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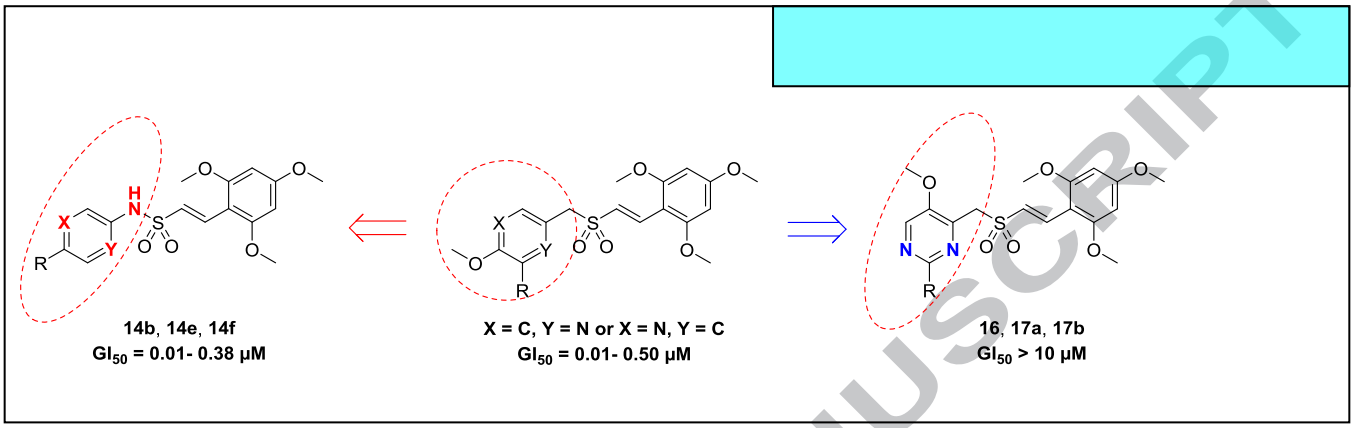
Graphical Abstract



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Synthesis and biological evaluation of heteroaryl styryl sulfone derivatives as anticancer agents

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ABSTRACT

Herein we disclose a series of novel heteroaryl styryl sulfone derivatives as potential anticancer agents. Structure-activity relationships of these newly synthesised compounds were explored with respect to the significance of the position and number of nitrogen atom of the heteroaryl ring for anti-proliferative activity in human cancer cell lines. A lead compound **14f** was tested against a panel of cancerous and untransformed cell lines, and found to be highly potent against cancer cells with minimal toxicity in the untransformed cells. Further mechanistic studies uncovered that **14f** caused cell-cycle arrest at the G2/M phase and induced apoptosis by targeting CDC25C and Mcl-1 proteins in A2780 ovarian cells.

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Rigosertib (ON01910.Na, **Fig. 1**) is a synthetic benzyl styryl sulfone that is currently in different phases of clinical trials for the treatment of myelodysplastic syndrome (MDS) and various other cancers.[1-5] It has shown efficacy with good tolerability through a range of dosing schedules by intravenous infusion.[6,7] However, it has poor oral bioavailability and ambiguous pharmacokinetics.[8] We have identified a new series of (*E*)-2/3-((styrylsulfonyl)methyl)pyridine derivatives as mechanistic mimetics of rigosertib. The lead drug candidates **8** and **18** (**Fig. 1**) showed potent anti-proliferative activity against various cancer cell lines and significant efficacy in xenografted tumour models.[9] Both compounds **8** and **18** possess superior cell permeability, metabolic stability and pharmacokinetic properties with good oral bioavailability to rigosertib.[9,10]

There are currently two plausible mechanisms of action proposed for rigosertib and its analogues. Firstly, rigosertib was found to target Polo-like kinase 1 (Plk1) in a substrate-dependent and ATP-independent manner.[11,12] Plk1 is an essential regulator of cell cycle progression as it regulates the activation of cyclin B1 and CDC25C phosphatase.[11,13] Secondly, rigosertib acts as a Ras mimetic. This is believed to be mediated by the binding of rigosertib to the Ras-binding domain (RBD) found in many Ras effector proteins, including the Raf and PI3K kinases.[14,15] As such, rigosertib can inhibit multiple Ras-driven signalling pathways. Previous data showed that rigosertib as well its mechanistic mimetics **8** and **18** gave rise to three major effects on cancer cells: (1) abnormal cell division containing irregular chromosomal segregation and cytokinesis; (2) mitotic (G2/M phase) arrest and apoptosis; and (3) reduced expression levels of CDC25C and cyclin D1.[11,16]

