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Syntheses, Characterization and Antioxidant Activity of Some Oxadiazoles

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Oxadiazole derivatives are an important class of heterocyclic compounds, specifically 2-amino substituted oxadiazoles with biological activities such as antioxidant, anticancer, antibacterial, antifungal, analgesic and antiinflammatory activities. The above observations prompted us to synthesize new oxadiazoles with various substitutions. The starting material 2-amino-5-(3'-acetamidophenyl)-1,3,4-oxadiazole (SBM-4) was synthesized by refluxing a mixture of 3-aminophenol and acetic anhydride in glacial acetic acid. This was followed by refluxing with ethyl chloroacetate and anhydrous K_2CO_3 in dry acetone, followed by refluxing with hydrazine hydrate in ethanol and the hydrazide was finally treated with CNBr in methanol. The parent compound was then converted to the oxadiazoles by reacting with various substituted aromatic aldehydes. The new compounds were evaluated for *in vitro* antioxidant activity using ascorbic acid as standard. Among the compounds tested, SBM-4a with 4"-chloro, SBM-4g with 2"-chloro and SBM-4h with 4"-methyl substitution at R showed good antioxidant activity.

Key Words: Syntheses, Oxadiazoles, Ascorbic acid, *In vitro,* Antioxidant activity.

INTRODUCTION

Heterocycles play an important role in biological processes because the side group of the most typical and essential constituent of living cells, DNA and RNA are based on aromatic heterocycles. Generally many drugs are obtained from plants and animals, but most drugs used in modern medicine are products of advances in synthetic organic chemistry and biotechnology. Oxadiazoles are 5-membered heterocyclic compounds with two nitrogen atoms and one oxygen atom. 1,3,4-Oxadiazoles are a class of heterocycles, which have attracted significant interest in medicinal chemistry and they have a wide range of pharmaceutical and biological activities. Molecules containing a 1,3,4-oxadiazole core have been shown to have a broad range of important biological activities including antimicrobial[1-3], pesticidal, antimycobacterial, antitumor[4,5], antiinflammatory[6-8], anticonvulsant, insecticidal, anticancer and antihypertensive properties. Among the 1,3,4-oxadiazoles, 2,5-unsymmetrical disubstituted derivatives have attracted considerable attention because of their biological and electrochemical properties.

Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Although there are several enzyme systems within the body that scavenge free radicals. The principle micronutrient (vitamin) antioxidants are Vitamin E, β-carotene and vitamin C. Additionally selenium a trace metal that is required for proper function of one of the body's antioxidant enzyme systems, is sometimes included in this category. The body cannot manufacture these micronutrients so they must be supplied in the diet.

EXPERIMENTAL

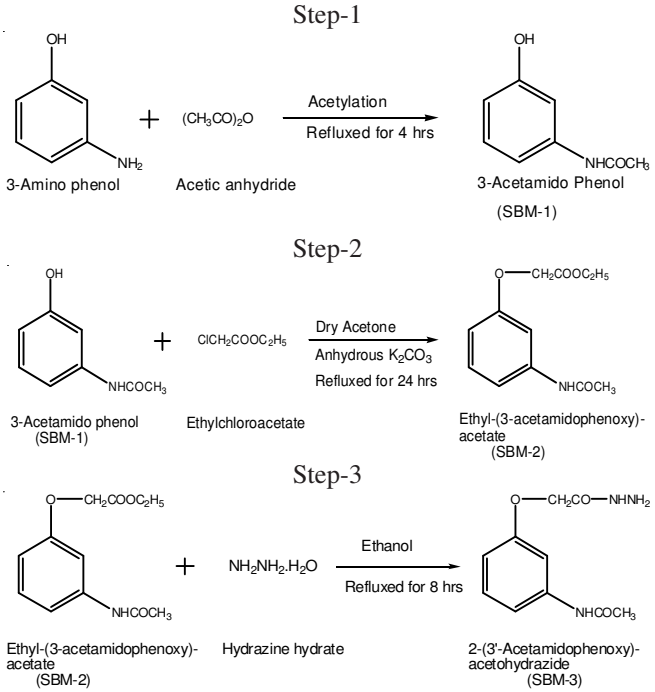
Step-1: Synthesis of 3-acetamido phenol (SBM-1): A mixture of 3-amino phenol (21.8 g, 0.2 mol), acetic anhydride (24 mL, 0.3 mol) and glacial acetic acid (60 mL) were refluxed for 4 h. Excess solvent was removed by distillation and the residual mixture was poured over crushed ice. The resultant product was recrystallized from water to yield pure 3-acetamido phenol (SBM-1). Yield (%): 71 %, m.p.: 152 ºC. IR (KBr, ν_{max}, cm^{-1}): 3594 (O-H), 3461 (NH stretch of amide), 3098 (Ar. C-H), 2961 (Ali. C-H), 1684 (C=O of amide), 1578 (NH bend), 1605 and 1468 (Ar. C=C), 898 (Ar. C-H bend).

