

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

D. Saipriya¹, Arun Prakash¹, Suvarna G Kini^{1,*}, Varadaraj Bhatt G¹, K Sreedhara Ranganath Pai², Subhankar Biswas², Mohammed Shameer K¹

¹Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, MAHE, Manipal, 576104, Karnataka, INDIA.

²Department of Pharmacology, Manipal College of Pharmaceutical Sciences, MAHE, Manipal, 576104, Karnataka, INDIA.

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, ¹H 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 IC₅₀ value of 2.517 μg/ml in comparison to the reference compound Cisplatin (17.2 μg/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 IC₅₀ value of 2.517 μg/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.

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 decades.¹ According to American Cancer society by 2050, 27 million new cancer cases will be registered. We all know that cancer is characterized by uncon-

trolled 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

Submission Date: 06-12-2017;

Revision Date: 17-05-2018;

Accepted Date: 14-08-2018

DOI:10.5530/ijper.52.4s.114

Correspondence:

Dr. Suvarna G Kini,

Manipal College of
Pharmaceutical Sciences,
MAHE, Manipal - 576104,
Karnataka, INDIA.

Phone: +919880713601

E-mail: suvarna.gk@manipal.edu



www.ijper.org

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 p53⁶ 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-viral²³ 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 NH₂ and endocyclic 'N' functions are suitable to enable reaction with common electrophilic agents to form a variety of fused heterocyclic compounds. Benzothiazoles having -NH₂, -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. ¹H NMR 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 IC₅₀ 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, ¹H 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 H₂SO₄ 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: C₁₆H₁₃FN₂OS, **IR** (cm⁻¹): 1641.42(C=N), 1602.85(C=C), 1307.74(C-O), 3074.53(aromatic C-H stretch), **¹H 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: C₁₆H₁₃FN₂OS, **IR** (cm⁻¹): 1645.28(C=N), 1604.77(C=C), 1307.74(C-O), 3062.96(aromatic C-H stretch), **¹H 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-2-yl) imino] methyl] phenol, SP3:

M.F: C₁₆H₁₄N₂O₂S, **IR** (cm⁻¹): 1645.28(C=N), 1602.85(C=C), 1307.74(C-O), 3070.68(aromatic C-H stretch), **¹H 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: C₁₉H₂₀N₂O₄S, **IR** (cm⁻¹): 1645.28(C=N), 1604.77(C=C), 1307.74(C-O), 3057.17(aromatic C-H stretch), **¹H 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: C₁₄H₁₂N₂O₂S, **IR** (cm⁻¹): 1637.56(C=N), 1608.63(C=C), 1309.67(C-O), 3076.49(aromatic C-H stretch), **¹H 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: C₁₇H₁₆N₂OS, **IR** (cm⁻¹): 1643.35(C=N), 1604.77(C=C), 1307.74(C-O), 3072.60(aromatic C-H stretch), **¹H 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-2-yl) methanimine, SP7

M.F: C₁₅H₁₁FN₂OS, **IR** (cm⁻¹): 1643.35(C=N), 1604.77(C=C), 1307.74(C-O), 3072.60(aromatic C-H stretch), **¹H 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: C₁₅H₁₁FN₂OS, **IR** (cm⁻¹): 1641.42(C=N), 1602.85(C=C), 1307.74(C-O), 3101.54(aromatic C-H stretch), **¹H 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: C₁₅H₁₁N₃O₃S, **IR** (cm⁻¹): 1641.44(C=N), 1602.85(C=C), 1307.74(C-O), 3097.68(aromatic C-H stretch), **¹H 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: C₁₈H₁₈N₂O₄S, **IR** (cm⁻¹): 1639.49(C=N), 1602.85(C=C), 1307.74(C-O), 3099.61(aromatic C-H stretch), **¹H 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: C₁₃H₁₀N₂O₂S, **IR** (cm⁻¹): 1641.42(C=N), 1606.70(C=C), 1307.74(C-O), 3101.54(aromatic C-H stretch), **¹H 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: C₁₆H₁₄N₂OS, **IR** (cm⁻¹): 1639.49(C=N), 1604.77(C=C), 1307.74(C-O), 3101.54(aromatic C-H stretch), **¹H NMR** (DMSO, δ, ppm): 3.76(6C-H),

