benzaldehyde (106 mg, 0.18 mmol, 1.0 equiv.) was added to a solution of 2-amino-4,6-diphenyl-pyridine-3-carbonitrile **13a** (50 mg, 0.18 mmol, 1.0 equiv.) and $NaBH_4$ (11 mg, 0.28 mmol, 1.5 equiv.) in 10 mL of dry THF. The reaction was stirred at room temperature for 48 h. The reaction was monitored *via* TLC (EtOAc/ hexane = 3:7) and, upon diagnosis, it was observed that no further spots had emerged. Starting materials were returned.

4.1.36. General procedure for the synthesis of 16a and 16b

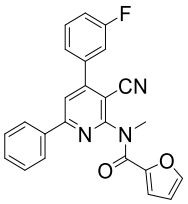
Within an oven dried, nitrogen purged, 25 mL one-necked round bottom flask KOtBu was added to an ice-cooled solution of compound **13a** or **1** in 5 mL of dry THF. Iodomethane was added to the reaction solution and the whole was vigorously stirred at room temperature until the reaction as complete (24 h). The solvent was removed, under reduced pressure, and the crude residue was partitioned between distilled water (25 mL) and DCM (25 mL). The organic layer was washed with brine (25 mL), dried over $MgSO_4$ and concentrated under reduced pressure.

4.1.37. *N*-(4-(3-bromophenyl)-3-cyano-6-phenylpyridin-2-yl)-*N*-methylfuran-2-carboxamide (16a)



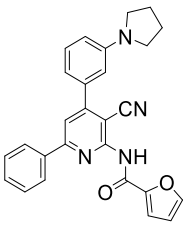
N-(3-cyano-4-(3-bromophenyl)-6-phenylpyridin-2-yl)-*N*-methylfuran-2-carboxamide **16a** was prepared from *N*-[3-cyano-4-(3-bromophenyl)-6-phenyl-2-pyridyl]furan-2-carboxamide **1** (50mg, 0.12 mmol, 1 equiv.), KOtBu (40 mg, 0.35 mmol, 1.5 equiv) and iodomethane (25 mg, 0.17 mmol, 1.5 equiv.), in accordance with the general procedure, as a white solid in 95 % (50.0 mg) yield. mp. 122 °C. IR (KBr, ν_{max}, cm^{-1}): 2926*m* (N-H), 2223*w* (C≡N), 1653*s* (3° amide, C=O), 1373*s* (arom. C-N), 1329*s* (arom. C-N), 1230*m* (C-N), 1179*m* (C-N), 690*s* (Ph-Br). ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 7.97 (*dd*, *J* = 6.6, 2.8 Hz, 2 arom. H), 7.74 (*d*, *J* = 10.4 Hz, 2 arom. H), 7.68 (*d*, *J* = 9.7 Hz, 1H), 7.56 (*d*, *J* = 7.8 Hz, 1H), 7.49 (*d*, *J* = 3.2 Hz, 3H), 7.42 (*t*, *J* = 7.9 Hz, 1H), 7.24 (*d*, *J* = 1.9 Hz, 1H), 7.02 (*d*, *J* = 3.5 Hz, 1H, CH), 6.41 (*dd*, *J* = 3.6, 1.7 Hz, 1H, CH), 3.69 (*s*, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$-*d*): δ_C 160.2, 159.9, 159.7, 154.6, 148.0, 144.5, 138.0, 136.7, 133.6, 131.6, 131.4, 130.9, 129.4, 127.7, 127.5, 123.5, 118.5, 117.9, 115.1, 112.0, 103.8, 36.7. MS (ESI, MeCN): ($C_{24}H_{16}BrN_3O_2$) $[M+H]^+$ found: 458.0631, calcd: 458.0426.

