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In vitro antioxidant activity of diazenyl schiff base molecules

Sarangi P. K. N.[1]*, Paidesetty S. K.[2] and Mohanta G. P.[3]

[1]Department of Pharmaceutical Chemistry, Sri Jayadev College of Pharmaceutical Sciences, Bhubaneswar, India.
[2]Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Siksha 'O' Anusandhan University, Bhubaneswar, India.
[3]Department of Pharmacy, Annamalai University, Annamalainagar, Tamil Nadu, India.

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A series of several diazenyl schiff base molecules were designed and synthesized through azo coupling of diazotised primary amines with the novel synthesized schiff base ligand *(E)-N-((2-chloroquinolin-3-yl)methylene)-4-phenylthiazol-2-amine*. All the synthesized molecules have been characterized by different spectral techniques for their structural confirmation. The results of *in vitro* antioxidant activity of the molecules by 2,2-diphenyl-1- picryl hydrazyl (DPPH) assay method revealed that the molecules (*NZ)-N-(((4-chlorophenyl) diazenyl) (2-chloroquinolin-3-yl) methylene) -4- phenylthiazol -2-amine* (5A) and *4-(((Z)-(2-chloroquinolin-3-yl)(4-phenylthiazol-2-ylimino)methyl)diazenyl)phenol* (5E) have shown potential free radical scavenging activity.

Key words: Schiff base, diazenyl, spectral, antioxidant.

INTRODUCTION

Oxidative stress is an imbalance between the reactive oxygen species (ROS) and the detoxifying biological process. Oxygen is one of the most essential molecules for life. As a strong oxidizing agent, it facilitates most of the metabolic processes in the body and in due process it generates free radicals. But when our endogenous supply of the antioxidants are insufficient, then the level of free radicals gets increased in our body causing internal cellular damage (Dubey and Batra, 2009). Free radicals are also generated by the external sources of environmental pollutants such as toxic metals, cigarette smoke and pesticides, which damage our body (Aseervatham et al., 2013). Accumulation of the free radicals leads to degenerative diseases such as Alzheimer's disease, Parkinson's disease, atherosclerosis, cancer and other aging problems (Ali et al., 2015). Antioxidants are capable of slowing and preventing the oxidation to build a control over the free radicals generation. Though the endogenous antioxidants are helping to reduce the accumulation of the free radicals,

*Corresponding author. E-mail: pknsarangi@gmail.com. Tel: +91 9437493396.

most of the time they are not sufficient to give protection against the reactive oxygen species, so we need to take the antioxidant rich foods such as fruits, vegetables, yogurt and green tea every day. In case of ageing and some disease conditions the dietary supplements with the endogenous antioxidants are unable to prevent oxidative stress. There comes the need for some potent synthetic antioxidants which play a vital role to prevent the production and accumulation of the reactive oxygen species (Raghavendra et al., 2013). On the basis of literature survey, the present investigation is mainly focused on the study of free radical scavenging activity of the reported diazenyl schiff base molecules.

MATERIALS AND METHODS

This experimental work includes the use of synthetic and analytical grade of chemicals procured from Sigma Aldrich, Hi Media Laboratories Pvt. Ltd. and Merck specialties Ltd. (Mumbai, India). The thin layer chromatographic (TLC) study of the synthesized molecules was done with appropriate solvent system to monitor the progress of reaction. The spectroscopic analysis of the synthesized molecules were performed by Fourier Transform/ InfraRed (FT/IR) (JASCO FT/IR 4100 Spectrophotometer) using KBr pellets, Liquid chromatography-mass spectrometry (LC-MS) (Shimadzu-Mass spectrophotometer) and ^{1}H NMR (Bruker^{1}HNMR 400 *MHz*) using tetramethylsilane as an internal standard. The elemental analysis for C, H, N and S were carried out on Perkin Elmer model 2400 CHNS/O analyzer. The melting points were determined by open capillary method (Elico). The solvatochromic analysis of the synthesized molecules was done using different solvents by UV-Vis spectrophotometer (JASCO V-630 Spectrophotometer). The chemical structures of the synthesized molecules were made using Chem Draw ultra 10.0 software.

Scheme

Synthesis of diazenyl schiff base derivatives (5A-E)

Synthesis of schiff base ligand (3) was prepared as per the procedure suggested by Hussain et al. 2014. To a solution of aromatic primary amine (3 mmol) and water (5 ml), a few drops of of concentrated H_2SO_4 (8-9 mmol) was added on an ice bath. The drop wise addition of a cold solution of $NaNO_2$ (0.207 g, 3 mmol) was made to it by maintaining the temperature of the reaction up to 5° c. To complete the diazotization reaction, the solution was kept for 15 min with occasional stirring. The above prepared ice cold solution of schiff base (3 mmol) with ethanol and 10% of 20 ml of aqueous NaOH, individual diazotised aromatic primary amines were added. The resultant mixture was stirred well and allowed to stand in an ice bath for 1 h by maintaining the pH at 5 to 6 with occasional and controlled addition of dilute HCl. Then the final products (5A-5E) obtained were filtered, washed repeatedly with water, dried and recrystallized of with ethanol (Sahoo et al., 2015).

