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Discovery of the cancer cell selective dual acting anti-cancer agent (*Z*)-2-(1*H*-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (A131)

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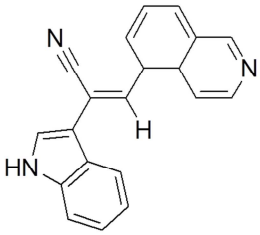
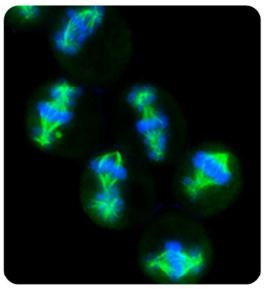


- Mitotic dysfunction in cancer cells
- Cancer cell selective cell death
- 74% tumor growth inhibition in mouse model of colon cancer

4a (A131)
Dual mechanism of action targeting mitosis and autophagy



Mitotic arrest, disorganized spindles, misaligned chromosomes in HeLa cells treated with **4a**

Discovery of the Cancer Cell Selective Dual Acting Anti-Cancer Agent (*Z*)-2-(1*H*-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (A131)

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We dedicate this work to the memory of Professor Sang Hyun Lee.

Running Title: Discovery of the Cancer Cell Selective Dual Inhibitor A131

Keywords: Antitumor; Antimitotic; Antiproliferative; Tumor-selective

Abstract

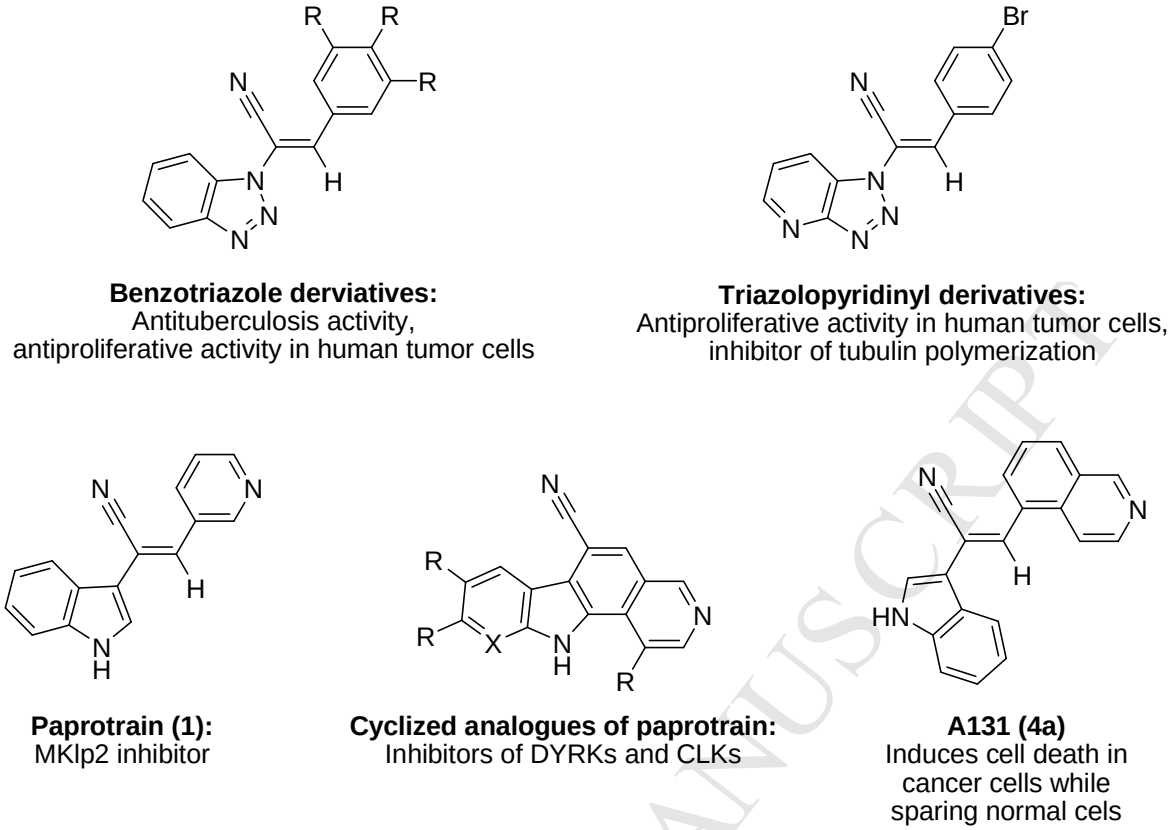
Selective targeting of cancer cells over normal cells is a key objective of targeted therapy. However few approaches achieve true mechanistic selectivity resulting in debilitating side effects and dose limitation. In this work we describe the discovery of A131 (**4a**), a new agent with an unprecedented dual mechanism of action targeting both mitosis and autophagy. Compound **4a** was first identified in a phenotypic screen in which HeLa cells treated with **4a** manifested mitotic arrest along with formation of multiple vesicles. Further investigations showed that **4a** causes an increase in mitotic marker pH3 and autophagy marker LC3. Importantly **4a** induces cell death in cancer cells while sparing normal cells which regrow after **4a** is removed. Dual activities against pH3 and LC3 markers are required for cancer cell selectivity. An extensive SAR investigation confirmed **4a** as the optimal dual inhibitor with potency against a panel of 30 cancer cell lines (average antiproliferative GI_{50} 1.5 μM). In a mouse model of paclitaxel-resistant colon cancer, **4a** showed 74% tumor growth inhibition when administered at a dose of 20 mg/kg IP twice a day.

Introduction

Success in the treatment of cancer is increased if selectivity between cancer cells and normal cells can be achieved. Agents which kill cancer cells while sparing normal cells can be given at higher doses leading to improved efficacy while balancing side effects. However it has been very challenging to find agents which have fundamentally differentiated mechanisms between cancer and normal cells [1].

Several compounds bearing an arylacrylonitrile structure have been shown to exhibit a range of anticancer biological activities (Fig. 1). A series of benzotriazole-acrylonitriles demonstrated antituberculosis activity as well as antiproliferative activity in human tumor cells [2-4]. Closely related triazolopyridinyl derivatives were subsequently found to be antimicrotubule agents [5]. In recent years, indoleacrylonitriles such as Paprotrain (**1**) were reported to be inhibitors of the kinesin MKlp2 [6-8]. Cyclization of paprotrain derivatives using photochemistry led to fused tricyclic compounds that showed inhibitory activities on kinases involved in neurodegenerative disorders [9].

We started our work studying **1**, an inhibitor of the kinesin MKlp2 which induces a unique cellular phenotype of binucleation [6]. We synthesised the novel analogue **4a** which was found to have unusual phenotypic properties in cancer cells but not in normal cells. Hence we embarked upon a detailed study of the chemistry and biology of **4a**. Herein we discuss the discovery of **4a**, subsequent SAR studies and characterisation in cells and in a mouse model of drug resistant cancer.



Benzotriazole derviatives:
Antituberculosis activity,
antiproliferative activity in human tumor cells

Triazolopyridinyl derivatives:
Antiproliferative activity in human tumor cells,
inhibitor of tubulin polymerization

Paprotrain (1):
MKlp2 inhibitor

Cyclized analogues of paprotrain:
Inhibitors of DYRKs and CLKs

A131 (4a)
Induces cell death in
cancer cells while
sparing normal cels

Fig. 1. Examples of arylacrylonitriles with biological activities.

Identification of compound 4a from phenotypic screening

We performed a phenotypic screen of a small in-house library of derivatives of **1** in HeLa cells, monitoring for visible perturbations to mitosis. One compound, **4a** (Scheme 1), presented a unique and interesting phenotype. In HeLa cells treated with **4a**, prolonged mitotic arrest was observed. At the same time, distinct large vacuole-like structures that filled out the cytoplasmic area were also visible using a high resolution microscope (Fig. 2).

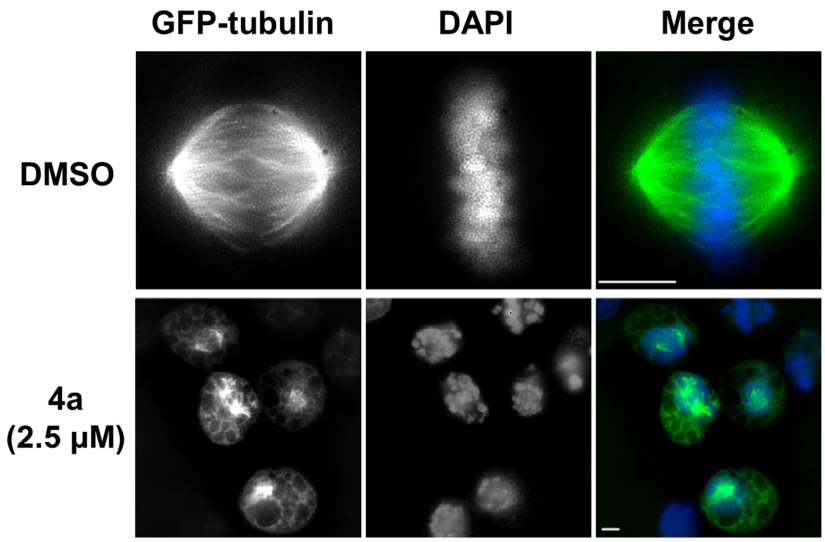


Fig. 2. HeLa cells stably expressing GFP-tubulin were treated with DMSO and **4a** for 20 h. White scale bar represents 5 μm.

Treatment with chloroquine, an effective late-stage inhibitor of autophagy *in vitro*, has been known to result in an accumulation of double-membraned structures called autophagosomes which can be observed using microscopy [10]. These structures contain cytoplasmic material that will be digested upon fusion with lysosomes [11]. To investigate if the vacuole-like structures induced by treatment with **4a** were related to autophagic vesicles, the levels of microtubule-associated protein light chain 3 (LC3) in **4a**-treated cells were evaluated by immunoblot. LC3 is a widely used marker for autophagy and it exists as two forms: LC3-I which is found in the cytosol and LC3-II which is conjugated with phosphatidylethanolamine and localized in the inner and outer membranes of the autophagosome [12, 13]. The amount of LC3-II is correlated with the number of autophagosomes [12].

As shown in Fig. 3, a concentration dependent increase in LC3-II levels without any significant changes to LC3-I levels was seen when HeLa cells were treated with increasing doses of **4a** for 6 h. Such effects were identical to that seen with chloroquine, however, compared to chloroquine, **4a** appears to be 4 to 10 times more potent as an inhibitor of

lysosome function, determined by the relative intensity ratio of LC3-II to LC-I compared to DMSO-treated control. Consistent with these findings, immunofluorescence imaging using antibodies against LC3 saw an increase in LC3 puncta formation following treatment with **4a**, but not with DMSO control (Fig. 4).

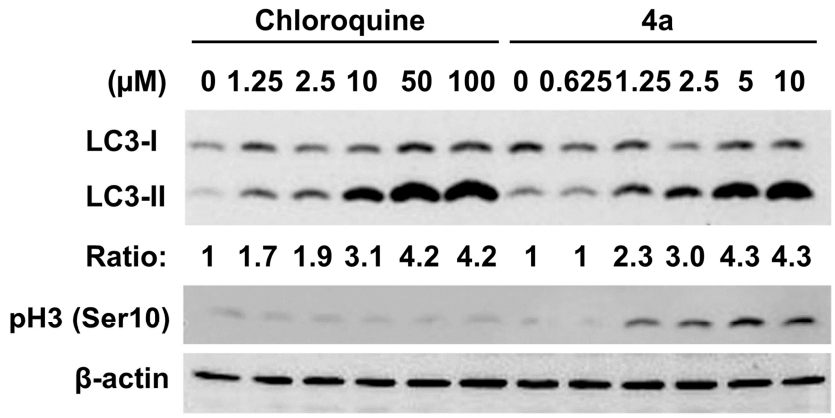


Fig. 3. Immunoblot of LC3 and pH3(Ser10) in HeLa cells following treatment with chloroquine and **4a** for 6 h. The relative band intensity ratios of LC3-II to LC3-I as compared to DMSO control (lane 1, 0 μM chloroquine and lane 7, 0 μM **4a**) are indicated.

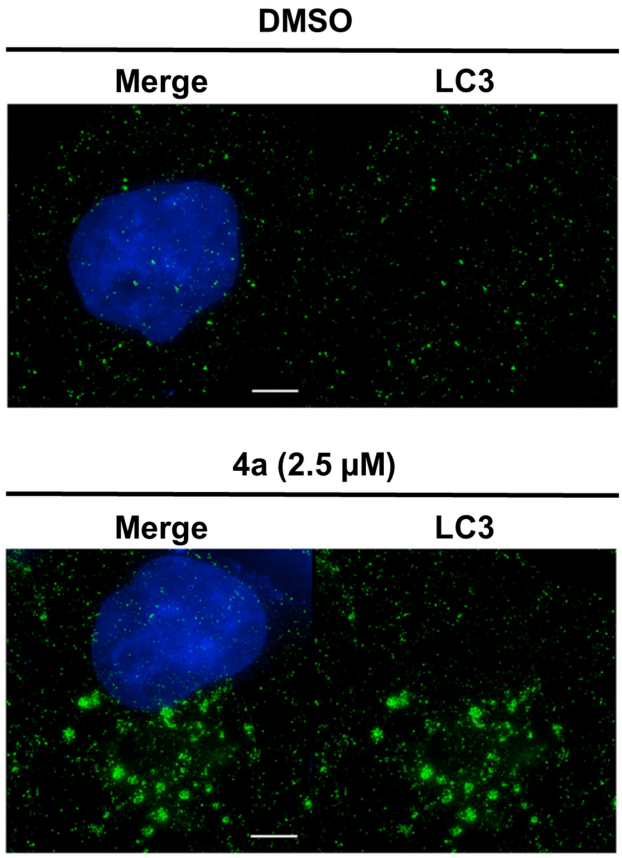


Fig. 4. Immunofluorescence analysis of HeLa cells following treatment with DMSO and **4a** for 6 h using antibodies against LC3 and counterstaining with DAPI to visualize the nucleus. Increased puncta formation is indicative of increase in LC3-II levels. White scale bar represent 5 μm.

Histone H3 phosphorylated at serine 10 (pH3(Ser10)) levels were also evaluated by immunoblot of **4a**-treated HeLa cells (Fig. 3, lower panel). Accumulation of pH3 was seen in a concentration-dependent manner with **4a** but not with chloroquine, signifying that **4a** has the ability to impede mitotic progression in HeLa cells while chloroquine does not. Indeed, examination of the mitotic spindles with immunofluorescence microscopy revealed that spindle defects were not observed with chloroquine treatment, only HeLa cells treated with **4a** were arrested in mitosis with grossly misaligned chromosomes and disorganized mitotic spindles (Fig. 5).

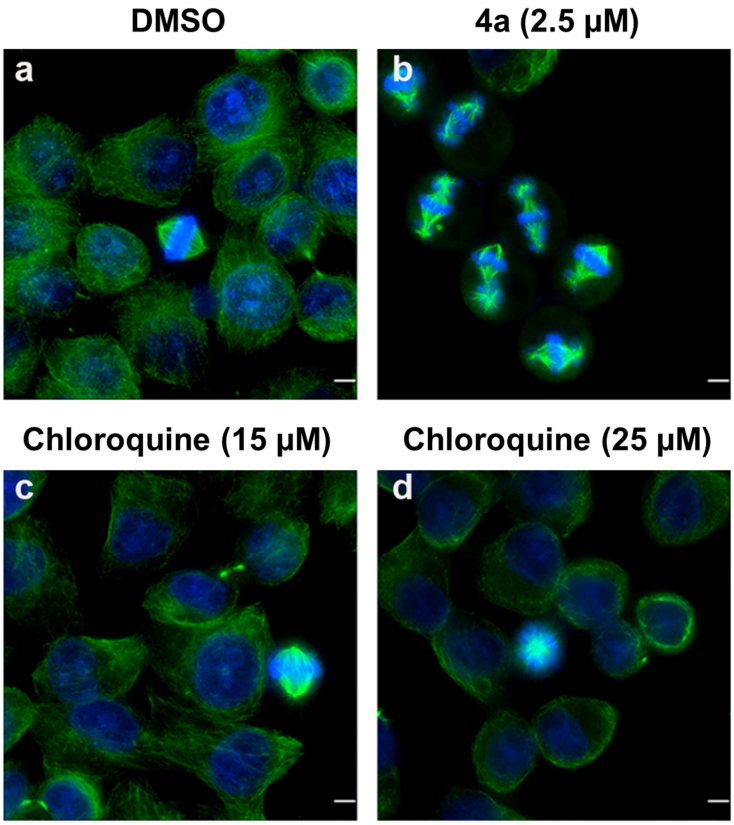


Fig. 5. Immunofluorescence analysis of HeLa cells following treatment with DMSO, chloroquine and **4a** for 6 h using antibodies against β-tubulin and counterstaining with DAPI to visualize the chromosomes. Only **4a**-treated HeLa cells arrested in mitosis with disorganized spindles and misaligned chromosomes. White scale bar represents 5 μm.

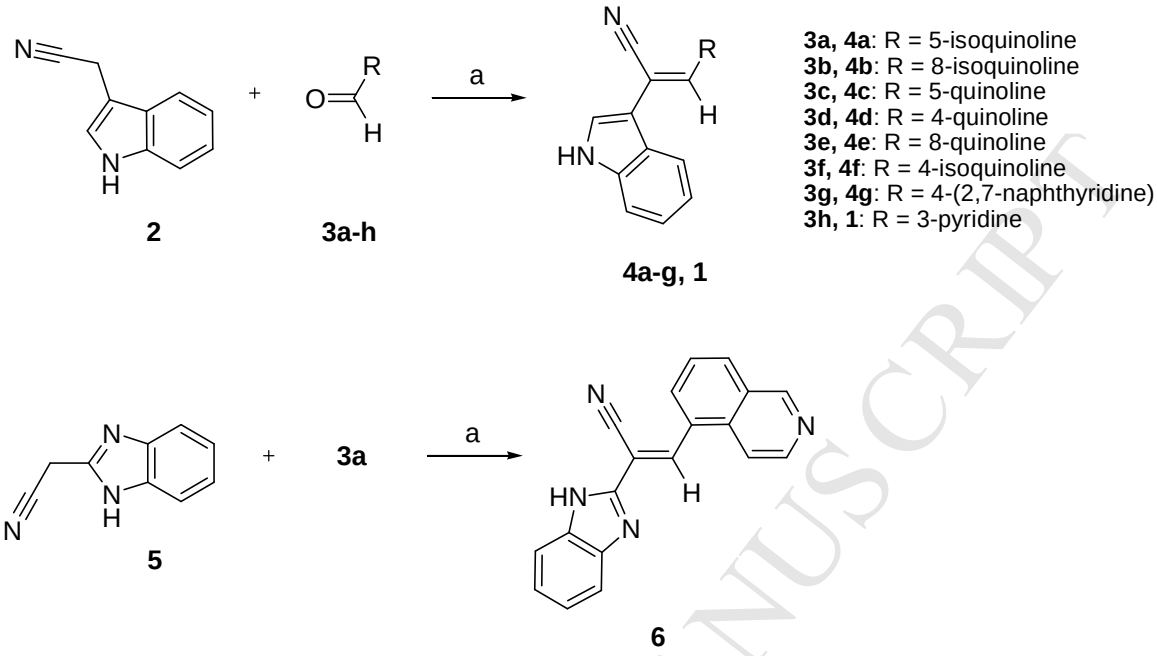
The intriguing dual phenotypic outcomes upon treatment of HeLa cells with **4a** led us to initiate a program of study to systematically investigate the structure activity relationships between analogues of **4a**, and to further characterise the most potent compounds in cellular models.

Strategy for SAR exploration

In this study, we aimed to establish a preliminary structure-activity relationship (SAR) around lead compound **4a** to elucidate the key chemical and structural features that are important for the dual activities of inhibiting mitotic progression and disrupting autophagy. Medicinal chemistry efforts focused on modifying the indole ring, introducing different substituents to the indole ring, altering the position of the nitrogen atom in the isoquinoline ring, as well as replacing the acrylonitrile moiety with alternative linkers. Synthesized compounds were evaluated using immunoblot analysis of the pH3 (Ser 10) and LC3 markers following treatment in transformed BJ fibroblast cells for 24 h at 5 μM. Relative intensity ratios of the bands for pH3 to DMSO treated control, as well as LC3-II to LC3-I compared to DMSO treated control, were determined. For each biomarker, a less than 2-fold induction is considered insignificant.

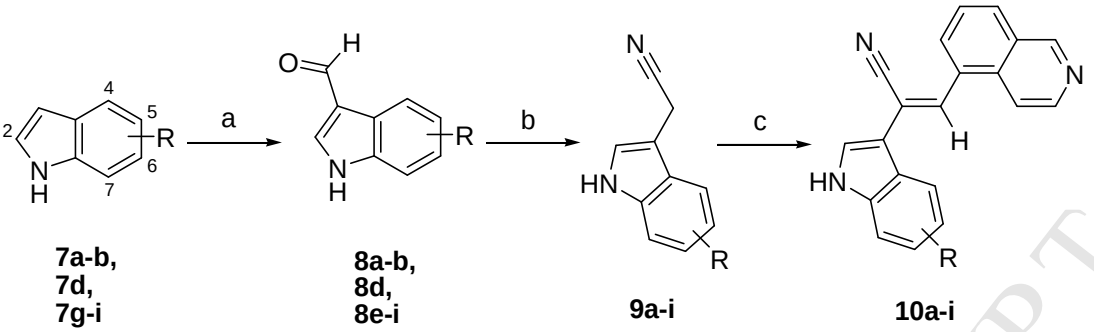
Chemistry

Scheme 1. Preparation of isomers and analogues of the isoquinoline ring **1**, **4a-g** and **6**.



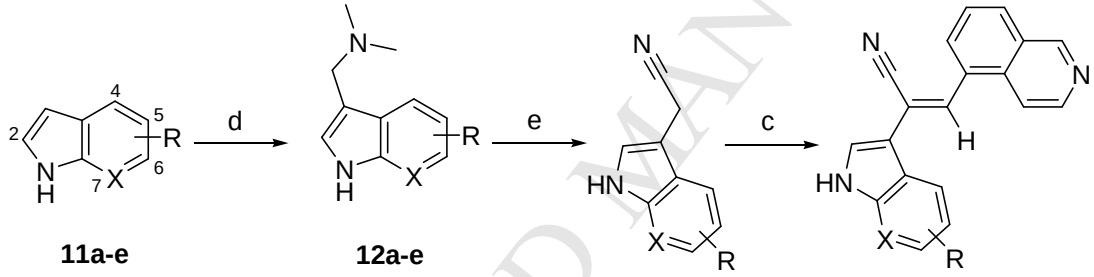
Reagents and Conditions: (a) CH_3ONa, MeOH, MW 95 °C, 8.5 min, 22-74%.

Scheme 2. Preparation of indole analogues **10a-i**, **14a-d**.



