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DESIGN, SYNTHESIS, CHARACTERIZATION AND *IN-VITRO* ANTICANCER STUDIES OF NOVEL SUBSTITUTED AZO LINKED SCHIFF BASE HYBRIDS

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Abstract: According to one pot microwave assisted synthesis, the versatile precursor 2- amino thiazole was prepared and utilized for the construction of new thiazole hybrids targeting MCF-7 cell lines. 2-amino thiazole was condensed with corresponding aldehydes to yield Schiff's base (2) intermediates followed by the diazo coupling reaction furnished the designed hybrids (3) contains azo-methine and diazo linkages in its structures. The newly synthesized compounds were confirmed on the basis of IR and H^1NMR spectral analytical data. All the synthesized compounds were evaluated for their *in-vitro* cytotoxicity activity against MCF-7cell lines using MTT assay method. The obtained results revealed the more promising compounds of the synthesised series, 3B and 3H with CTC50 value of 17.77±0.31µg/ml, 17.83±1.14 µg/ml.

Keywords: Thiazole, schiff's base, Azo compounds, Cytotoxicity, MCF-7, MTT assay.

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INTRODUCTION

On the basis of GLOBOCAN 2018 report, cancer is ranked no.1 and leading cause for mortality. Particularly in females, breast cancer is the most commonly diagnosed cancer and reported 11.6 % worldwide cancer death. When healthy cells in the breast grow abnormally, turns into a mass or sheet of cells called as tumor. A tumor can be either cancerous or benign. Cancerous tumour may grow and spread to other parts of the body through blood circulation and benign tumour can grow but not spread. [1-3]. Literature survey quoted 92 types of breast cancer cell lines based on the molecular features of cancer cells and classified through three important receptors i. e. , estrogen receptor (ER), progesterone receptor (PR), and human epithelial receptor 2 (HER2) in the cell lines. [4].

Researchers were attracted towards the hybrid molecules and their applications in medicinal chemistry and drug discovery over the past decades to generate therapeutically efficient compounds with low toxicity profiles. Schiff bases are nitrogen based organic compounds contain azomethine [-C=N-] linkage with broad range of reported biological activities such as antimalarial, antibacterial, antifungal, anticancer and antiviral. [5-7] The azo linkage [–N=N] influences the SAR of medicinal compounds and efficacious therapeutic utility of the compounds as antiseptic, antimicrobial, antidiabetic, antioxidant, anticancer [8]etc. Similarly, Thiazoles are major promising heterocyclic category found in many potent molecules used in the treatment of allergies, hypertension, inflammation, schizophrenia, bacterial, HIV infections, hypnotics more recently for the treatment of pain, as fibrinogen receptor antagonists with antithrombotic activity, as new inhibitors of bacterial DNA gyrase B and anticancer. [9-10] Hence, this research work was to evaluate the cytotoxicity activity of various synthesised hybrids of designed series by suitable *in-vitro* MTT assay method for the first time.

MATERIALS AND METHODS

The melting point of the synthesized compounds was determined by Melting Point apparatus (Legrand, India) at 10 °C/min temperature gradient. IR and ^{1}H NMR, spectra were recorded on a 400 MHz NMR spectrometer with TMS as an internal standard and $CDCl_3$ as an solvent. MS spectra were recorded on Shimadzu mass spectrometer. All reagents were purchased from Sigma Aldrich, Loba and Merck and were used without further purification. All the solvents used in the synthesis were analysis and synthesis grade.

General procedure for microwave assisted synthesis of 2- amino 4-anilido thiazole (1a)[11]

The starting material (2-amino 4-anilido thiazole) was prepared by the triturate acetanilide, thiourea and iodine (1:1:3). The powdered mass was subjected to microwave oven at 480v for

1 minute. The progress of the reaction was monitored by TLC using suitable solvent system ethyl acetate(10ml), ether(4ml) and ethanol(6ml).