(*E*)-N-[(2-chloroquinolin-3-yl)methylene]-4-phenylthiazol-2-amine, (3): Pale yellow color powder; Yield 82%; R_f: 0.6; m.p.: 207-10°C; UV-Vis (λ max, ethanol): 419 nm; IR (KBr, cm^{-1}): 1612 (C=N str.), 1527 (C=C str.), 1013(C-S str.), 717 (C-Cl str.), 3157 (C-H str. of azomethine); ^{1}H NMR (DMSO-*d*$_6$, δppm, 400 MHz): δ 7.43-7.79 (m, 5H, Ar H), 9.33 (s, 1H, Quinolinyl H-4), 8.07 (d, 1H, Quinolinyl H-5), 7.59 (m, 1H, Quinolinyl H-6), 7.78 (m, 1H, Quinolinyl H-7), 8.00 (d, 1H, Quinolinyl H-8), 8.13 (s, 1H, thiazolyl H-5), 9.005 (-CH=N-); LC-MS (RT, % area): 1.685, 93.62; *m/z*: 349.13 (M+1); Analysis for $C_{19}H_{12}ClN_3S$: Calcd: C,65.23; H, 3.46; N, 12.01; S, 9.17 Found: C, 65.28; H, 3.43; N, 12.09; S, 9.16%.

(NZ)-N-(((4-chlorophenyl) diazenyl) (2-chloroquinolin-3-yl) methylene) -4- phenylthiazol -2-amine (5A): Coffee red color powder; Yield 93%; R_f: 0.9; m.p.: 126-30°C; UV-Vis (λ max, Ethanol): 425 nm; IR (KBr, cm^{-1}): 1028 (C-S), 1617 (C=N str.), 1441 (-N=N-), 753 (C-Cl), 826 (1,4 disubstitution); 1H NMR (DMSO-d6, δppm, 400 MHz): 9.12 (s, 1H, Quinolinyl H-4), 8.03 (d, 1H, Quinolinyl H-5), 7.61 (m, 1H, Quinolinyl H-6), 7.78 (m, 1H, Quinolinyl H-7), 7.99 (d, 1H, Quinolinyl H-8), 7.52-7.76 (m, 5H, Ar H), 7.26-7.50 (m, 4H, diazenylAr H), 8.26 (s, 1H, thiazolyl H-5); LC-MS (RT, % area); 2.801, 91.72; m/z: 488.41 (M); Analysis for $C_{25}H_{15}Cl_2N_5S$: Calcd: C, 61.48; H, 3.10; N, 14.34; S, 6.57 Found: C, 61.23; H, 3.03; N, 14.39; S, 6.69%.

(NZ)-N-((2-chloroquinolin-3-yl)((4-nitrophenyl)diazenyl)methylene)-4-phenylthiazol-2-amine (5B): Yellowish brown color powder; Yield 97%; R_f: 0.6; m.p.: 116-19°C; UV-Vis (λ max, DMSO): 427 nm; IR (KBr, cm^{-1}): 998 (C-S), 1528, 1394 (NO_2 str.), 1444 (-N=N-), 753 (C-Cl), 840 (1,4 disubstitution); ^{1}H NMR (DMSO-d6, δppm, 400 MHz): 9.15 (s, 1H, Quinolinyl H-4), 8.03 (d, 1H, Quinolinyl H-5), 7.63 (m, 1H, Quinolinyl H-6), 7.79 (m, 1H, Quinolinyl H-7), 7.93 (d, 1H, Quinolinyl H-8), 7.55- 7.73 (m, 5H, Ar H), 7.29-8.15 (m, 4H, diazenylAr H), 8.19 (s, 1H, thiazolyl H-5); LC-MS (RT, % area); 1.821, 87.72; m/z: 498.71 (M); Analysis for $C_{25}H_{15}ClN_6O_2S$: Calcd: C, 60.18; H, 3.03; N, 16.84; S, 6.43 Found: C, 60.11; H, 3.11; N, 16.87; S, 6.39%.

(NZ)-N-((2-chloroquinolin-3-yl)((4-methoxyphenyl)diazenyl)methylene)-4-phenylthiazol-2-amine (5C): Brown color powder; Yield 88%; R_f: 0.6; m.p.: 123-27°C; UV-Vis (λ max, DMSO): 579 nm; IR (KBr, cm^{-1}): 1617 (C-N str.), 2922 (-CH2- str.), 1490 (-N=N-), 1028 (C-O-CH3), 751 (C-Cl); ^{1}H NMR (DMSO-d6, δppm, 400 MHz): 9.42 (s, 1H, Quinolinyl H-4), 7.99 (d, 1H, Quinolinyl H-5), 7.60 (m, 1H, Quinolinyl H-6), 7.78 (m, 1H, Quinolinyl H-7), 7.97 (d, 1H, Quinolinyl H-8), 7.41- 7.80 (m, 5H, Ar H), 6.90-7.15 (m, 4H, diazenylAr H), 8.22 (s, 1H, thiazolyl H-5), 3.70 (s, 3H, OCH3); LC-MS (RT, % area); 1.726, 87.72; m/z: 483.7 (M); Analysis for $C_{26}H_{18}ClN_5OS$: Calcd: C, 64.52; H, 3.75; N, 14.47; S, 6.63 Found: C, 64. 47; H, 3.83; N, 14.28; S, 6.49%.

