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Synthesis, characterization and *in-vitro* anti-oxidant activity of some novel 1, 3,4-thiadiazole derivatives

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ABSTRACT

*In the present study, we have synthesized 1, 3, 4-thiadiazole derivatives because of their diverse biological and clinical applications. This created interest in researchers who have synthesized variety of thiadiazole derivatives and screened them for their various biological activities viz. in vitro antioxidant, anticancer, anti-HIV, anthelmintic, antimycobacterial, and anti-inflammatory, antidiabetic, antimicrobial, trypanocidal as well antimalarial activities. The results revealed that, some of tested compounds showed potent antioxidant activity. Amongst all the compound **IIIA2** and **IIIA4** have shown good scavenging activity. The remaining tested compounds were found weakly active.*

Keywords: Synthesis; 1, 3, 4-Thiadiazole; Antioxidant activity, DPPH scavenging, Nitric oxide radical.

INTRODUCTION

Heterocyclic Compounds are the cyclic compounds having as ring members atoms of at least two different elements, e.g. quinolone, 1, 2-thiazole, bicycle [3.3.1] tetrasiloxane [1] .Usually they are indicated as counterparts of carbocyclic compounds, which have only ring atoms from the same element.The literature review showed that the thiadiazole nuclei have various biological activities like antioxidant [2], anticancer [3], antimicrobial [4], anti-inflammatory [5],antifungal [6] and antidepressant [7] activities. Numerous 1, 3, 4-thiadiazoles have been synthesized and reported to be biologically versatile compounds having bactericidal, fungicidal, muscle relaxant properties [8]. In the view of the facts mentioned above and as part of our initial efforts to discover potentially active new agents. Hence, we have screened some reported [9] 1, 3, 4-thiadiazole derivatives for their antioxidant activity.

MATERIALS AND METHODS

2.1. Synthesis

Melting points of all synthesized compounds were determined by open capillary tube method and were uncorrected. Purity of all synthesized compounds was checked by thin layer chromatography technique (0.2 mm thickness of silica gel GF plates) and iodine was used as visualizing agent. IR spectra were recorded on THERMO NICOLET iS10 FT-IR spectrometer using KBr disc method. Elemental analysis was performed using a Euro EA Elemental Analyser. Spectral and Elemental analysis was carried out at Central Analytical Instrument Facility (CAIF), Guwahati biotech park, spectra were recorded on 400-MHz BRUKER spectrometer in dimethylsulfoxide-d_6 as solvent and tetramethylsilane (TMS) as internal standard and chemical shift was expressed in δ or ppm, the coupling constants were given in Hz and analysis was carried out at Andhra University, Department Physics, Visakhapatnam.

2.1.1. Synthesis of 5-(2-hydroxyphenyl)-2-amino-[1, 3, 4]-thiadiazole 1(a, b, c) [10 , 11]:
A mixture of thiosemicarbazide (0.1mole), aryl carboxylic acid (0.1mole) and conc. Sulphuric acid (5ml) in 50 ml of ethanol was refluxed for 2-3hour.Reaction was monitored by TLC using mobile phase Chloroform: methanol (4:1). After completion of the reaction the reaction mixture was poured on to crushed ice.The solid separated out was filtered, washed with cold water and recrystallized from ethanol to give colourless crystals, Yield 90%, m.p.183-186^{o}C.

IR (KBr) *ν*/(cm^{-1}): 3514.22 (O-H, st.), 663.92, 688.65 (C-S-C, st.), 3428.26 (NH_2,N-H,st.), 1616.23 (C=N, st.), 1425.76 (Aryl C=C, st.), 3018.75(Aryl C-H, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 6.89-7.20(m, 4H, ArH), 10.32 (s, 1H, OH), 2.60-2.65 (bs, 2H, NH_2), Mass spectrum m/z: 193 (M^+).

2.1.2. Synthesis of 5-(3-chlorophenyl) -2-amino - [1, 3, 4]-thiadiazole (1b):
Yield 79%, m.p.165-167^0 C ; IR (KBr) *ν*/(cm^{-1}): 762.35 (C-Cl, st.), 682.35 (C-S-C, st.), 3447.21 (NH_2, N-H,st.), 1647.11 (C=N, st.),1491.70(C-N, st.), 1425.79 (Aryl C=C, st.),3025.25(Aryl C-H, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 6.95-7.35 (m, 4H, ArH), 2.48 (bs, 2H, NH_2), Mas spectrum m/z :211(M^+).

2.1.3. Synthesis of 5-(4-nitrophenyl) -2-amino - [1, 3, 4]-thiadiazole (1c).
Yield 88%, m.p.225-227^{0}C ; IR (KBr) *ν*/(cm^{-1}): 1375.53 , 1545.11, (NO_2, st.), 687.73 (C-S-C, st.), 34.50.78 (NH_2, N-H,st.), 1649.95 (C=N, st.), 1416.45 (Aryl C=C, st.),3087.72(Aryl C-H, st.); ^{1}H NMR (400MHz, DMSO-*d*6) δ 7.30-7.73(m, 4H, ArH), 2.59 (bs, 2H, NH_2), Mass spectrum m/z: 222 (M^+).

2.1.4. 2-chloro-*N*-substituted-phenyl-acetamide (II) [12]:
Aromatic amines (0.05mol) were dissolved in glacial acetic acid (25 ml) containing (25 ml) of saturated solution of sodium acetate. In case if the substance did not dissolve completely, the mixture was warmed and then the solution was cooled in ice-bath with stirring. To this chloroacetyl chloride (0.06mol) was added drop wise to avoid the vigorous reaction. After half an hour a white coloured product was separated and filtered. The product was washed with 50% aqueous acetic acid and finally with water. It was recrystallized from aqueous alcohol, m. p 128 ^{0}C, yield 85%.

2.1.5. *N*-(substuted-phenyl)-2-[5-(3-substituted-phenyl)-1, 3, 4-thiadiazol-2-yl amino]-acetamide (III A1-A12):
5-(2-hydroxyphenyl) -2-amino-[1, 3, 4]-thiadiazole **1**(**a, b, c**) (0.05mol) and 2-chloro-*N*-substituted-phenyl-acetamide **(II)** (0.05 mol) were mixed in 15 ml of 1,4-dioxane.To this (0.005 ml) of triethylamine (TEA) solution was added and the reaction mixture was refluxed for 3h. It was then cooled and poured into crushed ice. The solid separate out and filtered it. The filtered was washed with 10% K_2CO_3 and water.

2.1.6. Synthesis of 2-Chloro-*N*-[5-(Substituted-phenyl) - [1, 3, 4]-thiadiazol-2-yl]-acetamide (IV d, e, f) [13 , 14]:
To the mixture of appropriately substituted compound **I (a, b, c)** (10 mmol) in dry benzene (15ml) and 2 ml of dry pyridine, was cooled to 0-5^{0}C. Chloro-acetyl chloride (20 mmole) dissolved in dry benzene (10 ml) was added drop wise to the solution with constant stirring at room temperature. After complete addition, the reaction mixture was refluxed for about 6-8h. Benzene was removed *in vacuo*. The residue was poured over crushed ice. The precipitate was filtered, washed with water. The crude product was dried and crystallized from 1,4-dioxane to yield compound **(IV d, e, f)**; the purity of compounds was analyzed by TLC using benzene: acetone (9:1) as mobile phase. Yield 64.4%, m. p 210 - 212^{0}C.

