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FULL PAPER

Amino Alcohol Acrylonitriles as Activators of the Aryl hydrocarbon Receptor Pathway, An Unexpected MTT Phenotypic Screening Outcome

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Supporting information for this article is given via a link at the end of the document.

Abstract: Lead (Z)-N-(4-(2-cyano-2-(3,4-dichlorophenyl)vinyl)phenyl)acetamide, **1** showed MCF-7 GI_{50} = 30nM and 400-fold selective c.f. MCF10A (normal breast tissue). Acetamide moiety modification (**13a-g**) to introduce additional hydrophobic moieties was favoured with MCF-7 breast cancer cell activity enhanced at 1.3 nM. Other analogues were potent against the HT29 colon cancer cell line at 23 nM. Textbook SAR data was observed in the MCF-7 cell line via the *ortho* (**17a**), *meta* (**17b**) and *para* (**13f**). The amino alcohol -OH moiety was pivotal, but no stereochemical preference noted. But, these data did not fit our homology modelling expectations. Aberrant MTT ((3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) screening results and metabolic interference confirmed by sulforhodamine B (SRB) screening. Interfering analogues resulted in 120 and 80-fold CYP1A1 and CYP1A2 amplification, with no upregulation of SULT1A1. This is consistent with activation of the AhR pathway. Piperidine per-deuteration reduced metabolic inactivation. 3-OH / 4-OH piperidine analogues showed differential MTT and SRB activity supporting MTT assay metabolic inactivation. Data supports piperidine 3-OH, but not the 4-OH, as a CYP substrate. This family of β-amino alcohol substituted 3,4-dichlorophenylacetonitriles show broad activity modulated via the AhR pathway. By SRB analysis the most potent analogue was **23b**, (Z)-3-(4-(3-(4-phenylpiperidin-1-yl)-2-hydroxypropoxy)phenyl)-2-(3,4-dichlorophenyl)-acrylonitrile.

Introduction

Breast cancer is the most common cancer in women worldwide, and has the second highest mortality rate. In 2018, it was estimated that more than 600,000 women across the globe succumbed to this insidious disease.[1] Moreover, triple negative breast cancers that lack estrogen (ER), progesterone (PR) and HER-2 hormone receptors have no available targeted therapies, and resistance to standard treatment is common.[2] Combined with the sobering fact that metastatic breast cancer is essentially incurable, it is apparent that new targeted therapies are urgently required.[3,4]

The Aryl hydrocarbon receptor (AhR) is a member of the basic helix-loop-helix/Per-ARNT-SIM transcription factor family, and hijacking of its cytosol-to-nuclear pathway has been identified as a promising approach for a novel breast cancer treatment.[5-7] The AhR pathway localises in the cytosol, with the AhR complexing two molecules of heat shock protein 90 (Hsp90), co-chaperone p23 and the Aryl hydrocarbon interacting protein (AIP). Upon ligand binding by either an exogenous or endogenous ligand, AhR translocates into the cell nucleus, forms a heterodimer with the Aryl hydrocarbon receptor nuclear translocator (ARNT), and Hsp90 is released. The heterodimer then binds to xenobiotic response elements (XRE) located in the promotor region of critical genes, resulting in their transcriptional activation. The AhR targeted genes include the cytochrome P450 metabolising enzymes CYP1A1, CYP1A2 and CYP1B1. Depending upon the ligand, this metabolic activation may produce an inert product or a DNA damaging molecule that induces cell death (Figure 1).[8]

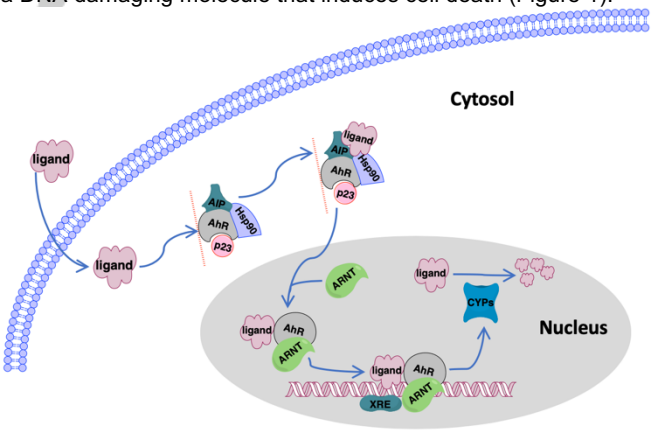

Figure 1: The AhR pathway showing ligand binding, nuclear translocation and cytochrome P450 (CYP) activation.

Upregulation of the AhR in the cytosol of inflammatory breast cancer cells, as well as its poor prognostication for patients with this disease, makes the AhR an attractive breast cancer treatment target.[9,10]

We have previously reported a range of dichlorinated phenyl acrylonitriles as potent and selective inhibitors of cell growth in the MCF-7 breast cancer cell line, mediating their effects via activation of the AhR pathway.[11,12] Two of these ligands, (*Z*)-*N*-(4-(2-cyano-2-(3,4-dichlorophenyl)vinyl)phenyl)acetamide (**1**) and ANI-7 (**2**) were found to selectively reduce cell viability in the

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MCF-7 breast cancer cell line, at 0.03 and 0.56 μM, respectively; representing more than 400-fold selectivity for **1**, and almost 70-fold selectivity for **2**, compared with the MCF10A normal breast cell line. Notably, these compounds also exhibited excellent decreased cell viability in the triple-negative breast cancer cell line MDAMB468 (0.042 μM for **1** and 0.23 μM for **2**), and good activity against the therapy-resistant triple-negative lines BT20 (2.5 μM for **1** and 2 μM for **2**) and MDAMB231 (5.1 μM for **1** and 17 μM for **2**). Other reported AhR ligands that show similar selectivity in breast carcinoma models are aminoflavone (**3**) and Phortress (**4**). Aminoflavone (**3**) and Phortress (**4**), as the pro-drugs, progressed to clinical trials; **3** for breast cancer (Phase II), and **4** for colon cancer (Phase I).[13,14]

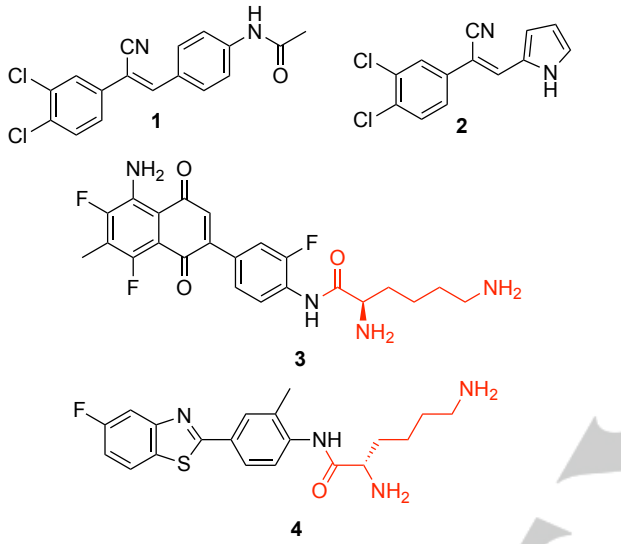

Figure 2: Chemical structures of selected known AhR ligands, (*Z*)-*N*-(4-(2-cyano-2-(3,4-dichlorophenyl)vinyl)phenyl)acetamide (**1**), ANI-7 (**2**), aminoflavone (**3**) and Phortress (**4**). Red highlights indicate the presence of the pro-moiety in **3** and **4**.

The mechanism of action of these cytotoxic AhR ligands requires the *in situ* formation of a nitrenium ion moiety, which enables highly localized DNA damage, and cell death to occur. This process demands a highly orchestrated interaction between the AhR ligand, the AhR, CYP1s and SULT1A1 to activate the 'dormant' amino moieties in these ligands.[6] Given our recent efforts with the phenyl acrylonitriles, we rationalized that enhanced breast cancer specificity and potency might result from increasing the number of amino moieties in these molecules. In turn, this allowed us to leverage against our in-house facile access to β-amino alcohols,[15] thus we hypothesized that addition of this moiety may provide additional activation of the AhR pathway.

β-Amino alcohols are known to be biologically active,[16-18] and introduction of sp^3 character (increased Fsp^3 character) is also known to improve drug solubility;[19] which has been an issue with some previous analogues.[11,12] The amino alcohol moiety is present in many clinically relevant compounds, including alprenolol (**5**), utilized for the treatment of angina pectoris, and febrifugine (**6**, Figure 3), a potent antimalarial agent. Additionally, we hoped to explore the effect of adding substituents to the phenyl ring, in an effort to further enhance the biological potency that was noted with the previously reported *N*-acetamide **1** against the MCF-7 breast cancer cell line. Herein we report our recent efforts in this area through the synthesis and biological analysis of a range of dichlorinated phenylacrylonitriles bearing a β-amino alcohol moiety. Critically we also report on an unexpected outcome from our MTT-based phenotypic screening.

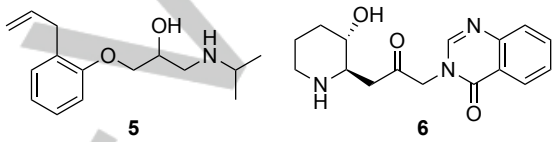

Figure 3: Two biologically active amino-alcohol containing drugs; alprenolol (**5**) and febrifugine (**6**).

Results and Discussion

To determine approach feasibility, a focused library was prepared and evaluated. Thus, the desired β-amino alcohols were accessed via flow chemistry introduction of *epi*-chlorohydrin (**7**) to 4-hydroxybenzaldehyde (**8**) which gave epoxide (**9**),[15] Knoevenagel condensation with 3,4-dichlorophenyl acetonitrile (**10**) in the basic ionic liquid benzytrimethyl ammonium hydroxide gave the key epoxide (**11**) (64% 2 steps; Scheme 1). With intermediate **11** in hand, we next examined the synthesis of a pilot library of β-amino alcohol acrylonitriles using amines **12a-g**, *Library A*. This library spanned a simple aniline (**13a**), a cyclohexyl derivative (**13b**), as well as the decorated aniline derivatives 4-chloro (**13c**), 4-bromo (**13d**), 4-methoxy (**13e**), 4-phenylpiperazine variant (**13f**), and dimethylamino (**13g**) moieties. This afforded a seven-component library in poor to moderate yields (18-43%). In all instances, excepting **13f**, the increased solubility and by-product profile required these analogues to be purified by flash chromatography (see Experimental).

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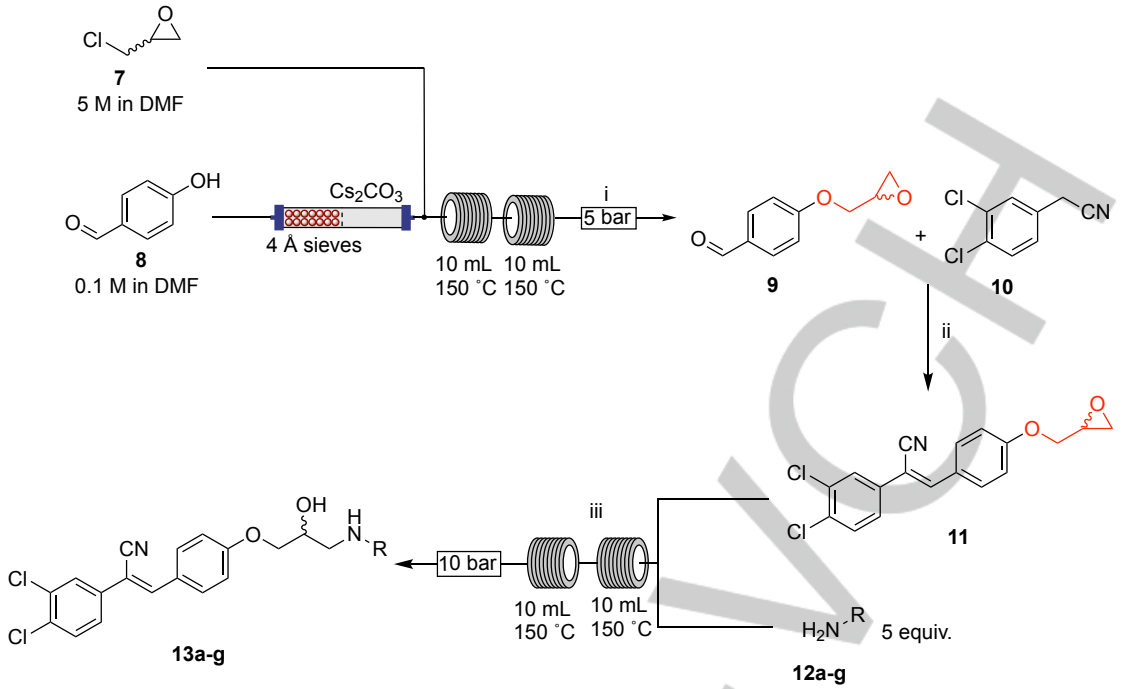
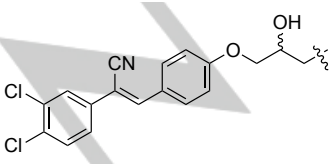
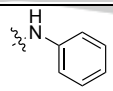
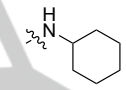
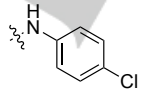
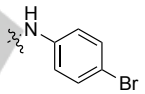
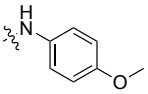
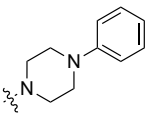


Scheme 1: Reagents and conditions: (*i*) Vapourtec Easy-MedChem, 5 M **7** (anhy. DMF), 0.1 M **8** (anhy. DMF), 105 °C, 5 bar, 1.0 mL.min^{-1} (t_r = 20 min); (*ii*) **10**, $PhCH_2NMe_3(OH)$, H_2O, 50 °C, 5h; (*iii*) RNH_2 (**12a-g**) 0.05M (EtOH), **11** 0.01 M (EtOH), 150 °C, 10 bar, 1.0 mL.min^{-1} (t_r = 20 min).

The effectiveness of *Library A* to selectively reduce the viability of breast cancer cells (MCF-7) relative to the normal breast cell line MCF10A or cells derived from another tumour type (HT29 colon) was evaluated in an MTT assay (see Table 1 for detail). Analogues **13a-13e** exhibited good activity against the MCF-7 cells (2.3 – 16 μM) (Table 1). Hydrophobic-substituted analogues **13b** and **13g** also revealed broad spectrum low micro-molar inhibition across the three cell lines tested. In particular, **13g** exhibited nanomolar activity against the colon cancer cell line HT29 (23 nM). Phenylpiperazine **13f**, however, showed low nano-molar potency in the MCF-7 cell line (1.3 nM), and sub- to low micro-molar growth inhibition (0.05 – 2.2 μM) in the remaining cell lines. Notably, this analogue demonstrates >260 times higher potency in the MCF-7 cells than the normal breast cells (MCF10A). This is in keeping with our earlier findings with the parent **1** and **2**.[11,12] Buoyed by this exciting development, we sought to explore the extreme potency of this derivative. It was noted that the addition of both electron-withdrawing (**13c** and **13d**) and electron-donating (**13e**) functional groups had little effect on the cell viability, while the presence of a hydrophobic functionality (**13b**, **13f** and **13g**) was favoured.

Table 1. GI_{50} values (μM) of *Library A* (**13a-g**) against HT29 (colon carcinoma), MCF-7 (breast carcinoma) and MCF10A (normal breast) cell lines, in an MTT assay. GI_{50} is the concentration at which 50% of the cell growth is inhibited.