(NZ)-N-(((4-bromo-3-methylphenyl)diazenyl) (2-chloroquinolin-3-yl) methylene)-4-phenylthiazol-2-amine (5D): Reddish brown color powder; Yield 91%; R_f: 0.7; m.p.: 115-17°C; UV-Vis (λ max, DMSO): 376 nm; IR (KBr, cm^{-1}): 1611 (C=C str./ C-N str.), 1480 (-N=N-), 862 (Tri substitution), 755 (C-Br); ^{1}H NMR (DMSO-d6, δppm, 400 MHz): 9.32 (s, 1H, Quinolinyl H-4), 8.09 (d, 1H, Quinolinyl H-5), 7.65 (m, 1H, Quinolinyl H-6), 7.73 (m, 1H, Quinolinyl H-7), 7.93 (d, 1H, Quinolinyl H-8), 7.49- 7.79 (m, 5H, Ar H), 7.03-7.48 (m, 3H, diazenylAr H), 8.14 (s, 1H, thiazolyl H-5), 2.45 (s, 3H, CH3); LC-MS (RT, % area); 1.926, 93.72; m/z: 546.17 (M); Analysis for $C_{26}H_{17}BrClN_5S$ Calcd: C, 57.10; H, 3.13; N, 12.81; S, 5.86 Found: C, 56.93; H, 3.21; N, 12.87; S, 5.74%.

4-(((Z)-(2-chloroquinolin-3-yl)(4-phenylthiazol-2-ylimino)methyl)diazenyl)phenol (5E): Yellowish brown color powder; Yield 81%; R_f: 0.8; m.p.: 168-70°C; UV-Vis (λ max, DMSO): 350 nm; IR (KBr, cm^{-1}): 3181 (OH str.) 1519 (C=C str str.), 1435 (-N=N-), 1273 (C-O str.), 756 (C-Cl); ^{1}H NMR (DMSO-d6, δppm, 400 MHz): 9.53 (s, 1H, OH), 9.28 (s, 1H, Quinolinyl H-4), 8.01 (d, 1H, Quinolinyl H-5), 7.58 (m, 1H, Quinolinyl H-6), 7.80 (m, 1H, Quinolinyl H-7), 7.99 (d, 1H, Quinolinyl H-8), 7.27- 7.75 (m, 5H, Ar H), 7.13-7.22 (m, 3H, diazenylAr H), 8.25 (s, 1H, thiazolyl H-5); LC-MS (RT, % area); 3.056, 93.72; m/z: 468.5 (M); Analysis for $C_{25}H_{16}ClN_5OS$: Calcd: C, 63.89; H, 3.43; N, 14.90; S, 6.82 Found: C, 63.91; H, 3.21; N, 14.83; S, 6.73%.

In vitro antioxidant activity of diazenyl schiff base molecules by DPPH assay method

The free radical scavenging activity of the selected newly synthesized molecules were measured by 2,2-diphenyl-1- picryl hydrazyl (DPPH) assay method (Sahoo and Kumar, 2015). The different concentrations of the synthesized molecules were prepared with methanol. The final volume of each test sample was adjusted up to 3 mL with methanol. To each of the test sample, 1 mL of freshly prepared 0.1mM DPPH in methanol was added. The test samples were vigorously shaken and kept in dark for 30 min. One milliliter of 0.1 mM of methanolic solution of DPPH was considered as control and 3 mL methanol was taken for blank. The antioxidant activity of synthesized molecules was compared with standard ascorbic acid. The optical density was measured at 517 nm and the inhibition concentration was calculated:

$$\% \text{ of inhibition} = \frac{A_{cont} - A_{test}}{A_{cont}} \times 100$$

Where A_{cont} = absorbance of control and A_{test} = absorbance of the test sample. All the experiments were carried out in triplicate and the values were expressed as mean ± SD.

Statistical analysis

The observed data on mean percent of inhibition for antioxidant activity of different synthesized molecules were subjected to one way- analysis of variance (ANOVA) for comparison of group means of different molecules. In the study of antioxidant activities of two of the molecules (**5A** and **5E**) of the scheme is given Figures 5 and 6 were compared with the standard ascorbic acid and among them through Post Hoc Bonferroni test. The test of significance, cut off value of p was taken as < 0.05. The statistical analysis was done using SPSS 16.0 software.

