Original Article

Design, Synthesis, Antioxidant and Anticancer Activity of Novel Schiff's Bases of 2-Amino Benzothiazole

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ABSTRACT

Introduction: Around 5,00,000 women are affected by cervical cancer and nearly half of them end up losing the battle of life with this deadly disease. So, there is an urgent need for the synthesis and development of new, small, synthetic molecules to tackle this challenge. Schiff's bases are derivatives of azomethine group (-CH=N-) and are highly reactive. Here a series of novel Schiff's bases were synthesized by single step process of condensing substituted 2-amino benzothiazole with different benzaldehydes. **Objectives:** To design, synthesize novel Schiff's bases of 2-amino benzothiazole and evaluate its anti-oxidant as well as anti-cancer activity. **Methods:** A total of 18 compounds were synthesized by single step process of condensing substituted 2-amino benzothiazole with different substituted benzaldehydes. These were characterized by FTIR, ^{1}H NMR, and Mass spectroscopy. The synthesized compounds were tested *in-vitro* for both antioxidant and antiproliferative activity. *In-silico* docking studies were performed on the crystal structure of the complex of caspase-3 with a nicotinic acid aldehyde inhibitor with PDB IDs 1RE1, 1RHM and 3DEH to study the interaction of the compounds with the receptor. **Results:** Majority of the derivatives displayed moderate to significant antiproliferative activity on HeLa cell line. Interestingly, the compound SP16 showed excellent activity with an IC_{50} value of 2.517μg/ml in comparison to the reference compound Cisplatin (17.2μg/ml). Compound SP7 and SP 15 showed favourable *in silico* interactions.**Conclusion:** A series of 18 novel Schiff's bases of 2-amino benzothiazoles compounds were designed, synthesized and evaluated for their biological activities. The compound SP16 showed excellent activity with an IC_{50} value of 2.517μg/ml in comparison to the reference compound Cisplatin and Compound SP7 and SP 15 showed favourable *in silico* interactions.

Key words: Schiff's base, Anti-cancer activity, HeLa, 1RE1, 1RHM, 3DEH, Antioxidant activity.

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INTRODUCTION

Cancer has emerged as a malignant devil which needs to be trounced, because according to WHO 14 million new cases and 8.2 million cancer related death were reported in 2012.[1] The number of new cases is expected to rise by about 70% in the next two decadeds.[1] According to American Cancer society by 2050, 27 million new cancer cases will be registered. We all know that cancer is characterized by uncontrolled and undesirable cell division. Unlike other diseases, it can affect all organs of the body like bones, muscles, brain, blood, liver and other organs. Cancer is addressed with different names based on the site of origin and out of all type of cancer, cervical cancer (CC) needs special attention in the context of India. Its high mortality rate in developing countries like India exemplifies health inequity. Around 5,00,000 women are

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affected by it and nearly half of them end up losing the battle of life with this deadly disease.[2] CC is characterized by extra growth of cervical cancer cells and is the third most common cancer among the women. Many epidemiological and biological studies point out infection with human papilloma viruses (HPV) as the most important etiological event in CC development.[3] HPV DNA has been discerned in more than 95% of CC biopsies, clearly suggesting a strong association of CC with HPV infection. Caspase 3,[4] E6 and E7 viral oncoprotein,[5] p53[6] apoptotic proteins have become the preferred target for the development of new anti-cervical cancer drugs. Currently available therapy for cervical cancer includes vaccines and drugs like cisplatin, topotecan, paclitaxel, gemcitabine and others. The main drawback of these therapies are its cost, effectiveness and severe side effects. Hence, there is an urgent need for the synthesis and development of new drug molecule. Development of free radical has a pivotal role in the development of many diseases like cancer, diabetes, cardiovascular diseases, autoimmune diseases.[7] Oxidative stress is the main reason for generation of free radicals. Hence, antioxidants have an important role in the prevention and treatment of these diseases. Literature study revealed many studies on the antioxidant activity of benzothiazoles.[8]

Schiff's bases are derivatives of azomethine group (-CH=N-) and are prepared by the condensation of the primary amine with aldehyde or ketone. These are one of the most extensively used compounds for industrial purpose as dyes, pigments, catalysts, intermediates in organic synthesis and as polymer stabilizer.[6-7] Schiff's bases have been utilized as starting materials in the preparation of a number of industrial and biologically active compounds like formazans, 4-thiazolidinones, benzoxazines, and so forth, via cycloaddition, ring closure and replacement reactions.[8]