General procedure for conventional synthesis of Schiff base ligand: (2a-2d)

Equimolar amount of aldehyde and 2-amino-4-anilido –thiazole were dissolved in ethanol separately and mixed together. Then the solution was stirred for an hour. After the completion of process, sodium acetate was sprinkled and the precipitated solid product was collected through the filtration and dried. The desired product was purified by distilled water.

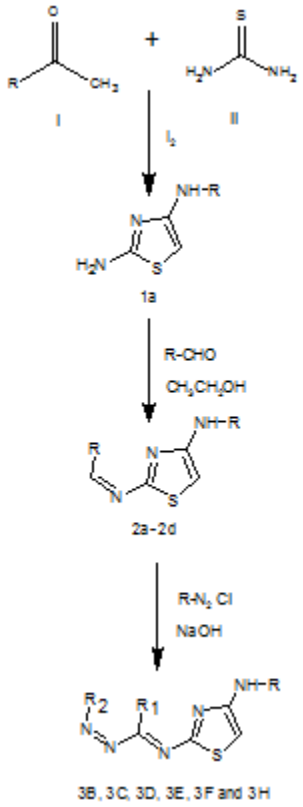
General procedure for synthesis of di azenyl derivatives: (3B, 3C, 3D, 3E, 3F, 3H) [12] Two to three drops of concentrated sulphuric acid was added to a solution of primary amine and water and kept on an ice bath. A cold solution of sodium nitrite was added dropwise to it by maintaining the temperature of the reaction below 5˚C. After addition, the solution was kept for 15 minutes with occasional stirring to complete the diazotisation reaction. To the above prepared diazotised compound, Schiff's base in ethanol and 10% of 20 ml of aqueous sodium hydroxide were poured. The resultant mixture was stirred and allowed to stand in an ice bath for 1 hour. During the process, pH was maintained 5-6 with occasional and controlled addition of dilute hydrochloric acid. Then the coloured products obtained were filtered and washed repeatedly with water then dried.

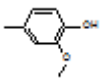
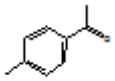
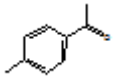
***In-vitro* cytotoxicity assessment:** [13-16]

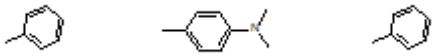
The evaluation was performed for the synthesised compounds 3B, 3C, 3D, 3E, 3F and 3H on Human Breast Cancer (MCF-7) cells to find a toxic concentration by MTT assay as per previous work.

RESULTS AND DISCUSSION

Synthesis of designed hybrids: (3B, 3C, 3D, 3E, 3F and 3H)



Compound	R	R_1	R_2
3B			
3C			
3D			
3E		—CH_3	
3F		—CH_3	

3H 

2-methoxy-5-((E)-((4-(phenylamino)thiazol-2-yl)imino)((Z)-phenyldiazenyl)methyl)phenol (3B). yield 72.3%, Yellow powder, m. p. 84-86°C, MS (C_{23} H_{19} N_5 O_2 S)[M]$^+$ 471, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.749ppm (singlet, N-H), 7.152- 7.757ppm(multiplet, Ar-H), 6.699- 6.721ppm (singlet, Thiazolyl), 3.089ppm (singlet, O-CH_3), 1.590ppm(singlet, O-H). IR: v 3053.11 Cm^{-1}(-NH), 1600.81-1787.89 Cm^{-1}(Ar-CH), 3197.76 Cm^{-1}(broad, O-H), 1938.33 Cm^{-1} (C=N), 1124cm^1(C-S), 3053cm^1(CH_3).

(E)-N^4-phenyl-N^2-(phenyl((Z)-phenyldiazenyl)methylene)thiazole-2,4-diamine (3C). yield 70.53% Brown color powder, m. p. 98-100°C, MS (C $_{24}H_{19}$ N_5 O S)[M]$^+$ 425, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.736ppm(singlet,N-H), 7.082- 7.819 ppm (multiplet, Ar-H), 6.693- 6.75ppm (singlet,Thiazolyl), 1.714ppm (singlet, $COCH_3$). IR: v 3294 Cm^{-1}(-NH), 1598-1947 Cm^{-1}(Ar-CH), 1039.56 Cm^{-1} (C=N), 1321cm^{-1}(C-S), 3060cm^{-1}(CH_3), 1664 cm^{-1} (C=O).