RESULTS AND DISCUSSION

The mixture of two reactants (**1)** and (**2)** in the presence of glacial acetic acid in ethanol gave schiff base (E)-N-((2-chloroquinolin-3-yl) methylene)-4- phenyl thiazol-2-amine (**3**) by nucleophilic addition reaction. The electron rich azomethine group of the schiff base ligand was undergone azo coupling reaction with a series of five diazotized primary aromatic amines (**4A-4E**) which act as electrophiles and gave some new diazenyl schiff based derivatives (**5A-5E**). The synthetic scheme is presented in Figure 1. The structures of prepared intermediates and final molecules have been confirmed by FT/IR, ^{1}HNMR, UV, LC-MS and elemental analysis. The short medium absorption band in all the compounds (**5A**-**5E**) appeared at the range of 1490 to 1435 cm^{-1} assigned to –N=N- group. The FT/IR spectral image of the compound **5E** is given in Figure 2.

The ^{1}H NMR analysis of the synthesized compounds showed the Quinolinyl H-4 singlet at a range of δ 8.83 to 9.42 ppm, thiazolyl H-5 singlet at a range δ 8.13 to 26 ppm and attached diazenyl aromatic protons at a range of δ 6.90 to 8.15 ppm. The ^{1}H NMR spectra of the compound **5E** is illustrated in Figure 3.

The predicted molecular weight of the synthesized compounds was confirmed by LC-MS. The compound (NZ)-N-((2-chloroquinolin-3-yl) ((4-methoxyphenyl)diazenyl)methylene)-4-phenylthiazol-2-amine (**5c)** having molecular ion peak 483.7 (M) showed in Figure 4 strongly reveals the predicted molecular formula $C_{26}H_{18}ClN_5OS$.

In vitro antioxidant activity of diazenyl schiff base molecules

The *in vitro* antioxidant activity of the diazenyl schiff base molecules **5A-5E** is presented in Tables 1 and 2.

The graphical presentation of free radical scavenging activity of diazenyl schiff base molecules (**5A-5E**) is given in Figures 5 and 6.

At each concentration of 5 to 600 µg/ml, there were significant difference among the molecules and the standard ascorbic acid at $p<0.05$. However with the increase of potency, all the molecules with the standard ascorbic acid registered increase in the mean percentage of inhibition.

At 600 µ/ml the molecule **5E** exhibited the mean percentage of inhibition of 87.88±0.87, which was very close to the performance of the standard ascorbic acid (85.83±0.83) at 50 µg/ml. Similarly **5A:** At 600 µg/ml have the mean percentage of inhibition 82.1 ± 0.87 which was also close to the performance of ascorbic acid (85.83 ± 0.83). Therefore the mean percentage of inhibition of **5A** and **5E** at 600 µg/ml and ascorbic acid (AA) at 50 µg/ml was compared through Post Hoc Bonferroni test. The result of antioxidant evaluation is given in Tables 1 and 2.

The mean percentage of inhibition of **5A** at 600 µg/ml was significantly lower than **5E** ($p=0.001$) and also lower than ascorbic acid at 50 µg/ml ($p=0.005$). The mean percentage of inhibition of **5E** at 600 µg/ml and that of ascorbic acid (**AA**) at 50 µg/ml were not significantly different ($p=0.547$). This implied the molecule **5E** at 600 µg/ml is giving comparable performance to ascorbic acid (**AA**) at 50 µg/ml. Literature survey revealed that phenolic molecules or nitrogen bearing heterocyclic rings have good free radical scavenging activity (Shridhar et al., 2016; Chinnagiri et al., 2013). The molecules (**5A** and **5E**) showed potential antioxidant activity and possess nitogen bearing heterocyclic ring. At the same time the molecule **5E** also possess the phenolic–OH group which may be responsible for exhibiting better antioxidant activity.

Conclusion

This part of research work comprises five diazenyl schiff base molecules (**5A**-**5E**) derived from the molecule (3) as explained in the scheme. The antioxidant activity of the synthesized molecules is investigated by DPPH method. The molecules (*NZ)-N-(((4-chlorophenyl) diazenyl) (2-*

