Heterocyclic Letters
Vol. 11| No.1|39-44| Nov-Jan |2021
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

ANTIOXIDANT EVALUATION OF NEW 1,3,4-OXADIAZOLE DERIVATIVES

Banylla Felicity Dkhar Gatphoh, B.C. Revanasiddappa*,

**Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences of Nitte -Deemed to be University, Paneer, Deralakatte, Mangalore-575 018,Karnataka, India*
Email: revan@nitte.edu.in

Abstract
A new series of 1,3,4-oxadiazoles **(AO1-8)** were synthesized by the reaction of p-toluic benzhydrazide **(1)** and substituted aromatic acids **(2)** in the presence of phosphorus oxychloride and tested for their *In-vitro* antioxidant potential. The structures of the newly synthesized compounds were assigned on the basis of IR, ^{1}H-NMR, and Mass spectral data.. The compound **AO2** bearing OH group showed potent DPPH scavenging, Nitric oxide scavenging activity when compared to other synthesized derivatives of 1,3,4-oxadiazoles. Ascorbic acid was used as standard antioxidnat agent for comparison purpose.

Keywords: Oxadiazole, p-toluic benzhydrazide, phosphorus oxychloride, anti-oxidant activity, DPPH assay.

Introduction
It is a fact reported that oxygen and nitrogen bearing molecules are principally utilize in medicine to prevent and treat various kinds of bacterial and fungal contamination along with the treatment of gastric ulcers, cancer, and so on[I]. The organic amalgam bearing nitrogen atom have reported to show greater effectiveness against various diseases[II].
1,3,4-Oxadiazole nucleus, enticed the chemists' wide attention in search of new curative molecules [III], owning to their chemistry as simple five-membered heterocycles bearing two nitrogen and one oxygen atom. Oxadiazoles have been effectively put into trails against many disorders and diseases hence, obtained an exceptional attention in the pharmaceutical fields due to their immense medicinal likelihoods. The 1,3,4-Oxadiazole nucleus have four isomeric forms such as 1,2,3- oxadiazole, 1,2,4-oxadiazole, 1,2,5- oxadiazole, and 1,3,4-oxadiazoles [IV]. Among the four possible isomers, 1,3,4-oxadiazoles and 1,2,4 -oxadiazoles are widely explored for various clinical applications. They have often been taken up to develop lead molecules and optimize the moiety to produce potentially less-toxic drugs [V].
Substituted 1,3,4- oxadiazole analogs have played an essential role in pharmaceutical and agrochemical fields. Diverse pharmacological and biological activities such as CNS depressant [VI], anticonvulsant [VII], antitubercular [VIII], herbicidal [IX], anti-inflammatory [X], viricidal [X1], neuroprotective [XII] effects have been reported by various researchers using oxadiazole

derivatives. Therefore, 1,3,4-oxadiazoles have fascinated researchers globally to work in this stretch of new drug development. Much indignation was undertaken to synthesize these compounds by engaging conservative methods, initiating new innovatory approaches and procedures, getting to the target molecules, and studying their pharmacological applications.

Materials and Methods

Melting points of the compounds were recorded by the open capillary method and are uncorrected. The ^{1}H-NMR spectra were recorded on Agilent FT-NMR Spectrometer (400 MHz, Model: 400MR DD_2) in $CDCl_3$ and DMSO with TMS as an internal standard and values are expressed in δ ppm. Waters LC-MS Mass spectrometer was used to record the mass spectra. IR spectra were obtained on Alpha Bruker IR Spectrometer with a KBr disc. All the compounds were checked for their homogeneity using silica gel-G plates.

Synthesis of 1,3,4-oxadiazole derivatives (AO1-O8)

p-toluic benzhydrazide(0.01mol) **(1)** and substituted aromatic acids **(2)** (0.01mol) was taken in round bottom flask and $POCl_3$ (8 mL) was added to the above reaction mixture and refluxed for 10-18hrs using an oil bath. After refluxing , the contents were poured into the crushed ice and stirred vigorously. The crude product obtained was neutralized with $NaHCO_3$ (15 mL), washed with water, filtered and recrystallized from alcohol. The physical data of the compounds **(AO1-8)**is given in Table-1.

2-(4-nitrophenyl)-5-p-tolyl-1,3,4-oxadiazole (AO1): Light yellow solid, % yield: 81.M.P: 189-191. FT-IR (KBr, v, cm^{-1}): 1110 (C-O-C), 1493 (C=C), 1606 (C=N), 2872 (C-H). ^{1}H-NMR (DMSO-d_6, 400 MHz,) δ ppm= 2.35 (s, CH_3, 3H), 6.84-8.03(m, Ar-H, 8H). MS (m/z): 281.27 (M+).

