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Synthesis and biological evaluation of benzyl styrylsulfonyl derivatives as potent anticancer mitotic inhibitors

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ABSTRACT

We herein report the synthesis, biological activity and structure activity relationship of derivatives of benzylstyrylsulfone, benzylstyrylsulfine and benzylsulfonyl-N-phenylacetamide.. A lead compound **7** represents a new class of mitotic inhibitors that demonstrates potent anti-proliferative activity and selectively induces cancer cell apoptosis while sparing non-transformed lung fibroblast.

Sodium (E)-{N-[2-methyloxy-5-(2,4,6-trimethoxy-styryl sulfonyl)methylenephenyl]amino}acetate (ON 01910.Na, Onconova Therapeutics Inc., Fig. 1) is a novel anticancer agent currently in phase I clinical trials in patients. ON 01910 is a cell-cycle inhibitor and selectively causes mitotic arrest by creating spindle abnormalities and abnormal centrosome localization and fragmentation leading to apoptosis in cancer cells. It has been shown to inhibit PLK1 pathway activity at a nanomolar range in a substrate-dependent and ATP-independent manner, although targeting other kinases has also been reported.[1],[2] This compound inhibits a broad spectrum of human tumour cells growth with GI$_{50}$ values in the nanomolar range and is active in a number of human xenografts in mice[3,4]. Currently, the drug is in several phase I and II clinical trials in adult patients with a variety of solid tumours as well as hematological malignancies.[5-8] Anti-tumour activity was observed in all phase I trials. Recent phase I studies in human B-cell chronic lymphocytic leukaemia (CLL) demonstrated that ON 01910.Na selectively induced apoptosis in all CLL samples tested and reduced PLK1 activity in the leukaemic cells .[3] ON 01910.Na is currently also being tested in phase I combination therapy in patients with solid tumors.

Our efforts to develop small molecule cell-cycle inhibitors for cancer therapy has resulted in the development of several classes of CDK and Aurora kinase inhibitors.[9-13] In the process of designing novel class PLK1 inhibitors we prepared and evaluated the biological activity of ON 01910 and several derivatives. The structure activity relationship established has provided guidance for us to rapidly progress our drug discovery programme. Here we report the synthesis and biological evaluation of analogue benzylstyrylsulfones, benzylstyrylsulfines and benzylsulfonyl-*N*-phenylacetamides (Type-I, -II and -III, Fig. 1). This study suggests that the benzylstyrylsulfinyl chemotype offers great potential for development of anti-cancer agents.

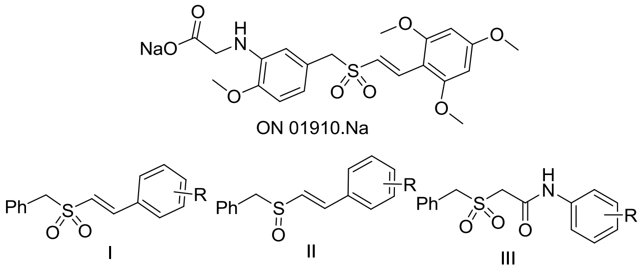

Fig. 1 Structures of ON 01910.Na and the designated derivatives

The synthetic chemistry employed to prepare type I-III compounds is outlined in Scheme 1. The synthesis started from 2-(4-methoxy-3-nitrobenzylthio)acetic acid **1**, which can be obtained by halogenation of 1-methoxy-4-methyl-2-nitrobenzene followed by treatment with 2-mercaptoacetic acid.[14,15] 2-(4-Methoxy-3-nitrobenzylsulfonyl)acetic acid **2** or 2-(4-methoxy-3-nitrobenzylsulfinyl)acetic acid **5** were obtained by