Moreover, it also shows a wide range of biological activities like antifungal, antibacterial,[12-15] antimalarial,[16] anticonvulsant,[17-18] antitumor,[19] fungicidal,[20-21] antileishmanial,[22] anti-inflammatory and anti-viral[23] properties. These compounds also play an important role in coordination chemistry and enzymatic reaction. 2-amino benzothiazoles containing Schiff bases are highly reactive compounds, they are widely utilized as reactant or reaction intermediates since NH_2 and endocyclic 'N' functions are suitable to enable reaction with common electrophilic agents to form a variety of fused heterocyclic compounds. Benzothiazoles having $–NH_2$, -Cl, -OH group show greater anticancer activity. Chloro (-Cl) substituted amino benzothiazoles were found to have more sensitivity to cancer cell lines as compared to fluoro (-F) substituted benzothiazoles.[24-25]

In this study, we have synthesized some novel Schiff's base derivatives by the reaction of substituted 2-amino benzothiazoles with various substituted benzaldehydes. All synthesized compounds were tested for antioxidant activity by DPPH free radical scavenging assay and also tested for anticancer activity on cervical cancer cell lines (HeLa) by using MTT assay. *In-silico* docking studies were performed by GLIDE (Schrodinger maestro, V: 10.3) against caspase-3 with a nicotinic acid aldehyde inhibitor with PDB ID 1RE1, 1RHM,[26] 3DEH.[27]

MATERIALS AND METHODS

Instruments

The purity of the compounds was checked by Thin Layer Chromatography (TLC) using ethyl acetate and hexane (1:1) as an eluent, FT-IR spectra were obtained using FT-IR 5000 spectrophotometer using KBr discs. H^1NMR spectra were recorded on Bruker UXNMR (300 MHz) in DMSO using TMS as an internal standard. Mass of synthesized compounds was identified by using LC-MS through (Atmospheric pressure control ionization)-APTI method. Melting point of the synthesized compounds was determined by using melting point apparatus. Microplate reader was used for measuring the absorbance to calculate the IC_{50} of the compound.

Synthesis

Eighteen novel Schiff's base derivatives were synthesized by one-step process which involves condensation of substituted 2-amino benzothiazoles with substituted benzaldehydes. All the synthesized compounds were stable with high melting points. Compounds were purified by recrystallization, and structure of all synthesized compounds were established with the help of FT-IR, ^{1}H NMR and Mass Spectroscopy data.

General Procedure for the Synthesis of Compounds SP1-SP18

To a solution of 0.005mol of substituted 2-amino benzothiazole in a minimum amount of ethanol, a solution of substituted benzaldehyde (0.005mol) in a minimum amount of ethanol was added to a 100ml round bottom flask. One drop of H_2SO_4 was added as a catalyst. The reaction mixture was kept for refluxing for 12h. The precipitate obtained was collected by filtration and washed with ice-cold water. It was dried well and recrystallized from ethanol and dried at room temp. The product of Schiff's bases were obtained (**SP1-SP18**) and whose scheme of synthesis is shown (**Scheme 1**) below

along with the physicochemical properties (**Table 1**), followed by spectral interpretation.

(E)-N-(6-ethoxy-1, 3-benzothiazol-2-yl)-1-(2-fluorophenyl) methanimine, SP1

M.F: $C_{16}H_{13}FN_2OS$, **IR** (cm^{-1}): 1641.42(C=N), 1602.85(C=C), 1307.74(C-O), 3074.53(aromatic C-H stretch), **^{1}H NMR** (DMSO, δ, ppm)**:** 1.32(3C-H Triplet), 4.03(2 C-H quadrant), 7.38(aromatic 4H Doublet of doublet), 8.32(N=CH singlet), 6.9 (aromatic 2H Doublet). LC-MS (ESI, Positive): m/z: $(M+H)^+$ = 300.35.

(*E*)-*N*-(6-ethoxy-1, 3-benzothiazol-2-yl)-1-(4-fluorophenyl) methanimine, SP2

M.F: $C_{16}H_{13}FN_2OS$, **IR** (cm^{-1}): 1645.28(C=N), 1604.77(C=C), 1307.74(C-O), 3062.96(aromatic C-H stretch), **^{1}H NMR** (DMSO, δ, ppm)**:** 1.34(3 C-H Triplet), 4.034(2 C-H quadrant), 7.40(aromatic 4H Doublet of doublet), 8.38(N=CH singlet), 6.92 (aromatic 2H Doublet). LC-MS (ESI, Positive): m/z: $(M+H)^+$ = 301.30.

3-{(*E*)-[(6-ethoxy-1, 3-benzothiazol-2yl) imino] methyl} phenol, SP3:

M.F: $C_{16}H_{14}N_2O_2S$, **IR** (cm^{-1}): 1645.28(C=N), 1602.85(C=C), 1307.74(C-O), 3070.68(aromatic C-H stretch), **^{1}H NMR** (DMSO, δ, ppm)**:**1.33(3 C-H Triplet), 4.015(2 C-H quadrant), 7.29(aromatic 4H Doublet of doublet), 8.34(N=CH singlet), 6.99 (aromatic 2H Doublet). LC-MS (ESI, Positive): m/z: $(M+H)^+$ = 299.19.

