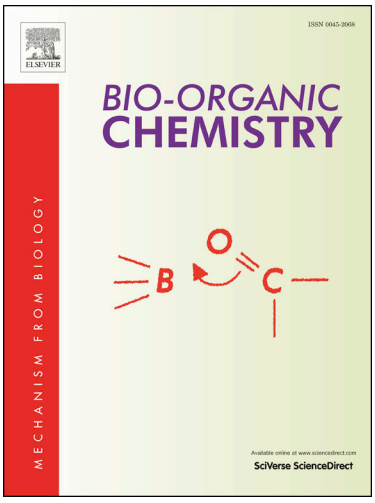
Journal Pre-proofs



Mechanistic investigations on substituted benzene sulphonamides as apoptosis inducing anticancer agents

Akhila Mettu, Venu Talla, Soujanya Thumma, Subhashini Naikal James Prameela

PII: S0045-2068(19)31166-6
DOI: https://doi.org/10.1016/j.bioorg.2019.103539
Reference: YBIOO 103539

To appear in: *Bioorganic Chemistry*

Received Date: 22 July 2019
Revised Date: 27 November 2019
Accepted Date: 21 December 2019

Please cite this article as: A. Mettu, V. Talla, S. Thumma, S. Naikal James Prameela, Mechanistic investigations on substituted benzene sulphonamides as apoptosis inducing anticancer agents, *Bioorganic Chemistry* (2019), doi: https://doi.org/10.1016/j.bioorg.2019.103539

Mechanistic investigations on substituted benzene sulphonamides as apoptosis inducing anticancer agents

Akhila Mettu,[a,c] Venu Talla,[b] Soujanya Thumma,[a] Subhashini Naikal James Prameela,*[c]

[a]Department of Pharmaceutical Chemistry, Gokaraju Rangaraju College of Pharmacy, Kukatpally, Hyderabad, India 500049.

[b]Department of Pharmacology, National Institute of Pharmaceutical Education and Research, Balanagar, Hyderabad, India 500037.

[c]Department of Chemistry, University College of Science, Osmania University, Hyderabad, India 500007.
Email: njsubhashini@yahoo.co.in

ABSTRACT

In an approach to develop potent cytotoxic compounds with targeted action, a systematic methodology was employed to design and initially synthesize parent compounds **A1, A8, A13** and **A14** followed by synthesis of further analogs of **A1** (**A2-A7**) and **A8** (**A9-A12**) with characterization by IR, NMR, mass and elemental techniques. These compounds were evaluated for their *in vitro* anti-proliferative activities against DU-145, MCF-7, HCT-15, HT-29 cell lines and apoptosis inducing potential via various mechanistic studies. Compounds **A2, A9, A10** exhibited significant cytotoxic activities compared to their parent compounds and standard drug 5-fluorouracil. Compound **A2** displayed superior cytotoxicity with IC_{50} values less than 1 μM in most of the tested cell lines. Further, compound **A2** also induced apoptosis in DU-145 cells as exemplified from DAPI staining, Annexin V-FITC assay, ROS generation and mitochondrial membrane alteration studies. The above studies depict the synthesized compound **A2** as potent anticancer agent with the ability to induce apoptosis in prostate cancerous cells.

Keywords: Benzene sulphonamide; Apoptosis; ROS; Anticancer; Annexin V.

INTRODUCTION

Research on cancer remains a continuous strive to unveil the novel drugs possessing targeted action with minimal side effects [1]. Generation of a tumor involves activation/deactivation of numerous pathways and proteins involved in cell growth and regulation [2,3]. Design and development of novel drugs for cancer therapy involves activation of proapoptotic factors and inhibition of antiapoptotic factors [4,5]. The various modes of cancerous cell death include the activation of intrinsic and extrinsic pathways of apoptosis, followed by release of caspase-3, release of cytochrome-c following mitochondrial stress, ROS mediated cell death and so on [6-9].

Pyrimidine is a renowned endogenous metabolite and its analogs have various therapeutic roles including anticancer action [10]. 5-Fluoro uracil, Tegafar are renowned pyrimidine antimetabolites for cancer therapy [11]. Various fused pyrimidines and aryl pyrimidines are reported in literature for their prominent anticancer activities [12-15]. Benzene sulphonamides are reported to have anticancer activity exhibited through multiple mechanisms, the most prominent ones being carbonic anhydrase inhibition and apoptotic induction [16,17]. Indisulam, SLC-0111 are the sulphonamide analogs possessing anticancer activity with induction of apoptosis [18,19]. Literature reports thioureido benzene sulphonamides [20], thiazolidone benzene sulphonamides [21], 7-chloro quinoline benzene sulphonamides [22], guanidinyl benzene sulphonamides [23] as anticancer compounds exhibited through various mechanisms.

Owing to the crucial role of pyrimidine analogs as antimetabolites and sulphonamide moiety as an essential pharmacophoric group in multiple anticancer drugs [19,24,25], we attempted to link substituted pyrimidine/ tetrahydro quinazoline with benzene sulphonamide and initially synthesized a set of four variously substituted benzene sulphonamide derivatives as parent compounds (**A1, A8, A13** and **A14**) employing four different schemes, and thereby evaluated for anticancer activity (Fig. 1). Subsequent analogs were synthesized from **A1** (**A2-A7)** and **A8** (**A9-A12**) and mechanistically investigated for their apoptotic potentials.

