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ARTICLE

Synthesis of 2-anilinopyridyl linked benzothiazole hydrazones as apoptosis inducing cytotoxic agents

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Abstract: A series of 2-anilinopyridyl linked benzothiazole-hydrazone conjugates (**5a-aa**) were designed synthesized and evaluated for their *in vitro* cytotoxic potency against a panel of cancer cell lines like mouse skin melanoma (B16F10), lung adenocarcinoma (A549**)**, breast adenocarcinoma (MCF-7), triple negative breast cancer (MDA-MB-231) and normal lung epithelial (L132). Preliminary screening results revealed that some of these conjugates like **5i** and **5l** exhibited significant antiproliferative effect against human breast cancer (MCF-7) with IC_{50} values of 1.03 and 1.69 µM respectively. Further, the detailed biological studies of this promising conjugate (**5i**) were carried out on the MCF-7 cells. The flow cytometric analysis revealed that this conjugate induce cell-cycle arrest in the G2/M phase in a dose dependent manner. Furthermore, in order to determine the effect of the conjugate on cell viability various cell based assays such as clonogenic assay, ethidium bromide staining, Hoechst staining, detection of ROS generation and annexin V–FITC/PI assays were performed. In these studies, apoptotic features were clearly observed indicating that this conjugate inhibited cell proliferation by apoptosis.

Introduction

Considering the increasing incidence of cancers associated with high mortality and tumour heterogeneity, its therapy is always a real challenge. Moreover poor prognosis, non-selectivity, acute toxicity and cellular drug resistance justifies the growing interest for designing, developing and the identification of newer chemotherapeutic agents for cancer treatment.[1] It is currently considered that deregulation of apoptosis can disrupt the delicate balance between cell proliferation and cell death and can lead to the development of cancer.[2,3] Literature reports are also suggestive of a link between apoptosis-inducing ability and antitumor efficacy of chemotherapeutic agents,[4] therefore further establishing the clinical significance of apoptosis towards overall tumour sensitivity. Apoptosis generally occurs through two pathways, the mitochondrial-intrinsic pathway and the death receptor pathway both resulting in characteristic morphological cellular changes such as cell shrinkage, blebbing of the plasma membrane prior to cell lysis, chromatin condensation and DNA fragmentation[5,6] which finally leads to cell death. Accordingly, proteins that regulate programmed cell death could represent potential cancer drug targets and apoptosis inducers represent potential anticancer agents, therefore their investigation provides an attractive approach for the discovery and development of anticancer agents.

Benzothiazole is a privileged bicyclic potent pharmacophore, exhibiting wide spectrum of biological activities like anticancer,[7] antibacterial,[8] antifungal,[9] anti-inflammatory,[10] anticonvulsant,[11] anti-viral,[12] antitubercular[13] and antidiabetic.[14] Thus, this scaffold is highly important in the medicinal chemistry and can be used as a template with new chemical group insertions and modifications, thus providing bioactive structural frameworks that could facilitate hit exploration in the early stage of drug discovery. E7010 (**2**) is a synthetic antimitotic sulphonamide based drug having acceptable toxicity profile, oral bioavailability, and is insensitive to multidrug resistance (MDR). Thus, proving to be a promising scaffold that could be further explored for the development of potential E7010 conjugates. Moreover, the azomethine (-NHN=CH-) group is also bioactive and is observed in many marketed anticancer drugs, e.g.: triapine (**1**) and some representative benzothiazole, 2-anilinopyridyl and hydrazone derivatives are illustrated in Figure. 1

In continuation to our research for the development of potential chemotherapeutic leads and considering the promising antiproliferative profile of benzothiazoles, hydrazones as well as E7010, and moreover based on our previous reported potential leads.[15,16] We herein, have designed some 2-anilino pyridyl linked benzothiazole

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hydrazone conjugates by replacing the sulphonamide group of the E7010 (**2**) with benzothiazole group of 2-anilinonicotinyl linked 2-amino benzothiazoles (**4**), the aminopyridylhydrazine linkage as observed in marketed drug triapine (**1**) is also incorporated in the conjugates (design strategy in Figure 2). Moreover this is in anticipation of hybridizing these bioactive pharmacophores with complementary functions to result in a hybrid with enhanced therapeutic effects[17] in comparison to the parent structural components.

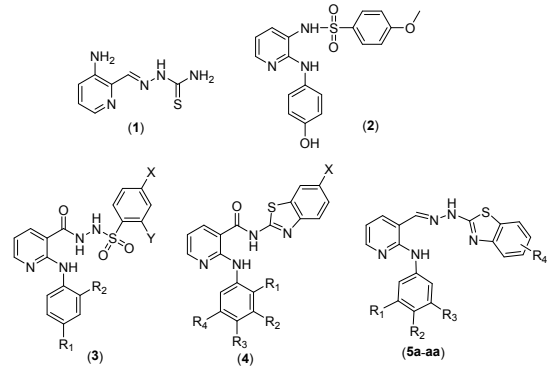


Figure 1. Chemical structures of some anticancer molecules: Triapine (**1**), E7010 (**2**), 2-anilino substituted nicotinyl arylsulfonylhydrazides (**3**),[15] 2-anilinonicotinyl linked 2-aminobenzothiazoles (**4**),[16] and synthesized 2-anilino pyridyl linked benzothiazole hydrazone conjugates (**5a-aa**) .

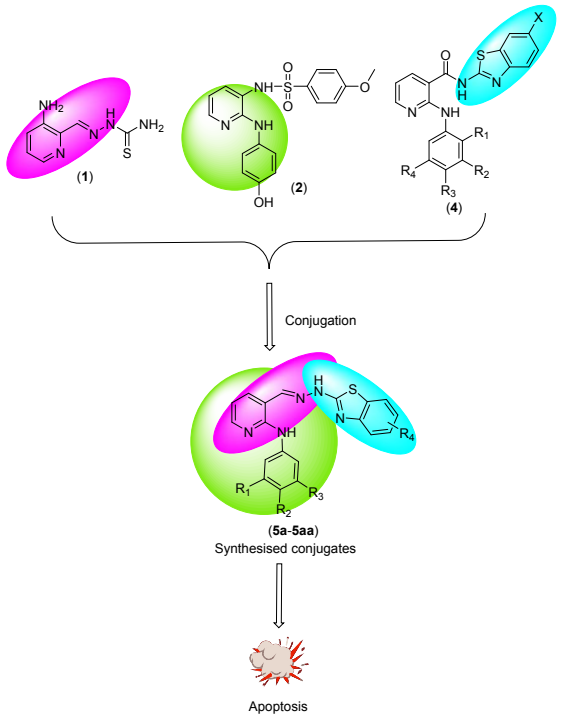


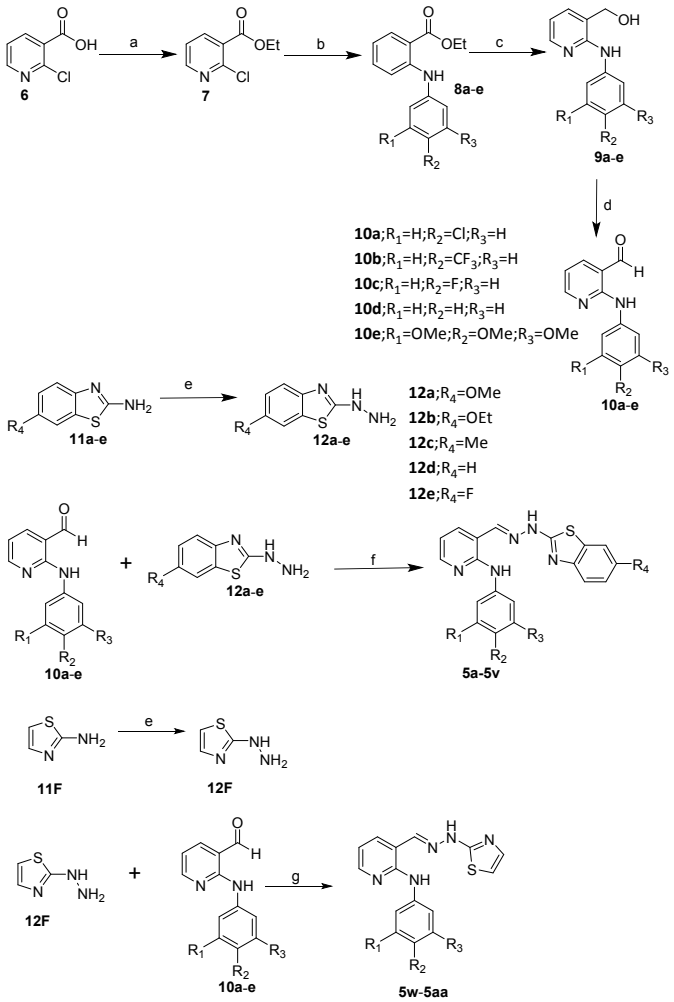
Figure 2. Design strategy of 2-anilino pyridyl linked benzothiazole hydrazone conjugates (**5a-aa**): Triapine (**1**), E7010 (**2**), 2-anilinonicotinyl linked 2-amino benzothiazoles (**4**) .

A library of compounds corresponding to this template with several substituents on both the pharmacophores has been synthesized to establish the SAR and to explore the possibility of developing new potent antitumor agents. Thus, we herein report the synthesis, cytotoxicity and structure-activity relationship (SAR) studies of 3-((2-(benzo[*d*]thiazol-2-yl)hydrazono)methyl)-N-phenylpyridin-2-amine (**5a-aa**). In addition, detailed biological investigations (for conjugate **5i**) such as clonogenic assay, Hoescht staining, acridine orange/ethidium bromide staining, measurement of reactive oxygen species (ROS), apoptosis detection assays and cell cycle analysis studies have been carried out and discussed herein.

Results and Discussion

Chemistry

The E7010 linked benzothiazole hydrazone conjugates (**5a-aa**) were synthesized as depicted in Scheme 1. The key intermediates, substituted 2-(phenylamino)nicotinaldehydes (**10a–e**) and 2-hydrazinylbenzo[*d*]thiazole (**12a-e**) were synthesised in sequential steps as discussed below.