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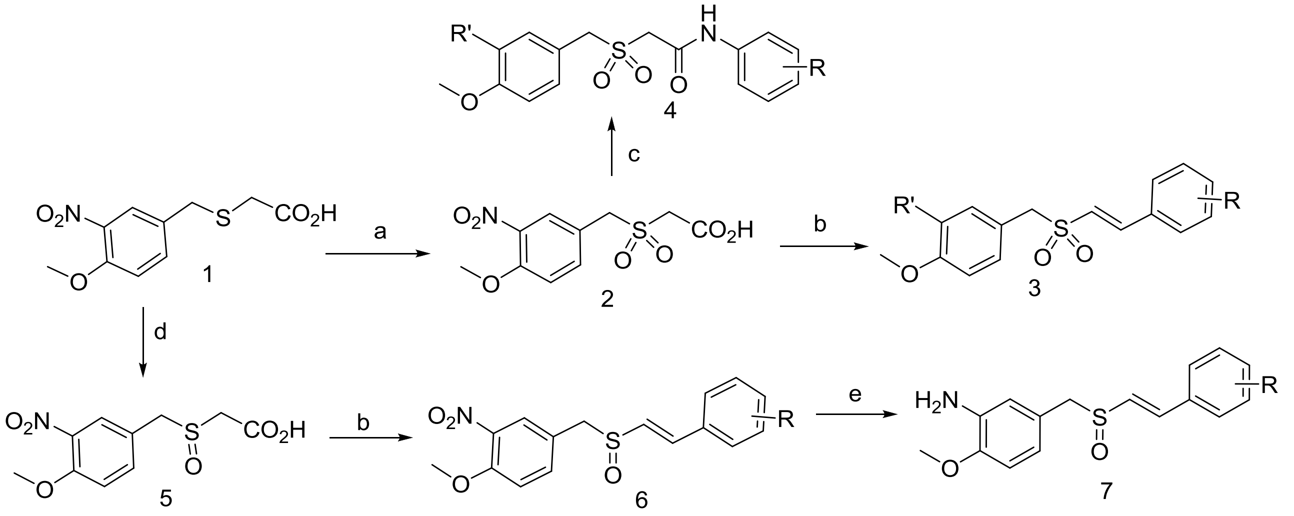
chemoselective oxidation of **1**. Doebner modification of Knoevenagel condensation[16] between **2** or **5** with substituted aromatic aldehyde in pyridine and catalytic amounts of piperidine yielded the corresponding (E)-1-methoxy-2-nitro-4-(styrylsulfonylmethyl)benzene **3a**, **3b**, **3c** and **3d** (R'=NO_2, Scheme 1 and Table 1) or (E)-1-methoxy-2-nitro-4-(styrylsulfinylmethyl)benzene **6** (R=2,4,6-trimethoxyl**)**. Reduction of **3a**, **3b**, **3c** or **3d,** as well as **6** resulted in their respective anilino derivatives **3e**, **3f**, **3g**, **3h** and **7**. Acylation of **3e** (R'=NH_2, R=2,4,6-$(OMe)_3$) with acylchloride afforded **3i** (R'=NHCOMe, R=2,4,6-$(OMe)_3$, Table 1). Treatment of **3e** with ethyl 2-bromoacetate in the presence of sodium acetate followed by hydrolysis in aqueous sodium carbonate yielded **3j** (R'=$NHCH_2COOH$, R=2,4,6-$(OMe)_3$, e.g. ON 01910).

To prepare chemotype-III sulfonyl-*N*-phenyl acetamides (Fig. 1) 2-(4-methoxy-3-nitrobenzylsulfonyl)acetic acid **2** was converted to 2-(4-methoxy-3-nitrobenzylsulfonyl)acetyl chloride. The later, treated with various substituted anilines, resulted in **4a,** **4b**, **4c**, **4d** and **4e**. Reduction of **4a** and **4b** in the presence of tin chloride generated **4f** and **4g** respectively.



