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Anti-oxidant activity of 2-amino-5-(3-arylsydnon-4-oyl) thiazoles

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ABSTRACT

In biochemistry and medicine, antioxidants are enzymes or other organic substances, such as vitamin E or β-carotene, that are capable of counteracting the damaging effects of oxidation in animal tissues". Because of these considerations and the wide variety seen in molecules that exhibit antioxidant ability through a multitude of mechanisms, AO activity may be interpreted in a variety of ways. Antioxidant activity of different 2-amino-5-(3-arylsydnon-4-oyl)thiazoles are studied by different methods like FRAP, β-carotene assay and DPPH assay and the results are presented.

Keywords— *Sydnones, Thiazoles, DPPH, FRAP, Beta-carotene assay*

1. INTRODUCTION

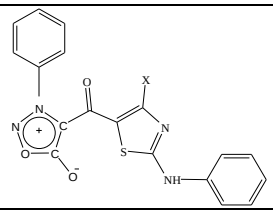
Antioxidants have significance in two broad areas in connection with humans: in respect of (i) chemicals and (ii) food and health. Thus, the exact implication of the word "antioxidant" seems to vary depending on the area of human, scientific or industrial discipline in which it is applied. In a food and health context, AO is a "synthetic or natural substances added to products to prevent or delay their deterioration by action of oxygen in air. In biochemistry and medicine, antioxidants are enzymes or other organic substances, such as vitamin E or β-carotene, that are capable of counteracting the damaging effects of oxidation in animal tissues". Because of these considerations and the wide variety seen in molecules that exhibit antioxidant ability through a multitude of mechanisms, AO activity may be interpreted in a variety of ways. As a consequence, a diverse set of methods are available for measuring AO activity. Methods for assaying and expressing AO activities involve either a direct or an indirect measurement of the rate or the extent of (i) the consumption of oxygen consumption, (ii) the disappearance of a probe or a substrate molecule, (iii) the formation of oxidation products or (iv) the formation or decay of probe free radicals.For the present study, we have chosen the FRAP assay, the β-carotene assay and the DPPH assay since our focus is on small-molecules that are expected to serve as synthetic AOs. Among these, the first and the third are SET type of assays whereas the second is a HAT type of assay.

2. RESULTS AND DISCUSSION

2.1 Antioxidant capacity by FRAP method

The method for measuring the Ferric Reducing Antioxidant power as modified by Rekha et al. was used for the present study. By this method, we first compared the AO activities of those sydnonoylthiazole in which a phenyl group was present on the C3 carbon of sydnone ring, a –NHPh substituent was present on the C-2 carbon of thiazole ring and the C4 thiazole ring carbon was variously substituted with $–NH_2$; -H or-Ph group. This was done in order to examine the effect of the C4 thiazole ring substituent on the AO activity, if any. As a result, the effect of the above substituents on C-4 position of thiazole on AO activity was expected to be revealed. The results are given below table 1 which showed that the presence of an amino group on the C4 carbon of the thiazole ring was beneficial for AO activity.

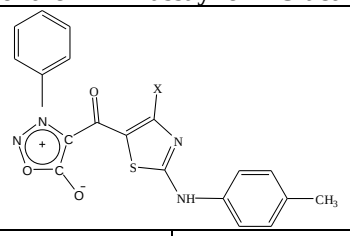
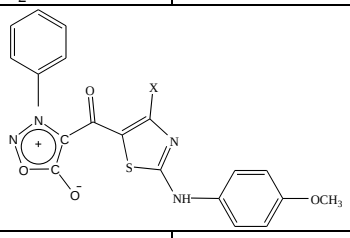
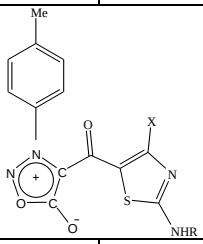
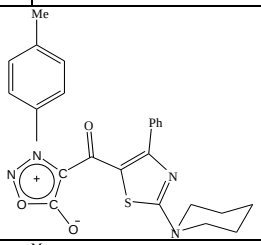
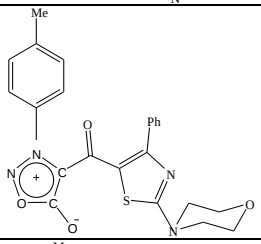
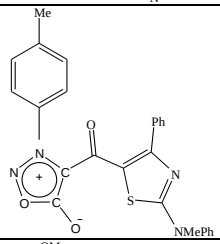
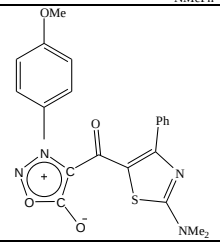
Table 1: Results of the FRAP assay for AO activity of sydnonoylthiazoles