R	HT29[a]	MCF-7[b]	MCF10A[c]
 13a	13±0.0	16±1.0	27±3.0
 13b	1.8±0.1	2.3±0.2	2.7±0.1
 13c	12±1.0	13±0.0	31±1.1
 13d	11±0.0	13±0.0	29±1.0
 13e	4.7±0.3	11±1.0	15±0.0
 13f	0.052±0.019	0.0013±0.0004	0.34±0.05
 13g	0.023±0.011	0.33±0.33	0.79±0.26

[a] Colon carcinoma; [b] breast carcinoma; [c] breast (normal); Data is presented as ±SEM (n=3).

Based on these data, we sought to further develop the SAR data associated with **13f**. We considered **13f** to comprise three distinct rapidly modifiable regions, the ether linkage (green), the alcohol moiety (blue) and the phenylpiperazine functionalities (red) (Figure 4). We have examined the SAR requirements of the dichlorophenyl moiety in earlier reports.[11]

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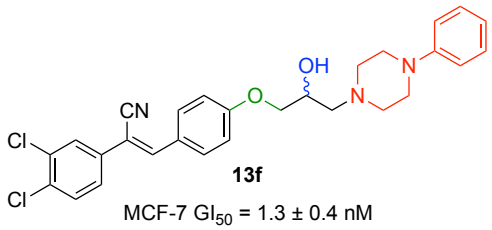


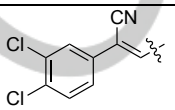
Figure 4: Chemical structure of Library A lead compound **13f**.

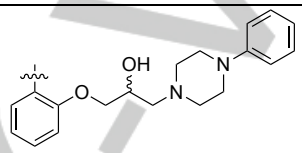
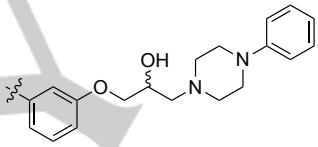
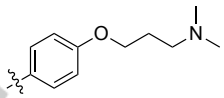
The initial modification explored was the *para*-ether linkage of **13f** through the synthesis of the corresponding *ortho*-**17a** and *meta*-**17b** analogues. This two-component library, *Library B*, was generated from the corresponding hydroxybenzaldehydes **14a** and **14b** as per Scheme 1 in good yields (72 and 74%). Knoevenagel condensation with **10** afforded the epoxides **16a** and **16b**. Epoxide-opening with 1-phenylpiperazine (**12f**) gave the desired **17a** and **17b**. MTT evaluation demonstrated that neither analogue showed significant activity in the cell lines tested, particularly the breast cancer cell line of interest, MCF-7 (Table 2). In this cell line, these analogues displayed an almost textbook activity response to ring substituent position changes, *viz*, inhibitory activity *para* > *meta* > *ortho* (**13f**, 1.3 nM; **17b**, 6.7 μM; **17a** 23 μM).

Concurrently we also examined the requirement of the alcohol moiety; as a function of ready access to starting materials, through the synthesis of the de-hydroxylated derivative of **13g**, **18**. As can be surmised from the cytotoxicity data in Table 2, removal of the hydroxy moiety resulted in a 10-fold decrease in potency in the MCF-7 cell line, and a similar loss across the remaining cell populations. The activity of these compounds was typically 3-fold more potent against the HT29 cell line than the target MCF-7 cell line. Only poor selectivity for MCF-7 vs. MCF10A was noted, unlike the lead compounds, **1** and **2**.

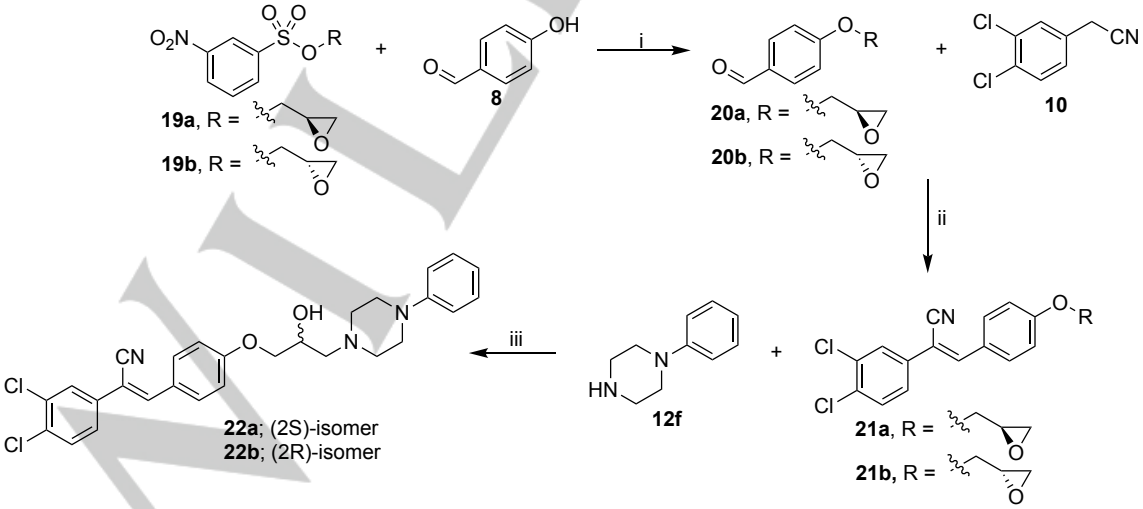
Given the critical nature of the -OH moiety, we next explored the stereochemical preference at this position through the synthesis of both enantiomers of **13f**. Coupling of commercially available (*S*)-(+)-glycidyl-3-nitrobenzenesulfonate (**19a**) or (*R*)-(-)-glycidyl-3-nitrobenzenesulfonate (**19b**) with **8** gave enantiomerically pure oxirane-benzaldehydes **20a** and **20b** respectively, in excellent yields (86 and 91%). Knoevenagel condensation with **10** gave the β-amino alcohol intermediates **21a** and **21b**. Oxirane ring opening with 1-phenylpiperazine (**12f**) under microwave conditions (ethanol, 20 min and 120 °C) afforded the desired (*S*)-**22a** and (*R*)-**22b** in good to excellent yields (59-83% and optical purity; electronic supporting information) (Scheme 2). No observable difference in bioactivity with **22a** and **22b** was evident (electronic supporting information).

Table 2: GI_{50} values (μM) of *Library B* (**17a**,**b** and **18**) against HT29 (colon carcinoma), MCF-7 (breast carcinoma) and MCF10A (normal breast) cell line, in an MTT assay. GI_{50} is the concentration at which 50% of the cell growth is inhibited.



R	HT29[a]	MCF-7[b]	MCF10A[c]
 **17a**	8.2±2.5	23±3.0	24±4.0
 **17b**	3.3±0.48	6.7±1.0	13±1.0
 **18**	0.70±0.01	2.3±0.1	2.5±0.6

[a] Colon carcinoma; [b] breast carcinoma; [c] breast (normal); Data is presented as ±SEM (n=3).



Scheme 2: Reagents and conditions: (*i*) 1.5 eq. Cs_2CO_3, DMF, 80 °C, 4 h; (*ii*) $PhCH_2NMe_3(OH)$, H_2O, 50 °C, 5 h; (*iii*) 2 eq. amine, EtOH, MW, 120 °C, 20 min.

Library C saw further modification of the phenylpiperazine moiety to determine the effect of this moiety. Using the above microwave protocol (Scheme 2), and the 12 analogues **23a**-**m** were afforded in moderate to good yields (39-87%). This library of compounds exhibited improved aqueous solubility over the previous analogues,[12] leading to a general trend of lower yields.

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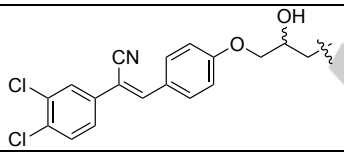
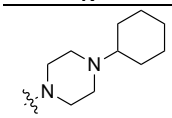
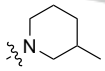
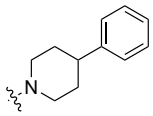
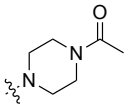
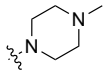
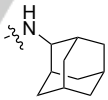
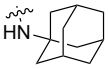
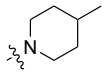
Subsequent MTT cytotoxicity screening in our cell line panel revealed all analogues, except **23b**, displayed low nano-molar to pico-molar activity against the MCF-7 cell line, with micro-molar to nano-molar in the remaining cell lines (Table 3).

The introduction of additional hydrophobic moieties with *Library C* analogues with **23a**, **23f** – **23m** saw apparent excellent activity and selectivity towards the MCF-7 breast cancer cells, with each analogue returning a >10-fold MCF-7 potency enhancement relative to **13f**. Addition of an extra nitrogen functionality (**23d**) showed a marginal potency reduction. Increase (**23k**) or reduction (**23j**) in the hydrophobic ring size had little impact on broad-spectrum cytotoxicity across the panel of cell lines evaluated, and with the exception of the slight loss of activity in the HT29 colon cancer cell line, neither did the addition of the adamantyl moiety (**23l** and **23m**). Addition of an oxygen, either in the saturated ring or proximal to it (**23c** and **23e**) resulted in a concomitant reduction in potency, but these compounds still retained low nano-molar potency (MCF-7) in the MTT assay.

Table 3: GI_{50} values (μM) of *Library C* analogues **23a-m** against HT29 (colon carcinoma), MCF-7 (breast carcinoma) and MCF10A (normal breast) cell line, in an MTT assay. GI_{50} is the concentration at which 50% of the cell growth is inhibited.



R	HT29[a]	MCF-7[b]	MCF10A[c]	R	HT29[a]	MCF-7[b]	MCF10A[c]
 **23a**	ND*	<0.0001	0.0±0.0	 **23h**	0.016±0.0005	<0.0001	0.20±0.00
 **23b**	1.5±0.27	2.4±0.28	2.9±0.13	 **23i**	0.00062	<0.0001	0.072±0.064
 **23c**	0.064±0.021	0.014±0.01	1.0±0.20	 **23j**	0.00059	<0.0001	0.032±0.0064
 **23d**	0.011±0.0005	0.00088	0.30±0.25	 **23k**	0.00010	<0.0001	0.14±0.047
 **23e**	0.0072±0.0027	0.0020	0.18±0.10	 **23l**	0.033±0.018	0.0±0.0019	0.11±0.022
 **23f**	ND	<0.0001	0.08±0.05	 **23m**	0.031±0.012	0.0±0.00	0.32±0.058
 **23g**	0.0025	<0.0001	0.11±0.050				

[a] Colon carcinoma; [b] breast carcinoma; [c] breast (normal); *ND denotes that the GI_{50} was not determined; Data is presented as ±SEM (n=3).

These data, excepting that noted with **23b**, was in part consistent with our prior observations that the ligand-binding PAS-B domain had shown that the binding pocket is lined with mostly hydrophobic residues, however these prior studies did not support the introduction of bulky substituents as the binding pocket was defined as compact and hydrophobic.[12,20] However a number of these analogues, e.g. **23a**, **23f-k**, returned undeterminable GI_{50} values, which was puzzling. Moreover, the magnitude in activity reduction with **23b** could not be explained by our PAS-B homology model.[12,20] Re-synthesis and re-examination of the *Library C* compounds screened by MTT assay showed significant activity variation. Subsequent morphological examination of the treated cells using phase contrast microscopy suggested that the visual morphology was inconsistent with the MTT determined cytotoxicity with all analogues excepting **23b**. As the MTT assay relies on mitochondrial dehydrogenase enzymes to reduce the tetrazolium moiety to a formazan precipitate,[21] it was hypothesized that a metabolite generated by the majority of the amino-alcohol products was interfering with this reaction. Microscopic examination of the MCF-7 cells after compound exposure (72 h, 1μM, **13f**) revealed viable cells with no evidence of cell death (Figure 5).

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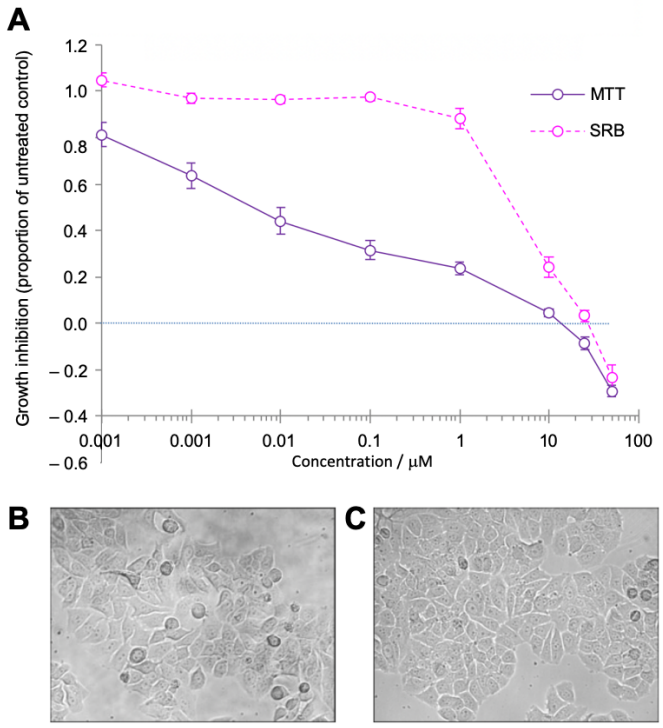

Figure 4: **A**. Comparison of the effect of **13f in** MTT and SRB assays in MCF-7 cells after 72 h treatment, data is ±SEM (n=3); **B**. Microscopy images of MCF-7 breast cancer cells treated with **13f** (1µM); **C.** As for B, with no added **13f** (untreated control cells).

Re-screening of **13f** and *Library C* in a sulforhodamine B (SRB) protein assay was conducted (Table 4). The SRB assay relies on the ability of the sulphur-based dye to bind to protein components of the cells.[22] Comparisons have been made between the SRB and MTT assays, with the former demonstrating greater sensitivity.[21,23] The outcome of this secondary assay was significantly different to that of the MTT assay, with the activity of all analogues starkly lower than previously noted, as shown in Figure 4 for **13f**. Only **23b** retained the activity in the SRB assay compared with the MTT assay.

The key point of differentiation between **13f** and **23b** is the N to C isosteric modification (**13f**, piperazine; **23b**, piperidine), and logically this is the effector of differential activity in these two assays. Phenylpiperazines are known to form highly reactive iminoquinone moieties capable of reacting with glutathione (Supplementary material),[24-26] which potentially rationalizes the differential reactivity of **13f** and **23b**, with the latter lacking the critical nitrogen to induce the iminoquinone formation. However, this does not explain the MTT *vs.* SRB assay differences observed with other analogues. In these instances, we hypothesize that *in vitro* CYP1 mediated oxidative activation results in the formation of an additional active metabolite that prevents the reduction of MTT,[16,27,28] and yielding aberrant assay outcomes. SRB cytotoxicity assay operates via an oxidative mechanism and is thus not affected by this reduction block.

Table 4: GI_{50} values (µM) of **13f** and *Library D* analogues (**23a-m**) against HT29 (colon carcinoma), MCF-7 (breast carcinoma) and MCF10A (normal breast) cell line, in an **MTT** assay and an SRB assay. GI_{50} is the concentration at which 50% of the cell growth is inhibited.