Scheme 1. Reagents and conditions: (a) H_2O_2, AcOH, 50 ^{o}C, 6 hr, 98 %; (b) Aromatic aldehyde, cat. piperidine, pyridine, rt, 36 hr, 19 – 50%; (c) i. Sulfurous dichloride, EtOAc, reflux, 40 min, 100 %; ii. Aniline, anhydrous THF, reflux, 3 hr, 80-91%; (d) H_2O_2, AcOH, 0 ^{o}C, 6 hr, 95 %; (e) Fe^0, AcOH/MeOH (1:2), reflux, 3 hr, 95-96 %

Table 1

Structure and growth inhibitory activity of selected compounds against human tumour cancer cells

Compd	Structure		Cytotoxicity, 72h MTT (GI_{50}, μM)[a]				
	R	R'	T47D	MDA-468	HCT-116	MCF-7	MRC-5
3a	2,4,6-$(OMe)_3$	NO_2	NT	NT	1.31	1.75	5.81
3b	2,3,4-$(OMe)_3$	NO_2	> 30	21.89	27.85	> 30	> 30
3c	2,6-$(OMe)_2$	NO_2	> 30	5.46	10.22	13.71	> 30
3d	3,5-$(OMe)_2$	NO_2	> 30	16.46	> 30	> 30	> 30
3e	2,4,6-$(OMe)_3$	NH_2	NT	0.03	< 0.01	< 0.01	0.08
3f	2,3,4-$(OMe)_3$	NH_2	5.90	3.48	5.31	5.95	> 30
3g	2,6-$(OMe)_2$	NH_2	0.05	0.06	0.02	0.04	17.09
3h	3,5-$(OMe)_2$	NH_2	0.53	0.52	0.54	0.49	> 30
3i	2,4,6-$(OMe)_3$	NHCOMe	NT	NT	0.15	0.35	0.81
3j	2,4,6-$(OMe)_3$	$NHCH_2CO_2H$	< 0.01	0.02	0.05	0.05	0.71
4a	H	NO_2	> 30	> 30	> 30	> 30	> 30
4b	2,4,6-$(Me)_3$	NO_2	> 30	> 30	7.91	> 30	27.96
4c	2,4,6-$(OMe)_3$	NO_2	> 30	> 30	18.80	> 30	> 30
4d	2,5-$(OMe)_2$	NO_2	> 30	> 30	> 30	> 30	> 30
4e	NO_2	NO_2	> 30	> 30	> 30	> 30	> 30
4f	H	NH_2	> 30	> 30	> 30	> 30	> 30
4g	2,4,6-$(OMe)_3$	NH_2	> 30	> 30	> 30	> 30	> 30
7	2,4,6-$(OMe)_3$	-	0.06	< 0.01	< 0.01	< 0.01	> 30

[a] Values are means of at least three independent determinations. NT: not tested

Anti-proliferative activity was assessed against colorectal carcinoma HCT-116, breast carcinoma MCF-7, MDA-468, MDA-231 and T74D using a standard 72-h MTT cytotoxicity assay.[17] The compounds were also tested against non-transformed lung fibroblast MRC-5. The results are summarized in Table 1.

Most (E)-2-methoxy-5-(styrylsulfonylmethyl)anilines, particularly **3e, 3g, 3h, 3i** and **3j,** exhibited potent anti-proliferative activity with GI$_{50}$ < 1 μM in cancer cells, except compound **3f** (R=2,3,4-(OMe)$_3$) which has only modest activity (GI$_{50}$ < 5 μM). **3e** and **3j** (ON 01910) were the most potent anti-proliferative agents with GI$_{50}$ below 0.1 μM; the 2,4,6-trimethoxy substituted styryl moiety seems important for the optimum potency observed. Interestingly, MRC-5 non-transformed cells appeared insensitive towards **3g** and **3h**, being < 280- and < 80-fold less cytotoxic compared with the cancer cells tested. Replacement of the amino group on benzyl moiety in **3f, 3g** and **3h** with nitro group resulting in corresponding analogue **3b, 3c** and **3d** abolished the activity. Compound **3a** which contained the favourable 2,4,6-trimethoxylstyryl moiety gained some degrees of activity compared to its analogues **3b-3d**. Replacement of the sulfonyl in **3e** with sulfinyl afforded (E)-2-methoxy-5-((2,4,6-trimethoxystyrylsulfinyl)methyl)aniline **7** which showed the excellent anti-tumour activity, being comparable to **3e**. Significantly, compound **7** selectively killed cancer cells while sparing non-transformed MRC-5 cells. Replacement of styrylsulfonyl with sulfonyl-*N*-phenylacetamide was poorly tolerated and compounds **4a-4g** were completely inactive in the assay

The established structure activity relationship is shown schematically in Fig. 2. The styrylbenzylsulfonyl or styrylbenzylsulfinyl moiety is essential for potency, replacement with sulfonyl-*N*-phenylacetamide showing a dramatic loss in activity. Substitutions on the styryl ring system were generally found to be tolerated, although 2,4,6-trimethoxyl was the most favourable function. Substitutions with electron donating group on the benzyl ring system are expected to be amenable to optimization. In addition to amines, hydroxyl, thiol or their ethereal function should result in a more highly potent compound.

