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Original article

## Investigation of anticancer potencies of newly generated Schiff base imidazolylphenylheterocyclic-2-ylmethylenethiazole-2-amines

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### ABSTRACT

A new series of multi-heterocyclic Schiff base was constructed starting from 4'-(imidazol-1-yl)-acetophenone which was converted to its 2-bromoethanone precursor which on cyclic condensation with thiourea yielded final thiazol-2-amine intermediate (**3**) to be reacted with substituted aldehydes to generate final imidazolylphenylheterocyclic-2-ylmethylenethiazole-2-amines (**4a–4i**). New Schiff base was investigated for their *in vitro* cytotoxic efficacies against a panel of three human cancer cell lines namely, MCF7 (human breast cancer), HCT116 (human colon cancer), and DU145 (human prostate cancer) and one normal skin fibroblast (SF). Most of these synthetic derivatives shown important cytotoxic actions against individual carcinoma cell line collections, but weak actions against SF, which is as anticipated. Observations of SAR suggested that the difference in the characteristics of substituents attached to the Schiff base function leads to the interesting variations within pharmacological effects of resultant molecular systems. Structural analysis performed using FT-IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR spectroscopy and CHN analysis for final potent anticancer Schiff base, which warrant further investigations.

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## 1. Introduction

Schiff bases, named after Hugo Schiff and their very first formation was accomplished in the nineteenth millennium [1]. They are known as azomethine or imine as nitrogen analogue an aldehyde or ketone structurally, where imine or azomethine group found to replace the carbonyl group [2]. Schiff base is very widely used and the most appreciated organic building blocks to have a diverse range of pharmacological importance as well as a versatile tool to explore in many other fields as biological, inorganic and analytical chemistry. They hold a spectrum of biological importance as antioxidant, anthelmintic, antitubercular, anti-inflammatory, anticancer, antimicrobial, anticonvulsant and so forth [3]. The active centers of cell constituents are supposed to get

interacted with azomethine's nitrogen atom *via* forming a hydrogen bond which interferes with normal cell processes [4] and results in the destruction of enzymatic activity of cancerous cells, thereby presents Schiff base as a potential target to discover anticancer chemotherapeutics. Hence, in the current research, we were directed to construct Schiff base derivatives involving the presence of three different types of heterocycles as imidazole, thiazole, and other heteroaromatics. Imidazole ring, highly polar heterocycle, is an important five-membered aromatic heterocycle widely present in natural products and synthetic molecules. It readily binds with different targeted enzymes in a biological system *via* ion–dipole, coordination, van der Waals forces,  $\pi$ – $\pi$  stacking, hydrophobic effects, cation– $\pi$ , and so on, because of its unique electronrich characteristic which establishes diverse weak interactions, thereby exhibiting broad bioactivities [5]. Hence, we have selected imidazole as a starting heterocyclic core to build the desired Schiff bases with intermediate thiazole entity which represents a versatile tool to generate anticancer chemotherapeutics [6]. Finally, different N, S and O-based heterocycles were

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attached to the Schiff base function because, such heterocyclic compounds have a considerable active role as antibacterial [7,8], anti-viral [9], anti-fungal [10], anti-inflammatory [11], and anti-tumor drugs [12–14].

Heterocycles represent a common architectural device of most promoted drugs and hence, we effort here to produce multi-heterocyclic systems to be examined for their *in vitro* anticancer results, because cancer provides being a major health issue in developed along with developing countries. With >100 types of cancer exist, 8.2 million people die each year from cancer, an estimated 13% of all deaths worldwide and 70% the increase in new cases of cancer expected over the next 2 decades [15]. In the attempts to distinguish a variety of chemical substances that may provide as leads for creating novel anticancer agents, nitrogen- and sulfur- that contains heterocycles are of specific attention [16–18] and the fact prompted us to generate anticancer multi-hetero-aromatic systems as imidazolylphenylheterocyclic-2-ylmethylene-thiazole-2-amines. Recently, we have observed many structures those represent similar features as ours with Schiff bases function linked to 2-aminothiazole nucleus presenting interesting pharmacological effects [19–22]. Hence, we attempt here to generate Schiff base derivatives holding all three other building block as diverse heterocycles which anticipated delivering significant anticancer effects.