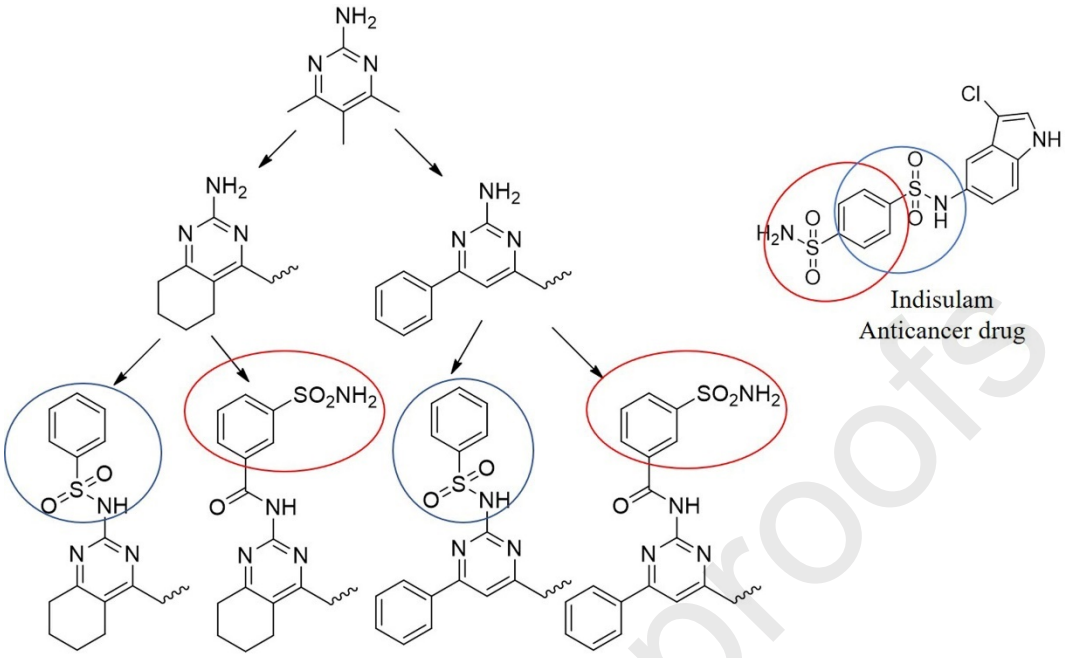


Fig. 1. Design of variously substituted benzene sulphonamides

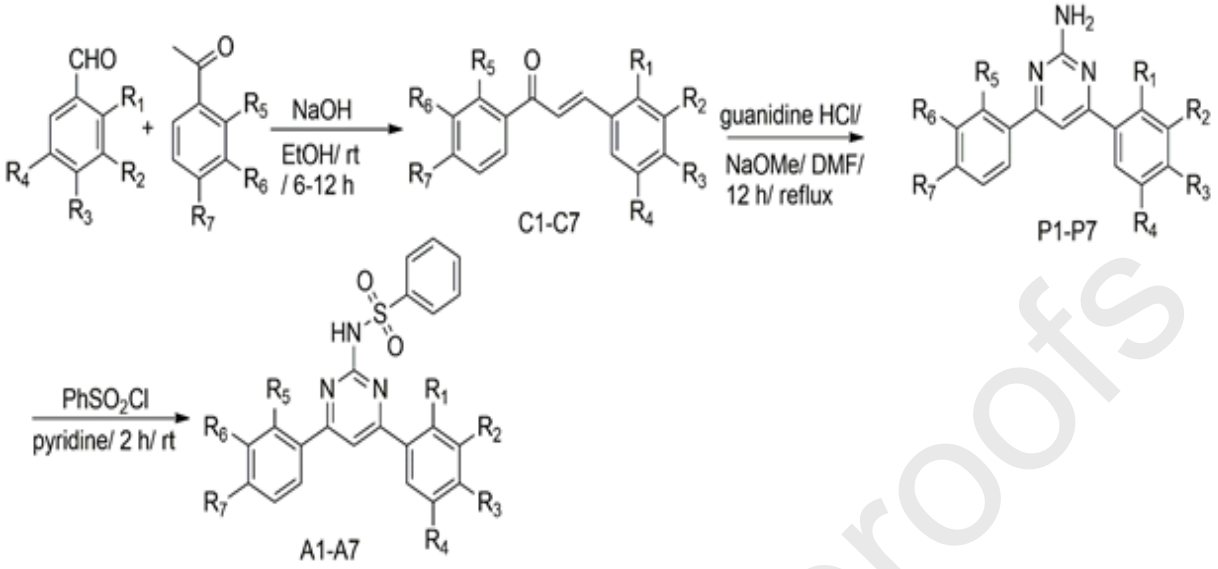
2. RESULTS AND DISCUSSION

2.1. Chemistry

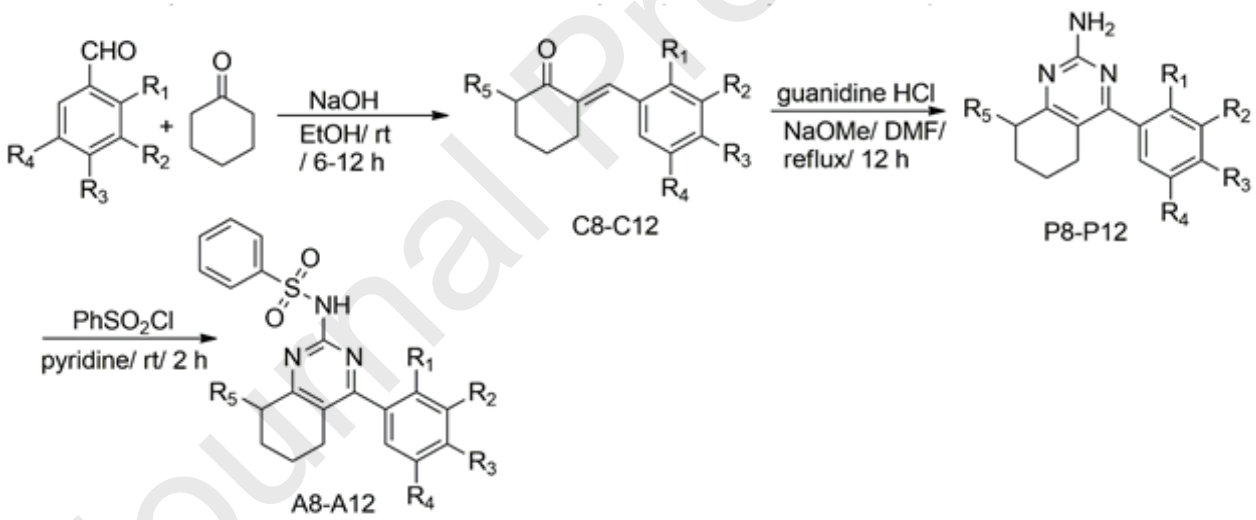
The scaffold was designed based on the indisulam anticancer drug. Two variants of pyrimidines were synthesized initially, one with attached aromatic groups and the other fused with cyclohexene. Then, the sulphonamide group was attached in two different ways, one substituted on the pyrimidine ring as sulphomoyl group (**A1-A12**) and other on the benzene ring as terminal sulphonamide (**A13, A14).** Overall, four variants of analogs were synthesized employing two common synthetic routes (Fig. 1).

Claisen-Schmidt condensation of substituted benzaldehydes with substituted acetophenones /cyclohexanone in 40% aq. NaOH and methanol afforded chalcones **C1-C12**. Reaction with cyclohexanone yielded mixture of products generating mono and di substituted alkenes. The required compound was separated by column chromatography. The IR spectrum showed the characteristic (C=O) peaks between 1680-1730 cm^{-1} and (C=C) peaks at 1600 to 1650 cm^{-1} along with molecular ion peaks [M^+] in mass spectrum. The chalcones were converted to 2-amino pyrimidines **P1-P12** by refluxing guanidine hydrochloride in DMF with sodium ethoxide as base. The IR spectra of compounds showed the presence of N-H, C=C, C=N, C-N stretching around 3300-3400 cm^{-1}, 1600-1650 cm^{-1}, 1690-1640 cm^{-1}, 1300-1350

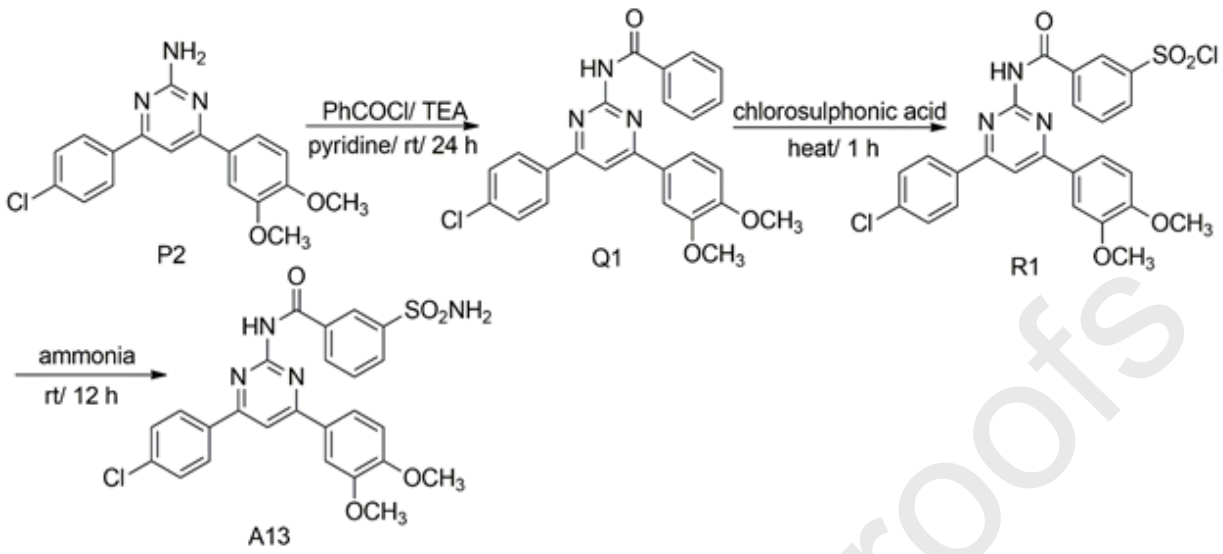
cm^{-1} respectively; ^1H NMR signals were observed at δ 5.0-5.5 ppm representing amino protons and δ 7.7-8.0 ppm indicating pyrimidine proton. The characteristic mass fragmentation pattern was observed at amino ($-NH_2$) group. In the third step, the scheme was bifurcated into two parts, one part involved the nucleophilic acyl substitution reaction between 2-amino pyrimidine with benzene sulphonyl chloride in pyridine to produce benzene sulphonamides **A1-A12** (Scheme 1 and 2, Table 1 and 2). The IR spectrum of these compounds showed characteristic N-H stretching at 3300-3600 cm^{-1} and 1300-1360, 1100-1150 cm^{-1} for O=S=O stretching; ^1H-NMR depicted the peaks for aliphatic methylene protons of cyclohexene ring at δ 1.6-1.7 and δ 2.6-2.7 ppm, methoxy protons at δ 3.5-4.0 ppm, aromatic protons at δ 6.8-8.3 ppm and the proton of sulphonamide group at δ 11.0-11.8 ppm. In another part, the amino group of pyrimidine (**P2, P9**) reacted with benzoyl chloride to generate benzamide group (**Q1, Q2**), which was further reacted with chloro sulphonyl chloride to obtain intermediates (**R1, R2**) and then refluxed with ammonium hydroxide to yield the final products (**A13, A14**) possessing terminal sulphonamide group (Scheme 3 and 4). The IR spectrum of these compounds showed absorptions bands characteristic for N-H stretching at 3500-3600 cm^{-1}; O=S=O stretching at 1360, 1150 cm^{-1}; C=O stretching at 1690-1720 cm^{-1}, ^1H-NMR confirmed the presence of two NH groups , free amino group at δ 5.5-6.0 ppm and NH group of benzamide at δ 9.5-9.8 ppm. Mass spectrums (molecular ion peaks) and elemental analysis (limits within 0.4) further confirmed the chemical structures of the final compounds.



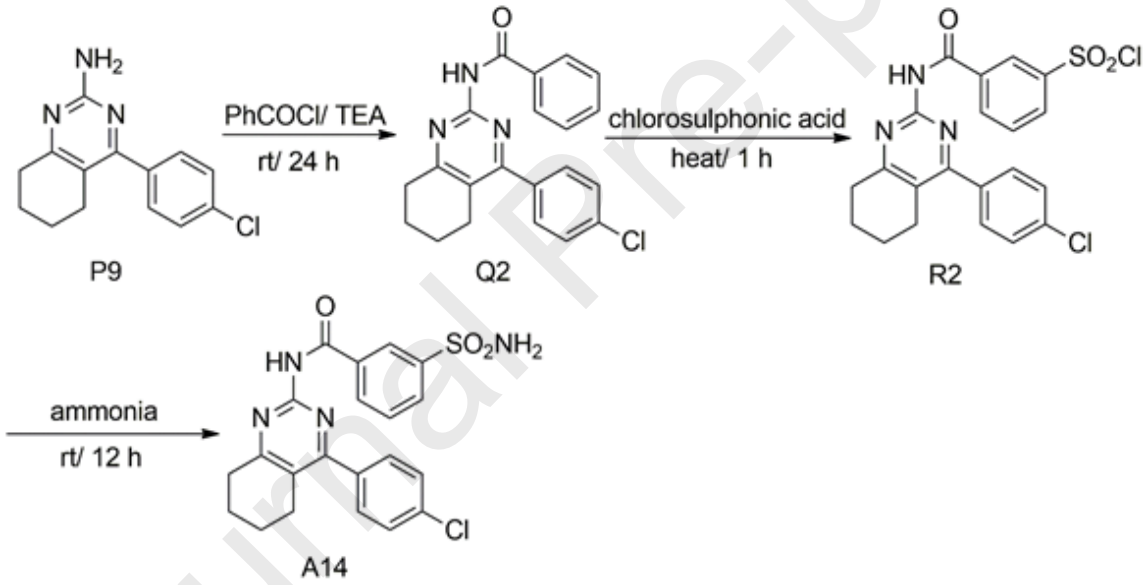
Scheme 1. Synthesis of 4,6-disubstituted pyrimidinyl benzene sulphonamides.