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 μ l 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% CO₂ atmosphere. The supernatant was removed and 50 μ l 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 CTC₅₀ (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

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

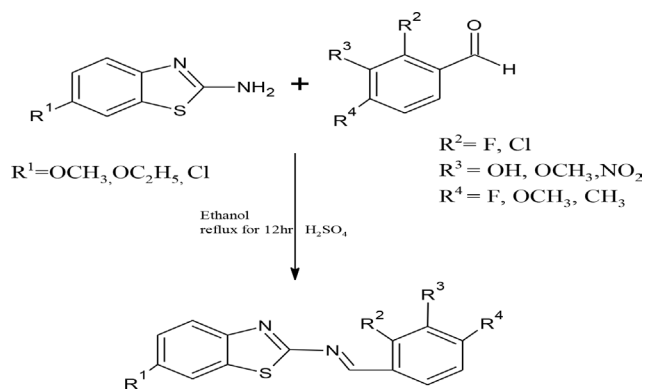
Molecular Docking Studies

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-crystallized 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, H¹ 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 μ g/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-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Among the 18 compounds screened, the compound **SP16** bearing methoxy group substitution showed more potent activity with an IC₅₀ value of (concentration of test substance to achieve 50% inhibition) **2.517 μ g/ml** than the standard (cisplatin) against HeLa cell line. The compounds **SP15** and **SP17** bearing hydroxyl and thiole substitution showed IC₅₀ < **25 μ g/ml**. Compound **SP13** bearing fluorine substitution showed IC₅₀ = **29.48 μ g/ml**. The results are presented in **Table 3**.

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

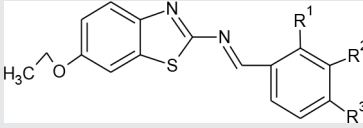
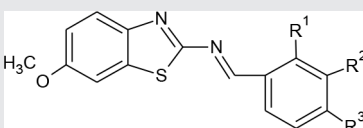
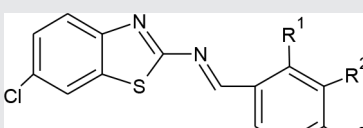
| Structure | Comp. Code | M.P | M.W | R _f | Log P | pKa | %yield |
|---|-------------|-------|--------|----------------|-------|------|--------|
|  | SP1 | 215°C | 300.35 | 0.5 | 3.78 | 3.61 | 33% |
| | SP2 | 218°C | 300.35 | 0.31 | 3.76 | 3.73 | 40% |
| | SP3 | 198°C | 298.35 | 0.57 | 3.36 | 3.84 | 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 (IC₅₀) of Synthesized Compounds SP1-SP18.

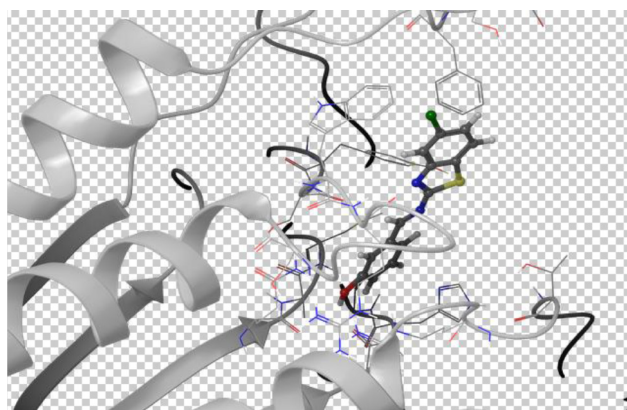
| Comp. Code | IC ₅₀ (µg/ml) |
|---------------|--------------------------|
| SP1 | >62.5 |
| SP2 | >62.5 |
| SP3 | >62.5 |
| SP4 | >62.5 |
| SP5 | >62.5 |
| SP6 | >62.5 |
| SP7 | 51.06 |
| SP8 | 89.15 |
| SP9 | >62.5 |
| SP10 | >62.5 |
| SP11 | >62.5 |
| SP12 | 53.3 |
| SP13 | >62.5 |
| SP14 | >62.5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

Table 3: IC₅₀ 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 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.