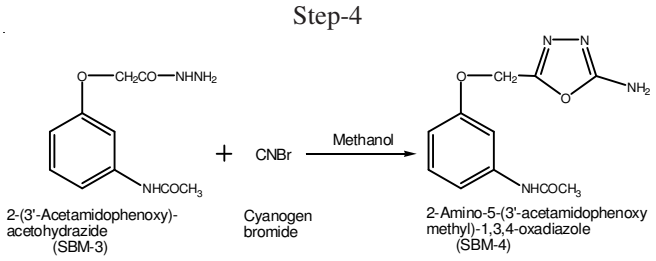
Step-2: Synthesis of ethyl-(3-acetamidophenoxy)-acetate (SBM-2): A mixture of 3-acetamido phenol (15.1 g, 0.1 mol), ethyl chloroacetate (10.5 mL, 0.1 mol) and anhydrous K_2CO_3 (20.7 g, 0.15 mol) in dry acetone (150 mL) was refluxed on a water bath for 24 h. The reaction mixture was cooled and filtered and from filtrate excess acetone was removed by distillation. Then the reaction mixture was poured into ice cold

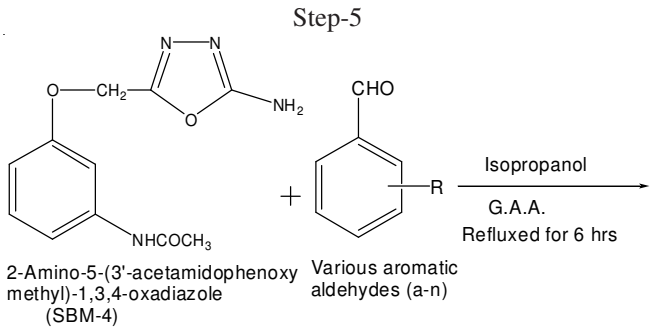
water and stirred well. The resultant product was recrystallized with ethanol (95 %) to yield pure ethyl-(3-acetamidophenoxy)-acetate (SBM-2). Yield (%): 62 %, m.p.: 70 ºC, IR (KBr, ν_{max}, cm^{-1}): 1752 (C=O of ester), 3460 (NH stretch of amide), 3109 (Ar. C-H), 2990 (Ali. C-H), 1684 (C=O of amide), 1560 (NH bend), 1218 (Ar-O-C), 1170 (O-C_2H_5), 1600 and 1469 (Ar. C=C), 830 (Ar. C-H bend).

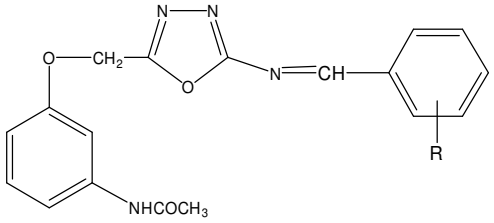
Step-3: Synthesis of 2-(3'-acetamidophenoxy)acetohydrazide (SBM-3): A mixture of ethyl-(3-acetamidophenoxy)-acetate (11.85 g, 0.05 mol) and hydrazine hydrate 99 % (10 mL, 0.2 mol) in ethanol (50 mL) was refluxed for 8 h. Excess of ethanol was removed by distillation. On cooling 2-(3'-acetamidophenoxy)-acetohydrazide initiated to separate. It was collected by filtration and recrystallized with ethanol (95 %) to yield pure 2-(3'-acetamidophenoxy)-acetohydrazide (SBM-3). Yield (%): 80 %, m.p.: 175 ºC, IR (KBr, ν_{max}, cm^{-1}): 3344 (NH_2 strech), 3470 (NH stretch of amide), 3078 (Ar. C-H), 2919 (Ali. C-H), 1680 (C=O of amide), 1556 (NH_2 bend), 1520 (NH bend), 1230 (Ar-O-C), 1605 and 1463 (Ar. C=C), 840 (Ar. C-H bend).

