Canadian Chemical Transactions

ISSN 2291-6458 (Print), ISSN 2291-6466 (Online)
Year 2014 | Volume 2 | Issue 4 | Page 518-525

Research Article

DOI:10.13179/canchemtrans.2014.02.04.0136

In Vitro Antioxidant Activity of 1-[5-(4-Methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine Derivatives

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Received: *May 31, 2014* ***Revised:*** *July 26, 2014* ***Accepted:*** *August 4, 2014* ***Published:*** *August 6, 2014*

Abstract: A series of new 1-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine derivatives **2(a-g)** were screened for their antioxidant activity by 2,2-diphenyl-1-picryl-hydrazyl (DPPH$^{\bullet}$), hydroxyl radical (OH$^{\bullet}$) and nitric oxide radical (NO$^{\bullet}$) methods. Butylated Hydroxytoluene (BHT) was used as standard. All the compounds showed activity, where compound **2c** was the best radical scavenger. These findings show that the 2,5-disubstituted-1,3,4-oxadiazole possesses antioxidant activity with different mechanism of actions towards the different free radicals tested.

Keywords: 1,3,4-Oxadiazole; DPPH; Hydroxyl radical; Nitric oxide

1. INTRODUCTION

Free radical contains an odd number of electrons which makes it unstable, short lived and highly reactive. Therefore, it reacts quickly with other compounds in order to capture the needed electron to gain stability. Generally, free radical attacks the nearest stable molecule, stealing its electron. When the attacked molecule loses its electron, it becomes a free radical itself, beginning a chain reaction cascade resulting in disruption of a living cell [1, 2]. There are two basic categories of antioxidants, namely, synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be phenolic compounds, nitrogen compounds as well as ascorbic acid [3, 4]. The primary antioxidants comprise essentially sterically hindered phenols and secondary aromatic amines [5]. These antioxidants act usually both through chain transfer and chain termination. The first step of the reactive radical's termination by this type of antioxidants is hydrogen atom transfer from the antioxidant molecule to the reactive radical intermediate [6]. In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma while lipid-soluble antioxidants protect cell membranes from lipid peroxidation [7]. These compounds may be synthesized in the body or obtained from the diet [8].

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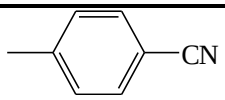
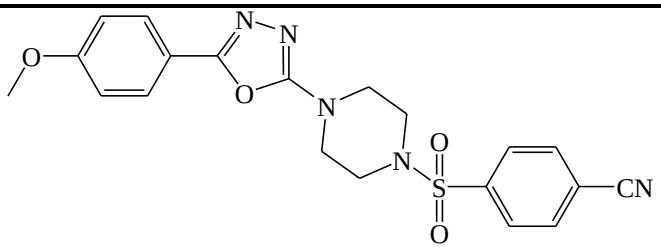
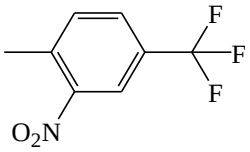
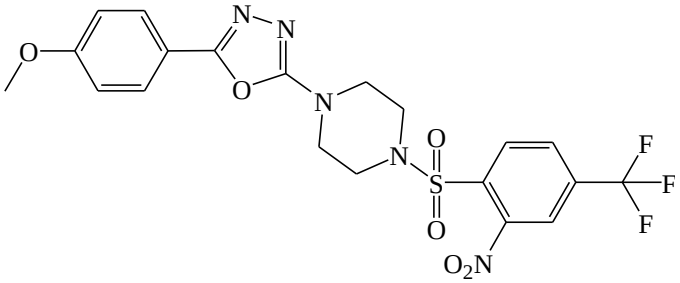
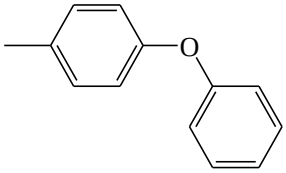
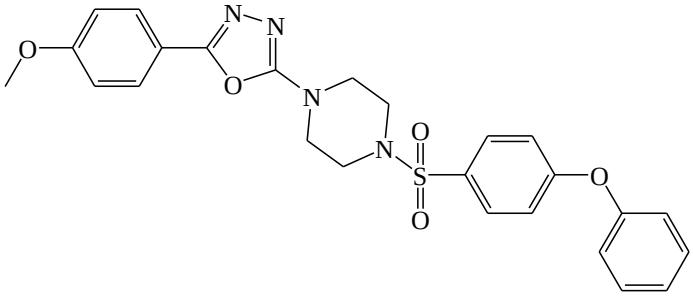
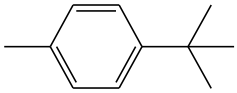
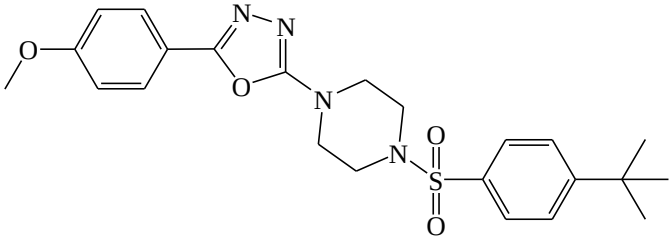
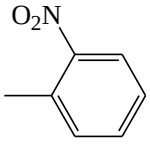
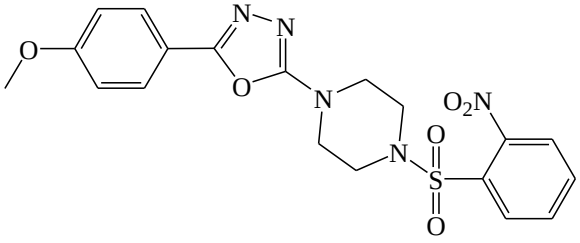
Table 1. Chemical structure of 1-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine derivatives **2(a-g)**

