

# Biological Evaluation of New Schiff Bases: Synthesized from 4-Amino-3,5-dimethyl-1,2,4-triazole, Phenathroline and Bipyridine Dicarboxaldehydes

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## Abstract

Four new Schiff bases with promising anticancer activity have been synthesized from 4-amino-3,5-dimethyl-1,2,4-triazole and di-pyridyl-aldehydes. Structures have been established by various spectroscopic methods. The compounds were tested *in vitro* to study their cytotoxicity and anti-oxidative activity in human lung carcinoma (A549), breast carcinoma (BT549), prostate adenocarcinoma (PC3) and mouse preadipocytes (3T3-L1) cells. Compound 1 was found to increase Glutathione (GSH) level slightly in all four cell lines. Compound 4 showed better selectivity and cytotoxicity against both BT549 and A549 cells compared to the anticancer drug tamoxifen. With the exception of compound 4 which reduced GSH levels in A549 and BT549, all other compounds maintained GSH levels in comparison to their respective controls.

## Keywords

[2,2'-Bipyridine]-5,5'-dicarboxaldehyde, Schiff Bases, Anticancer, Glutathione, Anti-Bacterial

## 1. Introduction

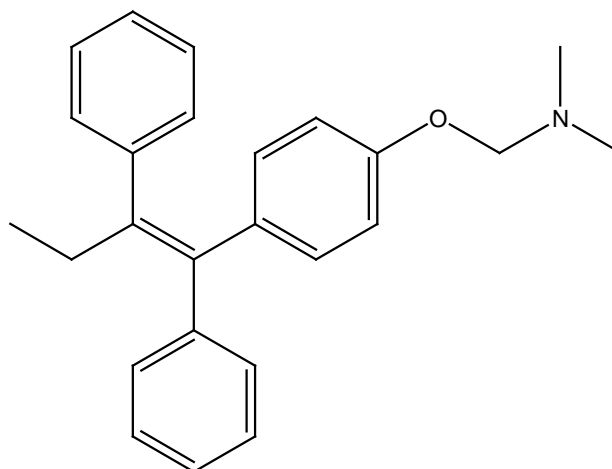
Cancer is the major cause of death in the present world. Approximately 14 million cancer cases was diagnosed and 8.2 million people died because of cancer in 2012 [1] [2]. These numbers are more likely to increase over the following decades [1]. Accordingly, developing new anticancer drugs has become an urgent need. Currently a number of Schiff base and their metal complexes have been investigated as effective scavengers of

reactive oxygen species (ROS) [3] [4], acting as antioxidants thereby reducing the incidence of certain cancers. Schiff bases form an important class of organic compounds with a wide variety of biological properties.

Chelating agents containing nitrogen are a group of compounds of continuing interests for their ability to interact with biological systems. It is known that the nitrogen rich 1,2,4-Triazole derivatives are a class of compounds that show antitumor [5], anticancer [6], antibacterial [5] [6], antiviral [5], anticonvulsant [5], antifungal [5] [6], anti-inflammatory [5] [6], antituberculous [5], analgesic [6], antimalarial [6] and antidepressant activities [6]. There are several drugs that contain 1,2,4-triazole nucleus such as Letrozole and Anastrozole (anticancer drug), Alprazolam (anxiolytic drug), Rizatriptan (antimigrane drug), Ribavirin (antiviral drug), Itraconazole and Fluconazole (antifungal drug).

Schiff bases and their metal complexes are usually considered one of the most biologically active chemical compounds. The imine bonds or Schiff bases are also found in different enzymes such as transaminases, transketolases and tryptophan synthase to mention a few. Schiff bases synthesized from aldehydes or amines containing heterocycles have been reported to have cytotoxic [7] [8], antiproliferative [9], antibacterial activity [8] [9] [10] [11], antiviral [8] [11], antifungal and anticancer activities [11]. For example, Schiff bases and Copper(II) complexes of Schiff bases synthesized from 4-amino-3,5-dimethyl-1,2,4-triazole have been reported to inhibit endocytosis [12] and protein tyrosine phosphatases [13] respectively. Triazole containing Schiff bases has also been reported to have antitumor activity [14]. Previous workers in our laboratory had synthesized new Schiff bases containing less flexible, structurally rigid 1,10-phenanthroline backbone, with O,N,S and F-containing amines and studied their antibacterial activity [10]. New Schiff bases have also been synthesized in our laboratory and their antibacterial properties are studied. Since 4-amino-3,5-dimethyl-1,2,4-triazole derivatives have been found to exhibit variety of inhibition properties, we have decided to synthesize various Schiff bases using this 4-amino-3,5-dimethyl-1,2,4-triazole with phenanthroline and bi-pyridyl aldehydes and study their anticancer and antioxidant activities. Particularly, we wanted to test the efficacy of our synthesized Schiff bases in maintaining the antioxidant potential of several cell lines by measuring the effects of the said compounds on levels of glutathione, a natural antioxidant, in cells. Also we have used tamoxifen (**Figure 1**), a breast cancer drug [15], as our internal standard in comparison to our synthesized Schiff bases for cell viability tests. The structure of tamoxifen is as follows.