## 2. Results and discussion

### 2.1. Chemistry

Scheme 1 represents the chemical steps followed to obtain desired Schiff base **4a–4i**. 4'-(Imidazol-1-yl)acetophenone was selected as a starting material to be treated with aqueous HBr with glacial acetic acid as well as bromine to give the first intermediate derivative 1-(4-(1H-imidazol-1-yl)phenyl)-2-bromoethanone (**2**). Intermediate **2** was subsequently reacted with thiourea in the presence base to generate final thiazole-2-amine intermediate **3**. FT-IR spectra of intermediate **2** revealed aromatic stretching at 2976  $\text{cm}^{-1}$ , carbonyl group characteristic peak at 1697  $\text{cm}^{-1}$  as well as C-Br peak at 691  $\text{cm}^{-1}$  which ensure its correct formation. In addition, FT-TR solid state spectrum obtained for intermediate **3** revealed  $\text{NH}_2$  characteristic peak at 3438  $\text{cm}^{-1}$  and thiazole ring peaks were observed as 1523 for C=N as well as 691 as C—S—C linkage.  $^1\text{H}$  NMR spectra for intermediate **2** revealed peak for  $\text{COCH}_2\text{Br}$  linkage as a singlet 4.80 ppm, whereas, a peak at 7.71 ppm was assigned to thiazole-H and such data confirmed the correct

**Table 1**  
Results of anticancer activity investigation for final Schiff base **4a–4i**.

| Entry       | MTT assay ( $\text{IC}_{50}$ $\mu\text{mol/L} \pm \text{SD}$ ) <sup>a</sup> |                     |                    |                 |
|-------------|---|---------------------|--------------------|-----------------|
|             | MCF7 <sup>b</sup>   | HCT116 <sup>c</sup> | DU145 <sup>d</sup> | SF <sup>e</sup> |
| <b>4a</b>   | 38.28 $\pm$ 2.03  | 72.10 $\pm$ 0.92    | 40.32 $\pm$ 2.80   | >50             |
| <b>4b</b>   | 54.90 $\pm$ 3.24  | 58.99 $\pm$ 2.04    | 49.87 $\pm$ 1.82   | >50             |
| <b>4c</b>   | 38.32 $\pm$ 1.89  | 46.56 $\pm$ 2.37    | 64.23 $\pm$ 1.78   | >50             |
| <b>4d</b>   | 67.34 $\pm$ 1.85  | 42.76 $\pm$ 3.21    | 70.12 $\pm$ 2.08   | >50             |
| <b>4e</b>   | 96.45 $\pm$ 3.56  | 82.96 $\pm$ 1.22    | 50.09 $\pm$ 0.41   | >50             |
| <b>4f</b>   | 86.67 $\pm$ 2.67  | 93.21 $\pm$ 2.07    | 27.54 $\pm$ 2.76   | >50             |
| <b>4g</b>   | 27.76 $\pm$ 2.14  | 82.45 $\pm$ 2.31    | 25.78 $\pm$ 1.65   | >50             |
| <b>4h</b>   | 56.98 $\pm$ 0.87  | 44.45 $\pm$ 1.09    | 57.23 $\pm$ 3.01   | >50             |
| <b>4i</b>   | 61.26 $\pm$ 2.19  | 36.34 $\pm$ 2.57    | 26.90 $\pm$ 0.84   | >50             |
| Doxorubicin | 11.12 $\pm$ 0.56  | 5.02 $\pm$ 0.34     | 4.68 $\pm$ 0.50    | >50             |

<sup>a</sup> Each value is the mean of three independent experiments.

<sup>b</sup> Human breast cancer.

<sup>c</sup> Human colon cancer.

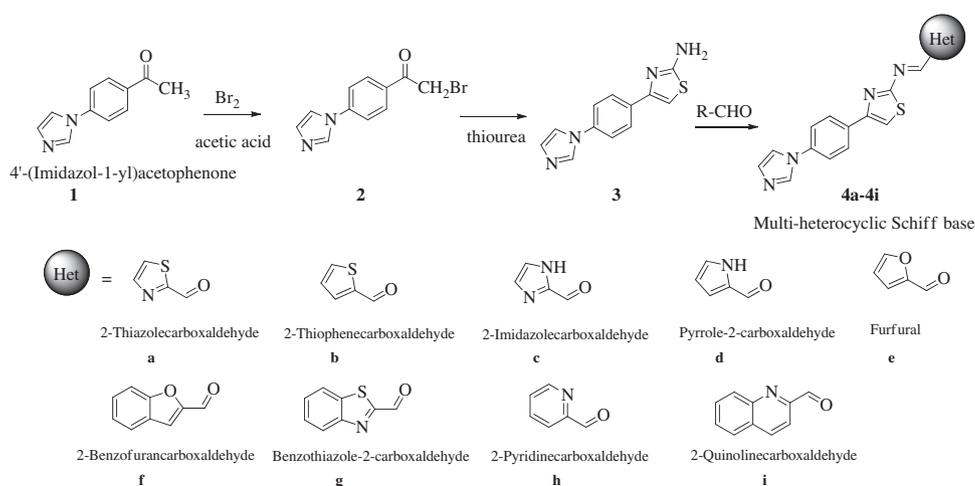
<sup>d</sup> Human prostate cancer.