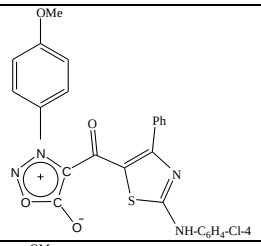
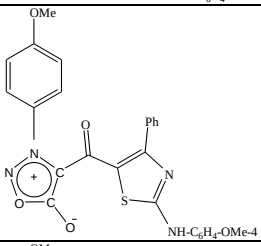
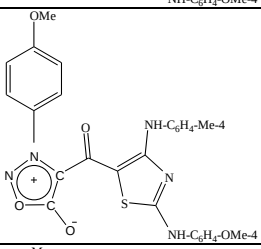
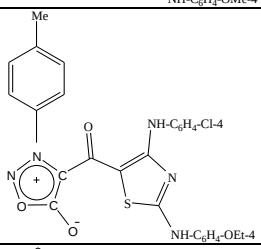
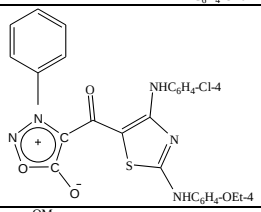
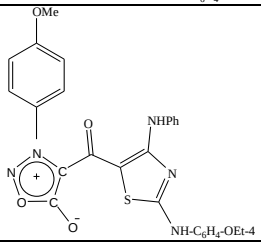


X	$FeSO_4$ equivalents (in μM)
-Ph	14.2
-H	18.7
$-NH_2$	21.5
Vitamin C (as reference standard in assays)	*14.8*

The above hypothesis was further extended for2-(N,N-disubstituted amino)-4-phenyl-5-(3-arylsydnon-4-oyl)thiazole and 5-(3-arylsydnon-4-oyl)-2-arylamino-4-phenylthiazoles , 2, 4-bis(arylamino)-5-(3-arylsydnon-4-oyl)thiazoles and the results obtained are shown below in Table 2.

Table. 2. Results of the FRAP assay for AO activity of sydnonoylthiazoles

		
Thiazole		$FeSO_4$ equivalents (in μM)
-Ph		16.1
-H		19.5
$-NH_2$		23.0
		
-Ph		17.8
-H		20.2
		
-Ph	-Ph	12.4
-H	-Ph	13.2
		10.9
		11.5
		11.6
		13.5

	14.5
	20.5
	14.7
	16.2
	18.2
	22.0
Vitamin C	*14.8*

Based on this investigation, it was surmised that even though a –NHAr substitution on the C4 carbon of the thiazole ring did not retard AO activity, a simple $–NH_2$ substituent was sufficient, since none of the 2,4-bis(arylamino)-5-(3-arylsydnon-4-oyl)thiazoles showed any superior FRAP AO activity values in comparison.

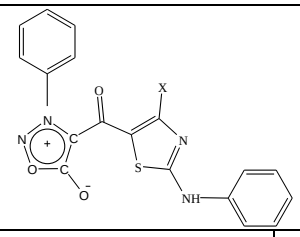
2.2 *β*-Carotene bleaching method

The *β*-Carotene bleaching method of Hidalgo was used to evaluate the antioxidant activity. The oxidative destruction of β-carotene by the degradation products of linoleic acid is measured spectrophotometrically at 450–470 nm using an aqueous emulsion of the linoleic acid which is the substrate, β-carotene which acts as the probe molecule and the antioxidant under assay.

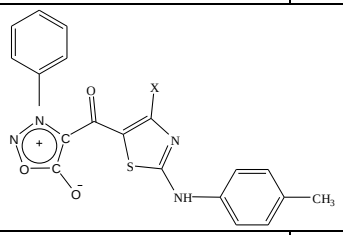
As in the case of the overall pattern followed in the determination of the AO activity based on the FRAP assay, in this investigation also, we had chosen to compare the AO activity of sydnonoylthiazoles based on *β*-carotene assay by first comparing the AO activities of those sydnonoylthiazoles in which the C3 carbon of the sydnone ring carried a selected, common aryl group such as phenyl, the C-2 carbon of the thiazole ring bore a –NHAr substituent, where the Ar group was either a C_6H_5-, C_6H_4-Me- or a C_6H_4-OMe-, and the C4 thiazole ring carbon, in each of the above three cases, was varied as a $-NH_2$; -H or a -Ph group. This was done so as to determine any possible effect of the C4 thiazole ring substituent on the AO activity. As a result, the effect of the above substituents on C-4 position of thiazole on AO activity may become apparent.