7a-b, 7d, 7g-i | **8a-b, 8d, 8e-i** | **9a-i** | **10a-i**

7a, 8a, 9a, 10a: R = 2-CH_3
7b, 8b, 9b, 10b: R = 4-Br
9c, 10c: R = 5-OCH_3
7d, 8d, 9d, 10d: R = 5-F
8e, 9e, 10e: R = 5-Cl
8f, 9f, 10f: R = 5-Br
7g, 8g, 9g, 10g: R = 5-$NHCOCH_3$
7h, 8h, 9h, 10h: R = 6-OCH_3
7i, 8i, 9i, 10i: R = 6-Br

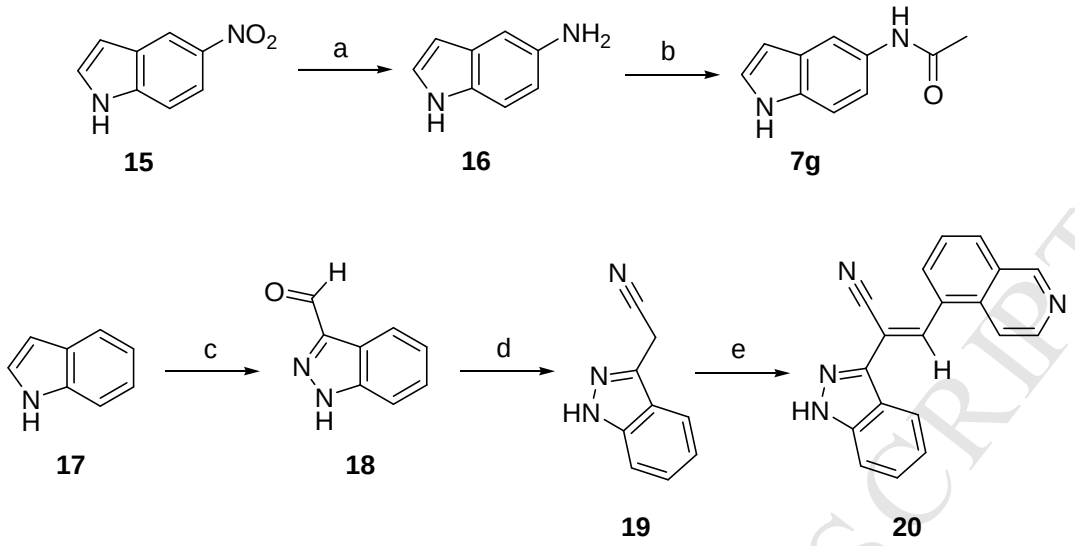


11a-e | **12a-e** | **13a-d** | **14a-d**

11a, 12a, 13a, 14a: X = CH, R = 5-NO_2
11b, 12b, 13b, 14b: X = CH, R = 7-OCH_3
11c, 12c, 13c, 14c: X = CH, R = 7-Br
11d, 12d, 13d, 14d: X = N, R = H (7-azaindole)
11e, 12e: X = CH, R = H

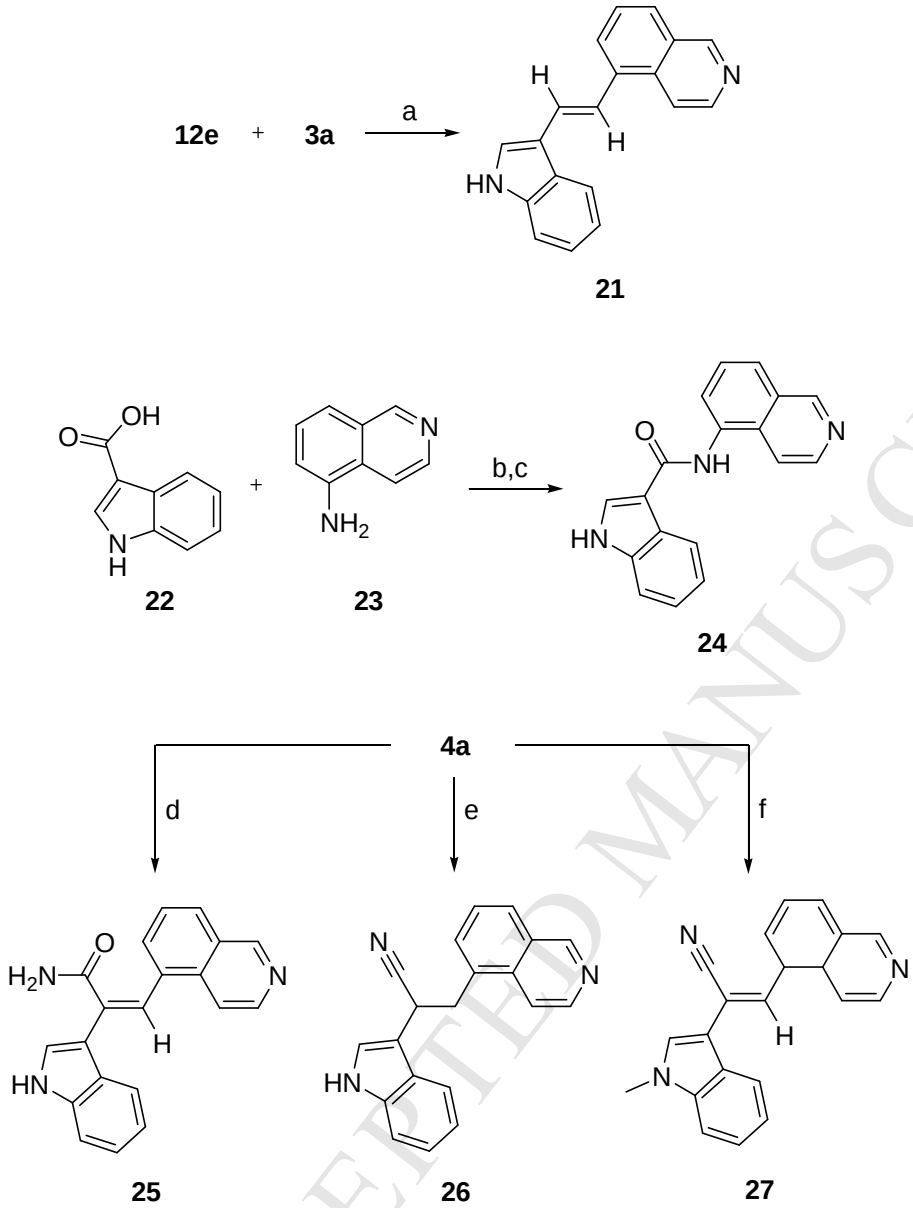
Reagents and Conditions: (a) $POCl_3$, DMF, 0 °C to RT, 1.5 h, 49-90%; (b) KCN, $NaBH_4$, MeOH/formamide, 55 °C, 16 h, 39-76%; (c) **3a**, CH_3ONa, MeOH, MW 95 °C, 8.5 min or sealed tube 75 °C, 16 h, 15-94%; (d) $(CH_3)_2NH$, HCHO, AcOH, H_2O, RT, 16 h, 66-97%; (e) KCN, CH_3I, EtOH/H_2O, RT, 16 h, 39-71%.

Scheme 3. Preparation of intermediate **7g** and indazole analogue **20**.



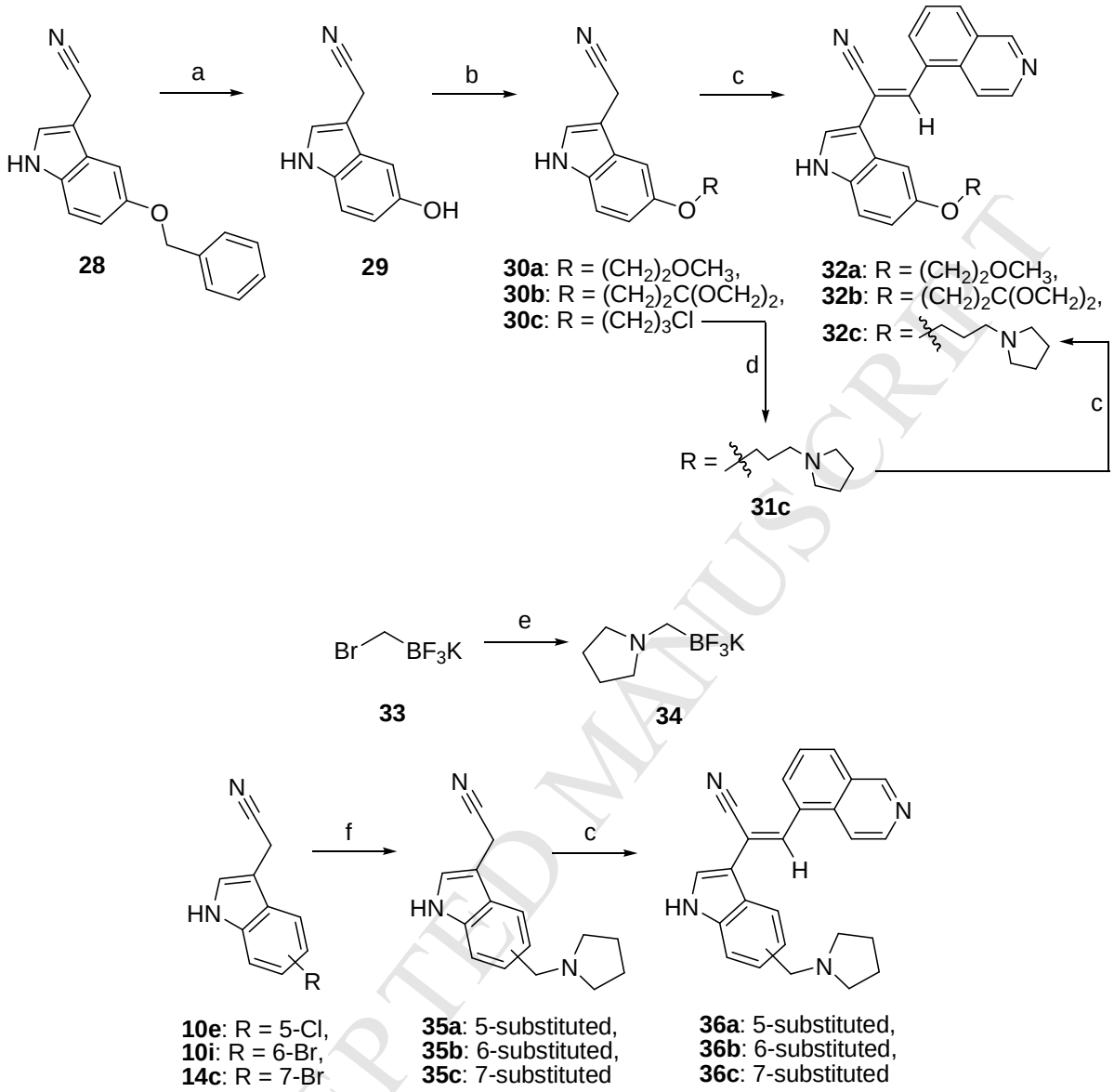
Reagents and Conditions: (a) H_2, Pd/C, EtOH, RT, 2.5 h, 53%; (b) CH_3COCl, Et_3N, DCM, rt, 3 h, 85%; (c) $NaNO_2$, HCl (aq), dioxane/H_2O, RT, 2 h, 7%; (d) KCN, $NaBH_4$, MeOH/formamide, 55 °C, 16 h, 60%; (e) **3a**, CH_3ONa, MeOH, sealed tube 75 °C, 16 h, 15-15%.

Scheme 4. Preparation of analogues **21**, **24-27**.



Reagents and Conditions: (a) PBu_3, MeCN, 90 °C, 22 h, 28%; (b) $(COCl)_2$, DCM, RT, 2 h; (c) Et_3N, THF, RT, 16 h, 42% (2 steps); (d) Pulverized KOH, *t*-BuOH, reflux, 18 h, 39%; (e) $NaBH_4$, THF/MeOH, 75 °C, 40 h, 20%; (f) CH_3I, NaH, THF, 0 °C to RT, 4 h, 88%.

Scheme 5. Preparation of analogues **32a-c**, **36a-c**.



Reagents and Conditions: (a) H_2, Pd/C, THF, RT, 7 h, 84%; (b) Alkyl halide, K_2CO_3, DMF, 50 °C, 16 h, 45-62%; (c) **3a**, CH_3ONa, MeOH, MW 95 °C, 8.5 min or sealed tube 75 °C, 16 h, 7-76%; (d) Pyrrolidine, K_2CO_3, NaI, MeCN, reflux, 40 h, 78%; (e) Pyrrolidine, THF, RT, 40 h, 16%; (f) **33**, $Pd(OAc)_2$, XPhos, Cs_2CO_3, THF/H_2O, 85 °C, 24 h, 53-59%.

The general approach for the synthesis of the acrylonitriles entails firstly the preparation of various indole-3-acetonitrile precursors followed by condensation with isoquinoline/quinoline/pyridine-5-carboxaldehydes (**3a-h)** in a Knoevenagel-like condensation reaction using sodium methoxide as the base to afford the final compounds (Scheme 1) [6]. The reaction was performed in anhydrous methanol in a sealed glass tube at 75 °C for 16 h in darkness. Microwave irradiation at a higher temperature of 95 °C allowed the reaction time to be shortened to 8.5 min. A number of the final compounds exhibited limited solubility in methanol. Reducing the solvent volume by evaporation and then chilling the mixture in an ice-bath facilitated precipitation of the desired products. For the more polar compounds that dissolved well in methanol, flash column chromatography and subsequent recrystallization furnished the desired products in good purity. X-ray single crystal analysis of **4a** revealed that the *Z*-configuration (heterocycles on opposite sides of the double bond) was obtained as the predominate isomer from the reaction. All compounds were obtained as single isomers except for **10a** and **10b**, which were isolated as inseparable mixtures with *Z*:*E* ratios of 88:12 and 75:25, respectively.

Two synthetic routes were employed for the preparation of the indole-3-acetonitrile precursors (Scheme 2). Starting from a wide variety of commercially available substituted indoles **7a-b**, **7d**, **7i** (reduction of 5-nitroindole (**15**) to 5-aminoindole (**16**) followed by acetylation allowed access to the acetamide-substituted indole **7g** (Scheme 3)), a formyl group was first introduced at the 3-position via the Vilsmeier-Haack reaction. This was followed by a one-pot sodium borohydride reduction and cyanide displacement to obtain the indole-3-acetonitrile intermediates **9a-i**. Alternatively, the starting indoles were subjected to a Mannich reaction to generate the gramines **12a-e**, which underwent subsequent substitution by the nucleophilic cyanide anion to afford the indole-3-acetonitrile intermediates **13a-d**. The key precursor to the synthesis of the indazole analogue **20** is the 3-formyl-indazole fragment

18. By reacting indole **17** with sodium nitrite under acidic conditions, this intermediate was successfully synthesized, but only in a meagre yield of 7%. Nevertheless, the subsequent nitrile transformation and condensation steps proceeded smoothly in good yields, rendering a straightforward overall route to indazole **20** (Scheme 3).

Following the procedure reported by Low and Magomedov [14], alkene-linked **21** was prepared through the direct coupling of gramine **12e** with isoquinoline-5-carboxaldehyde (**3a**) in an operationally simple and efficient phosphine-mediated reaction (Scheme 4). Amide bond formation between indole-3-carboxylic acid (**22**) and 5-amino-isoquinoline (**23**), using oxalyl chloride to generate the acyl chloride, afforded the amide-linked analogue **24** in moderate yield. Under reflux conditions in the presence of pulverized potassium hydroxide and *t*-butanol, the nitrile group in **4a** was transformed to a primary amide to furnish **25**, which was isolated as an inseparable 75:25 *Z*:*E* mixture. Reducing the double bond in **4a** using sodium borohydride provided **26**, while sodium hydride-assisted methylation of the indole nitrogen in **4a** yielded **27** in good yield.

A series of compounds exploring different alkoxy substituents on the indole 5-position (**32a-c**) were prepared according to the approach summarized in Scheme 5. The versatile hydroxy building block **29** was prepared from commercially available 5-benzyloxyindole-3-acetonitrile (**28**) in good yield using a chemoselective hydrogenation method described by Maegawa *et al* [15]. In this reaction, the catalytic activity of palladium on carbon was partially suppressed by the aproptic solvent THF to effect *O*-debenzylation without reducing the aliphatic nitrile. Alkylation of **29** gave the intermediates **30a-c**. The displacement of **30c** with pyrrolidine provided **31c** which led to more soluble compound **32c**.

To install an ionizable basic centre tethered to the indole ring by a short methylene linker, Molander's procedure was utilized [16]. Potassium bromotrifluoroborate (**33**) was stirred with pyrrolidine at room temperature for 40 h to afford potassium pyrrolidin-1-yl-methyl-

trifluoroborate (**34**). The ensuing Suzuki-Miyaura cross coupling with bromo/chloro-substituted indole-3-acetonitriles **10e**, **10i**, **14c** using palladium acetate, XPhos and cesium carbonate proceeded smoothly to afford intermediates **35a-c** which underwent Knoevenagel-like condensation with **3a** to furnish the more soluble analogues **36a-c**.

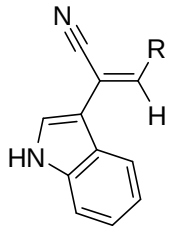
Results and Discussion

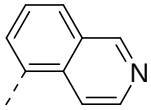
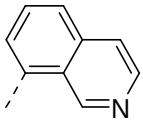
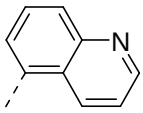
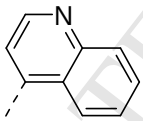
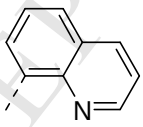
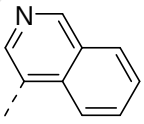
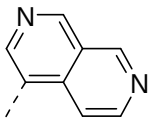
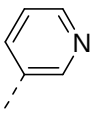
Structure-activity relationships

Investigation of the isoquinoline ring of **4a** was carried out with a series of quinoline and isoquinoline analogues exemplified in Table 1. Transposing the nitrogen atom to other regioisomeric positions on the fused bicyclic ring had a striking impact on both mitosis and LC3-II induction activity. Simply moving the nitrogen round the ring by one position to create the isomeric isoquinoline **4b** abolished activity against both pH3 and LC3-II biomarkers. This dramatic SAR likely indicates a highly specific interaction, possibly a hydrogen bond acceptor, being made by the nitrogen of the 5-isoquinoline ring with a cellular target, as opposed to a change in physicochemical properties reducing cell penetration by a large margin. Similarly, shifting the nitrogen one position further to give the 8-quinoline **4e** or to the other ring giving 4-isoquinoline **4f** and 4-quinoline **4d** also abolished activity for both pH3 and LC3-II biomarkers. On the other hand, the 5-quinoline **4c** exhibited a notable pH3-selective profile with strong anti-mitotic potency (more than 10-fold increase in pH3 levels) without LC3-II activity. Interestingly, introducing another nitrogen atom into the 5-isoquinoline ring to afford the 2,7-naphthyridin-4-yl analogue **4g** resulted in the loss of both pH3 and LC3-II activities. MKlp2 inhibitor Paprotrain (**1**), which bears a smaller monocyclic 3-pyridine ring, was inactive for both pathways, suggesting that the dual activities of **4a** on both markers is not due to MKlp2 inhibition. Evidently, the precise placement of the nitrogen

atom(s) in the bicyclic aromatic system is absolutely crucial to enable the dual mode of action of **4a**.

Table 1. Fold induction for pH3 and LC3 for isomers and analogues of the isoquinoline ring tested at a concentration of 5 μM.

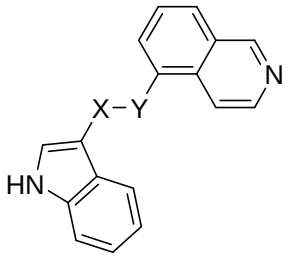


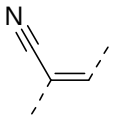
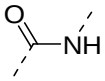
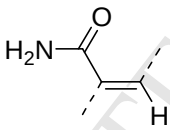
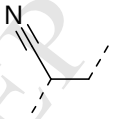
Compound	R	Fold induction (pH3 (Ser10))[a]	Fold induction (LC3-II / LC3-I)[a]
4a		**7.3**	**5.0**
4b		1.5	0.7
4c		**10.5**	0.7
4d		1.4	0.4
4e		1.3	0.6
4f		1.3	0.7
4g		1.4	1.0
1		1.2	1.1

[a] The band relative intensity ratios were determined for pH3 (Ser10) to DMSO treated control, as well as LC3-II to LC3-I as compared to DMSO treated control.

Next, we set out to modify the acrylonitrile bridge connecting the isoquinoline and indole rings (Table 2). Reducing the central double bond to allow free rotation of the two aromatic rings (**26**) abolished activity for both pH3 and LC3-II markers. This suggests that the central double bond could be responsible for constraining the aromatic rings in a configuration that is critical for both anti-mitotic and LC3-II induction functions. Removing the nitrile group in **4a** to afford a disubstituted alkene (**21**) retained strong LC3-II potency without activity for pH3. When an amide group was employed as the linkage between the aromatic rings (**24**), activity for both biomarkers was lost. Converting the nitrile group in **4a** to a larger primary amide moiety afforded **25** which was also not active at the concentration tested. Together, these observations suggest that the nitrile group is a key structural feature required for anti-mitotic activity whereas LC3-II potency is preserved with a simple alkene linker.

Table 2. Fold induction for pH3 and LC3 for linker analogues of compound **4a** tested at a concentration of 5 μM.



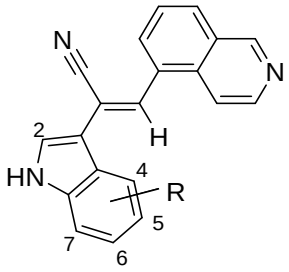
Compound	X-Y	Fold induction (pH3 (Ser10))[a]	Fold induction (LC3-II / LC3-I)[a]
4a		**7.3**	**5.0**
21		1.3	**5.4**
24		1.4	0.4
25		1.3	0.6
26		1.2	0.3

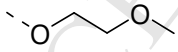
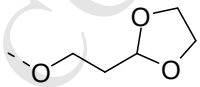
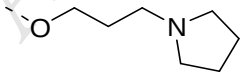
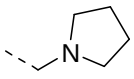
[a] The band relative intensity ratios were determined for pH3 (Ser10) to DMSO treated control, as well as LC3-II to LC3-I as compared to DMSO treated control.

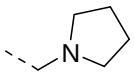
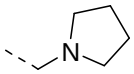
Exploration of the effects of different substituents at all the available positions of the indole ring was studied with a wide range of compounds (Table 3). Installing a methyl group on the indole C-2 (**10a**) was unfavourable as both pH3 and LC3-II activities were eliminated. Introducing a bromine atom at the 4-position also resulted in an inactive compound (**10b**). At

the 5-position, addition of a nitro group (**14a**) afforded a very poorly soluble compound that tested inactive for both pH3 and LC3-II markers. An acetamide moiety (**10g**) was not tolerated either as it failed to increase the levels of the two biomarkers. Varying effects were observed when different halogen atoms were introduced at the 5-position. Activity on both fronts was abolished with fluoro substituted **10d**. A small 2-fold increase in LC3-II/LC3-I was seen with chloro (**10e**) while pH3 and LC3-II induction improved to 2-fold and 4-fold, respectively, for 5-bromo compound **10f**. However, when the bromo substituent was located at positions 6 (**10i**) and 7 (**14c**), LC3-II activity was attenuated. Installing an electron donating methoxy group at positions 5, 6 and 7 afforded analogues **10c**, **10h** and **14b**, respectively, with moderate dual activities in the range of 2.5 to 4.5-fold induction of both biomarkers. Extending the 5-methoxy group into the longer methoxyethoxy side chain (**32a**) led to the decline of pH3 activity while maintaining LC3-II activity. Bulkier dioxolane (**32b**) suffered loss of activity against both pathways. Improving aqueous solubility was one of our goals, hence we made significant efforts to install a basic centre where possible. Interestingly, attaching a longer pyrrolidinopropyloxy side chain (**32c**), which we expected to be inactive given the result from the similar sized **32b**, demonstrated strong single activity for LC3-II. The anti-mitotic pH3 activities were abrogated but excellent LC3-II potency (6-fold induction) was achieved with this compound. Likewise, attaching a pendant basic methylpyrrolidine moiety to the 5, 6 and 7-positions (**36a**, **36b** and **36c**, respectively), also aimed at improving aqueous solubility, resulted in very similar LC3-II active but non-mitotic profiles.

