ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Synthesis and *In- vitro* antioxidant activity of substituted Pyridinyl 1, 3, 4 oxadiazole derivatives

M Vijey Aanandhi*, Mohammed Hashim Mansoori, S Shanmugapriya, Shiny George, P Shanmugasundaram

Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, VELS University, Chennai-600117, Tamilnadu, India.

ABSTRACT

A series of substituted pyridinyl 1, 3, 4 oxadiazole derivatives were synthesized from Schiff bases of nicotinic acid derivatives through chlorination followed by reaction with hydrazine hydrate and with the use various aldehydes. The synthesized compounds were characterized by elemental analysis, IR, ^{1}H NMR and Mass Spectra. All the compounds were screened for *in vitro* antioxidant activity by DPPH and Nitric oxide scavenging assay. Compounds substituted with electron donating groups like methoxy and hydroxyl showed higher antioxidant activity.

Keywords: Nicotinic acid, Schiff base, oxadiazole and antioxidant activity.

Corresponding author
Email: mvaanandhi@gmail.com

ISSN: 0975-8585

INTRODUCTION

From the past few decades the research on nicotinic acid derivative revealed that the derivative had wide range of therapeutic application such as antimicrobial[1], antimycobial [2], anti-inflammatory [3], anticancer [4], antiviral [5], anticonvulsant [6] and antioxidant activity [7]. The versatile applications of nicotinic acid have given zeal to design and synthesize the novel derivatives with the aim to achieve antioxidant activity. Oxadiazole derivatives also found to possess anthelmintic [8], antibacterial [9], anti-inflammatory [10] and antioxidant [11] activities.

In the present study nicotinic acid was converted to nicotinoyl chloride in the presence of phosphorous pentachloride and carbon tetrachloride. This was converted to nicotino hydrazide in presence of hydrazine hydrate and this was converted to Schiff base derivatives in presence of different aromatic aldehydes and further acetylation of schiff base in presence of acetic anhydride yielded 1,3,4 oxadiazole derivatives respectively (Scheme 1).

MATERIALS AND METHODS

Melting points were determined by open capillary method and were uncorrected. The reaction was monitored by TLC using solvent Chloroform: Methanol (50:50 ratio). FT-IR spectra was recorded on Shimadzu FT 8300, ^{1}H NMR were recorded at JEOL GSX400 and Mass spectra an JEOL GC Mate spectrometer. The elemental analysis was obtained on a CHN Rapid analyser and all compounds showed satisfactory elemental analysis.

EXPERIMENTAL

Synthesis of Nicotinoyl chloride (2)

A mixture of nicotinic acid (0.03 mol) and phosphorus pentachloride (0.05 ml) in anhydrous carbon tetra chloride (20 ml) was refluxed for two hours at 100°C. Solvent was distilled off and the acid chloride thus obtained was used for further reaction.

Synthesis of Nicotino Hydrazide (3)

In the acid chloride (0.03 mol) hydrazine hydrate was added (0.1 mol) drop wise at 0°C and the resultant mixture was separated out was washed with aqueous sodium carbonate (10%) and dried vaccum. It was recrystalized from methanol.

Synthesis of Schiff bases (4)

The nicotino hydrazide (0.01 mol) and aromatic aldehyde (0.01 mol) in tetra hydrofuran (25 ml) was heated gently for two hours at a temperature of 60°C. The reaction mixture was then poured into ice cold water and it was filtered. The pure compound recrystalized was from methanol.

ISSN: 0975-8585

Acetylation of Schiff base derivatives (5)

The mixture of Schiff base (0.01 mol) and acetic anhydride (10 ml) was dissolved in ethanol (25 ml) and the reaction mixture was refluxed for two hours at 100°C. The reaction mixture was then concentrated, allowed to cool, the solid product obtained was filtered, washed with water and recrystallized using methanol. The physical data of synthesized compounds are given in Table 1.

Spectral Analysis

1-(2-phenyl-5-(pyridin-3-yl)-1,3,4-oxadiazole-3 (2H)-yl) ethanone (A): IR (KBr): 3009, 1686, 2901, 1602, 1323, 1584, 1329 cm^{-1}; ^1H NMR (DMSO d_6): δ 7.3-9.2 (M, 9H), 8.1 (S, 1H), 2.4 (S, 3H); MS (relative intensity): m/z value 267.17 (10%), 103.41 (100%), 75.63 (75%), 84.18 (12%), 92.58 (14%), 109.40 (13%), 120.26 (87%), 126.85 (15%), 135.09 (16%), 147.39 (17%), 163.54 (16%), 171.15 (14%), 178.88 (10%), 186.44 (11%), 199.12 (14%), 207.31 (12%), 214.63 (14%), 232.20 (17%), 243.77 (11%),249.30 (25%), 255.35 (18%).