Glutathione (GSH) is a tripeptide whose reducing and nucleophilic properties have a vital role in metabolic pathways. GSH has been reported to be the main line of defense for the maintenance of the appropriate mitochondrial redox environment to avoid or repair oxidative modifications leading to mitochondrial dysfunction and cell death [16]. Thiol group of cysteine is oxidized when target molecules are reduced by GSH [16]. GSH also plays an important role in metabolism of xenobiotics, thiol disulfide exchange reactions, cellular signaling such as apoptosis, proliferation and cell-cycle regulation [16]. In this paper, we report the cell viability and oxidative properties of four Schiff



**Figure 1.** Structure of tamoxifen.

bases on three cancer cells and one obese cell lines.

## 2. Materials and Methods

### 2.1. Chemicals and Instrumentation

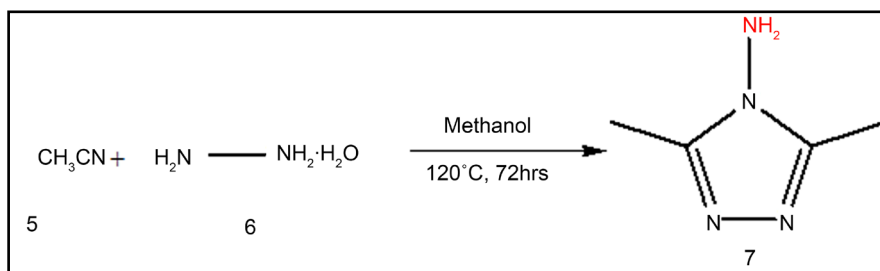
All chemicals were purchased from Fisher Scientific, Suwanee, GA and used without further purification. All NMR spectra were obtained on a 400 MHz varian NMR spectrometer. Chemical shifts are given in ppm with TMS as internal reference. All spectra also recorded at room temperature. Mass data was recorded on a Varian LC-MS with ESI.

### 2.2. Synthesis of 4-Amino-3,5-dimethyl-1,2,4-triazole (7)

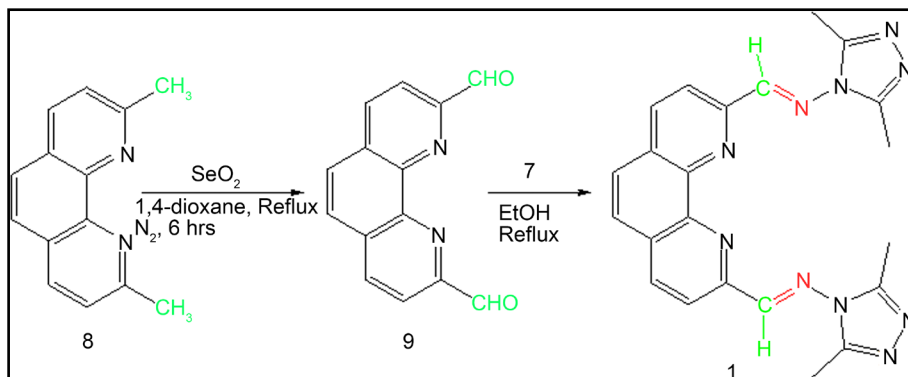
4-amino-3,5-dimethyl-1,2,4-triazole was synthesized from acetonitrile and hydrated hydrazine in methanol following literature procedure [17]. A mixture of 3.0 ml hydrated hydrazine, 1.5 ml acetonitrile and 1 ml methanol was heated in a 15 mL Teflon-lined autoclave at 120°C for 3 days (Scheme 1). The reaction mixture was cooled to room temperature slowly and finally cooled in ice bath. The white crystal product was filtered off. Yield 83%; <sup>1</sup>HNMR ( $\delta$ , DMSO-*d*<sub>6</sub>): 2.3 (s, 6H, CH<sub>3</sub>), 5.77 (s, 2H, NH<sub>2</sub>); <sup>13</sup>CNMR ( $\delta$ , DMSO-*d*<sub>6</sub>): 10.18, 151.78