Table 3. Fold induction for pH3 and LC3 for substitutions on the indole ring tested at a concentration of 5 μM.



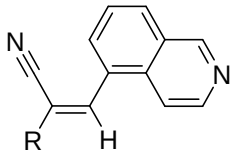
Compound	R	Position	Fold induction (pH3 (Ser10))[a]	Fold induction (LC3-II / LC3-I)[a]
4a	H	-	**7.3**	**5.0**
10a	Me	2	1.1	0.7
10b	Br	4	1.1	0.5
10c	OMe	5	**3.0**	**3.2**
10d	F	5	0.9	0.5
10e	Cl	5	1.4	**2.0**
10f	Br	5	**2.1**	**4.0**
14a	NO_2	5	0.9	0.6
10g	$NHCOCH_3$	5	1.2	0.5
32a		5	1.6	**3.2**
32b		5	1.0	0.7
32c		5	0.9	**6.0**
36a		5	1.0	**3.3**
10h	OMe	6	**4.3**	**2.6**

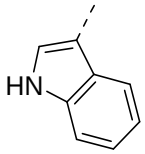
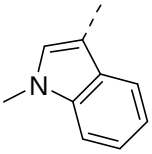
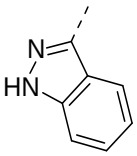
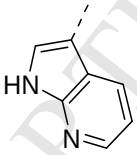
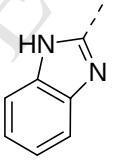
Compound	R	Position	Fold induction (pH3 (Ser10))[a]	Fold induction (LC3-II / LC3-I)[a]
10i	Br	6	1.6	0.7
36b		6	1.1	**5.7**
14b	OCH_3	7	**3.9**	**2.7**
14c	Br	7	1.4	**2.2**
36c		7	0.8	**3.7**

[a] The band relative intensity ratios were determined for pH3 (Ser10) to DMSO treated control, as well as LC3-II to LC3-I as compared to DMSO treated control.

Methylating the indole nitrogen (**27**) led to a conspicuous loss of both pH3 and LC3 activities, suggesting that the indole N-H could be making a key hydrogen bond interaction with its molecular target(s) responsible for its dual mode of action (Table 4). Efforts were also put into exploring potential bioisosteres of indole such as indazole, 7-azaindole and 2-benzimidazole in the hope of engendering compounds with improved potency. However, these endeavours ultimately proved disappointing. No activity was observed with the 2-benzimidazole analogue (**6**) while the indazole compound (**20**) showed only feeble LC3-II activity. 7-Azaindole **14d** was found to exhibit exceptionally poor solubility (poorly soluble even in DMSO). This is most likely because of its flat overall conformation facilitating tight packing within the crystal lattice enabling the formation of hydrogen bonds between the N-H of one azaindole molecule and the N-7 of another.

Table 4. Fold induction for pH3 and LC3 for other analogues of the indole ring tested at a concentration of 5 μM.



Compound	R	Fold induction (pH3 (Ser10))[a]	Fold induction (LC3-II / LC3-I)[a]
4a		**7.3**	**5.0**
27		1.1	1.6
20		1.4	**2.0**
14d		1.8	1.4
6		1.1	1.2

[a] The band relative intensity ratios were determined for pH3 (Ser10) to DMSO treated control, as well as LC3-II to LC3-I as compared to DMSO treated control.

Through this SAR study, compounds with dual activities on pH3 and LC3-II biomarkers (**10c**, **10f**, **10h**, **14b**), as well as compounds with single activity selective for LC3-II (**21**, **32a**, **32c**, **36a**, **36b**, **36c**) and a compound with single activity selective for pH3 (**4c**) have been obtained. Lead molecule **4a** exhibits a high degree of structural specificity for exerting the dual effects of mitosis inhibition and LC3-II induction. All of the structural features of **4a** are critical for its dual actions including the acrylonitrile linker, specific position of the isoquinoline nitrogen and the indole ring with optional 5-position substitution. The SAR landscape around the indole ring suggests a cliff for pH3 activity as substitution on the indole generally led to a considerable drop in pH3 potency. In contrast, LC3-II potency appears to be more tolerant of substitutions at the indole positions 5, 6 and 7 (Fig. 6). From this work we conclude that **4a** remains the most potent dual-acting compound identified thus far.

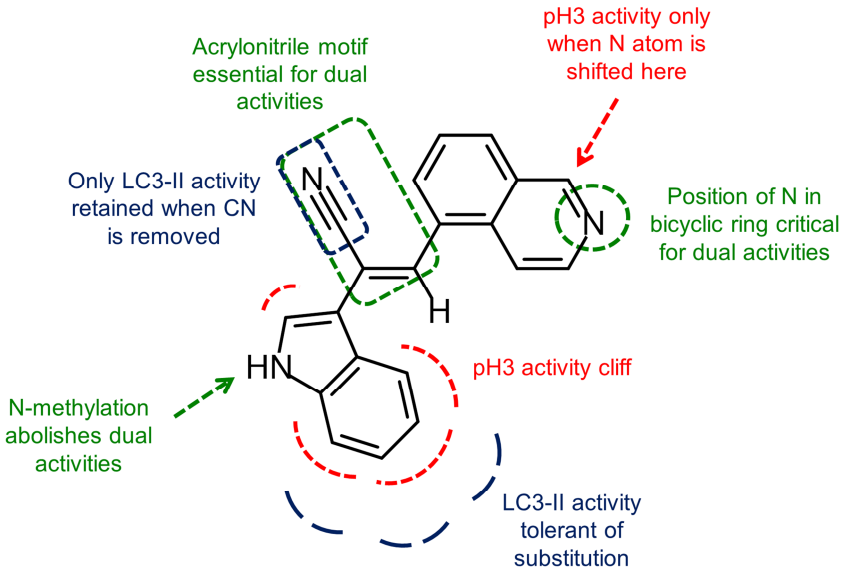
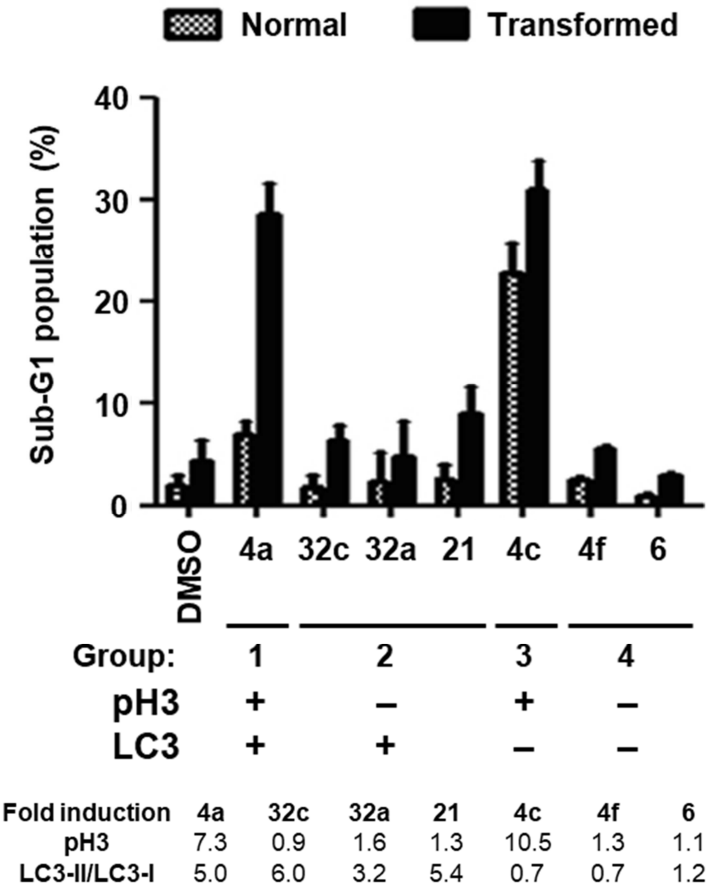


Fig. 6. Summary of key structure-activity relationship (SAR) findings.

Evaluation of cell death in isogenic normal and transformed BJ fibroblasts

Compounds were classified into four groups based on the SAR analysis: Group 1 compounds, which are active against both pH3 and LC3 markers; Group 2 compounds, which are active against LC3 only; Group 3 compounds, which are active against pH3 only; and Group 4 compounds, which are inactive against both pH3 and LC3 markers. Selected compounds from the various groups were evaluated for their effects on cell death in isogenic normal BJ cells and transformed BJ cells (Fig. 7). Following treatment with each compound at 5 μM for 48 h, the percentage of cells in the sub-G1 phase, an indicator of the extent of cell death, was determined. Compound **4a**, a group 1 compound with strong dual activities, exhibited a strong cell-killing effect in transformed cells but not in normal cells. This transformed cell selectivity was not seen in compounds from group 2 or group 3. Group 3 compound **4c** killed both transformed and normal cells with less selectivity than **4a** while group 2 and group 4 compounds did not kill either normal cells or transformed cells. This suggests that dual activity against both pH3 and LC3 markers are required for inducing selective cancer cell death.



Fold induction	4a	32c	32a	21	4c	4f	6
pH3	7.3	0.9	1.6	1.3	10.5	1.3	1.1
LC3-II/LC3-I	5.0	6.0	3.2	5.4	0.7	0.7	1.2

Fig. 7. Percentage of isogenic normal BJ cells and transformed BJ cells in the sub-G1 phase following treatment with the respective compounds at 5 µM for 48 h.

Analysis of tumor versus normal cells

Evaluation of the antiproliferative effects of preferred compound **4a** in a wide panel of cancer cell lines and in a small panel of normal cells indicated a higher sensitivity for cancer versus normal cells (Fig. 8A). Remarkably, a clear sensitivity of the vast majority of tumor cells over normal cells was seen. The mean GI_{50} from a panel of 30 cancer cell lines was 1.5 ± 0.9 μM and for a panel of 5 normal cells the mean was 6.5 ± 2.6 μM. Comparing between cell lines with different cell backgrounds is tenuous, hence to achieve a more objective comparison isogenic normal human BJ foreskin fibroblasts and transformed BJ fibroblasts may be compared. Compound **4a** showed good selectivity for transformed cells over normal cells (8.5 fold difference in IC_{50}s).

We further studied **4a** in two normal cells lines: the cardiomyocyte AC10 and mouse hepatocyte TAMH. We were pleased to find that **4a** was less toxic to these cells lines when tested at the high concentration of 50 μM (AC10 % viability at 50 μM = 77.6% ± 7.2% and TAMH % viability at 50 μM = 57.3% ± 4.6%).

This cancer cell selective effect was then investigated in apoptosis assays monitoring caspase-3/7 activation and cleaved PARP. Compound **4a** induced apoptosis in transformed BJ cells, but *not* in the normal BJ cell counterpart (Fig. 8B and C). Further assessment of **4a** in the AMES test indicated that the compound was not mutagenic (see Supplementary Information).

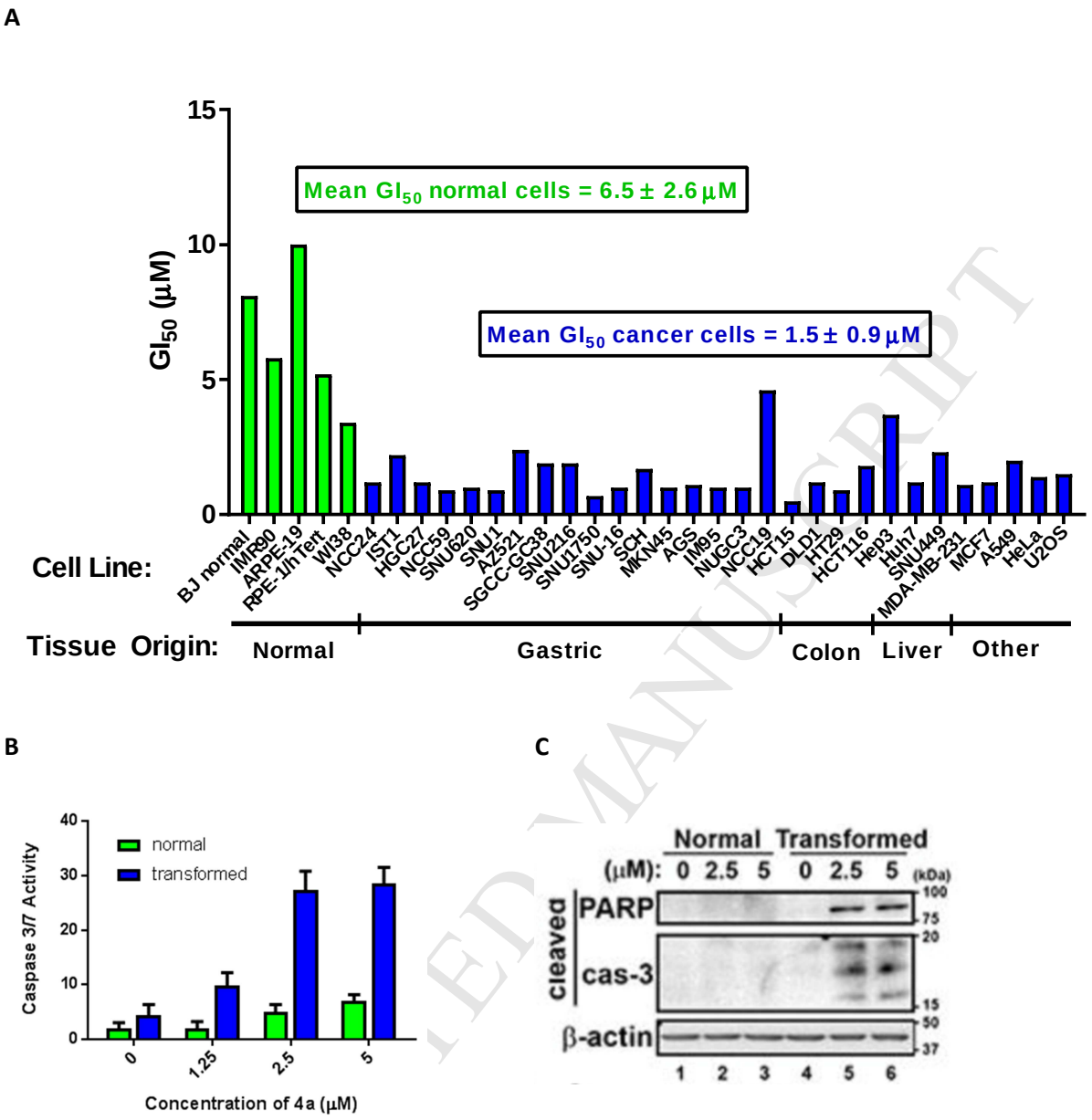


Fig. 8. Differential effects in normal versus transformed cells highlights cancer cell selective mechanism of **4a**. (A) MTT assay of human normal and cancer cell lines treated with **4a** for 72 h (n=3). Mean concentration values for **4a** to achieve 50% growth inhibition (GI_{50}) in each cell line are plotted with ± S.D. (B) (**b**) Normal and transformed BJ cells were treated for 48h with the indicated concentrations of **4a**. Selective increase in combined activity of caspase-3/7 in transformed BJ cells by **4a** treatment is shown. Mean values with ± S.D. are shown (n=3). Two-tailed unpaired t tests were performed to determine statistical significance. (C) Immunoblot analysis of cleaved PARP and caspase-3 (Cas-3) indicating **4a**-induced

apoptosis in transformed BJ cells (lanes 5, 6), but not normal counterparts (lanes 2, 3). Representative immunoblots shown (n=3).

To demonstrate that normal cells are not permanently affected following treatment of **4a**, a wash-out study was performed (Fig. 9). Normal BJ cells were treated with **4a** (5 μM) for 2, 4 and 11 days. When drug is washed out after 2 or 4 days the cells begin to proliferate again.

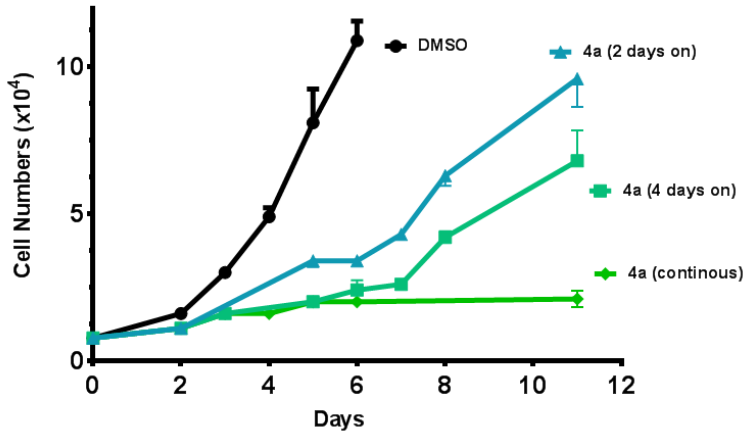


Fig. 9. Normal cell wash out shows cells regrow when drug removed. Normal BJ cells were synchronized at the G_1 phase by serum starvation (0.1% FBS) for 2 days. Subsequently, the cells were synchronously released in fresh media with 10% FBS and then treated with **4a** (5 μM) for 2, 4 or 11 days. After 2 or 4 days, **4a** was removed and cell proliferation continued in fresh media for up to 11 days. The total number of cells at various time points were calculated using automated cell counter (SCEPTOR, Merck) and mean values with ± S.D. are shown (n>6).

Antiproliferative effects of 4a in colony formation assays

The antiproliferative effects of **4a** on HeLa cells growing anchorage-independently in soft agar were next evaluated. Soft agar colony formation assays more accurately capture the features of *in vivo* tumors growing in three dimensions than cancer cell lines growing in an *in vitro* tissure culture [17]. As shown in Fig. 10, **4a** treatment resulted in a significant reduction in the number of HeLa colonies grown in soft agar in a dose-dependent manner. Notably, a 50% reduction in number of HeLa cell colonies was achieved with sub-micromolar concentrations of **4a**.

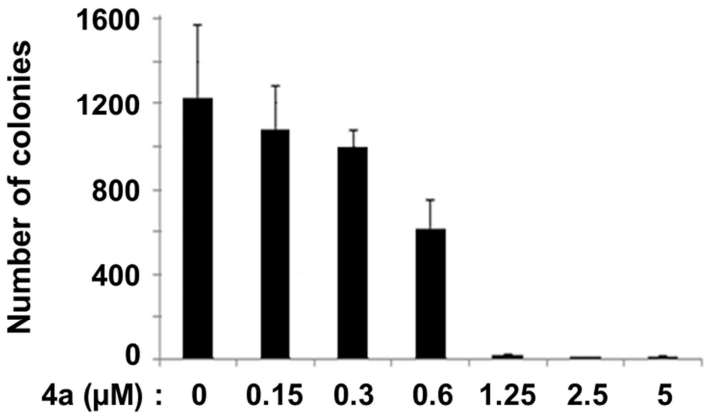


Fig. 10. Number of HeLa colonies in soft agar cultures with **4a** treatment at various concentrations.

Investigations into the Michael acceptor reactivity of 4a

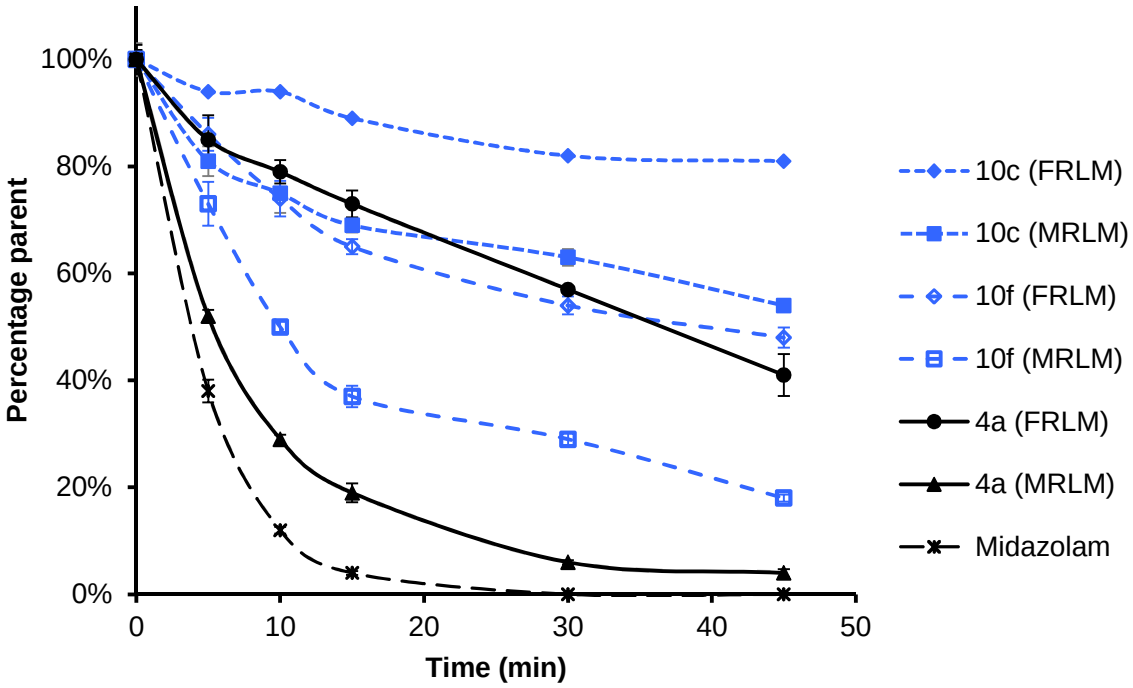
Owing to the acrylonitrile motif in **4a**, it could be possible that **4a** acts as a Michael acceptor as some acrylonitriles have been reported to form covalent adducts with thiols [18]. To evaluate the general Michael acceptor reactivity of **4a**, the NMR-based protocol described by Avonto *et al* was applied [19]. Using cysteamine (5.7 equivalents) as the electrophile-trapping agent, no adduct formation was observed upon incubation with **4a** in DMSO at room temperature up to 24 h (Supplementary Figure S4). Given the lack of reactivity with cysteamine and the strict SAR requirements for dual activity, it is unlikely **4a** exerts its action through a general electrophilic modification of proteins.

***In vitro* microsomal stability evaluation**

A few dual-acting compounds were shortlisted for *in vitro* rat liver microsomal stability testing. In the presence of male rat liver microsomes, **4a** was metabolized rapidly with a half-life ($t_{1/2}$) of 7.7 min (Fig. 11). However, metabolism in female rat liver microsomes was much slower ($t_{1/2}$ approximately 40 min). As such, the metabolic stability of **4a** is regarded to be gender dependent. As CYP3A and CYP2C are present in male rat liver microsomes but not in female rat microsomes, these two isoforms are most likely to be responsible for the metabolism of **4a**.