(*E*)-*N*-(6-ethoxy-1, 3-benzothiazol-2-yl)-1-(3, 4, 5-trimethoxyphenyl) methanimine, SP4

M.F: $C_{19}H_{20}N_2O_4S$, **IR** (cm^{-1}): 1645.28(C=N), 1604.77(C=C), 1307.74(C-O), 3057.17(aromatic C-H stretch), **^{1}H NMR** (DMSO, δ, ppm)**:** 1.32(3 C-H Triplet), 4.012(2 C-H quadrant), 7.30(aromatic 4H Doublet of doublet), 8.28(N=CH singlet), 6.89 (aromatic 2H Doublet). LC-MS (ESI, Positive): m/z: $(M+H)^+$ = 373.41.

(*E*)-*N*-(6-ethoxy-1, 3-benzothiazol-2-yl)-1-(thiophen-3-yl) methanimine, SP5

M.F: $C_{14}H_{12}N_2OS_2$, **IR** (cm^{-1}): 1637.56(C=N), 1608.63(C=C), 1309.67(C-O), 3076.49(aromatic C-H stretch), **^{1}H NMR** (DMSO, δ, ppm)**:** 1.33(3 C-H Triplet), 4.02(2 C-H quadrant), 7.39(aromatic 4H Doublet of doublet), 8.29(N=CH singlet), 6.921(aromatic 2H Doublet). LC-MS (ESI, Positive): m/z: $(M+H)^+$ = 289.38.

(*E*)-*N*-(6-ethoxy-1, 3-benzothiazol-2-yl)-1-(4-methylphenyl) methanimine, SP6

M.F: $C_{17}H_{16}N_2OS$, **IR** (cm^{-1}): 1643.35(C=N), 1604.77(C=C), 1307.74(C-O), 3072.60(aromatic C-H stretch), **^{1}H NMR** (DMSO, δ, ppm)**:** 1.30(3 C-H Triplet), 3.98(2 C-H quadrant), 7.28(aromatic 4H Doublet of doublet), 8.35(N=CH singlet), 6.91 (aromatic 2H Doublet). LC-MS (ESI, Positive): m/z: $(M+H)^+$ = 297.36.

(*E*)-1-(2-fluorophenyl)-*N*-(6-methoxy-1, 3benzothiazol-2yl) methanimine, SP7

M.F: $C_{15}H_{11}FN_2OS$, **IR** (cm^{-1}): 1643.35(C=N), 1604.77(C=C), 1307.74(C-O), 3072.60(aromatic C-H stretch), **^{1}H NMR** (DMSO, δ, ppm)**:** 3.82(3 C-H quadrant), 7.39 (aromatic 4H Doublet of doublet), 8.59(N=CH singlet), 6.89 (aromatic 2H Doublet). LC-MS (ESI, Positive): m/z: $(M+H)^+$ = 287.36.

(*E*)-1-(4-fluorophenyl)-*N*-(6-methoxy-1, 3-benzothiazol-2-yl) methanimine, SP8

M.F: $C_{15}H_{11}FN_2OS$, **IR** (cm^{-1}): 1641.42(C=N), 1602.85(C=C), 1307.74(C-O), 3101.54(aromatic C-H stretch), **^{1}H NMR** (DMSO, δ, ppm)**:** 3.76(3 C-H quadrant), 7.43(aromatic 4H Doublet of doublet), 8.43(N=CH singlet), 6.95 (aromatic 2H Doublet). LC-MS (ESI, Positive): m/z: $(M+H)^+$ = 287.40.

(*E*)-*N*-(6-methoxy-1, 3-benzothiazol-2-yl)-1-(3-nitrophenyl) methanimine, SP9

M.F: $C_{15}H_{11}N_3O_3S$, **IR** (cm^{-1}): 1641.44(C=N), 1602.85(C=C), 1307.74(C-O), 3097.68(aromatic C-H stretch), **^{1}H NMR** (DMSO, δ, ppm)**:** 3.69(3 C-H quadrant), 7.40(aromatic 4H Doublet of doublet), 8.42(N=CH singlet), 6.95 (aromatic 2H Doublet). LC-MS (ESI, Positive): m/z: $(M+H)^+$ = 314.39.

(*E*)-*N*-(6-methoxy-1, 3-benzothiazol-2-yl)-1-(3, 4, 5-trimethoxyphenyl) methanimine, SP10

M.F: $C_{18}H_{18}N_2O_4S$, **IR** (cm^{-1}): 1639.49(C=N), 1602.85(C=C), 1307.74(C-O), 3099.61(aromatic C-H stretch), **^{1}H NMR** (DMSO, δ, ppm)**:** 3.83(6 C-H), 7.41(aromatic 4H Doublet of doublet), 8.36(N=CH singlet), 6.93(aromatic 2H Doublet). LC-MS (ESI, Positive): m/z: $(M+H)^+$ = 359.46.