4-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenol (AO2) : White cream solid solid, % yield: 84. M.P: 208-210. FT-IR (KBr, v, cm^{-1}): 1103 (C-O-C), 1556 (C=C), 1606 (C=N), 2994 (C-H), 3318(OH). ^{1}H-NMR (DMSO-d_6, 400 MHz,) δ ppm= 2.38 (s, CH_3, 3H), 6.76-8.09(m, Ar-H, 8H), 10.28 (s, OH, 1H). MS (m/z): 252.27 (M+).

4-(5-p-tolyl-1,3,4-oxadiazol-2-yl)aniline (AO3): :Dark brown solid, % yield: 79. M.P: 228-230. FT-IR (KBr, v, cm^{-1}): 1021 (C-O-C), 1531 (C=C), 1611 (C=N), 3053 (C-H), 3312 (NH). ^{1}H-NMR (DMSO-d_6, 400 MHz,) δ ppm= 2.37 (s, CH_3, 3H), 6.76 (s, NH_2, 2H), 7.29-8.45(m, Ar-H, 8H). MS (m/z): 251.28 (M+).

2-phenyl-5-p-tolyl-1,3,4-oxadiazole (AO4) :White cream solid, % yield: 83. M.P: 214-216.FT-IR (KBr, v, cm^{-1}): 1016 (C-O-C), 1544 (C=C), 1623 (C=N), 3029 (C-H) ^{1}H-NMR (DMSO-d_6, 400 MHz,) δ ppm= 2.39 (s, CH_3, 3H), 7.40-8.11(m, Ar-H, 8H). MS (m/z): 236.27 (M+).

2-(5-p-tolyl-1,3,4-oxadiazol-2-yl)phenol (AO5): Light brown solid, % yield: 86. M.P: -172-174. FT-IR (KBr, v, cm^{-1}): 1087(C-O-C), 1532 (C=C), 1606 (C=N), 3092 (C-H), 3403(OH) ^{1}H-NMR (DMSO-d_6, 400 MHz,) δ ppm= 2.35 (s, CH_3, 3H), 6.84-8.03(m, Ar-H, 8H), 12.97(s, OH, 1H). MS (m/z): 252.27 (M+).

2-(2-bromophenyl)-5-p-tolyl-1,3,4-oxadiazole (AO6): White cream solid, % yield: 80. M.P: 240-242. FT-IR (KBr, v, cm^{-1}): 1049 (C-O-C), 1554 (C=C), 1584(C=N), 3071 (C-H^{1}H-NMR (DMSO-d_6, 400 MHz,) δ ppm=2.36 (s, CH_3, 3H), 7.43-8.07(m, Ar-H, 8H), MS (m/z): 315.16 (M+).

2-(3,5-dinitrophenyl)-5-p-tolyl-1,3,4-oxadiazole (AO7): Light yellow solid, % yield: 78. M.P: 194-196. FT-IR (KBr, v, cm^{-1}): 1053 (C-O-C), 1553 (C=C), 1593 (C=N), 3029 (C-H). ^{1}H-NMR (DMSO-d_6, 400 MHz,) δ ppm= 2.30 (s, CH_3, 3H), 7.20-9.19(m, Ar-H, 7H). MS (m/z): 326.26 (M+).

2-(2,4-dichlorophenyl)-5-p-tolyl-1,3,4-oxadiazole (AO8): Light yellow solid, % yield: 81. M.P: 256-258ºC. IR (KBr, v, cm^{-1}): 1069 (C-O-C), 1546 (C=C), 1607(C=N), 3029 (C-H).

^{1}H-NMR (DMSO-d_6, 400 MHz,) δ ppm= 2.40 (s, CH_3, 3H), 7.29-8.16(m, Ar-H, 7H). MS (m/z): 305.16 (M+).

Table I: Physical data of 1,3,4-oxadiazole derivatives (AO1-8)

Comp	Ar-COOH	Molecular Formula	Molecular Weight	MP (ºC)	Yield (%)
AO1	4-NO_2	$C_{15}H_{11}N_3O_3$	281.27	189-191	66
A02	4-OH	$C_{15}H_{12}N_2O_2$	252.27	208-210	68
A03	4-NH_2	$C_{15}H_{13}N_3O$	251.28	228-230	69
A04	C_6H_5	$C_{15}H_{12}N_2O$	236.27	214-216	71
A05	2-OH	$C_{15}H_{12}N_2O_2$	252.27	172-174	69
A06	2-Br	$C_{15}H_{11}BrN_2O$	315.16	240-242	68
A07	3,5-$(NO_2)_2$	$C_{15}H_{10}N_4O_5$	326.26	194-196	69
A08	2,4-$(Cl)_2$	$C_{15}H_{10}Cl_2N_2O$	305.16	256-258	72

In-vitro Antioxidnat activity

DPPH radical scavenging assay [X111]

Reagents prepared:

Reagent 1: 0.2mM solution of 2, 2-diphenyl-1- picrylhydrazyl (DPPH) in methanol

Test samples: 10-50 µg/ml concentration of compounds A01-A08.

