Antioxidant Capacity of 2-(3,5-diaryl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazoles

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Abstract: The antioxidant capacity of 2-(3,5-diaryl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazoles was evaluated. The values of antioxidant capacities of compounds 2d and 2e were found to be, respectively, 2,700 ± 150 and 3,135 ± 230 TE by the ORAC method, corresponding to a significant antioxidant capacity.

Keywords: Antioxidant, Antioxidant synthetic, Green chemistry, Thiazole, Trolox, ORAC.

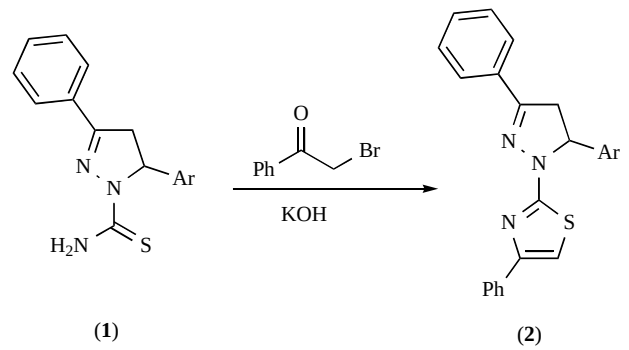
INTRODUCTION

Thiazole derivatives possess a broad spectrum of biological activities, which have attracted much recent attention from medicinal chemists [1-5]. Several drugs containing the thiazole nucleus, such as blenoxane, bleomycine and tiazofurin, are known antineoplastic agents [6].

The antioxidant capacity of several thiazolyl compounds has also been reported [7-9]. Recently, antioxidants have become a topic of increasing interest [10-13]. Characterization of the antioxidant capacity of natural and synthetic compounds with potential pharmacological activity is of great interest to medical and nutritional experts, to health and food science researchers and to the public in general [10-12]. Although there is no total antioxidant capacity assay based on a single, easily executable chemical reaction, there are numerous published methods that claim to measure total antioxidant capacity *in vitro* [10-12]. The ORAC method for determining antioxidant capacity uses: (a) AAPH (*an azo radical initiator*) which degrades the fluorescence of the probe; (b) the molecular probe fluorescein (FL) for monitoring the reaction progress and (c) the antioxidant of interest. In the presence of an antioxidant, the decrease in fluorescence is inhibited. The advantage of the ORAC approach is that it provides kinetics parameters (area under the curve) equally well for antioxidants that exhibit distinct lag phases and for those samples that have no lag phases [10-12]. In recent publication, we reported the antioxidant capacity of phenylpyrazole using the ORAC method [14]. In continuation of our research program, we report here the antioxidant evaluation of 2-(3,5-diaryl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazoles, synthetic derivatives compounds of facile preparation (Scheme **1**).

RESULTS AND DISCUSSION

A preliminary ORAC assay screening was carried out to determine which compounds exhibited the most significant antioxidant capacity. This led to the selection of 2-(5-(3-nitrophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazole (**2d**) and 2-(5-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazole (**2e**) for more careful quantification Table **1**, because the other compounds did not show measurable antioxidant potential by the ORAC method.



(**1**) (**2**)

Scheme 1. Preparation of 4-phenylthiazole **2a-2e.**

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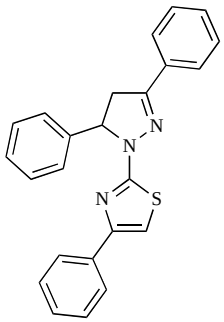
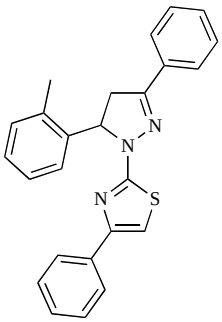
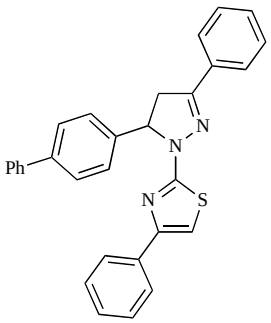
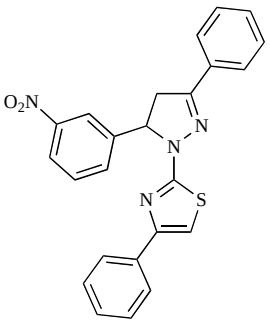
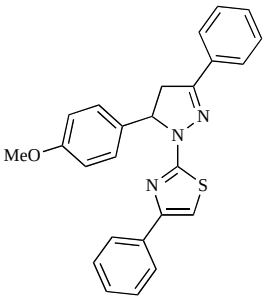
Table 1. Selected Experimental Compounds (2)