The results presented seemed to suggest that the presence of an amino group on the C4 carbon of the thiazole ring was beneficial for AO activity as assayed by the *β*-carotene bleaching method among the three presently examined structural classes wherein the C4 thiazole ring carbon carried either a $–NH_2$; -H or-Ph group.

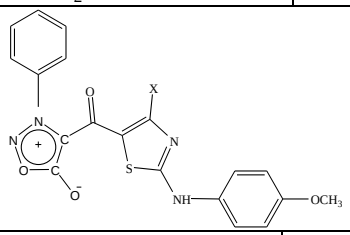
Table. 3. Results of the β-Carotene assay for AO activity of sydnonoylthiazoles



X	AO Activity %
-Ph	35
-H	41
$-NH_2$	49



X	AO Activity %
-Ph	39
-H	44
$-NH_2$	51



X	AO Activity %
-Ph	41
-H	43
$-NH_2$	54
BHA (as reference standard in assays)	*55*

In addition, the data in the above table indicates that the presence of a 4-methoxyphenylamino- substituent seemed to offer better activity among the 2-(4-phenylamino), 2-(4-methylphenylamino) and the 2-(4-methoxyphenylamino)-5-sydnonoylthiazoles.

2.3 Anti-Oxidant assay by DPPH method

DPPH, or 1,1-diphenyl-2-picrylhydrazyl (DPPH) is a stable, deep violet coloured free radical due to the delocalisation of the lone electron over the entire molecule with λ_{max} (EtOH) 520 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then DPPH gets converted to the reduced form **2** with the loss of this violet colour. An aliquot of methanol solution containing different concentrations of samples (80-320mg/L) and standard BHA (36-145mg/L) were added to DPPH (2.8 mL ,10^{-5} M) in methanol. Absorbance at 515 nm was measured. The DPPH concentration in the reaction medium was calculated from the calibration curve, determined by linear regression, according to the method of Brand-Williams et al.. The percentage DPPH Remaining at a particular time was calculated as:

$$\% \text{ DPPH Remaining} = 100 \times [Ab_c - Ab_s] / Ab_c$$

where
Ab = Absorbance;
c = control;
s= sample.

By this method, first we studied the AO activities of twelve 4-amino-2-arylamino-5-sydnonoylthiazoles. For each aminothiazole, the assay was conducted at four concentration levels of 0.2, 0.4, 0.6 and 0.8 mM. For each aminothiazole, the percentage of remaining DPPH was determined and a graph was plotted. In the graph, the concentration of the aminothiazoles, or the reference standard BHA, was plotted along the X-axis and the percentage of remaining DPPH was plotted along the Y-axis. The lesser the percentage of remaining DPPH, the greater would be the A.O activity. Thus, compounds represented by the curves that lie at the bottom of the plot area (representing a lower % of DPPH remaining) would be better antioxidants.The investigation pointed out that the AO activity decreased in the order of **BHA> 1 > 3> 2**, thereby indicating the presence of a $-NH_2$ group at the C-4 position of thiazole ring would impart a better AO activity.