Step-4: Synthesis of 2-amino-5-(3'-acetamidophenoxymethyl)-1,3,4-oxadiazole (SBM-4): 2-(3'-Acetamidophenoxy)-acetohydrazide (4.46 g, 0.02 mol) was added to a solution of cyanogen bromide (2.12 g, 0.02 mol) in 50 mL of methanol in such a way that the temperature should not rise above 40 ºC. The solution was then stirred for 1.5 h at 40 ºC and then it was refluxed at 70 ºC for 1.5 h, filtered hot and allowed to cool at room temp. Then the solution was neutralized with dil. ammonia. The resultant product was collected and recrystallized with methanol to yield pure 2-amino-5-(3'-acetamidophenoxymethyl)-1,3,4-oxadiazole (SBM-4). Yield (%): 54 %, m.p.: 198 ºC, IR (KBr, ν_{max}, cm^{-1}): 3394 (NH_2 strech), 3473 (NH stretch of amide), 3116 (Ar. C-H), 2901 (Ali. C-H), 1674 (C=O of amide), 1090 (C-O of Oxadiazole), 1640 (C=N), 1561 (NH bend), 1230 (Ar-O-C), 1460 (Ar. C=C), 830 (Ar. C-H bend).









2-[(substituted benzylidene) imino]-5-(3'-acetamidophenoxy methyl)-1,3,4-oxadiazoles (Schiff bases) SBM-4(a-n)

Sample code	R	Sample code	R
SBM-4a	4-chloro	SBM – 4h	4-methyl
SBM-4b	4-dimethylamino	SBM – 4i	2-hydroxy
SBM-4c	2-nitro	SBM – 4j	4-methoxy
SBM-4d	3, 4-dimethoxy	SBM – 4k	3-nitro
SBM-4e	4-hydroxy	SBM – 4l	3,4,5-trimethoxy
SBM-4f	4-hydroxy-3-methoxy	SBM – 4m	H
SBM-4g	2-chloro	SBM – 4n	4-nitro

Step-5: General method for the syntheses of 2-[(substituted benzylidene) imino]-5-(3'-acetamidophenoxy methyl)-1,3,4-oxadiazoles (SBM 4a-n) (Schiff bases): A mixture of 2-amino-5-(3'-acetamidophenoxy methyl)-1,3,4-oxadiazole (SBM-4) (0.001 mol), the required aryl aldehydes (0.001 mol) in isopropanol (15 mL) and catalytic amount of glacial acetic acid (0.5 mL) was subjected to reflux for 6 h. The reaction mixture was cooled to room temperature. The solid separated was filtered, washed with isopropanol and recrystallized with DMF : Water mixture (5:2). The new titled compounds formed were confirmed by melting point, TLC, IR and representative compounds by NMR and mass spectra.

***In vitro* Antioxidant activity (9):** The synthesized compounds were screened for *in vitro* antioxidant activity by free radical scavenging activity using Nitric oxide radical inhibition method.

Experimental design: Sodium nitroprusside (10 mM) in phosphate buffered saline was mixed with different concentrations (100-300 µg/mL) of synthesized compound were dissolved in DMSO and incubated at 250 ºC for 2.5 h. The same reaction mixture without the synthesized compound but the equivalent amount of DMSO served as the control. After

the incubation period, 0.5 mL of Griess reagent [1 % sulfanilamide, 2 % H_3PO_4 and 0.1 % naphthyl ethylenediamine] was added. The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm. (Shimadzu Spectrometer). Inhibition of nitrite formation by the synthesized compound and the standard antioxidant ascorbic acid were calculated relative to the control.

$$\text{Inhibition (\%)} = 100\ (1 - A_{test} / A_{control})$$

where, A = absorbance.

Melting points were determined in open capillaries and are uncorrected. Purity of the compounds was checked by TLC on silica gel plates. The solvent system used to carry out the TLC is toluene:acetonitrile (2:8). IR spectra were recorded in KBr on a Shimadzu FTIR-8700 spectrometer. ^{1}H NMR (ppm) in DMSO using TMS as reference on Bruker 400 AMX. Mass spectra of the compound coded SBM-4 was carried out.

RESULTS AND DISCUSSION

The physical data of all the syntheiszed compounds are given in Table-1.

