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Research article

Synthesis, characterization & biological evaluation of 1, 3, 4- oxadiazoles as antioxidant agents

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Abstract

A novel series of 1,3,4-oxadiazole, 5-[4-4′-chlorophenyl)-1,3-thiazole-2yl]-1,3,4-oxadiazole-2-thione (5) were synthesized as per literature method. It was further derivatized in to mannich bases 5-[4-4′-chloro thiazole-2yl]-3-substituted-1,3,4-oxadiazole-2-thione [5a-e]. Physical characterization of compounds which were done by identifying melting point, R_f value, percentage yield and solubility. The structures of these compounds were then identified using UV, FT-IR, ^{1}HNMR and MASS spectral analysis. The derivatives were then screened for antioxidant activity. The antioxidant characters of the synthesized compounds were found out by DPPH, Nitric oxide and Hydrogen peroxide scavenging assays. Compound '5c' showed maximum radical scavenging potential in all the three methods, due to presence of electron donating substituents like diethyl group.

Key words: 1,3,4-oxadiazole, Mannich bases, Antioxidant activity, DPPH assay, H_2O_2 Scavenging assay.

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1. Introduction

Among the five membered aromatic compounds which are hetrocyclic, 1,3,4-Oxadiazoles are critical compounds. There are four oxadiazoles that exist in different isomeric forms such as 1,2,4, 1,2,5, 1,2,3 and 1,3,4-oxadiazoles. Out of this 1,3,4-oxadiazole is widely being exhibit diverse biological activities [1] like antibacterial [2], antitubercular [3], vasodilatory [4], antifungal [5], anti-inflammatory [6], anticonvulsant [7], cytotoxic [8], anaesthetic [9], analgesic [10], hypolipidimic [11], anticancer [12], antioxidant [13], and ulcerogenic [14] activities. 1,3,4-oxadiazole derivatives and their mannich bases were reported to possess anticancer and antioxidant activity. Furthermore, certain thiazole derivatives are well known for their antioxidant.

Due to presence of an extra heteroatom there is inductive effect which makes oxadiazole a weak base. There are two pyridine like nitrogen (-N=) present in oxadiazole. The conjugated diene character in oxadiazole is mainly due to

reduction in aromaticity of oxadiazole ring. Replacement of hydrogen atom by nucleophiles has been seen in nucleophilic substituted oxadiazoles[15].

Free radicals are not stable, short lived and very reactive due to presence of odd number of electrons. So to gain stability it captures the odd electrons so that it reacts with other compounds free radicals generally capture the electrons of the nearest stable molecule. The living cells are disrupted by the chain reaction, that is, the attacked molecule lose it's electrons and become a free radical which attack the living cell. Nitrogen compounds, phenolic compounds are categorized as synthetic antioxidants. The sterically hindered phenols and secondary aromatic amines form the primary antioxidants compounds. The hydrogen atom migrate from the antioxidant molecule to radical intermediate is the first step of the radical termination [16].

Free radical scavenging is one of the best known mechanisms by which antioxidant inhibit lipid oxidation. The *in* vitro antioxidant activity can be performed by three methods, DPPH assay, Nitric oxide scavenging assay and Hydrogen peroxide radical scavenging assay. Some new 2-amino methyl thiazole derivatives (Figure 1) were prepared [17] and posses excellent antioxidant activity.

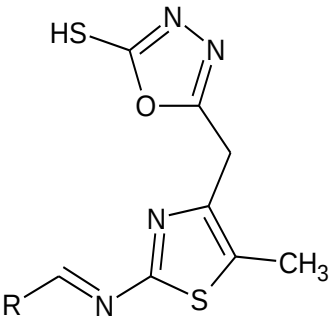


Figure 1. Structure of derivative

In view of the above studies, we considered it of interest to synthesize some new derivatives by incorporating thiazole moiety with mannich bases of 1,3,4-oxadiazole analogues and investigated their antioxidant property.