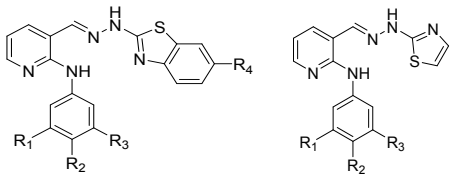


Scheme 1. Synthesis of 2-anilinopyridyl linked benzothiazole hydrazone conjugates; reagents and conditions: (a) Cat. H_2SO_4, EtOH, reflux, 2 h, 79%; (b) substituted anilines, ethylene glycol, 120 °C, 6 h, 73–81%; (c) LAH, THF, 0 °C, 3 h, 71–78%; (d) IBX, DMSO, rt, 30 min, 73–82%; (e) Hydrazine monohydrate, ethylene glycol, conc. HCl, reflux at 140 ° C, 3-4 h, 70-80%;(f) AcOH, EtOH, 3-4 h, reflux, 70–85% (g) AcOH, EtOH, 3-4 h, rt, 70–85% .

2-(Phenylamino)nicotinaldehyde synthesis involves 2-chloronicotinic acid (**6**), which was converted to ethyl-2-

chloronicotinate (**7**), by refluxing in ethanol for 2 hours in presence of catalytic amount of conc. H_2SO_4. The ester obtained was heated with substituted anilines in ethylene glycol at 140 °C for 6 h to provide the coupled product, ethyl 2-(phenylamino)nicotinate (**8a–e**). These esters were reduced by LAH in dry THF to give the corresponding alcohols (**9a-e**), which were selectively oxidized to 2-(phenylamino)nicotinaldehydes (**10a–e**) using 2-iodoxy benzoic acid in DMSO. On the other hand, 2-hydrazinyl benzothiazoles (**12a-e**) were synthesized by the reaction of hydrazine hydrate with substituted 2-amino benzothiazoles (**11a-e**) in ethylene glycol with catalytic amount of con. HCl.[18]

Table 1. Generic structure of conjugates **5a-aa**.



5a-v **5w-aa**

Compound	R_1	R_2	R_3	R_4
5a	H	Cl	H	OMe
5b	H	CF_3	H	OMe
5c	H	F	H	OMe
5d	H	H	H	OMe
5e	H	Cl	H	OEt
5f	H	CF_3	H	OEt
5g	H	F	H	OEt
5h	H	H	H	OEt
5i	H	Cl	H	Me
5j	H	CF_3	H	Me
5k	H	F	H	Me
5l	H	H	H	Me
5m	H	Cl	H	H
5n	H	CF_3	H	H
5o	H	F	H	H
5p	H	H	H	H
5q	OMe	OMe	OMe	H
5r	H	Cl	H	F
5s	H	CF_3	H	F
5t	H	F	H	F
5u	H	H	H	F
5v	OMe	OMe	OMe	F
5w	H	Cl	H	-
5x	H	CF_3	H	-
5y	H	F	H	-
5z	H	H	H	-
5aa	OMe	OMe	OMe	-

Finally, the desired 2-anilinopyridyl linked benzothiazole hydrazone conjugates (5a-aa) were obtained by condensation of 2-hydrazinyl benzothiazoles with 2-(phenylamino)nicotinaldehydes in the presence of catalytic amount of glacial acetic acid in ethanol and the product obtained was purified by recrystallisation in ethanol and all the conjugates were obtained in high yields.

Notably, they were synthesized with diverse substituents of both electron donating as well as withdrawing nature on each of the pharmacophores (Table 1). They were characterized by spectral analysis such as ^{1}H NMR, ^{13}C NMR, mass and HRMS.

Biological activity

Antiproliferative activity

All these conjugates **5a**-**aa** were evaluated for their cytotoxic activity against different cell lines including human breast adenocarcinoma (MCF-7), triple negative breast cancer (MDA-MB-231), lung adenocarcinoma (A549), mouse skin melanoma (B16F10) and normal lung epithelial (L132) cell lines.

Table 2. IC_{50} values[a] ± SD (in μM) for compounds in selected cancer cell lines.

Conjugates	B16F10[b]	A549[c]	MCF-7[d]	MDA-MB-231[e]	L132[f]
5a	1.25±0.2	>20	1.32±0.4	15.68±1.6	12.29±4.5
5b	2.19±0.1	6.56±0.21	2.50±0.8	12.80±0.2	13.82±4.3
5c	1.05±0.1	2.38±0.31	3.01±0.5	19.18±1.8	18.59±2.7
5d	1.19±0.3	2.19±0.46	1.20±0.4	>20	>20
5e	4.00±0.9	>20	>20	>20	>20
5f	2.04±0.6	>20	>20	>20	17.58±5.4
5g	1.10±0.1	12.02±1.5	1.68±0.7	19.38±2.7	13.73±2.9
5h	1.65±0.2	4.72±0.81	2.29±0.1	>20	15.44±5.8
5i	0.74±0.1	1.23±0.37	1.03±0.8	6.22±0.1	12.69±3.5
5j	1.07±0.3	2.65±0.39	2.28±0.1	18.61±15	12.37±14
5k	0.96±0.1	1.10±0.16	>20	15.03±01	13.0±4.3
5l	0.93±0.03	1.18±0.25	1.69±0.4	4.54±0.2	19.64±0.7
5m	0.96±0.1	4.97±0.84	1.64±0.17	6.61±1.31	11.76±2.7
5n	0.93±0.2	16.72±1.89	0.96±0.04	8.43±1.58	>20
5o	0.92±0.4	>20	2.38±0.45	9.98±0.42	14.56±3.6
5p	>20	>20	1.73±0.18	>20	>20
5q	>20	>20	2.49±0.34	>20	>20
5r	1.13±0.5	>20	>20	>20	>20
5s	0.92±0.3	>20	1.17±0.2	>20	>20
5t	>20	>20	>20	>20	>20
5u	>20	>20	>20	>20	>20
5v	3.01±0.8	>20	>20	>20	>20
5w	>20	>20	>20	>20	>20
5x	1.01±0.5	>20	>20	>20	>20
5y	>20	>20	>20	>20	>20
5z	>20	>20	>20	>20	>20
5aa	>20	>20	>20	>20	>20
E 7010		1.5±0.2	1.2±0.5	4.01±0.2	

[a]50% Inhibitory concentration after 48 h of drug treatment and the values are average of three individual experiments, [b]mouse skin melanoma, [c]lung adenocarcinoma, [d]breast adenocarcinoma, [e]triple negative breast cancer, [f]normal lung epithelial cells).

Inhibitory concentration (IC_{50}), a concentration required to inhibit proliferation of 50% cells after exposure to test compounds was determined using MTT assay. The IC_{50} values of different conjugates on the above cell lines are shown in Table 2 along with IC_{50} values of reference standard, E7010 (**2**). The preliminary results indicated that some of these conjugates were active and they (**5d**, **5i**, **5n** and **5s**) showed superior cytotoxic activity than E7010 (**2**), with IC_{50} values ranging from 0.9 to 1.3 µM. Wherein the conjugate **5i** showed maximum cell growth inhibition activity on MCF-7 cell line (IC_{50} 1.03 ± 0.08 µM). In addition, conjugate **5l** also showed significant activity on MCF-7 cells with an IC_{50} value of 1.69 ± 0.24 µM. Based on the promising results obtained with preliminary screening, conjugate **5i** was selected for detailed biological studies.

Several crucial structural requirements which contributed towards the potency were revealed by the SAR study. By exemplifying different substitution pattern on A and D ring, conjugates possessing both electron withdrawing as well as electron donating substituents were synthesized and their cytotoxicity was evaluated. Substituent like OEt (**5e**), F (**5t**, **5u**) at the 6th position of ring D proved to be slightly less beneficial towards the cytotoxicity, whereas presence of substituents like H (**5m**, **5n**), CH_3 (**5i**, **5j**, **5l**) and OMe groups (**5a**, **5d**) seems to enhance the cytotoxicity. The observed order of improved potency with the substituents on ring D may be represented as CH_3>H>OMe>OEt>F. It is noteworthy to observe that substituents like Cl (**5i**, **5m**) and H (**5d**, **5h**, and **5n**) at 4th position of the ring A proved beneficial towards activity and substituents like CF_3 (**5b**, **5j** and **5n**) and F (**5c**, **5g**, **5o**) were found to be well tolerated. However, the presence of trimethoxy group (**5q**, and **5v**) at ring A did not prove beneficial and absence of ring D (**5w-aa**) leads to complete loss of activity, thereby demonstrating the importance of benzene ring or ring D in terms of cytotoxicity. Intriguingly, conjugates **5i** and **5l**, the most active compounds in the series exhibited superior potency than the standard E7010 (**2**). The observed order of improved potency with the substituents on ring A may be represented as Cl>H>CF_3>F>3,4,5-triOMe. Therefore, it can be concluded that the presence of ring D and electron donating substituent on ring D and electron withdrawing substituent on ring A are beneficial for the cytotoxic activity and are pictorially represented in Figure 3.

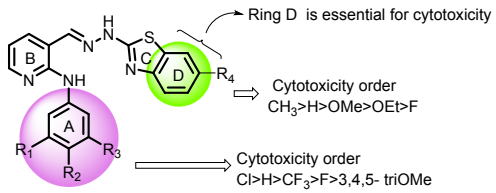


Figure 3. SAR analysis of 2-anilinopyridyl linked benzothiazole hydrazone conjugates.

Based on the promising results obtained with the preliminary screening, two cytotoxic conjugates **5i** and **5l** show significant activity on MCF-7 cells with an IC_{50} value of 1.03 and 1.69 µM. Conjugate **5i** was further subjected to investigations such as clonogenic assay, cell cycle analysis, reactive oxygen species (ROS) generation, ethidium bromide staining, Hoechst staining, change in mitochondrial membrane potential and apoptosis detection assay.

Clonogenic assay

Clonogenic assay was performed to assess the inhibitory effect of conjugate on colony forming capability of MCF-7 cells. These cells were allowed to grow in 6-well plate and treated with conjugate **5i**. The results clearly showed that there was a dose dependent growth inhibition effect of conjugate **5i** on MCF-7 cells. Moreover, the conjugate completely inhibited colony forming capability of single cancer cells at 5 and 10 µM as there were no visible colonies in the wells as shown in Figure 4.