### 2.3. Synthesis of 1,10-Phenanthroline-2,9-dicarboxaldehyde (9)

1,10-phenanthroline-2,9-dicarboxaldehyde (9) was synthesized from 2,9-dimethyl-1,10-phenanthroline hemihydrate (8) following previously reported procedure [18] [19]. Neocuproine hemihydrate (3.0 g, 0.0144 mol) was dissolved in 200 ml 1,4-dioxane containing 4% water and SeO<sub>2</sub> (6.0 g, 0.054 mol) was added and the reaction mixture was refluxed for 6 h (Scheme 2). Then the reaction mixture was filtered through thick celite pad. Light yellow product was precipitated on slow cooling to room temperature. The product was isolated and dried under vacuum. The crude product was purified by recrystallization in chloroform to get pure compound (Y: 70%). <sup>1</sup>HNMR ( $\delta$ , DMSO-*d*<sub>6</sub>):



**Scheme 1.** Synthesis of 4-amino-3,5-dimethyl-1,2,4-triazole.



**Scheme 2.** Synthesis of Schiff base 1 from 1,10-phenanthroline-2,9-dicarboxaldehyde.

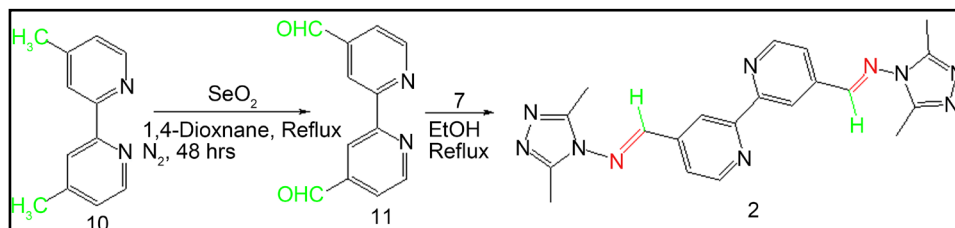
10.36 (s, 2H, CHO), 8.9 (d,  $J_{ortho} = 7.8\text{Hz}$ , 2H, Ar-H), 8.36 (m, 4H, Ar-H); <sup>13</sup>CNMR ( $\delta$ , DMSO-d<sub>6</sub>): 194.13, 152.68, 145.77, 138.86, 131.95, 129.76, 120.67.

#### 2.4. Synthesis of [2,2'-Bipyridine]-4,4'-dicarboxaldehyde (11)

[2,2'-Bipyridine]-4,4'-dicarboxaldehyde (11) was synthesized from 4,4'-dimethyl-2,2'-bipyridine (10) through direct oxidation by SeO<sub>2</sub> under nitrogen condition following literature procedure [20]. 4,4'-dimethyl-2,2'-bipyridine (2.0 g, 0.012 mol) was dissolved in 100 mL 1,4-dioxane containing 4% water and SeO<sub>2</sub> (4.8 g, 0.048 mol) was added and the reaction mixture was refluxed for 48 h (Scheme 3). The reaction was carried out under N<sub>2</sub> environment. Then the hot reaction mixture was filtered through a thick Celite pad. The filtrate was kept overnight in dark place to isolate dialdehyde as solid precipitate. The product was isolated and dried under vacuum. The product was further purified by recrystallization in chloroform (Y: 43%). <sup>1</sup>HNMR ( $\delta$ , DMSO-d<sub>6</sub>): 10.18 (s, 2H, CHO), 9.22 (d,  $J_{meta} = 2.32\text{Hz}$ , 2H, Ar-H), 8.65 (d,  $J_{ortho} = 8.6\text{Hz}$ , 2H, Ar-H), 8.45 (dd,  $J_{ortho} = 8.6\text{Hz}$ ,  $J_{meta} = 2.32\text{Hz}$ , 2H, Ar-H); <sup>13</sup>CNMR ( $\delta$ , DMSO-d<sub>6</sub>): 192.96, 158.62, 151.95, 138.36, 132.19, 122.55.