2. Materials and Methods

Chemistry

Synthetic Procedure

Step-1: Synthesis of P-Chloro Phenacyl Bromide. (1)

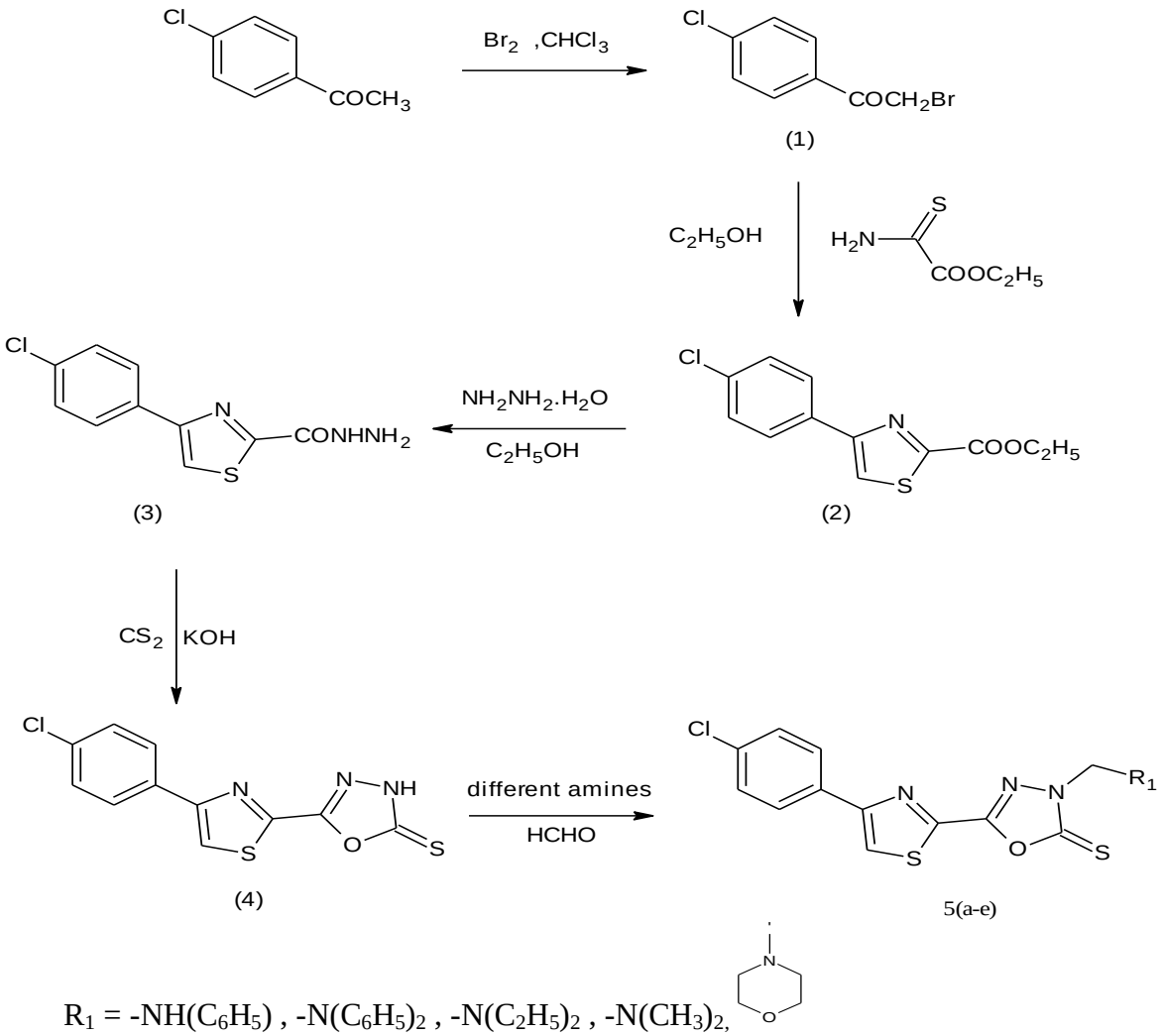
To a cold solution of p-chloro-acetophenones (0.01mol) in chloroform (25ml), bromine (0.012mol) in chloroform (10ml) was gradually added for about 30 minutes with continuous stirring were the temperature maintained is 0-5^{0}C. The mixture of reaction was brought to the room temperature after the addition was complete. For another 60 minutes the mixture was stirred till hydrogen gas evolution ceases. Under reduced pressure the solvent was eliminated and recrystallized the residue from ethanol to afford to get pure p-chloro-phencylbromide [18].