2.1.7. 2-(Substituted-amino)-*N*-[5-(Substituted-phenyl)-1, 3, 4-thiadiazol-2-yl]acetamide (V A13-A21):
The compound **IV (d, e, f)** 2-Chloro-*N*-[5-(Substituted-phenyl) - [1, 3, 4]-thiadiazol-2-yl]-acetamide (0.01 mol) was taken in about 25 ml of dry alcohol and 0.01 mol of thiourea /hydrazine hydrate / piperidine was added to it and the mixture was heated on water bath for 9 h. The content was cooled under tap water, filter, dried and recrystalized from alcohol. Purity of the compounds was analyzed by petroleum ether: acetone (9:1) as mobile phase.
The structures of synthesized compounds under investigation were supported by the Physical parameter, ^{1}H-NMR FTIR and MASS spectral measurement. The Physical parameter, ^{1}H-NMR, FTIR and Mass spectral data of the synthesized compounds spectra were recorded and assigned in Table 1, 2, 3.

2.2. Antioxidant Screening*: (In-Vitro):*
2.2.1. DPPH radical scavenging activity:
The nitrogen centered stable free radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) has often been used to characterize antioxidants. It is reversibly reduced and the odd electron in the DPPH free radical gives a strong absorption maximum at λ517 nm using SPECORD® 50 plus (analytic jena) spectrophotometer, which is purple in color. This property makes it suitable for spectrophotometer studies. A radical scavenging antioxidant reacts with DPPH stable free radical and converts it into 1, 1-diphenyl-2-picrylhydrazine. The resulting decolorization is stoichiometric with respect to the number of electrons captured. The change in the absorbance produced in this reaction has been used to measure antioxidant properties.

The hydrogen atom or electron donation ability of the compounds was measured from the bleaching of the purple colored methanol solution of DPPH radical. The spectrophotometric assay uses the stable radical DPPH as a reagent. To 4 ml of 0.004% (w/v) methanol solution of DPPH, 1 ml of various concentrations of the test compounds (4, 8, 10 μg/ml) in methanol were added. After a 30 min incubation period at room temperature, the absorbance was read against blank at λ 517 nm [15]. Ascorbic acid was used as the standard. The percent of inhibition (*I* %) of free radical production from DPPH was calculated by the following equation

$$I\% = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the control reaction (containing methanolic DPPH and ascorbic acid), A_{sample} is the absorbance of the test compound (containing methanolic DPPH and test compound). Tests were carried out in triplicate. The results were assigned in Table 4,Fig.1.

2.2.2. Nitric oxide scavenging activity:
The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 mL), phosphate buffer saline (pH 7.4, 1 ml) and test samples or standard, ascorbic acid solution in dimethyl sulphoxide (1 mL) at various concentrations (4, 8, 10 µg/ ml) was incubated at 25^0C for 150 min. After incubation, 0.5 mL of reaction mixture containing nitrite ion was removed, 1 ml of sulphanillic acid reagent was added to this, mixed well and allowed to stand for 5 min for completion of diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min in diffused light. A pink colored chromophore was formed. The absorbance was measured at λ 640 nm [16] using SPECORD® 50 plus (analytic jena) spectrophotometer. Ascorbic acid was used as standard. NO scavenging activity was calculated by the following equation

$$\text{\% of scavenging} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

where $A_{control}$ is the absorbance of the control reaction (containing all reagents and Ascorbic acid), A_{sample} is the absorbance of the test compound (containing all reagents and test compound). Tests were carried out in triplicate. The results were assigned in Table 5, Fig.2.

RESULTS AND DISCUSSION

3.1. Chemistry:
Treatment of aryl carboxylic acid in absolute ethanol with thiosemicarbazide afforded the corresponding 2-amino-5(substituted phenyl)-1, 3, 4-thiadiazole **I** (a, b and c). Molecular formula of the compounds (Table 1) derived from elemental analyses data are supported by their molecular weight.

The IR spectrum of **1a** showed characteristic absorption bands at 3428 cm^{-1} characteristic due to NH_2 functions in addition to the -OH absorption band at 3514 cm^{-1}, C-S-C absorption band at 688 cm^{-1}. Its ^{1}H NMR spectrum revealed the characteristic signal at δ 10.32 assigned to OH protons, two characteristic signals at δ 2.60 and 2.65 assigned to NH_2 protons which is exchangeable with D_2O, confirming the formation of thiadiazole. Also, its mass spectrum showed the molecular ion peak at *m/z* 193 [M^+] and the base peak at *m/z* 94.

The IR spectrum of **1b** showed characteristic absorption bands at 3447 cm^{-1} characteristic which is due to NH_2 functions in addition to the C-Cl absorption band at 762 cm^{-1}, C-S-C absorption band at 682 cm^{-1} and C=C (aromatic)absorption band at 1425 cm^{-1}. Its ^{1}H NMR spectrum revealed the characteristic signal at δ 2.48 assigned to

NH_2 protons which is exchangeable with D_2O, confirming the formation of thiadiazole. The mass spectrum showed the molecular ion peak at *m/z* 211 [M^+] and the base peak at *m/z* 42.