#### 2.5. Synthesis of [2,2'-Bipyridine]-5,5'-dicarboxaldehyde (13)

[2,2'-Bipyridine]-5,5'-dicarboxaldehyde (13) was synthesized from 5,5'-dimethyl-2,2'-bipyridine (12) through direct oxidation by SeO<sub>2</sub>. [2,2'-Bipyridine]-5,5'-dicarboxaldehyde was afforded from the treatment of 5.56 mmol 5,5'-dimethyl-2,2'-bipyridine with 20 mmol SeO<sub>2</sub> in 40 ml 1,4-dioxane by reflux for 48 h under N<sub>2</sub> environment (Scheme 4).



**Scheme 3.** Synthesis of Schiff base 2 from 2,2'-bipyridyl-4,4-dialdehyde.

Then the hot reaction mixture was filtered through a thick Celite pad. The filtrate was kept over night in dark place to isolate dialdehyde as solid precipitate. The dialdehyde was isolated and dried under vacuum. The pure compound was obtained by washing with hot chloroform (Y: 47%).  $^1\text{HNMR}$  ( $\delta$ , DMSO- $d_6$ ): 10.22 (s, 2H, CHO), 9.05 (d,  $J_{\text{ortho}} = 7.88\text{Hz}$ , 2H, Ar-H), 8.8 (s, 2H, Ar-H), 7.95 (d,  $J_{\text{ortho}} = 7.88\text{Hz}$ , 2H, Ar-H);  $^{13}\text{CNMR}$  ( $\delta$ , DMSO- $d_6$ ): 193.81, 156.33, 151.56, 143.31, 123.21, 119.88.

## 2.6. Synthesis of [2,2'-Bipyridine]-6,6'-dicarboxaldehyde (15)

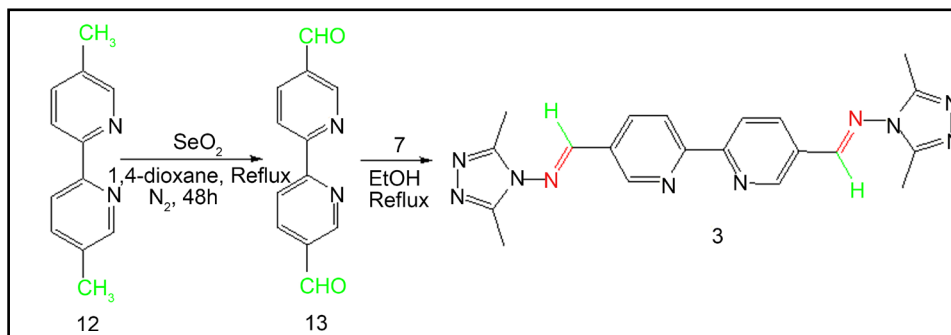
[2,2'-Bipyridine]-6,6'-dicarboxaldehyde (15) was synthesized from 6,6'-dimethyl-2,2'-bipyridine (14) through direct oxidation by  $\text{SeO}_2$  following literature procedure [21] [22]. 6,6'-dimethyl-2,2'-bipyridine (2.0 g, 0.012 mmol) was dissolved in 160 mL glacial acetic acid and  $\text{SeO}_2$  (12.0 g, 0.12 mol) was added and the reaction mixture was refluxed for 48 h (Scheme 5). Then the hot reaction mixture was filtered through a thick Celite pad. The filtrate was kept overnight in dark place to isolate dialdehyde as solid precipitate. The product was isolated and dried under vacuum. The crude product was dissolved in hot chloroform and then cooled to room temperature slowly. The impurities precipitated leaving the impurities in solution. The impurities were filtered off and the filtrate was evaporated under vacuum to get pure product (Y: 44%).  $^1\text{HNMR}$  ( $\delta$ , DMSO- $d_6$ ): 10.12 (s, 2H, CHO), 9.77 (d,  $J_{\text{ortho}} = 7.92\text{Hz}$ , 2H, Ar-H), 8.3 (t,  $J = 7.88$ , 2H, Ar-H), 8.05 (d,  $J_{\text{ortho}} = 7.64\text{Hz}$ , 2H, Ar-H);  $^{13}\text{CNMR}$  ( $\delta$ , DMSO- $d_6$ ): 193.85, 155.26, 152.55, 139.65, 125.6, 122.9.

