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Short communication

Cytotoxic 2-phenyacrylnitriles, the importance of the cyanide moiety and discovery of potent broad spectrum cytotoxic agents

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ABSTRACT

We previously reported the discovery of a simple conjugated cyano pharmacophore which had led to the development of (*Z*)-2-(3,4-dichlorophenyl)-3-(4-nitrophenyl)acrylonitrile (**1**), as a selective inhibitor of oestrogen receptor positive (ER+ve) human breast cancer cell line, MCF-7. Further exploration though modification of the acrylonitrile and aromatic substituents has highlighted key structural components necessary for broad spectrum cytotoxicity. The acrylic acid derivates (*Z*)-2-(3,4-dichlorophenyl)-3-(4-nitrophenyl)acrylic acid (**8**) and (*Z*)-2-(3,4-dichlorophenyl)-3-(4-methoxyphenyl)acrylic acid (**9**) were inactive; confirming the importance of the cyanide moiety. The most potent 2-phenylacrylonitriles synthesized were (*Z*)-2-(3,4-dichlorophenyl)-3-(1*H*-indol-3-yl)acrylonitrile (**3**) and (*Z*)-2-(3,4-dichlorophenyl)-3-(1*H*-indol-5-yl)acrylonitrile (**20**) with an average GI_{50} values of 1.4 and 0.53 μM respectively. Five additional (*Z*)-2-(3,4-dichlorophenyl)-3-(indolyl)acrylonitriles also displayed average GI_{50} values of ≤8.4 μM. In the case of indole **20**, this represents a 32-fold increase in broad spectrum cytotoxicity relative to the lead (**1**).

1. Introduction

In 2010 over 100,000 Australians were diagnosed with cancer and more than 43,000 died of this disease. Without better targeted therapies cancer will remain one of the leading causes of death in Australia [1]. Cancer is a family of diseases, that despite five decades of intense research still affects 1 in 3 people during the course of their lives. Current treatment strategies for cancer have limited efficacy, especially in the common malignancies such as breast, colon and lung cancer. Despite notable successes with new targeted therapies such as kinase inhibitors (particularly with haematologic malignancies), one-half of all cancer patients either, do not respond to therapy or relapse following initial response, and ultimately die from their metastatic disease [2–5]. For these patients the sole hope for survival lies in the development of better anticancer agents, both as targetted growth inhibitors of specific cancer types, e.g. oestrogen positive (ER+ve) breast cancer, but also as broad spectrum agents that are active across a panel of cancer types.

We recently reported a family of 2-phenylacrylonitriles that displayed novel anticancer activity [6]. With these 2-phenylacrylonitriles we developed a basic pharmacophore hypothesis to explain the observed SAR. This pharmacophore described an extended conjugation spanning two terminal aromatic rings with one of these rings containing an electron withdrawing group as important for the maintenance of cytotoxicity. Central to this conjugation was the presence of an acrylonitrile (cyanide) moiety (Fig. 1). Analogues such as **1** displayed high levels of specificity, ~500 fold relative to the other cell lines examined, towards the ER+ve breast cancer cell line MCF-7, but no single analogue displayed high potency against our panel of eleven cancer cell lines against which we routinely screen for cytotoxicity. In this work we further explore the SAR associated with this class of cytotoxic agents seeking to develop broad spectrum cytotoxic agents.

2. Chemistry

Our approach to lead compound development relies on the synthesis of highly focused small compound libraries combined with a robust biological screen. Herein each library was designed in an effort to answer specific questions about our original acrylonitrile pharmacophore and with compounds typically accessed via a facile Knoevenagel condensation between a phenylacrylonitrile and an appropriately substituted aldehyde (Scheme 1). In a typical synthesis 3,4-dichlorophenylacetonitrile was treated with one

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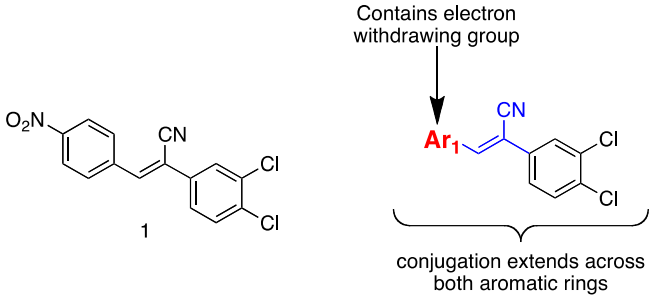


Fig. 1. (*Z*)-2-(3,4-Dichlorophenyl)-3-(4-nitrophenyl)acrylonitrile (**1**), a 0.127 ± 0.043 μM growth inhibitor of the oestrogen receptor positive (ER+ve) human breast cancer cell line, MCF-7 [6].

equivalent of an aromatic aldehyde in water with added 40% $PhCH_2NMe_3(OH)$ at 50 °C for 5 h, after which time the desired product was collected either by filtration and recrystallization or by extractive work up and flash chromatography (Scheme 1) [6,8–21]. *Library A* modified the Ar_1 moiety and explored the effect of the electron-withdrawing moiety in the second aromatic moiety, retaining the 3,4-dichlorophenylacrylo moiety present in **1** (Fig. 1).

Library B was specifically designed to determine the effect of −CN to $-CO_2H$ bioisosteric modification on the observed cytotoxicity of the two most potent compounds (**1** and **7**, Table 1) from our original series of acrylonitrile analogues and was accessed from the Knoevenagel condensation of 3,4-dichlorophenylacetic acid with 4-nitro- and 4-methoxy-benzaldehyde to afford the acrylic acid analogues **8** and **9** respectively.

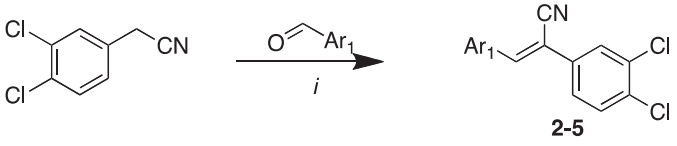
Library C was accessed using the same chemistry as described in Scheme 1 using four furan carboxaldehydes to yield substituted furans **10**–**13** (Table 3).

The final library, *Library D*, retained the 3,4-dichlorophenyl moiety from **1**, but modified the second aromatic ring through reaction with a family of indole carboxaldehydes (Table 4). As before all analogues were accessed in good to excellent yields.

3. Cytotoxicity

With the desired analogues synthesized, we examined their cytotoxicity against a panel of eleven human tumour cell lines: HT29 and SW480 (colon carcinoma), MCF-7 (breast carcinoma), A2780 (ovarian carcinoma), H460 (lung carcinoma), A431 (skin carcinoma), DU145 (prostate carcinoma), BE2-C (neuroblastoma), SJ-G2 (glioblastoma), MIA (pancreatic carcinoma) and SMA (spontaneous murine astrocytoma). All analogues were initially screened at a drug concentration of 25 μM. Those analogues returning good to excellent percentage growth inhibition across all cell lines, or those displaying cell line specificity were subjected to full dose response, GI_{50}, evaluation. These data are shown in Tables 1–4.

As part of our drug development program we do not screen against normal cell lines, as a single *in vitro* assay is a poor measure by which to evaluate risk and predict human toxicity. These approaches fail to provide a "go/no-go" step in the drug development process and when used in a prospective manner, they have not been highly predictive of *in vivo* toxicity [22]. A single *in vitro* screening platform is unlikely to provide the data required to evaluate risk and predict human toxicity. It is widely accepted that additional models using a tiered toxicity screening approach are required in order to define and predict clinical toxicity. As part of our drug development strategy small molecules that satisfy specific druggability characteristics as well as cytotoxicity will undergo further biological evaluation in animal models.



Scheme 1. Reagents and conditions: (i) Ar_1CHO (see Table 1 for details), 40% $PhCH_2NMe_3(OH)$ (cat), H_2O 50 °C 5 h.

4. Results and discussion

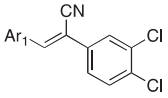
Using an approach of focused library synthesis, biological screening, re-design and library synthesis we commenced our 2-phenylacrylonitrile SAR investigations with the synthesis of a discrete four component library (*Library A*) [6,7]. Initially we sought to confirm the effect of increasing electron-withdrawing groups (EWG) on the first aromatic substituent (Ar_1, Fig. 1). The importance of the EWG was probed via the synthesis of **2**–**5** (Table 1, Scheme 1).