Table 1: Physical data of the synthesized compounds

Compd No.	Mol. Formula	Mol. Wt	m.p. ^0C	% Yield	Rf	Calculated			Found		
						C	H	N	C	H	N
1a	$C_8H_7N_3OS$	193.22	185-186	90	0.71	49.73	3.65	21.75	49.63	3.85	21.71
1b	$C_8H_6ClN_3S$	211.67	165-167	79	0.72	45.39	2.86	19.85	45.12	2.67	19.80
1c	$C_8H_6N_4O_2S$	222.22	225-227	88	0.70	43.24	2.72	25.21	43.20	2.67	25.20
IVd	$C_{10}H_8ClN_3O_2S$	269.70	210-212	61	0.78	44.53	2.99	15.58	44.55	3.09	15.51
IVe	$C_{10}H_7Cl_2N_3OS$	288.15	190-193	67	0.69	41.68	2.45	14.58	40.98	2.45	14.58
IVf	$C_{10}H_7ClN_4O_3S$	298.70	181-183	72	0.73	40.21	2.36	18.76	39.91	2.40	19.01
IIIA1	$C_{16}H_{14}N_4O_2S$	326.37	141-143	57	0.72	58.88	4.32	17.17	59.08	4.42	17.25
IIIA2	$C_{16}H_{15}N_5O_2S$	341.38	154-156	83	0.79	56.29	4.43	20.51	56.35	4.40	20.65
IIIA3	$C_{22}H_{18}N_4O_2S$	402.46	187-189	65	0.81	65.65	4.51	13.92	65.05	4.59	12.92
IIIA4	$C_{16}H_{13}N_7O_6S$	431.38	180-182	91	0.81	44.55	3.04	22.73	43.95	3.12	22.88
IIIA5	$C_{16}H_{13}ClN_4OS$	344.81	188-190	54	0.86	55.73	3.80	16.25	55.87	3.87	16.23
IIIA6	$C_{16}H_{14}ClN_5OS$	359.83	197-199	84	0.76	53.41	3.92	19.46	53.41	3.92	19.46
IIIA7	$C_{22}H_{17}ClN_4OS$	420.91	207-209	71	0.79	62.78	4.07	13.31	62.67	4.17	13.13
IIIA8	$C_{16}H_{12}ClN_7O_5S$	449.82	221-224	49	0.78	42.72	2.69	21.80	42.70	2.81	21.98
IIIA9	$C_{16}H_{13}N_5O_3S$	355.37	201-203	66	0.82	54.08	3.69	19.71	53.78	3.79	19.43
IIIA10	$C_{16}H_{14}N_6O_3S$	370.38	210-212	70	0.87	51.88	3.81	22.69	51.18	3.76	22.54
IIIA11	$C_{22}H_{17}N_5O_3S$	431.46	225-227	82	0.82	51.88	3.81	22.69	51.88	4.00	22.57
IIIA12	$C_{16}H_{12}N_8O_7S$	460.38	235-237	93	0.81	41.74	2.63	24.34	41.65	2.60	24.24
VA13	$C_{11}H_{11}N_5O_2S_2$	309.36	169-172	45	0.76	42.71	3.58	22.64	42.45	3.50	22.81
VA14	$C_{15}H_{18}N_4O_2S$	318.39	173-175	52	0.75	56.58	5.70	17.60	57.08	5.60	17.76
VA15	$C_{10}H_{11}N_5O_2S$	265.29	145-147	47	0.79	45.27	4.18	26.40	45.20	4.10	25.89
VA16	$C_{11}H_{10}ClN_5OS_2$	327.81	198-200	40	0.78	40.30	3.07	21.36	40.30	3.07	21.36
VA17	$C_{15}H_{17}ClN_4OS$	336.83	135-138	65	0.81	53.49	5.09	16.63	53.59	5.20	15.93
VA18	$C_{10}H_{10}ClN_5OS$	283.73	149-152	79	0.72	42.33	3.55	24.68	42.63	3.78	24.99
VA19	$C_{11}H_{10}N_6O_3S_2$	338.36	215-217	48	0.73	39.05	2.98	24.84	39.05	2.98	24.84
VA20	$C_{15}H_{17}N_5O_3S$	347.39	220-222	57	0.77	51.86	4.93	20.16	51.96	4.63	20.06
VA21	$C_{10}H_{10}N_6O_3S$	294.28	228-230	43	0.72	40.81	3.42	28.56	40.81	3.42	28.56

The IR spectrum of **1c** has exhibited characteristic absorption bands at 3450 , 682 and 1416 cm^{-1} due to NH_2 , C-S-C and C=C (aromatic) functions respectively .Two characteristic absorption band at 1375, 1545 cm^{-1} which are due to NO_2 function. It was also showed proton signals at: δ 2.59 (NH_2) and δ 7.30-7.73 (Ar-H), respectively. Mass spectrum **(1c)** of the compound exhibited its molecular ion (M+) at m/z 222 and the base peak at *m/z* 206.

For yielding the compound **II** (2-substituted-N-substituted-phenyl-acetamide) by stirring the aromatic amines with chloroacetyl chloride in the solution of glacial acetic acid and saturated solution of sodium acetate.

Compound **1 (a, b, c)** was refluxed for 3h with **II** in TEA and 1, 4-dioxan, yielding **III (A1-A12).**

The structures of the **IIIA1** was confirmed by the appearance of -OH, C=O, NH (aromatic), C-H (CH_2), C=C (aromatic) and C-S-C absorption bands at 3517, 1654, 3439, 2859, 1442 and 659 respectively. ^{1}HNMR spectrum of its showed proton signals at: δ 10.13 (OH), 4.69 (NH), 4.19 (CH_2), 6.88-7.89 (Ar-H), respectively. Mass spectrum of the compound exhibited its molecular ion (M+) at m/z 326 and the base peak at *m/z* 121.

The IR spectrum of **IIIA2** exhibited characteristic absorption bands at 3517 cm^{-1} which is due to OH functions in addition to the C=O absorption band at 1669 $cm^{-1}$and C-H (CH_2) absorption band at 3125 cm^{-1}. Its ^{1}H NMR spectrum revealed the characteristic signal at δ 10.42 assigned to OH protons, δ 3.23 for CH_2 protons and δ 6.89-7.79 assigned to aromatic protons respectively. The results of its mass spectrum showed the molecular ion peak at *m/z* 341 [M^+] and the base peak at *m/z* 142.

The structures of the products **IIIA3** and **III4** were confirmed by the appearance of -OH, C=O and C-H (CH_2) bands at 3525, 1670, 2540 cm^{-1} and 3515, 1708, 3110 stretching vibrations , respectively. Further the compound **IIIA4** was confirmed by the appearance of two characteristic absorption bands at 1307 and 1507 cm^{-1} due to NO_2 function. The ^{1}H NMR spectrum revealed the characteristic signal at δ 9.98, 10.26 assigned to OH proton respectively and δ 3.23, 3.57 assigned to CH_2 protons respectively. The mass spectrum showed the molecular ion peak at *m/z* 402 [M^+] and 431 [M^+] respectively along with the base peak at *m/z* 168 and 58.

Table 2: ^{1}H- NMR, FT-IR and MASS data of compounds (IIIA1-IIIA12)