TABLE-1
PHYSICAL DATA OF SYNTHESIZED COMPOUNDS

Comp.	m.f.	m.w. (g)	m.p. (ºC)	R_f value	Yield (%)
SBM -1	$C_8H_9NO_2$	151	152	0.61	71.0
SBM -2	$C_{12}H_{15}NO_4$	237	70	0.71	62.0
SBM -3	$C_{10}H_{13}N_3O_3$	223	175	0.37	80.0
SBM -4	$C_{11}H_{12}N_4O_3$	248	198	0.67	54.0
SBM -4a	$C_{18}H_{15}ClN_4O_3$	370	171	0.61	60.0
SBM -4b	$C_{20}H_{21}N_5O_3$	379	190	0.78	62.1
SBM -4c	$C_{18}H_{15}N_5O_5$	381	192	0.82	58.6
SBM -4d	$C_{20}H_{20}N_4O_5$	396	194	0.73	59.4
SBM -4e	$C_{18}H_{16}N_4O_4$	352	188	0.77	53.6
SBM -4f	$C_{19}H_{18}N_4O_5$	382	191	0.69	51.9
SBM -4g	$C_{18}H_{15}ClN_4O_3$	370	176	0.62	56.7
SBM -4h	$C_{19}H_{18}N_4O_3$	350	186	0.71	64.3
SBM -4i	$C_{18}H_{16}N_4O_4$	352	193	0.76	57.1
SBM -4j	$C_{19}H_{18}N_4O_4$	366	175	0.80	57.6
SBM -4k	$C_{18}H_{15}N_5O_5$	381	185	0.72	55.4
SBM -4l	$C_{21}H_{22}N_4O_6$	426	180	0.64	52.3
SBM -4m	$C_{18}H_{16}N_4O_3$	336	183	0.78	55.6
SBM -4n	$C_{18}H_{15}N_5O_5$	381	181	0.84	58.3

IR (KBr) cm^{-1}: Compound SBM-(**4a-4n**): 3598-3510 (O-H), 3470-3450 (NH stretch of amide), 3158-3060 (Ar. C-H), 3074-2912 (Ali. C-H), 1694-1658 (C=O of amide), 1098-1084 (C-O of oxadiazole), 1656-1628 (C=N), 1605-1583 (N=CH), 1596-1530 (NH bend), 1245-1211 (Ar-O-C), 1489-1412 (Ar. C=C), 920-823 (Ar. C-H bend), 1550 & 1378, 1546 & 1363, 1501 & 1354 (N=O), 1259-1240 (C-O-CH_3), 771-760 (C-Cl).

^{1}H NMR (DMSO) δ (ppm): Compound SBM-4: 2.01 (s, 3H, CH_3 at e); 5.08 (s, 2H, O-CH_2, at f); 6.73 (s, 2H, NH_2 at g); 7.14 (d, 2H, CH, ArH, at a, a'); 7.20 (t, 1H, CH, ArH, at b); 7.31 (d, 1H, CH ArH at c); 9.92 (s, 1H, NH at d).

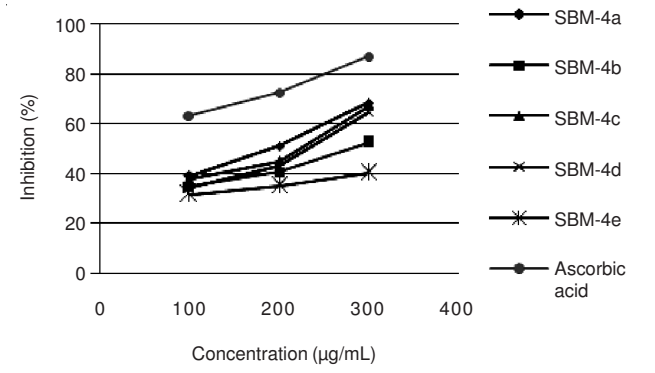
Compound SBM -4a: 2.07 (s,3H, CH_3 at e) ; 5.36 (s,2H, O-CH_2 at f); 7.14 (d, 2H, CH, ArH at a,a'); 7.18 (d, 4H, CH, ArH at h, h', i, i'); 7.251 (t, 1H, CH, ArH at b); 7.32 (d, 1H, CH, ArH at c); 9.91 (s, 1H, NH at d) ; 10.16 (s,1H, N=CH at g).