## 2.7. General Procedure for the Synthesis of Schiff Bases

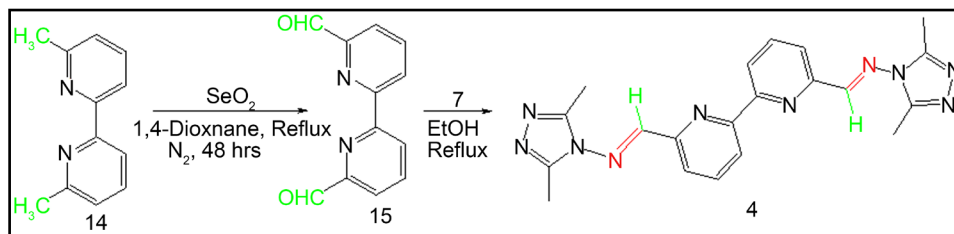
0.5 mmol aldehyde was dissolved in 20 ml hot ethanol or methanol. 1.1 mmol amine 1 was added to the hot solution followed by reflux for 2 h. The reaction mixture was cooled to room temperature and solvent was evaporated under vacuum. Product that obtained was washed with hot acetonitrile to get pure products. Addition of 2 - 3 drops of  $\text{H}_2\text{SO}_4$  increased the yield by at least 5%.

### 2.7.1. {9-[2-(2,5-Dimethyl-cyclopenta-2,4-dienyl)-vinyl]-[1,10]phenanthroline-2-ylmethylene}-(3,5-dimethyl-[1,2,4]triazol-4-yl)-amine (1)

$^1\text{HNMR}$  ( $\delta$ , DMSO- $d_6$ ): 9.13 (s, 2H, Ar-H), 8.75 (d,  $J_{\text{ortho}} = 8.4\text{Hz}$ , 2H, Ar-H), 8.55 (d,  $J_{\text{ortho}} = 8.4\text{Hz}$ , 2H, Ar-H), 8.19 (s, 2H, CH), 2.55 (s, 12H,  $\text{CH}_3$ );  $^{13}\text{CNMR}$  ( $\delta$ , DMSO- $d_6$ ): 160.28, 152.32, 148.55, 145.48, 138.39, 130.56, 128.85, 121.10, 11.01; ESI-MS  $m/z$  (% rel. abund.): 424  $[\text{M}]^+$  (74), 447  $[\text{M}+\text{Na}]^+$  (100).



**Scheme 4.** Synthesis of Schiff base 3 from 2,2'-bipyridyl-5,5-dialdehyde.



**Scheme 5.** Synthesis of Schiff base 4 from 2,2'-bipyridyl-6,6-dialdehyde.

### 2.7.2. {4'-[2-(2,5-Dimethyl-cyclopenta-2,4-dienyl)-vinyl]-[2,2']bipyridinyl-4-ylmethylene}-(3,5-dimethyl-[1,2,4]triazol-4-yl)-amine (2)

$^1\text{H NMR}$  ( $\delta$ , DMSO- $d_6$ ): 9.25 (d,  $J_{\text{meta}} = 2.52\text{Hz}$ , 2H, Ar-H), 9.05 (s, 2H, Ar-H), 8.66 (d,  $J_{\text{ortho}} = 8.52\text{Hz}$ , 2H, Ar-H), 8.55 (dd,  $J_{\text{ortho}} = 8.52\text{Hz}$ ,  $J_{\text{meta}} = 2.52\text{Hz}$ , 2H, Ar-H), 2.44 (s, 12H,  $\text{CH}_3$ );  $^{13}\text{CNMR}$  ( $\delta$ , DMSO- $d_6$ ): 160.53, 157.44, 150.87, 148.09, 136.83, 129.69, 121.88, 11.1; EI-MS  $m/z$  (% rel. abund.): 423 [ $\text{M} + \text{Na}$ ] $^+$  (100), 401 [ $\text{M}$ ] $^+$  (25).

### 2.7.3. {5'-[2-(2,5-Dimethyl-cyclopenta-2,4-dienyl)-vinyl]-[2,2']bipyridinyl-5-ylmethylene}-(3,5-dimethyl-[1,2,4]triazol-4-yl)-amine (3)

$^1\text{H NMR}$  ( $\delta$ ,  $\text{CD}_3\text{OD}$ ): 9.00 (m, 6H, Ar-H), 8.00 (s, 2H, CH), 2.60 (s, 12H,  $\text{CH}_3$ );  $^{13}\text{CNMR}$  ( $\delta$ ,  $\text{CD}_3\text{OD}$ ): 156.15, 157.06, 152.68, 150.15, 139.53, 124.87, 123.69, 11.12; EI-MS  $m/z$  (% rel. abund.): 401 [ $\text{M}$ ] $^+$  (100), 423 [ $\text{M} + \text{Na}$ ] $^+$ , (30).