Compd.No	^{1}H-NMR	IR (KBr) *ν*/(cm^{-1})	Mass(m/z)
IIIA1	^{1}H NMR (400MHz, DMSO-*d6*) δ 6.88-6.94 (m, 4H, ArH); 7.57-7.89 (m, 5H, ArH); 10.13 (s, 1H, OH); 4.19 (d, 2H, CH_2); 4.69 (t, 1H, aro. C-NH); 8.95 (s, 1H, CONH)	3517.52 (O-H, st.), 659.11, 697.43 (C-S-C, st.), 3439.40 (N-H, st.), 1612.58 (C=N, st.), 1484.09 (C-N, st.), 1654.84 (C=O,st.), 2859.23 (CH_2, C-H, st.), 3237.97(CON-H, st.) 1442.99 (Aryl C=C, st.), 3020.93 (Aryl C-H, st.)	**326(M^+)**
IIIA2	^{1}H NMR (400MHz, DMSO-*d6*) δ 6.89-7.52 (m, 4H, ArH); 7.76-7.79 (m, 5H, ArH); 10.42 (s, 1H, OH); 3.23 (d, 2H, CH_2); 4.29 (d, 1H, aro. C-NH); 8.58 (d, 1H, CONH); 3.83 (t, 1H, aro. C-NH)	3517.87 (O-H, st.), 659.14, 698.01 (C-S-C, st.), 3449.27 (N-H, st.), 1641.12 (C=N, st.), 1484.69 (C-N, st.), 1669.79 (C=O, st.), 3125.50 (CH_2, C-H, st.), 3234.81 (CON-H, st.), 1443.38(Aryl C=C,st.), 2973.65, 3010.85 (Aryl C-H, st.)	**341(M^+)**
IIIA3	^{1}H NMR (400MHz, DMSO-*d6*) δ 6.76-6.82 (m, 5H, ArH); 7.05-7.26 (m, 10H, ArH); 9.98 (s, 1H, OH); 3.57 (d, 2H, CH_2); 4.50 (t, 1H, aro. C-NH)	3525.40 (O-H, st.), 689.76,700 (C-S-C, st.), 3489.40 (N-H, st.), 1595.68 (C=N, st.), 1494.27 (C-N, st.), 1670.80 (C=O, st.), 2540.58 (CH_2, C-H, st.), 1457.87 (Aryl C=C, st.), 3040.77 (Aryl C-H, st.)	**402(M^+)**
IIIA4	^{1}H NMR (400MHz, DMSO-*d6*) δ 7.05-7.58 (m, 4H, ArH); 7.91-8.31 (m, 3H, ArH); 10.26 (s, 1H, OH); 3.55 (d, 2H, CH_2); 4.20 (d, 1H, aro. C-NH); 9.04 (d, 1H, CONH); 4.23 (t, 1H, aro. C-NH)	3515.27 (O-H, st.), 636.99 (C-S-C, st.), 3311.91 (N-H, st.), 1589.16 (C=N, st.), 1708.82 (C=O, st.), 3110.01 (CH_2, C-H, st.), 3230.73 (CON-H, st.) 1423.31 (Aryl C=C, st.), 3004.61(Aryl C-H, st.), 1307.61,1507.92 (NO_2)	**431(M^+)**
IIIA5	^{1}H NMR (400MHz, DMSO-*d6*) δ 6.90-7.48 (m, 4H, ArH); 7.49-7.52 (m, 5H, ArH); 4.23 (d, 2H, CH_2); 5.99 (t, 1H, aro. C-NH); 8.02 (s, 1H, aro.C-NH)	758.11 (C-Cl, st.), 650.18, 682.98 (C-S-C, st.), 3426.28 (N-H, st.), 491.93 (C=N, st.), 1700.62 (C=O, st.), 2868.17 (CH_2, C-H, st.), 3162.19 (CON-H, st.) 1418.12 (Aryl C=C, st.), 3094.00(Aryl C-H, st.),	**344(M^+)**
IIIA6	^{1}H NMR (400MHz, DMSO-*d6*) δ 7.53-7.56 (m, 4H, ArH); 7.91-8.16 (m, 5H, ArH); 3.64 (d, 2H, CH_2); 4.30 (t, 1H, aro.C-NH);9.18(d,1H, CONH); 4.29 (d, 1H, aro. C-NH)	762.11 (C-Cl, st.), 681.95 (C-S-C, st.), 3455.46 (N-H, st.), 1685.89 (C=O, st.), 2918.15 (CH_2, C-H, st.), 3128.79 (CON-H, st.) 1424.75, 1491.75 (Aryl C=C, st.),3082.11 (Aryl C-H, st.),	**359(M^+)**
IIIA7	^{1}H NMR (400MHz, DMSO-*d6*) δ 7.05-7.34 (m, 4H, ArH); 7.36-7.95 (m, 10H, ArH); 4.18 (d, 2H, CH_2); 5.57 (t, 1H, aro.C-NH)	761.98, 783.18 (C-Cl, st.), 682.69, 700.73 (C-S-C, st.), 3350.55 (N-H, st.), 1491.65(C-N,st.), 1681.86 (C=O, st.), 2945.49 (CH_2, C-H, st.), 1422.96 (Aryl C=C, st.).	**420(M^+)**
IIIA8	^{1}H NMR (400MHz, DMSO-*d6*) δ 7.53-7.93 (m, 4H, ArH); 8.30-8.33 (m, 3H, ArH); 3.39 (d, 2H, CH_2); 4.31 (d, 1H, aro.C-NH); 8.84 (d, 1H, CONH); 4.27 (s, 1H, aro.C-NH)	762.77 (C-Cl, st.), 628.90 , 682.20 (C-S-C, st.), 3321.12 (N-H,st.), 1492.15 (C-N, st.), 1696.96 (C=O, st.), 2995.25 (CH_2, C-H, st.), 1424.61(Aryl C=C, st.), 3094.06 (Aryl C-H, st.),1519.45(NO_2)	**449(M^+)**
IIIA9	^{1}H NMR (400MHz, DMSO-*d6*) δ 7.67-7.82 (m, 5H, ArH); 7.98-8.05 (m, 4H, ArH); 3.91 (d, 2H, CH_2); 4.81 (t, 1H, aro.C-NH); 9.13 (s, 1H, CONH)	1392.76, 1527.53 (NO_2), 697.90 (C-S-C, st.), 3440.22 (N-H, st.), 1631.34 (C=N, st.),1492.84 (C-N, st.), 1700.31 (C=O, st.), 3004.19 (CH_2, C-H, st.), 3200.12 (CO N-H) 1438.88 (Aryl C=C, st.), 2939.27 (Aryl C-H, st.).	**354(M^+-1)$^+$**
IIIA10	^{1}H NMR (400MHz, DMSO-*d6*) δ 6.90-7.43 (m, 5H, ArH);7.54-8.38 (m, 4H, ArH); 3.76 (d, 2H, CH_2); 4.93 (t, 1H, aro.C-NH); 8.44 (d, 1H, CONH); 4.95 (d, 1H, aro.C-NH)	1367.23, 1516.18 (NO_2), 680.81 (C-S-C, st.), 3350.42(N-H,st.), 1603.09 (C=N, st.),1477.08 (C-N, st.), 1649.03 (C=O, st.), 2925.46 (CH_2, C-H, st.), 3162.68 (CO N-H) 1403.38 (Aryl C=C, st.), 3022.23 (Aryl C-H, st.).	**370(M^+)**
IIIA11	^{1}H NMR (400MHz, DMSO-*d6*) δ 6.81-7.74 (m, 10H, ArH); 7.98-8.64 (m, 4H, ArH);3.94 (d, 2H, CH_2); 4.92 (t, 1H, aro.C-NH)	1310.04, 1338.05, 1365.98 (NO_2), 689.93 (C-S-C, st.), 3383.38, 3423.43(N-H,st.), 1602.18 (C=N, st.),1472.61 (C-N, st.), 1633.25,1731.60 (C=O, st.), 2975.03 (CH_2, C-H, st.), 3162.68 (CO N-H) 1438.84 (Aryl C=C, st.), 3026.22 (Aryl C-H, st.).	**431(M^+)**
IIIA12	^{1}H NMR (400MHz, DMSO-*d6*) δ 7.59-8.09 (m, 5H, ArH); 8.17-9.06 (m, 4H, ArH); 3.55 (d, 2H, CH_2); 3.91 (s, 1H, aro.C-NH); 9.29 (d, 1H, CONH); 4.34 (d, 1H, aro. C-NH)	1392.96, 1523.65 (NO_2), 670.05, 695.61 (C-S-C, st.), 3424.31(N-H, st.), 1624.38 (C=N, st.), 1731.62 (C=O, st.), **2979.91 (CH_2, C-H, st.)**, 3286.61 (CO N-H) 1427.22 (Aryl C=C, st.),	**460(M^+)**

Compounds **IIIA5**, **IIIA6**, **IIIA7** and **IIIA8** were showed the characteristic absorption band at 758, 762, 761 and 762 cm^{-1} respectively for C-Cl function, 3426, 3455, 3350 and 3321 stretching vibrations, respectively for NH group and at 2868, 2978, 2945 and 2995 cm^{-1} due to C-H (CH_2) stretching vibrations, respectively. Also ^{1}HNMR spectrum exhibited signal at δ 5.99, 4.30, 5.57 and 4.31, respectively, for NH proton. The signals were appears at δ 4.23, 3.64, 4.18 and 3.39 assigned to CH_2 protons. While the mass spectrum were showed the molecular ion peak at *m/z* 344 [M^+], 431 [M^+] , 359 [M^+] , 420 [M^+] and 449 [M^+] respectively and the base peak at *m/z* 134, 93, 197 and 255 respectively.