4.1.38. *N*-(4-(3-fluorophenyl)-3-cyano-6-phenylpyridin-2-yl)-*N*-methylfuran-2-carboxamide (16b)



N-(4-(3-fluorophenyl)-3-cyano-6-phenylpyridin-2-yl)-*N*-methylfuran-2-carboxamide **16b** was prepared from *N*-[3-cyano-4-(3-fluorophenyl)-6-phenyl-2-pyridyl]furan-2-carboxamide **13c** and (25 mg, 0.17 mmol, 2.0 equiv.) in accordance with the general procedure, as a white solid in 95 % (52.2 mg) yield. mp. 120 °C. IR (KBr, ν_{max}, cm^{-1}): 2926*m* (N-H), 2223*w* (C≡N), 1653*s* (3° amide, C=O), 1230*m* (C-N), 1179*m* (C-N), 1373*s* (arom. C-N), 1329*s* (arom. C-N), 763*s*. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 8.00 (*dd*, *J* = 6.8, 3.0 Hz, 2 arom. H), 7.80 (*s*, 1 arom. H), 7.60 – 7.47 (*m*, 4 arom. H), 7.42 (*dt*, *J* = 7.7, 1.3 Hz, 1 arom. H), 7.33 (*dt*, *J* = 9.2, 2.1 Hz, 1 arom. H), 7.29 – 7.20 (*m*, 2H), 7.01 (*d*, *J* = 3.6 Hz, 1H, CH), 6.42 (*dd*, *J* = 3.6, 1.8 Hz, 1H, CH), 3.70 (s, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$-*d*): δ_C 164.1, 161.6, 159.9, 159.7, 159.5, 154.6, 147.8, 144.7, 137.8, 136.5, 131.1, 129.1, 127.5, 124.4, 118.6, 118.4, 117.7, 117.5, 115.8, 115.6, 115.0, 111.7, 103.6, 36.5. MS (ESI, MeCN): ($C_{24}H_{16}FN_3O_2$) $[M+H]^+$ found: 398.1701, calcd.: 398.1227.

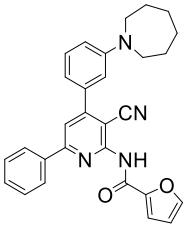
4.1.39. *N*-(3-cyano-6-phenyl-4-(3-(pyrrolidin-1-yl)phenyl)pyridin-2-yl)furan-2-carboxamide (18a)



An attempt to prepare *N*-(3-cyano-6-phenyl-4-(3-(pyrrolidin-1-yl)phenyl)pyridin-2-yl)furan-2-carboxamide **18a** proceeded in accordance with the following methodologies:

To an oven dried, nitrogen purged, 10 mL one-necked round bottom flask, 3 mol% of *S*-BINAP and 2 mol% of $Pd_2(dba)_3$ were pre-heated at 80 °C and stirred in 4 mL of toluene for 1 h. Pyrrolidine (20 mg, 0.28 mmol, 2.5 equiv.) and Cs_2CO_3 (56 mg, 0.16 mmol, 1.4 equiv.) were added and the reaction solution was heated at 80 °C for a further 15 mins. Pyrrolidine was distilled over KOH, prior to use. Lastly, *N*-(4-(3-bromophenyl)-3-cyano-6-phenylpyridin-2-yl)furan-2-carboxamide **1** (50 mg, 0.11, 1.0 equiv.), was added and the whole was refluxed and stirred for 48 h. Upon TLC analysis (EtOAc/ hexane = 3:7), it was noted that no further spots had emerged. Starting materials were returned.

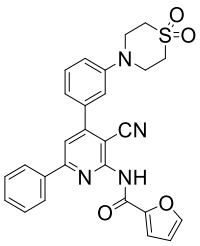
4.1.40. *N*-(4-(3-(azepan-1-yl)phenyl)-3-cyano-6-phenylpyridin-2-yl)furan-2-carboxamide (18b)



An attempt to prepare *N*-(4-(3-(azepan-1-yl)phenyl)-3-cyano-6-phenylpyridin-2-yl)furan-2-carboxamide **18b** proceeded in accordance with the following methodology: to an oven dried, nitrogen purged, 10 mL one-necked round bottom flask, 2 mol% of $Pd(PPh_3)_4$ was pre-heated at 80 °C and stirred in 5 mL of toluene for 0.5 h. Hexamethyleneimine (16 mg, 0.11 mmol, 1.2 equiv.), Na_2CO_3 (15 mg, 0.14 mmol, 1.6 equiv.) and KOtBu (16 mg, 0.14 mmol, 1.6 equiv.) were added and the reaction solution was heated at 80 °C for a further 15 mins. Lastly, *N*-(4-(3-bromophenyl)-3-cyano-6-phenylpyridin-2-yl)furan-2-

carboxamide **1** (40 mg, 0.09 mmol, 1.0 equiv.) was added and the reaction mixture was refluxed and stirred for 24 h. Upon TLC analysis (EtOAc/ hexane = 3:7), it was noted that no further spots had emerged. Starting materials were returned.