Control: DPPH without samples.

Standard: Ascorbic acid.

Procedure: Test samples at different concentrations between 10-50 µg/ml were prepared in DMSO and added to 100µL of 0.2mM solution of DPPH in methanol. The above reaction mixture was stirred vigorously upon the adding DPPH, which was then incubated at 25ºC for 30minutes. The absorbance was assessed of the control, test, and the standard at 517nm. The DPPH radical scavenging assays were analysed in triplicates and deliberated according using the following formula. The antioxidant capacity of the tested compounds was expressed as IC_{50} shown on Table-2.

$$\text{DPPH scavenging \%} = \left[\frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\right] \times 100$$

Nitric oxide radical scavenging assay [X111]

Reagents prepared:

Reagent 1: Sodium nitroprusside (1mL of 10mM)

Reagent 2: Griess's reagent containing 1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride.

Test samples: 10-50 µg/ml concentration of compounds A01-A08.

Control: Sodium nitroprusside and Griess's reagent without samples.

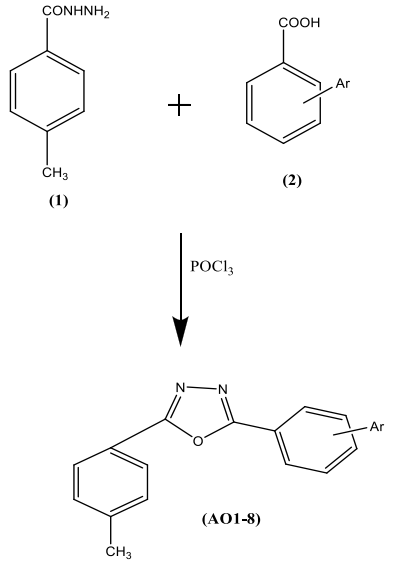
Standard: Ascorbic acid.

Procedure: Test samples at different concentrations between 10-50 µg/ml was prepared in DMSO and added to 1mL of 10mM Sodium nitroprusside. The reaction mixture was stirred vigorously upon addition of Sodium nitroprusside, which was then incubated at 25°C for 150 minutes. After150 minutes, 1mL of Griess's reagent was the added to the above solution. The absorbance was assessed of the control, test and standard at 546 nm. The NO radical scavenging assays were analysed in triplicates and deliberated according to the above formula. The antioxidant capacity of the tested compounds was expressed as IC_{50} shown on Table-2.

Results and Discussion

This work's objective was to synthesize new 1,3,4-oxadiazoles **(AO1-8)** by using $POCl_3$ as a cyclizing agent with p-toluic benzhydrazide and substituted aromatic acids. All the new compounds complied with the structures anticipated. The reaction sequence of the title compounds is shown in **Scheme-01.** Spectral data demonstrated the structure. The physicochemical data of the compounds is given in Table-1. The yield of the new compounds was found to be in appreciable limits. Purity of the compounds was checked by monitoring the TLC and compounds were further purified by recrystallization technique.

Scheme-01



DPPH(2,2-diphenyl-1-picrylhydrazyl), and nitric oxide scavenging assay were used to measure the antioxidant potential of the newly synthesized 1,3,4-oxadiazole derivatives. All the new compounds were tested at a concentration of 10-50µg/ml.. Ascorbic acid was used as standard in both the methods. IC_{50} values of the new compounds is showed in Table-2.

In the DPPH assay, most of the tested compounds exhibited moderate activity. The compound **(A08)** possessing 2,4-$(Cl)_2$ attached to the oxadiazole ring exhibited scavenging activity with IC_{50} values of 28.92 µg/ml compared to standard. The compounds **(A02, 06)** were found to interact with DPPH strongly may be due to the presence of electron-donating groups attached to the oxadiazole ring. The IC_{50} values for these compounds were 22-25µg/ml, while that of standard was 23 µg/ml.

Compound **AO2** results in the highest antioxidant activity, which shows the 1,3,4-oxadiazole portion's involvement along with the two aromatic rings in resonance stabilization of the phenolic radical formed.

In the nitric oxide assay, most of the tested compounds exhibited moderate activity Nitric oxide scavenging activity can be determined by the Griess IlIosvoy method. The capacity to scavenge nitric oxide was determined spectrophotometrically for the synthesized compound on the basis of inhibition. based on the results, we can conclude that electron-donating groups plays a major role in inhibiting nitric acid production. Compounds **AO2,05** showed good inhibitory action when compared to the standard. The electron donating groups are showing very good activity. The IC_{50} values for these compounds were 24-26µg/ml.