The IR spectrum of Compounds **IIIA9**, **IIIA10**, **IIIA11** and **IIIA12** were exhibited the characteristics absorption band at 3440, 3350, 3423 and 3424 cm^{-1} for NH stretching vibrations, respectively. Further it was found to be the absorption band at1700, 1649, 1731and 1731 cm^{-1} for C=O) stretching vibrations, respectively. The ^{1}H NMR spectrum revealed the characteristic signal at δ 4.81, 4.93, 4.92 and 4.34, respectively, for NH proton. Also the compounds were showed the presence of methylene (CH_2) group protons appeared at δ 3.91, 3.76, 3.94 and 3.55, respectively. The mass spectrum of all the above compounds exhibited the molecular ion peak at *m/z* 354[M -1] $^{+}$, 370[M^+], 431[M^+] and 460[M^+], respectively, and the base peak at *m/z* 142, 136, 221 and 183, respectively, corresponding to the molecular formula $C_{16}H_{13}N_5O_3S$, $C_{16}H_{14}N_6O_3S$, $C_{22}H_{17}N_5O_3S$ and $C_{16}H_{12}N_8O_7S$.

Table 3: ^{1}H- NMR, FT-IR and MASS data of compounds (VA13-VA21)

Compd.No	1H-NMR	IR (KBr) *v*/(cm^{-1})	Mass (m/z)
VA13	^{1}H NMR (400MHz, DMSO-*d6*) δ 6.90-7.52 (m, 4H, ArH); 10.12 (s, 1H, OH); 3.45 (d, 2H, CH_2); 6.16 (t, 1H, CH_2-NH); 9.22 (s, 1H, CONH); 2.87, 2.90 (d, 2H, NH_2)	3559.56 (O-H), 688.51 (C-S-C, st.), 3411.10 (N-H, st.), 1689.70 (C=O, st.), 3119.59 (CH_2, C-H, st.), 1122.86 (C=S) 1426.53 (Aryl C=C, st.), 3080.72 (Aryl C-H, st.), 3490.53(NH_2, st.)	**309(M^+)**
VA14	^{1}HNMR (400MHz, DMSO-*d6*) δ 6.88-7.51(m, 4H, ArH);10.15 (s, 1H, OH); 3.12 (s, 2H, CH_2); 8.69 (s,1H,CONH); 1.51-1.69 (m, 6H, piperidine), 2.60, 2.71 (t, 4H, piperidine)	3518.34 (O-H), 697.28, 659.15 (C-S-C, st.), 3237.91 (N-H, st.), 1655.19 (C=O, st.), 3040.95 (CH_2, C-H, st.), 1612.63 (C=N), 1324.90 (C-N, st. piperidine), 2860.33 (CH_2, C-H, st. piperidine)1445.93 (Aryl C=C, st.), 2998.01 (Aryl C-H, st.).	**318(M^+)**
VA15	^{1}H NMR (400MHz, DMSO-*d6*) δ 6.28-7.94(m, 4H, ArH); 9.64 (s, 1H, OH); 3.91 (d, 2H, CH_2); 2.54 (m, 1H, CH_2-NH); 8.85 (s, 1H, CONH); 2.45 (d, 2H, NH_2)	3521.96 (O-H), 680.47 (C-S-C, st.), 3420.28 (N-H, st.), 1640.57 (C=O, st.), 3088.23 (CH_2, C-H, st.), 1621.31 (C=N), 1464.43(C-N), 1449.63 (Aryl C=C, st.), 3014.04 (Aryl C-H, st.), 3453.36(NH_2, st.).	**265(M^+)**
VA16	^{1}H NMR (400MHz, DMSO-*d6*) δ 7.45-7.94(m, 4H, ArH); 3.55 (d, 2H, CH_2); 2.81 (m, 1H, CH_2-NH); 8.83 (s, 1H, CONH); 2. 60 (s, 2H, NH_2)	762.38 (C-Cl), 682.01 (C-S-C, st.), 3410.65 (N-H, st.), 1681.41 (C=O, st.), 2991.53 (CH_2, C-H, st.), 1591.77 (C=N), 1491.66 (C-N), 1128.66, 1175.69 (C=S), 1424.68 (Aryl C=C, st.), 2836.65 (Aryl C-H, st.), 3289.64 (NH_2, st.).	**328(M^++1)$^+$**
VA17	^{1}HNMR (400MHz, DMSO-*d6*) δ 7.33-7.43 (m, 4H, ArH); 3.72 (s, 2H, CH_2); 9.31 (s, 1H, CONH); 1.78-1.98 (m, 6H, piperidine), 2.57-2.69 (t, 4H, piperidine)	762.40 (C-Cl), 650.06, 681.85 (C-S-C, st.), 3410.63 (N-H, st.), 1655.19, 1700.08 (C=O, st.), 3083.19 (CH_2, C-H, st.), 1593.13 (C=N), 1491.75(C-N), 1423.33 (Aryl C=C, st.), 2991.53 (Aryl C-H, st.).	**336(M^+)**
VA18	^{1}H NMR (400MHz, DMSO-*d6*) δ 7.33-8.33 (m, 4H, ArH); 4.27 (d, 2H, CH_2); 2.49 (m, 1H,CH_2NH); 8.85 (s,1H,CONH); 1.99 (d, 2H, NH_2)	703.34 (C-Cl), 653.01 (C-S-C, st.), 3310.44 (N-H, st.), 1688.21 (C=O, st.), 3104.36 (CH_2, C-H, st.), 1624.25 (C=N), 1443.31 (Aryl C=C, st.), 3091.58 (Aryl C-H, st.).	**282(M^+-1)$^+$**
VA19	^{1}H NMR (400MHz, DMSO-*d6*) δ 7.01-8.45 (m, 4H, ArH); 3.55(d, 2H, CH_2); 2.49 (t, 1H, CH_2-NH); 8.81 (s, 1H, CONH); 1. 98 (d, 2H, NH_2)	1345.95,1392.86 (NO_2), 670.11 (C-S-C, st.), 3340.70 (N-H, st.), 1632.11,1731.44 (C=O, st.), 2770.99 (CH_2, C-H, st.), 1650.21 (C=N), 1345.95, 1392.86 (C-N), 1202.56, 1077.95, 1054.39 (C=S), 1441.47 (Aryl C=C, st.), 3039.76 (Aryl C-H, st.), 3400.35 (NH_2, st.).	**338(M^+)**
VA20	^{1}HNMR (400MHz, DMSO-*d6*) δ 7.76-8.15 (m, 4H, ArH); 3.31 (s, 2H, CH_2); 8.95 (s, 1H, CONH); 1.59-1.63 (m, 6H, piperidine), 2.51-2.62 (t, 4H, piperidine)	1398.11, 1515.18 (NO_2), 681.85 (C-S-C, st.), 1725.98 (C=O, st.), 3116.52 (CH_2, C-H, st.), 1653.50 (C=N), 1490.83 (Aryl C=C, st.), 3055.76 (Aryl C-H, st.).	**347(M^+)**
VA21	^{1}H NMR (400MHz, DMSO-*d6*) δ 7.43-7.91 (m, 4H, ArH); 3.68 (d, 2H, CH_2); 2.90 (m, 1H, CH_2-NH); 9.10 (s, 1H, CONH); 2.89 (d, 2H, NH_2)	1521.33 (NO_2), 650.50 (C-S-C, st.), 3450.05 (N-H,st.), 1626.76 (C=O, st.), 1570.14, 1606.78 (C=N), 1443.31 (Aryl C=C, st.), 3096.47 (Aryl C-H, st.), 3190.43 (CH_2 N-H), 3434.03 (NH_2, st.).	**294(M^+)**

To the mixture of compounds **I (a**, **b**, **c)**, added solution of chloro-acetyl chloride with constant stirring at room temperature. After complete addition, the reaction mixture was refluxed for about 6-8h. The precipitate was filtered, washed with water to yield compound **(IV d, e, f)**; Yield 64.4%, m. p 210 - 212^{0}C.