Compound	HT29[a]		MCF-7[b]		MCF10A[c]	
	SRB	MTT	SRB	MTT	SRB	MTT
13f	3.3±0.38	0.52±0.019	4.1±0.99	0.0013±0.0004	4.8±0.26	0.34±0.05
23a**	0.4	ND	1.2	<0.0001	2.0	0.0±0.0
23b	1.9±0.06	1.5±0.27	2.5±0.20	2.4±0.28	2.5±0.22	2.9±0.13
23c	8.6±2.2	0.64±0.21	5.3±2.6	0.014±0.01	16±0.33	1.0±0.20
23d	1.2±0.12	0.011±0.0005	2.3±0.38	0.00088	2.4±0.21	0.30±0.25
23e	3.1±0.13	0.0072±0.0027	5.3±0.91	0.0020	5.6±1.2	0.18±0.10
23f	0.66±0.24	ND	2.5±0.10	<0.0001	2.5±0.12	0.08±0.05
23g	1.7±0.23	0.0025	2.3±0.30	<0.0001	2.7±0.10	0.11±0.050
23h	2.4±1.3	0.016±0.0005	2.3±0.18	<0.0001	2.5±0.07	0.20±0.00
23i	0.36±0.07	0.00062	2.1±0.03	<0.0001	2.5±0.06	0.072±0.064
23j	1.5±0.57	0.00059	2.8±0.49	<0.0001	2.9±0.07	0.032±0.0064
23k	1.7±0.38	0.00010	2.8±0.23	<0.0001	3.1±0.07	0.14±0.047
23l	0.89±0.41	0.033±0.018	2.0±0.10	0.0±0.0019	2.5±0.22	0.11±0.022
23m	1.4±0.07	0.031±0.012	2.1±0.07	0.0±0.00	2.7±0.22	0.32±0.058

[a] Colon carcinoma; [b] breast carcinoma; [c] breast (normal); *ND denotes that the GI_{50} was not determined. **n=1 for SRB assay; All other data, MTT assay values are n=3; SRB values are n=3.

It is unknown whether this interferant occurs pre- or post-CYP1 activation in the cell lines evaluated. In an effort to elicit this information, mono-hydroxylated piperidine analogues of **13f**, *viz* **25a** and **25b** were synthesized as per Scheme 3, as potential CYP1 metabolism products and their activity evaluated in both assays. In a similar vein, we also prepared the per-deuterated piperidine analogue (**26**), noting that a CYP1 mediated transformation would be retarded by deuteration. In addition, each of the suspect analogues were stirred in the presence of MTT to examine the possibility that MTT itself was the reacting, and thus interfering species in these assays.

Combining the hydroxylated analogues **25a** and **25b** with MTT in a 'faux cell-free assay' as described previously,[29] and analysis of the plate contents at 8 different concentrations after incubation for 4 hours showed no change in the concentration of MTT, indicating that no reaction was occurring with the MTT reagent. Further examination of **25a**,**b** under standard MTT and SRB assay conditions revealed that the 4-OH **25b** showed no

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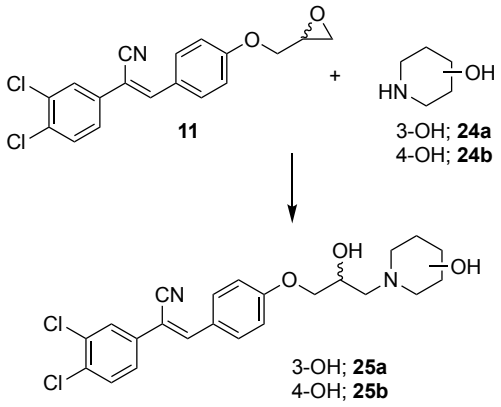
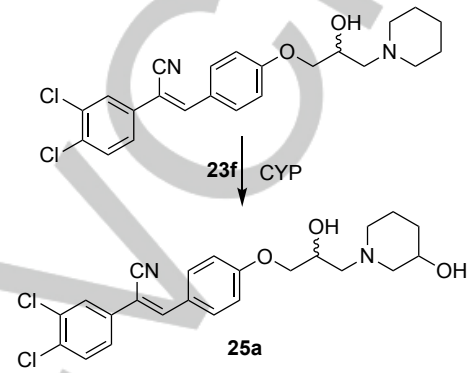
MTT *vs*. SRB assay disparity, while the effect was still evident for 3-OH **25a** (Table 5). This strongly suggest that the 4-moiety blocks the subsequent deactivation of the MTT assay, while the 3-OH moiety enables further reaction to deactivate MTT. This may be a function of the 3-OH **25a** remaining a CYP substrate and undergoing further metabolic activation of a MTT reactive species (Scheme 4). This is also consistent with **25a** being the major metabolite responsible for the deactivation of the MTT assay with this type of compound.



Scheme 3: Reagents and conditions: 1.5 eq. amine, EtOH, MW, 120 °C, 20 min.

(Table 5) exhibited a stark contrast to the previously observed results with the non-deuterated analogue **23f**. As shown in the results below, while **23f** demonstrated <0.0001 μM activity in the MCF-7 cell line, **26** exhibited much lower activity at 0.0031±0.0029 μM. This supports the hypothesis that a metabolite of these compounds is forming, as the deuteration would slow the rate of this formation. Gratifyingly, **26** exhibited an identical cell viability profile to **23f**, indicating that the substitution of hydrogen for deuterium had no effect on the activity of the analogue.



Scheme 4. Possible mechanism of activation of **26a** *vs*. **26b** explain the differential MTT vs. SRB assay data obtained.

Undeterred by the non-identification of the specific interfering compound, analysis of the per-deuterated piperidine analogue **26**

Table 5: GI_{50} values (μM) of **25a, 25b** and **26** against HT29 (colon carcinoma), MCF-7 (breast carcinoma) and MCF10A (normal breast) cell line, in an **MTT** assay and an SRB assay. Compound **23f** included as a reference compound. GI_{50} is the concentration at which 50% of the cell growth is inhibited. MTT assay values are n=3; SRB values are n=3.

Compound	HT29[a]		MCF-7[b]		MCF10A[c]	
	SRB	MTT	SRB	MTT	SRB	MTT
23f	0.66±0.24	ND*	2.5±0.10	<0.0001	2.5±0.12	0.08±0.05
25a	0.84±0.53	0.013±0.010	2.5±0.57	0.0016±0.0014	2.2±0.46	1.2±0.033
25b	1.0±0.62	0.20±0.06	3.1±1.5	1.9±0.94	3.1±0.033	1.9±0.32
26	0.29±0.0058	0.0034±0.0018	2.2±0.46	0.0031±0.0029	3.5±1.2	0.082±0.069

[a] Colon; [b] breast; [c] breast (normal); *ND denotes that the GI_{50} was not determined,Data presented as ±SEM viz, MTT assay (n=3) and SRB (n=3).

To further investigate the mechanism of action of these compounds and to determine whether they acted via the AhR pathway, gene expression studies were conducted with **13f**. It has been described that metabolism of aminoflavone (**3**) occurs via first a hydroxylation of the primary amines by a CYP, then a subsequent sulfonylation by sulfotransferase SULT1A1; before forming the final cytotoxic metabolite, a nitrenium ion.[30] As we have reported previously, the expression of enzymes such as CYP1A1 and CYP1A2 is increased following AhR ligation with our breast selective compounds. In the same study, it was also noted that basal expression of CYP1A1 and CYP1A2 did not predict for the activity of our compounds whilst the expression of SULT1A1 did; highlighting the inducible nature of CYP1s and the need for SULT1A1 activity.[31] In order to determine whether our current cohort of compounds were indeed acting via the AhR pathway, the expression of CYP1A1 and CYP1A2 following treatment for 4 h was evaluated, with the results portrayed as fold change compared to untreated cells (Figure 5).

As shown in Figure 5, treatment of the breast cancer cell line MCF-7 with 5 μM **13f** for 4 hours resulted in 120- and over 80-fold increased expression of CYP1A1 and CYP1A2 respectively; whilst no change was noted for the expression of AhR and SULT1A1. As previously observed, this expression profile is fully consistent with this compound acting via the AhR pathway.[31]

Conclusions

Rapid access to a series of diverse libraries of β-amino alcohol substituted 3,4-dichlorophenylacetonitriles was possible through a combination of flow and microwave chemistry approaches. This enabled the introduction of Fsp^3 enhancement and additional amine moieties, with the latter previously shown to be beneficial for AhR pathway activation. Phenotypic (MTT) evaluation of these compound libraries across a panel of two cancer and one normal cell line revealed a marked breast cancer selectivity for a number of the analogues produced. A particular focus was placed on the introduction of phenyl substituents based on the excellent activity and selectivity of the parent (**1**) (MCF-7 GI_{50} = 30nM and 400-fold selective compared to MCF10A (normal breast tissue)). Within the first library (**13a-g**), additional hydrophobic moieties were favoured, with **13f** the most MCF-7 potent (1.3 nM). Other analogues in this library also showed excellent activities against

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the colon cancer cell line HT29 (**13g**, GI_{50} = 23 nM). Within this series, textbook SAR data was observed with activity increasing against the MCF-7 cell line via the ortho (**17a**), meta (**17b**) and para (**13f**) analogues respectively. The pivotal nature of the amino alcohol -OH moiety was demonstrated with **13g** and **18**; and the lack of stereochemical requirement at this position noted with **22a** and **22b**. Intriguingly, increasing the hydrophobic nature of the terminal phenyl substituent afforded almost homeopathic levels of potency. Examination of this phenomenon revealed a by-product from cellular metabolism resulted in interference with the MTT assay, confirmed on alternative phenotypic screening via an SRB assay.

Our investigations confirmed that these analogues significantly upregulate both CYP1A1 and CYP1A2 with **13f** resulting in a 120- and 80-fold amplification of CYP1 expression, respectively. No equivalent upregulation of SULT1A1 was noted. This is consistent with these compounds acting via the AhR pathway. In addition, the rate of metabolic inactivation was slowed with the introduction of per-deuterated piperidine **26**. Further we note that the 3-OH **25a** and 4-OH **25b** showed differential activity in the MTT and SRB assay suggesting that it is a metabolic product from at least two oxidation steps that interferes with the MTT assay. In this instance we postulate that the 3-OH **25a** or a further metabolite thereof, adversely modulates the MTT-assay signal leading to false positives in this phenotypic assay. Notwithstanding this, we clearly demonstrate that these analogues are capable of AhR pathway upregulation, a feature recently postulated as a favourable outcome in the treatment of a range of cancers.[32]

Critically in this study the application of the SRB assay revealed that no true SAR data could be extracted, and this adds a significant note of caution as the MTT assay with this series of compounds clearly shown a bias towards significant overestimation of compound potency in the MCF-7 cell line.

In addition to identifying these analogues as potential novel breast cancer targeting compounds, this work also identified **23a**, **23f** and **23l** with sub-micromolar potency in killing the HT29 colon cancer cell line.

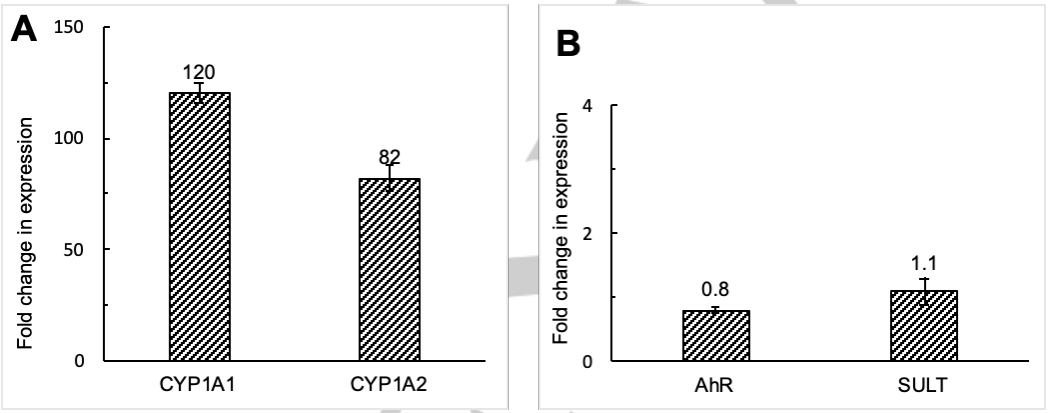

Figure 5: A. Gene expression analysis in MCF-7 cells in response to 5μM **13f** for 4h, demonstrating the upregulation of CYP1A1 and CYP1A2 by 120 and 82-fold over untreated cells respectively; **B.** As for **A** with AhR and SULT1A1 showing no change in expression level was observed for AhR and SULT1A1 .

Experimental Section

Biology

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 in a humidified atmosphere 5% CO_2 at 37 ºC. The cancer cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) (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). The non-cancer MCF10A cell line was cultured in DMEM:F12 (1:1) cell culture media, 5% heat inactivated horse serum, supplemented with penicillin (50 IU/mL), streptomycin (50 μg/mL), 20 mM Hepes, L-glutamine (2 mM), epidermal growth factor (20 ng/mL), hydrocortisone (500 ng/mL), cholera toxin (100 ng/mL), and insulin (10 μg/mL).

In vitro growth inhibition assay

Cells in logarithmic growth were transferred to 96-well plates. Cell viability was determined by plating cells in duplicate in 100 μL medium at a density of 2500-4000 cells/well in 96 well plates, *viz* MCF10A 2500 cells/well, HT29 3000 cells/well and MCF7 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 either the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) or sulforhodamine B (SRB) assay with absorbances read at 540 nm. Percentage growth inhibition was determined at a fixed drug concentration of 25 μM. A value of 100% is indicative of complete cell growth inhibition. Those analogues showing appreciable percentage growth inhibition underwent further dose response analysis allowing for the calculation of a GI_{50} value. An eight-point dose response curve was produced, using MS Excel software. Each data point is the mean ±S.E.M. calculated from four to five replicates, which were performed on separate occasions and separate cell line passages. From these dose-response curves, the GI_{50} value was calculated, representing 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.[33,34]

SRB: Briefly, cells from each 96 well plate were fixed by protein precipitation after the addition of 50 μL of 50% TCA. After an incubation of 1hr at 4 °C the plates were washed 5x in tap water and allowed to air dry. The cells were then stained with 50 μL of 0.4% SRB in 1% acetic acid for 15 minutes at room temperature. The plates were washed 4x in 1% acetic acid to remove unbound stain and allowed to air dry. The protein was then solubilized in 150 μL of 10mM unbuffered TRIS.

Morphological Assessment: Live cells were examined for morphological alterations after 72 h exposure with and without 1μM **13f** using phase

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contrast microscopy (Olympus CKX41 inverted microscope 100x magnification).

Gene Expression Analysis: For each cell population total RNA was extracted using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. One µg of RNA was reverse transcribed using the QuanitTect Reverse Transcription Kit (Qiagen) according to the manufacturer's instructions. Rotor-Gene SYBR Green PCR Kit (Qiagen) was used to perform qPCR for CYP1A1, CYP1A2, AhR and SULT1A1 on a Rotor-Gene 3000 Thermo -Cycler Instrument using β2-microglobulin as a housekeeping gene (Qiagen). The primer sequences were purchased from Qiagen as follows: AhR (QT02422938), CYP1A1 (QT00012341), CYP1A2 (QT00000917), CYP1B1 (QT00209496), SULT1A1 (QT01665489), AND β2M (QT00088935). HotStar Taq activation at 95°C for 5 minutes, 40 cycles of denaturation (95°C for 5 seconds), and annealing/extension (60ºC for 10 seconds). The comparative Ct value method was used for data analysis. Gene expression was examined in MCF-7 cells following treatment with 5 µM 13f for 4 h. Results show the fold change in gene expression relative to untreated control cells.