Scheme 2. Synthesis of 4-substituted 5,6,7,8-tetrahydro quinazolinyl benzene sulphonamides.



Scheme 3. Synthesis of N-(4-(4-chlorophenyl)-6-(3,4-dimethoxyphenyl)pyrimidin-2-yl)-3-sulfamoylbenzamide.



Scheme 4. Synthesis of *N*-(4-(4-chlorophenyl)-5,6,7,8-tetrahydroquinazolin-2-yl)-3-sulfamoylbenzamide.

2.2. Biology

2.2.1 Anticancer activity

To comprehend the effect the sulphonamide substitution, initially **A1, A8 (**parent compounds**)** with sulphomoyl group, **A13** and **A14 (**parent compounds**)** with terminal sulphonamide group were synthesized and evaluated for anticancer screening by MTT assay. Compounds **A1, A8** were moderately active and **A13, A14** were least active (Table 1,2,3). Therefore, further analogs of **A1** (pyrimidinyl series) were

developed with various substitutions (**A2-A7**) and similarly for **A8** (tetrahydro quinazolinyl series), further analogs (**A9-A12**) were developed. **A2** was the most potent compound in all the tested cell lines except MCF-7. Its potency was comparably higher than the tested standard drugs, 5-fluoro uracil (5-FU) against DU-145 (prostrate carcinoma), HCT-15, HT-29 (colon carcinoma) cell lines. Compound **A3** was the most potent one in MCF-7 (breast carcinoma) cell line. Compared to **A1;** compounds **A2, A3, A4, A6** were more active in DU-145 cell line; similarly, **A2, A3** in MCF-7; **A2, A3, A4** in HCT-15 cell line; **A2, A4** in HT-29 cell line. Compared with **A8**, compounds **A9, A10, A12** were more active in DU-145 cell line; **A10, A12** in HCT-15 cell line; **A9, A10** in HT-29 cell line. Overall, **A2**, **A9** and **A10** were comparatively more active WRT anticancer activity. This could be due to multiple methoxy substitutions on the phenyl rings. Further, compounds with methoxy substitutions were more active than chloro substituted ones, tri/di substitution predominated over mono substitution (**A6, A7, A11** were least active); dichloro compounds were more active than mono chloro compounds as elucidated in SAR studies.

Table 1

Structural features of compounds A1-A7 along with their IC_{50} values (μM) for anticancer activity obtained by MTT assay.

	Structural features of A1-A7							**Results of MTT assay (IC_{50}, μM)**			
#	**R^1**	**R^2**	**R^3**	**R^4**	**R^5**	**R^6**	**R^7**	**DU-145**	**MCF-7**	**HCT-15**	**HT-29**
A1 (parent compd)	H	H	Cl	H	H	OCH_3	OCH_3	18.45± 1.52	>20	16.87± 1.61	5.54± 0.59
A2	H	OCH_3	OCH_3	OCH_3	OCH_3	H	OCH_3	0.76± 0.11	8.56± 0.96	0.10± 0.04	0.22± 0.05
A3	OCH_3	H	OCH_3	H	H	OCH_3	OCH_3	4.29± 0.25	6.33± 0.54	3.41± 0.20	17.80± 0.23
A4	OCH_3	H	OCH_3	H	Cl	H	Cl	2.08± 0.25	>20	11.52± 4.27	0.86± 0.27
A5	H	OCH_3	OCH_3	H	Cl	H	Cl	>20	>20	>20	14.60± 0.96
A6	H	H	OCH_3	H	H	Cl	OCH_3	13.72± 4.29	>20	>20	>20
A7	H	H	OCH_3	H	H	Cl	Cl	>20	>20	>20	>20

Cytotoxicity value in μM between 0-10: most active; 10-20: moderately active; >20: least active. Each reading represents mean of three ±SD; MCF-7, breast carcinoma; HCT-15, HT-29, colon adenocarcinoma; DU-145, prostate carcinoma.

Table 2

Structural features of compounds A8-A12 along with their IC_{50} values (μM) for anticancer activity obtained by MTT assay.

	Structural features of A8-A12					**Results of MTT assay (IC_{50}, μM)**			
#	**R^1**	**R^2**	**R^3**	**R^4**	**R^5**	**DU-145**	**MCF-7**	**HCT-15**	**HT-29**
A8 (parent compd)	H	H	Cl	H	H	7.34± 0.37	10.40± 0.33	11.04± 0.23	14.43± 0.66
A9	H	OCH_3	OCH_3	OCH_3	3,4,5-trimethoxy benzylidene	3.43± 0.28	>20	14.84± 1.31	3.11± 0.45
A10	H	OCH_3	OCH_3	H	3,4-dimethoxy benzylidene	1.14± 0.08	>20	1.87± 0.41	4.99± 0.60
A11	OCH_3	H	OCH_3	H	3,4-dichloro benzylidene	>20	>20	>20	>20
A12	H	OCH_3	H	H	3,4-dimethoxy benzylidene	5.55± 0.29	19.59± 1.51	9.74± 0.42	14.38± 0.97

Cytotoxicity value in μM between 0-10: most active; 10-20: moderately active; >20: least active. Each reading represents mean of three ±SD; MCF-7, breast carcinoma; HCT-15, HT-29, colon adenocarcinoma; DU-145, prostate carcinoma.

Table 3

Cytotoxicity (IC_{50}) values in μM obtained by MTT assay.

	DU-145	**MCF-7**	**HCT-15**	**HT-29**
A13 (parent compd)	>20	>20	>20	>20
A14 (parent compd)	>20	>20	>20	>20
5-FU	1.15±0.07	5.12±1.33	1.02±0.4	4.2±1.2

Cytotoxicity value >20 μM represents least activity. Each reading represents mean of three ±SD; MCF-7, breast carcinoma;

HCT-15, HT-29, colon adenocarcinoma; DU-145, prostate carcinoma.5-FU, 5-fluoro uracil: positive control.

\2.2.2. Morphological changes observed through DAPI staining

Nucleomorphological changes like horse shoe shaped nuclei and nuclear fragmentation were observed upon treatment of DU-145 cells with compound **A2** for 24 h and further staining with DAPI. The effect was observed in concentration dependent manner (0.5, 1, 2, 4 µM) similar to the standard 5-FU. Nuclear morphology of control cells was devoid of any changes. (Fig. 2). These nuclear changes indicate the progression of apoptosis following drug treatment.

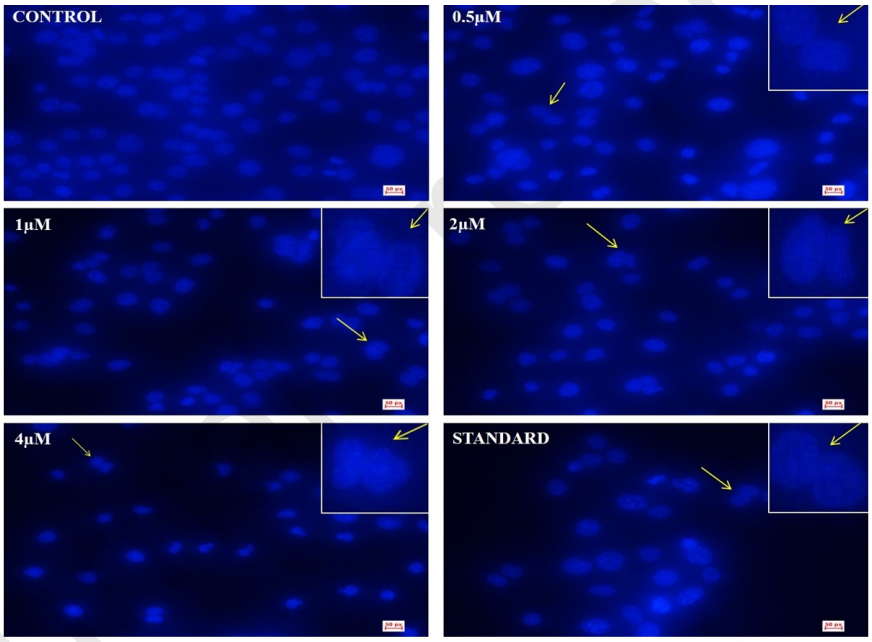


Fig. 2. Nucleomorphological changes induced by compound A2 in DU-145 cells. The cells were treated with increasing concentrations of drug along with standard for 24 h, stained with DAPI and analyzed by flow cytometry. Arrows represent the horse shoe shaped nuclear fragementation.

2.2.3. Apoptosis induction confirmed by Annexin V binding assay

To exploit the underlying mechanism of title compound **A2**, Annexin V/PI double staining assay was performed. The percentage of cells in various stages of apoptosis were noted after treatment with **A2** and the results were compared with control and standard. There was a rise in total percentage of apoptotic

and dead cell population from 6.70 % in control to 14.91 % at 0.5 µM and 35.46 % at 4 µM respectively in a dose dependent manner, while standard (5-FU, 1 µM) displayed 26.78 % of apoptotic population. The percentage of live cells declined in a dose dependent manner from 93.30% in control to 64.54 % at 4 µM, while the standard displayed 73.22% of live cells (Fig. 3.). The increase in apoptotic population indicates the ability of title compound **A2** to induce apoptosis mediated cytotoxicity.