Compound **IV (d, e, f)** was refluxed for 9h with thiourea /hydrazine hydrate / piperidine in alcohol, to yield **V (A13-A21).**

The FTIR spectrum of compound **VA13, VA14** and **VA15** showed a medium intensity band at 1622, 1612 and 1624 cm^{-1} that could correspond with (C=N) stretching in the vicinity of 1,3,4-thiadiazole ring[1]. In this spectrum there are two other characteristic bands at 3559, 3518, 3521 and 1689, 1655, 1640 cm^{-1} due to (O-H) and (C=O) stretching vibrations, respectively. Whereas the compound **VA13**, showed two absorption band at 1122 and 3490 cm^{-1} for (C=S) and (NH_2) stretching vibrations, respectively and in the compound **VA14** two absorption band was appeared at 1324 and 2860 cm^{-1} for (C-N, st. piperidine) and (CH_2, st. piperidine) stretching vibrations, respectively. Two characteristic band was found to be at 3453 and 3088 cm^{-1} stretching vibrations, respectively, indicated the presence of (N-H, NH_2, st.) and (C-H, CH_2, st.) functions in compound **VA15.** The ^{1}HNMR spectra of these compounds **VA13, VA14** and **VA15** showed the signal for the (O-H) group in the δ 10.12, 10.15 and 9.64 and those for the NH (amide) group at δ 9.22, 8.69 and 8.85, respectively. The mass spectrum were showed the molecular ion peak at *m/z* 309 [M^+], 318 [M^+], 265 [M^+], respectively, and the base peak at *m/z* 73, 128 and 127 respectively.

Table 4 - Antioxidant property of the synthesized compounds and Standard activity Using DPPH Scavenging Method-%DPPH Radical Scavenging activity

Compound No.	**% inhibition (Mean± S.D)**		
	DPPH scavenging (%)		
	4µg/ml	8µg/ml	10µg/ml
IIIA1	57.791±0.054	64.791±0.023	67.109±0.012
IIIA2	57.852±0.150	64.752±0.175	67.244±0.128
IIIA3	58.271±0.029	65.063±0.072	67.585±0.128
IIIA4	62.282±0.036	65.735±0.065	68.104±0.124
IIIA5	39.745±0.145	48.916±0.007	51.191±0.027
IIIA6	42.120±0.026	50.953±0.016	53.085±0.110
IIIA7	40.730±0.008	50.064±0.126	52.452±0.132
IIIA8	43.542±0.063	51.347±0.052	53.542±0.105
IIIA9	51.750±0.128	59.553±0.142	61.107±0.131
IIIA10	54.815±0.015	60.103±0.187	61.853±0.095
IIIA11	55.882±0.045	60.421±0.181	62.752±0.121
IIIA12	57.098±0.044	62.862±0.156	65.634±0.053
VA13	39.954±0.096	48.203±0.065	50.457±0.176
VA14	38.601±0.023	40.392±0.073	50.867±0.412
VA15	36.856±0.086	46.924±0.037	48.855±0.122
VA16	32.865±0.056	42.674±0.023	45.395±0.703
VA17	33.982±0.130	44.141±0.096	46.252±0.165
VA18	31.847±0.04	41.027±0.094	44.408±0.171
VA19	37.704±0.093	47.756±0.015	49.852±0.132
VA20	35.813±0.132	44.950±0.131	46.851±0.185
VA21	36.037±0.071	45.504±0.087	47.387±0.155
Standard	73.15± 0.045	80.954±0.039	83.826±0.081
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Values are mean ±SEM (n=3); Standard = Ascorbic ac id; (----) Showed no scavenging activity.

Table 5: Antioxidant property of the synthesized compounds and Standard activity using NO Scavenging Method-%NO Radical Scavenging activity

Compound No.	**% inhibition (Mean± S.D)**		
	Nitric oxide radical (NO) scavenging (%)		
	4 µg/ml	8µg/ml	10µg/ml
IIIA1	70.415±0.055	76.605±0.054	77.8915±0.145
IIIA2	69.460±0.015	75.968±0.170	78.998±0.084
IIIA3	59.906±0.163	69.275±0.155	75.985±0.145
IIIA4	56.834±0.015	69.157±0.134	73.125±0.128
IIIA5	58.205±0.164	68.003±0.160	70.885±0.142
IIIA6	66.757±0.135	69.828±0.150	72.864±0.096
IIIA7	64.454±0.012	67.454±0.124	71.309±0.130
IIIA8	65.837±0.143	68.784±0.070	70.553±0.074
IIIA9	52.542±0.089	63.746±0.120	66.836±0.025
IIIA10	61.438±0.097	65.158±0.023	69.05±0.054
IIIA11	58.758±0.124	64.103±0.110	68.754±0.055
IIIA12	46.167±0.053	53.590±0.125	64.128±0.063
VA13	52.369±0.075	59.869±0.023	61.987±0.098
VA14	58.634±0.134	60.347±0.109	62.389±0.163
VA15	38.168±0.086	49.458±0.166	61.108±0.186
VA16	48.275±0.067	53.794±0.180	58.746±0.071
VA17	41.549±0.132	49.130±0.065	58.907±0.107
VA18	45.706±0.132	49.358±0.183	56.843±0.134
VA19	34.706±0.127	45.654±0.108	48.445±0.120
VA20	38.920±0.115	47.125±0.195	49.264±0.142
VA21	32.563±0.176	44.369±0.113	46.867±0.134
Standard	76.246±0.017	81.460±0.137	84.794±0.080
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Values are mean ±SEM (n=3); Standard= Ascorbic aci d; (----) Showed no scavenging activity.