Better stability in male rat liver microsomes ($t_{1/2}$ approximately 20 min) was seen with 5-methoxy **10c** and 5-bromo **10f**, suggesting that the indole 5-position is a metabolically vulnerable site. Introducing a fluorine atom to block metabolism, a commonly used strategy [20], was not viable in this case as the resulting 5-fluoro analogue **10d** did not affect either pH3 or LC3 markers.

A



B

Compound	**4a**	**10c**	**10f**
$T_{1/2}$ (min) FRLM	40.2 (1.2)	33.4 (2.5)	43.8 (2.5)
$T_{1/2}$ (min) MRLM	7.7 (0.2)	21.8 (1.7)	19.6 (0.4)
CLint FRLM (µL/min/mg)	59 (3)	66 (7)	53 (3)
CLint MRLM (µL/min/mg)	299 (8)	106 (9)	118 (3)

Fig. 11. Rat microsomal stability data for **4a**, **10c** and **10f**. (A) Graph of percentage parent remaining against time upon incubation at 37°C for 45 min for female (FRLM) and male rat liver microsomes (MRLM). (B) Calculated pharmacokinetic parameters $T_{1/2}$ and Cl_{int} (mean (SD)).

Formulation of 4a for *in vivo* studies

The poor aqueous solubility of **4a** presented a major obstacle to further study of the compound *in vivo*. As described earlier, the SAR does not tolerate the installation of a basic centre to enhance solubility. We next turned to a formulation approach to address this issue. The hydrochloride salt of **4a** (**4a**.HCl) was first prepared. The goal was to develop an injectable solution of 1 mg/mL intended for various routes of administration in rodents (Supplementary Table S3). We aimed to achieve the targeted solubility with the simplest possible formulation employing pH adjustment, to leverage the ionizable weakly basic isoquinoline in **4a**, in combination with various pharmaceutically acceptable co-solvents [21]. Solubilities attained with different combinations of co-solvents at different pHs were quantified by HPLC analysis (Supplementary Table S4). Concentrations of over 1 mg/mL were obtained with several formulations and a formulation consisting of 10% DMSO, 50% PEG400 and 40% water pH 5.0 (Supplementary Table S4) was chosen for subsequent *in vivo* studies.

***In vivo* pharmacokinetics of 4a**

4a, the most potent dual-acting compound, was selected for pharmacokinetic and bioavailability evaluation in male Wistar rats (Fig. 12 and Table 5). The hydrochloride salt of **4a** was formulated as a 1 mg/ml solution in 10% DMSO, 50% PEG400, 40% water pH 5 buffer for *iv* and oral administration at doses of 2 mg/kg and 10 mg/kg, respectively. The area under curve (AUC) obtained following *iv* administration was found to have a small relative standard deviation of 16%. In contrast, AUC for oral administration showed greater variation. Absorption appeared to be variable between animals. For two out of three rats, T_{max} was approximately 20 min, suggesting rapid absorption of the compound following oral administration. The terminal half-life for *iv* administration was short (0.36 h), consistent with the findings of poor metabolic stability in rat liver microsomal stability studies (7.7 min in male rat liver microsomes). A significant first-pass effect arising from poor microsomal stability is expected for oral administration of **4a**.HCl as well. The oral bioavailability was calculated to be 43%.

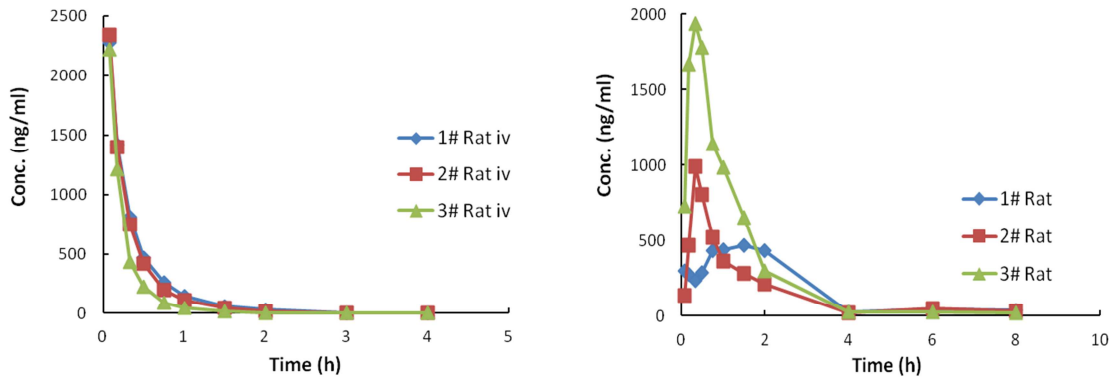


Fig. 12. Plasma concentration-time curves of **4a**.HCl in rats following (a) intravenous injection of 2 mg/kg **4a**.HCl and (b) oral administration of 10 mg/kg **4a**.HCl.

Table 5. Pharmacokinetic parameters of **4a**.HCl in rats.

Intravenous injection of 2 mg/kg **4a**.HCl[a]			
$t_{1/2}$ (h)	AUC_{0-t} (µg/L*h)	$AUC_{0-\infty}$ (µg/L*h)	MRT (h)
0.36 (0.04)	819.86 (134.45)	821.82 (134.30)	0.29 (0.08)

Oral administration of 10 mg/kg **4a**.HCl[a]						
$t_{1/2}$ (h)	AUC_{0-t} (µg/L*h)	$AUC_{0-\infty}$ (µg/L*h)	C_{max} (µg/L)	T_{max} (h)	MRT (h)	F (%)
1.6 (0.4)	1693.53 (656.29)	1768.93 (618.70)	1132.00 (747.54)	0.72 (0.68)	1.89 (0.59)	43

[a]Mean (± SD), n = 3 animals per study. *Iv* and *po* formulation vehicle: solution in PEG400, DMSO, water pH 5 (50:10:40 v/v/v)

Evaluation of 4a in a mouse xenograft model of colon cancer

Given its unique cancer cell selectivity, we evaluated lead compound **4a** in a mouse model of colon cancer. Mice bearing HCT15 tumors, a cell line which carries a K-Ras mutation (G13D) and is resistant to paclitaxel (PTX) [22], were treated with **4a** intraperitoneally (IP). Treatment with PTX did not result in any significant tumor growth inhibition (TGI). At a dose of 20 mg/kg IP twice per day the TGI of **4a** was 74% (Fig. 13). This data indicate that **4a** is efficacious in retarding tumor growth in the drug resistant cell line HCT15. This study supports further investigation of **4a** to ascertain the breadth of its antitumor effects.

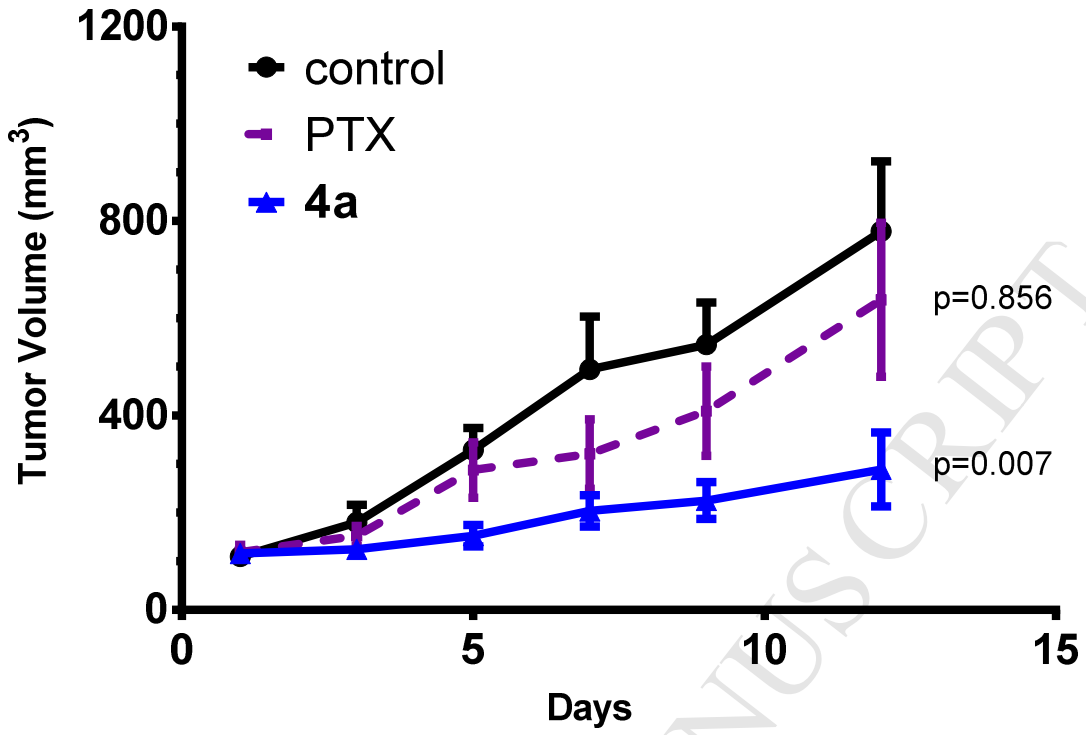


Fig. 13. Mice bearing established tumor xenografts were treated intraperitoneally (IP) twice daily with **4a** (20 mg/kg). Tumor volumes were calculated periodically as indicated. Paclitaxel (PTX) was administered intravenously (IV, 60 mg/kg) twice every 4 days. Mean tumor volumes ± SEM from 6 mice are shown. Two-way ANOVA was performed to determine statistical significance compared to vehicle control.

Conclusions

Following the phenotypic-driven discovery of **4a**, derived from **1**, an extensive SAR campaign explored three key areas of the molecule utilising the mitotic marker pH3 and the autophagy marker LC3. Very few compounds maintained the dual profile of **4a** and none could match its potency. A key benefit of the unique profile of **4a** is its cancer cell selectivity: in a number of cancer cell lines studied **4a** caused mitotic dysfunction and induced apoptosis whereas in normal cells studied **4a** did not induce cell death. When treatment of **4a** is withdrawn normal cells continue to grow. The mechanism behind this cancer cell selective

effect is further explored elsewhere [23]. In a mouse model of paclitaxel-resistant colon cancer **4a** inhibited 74% of tumour growth with no effects on mouse body weight. Further studies to improve the *in vivo* profile of **4a** will be reported in due course.

EXPERIMENTAL

Synthesis of compounds

All non-aqueous reactions were performed in oven-dried round bottom flasks under an inert nitrogen atmosphere unless stated otherwise. Commercially available AR grade solvents or anhydrous solvents packed in resealable bottles were used as received. All reaction temperatures stated in the procedures are external bath temperatures. Unless otherwise stated, commercial reagents were purchased from Sigma Aldrich, Alfa Aesar, TCI Chemicals, Combi-Blocks or Ark Pharm, and used as received without further purification. Yields refer to chromatographically and spectroscopically homogeneous materials, unless otherwise stated. Reaction progress was monitored by analytical thin layer chromatography (TLC) with 0.25 mm Merck pre-coated silica gel plates (60F-254) using UV light (254 nm) as visualizing agent, and ceric ammonium molybdate or potassium permanganate solutions as developing stains. Flash chromatography was performed on silica gel 60 (0.040 – 0.063 mm) purchased from SiliCycle or Merck. ^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectra were recorded on a Bruker Avance 400 Ultrashield (400 MHz) NMR spectrometer at ambient atmosphere. The deuterated solvents used were $CDCl_3$, DMSO-*d*6, and acetone-*d*6. Chemical shifts are reported in parts per million (ppm), and residual undeuterated solvent peaks were used as internal reference: proton (7.26 ppm for $CDCl_3$, 2.50 ppm for DMSO-*d*6, and 2.05 ppm for acetone-*d*6), carbon (77.16 ppm for $CDCl_3$, 39.52 ppm for DMSO-*d*6, and 29.84 ppm for acetone-*d*6). ^{1}H NMR coupling constants (*J*) are reported in Hertz (Hz), and multiplicities are presented as follows: s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad). Low resolution mass spectra were obtained on an Agilent 6130B Quadrupole LC/MS in ESI mode with an Agilent 1260 Infinity LC system using a ThermoScientific Hypersil 150 x 2.1mm 5 micron column or a Shimadzu LCMS-2020 in ESI mode. Purity of the compounds were assessed by high performance liquid chromatography by detection at 254 nm using an

Agilent 1200 series HPLC system with a Zorbax SB-C18 5 micron 4.6 x 250 mm column using a gradient elution starting from a 5% solution of acetonitrile / 1% trifluoroacetic acid (TFA) and a 95% solution of water / 1% TFA to a 100% solution of acetonitrile / 1% TFA at 0.5 mL per minute over 15 min. Purity of each compound was determined from the area of the major peak compared to the total area of peaks seen on the chromatogram.

General procedure A:

Microwave method: To an indole-3-acetonitrile derivative (1.0 equiv) dissolved in anhydrous methanol (4 mL for 2.31 mmol of starting material) in a dried microwave vial, sodium methoxide (1.7 equiv) was added and stirred at room temperature for 15 min protected from light. Quinoline/isoquinoline-carboxaldehyde derivative (1.2 equiv) was added and the mixture was subjected to microwave irradiation at 95 °C for 8.5 min. The reaction was cooled to room temperature and then chilled in an ice/salt bath. The resulting precipitate was filtered, washed with methanol, and dried under vacuum to afford a solid as the product.

Sealed tube method: To an indole-3-acetonitrile derivative (1.0 equiv), quinoline/isoquinoline-carboxaldehyde derivative (1.0 equiv), sodium methoxide (3.0 equiv) in a dried glass reaction tube, anhydrous methanol (15 mL for 7.72 mmol of indole-3-acetonitrile derivative) was added. The reaction tube was sealed and heated at 75 °C in an oil bath for 16 h protected from light. The reaction was allowed to cool to room temperature and then chilled in an ice/salt bath. The resulting precipitate was filtered, washed with methanol, and dried under vacuum to afford a solid as the product.

General procedure B:

An indole derivative (1.0 equiv) was dissolved in acetic acid (1.7 mL for 3.23 mmol of starting material) and water (1.7 mL for 3.23 mmol of starting material) and chilled to 0 °C in an ice bath. Formaldehyde 37% w/w in H_2O (1.25 equiv) and dimethylamine 40% w/w in H_2O (1.75 equiv) was then added. The reaction was allowed to warm to room temperature

and stirred for 16 h. The reaction was quenched by adding ice and basified to pH 12-14 with 5N NaOH. The resulting mixture was extracted with dichloromethane 3 times. The combined organic layer was washed with brine, dried over anhydrous sodium sulphate and evaporated under vacuum to obtain the gramine product which was used in the next step without further purification.

General procedure C:

A gramine derivative (1.0 equiv) was dissolved in ethanol (5.5 mL for 1.34 mmol of starting material) and water (0.55 mL for 1.34 mmol of starting material). The gramine solution was added to potassium cyanide (2.0 equiv) prepared in a separate flask. Iodomethane (2.6 equiv) was added and the reaction was stirred vigorously at room temperature for 16 h. Saturated sodium bicarbonate was added to quench the reaction, followed by extraction with ethyl acetate thrice. The combined organic layer was washed with brine, dried over anhydrous sodium sulphate and concentrated *in vacuo*. Purification by flash silica gel column chromatography afforded the indole-3-acetonitrile derivative product.

General procedure D:

To a dried two-neck round-bottom flask containing DMF (0.7 mL for 1.10 mmol of starting material) chilled in an ice bath, $POCl_3$ (1.95 equiv) was added slowly. After stirring for 20 min, a solution of an indole derivative (1.0 equiv) in DMF (3 mL for 1.10 mmol of starting material) was added dropwise. The reaction was allowed to warm to room temperature and allowed to stir for 1.5 h. The reaction was quenched by adding ice followed by 1N NaOH (40 mL) dropwise in an ice bath. The crude mixture was allowed to stand at room temperature and the precipitate formed was filtered to afford the 3-formyl-indole derivative product.

General procedure E:

To a flask containing a 3-formyl-indole derivative (1.0 equiv) and sodium borohydride (3.0 equiv), methanol (9 mL for 1.0 mmol of starting material) and formamide (9 mL for 1.0

mmol of starting material) were added and stirred at room temperature for 15 min. The mixture was added to potassium cyanide (10.0 equiv) prepared in a separate flask and stirred at 55 °C for 16 h. The reaction was quenched by adding brine and a few drops of 5N NaOH, followed by extraction with dichloromethane thrice. The combined organic layer was washed with brine, dried over anhydrous sodium sulphate and concentrated *in vacuo*. Purification by flash silica gel column chromatography afforded the indole-3-acetonitrile derivative product.

General procedure F:

To a solution of intermediate **29** (1.0 equiv) in DMF (5 mL for 0.99 mmol starting material), alkyl halide (3.0 equiv) and potassium carbonate (3.5 equiv) was added. The reaction was stirred at 50 °C for 16 h. The mixture was poured into water and extracted with ethyl acetate thrice. The combined organic layer was washed with brine, dried over anhydrous sodium sulphate and concentrated *in vacuo*. Purification by flash silica gel column chromatography afforded the 5-alkoxy-indole-3-acetonitrile product.

General procedure G:

To a dry reaction flask, bromo/chloro-indole-3-acetonitrile (1.0 equiv), intermediate **34** (1.2 equiv), XPhos (0.06 equiv), $Pd(OAc)_2$ (0.03 equiv) and (3.0 equiv) were added. The flask, with an attached condenser, was sealed with a septum and purged with nitrogen. A degassed mixture of 10:1 THF/H_2O (2.5 ml for 0.262 mmol of indole starting material) was added and the mixture was heated at 85 °C for 24 h. After the reaction was allowed to cool to room temperature, the mixture was poured into water and extracted with ethyl acetate thrice. The combined organic layer was washed with brine, dried over anhydrous sodium sulphate and concentrated *in vacuo*. Purification by flash silica gel column chromatography (85:15:0.015 dichloromethane/methanol/ammonium hydroxide solution) afforded the product.

(*Z*)-2-(1H-indol-3-yl)-3-(pyridin-3-yl)acrylonitrile (1)

Compound **1** (103 mg, 0.420 mmol) was prepared as an yellow solid from indole-3-acetonitrile (115 mg, 0.736 mmol) and 3-pyridine-carboxaldehyde (86.3 µl, 98.5 mg, 0.920 mmoles) according to general procedure A microwave method. Yield: 58%. HPLC RT 11.56 min; HPLC purity 99.65%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 11.80 (bs, 1H), 8.99 (s, 1H), 8.59 (d, *J* = 4.6 Hz, 1H), 8.34 (d, *J* = 8.0 Hz, 1H), 8.10 (d, *J* = 7.8 Hz, 1H), 7.83 (d, *J* = 10.9 Hz, 2H), 7.58 – 7.49 (m, 2H), 7.22 (dt, *J* = 14.8, 7.2 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 149.94, 149.58, 137.26, 134.53, 132.49, 130.79, 127.28, 123.67, 123.54, 122.63, 120.65, 119.60, 118.03, 112.52, 110.50, 108.07. MS (ESI): *m/z* 246.1 $[M+1]^{+}$.

(*Z*)-2-(1*H*-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (4a)

Compound **4a** (114 mg, 0.386 mmol) was prepared as a yellow solid from indole-3-acetonitrile (120 mg, 0.769 mmol), isoquinoline-5-carboxaldehyde (147 mg, 0.938 mmol) and sodium methoxide (71 mg, 1.307 mmol) according to general procedure A microwave method. Yield: 47%. Yellow crystals were obtained by recrystallization using acetone and methanol. HPLC RT 11.97 min; HPLC purity 99.23%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 9.41 (s, 1H), 8.60 (d, *J* = 6.0 Hz, 1H), 8.34 (s, 1H), 8.26 (d, *J* = 7.3 Hz, 1H), 8.22 (d, *J* = 8.1 Hz, 1H), 8.12 (d, J = 7.6 Hz, 1H), 8.05 (d, *J* = 6.0 Hz, 1H), 7.95 (s, 1H), 7.82 (t, *J* = 7.8 Hz, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.28 – 7.19 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 152.93, 143.69, 137.26, 133.54, 132.54, 131.29, 130.25, 128.91, 128.23, 127.44, 127.11, 123.83, 122.56, 120.69, 119.50, 117.96, 117.12, 112.56, 110.43, 110.41. LC-MS (ESI): *m/z* 296.1 $[M + H]^{+}$. HRMS (ESI): *m/z* calculated $[M + H]^{+}$ $C_{20}H_{14}N_3^{+}$ 296.1182, found 296.1190 (Diff = 2.6 ppm).

(*Z*)-2-(1*H*-indol-3-yl)-3-(isoquinolin-8-yl)acrylonitrile (4b)

Compound **4b** (37 mg, 0.124 mmol) was prepared as a bright yellow solid from indole-3-acetonitrile (89 mg, 0.572 mmol) and isoquinoline-8-carboxaldehyde (82 mg, 0.520 mmol) according to general procedure A microwave method. Yield: 22%. HPLC RT 12.04 min;

HPLC purity 99.64%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 11.83 (bs, 1H), 9.58 (s, 1H), 8.62 (d, *J* = 5.6 Hz, 1H), 8.54 (s, 1H), 8.21 – 8.12 (m, 1H), 8.07 (t, *J* = 7.7 Hz, 2H), 7.96 (s, 1H), 7.95 – 7.84 (m, 2H), 7.54 (d, *J* = 7.7 Hz, 1H), 7.24 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 149.13, 143.46, 137.20, 135.54, 133.05, 132.69, 130.18, 127.86, 127.70, 127.43, 125.87, 123.83, 122.59, 120.76, 120.71, 119.58, 117.92, 112.48, 110.94, 110.41. LC-MS (ESI): *m/z* 296.1 $[M + H]^+$.

(*Z*)-2-(1*H*-indol-3-yl)-3-(quinolin-5-yl)acrylonitrile (4c)

Compound **4c** (137 mg, 0.464 mmol) was prepared as a yellow solid from indole-3-acetonitrile (118 mg, 0.758 mmol) and quinoline-5-carboxaldehyde (147 mg, 0.932 mmol) according to general procedure A microwave method. Yield: 54%. HPLC RT 12.10 min; HPLC purity 99.59%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 8.99 (dd, *J* = 4.1, 1.5 Hz, 1H), 8.62 (d, *J* = 8.1 Hz, 1H), 8.38 (s, 1H), 8.14 – 8.10 (m, 2H), 8.07 (d, *J* = 7.2 Hz, 1H), 7.94 (s, 1H), 7.92 – 7.87 (m, 1H), 7.63 (dd, *J* = 8.6, 4.1 Hz, 1H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.33 – 7.14 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 150.87, 147.73, 137.24, 133.12, 132.90, 132.81, 130.24, 129.08, 127.36, 126.65, 126.28, 123.83, 122.54, 121.84, 120.65, 119.55, 117.99, 112.52, 110.46. LC-MS (ESI): *m/z* 296.1 $[M + H]^+$.