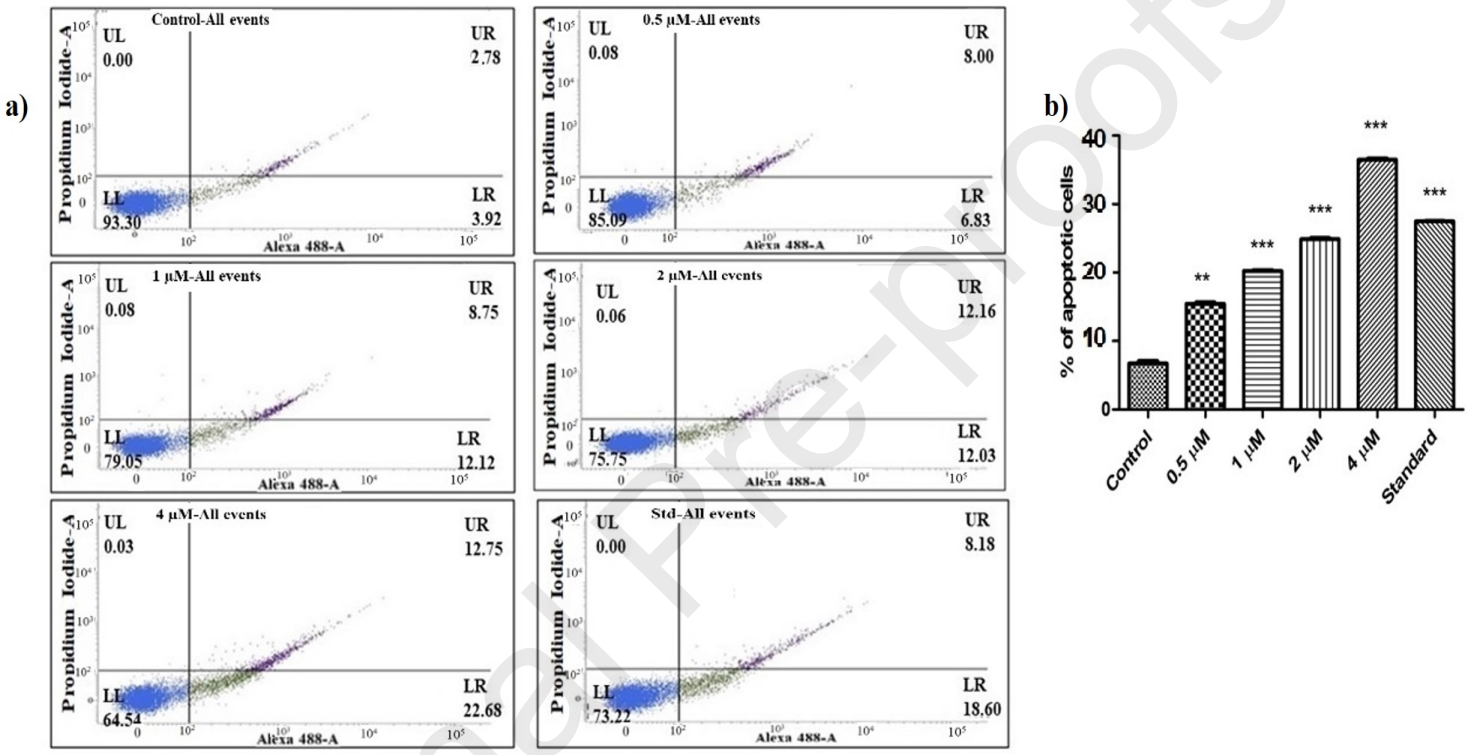


Fig. 3. Apoptosis induced by compound A2 in DU-145 cells. (a) The cells were incubated and treated with increasing concentrations of drug along with standard for 24 h. The treated cells were stained with Annexin V and PI and analyzed by flow cytometry (lower left (LL): live cells, lower right (LR): early apoptotic cells, upper right (UR): late apoptotic cells, upper left (UL): dead cells). (b) The bar graph represents total apoptotic and dead cell population at various concentrations. Data were expressed as mean ± SEM, ***p<0.001, **p<0.01 of two independent experiments.

2.2.4. Alteration of mitochondrial membrane potential in apoptotic cells identified by JC-1 stain.

JC-1 stain is an indicator of mitochondrial membrane potential in healthy cells due to accumulation of J-aggregates with excitation frequency at 590 nm. In apoptosis, there is a significant change in mitochondrial potential due to the opening of mitochondrial permeability transition pore (MPTP) leading to decoupling of respiratory chain and release of cytochrome c. This is evidenced by blue fluorescence (540 nm) due to the formation of J-monomers upon blue light excitation (490 nm). Flow cytometric analysis

displayed an increase in apoptotic population (depolarized cell population) from 23.11% in control to 39.54 % at 4 µM. The alteration of mitochondrial membrane potential upon treatment with **A2** was manifested by decrease in orange fluorescence and increase in blue fluorescence in dose dependent manner (Fig. 4).

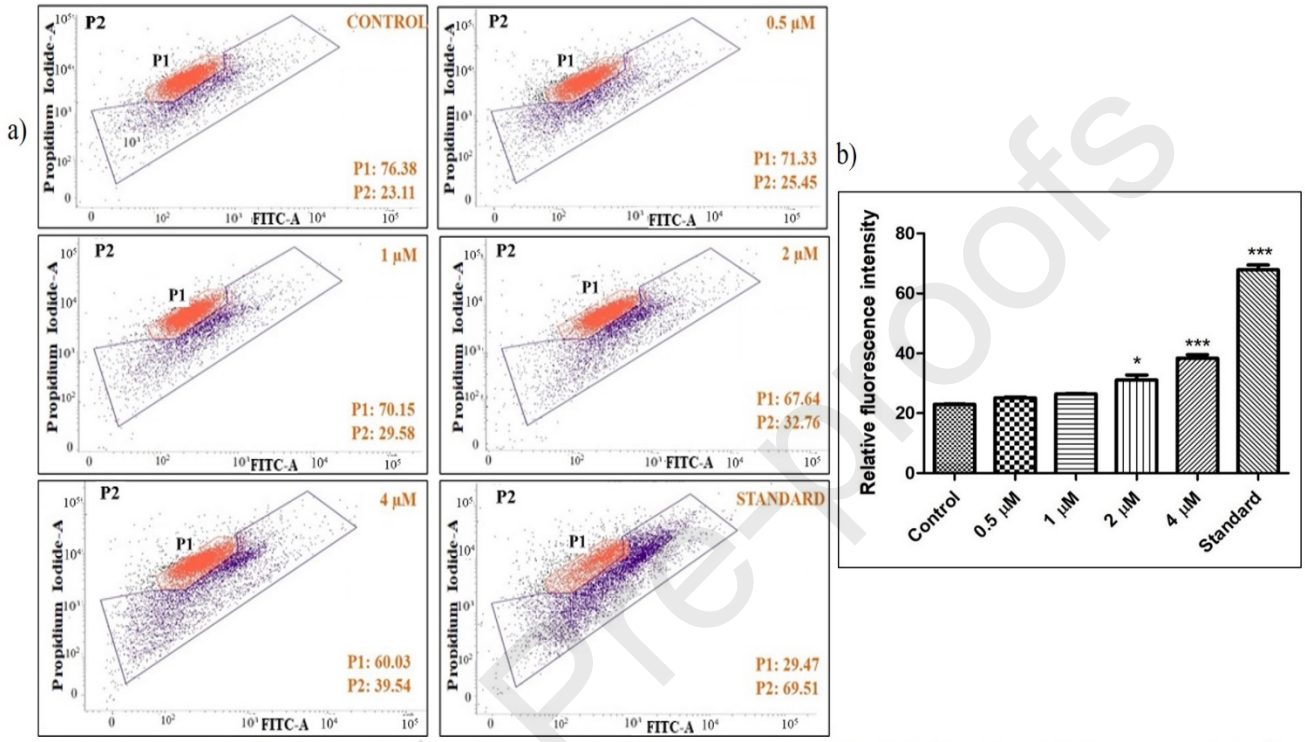


Fig. 4. Alteration of mitochondrial membrane potential by compound A2 in DU-145 cells. a) Cells were treated with increasing concentrations of compound A1 and compared with control and standard. The formation of J-monomers (blue, P1) and J-aggregates (orange, P2) was analyzed by flow cytometry. b) Graphical representation of dose dependent increase in fluorescence intensity depicting the J-monomer accumulation. Data were expressed as mean ± SEM, ***p<0.001, *p<0.05 of two independent experiments.

This could be due to increase in J-monomer formation upon apoptosis depicting the mitochondrial involvement in cell arrest probably via release of cytochrome c.

2.2.5. ROS generation by DCFDA staining

ROS is a reactive molecular entity involved in many biological pathways. When the levels of ROS increases in cancer cells, there would be an irreparable damage to DNA and proteins followed by activation of caspase-3 with apoptosis induction. tumor cells. In the present study, production of ROS was observed when DU-145 cells were treated with increasing concentrations (2.5, 5, 10 µM) of compound **A2** followed by DCFDA staining (Fig. 5.). The dose dependent shift in peaks towards right indicates the rise in fluorescence intensity. Similar effect was observed with standard 5-FU at 10 µM concentration.

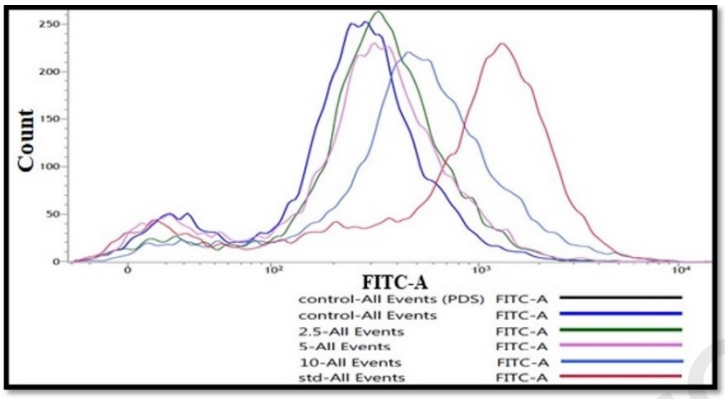


Fig. 5. Effect of compound A2 on ROS generation. DU-145 cells were treated with increasing concentrations of drug for 24 h and the results obtained were compared with control and standard. The ROS generated was stained with DCFDA and analysed by flow cytometer.

2.3. Structure Activity Relationship (SAR)

To understand the substituent effects on cytotoxicity, structure activity relationship studies were established (Table 1,2 and 3). The highlights are summarized below.