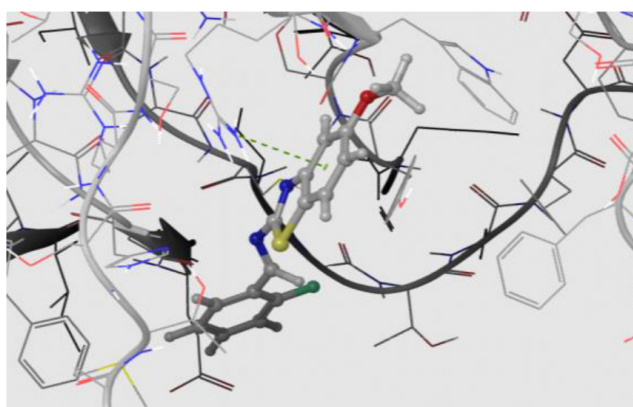
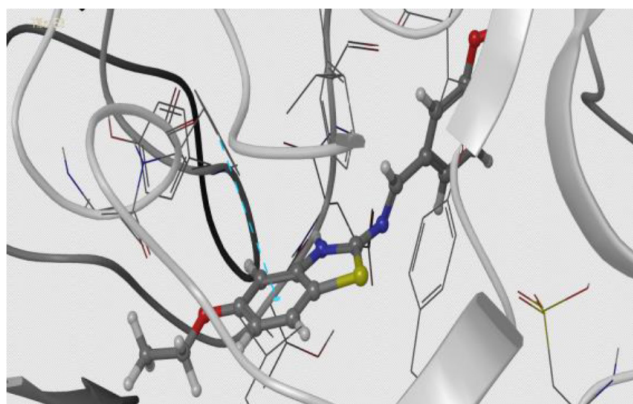
The binding interactions of compound **SP3** with the receptor (**Figure 1B**), shows that compound **SP3** interacts with THR A: 62, LYS C: 259 and THR C: 166 of receptor active site

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

**Figure 1A: Binding Interactions of Compound SP7 with receptor****Figure 1B: Binding Interactions of Compound SP3 with receptor.**

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.

ACKNOWLEDGEMENT

The authors are thankful to Manipal Academy of Higher education (Deemed to be University) and Manipal College of Pharmaceutical Sciences for providing necessary support and facilities to carry out the present research work. The authors are also thankful to Dr. Pritesh Bhat, Application Scientist, Schrodinger, Bengaluru, India in providing support for *in-silico* studies.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

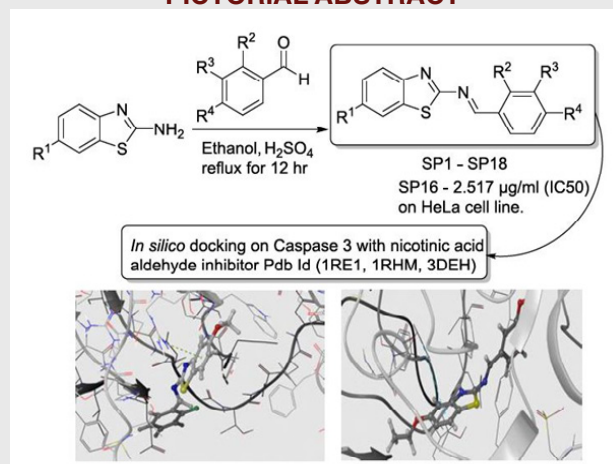
FTIR: Fourier Transform Infra Red; **H NMR**: Proton Nuclear Magnetic Resonance; **ESI**: Electron Spray Ionisation; **HeLa**: Cervical cancer Cells taken from Henrietta Lacks and named after her.; **WHO**: World Health Organisation; **CC**: Cervical Cancer; **HPV**: Human Papilloma Virus; **DNA**: Deoxy 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-picrylhydrazyl; **MTT**: 3-(4, 5-dimethylthiazol-2yl)-2, 5-diphenyltetrazolium bromide; **NCCS**: National Centre for Cell Sciences; **DMEM**: Dulbecco's Modified Eagle's Medium; **NBCS**: New Born Calf Serum.