(*Z*)-2-(1H-indol-3-yl)-3-(quinolin-4-yl)acrylonitrile (4d)

Compound **4d** (148 mg, 0.501 mmol) was prepared as a yellow solid from indole-3-acetonitrile (118 mg, 0.753 mmol) and quinoline-4-carboxaldehyde (144 mg, 0.919 mmol) according to general procedure A microwave method. Yield: 67%. HPLC RT 12.26 min; HPLC purity 99.40%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 11.91 (bs, 1H), 9.03 (d, *J* = 4.5 Hz, 1H), 8.38 (s, 1H), 8.23 (d, *J* = 8.2 Hz, 1H), 8.16 – 8.11(m, 2H), 8.00 (s, 1H), 7.91 (d, *J* = 4.5 Hz, 1H), 7.85 (t, *J* = 7.6 Hz, 1H), 7.70 (t, *J* = 7.6 Hz, 1H), 7.55 (d, *J* = 7.7 Hz, 1H), 7.30 – 7.22 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 150.28, 147.91, 140.35, 137.28, 131.01,

129.86, 129.61, 128.15, 127.19, 125.73, 124.48, 123.70, 122.77, 120.95, 120.06, 119.59, 117.41, 112.58, 112.28, 110.32. MS (ESI): *m/z* 296.3 $[M + H]^+$.

(*Z*)-2-(1*H*-indol-3-yl)-3-(quinolin-8-yl)acrylonitrile (4e)

Compound **4e** (109 mg, 0.369 mmol) was prepared as a dark orange solid from indole-3-acetonitrile (107 mg, 0.684 mmol) and quinoline-8-carboxaldehyde (132 mg, 0.842 mmol) according to general procedure A microwave method. Yield: 54%. HPLC RT 12.15 min; HPLC purity 96.85%. ^{1}H NMR (400 MHz, Acetone-*d*6) δ: 10.87 (bs, 1H), 9.11 (s, 1H), 9.02 (dd, *J* = 4.1, 1.8 Hz, 1H), 8.63 (d, *J* = 7.4 Hz, 1H), 8.42 (dd, *J* = 8.3, 1.7 Hz, 1H), 8.29 – 8.20 (m, 1H), 8.06 (dd, *J* = 8.2, 1.1 Hz, 1H), 7.85 (s, 1H), 7.77 (t, *J* = 7.8 Hz, 1H), 7.63 (dd, *J* = 8.3, 4.2 Hz, 1H), 7.60 – 7.57 (m, 1H), 7.34 – 7.24 (m, 2H). ^{13}C NMR (100 MHz, Acetone-*d*6) δ: 151.17, 147.12, 138.74, 137.42, 134.09, 134.03, 130.38, 129.40, 128.56, 127.44, 127.23, 125.26, 123.77, 122.88, 121.80, 120.70, 119.26, 113.58, 113.33, 108.32. LC-MS (ESI): *m/z* 296.1 $[M + H]^+$.

(*Z*)-2-(1*H*-indol-3-yl)-3-(isoquinolin-4-yl)acrylonitrile (4f)

Compound **4f** (116 mg, 0.393 mmol) was prepared as a yellow solid from indole-3-acetonitrile (107 mg, 0.687 mmol) and 4-isoquinoline-carboxaldehyde (132 mg, 0.838 mmol) according to general procedure A microwave method. Yield: 57%. HPLC RT 12.29 min; HPLC purity 98.24%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 11.89 (bs, 1H), 9.39 (s, 1H), 8.90 (d, *J* = 0.9 Hz, 1H), 8.31 (s, 1H), 8.24 (d, *J* = 8.0 Hz, 1H), 8.21 – 8.12 (m, 2H), 7.94 (s, 1H), 7.92 – 7.88 (m, 1H), 7.82 – 7.77 (m, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.24 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 153.25, 142.06, 137.21, 133.24, 131.38, 130.92, 128.28, 128.05, 127.66, 127.50, 126.38, 123.78, 123.43, 122.62, 120.74, 119.54, 117.86, 112.51, 111.00, 110.36. LC-MS (ESI): *m/z* 296.2 $[M + H]^+$.

(*Z*)-2-(1*H*-indol-3-yl)-3-(2,7-naphthyridin-4-yl)acrylonitrile (4g)

Compound **4g** (42 mg, 0.142 mmol) was prepared as an orange solid from indole-3-acetonitrile (93 mg, 0.595 mmol) and 2,7-napthyridine-4-carboxaldehyde (86 mg, 0.541 mmol) according to general procedure A microwave method. Yield: 24%. HPLC RT 12.53 min; HPLC purity 98.20%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 9.63 (d, *J* = 0.9 Hz, 1H), 9.59 (s, 1H), 9.16 (d, *J* = 0.9 Hz, 1H), 8.84 (d, *J* = 6.0 Hz, 1H), 8.25 (s, 1H), 8.17 – 8.13 (m, 1H), 8.11 (d, *J* = 6.0 Hz, 1H), 7.97 (s, 1H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.24 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 153.40, 147.51, 145.50, 137.39, 136.17, 128.89, 128.06, 125.48, 123.79, 122.64, 120.78, 119.60, 117.72, 116.46, 112.64, 111.68, 110.32. LC-MS (ESI): *m/z* 297.1 [M + H]$^{+}$.

(*Z*)-2-(1*H*-benzo[*d*]imidazol-2-yl)-3-(isoquinolin-5-yl)acrylonitrile (6)

Compound **6** (134 mg, 0.452 mmol) was prepared as a light yellow-orange solid from 2-benzimidazole acetonitrile (157 mg, 0.606 mmol) and isoquinoline-5-carboxaldehyde (157 mg, 0.667 mmol) according to general procedure A microwave method. Yield: 74%. HPLC RT 10.71 min; HPLC purity 96.03%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 13.31 (bs, 1H), 9.46 (d, *J* = 0.7 Hz, 1H), 8.97 (s, 1H), 8.70 (d, *J* = 6.0 Hz, 1H), 8.51 (d, *J* = 7.3 Hz, 1H), 8.35 (d, *J* = 8.2 Hz, 1H), 8.22 (d, *J* = 6.0 Hz, 1H), 7.94 – 7.86 (m, 1H), 7.70 – 7.68 (m, 2H), 7.35 – 7.24 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 153.26, 147.24, 144.15, 141.32, 133.70, 131.10, 130.88, 128.92, 128.18, 127.20, 123.21, 116.60, 115.80, 106.79. LC-MS (ESI): *m/z* 297.1 [M + H]$^{+}$.

(*Z*)-3-(isoquinolin-5-yl)-2-(2-methyl-1*H*-indol-3-yl)acrylonitrile (10a)

*Step 1: 2-methyl-1H-indole-3-carbaldehyde (**8a**)*

Intermediate **8a** (810 mg, 5.091 mmol) was prepared as a pink solid from 2-methylindole (1.046 g, 7.974 mmol) according to general procedure D. Yield: 63%. ^{1}H NMR (400 MHz, Acetone-*d*6) δ: 10.93 (bs, 1H), 10.18 (s, 1H), 8.18 – 8.15 (m, 1H), 7.45 – 7.35 (m, 1H), 7.24 – 7.10 (m, 2H), 2.75 (s, 3H).

*Step 2: 2-(2-methyl-1H-indol-3-yl)acetonitrile (**9a**)*

Intermediate **9a** (178 mg, 1.046 mmol) was prepared as a yellow solid from intermediate **8a** (276 mg, 1.736 mmol) according to general procedure E using 63:37 hexane/ethyl acetate as the eluent for flash silica gel column chromatography. Yield: 60%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.97 (s, 1H), 7.59 – 7.50 (m, 1H), 7.34 – 7.28 (m, 1H), 7.24 – 7.10 (m, 2H), 3.75 (s, 2H), 2.43 (s, 3H).

*Step 3: (Z)-3-(isoquinolin-5-yl)-2-(2-methyl-1H-indol-3-yl)acrylonitrile (**10a**)*

Compound **10a** (31 mg, 0.100 mmol) was prepared as a shiny yellow solid from intermediate **9a** (43 mg, 0.254 mmol) and isoquinoline-5-carboxaldehyde (68 mg, 0.432 mmol) according to general procedure A sealed tube method. Compound **10a** was obtained as an inseparable mixture of approximately 88:12 double bond geometry (calculated from ^{1}H NMR). Yield: 40%. HPLC RT 13.79, 13.96 min (overlapping peaks); HPLC peak areas 4.54%, 95.46%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 9.41 (d, *J* = 0.7 Hz, 1H), 8.60 (d, *J* = 6.0 Hz, 1H), 8.33 (d, *J* = 7.3 Hz, 1H), 8.25 (d, *J* = 8.2 Hz, 1H), 8.08 (s, 1H), 8.01 (d, *J* = 6.0 Hz, 1H), 7.89 – 7.81 (m, 1H), 7.78 (dd, *J* = 6.9, 1.2 Hz, 1H), 7.42 – 7.40 (m, 1H), 7.19 – 7.08 (m, 2H), 2.64 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 152.96, 143.75, 137.83, 136.03, 135.01, 133.46, 130.91, 130.37, 129.18, 128.20, 127.07, 125.93, 121.48, 120.04, 117.93, 117.87, 116.96, 111.20, 109.17, 106.36, 12.65. Chemical shifts were given for major isomer. LC-MS (ESI): *m/z* 310.1 $[M + H]^+$

(*Z*)-2-(4-bromo-1*H*-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (10b)

*Step 1: 4-bromo-1H-indole-3-carbaldehyde (**8b**)*

Intermediate **8b** (373 mg, 1.663 mmol) was prepared as an off-white solid from 4-bromoindole (663 mg, 3.383 mmol) according to general procedure D. Yield: 49%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 12.57 (bs, 1H), 10.68 (s, 1H), 8.30 (s, 1H), 7.57 (dd, *J* = 8.1, 0.8 Hz, 1H), 7.47 (dd, *J* = 7.7, 0.7 Hz, 1H), 7.17 (t, *J* = 7.9 Hz, 1H).

*Step 2: 2-(4-bromo-1H-indol-3-yl)acetonitrile (**9b**)*

Intermediate **9b** (122 mg, 0.519 mmol) was prepared as a brown solid from intermediate **8b** (235 mg, 1.049 mmol) according to general procedure E using 68:32 hexane/ethyl acetate as the eluent for flash silica gel column chromatography. Yield: 49%. ^{1}H NMR (400 MHz, Acetone-*d*6) δ: 10.64 (bs, 1H), 7.55 – 7.51 (m, 1H), 7.47 (dd, *J* = 8.2, 0.8 Hz, 1H), 7.26 (dd, *J* = 7.6, 0.8 Hz, 1H), 7.06 (t, *J* = 7.9 Hz, 1H), 4.22 (d, *J* = 0.9 Hz, 2H).

*Step 3: (Z)-2-(4-bromo-1H-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (**10b**)*

Compound **10b** (32 mg, 0.019 mmol) was prepared as a yellow solid from intermediate **9b** (52 mg, 0.221 mmol) and isoquinoline-5-carboxaldehyde (59 mg, 0.376 mmol) according to general procedure A sealed tube method. The reaction was evaporated under vacuum to remove the solvent and purified by silica gel flash column chromatography (100% ethyl acetate). The material obtained was dried under vacuum and washed with methanol to afford a yellow solid. Compound **10b** was obtained as an inseparable mixture of approximately 75:25 double bond geometry (calculated from ^{1}H NMR). Yield: 39%. HPLC RT 13.20, 13.36 min (overlapping peaks); HPLC peak areas 13.50%, 85.98%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 11.89 (bs, 1H), 9.42 (d, *J* = 0.5 Hz, 1H), 8.60 (d, *J* = 6.0 Hz, 1H), 8.32 (d, *J* = 7.2 Hz, 1H), 8.27 (d, *J* = 8.2 Hz, 1H), 8.10 – 8.07 (m, 2H), 7.90 (s, 1H), 7.89 – 7.83 (m, 1H), 7.54 (dd, *J* = 8.1, 0.7 Hz, 1H), 7.38 – 7.33 (m, 1H), 7.13 (t, *J* = 7.9 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 152.94, 143.73, 142.50, 137.40, 133.34, 130.56, 130.27, 129.57, 128.50, 128.15, 127.14, 124.10, 124.05, 123.27, 118.82, 116.99, 112.50, 111.88, 110.68, 109.29. Chemical shifts were given for the major isomer. LC-MS (ESI): *m/z* 374.0, 376.0 $[M + H]^{+}$.

(*Z*)-3-(isoquinolin-5-yl)-2-(5-methoxy-1*H*-indol-3-yl)acrylonitrile (10c)

Compound **10c** (602 mg, 1.849 mmol) was prepared as a yellow solid from 5-methoxy-indole-3-acetonitrile (525 mg, 2.819 mmol) and isoquinoline-5-carboxaldehyde (545 mg, 3.468 mmol) according to general procedure A microwave method. Yield: 66%. HPLC RT

11.98 min; HPLC purity 97.67%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 9.40 (s, 1H), 8.60 (d, *J* = 6.0 Hz, 1H), 8.30 (s, 1H), 8.26 – 8.21 (m, 2H), 8.04 (d, *J* = 6.0 Hz, 1H), 7.89 (s, 1H), 7.86 – 7.76 (m, 1H), 7.53 (d, *J* = 2.3 Hz, 1H), 7.43 (d, *J* = 8.9 Hz, 1H), 6.91 (dd, *J* = 8.9, 2.3 Hz, 1H), 3.82 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 154.55, 152.91, 143.64, 133.57, 132.44, 132.19, 131.47, 130.27, 128.81, 128.23, 127.67, 127.11, 124.26, 117.98, 117.18, 113.20, 112.41, 110.47, 110.15, 101.66, 55.51. LC-MS (ESI): *m/z* 326.1 $[M + H]^+$. HRMS (ESI): *m/z* calculated $[M + H]^+$ $C_{21}H_{16}N_3O^+$ 326.1288, found 326.1294 (Diff = 1.8 ppm)

(*Z*)-2-(5-fluoro-1*H*-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (10d)

*Step 1: 5-fluoro-1H-indole-3-carbaldehyde (**8d**)*

Intermediate **8d** (1.006 g, 6.166 mmol) was prepared as an orange solid from 5-fluoroindole (1.108 g, 8.199 mmol) according to general procedure D. Yield: 75%. ^{1}H NMR (400 MHz, Acetone-*d*6) δ: 11.23 (bs, 1H), 10.02 (s, 1H), 8.26 (d, *J* = 2.9 Hz, 1H), 7.88 (dd, *J* = 9.5, 2.6 Hz, 1H), 7.56 (dd, *J* = 8.9, 4.2 Hz, 1H), 7.08 (td, *J* = 9.1, 2.6 Hz, 1H). ^{19}F NMR (376 MHz, Acetone-*d*6) δ: -122.83.

*Step 2: 2-(5-fluoro-1H-indol-3-yl)acetonitrile (**9d**)*

Intermediate **9d** (342 mg, 1.964 mmol) was prepared as a brown oil from intermediate **8d** (505 mg, 3.098 mmol) according to general procedure E using 65:35 hexane/ethyl acetate as the eluent for flash silica gel column chromatography. Yield: 63%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.24 (bs, 1H), 7.32 (dd, *J* = 8.9, 4.3 Hz, 1H), 7.28 – 7.25 (m, 1H), 7.23 (dd, *J* = 9.2, 2.4 Hz, 1H), 7.00 (td, *J* = 9.0, 2.4 Hz, 1H), 3.79 (d, *J* = 0.9 Hz, 2H). ^{19}F NMR (376 MHz, $CDCl_3$) δ: -123.15.

*Step 3: (Z)-2-(5-fluoro-1H-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (**10d**)*

Compound **10d** (134 mg, 0.460 mmol) was prepared as a yellow solid from intermediate **9d** (79 mg, 0.455 mmol) and isoquinoline-5-carboxaldehyde (107 mg, 0.683 mmol) according to general procedure A sealed tube method. Yield: 94%. HPLC RT 12.22 min; HPLC purity

99.72%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 9.40 (d, *J* = 0.7 Hz, 1H), 8.63 – 8.56 (m, 1H), 8.33 (s, 1H), 8.23 (t, *J* = 8.0 Hz, 2H), 8.06 (d, *J* = 6.0 Hz, 1H), 8.02 (s, 1H), 7.88 (dd, *J* = 10.5, 2.4 Hz, 1H), 7.85 – 7.79 (m, 1H), 7.54 (dd, *J* = 8.9, 4.7 Hz, 1H), 7.11 (td, *J* = 9.1, 2.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 158.92, 156.60, 152.84, 143.59, 133.92, 133.52, 133.10, 131.33, 130.30, 129.14, 128.90, 128.17, 127.04, 124.03, 123.93, 117.82, 117.27, 113.68, 113.59, 110.81, 110.55, 110.46, 110.42, 109.99, 104.65, 104.40. ^{19}F NMR (376 MHz, DMSO-*d*6) δ: -122.46. LC-MS (ESI): *m/z* 314.1 $[M + H]^+$.

(*Z*)-2-(5-chloro-1*H*-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (10e)

*Step 1: 2-(5-chloro-1H-indol-3-yl)acetonitrile (**9e**)*

Intermediate **9e** (687 mg, 3.604 mmol) was prepared as a beige solid from 5-chloro-1*H*-indole-3-carboxaldehyde (959 mg, 5.338 mmol) according to general procedure E using 7:3 hexane/ethyl acetate as the eluent for flash silica gel column chromatography. Yield: 68%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.28 (bs, 1H), 7.55 (d, *J* = 1.9 Hz, 1H), 7.34 – 7.29 (m, 1H), 7.25 – 7.23 (m, 1H), 7.20 (dd, *J* = 8.7, 1.9 Hz, 1H), 3.79 (d, *J* = 1.0 Hz, 2H).

*Step 2: (Z)-2-(5-chloro-1H-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (**10e**)*

Compound **10e** (55 mg, 0.165 mmol) was prepared as a shiny yellow solid from intermediate **9e** (82 mg, 0.431 mmol) and isoquinoline-5-carboxaldehyde (75 mg, 0.474 mmol) according to general procedure A microwave method. Yield: 38%. HPLC RT 12.63 min; HPLC purity 100.00%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 9.40 (d, *J* = 0.6 Hz, 1H), 8.60 (d, *J* = 6.0 Hz, 1H), 8.35 (s, 1H), 8.23 (t, *J* = 7.8 Hz, 2H), 8.11 (d, *J* = 1.9 Hz, 1H), 8.08 – 8.01 (m, 2H), 7.86 – 7.77 (m, 1H), 7.55 (d, *J* = 8.7 Hz, 1H), 7.25 (dd, *J* = 8.7, 2.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 152.89, 143.62, 136.04, 133.66, 133.56, 131.36, 130.39, 129.22, 128.98, 128.20, 127.11, 125.20, 125.02, 122.39, 118.58, 117.92, 117.33, 114.22, 109.97, 109.78. LC-MS (ESI): *m/z* 330.0 $[M + H]^+$.

(*Z*)-2-(5-bromo-1*H*-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (10f)

*Step 1: 2-(5-bromo-1H-indol-3-yl)acetonitrile (**9f**)*

Intermediate **9f** (295 mg, 1.255 mmol) was prepared as a white solid from 5-bromo-1*H*-indole-3-carboxaldehyde (366 mg, 1.631 mmol) according to general procedure E using 65:35 hexane/ethyl acetate as the eluent for flash silica gel column chromatography. Yield: 76%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (bs, 1H), 7.76 – 7.69 (m, 1H), 7.34 (dd, *J* = 8.7, 1.8 Hz, 1H), 7.29 – 7.26 (m, 1H), 7.25 – 7.23 (m, 1H), 3.80 (d, *J* = 1.0 Hz, 2H).

*Step 2: (Z)-2-(5-bromo-1H-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (**10f**)*

Compound **10f** (31 mg, 0.083 mmol) was prepared as a yellow solid from intermediate **9f** (61 mg, 0.261 mmol) and isoquinoline-5-carboxaldehyde (45 mg, 0.287 mmol) according to general procedure A microwave method. Yield: 32%. HPLC RT 12.83 min; HPLC purity 100.00%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 9.40 (d, *J* = 0.8 Hz, 1H), 8.60 (d, *J* = 6.0 Hz, 1H), 8.35 (s, 1H), 8.28 – 8.17 (m, 3H), 8.06 (d, *J* = 6.0 Hz, 1H), 8.03 (s, 1H), 7.87 – 7.73 (m, 1H), 7.51 (d, *J* = 8.7 Hz, 1H), 7.36 (dd, *J* = 8.7, 1.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 152.88, 143.60, 136.41, 133.61, 133.54, 131.37, 130.36, 129.20, 128.94, 128.19, 127.09, 125.75, 124.87, 121.47, 117.93, 117.32, 114.71, 113.14, 109.80, 109.74. LC-MS (ESI): *m/z* 374.0, 376.0 $[M + H]^+$.

N-(3-((Z)-1-cyano-2-(isoquinolin-5-yl)vinyl)-1H-indol-5-yl)acetamide (10g)

*Step 1: 1H-indol-5-amine (**16**)*

5-nitro-1*H*-indole (6.3 g, 38.9 mmol, 1 equiv) was dissolved in ethanol (30 mL). 10% palladium on carbon (630 mg, 10 mmol%) was added. The reaction flask was shaken under hydrogen at 30 psi using the Parr Shaker Hydrogenation Apparatus for 2.5 h. The reaction was filtered over celite and washed with ethyl acetate. The filtrate was evaporated, concentrated *in vacuo* and purified by silica gel flash column chromatography (1:1 hexane/ethyl acetate) to afford intermediate **16** as a black solid (2.7 g, 20.4 mmol). Yield:

53%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.96 (bs, 1H), 7.22 – 7.17 (m, 1H), 7.13 (t, *J* = 2.8 Hz, 1H), 6.97 – 6.94 (m, 1H), 6.67 (dd, *J* = 8.5, 2.2 Hz, 1H), 6.39 – 6.37 (m, 1H), 3.49 (s, 2H).

*Step 2: N-(1H-indol-5-yl)acetamide (**7g**)*

To a solution of intermediate **16** (156 mg, 1.182 mmol, 1.0 equiv) and triethylamine (591 µL, 429 mg, 4.240 mmol, 3.6 equiv) in DCM (10 mL), acetyl chloride (101 µL, 111 mg, 1.418 mmol, 1.2 equiv) was added. The reaction was stirred at room temperature for 3 h. The mixture was poured into water and extracted with dichloromethane 3 times. The combined organic layer was washed with brine, dried over anhydrous sodium sulphate and concentrated *in vacuo*. Purification by flash silica gel column chromatography (7:3 hexane/ethyl acetate) afforded intermediate **7g** as a clear oil (177 mg, 1.016 mmol). Yield: 85%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (bs, 1H), 7.80 (s, 1H), 7.30 (d, *J* = 8.5 Hz, 2H), 7.20 (dd, *J* = 8.8, 2.0 Hz, 2H), 6.50 (s, 1H), 2.18 (s, 3H).

*Step 3: N-(3-formyl-1H-indol-5-yl)acetamide (**8g**)*

Intermediate **8g** (115 mg, 0.569 mmol) was prepared as a light yellow solid from intermediate **7g** (192 mg, 1.102 mmol) according to general procedure D. Following drop-wise addition of 1N NaOH (10x reaction volume), extraction with ethyl acetate was performed 3 times. The combined organic layer was washed with brine, dried over anhydrous sodium sulphate and concentrated *in vacuo*. Purification by flash silica gel column chromatography (100% ethyl acetate) afforded the product. Yield: 52%. ^{1}H NMR (400 MHz, Acetone-*d*6) δ: 11.08 (bs, 1H), 9.99 (s, 1H), 9.12 (bs, 1H), 8.38 (d, *J* = 1.7 Hz, 1H), 8.14 (d, *J* = 2.3 Hz, 1H), 7.71 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.44 (d, *J* = 8.8 Hz, 1H), 2.11 (s, 3H).