1-(2-(4-dimethylamino)phenyl)-5-(pyridine-3-yl)-1,3,4-oxadiazole-3(2H)-yl ethanone (B): IR (KBr): 3080, 1701, 2924, 1632, 1361, 1597, 1421 cm^{-1}; ^1H NMR (DMSO d_6): δ 6.6-9.2 (M, 8H), 8.1 (S, 1H), 2.4 (S, 3H), 2.9 (S, 6H); MS (relative intensity): m/z value 310.25 (24%), 249.10 (100%), 75.60 (40%), 88.76 (16%), 103.30 (55%), 112.85 (21%), 120.32 (46%), 135.68 (20%), 143.56 (22%), 158.31 (18%), 167.98 (21%), 175.87 (14%), 200.74 (28%), 216.36 (44%), 232.34 (60%), 242.55 (13%), 272.12 (18%), 297 (16%).

1-(2-(2-chlorophenyl)-5-(pyridine-3-yl)-1,3,4-oxadiazole-3(2H)-yl) ethanone (C): IR (KBr): 3058, 1673, 2885, 1638, 1360, 1594, 1419, 762 cm^{-1}; ^1H NMR (DMSO d_6): δ 7.2-9.2 (M, 8H), 8.1 (S, 1H), 2.4 (S, 3H); MS (relative intensity): m/z value 301.51 (29%), 249 (100%), 89.26 (33%), 103.22 (56%), 119.78 (54%), 127.70 (36%), 135.06 (48%), 147.29 (33%), 157.17 (34%), 165.89 (23%), 178.54 (30%), 191.29 (28%), 199.10 (31%), 216 (40%), 223.47 (33%), 232.15 (64%), 260.77 (30%), 266.31 (26%), 285.77 (28%), 295.77 (24%).

1-(2-(2-Nitrophenyl)-5-(pyridine-3-yl)-1,3,4-oxadiazole-3(2H)-yl) ethanone (D): IR (KBr): 3029, 1669, 2849, 1651, 1343, 1593, 1420, 1420 cm^{-1}; ^1H NMR (DMSO d_6): δ 7.6-9.2 (M, 8H), 8.1 (S, 1H), 2.4 (S, 3H); MS (relative intensity): m/z value 312.71 (40%), 247.65 (100%), 80.21 (48%), 86.64 (39%), 102.94 (54%), 108.92 (41%), 120.60 (44%), 135.59 (36%), 146.93 (40%), 155.80 (28%), 172.39 (32%), 185.89 (28%), 200.65 (49%), 232.28 (69%), 259.77 (33%), 271.58 (37%), 280.81 (29%), 300 (28%).

1-(2-(4-Methoxyphenyl)-5-(pyridine-3-yl)-1,3,4-oxadiazole-3(2H)-yl) ethanone (E): IR (KBr): 3072, 1701, 2924, 1632, 1361, 1597, 1421, 1293 cm^{-1}; ^1H NMR (DMSO d_6): δ 6.8-9.2 (M, 8H), 8.1 (S, 1H), 2.4 (S, 3H), 3.7 (S, 3H); MS (relative intensity): m/z value 297.33 (43%), 247.28 (100%), 76.65 (65%), 81.54 (60%), 85.20 (53%), 103.14 (79%), 111.11 (52%), 121.21 (49%), 137.93 (55%), 165.50 (56%), 177.86 (48%), 198.57 (58%), 207.59 (50%), 219.86 (44%), 231.37 (92%), 258.57 (40%), 272.54 (45%), 280.21 (52%).