(*E*)-*N*-(6-methoxy-1, 3-benzothiazol-2-yl)-1-(thiophen-3-yl) methanimine, SP11

M.F: $C_{13}H_{10}N_2OS_2$, **IR** (cm^{-1}): 1641.42(C=N), 1606.70(C=C), 1307.74(C-O), 3101.54(aromatic C-H stretch), **^{1}H NMR** (DMSO, δ, ppm)**:** 3.70(4C-H), 7.29(aromatic 4H Doublet of doublet), 8.26(N=CH singlet), 6.90(aromatic 2H Doublet). LC-MS (ESI, Positive): m/z: $(M+H)^+$ = 275.42.

(*E*)-*N*-(6-methoxy-1, 3-benzothiazol-2-yl)-1-(4-methylphenyl) methanimine, SP12

M.F: $C_{16}H_{14}N_2OS$, **IR** (cm^{-1}): 1639.49(C=N), 1604.77(C=C), 1307.74(C-O), 3101.54(aromatic C-H stretch), **^{1}H NMR** (DMSO, δ, ppm)**:** 3.76(6C-H),

7.43(aromatic 4H Doublet of doublet), 8.445(N=CH singlet), 6.95(aromatic 2H Doublet). LC-MS (ESI, Positive): m/z: $(M+H)^+$ = 283.40.

(*E*)-*N*-(6-chloro-1, 3-benzothiazol-2-yl)-1-(2-fluorophenyl) methanimine, SP13

M.F: $C_{14}H_8ClFN_2S$, **IR** (cm^{-1}): 1641.42(C=N), 1514.12(C=C), 3055.24(aromatic C-H stretch), **^{1}H NMR** (DMSO, δ, ppm)**:** 7.32(aromatic 4H Doublet of doublet), 8.38(N=CH singlet), 6.92(aromatic 2H Doublet). LC-MS (ESI, Positive): m/z: $(M+H)^+$ = 291.83.

(*E*)-*N*-(6-chloro-1, 3-benzothiazol-2-yl)-1-(2-chlorophenyl) methanimine, SP14

M.F: $C_{14}H_8Cl_2N_2S$, **IR** (cm^{-1}): 1649.14(C=N), 1529.55(C=C), 3055.24(aromatic C-H stretch), **^{1}H NMR** (DMSO, δ, ppm)**:** 7.01(aromatic 4H Doublet of doublet), 8.26(N=CH singlet), 6.90(aromatic 2H Doublet). LC-MS (ESI, Positive): m/z: $(M+H)^+$ = 308.26.

3-{(*E*)-[(6-chloro-1, 3-benzothiazol-2-yl) imino] methyl} phenol, SP15

M.F: $C_{14}H_9ClN_2OS$, **IR** (cm^{-1}): 1645.28(C=N), 1533.41(C=C), 3089.96(aromatic C-H stretch), **^{1}H NMR** (DMSO, δ, ppm)**:** 7.2(aromatic 4H Doublet of doublet), 8.28(N=CH singlet), 6.91(aromatic 2H Doublet). LC-MS (ESI, Positive): m/z: $(M+H)^+$ = 289.85.

(*E*)-*N*-(6-chloro-1, 3-benzothiazol-2-yl)-1-(2,5 dimethoxyphenyl) methanimine, SP16

M.F: $C_{16}H_{13}ClN_2O_2S$, **IR** (cm^{-1}): 1653.00(C=N), 1544.98(C=C), 3088.03(aromatic C-H stretch), **^{1}H NMR** (DMSO, δ, ppm)**:** 7.32(aromatic 4H Doublet of doublet), 8.38(N=CH singlet), 6.92(aromatic 2H Doublet). LC-MS (ESI, Positive): m/z: $(M+H)^+$ = 333.88.

(*E*)-*N*-(6-chloro-1, 3-benzothiazol-2-yl)-1-(thiophen-3-yl) methanimine, SP17

M.F: $C_{12}H_7ClN_2S_2$, **IR** (cm^{-1}): 1641.42(C=N), 1544.98(C=C), 3066.82(aromatic C-H stretch), **^{1}H NMR** (DMSO, δ, ppm)**:** 7.37(aromatic 4H Doublet of doublet), 8.73(N=CH singlet), 7.14(aromatic 2H Doublet). LC-MS (ESI, Positive): m/z: $(M+H)^+$ = 279.71.