Table-2: Antioxidant data of 1,3,4-oxadiazole derivatives (AO1-8)

	IC_{50} Value	
Comp	**DPPH assay**	**Nitric oxide assay**
AO1	38.20	36.8
AO2	**22.70**	**24.00**
AO3	54.70	56.47
AO4	46.03	47.63
AO5	31.71	**26.56**
AO6	**25.62**	34.5
AO7	43.64	41.32
AO8	**28.92**	**29.14**
STD (Ascorbic acid)	23.75	23.14

Conclusion

In conclusion a new series of 1,3,4-oxadiazoles were synthesized by a convenient method from p-toluic benzhydrazide and substituted aromatic acids and their antioxidant potential was studied. Some of the tested compounds showed moderate to significant antiradical potential. Hence, the compounds can be further evaluated for various other pathological activities lead by free radicals.

Acknowledgements

The authors of thankful to authorities of NGSM Institute of Pharmaceutical Sciences, Nitte-deemed to be university, Mangalore for providing all the necessary facilities. The authors are thankful to Institute of excellence, University of Mysore, Mysore for providing spectral data.

References

I. Saini MS, Kumar A, Dwivedi J, Singh R. A review: biological significances of heterocyclic compounds. Int. J. Pharm. Sci. Res. 2013; 4(3):66-77.

II. Asif M. A mini review: biological significances of nitrogen hetero atom containing heterocyclic compounds. Int. J. Bioorg. Chem. 2017; 2(3):146-52.

III. Waghamale S, Piste P. Pharmacological activities of triazole, oxadiazole and thiadiazole. Int J Pharm Bio Sci. 2013;4(3):310-32.

IV. Boström J, Hogner A, Llinàs A, Wellner E, Plowright AT. Oxadiazoles in medicinal chemistry. J. Med. Chem. 2012; 55(5):1817-30.

V. Zarghi A, Hajimahdi Z. Substituted oxadiazoles: A patent review (2010–2012). Expert opinion on Therapeutic Patents. 2013; 23(9):1209-32.

VI. Barthwal JP, Tandon SK, Agarwal VK, Dixit KS, Parmar SS. Relationship between CNS depressant and enzyme inhibitory properties of substituted quinazolone 1, 3, 4-oxadiazoles. J Pharma Sci. 1973; 62(4): 613-7.

VII. Yar MS, Akhter MW. Synthesis and anticonvulsant activity of substituted oxadiazole and thiadiazole derivatives. Acta Pol. Pharm. 2009; 66(4): 393-7.

VIII. Karabanovich G, Němeček J, Valášková L, Carazo A, Konečná K, Stolaříková J,

Hrabálek A, Pavliš O, Pávek P, Vávrová K, Roh J. S-substituted 3, 5-dinitrophenyl 1, 3, 4-oxadiazole-2-thiols and tetrazole-5-thiols as highly efficient antitubercular agents. Eur J Med Chem. 2017;126:369-83.

IX. Duan WG, Li XR, Mo QJ, Huang JX, Cen B, Xu XT, Lei FH. Synthesis and herbicidal activity of 5-dehydroabietyl-1, 3, 4-oxadiazole derivatives. Holzforschung. 2011; 65(2):191-7.

X. Nargund LV, Reddy GR, Hariprasad V. Anti-inflammatory activity of substituted 1, 3, 4-oxadiazoles. J Pharma Sci. 1994; 83(2): 246-8.

XI. El-Sayed WA, Fathi NM, Gad WA, El-Ashry ES. Synthesis and Antiviral Evaluation of Some 5-N-Arylaminomethyl-2-glycosylsulphanyl-1, 3, 4-oxadiazoles and their analogs against hepatitis a and herpes simplex viruses. J Carbohydrate Chem. 2008; 27(6):357-72.

XII. Mei WW, Ji SS, Xiao W, Wang XD, Jiang CS, Ma WQ, Zhang HY, Gong JX, Guo YW. Synthesis and biological evaluation of benzothiazol-based 1, 3, 4-oxadiazole derivatives as amyloid β-targeted compounds against Alzheimer's disease. Monatsheftefür Chemie. 2017; 148(10):1807-15.

XIII. Reshma S, Boja Poojary, Revanasiddappa BC, Hemanth K, Vijay KM.Novel [1,2,4]triazolo[3,4-b][1,3,4]thiadiazine derivatives embedded with benzimidazole moiety as potent antioxidants.J Chinese Chem Soc. 2020: 1-13.

Received on August 21, 2020