Syntheses of Aromatic Substituted Hydrazino-thiazole Derivatives to Clarify Structural Characterization and Antioxidant Activity between 3-Arylsydnonyl and Aryl Substituted Hydrazino-thiazoles

Mei-Hsiu SHIH,* Yu-Sheng SU, and Cheng-Ling WU

Department of Chemical and Materials Engineering, Southern Taiwan University; Tainan 710, Taiwan, R.O.C.
Received August 12, 2006; accepted May 21, 2007

This work clarifies the structural characterization and antioxidant activity between aromatic and 3-arylsydnonyl substituted hydrazino-thiazoles by further synthesizing a series of aromatic ring-substituted hydrazino-thiazole derivatives 8a—h and 9a—h. Hydrazino-thiazole derivatives 8a—h and 9a—h were obtained by reacting aromatic or heterocyclic aromatic aldehyde thiosemicarbazones 7a—h with cyclization reagents ethyl 2-chloroacetoacetate (2a) and 2-bromoacetophenone (2b), respectively. The ORTEP drawings of compounds 8g, 8h and 9f provide strong evidence of the structure of aromatic thiazole derivatives 8a—h and 9a—h. Undoubtedly, the structure of compounds 3e—h and 4e—h synthesized by the reaction of 3-aryl-4-formylsydnone thiosemicarbazones 1e—h with cyclization reagents 2a and 2b in the previous work should have the thiazole moiety, and not the thiazoline moiety. Both the new thiazole derivatives 8a—h and 9a—h and the 3-arylsydnonyl-substituted derivatives 3e—h and 4e—h were investigated to determine their antioxidant activity by two tests that have been highly documented—the direct scavenging effect on a stable free 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and the inhibition of the 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical. Results of this study demonstrate that not only the thiazole ring and the aryl ring has the contribution to the antioxidant activities, the sydnone ring of 3-arylsydnonyl moiety also has its considerable contribution.

Key words thiosemicarbazone; thiazole; thiazoline; antioxidant activity; 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS); 1,1-diphenyl-2-picrylhydrazyl (DPPH)

Thiosemicarbazones exhibit various biological activities and are extensively applied in medicine, especially in the treatment of tuberculosis.[1,2] Many compounds with a thiosemicarbazone moiety also exhibit biological activity.[3,4] Besides, thiazoles and their derivatives exhibit various biological activities, such as antimicrobial, anti-inflamatory, antiviral, antituberculosis and cytotoxic activities, among others.[5—14] Therefore, in our previous work, 3-aryl-4-formylsydnone thiosemicarbazones **1a**—**h** were synthesized and were applied to react with cyclization reagents such as ethyl 2-chloroacetoacetate (**2a**) and 2-bromoacetophenone (**2b**) to yield 3-arylsydnonyl-substituted derivatives **3a**—**h** and **4a**—**h** (Chart 1).[15] Among these, compounds **3e**—**h** and **4e**—**h** exhibit potent DPPH radical scavenging activity, which is comparable to that of vitamin E (Fig. 1).[15] The 2,3-dihydrothiazole moieties of compounds **3e**—**h** and **4e**—**h** should play an important role in scavenging radical activity. With the objective to understand the structure–activity relationships, a series of aromatic or heterocyclic aromatic ring-substituted thiazole derivatives **8a**—**h** and **9a**—**h** were synthe-

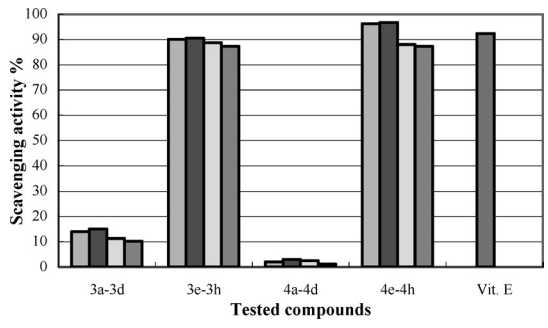
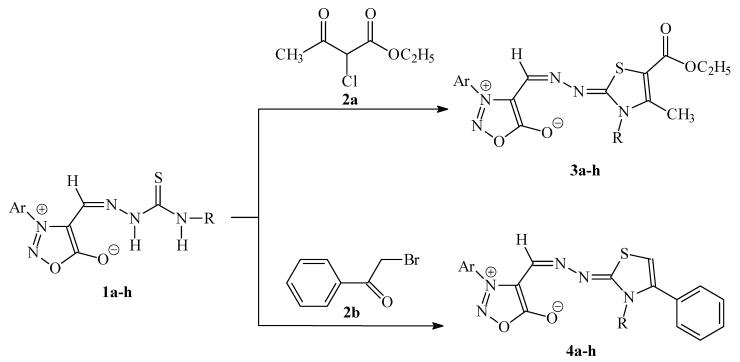


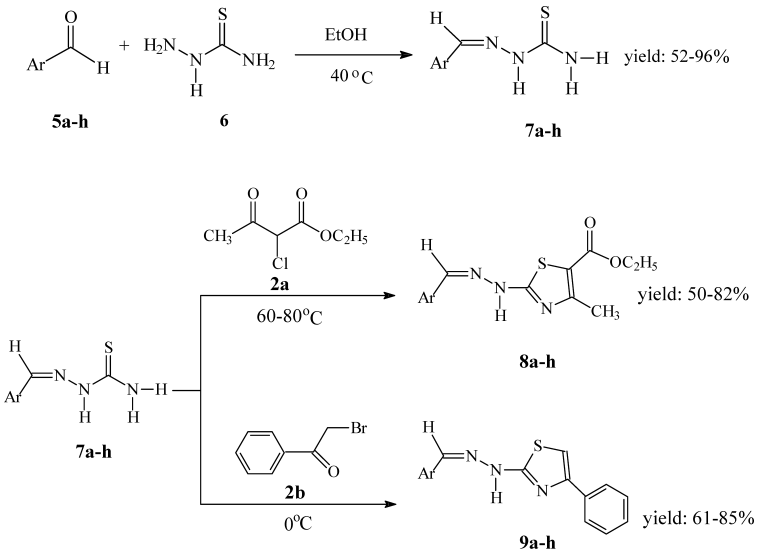
Fig. 1. Scavenging Activity of Compounds **3a**—**h** and **4a**—**h** (0.1 mM) Incubated 30 min with DPPH at 0.1 mM Concentration



1a, **1e**: Ar = C_6H_5; **1b**, **1f**: Ar = *p*-$CH_3C_6H_4$; **1c**, **1g**: Ar = *p*-$CH_3OC_6H_4$; **1d**, **1h**: Ar = *p*-$C_2H_5OC_6H_4$;
1a-d, 3a-d, 4a-d: R = C_6H_5; **1e-h, 3e-h, 4e-h**: R =H

Chart 1

* To whom correspondence should be addressed. e-mail: meihsius@mail.stut.edu.tw



5a: Ar = *p*-$CH_3C_6H_4$; **5b**: Ar = *p*-$CH_3OC_6H_4$; **5c**: Ar = C_6H_5; **5d**: Ar = *p*-ClC_6H_4; **5e**: Ar = *p*-CNC_6H_4; **5f**: Ar = 2-pyridyl; **5g**: Ar = 2-furyl; **5h**: Ar = 2-thiofuryl

Chart 2

sized (Chart 2), and their antioxidant activity were investigated *in vitro* by two methods-direct scavenging of a stable free 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical[16—18] and inhibition of the 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical.[19—21]

Results and Discussion

Synthetic Chemistry A series of aromatic aldehyde thiosemicarbazones **7a**—**h** were prepared in good yields by the reactions of aromatic or heterocyclic aromatic aldehydes **5a**—**h** with thiosemicarbazide (**6**). The thiosemicarbazones **7a**—**h** reacted with cyclization reagents ethyl 2-chloroacetoacetate (**2a**) and 2-bromoacetophenone (**2b**) to yield the corresponding heterocyclic substituted hydrazino-thiazoles **8a**—**h** and **9a**—**h**, respectively (Chart 2), with the aim to understand the structure–antioxidant activity relationships. All these products **8a**—**h** and **9a**—**h** were spectroscopically characterized. Among these new compounds, the crystals **8g**, **8h** and **9f** were analytically pure and suitable for X-Ray structure analyses. Figures 2, 3 and 4 display the ORTEP drawing of 4-methyl-2-(*N*′-furan-2-ylmethylene-hydrazino)thiazole-5-carboxylic acid ethyl ester (**8g**), 4-methyl-2-(*N*′-thiophen-2-ylmethylene-hydrazino)thiazole-5-carboxylic acid ethyl ester (**8h**) and 4-phenyl-2-(*N*′-pyridin-2-ylmethylene-hydrazino)-thiazole (**9f**). Table 1 presents crystal data of compounds **8g**, **8h** and **9f**.

In our earlier study,[15] thiosemicarbazones **1a**—**d** reacted with cyclization reagents **2a** and **2b** to yield thiazoline derivatives **3a**—**d** and **4a**—**d**, respectively. Based on the X-Ray ORTEP drawings of compounds **3b** and **4c**, we believed that compounds **3a**—**d** and **4a**—**d** have thiazoline moieties. Since no more X-Ray analysis evidence is available, compounds **3e**—**h** and **4e**—**h** were recognized to have similar structures with the 2,3-dihydro-thiazole moiety (Chart 1). In this work, the X-Ray analyses provide strong and forceful evidence for the structure of compounds **8g**, **8h** and **9f**, which have the thiazole moiety, but not 2,3-dihydro-thiazole moiety (Figs.