(*E*)-*N*-(6-chloro-1, 3-benzothiazol-2-yl)-1-(4-methylphenyl) methanimine, SP18

M.F: $C_{15}H_{11}ClN_2S$, **IR** (cm^{-1}): 1654.92(C=N), 1531.48(C=C), 3089.96(aromatic C-H stretch), **^{1}H NMR** (DMSO, δ, ppm)**:** 7.26(aromatic 4H Doublet of doublet), 8.83(N=CH singlet), 6.98(aromatic 2H Doublet). LC-MS (ESI, Positive): m/z: $(M+H)^+$ = 287.84.

In vitro antioxidant activity

All the synthesized novel Schiff's bases were tested for the antioxidant activity by *in vitro* 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay with concentrations ranging from 15.62-1000µg/ml. The assay is based on the measurement of the scavenging capacity of antioxidants towards the DPPH free radical. DPPH is a stable free radical which produces a violet purple solution in ethanol. Antioxidants react with DPPH and convert it into DPPHH and give a colourless solution.[28-29] As a result there will be decrease in absorbance due to the pairing of free electrons. The degree of decolouration indicates the scavenging potential of the antioxidant compounds.

$$\underset{\text{(Purple)}}{(DPPH) + (H\text{-}A)} \longrightarrow \underset{\text{(Colorless)}}{DPPH - H + (A)}$$

DPPH in 0.1mM of ethanol was prepared and 10µl of test sample solution was added to the 200µl of methanolic DPPH solution at different concentrations. The mixture was incubated at room temperature for 20 min and absorbance was measured at 517nm by using ascorbic acid as a standard. A blank was prepared without adding standard or test solution. Decrease in the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capacity to scavenge the DPPH radical was calculated using the following equation.[30]

$$\%\text{DPPH scavenged} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance in the presence of test compounds. The antioxidant activity of the test samples are expressed in comparison with ascorbic acid as standard.[31]

In vitro Cytotoxicity Studies

In vitro cytotoxicity studies of the synthesized compounds were performed by 3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide) (MTT) assay. The cell culture of HeLa (cervical cancer) was procured from National Centre for Cell Sciences (NCCS), Pune, India. The monolayer cell culture was trypsinized and cell count adjusted to 1.0 x 10^5 cells/ml using DMEM medium containing 10% NBCS. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, the monolayer once washed with medium and 100 µl of different extract concentrations was added to the cells in microtitre plates. Then the plates were incubated at 37°C for 3 days in 5% CO_2 atmosphere,

and microscopic examination was carried out and observations were noted every 24h. After 72 h, the extracted solutions in the wells were discarded and 50 µl of MTT in MEM–PR (Minimum essential medium without phenol red) was added to each well. The plates were gently shaken and incubated for 3h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 50 µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and CTC_{50} (concentration of drug or test extract needed to inhibit cell growth by 50%) values were generated from the dose-response curves for each cell line.

The pattern of all the cell lines as a group is used to rank compounds as toxic or non-toxic.[32]

$$\% \text{ Growth Inhibition} = \frac{\text{Mean OD of individual test group}}{\text{OD of control group}} \times 100$$

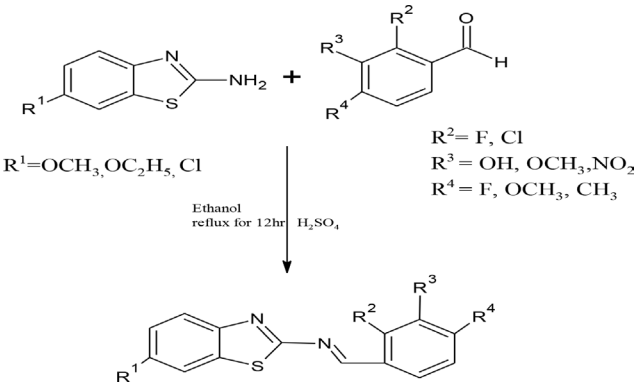
Molecular Docking Studies

Molecular docking study was performed on the synthesized novel ligands by using GLIDE (Schrodinger maestro, V: 10.3). Crystal structure of the caspase-3 with a nicotinic acid aldehyde inhibitor (PDB ID 1RE1, 1RHM, 3DEH) was downloaded from protein data bank (http://www.rcsb.org).The ligand was prepared by using Maestro build panel and the corresponding low energy 3D conformers of the ligand were prepared by LigPrep module. The protein was prepared using protein preparation wizard in which all the water molecules were removed and hydrogen atoms were added to the target. Receptor Grid generation was performed around the active site of the protein by selecting the co-crystalized ligand. Low energy conformation of the compound was selected and docked into the grid using standard precision docking protocol (SP). Dock pose of the ligand was analyzed for interactions with the receptor.