4.1.41. *N*-(3-cyano-4-(3-(1,1-dioxidothiomorpholino)phenyl)-6-phenylpyridin-2-yl)furan-2-carboxamide (18c)



An attempt to prepare *N*-(3-cyano-4-(3-(1,1-dioxidothiomorpholino)phenyl)-6-phenylpyridin-2-yl)furan-2-carboxamide **18c** proceeded in accordance with the following methodologies:

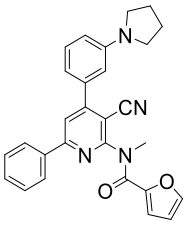
Procedure A: To a 5 mL microwave pressure vial, thiomorpholine 1,1-dioxide, Cs_2CO_3 (47 mg, 0.14, 2.0 equiv.) and $Pd(OAc)_2$ (5 mol%) were added to a solution of *N*-(4-(3-bromophenyl)-3-cyano-6-phenylpyridin-2-yl)furan-2-carboxamide **1** (53 mg, 0.12 mmol, 1.0 equiv.) in 2.0 mL of pyridine. The reaction vial was capped before being subjected to microwave mediated conditions (5 h, 160 °C). After cooling, the solvent was removed under high vacuum. The crude residue was partitioned between distilled water and DCM, dried over $MgSO_4$, and concentrated under reduced pressure. Upon TLC analysis (EtOAc/ hexane = 3:7), it was noted that no further spots had emerged. Starting materials were returned.

Procedure B: To an oven dried, nitrogen purged, 10 mL one-necked round bottom flask, 5 mol% of $Pd(OAc)_2$ thiomorpholine-1,1-dioxide (16 mg, 0.11 mmol, 1.2 equiv.) and KOtBu (16 mg, 0.14 mmol, 1.6 equiv.), were added to a solution of *N*-(4-(3-bromophenyl)-3-cyano-6-phenylpyridin-2-yl)furan-2-carboxamide **1** (40 mg, 0.09 mmol, 1.0 equiv.) in 4 mL of toluene. The reaction mixture was refluxed and stirred for 24 h. Upon TLC analysis (EtOAc/ hexane = 3:7), it was noted that no further spots had emerged. Starting materials were returned.

Procedure C: To an oven dried, nitrogen purged, 10 mL one-necked round bottom flask, 5 mol% of $Pd(OAc)_2$ was pre-heated at 80 °C and stirred in 4 mL of 1,4-dioxane for 0.5 h. Subsequently, thiomorpholine 1,1-dioxide (16 mg, 0.11 mmol, 1.2 equiv.), and KOtBu (16 mg, 0.14 mmol, 1.5 equiv.) were added and the reaction solution was heated at 80 °C for a further 15 mins. Lastly, *N*-(4-(3-bromophenyl)-3-cyano-6-phenylpyridin-2-yl)furan-2-carboxamide **1** (40 mg, 0.09 mmol, 1.0 equiv.) was added and the whole was refluxed and stirred for 24 h. Upon TLC analysis (EtOAc/ hexane = 3:7), it was noted that no further spots had emerged. Starting materials were returned.

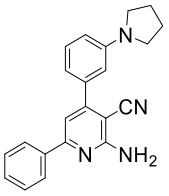
Procedure D: To an oven dried, nitrogen purged, 10 mL one-necked round bottom flask, 3 mol% of *S*-BINAP and 2 mol% of $Pd(OAc)_2$ was pre-heated at 80 °C and stirred in 4 mL of toluene for 1 h. Thiomorpholine 1,1-dioxide (16 mg, 0.11 mmol, 1.2 equiv.), Na_2CO_3 (15 mg, 0.14 mmol, 1.6 equiv.) and KOtBu (16 mg, 0.14 mmol, 1.6 equiv.) were added and the reaction solution was heated at 80 °C for a further 15 mins. Lastly, *N*-(4-(3-bromophenyl)-3-cyano-6-phenylpyridin-2-yl)furan-2-carboxamide **1** (40 mg, 0.09 mmol, 1.0 equiv.) was added and the whole was refluxed and stirred for 24 h. Upon TLC analysis (EtOAc/ hexane = 3:7), it was noted that no further spots had emerged. Starting materials were returned.