<sup>e</sup> Normal skin fibroblast cell.

formation of both intermediates. In further steps, intermediate **3** was reacted with various desired heterocyclic aldehyde entities (**a–i**) in the presence of organocatalyst piperidine with molecular sieve (4A) to furnish final thiazolyl Schiff bases **4a–4i** [24]. All FT-IR and  $^1\text{H}$  NMR data were in accurate accordance with the proposed structural features of the final compounds and  $^1\text{H}$  NMR for compound **4a** as a representative analogue; N=CH peak was observed at 8.91 ppm which apparently confirms the accurate synthesis. Mass spectral data were presented as M+1 molecular ion peaks and which were also in accordance with the molecular weights for the **4a–4i** and elemental analysis data were in a considerable range acceptable to confirm the correct formation of anticipated structures.

### 2.2. Pharmacology

The newly yielded multi-heterocomponent Schiff base **4a–4i** were subjected to the screening of their *in vitro* anticancer potencies against a panel of three cancerous cell lines as MCF7 (human breast cancer), HCT116 (human colon cancer), and DU145 (human prostate cancer) as well as one non-cancer normal skin fibroblast (SF) to inspect the cytotoxic character of the said molecules toward the healthy cell line. The results of anticancer screening are presented in Table 1 in terms of  $\text{IC}_{50}$ s in  $\mu\text{mol/L}$ , and the bioassay data suggested that these new Schiff base displayed a variable degree of anticancer activity with a broad scope of SAR



**Scheme 1.** Schematic presentation for the synthesis of imidazolylphenyl-heterocyclic-2-ylmethylene-thiazole-2-amines (**4a–4i**).

studies leading to a tool which can facilitate further rationale in drug designing. It was noticed that primary imidazole and intermediate thiazole rings were having an essential role to provide promising cancerous cell inhibitory effects, whereas, varying the type of coupled heterocyclic entity to the Schiff base function has a key role in determining the final anticancer potency of resultant molecule.