Basic skeleton: Compounds bearing benzene sulphonamide moiety (**A1-A12**) were more active than 3-sulphomoyl benzamide derivatives (**A13, A14**). 4,6-disubstituted pyrimidine nucleus (**A1-A7**) was comparatively more active than tetrahydro quinazolinyl nucleus (**A8-A12**).

Methoxy substitution: Increasing the methoxy groups significantly increased the cytotoxicity of the molecules. This was observed with compounds **A2, A3**, **A9** and **A10**. Increasing the methoxy groups in the range of 4-6 was more advantageous. This could be due to enhanced lipophilicity that allows the molecule to enter into the cytotoxic cells easily and thereby display their inhibitory potential.

Chloro substitution: Dichloro substitution was more predominate over mono chloro substitution. This is well exemplified with compounds **A4, A5, A11** with dichloro substitution being more active than parent analogs **A1** and **A8** with mono chloro substitution. Increase in chloro groups enhances the polarity

of the molecule and therefore could be the reason for enhanced activity observed with **A4** and **A5**. However, methoxy substituted compounds (**A2, A3, A9, A10**) were more active than chloro substituted ones.

Benzylidene substitution: Compounds bearing benzylidene group at 8th position on quinazoline ring (**A9-A12**) were more active than the parent compound **A8**. Trimethoxy benzylidene group (**A9**) and dimethoxy (**A10**) were more active than mono methoxy benzylidene group (**A12**). Further, dichloro substitution on benzylidene decreased the activity of the molecule (**A11**).

CONCLUSION

Four variants of benzene sulphonamides were developed and their effect on anticancer activity was analyzed. Preliminary anti-proliferative screening has identified compounds **A1, A8** as lead parent molecules. Further analogs of **A1** and **A8** were developed as 4,6-disubstituted pyrimidinyl benzene sulphonamides (**A2-A7**) and other as tetrahydro quinazolinyl benzene sulphonamides (**A9-A12**). Most of them have shown improvised activity profiles than parent compounds. **A2** was the most potent compound of the series and also potent than the standard drug, 5-fluorouracil with IC_{50} values less than 1 μM in DU-145, HCT-15, HT-29 cell lines. The drug also induced apoptosis in DU-145 cells as evidenced by various mechanistic studies on apoptosis. All of the above studies proved compound **A2** as excellent apoptosis inducing cytotoxic agent with potential source as lead for next generation.

4. MATERIALS AND METHODS

4.1. Chemistry

IR spectra were recorded by KBr pellet technique on Shimadzu IR affinity-1, FT-IR spectrophotometer (Schimadzu, Germany). Nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded using Bruker Avance spectrophotometer (Bruker Analytic, Karlsruhe, Germany; Bruker AG, Fallanden, Switzerland) with chemical shifts in parts per million (ppm, δ) downfield from TMS as an internal standard using $CDCl_3$ or DMSO solvent. Mass spectra were recorded with 6540 Qtof - Infinity 1290 (Agilent Technologies, India). Elemental analyses were carried out on Perkin-Elmer 2400 analyser (Waltham, MA, USA) and the results were found to be in good agreement within ±0.4 compared to their

theoretical valves. All the melting points were determined using BÜCHI Melting Point B-450 apparatus (Büchi Labortechnik, Switzerland) and were uncorrected. Commercially available reagents and solvents were procured from Sigma Aldrich/Merck and used without any purification unless stated. All the synthesized compounds were purified on Buchi Flash Chromatography using Merck silica gel 200-400#. Analytical thin-layer chromatography was carried out on Merck silica gel plates. All reactions were carried out nitrogen or under air atmosphere. General procedures for Chalcone synthesis (**C1-C12**) and pyrimidine synthesis (**P1-P12**) was performed in according to the procedures prescribed by Verma *et.al*.[26].

General procedure for synthesis of N-[4,6-diaryl pyrimidin-2-yl]benzene sulfonamides and N-[2,6-diaryl (5,6,7,8-tetrahydro-quinazolin-2-yl)]-benzenesulfonamides

To an ice cooled solution of intermediates (**P1-P12**, 1 mmol) in pyridine, benzene sulfonyl chloride (5 mmol) was slowly added. Then the reaction mixture was stirred at 0 °C to room temperature for 24 h. Further, dil. HCl was added and the obtained crystals were filtered, washed with water and recrystallized from ethanol to yield final compounds **A1-A12 (**Scheme 1 and 2; Table 1 and 2**)**.

N-[4-(4-chloro-phenyl)-6-(3,4-dimethoxy-phenyl)-pyrimidin-2-yl]-4-sulfamoyl-benzamide A1

Yellowish solid, yield 75%, mp 102 ^{o}C. FT-IR (KBr) cm^{-1}: 3649 (N-H stretching), 1350, 1150 (O=S=O stretching), 1616, 1454 (C=C aromatic stretching). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 3.86 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3) 7.05-7.07 (d, 1H, ArH), 7.09-7.11 (d, 1H, ArH), 7.44 (s, 1H, ArH), 7.53-7.55 (d, 2H, ArH), 7.77-7.96 (m, 3H, ArH), 8.05-8.07 (d, 2H, ArH), 8.19-8.21 (d, 2H, ArH), 8.35-8.37 (s, 1H, ArH), 11.46 (s, 1H, NH).^{13}C-NMR (75 MHz, $CDCl_3$): 167.7, 164.2, 160.3, 149.9, 148.6, 144.3, 134.2, 133.5, 132.4, 130.3, 129.5, 129.4, 128.9, 127.6, 122.8, 109.6, 100.2, 97.4, 56.4. ESI-MS: *m/z* 482 $(M+H)^{+}$. Anal. Calcd. for $C_{24}H_{20}ClN_3O_4S$: C, 59.81; H, 4.81; N, 8.72. Found: C, 59.85; H, 4.83; N, 8.61.

N-[4-(3,4-dimethoxy-phenyl)-6-(3,4,5-trimethoxy-phenyl)-pyrimidin-2-yl]-benzenesulfonamide A2

Yellowish solid, yield 45%, mp 220 ^{o}C. FT-IR (KBr) cm^{-1}: 3612 (N-H stretching), 1338, 1126 (O=S=O stretching), 1650, 1456 (C=C aromatic stretching). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 3.79 (s, 3H, OCH_3), 3.83 (s, 6H, OCH_3), 3.85 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3),

7.04-7.06 (d, 1H, ArH), 7.10-7.12 (d, 1H, ArH), 7.33-7.35 (d, 1H, ArH), 7.40 (d, 1H, ArH), 7.59-7.65 (m, 2H, ArH), 7.77 (s, 1H, ArH), 7.85-7.87 (dd, 1H, ArH), 8.05-8.07 (dd, 1H, ArH) , 8.14 (s, 1H, ArH), 8.20-8.22 (d, 1H, ArH), 11.86 (d, 1H, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 165.1, 163.7, 160.5, 153.5, 150.8, 149.6, 144.3, 137.7, 132.4, 131.5, 129.4, 128.7, 128.0, 122.8, 110.8, 110.2, 106.2, 104.7, 56.4, 56.0. ESI-MS: *m/z* 538 ($M + H^+$). Anal. Calcd. for $C_{27}H_{27}N_3O_7S$: C, 60.32; H, 5.06; N, 7.82. Found: C, 60.35; H, 5.07; N, 7.78.

N-[4-(3,4-dimethoxy-phenyl)-6-(2,4-dimethoxy-phenyl)-pyrimidin-2-yl]-benzenesulfonamide A3

Colorless solid, yield 45%, mp 160 ^{o}C, FT-IR (KBr) cm^{-1} : 3489 (N-H stretching), 1367, 1145 (O=S=O stretching) , 1607, 1467 (C=C aromatic stretching).^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 3.78 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 7.11-7.13 (d, 1H, ArH), 7.15 (s, 1H, ArH), 7.56 (s, 1H, ArH), 7.58-7.60 (d, 1H, ArH), 7.61-7.63 (d, 2H, ArH), 7.72-7.73 (m, 2H, ArH), 7.84-7.87 (t, 1H, ArH), 8.01-8.02 (d, 1H, ArH), 8.10 (s, 1H, ArH), 8.22-8.23 (d, 1H, ArH)), 11.66 (s, 1H, NH) . ^{13}C-NMR (75 MHz, $CDCl_3$): 167.3, 163.9, 161.7, 159.4, 158.2, 154.6, 150.3, 134.2, 132.4, 131.1, 130.4, 129.4, 129.5, 128.9, 127.6, 122.8, 105.6, 100.2, 97.4, 56.1. ESI-MS: *m/z* 530 ($M+Na^+$). Anal. Calcd. for $C_{26}H_{25}N_3O_6S$: C, 61.53; H, 4.96; N, 8.28. Found: C, 61.51; H, 4.94; N, 8.30.

N-(4-(2,4-dichlorophenyl)-6-(2,4-dimethoxyphenyl) pyrimidin-2-yl) benzenesulfonamide A4

Colorless solid, yield 60 %, mp 112 ^{o}C, FT-IR (KBr) cm^{-1}: 3365 (N-H stretching), 1314, 1156 (O=S=O stretching), 1649, 1445 (C=C aromatic stretching).^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 3.83 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 7.04-7.12 (d, 2H, ArH), 7.28-7.31 (d, 1H, ArH), 7.39 (s, 2H, ArH), 7.45-7.48 (d, 1H, ArH), 7.62-7.65 (m, 3H, ArH), 7.85-7.87 (dd, 2H, ArH), 8.14 (s, 1H, ArH), 11.58 (s, 1H, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 165.4, 163.2, 156.7, 153.5, 152.1, 149.1, 136.5, 133.3, 130.8, 129.9, 129.4, 128.8, 128.3, 126.2, 122.8, 110.9, 110.7, 110.0, 106.6, 100.2, 56.1. ESI-MS: *m/z* 515 (M^+). Anal. Calcd. for $C_{24}H_{19}Cl_2N_3O_4S$: C, 55.82; H, 3.71; N, 8.14. Found: C, 55.61; H, 3.94; N, 8.27.