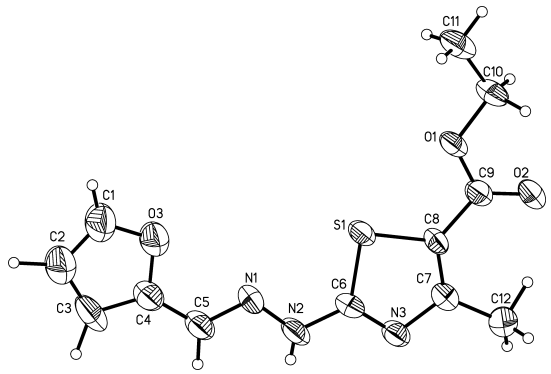


Fig. 2. ORTEP Drawing of Compound **8g**

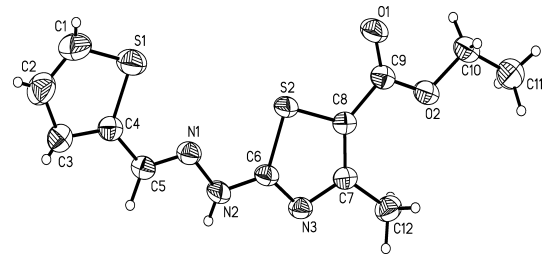


Fig. 3. ORTEP Drawing of Compound **8h**

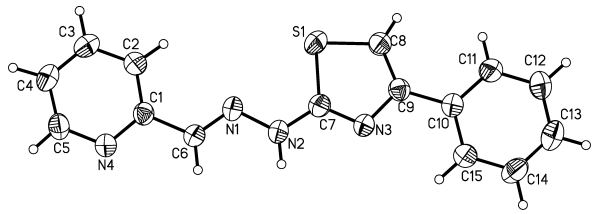


Fig. 4. ORTEP Drawing of Compound **9f**

Table 1. Crystal Data of Compounds **8g**, **8h** and **9f**

Compound	**8g**	**8h**	**9f**
Diffractometer	Bruker Smart Apex CCD	Bruker Smart Apex CCD	Bruker Smart Apex CCD
Formula	$C_{12}H_{13}N_3O_3S$	$C_{12}H_{13}N_3O_2S_2$	$C_{15}H_{12}N_4S$
Formula weight	279.32	295.37	280.3557
Crystal system	Monoclinic	Orthorhombic	Monoclinic
Space group	*C*2/*c*	$P2_12_12_1$	$P2_1/c$
a/Å	14.304(3)	4.8397(4)	8.8181(7)
b/Å	24.781(5)	11.0990(10)	10.8371(9)
c/Å	8.0877(16)	25.790(2)	28.596(2)
α/°	90.00	90.00	90.00
β/°	94.363(4)	90.00	90.304(2)
γ/°	90.00	90.00	90.00
V/Å^3	2858.4(10)	1385.3(2)	2732.7(4)
Z	8	4	4
D_{calc} (g cm^{-3})	1.298	1.416	1.363
F_{000}	1168.00	616.00	1168.00
μ (Mo,$K\alpha$)/cm^{-1}	2.34	3.85	2.31
Crystal size/mm	0.06×0.09×0.19	0.18×0.21×0.46	0.13×0.13×0.26
Temperature (K)	298	298	298
Scan type	Phi-omega scan	Phi-omega scan	Phi-omega scan
θ_{max}, deg	28.37	28.30	28.25
Reflections measured	Total: 3464	Total: 3333	Total: 6502
No. observations [I>2.00σ(I)]	2279	2763	5218
No. variables	221	224	457
Residuals: *R*; *Rw*	0.0904; 0.2015	0.0432; 0.1078	0.0684; 0.1453
GoF	1.151	1.060	1.174

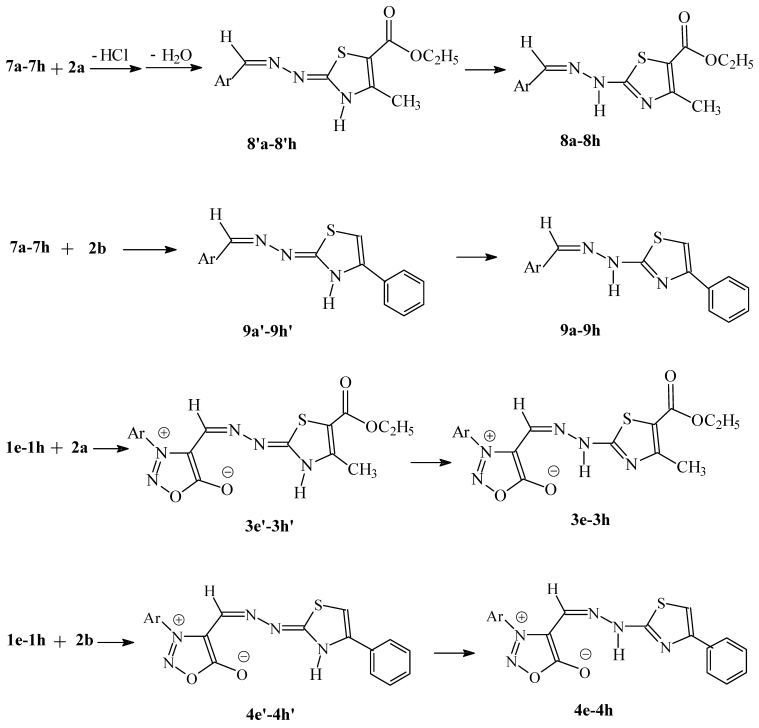


Chart 3. The Reactions of Thiosemicarbazones with Reagents **2a** and **2b**, Separately

2—4). Thiosemicarbazones **7a**—**h** first reacted with reagent **2a** to give the initial thiazoline derivatives **8a**′—**h**′, and then **8a**′—**h**′ *via* rearrangement to produce the more stable thiazoles **8a**—**h**. Similarly, thiazole derivatives **9a**—**h** were obtained by the reaction of thiosemicarbazones **7a**—**h** and reagent **2b** (Chart 3). Now, we believe that compounds **3e**—**h** and **4e**—**h**, which were synthesized by the reactions of thiosemicarbazones **1e**—**h** with reagent **2a** and **2b**, respectively, should be the structure with thiazole moiety (Chart 3).

Oxidation is well known to be a major cause of material degradation. Recently, oxygen-reactive species—in particular free radicals—have been recognized to be involved in several diseases. Overwhelming evidence reveals that free radicals cause oxidative damage to lipids, proteins and nucleic acids. Antioxidants, which can inhibit or delay the oxidation of an oxidisable substrate in a chain reaction, would therefore seem to be very important in the prevention of diseases, including cancer, arthritis, heart disease and brain dysfunc-

tion.[22—25] For all of these reasons, this work measures the antioxidant properties of synthesized compounds by using improved 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay[16—18] and 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) free radical inhibition assay.[19—21] Both approaches have been recommended as useful tools for evaluating the antioxidant capacities of compounds. A study of the antioxidant capacity of known and new hydrazino-thiazole derivatives **3e**—**h**, **4e**—**h**, **8a**—**h** and **9a**—**h** could help to elucidate their structure–activity relationships and support the development of new drugs to improve the treatment of various diseases.

Scavenging Effect of Antioxidant Activity on DPPH Radical[16—18] The model of the scavenging of the stable DPPH radical is extensively applied to evaluate antioxidant activities in less time than that is required by other methods. DPPH is a stable free radical that can accept an electron or hydrogen radical and must thus be converted to a stable, diamagnetic molecule. DPPH has an odd electron and so has a strong absorption band at 517 nm. When this electron becomes paired off, the absorption decreases stoichiometrically with respect to the number of electrons or hydrogen atoms taken up. Such a change in the absorbance by this reaction has been extensively adopted to test the capacity of several molecules to act as free radical scavengers. The scavenging effects of all the compounds **3e**—**h**, **4e**—**h, 8a**—**h** and **9a**—**h** on the DPPH radical were evaluated by the approach of Shimada, Leong and Braca under the same condition.[16—18] Various concentrations of the test compound in 1.5 ml methanol were added to a 1.5 ml (0.2 mM) solution of DPPH radical in methanol. (The final concentration of DPPH was 0.1 mM) The mixture was shaken vigorously and allowed to stand for 30 min; the absorbance of the resulting solution at 517 nm was measured using a Hitachi U-2001 spectrophotometer, and the percentage of activity was calculated. Vitamin E was used as a reference compound. All tests and analyses were performed on three replicates and the results averaged. The tests reveal that a greater concentration of the tested compound corresponded to greater radical scavenging activity. Figure 5 plots the profiles of the scavenging effect of compounds **8a**—**h** and **9a**—**h** on DPPH.

The scavenging activity of compounds **8a**—**h** and **9a**—**h** are perhaps due to the presence of an N–H group in the hydrazino moiety, which can donate a hydrogen atom to the DPPH radical. After donating a hydrogen atom, compounds **8a**—**h** and **9a**—**h** exist in a radical form, and the radical could delocalize to the thiazole ring and the *N*′-aryl ring to produce the stable resonance hybride shown in Chart 4. The electron conjugation in the structure stabilizes the radical, preventing it from participating in a destructive biochemical reaction. Based on our experimental result, the DPPH radical scavenging ability of compounds **9a**—**h** is better than that of compounds **8a**—**h** due to the electron resonance effect of phenyl group substituted at the thiazole ring in radical **9a**—**h**