ISSN: 0975-8585

1-(2-(3-Chloro-4-nitrophenyl)-5-(pyridine-3-yl)-1,3,4-oxadiazole-3(2H)-yl)ethanone (F): IR (KBr): 3000, 1700, 2900, 1640, 1375, 1596, 1423, 1468, 738 cm^{-1}; ^1H NMR (DMSO d_6): δ 7.6-9.2 (M, 7H), 8.1 (S, 1H), 2.4 (S, 3H); MS (relative intensity): m/z value 346.22 (56%), 120.99 (100%), 76.42 (96%), 86.36 (56%), 103.34 (84%), 135.42 (64%), 142.80 (36%), 164.42 (50%), 174.53 (48%), 187.34 (37%), 195.42 (68%), 216.27 (49%), 232.62 (61%), 247.28 (63%), 256.19 (40%), 265.56 (60%), 277.74 (32%), 293.11 (34%), 305.23 (44%), 321.77 (40%), 329 (34%).

1-(2-(3-nitrophenyl)-5-(pyridine-3-yl)-1,3,4-oxadiazole-3(2H)-yl)ethanone (G): IR (KBr): 3195, 1682, 2923, 1651, 1387, 15962, 1475 cm^{-1}; ^1H NMR (DMSO d_6): δ 7.2-9.2 (M, 8H), 8.1 (S, 1H), 2.4 (S, 3H); MS (relative intensity): m/z value 312.88 (14%), 58.38 (100%), 76.17 (12%), 103.76(15%), 115.75 (10%), 148.38 (8%), 208.82 (4%), 235.59 (6%), 249.62 (2%), 262.86 (4%), 277.32 (10%), 291.07 (8%), 302.05 (12%).

1-(2-(4-Hydroxy-3-methoxyphenyl)-5-(pyridine-3-yl)-1,3,4-oxadiazole-3(2H)-yl) ethanone (H): IR (KBr): 3063, 1698, 2957, 1651, 1374, 1596, 1475, 1202, 3383, 130 cm^{-1};
^1H NMR (DMSO d_6): δ 7.6-9.2 (M, 7H), 8.1 (S, 1H), 2.4 (S, 3H), 2.1(S, 1H), 3.8(S, 3H); MS (relative intensity): m/z value 313.09 (8%), 120.68 (100%), 76.17 (40%), 102.86 (55%), 58.40 (20%), 134.62 (2%), 233.57 (3%), 279.36 (6%), 296.44 (4%), 306.74 (10%).

1-(5-pyridine-3-yl)-2-p-tolyl-1,3,4-oxadiazole-3(2H)-yl) ethanone (I): IR (KBr): 3212, 1699, 3010, 1636, 1361, 1574, 1418, 1293 cm^{-1}; ^1H NMR (DMSO d_6): δ 7.3-9.2 (M, 8H), 8.1 (S, 1H), 2.3 (S, 3H), 2.4 (S, 3H); MS (relative intensity): m/z value 281.42 (2%), 103.69 (100%), 76.17 (80%), 120.70 (55%), 58.38 (18%), 89.03 (12%), 130.59 (14%), 145.44 (16%), 206.03 (3%), 219.92(10%), 238.81 (30%), 245.74 (6%).

1-(2-(3,4-Dimethoxyphenyl)-5-(pyridine-3-yl)-1,3,4-oxadiazole-3(2H)-yl)ethanone (J):
IR (KBr): 3210, 1698, 2915, 1652, 1373, 1596, 1417, 1301 cm^{-1}; ^1H NMR (DMSO d_6): δ 7.5-9.2 (M,7H), 8.1 (S,1H), 2.4 (S,3H), 3.6 (S,6H); MS (relative intensity): m/z value 327.92 (12%), 103.85 (100%), 76.15 (48%), 84.18 (8%), 119.65 (16%), 129.43 (10%), 154.19 (6%), 194.82 (10%), 208.78 (20%), 221.58 (16%), 235.63 (18%), 249.54 (12%), 279.33 (30%), 300.14 (35%), 313.09 (32%).

ANTIOXIDANT ACTIVITY

In the present study DPPH scavenging and Nitric oxide scavenging assay are two *in vitro* methods used for screening the antioxidant activity. The antioxidant activity of the synthesized compounds was expressed as IC_{50} values.

DPPH Assay method

The antioxidant activity using the DPPH assay was assessed by the method of Tagashira and Ohtake [12]. Test sample solution (200 µL) was added to 4 mL of 100 mmol L^{-1} ethanolic DPPH. After vortexing, the mixture was incubated for 10 minutes at room temperature and the absorbance at 517 nm was measured. The difference in absorbance between a test sample and

a control was considered as activity. The activity was shown as IC_{50} value (50% of inhibitory concentration in mg mL^{-1}). BHT was used as reference substance. The antioxidant activity data is given in Table 2.