Chemistry

General Methods

All reactions were performed using standard laboratory equipment and glassware. Solvents and reagents were purchased from Sigma Aldrich, Alfa Aesar or AK Scientific and used as received. Organic solvents were of bulk quality, and were distilled from glass prior to use. Organic solvent extracts were dried with magnesium sulfate ($MgSO_4$), and dried under reduced pressure with either Büchi or Heidolph rotary evaporators. Melting points were recorded in open capillaries on a Stuart SMP11 Melting Point Apparatus. Where available, literature values are provided and appropriately referenced. Electrospray mass spectra were recorded using 10% DMSO/H_2O or HPLC-grade methanol or acetonitrile as carrier solvents on an Agilent Technologies 1260 Infinity UPLC system with a 6120 Quadrupole LC/MS in electrospray ionization (ESI) positive and negative modes. TLC was performed on Merck silica gel 60 F254 pre-coated aluminum plates with a thickness of 0.2 mm. Column chromatography was performed under 'flash' conditions on Merck silica gel 60 (230-400 mesh).

Nuclear magnetic resonance (NMR) spectroscopy was performed on a Brüker Avance III 400 MHz spectrometer, where proton NMR (^{1}H NMR) spectra and carbon NMR (^{13}C NMR) spectra were acquired at 400 and 100 MHz respectively, or a Brüker Avance III 600 MHz spectrometer, where proton NMR (^{1}H NMR) spectra and carbon NMR (^{13}C NMR) spectra were acquired at 600 and 150 MHz respectively. All spectra were recorded in deuterated dimethyl sulfoxide (DMSO-d_6), deuterated acetone (acetone-d_6) or deuterated chloroform ($CDCl_3$) obtained from Cambridge Isotope Laboratories Inc. Chemical shifts (δ) were measured in parts per million (ppm) and referenced against the internal reference peaks. Coupling constants (*J*) were measured in Hertz (Hz). NMR assignments were determined through the interpretation of one- and two-dimensional spectra. Multiplicities are denoted as singlet (s), broad singlet (bs), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), triplet of doublets (td), doublet of triplets (dt) and multiplet (m). Peaks are listed in decreasing chemical shift in the following format: chemical shift (integration (^{1}H), multiplicity (^{1}H), coupling constant (^{1}H). The Biotage® initiator+ was used to perform microwave reactions. Optical rotation was performed on a Jasco P-2000 Polarimeter in a 10 x 100 mm cell, and the $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}.

General Procedure 1: Using a Vapourtec RS-400 equipped with fraction collection kit and auto-sampler, a 2.0 mL sample loop was charged with a 0.4 M solution of epoxide in toluene. An additional 2 mL sample loop was charged with a 2.8 M amine solution in ethanol (as stated). The solutions were flowed together and the resulting stream was then passed through two PFA coil reactors in series at 150 °C, 10 bar back pressure and 0.5 mL min^{-1} (residence time 40 min). The resulting reaction mixture was collected, concentrated *in vacuo* and purified as described below.

General Procedure 2: (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 1 eq.) was combined with the required amine (either 1.5 or 2 eq., as stated) and 20 mL ethanol. The solution was irradiated at 120 °C for 20 min. Upon chilling, the desired product was isolated via vacuum filtration.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)-acrylonitrile (**11**)*

4-Hydroxybenzaldehyde (**8**) (305 mg, 2.5 mmol) in 25 mL ACN was passed through a series of omnifit columns, the first containing molecular sieves, the second contained cesium carbonate and sand (50%:50% w/w) at a flow rate of 0.5 mL/min. The reactant stream was then met by a secondary stream containing epichlorohydrin (17 mL, with 20 mL DMF and 63 mL ACN, flow rate 0.5 mL/min), and together the reactant streams were passed through a Vapourtec static mixer reactor (20mL) heated at 105 °C and held at 5 bar back pressure.

The product solution was then evaporated under reduced pressure, then diluted with ethyl acetate (50 mL), extracted with water (3 x 50mL), and then brine (1 x 50 mL), dried over magnesium sulfate and the solvent removed under reduced pressure to afford 4-(oxiran-2-ylmethoxy)benzaldehyde (**9**) as a light brown oil (364 mg, 82%).

Alternatively, 4-hydroxybenzaldehyde (**8**, 2.019 g, 16.4 mmol, 1 eq.) and cesium carbonate (8.500 g, 49.3 mmol, 1.5 eq.) were combined with 15 mL DMF and 20 mL epichlorohydrin (**7**) in a round-bottomed flask. The reaction was heated to 75 °C and left to stir. After 3 h, the reaction was cooled to ambient temperature, diluted with EtOAc (50 mL), washed with water (3 x 30 mL, brine (2 x 30 mL), dried over $MgSO_4$ and concentrated under reduced pressure to afford 4-(oxiran-2-ylmethoxy)benzaldehyde (**9**) as a brown oil (2.604 g, 90%). 4-(Oxiran-2-ylmethoxy)benzaldehyde (**9**,12.177 g, 68 mmol, 1 eq.), was then added to a vigorously stirred solution of water (40 mL) and heated at 50 °C. 3,4-Dichlorophenylacetonitrile (**10**,12.109 g, 65 mmol, 1.05 eq.) was then slowly added forming a suspension. After 5–10 min of stirring, benzytrimethyl ammonium hydroxide (40 wt.% aq. solution) (25 mL) was added dropwise. After complete addition, the reaction was left to stir 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 solid. The resulting yellow solid was recrystallised from ethanol to afford the desired product as a yellow solid (15.805 g, 70%), m.p.: 121-124 °C; ^{1}H NMR (400 MHz, acetone-d_6) δ 8.02 (d, *J* = 8.8 Hz, 2H), 7.97 (s, 1H), 7.94 (d, *J* = 1.4 Hz, 1H), 7.73 – 7.68 (m, 2H), 7.15 (d, *J* = 8.8 Hz, 2H), 4.47 (dd, *J* = 11.4, 2.6 Hz, 1H), 4.00 (dd, *J* = 11.4, 6.4 Hz, 1H), 3.36 (td, *J* = 6.6, 2.6 Hz, 1H), 2.87 (dd, *J* = 4.8, 4.4 Hz, 1H), 2.75 (dd, *J* = 5.2, 2.4 Hz, 1H); ^{13}C NMR (101 MHz, acetone-d_6) δ 162.0, 144.6, 136.4, 133.5, 132.8, 132.5 (2C), 132.0, 128.2, 127.5, 126.5, 118.5, 116.0 (2C), 106.6, 70.4, 50.4, 44.4; IR υ_{max}/cm^{-1}: 3063 (C=C), 2210 (CN), 1255 (arom. C-O), 811 (C-Cl); LRMS: (ESI$^-$) *m/z*: 368 ($C_{18}H_{12}Cl_2NNaO_2$) [M+Na-H]; HRMS: Material decomposed.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-(2-hydroxy-3-(phenylamino)-propoxy)phenyl)acrylonitrile (**13a**)*

Prepared according to general procedure 1 with (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq) and aniline (**12a**, 456 µL, 5 mmol, 5 eq.). Column chromatography (0-100% EtOAc in hexanes) afforded the desired compound as a pale brown solid (78 mg, 18%), m.p.: 130-133 °C. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.02 (d, *J* = 8.8 Hz, 2H), 7.98 (s, 1H), 7.94 (d, *J* = 1.6 Hz, 1H), 7.713 – 7.706 (m, 2H), 7.14 (d, *J* = 8.8 Hz, 2H), 7.10 (dd, *J* = 8.5, 7.4 Hz, 2H), 6.70 (d, *J* = 7.7 Hz, 2H), 6.60 (t, *J* = 7.6 Hz, 1H), 4.96 (s, 1H), 4.43 (d, *J* = 4.6 Hz, 1H), 4.27 – 4.21 (m, 2H), 4.21 – 4.15 (m, 1H), 3.50 – 3.40 (m, 1H), 3.28 (dd, *J* = 12.9, 5.7 Hz, 1H); ^{13}C NMR (101 MHz, acetone-d_6) δ 162.4, 149.9, 144.7, 136.5, 133.5, 132.8, 132.5 (2C), 132.0, 129.8 (2C), 128.2, 127.2, 126.5, 118.5, 117.4, 116.0 (2C), 113.5 (2C), 106.3, 71.7, 69.1, 47.3; IR υ_{max}/cm^{-1}: 3497 (NH), 3390 (br, OH), 3050 (C=C), 2210 (CN), 1598 (NH), 1253 (C-O), 815 cm^{-1} (C-Cl). LRMS: (ESI$^+$) m/z: 439 ($C_{24}H_{21}Cl_2N_2O_2$) [M+H]; HRMS: Exact mass calculated for $C_{24}H_{21}Cl_2N_2O_2$ [M+H], 439.0975. Found 439.0976.

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*(Z)-3-(4-(3-(Cyclohexylamino)-2-hydroxypropoxy)phenyl)-2-(3,4-dichlorophenyl)-acrylonitrile (**13b**)*

Prepared according to general procedure 1 with (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq) and cyclohexylamine (**12b**, 458 μL, 5 mmol, 5 eq.). Column chromatography (0-100% EtOAc in hexanes) afforded the desired compound as a pale yellow solid (119 mg, 27%), m.p.: 112-114 ˚C. ^{1}H NMR (600 MHz, acetone-d_6) δ 8.01 (d, *J* = 8.8 Hz, 2H), 7.97 (s, 1H), 7.94 (d, *J* = 2.0 Hz, 1H), 7.73 – 7.68 (m, 2H), 7.14 – 7.10 (m, 2H), 4.16 (dd, *J* = 9.7, 4.5 Hz, 1H), 4.09 (dd, *J* = 9.7, 6.0 Hz, 1H), 4.03 (dd, *J* = 12.0, 5.4 Hz, 1H), 2.92 (dd, *J* = 11.9, 4.6 Hz, 1H), 2.77 (dd, *J* = 11.9, 7.1 Hz, 1H), 2.48 (ddd, *J* = 10.1, 7.0, 3.8 Hz, 1H), 1.91 – 1.90 (m, 2H), 1.73 – 1.70 (m, 2H), 1.60 – 1.57 (m, 1H), 1.28 (tt, *J* = 15.7, 3.3 Hz, 2H), 1.21 – 1.16 (m, 1H), 1.15 – 1.07 (m, 2H); ^{13}C NMR (151 MHz, acetone-d_6) δ 162.5, 144.7, 136.5, 133.5, 132.7, 132.5 (2C), 132.0, 128.2, 127.1, 126.5, 118.5, 116.0 (2C), 106.2, 72.0 (2C), 69.4, 57.5, 50.0 (2C), 34.1 (d, J = 21.1 Hz), 26.9, 25.6; IR υ_{max}/cm^{-1}: 3305 (NH), 3119 (br, OH), 2922 (C=C), 2853 (C-O-C), 2209 (CN), 1597 (NH), 1256 (C-O), 823 cm^{-1} (C-Cl).; LRMS: (ESI$^+$) m/z: 445 ($C_{24}H_{27}Cl_2N_2O_2$) [M+H]; HRMS: Exact mass calculated for $C_{24}H_{27}Cl_2N_2O_2$ [M+H], 445.1444. Found 445.1447.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-(2-hydroxy-3-((4-chlorophenyl)-amino)propoxy)-phenyl)acrylonitrile (**13c**)*

Prepared according to general procedure 1 with (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.) and 4-chloroaniline (**12c**, 446 μL, 5 mmol, 5 eq.). Column chromatography (0-100% EtOAc in hexanes) afforded the desired compound as a pale yellow solid (198 mg, 42%), m.p.: 110-113 °C. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.01 (d, *J* = 8.8 Hz, 2H), 7.97 (s, 1H), 7.94 (d, *J* = 1.6 Hz, 1H), 7.71 – 7.70 (m, 2H), 7.14 (d, *J* = 8.8 Hz, 2H), 7.09 (d, *J* = 8.8 Hz, 2H), 6.72 (d, *J* = 8.8 Hz, 2H), 5.19 (t, *J* = 5.6 Hz, NH), 4.49 (d, *J* = 4.4 Hz, 1H, OH), 4.23 – 4.17 (m, 3H), 3.45 (ddd, *J* = 13.1, 6.6, 4.6 Hz, 1H), 3.31 – 3.25 (m, 1H); ^{13}C NMR (101 MHz, acetone-d_6) δ 162.3, 148.8, 144.7, 136.5, 133.5, 132.8, 132.5 (2C), 132.0, 129.6 (2C), 128.2, 127.2, 126.5, 121.2, 118.5, 116.0 (2C), 114.7 (2C), 106.4, 71.5, 69.0, 47.4; IR υ_{max}/cm^{-1}: 3385 (br, OH), 2926 (C=C), 2210 (CN), 1586 (NH), 1257 (C-O), 828 (C-Cl); LRMS: (ESI$^+$) m/z: 473 ($C_{24}H_{20}Cl_3N_2O_2$) [M+H]; HRMS: Exact mass calculated for $C_{24}H_{20}Cl_3N_2O_2$ [M+H], 473.0585. Found 473.0575.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-(2-hydroxy-3-((4-bromophenyl)amino)propoxy)-phenyl)acrylonitrile (**13d**)*

Prepared according to general procedure 1 with (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.) and 4-bromoaniline (**12d**, 573 μL, 5 mmol, 5 eq.). Column chromatography (0-100% EtOAc in hexanes) afforded the desired compound as a yellow solid (225 mg, 43%), m.p.: 121-124 ˚C. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.00 (d, J = 7.6 Hz, 2H), 7.95 (d, *J* = 2.8 Hz, 1H), 7.92 (s, 1H), 7.69 – 7.68 (m, 2H), 7.21 (d, *J* = 8.8 Hz, 2H), 7.13 – 7.11 (m, 2H), 6.67 (d, *J* = 8.8 Hz, 2H), 5.21 (bs, NH), 4.49 (d, *J* = 4.5 Hz, 1H), 4.25 – 4.15 (m, 3H), 3.47 – 3.41 (m, 1H), 3.31 – 3.24 (m, 1H); ^{13}C NMR (101 MHz, acetone-d_6) δ 162.3, 149.1, 144.6, 136.4, 133.5, 132.7, 132.44, 132.40, 131.9, 128.1, 127.2, 126.5, 118.4, 115.9, 115.2, 108.1, 106.3, 71.5, 68.9, 47.2; IR υ_{max}/cm^{-1}: 3380 (br, OH), 3264 (C=C), 2878 (O-CH$_3$), 2210 (CN), 1594 (NH), 1246 (C-O), 812 (C-Cl); LRMS: (ESI$^+$) m/z: 517 ($C_{24}H_{20}BrCl_2N_2O_2$) [M+H]; HRMS: Exact mass calculated for $C_{24}H_{20}{}^{79}BrCl_2N_2O_2$ [M+H], 517.0080. Found 517.0086. Exact mass calculated for $C_{24}H_{20}{}^{81}BrCl_2N_2O_2$ [M+H], 519.0060. Found 519.0062.