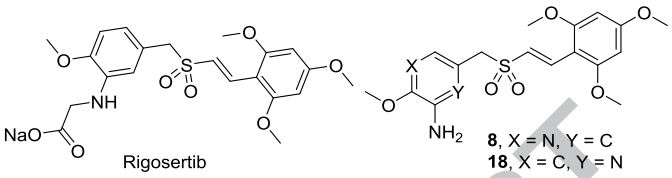
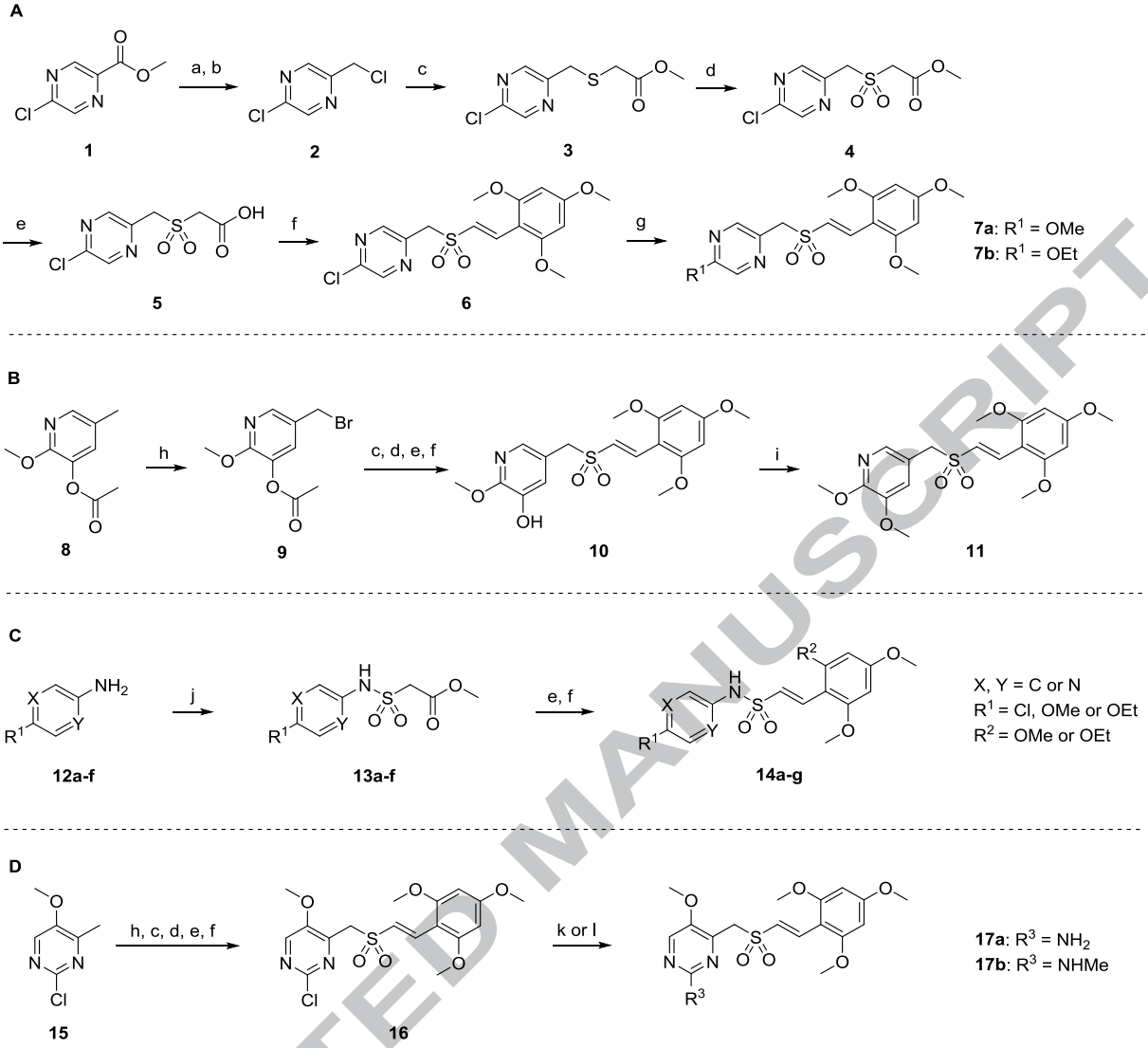


Figure 1. Structures of rigosertib and (*E*)-2/3-((styrylsulfonyl)methyl) pyridines **8** and **18**.