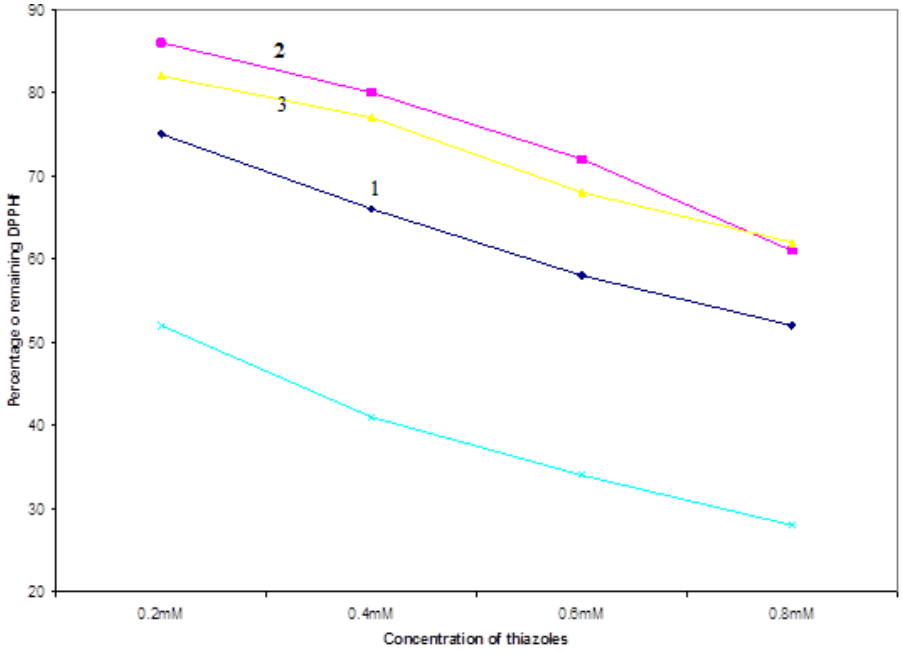
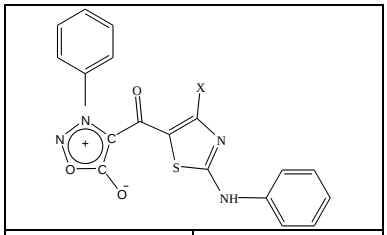


Fig.1: Results of the DPPH Assay for AO activity of sydnonoylthiazoles



Comp. No:	X
1	-Ph
2	-H
3	-NH_2

To further confirm the generality of this observation another set of three 2-(4-methoxyphenylamino)sydnonoylthiazoles **2a, 3a** and **1a** were taken**,** in which the N-3 of the sydnon ring carried a phenyl group and the C-2 of the thiazole ring bore a 4-methoxyphenylamino group. As it can be seen from the curves presented below, the general trend observed seemed to hold here as well and the decreasing gradation in the AO activity was seen to be in the order **BHA**> **1a**> **3a**> **2a**. A similar observation has been made in the case other 2-(arylamino)-5-(3-arylsydnon-4-oyl)thiazoles.

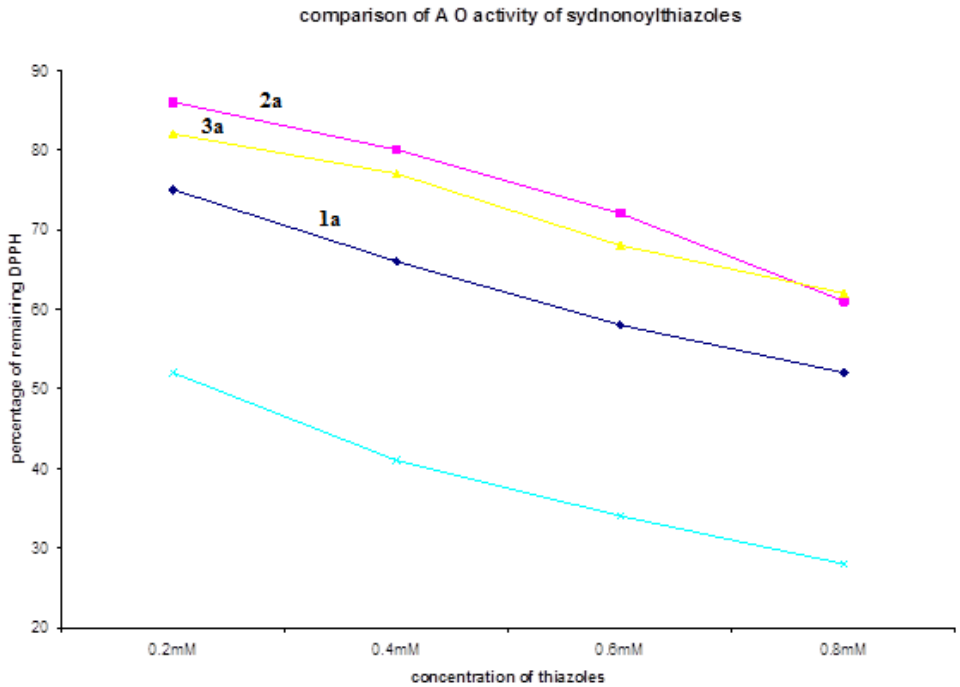
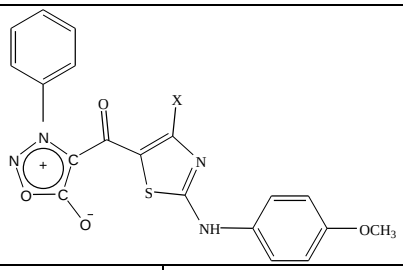