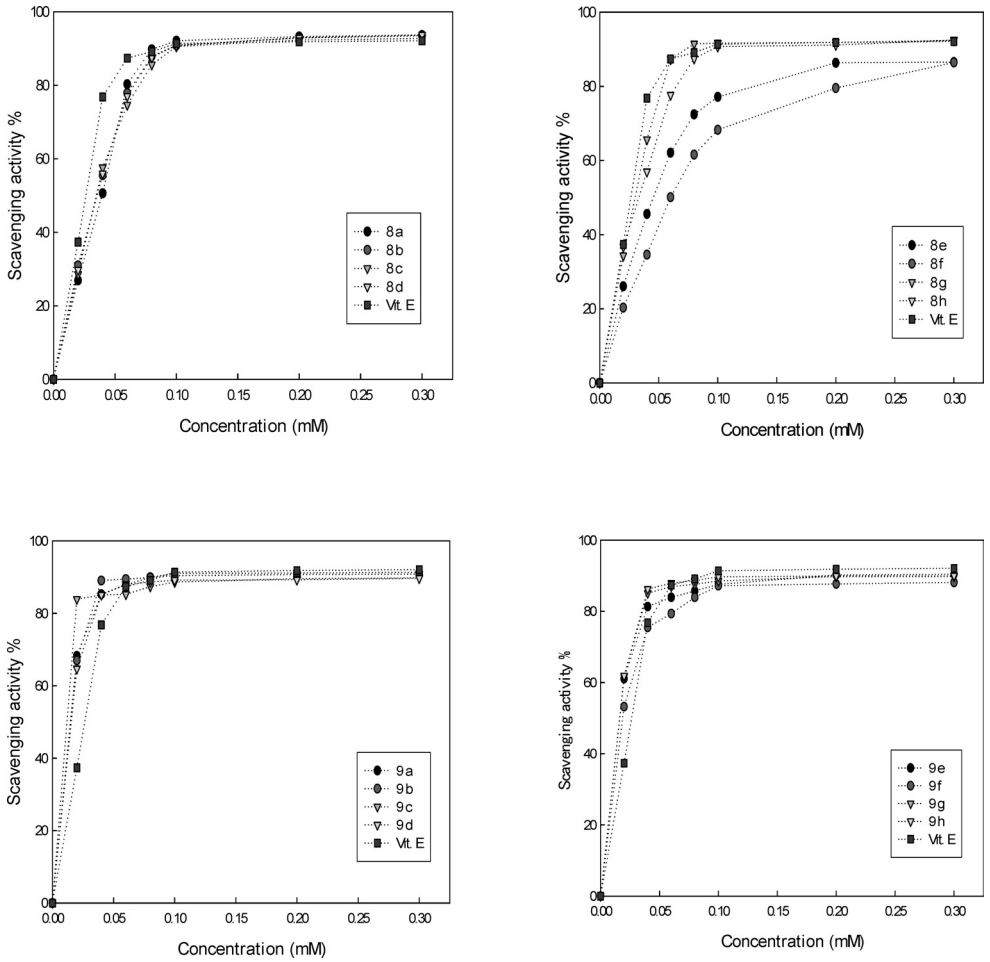
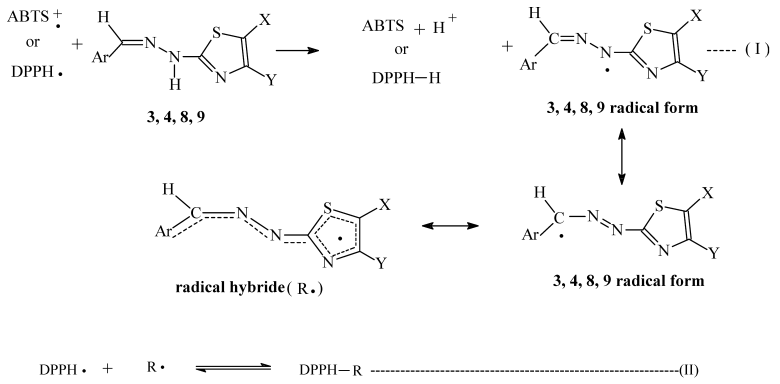


Fig. 5. Scavenging Activity of Compounds **8a**—**d**, **8e**—**h**, **9a**—**d** and **9e**—**h** on DPPH Radical



3e, **4e**: Ar = 3-C_6H_5-sydnonyl-4-yl; **3f**, **4f**: Ar = 3-(*p*-$CH_3C_6H_4$)sydnonyl-4-yl; **3g**, **4g**: Ar = 3-(*p*-$CH_3OC_6H_4$) sydnonyl-4-yl; **3h**, **4h**: Ar = 3-(*p*-$C_2H_5OC_6H_4$)sydnonyl-4-yl; **8a**, **9a**: Ar = *p*-$CH_3C_6H_4$; **8b**, **9b**: Ar = *p*-$CH_3OC_6H_4$; **8c**, **9c**: Ar = C_6H_5; **8d**, **9d**: Ar = *p*-ClC_6H_4; **8e**, **9e**: Ar = *p*-CNC_6H_4; **8f**, **9f**: Ar = 2-pyridyl; **8g**, **9g**: Ar = 2-furyl; **8h**, **9h**: Ar = 2-thiofuryl

Chart 4. The Reactions of Compounds **3e**—**h**, **4e**—**h**, **8a**—**h** and **9a**—**h** with DPPH and ABTS Radical Cation

making the radical more stable. Compounds **9a**—**h** caused instantaneous decrease in the absorbance of DPPH, in a way similar to that of vitamin E, suggesting a high radical scavenger activity, whereas compounds **8e** and **8f** caused a more gradual decrease in absorbance, indicating a slower reactivity as compared with the reference compound. The electron-withdrawing resonance effect of CN substituent and the electronegativity inductive effect of pyridyl group make the radicals **8e** and **8f** less stable, respectively, relative to the others. Therefore, compounds **8e** and **8f** behave slightly worse DPPH radical scavenging ability than the other compounds.

The reaction of the DPPH radical may be based either on a charge transfer with tested compounds perhaps initiated by DPPH radical (eq. (I)) or on a combination of the DPPH radical with thiazole radical formed during the DPPH radical scavenging assay (eq. (II)). Because of their steric hindrance, a reaction of DPPH molecules with each other is not possible. The reaction mechanism of thiazoles **3e**—**h**, **4e**—**h**, **8a**—**h** and **9a**—**h** with DPPH radical was suggested at Chart 4. In the DPPH radical scavenging effect assay, when the concentration of thiazoles **3**, **4**, **8**, and **9** was higher than that of DPPH radical, the quantity of thiazoles was enough to consume the DPPH radical and the stoichiometry of this reaction was 1 : 1 shown in Chart 4, eq. (I). When the concentration of the tested compounds was lower than that of DPPH radical, the residual DPPH radical might combine with the resulting thiazole radical **3**, **4**, **8**, and **9** shown in Chart 4, eq. (II), and the stoichiometry of this reaction seemed to be higher than 1 : 1 in some case.

Besides that, 3-arylsydnonyl-substituted thiazoles **3e**—**h** and **4e**—**h** behaved the strong DPPH radical scavenging activity, which had been published before.[15] In this paper, we also re-examined their DPPH radical scavenging activity in the same condition as that of compounds **8a**—**h** and **9a**—**h**. The DPPH radical scavenging ability of compounds **4e**—**h** is also better than that of compounds **3e**—**h** due to the electron resonance effect of phenyl group substituted at the thiazole ring in radical **4e**—**h** making the radical more stable. All the compounds **4e**—**h** and **9a**—**h**, with phenyl group substituted at the thiazole ring, behave the very strong DPPH radical scavenging activity, and the structural difference between them was that the formal was substituted with 3-arylsydnonyl moiety, the latter only with aryl group. The result of DPPH radical scavenging activity of all compounds **3**, **4**, **8** and **9** was summarized by IC_{50} shown in Table 2. Base on the IC_{50} value suggests that the DPPH radical scavenging activities of compounds **4e**—**h** and **9a**—**h** substituted with phenyl group on the thiazole moiety are better than that of compounds **3e**—**h** and **8a**—**h** substituted with carboxylic acid ethyl ester, respectively. Moreover, the DPPH radical scavenging activities are in the order **4e**—**h**>**9a**—**h**, **3e**—**h**>**8a**—**h**, and it seems that the free radical (shown in Chart 4) can delocalize to the 3-arylsydnone ring in compounds **4e**—**h** and **3e**—**h** more than to the aryl ring in compounds **9a**—**h** and **8a**—**h**. In order to further reveal the contribution to antioxidant activity between 3-arylsydnonyl and aryl ring, using the other method, especially concerned with the reaction rate, was necessary. In this paper, measurement of lag time of antioxidant activity in ABTS/H_2O_2/HRP system was applied to test all the synthesized thiazole derivatives.

Table 2. IC_{50} of the DPPH Radical Scavenging Activity of Investigated Compounds

Compound	IC_{50} (mM)	Compound	IC_{50} (mM)
3e	0.0253	**4e**	0.0148
3f	0.0256	**4f**	0.0156
3g	0.0294	**4g**	0.0223
3h	0.0276	**4h**	0.0206
8a	0.0381	**9a**	0.0257
8b	0.0359	**9b**	0.0258
8c	0.0369	**9c**	0.0243
8d	0.0364	**9d**	0.0245
8e	0.0460	**9e**	0.0268
8f	0.0602	**9f**	0.0298
8g	0.0306	**9g**	0.0256
8h	0.0331	**9h**	0.0257
α-Tocopherol	0.0263		

Measurement of Lag Time of Antioxidant Activity in ABTS/H_2O_2/HRP System[19—21] The other approach for measuring the antioxidant activity of compounds **3e**—**h**, **4e**—**h**, **8a**—**h** and **9a**—**h** was based on the inhibition of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation, which has a characteristic absorption spectrum with a maximum at 414 nm. Antioxidant compounds suppress the absorbance of the ABTS radical cation to an extent and on a time scale that depend on the antioxidant capacity

of the substance under investigation. Various analytical strategies are apparent. They include, (a) decolorization assay, (b) inhibition assay (fixed time point), (c) inhibition assay (reaction rate) and (d) lag phase measurement.[20] This study describes a procedure for measuring the lag time that is caused by adding various amounts of antioxidant to a standard ABTS/H_2O_2/horseradish peroxidase system.