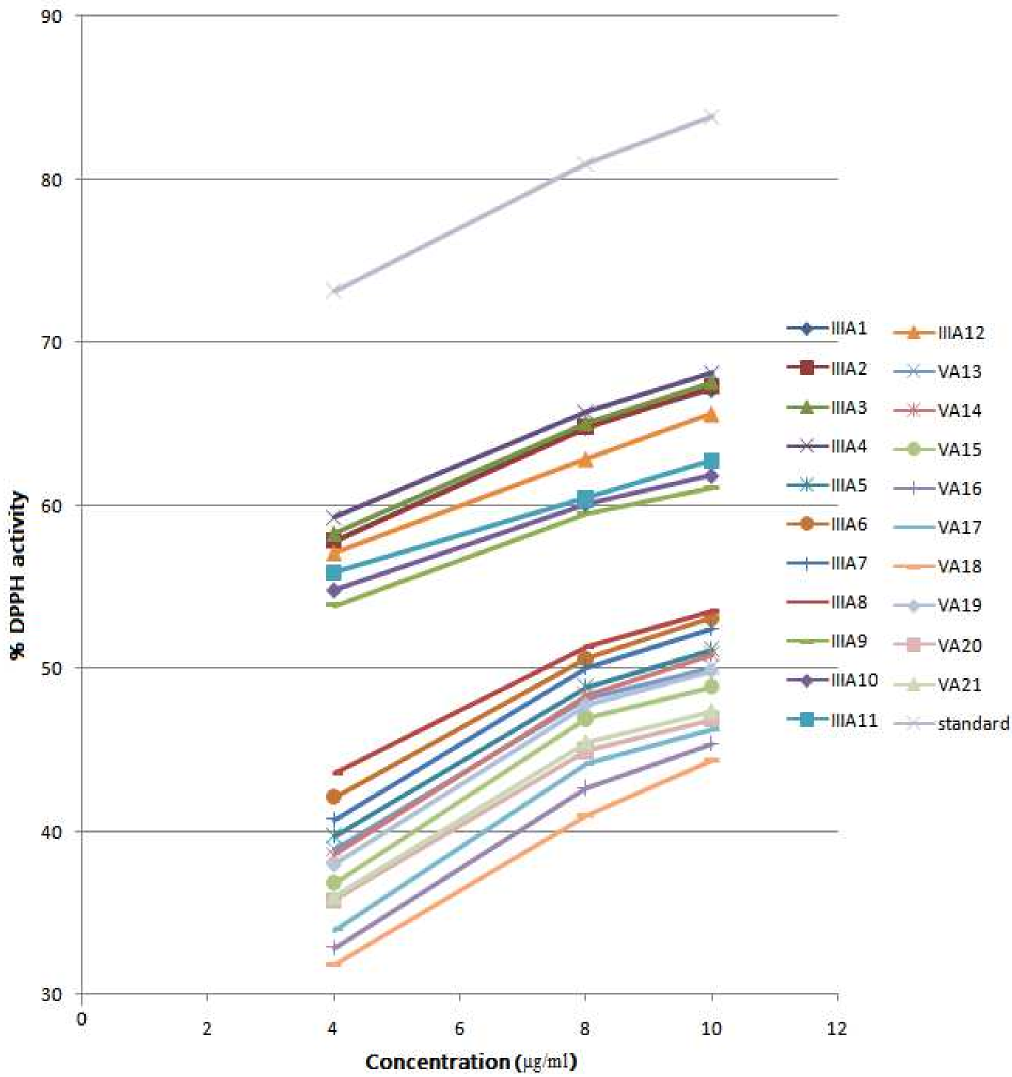


Figure 1: Free Radical Scavenging Activity of Compound (IIIA1-IIIA12, VA13-VA21) by DPPH Method

The structures of compounds **VA16, VA17 and VA18** were assigned by IR and ^{1}H NMR spectroscopic data, which are consistent with the proposed molecular structures. IR spectra of compound **VA16, VA17** and **VA18** showed characteristic bands for NH, CH–aliphatic, C-Cl and C=O groups. ^{1}H-NMR spectrum of compound **VA16, VA17** and **VA18** showed signals for CONH at δ 9.31, 8.85 and 8.81, respectively, for CH_2 at δ 3.72, 4.27 and 3.55, respectively. The primary amino group in compound **VA16** and **VA18** were depicted by the presence of NH function at δ 2.60 and 1.99, respectively. The appearance of multiplate at range δ 1.78-2.69 confirmed the presence of the pyrrolidine ring system in compound **VA17**. Mass spectrum of compounds **VA16**, **VA17** and **VA18** were showed the molecular ion peak at *m/z*: 328 $[M+1]^+$, 336 and 282 $[M-1]^+$, with a base peak at *m/z*: 100, 126 and 157 respectively.

The structures of compounds **VA19**, **VA20** and **VA21** were assigned by IR and ^{1}H NMR spectroscopic data, which are consistent with the proposed molecular structures. The primary amino group in compounds **VA19** and **VA21** was depicted by the presence of NH asymmetric stretch at 3400 and 3434 cm^{-1}. The IR bending vibration corresponding to C=S of compound **VA19** appeared at 1202 cm^{-1}. The presence of heterocyclic pyrrolidine moiety in compound **VA20** was demonstrated by the presence of C-N at 1345 cm^{-1}. The appearance of C=O stretch in the range of 1626 -1725 cm^{-1} indicated the formation of secondary amides (**VA19 -VA21**) by the reaction of hydrazine/ thiourea/ piperidine with the 2-chloro-*N*-[5-(2-nitrophenyl)-1,3,4-thiadiazol-2-yl] acetamide. The appearance of singlet at δ 8.81, 8.95 and 9.10,respectively,corresponds to the proton of CONH in the NMR of all the compounds indicated the presence of secondary amide to the 2nd position of synthesized 1, 3, 4-thiadiazoles moiety (**VA19-VA21**). Mass spectrum of compounds **VA19**, **VA20** and **VA21** were showed the molecular ion peak at *m/z*: 338 [M $^{+}$], 347 [M $^{+}$] and 294 [M^{+}], respectively, with a base peak at *m/z*: 181, 225 and 88 respectively.