### 2.7.4. {6'-[2-(2,5-Dimethyl-cyclopenta-2,4-dienyl)-vinyl]-[2,2']bipyridinyl-6-ylmethylene}-(3,5-dimethyl-[1,2,4]triazol-4-yl)-amine (4)

$^1\text{H NMR}$  ( $\delta$ ,  $\text{CDCl}_3$ ): 8.70 (m, 4H, 2 Ar-H, 2 CH), 8.30 (d,  $J_{\text{ortho}} = 7.92\text{Hz}$ , 2H, Ar-H), 8.07 (t,  $J = 7.52\text{Hz}$ , 2H, Ar-H), 2.8 (s, 12H,  $\text{CH}_3$ );  $^{13}\text{CNMR}$  ( $\delta$ ,  $\text{CDCl}_3$ ): 160.26, 155.37, 150.99, 148.46, 138.18, 123.69, 121.84, 11.96; EI-MS  $m/z$  (% rel. abund.): 423 [ $\text{M} + \text{Na}$ ] $^+$  (100), 401 [ $\text{M} + \text{H}$ ] $^+$  (20).

## 2.8. Biological Materials

All the cell lines were purchased from American Type Culture Collection (ATCC), Manassas, VA. Dulbecco's Modified Eagle's Medium (DMEM), bovine calf serum (BCS) and penicillin-streptomycin were purchased from GIBCO, New York, USA. MTT assay kit, Glutathione assay kit and reduced glutathione were purchased from Calbiochem, California, USA.

## 2.9. Cell Culture

All four different cell lines were cultured under 5% CO<sub>2</sub> atmosphere at 37°C in DMEM supplemented with 10% (v/v) BCS and 1% (v/v) penicillin-streptomycin following literature procedure [23]. Media was changed in every two days. At 80% confluence in T-75 flasks, cells were trypsinized with 3 ml of Trypsin-EDTA solution and seeded in either 6 or 96 well plates.

## 2.10. MTT Assay

Cell viability was determined as previously reported [23] [24]. Briefly,  $1 \times 10^4$  cells/well were seeded in 96-well plates and treated with 100  $\mu$ L different doses (10, 20, 30, 40, 50, 60, 70, 80, 90, 100 and 200  $\mu$ M) of the synthesized Schiff bases dissolved in DMSO. The plates were then incubated in an incubator (Thermo Scientific, Marietta, OH) at 37°C, 5% CO<sub>2</sub> for 24 h. following the incubation 0.5 mg/ml MTT (3-4,5-dimethylthiazol-2-yl-2,3-diphenyl tetrazolium bromide) were added to the wells and incubated at 37°C for 4 hours. Supernatants were aspirated and the formazan crystals were dissolved in 100  $\mu$ L DMSO and the absorbance read at 570 nm in a synergic microplate reader (BioTek, Winooski, VT). The percentage of cell viability was calculated using previously reported formula [20]. The experiments were repeated 3 $\times$  with each treatment at 4 replicates.

## 2.11. Glutathione (GSH) Assay

$2 \times 10^6$  cells/well for each cell lines were seeded in 6-well plates and cells were treated with 25, 50, 75 and 100  $\mu$ M for each of synthesized Schiff bases and incubated for 24 h at 37°C under 5% CO<sub>2</sub>. Supernatants were aspirated and 500  $\mu$ L of Trypsin-EDTA was added and incubated for 5 - 10 minutes at 37°C to detach cells from wells. 1.50 ml DMEM was added and the cell solutions (2 ml) were transferred to a 2 ml Eppendorf tubes. Cells were pelleted at 3000  $\times g$  for 10 minutes at 4°C in a refrigerated centrifuge (Thermo Scientific, Marietta, OH). Supernatants were discarded and cells were resuspended in 200  $\mu$ L of 5% metaphosphoric acid (MPA). Reduced GSH was analyzed according to previously reported method [25]. The cells were sonicated under ice for 30 seconds using a cell dismembrator (Fisher Scientific, Suwanee, GA) at a setting of 3. Samples were then centrifuged at 4°C at 4000  $\times g$  for 8 minutes and the supernatant was used for the GSH analysis as described by the GSH Assay Kit (Cat# 354102). A standard curve was prepared to calculate GSH levels expressed as nmol GSH/ $10^6$  cells [25] [26]. The experiments were repeated 2 $\times$  with each treatment at 4 replicates.