REFERENCES

1. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al. Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11. GLOBOCAN. 2012;1:0.
2. Doorbar J. Molecular biology of human papillomavirus infection and cervical cancer. Clin Sci. 2006;110(5):525-41.
3. Shuai Z, Chen-Ming H, Li-Hong B. Glutathione S-transferase polymorphism interactions with smoking status and HPV infection in cervical cancer risk: an evidence-based meta-analysis. PLoS ONE. 2013;8(12):e8349.
4. Qian H, Jing P, Weiping L, Xiaoli H, Ling C, Xinlian C, et al. Elevated cleaved caspase-3 is associated with shortened overall survival in several cancer types. Int J Clin Exp Pathol. 2014;7(8):5057-70.
5. Nishimura A, Nakahara T, Ueno T, Sasaki K, Yoshida S, Kyo S, et al. Requirement of E7 oncoprotein for viability of HeLa cells. Microbes Infect. 2006;8(4):984-93.
6. Scheffner M, Huibregtse MJ, Vierstra RD, Howley PM. The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. Cell. 1993;75(3):495-505.
7. Cressier D, Prouillac C, Hernandez P, Amourette C, Diserbo M, Lion C, et al. Bioorganic and Medicinal Chemistry Synthesis, antioxidant properties and radioprotective effects of new benzothiazoles and thiaziazoles. Bioorganic Med Chem. 2009;17(14):5275-84.
8. Sunil K. The importance of antioxidant and their role in pharmaceutical science. Asian J of Res in Chem and Pharmaceutical Sci. 2014;1(1):27-44.
9. Abudief AM, Abu-dief AM, Mohamed IMA. A review on versatile applications of transition metal complexes incorporating Schiff bases. Beni-Suef Univ J Basic Appl Sci. 2015;4(2):119-33.
10. El-halim HFA, Omar MM, Mohamed GG: Molecular and Biomolecular Spectroscopy Synthesis, structural, thermal studies and biological activity of a tridentate Schiff base ligand and their transition metal complexes. Spectrochim Acta Part. 2011;78(1):36-44.
11. Ribeiro MDMC, Gonc JM, Silva ALR, Oliveira PCFC, Schr B, Ribeiro MAV. Molecular thermochemical study of Ni (II), Cu (II) and Zn (II) complexes with N , N -bis (salicylaldehyde) ethylenediamine. J Mol Catalysis A Chemi. 2004;224(1-2):207-12.
12. Abu-khadra AS, Farag RS, Abdel-hady AEM. Synthesis, Characterization and Antimicrobial Activity of Schiff Base Phenylsulfonyl) Acetamide Metal Complexes. Am J Analyt Chem. 2016;7(3):233-45.
13. Zaltariov M, Cazacu M, Avadanei M, Shova S, Balan M, Vornicu N, et al. Synthesis, characterization and antimicrobial activity of new Cu(II) and Zn(II) complexes with Schiff bases derived from trimethylsilyl-propyl-p-aminobenzoate. Polyhedron. 2015;100:121-31.
14. Wadher SJ, Puranik MP, Karande NA, Yeole PG. Synthesis and Biological Evaluation of Schiff base of Dapsone and their derivative as Antimicrobial agents. Int J Pharm Tech Res. 2009;1(1):22-33.
15. Christie CA, Shiju C, Fen TFA. European journal of pharmaceutical synthesis, spectroscopic characterization and biological activities of Co (ii), Ni (ii), Cu (ii) and Zn (ii) complexes of schiff base derived from isatin monohydrazone and furfuraldehyde. Int J Pharm Pharm Sci. 2016;2(4):177-81.
16. Sharma R, Goswami A, Rudrapal M, Sharma D, Sharma HK, Chetia D. In Vitro Evaluation of the Antimalarial Activity of a Designed Novel Quinuclidine Derivative. Curr Sci. 2016;111(12):20-28.
17. Paneersalvam P, Raj T, Ishar MPS, Singh B, Sharma V, Rather BA. Anticonvulsant Activity of Schiff Bases of 3-Amino-6,8-dibromo-2-phenylquinazolin-4(3H)-ones. Indian J Pharm Sci. 2010;72(3):375-8.
18. Chinnasamy R, Sundararajan R, Govindaraj S. Synthesis, characterization, and analgesic activity of novel schiff base of isatin derivatives. J Adv Pharm Technol Res. 2010;1(3):342.
19. Arulmurugan S, Kavitha HP, Venkatraman BR. Biological activities of schiff base and its complexes : a review. Rasayan J Chem. 2010;3(3):385-410.
20. Aggarwal N, Kumar R, Dureja P, Rawat DS. Schiff bases as potential fungicides and nitrification inhibitors. J Agric Food Chem. 2009;57(18):8520-5.
21. Joseyphus RS, Nair MS. Antibacterial and Antifungal Studies on Some Schiff Base Complexes of Zinc (II). Korean J Mycol. 2008;36(2):93-8.
22. Süleymanoğlu N, Ustabaş R, Direkel Ş, Alpaslan YB, Ünver Y. 1,2,4-triazole derivative with Schiff base; thiol-thione tautomerism, DFT study and antileishmanial activity. J Mol Struct. 2017;1150:82-7.
23. Kumar KS, Ganguly S, Veerasamy R, Clercq ED. Synthesis, antiviral activity and cytotoxicity evaluation of Schiff bases of some 2-phenyl quinazoline-4(3)H-ones. Eur J Med Chem. 2010;45(11):5474-9.
24. Yadav PS, P SG. Benzothiazole: Different Methods of Synthesis and Diverse Biological Activities. Int J Pharm Sci Drug Res. 2011;3(1):1-7.
25. Ali R, Siddiqui N. Biological Aspects of Emerging Benzothiazoles : A Short Review. Journal of Chemistry. 2013.
26. Becker JW, Rotonda J, Soisson SM, Aspiotis R, Bayly C, Gallant M, et al. Reducing the Peptidyl Features of Caspase-3 Inhibitors : A Structural Analysis. J Med Chem. 2004;47(10):2466-74.