ABTS is a peroxidase substrate, when oxidized in the presence of H_2O_2 in a typical peroxidative reaction, generates a blue-green metastable radical with long half-life and an absorption maximum at 414 nm. The accumulation of the ABTS radical catalyzed by peroxidase can be inhibited by the presence of antioxidants in the reaction medium. This inhibition delays the accumulation of the ABTS radical and causes a lag time. The longer the lag time of the tested compound, the better the antioxidant capacity it is. Hence, we studied the antioxidant activity of the synthesized compounds **3e**—**h**, **4e**—**h**, **8a**—**h** and **9a**—**h** and the relation to α-tocopherol in the ABTS/H_2O_2/horseradish peroxidase system, using the Arnao and Rice–Evans method.[19—21] All the reagents are added together, and the reaction is started by the addition of hydrogen peroxide. Spectrophotometric measurements were made using a UV–visible absorption spectrometer Bio-Tek MQ 200R. The temperature was controlled at 25 °C. The reactions were monitored at a wavelength of 414 nm. All tests and analyses were undertaken on three replicates and the results averaged. The time required to develop a blue-green color is monitored. Figure 6 displays the different time courses of ABTS oxidation at various α-tocopherol concentrations in the assay. Notably, the presence of α-tocopherol causes a lag time that is proportional to the α-tocopherol concentration to appear. The lag time before the steady state is reached is proportional to the concentration of the antioxidant.

The lag time of the antioxidant activity of all the compounds **3e**—**h**, **4e**—**h**, **8a**—**h** and **9a**—**h** in the ABTS/H_2O_2/HRP system was measured using the same procedure as α-tocopherol. First, the tested compound was treated at different concentration, such as 0, 3, 6, 9, 12 nmol in the same ABTS oxidation condition as α-tocopherol, and the absorption at 414 nm was measured relative to time course. The resulting data curve showed that in the presence of tested compound a lag time appeared, which was proportional to the compound concentration. Then, the time course of ABTS radical inhibition with 6 nmol of each compound was chosen to plot in the figure to compare the lag time and antioxidant activity. Figure 7 displays different time courses of the ABTS oxidation by 6 nmol of compounds **3e**—**h**, **4e**—**h**, **8a**—**h** and **9a**—**h** in the assay. Table 3 lists the lag time and lag ratio to α-tocopherol for 6 nmol of antioxidant compounds that are assayed at pH 7.4.

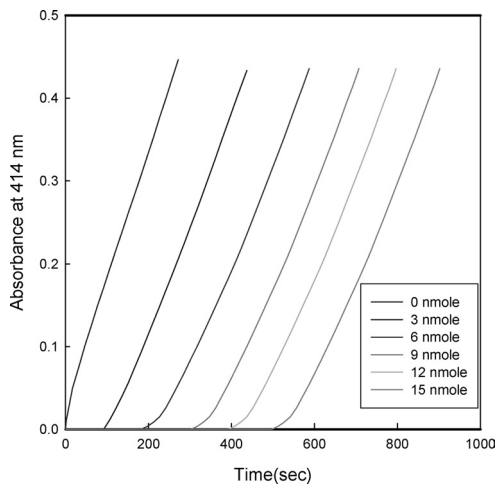


Fig. 6. Time Course of ABTS Radical Accumulation in the Presence of Peroxidase (2.5 nM), ABTS (2 mM) and H_2O_2 (0.1 mM) in 50 mM Potassium Phosphate Buffer (pH 7.4)

The reaction was followed by measuring the $\Delta Abs_{414\,nm}$ and time courses of the same reaction with 0, 3, 6, 9, 12, 15 nmol of α-tocopherol added, respectively.

Because the longer the lag time of the tested compound, the better the antioxidant capacity it is. The results reveal that the antioxidant activity of 3-arylsydnonyl-substituted thiazoles **4e**—**h** is better than that of compounds **9a**—**h**. Similarly, compounds **3e**—**h** is also better than compounds **8a**—**h**. The result of antioxidant activity by ABTS method is consistent with that by DPPH method. Synthesized thiazoles react with ABTS radical cation to generate ABTS and monodehydrothiazoles, which are radicals, as shown in Chart 4. The more stable the radical, the more easily the radical formed it is. Therefore, compounds **3e**—**h** and **4e**—**h** are more inhibitive to ABTS radicals than compounds **8a**—**h** and **9a**—**h**, respectively.

Nešpûrek, S. and other researchers have characterized sydnone skeleton.[26—28] The general structural formula of sydnone is presented by (A), (B) and (C) in Chart 5. The ring has a semi-aromatic character, the N(2)-N(3)-C(4)-C(5) portion together with the exocyclic oxygen atom, O(6), are conjugate like structure (I). Atom O(1) does not participate in conjugation and retains as its "individuality" more than the other atoms of the mesoionic system. The sydnone ring carries a negative charge on position C(4), and 3-substituted-sydnon-4-yl group has the electron-releasing property (Chart 5). Therefore, we could recognize that 3-arylsyndnon-4-yl group possesses greater electron-releasing ability than aryl ring does, because it simultaneously has the aryl ring and the sydnone ring. The free radical in **4e**—**h** could delocalize to the sydnone and the aryl ring (Chart 5), and could be stabilized by the electron-releasing ability of 3-arylsydnon-4-yl moiety, so the lag time of compounds **4e**—**h** was longer than that of compounds **9a**—**h**, which were substituted only with aryl ring. Similary, the lag time of compounds **3e**—**h** was also longer than that of compounds **8a**—**h**. Therefore, we may conclude that not only the aryl ring has the contribution to he antioxidant activity, the sydnonyl moiety also has its indelible contribution.

Conclusion

In summary, this work clarifies the structural characterization and antioxidant activity between aromatic and 3-arylsydnonyl-substituted hydrazino-thiazoles by further synthesizing a series of aromatic or heterocyclic aromatic ring-substituted hydrazino-thiazole derivatives **8a**—**h** and **9a**—**h**. Hydrazino-thiazoles **8a**—**h** and **9a**—**h** were obtained by reacting heterocyclic aromatic aldehyde thiosemicarbazones **7a**—**h** with cyclization reagents **2a** and **2b**, respectively. The ORTEP drawings of compounds **8g**, **8h** and **9f** provide strong evidence of the structure of heterocyclic or aromatic thiazole derivatives **8a**—**h** and **9a**—**h**. Undoubtedly, the structure of compounds **3e**—**h** and **4e**—**h** synthesized by the reaction of 3-aryl-4-

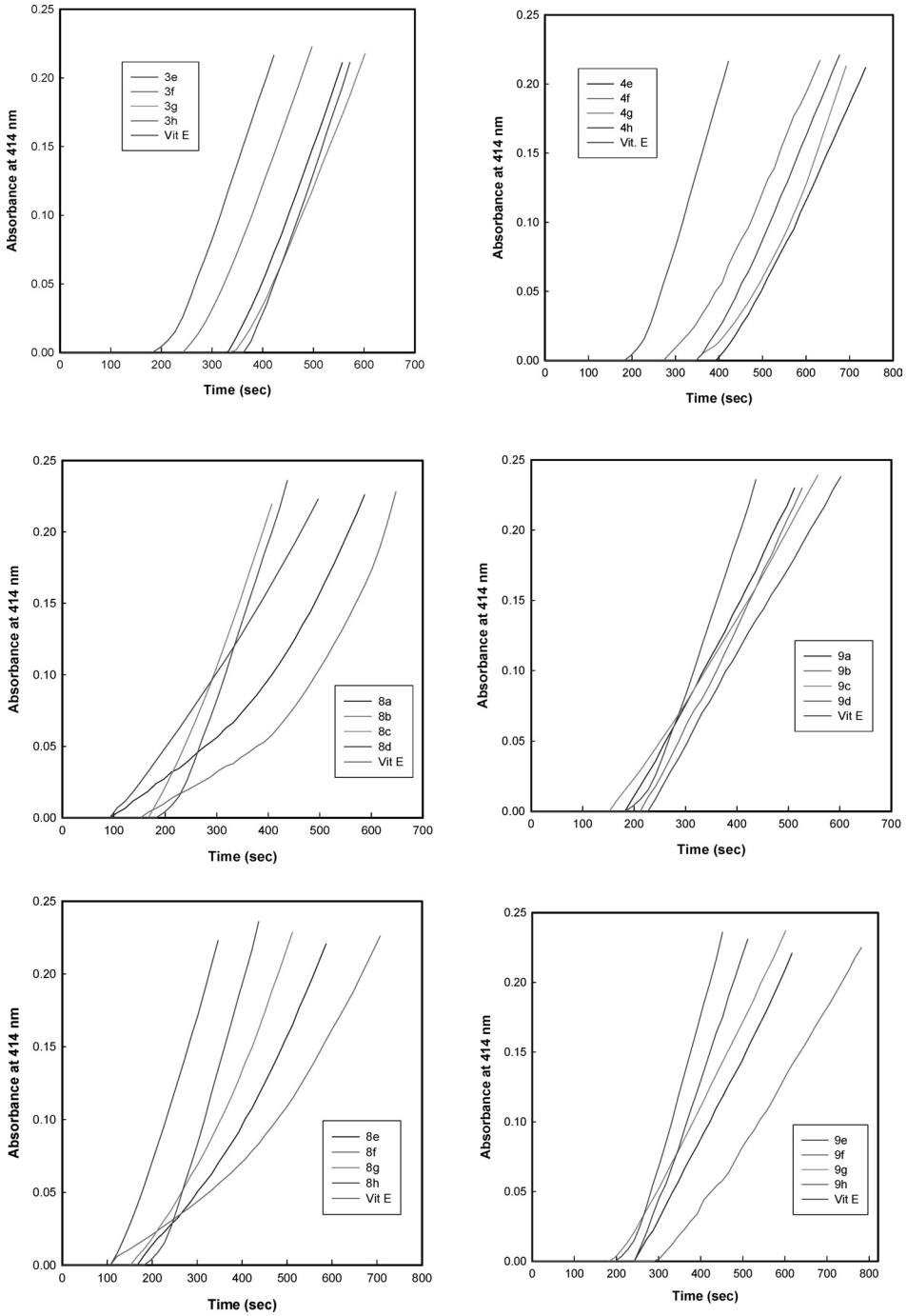


Fig. 7. Time Course of ABTS Radical Inhibition with 6 nmol of Compounds **3e**—**h**, **4e**—**h**, **8a**—**h** and **9a**—**h**