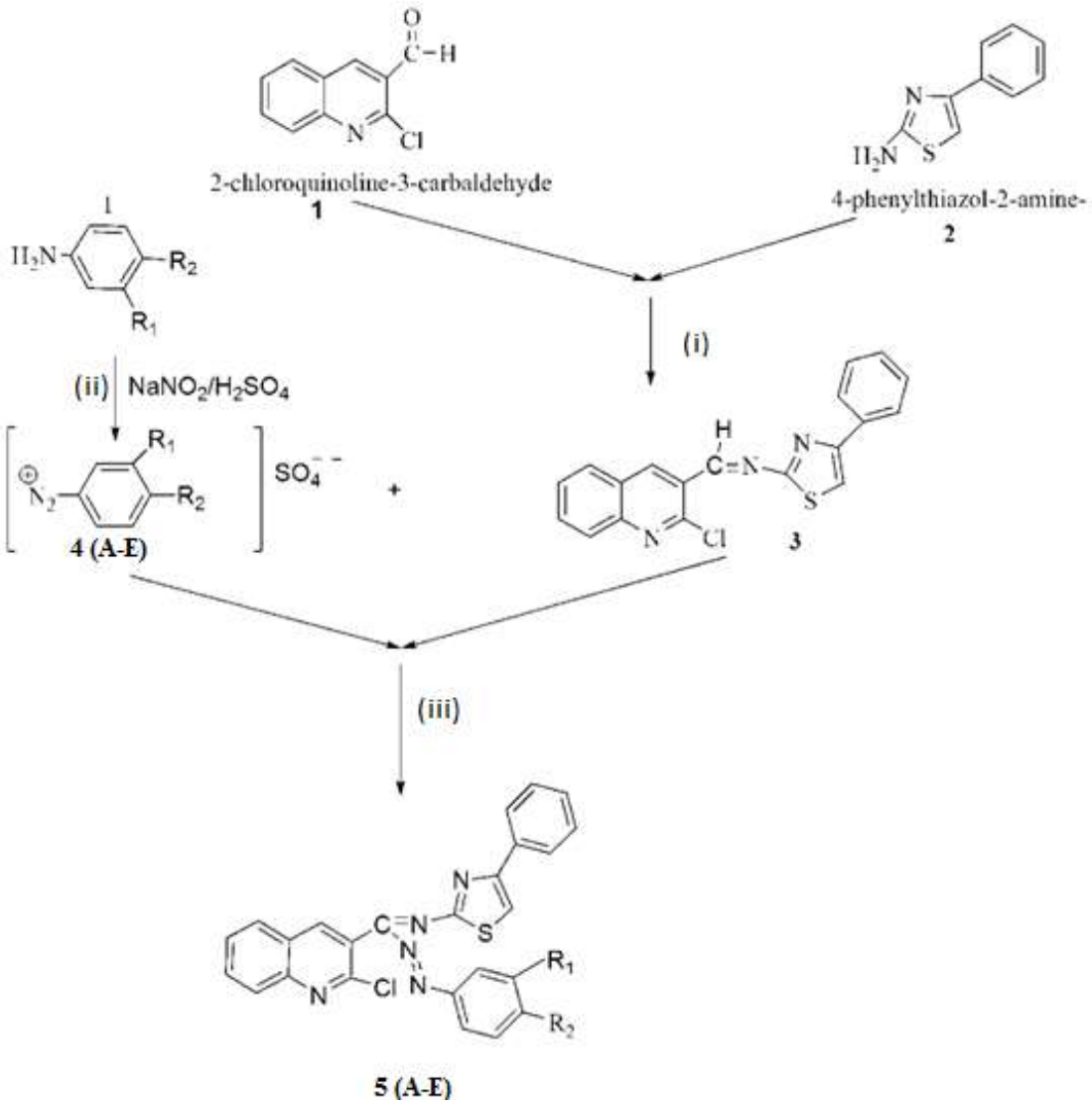

Figure 1. Synthetic scheme of diazenyl schiff base molecules. 5A (R_1=H, R_2=Cl), 5B (R_1=H, R_2=NO_2), 5C (R_1=H, R_2=OCH_3), 5D (R_1=Br, R_2=CH_3), 5E (R_1= H, R_2=OH) Reaction: - i. Ethanol/ Glacial acetic acid reflux 2h, ii. $NaNO_2/H_2SO_4$ (0-5˚C), iii. 10% NaOH coupling reaction.

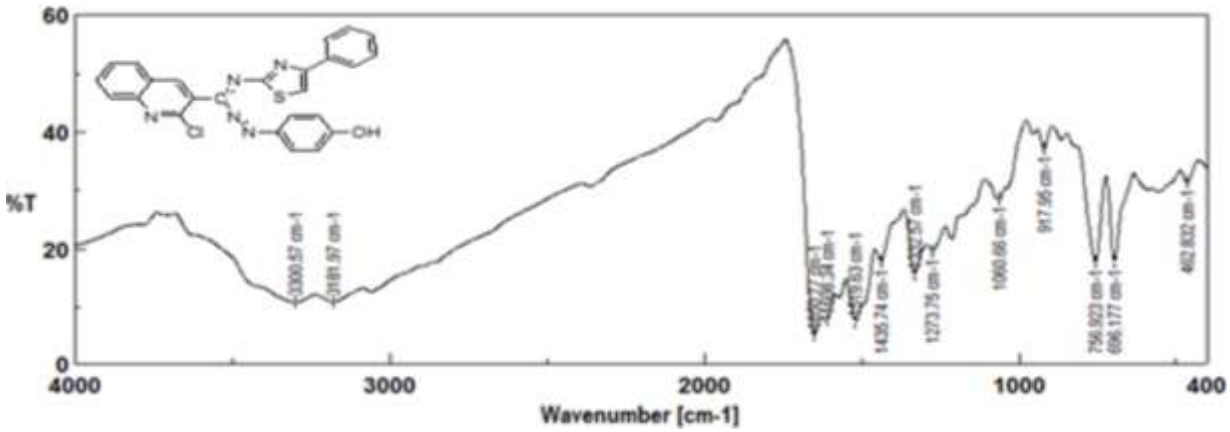

Figure 2. FT/IR spectra of (((Z)-(2-chloroquinolin-3-yl)(4-phenylthiazol-2-ylimino)methyl)diazenyl)phenol (**5E**).