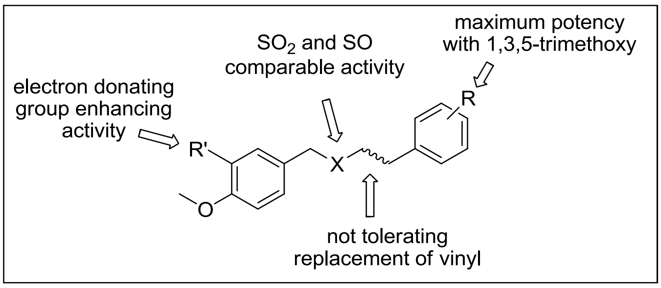


Fig. 2. Summary of structure activity relationship

The primary cellular mode of action of **7** was investigated when compared with **3j**. The time-dependent growth inhibitory activity was examined in MDA468 cells. As shown in Table 2, both compounds exhibited comparable cytotoxicity and increased potency with extended time. We next examined the cell-cycle effects.[18] Analyses by flow cytometry exposed severe perturbation of cell-cycle progression following treatment of cells with ≥ 0.5 μM of **3j** or **7** (Fig. 3). 20 h post treatment of MDA-468 cells with **3j** at 0.5 μM (GI$_{50}$) and 1 μM (2xGI$_{50}$) resulted in accumulation of G2/M events – 58% and 84% respectively. This was consistent with ON 01910 mechanism of action described previously.[15,3] Compound **7** demonstrated a similar cell-cycle profile; treatment of the cells with **7** at 0.5 μM (GI$_{50}$) and 1.0 μM (2xGI$_{50}$) causing 77 % and 85 % cells with G2/M DNA content respectively.

Induction of apoptosis by the compounds was further analyzed by annexinV/PI double staining[18] in MDA-468 cells following treatment with either **3j** or **7** at 0.5 μM, 1 μM and 10 μM for a period of 20 hr. Both **3j** and **7** induced significant numbers of apoptotic cells, effectively starting from 0.5 μM in a dose-dependent manner (Fig. 4).

Table 2
Time course MTT assay in MDA-468 cells

Time (hr)	Cytotoxicy, MTT (GI$_{50}$, μM)[a]	
	3j	**7**
24	0.601 ± 0.065	0.559 ± 0.095
48	0.302 ± 0.021	0.137 ± 0.023
72	0.014 ± 0.003	< 0.01

[a] Represent as the mean of three independent assay ± s.d

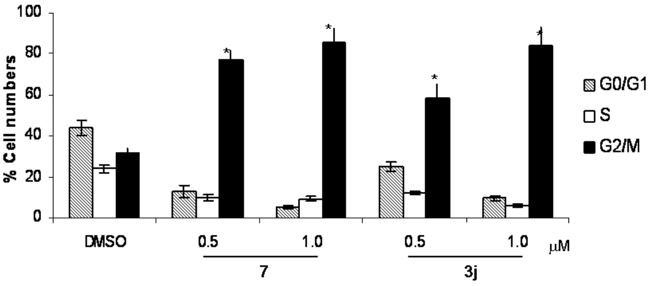


Figure 3. Cell cycle analysis of MDA-468 cells following treatment with compound **3j** or **7** for a period of 20 hr at the concentrations shown. Vertical bars represent the mean ± s.d. of three independent experiments. Values significantly ($p < 0.05$) different from DMSO vehicle control are marked with an asterisk (*).