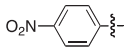
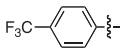
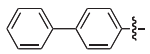
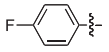
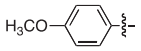
Of the *Library A* analogues, the 4-Ph (**5**) was insoluble in the testing media precluding evaluation. In *Library A* the 4-CF_3 moiety of **2** most closely mimicked the electron withdrawing effects of the 4-NO_2 moiety of **1**, and returned modest levels of growth inhibition with an average GI_{50} ~ 41 μM (c.f. **1**, average GI_{50} = 16.8 μM). The 4-CF_3 (**2**) was equipotent with furan (**4**) (average GI_{50} = 39 μM), but a 3-fold reduction in activity relative to **1**. Indole (**3**) was highly potent displaying excellent broad spectrum growth inhibition, with an average GI_{50} value of 1.4 μM. Indole (**3**) was 12 fold more potent than **1**. Of the analogues shown in Table 1, the 4-OCH_3 (**7**) displayed the highest level of broad spectrum cytotoxicity with an average GI_{50} value of 1.08 μM. The 4-OCH_3 (**7**) was 16 fold more potent than **1**. The indole (**3**), furan (**4**), phenyl (**5**) and 4-OCH_3 (**7**) lack the electron withdrawing capability, but do place a considerable degree of electron density in a similar region to the $-CF_3$ and $-NO_2$ moieties of (**1**) and (**2**) which may account for the observed activity.

While the 4-CF_3 (**2**) was equipotent with the furan (**4**) its activity compared very poorly to both the lead (**1**) and our previously reported 4-F analogue (**6**; average GI_{50} value of 11 μM). The enhanced potency of **7** with the electron donating $-OCH_3$ moiety strongly suggested that the enhanced cytotoxicity was not one purely associated with the EWG effects at C4′, but that there was also possibly a steric component. This was reinforced on examination of the cytotoxicity of (**3**) and (**4**) which despite possessing no Ar_1-EWG returned excellent and modest average GI_{50} values of 1.4 and 36 μM respectively. We note that indole (**3**) was significantly more cytotoxic than furan (**4**). This may be due to the relative orientation of the lone pairs of electrons and/or the increased steric bulk associated with the indole moiety. Regardless, (**3**) and (**4**) showed clear evidence of growth inhibition which is contrary to our initial 2-phenylacrylonitrile hypothesis [6]. From within *Library A* only (**4**) displayed any selectivity towards the MCF-7 breast cancer cell line, and this was a modest 5-fold. Indole (**3**) and 4-OCH_3 (**7**) were the most potent acrylonitriles to emerge from our laboratory thus far, but failed to display any cell line specificity, unlike the lead acrylonitrile (**1**).

To investigate the role of the cyanide moiety within the acrylonitrile unit, two acrylic acid analogues were synthesized (**8** and **9**) from 3,4-dichlorophenylacetic acid and 4-methoxybenzaldehyde and 4-nitrobenzaldehyde (Scheme 2). Cytotoxicity screening showed that neither of the two acrylic acid derivatives (**8**) and (**9**) was active at 25 μM drug concentration (*Library B*, Table 2). This compared very poorly with the corresponding acrylonitrile analogues (**1**) and (**7**) (Table 1) which, against our original panel of 10 human cancer cell lines, returned an average GI_{50} value of 11 and

Table 1
Evaluation of the cytotoxicity, GI_{50} values, of (*Z*)-2-(3,4-dichlorophenyl)-3-(aromatic)acrylonitriles derivatives **2**–**5** (*Library A*), against a panel of eleven cancer cell lines. GI_{50} is the concentration of drug that reduces cell growth by 50%.



Ar_1	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]	SMA[j]
 **1**	3.1 ± 1.8	8.4 ± 3.3	0.127 ± 0.04	12 ± 1	69 ± 2	7.1 ± 0.1	18 ± 3	8.9 ± 0.7	14 ± 1	27 ± 4	n.d.[k]
 **2**	35 ± 6	49 ± 13	31 ± 6	41 ± 9	36 ± 1	53 ± 14	45 ± 13	43 ± 9	41 ± 10	35 ± 8	45 ± 13
 **3**	0.85 ± 0.1	2.1 ± 0.1	1.3 ± 0.0	0.90 ± 0.1	1.0 ± 0.1	0.8 ± 0.0	3.1 ± 0.1	1.0 ± 0.1	1.7 ± 0.1	1.6 ± 0.2	1.3 ± 0.2
 **4**	36 ± 0	45 ± 4	7 ± 0	38 ± 2	52 ± 24	39 ± 4	42 ± 6	38 ± 1	50 ± 6	46 ± 6	36 ± 0
 **5**	–	–	–	–	–	–	–	–	–	–	–[l]
 **6**	9.3 ± 2.4	5.6 ± 0.6	6.5 ± 1.0	9.5 ± 1	10 ± 0	10 ± 0	18 ± 2	13 ± 2	16 ± 1	11 ± 1	−6
 **7**	0.52 ± 0.05	1.4 ± 0.1	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.6 ± 0.1	1.4 ± 0.1	2.7 ± 2.0	1.5 ± 0.2	0.7 ± 0.0	−6

[a] HT29 and SW480 (colon carcinoma).
[b] MCF-7 (breast carcinoma).
[c] A2780 (ovarian carcinoma).
[d] H460 (lung carcinoma).
[e] A431 (skin carcinoma).
[f] DU145 (prostate carcinoma).
[g] BEC-2 (neuroblastoma).
[h] SJ-G2 (glioblastoma).
[i] MIA (pancreatic carcinoma).
[j] SMA (spontaneous murine astrocytoma).
[k] n.d. = not determined.
[l] Insoluble in testing media.

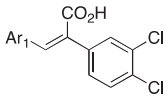
1.1 μM respectively [6]. While it was possible that this reduction in cytotoxicity was merely a reflection of the change in cell permeability, but with other carboxylate containing cytotoxics we have not experienced such changes in cell permeability [23,24]. We believe that these data strongly suggested a pivotal role for the −CN moiety in preventing cell growth.

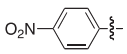
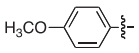
Of the *Library A* active analogues we next examined the effects of subtle modifications of the furan moiety. Synthesis was conducted as per Scheme 1. This new library, *Library C*, comprised four furan analogues (**10**–**13**) and these were screened against our panel of eleven cancer cell lines. These data are presented in Table 3. As can be seen from the data presented, none of these furan analogues (**10**–**13**) returned growth inhibition at 25 μM drug concentration at a level that warranted determination of a full GI_{50} determination. Of the two most active furans (**12** and **13**) the 5-methyl (**13**) is marginally the most potent (Table 3). However the level of activity in *Library C* was not deemed promising enough to warrant the synthesis of additional members, rather our attention turned to the most active compound produced thus far, the indole (**3**).

Taking indole (**3**) as our new lead compound we developed *Library D* in which the 3,4-dichlorophenyl moiety was retained and modifications were made to the indole nucleus. Synthesis was conducted as per Scheme 1 and the results of cytotoxicity screening are presented in Table 4.

With the exception of the 5-cyanoindole (**18**) all *Library D* analogues returned excellent levels of growth inhibition with average GI_{50} values between 0.53 and 8.4 μM. The 2-methylindole (**14**) was marginally more potent (average GI_{50} = 2.9 μM) than the corresponding 5-methylindole (**15**) (average GI_{50} = 4.0 μM), suggesting a positional preference for a small alkyl moiety. Examination of the 5-Cl, 5-Br and 5-CN indoles (**16**–**18**) showed a clear trend of diminishing potency as the size/EWG potential of the 5-moiety increased, with average GI_{50} values of 8.2 and 8.4 μM for (**16**) and (**17**) with (**18**) essentially inactive. Interestingly the benzo[*g*]indole (**19**) which positions the indole substituents at C6/C7, saw a restoration of activity with an average GI_{50} = 5.3 μM, demonstrating that 5-substituents are less well tolerated and like our initial *Library A* EWGs are not as well

Table 2
Evaluation of the cytotoxicity of (*Z*)-2-(3,4-dichlorophenyl)-3-(aromatic)acrylic acid derivatives **8** and **9** against a panel of eleven cancer cell lines. Values are the % growth inhibition measured at 25 μM drug concentration.