A molecule with a benzothiazole entity (**4g**) connected to the azomethine function showed the highest anticancer activity against MCF-7 cell line with  $IC_{50}$  of  $27.76 \pm 2.14 \mu\text{mol/L}$  which can be comparable to that of control drug doxorubicin with  $11.12 \pm 0.56 \mu\text{mol/L}$  of  $IC_{50}$ . Moreover, the presence of two heteroatoms as S and N was found beneficial to gain activity against MCF-7 as compounds **4a** with thiazole ring and **4c** with imidazole moiety presented  $38.28 \pm 2.03 \mu\text{mol/L}$  and  $38.32 \pm 1.89 \mu\text{mol/L}$  of  $IC_{50}$ s, respectively. All these three derivatives indicated low cytotoxic character toward normal skin fibroblast cell with  $>50 \mu\text{mol/L}$  of  $IC_{50}$ . It will be suffice to mention here that S heteroatom has a good role to play for the anticancer potency against MCF-7 because a compound with S-heterocyclic entity in terms of thiophene (**4b**) demonstrated good anticancer action with  $54.90 \pm 3.24 \mu\text{mol/L}$  of  $IC_{50}$ , whereas, compounds (**4e** and **4f**) bearing oxygenated heterocycles showed poor anticancer potencies with  $IC_{50}$ s  $>85 \mu\text{mol/L}$ . Finally, molecules holding single N atom as **4d**, **4h** and **4i** appeared to have moderate anticancer effects with  $IC_{50}$ s,  $67.34 \pm 1.85 \mu\text{mol/L}$ ,  $56.98 \pm 0.87 \mu\text{mol/L}$  and  $61.26 \pm 2.19 \mu\text{mol/L}$ , respectively. Furthermore, quinolone ring (**4i**) had the most influence on HCT116 cell line with  $36.34 \pm 2.57 \mu\text{mol/L}$  of the  $IC_{50}$  level and  $>50 \mu\text{mol/L}$  of  $IC_{50}$  against non-cancer skin fibroblast cell being the most active analogue of the series against the mentioned cancerous cell line. Similar to the activity results observed against MCF-7, nitrogen-based heterocycles were found remarkably active against HCT116 cell line as compounds **4c**, **4d**, and **4h** with imidazole, pyrrole and pyridine rings demonstrated  $46.56 \pm 2.37 \mu\text{mol/L}$ ,  $42.76 \pm 3.21 \mu\text{mol/L}$  and  $44.45 \pm 1.09 \mu\text{mol/L}$ , respectively, however their cytotoxicity towards SF was observed beyond  $50 \mu\text{mol/L}$ , thus suggesting these N-heterocyclic candidates as potentially active anticancer agent against human colon cancer. The above data suggested that heterocycle with single N atom were active against human colon cancer than those carrying two N atoms. In fact, the presence of an additional heteroatom in the form of S lead to the reduced activity of the molecule as **4g** and **4a** showed  $>70 \mu\text{mol/L}$  of  $IC_{50}$ s against HCT116 cell line, respectively. However, molecules bearing only a single sulfur atom as compound **4b** observed to have moderate anticancer activity against HCT116 cell line with  $IC_{50}$  of  $58.99 \pm 2.04 \mu\text{mol/L}$ . Lastly, with O-based heterocycles, similar to activity recorded against MCF-7, compounds **4e** and **4f** were found least sensitive against HCT116 cell line with  $>80 \mu\text{mol/L}$  of  $IC_{50}$ s. Presence of an additional aromatic ring along with a heterocyclic core in terms of benzofuran (**4f**), benzothiazole (**4g**) and quinolone (**4i**) was a key to have successive anticancer action against DU-145 prostate cancer cell line with  $IC_{50}$ s,  $27.54 \pm 2.76 \mu\text{mol/L}$ ,  $25.78 \pm 1.65 \mu\text{mol/L}$  and  $26.90 \pm 0.84 \mu\text{mol/L}$ , respectively and it was found that heterocyclic with two different heteroatoms (**4g**) was most active. Similarly, further data recorded against DU-145 cell line proved that S has an excellent role to play as molecules with thiazole (**4a**) and thiophene (**4b**) entities showed  $40.32 \pm 2.80 \mu\text{mol/L}$  and  $49.87 \pm 1.82 \mu\text{mol/L}$  of  $IC_{50}$ s, respectively; and  $>50$  of  $IC_{50}$ s toward SF cells presenting themselves as lead anticancer candidates against prostate cancer. However, with an exception to the activity data for MCF-7 and HCT116, a compound with O heteroatom in the form of furan (**4e**) was as active as that with thiophene ring towards DU-145 cell line with  $50.09 \pm 0.41 \mu\text{mol/L}$  of  $IC_{50}$ . Finally, a derivative with a pyridine (**4h**) ring has good action than imidazole (**4c**:  $IC_{50}$ ,  $64.23 \pm$

$1.78 \mu\text{mol/L}$ ) and pyrrole (**4d**:  $70.12 \pm 2.08 \mu\text{mol/L}$ ) with  $57.23 \pm 3.01$  of  $IC_{50}$  against DU-145 cell line. Overall, many of the final Schiff base derivatives showed  $<50 \mu\text{mol/L}$  of  $IC_{50}$ s against all three cancerous cell lines and  $>50 \mu\text{mol/L}$  of  $IC_{50}$ s against non-cancer cells which present them as leading candidates for anticancer drug discovery.