Step 4: N-(3-(cyanomethyl)-1*H*-indol-5-yl)acetamide *(**9g**)*

Intermediate **9g** (42 mg, 0.197 mmol) was prepared as a pale yellow solid from Intermediate **8g** (109 mg, 0.539 mmol) according to general procedure E using 15:85 hexane/ethyl acetate as the eluent for flash silica gel column chromatography. Yield: 36%. ^{1}H NMR (400 MHz,

Acetone-*d*6) δ: 10.25 (bs, 1H), 9.10 (bs, 1H), 7.95 (d, *J* = 1.7 Hz, 1H), 7.41 – 7.32 (m, 3H), 3.94 (d, *J* = 0.8 Hz, 2H), 2.10 (s, 3H).

*Step 5: N-(3-((Z)-1-cyano-2-(isoquinolin-5-yl)vinyl)-1H-indol-5-yl)acetamide (**10g**)*

Compound **10g** (5 mg, 0.014 mmol) was prepared as a yellow solid from intermediate **9g** (42 mg, 0.197 mmol) and isoquinoline-5-carboxaldehyde (35 mg, 0.223 mmol) according to general procedure A sealed tube method. The reaction was concentrated *in vacuo* and purified by silica gel flash column chromatography (9:1 ethyl acetate/methanol). The crude material obtained was dried under vacuum and triturated with small amounts of methanol to obtain a yellow precipitate. Yield: 7%. HPLC RT 10.87 min; HPLC purity 96.20%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 11.74 (s, 1H), 9.92 (s, 1H), 9.41 (s, 1H), 8.61 (d, *J* = 6.0 Hz, 1H), 8.42 (s, 1H), 8.30 (d, *J* = 7.3 Hz, 1H), 8.23 (d, *J* = 8.2 Hz, 1H), 8.21 (s, 1H), 8.09 (d, *J* = 6.0 Hz, 1H), 7.88 (s, 1H), 7.86 – 7.77 (m, 1H), 7.45 (d, *J* = 1.0 Hz, 2H), 2.05 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 168.11, 153.13, 143.89, 133.73, 133.59, 133.34, 131.93, 130.98, 130.20, 129.13, 128.34, 127.86, 127.26, 123.74, 117.88, 116.87, 116.12, 112.48, 110.55, 110.29, 109.52, 24.03. LC-MS (ESI): *m/z* 353.1 $[M + H]^{+}$.

(*Z*)-3-(isoquinolin-5-yl)-2-(6-methoxy-1*H*-indol-3-yl)acrylonitrile (10h)

*Step 1: 6-methoxy-1H-indole-3-carbaldehyde (**8h**)*

Intermediate **8h** (1.579 g, 9.011 mmol) was prepared as a black solid from 6-methoxyindole (2.244 g, 15.25 mmol) according to general procedure D. Yield: 59%. ^{1}H NMR (400 MHz, Acetone-*d*6) δ: 10.95 (bs, 1H), 9.97 (s, 1H), 8.08 – 8.05 (m, 2H), 7.06 (d, *J* = 2.3 Hz, 1H), 6.90 (dd, *J* = 8.7, 2.3 Hz, 1H), 3.82 (s, 3H).

*Step 2: 2-(6-methoxy-1H-indol-3-yl)acetonitrile (**9h**)*

Intermediate **9h** (268 mg, 1.438 mmol) was prepared as a beige solid from intermediate **8h** (561 mg, 3.201 mmol) according to general procedure E using 65:35 hexane/ethyl acetate as the eluent for flash silica gel column chromatography. Yield: 45%. ^{1}H NMR (400 MHz,

$CDCl_3$) δ: 8.08 (bs, 1H), 7.45 (d, *J* = 8.4 Hz, 1H), 7.10 – 7.08 (m, 1H), 6.87 – 6.84 (m, 2H), 3.85 (s, 3H), 3.79 (d, *J* = 1.1 Hz, 2H).

*Step 3: (Z)-3-(isoquinolin-5-yl)-2-(6-methoxy-1H-indol-3-yl)acrylonitrile (**10h**)*

Compound **10h** (89 mg, 0.274 mmol) was prepared as a yellow solid from intermediate **9h** (58 mg, 0.313 mmol) and isoquinoline-5-carboxaldehyde (49 mg, 0.313 mmol) according to general procedure A sealed tube method. Yield: 88%. HPLC RT 12.10 min; HPLC purity 98.37%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 9.40 (d, *J* = 0.7 Hz, 1H), 8.60 (d, *J* = 6.0 Hz, 1H), 8.28 (s, 1H), 8.26 – 8.19 (m, 2H), 8.03 (d, *J* = 6.0 Hz, 1H), 8.00 (d, *J* = 8.8 Hz, 1H), 7.84 – 7.79 (m, 1H), 7.79 (s, 1H), 7.02 (s, 1H), 6.86 (dd, *J* = 8.8, 2.4 Hz, 1H), 3.82 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 156.30, 152.94, 143.69, 138.28, 133.56, 131.90, 131.37, 130.28, 128.88, 128.25, 127.14, 126.34, 120.33, 117.99, 117.94, 117.16, 110.78, 110.67, 110.56, 95.38, 55.26. LC-MS (ESI): *m/z* 326.1 $[M + H]^+$.

(*Z*)-2-(6-bromo-1*H*-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (10i)

*Step 1: 6-bromo-1H-indole-3-carbaldehyde (**8i**)*

Intermediate **8i** (7.800 g, 34.81 mmol) was prepared as a brown solid from 6-bromoindole (7.533 g, 38.42 mmol) according to general procedure D. Yield: 90%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.85 (s, 1H), 8.03 (d, *J* = 8.4 Hz, 1H), 7.77 (s, 1H), 7.54 – 7.53 (m, 1H), 7.30 (dd, *J* = 8.4, 1.7 Hz, 1H).

*Step 2: 2-(6-bromo-1H-indol-3-yl)acetonitrile (**9i**)*

Intermediate **9i** (347 mg, 1.476 mmol) was prepared as a beige solid from intermediate **8i** (843 mg, 3.764 mmol) according to general procedure E using 7:3 hexane/ethyl acetate as the eluent for flash silica gel column chromatography. Yield: 39%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.34 (bs, 1H), 7.55 (d, *J* = 1.6 Hz, 1H), 7.44 (d, *J* = 8.5 Hz, 1H), 7.28 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.20 – 7.17 (m, 1H), 3.81 (d, *J* = 1.0 Hz, 2H).

*Step 3: (Z)-2-(6-bromo-1H-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (**10i**)*

Compound **10i** (99 mg, 0.265 mmol) was prepared as a yellow solid from intermediate **9i** (71 mg, 0.301 mmol) and isoquinoline-5-carboxaldehyde (47 mg, 0.301 mmol) according to general procedure A sealed tube method. Yield: 87%. HPLC RT 13.00 min; HPLC purity 96.14%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 9.40 (d, *J* = 0.5 Hz, 1H), 8.60 (d, *J* = 6.0 Hz, 1H), 8.33 (s, 1H), 8.26 (d, *J* = 7.3 Hz, 1H), 8.22 (d, *J* = 8.2 Hz, 1H), 8.07 – 8.04 (m, 2H), 7.99 (s, 1H), 7.85 – 7.79 (m, 1H), 7.73 (d, *J* = 1.7 Hz, 1H), 7.32 (dd, *J* = 8.6, 1.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 152.92, 143.67, 138.53, 133.52, 133.15, 131.18, 130.29, 129.00, 128.82, 128.21, 127.10, 123.28, 123.01, 121.17, 117.87, 117.16, 115.27, 115.02, 110.48, 109.87. LC-MS (ESI): *m/z* 374.0, 376.0 $[M + H]^+$.

(*Z*)-3-(isoquinolin-5-yl)-2-(5-nitro-1*H*-indol-3-yl)acrylonitrile (14a)

*Step 1: N,N-dimethyl(5-nitro-1H-indol-3-yl)methanamine (**12a**)*

Intermediate **12a** (1.62 g, 7.389 mmol) was prepared as a yellow solid from 5-nitroindole (1.72 g, 10.60 mmol) according to general procedure B. Yield: 70%. ^{1}H NMR (400 MHz, MeOD) δ: 8.67 – 8.63 (m, 1H), 8.05 (dd, *J* = 9.0, 2.2 Hz, 1H), 7.48 (dd, *J* = 9.0, 0.4 Hz, 1H), 7.43 (s, 1H), 3.72 (s, 2H), 2.29 (s, 6H).

*Step 2: 2-(5-nitro-1H-indol-3-yl)acetonitrile (**13a**)*

Intermediate **13a** (294 mg, 1.461 mmol) was prepared as yellow-orange solid from intermediate **12a** (622 mg, 2.839 mmol) according to general procedure C using 3:2 hexane/ethyl acetate as the eluent for flash silica gel column chromatography. Yield: 51%. ^{1}H NMR (400 MHz, Acetone-*d*6) δ: 10.99 (bs, 1H), 8.69 (d, *J* = 2.2 Hz, 1H), 8.10 (dd, *J* = 9.0, 2.2 Hz, 1H), 7.68 (s, 1H), 7.65 (dd, *J* = 9.0, 0.4 Hz, 1H), 4.18 (d, *J* = 1.0 Hz, 2H).

*Step 3: (Z)-3-(isoquinolin-5-yl)-2-(5-nitro-1H-indol-3-yl)acrylonitrile (**14a**)*

Compound **14a** (144 mg, 0.422 mmol) was prepared as a yellow solid from intermediate **13a** (130 mg, 0.648 mmol) and isoquinoline-5-carboxaldehyde (112 mg, 0.713 mmol) according to general procedure A microwave method. Yield: 65%. HPLC purity not determined due to

poor solubility. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 9.42 (d, *J* = 0.6 Hz, 1H), 9.00 (d, *J* = 2.1 Hz, 1H), 8.61 (d, *J* = 6.0 Hz, 1H), 8.50 (s, 1H), 8.32 (d, *J* = 7.3 Hz, 1H), 8.29 (s, 1H), 8.26 (d, *J* = 8.2 Hz, 1H), 8.16 – 8.07 (m, 2H), 7.90 – 7.78 (m, 1H), 7.71 (d, *J* = 9.0 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 152.97, 143.70, 141.62, 140.22, 135.95, 133.55, 130.86, 130.52, 130.50, 129.42, 128.19, 127.13, 123.31, 117.81, 117.59, 117.18, 116.24, 113.10, 112.33, 108.62. LC-MS (ESI): *m/z* 341.1 $[M + H]^{+}$.

(*Z*)-3-(isoquinolin-5-yl)-2-(7-methoxy-1*H*-indol-3-yl)acrylonitrile (14b)

*Step 1: (7-methoxy-1H-indol-3-yl)-N,N-dimethylmethanamine (**12b**)*

Intermediate **12b** (574 mg, 2.810 mmol) was prepared as a dark yellow oil from 7-methoxyindole (427 mg, 2.897 mmol) according to general procedure B. Yield: 97%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.40 (bs, 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 7.14 (d, *J* = 2.3 Hz, 1H), 7.05 (t, *J* = 7.9 Hz, 1H), 6.65 (d, *J* = 7.6 Hz, 1H), 3.96 (s, 3H), 3.66 (s, 2H), 2.30 (s, 6H).

*Step 2: 2-(7-methoxy-1H-indol-3-yl)acetonitrile (**13b**)*

Intermediate **13b** (138 mg, 0.739 mmol) was prepared as a white solid from intermediate **12b** (392 mg, 1.919 mmol) according to general procedure C using 3:1 hexane/ethyl acetate as the eluent for flash silica gel column chromatography. Yield: 39%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.38 (bs, 1H), 7.20 – 7.18 (m, 2H), 7.10 (t, *J* = 7.8 Hz, 1H), 6.70 (d, *J* = 7.7 Hz, 1H), 3.97 (s, 3H), 3.82 (s, 2H).

*Step 3: (Z)-3-(isoquinolin-5-yl)-2-(7-methoxy-1H-indol-3-yl)acrylonitrile (**14b**)*

Compound **14b** (105 mg, 0.323 mmol) was prepared as a yellow solid from intermediate **13b** (73 mg, 0.393 mmol) and isoquinoline-5-carboxaldehyde (62 mg, 0.393 mmol) according to general procedure A sealed tube method. Yield: 82%. HPLC RT 11.55 min; HPLC purity 95.93%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 9.40 (d, *J* = 0.5 Hz, 1H), 8.60 (d, *J* = 6.0 Hz, 1H), 8.31 (s, 1H), 8.26 (d, *J* = 7.3 Hz, 1H), 8.22 (d, *J* = 8.2 Hz, 1H), 8.04 (d, *J* = 6.0 Hz, 1H), 7.86 – 7.78 (m, 2H), 7.69 (d, *J* = 8.0 Hz, 1H), 7.14 (t, *J* = 8.0 Hz, 1H), 6.83 (d, *J* = 7.7 Hz, 1H),

3.97 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 152.92, 146.53, 143.69, 133.54, 132.59, 131.24, 130.21, 128.90, 128.22, 127.45, 127.10, 126.71, 125.33, 121.41, 117.97, 117.09, 112.00, 110.91, 110.35, 103.07, 55.32. LC-MS (ESI): *m/z* 326.1 $[M + H]^+$.

(Z)-2-(7-bromo-1*H*-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (14c)

*Step 1: (7-bromo-1H-indol-3-yl)-N,N-dimethylmethanamine (**12c**)*

Intermediate **12c** (610 mg, 2.410 mmol) was prepared as a brown solid from 7-bromoindole (546 mg, 2.785 mmol) according to general procedure B. Yield: 86%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.55 (dd, *J* = 8.0, 0.8 Hz, 1H), 7.30 (d, *J* = 7.6 Hz, 1H), 7.25 (s, 1H), 6.97 (t, *J* = 7.8 Hz, 1H), 3.67 (s, 2H), 2.29 (s, 6H).

*Step 2: 2-(7-bromo-1H-indol-3-yl)acetonitrile (**13c**)*

Intermediate **13c** (224 mg, 0.951 mmol) was prepared as an off-white solid from intermediate **12c** (592 mg, 2.339 mmol) according to general procedure C using 4:1 hexane/ethyl acetate as the eluent for flash silica gel column chromatography. Yield: 41%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.37 (bs, 1H), 7.55 (d, *J* = 8.0 Hz, 1H), 7.45 – 7.38 (m, 1H), 7.32 – 7.28 (m, 1H), 7.08 (t, *J* = 7.8 Hz, 1H), 3.83 (d, *J* = 1.0 Hz, 2H).

*Step 3: (Z)-2-(7-bromo-1H-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (**14c**)*

Compound **14c** (66 mg, 0.176 mmol) was prepared as a shiny yellow solid from intermediate **13c** (46 mg, 0.197 mmol) and isoquinoline-5-carboxaldehyde (31 mg, 0.197 mmol) according to general procedure A sealed tube method. Yield: 90%. HPLC RT 12.92 min; HPLC purity 99.05%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 9.40 (d, *J* = 0.8 Hz, 1H), 8.60 (d, *J* = 6.0 Hz, 1H), 8.38 (s, 1H), 8.28 (d, *J* = 7.3 Hz, 1H), 8.23 (d, *J* = 8.2 Hz, 1H), 8.12 (dd, *J* = 8.1, 0.7 Hz, 1H), 8.07 (d, *J* = 6.0 Hz, 1H), 7.99 (s, 1H), 7.86 – 7.79 (m, 1H), 7.48 (d, *J* = 7.1 Hz, 1H), 7.15 (t, *J* = 7.8 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 152.94, 143.71, 135.86, 134.00, 133.55, 131.11, 130.33, 129.12, 128.63, 128.22, 127.12, 125.69, 124.99, 121.85, 118.89, 117.88, 117.19, 111.42, 109.70, 105.32. LC-MS (ESI): *m/z* 374.0, 376.0 $[M + H]^+$.

(*Z*)-3-(isoquinolin-5-yl)-2-(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)acrylonitrile (14d)

Step 1: N,N-dimethyl(1*H*-pyrrolo[2,3-*b*]pyridin-3-yl)methanamine (**12d**)

Intermediate **12d** (443 mg, 2.528 mmol) was prepared as a white solid from 7-azaindole (346 mg, 2.925 mmol) according to general procedure B. Yield: 86%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.44 (bs, 1H), 8.31 (dd, *J* = 4.7, 1.3 Hz, 1H), 8.05 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.27 (s, 1H), 7.08 (dd, *J* = 7.8, 4.8 Hz, 1H), 3.61 (s, 2H), 2.27 (s, 6H).

*Step 2: 2-(1H-pyrrolo[2,3-b]pyridin-3-yl)acetonitrile (**13d**)*

Intermediate **13d** (149 mg, 0.951 mmol) was prepared as a white solid from intermediate **12d** (235 mg, 1.344 mmol) according to general procedure C using 65:35 hexane/ethyl acetate as the eluent for flash silica gel column chromatography. Yield: 71%. ^{1}H NMR (400 MHz, MeOD) δ: 8.24 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.08 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.41 (s, 1H), 7.16 (dd, *J* = 7.9, 4.8 Hz, 1H), 3.99 (d, *J* = 1.0 Hz, 2H).

Step 3: *(Z)-3-(isoquinolin-5-yl)-2-(1H-pyrrolo[2,3-b]pyridin-3-yl)acrylonitrile (**14d**)*

Compound **14d** (78 mg, 0.263 mmol) was prepared as a pale yellow solid from intermediate **13d** (57 mg, 0.365 mmol) and isoquinoline-5-carboxaldehyde (57 mg, 0.365 mmol) according to general procedure A microwave method. Yield: 72%. HPLC purity not determined due to poor solubility. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 12.37 (s, 1H), 9.41 (s, 1H), 8.60 (d, *J* = 6.0 Hz, 1H), 8.55 (dd, *J* = 8.0, 1.4 Hz, 1H), 8.41 (s, 1H), 8.37 (dd, *J* = 4.6, 1.4 Hz, 1H), 8.27 (d, *J* = 7.2 Hz, 1H), 8.24 (d, *J* = 8.2 Hz, 1H), 8.09 – 8.07 (m, 2H), 7.87 – 7.78 (m, 1H), 7.26 (dd, *J* = 8.0, 4.7 Hz, 1H). LC-MS (ESI): *m/z* 297.1 $[M + H]^+$.

(*Z*)-2-(1*H*-indazol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (20)

*Step 1: 1H-indazole-3-carbaldehyde (**18**)*

An aqueous solution of 0.25 M $NaNO_2$ (200 ml, 50 mmol, 1.05 equiv) was acidified with dilute HCl to pH 2.5. To this solution, dioxane (30ml, 15% v/v) was added. The flask was protected from light by wrapping with aluminium foil. Indole (5.578g, 47.62 mmol, 1.0 equiv)

was added slowly in small portions. The mixture was stirred vigorously at room temperature for 2 h. The liquid in the reaction flask was decanted out and extracted with ethyl acetate 3 times. The combined organic layer was washed with brine, dried over anhydrous sodium sulphate, concentrated *in vacuo* and purified by flash silica gel column chromatography (7:3 hexane/ethyl acetate) to afford intermediate **18** as a brown solid (514 mg, 3.517 mmol). Yield: 7%. ^{1}H NMR (400 MHz, Acetone-*d*6) δ: 10.23 (s, 1H), 8.23 (dt, *J* = 8.1, 1.0 Hz, 1H), 7.74 (dt, *J* = 8.5, 0.9 Hz, 1H), 7.55 – 7.48 (m, 1H), 7.41 – 7.36 (m, 1H).

*Step 2: 2-(1H-indazol-3-yl)acetonitrile (**19**)*

Intermediate **19** (330 mg, 2.100 mmol) was prepared as a brown oil from intermediate **18** (513 mg, 3.513 mmol) according to general procedure E using 63:27 hexane/ethyl acetate as the eluent for flash silica gel column chromatography. Yield: 60%. ^{1}H NMR (400 MHz, Acetone-*d*6) δ: 12.25 (s, 1H), 7.86 (dt, *J* = 8.2, 0.9 Hz, 1H), 7.60 (dt, *J* = 8.5, 0.8 Hz, 1H), 7.45 – 7.39 (m, 1H), 7.23 – 7.17 (m, 1H), 4.28 (s, 2H).

*Step 3: (Z)-2-(1H-indazol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (**20**)*

Compound **20** (30 mg, 0.101 mmol) was prepared as a light yellow-orange solid from intermediate **19** (104 mg, 0.663 mmol) and isoquinoline-5-carboxaldehyde (130 mg, 0.829 mmol) according to general procedure A sealed tube method. After the reaction was cooled to room temperature, the mixture was filtered and the evaporated under vacuum and the resulting material was recrystallized with methanol to afford **20** as a light yellow-orange solid. Yield: 15%. HPLC RT 11.55 min; HPLC purity 95.60%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 9.43 (d, *J* = 0.7 Hz, 1H), 8.70 (s, 1H), 8.62 (d, *J* = 6.0 Hz, 1H), 8.36 (d, *J* = 7.3 Hz, 1H), 8.28 (d, *J* = 8.2 Hz, 1H), 8.24 (d, *J* = 8.3 Hz, 1H), 8.04 (d, *J* = 6.0 Hz, 1H), 7.89 – 7.82 (m, 1H), 7.68 (d, *J* = 8.4 Hz, 1H), 7.45 – 7.39 (m, 1H), 7.26 (t, *J* = 7.5 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 152.98, 143.85, 142.72, 138.12, 137.53, 133.49, 130.63, 130.51, 129.68,

128.17, 127.09, 126.18, 121.65, 120.20, 119.80, 117.04, 116.96, 111.59, 110.00. LC-MS (ESI): *m/z* 297.1 $[M + H]^+$.

5-((*E*)-2-(1*H*-indol-3-yl)vinyl)isoquinoline (21)

*Step 1: (1H-indol-3-yl)-N,N-dimethylmethanamine (**12e**)*

Intermediate **12e** (374 mg, 2.146 mmol) was prepared as an off-white solid from indole (379 mg, 3.232 mmol) according to general procedure B. Yield: 66%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.21 (bs, 1H), 7.75 – 7.67 (m, 1H), 7.39 – 7.33 (m, 1H), 7.22 – 7.17 (m, 1H), 7.15 – 7.10 (m, 2H), 3.65 (s, 2H), 2.30 (s, 6H).