Ar_1	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]	SMA[j]
 **8**	28 ± 1	<10	11 ± 5	<10	<10	<10	<10	<10	<10	<10	<10
 **9**	11 ± 1	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10

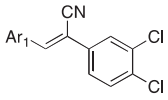
[a] HT29 and SW480 (colon carcinoma).
[b] MCF-7 (breast carcinoma).
[c] A2780 (ovarian carcinoma).
[d] H460 (lung carcinoma).
[e] A431 (skin carcinoma).
[f] DU145 (prostate carcinoma).
[g] BEC-2 (neuroblastoma).
[h] SJ-G2 (glioblastoma).
[i] MIA (pancreatic carcinoma).
[j] SMA (spontaneous murine astrocytoma).

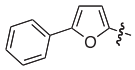
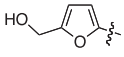
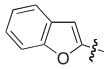
tolerated as our original hypothesis suggested. To further evaluate this we synthesized indole (**20**), (*Z*)-2-(3,4-dichlorophenyl)-3-(1*H*-indol-5-yl)acrylonitrile saw an inversion of the indole moiety relative to lead (**3**), with the addition to the acrylonitrile moiety occurring via C5 of the indole (with **3**, the addition occurs via the indole C3) (Fig. 2). This minor change gave rise to the most potent broad spectrum cytotoxic analogue in the libraries developed to date with an average GI_{50} = 0.53 μM. This is a 32 fold increase in cytotoxicity relative to **1**.

5. Conclusion

In our initial series of 2-phenylacrylonitriles we demonstrated high levels of specificity towards the ER+ve breast cancer cell line,

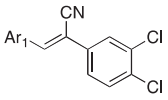
Table 3
Cell death of (*Z*)-2-(3,4-dichlorophenyl)-3-(furanyl)acrylonitriles derivatives **10**–**13** (*Library B*) against a panel of eleven cancer cell lines. Values are the % growth inhibition measured at 25 μM drug concentration.

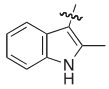
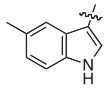
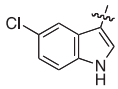
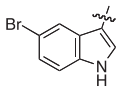
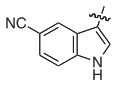
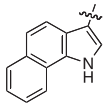
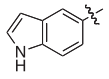


Ar_1	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]	SMA[j]
 **10**	15 ± 2	<10	<10	13 ± 1	11 ± 2	<10	<10	<10	<10	<10	<10
 **11**	41 ± 4	<10	27 ± 4	25 ± 6	30 ± 5	<10	<10	<10	27 ± 1	<10	16 ± 2
 **12**	43 ± 5	43 ± 1	40 ± 2	49 ± 4	36 ± 3	54 ± 5	14 ± 4	20 ± 8	46 ± 24	47 ± 1	55 ± 18
 **13**	58 ± 3	47 ± 1	68 ± 0	43 ± 3	40 ± 2	46 ± 5	<10	50 ± 2	32 ± 2	36 ± 1	33 ± 3

[a] HT29 and SW480 (colon carcinoma).
[b] MCF-7 (breast carcinoma).
[c] A2780 (ovarian carcinoma).
[d] H460 (lung carcinoma).
[e] A431 (skin carcinoma).
[f] DU145 (prostate carcinoma).
[g] BEC-2 (neuroblastoma).
[h] SJ-G2 (glioblastoma).
[i] MIA (pancreatic carcinoma).
[j] SMA (spontaneous murine astrocytoma).

Table 4
Evaluation of the cytotoxicity, GI_{50} values, of (*Z*)-2-(3,4-dichlorophenyl)-3-(indolyl)acrylonitriles derivatives **14**–**20** (*Library D*), against a panel of eleven cancer cell lines. GI_{50} is the concentration of drug that reduces cell growth by 50%. Values in parentheses and italics are the % growth inhibition measured at 25 μM drug concentration.

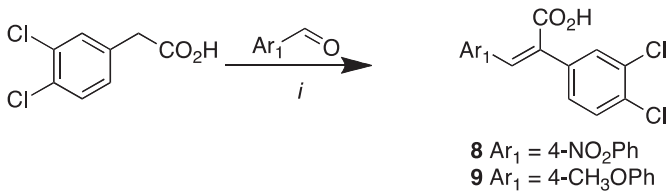


Ar_1	HT29[a]	SW480[a]	MCF-7[b]	A2780[c]	H460[d]	A431[e]	DU145[f]	BE2-C[g]	SJ-G2[h]	MIA[i]	SMA[j]
 **14**	1.9 ± 0.06	3.8 ± 0.35	2.8 ± 0.20	2.2 ± 0.37	2.2 ± 0.20	2.1 ± 0.07	5.6 ± 0.61	2.0 ± 0.03	4.0 ± 0.50	2.4 ± 0.22	2.9 ± 0.24
 **15**	2.8 ± 0.26	4.7 ± 0.58	3.7 ± 0.23	3.2 ± 0.20	3.0 ± 0.12	3.0 ± 0.10	7.4 ± 1.03	3.4 ± 0.23	5.3 ± 0.58	3.7 ± 0.09	4.1 ± 0.27
 **16**	7.1 ± 0.19	8.8 ± 1.33	2.3 ± 1.46	7.4 ± 0.49	8.7 ± 0.73	7.7 ± 0.20	12 ± 0.50	8.6 ± 0.69	9.7 ± 0.88	11 ± 0.17	6.7 ± 0.15
 **17**	7.0 ± 0.12	8.2 ± 1.01	6.9 ± 0.94	6.8 ± 0.68	7.5 ± 0.29	7.3 ± 0.38	13 ± 1.00	7.0 ± 0.52	10 ± 0.29	9.0 ± 0.52	9.2 ± 0.73
 **18**	(*57 ± 5*)	(*43 ± 2*)	(*38 ± 8*)	(*47 ± 4*)	(*33 ± 3*)	(*34 ± 2*)	(*18 ± 7*)	(*50 ± 5*)	(*44 ± 2*)	(*43 ± 2*)	(*44 ± 7*)
 **19**	4.2 ± 0.40	7.5 ± 0.31	4.0 ± 0.15	3.7 ± 0.27	5.0 ± 0.69	3.0 ± 0.50	8.7 ± 1.20	3.8 ± 0.80	6.8 ± 0.72	6.1 ± 0.90	5.9 ± 0.55
 **20**	0.36 ± 0.01	0.67 ± 0.03	0.23 ± 0.02	0.41 ± 0.04	0.39 ± 0.02	0.37 ± 0.08	1.04 ± 0.28	0.33 ± 0.09	0.83 ± 0.18	0.50 ± 0.08	0.74 ± 0.06

[a] HT29 and SW480 (colon carcinoma).
[b] MCF-7 (breast carcinoma).
[c] A2780 (ovarian carcinoma).
[d] H460 (lung carcinoma).
[e] A431 (skin carcinoma).
[f] DU145 (prostate carcinoma).
[g] BEC-2 (neuroblastoma).
[h] SJ-G2 (glioblastoma).
[i] MIA (pancreatic carcinoma).
[j] SMA (spontaneous murine astrocytoma).

MCF-7, but limited broad spectrum efficacy [6]. Preliminary conclusions based on focused libraries developed herein suggest that EWGs on Ar_1 are not pivotal to cytotoxicity in this class of compound. We hypothesize that the presence and positioning of a lone pair of electrons capable of participating in hydrogen bonding interactions may be of importance.

Of particular note were the two indole analogues (**3**) and (**20**) which return average GI_{50} values of 1.4 and 0.53 μM respectively. Indoles (**3**) and (**20**) lack a powerful EWG but are, 12 and 32 fold respectively, more active than the lead (**1**). Moreover we have also demonstrated the pivotal nature of the −CN moiety (of the acrylonitrile) as conversion to the corresponding −COOH (acrylic acid) gave compounds bereft of activity against the eleven human cancer cell lines evaluated. These new indolylacrylonitriles hold considerable promise as broad spectrum anticancer agents and we will report new developments in due course.



Scheme 2. Reagents and conditions: (i) 4-$CH_3OPhCHO$ or 4-NO_2PhCHO, Et_3N, Ac_2O, N_2, 140 °C.