Step-2: Synthesis of 4-(4′-Chloro-phenacyl)-1,3-Thiazole-2-Carboxylate. (2)

In a round bottom flask a mixture of ethyl thiooxamate (1 equivalent weight), p-chloro phencylbromide (1.1 equivalent weight) and ethanol 10-15ml were taken, and refluxed for 2 hr, the ethanol was distilled off under vacuum and it was neutralized with sodium bicarbonate.

Ethyl acetate is used to extract the mixture, and then washed with water. Under vacuum the solvent was eliminated. Crude product obtained was recrystallized from ethanol [l9].

Scheme of synthesis:



Step-3: Synthesis of 4-(4′-Chloro Phenacyl)-1,3-Thiazole-2-Carbo hydrazide. (3)

Compound-3 was prepared by refluxing 4-(4′-chlorophenacyl)-1,3-thiazole-2-carboxylate (0.015 mol) with hydrazine hydrate (1.6 mL) in ethanol (20 mL) for 5 h. After cooling the mixture the product obtained was recrystallized from DMF: ethanol mixture (6:1) [20, 21].

Step-4: Synthesis of 5-[4-(4′-Chloro Phenacyl)-1,3-Thiazole-2yl] -1,3,4-Oxadiazole-2-Thione. (4)

Potassium hydroxide (0.04mol), ethanol (25ml), 4-(4′-chlorophenyl) thiazole-2-carbohydrazide (0.02mol) were mixed together. With stirring carbon disulfide (14ml) was added. For 8 hours the reaction mixture was heated. Under reduced pressure solvent was eliminated. The residue was filtered after washing with water. Filtrate was cooled and using dilute hydrochloric acid it is neutralized to pH 6. The final product obtained was filtered, washed with water, dried and recrystallized using ethanol [20, 21].

Step-5: Synthesis of 5-[4-(4′-Chloro Phenacyl)-1,3-Thiazole-2yl]-3-(2-

Methyl Substituted) -1,3,4-Oxadiazole-2-Thione. (5a-e)

Formaldehyde 40% (0.003mol) was added to a solution of 5-[4-(4′-chlorophenacyl)-1,3-thiazole-2yl]-3-(2-methyl substituted) -1,3,4-oxadiazole-2-thione (0.003 mol) in absolute ethanol (10ml). An ethanolic solution (2ml) of the different primary or secondary amine (0.003 mol) was added portion wise to the above reaction mixture, stirred for 3 hours at room temperature, and left overnight in a refrigerator. The precipitate formed was filtered, dried, and crystallized from DMF: Water (5:2) [21].

Pharmacological Study Results

Antioxidant activity results:

DPPH assay (2, 2-diphenyl -1-picrylhydrazyl)

The DPPH assay was used to study radical scavenging activity of test samples according to Scherer and Godoy [22]. The measurement is taken at 517 nm. After the addition of an antioxidant the decrease in the absorption of the DPPH solution was taken. Standard taken is Ascorbic acid.