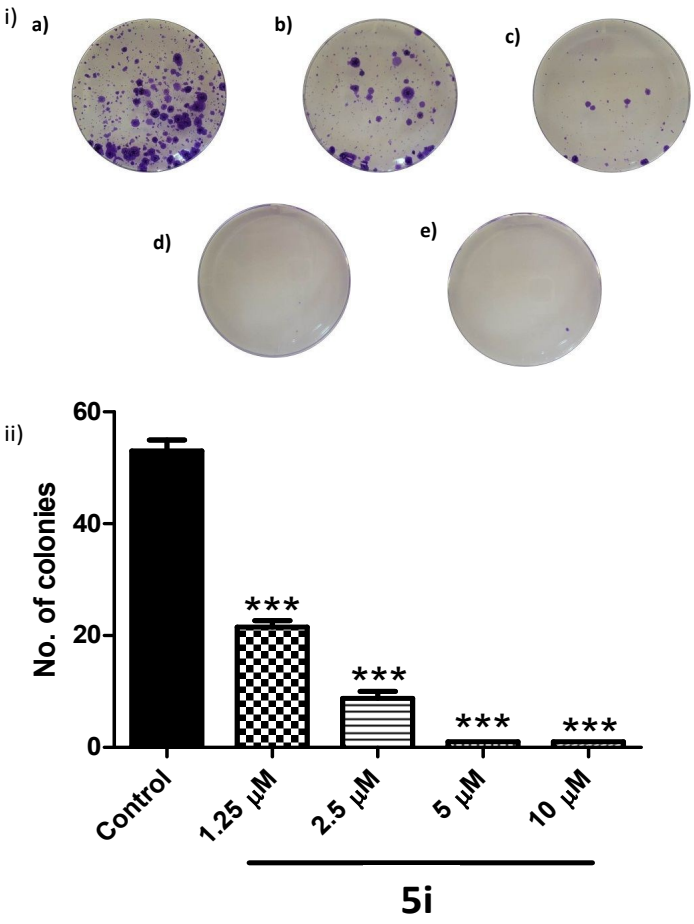


Figure 4. Effect of conjugate **5i** on colony forming capabilities of MCF-7 cells. i) a) Control cells (MCF-7), b) **5i** (1.25 µM), c) **5i** (2.5 µM), d) **5i** (5 µM), e) **5i** (10 µM) ii) Number of colonies after treatment of the cells with increasing concentration of the compound **5i**.

Acridine orange/ethidium bromide (AO/EB) staining

To further confirm the cytotoxic activity of these conjugates, acridine orange/ethidium bromide (AO/EB) dual staining was performed to visualize nuclear changes and apoptotic body formation, to differentiate between live and dead cells. The exposure of cells with conjugate **5i** resulted in alteration of cellular morphology. Florescent microscopic images revealed altered morphological characteristics in form of apoptotic body formation, cell shrinkage, cell fragments and membrane

blebbing suggesting induction of apoptosis by treatment with conjugate **5i** in MCF-7 cells as shown in Figure 5.

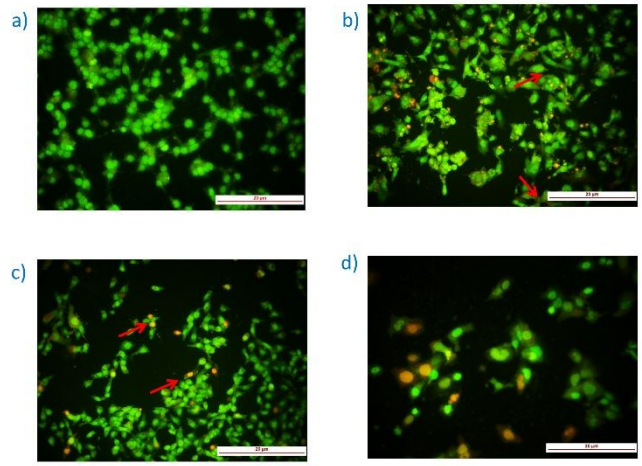


Figure 5. Acridine orange ethidium bromide (AO/EB) dual staining in MCF-7 cell line showing apoptosis. a) Untreated cells (MCF-7), b) **5i** (1.25 µM), c) **5i** (2.5 µM), d) **5i** (5 µM). The treated cells showed induction in apoptosis as revealed by crescent shaped cells stained with yellow green acridine orange (Early apoptotic) and orange colored cells stained with ethidium bromide (Late apoptotic cells) as represented by arrows.

Hoechst staining

Nuclear damage is a critical prerequisite for cellular death. To examine nuclear damaging potential of these conjugates, nuclear morphology was studied using Hoechst 33342 staining method. Treated and untreated cells were washed with PBS twice followed by staining with Hoechst dye to stain the nuclei. The stained nuclei were observed under fluorescent microscope using DAPI filter at 400X magnification. The untreated control group showed no alteration in nuclear morphology while exposure of cells with conjugate **5i** produced clearly visible nuclear fragments as shown in Figure 6 (marked by arrows). The results clearly demonstrated that cells treated with conjugate **5i** showed condensed, fragmented and sickle shaped nuclei which are characteristic features of cells undergoing apoptosis.

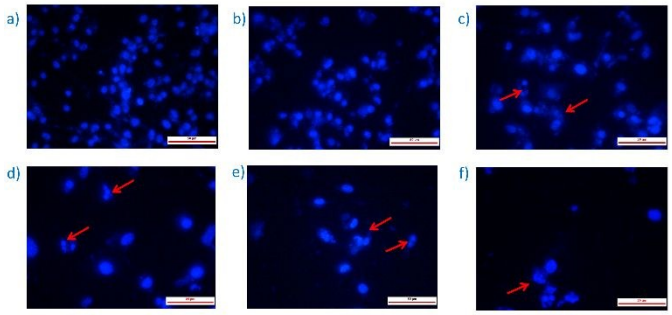


Figure 6. Hoechst staining in MCF-7 cell line. a) Control cells (MCF-7), b) **5i** (0.625 µM), c) **5i** (1.25 µM), d) **5i** (2.5 µM), e) **5i** (5 µM) and f) **5i** (10 µM). The cells treated with compound **5i** showed nuclear condensation and sickle shaped nuclei as represented by arrows suggesting the nuclear damaging potential of the compound.

Detection of intracellular reactive oxygen species (ROS) by DCFH-DA

Elevation in intracellular ROS is known to be involved in induction of apoptosis in cancer cells. Intracellular levels of ROS were estimated by using DCFH-DA staining method. MCF-7 cells were treated with conjugate **5i** at 0.625, 1.25, 2.5, 5, and 10 µM for 6 hours.

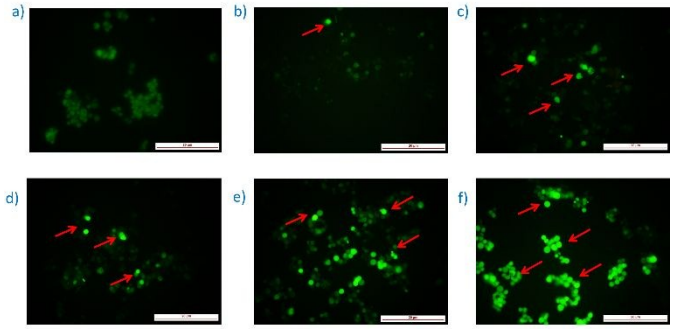


Figure 7. Determination of ROS generation in MCF-7. Fluorescence images are obtained from the cells treated with the conjugate 5i for 24 h and then observed the production of ROS by DCFH-DA. a) Control cells (MCF-7), b) 5i (0.625 µM), c) **5i** (1.25 µM), d) **5i** (2.5 µM), e) **5i** (5 µM) and f) **5i** (10 µM). There was a concentration dependent increase in the bright green florescence as compared to untreated cells which indicated that the conjugate **5i** increased intracellular ROS in MCF-7 in a concentration dependent manner. The increased ROS in the MCF-7 cells could be one of the potential mechanisms behind cytotoxic potential of the conjugates.

The results showed increased intensity in green florescence in a dose dependent manner when compared to control indicating induction of increase in intracellular ROS production by conjugate **5i** (Figure 7) which could ultimately lead to apoptosis.

Measurement of mitochondrial membrane potential (ΔΨm)

Furthermore, we evaluated ROS-induced mitochondrial membrane potential (MMP) alteration in MCF-7 cells, as alteration in MMP (ΔΨm) is implicated as one of the major pathways for cellular death. To further confirm changes in MMP (ΔΨm) due to increased ROS we used JC-1 staining method. JC-1 is a fluorescent dye which gives a red colour as monomer while in the presence of altered MMP it gets aggregated and imparts green coloration. Treatment of cells for 24 hours showed increased green signal as shown in the Figure 8 clearly indicating the alteration of MMP (ΔΨm) by different concentrations of conjugate **5i**. Moreover at 10 µM concentration almost all cells stained with green colour confirming alteration in MMP.

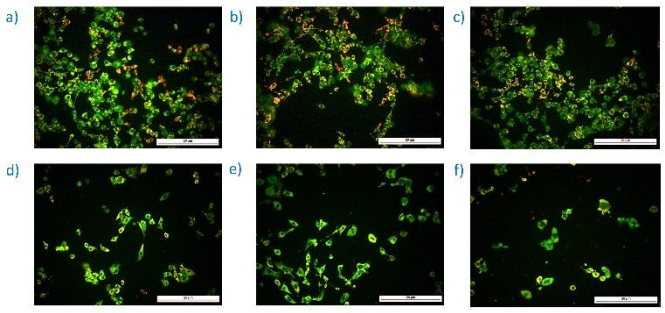


Figure 8. MCF-7 cells treated with test compound **5i** triggers mitochondrial injury. Drop in membrane potential (ΔΨm) was assessed by JC-1 staining and the images were captured by a fluorescent microscope. a) Control cells (MCF-7), b) **5i** (0.625 µM), c) **5i** (1.25 µM), d) **5i** (2.5 µM), e) **5i** (5 µM) and f) **5i** (10 µM). The treated cells showed a

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drop in mitochondrial membrane potential (ΔΨm) which was evident by increase in green florescence after treatment of the cells with compound **5i**. Further, the drop in ΔΨm was found to be in a concentration dependent fashion.

Detection of apoptosis by annexin V-FITC/propidium iodide staining

The arrest of cell growth in a specific phase of cell cycle may lead to apoptosis of cells.[24] To evaluate the extent of apoptosis carried out by conjugate **5i**, we used florescent conjugate of annexin V with FITC along with PI. The results obtained with annexin V assay showed 91.37% cells were alive in untreated control group. On the other hand, population of early apoptotic cells increased from 2.80% to 8.39 and 10.3% by treatment with conjugate **5i** with 1 and 3 µM, respectively.