1-(4-((Z)-((E)-phenyl((4-(phenylamino)thioazol-2-l)imino)methyl)diazenyl)phenyl)ethanone (3D). yield 73.20%, Brown color powder, m. p. 76- 78°C, MS (C_{22} H_{17} N_5 S)[M]+383, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.743ppm (singlet, N-H), 7.106- 7.752ppm(multiplet, Ar-H), 6.697-6.720ppm(singlet, Thiazolyl). IR: v 3371 Cm^{-1}(-NH), 2109-2190 Cm^{-1}(Ar-CH), 1122.49 Cm^{-1} (C=N), 1400cm^{-1}(C-S).

(E)-N^4-phenyl-N^2-(1-((Z)-phenyldiazenyl)ethylidene)thiazole-2,4-diamine (3E). yield 80.09%, Orange color powder, m. p. 85-87°C, MS (C $_{19}H_{17}$ N_5 O S)[M]$^+$363, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.737ppm (singlet, N-H), 7.081- 7.814ppm (multiplet, Ar-H), 6.629ppm (singlet, Thiazolyl), 1.705ppm (singlet, $COCH_3$), 3.089ppm (singlet, O-CH_3), 2.004ppm (singlet, Alkyl CH_3). IR: v 3296 Cm^{-1}(-NH), 1866-2260 Cm^{-1}(Ar-CH), 1130 Cm^{-1} (C=N), 1323cm^{-1}(C-S), 3058cm^{-1}(CH_3), 1664 cm^{-1} (C=O).

1-(4-((Z)-((E)-1-((4-(phenylamino)thiazol-2-yl)imino)ethyl)diazenyl)phenyl)ethanone (3F). yield 76.32%, Orange powder, m. p. 73-75°C, MS (C_{17} H_{15} N_5 S)[M]$^+$321, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.679ppm (singlet,N-H), 7.113- 7.522ppm (multiplet, Ar-H), 6.629ppm (singlet, Thiazolyl), 3.086ppm (singlet, Alkyl CH_3). IR: v 3195 Cm^{-1}(-NH), 1722-1955 Cm^{-1}(Ar-CH), 1070 Cm^{-1} (C=N), 1244cm^{-1}(C-S), 3053cm^{-1}(CH_3).

1-(4-((Z)-((E)-(4-(dimethylamino)phenyl)((4-(phenylamino)thiazol-2-yl) imino) methyl) diazenyl) phenyl) ethanone (3H). yield 61.06%, Reddish brown color powder, m. p. 94-96°C, MS (C_{24} H_{22} N_6 S)[M]$^+$426, ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.757ppm (singlet, N-H), 7.157- 7.764ppm

(multiplet, Ar-H), 6.698ppm (singlet, Thiazolyl), 3.086ppm (singlet, Alkyl CH_3). IR: v 3396.41Cm^{-1}(-NH), 1598-1947 Cm^{-1}(Ar-CH), 1039.56 Cm^{-1} (C=N), 1334cm^{-1}(C-S), 3053cm^{-1}(CH_3), 1340.43Cm^{-1} (C-N).

***In-vitro* anti cancer activity:**

All the prepared compounds were screened for their in vitro cytotoxicity activity against MCF-7 human breast cancer cell lines by MTT assay. The cells were exposed to different concentrations ranging from 1000µg/ml to 7.8µg/ml to determine the percentage growth inhibition on MCF-7 cells. The test compounds, 3B, 3H, 3F, 3C, 3D and 3E exhibited a CTC_{50} value of 17.77±0.31µg/ml, 17.83±1.14 µg/ml,.25.61±0.44µg/ml, 237.78±3.46µg/ml, 120.28±3.52µg/ml and 147.80±4.73µg/ml on MCF-7 cells respectively. (Table no.1 and Figure no.1.) The results revealed that compounds 3B and 3H were showed the more promising activity against the MCF-7 cell lines.