Nitric oxide scavenging activity assay

Nitric oxide radical scavenging activity was determined according to the method reported by Garrat [13]. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions. 2 mL of 10 mM sodium nitroprusside in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of test solution at various concentrations and the mixture incubated at 25^{o}C for 150 min. From the incubated mixture 0.5 mL was taken out and added into 1.0 mL sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. finally, 1.0 mL naphthylethylene diamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min before measuring the absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated and data is given in Table 3.

RESULTS AND DISCUSSION

All the targeted compounds were synthesized in good yield. The melting points of all synthesized compounds were found in open capillary tubes and readings were uncorrected. The synthesized compounds were screened for their *in vitro* antioxidant activity by DPPH, Nitric oxide scavenging method shows good antioxidant activity, out of all the synthesized compounds 1-(2-(4-Methoxyphenyl)-5-(pyridine-3-yl)-1,3,4-oxadiazole-3(2H)-yl) ethanone (E), 1-(2-(4-Hydroxy-3-methoxyphenyl)-5-(pyridine-3-yl)-1,3,4-oxadiazole-3(2H)-yl) ethanone (H) followed by I and J showed significant antioxidant activity, in these two methods except the compound F and G which showed less when compared to that of the standard butylated hydroxyl toluene. The compounds (E, H, I and J) substituted with electron donating groups like Methoxy and hydroxyl showed higher antioxidant activity compared to others.

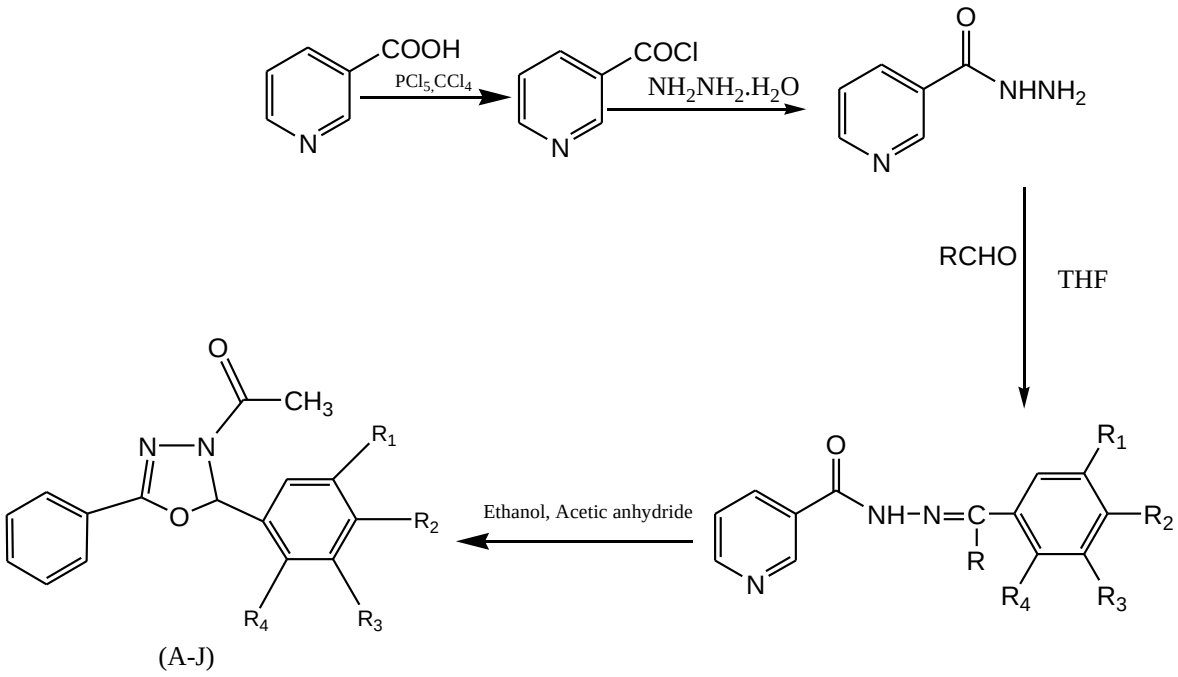
CONCLUSION

A series of pyridyl 1,3,4 oxadiazole derivatives were synthesized by reaction of nicotinoyl chloride with various aromatic aldehydes and further acetylation. Synthesized compounds were screened for *in vitro* antioxidant assay and possess significant activity. The results revealed that the test compound is electron donor and could react with free radical chain reaction.