Procedure

Using 2,2-diphenyl-1-picrylhydrazyl radical(DPPH) antioxidant potency of the test samples and standard were evaluated. 3.9mL of DPPH in methanol solution (0.2mM) is added to methanol solutions of samples or standards (0.1ml) in concentrations of (20, 40, 60, 80, 100 microgram/ml). 0.1mL of methanol added to 3.9 mL of DPPH solution is the control. Triplicate of the procedure were done. In the dark, 90-min incubation is done at room temperature, then absorbance was measured at 517 nm. Without test compound, but an equivalent amount of methanol taken is the control [22].

Ascorbic acid was used as standard. The results obtained from DPPH assay is shown in table 2. IC_{50} values for standard and test samples were determined using ED50 plus V 1.0 software. Percentage inhibition is calculated using the formula;

% scavenging activity = $\frac{control-test}{control} X100$

Nitric Oxide Scavenging Activity

Nitric oxide scavenging activity can be estimated by the use of Griessre action (Garrat, 1964). The compound sodium nitroprusside produce NO by decomposing in aqueous solution at pH 7.2. In aerobic condition, NO reacts with oxygen to produce stable products (nitrate and nitrite). The quantities of which can be determined using Griess reagent. The completion between scavengers of nitric oxide and oxygen leads to decrease in production of nitrite ions.

Procedure

Griess reaction is used to measured Nitric oxide scavenging activity, spectrophoto-metrically. Different concentration of test sample (20, 40, 60, 80, 100 µg mL^{-1}) in methanol is mixed with sodium nitro prusside (5mmolL^{-1}) in phosphate buffered saline (pH-7.4) and incubated for 30 minutes at 25°C. Instead of test sample equivalent amount of methanol is taken and used as control. 1.5mL of Griess reagent is used to dilute 1.5mL of the incubated solution after 30 minutes. Absorbance was evaluated at 517nm with reference to standard and percentage-scavenging property was evaluated [23].

Ascorbic acid was used as standard. The results obtained from nitric oxide scavenging assay is shown in table 3. IC_{50} values for standard and test samples were determined using ED50 plus V 1.0 software. Percentage inhibition is calculated using the formula;

% scavenging activity = $\frac{control-test}{control} X100$

Hydrogen Peroxide Radical Scavenging Assay

By the action of oxidase enzymes, *in vivo* hydrogen peroxide is generated. Through the reduction product called hydroxyl radical (OH•) hydrogen peroxide is scavenged.

Here hydrogen peroxide is scavenged by the test sample, which is the basis of this method. Using phosphate buffer solution (pH 7.4), hydrogen peroxide solution (2mmol/l) was prepared. To hydrogen peroxide solution (0.6 ml) test samples (20, 40, 60, 80, 100 µg/ml) were added. After 10 min absorbance of hydrogen peroxide was evaluated against blank at 230 nm which containing phosphate buffer without hydrogen peroxide. The reference compound is ascorbic acid, which is used for comparison [24].

Ascorbic acid was used as standard. The results obtained from hydrogen peroxide assay is shown in table 4. IC_{50} values for standard and test samples were determined using ED50 plus V 1.0 software. Percentage inhibition is calculated using the formula;

% scavenging activity = $\frac{control-test}{control} X100$

Data are the mean of three or more experiments and reported as mean ± standard error of the mean (SEM)

3. Result and Discussion

Physical data: Physical data like Molecular formula, Melting point, Solubility and R_f value were evaluated for the mannich derivatives and is shown in table 1.

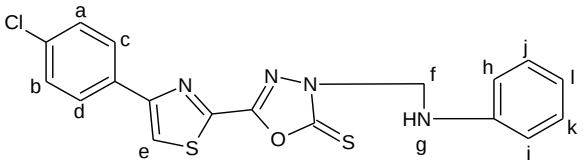
Spectral Analysis:

The spectral analysis like UV, IR, ^{1}H NMR and Mass were done to identify the structure of the mannich derivatives. The instrument model for UV spectra is SHIMADZU UV-2600. IR spectra were analyzed using BRUKER VERTEX FT-IR spectrophotometer. BRUKER 400Mhz is used to obtain ^{1}HNMR. MASS spectra were obtained using the instrument, LCMS THERMO LCQ DECA XP.