### 3. Conclusion

To conclude, a successful attempt has been made to furnish a new series holding multi-hetero component resides with tremendous anticancer action against three different cancerous cell lines. Imidazole and thiazole core were kept constant in all final molecules to get a regular anticancer data with a variable degree of efficacy observed while varying heterocyclic moieties connected to the Schiff base link. The correct formation of the desired molecules was ensured utilizing different analytical techniques followed by the inspection of their *in vitro* anticancer effects, in which, all compounds presented  $<100 \mu\text{mol/L}$  of  $IC_{50}$ s. Core imidazole liked to the thiazole ring was very beneficial, as final results also displayed anticipated potencies with thiazole core lined to the Schiff base function. The presence of particular heteroatom, as well as presence of an additional benzene ring, had a significant effect against MCF-7 and DU-145, and heterocycles with S atoms were more active, whereas, against HCT116, N-heterocycles played a major role. Overall, **4a**, **4g**, and **4i** showed tremendous activity against all cancerous cell lines. In addition, all compounds presented  $>50 \mu\text{mol/L}$  of  $IC_{50}$ s against non-cancer normal skin fibroblast (SF) cells which indicated tolerable cytotoxic nature of these Schiff bases toward healthy cells. As some substituent were found inactive, for example, those with O heteroatom, more studies are obviously warranted to replace basic core from imidazole and thiazole to other heteroatom-containing rings to examine their overall influence on anticancer effects.

### 4. Experimental

Reichert Thermover instrument was utilized to record melting points of the final compounds which were uncorrected. FT-IR spectra were recorded using KBr on Perkin Elmer RXI spectrometer. Proton and carbon NMR data were recorded on Bruker AVANCE III 400 instrument spectrometer ( $^1\text{H}$  NMR, 400 MHz;  $^{13}\text{C}$  NMR, 100 MHz) in the presence of TMS internal standard and with DMSO. Mass spectra have been registered on JEOL-Accu TOF JMS-T100LC DART-MS spectrometer. Microanalytical data were collected using Carlo Erba analyzer model 1108. TLC plates were used to monitor the reaction as well as to identify the purity of the prepared products.

#### 4.1. Synthetic procedure for 1-(4-(1H-imidazol-1-yl)phenyl)-2-bromoethanone (**2**)

In a flask charged with 4'-(imidazol-1-yl)acetophenone (0.01 mol) with glacial acetic acid (20 mL) and aqueous hydrobromic acid (HBr) [48% (w/w)]. The resulting mixture was immersed in an ice-salt mixture ( $0-5^\circ\text{C}$ ), and bromine (0.01 mol) was added, and temperature of the reaction mixture does not exceed  $5^\circ\text{C}$ . After completion of addition, it was stirred at room temperature for 2–3 h. The progress of the reaction was monitored by using TLC. After the completion of the reaction, the suspension was poured onto crushed ice. The colorless precipitate was filtered, repeatedly washed with water and dried at room temperature. Yield: 55%–57%, m.p:  $147-149^\circ\text{C}$ ; FT-IR (KBr)  $\text{cm}^{-1}$ : 2976 (Ar-stretching), 1697 (C=O), 691 (C–Br);  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  8.38 (s, 1H), 8.13 (d, 1H,  $J = 7.6$  Hz.), 8.02 – 7.96 (m, 2H), 7.94 – 7.87 (m, 2H), 7.84 (d, 1H,  $J = 7.5$  Hz.), 4.80 (s, 2H).  $^{13}\text{C}$  NMR

(100 MHz, DMSO- $d_6$ ):  $\delta$  181.34, 150.12, 148.34, 137.6, 134.2, 132.7, 129.9, 125.4, 123.7, 122.5, 34.23.

#### 4.2. Synthetic procedure for 4-(4-(1H-imidazol-1-yl)phenyl)thiazol-2-amine (3)

In a flask charged with compound 2 (0.01 mol), thiourea (0.01 mol) and triethylamine (0.01 mol) and the reaction mixture was refluxed for 10–12 h in 30 mL acetonitrile until the complete consumption of starting material as detected by TLC. The reaction mixture was then cooled, poured onto ice. The precipitate was filtered, dried and recrystallised from ethanol. Yield: 45%–53%, m. p: 160–163 °C; FT-IR (KBr)  $\text{cm}^{-1}$ : 3438 ( $\text{NH}_2$ ), 1577 (aromatic C=C stretch), 1523 (C=N) thiazole, 1154 (C–N), 691 (C–S–C) thiazole;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  5.08 (s, 1H,  $\text{NH}_2$ ), 8.13 (d, 1H,  $J=7.5$  Hz.), 7.98–7.91 (m, 2H), 7.84 (d, 1H,  $J=7.5$  Hz.), 7.71 (s, 1H), 7.61 (s, 2H), 7.48–7.42 (m, 2H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  167.52, 153.71, 147.89, 146.21, 138.7, 134.6, 131.8, 128.7, 125.6, 124.6, 122.8, 121.9. Anal Calcd. for  $\text{C}_{22}\text{H}_{22}\text{N}_2\text{O}_3$ : C, 72.91; H, 6.12; N, 7.73; Found: C, 72.76; H, 6.29; N, 7.62.