(*Z)-2-(3,4-Dichlorophenyl)-3-(4-(2-hydroxy-3-((4-methoxyphenyl)amino)propoxy)-phenyl)acrylonitrile (**13e**)*

Prepared according to general procedure 1 with (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.) and 4-methoxyaniline (**12e**, 575 μL, 5 mmol, 5 eq.). Column chromatography (0-100% EtOAc in hexanes) afforded the desired compound as a pale yellow solid (153 mg, 33%), m.p.: 130-133 °C. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.01 (d, *J* = 8.8 Hz, 2H), 7.97 (s, 1H), 7.94 (d, *J* = 1.5 Hz, 1H), 7.70 – 7.70 (m, 2H), 7.13 (d, *J* = 8.8 Hz, 2H), 6.76 – 6.73 (m, 2H), 6.68 – 6.66 (m, 2H), 4.59 (bs, NH), 4.41 (d, *J* = 4.5 Hz, OH), 4.25 – 4.14 (m, 3H), 3.68 (s, 3H), 3.39 (dd, *J* = 12.6, 3.6 Hz, 1H), 3.22 (dd, *J* = 12.6, 6.0 Hz, 1H); ^{13}C NMR (101 MHz, acetone-d_6) δ 162.4, 152.8, 144.7, 144.1, 136.5, 133.5, 132.7, 132.5 (2C), 132.0, 128.2, 127.2, 126.5, 118.5, 116.0 (2C), 115.5 (2C), 114.8 (2C), 106.3, 71.8, 69.2, 55.8, 48.3; IR υ_{max}/cm^{-1}: 3260 (br, OH), 3050 (C=C), 2829 (O-CH$_3$), 2210 (CN), 1596 (NH), 1272 (C-O), 820 (C-Cl); LRMS: (ESI+) m/z: 469 ($C_{25}H_{23}Cl_2N_2O_3$) [M+H]; (ESI$^-$) m/z: 513 ($C_{26}H_{24}Cl_2N_2O_5$) [M+FA-H]; HRMS: Exact mass calculated for $C_{25}H_{23}Cl_2N_2O_3$ [M+H], 469.1081. Found 469.1083.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-(2-hydroxy-3-(4-phenyl-piperazin-1-yl)propoxy)-phenyl)acrylonitrile (**13f**)*

Prepared according to general procedure 1 with (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.) and 1-phenylpiperazine (**12f**, 788 μL, 5 mmol, 5 eq.). The resulting mixture was filtered under vacuum and washed with cold EtOH (2 x 10 mL) to afford the desired compound as a pale yellow solid (106 mg, 21%); Prepared according to general procedure 2 (with 346 mg, 1 mmol epoxide) and 1-phenylpiperazine (17, 316 μL, 2 mmol, 2 eq.) to afford the desired compound as a pale yellow solid (430 mg, 88%), m.p.: 175-178 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.10 (s, 1H), 8.01 (d, *J* = 2.2 Hz, 1H), 7.97 (d, *J* = 8.9 Hz, 2H), 7.77 (d, *J* = 8.5 Hz, 1H), 7.69 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.20 (dd, *J* = 8.5, 7.4 Hz, 2H), 7.15 (d, *J* = 8.9 Hz, 2H), 6.92 (d, *J* = 8.0 Hz, 2H), 6.76 (t, *J* = 7.2 Hz, 1H), 4.98 (d, *J* = 4.7 Hz, 1H), 4.12 (dd, *J* = 9.3, 2.9 Hz, 1H), 4.03 – 4.00 (m, 2H), 3.12 (t, *J* = 4.9 Hz, 4H), 2.63 (dt, *J* = 10.0, 4.8 Hz, 2H), 2.63 – 2.58 (m, 2H), 2.54 (s, 1H), 2.44 (dd, *J* = 12.7, 6.1 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.2, 151.1, 144.3, 134.9, 132.0, 131.5 (2C), 131.2, 131.1, 128.89 (2C), 128.86, 127.0, 125.80, 125.78, 118.7, 117.9, 115.31 (2C), 115.27, 115.1 (2C), 104.4, 71.4, 66.5, 60.9, 53.5, 48.3; IR υ_{max}/cm^{-1}: 3392 (br, OH), 2825 (N-CH$_2$), 2210 (CN), 1245 (C-O), 818 (C-Cl); LRMS: (ESI$^+$) m/z: 508 ($C_{28}H_{28}Cl_2N_3O_2$) [M+H]; HRMS: Exact mass calculated for $C_{28}H_{28}Cl_2N_3O_2$ [M+H], 508.1553. Found 508.1557.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-(3-(dimethylamino)propoxy)-phenyl)acrylonitrile (**13g**)*

Prepared according to general procedure 2 from (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.) and dimethylamine (**12g**, 68 mg, 1.5 mmol, 1.5 eq.; 3 mL of a 2.0 M solution in THF) to afford the desired compound as a pale yellow solid (97 mg, 25%), m.p.: 99-101 ˚C. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.01 (d, *J* = 8.4 Hz, 2H), 7.97 – 7.94 (m, 2H), 7.70 (s, 2H), 7.13 (d, *J* = 8.4 Hz, 2H), 4.17 (dd, *J* = 11.3, 5.6 Hz, 1H), 4.07 (d, *J* = 6.0 Hz, 2H), 2.48 (s, 2H), 2.28 (s, 6H); ^{13}C NMR (101 MHz, acetone-d$_6$) δ 162.6, 144.7, 136.5, 133.5, 132.7, 132.5 (2C), 132.0, 128.2, 127.1, 126.5, 118.5, 115.9 (2C), 106.2, 72.2, 67.7, 63.0, 46.2 (2C); IR υ_{max}/cm^{-1}: 3350 (OH), 2939 (C=H), 2208 (CN), 1692 (C-O), 812 (C-Cl); LRMS: (ESI$^+$) m/z: 391 ($C_{20}H_{21}Cl_2N_2O_2$) [M+H]; HRMS: Exact mass calculated for $C_{20}H_{21}Cl_2N_2O_2$ [M+H], 391.0975. Found 391.0977.

*2-(Oxiran-2-ylmethoxy)benzaldehyde (**15a**)*

2-Hydroxybenzaldehyde (**14a**, 300 μL, 2.86 mmol) in 50 mL ACN was passed through a series of omnifit columns, the first containing molecular sieves, the second contained cesium carbonate and sand (50%:50% w/w) at a flow rate of 0.5 mL/min. The reactant stream was then met by a secondary stream containing epichlorohydrin (17 mL, with 20 mL DMF and 63 mL ACN, flow rate 0.5 mL/min), and together the reactant streams were passed through a Vapourtec static mixer reactor (20mL) heated at 105 °C and held at 5 bar back pressure.

The product solution was then evaporated under reduced pressure, then diluted with ethyl acetate (50 mL), extracted with water (3 x 50mL), and then brine (1 x 50 mL), dried over magnesium sulfate and the solvent removed under reduced pressure to afford 2-(oxiran-2-ylmethoxy)benzaldehyde (**15a**) as a light brown oil (379 mg, 74%).

Alternatively, 2-hydroxybenzaldehyde (**14a**, 436 μL, 4.10 mmol, 1 eq.) and caesium carbonate (1.940 g, 6.14 mmol, 1.5 eq.) was combined with 15 mL DMF and 6 mL epichlorohydrin (**7**) in a round-bottomed flask. The reaction was heated to 75 °C and left to stir. After 3 h, the reaction was cooled to ambient temperature, diluted with EtOAc (50 mL), washed with water (3 x 30 mL, brine (2 x 30 mL)), dried over $MgSO_4$ and concentrated

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under reduced pressure to afford the desired product as a yellow oil (**15a**; 634 mg, 86%). LRMS: (ESI$^+$) m/z: 179 ($C_{10}H_{11}O_3$) [M+H]. The material was carried directly through without further characterization.

*(Z)-2-(3,4-Dichlorophenyl)-3-(2-(oxiran-2-ylmethoxy)phenyl)-acrylonitrile (**16a**)*

2-(Oxiran-2-ylmethoxy)benzaldehyde (**15a**, 693 mg, 3.9 mmol, 1.05 eq.) was combined with 10 mL water in a round-bottomed flask and heated to 50 °C. The solution was stirred for 10 min, then 3,4-dichlorophenylacetonitrile (**10**, 689 mg, 3.7 mmol, 1 eq.) was added to the stirring solution. Benzytrimethyl ammonium hydroxide (40 wt.% aq. solution) (7 mL) was added dropwise and the reaction left to stir at 50 °C for 5 h. The reaction mixture was then filtered under vacuum and the resulting solid was recrystallised from ethanol to afford the desired product as a pale brown solid (380 mg, 30%). m.p.: 101-104 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.96 (d, J = 2.1 Hz, 1H), 7.91 (d, J = 7.3 Hz, 1H), 7.78 (d, J = 8.5 Hz, 1H), 7.70 (dd, J = 8.5, 2.1 Hz, 1H), 7.51 (dd, J = 11.5, 4.2 Hz, 1H), 7.19 (d, J = 8.4 Hz, 1H), 7.13 (t, J = 7.5 Hz, 1H), 4.47 (dd, J = 11.6, 2.3 Hz, 1H), 3.99 (dd, J = 11.6, 6.4 Hz, 1H), 2.86 (t, J = 4.6 Hz, 1H), 2.74 (dd, J = 5.0, 2.6 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 156.8, 140.4, 134.5, 132.7, 132.1, 131.8, 131.4, 128.5, 127.5, 126.1, 122.6, 121.0, 117.2, 113.0, 109.4, 69.7, 49.6. 43.8; IR υ_{max}/cm^{-1}: 3042 (C=C), 2220 (CN), 1252 (arom. C-O), 821 (C-Cl); LRMS: (ESI$^+$) m/z: 346 ($C_{18}H_{14}Cl_2NO_2$) [M+H]; HRMS: Material decomposed.

*(Z)-2-(3,4-Dichlorophenyl)-3-(2-(2-hydroxy-3-(4-phenyl-piperazin-1-yl)propoxy)phenyl)-acrylonitrile (**17a**)*

Prepared according to general procedure 2 with (*Z*)-2-(3,4-dichlorophenyl)-3-(2-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**16a**, 150 mg, 0.43 mmol, 1 eq.) and 1-phenylpiperazine (**12f**, 137 μL, 0.87 mmol, 2 eq.) to afford the desired compound as a yellow solid (178 mg, 82%), m.p.: 128-130 °C. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.27 (s, 1H), 8.11 (dd, J = 7.8, 1.2 Hz, 1H), 7.92 (t, J = 1.2 Hz, 1H), 7.72 (d, J = 1.2 Hz, 2H), 7.53 – 7.49 (m, 1H), 7.23 – 7.19 (m, 3H), 7.12 (t, J = 7.6 Hz, 1H), 6.93 (d, J = 7.9 Hz, 2H), 6.77 (t, J = 7.3 Hz, 1H), 4.25 (d, J = 8.9 Hz, 1H), 4.22 – 4.14 (m, 2H), 3.17 (t, J = 5.0 Hz, 4H), 2.72 – 2.61 (m, 6H); ^{13}C NMR (101 MHz, acetone-d_6) δ 168.5, 162.2, 150.2, 146.0, 143.33, 143.29, 142.9, 141.8, 139.4 (2C), 138.8, 138.2, 136.4, 133.5, 131.4, 129.6, 127.7, 126.2 (2C), 123.5, 119.5, 82.6, 77.3, 71.6, 64.3 (2C), 59.5 (2C); IR υ_{max}/cm^{-1}: 3433 (OH), 2814 (N-CH$_2$), 2214 (CN), 1228 (C-O), 830 (C-Cl); LRMS: (ESI$^+$) m/z: 508 ($C_{28}H_{28}Cl_2N_3O_2$) [M+H]; HRMS: Exact mass calculated for $C_{28}H_{28}Cl_2N_3O_2$ [M+H], 508.1553. Found 508.1557.

*3-(Oxiran-2-ylmethoxy)benzaldehyde (**15b**)*

3-Hydroxybenzaldehyde (**14b**, 389 mg, 3.1 mmol) in 25 mL ACN was passed through a series of omnifit columns, the first containing molecular sieves, the second contained caesium carbonate and sand (50%:50% w/w) at a flow rate of 0.5 mL/min. The reactant stream was then met by a secondary stream containing epichlorohydrin (17 mL, with 20 mL DMF and 63 mL ACN, flow rate 0.5 mL/min), and together the reactant streams were passed through a Vapourtec static mixer reactor (20mL) heated at 105 °C and held at 5 bar back pressure.

The product solution was then evaporated under reduced pressure, then diluted with ethyl acetate (50 mL), extracted with water (3 x 50mL), and then brine (1 x 50 mL), dried over magnesium sulfate and the solvent removed under reduced pressure to afford 3-(oxiran-2-ylmethoxy)benzaldehyde (**15b**) as a light brown oil (403 mg, 72%).

Alternatively, 3-hydroxybenzaldehyde (**14b**, 500 mg, 4.10 mmol, 1 eq.) and caesium carbonate (1.940 g, 6.14 mmol, 1.5 eq.) were combined with 15 mL DMF and 6 mL epichlorohydrin (**7**) in a round-bottomed flask. The reaction was heated to 75 °C and left to stir. After 3 h, the reaction was cooled to ambient temperature, diluted with EtOAc (50 mL), washed with water (3 x 30 mL, brine (2 x 30 mL)), dried over $MgSO_4$ and concentrated under reduced pressure to afford 3-(oxiran-2-ylmethoxy)benzaldehyde (**15b**) as a pale yellow oil (660 mg, 90%). LRMS: (ESI$^+$) m/z: 179 ($C_{10}H_{11}O_3$) [M+H]. The material was carried directly through without further characterization.

*(Z)-2-(3,4-Dichlorophenyl)-3-(3-(oxiran-2-ylmethoxy)phenyl)-acrylonitrile (**16b**)*

3-(Oxiran-2-ylmethoxy)benzaldehyde (**15b**, 429 mg, 2.41 mmol, 1.05 eq.) was then combined with 10 mL water in a round-bottomed flask and heated to 50 °C. The solution was stirred for 10 min, then 3,4-dichlorophenylacetonitrile (**10**, 426 mg, 2.29 mmol, 1 eq.) was added to the stirring solution. Benzyltrimethyl ammonium hydroxide (40 wt.% aq. solution) (7 mL) was added dropwise and the reaction left to stir at 50 °C for 5 h. The reaction mixture was then filtered under vacuum and the resulting solid was recrystallised from ethanol to afford the desired product as a crystalline golden solid (495 mg, 59%); m.p.: 126-128 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.16 (s, 1H), 8.05 (d, J = 2.1 Hz, 1H), 7.80 (d, J = 8.5 Hz, 1H), 7.72 (dd, J = 8.5, 2.1 Hz, 1H), 7.56 (d, J = 7.3 Hz, 2H), 7.48 (t, J = 8.0 Hz, 1H), 7.16 – 7.14 (m, 1H), 4.39 (dd, J = 11.3, 2.6 Hz, 1H), 3.90 (dd, J = 11.3, 6.5 Hz, 1H), 3.38 (dd, J = 6.5, 3.9 Hz, 1H), 2.87 (t, J = 4.6 Hz, 1H), 2.73 (dd, J = 4.9, 2.6 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.4, 144.6, 134.6, 134.4, 132.1, 131.8, 131.3, 130.2, 127.4, 126.2, 122.0, 117.4, 117.3, 115.2, 108.2, 69.1, 49.6, 43.8; IR υ_{max}/cm^{-1}: 3076 (C=C), 2218 (CN), 1272 (arom. C-O), 830 (C-Cl); LRMS: (ESI$^+$) m/z: 346 ($C_{18}H_{14}Cl_2NO_2$) [M+H]; HRMS: Material decomposed.