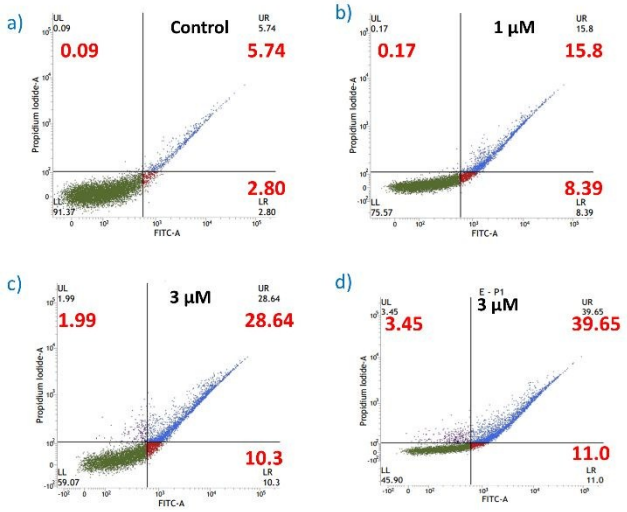

Figure 9. Newly synthesized conjugates induce apoptosis in MCF-7 cells. Cells were treated with **5i** and E7010 (as positive control) for 24 h. Cells were labelled with annexin V FITC, PI and analysed by flow cytometry. a) Control cells, b) **5i** (1 µM), c) **5i** (3 µM) and d) E7010 (3 µM). The compound **5i** increased almost 3-fold and 3.5-fold increase in the early apoptotic cells at a concentration of 1 and 3 µM, respectively. In addition, **5i** also caused almost 5-fold increase in the late apoptotic/necrotic cells at 3 µM as compared to untreated cells.

In addition, late apoptotic/necrotic cells increased from 5.74% to 15.80 and 28.64% by exposure of cells to conjugate **5i** at 1 and 3 µM, respectively. Moreover E7010 caused increase in early apoptotic cells from 2.80 to 11.0% at concentrations of 3 µM. Therefore the percentage increase in number of early and late apoptotic cells confirmed that conjugates induced apoptosis in MCF-7 cells (Figure 9).

Cell cycle analysis

To evaluate the role of cell cycle arrest in imparting cytotoxic potential to compounds, MCF-7 cells were treated with different concentration of conjugate **5i** followed by observation of cell cycle distribution by flow cytometer. The results clearly showed G2/M phase arrest when compared to control as shown in Figure 10. Following treatment for 24 hours with conjugate **5i** at 3 µM concentration reduced the fraction of cells in G1 phase from 76.61% to 64.56%. However, at the same time, an increased fraction of cells present in S and G2/M phase was observed. Conjugate **5i** increased the fraction of cells present in G2/M phase from 15.94% to 22.78% at 3 µM concentration respectively. The results obtained with cell cycle analysis revealed that the growth inhibition induced by these compounds was mainly due to G2/M phase arrest in MCF-7 cells (Figure 10, Table 2).

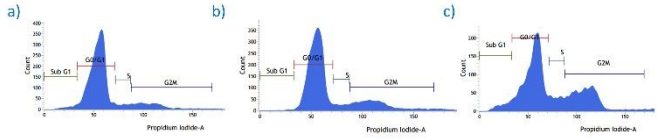

Figure 10. Cell cycle analysis of MCF-7 cells treated with conjugate **5i** at indicated concentrations for 24 h. a) Control cells (MCF-7), b) **5i** (1µM), c) **5i** (3 µM). The untreated cells showed normal cell cycle behaviour while on the other hand, the treated cells showed a G2/M specific cell growth arrest. The increase in the population in G2/M phased indicated that the conjugate 5i halted the cell growth and arrested the cell cycle in G2/M phase.

Table 2. Flow cytometric analysis in MCF-7 breast cancer cell line after treatment with conjugate 5i.

Conjugate	Sub G1	G0/G1	S	G2/M
Control	2.76	77.04	4.43	14.03
5i(1µm)	0.66	76.61	5.00	15.94
5i(3µm)	3.42	64.56	7.02	22.78

Conclusion

In summary, a library of 2-anilinopyridyl linked benzothiazole hydrazone conjugates (**5a**-**aa**) were synthesised and evaluated for their anticancer potential. The majority of these compounds demonstrated significant antiproliferative activities against tested cancer cell lines. SAR study revealed the crucial structural requirements of the conjugates like presence of ring D and electron donating substituents on ring D and electron withdrawing substituents on ring A, which contributed towards the potency. Amongst the synthesised conjugates, **5i** and **5l** that contain CH_3 group on ring D and Cl and H group on ring A were found to be the most active with an IC_{50} value of 1.03 and 1.69 µM against MCF-7 cells. Moreover, cell cycle analysis indicates that conjugate **5i** arrest MCF-7 cells in S and G2/M phase. Subsequent cell viability studies such as clonogenic assay, acridine orange/ethidium bromide staining, Hoescht staining, generation of ROS, changes in mitochondrial membrane potential, annexin V–FITC/PI assay confirms that this conjugate induce apoptosis. Based on these results, conjugate **5i** could be considered as potential lead in the development of chemical libraries that would serve as a source of potential drugs for treating breast cancer.

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Experimental section

Chemistry

Materials and methods

All chemicals and reagents were obtained from Aldrich (Sigma-Aldrich), St. Louis, MO, USA, Lancaster (Alfa Aesar, Johnson Matthey Company, Ward Hill, MA, USA), or Spectrochem Pvt. Ltd (Mumbai, India) and were used without further purification. Reactions were monitored by TLC performed on a silica gel glass plate containing 60 GF-254, and visualization was done by iodine indicators or UV light. Column chromatography was performed with Merck 60–120 mesh silica gel. ^{1}H and ^{13}C NMR spectra were recorded in TFA-D + $CDCl_3$ by using Avance and Varian instruments. Chemical shifts are expressed in parts per million (δ in ppm) downfield from internal TMS and coupling constants are expressed in Hz. ^{1}H NMR spectroscopic data are reported in the following order: multiplicity (s, singlet; brs, broad singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet), coupling constants in Hz, number of protons. ESI mass spectra were recorded on Micro mass Quattro LC using ESI+ software with capillary voltage 3.98 kV and an ESI mode positive ion trap detector. Melting points were determined with Electrothermal melting point apparatus, and are uncorrected.

2-(Arylamino)nicotinaldehydes (**10a–e**) intermediates were prepared by using the reported protocol.[17] Likewise, 2-hydrazinylbenzo[d]thiazoles (**12a**-**e**) were prepared by using the reported protocol.[18]

General procedure for the synthesis of 3-((2-(benzo[d]thiazol-2-yl)hydrazono)methyl)-N-phenylpyridin-2-amines (5a-aa):

2-(arylamino)nicotinaldehyde (**10a-e**) and 2-hydrazinylbenzo[d]thiazole (**12a-e**) were dissolved in EtOH (10 mL) and glacial acetic acid (2–3 drops) was added. The reaction mixture was held at reflux for 3–4 h (reaction monitored by TLC) and the precipitate formed on cooling was collected by filtration and recrystallised in EtOH to obtain the pure product (**5a-aa**).

N-(4-Chlorophenyl)-3-((2-(6-methoxybenzo[d]thiazol-2-yl)hydrazono)methyl)pyridin-2-amine (5a)*:*

Compound 5a was prepared according to the above explained general method by employing 2-((4-chlorophenyl)amino)nicotinaldehyde (**10a**, 100 mg, 0.43 mmol) and 2-hydrazinyl-6-methoxybenzo[*d*]thiazole (**12a**, 84mg, 0.43mmol) to afford **5a** as yellow solid, yield: 82%, Mp: 336-339 °C, ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) *δ* 8.76 (s, 1H), 8.34 (d, *J* = 7.0 Hz, 1H), 7.99 (d, *J* = 5.4 Hz, 1H), 7.62 (m, 3H), 7.42 (d, *J* = 7.7 Hz, 2H), 7.26 (m, 3H), 3.91 (s, 3H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) *δ* 167.10, 158.76, 149.72, 149.37, 148.85, 138.32, 137.44, 131.86, 131.40, 130.06, 127.79, 123.96, 117.80, 117.02, 116.15, 114.83, 106.96, 56.42. MS (ESI): *m/z* 410 $[M+H]^+$; HRMS calculated for $C_{20}H_{17}N_5OClS$ $[M+H]^+$ 410.08369, found 410.08239.

3-((2-(6-Methoxybenzo[d]thiazol-2-yl)hydrazono)methyl)-N-(4-(trifluoromethyl)phenyl)pyridin-2-amine (5b):

Compound **5b** was prepared according to the above explained general method by employing 2-((4-(trifluoromethyl)phenyl)amino)nicotinaldehyde (**10b**, 100 mg, 0.37 mmol) and 2-hydrazinyl-6-methoxybenzo[d]thiazole (**12a**, 73 mg, 0.37 mmol) to afford **5b** yellow solid, yield: 85%, Mp: 324-327 °C,^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) *δ* 8.85 (m, 1H), 8.43 (m, 1H), 8.06 (m, 1H), 7.93 (m, 1H), 7.82 (m, 3H), 7.69 (m, 1H), 7.32 (m, 3H) 3.94 (s, 3H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) *δ* 158.87, 157.91, 149.90, 149.45, 149.16, 138.44, 132.61, 130.06, 127.83, 124.11, 123.72, 117.95, 117.08, 115.30, 107.19, 56.48. MS (ESI): m/z 444 $[M+H]^+$; HRMS calcd for $C_{21}H_{16}N_5OF_3S$ $[M+H]^+$ 444.1100, found 444.1080.