In an attempt to discover further novel series of styryl sulfones for potential drug development, we designed and synthesised a series of styrylsulfonyl derivatives with different *N*-containing heteroaryl systems. Introduction of additional nitrogen atom(s) into the methylpyridinyl moiety of molecules may result in not only favourable pharmacological properties, but also new intellectual property. Herein, we report the synthesis of these novel compounds, their SAR analysis with respect to the alterations of heteroaryl rings and the adjacent methylene group, and insights into the mechanism of action. These data provide valuable clues for further optimising this class as potential anticancer agents.



Scheme 1. General synthetic routes to (*E*)-2-(((2,4,6-trimethoxystyryl)sulfonyl)methyl)pyrazine derivatives (**A**), (*E*)-2,3-dimethoxy-5-(((2,4,6-trimethoxystyryl) sulfonyl)methyl)pyridine (**B**), (*E*)-*N*-(hetero)aryl-2-(2,4,6-trialkoxyphenyl)ethene-1-sulfonamide derivatives (**C**) and (*E*)-5-methoxy-4-(((2,4,6-trimethoxystyryl) sulfonyl)methyl)pyrimidine derivatives (**D**). Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C, o/n, 66%; (b) $SOCl_2$, DCM, rt, 1 h, 81%; (c) methyl thioglycolate, Na_2CO_3, MeOH, rt, 12 h, 62-87%; (d) H_2O_2, acetic acid, 60 °C, 12 h, 78-93%; (e) Na_2CO_3, H_2O/MeOH (1:1), rt, 12 h, 61-94%; (f) 2,4,6-trimethoxybenzaldehyde or 2-ethoxy-4,6-dimethoxybenzaldehyde, benzoic acid, piperidine, toluene, reflux, 3 h, 38-47%; (g) MeONa (or EtONa), MeOH (or EtOH), 90 °C, 2 h, 68-72%; (h) NBS, AIBN, CH_2Cl_2, reflux, 18 h, 36-47%; (i) MeI, K_2CO_3, DMF, rt, 12 h, 65%; (j) methyl 2-(chlorosulfonyl)acetate, Et_3N, DCM, rt, 3 h, 74-82%; (k) 32% aqueous ammonia, reflux, o/n, 10%; (l) $MeNH_2$·HCl, Et_3N, 2-methoxyethanol, microwave, 150 °C, 1 h, 79%.

The general synthetic route to designed derivatives is outlined in **Scheme 1**. The synthesis of **7** started with the reduction of ester **1**, and the subsequent chlorination gave 2-chloro-5-(chloromethyl)pyrazine **2**. Replacement of chlorine of the methyl group with methyl thioglycolate yielded thioether **3**, which was further oxidised to give sulfone **4**. After hydrolysis, Doebner modification of Knoevenagel condensation between **5** and 2,4,6-trimethoxybenzaldehyde was carried out to generate **6**, which was reacted with sodium alkoxide to yield **7a** and **7b**.

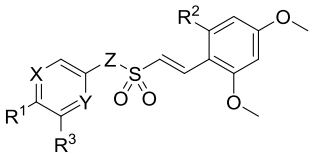
Above synthetic route was employed to prepare **10** and **16** following the bromination of the methyl group of **8** and **15** with *N*-bromosuccinimide. 3-Pyridinol **10** was methylated with iodomethane to give **11**; chloride **16** was reacted with aqueous ammonia or methylamine hydrochloride to produce **17a** and **17b** respectively. Preparation of **14a-g** started with the coupling of an appropriate amine **12** with methyl 2-(chlorosulfonyl)acetate to give the corresponding sulfonamide **13**, which was followed by saponification and condensation to yield **14**.

These newly synthesised molecules were tested for their anti-proliferative activity against two tumour cell lines derived from ovarian carcinoma A2780 and colorectal carcinoma HCT-116 using a 72 h MTT cell viability assay. Rigosertib served as a positive control, and the results are presented in **Table 1**.