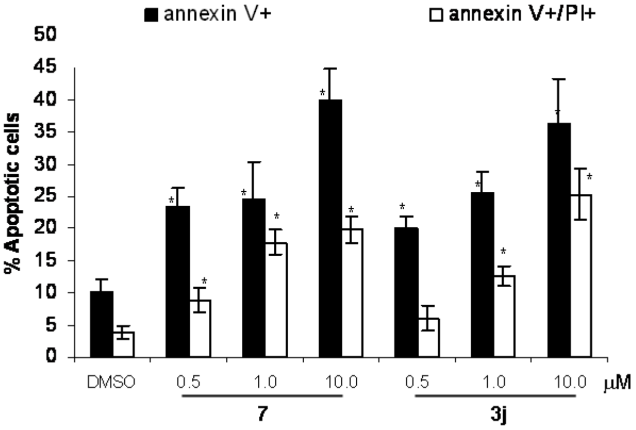


Figure 4. Apoptosis of MDA-468 cells following treatment with **3j** or **7** for 20 hr at concentrations indicated. The percentage of cells undergoing apoptosis was defined as the sum of early apoptosis (annexin V-positive cells) and late apoptosis (annexin V-positive and PI-positive cells). Vertical bars represent the mean ± s.d. of three independent experiments. Values significantly ($p < 0.05$) different from DMSO vehicle control are marked with an asterisk (*).

In conclusion, a series of benzylstyrylsulfonyl derivatives and benzylsulfonyl-*N*-phenylacetamides were prepared[19] and the structure activity relationships were established. (E)-2-methoxy-5-((2,4,6-trimethoxystyrylsulfinyl)methyl)aniline **7** possessed potent anti-proliferative activity against cancer cell lines, being comparable to **3j**. Compound **7** showed similar cell-cycle effects to **3j** and was capable of inducing cancer cells to apoptosis.