Compound	R	Structure
2a		
2b		
2c		
2d		
2e		

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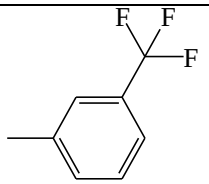
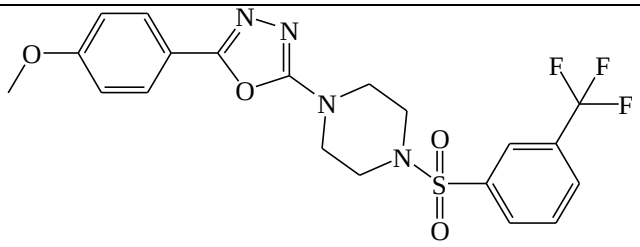
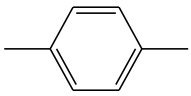
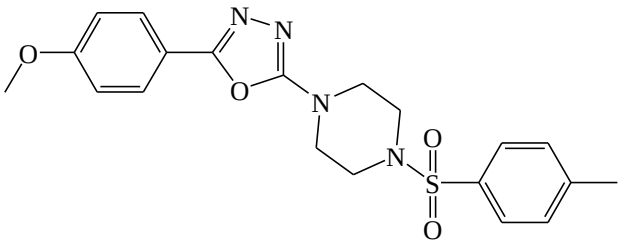
2f		
2g		

Phenolic derivatives are one of the groups of antioxidants that have been studied by many research groups. A great number of examples have been described in the literature, such as caffeic acid and its analogues, which are known to have antiviral and antiatherosclerotic properties [9], resveratrol with known anticancer and heart protecting effects [10] and olive oil phenols, particularly hydroxyl tyrosol which inhibits human low-density lipoprotein (LDL) oxidation (a critical step in atherosclerosis) [11] inhibits platelet aggregation [12] and exhibits anti-inflammatory [13] and anticancer properties [14]. Phenols have been utilized extensively for food preservation. The main structural feature responsible for the antioxidative and free radical scavenging activity of phenolic derivatives is the phenolic OH-group. Phenols are able to donate the hydrogen atom of the phenolic OH to the free radicals, thus stopping the propagation chain during the oxidation process. In this respect, the present paper reports the antioxidant activity of 1-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine derivatives.