*(Z)-2-(3,4-Dichlorophenyl)-3-(3-(2-hydroxy-3-(4-phenyl-piperazin-1-yl)propoxy)phenyl)-acrylonitrile (**17b**)*

Prepared according to general procedure 2 with (*Z*)-2-(3,4-dichlorophenyl)-3-(3-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**16b**, 205 mg, 0.59 mmol, 1 eq.) and 1-phenylpiperazine (**12f**, 187 μL, 1.18 mmol, 2 eq.) to afford the desired compound as a creamy-coloured solid (137 mg, 63%), m.p.: 132-134 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.17 (s, 1H), 8.05 (s, 1H), 7.78 (d, J = 8.2 Hz, 1H), 7.71 (d, J = 8.1 Hz, 1H), 7.54 (d, J = 12.3 Hz, 2H), 7.48 (d, J = 7.7 Hz, 1H), 7.16 (dd, J = 16.9, 7.5 Hz, 3H), 6.90 (d, J = 7.7 Hz, 2H), 6.77 (d, J = 6.2 Hz, 1H), 4.99 (s, 1H), 4.08 (d, J = 8.3 Hz, 2H), 3.97 (s, 1H), 3.12 (s, 4H), 2.62 (s, 5H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 158.9, 144.8, 134.6, 134.4, 132.1, 131.8, 131.3, 130.3, 130.2, 128.9, 127.4, 126.2, 121.8, 118.8, 117.8, 117.4, 115.4, 114.0, 108.1, 71.2, 69.2, 53.5, 49.6, 48.2; IR υ_{max}/cm^{-1}: 3190 (O-H), 2978 (C=C), 2216 (CN), 1274 (arom. C-O), 819 (C-Cl); LRMS: (ESI$^+$) m/z: 508 ($C_{28}H_{28}Cl_2N_3O_2$) [M+H]; HRMS: Exact mass calculated for $C_{28}H_{28}Cl_2N_3O_2$ [M+H], 508.1553. Found 508.1544.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-(3-(dimethylamino)propoxy)-phenyl)acrylonitrile (**18**)*

Prepared according to general procedure 2 from 3,4-dichlorophenylacetonitrile (**10**, 346 mg, 1 mmol, 1 eq.) and 4-(3-(dimethylamino)propoxy)benzaldehyde (514 mg, 2 mmol, 2 eq.) to afford the desired compound as a pale brown solid (76 mg, 20%), m.p.: 89-92 ˚C. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.01 (d, J = 8.8 Hz, 2H), 7.97 (s, 1H), 7.94 (d, J = 1.6 Hz, 1H), 7.71 – 7.70 (m, 2H), 7.10 (d, J = 8.9 Hz, 2H), 4.16 (t, J = 6.4 Hz, 2H), 2.42 (t, J = 7.0 Hz, 2H), 2.18 (s, 6H), 1.93 (dd, J = 13.4, 6.7 Hz, 2H); ^{13}C NMR (101 MHz, acetone-d_6) δ 162.6, 144.8, 136.5, 133.9, 132.5 (2C), 132.0, 130.0, 128.2, 127.0, 126.5, 118.5, 115.8 (2C), 106.1, 67.2, 56.7, 45.7 (2C), 28.1; IR υ_{max}/cm^{-1}: 2953 (C=H), 2216 (CN), 1248 (C-O), 727 (C-Cl); LRMS: (ESI$^+$) m/z: 375 ($C_{20}H_{20}Cl_2N_2O$) [M+H]; HRMS: Exact mass calculated for $C_{20}H_{21}Cl_2N_2O$ [M+H], 375.1025. Found 375.1013.

*(S)-4-(Oxiran-2-ylmethoxy)benzaldehyde (**20a**)*

(*S*)-(+)-Glycidyl-3-nitrobenzenesulfonate (**19a**, 493 mg, 1.9 mmol, 1.1 eq.), 4-hydroxybenzaldehyde (**8**, 214 mg, 1.75 mmol, 1 eq.) and cesium carbonate (847 mg, 2.6 mmol, 1.5 eq.) were combined in a round-bottomed flask with 20 mL DMF. The solution was heated to 80 °C and stirred for 4 h. The solution was cooled to RT, extracted with ethyl acetate (50 mL) and washed with water (5 x 30 mL) and brine (30 mL). The organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure to afford the desired product as a brown oil (286 mg, 86%).^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (s, 1H), 7.84 (d, J = 8.7 Hz, 2H), 7.03 (d, J = 8.7 Hz, 2H), 4.34 (dd, J = 11.1, 2.9 Hz, 1H), 4.01 (dd, J = 11.1, 5.8 Hz, 1H), 3.38 (ddd, J = 6.7, 4.8, 2.0 Hz, 1H), 2.94 (t, J = 4.5 Hz, 1H), 2.78 (dd, J = 4.7, 2.6 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.9, 136.5, 132.1, 130.5, 115.1, 69.1, 50.0, 44.7; IR υ_{max}/cm^{-1}: 2834 (C-C), 1686 (C=O), 1250 (C-

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O); LRMS: (ESI$^+$) m/z: 179 ($C_{10}H_{11}O_3$) [M+H]; Material was not completely dried, contained acetone.

*(S,Z)-2-(3,4-Dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)-acrylonitrile (**21a**)*

(*S*)-4-(Oxiran-2-ylmethoxy)benzaldehyde (**20a**, 220 mg, 1.23 mmol, 1.05 eq.) was combined with 10 mL water in a round-bottomed flask and heated to 50 °C. The solution was stirred for 10 min, then 3,4-dichlorophenylacetonitrile (**10**, 219 mg, 1.18 mmol, 1 eq.) was added to the stirring solution. Benzytrimethyl ammonium hydroxide (40 wt.% aq. solution) (7 mL) was added dropwise and the reaction left to stir at 50 °C for 5 h. The reaction mixture was then filtered under vacuum and the resulting solid was recrystallised from ethanol to afford the desired product as a yellow solid (216 mg, 53%), m.p.: 112-115 °C. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.02 (d, *J* = 8.9 Hz, 2H), 7.98 (s, 1H), 7.96 – 7.93 (m, 1H), 7.75 – 7.67 (m, 2H), 7.15 (d, *J* = 8.9 Hz, 2H), 4.47 (dd, *J* = 11.4, 2.6 Hz, 1H), 4.00 (dd, *J* = 11.4, 6.4 Hz, 1H), 3.36 (ddd, *J* = 6.7, 5.2, 2.6 Hz, 1H), 2.87 (dd, *J* = 5.0, 4.3 Hz, 1H), 2.75 (dd, *J* = 5.1, 2.6 Hz, 1H); ^{13}C NMR (101 MHz, acetone-d_6) δ 162.0, 144.7, 136.5, 133.6, 132.8, 132.5 (2C), 132.0, 128.2, 127.5, 126.6, 118.5, 116.0 (2C), 106.6, 70.4, 50.4, 44.4 IR υ$_{max}$/cm^{-1}: 3093 (C=C), 2213 (CN), 1257 (arom. C-O), 824 (C-Cl); LRMS: (ESI$^+$) m/z: 346 ($C_{18}H_{14}Cl_2NO_2$) [M+H], HRMS: Material decomposed.

*(S,Z)-2-(3,4-Dichlorophenyl)-3-(4-(2-hydroxy-3-(4-phenylpiperazin-1-yl)propoxy)phenyl)-acrylonitrile (**22a**)*

Prepared according to general procedure 2 with (*S,Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**21a**, 134 mg, 0.39 mmol, 1 eq.) and 1-phenylpiperazine (**12f**, 122 μL, 0.77 mmol, 2 eq.) to afford the desired compound as a pale yellow solid (116 mg, 59%), m.p.: 157-159 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (s, 1H), 8.01 (d, *J* = 1.5 Hz, 1H), 7.96 (d, *J* = 8.7 Hz, 2H), 7.76 (d, *J* = 8.4 Hz, 1H), 7.69 (dd, *J* = 8.4, 1.5 Hz, 1H), 7.20 (t, *J* = 7.7 Hz, 2H), 7.15 (d, *J* = 8.7 Hz, 2H), 6.92 (d, *J* = 8.1 Hz, 2H), 6.76 (t, *J* = 7.1 Hz, 1H), 4.99 (s, 1H), 4.13 – 4.11 (m, 1H), 4.02 – 3.98 (m, 2H), 3.12 (m, 4H), 2.65 – 2.57 (m, 4H), 2.44 (dd, *J* = 12.8, 5.8 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.2, 151.1, 144.4, 135.0, 132.1, 131.5 (2C), 131.2, 131.1, 128.9 (2C), 127.0, 125.82, 125.80, 118.8, 117.9, 115.3 (2C), 115.2 (2C), 104.4, 71.4, 66.5, 60.9, 53.5 (2C), 48.3 (2C); IR υ$_{max}$/cm^{-1}: 3392 (br, OH), 2825 (N-CH$_2$), 2210 (CN), 1245 (C-O), 818 (C-Cl); LRMS: (ESI$^+$) m/z: 508 ($C_{28}H_{28}Cl_2N_3O_2$) [M+H]; CHIRAL HPLC: Column: Phemonex Lux Cellulose 2 (250 x 4.6 mm); Conditions: 80% hexane + 0.1% DEA, 20% IPA + 0.1% DEA. Runtime: 30 min; Peak retention time: 15.658 mins; Area (%): 100.0%. Optical rotation: [α]$_D$ 0.235 (c 0.514 in DMSO). HRMS: Exact mass calculated for $C_{28}H_{28}Cl_2N_3O_2$ [M+H], 508.1553. Found 508.1539.

*(R)-4-(Oxiran-2-ylmethoxy)benzaldehyde (**20b**)*

(*R*)-(-)-Glycidyl-3-nitrobenzenesulfonate (**19b**, 508 mg, 1.9 mmol, 1.1 eq.), 4-hydroxybenzaldehyde (**8**, 215 mg, 1.75 mmol, 1 eq.) and caesium carbonate (847 mg, 2.6 mmol, 1.5 eq.) were combined in a round-bottomed flask with 20 mL DMF. The solution was heated to 80 °C and stirred for 4 h. The solution was cooled to ambient temperature, extracted with ethyl acetate (50 mL) and washed with water (5 x 30 mL) and brine (30 mL). The organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure to afford the desired product as a brown oil (286 mg, 86%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.89 (s, 1H), 7.83 (dd, *J* = 8.8, 2.6 Hz, 2H)), 7.03 (dd, *J* = 8.8, 2.6 Hz, 2H), 4.34 (dd, *J* = 11.1, 2.9 Hz, 1H), 4.02 (dd, *J* = 11.1, 5.9 Hz, 1H), 3.38 (ddd, *J* = 5.8, 2.9, 1.3 Hz, 1H), 2.99 – 2.86 (m, 1H), 2.78 (dd, *J* = 4.8, 2.6 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 190.9, 163.5, 132.1 (2C), 130.5, 115.0 (2C), 69.1, 50.0, 44.7; IR υ$_{max}$/cm^{-1}: 2825 (C-C), 1686 (C=O), 1250 (C-O); LRMS: (ESI$^+$) m/z: 179 ($C_{10}H_{11}O_3$) [M+H].

*(R,Z)-2-(3,4-Dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)-acrylonitrile (**21b**)*

(*R*)-4-(oxiran-2-ylmethoxy)benzaldehyde (**20b**, 1.603 g, 8.77 mmol, 1.1 eq.) was combined with 15 mL water in a round-bottomed flask and heated to 50 °C. The solution was stirred for 10 min, then 3,4-dichlorophenylacetonitrile (**10**, 1.483 g, 7.97 mmol, 1 eq.) was added to the stirring solution. Benzyltrimethyl ammonium hydroxide (40 wt.% aq. solution) (10 mL) was added dropwise and the reaction left to stir at 50 °C for 5 h. The reaction mixture was then filtered under vacuum and the resulting solid was recrystallised from ethanol to afford the desired product as a yellow solid (1.720 g, 62%), m.p.: 111-113 °C. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.04 (d, *J* = 2.5 Hz, 1H), 8.02 (d, *J* = 8.9 Hz, 2H), 7.97 (d, *J* = 0.8 Hz, 1H), 7.71 (d, *J* = 1.9 Hz, 2H), 7.15 (d, *J* = 8.9 Hz, 2H), 4.47 (dd, *J* = 11.4, 2.6 Hz, 1H), 3.99 (dd, *J* = 11.4, 6.5 Hz, 1H), 3.37 (ddd, *J* = 6.6, 2.6, 1.7 Hz, 1H), 2.87 (dd, *J* = 5.0, 4.3 Hz, 1H), 2.75 (dd, *J* = 5.1, 2.6 Hz, 1H); ^{13}C NMR (101 MHz, acetone-d_6) δ 162.0, 144.6, 136.4, 133.5, 132.8, 132.5 (2C), 132.0, 128.2, 127.5, 126.5, 118.5, 115.9 (2C), 106.5, 70.4, 50.4, 44.4; IR υ$_{max}$/cm^{-1}: 3390 (OH), 3094 (C=C), 2213 (CN), 1257 (arom. C-O), 825 (C-Cl); LRMS: (ESI$^+$) m/z: 346 ($C_{18}H_{14}Cl_2NO_2$) [M+H], HRMS: Material decomposed.

*(R,Z)-2-(3,4-Dichlorophenyl)-3-(4-(2-hydroxy-3-(4-phenylpiperazin-1-yl)propoxy)phenyl)-acrylonitrile (**22b**)*

Prepared according to general procedure 2 from (*S,Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**21b**, 213 mg, 0.62 mmol, 1 eq.) and 1-phenylpiperazine (**12f**, 194 μL, 1.22 mmol, 2 eq.) to afford the desired compound as a pale yellow solid (312 mg, 82%), m.p.: 163-165 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (s, 1H), 8.00 (d, *J* = 2.0 Hz, 1H), 7.96 (d, *J* = 8.8 Hz, 2H), 7.76 (d, *J* = 8.5 Hz, 1H), 7.68 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.20 (t, *J* = 7.9 Hz, 2H), 7.14 (d, *J* = 8.8 Hz, 2H), 6.92 (d, *J* = 8.2 Hz, 2H), 6.76 (t, *J* = 7.2 Hz, 1H), 4.98 (d, *J* = 4.2 Hz, 1H), 4.13 – 4.11 (m, 1H), 4.02 – 3.99 (m, 2H), 3.12 (t, *J* = 4.7 Hz, 4H), 2.65 – 2.57 (m, 4H), 2.44 (dd, *J* = 12.6, 5.9 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.2, 151.1, 144.3, 134.9, 132.0, 131.5 (2C), 131.2, 131.1, 128.9 (2C), 127.0, 125.80, 125.77, 118.7, 118.0, 115.3 (2C), 115.1 (2C), 104.4, 71.4, 66.5, 60.9, 53.5 (2C), 48.3 (2C); IR υ$_{max}$/cm^{-1}: 2825 (N-CH$_2$), 2210 (CN), 1245 (C-O), 818 (C-Cl); LRMS: (ESI$^+$) m/z: 508 ($C_{28}H_{28}Cl_2N_3O_2$) [M+H]; CHIRAL HPLC: Column: Phemonex Lux Cellulose 2 (250 x 4.6 mm); Conditions: 80% hexane + 0.1% DEA, 20% IPA + 0.1% DEA. Runtime: 30 min; Peak retention time: 17.213 mins; Area (%): 100.0%. Optical rotation: [α]$_D$ -0.225 (c 0.472 in DMSO). HRMS: Exact mass calculated for $C_{28}H_{28}Cl_2N_3O_2$ [M+H], 508.1553. Found 508.1558.