***N*-(4-Fluorophenyl)-3-((2-(6-methoxybenzo[d]thiazol-2-yl) hydrazono)methyl)pyridin-2-amine (5c):**

Compound **5c** was prepared according to the above explained general method by employing 2-((4-fluorophenyl)amino)nicotinaldehyde (**10c**, 100 mg, 0.46 mmol) and 2-hydrazinyl-6-methoxybenzo[*d*]thiazole (**12a**, 90 mg, 0.46 mmol) to afford **5c** yellow solid, yield: 85%, Mp: 316-319 °C, ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) *δ* 8.77 (s, 1H), 8.33 (d, *J* = 7.3 Hz, 1H), 7.98 (d, *J* = 5.4 Hz, 1H), 7.64 (d, *J* = 8.8 Hz, 1H), 7.51–7.39 (m, 2H), 7.35–7.25 (m, 3H), 7.20 (d, *J* = 10.7 Hz, 2H), 3.89 (s, 3H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 167.11, 158.67, 149.54, 148.52, 138.36, 131.47, 128.97, 128.85, 123.95, 118.80, 118.49, 117.60, 117.02, 116.45, 115.98, 114.55, 112.67, 106.79, 56.34. MS (ESI): *m/z* 394 $[M+H]^+$; HRMS calcd for $C_{20}H_{16}N_5OFS$ $[M+H]^+$ 394.11324, found 394.11167

3-((2-(6-Methoxybenzo[d]thiazol-2-yl)hydrazono)methyl)-N-phenylpyridin-2-amine (5d):

Compound **5d** was prepared according to the above explained general method by employing 2-(phenylamino)nicotinaldehyde (**10d**, 100 mg, 0.50 mmol) and 2-hydrazinyl-6-methoxybenzo[*d*]thiazole (**12a**, 98 mg, 0.50 mmol) to afford **5d** yellow solid, yield: 92%, Mp: 336-338 °C, ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) *δ* 8.77 (s, 1H), 8.32 (d, *J* = 7.4 Hz, 1H), 7.97 (d, *J* = 5.9 Hz, 1H), 7.67–7.57 (m, 4H), 7.45 (d, *J* = 7.7 Hz, 2H), 7.20 (td, *J* = 5.4, 2.5 Hz, 3H), 3.88 (s, 3H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) *δ* 167.19, 158.65, 149.62, 149.32, 148.55, 138.31, 131.58, 131.48, 130.84, 126.23, 123.99, 117.59, 117.00, 115.98, 114.39, 108.90, 56.34. MS (ESI): m/z 376 $[M+H]^+$; HRMS calcd for $C_{20}H_{17}N_5OS$ $[M+H]^+$ 376.12266, found 376.12159.

N-(4-Chlorophenyl)-3-((2-(6-ethoxybenzo[d]thiazol-2-yl) hydrazono)methyl)pyridin-2-amine (5e):

Compound **5e** was prepared according to the above explained general method by employing 2-((4-chlorophenyl)amino)nicotinaldehyde (**10a**, 100 mg, 0.43 mmol) and 6-ethoxy-2-hydrazinylbenzo[d]thiazole (**12b**, 90mg, 0.43mmol) to afford **5e** yellow solid, yield: 85%, Mp: 312-315^{0}C , ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) *δ* 8.75 (s, 1H), 8.33 (d, *J* = 7.5 Hz, 1H), 7.99 (d, *J* = 6.3 Hz, 1H), 7.66–7.58 (m, 3H), 7.42 (d, *J* = 8.5 Hz, 2H), 7.25 (dt, *J* = 18.9, 5.6 Hz, 3H), 4.12 (q, *J* = 6.8 Hz, 2H), 1.46 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) *δ* 167.04, 158.11, 149.66, 148.84, 138.28, 137.43,

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131.86, 131.27, 130.07, 127.79, 123.90, 118.41, 116.98, 114.83, 107.57, 65.38, 14.65. MS (ESI): *m/z* 424 $[M+H]^+$; HRMS calcd for $C_{21}H_{18}N_5OClS$ $[M+H]^+$ 424.09934, found 424.09808.

3-((2-(6-Ethoxybenzo[d]thiazol-2-yl)hydrazono)methyl)-N-(4-(trifluoromethyl)phenyl)pyridin-2-amine (5f):

Compound **5f** was prepared according to the above explained general method by employing 2-((4-(trifluoromethyl)phenyl)amino)nicotinaldehyde (**10b**, 100 mg, 0.37 mmol) and 6-ethoxy-2-hydrazinylbenzo[d]thiazole (**12b**, 78mg, 0.37mmol) to afford **5f** yellow solid, yield: 85%, Mp: 242-244 °C, ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.78 (s, 1H), 8.37 (d, *J* = 6.8 Hz, 1H), 8.00 (d, *J* = 5.4 Hz, 1H), 7.70-7.85 (m, 4H), 7.62 (d, *J* = 9.3 Hz, 1H), 7.26 – 7.17 (m, 3H), 4.09 (q, *J* = 7.0 Hz, 2H), 1.46 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 167.03, 158.08, 149.38, 149.17, 148.67, 138.49, 134.31, 132.72, 132.33, 131.28, 129.89, 123.89, 123.50, 118.21, 117.03, 115.15, 107.39, 65.13, 14.64. MS (ESI): m/z 458 $[M+H]^+$; HRMS calcd for $C_{22}H_{18}N_5OF_3S$ $[M+H]^+$ 458.12569, found 458.12347.

3-((2-(6-Ethoxybenzo[d]thiazol-2-yl)hydrazono)methyl)-N-(4-fluorophenyl)pyridin-2-amine (5g):

Compound **5g** was prepared according to the above explained general method by employing 2-((4-fluorophenyl)amino)nicotinaldehyde (**10c**, 100 mg, 0.46 mmol) and 6-ethoxy-2-hydrazinylbenzo[d]thiazole (**12b**, 96mg, 0.46mmol) to afford **5g** yellow solid, yield: 87%, Mp: 272-275 °C , ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.76 (s, 1H), 8.33 (d, *J* = 7.4 Hz, 1H), 7.97 (d, *J* = 6.1 Hz, 1H), 7.61 (d, *J* = 8.8 Hz, 1H), 7.51 – 7.42 (m, 2H), 7.32 (t, *J* = 7.4 Hz, 2H), 7.27 (d, *J* = 2.1 Hz, 1H), 7.16-7.24 (m, 2H), 4.11 (q, *J* = 13.9, 6.9 Hz, 2H), 1.46 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 167.02, 159.31, 158.74, 158.09, 149.64, 148.77, 138.16, 131.29, 129.01, 128.79, 127.40, 123.92, 120.33, 118.94, 118.67, 118.32, 116.93, 116.56, 116.22, 114.67, 112.79, 107.55, 65.32, 14.52. MS (ESI): m/z 408 $[M+H]^+$; HRMS calcd for $C_{21}H_{18}N_5OFS$ $[M+H]^+$ 408.12889, found 408.12663.

3-((2-(6-Ethoxybenzo[d]thiazol-2-yl)hydrazono)methyl)-N-phenylpyridin-2-amine (5h):

Compound **5h** was prepared according to the above explained general method by employing 2-(phenylamino)nicotinaldehyde (**10d**, 100 mg, 0.50 mmol) and 6-ethoxy-2-hydrazinylbenzo[d]thiazole (**12b**, 104 mg, 0.50 mmol) to afford **5h** yellow solid, yield: 92%, Mp: 274-276 °C, ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.76 (s, 1H), 8.32 (d, *J* = 7.0 Hz, 1H), 7.97 (d, *J* = 6.1 Hz, 1H), 7.70–7.56 (m, 4H), 7.48–7.41 (m, 2H), 7.18 (d, *J* = 10.5 Hz, 3H), 4.08 (q, *J* = 6.9 Hz, 2H), 1.45 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 167.15, 158.74, 158.18, 157.97, 149.42, 148.41, 138.30, 131.76, 131.53, 130.75, 126.22, 123.99, 118.02, 116.93, 116.53, 116.01, 114.35, 107.29, 64.98, 14.66. MS (ESI): m/z 390 $[M+H]^+$; HRMS calcd for $C_{21}H_{20}N_5OS$ $[M+H]^+$ 390.13831, found 390.13686.

N-(4-Chlorophenyl)-3-((2-(6-methylbenzo[d]thiazol-2-yl)hydrazono)methyl)pyridin-2-amine (5i):

Compound **5i** was prepared according to the above explained general method by employing 2-((4-chlorophenyl)amino)nicotinaldehyde (**10a**, 100 mg, 0.43 mmol) and 2-hydrazinyl-6-methylbenzo[d]thiazole (**12c**, 77mg, 0.43mmol) to afford **5i** pale yellow solid, yield: 87%, Mp: 282-284 °C, ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.77 (s, 1H), 8.34 (d, *J* = 7.1 Hz, 1H), 7.99 (d, *J* = 5.6 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 4H), 7.43 (t, *J* = 10.3 Hz, 3H), 7.22 (t, *J* = 6.8 Hz, 1H), 2.49 (s, 3H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 167.82, 149.78, 149.29, 148.81, 138.33, 137.92, 137.23, 135.43, 131.76, 131.06, 130.12, 127.68, 122.70, 122.49, 116.10, 115.78, 114.76, 21.42. MS (ESI): *m/z* 394 $[M+H]^+$; HRMS calcd for $C_{20}H_{16}N_5ClS$ $[M+H]^+$ 394.08877, found 394.08711.

3-((2-(6-Methylbenzo[d]thiazol-2-yl)hydrazono)methyl)-N-(4-(trifluoromethyl)phenyl)pyridin-2-amine (5j):

Compound **5j** was prepared according to the above explained general method by employing 2-((4-(trifluoromethyl)phenyl)amino)nicotinaldehyde (**10b**, 100 mg, 0.37 mmol) and 2-hydrazinyl-6-methylbenzo[d]thiazole (**12c**, 67mg, 0.37mmol) to afford **5j** pale yellow solid, yield: 82%, Mp: 296-298 °C, ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.79 (s, 1H), 8.38 (d, *J* = 7.2 Hz, 1H), 8.00 (d, *J* = 5.8 Hz, 1H), 7.83 (t, *J* = 8.2 Hz, 1H), 7.7-7.8 (m, 3H), 7.63-7.54 (m, 2H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.24-7.3 (m, 1H), 2.49 (s, 3H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 167.83, 149.71, 149.19, 148.87, 138.48, 137.95, 135.41, 132.66, 132.36, 131.09, 129.83, 127.50, 123.46, 122.71, 122.49, 116.22, 115.78, 115.12, 21.42. MS (ESI): *m/z* 428 $[M+H]^+$; HRMS calcd for $C_{21}H_{16}N_5F_3S$ $[M+H]^+$ 428.11513, found 428.11233.