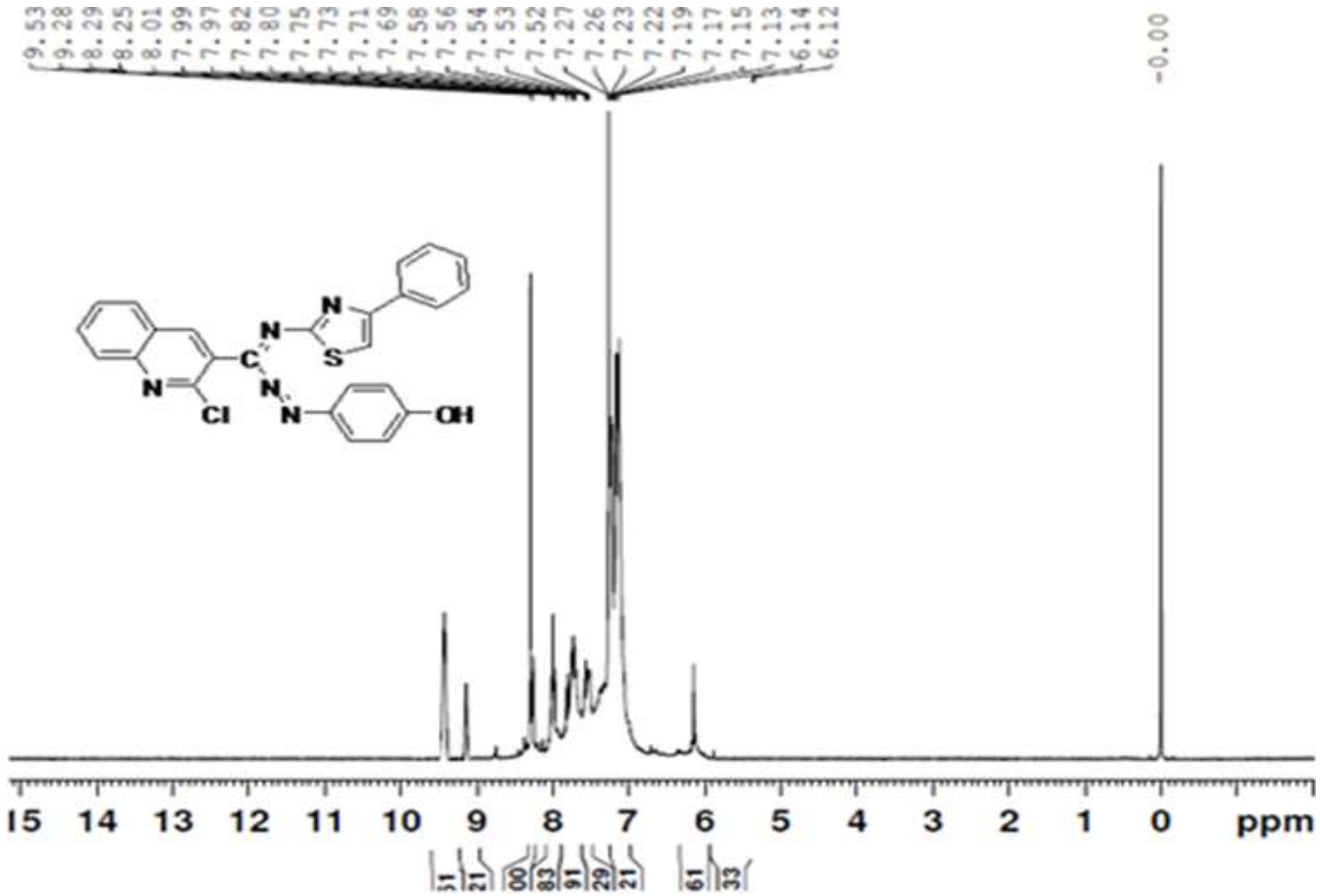

Figure 3. ^{1}H NMR of (((Z)-(2-chloroquinolin-3-yl)(4-phenylthiazol-2-ylimino)methyl)diazenyl) phenol (**5E**).