Fig. 2: Results of the DPPH Assay for AO activity of sydnonoylthiazoles



Comp. No:	X
2a	-Ph
3a	-H
1a	-NH_2

So when we compared the AO activity of 2-arylamino-4-phenyl-5-(3-arylsydnon-4-oyl)thiazoles and 2-(N,N-disubstituted amino)-4-phenyl-5-(3-arylsydnon-4-oyl)thiazoles, it wascan be seen that 2-arylamino-4-phenyl-5-(3-arylsydnon-4-oyl)thiazoles exhibited a higher AO activity. In a further continuation of these studies, we had also compared the AO activity of 2,4-bis(arylamino)-5-(3-arylsydnon-4-oyl)thiazoles with the AO activities of the other classes of sydnonoylthiazoles and it was observed that 2,4-bis(arylamino)-5-(3-arylsydnon-4-oyl)thiazoles showed a lower AO activity than that of 4-amino-2-arylamino-5-(3-arylsydnon-4-oyl)thiazoles, but a higher AO activity than 2-arylamino-5(3-arylsydnon-4-oyl)thiazoles.

3. CONCLUSIONS

The AO activity, as determined by the FRAP assay showed that (i) a 2-alkoxyphenylamino substituent on the C2 carbon of the thiazole ring promoted AO activity, (ii) a disubstituted amino group on C2 carbon of the thiazole ring was seen to be unfavorable, (iii) a 4-amino substituent on the C4 carbon of the thiazole ring was desirable since among the compounds screened, 4-aminothiazole derivatives were showed relatively higher activity in general; though a 4-H or 4-Ar substitutions was not damaging and (iv) an alkoxyphenyl substituent on the C3 carbon of the sydnone ring was preferable for good AO activity, but not essential; Ph and 4-ClC_6H_4- groups were also not deleterious.

The study based on β-carotene bleaching assay showed that (i) a 2-alkoxyphenylamino substituent on the C2 carbon of the thiazole ring seemed to promote AO activity; (ii) a disubstituted amino group on C2 carbon of the thiazole ring had no advantages over a monosubstituted amino group; (iii) a 4-amino substituent on the C4 carbon of the thiazole ring was to be favoured and (iv) one or more alkoxyphenyl substituent on the basic molecular framework conferred superior AO activity.

From DPPH method, it is clear that AO activities of the synthesized sydnonoylthiazoles were higher than that of corresponding thiazoles as well as sydnone derivatives and a combination of the sydnone with a 2-amino-5-ketothiazole motif was advantageous. Moreover, some of the aminosydnonoylthisazoles now synthesized exhibited nearly twice the activity of the reference standard vitamin C.The above comparison of the AO activities of 4-amino-2-arylamino-5-sydnonoylthiazoles**,** 2,4-bis(arylamino)-5-sydnonoylthiazoles**,** 2-arylamino-5-sydnonoylthiazoles, 2-arylamino-4-phenyl-5-sydnonoylthiazoles and 2-(N,N-disubstituted amino)-4-phenyl-5-sydnonoylthiazoles allowed us to conclude that in relation to the AO activity of the standard reference BHA, the AO activities of the newly synthesized sydnonoylthiazoles were in the decreasing order of BHA > 4-amino-2-arylamino-5-sydnonoylthiazoles > 2,4-bis(arylamino)-5-sydnonoylthiazoles > 2-arylamino-5-sydnonoylthiazoles > 2-arylamino-4-phenyl-5-sydnonoylthiazoles >2-(N,N-disubstitutedamino)-4-phenyl-5-sydnonoylthiazoles.

4. REFERENCES

[1] Webster's Third New International dictionary, Unabridged; Merriam-Webster: 2002; http://unabridged.merriam-webster.com
[2] Annon., http://cancerweb.ncl.ac.uk/cgi-bin/omd?query)antioxidants
[3] Taga et al., 1984; Fukumoto and Mazza, 2000.