Step 2: *5-((E)-2-(1H-indol-3-yl)vinyl)isoquinoline (**21**)*

In a dry sealed tube, intermediate **12e** (92 mg, 0.530 mmol, 1.0 equiv) and isoquinoline-5-carboxaldehyde (83 mg, 0.530 mmol, 1.0 equiv) was dissolved in anhydrous acetonitrile (3 mL). Tributylphosphine (301 µL, 244mg, 1.195 mmol, 2.25 equiv) was added and the sealed reaction was heated at 90 °C in an oil bath for 22 h, protected from light. After the reaction was allowed to cool to room temperature, the solvent was removed under vacuum. The crude material was washed with methanol and diethyl ether to afford **21** as a yellow precipitate which was filtered and dried under vacuum (40 mg, 0.148 mmol). Yield 28%. HPLC RT 12.20 min; HPLC purity 98.96%. ^{1}H NMR (400 MHz, Acetone-*d*6) δ: 10.57 (s, 1H), 9.28 (d, *J* = 0.8 Hz, 1H), 8.56 (d, *J* = 6.0 Hz, 1H), 8.22 (d, *J* = 6.0 Hz, 1H), 8.17 – 8.11 (m, 2H), 7.98 (d, *J* = 8.2 Hz, 1H), 7.90 (d, *J* = 16.2 Hz, 1H), 7.78 (d, *J* = 2.5 Hz, 1H), 7.69 (t, *J* = 7.7 Hz, 1H), 7.62 (d, *J* = 16.2 Hz, 1H), 7.53 – 7.47 (m, 1H), 7.29 – 7.14 (m, 2H). ^{13}C NMR (100 MHz, Acetone-*d*6) δ: 154.07, 151.28, 147.70, 143.48, 138.48, 136.75, 129.59, 128.49, 127.67, 126.60, 124.40, 123.17, 121.19, 120.89, 116.74, 116.54, 115.67, 112.82. LC-MS (ESI): *m/z* 272.1 $[M + H]^+$.

***N*-(isoquinolin-5-yl)-1*H*-indole-3-carboxamide (24)**

To a suspension of 1*H*-indole-3-carboxylic acid (135 mg, 0.838 mmol, 1.0 equiv) in dichloromethane (10 mL), oxalyl chloride 2.0 M solution in dichloromethane (approximately 1.5 mL) was added, followed by one drop of DMF. The reaction was stirred at room temperature for 2 h which turned into a dark yellow solution. The solution was evaporated under vacuum to afford the crude acid chloride. Dichloromethane was added to the crude acid chloride and evaporated under vacuum again for 2 times. The acid chloride was then re-dissolved in THF and added slowly to a solution of 5-aminoisoquinoline (145 mg, 1.006 mmol, 1.2 equiv) and triethylamine (292 µL, 212 mg, 2.096 mmol, 2.5 equiv) in THF. The reaction was stirred at room temp for 16 h. The mixture was poured into water and extracted with ethyl acetate 3 times. The combined organic layer was washed with brine, dried over anhydrous sodium sulphate, concentrated *in vacuo* and purified by flash silica gel column chromatography (9:1 ethyl acetate/methanol) to yield a yellow oil which was recrystallized with methanol to afford **24** as a brown solid (101 mg, 0.352 mmol). Yield: 42%. HPLC RT 10.79 min; HPLC purity 95.43%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 11.80 (s, 1H), 9.98 (s, 1H), 9.35 (d, *J* = 0.7 Hz, 1H), 8.53 (d, *J* = 6.0 Hz, 1H), 8.44 (s, 1H), 8.20 – 8.14 (m, 1H), 7.99 (t, *J* = 7.0 Hz, 2H), 7.94 (d, *J* = 6.0 Hz, 1H), 7.72 (t, *J* = 7.8 Hz, 1H), 7.53 – 7.47 (m, 1H), 7.22 – 7.13 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 163.86, 152.45, 142.51, 136.24, 133.55, 131.11, 129.15, 128.87, 127.17, 126.48, 126.43, 124.59, 122.16, 121.02, 120.71, 116.41, 111.96, 109.97. LC-MS (ESI): *m/z* 288.1 $[M + H]^{+}$.

(*Z*)-2-(1*H*-indol-3-yl)-3-(isoquinolin-5-yl)acrylamide (25)

To a solution of **4a** (89 mg, 0.302 mmol, 1.0 equiv) in 4.5 ml of t-butanol, pulverized potassium hydroxide (305 mg, 5.436 mmol, 18.0 equiv) was added. The reaction was refluxed for 18 h, protected from light. After cooling to room temperature, the solvent was removed under vacuum. The crude material obtained was dissolved in ethyl acetate which was washed with water, saturated ammonium chloride and then brine. The organic layer was

dried over anhydrous sodium sulphate, concentrated *in vacuo* and purified by flash silica gel column chromatography (92:8 ethyl acetate/methanol) to afford **25** as a yellow solid (37 mg, 0.119 mmol). Compound **25** was obtained as an inseparable mixture of approximately 86:14 double bond geometry (calculated from ^{1}H NMR). Yield: 39%. HPLC RT 10.59, 10.77 min (overlapping peaks); HPLC peak areas 25.93%, 74.07%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 11.46 (s, 1H), 9.32 (s, 1H), 8.56 (d, *J* = 5.6 Hz, 1H), 8.04 – 8.00 (m, 3H), 7.99 (d, *J* = 7.8 Hz, 1H), 7.68 – 7.66 (m, 2H), 7.61 (d, *J* = 2.4 Hz, 1H), 7.49 – 7.47 (m, 2H), 7.32 (s, 1H), 7.21 – 7.12 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 170.96, 152.64, 142.97, 137.54, 136.93, 133.75, 133.31, 129.05, 127.03, 126.47, 125.94, 124.65, 121.72, 119.97, 119.91, 117.48, 116.72, 113.08, 112.05. Chemical shifts were given for the major isomer. LC-MS (ESI): *m/z* 314.1 $[M + H]^{+}$.

2-(1*H*-indol-3-yl)-3-(isoquinolin-5-yl)propanenitrile (26)

To **4a** (100 mg, 0.338 mmol, 1.0 equiv) dissolved in mixture of THF (6 ml) and methanol (1.2 ml) in a dried reaction tube, sodium borohydride (115 mg, 3.041 mmol, 9.0 equiv) was added. The reaction tube was sealed and heated at 75 °C in an oil bath for 40 h. The reaction was allowed to cool to room temperature and the solvents evaporated under vacuum. The crude material was dissolved in ethyl acetate and washed with water 3 times, followed by brine once. The organic layer was dried over anhydrous sodium sulphate and concentrated *in vacuo*. Purification by flash silica gel column chromatography (7:3 ethyl acetate/hexane) yielded a beige solid which was triturated with diethyl ether to obtain **26** as an off-white precipitate (20 mg, 0.067 mmol). Yield: 20%. HPLC RT 13.30 min; HPLC purity 98.98%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 11.17 (s, 1H), 9.32 (s, 1H), 8.50 (d, *J* = 5.9 Hz, 1H), 8.05 (d, *J* = 8.2 Hz, 1H), 7.96 (d, *J* = 6.0 Hz, 1H), 7.76 (d, *J* = 6.3 Hz, 1H), 7.71 (d, *J* = 7.9 Hz, 1H), 7.64 (dd, *J* = 8.0, 7.2 Hz, 1H), 7.44 – 7.36 (m, 2H), 7.19 – 7.10 (m, 1H), 7.11 – 7.03 (m, 1H), 4.82 (dd, *J* = 9.1, 6.6 Hz, 1H), 3.85 – 3.71 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ:

152.93, 143.07, 136.30, 133.97, 133.04, 131.70, 128.47, 127.12, 126.90, 125.17, 123.92, 121.62, 121.00, 119.08, 118.28, 116.66, 111.87, 108.55, 34.70, 29.18. LC-MS (ESI): *m/z* 298.1 $[M + H]^+$.

(*Z*)-3-(isoquinolin-5-yl)-2-(1-methyl-1*H*-indol-3-yl)acrylonitrile (27)

To a solution of **4a** (74 mg, 0.249 mmol, 1.0 equiv) in THF (8 mL) cooled to 0 °C in an ice bath, sodium hydride (60% dispersion in mineral oil) (13 mg, 0.324 mmol, 1.3 equiv) was added, and allowed to stir at 0 °C for 10 min. Iodomethane (26 µL, 58 mg, 0.411 mmol, 1.65 equiv) was then added. The reaction was allowed to warm to room temperature and stirred for 4 h protected from light. The solvent was evaporated under vacuum. Saturated aqueous ammonium chloride was added to the resulting crude mixture and extracted with ethyl acetate 3 times. The combined organic layer was washed with brine, dried over anhydrous sodium sulphate, concentrated *in vacuo* and purified by flash silica gel column chromatography (6:4 hexane/ethyl acetate) to afford **27** as a yellow solid (68 mg, 0.220 mmol). Yield: 88%. HPLC RT 12.87 min; HPLC purity 96.46%. Yellow crystals were obtained by recrystallization using acetone and methanol. ^{1}H NMR (400 MHz, Acetone-*d*6) δ: 9.37 (d, *J* = 0.9 Hz, 1H), 8.60 (d, *J* = 6.0 Hz, 1H), 8.43 – 8.27 (m, 2H), 8.23 – 8.15 (m, 2H), 8.06 (dt, *J* = 6.0, 0.9 Hz, 1H), 7.86 – 7.76 (m, 2H), 7.57 (dt, *J* = 8.3, 0.9 Hz, 1H), 7.38 – 7.34 (m, 1H), 7.31 – 7.26 (m, 1H), 3.98 (s, 3H). ^{13}C NMR (100 MHz, Acetone-*d*6) δ: 154.00, 144.85, 139.18, 135.03, 132.96, 132.59, 131.99, 131.14, 129.76, 129.72, 127.89, 125.65, 123.79, 121.97, 120.87, 118.56, 117.71, 111.79, 111.56, 111.44, 33.35. LC-MS (ESI): *m/z* 310.1 $[M + H]^+$.

(*Z*)-2-(5-(2-methoxyethoxy)-1*H*-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (32a)

Step 1: *2-(5-hydroxy-1H-indol-3-yl)acetonitrile (**29**)*

5-Benzyloxyindole-3-acetonitrile (2.96 g, 11.28 mmol, 1.0 equiv) was dissolved in THF (18 mL). 10% palladium on carbon (296 mg, 10 mmol%) was added. The reaction was shaken under hydrogen at 30 psi using the Parr Shaker Hydrogenation Apparatus for 7 h. The

mixture was filtered over celite and washed with ethyl acetate. The filtrate was evaporated, concentrated *in vacuo* and purified by silica gel flash column chromatography (1:1 hexane/ethyl acetate) to afford intermediate **29** as an off-white solid (1.65 g, 9.57 mmol). Yield: 84%. ^{1}H NMR (400 MHz, Acetone-*d*6) δ: 10.04 (bs, 1H), 7.76 (s, 1H), 7.30 – 7.28 (m, 1H), 7.26 (dd, *J* = 8.7, 0.4 Hz, 1H), 7.01 (d, *J* = 2.3 Hz, 1H), 6.77 (dd, *J* = 8.7, 2.3 Hz, 1H), 3.90 (d, *J* = 0.9 Hz, 2H).

*Step 2: 2-(5-(2-methoxyethoxy)-1H-indol-3-yl)acetonitrile (**30a**)*

Intermediate **30a** (76 mg, 0.330 mmol) was prepared as light-yellow oil from intermediate **29** (91 mg, 0.530 mmol) and 2-bromoethyl-methyl-ether (150 µL, 221 mg, 1.591 mmol) according to general procedure F using 3:2 hexane/ethyl acetate as the eluent for flash silica gel column chromatography. Yield: 62%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.22 (bs, 1H), 7.27 (d, *J* = 8.7 Hz, 1H), 7.18 – 7.15 (m, 1H), 7.02 (d, *J* = 2.4 Hz, 1H), 6.94 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.21 – 4.16 (m, 2H), 3.81 – 3.78 (m, 2H), 3.78 (d, *J* = 1.0 Hz, 2H), 3.48 (s, 3H).

*Step 3: (Z)-2-(5-(2-methoxyethoxy)-1H-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (**32a**)*

Compound **32a** (34 mg, 0.092 mmol) was prepared as a yellow solid from intermediate **30a** (75 mg, 0.326 mmol) and isoquinoline-5-carboxaldehyde (67 mg, 0.423 mmol) according to general procedure A microwave method. Yield: 28%. HPLC RT 11.27 min; HPLC purity 98.55%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 9.40 (d, *J* = 0.7 Hz, 1H), 8.59 (d, *J* = 6.0 Hz, 1H), 8.29 (s, 1H), 8.23 (t, *J* = 7.5 Hz, 2H), 8.03 (d, *J* = 6.0 Hz, 1H), 7.88 (s, 1H), 7.85 – 7.76 (m, 1H), 7.55 (d, *J* = 2.3 Hz, 1H), 7.43 (d, *J* = 8.8 Hz, 1H), 6.92 (dd, *J* = 8.9, 2.3 Hz, 1H), 4.21 – 4.09 (m, 2H), 3.83 – 3.58 (m, 2H), 3.31 (s, 3H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 153.71, 152.91, 143.61, 133.57, 132.54, 132.29, 131.53, 130.30, 128.80, 128.23, 127.73, 127.12, 124.25, 117.97, 117.24, 113.21, 112.89, 110.50, 110.18, 102.70, 70.62, 67.54, 58.17. LC-MS (ESI): *m/z* 370.1 $[M + H]^+$.

(*Z*)-2-(5-(2-(1,3-dioxolan-2-yl)ethoxy)-1*H*-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (32b)

*Step 1: 2-(5-(2-(1,3-dioxolan-2-yl)ethoxy)-1H-indol-3-yl)acetonitrile (**30b**)*

Intermediate **30b** (121 mg, 0.444 mmol) was prepared as a light-yellow oil from intermediate **29** (170 mg, 0.989 mmol) and 2-(2-bromoethyl)-1,3-dioxolane (348 µL, 537 mg, 2.966 mmol) according to general procedure F using 3:2 hexane/ethyl acetate as the eluent for flash silica gel column chromatography. Yield: 45%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.22 (bs, 1H), 7.27 (d, *J* = 8.7 Hz, 1H), 7.18 – 7.14 (m, 1H), 7.01 (d, *J* = 2.3 Hz, 1H), 6.90 (dd, *J* = 8.8, 2.4 Hz, 1H), 5.14 (t, *J* = 4.9 Hz, 1H), 4.18 (t, *J* = 6.5 Hz, 2H), 4.04 – 3.97 (m, 2H), 3.94 – 3.85 (m, 2H), 3.77 (d, *J* = 0.9 Hz, 2H), 2.22 – 2.18 (m, 2H).

*Step 2: (Z)-2-(5-(2-(1,3-dioxolan-2-yl)ethoxy)-1H-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (**32b**)*

Compound **32b** (42 mg, 0.101 mmol) was prepared as a yellow solid from intermediate **30b** (116 mg, 0.426 mmol) and isoquinoline-5-carboxaldehyde (107 mg, 0.682 mmol) according to general procedure A microwave method. Yield: 24%. HPLC RT 10.82 min; HPLC purity 98.08%. ^{1}H NMR (400 MHz, DMSO-*d*6) δ: 9.40 (d, *J* = 0.6 Hz, 1H), 8.59 (d, *J* = 6.0 Hz, 1H), 8.28 (s, 1H), 8.25 – 8.21 (m, 2H), 8.03 (d, *J* = 6.0 Hz, 1H), 7.89 (s, 1H), 7.84 – 7.80 (m, 1H), 7.53 (d, *J* = 2.3 Hz, 1H), 7.44 (d, *J* = 8.7 Hz, 1H), 6.90 (dd, *J* = 8.9, 2.3 Hz, 1H), 5.01 (t, *J* = 5.0 Hz, 1H), 4.14 (t, *J* = 6.6 Hz, 2H), 3.90 – 3.82 (m, 2H), 3.81 – 3.71 (m, 2H), 2.07 – 2.03 (m, 2H). ^{13}C NMR (100 MHz, DMSO-*d*6) δ: 153.59, 152.92, 143.60, 133.57, 132.46, 132.40, 131.52, 130.29, 128.79, 128.24, 127.87, 127.13, 124.33, 117.99, 117.22, 113.32, 112.84, 110.47, 110.13, 102.64, 101.31, 64.22, 64.10, 33.52. LC-MS (ESI): *m/z* 412.2 $[M + H]^+$.

(*Z*)-2-(5-(3-(pyrrolidin-1-yl)propoxy)-1*H*-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (32c)

*Step 1: 2-(5-(3-chloropropoxy)-1H-indol-3-yl)acetonitrile (**30c**)*

Compound **30c** (150 mg, 0.603 mmol) was prepared as dark-yellow oil from intermediate **29** (207 mg, 1.200 mmol) 1-chloro-3-iodopropane (258 µL, 490 mg, 2.70 mmol) according to general procedure F using 65:35 hexane/ethyl acetate as the eluent for flash silica gel column chromatography. Yield: 50%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.08 (bs, 1H), 7.29 (d, *J* = 8.8 Hz, 1H), 7.21 – 7.19 (m, 1H), 7.03 (d, *J* = 2.3 Hz, 1H), 6.91 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.18 (t, *J* = 5.8 Hz, 2H), 3.77 – 3.80 (m, 4H), 2.32 – 2.21 (m, 2H).

*Step 2: 2-(5-(3-(pyrrolidin-1-yl)propoxy)-1H-indol-3-yl)acetonitrile (**31c**)*

To a solution of intermediate **30c** (149 mg, 0.599 mmol, 1.0 equiv) in acetonitrile (5 mL), potassium carbonate (306 mg, 2.214 mmol, 3.7 equiv), sodium iodide (135 mg, 0.899 mmol, 1.5 equiv) and pyrrolidine (149 µL, 128 mg, 1.799 mmol, 3.0 equiv) were added. The mixture was heated to reflux for 40 h. After cooling to room temperature, the reaction was filtered and washed with small amounts of ethyl acetate. The filtrate and washings were concentrated *in vacuo* and purified by flash silica gel column chromatography (9:1:0.01 dichloromethane/methanol/ammonium hydroxide solution) to afford intermediate **31c** as a dark-yellow oil (132 mg, 0.466 mmol). Yield: 78%. ^{1}H NMR (400 MHz, MeOD) δ: 7.37 – 7.31 (m, 1H), 7.26 (s, 1H), 7.17 (d, *J* = 2.3 Hz, 1H), 6.90 (dd, *J* = 8.8, 2.4 Hz, 1H), 4.17 (t, *J* = 5.8 Hz, 2H), 3.96 (d, *J* = 0.8 Hz, 2H), 3.44 – 3.29 (m, 6H), 2.27 – 2.20 (m, 2H), 2.13 – 2.05 (m, 4H).

*Step 3: (Z)-2-(5-(3-(pyrrolidin-1-yl)propoxy)-1H-indol-3-yl)-3-(isoquinolin-5-yl)acrylonitrile (**32c**)*

Compound **32c** (8 mg, 0.019 mmol) was prepared as a yellow solid from intermediate **31c** (84 mg, 0.295 mmol) and isoquinoline-5-carboxaldehyde (70 mg, 0.442 mmol) according to general procedure A microwave method. The reaction was evaporated under vacuum to remove the solvent and purified by silica gel flash column chromatography (85:15:0.015 dichloromethane/methanol/ ammonium hydroxide solution). The material obtained was dried

under vacuum and triturated with small amounts of methanol and acetone to obtain a yellow precipitate. Yield: 7%. HPLC RT 10.63 min; HPLC purity 95.80%. ^{1}H NMR (400 MHz, MeOD) δ: 9.32 (s, 1H), 8.54 (d, *J* = 6.0 Hz, 1H), 8.32 (d, *J* = 7.3 Hz, 1H), 8.21 – 8.19 (m, 2H), 8.05 (d, *J* = 6.1 Hz, 1H), 7.86 – 7.79 (m, 1H), 7.77 (s, 1H), 7.57 (d, *J* = 2.2 Hz, 1H), 7.43 (d, *J* = 8.9 Hz, 1H), 6.97 (dd, *J* = 8.9, 2.3 Hz, 1H), 4.20 (t, *J* = 5.7 Hz, 2H), 3.49 – 3.36 (m, 6H), 2.27 – 2.24 (m, 2H), 2.11 – 2.08 (m, 4H). ^{13}C NMR (100 MHz, MeOD) δ: 155.19, 153.95, 143.72, 135.85, 134.38, 133.25, 133.02, 132.25, 130.27, 128.61, 128.35, 126.02, 118.96, 118.76, 114.15, 114.09, 112.73, 112.21, 104.10, 66.79, 55.46, 54.13, 27.35, 23.97. LC-MS (ESI): *m/z* 423.2 $[M + H]^{+}$.

(*Z*)-3-(isoquinolin-5-yl)-2-(5-((pyrrolidin-1-yl)methyl)-1*H*-indol-3-yl)acrylonitrile (36a)

*Step 1: Potassium ((pyrrolidin-1-yl)methyl)trifluoroborate (**34**)*

To pyrrolidine (494 µL, 428 mg, 6.014 mmol, 1.05 equiv) in dry THF (6.5 ml), potassium (bromomethyl) trifluoroborate (1150 mg, 5.728 mmol, 1.0 equiv) was added. The mixture was allowed to stir at room temperature for 40 h. The solvent was evaporated under vacuum and the resulting crude material was dissolved in acetone. Potassium carbonate (792 mg, 5.728 mmol, 1.0 equiv) was added and stirred at room temperature for 1 h. The mixture was filtered through a pad of Celite to remove the insoluble salts, and the filtrate was evaporated under vacuum to yield a dark brown gum. Recrystallization with acetone afforded intermediate **34** as orange needle-like solids (171 mg, 0.895 mmol). Yield: 16%. ^{13}C NMR (100 MHz, Acetone-*d*6) δ 57.09, 23.66. ^{19}F NMR (376 MHz, Acetone-*d*6) δ -144.14.

*Step 2: 2-(5-((pyrrolidin-1-yl)methyl)-1H-indol-3-yl)acetonitrile (**35a**)*

Intermediate **35a** (37 mg, 0.154 mmol) was prepared as a light-yellow oil from intermediate **10e** (50 mg, 0.262 mmol) according to general procedure G. Yield: 59%. ^{1}H NMR (400 MHz, MeOD) δ: 7.58 (d, *J* = 0.9 Hz, 1H), 7.38 (dd, *J* = 8.4, 0.5 Hz, 1H), 7.26 (s, 1H), 7.20 (dd, *J* =

8.4, 1.6 Hz, 1H), 3.95 (d, *J* = 0.9 Hz, 2H), 3.84 (s, 2H), 2.78 – 2.58 (m, 4H), 1.88 – 1.75 (m, 4H).

*Step 3: (Z)-3-(isoquinolin-5-yl)-2-(5-((pyrrolidin-1-yl)methyl)-1H-indol-3-yl)acrylonitrile (**36a**)*

Compound **36a** (27 mg, 0.071 mmol) was prepared as a yellow solid from intermediate **35a** (38 mg, 0.157 mmol) and isoquinoline-5-carboxaldehyde (27 mg, 0.173 mmol) according to general procedure A sealed tube method. The reaction was evaporated under vacuum to remove the solvent and purified by silica gel flash column chromatography (85:15:0.015 dichloromethane/methanol/ammonium hydroxide solution) to yield a dark yellow oil. Recrystallization with dichloromethane/methanol afforded **36a** as a yellow solid. Yield: 45%. HPLC RT 12.12 min; HPLC purity 99.59%. ^{1}H NMR (400 MHz, MeOD) δ: 9.31 (s, 1H), 8.53 (d, *J* = 6.1 Hz, 1H), 8.34 – 8.26 (m, 2H), 8.18 (d, *J* = 8.3 Hz, 1H), 8.09 – 8.05 (m, 2H), 7.85 – 7.76 (m, 1H), 7.75 (s, 1H), 7.47 (d, *J* = 8.3 Hz, 1H), 7.27 (dd, *J* = 8.4, 1.5 Hz, 1H), 3.81 (s, 2H), 2.67 – 2.57 (m, 4H), 1.84 – 1.81 (m, 4H). ^{13}C NMR (100 MHz, MeOD) δ: 153.89, 143.72, 138.59, 135.85, 133.28, 133.22, 132.28, 131.90, 130.25, 130.21, 128.63, 128.59, 125.89, 125.57, 121.47, 118.89, 113.08, 112.97, 112.70, 61.98, 54.88, 24.10. LC-MS (ESI): *m/z* 379.1 $[M + H]^{+}$.