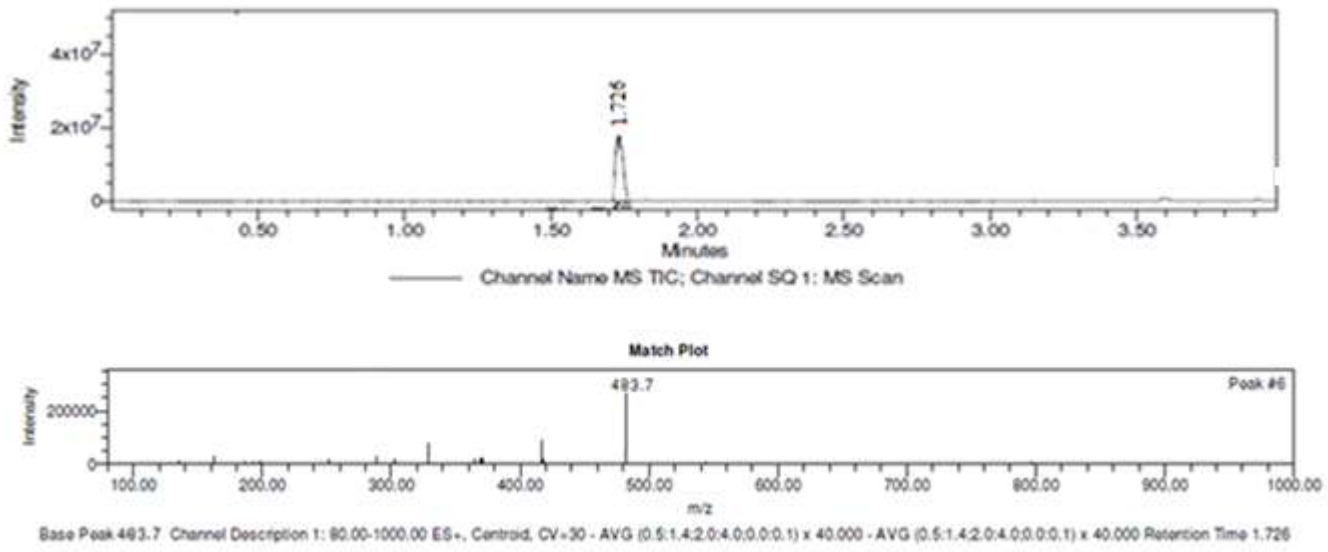

Figure 4. LC-MS of -N-((2-chloroquinolin-3-yl)((4-methoxyphenyl)diazenyl) methylene)-4-phenylthiazol-2-amine **(5C**)

Table 1. *In vitro* antioxidant activity of diazenyl schiff base molecules.

Molecules (n=3)	Concentration (µg/ml)						
	5	10	50	100	200	400	600
	% Inhibition						
5A	41.56±0.87	44.97±0.87	49.25±0.87	54.88±0.87	62.37±0.87	71.89±0.87	82.1±0.87
5B	29.07±0.87	31.9±0.87	35.67±0.87	39.81±0.87	45.38±0.87	56.72±0.87	64.52±0.87
5C	28.51±0.87	29.45±0.87	33.41±0.87	39.16±0.87	46.48±0.87	56.86±0.87	65.78±0.87
5D	27.68±0.87	28.28±0.87	32.01±0.87	38.25±0.87	44.76±0.87	53.35±0.87	61.81±0.87
5E	49.33±0.87	51.26±0.87	56.2±0.87	60.78±0.87	67.41±0.87	77.79±0.87	86.88±0.87
Ascorbic acid	73.37±0.66	79.62±0.7	85.83±0.83	95.96±0.61	99.18±0.27	99.4±0.3	99.48±0.39
ANOVA 'p' value	0.000	0.000	0.000	0.000	0.000	0.000	0.000

p< 0.05 indicates significant different among group means at different level of concentration. Data are expressed mean ± SD.

Table 2. *In vitro* antioxidant activity of diazenyl schiff base molecules.

(I) Molecules	(J) Molecules	Mean Difference (I-J) ± SE
5A at 600	5E at 600	-4.78±0.7**
	Ascorbic acid at 50	-3.73±0.7**
5E at 600	Ascorbic acid at 50	1.05±0.7

***** p<0.05, ** p<0.01

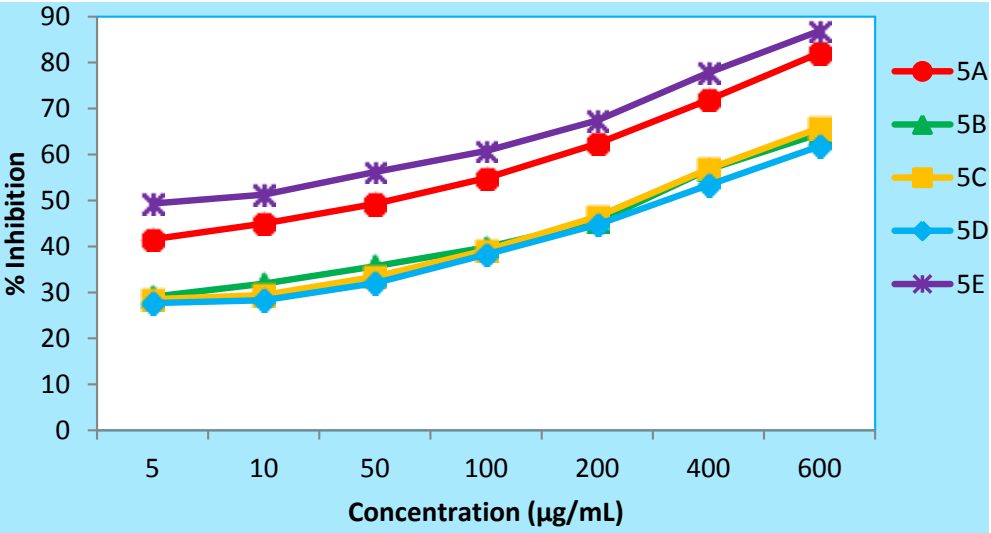

Figure 5. Trend of mean percentage of inhibition of antioxidant activities of diazenyl schiff base molecules (**5A-5E**) in different concentrations. Data are expressed in mean ± 95% CI.