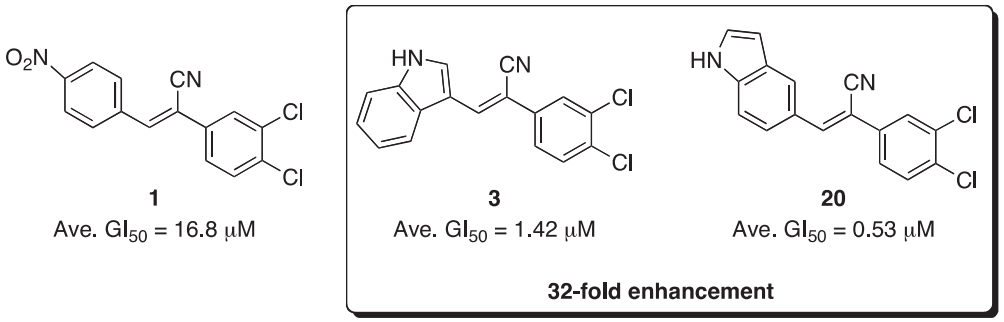


Fig. 2. Chemical structures of the acrylonitrile lead (**1**) and indoles (**3**) and (**20**) and their average GI_{50} values across the cell lines evaluated herein. Simple modifications have affected a 32-fold potency enhancement.

6. Experimental

6.1. Biology

6.1.1. Cell culture and stock solutions

Stock solutions were prepared as follows and stored at −20 °C: drugs were stored as 40 mM solutions in DMSO. All cell lines were cultured at 37 °C, under 5% CO_2 in air and were maintained in Dulbecco's modified Eagle's medium (Trace Biosciences, Australia) supplemented with 10% foetal bovine serum, 10 mM sodium bicarbonate penicillin (100 IU/mL), streptomycin (100 μg/mL), and glutamine (4 mM).

6.1.2. In vitro growth inhibition assay

Cells in logarithmic growth were transferred to 96-well plates. Cytotoxicity was determined by plating cells in duplicate in 100 mL medium at a density of 2500–4000 cells/well. On day 0, (24 h after plating) when the cells were in logarithmic growth, 100 μL medium with or without the test agent was added to each well. After 72 h drug exposure growth inhibitory effects were evaluated using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay and absorbance read at 540 nm. Percentage growth inhibition was determined at a fixed drug concentration of 25 μM. A value of 100% is indicative of total cell growth inhibition. Those analogues showing appreciable percentage growth inhibition underwent further dose response analysis allowing for the calculation of a GI_{50} value. This value is the drug concentration at which cell growth is 50% inhibited based on the difference between the optical density values on day 0 and those at the end of drug exposure [25,26].

6.2. Chemistry

6.2.1. General experimental – general methods

All reagents were purchased from Sigma–Aldrich, Matrix Scientific or Lancaster Synthesis and were used without purification. With the exception of THF (anhydrous > 99%) obtained from Sigma–Aldrich, all solvents were re-distilled from glass prior to use.

^1H and ^{13}C NMR spectra were recorded on a Bruker Avance™ AMX 300 MHz spectrometer at 300.13 and 75.48 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) measured relative to the internal standards, and coupling constants (J) are expressed in Hertz (Hz). Mass spectra were recorded on a Shimadzu LCMS 2010 EV using a mobile phase of 1:1 acetonitrile:H_2O with 0.1% formic acid. Analyses indicated by the symbols of the elements or functions were within ±0.4% of the theoretical values.

Melting points were recorded on a Stuart Scientific melting point apparatus (UK) and are uncorrected. Thin layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} pre-coated aluminium plates with a thickness of 0.2 mm. Column chromatography was performed under 'flash' conditions on Merck silica gel 60 (230–400 mesh) or using the Biotage SP4 flash purification system with a 100 g pre-packed snap column.

A CEM Discover® BenchMate microwave (120 °C, 200 W, 1 h) was used to perform several refluxes.

6.2.2. (Z)-2-(3,4-Dichlorophenyl)-3-(4-nitrophenyl)acrylonitrile (**1**)

4-Nitrobenzaldehyde (263 mg, 1.74 mmol), was added to a vigorously stirring solution of water (10 mL) and heated to 50 °C. Once the aldehyde was seen to dissolve, 3,4-dichlorophenylacetonitrile (307 mg, 1.65 mmol) was slowly added resulting in the formation of a suspension and the reaction mixture was stirred for a further 10 min. After this time, 40% *N*,*N*,*N*-trimethyl(phenyl)methanammonium hydroxide, [$PhCH_2NMe_3(OH)$], in water (7 mL) was added drop-wise to the reaction mixture and once addition was complete, the reaction vessel was sealed and stirring was continued at 50 °C for 5 h. After this period, the solution was filtered hot, washed with warm water and dried under suction to yield a brown solid. The crude solid was subsequently recrystallized from EtOH to afford (**1**) as a purple solid (75%), m.p. 133–134 °C; ^1H NMR ($CDCl_3$): δ 8.34 (d, J = 8.8 Hz, 2H, H-3′ and H-5′), 8.03 (d, J = 8.8 Hz, 2H, H-2′ and H-6′), 7.81–7.80 (m, 1H, H-5), 7.58–7.56 (m, 2H, H-2 and H-6), 7.26 (s, 1H, CH=C); ^{13}C NMR ($CDCl_3$) (75 MHz): δ; 148.6, 140.1, 138.9, 134.7, 133.9, 133.3, 131.3, 130.1, 128.0, 125.4, 124.3, 116.4, 113.7; IR (KBr) ν_{max}/cm^{-1}: 2215 (CN), 1674 (C=C), 1592 (Ar), 1513 (NO), 1345 (NO); m/z (APCI M + H) 289 ($NO_2 \rightarrow NH_2$); HRMS (APCI M + H): Calculated for $C_{15}H_{10}Cl_2N_2$; Exact Mass: 288.0221, found: 289.0263. Anal. $C_{15}H_{10}Cl_2N_2$ (C, H, N).

6.2.3. (Z)-2-(3,4-Dichlorophenyl)-3-(4-(trifluoromethyl)phenyl) acrylonitrile (**2**)

Synthesized using the general procedure as for (**1**), from 4-(trifluoromethyl)benzaldehyde and 3,4-dichlorophenylacetonitrile to afford (**2**) as a white solid (89%), m.p. 120–122 °C; ^1H NMR (DMSO-d_6): δ 8.27 (s, 1H, CH=C), 8.09–8.05 (m, 3H, H-2′, H-6′ and H-5), 7.90 (d, J = 8.2 Hz, 2H, H-3′ and H-5′), 7.79–7.70 (m, 2H, H-2 and H-6); ^{13}C NMR (DMSO-d_6): δ 142.9, 137.2, 133.8, 132.8, 132.2, 131.2, 130.0, 129.8 (2× Ar), 127.5, 126.3, 125.8 (2× Ar), 125.7, 116.7, 110.6; IR (KBr) ν_{max}/cm^{-1}: 2215 (CN), 1618 (C=C), 1478 (Ar); m/z (APCI M − H) 341; HRMS (ESI M − H): Calculated for $C_{16}H_8Cl_2F_3N$; Exact Mass: 340.9986, found: 341.0126. Anal. $C_{16}H_8Cl_2F_3N$ (C, H, N).

6.2.4. (Z)-2-(3,4-Dichlorophenyl)-3-(1H-indol-3-yl)acrylonitrile (**3**)

Synthesized using the general procedure as for (**1**), from 1*H*-indole-3-carbaldehyde to afford (**3**) as a yellow solid (48%), m.p. 225–226 °C; ^1H NMR (DMSO-d_6): δ 12.03 (br, NH), 8.39 (s, 1H, CH=C), 8.34 (s, 1H, H-2′), 8.12–8.09 (m, 1H, H-4′), 8.03–8.02 (m, 1H, H-5), 7.70–7.61 (m, 2H, H-5′ and H-6′), 7.52–7.49 (m, 1H, H-7′), 7.26–7.16 (m, 2H, H-2 and H-6); ^{13}C NMR (DMSO-d_6): δ 136.3, 135.7, 135.2,

131.9, 130.9, 129.8, 127.8, 127.2, 126.1, 125.0, 122.8, 120.7, 119.2, 118.9, 112.2, 110.7, 99.6; IR (KBr) ν_{max}/cm^{-1}: 3311 (NH), 2212 (CN), 1628 (C═C), 1574 (Ar), 732 (Ar–Cl); *m/z* (APCI M − H) 311; HRMS (ESI M − H): Calculated for $C_{17}H_{10}Cl_2N_2$; Exact Mass: 312.0221, found: 311.0160. Anal. $C_{17}H_{10}Cl_2N_2$ (C, H, N).