Table 1. Physical characteristics of compounds

Sample Code	Physical data				
	Molecular Formula	**Melting Point**	**Solubility**	**% yield**	**R_f value**
5a	$C_{18}H_{13}ClN_4OS_2$	97^{0}C -99^{0}C	Ethanol &DMSO	59%	0.69
5b	$C_{24}H_{17}ClN_4OS_2$	197^{0}C -199^{0}C	Ethanol &DMSO	49.5%	0.75
5c	$C_{16}H_{17}ClN_4OS_2$	105^{0}C -108^{0}C	Ethanol &DMSO	44%	0.81
5d	$C_{14}H_{13}ClN_4OS_2$	86^{0}C -90^{0}C	Ethanol &DMSO	53%	0.65
5e	$C_{16}H_{15}ClN_4O_2S_2$	124^{0}C -126^{0}C	Ethanol &DMSO	51%	0.89

Spectral data of compound 5a



IR Spectra (cm^{-1}): 3286.64(- NH structure); 3983.76 (Ar- CH); 1648.10(HC=N); 1627.6 6 (-NHbent);1499.83(Ar C=C);1260.12(C=S); 1218.18(C-O-C);1178.08(C-N);733.74(C-Cl); 814.20(C-S).

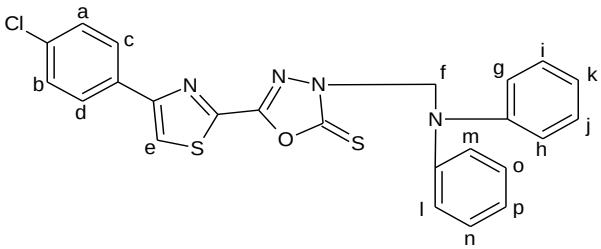
NMR Spectra: ^{1}H NMR, δ(ppm)(DMSO): 7.22(d, 2H, Ar CH at a,b); 7.39(d, 2H, Ar CH at c, d); 8.11(s, 1H, CH of thiazole at e); 5.11(s, 2H, CH_2 at f); 3.81(s, 1H, NH at g); 6.51(d,2H, Ar CH at h,i); 6.90 (t, 2H, Ar CH at j,k); 6.71(t, 1H, Ar CH at l); solvent peaks at 2.49 and 3.31.

UV-λmax (nm): 347.

Mass spectra: Molecular weight=400

Spectral data of compound 5b

IR SPECTRA (cm^{-1}): 3079.82(Ar-CH str); 2918.98(Ali- CH); 1588.12 & 1508(Ar C=C); 1658.46(C=N); 1221.34(C-O-C); 1266.12 (C=S); 828.40(C -S); 778.75(C-Cl).

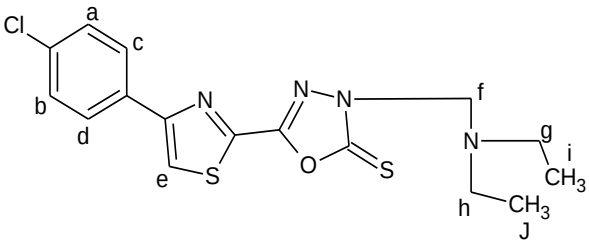


NMR Spectra: ^{1}H NMR, δ(ppm)(DMSO): 7.29(d, 2H, Ar CH at a, b); 7.38(d, 2H, Ar CH at c, d); 8.12(s, 1H, CH of thiazole at e); 5.17(s, 2H, CH_2 at f); 7.11(d, 4H, Ar CH at l, g, m, h); 6.91(t, 4H, Ar CH at n, o, j, i); 6.82(t, 2H, Ar CH at p, k). Solvent peaks at 2.49 and 3.31.

UV-λmax (nm): 397.