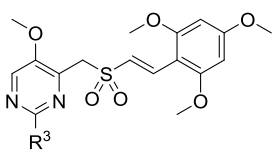
Pyrazine derivative **6** displayed moderate to weak anti-proliferative activity against A2780 and HCT-116 cells (GI_{50} = 4.76 μM and > 10 μM, respectively). Replacement of the bridging methylene between the pyrazine and sulfone moieties of **6** (Z = CH_2) with a secondary amino group (**14g**, Z = NH) abolished the activity (GI_{50} > 10 μM for both cell lines), while conversion of the chloride on **6** (R^1 = Cl) into an alkyloxy group (**7a**, R^1 = OMe; **7b**, R^1 = OEt) dramatically boosted the potency against both cell lines with the GI_{50} values in the range of 0.49-1.28 μM. Replacement of the pyrazine ring of **7a** (Y = N) with a pyridine ring (**11**, Y = CH) reduced the potency by approximately 2 folds against both cell lines. On the other hand, replacement of the R^3 of the comparator **8** (R^3 = NH_2) with methoxy

Table 1.

Anti-proliferative activity of the synthesised compounds against human tumour cancer cells



General Structure I: **6, 7a-b, 11, 14a-g**



General Structure II: **16, 17a-b**

Compd	Structure						72 h MTT growth inhibition, GI_{50} (μM)[a]	
	X	Y	Z	R^1	R^2	R^3	A2780	HCT-116
6	N	N	CH_2	Cl	OMe	H	4.76 ± 1.91	> 10
7a	N	N	CH_2	OMe	OMe	H	0.49 ± 0.01	0.95 ± 0.16
7b	N	N	CH_2	OEt	OMe	H	0.54 ± 0.12	1.28 ± 0.24
11	N	CH	CH_2	OMe	OMe	OMe	0.93 ± 0.11	2.81 ± 0.30
14a	N	CH	NH	Cl	OMe	H	2.60 ± 0.53	5.64 ± 0.68
14b	N	CH	NH	OMe	OMe	H	0.38 ± 0.04	1.00 ± 0.10
14c	N	CH	NH	OMe	OEt	H	0.47 ± 0.12	0.42 ± 0.07
14d	CH	CH	NH	Cl	OMe	H	> 10	> 10
14e	CH	CH	NH	OMe	OMe	H	0.01 ± 0.01	0.01 ± 0.01
14f	CH	CH	NH	OEt	OMe	H	0.31 ± 0.02	0.35 ± 0.03
14g	N	N	NH	Cl	OMe	H	> 10	> 10
16	-	-	-	-	-	Cl	> 10	> 10
17a	-	-	-	-	-	NH_2	> 10	> 10
17b	-	-	-	-	-	NHMe	7.54 ± 0.80	> 10
Rigosertib	CH	CH	CH_2	OMe	OMe	$NHCH_2COONa$	0.04 ± 0.01	0.06 ± 0.01
8	N	CH	CH_2	OMe	OMe	NH_2	0.01 ± 0.01	0.01 ± 0.01

[a] GI_{50} values are presented as mean ± standard deviation derived from at least two replicates.

(**11**, R^3 = OMe) significantly decreased anti-proliferative activity by ~100 folds, indicating the vital role of the amino group. These results indicate that the position and number of the heteroaryl nitrogen atom significantly affect the anti-proliferative activities, and that alkyloxy substitutions at R^1 position also contribute to the potency of pyrazine analogues. Surprisingly, all pyrimidine derivatives (**16**, **17a** and **17b**) were not active against both A2780 and HCT-116 cells regardless of the nature of substitutions on the pyrimidine ring (R^3 = Cl, NH_2 or NHMe).

(*E*)-*N*-Phenyl-2-(2,4,6-trimethoxyphenyl)ethene-1-sulfonamide and (*E*)-*N*-(pyridin-3-yl)-2-(2,4,6-trimethoxyphenyl)-ethene-1-sulfonamide series *i.e.* **14a**-**14f**, where Z = NH, show similar SAR profiles. **14a** (R^1 = Cl) exhibited moderate cytotoxicity (GI_{50} = 2.60 μM against A2780 and GI_{50} = 5.64 μM against HCT-116). Replacement of chloride with methoxy resulting in **14b** (R^1 = OMe) dramatically enhanced cellular potency with GI_{50} values of 0.38 μM (A2780) and 1.00 μM (HCT-116), which are approximately 5-7 folds more potent than **14a**. The potency was retained when the R^2 position of the styryl ring of **14b** (R^2 = OMe) was altered to the ethoxy (**14c**, R^2 = OEt), suggesting that the less bulky alkyloxy group is tolerated at R^2 position. Amongst all the heteroaryl styryl sulfones synthesised, aniline derivative **14e (**X = Y = CH, Z = NH, R^1 = OMe) displayed the highest potency with a GI_{50} value of 0.01 μM against both cell lines, and is 38-fold and 100-fold more potent against A2780 and HCT-116 cells respectively than its pyridinyl counterpart **14b**, suggesting the favorable carbon atom over nitrogen atom at the X position. As expected, the corresponding ethoxy molecule **14f** had a slightly reduced activity (GI_{50} = 0.31 μM against A2780 and GI_{50} = 0.35 μM against HCT-116)