Table.1. The *in-vitro* cytotoxic activity of synthesized compounds by MTT assay

Sl. No	Name of Test Substance	TestConc. (g/ml)	% Cytotoxicity	CTC_{50} (g/ml)
1.	3B	1000 500 250 125 62.5 31.25 15.6 7.8	94.97±0.24 93.21±0.24 92.52±0.16 85.97±1.12 73.04±0.44 61.72±0.25 48.10±0.32 31.62±0.33	**17.77±0.31**
		1000 500 250 125	95.73±0.28 94.05±0.16 92.42±0.34 84.91±0.42	**17.83±1.14**

2.	3H	62.5 31.25 15.6 7.8	71.72±0.44 54.50±0.82 49.24±0.48 32.70±0.65	
3.	3F	1000 500 250 125 62.5 31.258 15.6 7.8	93.26±0.32 92.42±0.28 83.86±0.09 82.89±0.20 80.09±0.28 56.32±0.80 38.84±0.28 33.04±0.41	**25.61±0.44**
4.	3C	1000 500 250 125 62.5 31.25 15.6 7.8	70.58±0.67 53.18±0.53 51.15±0.42 39.48±1.26 35.64±0.96 26.83±1.72 15.79±0.44 2.52±0.84	**237.78±3.46**
		1000 500 250	62.54±1.26 56.32±0.64 53.04±0.63	

5.	3D	125 62.5 31.25 15.6 7.8	50.87±0.64 39.13±1.08 21.94±0.67 14.40±0.64 6.01±0.64	**120.28±3.52**
6.	**3E**	**1000** **500** **250** **125** **62.5** **31.25** **15.6** **7.8**	**66.18±0.74** **59.26±1.52** **56.81±1.17** **48.43±0.63** **39.48±0.74** **35.15±0.53** **23.48±0.55** **1.68±0.42**	**147.80±4.73**

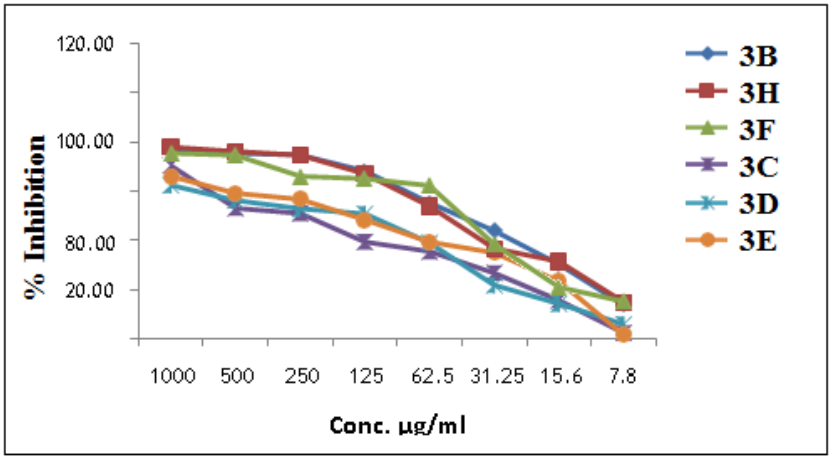


Figure. 2. The graphical representation of *in-vitro* cytotoxic activity of synthesized compounds by MTT assay

CONCLUSION

Six new derivatives of targeted series were prepared through base catalysed diazo coupling reaction. All final compounds were screened for their in-vitro cytotoxicity activity by MTT assay method. All the above synthesized compounds were found to be active against the selected MCF-7 cell lines. Among six compounds, the prominent anticancer activity was observed with 3B, 3H and 3F with CTC_{50} value of 17.77±0.31µg/ml, 17.83±1.14 µg/ml and 25.61±0.44µg/ml. The outcomes from the findings recommend that the designed series exhibited promising cytotoxic effect against MCF-7 cell lines and also it might be successful against cancerous cells. So the study can be extended further for i*n-vivo* anticancer methods.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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