formylsydnone thiosemicarbazones **1e**—**h** with cyclization reagents **2a** and **2b** in the previous work should have the thiazole moiety, and not the thiazoline moiety. With a view to understand the structure–activity relationships, both new hydrazino-thiazole derivatives **8a**—**h** and **9a**—**h** and sydnonyl-substituted hydrazino-thiazoles **3e**—**h** and **4e**—**h** were studied to determine their antioxidant activity by two tests—the direct scavenging of a stable free 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and the inhibition of the 2,2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical. The result of DPPH free radical–scavenging assay indicated that the electron releasing resonance effect of phenyl group substituted at the thiazole ring made the radicals **4e**—**h** and **9a**—**h** more stable, and the electron withdrawing effect of 5-carboxylic acid ethyl ester made the radicals **3e**—**h** and **8a**—**h** less stable and decreased the DPPH radical scavenging ability. Both compounds **4e**—**h** and **9a**—**h** behave very strong DPPH radical scavenging activity, and the activity of **4e**—**h** is slightly stronger than that of **9a**—**h** by IC_{50} of DPPH assay. The structural difference between them was that the formal was substituted with 3-arylsydnon-4-yl moiety, the latter only with aryl ring. In the ABTS method, antioxidant activity of compounds **4e**—**h** was also better than that of compounds **9a**—**h**. Similary, compounds **3e**—**h** was also better than that of compounds **8a**—**h**. The electron-donating resonance effect of 3-arylsydnon-4-yl group really stabilized

hydrazino-thiazole radicals stronger than the only aryl ring did. Accordingly, not only the thiazole ring and the aryl ring have the contribution to the antioxidant activity, the sydnone ring in the 3-arylsydnonyl moiety might also contribute substantially *in vitro* by the two method–DPPH and ABTS assay.

Experimental

General All melting points were determined on an England Electrothermal Digital Melting Point apparatus and are uncorrected. IR spectra were recorded using a MATTSON/SATELLITE 5000 FT-IR spectrophotometer. Mass spectra were measured on a VG Quattro GC/MS/MS/DS spectrometer. ^{1}H-NMR spectra were obtained on a Bruker AMX-200 NMR spectrometer, using TMS as an internal standard. ^{13}C-NMR spectra were carried out with complete ^{1}H decoupling and assignments were made through additional DEPT experiments. Elemental analyses were taken with a Heraeus CHN-O-Rapid Analyzer or an Elementar Vario EL-III Analyzer. X-Ray spectra were performed on a Bruker AXS SMART APEX CCD diffractometer. Horseradish peroxidase (type VI, RZ=3.0) and ABTS in the crystallized diammonium salt form were purchased from Sigma (U.S.A.). The concentration of enzyme horseradish peroxidase and ABTS were determined by measuring their absorbance using $\varepsilon_{403\,nm}=100000\,M^{-1}\,cm^{-1}$ for enzyme and $\varepsilon_{340\,nm}=36000\,M^{-1}\,cm^{-1}$ for ABTS. α-Tocopherol, tergitol NP-40, potassium phosphate monobasic and potassium phosphate dibasic were also obtained from Sigma (U.S.A.). The buffer used is 50 mM potassium phosphate (pH 7.4). H_2O_2 (30%, v/v) was obtained from Riedel-DeHäen (Germany).

Table 3. Lag Time and Lag Ratio for Antioxidant Compounds Assayed at pH 7.4

Compounds	Lag time (s)	Lag ratio
α-Tocopherol	182	1
3e	317	1.74
3f	242	1.33
3g	332	1.82
3h	362	1.99
4e	392	2.15
4f	288	1.58
4g	377	2.07
4h	362	1.99
8a	92	0.51
8b	152	0.84
8c	167	0.92
8d	92	0.51
8e	167	0.92
8f	107	0.59
8g	152	0.84
8h	107	0.59
9a	182	1.00
9b	212	1.16
9c	167	0.92
9d	227	1.24
9e	227	1.24
9f	287	1.58
9g	182	1.00
9h	242	1.33

The amount of antioxidant used in the assays was 6 nmol. The reaction mixture contained 2.5 nM peroxidase, 2 mM ABTS and 0.1 mM H_2O_2 in 50 mM phosphate buffer (pH 7.4).

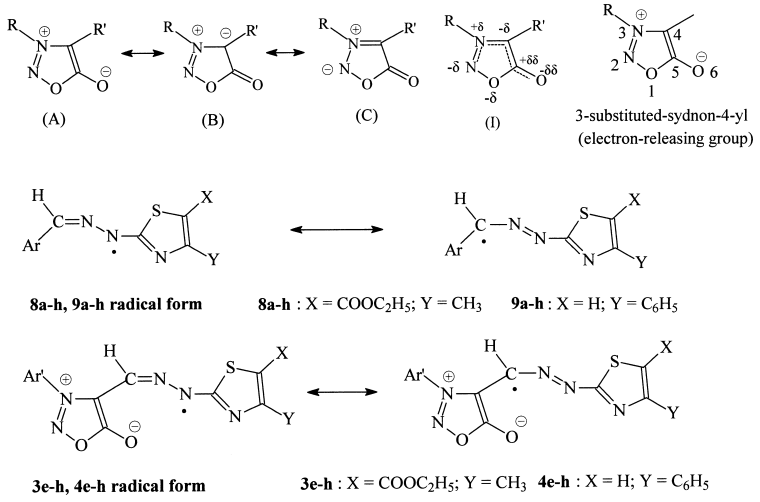
Syntheses of Aromatic Aldehyde Thiosemicarbazones (7a—h) Thiosemicarbazide (**6**, 1.365 g, 15.0 mmol) was slowly added to a solution of 4-methylbenzaldehyde (**5a**, 1.20 g, 10.0 mmol) in absolute ethanol (10 ml). The mixed solution was heated at 45 °C for 10 h and then cooled. The precipitating white solid (1.437 g) was collected by filtration and recrystallized from dichloromethane/ethanol to produce 1.202 g of 4-methylbenzaldehyde thiosemicarbazone (**7a**) as white powder in a yield of 62%. The chemical and physical spectral characteristics of these products **7a**—**h** are given below.

4-Methylbenzaldehyde Thiosemicarbazone (**7a**): White powder from CH_2Cl_2/EtOH; yield 62%; mp 168—169 °C; IR (KBr): 3401, 3240, 3156, 3026, 2989, 1598, 1539, 1509 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 2.30 (3H, s), 7.20 (2H, d, J=7.9 Hz), 7.66 (2H, d, J=7.9 Hz), 7.92 (1H, s), 8.00 (1H, s), 8.14 (1H, s), 11.35 (1H, s); FAB-MS *m/z*: 194 (M^++H); *Anal*. Calcd for $C_9H_{11}N_3S$: C, 55.93; H, 5.74; N, 21.74. Found: C, 55.84; H, 5.66; N, 21.53.

4-Methoxybenzaldehyde Thiosemicarbazone (**7b**): White powder from CH_2Cl_2/EtOH; yield 84%; mp 170—171 °C; IR (KBr): 3404, 3289, 3150, 2972, 1599, 1535, 1511, 1244, 1172 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 3.77 (3H, s), 6.94 (2H, d, J=8.7 Hz), 7.72 (2H, d, J=8.7 Hz), 7.89 (1H, s), 7.98 (1H, s), 8.09 (1H, s), 11.30 (1H, s); FAB-MS *m/z*: 210 (M^++H); *Anal*. Calcd for $C_9H_{11}N_3OS$: C, 51.66; H, 5.30; N, 20.08. Found: C, 51.44; H, 5.28; N, 19.95.

Benzaldehyde Thiosemicarbazone (**7c**): White powder from CH_2Cl_2/EtOH; yield 52%; mp 160—161 °C; IR (KBr): 3401, 3229, 3144, 3019, 1600, 1584, 1527, 1285, 1099 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 7.37—7.41 (3H, m), 7.75—7.80 (2H, m), 7.98 (1H, s), 8.04 (1H, s), 8.18 (1H, s), 11.42 (1H, s); FAB-MS *m/z*: 180 (M^++H); *Anal*. Calcd for $C_8H_9N_3S$: C, 53.61; H, 5.06; N, 23.44. Found: C, 53.45; H, 4.96; N, 23.24.

4-Chlorobenzaldehyde Thiosemicarbazone (**7d**): White powder from CH_2Cl_2/EtOH; yield 76%; mp 196—197 °C; IR (KBr): 3437, 3281, 3165, 1601, 1525, 1490, 1282, 1090 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 7.44 (2H, d, J=8.5 Hz), 7.82 (2H, d, J=8.5 Hz), 8.01 (1H, s), 8.05 (1H, s), 8.22 (1H, s), 11.46 (1H, s); FAB-MS *m/z* (%): 216 (M^++2+H), 214 (M^++H); *Anal*. Calcd for $C_8H_8N_3ClS$: C, 44.97; H, 3.77; N, 19.66. Found: C, 44.81; H, 3.73;



8a, 9a: Ar = *p*-$CH_3C_6H_4$; **8b, 9b**: Ar = *p*-$CH_3OC_6H_4$; **8c, 9c**: Ar = C_6H_5; **8d, 9d**: Ar = *p*-ClC_6H_4;
8e, 9e: Ar = *p*-CNC_6H_4; **8f, 9f**: Ar = 2-pyridyl; **8g, 9g**: Ar = 2-furyl; **8h, 9h**: Ar = 2-thiofuryl
3e, **4e**: Ar' = C_6H_5; **3f**, **4f**: Ar' = *p*-$CH_3C_6H_4$; **3g**, **4g**: Ar' = *p*-$CH_3OC_6H_4$; **3h**, **4h**: Ar' = *p*-$C_2H_5OC_6H_4$;

Chart 5. The Properties of Sydnone Ring and the Difference of Electron Density among Radicals **8a**—**h**, **9a**—**h**, **3e**—**h** and **4e**—**h**

N, 19.69.