Table 2.

Anti-proliferative activity of **14f** against a panel of human cell lines

Human cell line		72 h MTT assay, GI_{50} (μM)[a]
Origin	Destinatio	**14f**
Breast	MCF-7	0.53 ± 0.05
Colon	HCT-116	0.35 ± 0.03
	HT-29	0.58 ± 0.02
	Colo 205	0.59 ± 0.03
	KM12	0.34 ± 0.00
Ovarian	A2780	0.31 ± 0.02
Pancreatic	PANC-1	0.30 ± 0.01
Prostate	PC3	0.60 ± 0.01
	LNCAP	0.52 ± 0.15
	C4-2B	0.45 ± 0.01
	22-RV1	0.53 ± 0.03
	DU145	0.52 ± 0.16
Medulloblastoma	DAOY	0.34 ± 0.01
Untransformed	WI-38	> 10
	MRC-5	> 10

[a] GI_{50} values are presented as mean ± standard deviation derived from at least two replicates.

whereas the chloride counterpart **14d** (**R**^{1} = Cl) was inactive (GI_{50} > 10 μM for both cell lines).

Taken together, alkyloxy substitutions at R^1 position of pyrazine, pyridinylamine and aniline derivatives significantly improved toxicity against A2780 and HCT-116 cells. The position and number of the nitrogen atom in the heterocyclic ring play an essential role in the cytotoxicity.

To further assess anti-proliferative activities and cellular mode of action, **14f**, one of the most active compounds, was tested against a panel of 12 human cancer cell lines and 2 untransformed cell lines. The results are summarised in **Table 2**. **14f** exhibited potent activity against all cancer cell lines with GI_{50} values ranging from 0.30 to 0.60 μM. It was particularly active against pancreatic cancer cells (PANC-1) and ovarian cancer cells (A2780) with a GI_{50} value of 0.30 and 0.31 μM respectively. The GI_{50} values of **14f** for untransformed cell lines (WI-38 and MRC-5) were greater than 10 μM, showing high selectivity of this compound against cancer cell lines over normal cell lines.

In order to examine whether the anti-proliferative effects of **14f** were a consequence of inhibition of cell-cycle progression, A2780 cells were exposed to **14f** (or rigosertib) for 24 h at the concentrations of GI_{50} and 5×GI_{50} μM and analysed by flow cytometry. As shown in **Fig. 2**, significant disturbance of cell-cycle progression was observed. **14f** caused a marked increase in the population of G2/M-phase cells and a large accumulation of sub-G1-phase cells at 5×GI_{50} concentration, which was companied by a substantial loss of G1-phase cells and a minor decrease of S-phase cells. Rigosertib showed similar cell-cycle effects, but to a lesser extent.

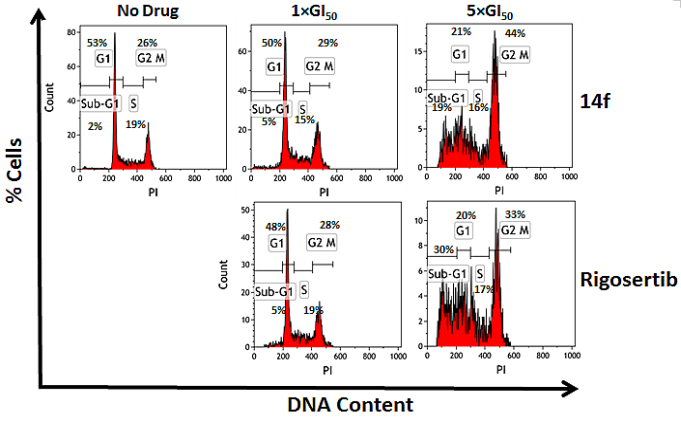


Figure 2. Cell cycle analysis of A2780 cells after treatment with **14f** or rigosertib for 24 h.