*6.2.5. (Z)-2-(3,4-Dichlorophenyl)-3-(furan-2-yl)acrylonitrile (**4**)*

Synthesized using the general procedure as for (**1**), from furan-2-carbaldehyde to afford (**4**) as a light orange solid (75%), m.p. 126–128 °C; ^{1}H NMR ($CDCl_3$): δ 7.72 (s, 1H, H-5′), 7.63 (s, 1H, CH═C), 7.48 (m, 2H, H-5 and H-2), 7.36 (s, 1H, H-6), 7.23 (d, *J* = 3.4 Hz, 1H, H-3′), 6.61 (dd, *J* = 3.2, 1.4 Hz, 1H, H-4′); ^{13}C NMR ($CDCl_3$): δ 149.6, 145.6, 133.7, 133.1, 130.9, 129.2, 128.9, 127.2, 124.7, 117.0, 116.4, 113.0, 105.1; IR (KBr) ν_{max}/cm^{-1}: 2215 (CN), 1617 (C═C), 1466 (Ar); *m/z* (APCI M − H) 363; HRMS (ESI M − H): Calculated for $C_{13}H_7Cl_2NO$; Exact Mass: 262.9905, found: 263.0243. Anal. $C_{13}H_7Cl_2NO$ (C, H, N).

*6.2.6. (Z)-3-(Biphenyl-4-yl)-2-(3,4-dichlorophenyl)acrylonitrile (**5**)*

Synthesized using the general procedure as for (**1**), from biphenyl-4-carbaldehyde to afford (**5**) as a white solid (61%), m.p. 230–232 °C; ^{1}H NMR (DMSO-d_6): δ 8.23 (s, 1H, CH═C), 8.08–8.05 (m, 3H, H-2, H-3′ and H-5′), 7.89 (d, *J* = 8.2 Hz, 2H, H-2′ and H-6′), 7.81–7.73 (m, 4H, H-2″, H-3″, H-5″ and H-6″), 7.53–7.49 (m, 2H, H-5, H-4″), 7.45–7.42 (m, 1H, H-6); ^{13}C NMR (DMSO-d_6): δ 144.6, 143.0, 139.3, 135.0, 132.8, 132.6, 132.2, 131.8, 130.5, 129.6, 128.7, 127.8, 127.6, 127.3, 126.6, 118.0, 107.9; IR (KBr) ν_{max}/cm^{-1}: 2216 (CN), 1593 (Ar), 1474 (Ar), 1448 (Ar); *m/z* (APCI M − H) 349; HRMS (ESI M − H): Calculated for $C_{21}H_{13}Cl_2N$; Exact Mass: 349.0425, found: 349.0397. Anal. $C_{21}H_{13}Cl_2N$ (C, H, N).

*6.2.7. (Z)-2-(3,4-Dichlorophenyl)-3-(4-fluorophenyl) acrylonitrile (**6**)*

Synthesized using the general procedure as for (**1**), from 4-fluorobenzaldehyde and 4-chlorophenyl acetonitrile to afford (**6**) as a white solid (94%), m.p. 156–157 °C (Lit. [4] 155 °C); ^{1}H NMR ($CDCl_3$): δ 7.93–7.88 (m, 2H, H-2′ and H-6′), 7.75–7.74 (m, 1H, H-5), 7.51–7.48 (m, 3H, H-3′, H-5′ and CH═C), 7.20–7.15 (m, 2H, H-2 and H-6); ^{13}C NMR ($CDCl_3$): δ 141.5, 133.7, 133.0 (2× Ar), 132.9, 131.1, 131.0, 130.5, 128.9, 127.1, 124.6 (2× Ar), 116.6, 115.9, 115.7; IR (KBr) ν_{max}/cm^{-1}: 2213 (CN), 1636 (C═C), 1596 (Ar), 809 (Ar–Cl); *m/z* (APCI M − H) 291; HRMS (APCI M − H): Calculated for $C_{15}H_8Cl_2FN$; Exact Mass: 291.0018, found: 291.0029. Anal. $C_{15}H_8Cl_2FN$ (C, H, N).

*6.2.8. (Z)-2-(3,4-Dichlorophenyl)-3-(4-methoxyphenyl) acrylonitrile (**7**)*

Synthesized using the general procedure as for (**1**), from 4-methoxybenzaldehyde and 3,4-dichlorophenylacetonitrile to afford (**7**) as a yellow solid (79%), m.p. 166–167 °C (Lit. [4] 142 °C); ^{1}H NMR (DMSO-d_6): 8.10 (s, 1H, CH═C), 8.00–7.96 (m, 3H, H-5, H-2′ and H-6′), 7.77–7.75 (m, 1H, H-2), 7.70–7.67 (m, 1H, H-6), 7.13 (d, *J* = 8.9 Hz, 2H, H-3′ and H-5′), 3.85 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6): δ 162.1, 144.8, 135.4, 132.5, 132.0 (2× Ar), 131.7, 131.6, 127.4, 126.3, 126.2, 118.3, 115.1 (2× Ar), 104.9, 55.6; IR (KBr) ν_{max}/cm^{-1}: 2212 (CN), 1638 (C═C), 1609 (Ar), 1593 (Ar), 1513 (Ar); *m/z* (APCI M − H) 303; HRMS (APCI M − H): Calculated for $C_{16}H_{11}Cl_2NO$; Exact Mass: 303.0296, found: 303.0299. Anal. $C_{16}H_{11}Cl_2NO$ (C, H, N).

*6.2.9. (Z)-2-(3,4-Dichlorophenyl)-3-(4-nitrophenyl)acrylic acid (**8**)*

Triethylamine (1.51 g, 15 mmol) was added to a solution of 4-nitrobenzaldehyde (1.51 g, 10 mmol) and 2-(3,4-dichlorophenyl) acetic acid (2.29 g, 11 mmol) in Ac_2O (5 mL). The solution was heated at 140 °C under a nitrogen atmosphere for 1 h. After this period, the solvent was removed *in vacuo* to yield a yellow oil which was purified by flash silica chromatography (1:9 EtOAc:Hexanes) to afford (**8**) as a yellow solid (1.76 g, 52%), m.p. 151–153 °C; ^{1}H NMR (DMSO-d_6): δ 8.15 (d, *J* = 8.8 Hz, 2H, H-3′ and H-5′), 7.90 (d, *J* = 8.8 Hz, 2H, H-2′ and H-6′), 7.83 (m, 1H, H-5), 7.61 (m, 2H, H-2 and H-6), 6.73 (s, 1H, CH═C); ^{13}C NMR (DMSO-d_6): δ 170.5, 145.6, 145.2, 143.9, 139.1, 131.0, 130.4, 130.0, 128.9 (2× Ar), 128.0, 126.4, 123.2 (2× Ar), 119.1; IR (KBr) ν_{max}/cm^{-1}: 3347 (OH), 1594 (C═O), 1337 (N–O); *m/z* (APCI M − H) 336; HRMS (ESI M − H): Calculated for $C_{15}H_9Cl_2NO_4$; Exact Mass: 335.9836, found: 335.9919. Anal. $C_{15}H_9Cl_2NO_4$ (C, H, N).