4-Cyanobenzaldehyde Thiosemicarbazone (**7e**): White powder from CH_2Cl_2/EtOH; yield 96%; mp 225—226 °C; IR (KBr): 3418, 3249, 3151, 3013, 2220, 1601, 1535, 1503, 1464, 1361, 1295, 1099 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 7.83 (2H, d, J=8.4 Hz), 8.00 (2H, d, J=8.4 Hz), 8.05 (1H, s), 8.20 (1H, s), 8.34 (1H, s), 11.63 (1H, s); FAB-MS m/z (%): 205 (M^++H); *Anal*. Calcd for $C_9H_8N_4S$: C, 52.93; H, 3.95; N, 27.43. Found: C, 52.85; H, 3.91; N, 27.44.

Pyridine-2-carbaldehyde Thiosemicarbazone (**7f**): Yellow powder from CH_2Cl_2/EtOH; yield 71%; mp 199—201 °C; IR (KBr): 3417, 3244, 3156, 1611, 1525, 1468 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 7.35 (1H, dd, J=8.0, 5.2 Hz), 7.80 (1H, t, J=8.0 Hz), 8.07 (1H, s), 8.15 (1H, s), 8.25 (1H, d, J=8.0 Hz), 8.33 (1H, s), 8.54 (1H, d, J=5.2 Hz), 11.62 (1H, s); FAB-MS m/z (%): 181 (M^++H); *Anal*. Calcd for $C_7H_8N_4S$: C, 46.65; H, 4.47; N, 31.09. Found: C, 46.64; H, 4.44; N, 31.02.

Furan-2-carbaldehyde Thiosemicarbazone (**7g**): White powder from CH_2Cl_2/EtOH; yield 66%; mp 189—190 °C; IR (KBr): 3412, 3219, 3138, 3016, 1608, 1582, 1528, 1475, 1343, 1277, 1093 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 6.60 (1H, dd, J=3.9, 1.9 Hz), 6.95 (1H, d, J=3.9 Hz), 7.61 (1H, s), 7.79 (1H, d, J=1.9 Hz), 7.95 (1H, s), 8.19 (1H, s), 11.41 (1H, s); FAB-MS m/z (%): 170 (M^++H); *Anal*. Calcd for $C_6H_7N_3OS$: C, 42.59; H, 4.17; N, 24.83. Found: C, 42.38; H, 4.11; N, 24.81**.**

Thiophene-2-carbaldehyde Thiosemicarbazone (**7h**): Yellow powder from CH_2Cl_2/EtOH; yield 68%; mp 185—186 °C; IR (KBr): 3412, 3229, 3145, 3018, 2983, 1611, 1578, 1536, 1509, 1475, 1278, 1100 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 7.09 (1H, dd, J=4.9, 3.9 Hz), 7.43 (1H, d, J=3.9 Hz), 7.53 (1H, s), 7.63 (1H, d, J=4.9 Hz), 8.18 (1H, s), 8.23 (1H, s), 11.43 (1H, s); FAB-MS m/z (%): 186 (M^++H); *Anal*. Calcd for $C_6H_7N_3S_2$: C, 38.90; H, 3.81; N, 22.68. Found: C, 38.88; H, 3.72; N, 22.70.

Cyclization of Aromatic Aldehyde Thiosemicarbazones (7a—h) with Ethyl 2-Chloroacetoacetate (2a) To the solution of 4-methylbenzaldehyde thiosemicarbazone (**7a**, 386 mg, 2 mmol) in absolute ethanol (6 ml), sodium acetate (492 mg, 6 mmol) was slowly added. Acetic acid (1 ml) was added as a catalyst and then ethyl 2-chloroacetoacetate (**2a**, 494 mg, 3 mmol) was slowly added to the above solution. The mixed solution was heated at 70 °C for about 9 h until the reaction was completed. The system was then allowed to reach room temperature. The precipitating solid was collected by filtration and washed with ice-cold water and cold ethanol. The collected solid (451 mg) was recrystallized from dichloromethane/ethanol to afford 376 mg (1.24 mmol) of 4-methyl-2-[N'-(4-methylbenzylidene)hydrazino]thiazole-5-carboxylic acid ethyl ester (**8a**) as yellow powder in a yield of 62%. The chemical and physical spectral characteristics of these products **8a**—**h** are given below.

4-Methyl-2-[N'-(4-methylbenzylidene)hydrazino]thiazole-5-carboxylic Acid Ethyl Ester (**8a**): Yellow powder from CH_2Cl_2/EtOH; yield 62%; mp 189—191 °C; IR (KBr): 3192, 3101, 2980, 1664, 1567, 1524, 1429, 1367, 1273, 1091; ^{1}H-NMR (DMSO-d_6) δ: 1.25 (3H, t, J=7.2 Hz), 2.32 (3H, s), 2.45 (3H, s), 4.18 (2H, q, J=7.2 Hz), 7.23 (2H, d, J=8.0 Hz), 7.56 (2H, d, J=8.0 Hz), 8.03 (1H, s), 12.37 (1H, s); ^{13}C-NMR (DMSO-d_6) δ: 14.46, 17.25, 21.17, 60.21, 109.15, 126.80, 129.59, 131.47, 139.76, 144.64, 158.36, 162.08, 169.34; FAB-MS m/z (%): 304 (M^++H); *Anal*. Calcd for $C_{15}H_{17}N_3O_2S$: C, 59.39; H, 5.65; N, 13.85 Found: C, 59.28; H, 5.64; N, 13.81.

4-Methyl-2-[N'-(4-methoxybenzylidene)hydrazino]thiazole-5-carboxylic Acid Ethyl Ester (**8b**): Yellow powder from CH_2Cl_2/EtOH; yield 65%; mp 182—184 °C; IR (KBr): 3166, 3056, 2923, 2837, 1699, 1604, 1581, 1512, 1432, 1372, 1310, 1257, 1082; ^{1}H-NMR (DMSO-d_6) δ: 1.25 (3H, t, J=7.1 Hz), 2.45 (3H, s), 3.79 (3H, s), 4.18 (2H, q, J=7.1 Hz), 6.99 (2H, d, J=8.4 Hz), 7.61 (2H, d, J=8.4 Hz), 8.02 (1H, s), 12.32 (1H, s); ^{13}C-NMR (DMSO-d_6) δ: 14.49, 17.28, 55.45, 60.21, 108.93, 114.53, 126.80, 128.45, 144.56, 158.36, 160.87, 162.13, 169.34; FAB-MS m/z (%): 320 (M^++H); *Anal*. Calcd for $C_{15}H_{17}N_3O_3S$: C, 56.41; H, 5.37; N, 13.16. Found: C, 56.39; H, 5.33; N, 13.16.

4-Methyl-2-(*N*′-benzylidene-hydrazino)thiazole-5-carboxylic Acid Ethyl Ester (**8c**): Yellow powder from CH_2Cl_2/EtOH; yield 50%; mp 194—197 °C; IR (KBr): 3193, 3099, 2978, 1664, 1562, 1528, 1423, 1369, 1274, 1090; ^{1}H-NMR (DMSO-d_6) δ: 1.27 (3H, t, J=6.9 Hz), 2.46 (3H, s), 4.21 (2H, q, J=6.9 Hz), 7.42—7.50 (3H, m), 7.67—7.72 (2H, m), 8.09 (1H, s), 12.47 (1H, s); ^{13}C-NMR (DMSO-d_6) δ: 14.46, 17.24, 60.26, 109.31, 126.83, 128.99, 129.94, 134.18, 144.52, 158.25, 162.06, 169.39; FAB-MS m/z (%): 290 (M^++H); *Anal*. Calcd for $C_{14}H_{15}N_3O_2S$: C, 58.11; H, 5.23; N, 14.52. Found: C, 58.05; H, 5.16; N, 14.45.

4-Methyl-2-[N'-(4-chlorobenzylidene)hydrazino]thiazole-5-carboxylic Acid Ethyl Ester (**8d**): Yellow powder from CH_2Cl_2/EtOH; yield 77%; mp 206—208 °C; IR (KBr): 3198, 3087, 2980, 1676, 1553, 1490, 1422, 1371, 1272, 1091; ^{1}H-NMR (DMSO-d_6) δ: 1.24 (3H, t, J=7.1 Hz), 2.45 (3H, s), 4.18 (2H, q, J=7.1 Hz), 7.47 (2H, d, J=8.5 Hz), 7.68 (2H, d, J=8.5 Hz), 8.05 (1H, s), 12.49 (1H, s); ^{13}C-NMR (DMSO-d_6) δ: 14.67, 17.39, 60.49, 109.56, 128.61, 129.27, 133.35, 134.56, 143.46, 158.29, 162.23, 169.51; FAB-MS m/z (%): 326 (M^++2+H), 324 (M^++H); *Anal*. Calcd for $C_{14}H_{14}N_3O_2ClS$: C, 51.93; H, 4.36; N, 12.98. Found: C, 51.96; H, 4.38; N, 12.95.

4-Methyl-2-[N'-(4-cyanobenzylidene)hydrazino]thiazole-5-carboxylic Acid Ethyl Ester (**8e**): Yellow powder from CH_2Cl_2/EtOH; yield 71%; mp 227—228 °C; IR (KBr): 3187, 3085, 2980, 2225, 1678, 1556, 1427, 1376, 1277, 1093; ^{1}H-NMR (DMSO-d_6) δ: 1.25 (3H, t, J=6.9 Hz), 2.48 (3H, s), 4.18 (2H, q, J=6.9 Hz), 7.75—7.93 (4H, m), 8.09 (1H, s), 11.82 (1H, s); ^{13}C-NMR (DMSO-d_6) δ: 14.44, 17.11, 60.40, 110.35, 111.57, 118.89, 127.31, 132.88, 138.61, 142.39, 158.82, 161.92, 169.26; FAB-MS m/z (%): 315 (M^++H); *Anal*. Calcd for $C_{15}H_{14}N_4O_2S$: C, 57.31; H, 4.49; N, 17.82. Found: C, 57.27; H, 4.49; N, 17.80.