27. Du J, Wu J, Zhang H, Zhang Y, Qiu B, Wu F, et al. Isoquinoline-1, 3, 4-trione Derivatives Inactivate Caspase-3 by Generation of Reactive Oxygen Species. *J Biol Chem*. 2008;283(44):30205-15.
28. Paul A. Synthesis and Evaluation of Antioxidant Activity of Semicarbazone Derivatives Synthesis and Evaluation of Antioxidant Activity of Semicarbazone Derivatives. *Int J Pharm Sci Drug Res*. 2011;3(2):150-4.
29. Likhari R, Perumal P, Kolhe N, Bhaskar VH, Daroi P, No- P, et al. Synthesis and antioxidant activity of novel 2-aryl substituted benzothiazole derivatives. *Int J Curr Pharm Res*. 2015;7: 34-7
30. Shanthalakshmi K, Bhat M, SI B. Synthesis of Benzothiazole Schiff's Bases and screening for the Anti- Oxidant Activity. *J Chem Pharm Res*. 2016;8(10):240-3.
31. Garcia EJ, Cadorin OTL, Alencar SMD, Reis A, Loguercio AD, Miranda GRH. Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. *Braz Dent J*. 2012;23(1):22-7.
32. Walter T. Rapid colorimetric assay for cell growth and survival Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability. *J Immunol Methods*. 1986;89(2):271-7.

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, ¹H 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 IC₅₀ 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.

About Authors



Suvarna G Kini is Professor in the Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, MAHE, Manipal. She completed her B.Pharm and M.Pharm from GCP, Bangaluru and PhD from MAHE, Manipal. She has about 27 years of teaching experience. Her area of research is in drug design using CADD, synthesis of heterocyclic compounds and their evaluation against cancer, TB, HIV, hypertension, bacterial, fungal and viral diseases and Pharma Analysis. She is mostly interested in target based drug design. She has published around 40 papers in highly impact factor national and international medicinal chemistry journals.



Varadaraj Bhat G is Associate Professor in the Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, MAHE, Manipal. His research areas are in drug discovery and Pharma analysis. He has about 20 years of teaching experience. He has about 25 publications to his credit published in highly impact factor national and international journals.



K. Sreedhara Ranganath Pai, M. Pharm, PhD. Professor and Head, Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education (MAHE), Manipal, has 20 years of teaching and research experience. Area of research is cancer biology, chemical biology, metabolic disorders and Computer aided drug design.



Mr. Subhankar Biswas completed his Master of Pharmacy in 2014 from Manipal College of Pharmaceutical Sciences with specializing in Pharmacology. Currently he is working as a doctoral student in the Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal-576104, Karnataka, India

Cite this article: Saipriya D, Prakash A, Kini SG, Bhatt VG, Pai KSR, Biswas S, Shameer MK. Design, Synthesis, Antioxidant and Anticancer Activity of Novel Schiff's Bases of 2-Amino Benzothiazole. *Indian J of Pharmaceutical Education and Research*. 2018;5 [4 Suppl 2]:s333-s342.