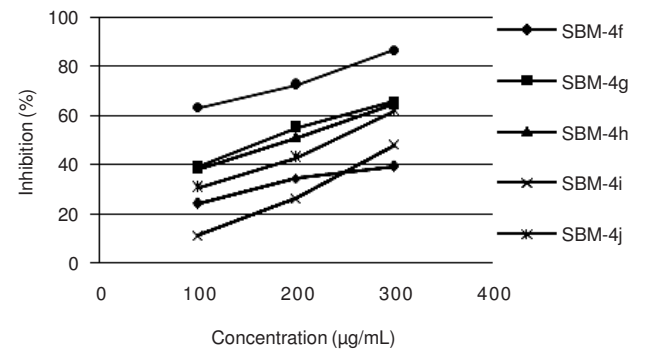
Compound SBM-4h: 2.08 (s,3H, CH_3 at e) ; 3.38 (s,3H, Ar-CH_3 at j); 5.08 (s,2H, O-CH_2 at f); 6.92 (d, 2H, CH, ArH at a,a'); 7.18 (d, 4H, CH, ArH at h, h', i, i'); 7.31 (t, 1H, CH, ArH at b); 7.41 (d, 1H, CH, ArH at c); 9.50 (s, 1H, NH at d) ; 10.09 (s,1H, N=CH at g).

***In vitro* antioxidant activity data:** Antioxidant activity[9] was carried out using Nitric oxide radical inhibition method. All the title compounds (SBM-**4a-n**) were screened for *in vitro* antioxidant activity. The results of the antioxidant activity of the compounds are shown in the Table-2 and Fig. 1. Among all the compounds tested, SBM-**4a** with 4'-chloro substitution at R, SBM-4g with 2'-chloro substitution at R and SBM-4h with 4'- methyl substitution at R showed good antioxidant activity. The remaining compounds exhibited mild to moderate activities compared to the standard ascorbic acid.

TABLE-2
In vitro ANTIOXIDANT ACTIVITY DATA

Comp. no	Inhibition (%) 100 µgm	200 µgm	300 µgm	Avg	IC_{50} (µ gm/mL)
SBM-4a	39.17	50.86	68.26	52.76	189.54
SBM-4b	34.79	40.59	52.29	42.56	234.96
SBM-4c	37.74	44.96	66.84	49.85	200.60
SBM-4d	34.08	42.83	64.60	47.17	211.99
SBM-4e	31.54	35.10	40.18	35.61	280.82
SBM-4f	24.11	34.38	39.17	32.55	307.22
SBM-4g	39.37	54.93	65.62	53.31	187.58
SBM-4h	38.15	50.97	64.70	51.27	195.04
SBM-4i	10.99	26.14	48.12	28.42	351.86
SBM-4j	30.42	42.62	61.85	44.96	222.42
SBM-4k	14.95	29.09	34.49	26.18	381.97
SBM-4l	20.45	30.21	32.96	27.87	358.81
SBM-4m	33.27	37.74	45.37	38.79	257.79
SBM-4n	33.77	39.27	60.22	44.42	225.12
Ascorbic acid	63.17	72.33	86.67	74.06	135.03





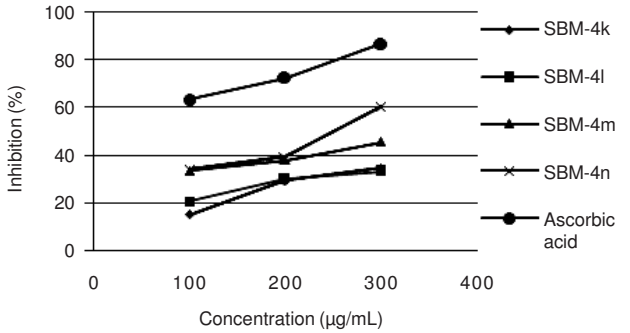


Fig. 1. Graphical representation of *in vitro* antioxidant activity of oxadiazoles

The purpose of the present work was to synthesize a series of desired title compounds from 2-amino-5-(3'-acetamido phenoxy methyl)-1,3,4-oxadiazole (SBM-4) by reacting with various substituted aromatic aldehydes.

Thus, present investigation was planned to synthesize a novel class of oxadiazoles using four steps and test their effect on antioxidant activity.

The *invitro* antioxidant activity results reveals that both electron donating and electron withdrawing groups on the aldehydic phenyl ring of the compounds influenced the activity. Among the compounds tested, SBM-4a with 4"-chloro, SBM-4g with 2"-chloro and SBM-4h with 4"-methyl substitution at R showed good antioxidant activity. The remaining compounds exhibited mild to moderate activities compared to the standard ascorbic acid.

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