4.1.42. *N*-(3-cyano-6-phenyl-4-(3-(pyrrolidin-1-yl)phenyl)pyridin-2-yl)-*N*-methylfuran-2-carboxamide (19a)



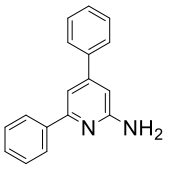
An attempt to prepare *N*-(3-cyano-6-phenyl-4-(3-(pyrrolidin-1-yl)phenyl)pyridin-2-yl)-*N*-methylfuran-2-carboxamide proceeded in accordance with the following methodology: within a 25 mL oven dried, nitrogen purged, ice-cooled, one-necked round bottom flask, a 2.5 M solution of butyllithium in hexane (1.2 equiv.) was added dropwise to a mixture of *N*-(4-(3-fluorophenyl)-3-cyano-6-phenylpyridin-2-yl)-*N*-methylfuran-2-carboxamide **16b** (50 mg, 0.13 mmol, 1.0 equiv.) and pyrrolidine (1.2 equiv.) in 10 mL of THF. Pyrrolidine was distilled over KOH, prior to use. The reaction mixture was allowed to warm to room temperature before being stirred vigorously and subsequently quenched with sat. NH_4Cl (aq.) after 48 h. Upon TLC analysis (EtOAc/ hexane = 3:7), it was noted that no further spots had emerged. Starting materials were returned.

4.1.43. 2-Amino-6-phenyl-4-(3-(pyrrolidin-1-yl)phenyl)nicotinonitrile (20a)



An attempt to prepare 2-amino-6-phenyl-4-(3-(pyrrolidin-1-yl)phenyl)nicotinonitrile **20a** proceeded in accordance with the following methodology: to an oven dried, nitrogen purged, 25 mL one-necked round bottom flask, 2-amino-4-(3-bromophenyl)-6-phenylnicotinonitrile **8f** (100 mg, 0.28 mmol, 1.0 equiv.), CuI (37 mg, 0.19 mmol, 0.7 equiv.), K_3PO_4 (121 mg, 0.57 mmol, 2.0 equiv.), and pyrrolidine (22 mg, 0.31 mmol, 1.1 equiv.) were added to a solution of ethylene glycol (35 mg, 0.57 mmol, 2.0 equiv.) in 10 mL of propan-2-ol. The reaction mixture was stirred at room temperature for 24 h. Upon TLC analysis (EtOAc/ hexane = 3:7), it was noted that no further spots had emerged. Starting materials were returned.

4.1.44. 4,6-Diphenylpyridin-2-amine (21a)



An attempt to prepare 4,6-diphenylpyridin-2-amine **21a** proceeded in accordance with the following methodology: to an oven dried, nitrogen purged, 50 mL one-necked round rottom flask, a solution of 1-(cyanomethyl)pyridin-1-ium **6a** (100 mg, 0.65 mmol, 1.2 equiv.), acetophenone (65 mg, 0.54 mmol, 1.0 equiv.), benzaldehyde (57 mg, 0.54 mmol, 1.0 equiv.), ammonium acetate (33 mg, 0.43 mmol, 0.8 equiv.) and 1.7 mL of AcOH were refluxed for 48 h. Upon TLC analysis (EtOAc/ hexane = 3:7), it was noted that no further spots had emerged. Starting materials were returned.

4.2. KISS1R functional assay – antagonist effect

Eurofins, Cerep: GPCR functional assay (Catalogue ref. G275-4271).[34] Rat basophil leukemia cells were suspended in HBSS buffer (Invitrogen) complemented with 20 mM Hepes, then distributed in microplates at a density of $1x10^6$ cells/well. The fluorescent probe (Fluo8 Direct, AAT Bioquest) mixed with probenicid in HBSS buffer (Invitrogen) complemented with 20 mM Hepes (Invitrogen; pH 7.4) was then added into each well and equilibrated with the cells for 60 min at 30°C. Thereafter,

the assay plates were positioned within a microplate reader (FlipR Tetra, Molecular Device) which was used for the addition of test compounds **1**, **2** or HBSS buffer (basal control), then 5 min later 10 nM Metastin (45-54), and the measurements of changes in fluorescence intensity which varies proportionally to the free cytosolic Ca^{2+} ion concentration. The results are expressed as a percent inhibition of the control response to Metastin (45-54) at 10 nM. Test compounds were dissolved in DMSO (concentration: 10 mM) with a testing top concentration of 10 µM (log1/2 dilution).