Mass spectra: Molecular weight=477

Spectral data of compound 5c



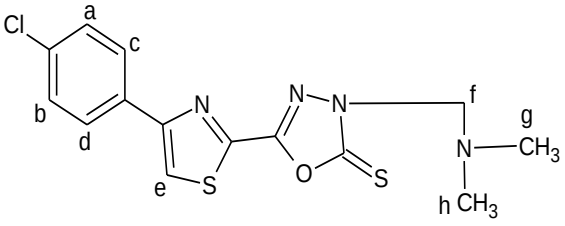
IR Spectra (cm^{-1}): 3065.79(Ar-CH structure); 2916.36(Ali- CH); 1524.65 & 1584.40(Ar C=C); 1679.33(C=N); 1270.18(C=S); 1207.15(C-O-C); 751.89(C-S); 721.65(C-Cl).

NMR Spectra: ^{1}H NMR, δ(ppm) (DMSO): 7.32(d, 2H, Ar CH at a, b); 7.12(d, 2H, Ar CH at c, d); 6.90(s, 1H, CH of thiazole at e); 5.47(s, 2H, CH_2 at f); 2.95(m, 4H, CH_2-N-CH_2 at g, h); 1.11(t, 6H, CH_3 at i, j) solvent peaks at 2.49 and 3.31.

UV-λmax (nm): 270

Mass spectra: Molecular weight=380

Spectral data of compound 5d



IR Spectra (cm^{-1}): 3076.74(Ar-CH str); 2 926(Ali- CH); 1516.24 & 1581.11(Ar C=C); 1648.25(C=N); 1160.27(C-O-C); 1251.70 (C=S); 1040(C-N); 754.08(C-S); 695.15(C-Cl).

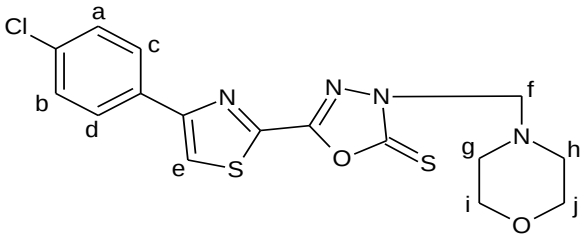
NMR Spectra: ^{1}H NMR, δ(ppm) (DMSO): 7.31(d, 2H, Ar CH at a, b); 7.22(d, 2H, Ar CH

at c, d); 6.89(s, 1H, CH of thiazole at e); 5.46(s, 2H, CH_2 at f); 2.01(s, 6H, CH_3-N-CH_3 at g, h); solvent peaks at 2.49 and 3.31.

UV-λmax (nm): 266

Mass spectra: Molecular weight=352

C Spectral data of compound 5e



IR spectra (cm^{-1}): 3052.46(Ar-CH str); 2900.76(Ali- CH); 1513.60 & 1600.48(Ar C=C); 1122.24(C-N); 1228.20(C-O-C); 1663.48(C=N); 1270.14(C =S); 817.26(C-S); 764.88(C-Cl).

NMR Spectra: ^{1}H NMR,δ(ppm)(DMSO): 8.24(d, 2H, Ar CH at a, b); 7.70(d, 2H, ArCH at c, d); 6.91(s, 1H, CH of thiazole at e); 5.49(s, 2H, CH_2 at f); 2.16(t, 4H, CH_2-N-CH_2 at g, h); 3.71(t, 4H, CH_2-O-CH_2 at i, j) solvent peaks at 2.49 and 3.31.

UV-λmax (nm): 281

Mass spectra: Molecular weight=394

Antioxidant activity results:

The derivatives prepared were evaluated for antioxidant activity by three methods like DPPH assay, Nitric oxide scavenging assay and Hydrogen peroxide scavenging assay. The compounds were tested in five different concentrations.