*(Z)-3-(4-(3-(4-Cyclohexylpiperazin-1-yl)-2-hydroxypropoxy)-phenyl)-2-(3,4-dichlorophenyl)-acrylonitrile (**23a**)*

Prepared according to general procedure 2 from (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.) and 1-cyclohexylpiperazine (**12h**, 340 mg, 2 mmol, 2 eq.) to afford the desired compound as a yellow solid (277 mg, 54%), m.p.: 137-139 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (s, 1H), 8.00 (d, *J* = 2.1 Hz, 1H), 7.96 (d, *J* = 8.8 Hz, 2H), 7.76 (d, *J* = 8.5 Hz, 1H), 7.69 (d, *J* = 2.1 Hz, 1H), 7.13 (d, *J* = 8.8 Hz, 2H), 4.88 (d, *J* = 4.1 Hz, 1H), 4.10 – 4.06 (m, 1H), 3.95 (d, *J* = 6.2 Hz, 2H), 2.46 – 2.31 (m, 10H), 2.16 (s, 1H), 1.74 – 1.70 (m, 4H), 1.55 (d, *J* = 11.7 Hz, 1H), 1.23 – 1.04 (m, 5H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.2, 144.3, 135.0, 132.0 (2 overlapping signals), 131.5 (2C), 131.2, 131.1, 127.0, 125.8, 117.9 (2C), 115.1, 104.3, 71.5, 66.4, 62.5, 61.1, 54.1 (2C), 48.5 (2C), 28.4, 25.9 (2C), 25.3 (2C); IR υ$_{max}$/cm^{-1}: 3498 (OH), 2812 (N-CH$_2$), 2212 (CN), 1269 (C-O), 813 (C-Cl); LRMS: (ESI$^+$) m/z: 514 ($C_{28}H_{34}Cl_2N_3O_2$) [M+H]; HRMS: Exact mass calculated for $C_{28}H_{34}Cl_2N_3O_2$ [M+H], 514.2023. Found 514.2027.

*(Z)-3-(4-(3-(4-Phenylpiperidin-1-yl)-2-hydroxypropoxy)phenyl)-2-(3,4-dichlorophenyl)-acrylonitrile (**23b**)*

Prepared according to general procedure 2 from (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.) and 4-phenylpiperidine (**12i**, 340 mg, 2 mmol, 2 eq.) to afford the desired compound as a pale yellow solid (448 mg, 87%), m.p.: 170-172 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (s, 1H), 8.01 (d, *J* = 2.2 Hz, 1H), 7.97 (d, *J* = 8.8 Hz, 2H), 7.76 (d, *J* = 8.5 Hz, 1H), 7.68 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.30 – 7.22 (m, 4H), 7.18 – 7.14 (m, 3H), 4.91 (d, *J* = 4.1 Hz, 1H), 4.13 – 4.11 (m, 1H), 4.00 - 3.98 (m, 2H), 3.00 (dd, *J* = 26.9, 10.9 Hz, 2H), 2.49 – 2.35 (m, 3H), 2.12 (dt, *J* = 11.3, 8.4 Hz, 2H), 1.70 – 1.60 (m, 4H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.2, 146.3, 144.3, 135.0, 132.0 (2 overlapping signals), 131.5 (2C), 131.2, 131.1, 128.3 (2C), 127.0, 126.7 (2C), 126.0, 125.8, 117.9, 115.2 (2C), 104.3, 71.5, 66.6, 61.3, 54.6 (d, *J* = 20.8 Hz, 2C), 41.8, 33.2 (d, *J* = 3.0 Hz, 2C); IR υ$_{max}$/cm^{-1}: 3422 (OH),

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2808 (N-CH_2), 2207 (CN), 1263 (C-O), 810 (C-Cl); LRMS: (ESI^+) m/z: 507 ($C_{29}H_{29}Cl_2N_2O_2$) [M+H]; HRMS: Exact mass calculated for $C_{29}H_{29}Cl_2N_2O_2$ [M+H], 507.1601. Found 507.1620.

*(Z)-3-(4-(3-(4-Acetylpiperazin-1-yl)-2-hydroxypropoxy)phenyl)-2-(3,4-dichlorophenyl)-acrylonitrile (**23c**)*

Prepared according to general procedure 2 from (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.) and *N*-acetylpiperazine (**12j**, 256 mg, 2 mmol, 2 eq.) to afford the desired compound as a pale yellow solid (346 mg, 73%), m.p.: 88-91 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (s, 1H), 8.01 (d, *J* = 2.2 Hz, 1H), 7.96 (d, *J* = 8.9 Hz, 2H), 7.77 (d, *J* = 8.5 Hz, 1H), 7.69 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.14 (d, *J* = 8.9 Hz, 2H), 4.97 (d, *J* = 4.3 Hz, 1H), 4.10 – 4.07 (m, 1H), 4.00 – 3.96 (m, 2H), 3.42 – 3.39 (m, 4H), 2.48 – 2.37 (m, 10H), 1.97 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.1, 161.2, 144.4, 135.0, 132.1, 131.5 (2C), 131.3, 131.2, 127.0, 125.84, 125.81, 117.9, 115.2 (2C), 104.4, 71.3, 66.5, 60.8, 53.7* (2C), 53.2* (2C), 45.8* (2C), 40.9* (2C), 21.2. *Isomerization confirmed by 2D correlations; IR υ_{max}/cm^{-1}: 3367 (br, OH), 2819 (N-CH_2), 2212 (CN), 1182 (C-O), 815 (C-Cl); LRMS (ESI^+) m/z: 474 ($C_{24}H_{26}Cl_2N_3O_3$) [M+H]; HRMS: Exact mass calculated for $C_{24}H_{26}Cl_2N_3O_3$ [M+H], 474.1352. Found 474.1332.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-(2-hydroxy-3-(4-methylpiperazin-1-yl)propoxy)phenyl)-acrylonitrile (**23d**)*

Prepared according to general procedure 2 from (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.) and *N*-methylpiperazine (**12k**, 222 μL, 2 mmol, 2 eq.) to afford the desired compound as a yellow solid (221 mg, 50%), m.p.: 111-114 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (s, 1H), 8.00 (d, *J* = 2.1 Hz, 1H), 7.96 (d, J = 8.8 Hz, 2H), 7.76 (d, *J* = 8.5 Hz, 1H), 7.68 (dd, *J* = 8.5, 2.1 Hz, 1H), 7.13 (d, J = 8.8 Hz, 2H), 4.90 (d, *J* = 3.7 Hz, OH), 4.09 – 4.06 (m, 1H), 3.99 – 3.95 (m, 2H), 2.46 – 2.42 (m, 3H), 2.38 – 2.23 (m, 4H), 2.13 (s, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.2, 144.3, 134.9, 132.0, 131.5 (2C), 131.2, 131.1, 127.0, 125.8, 117.9, 115.2, 115.1 (2C), 104.4, 71.4, 66.4, 60.9, 54.8 (2C), 53.4 (2C), 45.8; IR υ_{max}/cm^{-1}: 3070 (br, OH), 2933 (N-CH_2), 2210 (CN), 1183 (C-O), 870 (C-Cl); LRMS (ESI^+) m/z: 446 ($C_{23}H_{26}Cl_2N_3O_2$) [M+H]; Exact mass calculated for $C_{23}H_{26}Cl_2N_3O_2$ [M+H], 446.1397. Found 446.1383.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-(2-hydroxy-3-morpholinopropoxy)phenyl)acrylonitrile (**23e**)*

Prepared according to general procedure 2 from (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.) and morpholine (**12l**, 172 μL, 2 mmol, 2 eq.) to afford the desired compound as a yellow solid (192 mg, 44%), m.p.: 103-105 °C. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.01 (d, *J* = 8.9 Hz, 2H), 7.97 (s, 1H), 7.94 (d, *J* = 1.6 Hz, 1H), 7.71 – 7.70 (m, 2H), 7.13 (d, *J* = 8.9 Hz, 2H), 4.20 (dd, *J* = 9.1, 3.4 Hz, 1H), 4.17 – 4.13 (m, 1H), 4.09 (dd, *J* = 9.0, 5.4 Hz, 1H), 3.64 (s, 4H), 2.61 – 2.51 (m, 6H); ^{13}C NMR (101 MHz, acetone-d_6) δ 162.5, 144.7, 136.5, 133.5, 132.7, 132.5 (2C), 132.0, 128.2, 127.1, 126.5, 118.5, 116.0 (2C), 106.3, 72.1, 67.4 (2C), 67.3, 62.3, 55.1 (2C); IR υ_{max}/cm^{-1}: 3360 (br, OH), 2861 (N-CH_2), 2214 (CN), 1270 (C-O), 814 (C-Cl); LRMS: (ESI^+) m/z: 433 ($C_{22}H_{23}Cl_2N_2O_3$) [M+H]; HRMS: Exact mass calculated for $C_{22}H_{23}Cl_2N_2O_3$ [M+H], 433.1080. Found 433.1067.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-(2-hydroxy-3-(piperidin-1-yl)propoxy)phenyl)acrylonitrile (**23f**)*

Prepared according to general procedure 2 from (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.) and piperidine (**12m**, 198 μL, 2 mmol, 2 eq.) to afford the desired compound as a yellow solid (169 mg, 30%), m.p.: 111-115 °C. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.01 (d, *J* = 8.8 Hz, 2H), 7.97 (s, 1H), 7.94 (d, *J* = 1.6 Hz, 1H), 7.73 – 7.68 (m, 2H), 7.13 (d, *J* = 8.8 Hz, 2H), 4.18 – 4.15 (m, 1H), 4.12 – 4.05 (m, 2H), 2.53 – 2.44 (m, 6H), 1.57 (dt, *J* = 10.7, 5.4 Hz, 4H), 1.46 – 1.41 (m, 2H); ^{13}C NMR (101 MHz, acetone-d_6) δ 162.6, 144.8, 136.5, 133.5, 132.7, 132.5 (2C), 132.0, 128.2, 127.1, 126.5, 118.5, 116.0 (2C), 106.2, 72.3, 67.2, 62.4, 55.8 (2C), 26.89 (2C), 25.0; IR υ_{max}/cm^{-1}: 3300 (b, OH), 2853 (N-CH_2), 2214 (CN), 1264 (C-O), 816 (C-Cl); LRMS: (ESI^+) m/z: 431 ($C_{23}H_{25}Cl_2N_2O_2$) [M+H]; HRMS: Exact mass calculated for $C_{23}H_{25}Cl_2N_2O_2$ [M+H], 431.1288. Found 431.1275.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-(2-hydroxy-3-(4-methylpiperidin-1-yl)propoxy)phenyl)-acrylonitrile (**23g**)*

Prepared according to general procedure 2 from (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.) and 4-methylpiperidine (**12n**, 178 μL, 1.5 mmol, 1.5 eq.) to afford the desired compound as a pale yellow solid (216 mg, 57%), m.p.: 79-81 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (s, 1H), 8.00 (d, *J* = 2.1 Hz, 1H), 7.96 (d, *J* = 8.8 Hz, 2H), 7.76 (d, *J* = 8.5 Hz, 1H), 7.68 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.13 (d, *J* = 8.8 Hz, 2H), 4.85 (d, *J* = 3.4 Hz, 1H), 4.08 (d, *J* = 6.5 Hz, 1H), 3.95 (d, *J* = 6.6 Hz, 2H), 2.84 (dd, *J* = 25.6, 10.6 Hz, 2H), 2.37 (ddd, *J* = 32.9, 12.6, 5.7 Hz, 2H), 1.97 (q, *J* = 9.4 Hz, 2H), 1.55 (d, *J* = 12.6 Hz, 2H), 1.38 – 1.22 (m, 1H), 1.12 (qd, *J* = 12.0, 3.5 Hz, 2H), 0.87 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.2, 144.3, 134.9, 132.0, 131.5 (2C), 131.2, 131.1, 127.0, 125.8, 117.9, 115.2, 115.1 (2C), 104.3, 71.5, 66.5, 61.3, 54.2, (d, *J* = 21.3 Hz, 2C), 34.1 (d, *J* = 4.6 Hz, 2C), 30.2, 21.9; IR υ_{max}/cm^{-1}: 3300 (OH), 2981 (C=C), 2212 (CN), 1180 (C-O), 815 (C-Cl); LRMS: (ESI^+) m/z: 445 ($C_{24}H_{27}Cl_2N_2O_2$) [M+H]; HRMS: Exact mass calculated for $C_{24}H_{27}Cl_2N_2O_2$ [M+H], 445.1444. Found 445.1461.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-(2-hydroxy-3-(3-methylpiperidin-1-yl)propoxy)phenyl)-acrylonitrile (**23h**)*

Prepared according to general procedure 2 from (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.) and 3-methylpiperidine (**12o**, 176 υL, 1.5 mmol, 1.5 eq.) to afford the desired compound as a pale yellow solid (279 mg, 65%), m.p.: 103-106 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (s, 1H), 8.01 (t, *J* = 3.0 Hz, 1H), 7.96 (d, *J* = 8.9 Hz, 2H), 7.77 (dd, *J* = 8.5, 2.6 Hz, 1H), 7.69 (dd, J = 8.5, 2.3 Hz, 1H), 7.14 (t, *J* = 6.2 Hz, 2H), 4.88 (s, 1H), 4.08 (t, J = 6.4 Hz, 1H), 4.02 – 3.88 (m, 2H), 2.91 – 2.71 (m, 2H), 2.39 (d, *J* = 20.5 Hz, 2H), 2.03 – 1.87 (m, 1H), 1.69 – 1.53 (m, 3H), 1.45 (dd, *J* = 24.5, 12.4 Hz, 1H), 0.82 (dd, *J* = 6.4, 2.8 Hz, 3H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.2, 144.3, 135.0, 132.0, 131.5 (2C), 131.2, 131.1, 127.0, 125.78, 125.76, 117.9, 115.1 (2C), 104.3, 71.5, 66.4 (d, *J* = 1.7 Hz), 62.3 (d, J = 12.5 Hz), 61.4 (d, *J* = 3.4 Hz), 54.3, (d, *J* = 12.9 Hz, 2C), 32.5, 30.7 (d, *J* = 2.4 Hz), 25.1, 19.6; IR υ_{max}/cm^{-1}: 3270 (OH), 2926 (C=C), 2215 (CN), 1180 (C-O), 814 (C-Cl); LRMS: (ESI^+) m/z: 445 ($C_{24}H_{27}Cl_2N_2O_2$) [M+H]; HRMS: Exact mass calculated for $C_{24}H_{27}Cl_2N_2O_2$ [M+H], 445.1444. Found 445.1448.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-(2-hydroxy-3-(2-methyl-piperidin-1-yl)propoxy)phenyl)-acrylonitrile (**23i**)*

Prepared according to general procedure 2 from (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.) and 2-methylpiperidine (**12p**, 177 μL, 1.5 mmol, 1.5 eq.) to afford the desired compound as a pale yellow solid (165 mg, 39%), m.p.: 94-96 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (s, 1H), 8.00 (s, 1H), 7.96 (d, *J* = 7.8 Hz, 2H), 7.76 (d, *J* = 8.1 Hz, 1H), 7.68 (d, *J* = 7.4 Hz, 1H), 7.14 (s, 2H), 4.83 (d, *J* = 37.4 Hz, 1H), 4.10 – 3.90 (m, 3H), 3.45 – 3.33 (m, 2H), 2.77 (dd, *J* = 51.7, 42.7 Hz, 2H), 2.42 – 2.03 (m, 2H), 1.57 – 1.45 (m, 3H), 1.23 (s, 2H), 1.00 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.2 (d, *J* = 7.0 Hz), 144.3, 135.0, 132.0, 131.5 (2C), 131.2, 131.1, 127.0, 125.8, 125.7 (d, *J* = 3.6 Hz), 117.9, 115.1 (d, *J* = 2.9 Hz, 2C), 104.3, 71.2 (d, *J* = 5.8 Hz), 69.3 (d, *J* = 18.0 Hz), 56.5 (d, *J* = 80.5 Hz), 56.0 (d, *J* = 18.4 Hz), 52.9 (d, *J* = 21.0 Hz), 34.10 (d, *J* = 28.1 Hz, 25.8 (d, *J* = 4.4 Hz), 23.1 (d, *J* = 38.4 Hz); IR υ_{max}/cm^{-1}: 3380 (OH), 2926 (C=C), 2221 (CN), 1180 (C-O), 815 (C-Cl). LRMS: (ESI^+) m/z: 445 ($C_{24}H_{27}Cl_2N_2O_2$) [M+H]; HRMS: Exact mass calculated for $C_{24}H_{27}Cl_2N_2O_2$ [M+H], 445.1444. Found 445.1447.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-(2-hydroxy-3-(pyrrolidin-1-yl)propoxy)phenyl)acrylonitrile (**23j**)*

Prepared according to general procedure 2 from (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.) and pyrrolidine (**12q**, 123 μL, 1.5 mmol, 1.5 eq.) to afford the desired compound as a yellow solid (169 mg, 42%), m.p.: 90-93 °C. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.01 (d, *J* = 8.8 Hz, 2H), 7.96 (s, 1H), 7.93 (d, *J* = 1.5 Hz, 1H), 7.71 – 7.70 (m, 2H), 7.12 (d, *J* = 8.8 Hz, 2H), 4.19 – 4.16 (m, 1H), 4.10 – 4.05 (m, 2H), 3.56 (q, *J* = 7.0 Hz, 1H), 2.65 (d, *J* =

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5.6 Hz, 1H), 2.61 – 2.56 (m, 3H), 1.74 (s, 3H), 1.12 (t, *J* = 7.0 Hz, 1H); ^{13}C NMR (101 MHz, acetone-d_6) δ 162.6, 144.7, 136.5, 133.5, 132.7, 132.5 (2C), 132.0, 128.2, 127.1, 126.5, 118.5, 116.0 (2C), 106.2, 72.2, 68.7, 59.5, 55.1 (2C), 24.3 (2C); IR υ_{max}/cm^{-1}: 3340 (OH), 2931 (C=C), 2211 (CN), 1183 (C-O), 815 (C-Cl). LRMS: (ESI$^+$) m/z: 417 ($C_{22}H_{23}Cl_2N_2O_2$) [M+H]; HRMS: Exact mass calculated for $C_{22}H_{23}Cl_2N_2O_2$ [M+H], 417.1131. Found 417.1136.