N-(4-Fluorophenyl)-3-((2-(6-methylbenzo[d]thiazol-2-yl)hydrazono)methyl)pyridin-2-amine (5k):

Compound **5k** was prepared according to the above explained general method by employing 2-((4-fluorophenyl)amino)nicotinaldehyde (**10c**, 100 mg, 0.46 mmol) and 2-hydrazinyl-6-methylbenzo[d]thiazole (**12c**, 83mg, 0.46mmol) to afford **5k** yellow solid, yield: 85%, Mp: 302-304 °C, ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.77 (s, 1H), 8.33 (d, *J* = 6.9 Hz, 1H), 7.97 (d, *J* = 5.5 Hz, 1H), 7.63-7.54 (m, 2H), 7.46 (dd, *J* = 7.8, 5.6 Hz, 3H), 7.31 (t, *J* = 8.3 Hz, 2H), 7.23-7.16 (m, 1H), 2.49 (s, 3H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 167.80 (d, *J* = 253 Hz), 163.71, 149.84, 149.66, 148.73, 138.34, 137.88, 135.43, 131.05, 128.91(d, *J* = 8.8 Hz), 122.69, 122.49, 120.24, 118.85, 116.00, 115.78, 114.59, 21.43. MS (ESI): m/z 378 $[M+H]^+$; HRMS calcd for $C_{20}H_{16}N_5FS$ $[M+H]^+$ 378.11832, found 378.11728.

3-((2-(6-Methylbenzo[d]thiazol-2-yl)hydrazono)methyl)-N-phenylpyridin-2-amine (5l):

Compound **5l** was prepared according to the above explained general method by employing 2-(phenylamino)nicotinaldehyde (**10d**, 100 mg, 0.50 mmol) and 2-hydrazinyl-6-methylbenzo[d]thiazole (**12c**, 91mg, 0.50mmol) to afford **5l** yellow solid, yield: 92%, Mp: 284-286 °C , ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.77 (s, 1H), 8.33 (d, *J* = 7.2 Hz, 1H), 7.96 (d, *J* = 5.9 Hz, 1H), 7.55-7.67 (m, 5H), 7.45 (d, *J* = 7.7 Hz, 3H), 7.19 (t,

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J = 6.9 Hz, 1H), 2.49 (s, 3H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 167.87, 149.90, 149.34, 148.74, 138.28, 137.83, 135.45, 131.62, 131.02, 130.88, 126.15, 122.71, 122.54, 116.00, 115.76, 114.43, 21.42. MS (ESI): m/z 360 $[M+H]^+$; HRMS calcd for $C_{20}H_{17}N_5S$ $[M+H]^+$ 360.12774, found 360.12656.

3-((2-(Benzo[d]thiazol-2-yl)hydrazono)methyl)-N-(4-chlorophenyl)pyridin-2-amine (5m):

Compound **5m** was prepared according to the above explained general method by employing 2-((4-chlorophenyl)amino)nicotinaldehyde (**10a**, 100 mg, 0.43 mmol) and 2-hydrazinylbenzo[d]thiazole (**12d**, 71 mg, 0.43 mmol) to afford **5m** yellow solid, yield: 85%, Mp: 296-298 °C, ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.79 (s, 1H), 8.36 (d, *J* = 7.3 Hz, 1H), 7.99 (d, *J* = 5.8 Hz, 1H), 7.81-7.48 (m, 6H), 7.42 (d, *J* = 8.2 Hz, 2H), 7.2-7.3 (m, 1H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 168.31, 150.23, 149.36, 148.98, 138.43, 137.62, 137.31, 131.80, 130.07, 129.92, 127.74, 127.03, 122.88, 122.44, 116.19, 116.07, 114.80. MS (ESI): *m/z* 380$[M+H]^+$; HRMS calcd for $C_{19}H_{15}N_5ClS$ $[M+H]^+$ 380.07312, found 380.07409.

3-((2-(Benzo[d]thiazol-2-yl)hydrazono)methyl)-N-(4-(trifluoromethyl)phenyl)pyridin-2-amine (5n):

Compound **5n** was prepared according to the above explained general method by employing 2-((4-(trifluoromethyl)phenyl)amino)nicotinaldehyde (**10b**, 100 mg, 0.37 mmol) and 2-hydrazinylbenzo[d]thiazole (**12d**, 62mg, 0.37mmol) to afford **5n** yellow solid, yield: 85%, Mp: 264-266 °C, ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.82 (s, 1H), 8.39 (d, *J* = 6.8 Hz, 1H), 8.00 (d, *J* = 5.4 Hz, 1H), 7.83 (t, *J* = 8.0 Hz, 1H), 7.65-7.8(m, 6H), 7.52 (t, *J* = 7.6 Hz, 1H), 7.26 (t, *J* = 7.0 Hz, 1H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 168.34, 150.10, 149.23, 148.84, 138.61, 137.65, 134.36, 133.91, 132.66, 132.36, 129.90, 127.47, 127.00, 123.49, 122.88, 122.45, 116.21, 115.13. MS (ESI): m/z 414 $[M+H]^+$; HRMS calcd for $C_{20}H_{14}N_5F_3S$ $[M+H]^+$ 414.09948, found 414.09889.

3-((2-(Benzo[d]thiazol-2-yl)hydrazono)methyl)-N-(4-fluorophenyl)pyridin-2-amine (5o):

Compound **5o** was prepared according to the above explained general method by employing 2-((4-fluorophenyl)amino)nicotinaldehyde (**10c**, 100 mg, 0.46mmol) and 2-hydrazinylbenzo[d]thiazole (**12d**, 76 mg, 0.46 mmol) to afford **5o** yellow solid, yield: 87%, Mp: 254-257 °C , ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.79 (s, 1H), 8.35 (d, *J* = 7.4 Hz, 1H), 7.97 (d, *J* = 5.7 Hz, 1H), 7.74 (dd, *J* = 11.5, 8.4 Hz, 2H), 7.65 (t, *J* = 7.7 Hz, 1H), 7.56-7.44 (m, 3H), 7.29 (dd, *J* = 14.5, 6.0 Hz, 2H), 7.21 (t, *J* = 7.0 Hz, 1H) ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 168.30, 150.26, 149.71, 148.89, 138.40, 137.62, 129.89, 128.96 (d, *J* = 9.0 Hz), 127.40 (d, *J* = 2.5 Hz), 126.99, 122.87, 122.45, 120.27, 118.56, 116.18, 115.96, 114.62. MS (ESI): *m/z* 364 $[M+H]^+$; HRMS calcd for $C_{19}H_{14}N_5FS$ $[M+H]^+$ 364.10267, found 364.10228.

3-((2-(Benzo[d]thiazol-2-yl)hydrazono)methyl)-N-phenylpyridin-2-amine (5p):

Compound **5p** was prepared according to the above explained general method by employing 2-(phenylamino)nicotinaldehyde (**10d**, 100 mg, 0.50 mmol) and 2-hydrazinylbenzo[d]thiazole (**12d**, 83 mg, 0.50 mmol) to afford **5p** yellow solid, yield: 90%, Mp: 278-280 °C, ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.80 (s, 1H), 8.34 (d, *J* = 6.8 Hz, 1H), 7.96 (d, *J* = 5.4 Hz, 1H), 7.79-7.71 (m, 2H), 7.58-7.68 (m, 4H), 7.51 (t, *J* = 7.9 Hz, 1H), 7.45 (d, *J* = 6.5 Hz, 2H), 7.22-7.16 (t, *J* = 7.8 Hz, 1H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 168.35, 150.31, 149.38, 148.87, 138.38, 137.66, 131.63, 130.90, 129.84, 126.92, 126.19, 122.88, 122.49, 116.17, 115.94, 114.43. MS (ESI): *m/z* 346 $[M+H]^+$; HRMS calcd for $C_{19}H_{16}N_5S$ $[M+H]^+$ 346.11227, found 346.11209.

3-((2-(Benzo[d]thiazol-2-yl)hydrazono)methyl)-N-(3,4,5-trimethoxyphenyl)pyridin-2-amine (5q):

Compound **5q** was prepared according to the above explained general method by employing 2-((3,4,5-trimethoxyphenyl)amino)nicotinaldehyde (**10e**, 100 mg, 0.34 mmol) and 2-hydrazinylbenzo[d]thiazole (**12d**, 57 mg, 0.34 mmol) to afford **5q** yellow solid, yield: 92%, Mp: 249-252 °C , ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.78 (s, 1H), 8.35 (d, *J* = 7.0 Hz, 1H), 8.00 (d, *J* = 5.4 Hz, 1H), 7.79 (d, *J* = 7.8 Hz, 1H), 7.62-7.72 (m, 2H), 7.43-7.53 (m, 1H), 7.15-7.25 (m, 1H), 6.71 (s, 2H), 3.97 (s, 3H), 3.87 (s, 6H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 168.15, 154.90, 153.80, 150.28, 149.56, 148.91, 138.49, 138.16, 137.58, 129.89, 127.87, 127.00, 122.92, 122.43, 116.13, 115.87, 114.60, 104.06, 61.49, 56.58. MS (ESI): *m/z* 436 $[M+H]^+$; HRMS calcd for $C_{22}H_{22}N_5O_3S$ $[M+H]^+$ 436.14379, found 436.14305.

***N*-(4-Chlorophenyl)-3-((2-(6-fluorobenzo[d]thiazol-2-yl)hydrazono)methyl)pyridin-2-amine (5r):**

Compound **5r** was prepared according to the above explained general method by employing 2-((4-chlorophenyl)amino)nicotinaldehyde (**10a**, 100 mg, 0.43 mmol) and 6-fluoro-2-hydrazinylbenzo[d]thiazole (**12e**, 79 mg, 0.43 mmol) to afford **5r** yellow solid, yield: 87%, Mp: 318-320 °C, ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.78 (s, 1H), 8.38-8.33 (m, 1H), 8.02-7.97 (m, 1H), 7.72 (dd, *J* = 9.0, 4.1 Hz, 1H), 7.59 (d, *J* = 8.7 Hz, 2H), 7.50 (dd, *J* = 7.2, 2.3 Hz, 1H), 7.40 (d, *J* = 8.7 Hz, 3H), 7.26-7.18 (m, 1H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 168.25, 159.25, 150.44, 149.37, 149.04, 138.42, 137.39, 134.11, 131.82, 130.01, 127.74, 124.06, 123.78, 118.35, 118.03, 117.61, 117.49, 116.02, 114.83, 110.35, 109.98. MS (ESI): *m/z* 398$[M+H]^+$; HRMS calcd for $C_{19}H_{14}N_5FClS$ $[M+H]^+$ 398.06374, found 398.06370.