2. EXPERIMENTAL

All solvents and reagents were purchased from Sigma-Aldrich, India**.**

2.1. Antioxidant activity

2.2.1. DPPH radical scavenging assay

DPPH radical scavenging activity was carried out according to Scherer R et al. method. [15] Briefly, 1ml of DPPH solution (0.1mM in 95% ethanol) was mixed with different aliquots of 2-10µg of the tannin sample. After vigorous shaking, the mixture was allowed to stand for 20 min at room temperature. Absorbance of the resulting solution was measured at 517 nm with a UV-VIS spectrophotometer (HITACHI, U-2900) Butylated hydroxyl toluene (BHT) was used as positive control. Radical scavenging potential was expressed as EC_{50} value, which represents the sample concentration at which 50 % of the DPPH radicals were scavenged.

2.2.2. Hydroxyl radical scavenging assay

The reaction mixture containing different aliquots of 2-10 µg of the tannins, deoxyribose (10mM), H_2O_2 (10mM), $FeCl_3$ (5mM), EDTA (1mM) and ascorbic acid (5mM) in potassium phosphate buffer (50mM,pH 7.4) was allowed to stand for 60 min at 37 ^oC [16]. The reaction product was boiled in Trichloroacetic acid (TCA, 5%) and Thiobarbituric acid (TBA, 0.2%) for 15 min and then cooled. The absorbance was measured at 535nm against the reagent blank and inhibition of the oxidation of

ISSN 2291-6458 (Print), ISSN 2291-6466 (Online)
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deoxyribose was calculated against the control using UV-VIS spectrophotometer (HITACHI, U-2900). BHT was used as positive control and radical scavenging potential was expressed as EC_{50} value.

2.2.3. Nitric oxide radical scavenging activity

Nitric oxide was generated from sodium nitroprusside and measured by Griess reaction [17]. Sodium nitroprusside in phosphate buffer at physiological pH spontaneously generates nitric oxide, which in turn reacts with oxygen to produce nitrite ions that can be estimated by the Griess reagent [18]. Nitric oxide scavengers compete with oxygen, leading to reduced production of nitric oxide. Sodium nitroprusside (5mM) in phosphate buffered saline was mixed with different aliquots of 2-10 µg of tannins and incubated at 25 ºC for 3 hrs. The absorbance of the color formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm and referred to the absorbance of BHT treated in the same way with the Griess reagent. The radical scavenging potential was calculated and expressed as EC_{50} value.

Table 2. DPPH radical scavenging activity of the tested compounds

Compound	Scavenging effect (%)					IC_{50} (µg/ml)
	Concentration of the tested compounds (µg/ml)					
	20	40	60	80	100	
2a	64.9	65.8	66.0	67.8	76.9	39.0
2b	65.6	66.9	68.5	69.8	76.9	35.0
2c	70.8	73.9	78.9	89.9	93.8	46.0
2d	67.2	67.8	68.9	72.9	76.8	39.0
2e	70.9	76.9	88.6	90.3	92.7	45.5
2f	70.9	76.5	87.4	88.4	89.9	44.5
2g	50.9	71.8	77.9	83.9	90.8	45.0
BHT	75.9	80.9	90.8	93.7	96.8	47.5

3. RESULTS AND DISCUSSION

The chemical structures of all the synthesized compounds are tabulated in Table 1. The synthetic routes for the target compounds are illustrated in Scheme 1 [19]. The *in vitro* scavenging assay was performed spectrophotometrically with BHT as positive control. Percentages of radical scavenging activity were tabulated in Tables 2-4. All the compounds showed antioxidant activity. The percentage scavenging effect of the compound **2c** at 100 µg/ml is 92.9 %. The compound **2c** showed higher radical inhibition activity. Antioxidant activity of new 4-aryl-1-(1,4-benzodioxane-2-carbonyl)piperazines has been reported [20].

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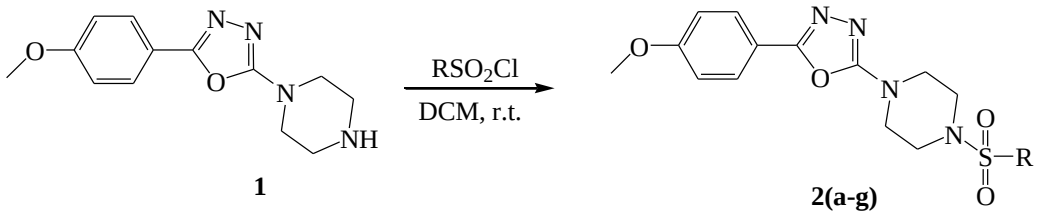