N-[4-(2,4-dichloro-phenyl)-6-(3,4-dimethoxy-phenyl)-pyrimidin-2-yl]-benzenesulfonamide A5

Yellowish solid, yield 67%, mp 108 ^{o}C, FT-IR (KBr) cm^{-1}: 3371 (N-H stretching), 1312, 1162 (O=S=O stretching), 1656, 1459 (C=C aromatic stretching). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 3.84 (s, 3H,

OCH_3), 3.86 (s, 3H, OCH_3), 7.02-7.11 (dd, 1H, ArH), 7.28-7.48 (m, 1H, ArH), 7.56-7.65 (d, 2H, ArH), 7.77-7.89 (m, 3H, ArH), 8.02-8.05 (d, 2H, ArH), 8.19 (d, 2H, ArH), 8.38 (s, 1H, ArH), 11.92 (s, 1H, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 164.4, 161.2, 160.0, 157.6, 154.6, 147.3, 136.4, 134.2, 132.4, 130.4, 129.9, 129.0, 128.5, 128.2, 127.6, 126.2, 122.8, 111.5, 108.9, 100.6, 56.1. ESI-MS: *m/z* 516 ($M+H^+$). Anal. Calcd. for $C_{24}H_{19}Cl_2N_3O_4S$: C, 61.53; H, 4.96; N, 8.28. Found: C, 61.51; H, 4.94; N, 8.30.

N-[4-(4-methoxy phenyl)-6-(4-methoxy-phenyl)-pyrimidin-2-yl]-benzenesulfonamide A6

Colorless solid, yield 72 %, mp 242 ^oC, FT-IR (KBr) cm^{-1}: 3592 (N-H stretching), 1378, 1143 (O=S=O stretching), 1650, 1489 (C=C aromatic stretching). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 3.83 (s, 6H, OCH_3), 7.05-7.08 (d, 4H, ArH), 7.58-7.60 (d, 3H, ArH), 8.00 (s, 1H, ArH), 8.03-8.06 (m, 2H, ArH), 8.13-8.16 (d, 4H, ArH), 11.74 (s, 1H, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 166.6, 163.3, 159.3, 146.4, 131.6, 129.4, 128.3, 128.8, 128.1, 127.8, 101.2, 56.1. ESI-MS: *m/z* 447 (M^+). Anal. Calcd. for $C_{24}H_{21}N_3O_4S$: C, 64.41; H, 4.73; N, 9.39. Found: C, 64.56; H, 4.65; N, 9.40.

N-(4-(3,4-dichlorophenyl)-6-(4-methoxy phenyl) pyrimidin-2-yl) benzenesulfonamide A7

Yellowish solid, yield 57 %, mp 163 ^oC, FT-IR (KBr) cm^{-1}: 3584 (N-H stretching), 1369, 1139 (O=S=O stretching), 1642, 1476 (C=C aromatic stretching). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 3.89 (s, 3H, OCH_3), 6.68-6.70 (d, 2H, ArH), 7.01-7.03 (d, 2H, ArH), 7.68-7.79 (m, 6H, ArH), 7.97-8.00 (d, 2H, ArH), 8.21-8.23 (d, 1H, ArH), 11.32 (s, 1H, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 165.7, 163.9, 160.2, 158.8, 145.1, 134.7, 132.8, 132.4, 131.6, 130.5, 129.4, 128.5, 128.3, 127.2, 127.0, 114.8, 101.5, 108.9, 55.4. ESI-MS: *m/z* 487 (M^+). Anal. Calcd. for $C_{23}H_{17}Cl_2N_3O_3S$: C, 56.80; H, 3.52; N, 8.64. Found: C, 56.76; H, 3.59; N, 8.71.

N [4-(4- chloro -phenyl)-5,6,7,8-tetrahydro-quinazolin-2-yl]-benzenesulfonamide A8

Yellowish solid, yield 65 %, mp 130 ^oC, FT-IR (KBr) cm^{-1}: 3678 (N-H stretching), 1350, 1150 (O=S=O stretching), 1624, 1431 (C=C aromatic stretching). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.61-1.82 (m, 4H, CH_2), 2.42-2.55 (m, 4H, CH_2), 7.05-7.15 (d, 2H, ArH), 7.38-7.51 (m, 3H, ArH), 7.81-7.89 (d, 2H, ArH), 8.05-8.07 (d, 2H, ArH), 11.25 (s, 1H, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 167.8, 164.7, 144.4,

134.2, 132.4, 131.3, 129.4, 129.0, 128.7, 127.5, 126.9, 123.3, 29.8, 28.7, 24.7, 23.9. ESI-MS: *m/z* 422 (M + Na^+). Anal. Calcd. for $C_{20}H_{18}ClN_3O_2S$: C, 60.07; H, 4.54; N, 10.51. Found: C, 62.51; H, 4.59; N, 10.67.

N-[8-(3,4,5-timethoxy benzylidene)-2-(3,4,5-trimethoxy phenyl)-5,6,7,8-tetrahydro-quinazolin-4-yl]-benzenesulfonamide A9

Yellow solid, yield 45%, mp 195 °C, FT-IR (KBr) cm^{-1}: 3649 (N-H stretching), 1336, 1128 (O=S=O stretching), 1630, 1446 (C=C aromatic stretching). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.69-1.92 (m, 4H, CH_2), 2.85-2.88 (m, 2H, CH_2), 3.89 (s, 6H, OCH_3), 3.91(s, 6H, OCH_3), 3.92 (s, 6H, OCH_3), 6.66 (s, 2H, ArH), 6.74 (s, 2H, ArH), 7.45-7.60 (m, 3H, ArH), 7.98 (s, 1H, =CH), 8.16-8.19 (d, 2H, ArH), 11.23 (s, 1H, NH).^{13}C-NMR (75 MHz, $CDCl_3$): 167.6, 163.4, 162.2, 153.2, 153.0, 139.7, 139.4, 138.8, 138.4, 132.4, 130.1, 129.4, 129.0, 127.3, 124.8, 115.8, 104.6, 103.7, 60.5, 56.1, 30.6, 27.8, 23.7. ESI-MS: *m/z* 634 (M + H^+). Anal. Calcd. for $C_{33}H_{35}N_3O_8S$: C, 62.54; H, 5.57; N, 6.63. Found: C, 62.51; H, 5.49; N,

N-[6-(3,4-dimethoxy-benzylidene)-2-(3,4-dimethoxy-phenyl)-5,6,7,8-tetrahydro-quinazolin-4-yl]-benzenesulfonamide A10

Yellowish solid, yield 45%, mp 110 °C, FT-IR (KBr) cm^{-1}: 3673 (N-H stretching), 1372, 1156 (O=S=O stretching), 1606, 1478 (C=C aromatic stretching). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.57-1.59 (m, 2H, CH_2), 1.78-1.80 (m, 2H, CH_2), 2.67-2.74 (m, 2H, CH_2), 3.79 (s, 6H, OCH_3), 3.81 (s, 6H, OCH_3), 7.00-7.03 (d, 4H, ArH), 7.38-7.62 (m, 5H, ArH), 7.76 (s, 1H, =CH), 7.98-8.00 (d, 2H, ArH), 11.58 (s, 1H, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 166.7, 166.5, 161.9, 150.3, 149.8, 149.7, 149.1, 144.3, 139.7, 134.3, 131.4, 129.4, 127.6, 126.8, 122.5, 121.7, 121.3, 120.2, 117.6, 114.9, 109.6, 56.1, 30.3, 27.6, 24.4. ESI-MS: *m/z* 573 (M^+). Anal. Calcd. for $C_{31}H_{31}N_3O_6S$: C, 64.90; H, 5.45; N, 7.32. Found: C, 64.81; H, 5.55; N, 7.45.

N-[6-(3,4-dichloro-benzylidene)-2-(3,4-dichloro-phenyl)-5,6,7,8-tetrahydro-quinazolin-4-yl]-benzenesulfonamide A11

Colorless solid, yield 45%, mp 230 °C, FT-IR (KBr) cm^{-1}: 3493 (N-H stretching), 1369, 1143 (O=S=O stretching), 1655, 1467 (C=C aromatic stretching). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.59-1.60 (m, 4H, CH_2), 2.63-2.72 (m, 2H, CH_2), 6.60 (s, 1H, ArH), 6.76 (s, 1H, ArH), 7.36-7.59 (m, 6H, ArH), 7.72-7.76 (m, 3H, ArH), 7.99 (s, 1H, =CH), 9.82 (s, 1H, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 166.3, 166.1, 163.5,

144.3, 134.5, 133.8, 133.6, 132.7, 132.5, 132.3, 131.1, 130.6, 130.2, 129.4, 129.0, 128.6, 127.8, 127.3, 127.1, 122.5, 30.5, 27.6, 24.8. ESI-MS: *m/z* 591 (M^+). Anal. Calcd. for $C_{31}H_{31}N_3O_6S$: C, 62.10; H, 5.45; N, 9.88. Found: C, 62.11; H, 5.43; N, 9.87.