## 2.12. Antibacterial Screening Test

Antibacterial screening test was done using the disc diffusion method [10] [27] [28]. Previously reported procedure [10] was followed to prepare media plate and culture bacteria. Stock solution (10 mg/ml DMSO) was prepared for both synthesized compounds and antibacterial drug tetracycline. Stock solution soaked sterile 6 mm whatman filter paper was placed in the middle of bacteria spread media plate and incubated

for 24 h at 37°C. Zone of inhibition was measured after 24 h incubation.

### 3. Results and Discussion

#### 3.1. Chemistry

The starting amine 7 was synthesized following previously reported procedure (**Scheme 1**) [17]. 2,9-phenanthroline-1,10-dialdehyde (9) was also synthesized using literature procedure [18] [19]. The dialdehyde was then reacted with 1 to produce Schiff base 1 (**Scheme 2**). All product structures were determined by spectral methods. The dialdehyde 11 and 15 have been prepared using previously published procedure [20] [21] [22] following SeO<sub>2</sub> oxidation. Dialdehyde 13 has been synthesized previously *via* multiple step reactions such as bromination of 5,5'-dimethyl-2,2'-bipyridine followed by hydrolysis in acetic acid [29]. In this paper we are reporting an efficient SeO<sub>2</sub> oxidation reaction to synthesize dialdehyde 13. Procedure involves the use of 1:4 equivalents of the precursor to SeO<sub>2</sub> ratio producing the best yield (47%). Bipyridylaldehydes (11, 13 and 15) were then subjected to react with amine 7 to produce corresponding Schiff bases 2, 3 and 4 (**Schemes 3-5**). The synthesis of Schiff bases were carried out in both methanol as well as in ethanol. Higher yields of all four Schiff bases were obtained in ethanol. No reaction occurred in tetrahydrofuran and in chloroform. Solvent Polarity and reflux temperature may have had tremendous effect on Schiff base formation. Addition of 2 - 3 drops of sulfuric acid to the reaction mixture further increased the yield of Schiff bases due to the enhanced nucleophilic attack by amine group of 4-amino-3,5-dimethyl-1,2,4-triazole to carbonyl carbon upon protonation of the carbonyl group. Percent yields of Schiff bases for different reaction conditions are listed in **Table 1**.

#### 3.2. Cell Viability Activities *in Vitro*

The synthesized Schiff bases were tested to determine their effects on cell viability in A549, BT549, PC3 and 3T3-L1 cell lines using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay [23] [24] (**Table 2**). Briefly cells were treated with the respective compounds dissolved in DMSO. Controls containing 2% DMSO was used as the negative control. The anticancer drug tamoxifen was used as positive control. IC<sub>50</sub> for tamoxifen for the A549, BT549, PC3 and 3T3-L1 cell lines were 55, 55, 60 and 35 μM respectively. Compound 4 decreased cell viability in A549 and BT549 cell lines with IC<sub>50</sub> values of 70 μM and 50 μM respectively.

**Table 1.** Percent yield of Schiff bases 1, 2, 3, and 4 in different reaction condition.

Compound	% yield in			
	Methanol	Methanol + 2 - 3 drops H <sub>2</sub> SO <sub>4</sub>	Ethanol	Ethanol + 2 - 3 drops H <sub>2</sub> SO <sub>4</sub>
1	58	63	70	81
2	51	61	73	80
3	63	74	69	76
4	68	81	78	93



**Table 2.** Cytotoxic effects of 1, 2, 3 and 4 against A549, BT549, PC3 and 3T3-L1 cell lines.

Compound	IC <sub>50</sub> (μM)			
	A549	BT549	PC3	3T3-L1
1	>200	>200	>200	>200
2	>200	>200	>200	>200
3	>200	>200	>200	>200
4	70	50	>200	>200
Tamoxifen	55	55	60	35

However, cell viability did not significantly decrease for compound 4 following exposure to the PC3 and 3T3-L1 cells respectively. On the other hand, tamoxifen was found to have more cytotoxicity against 3T3-L1 cells compared to the A549, BT549 and 3T3-L1 cell lines. In addition compound 4 was found to have a better anticancer activity against BT549 with an IC<sub>50</sub> of 50 μM compared to anticancer drug tamoxifen with an IC<sub>50</sub> of 55 μM. Compound 1, 2 and 3 did not show any significant effects on cell viability in any of the four cell lines tested. These findings suggest that apart from compound 4 which showed some cytotoxic effects to cell lines A549 and BT549, all the others did not affect cell lines tested as well as their anti-proliferative activities.