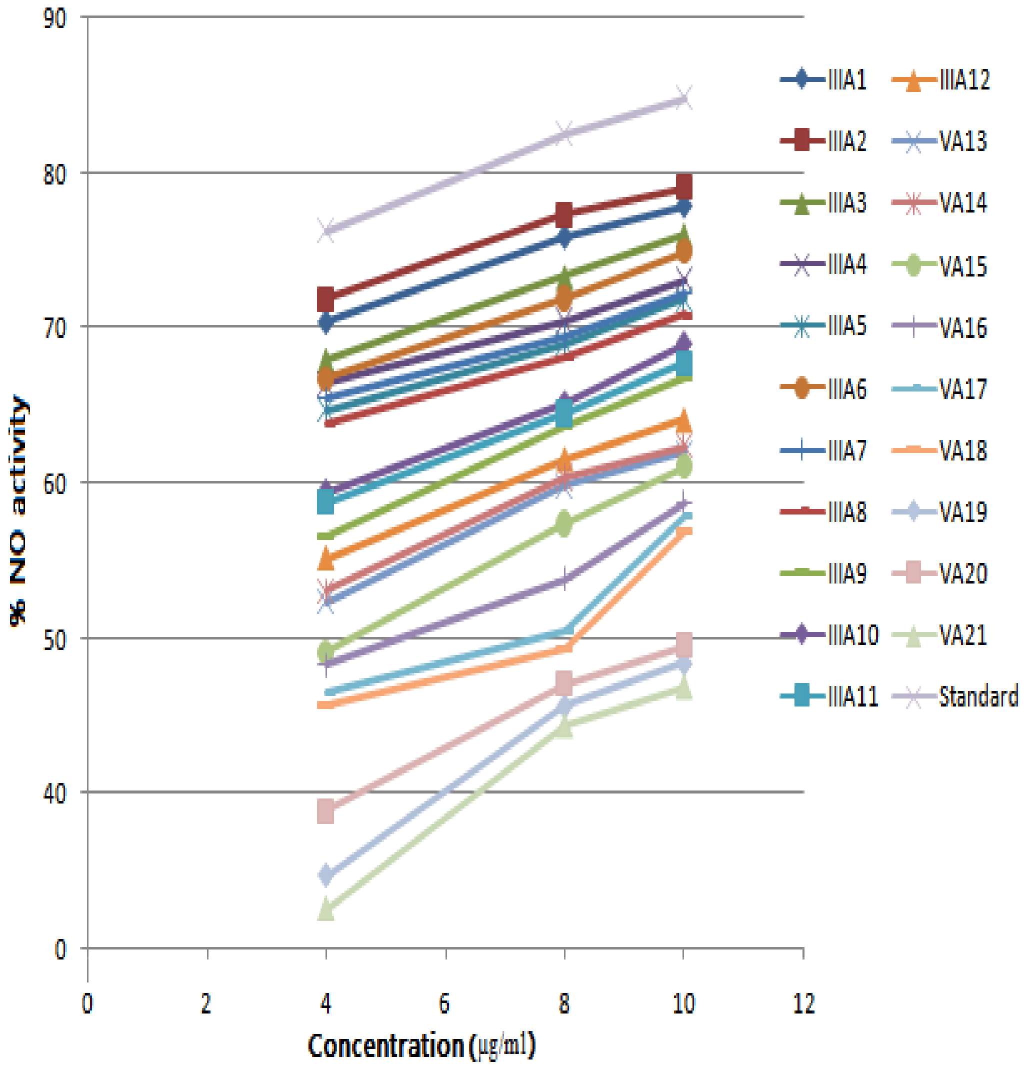


Figure 2: Free Radical Scavenging Activity of Compound (IIIA1-IIIA12, VA13-VA21) NO Method

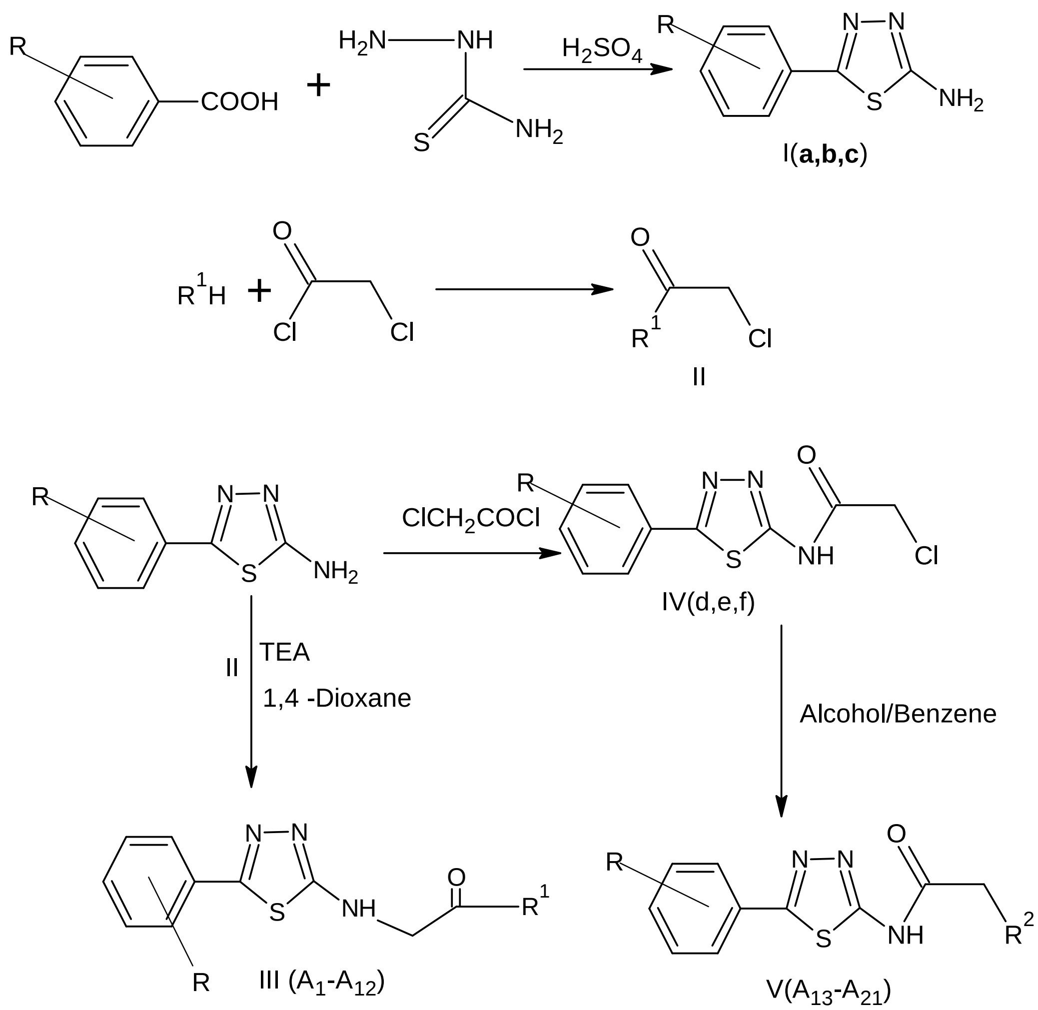
3.2. Antioxidant activity (*in-vitro*):

The compounds **IIIA1-IIIA12** and **IV13-IV21** were tested for anti-oxidant property by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide methods at three different concentrations 4 µg/ml, 8µg/ml and 10 µg/ml. The observed data on the anti-oxidant activity of the compounds controlled drug were shown in (Table: 4 and 5, Figure: 1 and 2).

3.2.1. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging method:

All the synthesized compounds were tested for antioxidant activity against nitric oxide free radical. When comparison is made between **IIIA4** (68.10%) , **IIIA3** (**67.585**%), and **IIIA2** (**67.22**%) at 10µg/ml, it was found to be presence of the o-hydroxyl and p-chloro group make the compounds more potent as well as showed almost equal percentage of inhibition compared to the standard ascorbic acid. Compounds **IIIA1, IIIA12** and **IIIA11** exhibited comparable percentage of inhibition with the standard. Compounds **IIIA9** and **IIIA10** were found to be moderate antioxidant. All other compounds were found to be weak antioxidant activity against **DPPH** free radical. Compounds **VA13-VA21**, respectively, showed poor percentage of inhibition compare to that of standard. An increase in concentration results in an increase in DPPH• scavenging activity (Table 4, Figure:1).

SCHEME



3.2.2. Nitric oxide scavenging method:

Among the compounds tested for antioxidant activity, **(IIIA2)** exhibited the highest antioxidant activity with the % Inhibition value of 78.99, while % Inhibition of reference compound ascorbic acid was found to be 84.79. Other moderately active compounds, **IIIA1** and **(IIIA3**) showed the % inhibition values of 77.89 and 75.98, respectively. The compounds showed activity which is comparable with control against bacterial strains in increasing order of *o*- OH> *o*-Cl > *o*-NO_2 (Table5, Figure: 2).

CONCLUSION

The antioxidant data given for the compounds allowed us to state that the variation of antioxidant activity may be associated with the nature of tested microorganisms and also is due to the chemical structure of the tested compounds. Performed SAR observation has showed the importance of electronic environment on antioxidant activity. The presence of hydroxyl (OH) and halogens (especially chloro) substituent on the aromatic ring have increased the activity of the compounds compared to those with other substituent which may be due to the presence of the versatile pharmacophore.

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