Induction of apoptosis was further confirmed using annexin V/propidium iodide (PI) staining assay following treatment of A2780 cells with **14f** or rigosertib for 24 h. As illustrated in **Fig. 3**, both compounds induced apoptotic cell death (annexin V+/PI- and annexin V+/PI+) in comparison with the control. Again, **14f** was at least equal or more effective in inducing apoptosis compared to rigosertib.

Given that **14f** caused G2/M arrest and induced apoptosis, the cellular mechanism of action was further evaluated using Western blotting. CDC25C is an essential substrate of Plk1, and

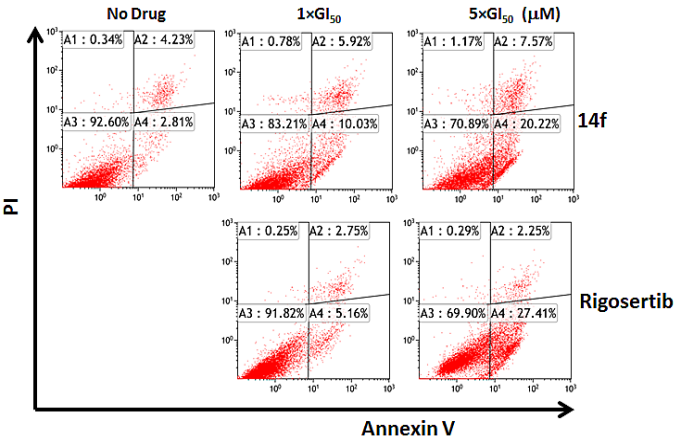


Figure 3. Induction of apoptosis of **14f** in A2780 cells after treatment with **14f** (or rigosertib) for 24 h by annexin V/PI assay.

phosphorylated CDC25C directs dephosphorylation of CDC2/cyclin B1 complex, leading to entry into mitosis.[17,18] We have previously reported that (*E*)-2/3-((styrylsulfonyl)methyl) pyridine derivatives **8** and **18** inhibited CDC25C, and that there was a good correlation between the level of CDC25C and growth inhibition of cancer cells treated with these compounds.[9] As shown in **Fig. 4**, the level of p-CDC25C was significantly reduced by **14f**, confirming its cellular inhibition of Plk1. The reduction in CDC25C expression might be modulated by CHK2 and p53 activities due to Plk1 inhibition.[19,20] These two proteins can promote the down-regulation of CDC25C.[21,22] Significantly, **14f** was capable of down-regulating anti-apoptotic protein myeloid cell leukaemia 1 (Mcl-1) and of causing cleavage of poly (ADP-ribose) polymerase (PARP), both of which are associated with the apoptosis observed in A2780 cells. These results clearly demonstrated the mode of apoptotic cell death induced by **14f**. Rigosertib exerted similar effects on these proteins.

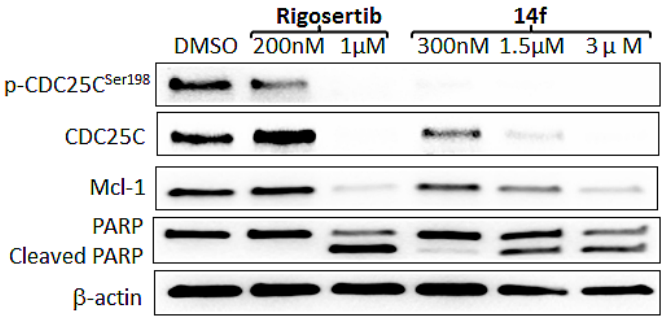


Figure 4. Cellular mode of action investigation of **14f** in A2780 cells after treatment with **14f** for 24 h by western blot analysis.

In summary, we have identified a series of new *N*-containing heteroaryl styryl sulfone derivatives and their SARs were analysed. We have demonstrated that the position and number of heteroaryl nitrogen atom influenced their anti-proliferative activities. **14f** exhibited high potency and was selectively cytotoxic against human cancer cells with minimal effects on untransformed cells. Cellular mechanistic studies showed that **14f** arrested cells at the G2/M phase, down-regulated Mcl-1 and caused PARP cleavage, thus inducing apoptosis in cancer cells. This work offers a valuable direction for further modification on these heteroaryl styryl sulfone scaffolds for potential drug development.

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Supplementary Data

Supplementary data associated with this article can be found in the online version.

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