*6.2.10. (Z)-2-(3,4-Dichlorophenyl)-3-(4-methoxyphenyl)acrylic acid (**9**)*

Synthesized using the general procedure as for (**8**), from 4-methoxybenzaldehyde to afford (**9**) as a yellow solid (15%), m.p. 221–223 °C; ^{1}H NMR (DMSO-d_6): δ 7.75 (s, 1H, CH═C), 7.63 (d, *J* = 8.2 Hz, 1H, H-5), 7.46 (d, *J* = 2.0 Hz, 1H, H-2), 7.15 (dd, *J* = 2.0, 8.2 Hz, 1H, H-6), 7.04 (d, *J* = 8.9 Hz, 2H, H-2′ and H-6′), 6.82 (d, *J* = 8.9 Hz, 2H, H-3′ and H-5′), 3.71 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6): δ 167.7, 160.1, 139.9, 137.5, 132.1 (2× Ar), 132.0, 131.6, 131.1, 130.7, 130.1, 128.1, 126.1, 114.0 (2× Ar), 55.1; IR (KBr) ν_{max}/cm^{-1}: 3448 (OH), 1663 (C═O), 1172 (C–O); *m/z* (APCI M − H) 321; HRMS (ESI M − H): Calculated for $C_{16}H_{12}Cl_2O_3$; Exact Mass: 321.0091, found: 321.0184. Anal. $C_{16}H_{12}Cl_2O_3$ (C, H, N).

*6.2.11. (Z)-2-(3,4-Dichlorophenyl)-3-(5-phenylfuran-2-yl) acrylonitrile (**10**)*

Synthesized using the general procedure as for (**1**), from 5-phenyl-2-furaldehyde and 3,4-dichlorophenylacetonitrile to afford (**10**) as a yellow solid (94%), m.p. 141–143 °C; ^{1}H NMR (DMSO-d_6): δ 8.00 (s, 1H, CH═C), 7.95–7.94 (m, 1H, H-2″), 7.90–7.87 (m, 2H, H-6″ and H-3″), 7.72–7.62 (m, 2H, H-5″ and H-5), 7.51–7.36 (m, 3H, H-4″, H-2, and H-6), 7.27–7.25 (m, 1H, H-4′), 7.18–7.17 (m, 1H, H-3′); ^{13}C NMR (DMSO-d_6): δ 156.3, 148.6, 134.2, 132.0, 131.1, 131.1, 131.0, 129.0, 128.9, 128.8, 126.6, 125.5, 124.4, 121.6, 117.5, 109.2, 102.2; IR (KBr) ν_{max}/cm^{-1}: 2214 (CN), 1630 (C═C), 1578 (Ar), 1507 (Ar), 1448 (Ar), 798 (Ar–Cl); *m/z* (APCI M − H) 339; HRMS (APCI M − H): Calculated for $C_{19}H_{11}Cl_2NO$; Exact Mass: 339.0218, found: 339.0219. Anal. $C_{19}H_{11}Cl_2NO$ (C, H, N).

*6.2.12. (Z)-2-(3,4-Dichlorophenyl)-3-(5-(hydroxymethyl)furan-2-yl)acrylonitrile (**11**)*

Synthesized using the general procedure as for (**1**), from 5-(hydroxymethyl)-2-furaldehyde and 3,4-dichlorophenylacetonitrile to afford (**11**) as a yellow solid (78%), m.p. 145–147 °C; ^{1}H NMR ($CDCl_3$): δ 7.70–7.69 (m, 1H, H-2), 7.51–7.42 (m, 2H, H-5 and H-6), 7.32 (s, 1H, CH═C), 7.14 (d, *J* = 3.5 Hz, 1H, H-4′), 6.50 (d, *J* = 3.5 Hz, 1H, H-3′), 4.71 (s, 2H, CH_2), 2.49 (br, OH); ^{13}C NMR ($CDCl_3$): δ 157.1, 148.8, 133.1, 133.0, 132.5, 130.5, 128.2, 126.6, 124.1, 117.1, 116.6, 110.2, 104.3, 56.9; IR (KBr) ν_{max}/cm^{-1}: 3425 (OH), 2213 (CN), 1625 (C═C), 1513 (Ar), 796 (Ar–Cl); *m/z* (APCI M − H) 293; HRMS (ESI M − H): Calculated for $C_{14}H_9Cl_2NO_2$; Exact Mass: 293.0010, found: 293.0013. Anal. $C_{14}H_9Cl_2NO_2$ (C, H, N).

*6.2.13. (Z)-3-(Benzofuran-2-yl)-2-(3,4-dichlorophenyl) acrylonitrile (**12**)*

Synthesized using the general procedure as for (**1**), from 1-benzofuran-2-carbaldehyde and 3,4-dichlorophenylacetonitrile to afford (**12**) as a yellow solid (60%), m.p. 203–205 °C; ^{1}H NMR (DMSO-d_6): δ 8.21 (s, 1H, H-3′), 8.06 (s, 1H, CH═C), 7.81–7.64 (m, 4H, H-7′, H-4′, H-2, H-6), 7.55 (s, 1H, H-5), 7.50–7.45 (m, 1H, H-6′), 7.36–7.31 (m, 1H, H-5′); ^{13}C NMR (DMSO-d_6): δ 155.0, 150.7, 133.9, 132.1, 131.9, 131.3, 130.2, 127.7, 127.5, 127.2, 126.0, 124.0, 122.7, 116.7, 114.4, 111.5, 106.5; IR (KBr) ν_{max}/cm^{-1}: 2220 (CN), 1632 (C═C), 1578 (Ar), 1509 (Ar), 790 (Ar–Cl); *m/z* (APCI M − H) 313; HRMS (APCI M − H): Calculated for $C_{17}H_9Cl_2NO$; Exact Mass: 313.0061, found: 313.0064. Anal. $C_{17}H_9Cl_2NO$ (C, H, N).

*6.2.14. (Z)-2-(3,4-Dichlorophenyl)-3-(5-methylfuran-2-yl) acrylonitrile (**13**)*

Synthesized using the general procedure as for (**1**), from 5-methyl-2-furaldehyde and 3,4-dichlorophenylacetonitrile to afford (**13**) as a yellow solid (95%), m.p. 154–156 °C; ^{1}H NMR (acetone-d_6): δ 7.91–7.89 (m, 1H, H-2), 7.77 (s, 1H, CH=C), 7.68–7.67 (m, 2H, H-5 and H-6), 7.15–7.14 (m, 1H, H-3′), 6.39–6.37 (m, 1H, H-4′), 2.42 (s, 3H, CH_3); ^{13}C NMR (acetone-d_6): δ 156.4, 148.2, 134.4, 132.1, 131.1, 130.6, 128.7, 126.3, 124.7, 118.6, 116.5, 109.3, 101.8, 12.4; IR (KBr) ν_{max}/cm^{-1}: 2212 (CN), 1629 (C=C), 1545 (Ar), 1520 (Ar), 785 (Ar–Cl); *m*/*z* (APCI M − H) 277; HRMS (APCI M − H): Calculated for $C_{14}H_9Cl_2NO$; Exact Mass: 277.0061, found: 277.0065. Anal. $C_{14}H_9Cl_2NO$ (C, H, N).

*6.2.15. (Z)-2-(3,4-Dichlorophenyl)-3-(2-methyl-1H-indol-3-yl) acrylonitrile (**14**)*

Synthesized using the general procedure as for (**1**), from 2-methyl-1*H*-indole-3-carbaldehyde and 3,4-dichlorophenylacetonitrile to afford (**14**) as a yellow solid (78%), m.p. 217–218 °C; ^{1}H NMR (acetone-d_6): δ 11.10 (br, NH), 8.50 (1, 1H, CH=C), 8.31 (s, 1H, H-2), 7.96 (d, *J* = 2.2 Hz, H-4′), 7.81 (m, 1H, H-4′), 7.72 (dd, *J* = 8.5, 2.2 Hz, 1H, H-5′), 7.63 (d, *J* = 8.5 Hz, 1H, H-6′), 7.44 (d, *J* = 8.3 Hz, 1H, H-5), 7.09 (dd, *J* = 8.3, 1.2 Hz, 1H, H-6), 2.45 (s, 3H, CH_3); ^{13}C NMR (acetone-d_6): δ 135.3, 135.1, 133.9, 132.0, 130.4, 130.0, 129.8, 127.4, 127.2, 125.9, 124.3, 124.2, 118.6, 117.5, 111.3, 110.4, 99.8, 20.3; IR (KBr) ν_{max}/cm^{-1}: 3333 (NH), 2196 (CN), 1639 (C=C), 1578 (Ar), 1507 (Ar), 790 (Ar–Cl); *m*/*z* (ESI M − H) 325; HRMS (ESI M − H): Calculated for $C_{18}H_{12}Cl_2N_2$; Exact Mass: 326.0378, found: 326.0378. Anal. $C_{18}H_{12}Cl_2N_2$ (C, H, N).