3-((2-(6-Fluorobenzo[d]thiazol-2-yl)hydrazono)methyl)-N-(4-(trifluoromethyl)phenyl)pyridin-2-amine (5s):

Compound **5s** was prepared according to the above explained general method by employing 2-((4-(trifluoromethyl)phenyl)amino)nicotinaldehyde (**10b**, 100 mg, 0.37 mmol) and 6-fluoro-2-hydrazinylbenzo[d]thiazole (**12e**, 69 mg, 0.37 mmol) to afford **5s** yellow solid, yield: 85%, Mp: 307-309 °C, ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.79 (s, 1H), 8.38 (d, *J* = 7.2 Hz, 1H), 8.02 (d, *J* = 5.8 Hz, 1H), 7.81 (t, *J* = 7.8 Hz, 1H), 7.65-7.75 (m, 4H), 7.48 (dd, *J* = 7.2, 2.3 Hz, 1H), 7.37 (td, *J*

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= 8.7, 2.4 Hz, 1H), 7.23-7.28 (m, 1H). ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 168.41, 160.64, 149.97, 149.20, 148.70, 138.82, 134.45, 134.18, 133.73, 132.76, 132.27, 129.87, 127.39, 127.34, 124.01, 123.87, 123.45, 121.27, 120.43, 118.14, 117.81, 117.62, 116.10, 110.34, 109.91, 109.08. MS (ESI): *m/z* 432 $[M+H]^+$; HRMS calcd for $C_{20}H_{14}N_5F_4S$ $[M+H]^+$ 432.09006, found 432.09001.

3-((2-(6-Fluorobenzo[d]thiazol-2-yl)hydrazono)methyl)-N-(4-fluorophenyl)pyridin-2-amine 5t: Compound **5t** was prepared according to the above explained general method by employing 2-((4-fluorophenyl)amino)nicotinaldehyde (**10c**, 100 mg, 0.46 mmol) and 6-fluoro-2-hydrazinylbenzo[d]thiazole (**12e**, 85mg, 0.46mmol) to afford **5t**, yellow solid yield: 87%, Mp: 316-319 ^oC, ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.78 (s, 1H), 8.34 (d, *J* = 6.9 Hz, 1H), 7.99 (d, *J* = 5.7 Hz, 1H), 7.72 (dd, *J* = 9.0, 4.1 Hz, 1H), 7.50-7.42 (m, 3H), 7.37 (td, *J* = 8.8, 2.4 Hz, 1H), 7.33-7.26 (m, 2H), 7.24-7.16 (m, 1H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 168.35, 165.35, 162.36, 150.24, 149.66, 148.72, 138.58, 134.34, 128.93, 128.81, 127.46, 123.82, 118.48, 118.17, 117.84, 117.60, 117.48, 114.55, 112.82, 110.28, 109.91, 109.09. MS (ESI): *m/z* 410 $[M+H]^+$; HRMS calcd for $C_{19}H_{14}N_5F_2S$ $[M+H]^+$ 382.09325, found 382.09338.

3-((2-(6-Fluorobenzo[d]thiazol-2-yl)hydrazono)methyl)-N-phenyl pyridin-2-amine 5u:
Compound **5u** was prepared according to the above explained general method by employing 2-(phenylamino)nicotinaldehyde (**10d**, 100 mg, 0.50 mmol) and 6-fluoro-2-hydrazinylbenzo[d]thiazole (**12e**, 92mg, 0.50mmol) to afford **5u** pale yellow solid, yield: 90%, Mp: 286-288 ^oC , ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.79 (s, 1H), 8.34 (d, *J* = 6.5 Hz, 1H), 7.97 (d, *J* = 6.5, 1H), 7.71 (dd, *J* = 9.0, 4.0 Hz, 1H), 7.62 (d, *J* = 7.2 Hz, 2H), 7.49 (dd, *J* = 7.2, 2.3 Hz, 1H), 7.47-7.42 (m, 2H), 7.38 (td, *J* = 8.8, 2.4 Hz, 1H), 7.20 (t, *J* = 8.8Hz, 1H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 168.33, 159.22, 150.62, 149.44, 149.06, 138.39, 134.07, 131.71, 131.48, 131.03, 126.21, 123.92, 123.78, 118.33, 118.01, 117.57, 117.45, 115.93, 114.52, 110.37, 110.00. MS (ESI): *m/z* 364 $[M+H]^+$; HRMS calcd for $C_{19}H_{15}N_5FS$ $[M+H]^+$ 364.10267, found 364.10271.

3-((2-(6-Fluorobenzo[d]thiazol-2-yl)hydrazono)methyl)-N-(3,4,5-trimethoxyphenyl)pyridin-2-amine (5v):
Compound **5v** was prepared according to the above explained general method by employing 2-((3,4,5-trimethoxyphenyl)amino)nicotinaldehyde (**10e**, 100 mg, 0.34 mmol) and 6-fluoro-2-hydrazinylbenzo[d]thiazole (**12e**, 64mg, 0.34mmol) to afford **5v** yellow solid, yield: 92%, Mp: 264-266 ^oC, ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.77 (s, 1H), 8.35 (d, *J* = 7.4 Hz, 1H), 7.99 (d, *J* = 6.0 Hz, 1H), 7.70 (dd, *J* = 9.0, 4.0 Hz, 1H), 7.52 (dd, *J* = 7.2, 2.2 Hz, 1H), 7.38 (td, *J* = 8.8, 2.3 Hz, 1H), 7.21 (t, *J* = 6.9 Hz, 1H), 6.71 (s, 2H), 3.97 (s, 3H), 3.87 (s, 6H). ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 169.13, 168.21, 160.59, 159.28, 154.95, 150.56, 149.62, 149.12, 138.44, 138.12, 134.00, 127.93, 123.98, 123.84, 118.37, 118.10, 117.54, 117.40, 115.93, 114.72, 110.43, 110.05, 61.55, 56.49. MS (ESI): *m/z* 454 $[M+H]^+$; HRMS calcd for $C_{22}H_{21}N_5O_4FS$ $[M+H]^+$ 454.13436, found 454.13448.

N-(4-Chlorophenyl)-3-((2-(thiazol-2-yl)hydrazono)methyl)pyridin-2-amine (5w):
Compound **5w** was prepared according to the above explained general method by employing 2-((4-chlorophenyl)amino)nicotinaldehyde (**10a**, 100 mg, 0.43 mmol) and 2-hydrazinylthiazole (**12f**, 50 mg, 0.43 mmol) to afford **5w** yellow solid, yield: 85%, Mp: 290-294 ^oC ,^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.70 (s, 1H), 8.33 (d, *J* = 6.6 Hz, 1H), 7.97 (d, *J* = 6.3 Hz, 1H), 7.52-7.62 (m, 3H), 7.37 (d, *J* = 8.6 Hz, 2H), 7.21 (t, *J* = 6.7 Hz, 1H), 6.94 (d, *J* = 4.3 Hz, 1H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 168.95, 149.23, 148.94, 148.64, 138.08, 137.28, 131.77, 130.03, 129.09, 127.52, 116.22, 114.78, 109.56. MS (ESI): *m/z* 330$[M+H]^+$; HRMS calcd for $C_{15}H_{13}N_5ClS$ $[M+H]^+$ 330.05747, found 330.05741.

3-((2-(Thiazol-2-yl)hydrazono)methyl)-N-(4-(trifluoromethyl) phenyl)pyridin-2-amine (5x):
Compound **5x** was prepared according to the above explained general method by employing 2-((4-(trifluoromethyl)phenyl)amino)nicotinaldehyde (**10b**, 100 mg, 0.37 mmol) and 2-hydrazinylthiazole (**12f**, 43mg, 0.37 mmol) to afford **5x** yellow solid, yield: 82%, Mp: 280-282 ^oC , ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.72 (s, 1H), 8.37 (d, *J* = 6.6 Hz, 1H), 7.98 (d, *J* = 5.1 Hz, 1H), 7.80 (t, *J* = 8.9 Hz, 1H), 7.74-7.63 (m, 3H), 7.59 (d, *J* = 4.4 Hz, 1H), 7.23 (d, *J* = 6.6 Hz, 1H), 6.94 (d, *J* = 4.3 Hz, 1H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 168.95, 148.85, 148.70, 138.22, 133.93, 132.55, 132.37, 129.77, 129.07, 127.43, 123.37, 115.14, 109.62. MS (ESI): *m/z* 364 $[M+H]^+$; HRMS calcd for $C_{16}H_{13}N_5F_3S$ $[M+H]^+$ 364.08420, found 364.08383.

***N*-(4-Fluorophenyl)-3-((2-(thiazol-2-yl)hydrazono)methyl)pyridin-2-amine 5y :**
Compound **5y** was prepared according to the above explained general method by employing 2-((4-fluorophenyl)amino)nicotinaldehyde (**10c**, 100 mg, 0.46 mmol) and 2-hydrazinylthiazole (**12f**, 53 mg, 0.46 mmol) to afford **5y** yellow solid, yield: 87%, Mp: 284-286 ^oC, ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.70 (s, 1H), 8.32 (d, *J* = 7.4 Hz, 1H), 7.95 (d, *J* = 6.2 Hz, 1H), 7.59 (d, *J* = 4.1 Hz, 1H), 7.46-7.37 (m, 2H), 7.27 (t, *J* = 8.1 Hz, 2H), 7.19 (t, *J* = 6.8 Hz, 1H), 6.93 (d, *J* = 3.8 Hz, 1H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 168.95, 149.57, 148.94, 148.50, 138.07, 129.08, 128.75, 128.63, 118.81, 118.50, 116.10, 114.58, 109.49. MS (ESI): *m/z* 313 $[M]^+$; HRMS calcd for $C_{15}H_{12}N_5FS$ $[M+H]^+$ 313.07920, found 313.07722.