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18. Cell cycle analysis: Exponentially growing cells were seeded at a density of 4×10^5 and incubated at 37 ^{o}C in a humidified, 5 % CO_2 atmosphere overnight. Following 20h incubation with compound at appropriate concentrations, the cells were collected. Cell pellets were washed once with cold PBS and re-suspended in 0.4 ml hypotonic fluorochrome solution. Cell cycle status was analysed using a Beckman Coulter EPICS-XL MCL^{TM} flow cytometer and data analyzed using EXPO32^{TM} software.
    AnnexinV/propidium iodide (PI) staining: was used to quantitatively determine the percentage of apoptotic cells. Cells (4 x 10^5) per well were treated with compounds after overnight culture. Sample preparation, staining, and analysis were performed following the protocol provided by BD (BD Bioscience).
19. Synthesis of lead compound **7**: 2-(4-Methoxy-3-nitrobenzylthio) acetic acid (**5**): To a solution of 2-mercaptoacetic acid (0.35 mL, 5 mmol) in 100 mL of methanol, sodium carbonate (0.40 g) and 4-(bromomethyl)-1-methoxy-2-nitrobenzene (0.62 g, 2.5 mmol) was added, and the mixture was refluxed for 1 hr, cooled, and spilled over ice (300 g). The pH was adjusted to 3 by addition of 2N HCl aq. to give a yellow precipitate. Recrystallisation from Pet/EtOAc afforded **5** as pale yellow crystals (0.56 g, 88 % yield). mp 128 - 131^{o}C; ^{1}H-NMR (DMSO-D6) δ 3.14 (s, 2H, CH_2), 3.83 (s, 2H, CH_2), 3.91 (s, 3H, OCH_3) 7.33 (d, J = 8.4 Hz, 1H, Ph-H), 7.61 (dd, J = 8.4, 2.0 Hz, 1H, Ph-H), 7.83 (d, J = 2.0 Hz, 1H, Ph-H), 12.62 (s, 1H, CO_2H). ^{13}C-NMR (DMSO-D_6) δ 33.04, 34.39, 57.15, 114.86, 125.58, 130.97, 135.45, 139.29, 151.54, 171.65. HRMS (ESI$^-$) *m/z* 256.0065 [M-1]$^-$, $C_{10}H_{11}NO_5S$ requires 257.0358.
    2-(4-Methoxy-3-nitrobenzylsulfinyl) acetic acid (**6**): Solution of 2-(4-methoxy-3-nitrobenzylthio) acetic acid (0.26 g, 1 mmol) in 20 mL of acetic acid was cooled to 0 ^{o}C on an ice bath. Hydrogen peroxide 35 % w/v (0.15 mL, 1.5 mmol) was added and the mixture was stirred at 0 ^{o}C for 6 hrs. After concentration the reaction mixture was purified by flash chromatography using EtOAc to yield **6** as a pale yellow solid (0.26 g, 95 % yield); mp 67 - 68 ^{o}C; ^{1}H-NMR (DMSO-D_6) δ 3.52 (d, J = 14.4 Hz, 1H, CH_2), 3.88 (d, J = 14.4 Hz, 1H, CH_2), 3.94 (s, 3H, OCH_3), 4.11 (d, J = 12.8 Hz, 1H, CH2), 4.28 (d, J = 12.8 Hz, 1H, CH2), 7.40 (d, J = 8.8 Hz, 1H, Ph-H), 7.61 (dd, J = 8.8, 2.17 Hz, 1H, Ph-H), 7.85 (d, J = 2.17 Hz, 1H, Ph-H). ^{13}C-NMR (DMSO-D_6) δ 55.12, 55.82, 57.24, 115.02, 123.69, 127.08, 136.97, 139.33, 152.35, 167.91. HRMS (ESI$^-$) *m/z* 272.0130 [M -1]$^-$, $C_{10}H_{11}NO_6S$ requires 273.0307.
    (E)-2-Methoxy-5-((2,4,6-trimethoxystyrylsulfinyl)methyl)aniline (**7**): 2-(4-methoxy-3-nitrobenzylsulfinyl)acetic acid (0.55 g, 2 mmol) and 2,4,6-trimethoxybenzaldehyde (0.49 g, 2.5 mmol) were dissolved in mixture of anhydrous pyridine (10 mL) and anhydrous Piperidine (few drops). After stirring at rt for 36 hr the mixture was evaporated to give gummy brown residue which was dissolved in ethyl acetate (20 mL) and washed with 2N NaOH aq. (10 mL), 2N HCl aq. (10 mL), and distilled water (10 mL). After being dried over anhydrous $MgSO_4$, the organic solution was evaporated to afford a mixture of (E)-1,3,5-trimethoxy-2-(2-(4-methoxy-3nitrobenzylsulfinyl)vinyl)benzene. Without further purification the compound was dissolved in hot methanol (10 mL) and treated with Iron powder (10 mmol) in acetic acid (5 mL). After refluxing for 3 hr, the mixture was treated with ammonia solution (2N aq.) and extracted with EtOAc (20 mL). The organic layer was concentrated and purified by flash chromatography (EtOAc) to give **7** as a pale brown solid (0.30 g, 40 % yield); mp 117 – 119 ^{o}C; ^{1}H-NMR (Acetone-D_6) δ 3.16 (s, 3H, OCH_3), 3.85 (s, 2H, CH_2), 3.87 (s, 3H, OCH_3), 3.90 (s, 6H, OCH_3x2), 6.29 (s, 2H, Ph-H), 6.61(dd, J = 8.0, 2.0 Hz, 1H, Ph-H), 6.73 (d, J = 2.0 Hz, 1H, Ph-H), 6.80 (d, J = 8.0 Hz, 1H, Ph-H), 7.26 (d, J = 15.6 Hz, 1H, CH), 7.45 (d, J = 15.6 Hz, 1H, CH). ^{13}C-NMR (Acetone-D_6) δ 54.90, 54.99, 55.31, 61.28, 90.61, 105.50, 110.17, 115.84, 118.91, 123.50, 125.30, 131.25, 137.80, 147.50, 160.34, 162.50. HRMS (ESI$^+$) *m/z* 377.9233 [M+1]$^+$, $C_{19}H_{23}NO_5S$ requires 377.1297. Anal. RP-HPLC (Kromasil C_{18} column, 250 x 4.6 mm, H_2O/CH_3CN containing 0.3 % CF_3COOH): t_R = 2.4 min, purity > 99 %.