Scheme 1

Table 3. Nitric oxide radical scavenging activity of the tested compounds

Compound	Scavenging effect (%)					IC_{50} (µg/ml)
	Concentration of the tested compounds (µg/ml)					
	20	40	60	80	100	
2a	52.9	75.9	83.9	86.9	90.2	42.0
2b	60.9	73.9	84.5	87.9	89.9	42.0
2c	74.9	76.9	84.4	92.4	93.9	47.5
2d	72.9	74.8	85.9	87.9	89.9	45.0
2e	74.9	76.9	89.6	90.3	92.7	46.0
2f	71.9	77.8	83.5	90.8	92.9	45.0
2g	64.9	79.8	86.9	89.9	92.8	45.0
BHT	76.9	80.8	90.9	93.9	94.9	48.0

Natural antioxidants are characterized by their ability to scavenge free radicals. Proton-radical scavenging action is an important attribute of antioxidants, which is measured by the DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay. DPPH, a protonated radical has significant absorbance maxima at 517 nm which decreases in the presence of antioxidant due to the scavenging of the proton radical [21]. Hydrogen donating ability of the antioxidant molecule contributes to its free radical scavenging potential. The DPPH radical scavenging activity shown by the tannins is because of its H-donating capacity. In the present investigation showed high DPPH radical scavenging activity at the lowest concentration and are comparable with BHT. However, increase in activity was marginal with increase in concentration. Compound **2c** showed good antioxidant activity in scavenging DPPH radical assay with an IC_{50} value of 45.5 µg/ml when compared to 47.5 µg/ml recorded with BHT.

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology and is capable of damaging

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Table 4. Hydroxyl radical scavenging activity of the tested compounds

Compound	Scavenging effect (%)					IC_{50} (µg/ml)
	Concentration of the tested compounds (µg/ml)					
	20	40	60	80	100	
2a	62.7	71.8	79.9	84.8	90.9	45.0
2b	63.6	70.9	75.5	87.9	92.9	43.0
2c	67.9	75.9	82.9	89.9	95.8	47.0
2d	66.9	72.8	78.9	86.9	91.8	45.0
2e	64.2	74.3	84.6	88.3	93.7	45.0
2f	65.3	73.5	83.4	90.3	93.9	45.0
2g	61.5	77.8	81.5	86.7	92.8	45.0
BHT	69.8	78.9	89.8	93.7	96.9	49.0

biomolecules found in living cells [22]. Hydroxyl radical has the capacity to cause breakage of DNA, which results in cytotoxicity, carcinogenesis and mutagenesis. In addition, this radical species is considered to be one of the quick initiators of the peroxidation of the lipid, removing hydrogen atoms from unsaturated fatty acids. Compound **2c** showed significant activity in scavenging hydroxyl radical assay with an IC_{50} value of 45.0 µg/ml when compared to 49.0 µg/ml recorded with BHT.

Under physiological condition, nitric oxide (NO) plays important role as a neurotransmitter, vasodilator and in the immunological system it fights against tumor cells and infectious agents. During inflammatory reactions, NO is produced by the inducible enzyme NO synthase (iNOS) in cells like macrophages, hepatocytes and renal cells after the stimulation with lipopolysaccharide (LPS), tumor necrosis factor (TNF-α), interleukin (IL-1) or interferon (INF-γ) and acts as a defense and regulatory signal molecule. However, NO is pathogenic when present in excess. NO per se as a reactive radical, directly damages normal tissues [23]. Further, nitric oxide can also react with superoxide anion radical to form an even stronger oxidant peroxy nitrite [24]. Compound **2c** showed significant activity in scavenging nitric oxide radical assay with an IC_{50} value of 47.5 µg/ml when compared to 48.0 µg/ml recorded with BHT.

5. CONCLUSION

In conclusion, a series of 1-[5-(4-methoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-piperazine derivatives **2(a-g)** were screened for their antioxidant activity have been evaluated. All the compounds showed radical scavenging activity, where compound **2c** was the best radical scavenger.

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ACKNOWLEDGEMENTS

One of the authors (NDR) grateful to JSS College of Arts, Commerce and Science, Mysore, to carryout antioxidant activity.

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The authors declare no conflict of interest

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