Compound 2a	Compound 2b	Compound 2c	Compound 2d*	Compound 2e*
				

*Compound with antioxidant capacity.

The values of antioxidant capacities of 2-(5-(3-nitrophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazole (**2d**) and 2-(5-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazole (2e) were found to be, respectively, 2,700 ± 150 and 3,135 ± 230 µmol eq. Trolox/g (TE) by the ORAC method (Table **2**), corresponding to quite good antioxidant capacity (Trolox itself is 4000 µmol/g). Of course, it should be kept in mind that *in vitro* antioxidant assays only represent the observed response in the given reaction medium, meaning that the values of the apparent antioxidant activities might also be influenced by differences in the chemical reaction between the free radical used in the assay and the substance being tested.

ORAC Methodology, Antioxidant Evaluation

This method was employed because it uses a physiological relevant free radical as well as its reaction medium. The automatic ORAC assay was described by Ou *et al.* [10]. In this method, an azo initiator (AAPH) decomposing at 37°C abstracts hydrogen from sodium fluorescein, reducing its fluorescence (485/20 nm excitation filter and 528/20 nm emission filter). When a test compound with antioxidant capacity is added, the reaction with fluorescein and the resultant decrease in its fluorescence are delayed until the antioxidant capacity of the test compound is completely exhausted. The putative antioxidants were solubilized in acetone and diluted in 7% randomly methylated β-cyclodextrin solution. After appropriate dilution, 25 µL of these solutions were transferred to microplates in triplicate and 150 µL of 40 nM fluorescein diluted in 75mM phosphate buffer pH 7.0, were added. On the same microplate a control (25 µL of solvent + 150 µL of 40 nM fluorescein) and a Trolox (standard) curve (150 µL fluorescein 40 nM + 25 µL aliquot from a Trolox® solution of known concentration) were made. To maintain the plate temperature, 300 µL of water were added around the wells. After the incubation (37°C/30 min), 25 µL of AAPH (153 mM in 75 mM phosphate buffer, pH 7.0) were added and the plate shaken during 10 seconds at maximum intensity. The plate reader (Synergy – BIOTEK Multidetection microplate reader, Winooski, VT) was programmed to record the fluorescence at each cycle from 1 minute after addition of AAPH up to 60 minutes and the area under the fluorescence curve integrated over time by using Gen5 software. All area values were corrected for the control (25 µL of solvent + 150 µL of 40 nM fluorescein + 25 µL AAPH) to obtain the net area under the curve (net AUC) for each sample.

Table 2. ORAC Antioxidant Capacity as the Concentration of Compound 2d and 2e (Values were Expressed as Mean ± Standard Deviation)

Compound	Antioxidant Capacity	Correlation Coefficient (r^2)
2d	2,700 ± 150 µmol eq. trolox/g	0,9722
2e	3,135 ± 230 µmol eq. trolox/g	0,9849

Using the Trolox® calibration curve, expressed as net area under the curve versus concentration (µM), the antioxidant capacity of each sample can be expressed in terms of its µmol equivalents of Trolox/g ± standard deviation or Trolox® equivalent (TE) (Table **2**). This procedure was carried out in triplicate for each sample at each concentration. Thus, higher values of TE mean higher antioxidant capacity of the sample.