4.3. Tissue culture

Human breast carcinoma cells, MDA-MB-231 and MCF-7 cells lines were supplied by Prof. Giamas, Giamas Lab, University of Sussex and cultured in high glucose, DMEM (Sigma-Aldrich) supplemented with 10 % FBS (Sigma-Aldrich), 2 mM L-glutamine, and 5 % penicillin-streptomycin (Sigma-Aldrich). Cells were grown under sterile conditions in a humidified incubator at at 37 °C and 5 % CO_2. Cells were maintained in culture for no more than 24 passages.

4.3.1. Incucyte, P-234 Cell Proliferation Assay

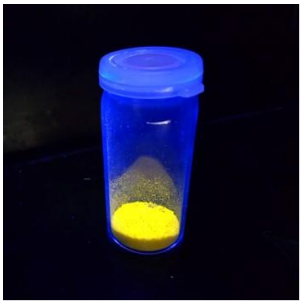
For WST-1 cell proliferation assays 2.5 x 10^3 MCF-7 and MDA-MB-231 cells were seeded in 96-well culture plates (Sigma-Aldrich) overnight (12 h) prior to the addition of P-234 (Tocris) in 200 µL/ well volume: 0 nM, 10 nM, 100 nM. of P-234 (Tocris). Plates were incubated in an Incucyte ZOOM (Essen Bioscience) for 48 h with images captured ever 4 h. By quantitative kinetic processing, from time-lapse image acquisition, the confluency (%) of the cell cultures were obtained and measured against time.

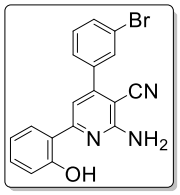
4.3.2. WST-1, P-234 Cell Proliferation Assay

For WST-1 cell proliferation assays 2.5 x 10^3 MCF-7 and MDA-MB-231 cells were seeded in 96-well culture plates (Sigma-Aldrich) overnight (12 h) prior to the addition of P-234 (Tocris) in 200 µL/ well volume: 0 nM, 10 nM, 100 nM, and 1000 nM. After 24 h, 48 h and 72 h, 10 µL/well of cell proliferation reagent WST-1 (RocheDiagnostics GmbH) was added and the cells were incubated for 1 h, according to manufacturer's instructions. The absorbance of each sample was then determined using an infinite plate reader (Tecan) at 440 nm. The absorbance of the samples against a background control were recorded.

5. 2-Aminopyridine-3-carbonitrile Fluorescent Turn-Off Chemosensor

Compounds **8h**, **8i**, and **8k**, bearing a 2'-hydroxy moiety on their 4-phenyl ring presented an intense fluorescence when subjected with UV (365 nm), as shown in Figure 12. The design and synthesis of small pyridine-based molecules, that exhibit fluorescence, are a prime focus of current research in the field of chemosensors aimed at metal iron coordination.[63,64] It was reasoned that the intense yellow fluorescence, of **8k**, was due to the electron-donating properties of the hydroxy and amino groups, in synergy with a large conjugated system.





8k

Figure 12. i) Picture taken of compound **8k** when illuminated under UV lamp (365 nm). ii) Structure of compound **8k**.

Spectrofluorimetric quenching of compound **8** with an analyte (i.e., Fe^{3+} or Fe^{2+}) could afford a new generation of iron coordinating chemosensors. Compound **8i** was dissolved in MeOH and preliminary studies indicated that its fluorescence was quenched with either Fe^{3+} or Fe^{2+} ions; data not showed. An absorbance spectrum of aforesaid ion solution established **8i** and Fe^{3+}/Fe^{2+} coordination. The 2'hydroxy functionalised substrates constitute interesting building blocks for further work towards a potent chemosensor. Further work towards developing a fluorescent turn-off chemosensor is underway and will be reported on in due course.

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