RESULT AND DISCUSSION

Chemistry

With a plan to develop novel benzothiazole Schiff's bases with antioxidant and anticytotoxic activity from easily available starting material, here in this paper we report the synthesis of some novel benzothiazole Schiff's bases derivatives (**Scheme 1**). Synthesized compounds were characterized by FT-IR, H^1 NMR, and LC-MS spectral data. We have synthesized eighteen



Scheme 1: General Scheme for synthesis of Schiff bases.

novel Schiff base derivatives by one step process which involves condensation of substituted 2-amino benzothiazoles with substituted benzaldehydes. The compounds were further purified by recrystallization. All the compounds were stable with high melting points, and these compounds were insoluble in common organic solvents other than DMSO. (Physicochemical properties of the synthesized compounds are reported in **Table 1**)

In vitro antioxidant activity

Antioxidant activity is expedient for performing many related biological activities, including anti-inflammatory, antiallergic, anticancer and antidiabetic. All the synthesized compounds were screened for their antioxidant activity. DPPH assay was performed to measure the ability of the compounds to scavenge the DPPH free radicals. Colour change produced by free radical scavenging, results a change in absorbance.

The assay was performed in concentrations ranging from 15.62-1000 µg/ml by using ascorbic acid as standard. Among the compounds **SP7, SP12, SP15, SP16, SP18** showed moderate activity. But the result was not good as compared to that of standard (ascorbic acid) (The results are reported in **Table 2**).

In vitro Cytotoxicity Studies

In vitro cytotoxicity studies of the synthesized compounds were performed by 3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide) (MTT) assay. Among the 18 compounds screened, the compound **SP16** bearing methoxy group substitution showed more potent activity with an IC_{50} value of (concentration of test substance to achieve 50% inhibition) **2.517µg/ml** than the standard (cisplatin) against HeLa cell line.The compounds **SP15** and **SP17** bearing hydroxyl and thiole substitution showed IC_{50} **< 25 µg/ml.** Compound **SP13** bearing fluorine substitution showed IC_{50}= **29.48 µg/ml.** The results are presented in **Table 3**.

Table 1: Physicochemical properties of synthesized Schiff bases SP1- SP18.

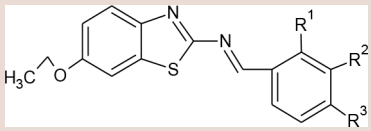
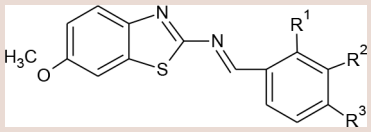
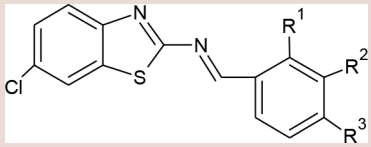
Structure	Comp. Code	M.P	M.W	R_f	Log P	pKa	%yield
	**SP1**	215°C	300.35	0.5	3.78	3.61	33%
	SP2	218°C	300.35	0.31	3.76	3.73	40%
	SP3	198°C	298.35	0.57	3.36	3.84	35%
	SP4	195°C	372.43	0.54	3.43	4.45	25.3%
	SP5	220°C	288.38	0.48	3.39	3.29	60%
	SP6	205°C	296.38	0.42	3.96	3.74	52.6%
	SP7	220°C	286.32	0.45	3.43	3.53	38%
	SP8	223°C	286.32	0.47	3.44	3.64	30%
	SP9	221°C	313.33	0.57	2.83	3.47	32%
	SP10	230°C	358.4	0.51	3.11	4.32	20.6%
	SP11	225°C	274.36	0.46	3.08	3.2	40%
	SP12	238°C	282.36	0.40	3.64	3.65	56.8%
	**SP13**	198°C	290.74	0.54	4.03	2.07	40%
	SP14	192°C	307.19	0.42	4.34	1.93	39%
	SP15	208°C	288.75	0.56	3.62	2.6	35%
	SP16	215°C	332.80	0.5	3.76	3.4	55%
	SP17	205°C	278.78	0.63	3.58	2.06	53.5%
	SP18	220°C	286.77	0.5	4.37	2.47	59.7%

Table 2: Antioxidant activity (IC_{50}) of Synthesized Compounds SP1-SP18.

Comp. Code	IC_{50} (µg/ml)
SP1	>62.5
SP2	>62.5
SP3	>62.5
SP4	>62.5
SP5	>62.5
SP6	>62.5
SP7	**51.06**
SP8	89.15
SP9	>62.5
SP10	>62.5
SP11	>62.5
SP12	**53.3**
SP13	>62.5
SP14	>62.5
SP15	**46.9**
SP16	**38.19**
SP17	>62.5
SP18	**62.5**
Ascorbic acid	0.064

Table 3: IC_{50} values for tested compounds against HeLa cell lines of Compounds SP1-SP18.