4-Methyl-2-(*N*′-pyridin-2-ylmethylene-hydrazino)thiazole-5-carboxylic Acid Ethyl Ester (**8f**): White power from CH_2Cl_2/EtOH; yield 82%; mp 229—231 °C; IR (KBr): 3147, 3040, 2987, 2939, 1685, 1563, 1474, 1426, 1370, 1324, 1308, 1283, 1096; ^{1}H-NMR (DMSO-d_6) δ: 1.28 (3H, t, J=7.1 Hz), 2.50 (3H, s), 4.22 (2H, q, J=7.1 Hz), 7.40 (1H, t, J=4.7 Hz), 7.80—8.00 (2H, m), 8.10 (1H, s), 8.60 (1H, d, J=4.4 Hz), 12.71 (1H, s); ^{13}C-NMR (DMSO-d_6) δ: 14.65, 17.67, 60.60, 110.87 120.04, 124.51, 137.26, 144.37, 149.92, 153.11, 159.57, 162.18, 169.45; FAB-MS m/z (%): 291 (M^++H); *Anal*. Calcd for $C_{13}H_{14}N_4O_2S$: C, 53.78; H, 4.86; N, 19.30. Found: C, 53.57; H, 4.82; N, 19.13.

4-Methyl-2-(*N*′-furan-2-ylmethylene-hydrazino)thiazole-5-carboxylic Acid Ethyl Ester (**8g**): Yellow crystals from CH_2Cl_2/EtOH; yield 56%; mp 194—196 °C; IR (KBr): 3208, 3097, 2980, 1668, 1561, 1528, 1410, 1371, 1318, 1277, 1091; ^{1}H-NMR (DMSO-d_6) δ: 1.26 (3H, t, J=7.3 Hz), 2.49 (3H, s), 4.19 (2H, q, J=7.3 Hz), 6.63 (1H, dd, J=3.3, 1.5 Hz), 6.89 (1H, d, J=3.3 Hz), 7.83 (1H, d, J=1.5 Hz), 9.97 (1H, s), 12.4 (1H, s); ^{13}C-NMR (DMSO-d_6) δ: 14.41, 17.08, 60.27, 109.08, 112.36, 113.49, 134.77, 145.19, 149.27, 157.85, 162.02, 168.99; FAB-MS m/z (%): 280 (M^++H); *Anal*. Calcd for $C_{12}H_{13}N_3O_3S$: C, 51.60; H, 4.69; N, 15.04. Found: C, 51.60; H, 4.68; N, 15.07. X-Ray analytical data are listed in Table 1. Further details have been deposited at the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 615373.

4-Methyl-2-(*N*′-thiophen-2-ylmethylene-hydrazino)thiazole-5-carboxylic Acid Ethyl Ester (**8h**): Yellow crystals from CH_2Cl_2/EtOH; yield 70%; mp 209—210 °C; IR (KBr): 3191, 3096, 2984, 1665, 1561, 1515, 1413, 1369, 1314, 1268, 1091; ^{1}H-NMR (DMSO-d_6) δ: 1.26 (3H, t, J=7.2 Hz), 2.46 (3H, s), 4.19 (2H, q, J=7.2 Hz), 7.12 (1H, dd, J=5.1, 3.7 Hz), 7.42 (1H, d, J=3.7 Hz), 7.63 (1H, d, J=5.1 Hz), 8.28 (1H, s), 12.42 (1H, s); ^{13}C-NMR (DMSO-d_6) δ: 14.47, 17.00, 60.30, 108.70, 128.10, 128.68, 130.35, 138.98, 140.19, 157.57, 162.03, 168.72; FAB-MS m/z (%): 296 (M^++H); *Anal*. Calcd for $C_{12}H_{13}N_3O_2S_2$: C, 48.80; H, 4.44; N, 14.23. Found: C, 48.74; H, 4.45; N, 14.05. X-Ray analytical data are listed in Table 1. Further details have been deposited at the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 615372.

Cyclization of Aromatic Aldehyde Thiosemicarbazones (7a—h) with 2-Bromoacetophenone (2b) Sodium acetate (492 mg, 6 mmol) was slowly added to an iced-cold solution of 4-methylbenzaldehyde thiosemicarbazone (**7a**, 386 mg, 2 mmol) in absolute ethanol (2 ml). Acetic acid (0.5 ml) was added as a catalyst and then 2-bromoacetophenone (**2b**, 418 mg, 2.1 mmol) was slowly added to the above solution. The mixed solution was stirred at 0 °C for about 5 h until the reaction was completed. The precipitate was collected by filtration and washed with ice-cold water and cold ethanol. The collected solid (573 mg) was recrystallized from dichloromethane/ethanol to afford 498 mg (1.70 mmol) of 4-phenyl-2-[N'-(4-methylbenzylidene)hydrazino]thiazole (**9a**) as a reddish orange powder in a yield of 85%. The chemical and physical spectral characteristics of these products **9a—h** are given below.

4-Phenyl-2-[N'-(4-methylbenzylidene)hydrazino]thiazole (**9a**): Reddish orange powder from CH_2Cl_2/EtOH; yield 85%; mp 195—196 °C; IR (KBr): 3279, 3109, 3026, 2916, 1555, 1510, 1479, 1344, 1268, 1129, 1050, 711 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 2.32 (3H, s), 7.23 (2H, d, J=8.0 Hz), 7.28—7.40 (4H, m), 7.54 (2H, d, J=8.0 Hz), 7.85 (2H, d, J=8.3 Hz), 8.00 (1H, s), 12.08 (1H, s); ^{13}C-NMR (DMSO-d_6) δ: 21.16, 103.68, 125.70, 126.42, 127.68, 128.77, 129.60, 131.91, 134.91, 139.12, 141.57, 150.72, 168.49; FAB-MS m/z (%): 294 (M^++H); *Anal*. Calcd for $C_{17}H_{15}N_3S$: C, 69.60; H, 5.15; N, 14.32. Found: C, 69.60; H, 5.29; N, 14.23.

4-Phenyl-2-[N'-(4-methoxybenzylidene)hydrazino]thiazole (**9b**): Brown

powder from CH_2Cl_2/EtOH; yield 74%; mp 190—191 °C; IR (KBr): 3193, 2963, 2934, 1610, 1567, 1512, 1358, 1254, 1171, 1027, 721 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 3.78 (3H, s), 6.99 (2H, d, J=8.8 Hz), 7.27—7.43 (4H, m), 7.59 (2H, d, J=8.8 Hz), 7.85 (2H, d, J=8.4 Hz), 7.98 (1H, s), 12.00 (1H, s); ^{13}C-NMR (DMSO-d_6) δ: 55.39, 103.48, 114.50, 125.68, 127.24, 127.64, 127.96, 128.74, 134.94, 141.46, 150.68, 160.42, 168.55; FAB-MS m/z (%): 310 (M^++H); *Anal*. Calcd for $C_{17}H_{15}N_3OS$: C, 66.00; H, 4.89; N, 13.58. Found: C, 66.07; H, 5.02; N, 13.50.

4-Phenyl-2-(*N*′-benzylidene-hydrazino)thiazole (**9c**): Pale brown powder from CH_2Cl_2/EtOH; yield 71%; mp 186—187 °C; IR (KBr): 3305, 3104, 3057, 1560, 1487, 1428, 1276, 1126, 1054, 691 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 7.28—7.43 (7H, m), 7.63—7.68 (2H, m), 7.86 (2H, d, J=8.4 Hz), 8.04 (1H, s), 12.17 (1H, s); ^{13}C-NMR (DMSO-d_6) δ: 103.83, 125.70, 126.42, 127.69, 128.77, 128.98, 129.39, 134.61, 134.88, 141.40, 150.74, 168.44; FAB-MS m/z (%): 280 (M^++H); *Anal*. Calcd for $C_{16}H_{13}N_3S$: C, 68.79; H, 4.69; N, 15.04. Found: C, 68.44; H, 4.71; N, 14.84.

4-Phenyl-2-[*N*′-(4-chlorobenzylidene)hydrazino]thiazole (**9d**): Yellow powder from CH_2Cl_2/EtOH; yield 77%; mp 197—198 °C; IR (KBr): 3141, 3096, 3065, 1561, 1531, 1490, 1432, 1359, 1270, 1132, 1091, 1055, 819, 720, 696 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 7.28—7.44 (4H, m), 7.47 (2H, d, J=8.6 Hz), 7.66 (2H, d, J=8.6 Hz), 7.85 (2H, d, J=8.4 Hz), 8.01 (1H, s), 12.24 (1H, s); ^{13}C-NMR (DMSO-d_6) δ: 103.99, 125.69, 127.73, 128.00, 128.78, 129.08, 133.55, 133.76, 134.80, 140.08, 150.77, 168.26; FAB-MS m/z (%): 316 (M^++2+H), 314 (M^++H); *Anal*. Calcd for $C_{16}H_{12}N_3ClS$: C, 61.24; H, 3.85; N, 13.39. Found: C, 61.32; H, 3.85; N, 13.35.

4-Phenyl-2-[*N*′-(4-cyanobenzylidene)hydrazino]thiazole (**9e**): Yellow powder from CH_2Cl_2/EtOH; yield 72%; mp 225—226 °C; IR (KBr): 3273, 3111, 2224, 1561, 1504, 1354, 1278, 1147, 1053, 720 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 7.29—7.44 (4H, m), 7.78—7.87 (6H, m), 8.06 (1H, s), 12.50 (1H, s); ^{13}C-NMR (DMSO-d_6) δ: 104.49, 111.03, 119.01, 125.71, 126.87, 127.81, 128.81, 132.91, 134.70, 139.08, 139.22, 150.82, 168.02; FAB-MS m/z (%): 305 (M^++H); *Anal*. Calcd for $C_{17}H_{12}N_4S$: C, 67.08; H, 3.97; N, 18.41. Found: C, 66.94; H, 3.97; N, 18.38.