N-[6-(4-methoxy-benzylidene)-2-(4-methoxy-phenyl)-5,6,7,8-tetrahydro-quinazolin-4-yl]-benzenesulfonamide A12

Colorless solid, yield 45%, mp 165 ^oC. FT-IR (KBr) cm^{-1}: 3567 (N-H stretching), 1310, 1162 (O=S=O stretching), 1630, 1467 (C=C aromatic stretching). ^1H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.52-1.78 (m, 4H, CH_2), 2.73-2.87 (m, 2H, CH_2), 3.11 (s, 6H, OCH_3), 6.38-6.41 (d, 1H, ArH), 6.56-6.64 (d, 2H, ArH), 6.78-6.81 (d, 1H, ArH), 7.01-7.13 (dd, 2H, ArH), 7.25-7.27 (d, 1H, ArH), 7.45-7.60 (m, 5H, ArH), 7.69-7.77 (d, 1H, ArH), 8.02 (s, 1H, =CH), 11.62 (s, 1H, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 164.8, 164.3, 161.2, 159.6, 144.7, 135.3, 134.2, 132.7, 131.1, 130.4, 129.4, 129.0, 128.5, 128.1, 127.6, 125.4, 117.8, 114.2, 56.1, 34.6, 31.3, 29.8. ESI-MS: *m/z* 516. ($M+H^+$). Anal. Calcd. for $C_{31}H_{31}N_3O_6S$: C, 67.82; H, 5.30; N, 8.18. Found: C, 67.95; H, 5.42; N, 8.25.

*Procedure for synthesis of N-[4-(4-chloro-phenyl)-6-(3,4-dimethoxy-phenyl)-pyrimidin-2-yl]-4-sulfamoyl-benzamide (**A13**) and N-[4-(4-chloro-phenyl)-5,6,7,8-tetrahydro-quinazolin-2-yl]-4-sulfamoyl-benzamide (**A14**)*

To the mixture of ice cooled solution of pyrimidines (**P2, P9**; 1 mmol) in pyridine, triethylamine (5 mmol) and benzoyl chloride (5 mmol) were added and the reaction was allowed to stir at room temperature for about 24 h. To the reaction mixture, dil. HCl was added and the obtained solid was filtered, washed with water and recrystallized from ethanol to produce **Q1** and **Q2**. The products were confirmed by their molecular ion (M^+) peaks observed in mass spectrums [27].

A mixture of ice cooled benzamides (**Q1, Q2**; 1 mmol) in chloro sulfonic acid (0.33 mL, 5 mmol) was heated on water bath for 1 h then poured onto crushed ice and the obtained solid was filtered, washed with water and recrystallized from ethanol to produce **R1** and **R2**. Further, the benzamido sulphonyl chloride (**R1, R2**; 1mmol) was refluxed with ammonium hydroxide (2 mL) for 1 h. After cooling to room

temperature, the obtained solid was filtered and washed with water to obtain the final products **A13** and **A14** (Scheme 3 and 4).

A13: Yellowish solid, yield 41%, mp 124 ^{o}C. FT-IR (KBr) cm^{-1}: 3565 (N-H stretching), 1321, 1165 (O=S=O stretching), 1604, 1458 (C=C aromatic stretching). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 3.85 (s, 6H, OCH_3), 5.55 (s, 2H, NH_2), 6.81-6.90 (d, 2H, ArH), 7.12-7.15 (d, 2H, ArH), 7.24 (s, 1H, ArH), 7.72-7.76 (d, 2H, ArH), 7.88-7.99 (m, 3H, ArH), 8.32-8.38 (d, 2H, ArH), 9.62 (s, 1H, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 166.8, 164.5, 164.3, 159.4, 151.6, 149.6, 146.8, 137.3, 135.6, 131.8, 130.2, 129.4, 128.6, 127.5, 126.1, 121.3, 110.5, 107.6, 101.7, 56.2. ESI-MS: *m/z* 526 $(M+H)^+$. Anal. Calcd. for $C_{25}H_{21}ClN_4O_5S$: C, 59.81; H, 4.81; N, 8.72. Found: C, 59.85; H, 4.83; N, 8.61.

A14: Yellowish solid, yield 47 %, mp 116 ^{o}C, FT-IR (KBr) cm^{-1}: 3630 (N-H stretching), 1360, 1150 (O=S=O stretching), 1641, 1466 (C=C aromatic stretching). ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.65-1.92 (m, 4H, CH_2), 2.44-2.55 (t, 2H, CH_2), 2.65-2.71 (t, 2H, CH_2), 5.38 (s, 2H, NH_2) 7.48-7.68 (d, 2H, ArH), 7.72-7.83 (d, 2H, ArH), 7.92-8.00 (d, 2H, ArH), 8.32-8.48 (d, 2H, ArH), 9.52 (s, 1H, NH). ^{13}C-NMR (75 MHz, $CDCl_3$): 165.2, 163.8, 159.4, 146.9, 136.8, 133.3, 132.3, 130.5, 129.6, 128.6, 127.6, 127.4, 122.7, 29.6, 28.2, 24.3, 23.7. ESI-MS: *m/z* 463 $(M + Na^+)$. Anal. Calcd. for $C_{21}H_{19}ClN_4O_3S$: C, 56.95; H, 4.32; N, 12.65. Found: C, 56.86; H, 4.23; N, 12.67.

4.2. Biology

4.2.1. Cytotoxicity assay

Cytotoxicity studies on the test compounds was carried by MTT assay. DU-145, MCF-7, HCT-15, HT 29, cell lines were seeded at a density of 4 x 10^3, 3.7 × 10^4, 3.5 × 10^4 cells per well in 100 µL of respective media and were allowed to attach overnight for 24 h. The cells were treated with test compounds (**A1-A14**) at different concentrations for a period of 48 h. After the treatment, MTT (0.5 mg/ml) was added and incubated at 37 ^{o}C for 4 h. The insoluble purple formazan crystals were dissolved in 100 µL DMSO and cell viability was assessed by measuring the absorbance at 570 nm in a multi detection plate reader

(Spectramax M4, Molecular devices, USA). The IC_{50} values shown are the means and standard deviation of at least three independent experiments performed in duplicate [28].

4.2.2. DAPI staining

To evaluate the morphological changes following treatment with compound **A2**, DAPI staining was performed. DU-145 cells were grown in 24-well plates at a seeding density of 5×10^4 cells/well for 24 h and then treated with compound **A2** at various concentrations (0.5, 1, 2, 4 μM). After 24 h, untreated cells, cells treated with PS01 and standard 5-FU (1 μM) were washed thrice with PBS. Then, 200 μL of 0.1 % triton X was added for permeabilization and stained with 1 μM of DAPI at 37 ^oC for 10 min. The stained cells with fragmented nuclei/ condensed chromatin were observed under fluorescence microscope with excitation at 359 nm and emission at 461 nm using DAPI filter at 200X magnification.

4.2.3. FITC- Annexin V apoptosis detection

Annexin V is labeled with a fluorescent tag, FITC, to detect and quantify the apoptotic cells generated by treatment with compound **A2**. DU-145 cells (1×10^6) were seeded in 12-well plates and allowed to grow for 24 h. After cell attachment, the supernatant liquid was replaced by new 10% FBS/ RPMI 1640 medium containing DMSO alone for negative control, varying concentrations of compound **A2** (0.5, 1, 2, 4 μM) and standard 5-FU (1 μM). After 24 h of treatment, cells from the supernatant and adherent monolayer cells were harvested by trypsinization, washed with PBS and centrifuged at 3000 rpm. Then cells were processed with Annexin V- assay kit (FITC Annexin V Apoptosis Detection Kit, BD Pharmingen, USA) according to the manufacturer's instruction [29,30]. Further, flow cytometric analysis was performed using a flow cytometer (BD FACS Verse USA).

4.2.4. Analysis of mitochondrial membrane potential (MMP)

Apoptosis induces changes in mitochondrial membrane permeability that allows accumulation of JC-1 dye and thereby decrease the red/ green fluorescence intensity ratio. DU-145 cells were plated at a seeding density of 1×10^6 cells/well in a 12-well plate. They were treated with DMSO as negative control and varying concentrations of compound **A2** (0.5, 1, 2, 4) and standard 5-FU (1 μM) for 24 h. After

treatment, cells were incubated with JC-1 (5 μM) for 30 min at 37 ^{o}C. Finally, cells were harvested, collected in tubes, washed with PBS and analyzed using flow cytometer [31].

4.2.5. Detection of intracellular ROS

2, 7'-dichlorofluorescindiacetate (DCFDA), is a cell permeable fluorogenic dye that measures hydroxyl, peroxyl and other reactive oxygen species (ROS) activity within the cell. DU-145 cells were seeded in 12-well plate at density of 1×10^{6} cells/well and incubated for 24 h at 37 ^{o}C. After incubation, cells were treated with DMSO for negative control or with varying concentrations of compound **A2** (2.5, 5, 10 μM) concentrations along with standard 5-FU (10 μM). After 24 h of treatment, 400μL of DCFDA working solution was added per well containing 5μM of DCFDA and incubated for 30 min. Then, DCFDA working solution was removed and the cells were trypsinised and centrifuged at 5000 rpm for 5 min. The obtained pellet was rinsed with PBS and centrifuged at 3000 rpm for 3 min [32, 33]. Finally, 400μL of PBS was added and analyzed by flow cytometer (BD FAC Verse, USA).

2.9. Statistical Analysis

All the results were expressed as mean ± SD and IC_{50}, standard deviation and P-value were evaluated by one way analysis of variance (ANOVA). $P < 0.05$ was the allowed standard deviation. All the experiments were independently repeated thrice and the readings from the triplicates were combined and analyzed.

Conflict of Interest:

There are no conflicts to declare.

Acknowledgements

This research was supported by Women Scientist Scheme (WOS-A), Department of Science and Technology (**DST**), New Delhi, India (Grant no. **SR/WOS-A/CS-1003/2014**).

REFERENCES

1) F. U. Hartl, A. Bracher, M. H. Hartl, Nature 475 (2011) 324-432.
2) J. N. Moloney, T. G. Cotter, Semin. Cell Dev. Biol. 80 (2018) 50-64.