S.no	Compound code	HeLa cell line IC_{50}
1	SP1	178.9
2	SP2	188.4
3	SP3	174.6
4	SP4	176.6
5	SP5	>200
6	SP6	117.7
7	SP7	65.95
8	SP8	101.1
9	SP9	116.6
10	SP10	103.5
11	SP11	179.9
12	SP12	142.1
13	SP13	29.48
14	SP14	>200
15	SP15	22.31
16	**SP16**	**2.517**
17	SP17	22.2
18	SP18	72.5
Standard	Cisplatin	17.2

Table 4: Dock score of the Synthesized Novel Schiff base Ligands (SP1- SP18) on PDB ID 1RE1, 1RHM, 3DEH.

Compound Code	Dock Score		
	Target 1RE1	Target 1RHM	Target 3DEH.
SP1	-4.108	-4.862	-5.928
SP2	-3.85	-4..001	-5.527
SP3	-4.656	-5.105	**-7.125**
SP4	-2.434	-4.817	-5.686
SP5	-3.742	-3.808	-5.407
SP6	-3.638	-3.749	-5.456
SP7	**-5.265**	-3.668	-6.148
SP8	-4.207	-3.929	-5.937
SP9	-4.305	-4.4	-5.604
SP10	-3.413	-3.104	-5.188
SP11	-4.468	-4.114	-5.363
SP12	-4.121	-3.993	-5.47
SP13	-4.835	-3.938	-6.274
SP14	-4.692	-3.839	-5.648
SP15	-4.946	**-5.116**	-5.993
SP16	-4.204	-4.958	-5.819
SP17	-3.603	-4.18	-5.531
SP18	-3.558	-4.388	-6.01

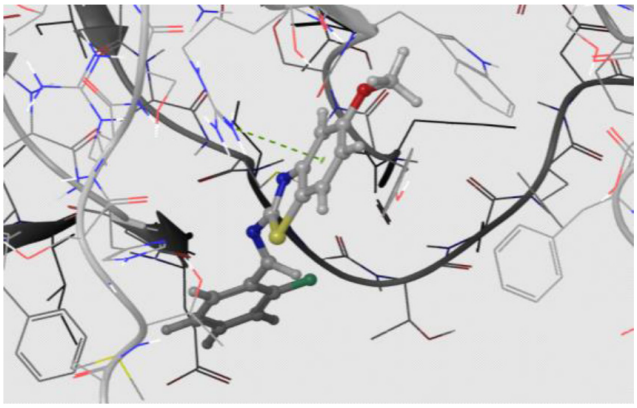


Figure 1A: Binding Interactions of Compound SP7 with receptor

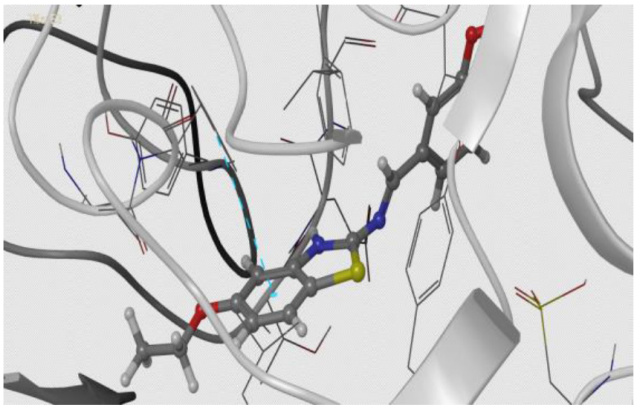


Figure 1B: Binding Interactions of Compound SP3 with receptor.

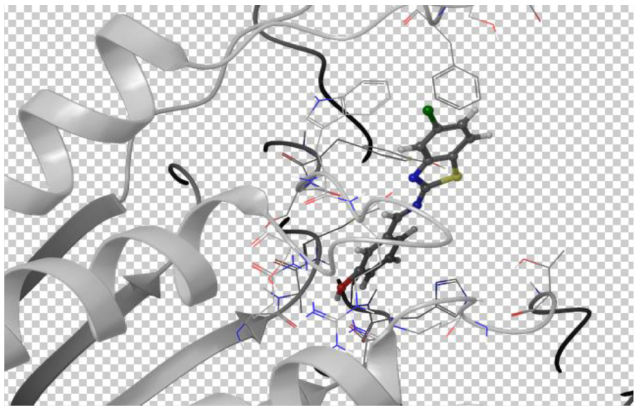


Figure 1C: Binding Interactions of Compound SP15 with receptor.

Molecular Docking Studies

In order to understand the detailed interaction between novel compounds and the receptor, Molecular docking studies were performed on the synthesized novel ligands by using GLIDE (Schrodinger maestro, V:10.3). Crystal structure of the caspase-3 with a nicotinic acid aldehyde inhibitor (PDB ID 1RE1, 1RHM, 3DEH) was downloaded from protein data bank (http://www.rcsb.org). From literature search we found this target being used for docking studies.