#### 4.3. General method for the preparation of imidazolylphenyl-heterocyclic-2-ylmethylene-thiazole-2-amines (4a–4i)

A mixture of substituted compound 3 (0.01 mol), substituted heterocyclic aldehyde (0.01 mol) and piperidine (0.01 mol) was refluxed in 30 mL ethanol for 6–8 h. The reaction mixture was poured into an ice–water mixture to get a product, which was filtered, dried, and recrystallized using ethanol to give the final compounds 4a–4i, for example, 4-(4-(1H-imidazol-1-yl)phenyl)-N-(thiazol-2-ylmethylene)thiazol-2-amine (4a): Yield 65%. m.p. 213–214 °C; FT-IR (KBr)  $\text{cm}^{-1}$ : 3067 (aromatic C–H stretch), 1608 (N=CH stretch, azomethine), 1572 (aromatic C=C stretch), 1509 (C=N) thiazole, 1147 (C–N), 685 (C–S–C) thiazole;  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ ):  $\delta$  8.91 (s, 1H, N=CH); 8.38 (s, 1H), 8.13 (d, 1H,  $J=7.5$  Hz), 8.05 (d, 1H,  $J=7.5$  Hz), 7.99–7.92 (m, 2H), 7.84 (d, 1H,  $J=7.5$  Hz), 7.75 (s, 1H), 7.55 (d, 1H,  $J=7.5$  Hz), 7.49–7.43 (m, 2H).  $^{13}\text{C}$  NMR (100 MHz, DMSO- $d_6$ ):  $\delta$  164.71, 164.03, 152.22, 147.72, 146.81, 144.11, 135.26, 131.25, 130.19, 125.67, 123.88, 122.45, 121.37, 118.30, 113.17, 112.61. EI-MS  $m/z$  ( $M^+$ ): 338.74; Anal Calcd. for  $\text{C}_{16}\text{H}_{11}\text{N}_5\text{S}_2$ : C, 56.95; H, 3.29; N, 20.76; Found: C, 56.88; H, 3.35; N, 20.64.

#### 4.4. In vitro evaluation of anticancer activity

Human cancer cell lines namely, MCF7 (human breast cancer), HCT116 (human colon cancer), and DU145 (human prostate cancer) and one normal skin fibroblast (SF), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], DMEM, trypsin–EDTA, RPMI and 96-well flat bottom tissue culture plates were obtained from the commercial suppliers. Cancerous were grown in DMEM medium, and SF cells were grown in RPMI medium supplemented with 10% fetal bovine serum, 200  $\mu\text{g}/\text{mL}$  streptomycin, 100  $\mu\text{g}/\text{mL}$  penicillin, 2  $\text{mmol}/\text{L}$   $\text{l}$ -glutamine, and culture was maintained with 5%  $\text{CO}_2$ . A stock solution of for compounds to be tested was prepared in DMSO with sterile PBS to get required concentration. A standard MTT colorimetric assay was performed for investigating the anticancer potential of 4a–4i because MTT is a photosensitive compound and is consumed by living organisms and a mitochondrial dehydrogenase enzyme performs its reduction to a purple formazan product that is impermeable to the cell membrane. DMSO solubilization of test compounds yields liberation and resulting formazan product with purple color is directly related to the cell viability. Cells placed in 96-well plates were incubated with test compounds and a controlled drug (doxorubicin) with variable dilutions at 37 °C for 24 h in DMEM medium carrying 10% FBS medium. After that, 90  $\mu\text{L}$

of fresh serum free media and 10  $\mu\text{L}$  of MTT reagent (5  $\text{mg}/\text{mL}$ ) was placed in the place of old media followed by the incubation at 37 °C for 4 h and lastly, this media was changed to 200  $\mu\text{L}$  of DMSO followed by last incubation at 37 °C for 10 min. The absorbance at 570 nm was calculated on a spectrophotometer (Spectra Max, Molecular devices)  $\text{IC}_{50}$  principles were identified from plot: % cell viability (from control) versus concentration [23].

#### Conflicts of interest

The authors report no conflicts of interest.

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#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ccllet.2016.10.021>.

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