*6.2.16. (Z)-2-(3,4-Dichlorophenyl)-3-(5-methyl-1H-indol-3-yl) acrylonitrile (**15**)*

Synthesized using the general procedure as for (**1**), from 5-methyl-1*H*-indole-3-carbaldehyde and 3,4-dichlorophenylacetonitrile to afford (**15**) as a yellow solid (51%), m.p. 207–209 °C; ^{1}H NMR (DMSO-d_6): δ 11.96 (br, NH), 8.37 (s, 1H, CH=C), 8.34 (s, 1H, H-2′), 8.07 (m, 1H, H-4′), 7.93 (s, H-2), 7.72–7.66 (m, 2H, H-6′ and H-7′), 7.41 (d, *J* = 8.3 Hz, 1H, H-5), 7.08 (dd, *J* = 8.3, 1.0 Hz, 1H, H-6), 2.45 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6): δ 136.3, 135.2, 134.1, 131.9, 130.9, 129.8, 129.7, 127.8, 127.5, 126.1, 125.0, 124.5, 119.5, 118.5, 112.0, 110.3, 99.1, 21.3; IR (KBr) ν_{max}/cm^{-1}: 3333 (NH), 2207 (CN), 1633 (C=C), 1577 (Ar), 1507 (Ar), 1471 (Ar), 786 (Ar–Cl); *m*/*z* (ESI M − H) 325; HRMS (ESI M − H): Calculated for $C_{18}H_{12}Cl_2N_2$; Exact Mass: 326.0378, found: 326.0378. Anal. $C_{18}H_{12}Cl_2N_2$ (C, H, N).

*6.2.17. (Z)-3-(5-Chloro-1H-indol-3-yl)-2-(3,4-dichlorophenyl) acrylonitrile (**16**)*

Synthesized using the general procedure as for (**1**), from 5-chloro-1*H*-indole-3-carbaldehyde and 3,4-dichlorophenylacetonitrile to afford (**16**) as a yellow solid (21%), m.p. 273–274 °C; ^{1}H NMR (DMSO-d_6): δ 12.22 (br, NH), 8.46 (s, 1H, CH=C), 8.40 (s, 1H, H-2′), 8.31 (d, *J* = 1.8 Hz, 1H, H-2), 8.13 (s, 1H, H-4′), 7.74–7.67 (m, 2H, H-6′ and H-7′), 7.54 (d, *J* = 8.6 Hz, 1H, H-5), 7.25 (dd, *J* = 8.6, 1.8 Hz, 1H, H-6); ^{13}C NMR (DMSO-d_6): δ 135.8, 134.9, 134.2, 131.9, 131.0, 130.0, 129.0, 128.5, 126.2, 125.8, 125.2, 122.9, 119.1, 118.5, 113.9, 110.4, 100.3; IR (KBr) ν_{max}/cm^{-1}: 3299 (NH), 2207 (CN), 1636 (C=C), 1591 (Ar), 1510 (Ar), 1471 (Ar) 792 (Ar–Cl); *m*/*z* (ESI M − H) 345; HRMS (ESI M − H): Calculated for $C_{17}H_9Cl_3N_2$; Exact Mass: 345.9831, found: 345.9839. Anal. $C_{17}H_9Cl_3N_2$ (C, H, N).

*6.2.18. (Z)-3-(5-Bromo-1H-indol-3-yl)-2-(3,4-dichlorophenyl) acrylonitrile (**17**)*

Synthesized using the general procedure as for (**1**), from 5-bromo-1*H*-indole-3-carbaldehyde and 3,4-dichlorophenylacetonitrile to afford (**17**) as a yellow solid (30%), m.p. 268–270 °C; ^{1}H NMR (DMSO-d_6): δ 12.14 (br, NH), 8.45–8.34 (m, 3H, CH=C, H-2′, and H-2), 8.12 (s, 1H, H-4′), 7.72–7.69 (m, 2H, H-6′ and H-7′), 7.50 (d, *J* = 8.6 Hz, 1H, H-5), 7.37 (dd, *J* = 8.6, 1.8 Hz, 1H, H-6); ^{13}C NMR (DMSO-d_6): δ 135.7, 134.9, 134.5, 131.9, 130.9, 130.0, 129.1, 128.8, 126.2, 125.5, 125.2, 121.5, 119.2, 114.3, 113.8, 110.3, 100.4; IR (KBr) ν_{max}/cm^{-1}: 3334 (NH), 2211 (CN), 1654 (C=C), 1592 (Ar); *m*/*z* (ESI M − H) 389; HRMS (ESI M − H): Calculated for $C_{17}H_9BrCl_2N_2$; Exact Mass: 389.9326, found: 389.9396. Anal. $C_{17}H_9BrCl_2N_2$ (C, H, N).

*6.2.19. (Z)-3-(2-Cyano-2-(3,4-dichlorophenyl)vinyl)-1H-indole-5-carbonitrile (**18**)*

Synthesized using the general procedure as for (**1**), from 3-formyl-1*H*-indole-5-carbonitrile and 3,4-dichlorophenylacetonitrile to afford (**18**) as a yellow solid (60%), m.p. >300 °C; ^{1}H NMR (DMSO-d_6): δ 8.73 (s, 1H, CH=C), 8.51 (s, 1H, H-2′), 8.39 (s, 1H, H-2), 8.09 (s, 1H, H-4′), 7.71–7.63 (m, 3H, H-6′, H-7′, and H-5), 7.55 (dd, *J* = 8.4, 1.3 Hz, 1H, H-6); ^{13}C NMR (DMSO-d_6): δ 137.5, 135.1, 134.6, 132.0, 131.0, 130.4, 129.8, 127.1, 126.3, 125.5, 125.3, 124.9, 120.3, 118.9, 113.7, 111.1, 102.9, 101.7; IR (KBr) ν_{max}/cm^{-1}: 3296 (NH), 2222 (CN), 2207 (CN), 1623 (C=C), 1473 (Ar), 809 (Ar–Cl); *m*/*z* (ESI M − H) 336; HRMS (ESI M − H): Calculated for $C_{18}H_9Cl_2N_3$; Exact Mass: 337.0174, found: 337.0223. Anal. $C_{18}H_9Cl_2N_3$ (C, H, N).

*6.2.20. (Z)-3-(1H-Benzo[g]indol-3-yl)-2-(3,4-dichlorophenyl) acrylonitrile (**19**)*

Synthesized using the general procedure as for (**1**), from 1*H*-benzo[*g*]indole-3-carbaldehyde and 3,4-dichlorophenylacetonitrile to afford (**19**) as a yellow solid (24%), m.p. 254–256 °C; ^{1}H NMR (DMSO-d_6): δ 8.46–8.39 (m, 3H, CH=C, H-6′, and H-9′), 8.24 (d, *J* = 8.8 Hz, 1H, H-7′), 8.11 (s, 1H, H-2′), 7.99–7.97 (m, 1H, H-8′), 7.75–7.57 (m, 4H, H-2, H-5, H-4′, and H-5′), 7.50–7.45 (m, 1H, H-6); ^{13}C NMR (DMSO-d_6): δ 136.4, 135.0, 132.0, 131.1, 130.6, 130.4, 130.1, 128.4, 126.3, 126.0, 125.3, 125.2, 124.5, 123.6, 121.7, 121.5, 120.6, 119.3, 118.8, 112.4, 100.7; IR (KBr) ν_{max}/cm^{-1}: 3316 (NH), 2196 (CN), 1639 (C=C), 1588 (Ar), 1516 (Ar), 1473 (Ar), 744 (Ar–Cl); *m*/*z* (ESI M − H) 361; HRMS (ESI M − H): Calculated for $C_{21}H_{12}Cl_2N_2$; Exact Mass: 362.0378, found: 362.0399. Anal. $C_{21}H_{12}Cl_2N_2$ (C, H, N).