From 2D-presentation of the binding interactions of compound **SP7** with the receptor (**Figure 1A**), it was observed that compound **SP7** interacts with ARG B: 348 of receptor active site.

The binding interactions of compound **SP3** with the receptor (**Figure 1B**), shows that compound **SP3** interacts with THR A: 62, LYS C: 259 and THR C: 166 of receptor active site

The binding interactions of compound **SP15** with receptor (**Figure 1C**), shows that **SP15** interacts with SER A: 236, ARG A: 179 and GLU A: 283 of receptor active site.

These results of molecular docking studies suggest that compounds **SP7, SP15** and **SP3** had high binding potency with the three different receptor domains of caspase-3 and is presented in **Table 4.**

CONCLUSION

A series of 18 novel Schiff's bases of 2-amino benzothiazoles compounds were designed, synthesized and evaluated for their biological activities. The antioxidant activity of the compounds was tested by DPPH assay. Assay was performed in concentrations ranging from 15.62- 1000µg/ml by using ascorbic acid as a standard. Among the tested compounds, **SP7, SP12, SP15, SP16, SP18** showed moderate antioxidant activity. But

the result was not good as compared to that of standard (ascorbic acid).

In-vitro MTT assay was performed on HeLa cell lines to validate the cytotoxic activity against cervical cancer cells. The compounds **SP13**, **SP15**, **SP17** and **SP18** showed good anticancer activity against HeLa cell lines, and compound **SP16** exhibited very good activity indicating better than the standard drug Cisplatin. Generally, chlorine substitution at 6 position of benzothiazole increases the activity which was further confirmed by *in-silico* docking studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

FTIR: Fourier Transform Infra Red; **H NMR:** Proton Nuclear Magnetic Resonance; **ESI:** Electron Spray Ionisation; **HeLa:** Cervical cancer Cells taken from Henrietta Lacks and named after her.; **WHO:** World Health Organisation; **CC:** Cervical Cancer; **HPV:** Human Papilloma Virus; **DNA:** Deoxy Ribose Nucleic acid; **DMSO :** DiMethylSulphoxide; **LCMS:** Liquid Chromatography and Mass Spectroscopy; **APTI:** Atmospheric pressure control ionization; **TMS:** Tetra Methyl Silane; **H2SO4 :** Concentrated Sulphuric Acid; **DPPH:** 1, 1-diphenyl-2-pic¬rylhydrazyl; **MTT:** 3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide; N**CCS :** National Centre for Cell Sciences; **DMEM:** Dulbecco's Modified Eagle's Medium; **NBCS:** New Born Calf Serum.

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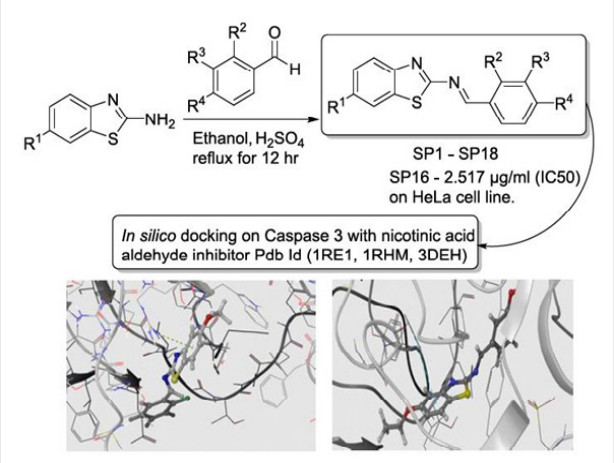
PICTORIAL ABSTRACT



SUMMARY

- A series of novel Schiff's bases were synthesized by single step process of condensing substituted 2-amino benzothiazole with different substituted benzaldehydes. A total of 18 compounds were synthesized and characterized by FTIR, ^{1}H NMR, and Mass spectroscopy. The synthesized compounds were tested *in-vitro* for both antioxidant and antiproliferative activity. Unfortunately, none of the compound showed comparable antioxidant activity with that of ascorbic acid (0.063µg/ml,) even though the majority of the derivatives displayed moderate to significant antiproliferative activity on HeLa cell line. Interestingly, the compound SP16 showed excellent activity with an IC_{50} value of 2.517µg/ml in comparison to the reference compound Cisplatin (17.2µg/ml). *In-silico* docking studies were performed to identify the interactions and binding mode of the synthesized Schiff bases on the crystal structure of the complex of caspase-3 with a nicotinic acid aldehyde inhibitor with PDB IDs 1RE1, 1RHM and 3DEH. Compound SP7 and SP15 showed favourable *in silico* interactions.

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