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Synthesis and evaluation of phenyl substituted sydnones as potential DPPH-radical scavengers†

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A series of phenyl substituted sydnones has been synthesized and their radical-scavenging activity has been studied on DPPH free radical. Out of eighteen compounds screened, nine compounds show interesting activity. A mechanism is presented whereby sydnones scavenge DPPH radical through donating H-atom at 4th-position. Its strong radical-scavenging activity mainly arises from 1, 2, 3-oxadiazolium-5-olate ring. Different substituents and their positions on the phenyl ring differently influence DPPH scavenging activity and therefore, may provide clues to design and develop better free-radical scavenging sydnones with multiple activities.

Keywords: Sydnones, DPPH radical, oxidative stress

Hyper physiological burden of free radicals causes imbalance in homeostatic phenomena between oxidants and antioxidants in the body. This imbalance leads to oxidative stress that is being suggested as the root cause of aging and various human diseases like atherosclerosis, stroke, diabetes, cancer and neurodegenerative diseases such as Alzheimer's disease and Parkinson's. Researches in the recent past have accumulated enormous evidence advocating enrichment of body systems with antioxidants to correct vitiated homeostasis and prevent the onset as well as treat the diseases caused and/or fostered due to free-radicals and related oxidative stress. Therefore, molecules possessing free-radical scavenging activities are being widely proposed as bases for the development of new approaches for pharmacological regulation of oxidative-antioxidative homeostatic imbalance[1,2].

Sydnones are unique, dipolar, heteroaromatic members of the general class of mesoionic compounds[3]. Their derivatives are associated with an array of physiological activities, like anti-inflammatory[4,5], analgesic[6], antipyretic[7], antitumor[8] and antiarthritic[9]. It is also reported that the ionic resonance structures of the heterocyclic ring of sydnones promote significant interactions with biological molecules[7], which also fulfill many of the spatial and electronic requirements ascribed to their biological activities[10].

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a widely used free radical for identifying free-radical scavengers of vivid origin. These free-radical scavengers, tested on DPPH, have been found extremely effective in cell systems of oxidative stress, used to test anticancer agents[11]. The oxidative modifications especially of low-density lipoprotein (LDL), play critical role in atherogenesis, therefore, antioxidants mitigating oxidative modification of LDL possess antiatherogenic potential[12]. It has been observed that, structural requirements for an antioxidant to mitigate LDL oxidation under different conditions are the same as to scavenge DPPH radical[13]. Similarly, increased levels of advanced glycation end-products (AGEs) are well known to be the cause of aging and diabetic complications. In this regard also, it has been reported that the AGEs formation inhibitory activities of several antioxidants are in accordance with their DPPH radical scavenging activity[14]. Therefore, DPPH as free-radical has became an useful, primary and simple test model in designing free-radical scavengers for the development of anticancer, antiatherosclarotic, antidiabetic therapeutics, *etc*. This report presents DPPH scavenging activity of a series of phenyl sydnones (3-aryl-5-oxido-1, 2, 3-oxadiazoliums), their structure-activity relationship and possible mechanism for DPPH scavenging.

Sydnone derivatives were prepared in three steps. The first step, a condensation, involved neutralizing an aqueous solution of chloroacetic acid with an

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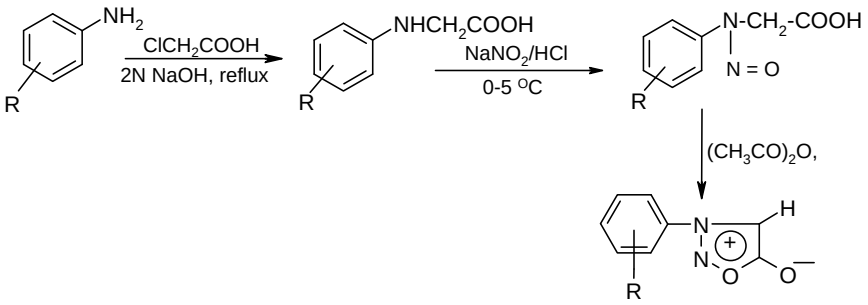