Table 2. DPPH assay results

Sample	% Scavenging Activity At Different Concentrations					**IC_{50}**
	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	
5a	39.94±0.521	59.14±0.652	61.38±0.631	63.59±0.245	65.34±0.534	29.7
5b	46.63±0.342	49.7±0.352	57.51±0.421	60.51±0.634	62.65±0.453	43.3
5c	44.86±0.245	62.22±0.214	64.66±0.341	65.82±0.372	67.76±0.215	**26.7**
5d	44.64±0.234	53.89±0.123	62.73±0.223	64.02±0.321	66.92±0.431	27.1
5e	47.34±0.235	48.16±0.516	49.54±0.461	52.98±0.371	55.75±0.297	61.3
Standard	49.38±0.515	67.03±0.541	75.78±0.223	91.92±0.561	95.34±0.111	**21.3**

Standard drug used is ascorbic acid. IC_{50} values in µg/ml for samples were determined using ED50 plus V 1.0 software. Data are the mean of three or more experiments and reported as mean ± standard error of the mean(SEM).

Table 3. Nitric oxide scavenging assay results:

Sample	% Scavenging Activity At Different Concentrations					**IC_{50}**
	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	
5a	34.83±0.527	40.63±0.654	43.87±0.691	52.15±0.215	53.11±0.514	72.1
5b	27.34±0.372	29.81±0.352	38.25±0.421	42.55±0.639	50.54±0.450	98.3
5c	33.57±0.243	44.97±0.211	48.69±0.348	52.35±0.442	53.15±0.218	**66.2**
5d	33.28±0.232	44.40±0.128	45.70±0.224	52.01±0.331	54.29±0.481	69.8
5e	26.67±0.295	29.30±0.506	44.95±0.411	51.98±0.381	52.07±0.297	70.6
Standard	47.53±0.624	63.44±0.521	84.28±0.623	90.53±0.411	93.56±0.221	**25.2**

Standard drug used is ascorbic acid. IC_{50} values in µg/ml for samples were determined using ED50 plus V 1.0 software. Data are the mean of three or more experiments and reported as mean ± standard error of the mean(SEM).

Table 4. Hydrogen peroxide radical scavenging assay results:

Sample	% Scavenging Activity At Different Concentrations					**IC_{50}**
	20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	
5a	35.75±0.612	44.97±0.237	55.19±0.226	65.93±0.662	67.14±0.653	47.1
5b	34.01±0.563	43.51±0.464	58.83±0.152	60.48±0.353	62.50±0.452	49.1
5c	34.24±0.263	46.06±0.533	58.82±0.623	62.12±0.621	63.63±0.236	**43.3**
5d	33.93±0.235	46.81±0.516	56.52±0.532	59.89±0.623	61.39±0.425	45.6
5e	34.48±0.342	44.88±0.345	55.57±0.173	56.61±0.535	58.63±0.654	50.6
Standard	44.53±0.526	64.65±0.653	71.47±0.36	89.22±0.621	96.19±0.456	**26.9**

Standard drug used is ascorbic acid. IC_{50}values in µg/ml for samples were determined using ED50 plus V 1.0 software.Data are the mean of three or more experiments and reported as mean ± standard error of the mean(SEM)

Conclusion

The physical and spectral analysis of the mannich derivatives were carried out. In vitro antioxidant activity was carried out by three methods. In DPPH assay compound 5c with IC_{50} value of 26.7µg/ml showed significant activity when compared to ascorbic acid with IC_{50} 21.3µg/ml. In Nitric oxide and Hydrogen peroxide scavenging methods compound 5c showed maximum activity when compared to other derivatives but was not significant to the result obtained in DPPH method. It is well established that organic molecules incorporating an electron donating group can act as free radical trapping agents and are capable of opposing oxidative challenges. Compound 5c showed maximum antioxidant activity in all the three methods. This may be due to the presence of chloro substitution at 4' position of phenyl ring and the diethyl group attached to amine. Both chloro and diethyl group are electron releasing which are important in radical scavenging activity.

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