chloroquinolin-3-yl) methylene) -4- phenylthiazol -2-amine (**5A**) and *4-(((Z)-(2-chloroquinolin-3-yl)(4-phenylthiazol-2-ylimino)methyl)diazenyl)phenol* (**5E**) showed potential antioxidant activity which justified that the existence of phenolic -OH group and heterocyclic nitrogen bearing molecules can promote free radical scavenging activity for which these novel molecules may have gone for further investigations to establish them as potent molecules for the treatment of oxidative stress related diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

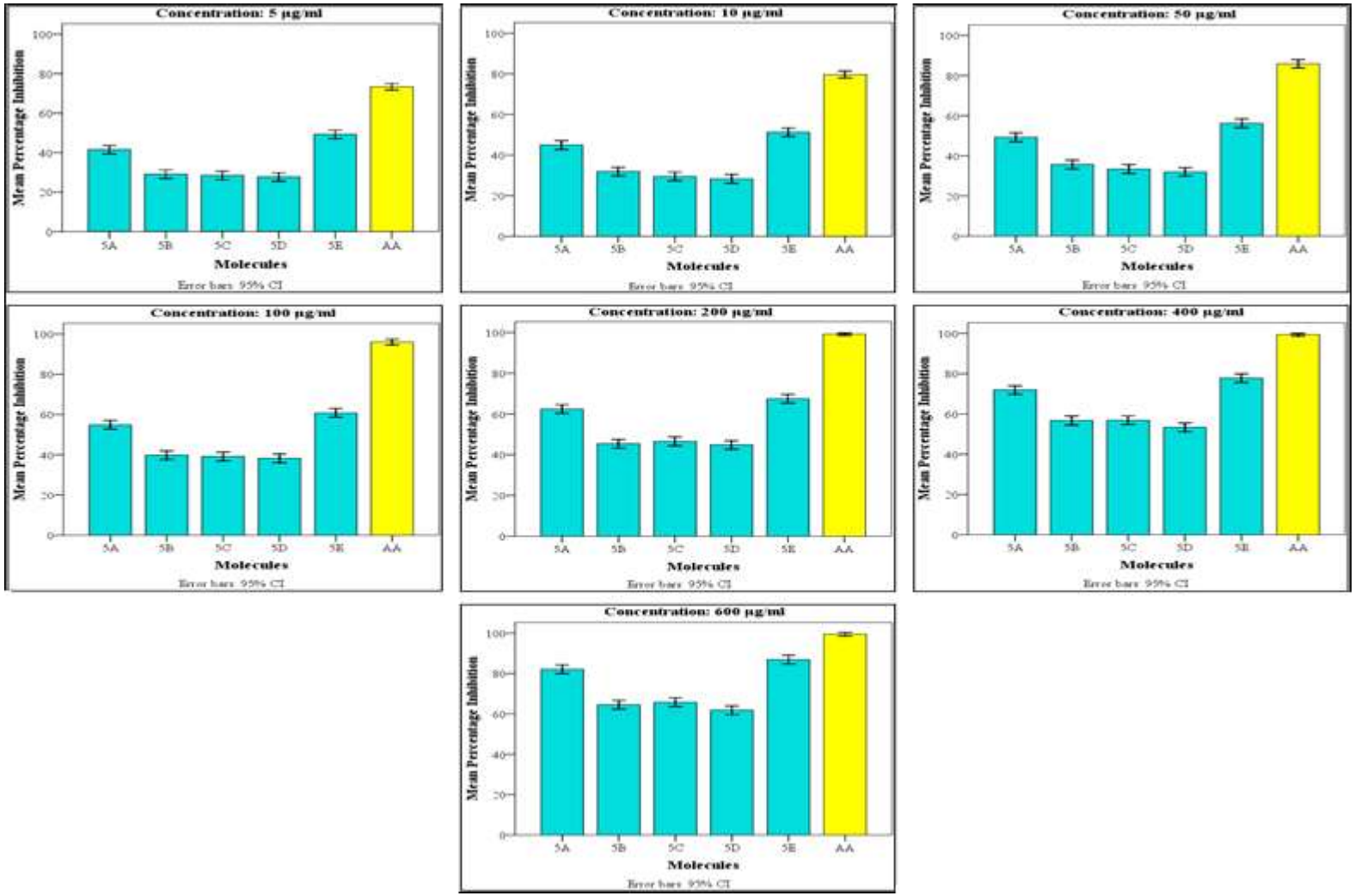

Figure 6. Antioxidant activities of the diazenyl schiff based molecules **5A-5E** and standard Ascorbic acid (**AA**) by DPPH model. Data are expressed as mean percentage of inhibition ± 95% CI at different concentration

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