Scheme I

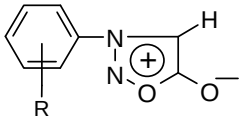
equimolar equivalent of 2*N* NaOH and adding this solution to an aqueous solution of amines over a period of 4 hr. This reaction mixture was heated for 2-48 hr and the clear liquor was then vacuum filtered while hot, to remove any decomposition products and refrigerated overnight. The resulting crystals were again filtered out to obtain phenyl glycines. In the second step, the resulting substituted phenyl glycines were mechanically stirred at 0-5ºC. An aqueous solution of sodium nitrite was then slowly added to the phenyl glycines over a period of 40 min and the solid was vacuum filtered, washed with water and dried overnight to obtain *N*-substituted-*N*-nitroso amino acids. The third step involved, cyclodehydration of *N*-substituted-*N*-nitroso amino acids with acetic anhydride[15,16] to yield sydnones **1-18** (**Scheme I**). All the compounds were recrystalized from ethanol and tested for DPPH radical scavenging activity[17].

Results and Discussion

It was observed that, 3-phenylsydnone **1** poorly scavenged DPPH radical (**Table I**), however, a methyl substitution at 2nd and 3rd position of the phenyl ring of sydnone improved the radical scavenging activity **2** and **3**. Surprisingly, it was observed that methyl substitution at 4th position could not improve radical scavenging activity of 3-phenyl sydnone **4**. Similar observations were made with methoxyl substitutions also, where substitution at 2nd and 3rd position increased the activity **5** and **6** and substitution at 4th position **7** could not influence DPPH scavenging activity of 3-aryl sydnones. Furthermore, dimethyl substitution at 2nd and 4th positions also, could not improve the activity **16**. However, dimethoxy substitution at 2nd and 5th positions of the phenyl ring increased the DPPH radical scavenging **17**. It was interesting to note that chloro substitution at the 5th position of phenyl ring of 3-(2, 4-dimethoxyphenyl) sydnone **18** enhanced the DPPH scavenging activity more than twice that of 3-(2,5-dimethoxyphenyl) sydnone **17** (**Table II**).

Furthermore, it was observed that, nitro-substitution at the 4th position of phenyl ring of sydnone **11** displayed more potent radical scavenging activity than substitution at 2nd position. Carboxyl substitution at 2nd position of phenyl ring of sydnone **8** displayed maximum radical scavenging activities

Table I — Free-radical scavenging activity of sydnones**.** Data presents values of DPPH scavenged from 25 µg concentration of each compound in the reaction medium in primary screening and values represent mean, SD of at least three readings.



1-18

Compd	R	% DPPH Scavenging activity
1	H	7.68 ± 0.31
2	2-CH_3	12.76 ± 0.32
3	3-CH_3	34.95 ± 1.29
4	4-CH_3	8.55 ± 2.82
5	2-OCH_3	26.36 ± 0.52
6	3-OCH_3	28.05 ± 1.78
7	4-OCH_3	7.95 ± 2.94
8	2-COOH	69.44 ± 1.85
9	4-COOH	14.82 ± 4.99
10	2-NO_2	34.22 ± 1.78
11	4-NO_2	54.10 ± 3.94
12	3-Cl	10.46 ± 0.04
13	4-Cl	35.90 ± 0.86
14	4-Br	5.84 ± 2.47
15	4-F	8.44 ± 0.17
16	2, 4-$(CH_3)_2$	9.18 ± 1.68
17	2, 5-$(OCH_3)_2$	38.8 ± 4.23
18	2, 4-$(OCH_3)_2$, 5-Cl	55.24 ± 0.24

among the eighteen sydnones tested (**Tables I** and **II**). In case of halides, only chloro substitution **12** and **13** could display mild to moderate radical scavenging activity respectively.

It has been reported that, alkyl and aryl substitution at the 3rd position of sydnone ring considerably affects the charge density pattern around the sydnone nucleus[18]. It appears from this report therefore, that substitution of different groups on phenyl ring of sydnones like methyl, methoxy, carboxyl, nitro and halides affects differently the charge density pattern around the sydnone nucleus and hence, impart different radical-scavenging potentials with respect to their positions on the phenyl ring attached to sydnone molecule.