4-Phenyl-2-(*N*′-pyridin-2-ylmethylene-hydrazino)thiazole (**9f**): Brown powder from CH_2Cl_2/EtOH; yield 81%; mp 181—182 °C; IR (KBr): 3167, 3061, 2959, 2843, 1595, 1572, 1482, 1434, 1357, 1276, 1157, 1137, 702 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 7.28—7.45 (5H, m), 7.70—7.93 (4H, m), 8.06 (1H, s), 8.56 (1H, d, J=4.6 Hz), 12.41 (1H, s); ^{13}C-NMR (DMSO-d_6) δ: 104.33, 119.32, 123.77, 125.68, 127.76, 128.78, 134.71, 136.91, 141.69, 149.60, 150.77, 153.37, 167.94; FAB-MS m/z (%): 281 (M^++H); *Anal*. Calcd for $C_{15}H_{12}N_4S$: C, 64.27; H, 4.31; N, 19.98. Found: C, 64.02; H, 4.35; N, 19.87. X-Ray analytical data are listed in Table 1. Further details have been deposited at the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 615374.

4-Phenyl-2-(*N*′-furan-2-ylmethylene-hydrazino)thiazole (**9g**): Pale brown powder from CH_2Cl_2/EtOH; yield 61%; mp 168—169 °C; IR (KBr): 3140, 3116, 3064, 1560, 1552, 1477, 1415, 1343, 1278, 1117, 1013, 755, 707 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 6.59 (1H, dd, J=3.2, 1.8 Hz), 6.79 (1H, d, J=3.3 Hz), 7.28—7.43 (4H, m), 7.79—7.91 (4H, m), 12.10 (1H, s); ^{13}C-NMR (DMSO-d_6) δ: 103.77, 112.20, 112.25, 125.66, 127.68, 128.74, 131.71, 134.79, 144.61, 149.52, 150.60, 168.05; FAB-MS m/z (%): 270 (M^++H); *Anal*. Calcd for $C_{14}H_{11}N_3OS$: C, 62.44; H, 4.12; N, 15.60. Found: C, 62.31; H, 4.14; N, 15.50.

4-Phenyl-2-(*N*′-thiophen-2-ylmethylene-hydrazino)thiazole (**9h**): Brown powder from CH_2Cl_2/EtOH; yield 71%; mp 173—174 °C; IR (KBr): 3168, 3101, 3077, 3056, 3027, 1601, 1581, 1441, 1418, 1363, 1337, 1279, 1056, 724, 695 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ: 7.09 (1H, dd, J=5.0, 3.6 Hz), 7.29—7.44 (5H, m), 7.57 (1H, d, J=5.0 Hz), 7.85 (2H, d, J=8.5 Hz), 8.22 (1H, s), 12.12 (1H, s); ^{13}C-NMR (DMSO-d_6) δ: 103.79, 125.67, 127.69, 127.78, 128.00, 128.77, 129.22, 134.79, 136.78, 139.37, 150.55, 168.01; FAB-MS m/z (%): 286 (M^++H); *Anal*. Calcd for $C_{14}H_{11}N_3S_2$: C, 58.92; H, 3.89; N, 14.72. Found: C, 58.89; H, 3.84; N, 14.66.

Scavenging Effect on 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical The scavenging effect of the synthesized compounds **8a**—**9h** on the DPPH radical was evaluated according to the method of Shimada, Leong and Braca.[16—18] Various concentrations of the test compound in 1.5 ml methanol were added to a 1.5 ml (0.2 mM) solution of DPPH radical in methanol (final concentration of DPPH was 0.1 mM). The mixture was shaken vigorously and allowed to stand for 30 min; absorbance at 517 nm was determined (Hitachi U-2001 Spectrophotometer), and the percentage of activity was calculated. Vitamin E was used as a reference compound. All tests and analyses were undertaken on three replicates and the results averaged.

scavenging activity (%)={[(Ab+As)−Am]/Ab}×100%

Ab: absorbance of 0.1 mM DPPH methanol solution at 517 nm;

As: absorbance of various concentration solution of test compound at 517 nm;

Am: absorbance of mixture methanol solution at 517 nm

Measurement of Lag Time of Antioxidant Activity in ABTS/H_2O_2/HRP System To a 96-well microplate was transferred 151 μl of potassium phosphate buffer (50 mM, pH 7.4) containing 2% NP-40 (Nonidet P-40), 35 μl 0f 20 nM horseradish peroxidase in potassium phosphate, 10 μl of various concentration (3, 6, 9 nmol, *etc*) of tested compound in 1-methyl-2-pyrrolidon and 56 μl of 10 mM ABTS in potassium phosphate buffer, with vortexing. The reaction was initiated by adding 28 μl of 1.0 mM H_2O_2 in potassium phosphate buffer. The clock was started, and the absorbance at 414 nm was determined using a Bio-Tek MQ 200R spectrophotometer at 6 s interval for 15 min. The incubation volume was thus 280 μl, and the reagents were at the desired concentration (2.5 nM horseradish peroxidase, 2 mM ABTS, 0.1 mM H_2O_2 in 50 mM potassium phosphate). The blank was also performed simultaneously. All tests and analyses were conducted on three replicates and the results averaged.

Acknowledgments Financial support of this work by the National Science Council of the Republic of China (NSC-95-2113-M-218-001) and Southern Taiwan University is highly appreciated.

References

1) Shukla H. K., Desai N. C., Astik R. R., Thaker K. A., *J. Indian Chem. Soc.*, **1984**, 168—169.
2) Desai N. C., Shukla H. K., Parekh B. P., Thaker K. A., *J. Indian Chem. Soc.*, **1984**, 455—457.
3) Marusawa H., Setoi H., Kuroda A., Sawada A., Seki J., Motoyama Y., Tanaka H., *Bioorg. Med. Chem.*, **7**, 2635—2645 (1999).
4) Tarasconi P., Capacchi S., Pelosi G., Cornia M., Albertini R., Bonati A., Dall'Aglio P. P., Lunghi P., Pinelli S., *Bioorg. Med. Chem.*, **8**, 157—162 (2000).
5) Mahler G., Serra G., Dematteis S., Saldaña J., Domínguez L., Manta E., *Bioorg. Med. Chem. Lett.*, **16**, 1309—1311 (2006).
6) Bolos C. A., Papazisis K. T., Kortsaris A. H., Voyatzi S., Zambouli D., Kyriakidis D. A., *J. Inorg. Biochem.*, **88**, 25—36 (2002).
7) Tapia R. A., Alegria L., Pessoa C. D., Salas C., Cortés M. J., Valderrama J. A., Sarciron M. E., Pautet F., Walchshofer N., Fillion H., *Bioorg. Med. Chem.*, **11**, 2175—2182 (2003).
8) Oh C. H., Cho H. W., Baek D., Cho J. H., *Eur. J. Med. Chem.*, **37**, 743—754 (2002).
9) Holla B. S., Malini K. V., Rao B. S., Sarojini B. K., Kumari N. S., *Eur. J. Med. Chem.*, **38**, 313—318 (2003).
10) Andreani A., Granaiola M., Leoni A., Locatelli A., Morigi R., Rambaldi M., *Eur. J. Med. Chem.*, **36**, 743—746 (2001).
11) Bonde C. G., Gaikwad N. J., *Bioorg. Med. Chem.*, **12**, 2151—2161 (2004).
12) Pontillo J., Chen C., *Bioorg. Med. Chem. Lett.*, **15**, 1407—1411 (2005).
13) Pontiki E., Hadjipavlou-Litina D., Chaviara A. T., Bolos C. A., *Bioorg. Med. Chem. Lett.*, **16**, 2234—2237 (2006).
14) Barros-García F. J., Bernalte-García A., Higes-Rolando F. J., Luna-Giles F., Maldonado-Rogado M. A., Vinuelas-Zahínos E., *Inorg. Chim. Acta*, **357**, 3574—3582 (2004).
15) Shih M. H., Ke F. Y., *Bioorg. Med. Chem.*, **12**, 4633—4643 (2004).
16) Shimada K., Fujikawa K., Yahara K., Nakamura T., *J. Agric. Food Chem.*, **40**, 945—948 (1992).
17) Leong L. P., Shui G., *Food Chem.*, **76**, 69—75 (2002).
18) Braca A., Sortino C., Politi M., Morelli I., Mendez J., *J. Ethnopharmacol.*, **79**, 379—381 (2002).
19) Arnao M. B., Cano A., Hernández-Ruiz J., García-Cánovas F., Acosta M., *Anal. Biochem.*, **236**, 255—261 (1996).
20) Rice-Evans C., Miller N. J., *Methods Enzymol.*, **234**, 279—293 (1994).
21) Arnao M. B., Casas J. L., del Río J. A., Acosta M., García-Cánovas F., *Anal. Biochem.*, **185**, 335—338 (1990).
22) Pratico D., Delanty N., *Am. J. Med.*, **109**, 577—585 (2000).
23) Wang H., Cao G., Prior R. L., *J. Agric. Food Chem.*, **44**, 701—705 (1996).
24) Aruoma O. I., *Free Radic. Biol. Med.*, **20**, 675—705 (1996).
25) Aruoma O. I., Spencer J. P. E., Mahmood N., *Food Chem. Toxicol.*, **37**, 1043—1053 (1999).
26) Fan J. M., Wang Y., Ueng C. H., *J. Phys. Chem.*, **97**, 8193—8199 (1993).
27) Nešpûrek S., Böhm S., Kuthan J., *J. Mol. Struct.*, **136**, 261—273 (1986).
28) Nešpûrek S., Hašek J., Šorm M., Huml K., *J. Mol. Struct.*, **82**, 95—105 (1982).