ACKNOWLEDGEMENT

The authors are thankful to the Head of School of Pharmaceutical Sciences, Vels University for providing necessary facilities for this research work.

ISSN: 0975-8585



Scheme

Table 1 Physical data of synthesized compounds

Compound	R_1	R_2	R_3	R_4	Mol. Formula	M.P (ºC)	Yield (%)	Elemental analysis (%) found		
								C	H	N
A	H	H	H	H	$C_{15}H_{13}N_3O_2$	145	62	67.40	4.90	15.72
B	H	$N(CH_3)_2$	H	H	$C_{17}H_{18}N_4O_2$	170	65	65.79	5.85	18.05
C	H	H	H	Cl	$C_{15}H_{12}ClN_3O_2$	180	63	59.71	4.01	13.93
D	H	H	H	NO_2	$C_{15}H_{12}N_4O_4$	190	67	57.69	3.87	17.94
E	H	OCH_3	H	H	$C_{16}H_{15}N_3O_3$	173	61	64.64	5.09	14.13
F	Cl	NO_2	H	H	$C_{15}H_{11}ClN_3NO_2O_2$	182	68	22.01	1.35	5.13
G	H	H	NO_2	H	$C_{15}H_{12}N_4O_4$	193	66	57.69	3.87	17.94
H	H	OH	OCH_3	H	$C_{16}H_{15}N_3O_4$	150	64	61.34	4.83	13.41
I	H	CH_3	H	H	$C_{16}H_{15}N_3O_2$	157	65	68.31	5.37	14.94
J	OCH_3	OCH_3	H	H	$C_{17}H_{17}N_3O_4$	140	63	62.38	5.23	12.84

ISSN: 0975-8585

Table 2 Antioxidant activity by DPPH radical scavenging method

S. No.	Compounds	%RSC				IC_{50}
		25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	
1.	**A**	15.50±0.213	33.72±0.477	51.46±0.450	63.80±0.531	73
2.	**B**	19.72±0.603	34.25±0.567	41.25±0.458	58.30±0.558	88
3.	**C**	16.20±0.580	28.16±0.492	38.50±0.406	51.14±0.693	99
4.	**D**	19.25±0.219	35.30±0.350	45.35±0.262	59.31±0.641	84
5.	**E**	24.91±0.608	63.18±0.529	73.26±0.542	82.10±0.726	42
6.	**F**	12.86±0.437	24.92±0.609	36.21±0.814	53.12±0.832	96
7.	**G**	15.50±0.144	30.85±0.298	43.51±0.403	54.69±0..249	91
8.	**H**	17.50±0.393	31.07±0.430	45.97±0.393	61.59±0.572	83
9.	**I**	35.06±0.684	67.19±0.510	82.13±0.457	90.16±0.593	37
10.	**J**	38.23±0.606	71.15±0.433	86.26±0.524	91.86±0.588	34
11.	**BHT**	12.35±0.360	25.72±0.079	58.81±0.303	97.32±0.553	<50

Table 3 Antioxidant activity by Nitric oxide radical scavenging assay

S. No.	Compounds	%RSC				IC_{50}
		25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	
1.	**A**	9.21±0.17	15.69±0.21	24.81±0.29	41.06±0.06	>100
2.	**B**	20.91±0.28	31.15±0.41	42.28±0.18	53.02±0.07	93
3.	**C**	16.91±0.34	30.58±0.19	41.58±0.22	52.63±0.27	95
4.	**D**	21.46±0.18	32.44±0.13	45.12±0.42	56.42±0.31	89
5.	**E**	28.49±0.19	54.81±0.21	74.48±0.28	84.19±0.15	41
6.	**F**	12.49±0.06	19.96±0.09	35.82±0.05	59.96±0.03	91
7.	**G**	17.90±0..08	32.10±0.18	44.08±0.13	67.15±0.11	82
8.	**H**	32.71±0.07	51.65±0.16	68.92±0.22	82.15±0.26	46
9.	**I**	36.24±0.09	60.81±0.21	74.39±0.25	85.56±0.17	40
10.	**J**	41.43±0.23	66.28±0.16	79.80±0.09	91.73±0.25	44
11.	**BHT**	13.30±0.04	27.65±0.17	59.61±0.32	96.98±0.61	<50

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