*6.2.21. (Z)-2-(3,4-Dichlorophenyl)-3-(1H-indol-5-yl)acrylonitrile (**20**)*

Synthesized using the general procedure as for (**1**), from 1*H*-indole-5-carbaldehyde and 3,4-dichlorophenylacetonitrile to afford (**20**) as a yellow solid (70%), m.p. 185–186 °C; ^{1}H NMR (acetone-d_6): δ 10.7 (br, NH), 8.31 (s, 1H, H-4′), 8.10 (s, 1H, CH=C), 7.96–7.89 (m, 2H, H-6′ and H-7′), 7.75–7.57 (m, 3H, H-2, H-5, and H-6), 7.47 (d, *J* = 3.2 Hz, 1H, H-2′), 6.63 (d, *J* = 3.2 Hz, 1H, H-3′); ^{13}C NMR (acetone-d_6): δ 145.7, 135.5, 132.0, 130.9, 130.5, 126.6, 126.1, 124.9, 124.5, 123.2, 122.2, 117.6, 111.4, 111.3, 102.3, 102.2; IR (KBr) ν_{max}/cm^{-1}: 3338 (NH), 2207 (CN), 1653 (C=C), 1569 (Ar), 1474 (Ar); *m*/*z* (ESI M − H) 311; HRMS (ESI M − H): Calculated for $C_{17}H_{10}Cl_2N_2$; Exact Mass: 312.0221, found: 312.0286. Anal. $C_{17}H_{10}Cl_2N_2$ (C, H, N).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2012.09.019.

References

[1] www.cancer.org.au/Newsmedia/factsfigures.htm, (accessed 24.02.12).
[2] R. Griffith, M.N. Brown, A. McCluskey, L.K. Ashman, Small molecule inhibitors of protein kinases in cancer – how to overcome resistance, Mini Rev. Med. Chem. 6 (2006) 1101–1110.

[3] J. Zhang, P.L. Yang, N.S. Gray, Targeting cancer with small molecule kinase inhibitors, Nat. Rev. Cancer 9 (2009) 28–39.
[4] P.A. Janne, N. Gray, J. Settleman, Factors underlying sensitivity of cancers to small-molecule kinase inhibitors, Nat. Rev. Drug Discov. 8 (2009) 709–723.
[5] S.K. Grant, Therapeutic protein kinase inhibitors, Cell. Mol. Life Sci. 66 (2009) 1163–1177.
[6] M. Tarleton, M.J. Robertson, J. Gilbert, J.A. Sakoff, A. McCluskey, Library synthesis and cytotoxicity of a family of 2-phenylacrylonitriles and discovery of an estrogen dependent lead compound, MedChemComm 2 (2011) 31–37.
[7] A. Ali, M. Bliese, J.A.M. Rasmussen, R.M. Sargent, S. Saubern, D.G. Sawutz, J.S. Wilkie, D.A. Winkler, K.N. Winzenberg, R.C.J. Woodgate, Discovery of (*Z*)-2-phenyl-3-(1*H*-pyrrol-2-yl)acrylonitrile derivatives active against *Haemonchus contortus* and *Ctenocephalides felis* (Cat flea), Bioorg. Med. Chem. Lett. 17 (2007) 993–997.
[8] T. Hill, L.R. Odell, J.K. Edwards, M.E. Graham, A.B. McGeachie, J. Rusak, A. Quan, R. Abagyan, J.L. Scott, P.J. Robinson, A. McCluskey, J. Med. Chem. 48 (2005) 7781–7788.
[9] T.A. Hill, L.R. Odell, A. Quan, G. Ferguson, P.J. Robinson, A. McCluskey, Long chain amines and long chain ammonium salts as novel inhibitors of dynamin GTPase activity, Bioorg. Med. Chem. Lett. 14 (2004) 3275–3278.
[10] T.A. Hill, C.P. Gordon, A.B. McGeachie, B. Venn-Brown, L.R. Odell, N. Chau, A. Quan, A. Mariana, J.A. Sakoff, M. Chircop, P.J. Robinson, A. McCluskey, Inhibition of dynamin mediated endocytosis by the dynoles – synthesis and functional activity of a family of indoles, J. Med. Chem. 52 (2009) 3762–3773.
[11] G. Alberghina, M.E. Amato, F.A. Bottino, A. Corsaro, S. Fisichella, Proton NMR, ultraviolet, and infrared spectra of some (*Z*)-α-(phenyl)-β-(2-furyl), -(2-pyrrolyl), and -(*N*-methyl-2-pyrrolyl)acrylonitriles, J. Heterocycl. Chem. 23 (1986) 1747–1752.
[12] W. Herz, J. Brasch, Pyrroles. XII. Reaction of pyrrolealdehydes with arylacetonitriles, J. Org. Chem. 23 (1958) 711–714.
[13] K.N. Winzenberg, S. Saubern, D.G. Sawutz, PCT Int. Appl. WO 06/055565, 2006.
[14] K. Wang, D. Dabin Kim, A. Dömling, Cyanoacetamide MCR (III): three-component Gewald reactions revisited, J. Comb. Chem. 12 (2010) 111–118.
[15] M. Radi, L. Botta, G. Casaluce, M. Bernardini, M. Botta, Practical one-pot two-step protocol for the microwave-assisted synthesis of a highly functionalized rhodanine derivatives, J. Comb. Chem. 12 (2010) 200–205.
[16] A. McCluskey, P.J. Robinson, T. Hill, J.L. Scott, J.K. Edwards, Green chemistry approaches to the Knoevenagel condensation: comparison of ethanol, water and solvent free (dry grind) approaches, Tetrahedron Lett. 43 (2002) 3117–3120.
[17] T. Inokuchi, H. Kawafuchi, *E*- or *Z*-selective Knoevenagel condensation of acetoacetic derivatives: effect of acylated substituent, that is, TEMPO and amines, as an auxiliary, and new access to trisubstituted *E*- and *Z*-2-alkenals and furans, J. Org. Chem. 71 (2006) 947–953.
[18] F.S. Prout, F.A. Abdel-Latif, M.R. Kamal, Catalyst study of the Knoevenagel condensation, J. Chem. Eng. Data 8 (1963) 597–599.
[19] S.V. Ryabukhin, A.S. Plaskon, D.M. Volochnyuk, S.E. Pipko, A.N. Shivanyuk, A.A. Tolmachev, Combinatorial Knoevenagel reactions, J. Comb. Chem. 9 (2007) 1073–1078.
[20] A. Carta, I. Briguglio, S. Piras, G. Boatto, P. La Colla, R. Loddo, M. Tolomeo, S. Grimaudo, A.D. Cristina, R.M. Pipitone, E. Laurini, M.S. Paneni, P. Posocco, M. Fermeglia, S. Pricl, 3-Aryl-2-[1*H*-benzotriazol-1-yl]acrylonitriles: a novel class of tubulin inhibitors, Eur. J. Med. Chem. 46 (2011) 4151–4167.
[21] A. Gazit, N. Osherov, C. Gilon, A. Levitzki, Tyrphostins. 6. Dimeric benzlidenemalononitrile tyrphostins: potent inhibitors of EGF receptor tyrosine kinase *in vivo*, J. Med. Chem. 39 (1996) 4905–4911.
[22] J.M. McKim Jr., Building a tiered approach to in vitro predictive toxicity screening: a focus on assays with *in vivo* relevance, Comb. Chem. High Throughput Screen. 13 (2010) 188–206.
[23] T.A. Hill, S.G. Stewart, C.P. Gordon, S.P. Ackland, J. Gilbert, B. Sauer, J.A. Sakoff, A. McCluskey, Norcantharidin analogues: synthesis, anticancer activity and protein phosphatase 1 and 2A inhibition, ChemMedChem 3 (2008) 1878–1892.
[24] T.A. Hill, S.G. Stewart, B. Sauer, J. Gilbert, S.P. Ackland, J.A. Sakoff, A. McCluskey, Heterocyclic substituted cantharidin and norcantharidin analogues – synthesis, protein phosphatase (1 and 2A) inhibition, and anti-cancer activity, Bioorg. Med. Chem. Lett. 17 (2007) 3392–3397.
[25] A.M. Bergman, V.W. Ruiz van Haperen, G. Veerman, C.M. Kuiper, G.J. Peters, Synergistic interaction between cisplatin and gemcitabine in vitro, Clin. Cancer Res. 2 (1996) 521–530.
[26] J.A. Sakoff, S.P. Ackland, Thymidylate synthase inhibition induces S-phase arrest, biphasic mitochondrial alterations and caspase-dependent apoptosis in leukaemia cells, Cancer Chemother. Pharmcol. 46 (2000) 477–487.