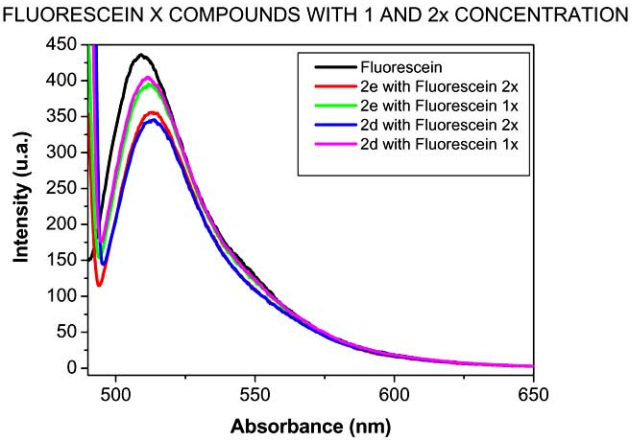


Fig. (1). Absorbance (nm) of compounds **2d** and **2e** in acetone.

Finally, care was taken to demonstrate that compounds **2a - 2e** do not interfere with the fluorimetric analysis itself. All five compounds fluoresce at about 450nm when exited

below 425nm, where they absorb (Fig. **1**). Thus competitive absorption and emission can be ruled out under the conditions of excitation of fluorescein 485nm. Quenching of the fluorescence of fluorescein by compounds **2a - 2e** was also ruled out by demonstrating that the effect of addition of up to a two-fold excess of these compounds in acetone to an aqueous solution of fluorescein had the made effect (due merely to dilution of the solution) as addition of acetone alone.

A it empts were made to quantify the antioxidant capacity by the DPPH method but, unfortunately, due to the lack of solubility of these compounds in the reaction media tested (methanol, ethanol and isopropanol), this was not possible.

Synthesis and Structure Confirmation

The 1-thiocarbamoyl-3,5-diaryl-4,5-dihydro-1*H* pyrazoles (**1**) were prepared according literature [15]. The 2-(3,5-diaryl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazoles (**2**) were prepared from the 1-thiocarbamoyl-4,5-dihydro-1*H*-pyrazole (**1)** by reaction with phenacyl bromine in ethanol in the presence of potassium hydroxide (Scheme **1**) [16].

The structure of compounds (**2**) were confirmed by NMR and mass spectra. NMR spectra were recorded on a Bruker DPX 500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C) at 300 K. Low resolution mass spectra were obtained on a Varian Saturn 2200 GC/MS spectrometer operating at 70 eV.

Selected Experimental Data for Compounds 2a–e

*2-(3,5-Diphenyl-4,5-dihydro-1H-pyrazol-1-yl)-4-phenylthiazole (**2a**):* Yield (70%); yellow solid; mp 213–215°C; IR (KBr): ν (cm^{-1}) 3115–3028, 1953–1756, 1541, 1520, 1492, 1443, 707, 694; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 3.33 (dd, 1H, *J*=6.7 Hz, *J*=17.4 Hz), 3.90 (dd, 1H, *J*=12.0 Hz, *J*=17.4 Hz), 5.68 (dd, 1H, *J*= 6.7Hz, *J*=12.0 Hz), 6.80 (s, 1H), 7.20–7.77 (m, 15H, ArH); ^{13}C NMR ($CDCl_3$, 126 MHz): δ (ppm) 43.4, 64.7, 103.3, 125.8, 126.3, 126.6, 127.4, 127.7, 128.4, 128.6, 128.7, 129.7, 131.5, 135.0, 141.8, 151.5, 151.5, 164.9.