### 3.3. Effects on Glutathione (GSH) Level

Oxidative stress causes cancer [30], Alzheimer [31], Parkinson [32], infection [33] and heart failure [34]. Glutathione (GSH) plays an important role in the antioxidant status of cells. We measured GSH levels in A549, BT549, PC3 and 3T3-L1 after 24 h exposure to our synthesized compounds. **Table 3** shows the levels of GSH in controls and treated samples for all the four cell lines. Compound 1 increased GSH level slightly for all the doses tested for four cell lines. The increases in GSH for compound 1 from 10 μM to 100 μM were 1.17, 0.98, 1.51 and 1.89 nMole/106 cells for A549, BT549, PC3 and 3T3-L1 cell lines respectively. Compounds 2 and 3 did not have any significant effect on GSH levels for the doses tested. Furthermore, Compound 4 did not have significant effect on GSH in PC3 and 3T3-L1 cells for the doses tested from 0 - 75 μM. However, a slight drop in GSH level was noticed for both the PC3 and 3T3-L1 cells following exposure to 100 μM. GSH levels also dropped significantly for A549 and BT549 for the said compound for all the doses tested. The observed decreases in GSH for the cells for 4 may be due to its cytotoxic effects. Thus, most of the tested Schiff bases did not cause the loss of GSH as shown in **Table 3**. The above findings may indicate the abilities of these compounds in maintaining and preserving any oxidative damage to the cells.

### 3.4. Antibacterial Studies

Antibacterial screening test of all four Schiff bases were analyzed using both gram positive and negative bacteria. None of the tested Schiff bases was active against *E. coli* MC4100, *E. coli* DH5α, *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus subtilis*. Zone of inhibitions were zero for all Schiff bases tested in all 5 bacteria strains.

**Table 3.** GSH level in A549, BT549, PC3 and 3T3-L1 cells after 24 exposure to 2% DMSO and compounds 1, 2, 3 and 4.

Compound	Concentration ( $\mu\text{M}$ )	GSH level (nmol/ $10^6$ cells) in			
		A459	BT549	PC3	3T3-L1
1	10	12.88	14.83	8.08	18.42
	25	13.04	14.99	8.34	18.79
	50	13.37	15.18	8.71	19.24
	75	13.88	15.43	9.11	19.88
	100	14.05	15.81	9.59	20.31
2	10	11.66	14.69	9.01	19.13
	25	11.69	14.58	9.07	19.09
	50	11.68	14.65	8.94	19.18
	75	11.63	14.72	8.99	19.18
	100	11.67	14.60	8.91	19.15
3	10	13.17	15.17	8.6	19.01
	25	13.14	15.03	8.62	18.97
	50	13.16	15.18	8.71	18.91
	75	13.21	15.24	8.64	18.99
	100	13.18	15.21	8.64	19.01
4	10	12.53	14.39	8.22	19.21
	25	11.97	11.95	8.15	19.29
	50	6.08	6.18	8.11	19.31
	75	2.98	2.06	8.19	19.15
	100	1.91	0.79	7.27	17.88
DMSO	2%	12.84	14.94	8.39	19.07

#### 4. Conclusion

Four Schiff bases have been synthesized successfully and reaction condition for Schiff base preparation has been optimized to better yield. Addition of 2 - 3 drops of sulfuric acid increases the yield by 5% - 15%. Compound 1 containing rigid phenanthroline backbone may act as an antioxidant since it can increase and maintain GSH levels of cells. Compound 4 exhibited promising anticancer activity with excellent selectivity towards A549 and BT549 over PC3 and 3T3-L1 as opposed to the anticancer drug tamoxifen which killed the cells randomly. To use 4 as an anticancer drug and in clinical studies further studies are anticipated. In conclusion, exposure of several cell lines to different doses of Schiff bases with the exception of one (*i.e.* compound 4) did not cause significant losses in intracellular levels of GSH compared to their respective controls. The decrease in GSH levels for compound 4 may be due to its cytotoxicity.

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