3) J. Paluncic, Z. Kovacevic, P. J. Jansson, D. Kalinowski, A. M. Merlot, M. L. Huang, H. C. Lok, S. Sahni, D. J. Lane, D. R. Richardson, Biochim. Biophys. Acta. 1863 (2016) 770-784.
4) R. S. Wong, J. Exp. Clin. Cancer Res. 30 (2011) 87.
5) G. Pistritto, D. Trisciuoglio, C. Ceci, A. Garufi, G. D'Orazi, Aging (Albany NY) 8 (2016) 603–619.
6) A. Gross, J. M. McDonnell, S. J. Korsmeyer, Genes Dev. 13 (1999) 1899–1911.
7) I. M. Ghobrial, T. E. Witzig, A. A. Adjei, CA Cancer J. Clin. 55 (2005) 178–194.
8) S. Galadari, A. Rahman, S. Pallichankandy, F. Thayyullathil, Free Radic. Biol. Med. 104 (2017) 144-164.
9) Z. Zou, H. Chang, H. Li, S. Wang, Apoptosis 22 (2017) 1321-1335.
10) X. Xu, J. Wang, Q. Yao, Bioorg. Med. Chem. Lett. 25 (2015) 241-244.
11) Y. M. El Sayed, W. Sadee, Cancer Res 43 (1983) 4039-4044.
12) X. J. Song, Y. Shao, X. Gao, Chin. Chem. Lett. 22 (2011) 1036-1038.
13) Z. Liu, Y. Wang, H. Lin, D. Zuo, L. Wang, Y. Zhao, P. Gong, Eur. J. Med. Chem. 85 (2014) 215-227.
14) D. A. Ibrahim, N. S. Ismail, Eur. J. Med. Chem. 46 (2011) 5825-5832.
15) B. Barlaam, S. Cosulich, S. Degorce, M. Fitzek, F. Giordanetto, S. Green, T. Inghardt, L. Hennequin, U. Hancox, C. Lambert-van der Brempt, R. Morgentin, S. Pass, P. Plé, T. Saleh, L. Ward, Bioorg. Med. Chem. Lett. 24 (2014) 3928-3935.
16) Y. H. Kim, K. J. Shin, T. G. Lee, E. Kim, M. S. Lee, S. H. Ryu, P. G. Suh, Biochem. Pharmacol. 69 (2005) 1333-1341.
17) A. Casini, A. Scozzafava, A. Mastrolorenzo, L. T. Supuran, Curr. Cancer Drug Targets 2 (2002) 55-75.
18) D. C. Talbot, J. von Pawel, E. Cattell, S.M. Yule, C. Johnston, A.S. Zandvliet, A. D. Huitema, C. J. Norbury, P. Ellis, L. Bosquee, M. Reck, Clin. Cancer Res. 13 (2007) 1816-1822.
19) C. T. Supuran, Expert Opin. Investig. Drugs 27 (2018) 963-970.
20) M. M. Ghorab, M. S. Al said, M. S. Al-Dossary, Y. M. Nissan, S. M. Attia, Chem. Cent. J. 10 (2016) 19.
21) K. O. Mohamed, Y. M. Nissan, A. A. El-Malah, W. A. Ahmed, D. M. Ibrahim, T. M. Sakr, Eur. J. Med. Chem. 135 (2017) 424-433.
22) S. Mohammed, M. M. Al-Dosari, M. M. Ghorab; M.S. AlSaid, Y. M. Nissan, A. B. Ahmed, Eur. J. Med. Chem. 69 (2013) 373-383.
23) M. G. Mostafa, M. G. El-Gazzar, M.S. Alsaid, Int. J. Mol. Sci. 15 (2014) 5582-5595.

24) M.V. Kachaeva, D. M. Hodyna, I.V. Semenyuta, S. G. Pilyo, V. M. Prokopenko, V. V. Kovalishyn, L. O. Metelytsia, V. S. Brovarets, Comput. Biol. Chem. 74 (2018) 294-303.

25) K. P. Rakesh, S. M. Wang, J. Leng, L. Ravindar, A. M. Asiri, H. M. Marwani, H. L. Qin, Anticancer Agents Med. Chem. 18 (2018) 488-505.

26) K. F. Hussain, A. Ashawa, B. L. Verma, Arabian J. Chem. 9 (1997) 86-90.

27) V. Theodorou, M. Gogou, A. Giannoussi, K. Skobridis, ARKIVOC IV (2014) 11-23.

28) Y. Priyadarshini Devi, A. Uma, M. N. Lakshmi, C. Kalyani, Int. J. Res. Appl. Nat. Soc. Sci. 2 (2014) 269-272.

29) I. Vermes, C. Haanen, H. Steffens-Nakken, C. Teutelinger, J. Immunol. Methods 184 (1995) 39–51.

30) M. Van Engeland, L. J. W. Nieland, F. C. S. Ramaekers, B. Schutte, C. P. M. Reutelingsperger, Cytometry 31 (1998) 1–9.

31) A. Cossarizza, M. Baccaranicontri, G. Kalashnikova, C. Franceschi, Biochem. Biophys. Res. Comm. 197 (1993) 40-45.

32) C. Focaccetti, A. Bruno, E. Magnani, D. Bartolini, E. Principi, K. Dallaglio, E. O. Bucci, G. Finzi, F. Sessa, D. M. Noonan, A. Albini,, PLoS One 10 (2015) e0115686.

33) D. Trachootham, W. Lu, M. A. Ogasawara, R. D. Nilsa, P. Huang, Antioxid. Redox Signal 10 (2008) 1343–1374.

Mechanistic investigations on substituted benzene sulphonamides as apoptosis inducing anticancer agents

Akhila Mettu,[a,c] Venu Talla,[b] Soujanya Thumma,[a] Subhashini Naikal James Prameela,*[c]

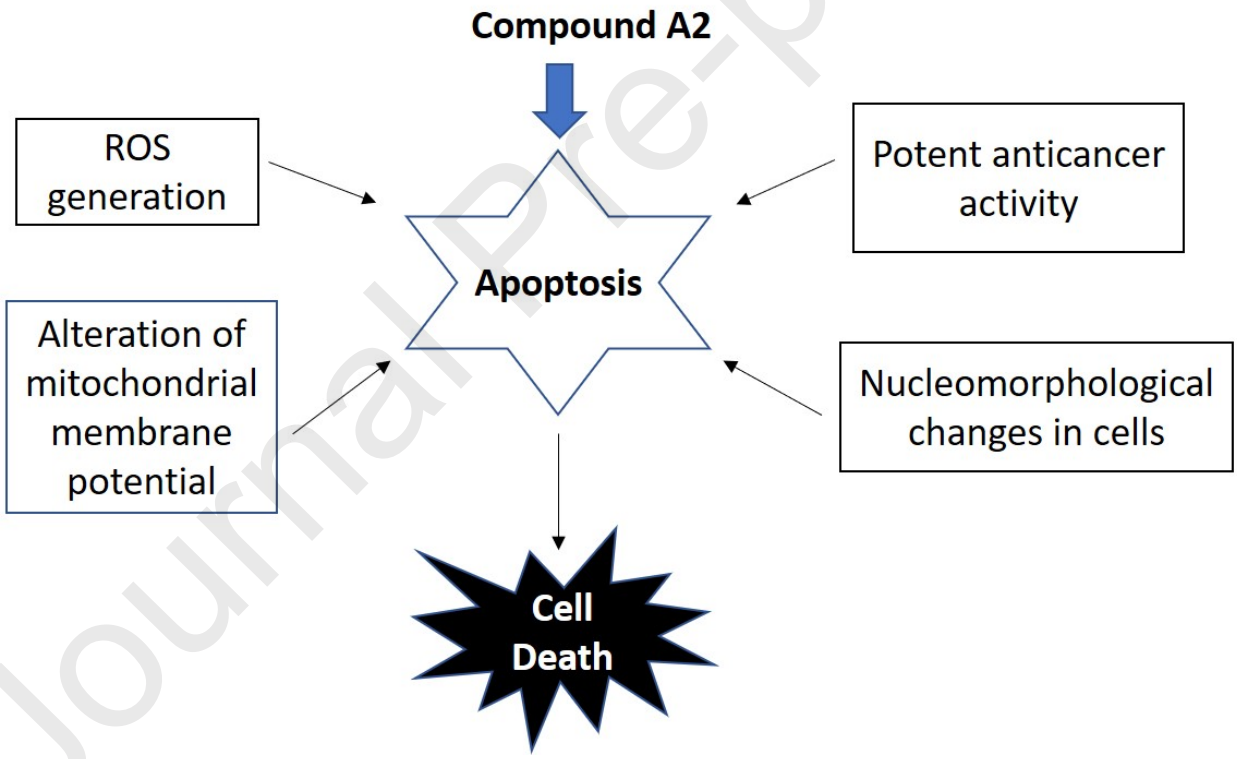
[a]Department of Pharmaceutical Chemistry, Gokaraju Rangaraju College of Pharmacy, Kukatpally, Hyderabad, India 500049.

[b]Department of Pharmacology, National Institute of Pharmaceutical Education and Research, Balanagar, Hyderabad, India 500037.

[c]Department of Chemistry, University College of Science, Osmania University, Hyderabad, India 500007.

Email: njsubhashini@yahoo.co.in

Graphical Abstract



Mechanistic investigations on substituted benzene sulphonamides as apoptosis inducing anticancer agents

Akhila Mettu,[a,c] Venu Talla,[b] Soujanya Thumma,[a] Subhashini Naikal James Prameela,*[c]

[a]Department of Pharmaceutical Chemistry, Gokaraju Rangaraju College of Pharmacy, Kukatpally, Hyderabad, India 500049.

[b]Department of Pharmacology, National Institute of Pharmaceutical Education and Research, Balanagar, Hyderabad, India 500037.

[c]Department of Chemistry, University College of Science, Osmania University, Hyderabad, India 500007.

Email: njsubhashini@yahoo.co.in

Highlights

- Four variants of benzene sulphonamides A1-A14 were designed, synthesized and characterized analytically.
- Compounds were excellent anti-proliferative agents tested on four cell lines.
- Apoptotic mechanisms were exemplified in DU-145 cells by various mechanistic studies.
- Compounds A2 was the most potent compound of the series.
- Structure activity relationships were established.