*2-[5-(2-Methylphenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl]-4-phenylthiazole (**2b**):* Yield (74%); yellow solid; mp 172–174°C; IR (KBr): ν (cm^{-1}) 3117–2915, 1943–1875, 1542, 1489, 1440, 713, 683; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 2.55 (s, 3H), 3.15 (dd, 1H, *J*=7.0 Hz, *J*=17.3 Hz), 3.84 (dd, 1H, *J*=12.2 Hz, *J*=17.3 Hz), 5.82 (dd, 1H, *J*=6.9 Hz, *J*=12.2 Hz), 6.78 (s, 1H), 7.10–7.74 (m, 14H, ArH); ^{13}C NMR ($CDCl_3$, 126 MHz): δ (ppm) 19.6, 42.7, 61.4, 103.3, 125.7, 125.8, 126.3, 126.6, 127.3, 127.4, 128.3, 128.6, 129.6, 130.4, 131.5, 134.7, 135.0, 139.9, 151.3, 151.5, 164.7; Mass: calcd 395.15, found 396.2; LC/MS/MS: *m/z* 396.1 (M+1, 100), 222.3, 175.2, 148.1, 104.1.

*2-[5-(4-Biphenylyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl]-4-phenylthiazole (**2c**):* Yield (65%), yellow solid; mp 161–163°C; IR (KBr): ν (cm^{-1}) 3124–2911, 1982–1680, 1546, 1482, 1444, 708, 693; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 3.41 (dd, 1H, *J*=6.4 Hz, *J*=17.5 Hz), 3.97 (dd, 1H, *J*=12.0 Hz, *J*=17.5 Hz), 5.91 (dd, 1H, *J*=6.4 Hz, *J*=12.0 Hz), 6.82 (s, 1H), 7.22–7.81 (m, 19H, ArH); ^{13}C NMR ($CDCl_3$, 126 MHz): δ (ppm) 43.6, 64.5, 103.3, 126.0, 126.5, 127.1, 127.1, 127.3, 127.5, 128.4, 128.7, 128.7, 129.0, 130.0, 140.7, 140.7, 147.5, 154.3, 165.0;

*2-[5-(3-Nitrophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl]-4-phenylthiazole (**2d**):* Yield (69%), white solid; mp 172–174°C; IR (KBr): ν (cm^{-1}) 3410, 3246–3060, 1961–1760, 1596, 1530, 1462, 1442, 1346, 686; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 3.36 (dd, 1H, *J*=7.5 Hz, *J*=17.5 Hz), 4.00 (dd, 1H, *J*=12.1 Hz, *J*=17.5 Hz), 5.75 (dd, 1H, *J*=7.5 Hz, *J*=12.1 Hz), 6.86 (s, 1H), 7.21–8.16 (m, 14H, ArH); ^{13}C NMR ($CDCl_3$, 126 MHz): δ (ppm) 43.2, 64.1, 103.9, 122.3, 122.8, 125.7, 126.4, 127.6, 128.5, 128.8, 129.7, 130.1, 131.0, 132.8, 134.6, 148.2, 151.4, 151.5, 164.9;

*2-[5-(4-Methoxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl]-4-phenylthiazole (**2e**):* Yield (81%), yellow solid; mp 182–184°C; IR (KBr): ν (cm^{-1}) 3123–2833, 1959–1647, 1544, 1515, 1443, 1249, 789, 672; ^{1}H NMR ($CDCl_3$, 500 MHz): δ (ppm) 3.29 (dd, 1H, *J*=6.6 Hz, *J*=17.4 Hz), 3.75 (s, 3H), 3.83 (dd, 1H, *J*=12.0 Hz, *J*=17.4 Hz), 5.61 (dd, 1H, *J*=6.6 Hz, *J*=12.0 Hz), 6,78 (s, 1H), 6.84–7.76 (m, 14H, ArH); ^{13}C NMR ($CDCl_3$, 126 MHz): δ (ppm) 43.3, 55.2, 64.1, 103.3, 114.0, 125.8, 126.3, 127.4, 127.9, 128.3, 128.6, 129.6, 131.5, 133.8, 135.0, 151.4, 151.5, 159.0, 164.9.

CONCLUSIONS

In conclusion, 2-(5-(3-nitrophenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazole (**2d**) and 2-(5-(4-methoxyphenyl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)-4-phenylthiazole (**2e**) showed good antioxidant capacity compared comparable to that of Trolox®. More investigations are currently in progress to explore the reaction and relationship structure-activity of 4-phenylthiazoles.

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