Recently, Wang *et al.*[19] has proposed two mechanisms for antioxidants to scavenge DPPH radical: first is a direct H-atom abstraction process (Eqn.1) and the second, concerted electron-transfer process (Eqn. 2).

$$DPPH^{\bullet}+RXH \rightarrow DPPHH+RX^{\bullet} \quad \ldots(1)$$

$$DPPH^{\bullet}+RXH \rightarrow DPPH^{-}+RXH^{\bullet+} \rightarrow DPPHH+RX^{\bullet} \quad \ldots(2)$$

In which, X represents O, N, S or C.

Table II — Concentrations of the potent compounds required to scavenge 50% DPPH radical ($SC_{50\%}$) in reaction medium. Values were determined by linear regression analysis using at least five different concentrations in triplicate and represent mean of the data

Compd	$SC_{50\%}$ (μM)
8	0.11
11	0.21
17	0.44
18	0.16

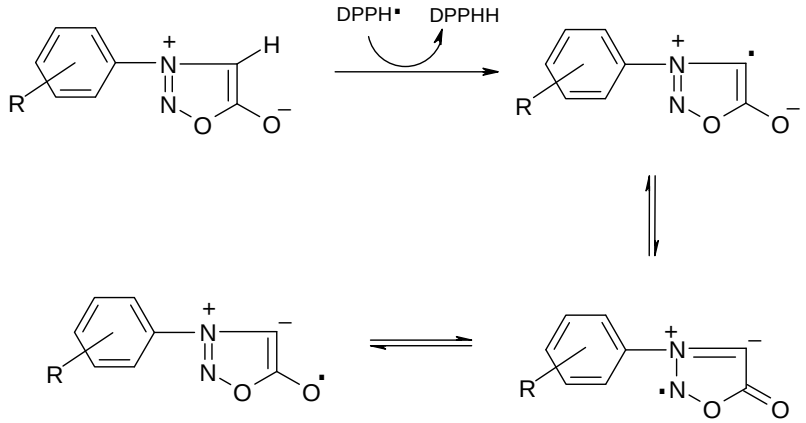
On the basis of this mechanism, we propose that, sydnone scavenges DPPH radical through donating the H-atom at 4th position (**Scheme II**) and its strong radical-scavenging activity mainly arises from 1,2,3-oxadiazolium-5-olate ring. Furthermore, different substituents attached to sydnone nucleus may influence considerably DPPH radical scavenging, therefore, may provide new clues to design and modify sydnones to give better radical-scavenging activity for future development as therapeutic agents for diseases of oxidative stress origin.

Experimental Section

General. The spectra were recorded with the following instruments: IR, Perkin-Elmer spectrum RX I FT-IR; NMR, Gemini-200 MHz; EIMS, VG-Micro mass 7070H. Column Chromatography was performed with silica gel (Acme, 100-200). Monitoring of reactions was carried out by using Merck 60 F 254 silica gel, glass-supported TLC plates and visualization with UV light (254, 365 nm). Melting points were measured with a Buchi-510 apparatus and are uncorrected.

Determination of the scavenging effect on 1,1-di-phenyl-2-pycryl-hydrazyl radical (DPPH•)

Assay was modified suitable for micro plate reading. In brief, in a 96-well micro plates, 25 μL test sample dissolved in dimethyl sulfoxide (DMSO, AR grade), 125 μL of 0.1 *M* tris-HCl buffer (*p*H 7.4) and 125 μL of 0.5 mm DPPH (1,1-diphenyl-2-picrylhydrazyl, sigma) dissolved in absolute ethyl alcohol were mixed and shaken well. After incubating 20 min in dark, the absorbance was recorded spectrophotometrically ($SPECTRA_{MAX}$ $PLUS^{384}$, Molecular Devices, USA) at 517 nm. The free radical



Scheme II

scavenging potential was determined as the percent decolorization of DPPH due to the test samples and calculated as (1-B/A) × 100, where A is absorbance of DPPH control with solvent and B absorbance of decolorized DPPH in the presence of test compound. The SC_{50} (50% radical scavenging concentration) of the test compound values were calculated by linear regression analysis. Probucol and trolox were taken as reference compounds. All the analysis was done in triplicate.

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