*(Z)-3-(4-(3-(Azepan-1-yl)-2-hydroxypropoxy)phenyl)-2-(3,4-dichlorophenyl)acrylonitrile (**23k**)*

Prepared according to general procedure 2 from (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.) and azepane (**12r**, 169 μL, 1.5 mmol, 1.5 eq.) to afford the desired compound as a pale yellow solid (177 mg, 42%), m.p.: 76-78 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (s, 1H), 8.02 – 8.01 (m, 1H), 7.96 (d, *J* = 8.9 Hz, 2H), 7.77 (dd, *J* = 8.5, 2.5 Hz, 1H), 7.69 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.14 (d, *J* = 8.9 Hz, 2H), 4.82 (s, 1H), 4.12 (dd, *J* = 10.1, 3.3 Hz, 1H), 3.98 (dd, *J* = 10.1, 6.1 Hz, 1H), 3.87 (s, 1H), 2.65 (m, 3H), 2.57 – 2.54 (m, 1H), 1.53 (s, 6H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.3, 144.4, 135.0, 132.0, 131.5 (2C), 131.2, 131.1, 127.0, 125.8, 125.7, 117.9, 115.1 (2C), 104.3, 71.4, 67.3, 60.3, 55.6 (2C), 28.1 (2C), 26.6 (2C); IR υ_{max}/cm^{-1}: 3366 (OH), 2926 (C=C), 2215 (CN), 1180 (C-O), 816 (C-Cl). LRMS: (ESI$^+$) m/z: 445 ($C_{24}H_{27}Cl_2N_2O_2$) [M+H]; HRMS: Exact mass calculated for $C_{24}H_{27}Cl_2N_2O_2$ [M+H], 445.1444. Found 445.1450.

*(Z)-3-(4-(3-((1S,3S)-Adamantan-1-ylamino)-2-hydroxy-propoxy)phenyl)-2-(3,4-dichlorophenyl)acrylonitrile (**23l**)*

Prepared according to general procedure 2 from (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.) and 1-adamantylamine (**12s**, 303 mg, 2 mmol, 2 eq.) to afford the desired compound as a pale yellow solid (307 mg, 64%), m.p.: 170-172 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.10 (s, 1H), 8.01 (d, *J* = 2.2 Hz, 1H), 7.96 (d, *J* = 8.8 Hz, 2H), 7.77 (d, *J* = 8.5 Hz, 1H), 7.69 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.13 (d, *J* = 8.8 Hz, 2H), 4.97 (bs, OH), 4.09 (dd, *J* = 9.9, 4.3 Hz, 1H), 3.97 (dd, *J* = 10.0, 6.2 Hz, 1H), 3.83 – 3.74 (m, 1H), 2.65 – 2.59 (m, 2H), 2.00 (s, 3H), 1.63 – 1.56 (m, 12H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.2, 144.4, 135.0, 132.0, 131.5 (2C), 131.2, 131.1, 127.0, 125.8, 125.7, 117.9, 115.1 (2C), 104.3, 71.1, 69.1, 43.0, 42.4, 36.3 (6C), 29.0 (3C); IR υ_{max}/cm^{-1}: 2903 (CH_2), 2215 (CN), 1142 (C-O), 816 (C-Cl); LRMS (ESI) m/z (%): LRMS (ESI$^+$): 497 ($C_{28}H_{31}Cl_2N_2O_2$) [M+H]; HRMS: Exact mass calculated for $C_{28}H_{31}Cl_2N_2O_2$ [M+H], 497.1757. Found 497.1742.

*(Z)-3-(4-(3-((1R,3S,5r,7r)-Adamantan-2-ylamino)-2-hydroxy-propoxy)phenyl)-2-(3,4-dichlorophenyl)acrylonitrile (**23m**)*

2-Adamantylamine hydrochloride (**12t**, 513 mg, 2.66 mmol, 1 eq.) was combined with sodium hydroxide (126 mg, 3.14 mmol, 1.2 eq.) and water (30 mL). Stirred at ambient temperature for 5 min. The solution was diluted with ethyl acetate (20 mL), washed with water (3 x 20 mL) dried over $MgSO_4$ and concentrated under reduced pressure. The resulting white powder was then carried directly through to the next step. Prepared according to general procedure 2 from (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)-acrylonitrile (**11**, 500 mg, 1.44 mmol, 1 eq.) and 2-adamantylamine (**12ta**, 328 mg, 2.17 mmol, 1.5 eq.) to afford the desired compound as a pale yellow solid (325 mg, 60%), m.p.: 123-126 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (s, 1H), 8.00 (d, *J* = 2.0 Hz, 1H), 7.96 (d, *J* = 8.8 Hz, 2H), 7.76 (d, *J* = 8.5 Hz, 1H), 7.68 (dd, *J* = 8.5, 2.0 Hz, 1H), 7.13 (d, *J* = 8.8 Hz, 2H), 5.02 (d, *J* = 4.4 Hz, 1H), 4.11 (dd, *J* = 9.9, 4.3 Hz, 1H), 4.00 (dd, *J* = 9.9, 6.0 Hz, 1H), 3.90 (dd, *J* = 8.7, 3.8 Hz, 1H), 2.70 – 2.57 (m, 3H), 2.01 (d, *J* = 12.2 Hz, 2H), 1.79 – 1.77 (m, 5H), 1.70 – 1.66 (m, 6H), 1.37 (d, *J* = 11.8 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 161.2, 144.3, 135.0, 132.0, 131.5 (2C), 131.2, 131.1, 127.0, 125.8, 125.7, 117.9, 115.1 (2C), 104.3, 71.1, 68.4, 61.3, 49.6, 37.5, 39.91, 39.89, 31.6, 31.5, 30.79, 30.77, 27.3, 27.2; IR υ_{max}/cm^{-1}: 2903 (CH_2), 2215 (CN), 1141 (C-O), 818 (C-Cl); LRMS (ESI) m/z (%): LRMS (ESI$^+$): 497 ($C_{28}H_{31}Cl_2N_2O_2$) [M+H]; HRMS: Exact mass calculated for $C_{28}H_{31}Cl_2N_2O_2$ [M+H], 497.1757. Found 497.1742.

*Triethylammonium(Z)-1-(3-(4-(2-cyano-2-(3,4-dichlorophenyl)vinyl)phenoxy)-2-hydroxypropyl)piperidin-3-olate (**25a**)*

Prepared according to general procedure 2 from (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.), 3-hydroxypiperidine hydrochloride (**12u**, 262 mg, 2 mmol, 1 eq.) and triethylamine (280 μL, 2 mmol, 2 eq.). Reaction mixture was evaporated to dryness, loaded onto silica and purified by flash column chromatography (0-10% MeOH in DCM with 1% NH_4OH) to afford the desired compound as a yellow crystalline solid (trimethylamine salt) (327 mg, 60%), m.p.: 178-180 °C. ^{1}H NMR (600 MHz, MeOD) δ 7.96 (s, 2H), 7.86 – 7.82 (m, 2H), 7.63 – 7.61 (m, 2H), 7.09 (s, 2H), 4.59 (s, OH), 4.28 (s, 1H), 4.08 (d, *J* = 18.3 Hz, 2H), 3.90 – 3.86 (m, 1H), 3.20 (s, 6H)*, 3.10 (s, 1H), 2.95 (s, 3H), 2.69 (s, 2H), 1.94 (s, 1H), 1.86 (s, 1H), 1.67 (s, 1H), 1.49 (s, 1H), 1.32 (d, *J* = 3.3 Hz, 9H)*; ^{13}C NMR (151 MHz, MeOD) δ 162.5, 144.9 (d, *J* = 1.0 Hz), 136.6, 134.2, 133.5, 132.7 (2C), 132.2, 128.4, 127.94 (d, *J* = 4.4 Hz), 126.4, 118.9, 116.1, 107.0, 71.9 (2 overlapping signals), 71.8, 67.2, 61.7, 55.1 (2 overlapping signals), 49.6, 47.9*, 9.25*; *denotes peaks associated with trimethylamine salt; IR υ_{max}/cm^{-1}: 3308 (OH), 2939 (C=C), 2215 (CN), 1182 (C-O), 816 (C-Cl). LRMS: (ESI$^+$) m/z: 447 ($C_{23}H_{25}Cl_2N_2O_3$) [M+H]; HRMS: Exact mass calculated for $C_{23}H_{25}Cl_2N_2O_3$ [M+H], 447.1237. Found 447.1263.

*(Z)-2-(3,4-dichlorophenyl)-3-(4-(2-hydroxy-3-(4-hydroxy-piperidin-1-yl)propoxy)phenyl)-acrylonitrile (**25b**)*

Prepared according to general procedure 2 from (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**, 346 mg, 1 mmol, 1 eq.) and 4-hydroxypiperidine (**12v**, 101 mg, 1 mmol, 1 eq.) to afford the desired compound as a pale yellow solid (254 mg, 60%), m.p.: 101-103 °C. 1H NMR (400 MHz, DMSO) δ 8.10 (s, 1H), 8.00 (d, *J* = 2.2 Hz, 1H), 7.96 (d, J = 8.8 Hz, 2H), 7.76 (d, *J* = 8.5 Hz, 1H), 7.68 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.13 (d, *J* = 8.8 Hz, 2H), 4.86 (s, 1H), 4.52 (d, *J* = 2.8 Hz, 1H), 4.08 (d, *J* = 6.6 Hz, 1H), 4.01 – 3.87 (m, 2H), 3.47 – 3.39 (m, 1H), 2.83 – 2.64 (m, 2H), 2.37 (ddd, *J* = 32.4, 12.7, 5.8 Hz, 2H), 2.09 (dd, *J* = 21.6, 10.6 Hz, 2H), 1.77 – 1.60 (m, 2H), 1.38 (dd, *J* = 19.0, 9.3 Hz, 2H); ^{13}C NMR (151 MHz, Acetone) δ 151.5, 134.6, 125.3, 122.3, 121.8 (2C), 121.5, 121.4, 117.3, 116.3, 116.1, 108.2, 105.4 (2C), 94.6, 61.8, 56.9 (2 overlapping signals), 51.2, 42.1 (d, *J* = 27.2 Hz, 2C), 24.9 (d, *J* = 4.1 Hz, 2C); IR υ_{max}/cm^{-1}: 3314 (OH), 2933 (C=C), 2215 (CN), 1183 (C-O), 815 (C-Cl). LRMS: (ESI$^+$) m/z: 447 ($C_{23}H_{25}Cl_2N_2O_3$) [M+H]; HRMS: Exact mass calculated for $C_{23}H_{25}Cl_2N_2O_3$ [M+H], 447.1237. Found 447.1274.

*(Z)-2-(3,4-Dichlorophenyl)-3-(4-(2-hydroxy-3-(D-11-piperidin-1-yl)propoxy)phenyl)-acrylonitrile (**26**)*

Prepared according to general procedure 2 from (*Z*)-2-(3,4-dichlorophenyl)-3-(4-(oxiran-2-ylmethoxy)phenyl)acrylonitrile (**11**) (346 mg, 1 mmol, 1 eq.) and piperidine-d_{11} (**12w**, 148 μL, 1.5 mmol, 1.5 eq.) to afford the desired compound as a pale yellow solid (345 mg, 82%), m.p.: 103-105 °C. ^{1}H NMR (600 MHz, DMSO-d_6) δ 8.10 (s, 1H), 8.01 (d, *J* = 1.7 Hz, 1H), 7.96 (d, *J* = 8.7 Hz, 2H), 7.77 (d, *J* = 8.5 Hz, 1H), 7.69 (dd, *J* = 8.5, 1.9 Hz, 1H), 7.13 (d, *J* = 8.7 Hz, 2H), 4.85 (d, *J* = 3.0 Hz, 1H), 4.08 (t, *J* = 6.4 Hz, 1H), 3.97 – 3.95 (m, 2H), 2.39 (dd, *J* = 12.6, 5.8 Hz, 1H), 2.32 (dd, *J* = 12.6, 5.8 Hz, 1H); ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.2, 144.3, 135.0, 132.0, 131.5 (2C), 131.2, 131.1, 127.0, 125.78, 125.75, 117.9, 115.1 (2C), 104.3, 71.5, 66.4, 61.6; IR υ_{max}/cm^{-1}: 3300 (OH), 2931 (N-CH_2), 2211 (CN), 1182 (C-O), 815 (C-Cl); LRMS (ESI$^+$) m/z: 441 ($C_{23}H_{15}D_{10}{}^{35}Cl_2N_2O_2$) [M+H]; HRMS: Exact mass calculated for $C_{23}H_{15}D_{10}Cl_2N_2O_2$ [M+H], 441.1922. Found 441.1925.

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Keywords: dichlorophenylacrylonitriles • aryl hydrocarbon receptor 2 • breast cancer • MTT assay 4 • SRB assay

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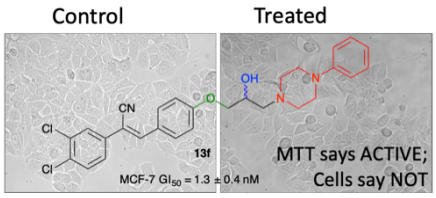

Microscopy examination reveals and unexpected interference in the MTT assay screening of a range of dichlorophenylacrylonitrles amino alcohols. SRB assays reveal significantly reduced cell viability.

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