(*Z*)-3-(isoquinolin-5-yl)-2-(6-((pyrrolidin-1-yl)methyl)-1*H*-indol-3-yl)acrylonitrile (36b)

*Step 1: 2-(6-((pyrrolidin-1-yl)methyl)-1H-indol-3-yl)acetonitrile (**35b**)*

Intermediate **35b** (30 mg, 0.125 mmol) was prepared as a light-yellow oil from intermediate **10i** (55 mg, 0.236 mmol) according to general procedure G. Yield: 53%. ^{1}H NMR (400 MHz, MeOD) δ: 7.56 (dd, *J* = 8.2, 0.5 Hz, 1H), 7.39 (d, *J* = 0.7 Hz, 1H), 7.25 (s, 1H), 7.11 (dd, *J* = 8.2, 1.4 Hz, 1H), 3.93 (d, *J* = 0.9 Hz, 2H), 3.77 (s, 2H), 2.64 – 2.60 (m, 4H), 1.87 – 1.78 (m, 4H).

*Step 2: (Z)-3-(isoquinolin-5-yl)-2-(6-((pyrrolidin-1-yl)methyl)-1H-indol-3-yl)acrylonitrile (**36b**)*

Compound **36b** (47 mg, 0.095 mmol) was prepared as a yellow solid from intermediate **35b** (30 mg, 0.125 mmol) and isoquinoline-5-carboxaldehyde (22 mg, 0.137 mmol) according to general procedure A sealed tube method. The reaction was evaporated under vacuum to remove the solvent and purified by silica gel flash column chromatography (85:15:0.015 dichloromethane/methanol/ammonium hydroxide solution). The material obtained was dried under vacuum and triturated with a small amount of methanol to obtain **36b** as a yellow precipitate. Yield: 76%. HPLC RT 12.98 min; HPLC purity 99.41%. ^{1}H NMR (400 MHz, MeOD) δ: 9.32 (s, 1H), 8.54 (d, *J* = 6.1 Hz, 1H), 8.33 (d, *J* = 7.3 Hz, 1H), 8.27 (s, 1H), 8.19 (d, *J* = 8.3 Hz, 1H), 8.07 – 8.04 (m, 2H), 7.84 – 7.78 (m, 1H), 7.77 (s, 1H), 7.52 (s, 1H), 7.27 (dd, *J* = 8.3, 1.4 Hz, 1H), 3.84 (s, 2H), 2.69 – 2.66 (m, 4H), 1.90 – 1.85 (m, 4H). ^{13}C NMR (100 MHz, MeOD) δ: 153.90, 143.75, 139.24, 135.84, 133.78, 133.08, 133.06, 132.21, 130.24, 130.23, 128.59, 128.49, 124.89, 123.89, 120.46, 118.86, 118.76, 114.25, 112.84, 112.65, 61.65, 54.90, 24.10. LC-MS (ESI): *m/z* 379.2 $[M + H]^+$.

(*Z*)-3-(isoquinolin-5-yl)-2-(7-((pyrrolidin-1-yl)methyl)-1*H*-indol-3-yl)acrylonitrile (36c)

*Step 1: 2-(7-((pyrrolidin-1-yl)methyl)-1H-indol-3-yl)acetonitrile (**35c**)*

Intermediate **35c** (32 mg, 0.134 mmol) was prepared as a light-yellow oil from intermediate **14c** (70 mg, 0.302 mmol) according to general procedure G. Yield: 53%. ^{1}H NMR (400 MHz, MeOD) δ: 7.55 (dd, *J* = 7.8, 1.1 Hz, 1H), 7.29 (s, 1H), 7.14 (d, *J* = 6.6 Hz, 1H), 7.11 – 7.05 (m, 1H), 4.01 (s, 2H), 3.95 (d, *J* = 0.9 Hz, 2H), 2.76 – 2.65 (m, 4H), 1.90 – 1.77 (m, 4H).

*Step 2: (Z)-3-(isoquinolin-5-yl)-2-(7-((pyrrolidin-1-yl)methyl)-1H-indol-3-yl)acrylonitrile (**36c**)*

Compound **36c** (18 mg, 0.048 mmol) was prepared as a yellow solid from intermediate **35c** (32 mg, 0.134 mmol) and isoquinoline-5-carboxaldehyde (24 mg, 0.154 mmol) according to

general procedure A sealed tube method. The reaction was evaporated under vacuum to remove the solvent and purified by silica gel flash column chromatography (87:13:0.008 dichloromethane/methanol/ammonium hydroxide solution) to yield a yellow sticky solid. Recrystallization with dichloromethane/methanol afforded **36c** as a yellow precipitate. Yield: 35%. HPLC RT 12.47 min; HPLC purity 98.79%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.55 (bs, 1H), 9.32 (s, 1H), 8.62 (d, *J* = 5.5 Hz, 1H), 8.34 (d, *J* = 7.3 Hz, 1H), 8.18 (s, 1H), 8.03 (d, *J* = 8.2 Hz, 1H), 7.95 (d, *J* = 8.0 Hz, 1H), 7.86 (d, *J* = 5.9 Hz, 1H), 7.75 – 7.71 (m, 1H), 7.70 (s, 1H), 7.25 – 7.20 (m, 1H), 7.12 (d, *J* = 7.1 Hz, 1H), 4.04 (s, 2H), 2.64 – 2.59 (m, 4H), 1.90 – 1.86 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.41, 144.03, 137.04, 134.49, 132.32, 131.46, 130.57, 129.07, 128.91, 127.29, 126.39, 124.36, 122.34, 121.41, 118.82, 118.13, 116.75, 112.56, 111.41, 59.22, 54.42, 23.89. LC-MS (ESI): *m/z* 379.1 $[M + H]^+$.

Biology

Cell proliferation inhibition assays

Human BJ foreskin fibroblasts were a gift from Dr. Mathijs Voorhoeve [24] and were fully transformed with hTert, small t, shRNAs against p53 and pRb, as well as H-RasV12-ER [23]. All other cells were purchased from ATCC (Rockville, MD) and cultured according to the instructions (see Supplementary Table S4). Cells were grown at 37 °C, 5% CO_2. The media was supplemented with 10% fetal bovine serum (Gibco, sterile-filtered), 50 µg/mL penicillin and 50 µg/mL streptomycin. Cells were sub-cultured at 80-90% confluency and used within 12-20 passages. Cells were seeded at 2.5×10^3 cells per well, 100 µL media per well for 24 h. The media was then removed and aliquots of test compound (initially prepared as stock solutions in DMSO at 200-fold higher concentrations) were added to the wells. The final DMSO concentration was kept at 0.5% v/v. Treated cells were incubated for 72 h. Cell viability was assessed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium

bromide) (Sigma-Aldrich). MTT was reconstituted in phosphate buffered saline pH 7.4 to 2 mg/mL and diluted to 0.5 mg/mL with serum-free media before use. At the end of the incubation period, the media was removed and replaced with 200 µL of 0.5 mg/mL MTT media. Treated plates were further incubated for 3 h. The MTT media was removed and 100 µL of DMSO was added to each well to dissolve the purple formazan crystals. Absorbance readings were taken on a micro plate reader (Tecan Infinite M200 Pro) at 570 nm. Cell viability was assessed from the readings of treated wells compared to control wells (absence of test compound) with correction of background absorbance. Percentage viability readings for each test compound were plotted against log concentration on GraphPad Prism (Version 5.0, GraphPad Software, San Diego, CA), with constraints set at ≥ 0 and $\leq 100\%$. A sigmoidal curve was generated from which the IC_{50} (concentration required to reduce viability by 50% compared to untreated/control cells) was obtained. Three independent determinations of IC_{50} were made for each compound.

Determination of Toxicity in Transforming growth factor-alpha mouse hepatocyte (TAMH) Cells

One day before drug treatment, TAMH cells were seeded at a cell density of 12,000 cells/well (60,000 cells/mL) in a 96-well plate (NUNC). Cells were then treated with test compounds starting from a concentration of 50 µM (0.5% DMSO concentration: 0.5 µl of test compound in 99.5 µL of media in each well; stock concentration of test compounds is 10 mM). Treated cells were then incubated at 37 °C for 24 hours. Cell viability was then determined with CellTiter-Glo® Cell Viability Assay (Promega Corporation) as per manufacturer's instructions. The cell-reagent mixture was transferred to a solid white flat-bottom 96-well plate (Greiner). Luminescence was then recorded with an integration time of 0.25 seconds (Tecan Infinite® M200 Microplate reader). Either percentage inhibition at the top concentration or an IC_{50} was calculated.

Determination of Toxicity in AC10 Human Cardiomyocyte Cells

One day before drug treatment, AC10 cells were seeded at a cell density of 5,000 cells/well (75,000 cells/mL) in a 96-well plate (NUNC). Cells were then treated as for TAMH assay.

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Determination of Caspase-3/7 Activity

Activity of caspase-3/7 was determined using the caspase-Glo 3/7 Assay Kit (Promega) and normalized to the number of viable cells as determined by MTT assay.

Immunoblotting

HeLa cells were treated with different concentrations of **4a** (0.625, 1.25, 2.5, 5 and 10 µM**)**, chloroquine (1.25, 2.5, 10, 50 and 100 µM) or DMSO for 6 h. The cells were then harvested and lysed in 1% triton lysis buffer (25 mM Tris HCl, (pH 8.0), 150 mM NaCl, 1% triton X-100, 1 mM dithiothreitol, protease inhibitor mix (Complete Mini, Roche) and phosphatase inhibitor (PhosphoStop, Roche)). The proteins in total lysates were boiled with sample buffer (60 mM Tris HCl (pH 6.8), 25% glycerol, 2% SDS, 14.4 mM 2-mercaptoethanol, 0.1% bromophenol blue) and resolved by 14% SDS-PAGE and subjected to immunoblot analysis using specific antibodies against pH3 (Ser10) (Cell Signaling, Cat#3377), specific antibodies against LC3 (Cell Signaling, Cat#4108), anti-β-actin (Millipore, Cat#MAB1501) and anti-cleaved PARP (Abcam, #ab32064). The same protocol was used for transformed BJ cells treated with various test compounds at a concentration of 5 µM for 24 h.

Immunofluorescence analysis

HeLa cells were treated with **4a**, chloroquine or DMSO for 6 h. The treated cells were fixed in methanol and subjected to immunofluorescence analysis using anti-β-tubulin antibodies and counterstaining with DAPI. Images were acquired at room temperature.

Soft agar colony formation assays

HeLa cells (1×10^4 cells/well) were suspended in 2 mL of soft agar at a final concentration of 0.4%. The suspension was added on top of a 2 mL layer of 1% agar in 6-well plates. **4a**, at final concentrations of 0.15, 0.3, 0.6, 1.25, 2.5 and 5 µM was added to the different wells. DMSO was used as the control. Seven days after the addition of **4a**, the number of colonies per plate was counted in an unbiased manner using MATLAB. The experiment was performed in triplicates.

Determination of Michael acceptor reactivity by NMR

The procedure reported by Avonto *et al* was followed with slight modifications. **4a** (4 mg) was dissolved in 0.6ml of DMSO-*d*6. The ^{1}H NMR spectra was immediately recorded on a Bruker Avance 400 Ultrashield (400 MHz) NMR spectrometer. Cysteamine (6 mg) was then added and the mixture was vortexed for 1 min. The ^{1}H NMR spectra was then recorded again, and also at 1 h, 3h and 24 h.

Determination of *in vitro* metabolic stability in female (FRLM) and male rat liver microsomes (MRLM).

Incubations were conducted in triplicates for the respective pooled female and male rat liver microsomes (BD Gentest Corp, Woburn, MA). Incubation mixtures consisted of 7.5 µL of 20 mg/mL FRLM or MRLM (final concentration: 0.3 mg microsome protein/mL), 2.5 µL of 600 µM **4a**/**10c**/**10f** in acetonitrile (final: 3 µM), 440 µL of 0.1 M phosphate buffer (pH 7.4). The mixture was first shaken for 5 min for pre-incubation in a shaking water bath at 37 ºC. Reaction was initiated by adding 50 µL of 10 mM NADPH to obtain a final concentration of 1mM NADPH in the mixture. The total volume of the reaction mixture was 500 µL. For metabolic stability studies, aliquots of 50 µL of the incubation sample mixture were collected at 0, 5, 10, 15, 30, and 45 min. Following the collection of samples, the reaction was terminated with 100 µL of chilled acetonitrile containing the internal standard (1.5 µM **10c**/**4a**). The mixture was then centrifuged at 10,000 X g to remove the protein and the

supernatant was subsequently subjected to LC-MS/MS analysis. Positive control (PC) samples were prepared as described above, except that the test compound was replaced with the known P450 substrate (Midazolam, 3 μM). The samples were assayed for the degradation of midazolam to evaluate the adequacy of the experimental conditions for drug metabolism study. Negative control samples were also prepared as described above but without NADPH.

Formulation studies

To 1 mg of **4a**.HCl, components of each vehicle were added in the following order: organic solvent (DMSO / ethanol / NMP), followed by PEG400, and finally water. The total volume of each formulation was 0.5 ml. The contents were mixed using a vortex mixer after each addition step. After vortex mixing was performed for the last addition step, the mixture was sonicated for 15 min in a room temperature water bath. Each mixture was allowed to stand at room temperature for 3 h and then centrifuged at 5000 RPM for 5 min. The supernatant was passed through a 0.45 micron syringe filter. To 100 μL of this filtrate, 300 μL of methanol was added followed by 30 μL of internal standard (IS) prepared as a 1 mg/mL 10% v/v DMSO in methanol solution. The samples were subjected to HPLC analysis and the solubility of each formulation was estimated by interpolating the area ratio of the **4a**.HCl peak to the internal standard peak from a standard curve established using solutions of **4a**.HCl of known concentrations.

Pharmacokinetics

Male Wistar rats (aged 8 weeks, weighing around 300g) were used in study. The animal studies were performed as per approved internal protocols for animal care and use. Two days before the pharmacokinetic study, a cannula was inserted into the jugular vein of each rat after the animal was anaesthetized. The *iv* and *po* doses of **4a**.HCl were 2 mg/kg and 10 mg/kg respectively. The doses were administered as a solution (10% DMSO, 50% PEG400, 40% water pH 5.0). After dosing, blood samples were collected from the catheter at various

time points (0 – 4 h for *iv*, 0 – 24h for *po*). The blood was collected in tubes containing heparin as anticoagulant (10 I.U final concentration), and centrifuged at 4000 rpm for 5 min. The plasma was separated and stored at -80 °C until analysis by LCMSMS. PK parameters were estimated with non-compartmental methods using WinNonlin (ver 4.1, Pharsight, CA).

In vivo mouse xenograft model

BALB/c athymic female nude mice (nu/nu, 5-7 weeks) (InVivos) were kept under specific pathogen-free conditions. The care and use of mice was approved by the Duke-NUS IACUC in accordance with protocol 2015/SHS/1030. HCT15 human colon cancer cells ($5x10^6$) were subcutaneously injected into the flanks of mice. When the mean tumor volume reached 100–300 mm^3 (Day 1), the mice were randomly divided into experimental groups of 6 mice ensuring that each treatment group has similar mean tumor burden and standard deviation. The animals were treated with intraperitoneal (IP) injection of **4a** (20 mg/kg) or vehicle control twice per day for 12 days. **4a** was dissolved in DMSO followed by the addition of PEG400 and deionized water (pH 5.0) (final concentrations, 10% DMSO, 50% PEG400). Paclitaxel (Cayman Chemical) was dissolved in ethanol:Tween 80 = 1:3 (v/v) solution followed by the addition of a 5% glucose solution (final ratio, ethanol:Tween 80:5% glucose = 5:15:80) and injected via the tail vein (IV). Tumor dimensions were measured using calipers, and tumor volume (mm^3) was calculated using the formula width2 × length/2 in a blinded fashion.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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REFERENCES

[1] B.W. Stewart, C.P. Wild, World Cancer Report 2014, World Health Organization, International Agency for Research on Cancer, 2014.
[2] A. Carta, M. Palomba, G. Boatto, B. Busonera, M. Murreddu, R. Loddo, Synthesis and antiproliferative activity of 3-aryl-2-[1H(2H)-benzotriazol-1(2)-yl]acrylonitriles variously substituted: Part 4, Farmaco, 59 (2004) 637-644.
[3] P. Sanna, A. Carta, L. Gherardini, M. Esmail, R. Nikookar, Synthesis and antimycobacterial activity of 3-aryl-, 3-cyclohexyl- and 3-heteroaryl- substituted-2-(1H(2H)-benzotriazol-1(2)-yl)prop-2-enenitriles, prop-2-enamides and propenoic acids. II, Farmaco, 57 (2002) 79-87.
[4] P. Sanna, A. Carta, M.E. Nikookar, Synthesis and antitubercular activity of 3-aryl substituted-2-[1H(2H)benzotriazol-1(2)-yl]acrylonitriles, Eur. J. Med. Chem., 35 (2000) 535-543.
[5] I. Briguglio, E. Laurini, M.A. Pirisi, S. Piras, P. Corona, M. Fermeglia, S. Pricl, A. Carta, Triazolopyridinyl-acrylonitrile derivatives as antimicrotubule agents: Synthesis, in vitro and in silico characterization of antiproliferative activity, inhibition of tubulin polymerization and binding thermodynamics, Eur. J. Med. Chem., 141 (2017) 460-472.
[6] S. Tcherniuk, D.A. Skoufias, C. Labriere, O. Rath, F. Gueritte, C. Guillou, F. Kozielski, Relocation of Aurora B and survivin from centromeres to the central spindle impaired by a kinesin-specific MKLP-2 inhibitor, Angewandte Chemie, 49 (2010) 8228-8231.
[7] C. Guillou, F. Kozielski, C. Labriere, F. Gueritte, S. Tcherniuk, D.A. Skoufias, C. Thal, H. Husson. Use of Derivatives of Indoles for the Treatment of Cancer. WO2010150211, 2010.
[8] C. Labriere, S.K. Talapatra, S. Thoret, C. Bougeret, F. Kozielski, C. Guillou, New MKLP-2 inhibitors in the paprotrain series: Design, synthesis and biological evaluations, Bioorg. Med. Chem., 24 (2016) 721-734.

[9] C. Labriere, O. Lozach, M. Blairvacq, L. Meijer, C. Guillou, Further investigation of Paprotrain: Towards the conception of selective and multi-targeted CNS kinase inhibitors, Eur. J. Med. Chem., 124 (2016) 920-934.
[10] R.K. Amaravadi, C.B. Thompson, The roles of therapy-induced autophagy and necrosis in cancer treatment, Clinical cancer research : an official journal of the American Association for Cancer Research, 13 (2007) 7271-7279.
[11] B. Levine, G. Kroemer, Autophagy in the Pathogenesis of Disease, Cell, 132 (2008) 27-42.
[12] Y. Kabeya, N. Mizushima, T.i. Ueno, A. Yamamoto, T. Kirisako, T. Noda, E. Kominami, Y. Ohsumi, T. Yoshimori, LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing, EMBO J., 19 (2000) 5720-5728.
[13] Y. Kabeya, N. Mizushima, A. Yamamoto, S. Oshitani-Okamoto, Y. Ohsumi, T. Yoshimori, LC3, GABARAP and GATE16 localize to autophagosomal membrane depending on form-II formation, J. Cell Sci., 117 (2004) 2805-2812.
[14] K.H. Low, N.A. Magomedov, Phosphine-Mediated Coupling of Gramines with Aldehydes: A Remarkably Simple Synthesis of 3-Vinylindoles, Org. Lett., 7 (2005) 2003-2005.
[15] T. Maegawa, Y. Fujita, A. Sakurai, A. Akashi, M. Sato, K. Oono, H. Sajiki, Pd/C(en) catalyzed chemoselective hydrogenation in the presence of aryl nitriles, Chem. Pharm. Bull. (Tokyo), 55 (2007) 837-839.
[16] G.A. Molander, P.E. Gormisky, D.L. Sandrock, Scope of Aminomethylations via Suzuki−Miyaura Cross-Coupling of Organotrifluoroborates, J. Org. Chem., 73 (2008) 2052-2057.
[17] J.A. Hickman, R. Graeser, R. de Hoogt, S. Vidic, C. Brito, M. Gutekunst, H. van der Kuip, Three-dimensional models of cancer for pharmacology and cancer cell biology: Capturing tumor complexity in vitro/ex vivo, Biotechnol. J., 9 (2014) 1115-1128.
[18] I.M. Serafimova, M.A. Pufall, S. Krishnan, K. Duda, M.S. Cohen, R.L. Maglathlin, J.M. McFarland, R.M. Miller, M. Frödin, J. Taunton, Reversible targeting of noncatalytic cysteines with chemically tuned electrophiles, Nat. Chem. Biol., 8 (2012) 471-476.
[19] C. Avonto, O. Taglialatela-Scafati, F. Pollastro, A. Minassi, V. Di Marzo, L. De Petrocellis, G. Appendino, An NMR Spectroscopic Method to Identify and Classify Thiol-Trapping Agents: Revival of Michael Acceptors for Drug Discovery?, Angew. Chem. Int. Ed. Engl., 50 (2011) 467-471.
[20] B.K. Park, N.R. Kitteringham, P.M. O'Neill, Metabolism of Fluorine-Containing Drugs, Annu. Rev. Pharmacol. Toxicol., 41 (2001) 443-470.
[21] R.G. Strickley, Solubilizing excipients in oral and injectable formulations, Pharm. Res., 21 (2004) 201-230.
[22] D. Sampath, L.M. Greenberger, C. Beyer, M. Hari, H. Liu, M. Baxter, S. Yang, C. Rios, C. Discafani, Preclinical pharmacologic evaluation of MST-997, an orally active taxane with superior in vitro and in vivo efficacy in paclitaxel-and docetaxel-resistant tumor models, Clin. Cancer Res., 12 (2006) 3459-3469.
[23] M. Kitagawa, P.J. Liao, K.H. Lee, J. Wong, C.S. See, N. Minami, O. Sampetrean, H. Saya, D. Lingyun, N. Prabhu, G.K. Diam, R. Sobota, A. Larsson, P. Nordlund, F. McCormick, S. Ghosh, D.M. Epstein, B.W. Dymock, S.H. Lee, Dual blockade of the lipid kinase PIP4Ks and mitotic pathways leads to cancer-selective lethality, Nat Commun, 8 (2017) 2200.
[24] P.M. Voorhoeve, R. Agami, The tumor-suppressive functions of the human INK4A locus, Cancer Cell, 4 (2003) 311-319.

Highlights

- Compound **4a** is a new agent with a dual mode of action targeting both mitosis and autophagy.
- Structure-activity relationships were explored utilizing mitotic marker pH3 and autophagy marker LC3.
- **4a** induces cell death in cancer cells while sparing normal cells which regrow after **4a** is removed.
- **4a** showed antiproliferative potency against a panel of 30 cancer cell lines.
- **4a** suppressed tumor growth in a mouse model of paclitaxel-resistant colon cancer.