***N*-Phenyl-3-((2-(thiazol-2-yl)hydrazono)methyl)pyridin-2-amine (5z):**
Compound **5z** was prepared according to the above explained general method by employing 2-(phenylamino)nicotinaldehyde (**10d**, 100 mg, 0.50 mmol) and 2-hydrazinylthiazole (**12f**, 58 mg, 0.50 mmol) to afford **5z** yellow solid, yield: 90%, Mp: 286-290 ^oC , ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.70 (s, 1H), 8.31 (d, *J* = 7.6 Hz, 1H), 7.93 (d, *J* = 6.3 Hz, 1H), 7.55-7.65 (m, 4H),

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7.40 (d, *J* = 6.2 Hz, 2H), 7.17 (t, *J* = 6.5 Hz, 1H), 6.93 (d, *J* = 4.4 Hz, 1H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 168.96, 149.28, 149.02, 148.58, 137.90, 131.63, 131.49, 130.88, 128.99, 125.97, 116.15, 114.45, 109.60. MS (ESI): *m/z* 296[M+H]$^+$; HRMS calcd for $C_{15}H_{14}N_5S$ [M+H]$^+$ 296.09635, found 296.09644.

3-((2-(Thiazol-2-yl)hydrazono)methyl)-N-(3,4,5-trimethoxyphenyl) pyridin-2-amine (5aa):

Compound **5aa** was prepared according to the above explained general method by employing 2-((3,4,5-trimethoxyphenyl)amino)nicotinaldehyde (**10e**, 100 mg, 0.34 mmol) and 2-hydrazinylthiazole (**12f**, 40 mg, 0.34 mmol) to afford **5aa** yellow solid, yield: 95%, Mp: 244-246 ^{o}C , ^{1}H NMR (300 MHz, TFA-d + $CDCl_3$) δ 8.68 (s, 1H), 8.32 (d, *J* = 7.5 Hz, 1H), 7.96 (d, *J* = 6.2 Hz, 1H), 7.57 (d, *J* = 4.4 Hz, 1H), 7.20 (dd, *J* = 13.2, 5.9 Hz, 1H), 6.95 (d, *J* = 4.3 Hz, 1H), 6.67 (s, 2H), 3.94 (s, 3H), 3.85 (s, 6H); ^{13}C NMR (75 MHz, TFA-d + $CDCl_3$) δ 168.85, 154.86, 149.43, 148.90, 148.55, 138.05, 128.95, 127.97, 116.01, 114.59, 109.65, 103.95, 61.50, 56.47. MS (ESI): m/z 386 [M+H]$^+$; HRMS calcd for $C_{18}H_{20}N_5O_3S$ [M+H]$^+$ 386.12814, found 386.12827.

Experimental section

Biology

Cytotoxic activity

Cell culture

The human breast adenocarcinoma (MCF-7), triple negative breast cancer (MDA-MB-231), lung adenocarcinoma (A549), and mouse melanoma (B16F10) cells were obtained from National Centre for Cell Science (NCCS, Pune). Dulbecco's Modified Eagle Medium (DMEM), Rosewell Park Memorial Institute Medium 1640 (RPMI 1640), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma Aldrich (St. Louis, USA). Fetal Bovine Serum (FBS), Penicillin-Streptomycin mixture and Trypsin-EDTA were obtained from Gibco, USA. Cells were grown in respective media supplemented with 10% FBS and 1% penicillin-streptomycin mixture and allowed to grow in an incubator maintained at 37 °C with 90% humidity and 5% CO_2. The cells were treated with compounds dissolved in DMSO while untreated controls were exposed to corresponding volumes of DMSO.

MTT assay

Cytotoxicity of synthesized compounds was evaluated using MTT assay which involves conversion of yellow colour MTT to purple formazan crystals by live cells.[19] Briefly, 3000-4000 cells were seeded in 100 µl of DMEM supplemented with 10% FBS and 1% antibiotic-antimycotic mixture in each well of 96 microtiter plate and incubated at 37 °C and 5% CO_2 environment in an incubator. The seeded cells were exposed to gradient concentrations (0.625-20 µM) of compounds along with proper vehicle control for 48 hours. After 48 hours of incubation, media was discarded and cells were washed twice with PBS. 100 µL of MTT (0.5 mg/ml) was added to each well and incubated for next 4 hours at 37 °C. Subsequently, supernatant was discarded and formazan crystals were dissolved in 200 µL of DMSO. The absorbance was taken at 570 nm using spectrophotometer (Molecular devices, USA).

Clonogenic assay

Clonogenic assay (colony formation assay) is an *in vitro* cell survival assay technique which is based on the ability of a single cell to grow and form colonies. The assay represents a valuable technique to assess the retained capacity of cells after treatment with cytotoxic agents, to eventually produce large number of progeny. Clonogenic assay was performed to assess the effect of compounds on colony forming capability of MCF-7 cells as described by Franken *et al* with slight modifications.[20] Briefly, the cells were plated at a density of 200 cells/well in a 6-well plate followed by their exposure to different concentrations of conjugate **5i** for 24 hours. Subsequently, cells were allowed to grow for 14 days after giving fresh media. The growth medium was changed with fresh medium every 3rd day to supplement the cells with required nutrients and growth factors. After 14 days, grown colonies were fixed and stained with crystal violet (1% in methanol) and number of stained colonies was counted.

Detection of intracellular reactive oxygen species (ROS) by DCFDA

Increased intracellular levels of reactive oxygen species (ROS) has been implicated as a major protagonist for induction of apoptosis in cancer cells.[21] To measure the levels of intracellular ROS induced by test compound, an oxidant-sensitive florescent probe, 2',7'-dichlorofluorescindiacetate (DCFH-DA) was used which gets converted to highly florescent derivative when oxidized by intracellular ROS.[22] MCF-7 cells were plated in 12-well plate and allowed to grow for 24 hours. Cells were treated with increasing concentration of conjugate 5i (0.625, 1.25, 2.5, 5, 10 µM) for 4 hours. After 4 hours of incubation, treatment medium was discarded and cells were washed with PBS followed by incubation of cells with DCFH-DA (10 µM) at 37 °C for 20 min. The oxidation of DCFH-DA to florescent DCF mediated by intracellular ROS was observed under florescent microscope (Nikon, Japan) with excitation (498 nm) and emission (530 nm) wavelength.

Measurement of mitochondrial membrane potential (ΔΨm)

Loss of mitochondrial membrane potential (ΔΨm) is implicated as one of the major pathways for cellular death as maintenance of mitochondrial membrane potential (ΔΨm) is crucial to sustain mitochondrial integrity and its function [23]. To investigate the loss of mitochondrial membrane potential (ΔΨm) MCF-7 cells (1 X 10^5 cells/well) were plated in 6-well plate and allowed to grow for 24 hours. The cells were then incubated with different concentrations (0.625, 1.25, 2.5, 5, 10 µM) of conjugate **5i** for 24 hours. Subsequently, cells were harvested, washed with PBS and resuspended in PBS containing JC-1 dye (5 µg/ml) and incubated in dark for 30 minutes. Cells were washed with PBS twice and observed under florescent microscope (Nikon, Japan).

Acridine orange/ethidium bromide (AO/EB) staining

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AO/EB staining method was used to visualize changes in cellular morphology such as chromatin condensation and apoptotic body formation which are characteristic features of cells undergoing apoptosis.[24] MCF-7 cells were grown in 24-well plate and treated with gradient concentration of conjugate **5i** (0.625, 1.25, 2.5 µM) for 24 hours at 37°C in an atmosphere of 5% CO_2. After 24 hours of treatment, cells were washed with PBS after discarding treatment medium and were stained with acridine orange/ethidium bromide (10 µg/ml each) followed by observation under fluorescent microscope (Nikon, Japan) with excitation (488 nm) and emission (550 nm) at 200X magnification.

Hoechst staining

Nuclear fragmentation and nuclear shrinkage are characteristic features of cells undergoing apoptosis.[25] To evaluate the nuclear damaging potential of compounds, Hoechst 33342 staining method was used. Briefly, human breast adenocarcinoma (MCF-7) cells were seeded at a density of 5 X 10^4 cells/well and allowed to adhere for 24 hours. Cells were treated with conjugate **5i** (0.625, 1.25, 2.5, 5, 10 µM) for 24 hours. Next, cells were washed and stained with Hoechst 33342 (5 µg/ml) for 30 minutes at room temperature. Stained cells were examined under florescent microscope (Nikon, Japan) using excitation (350 nm) and emission (460 nm) at 400X magnification.

Cell cycle analysis

The antiproliferative activity of test compounds is linked to differential distribution of population of cells into different phases of cell cycle. Most of the anticancer compounds arrest cell cycle at a particular checkpoint.[26] The potential of compound to arrest growth of the cells was evaluated by using propidium iodide (PI) DNA staining method. Briefly, MCF-7 (1 X 10^5 cells/well) cells were seeded in each well of 6-well plate. After treating the cells with conjugate **5i** (1 and 3 µM) for 24 hours, cells were harvested, washed with PBS and fixed in ice-cold 70% ethanol overnight. Cells were then centrifuged and suspended in cell cycle buffer containing PI, RNase A and Triton X-100 for 15 minutes followed by estimation of cell cycle arrest by flow cytometer (BD FACSVerse™, USA). The histograms were drawn with population of cells in each phase of cell cycle after counting a minimum of 10,000 events excluding cellular debris by proper gating.

Annexin V-FITC/PI staining

To determine the extent of apoptosis and necrosis, cells were stained with annexin V conjugated with FITC and PI using apoptosis detection kit (Invitrogen, USA) according to manufacturer's protocol. Annexin V has high affinity for phosphatidylserine moiety exposed on outer membrane of apoptotic cells while PI which is membrane impermeable binds to cells with ruptured membrane (late apoptotic, necrotic cells)[27]. MCF-7 cells (1 X 10^5 cells/well) were seeded in 6-well plate and were exposed to different concentrations (1 and 3 µM) of conjugate **5i**. After 24 hours of incubation cells were harvested using trypsin-EDTA and washed twice with PBS and resuspended in 100 µL of 1X binding buffer. Next, 5 µL of FITC conjugated annexin V and 1 µL of PI was added to it and incubated for 15 minutes in dark. After addition of 400 µL of 1X binding buffer, cells were kept on ice and immediately analysed by flow cytometer (BD FACSVerse™, USA) for detection of population of cells undergoing apoptosis. The cell population was divided into live (annexin V-negative, PI-negative), early apoptotic (annexin V-positive, PI-negative), late apoptotic (annexin V-positive, PI-positive) and necrotic (annexin V-negative, PI-positive) cells after counting minimum of 10,000 events.

Conflicts of interest

There are no conflicts to declare.

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Synthesis of 2-anilinopyridyl linked benzothiazole hydrazones as apoptosis inducing cytotoxic agents

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