Efficient Microwave-Assisted Synthesis and Antioxidant Activity of 4-Arylidene-2-phenyl-1*H*-imidazol-5(4*H*)-ones

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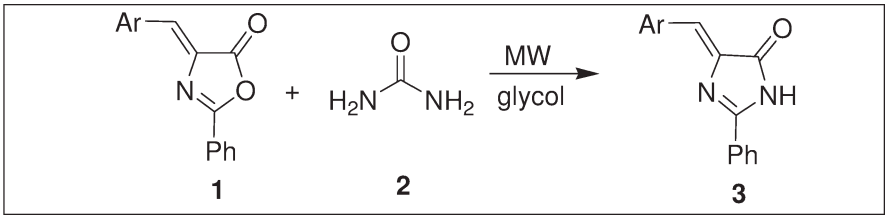

The efficient synthesis of 4-arylidene-2-phenyl-1*H*-imidazol-5(4*H*)-ones was achieved via microwave-assisted reactions of 4-arylmethylene-2-phenyloxazol-5(4*H*)-ones with urea in glycol. This approach provides a facile shortcut for the synthesis of this type of compounds with short reaction time, high yields, broad substrate scope and easy operation. Besides, the synthesized compounds were subject to the test of antioxidant activity, which is represented by their capacities for scavenging 1,1-diphenyl-2-picrylhydrazyl, hydroxyl and superoxide anion free radicals. Bioassay of these compounds resulted in the finding of several 4-arylidene-2-phenyl-1*H*-imidazol-5(4*H*)-ones with significant antioxidant activity.

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INTRODUCTION

4-Arylidene-2-phenyl-1*H*-imidazol-5(4*H*)-ones (Fig. 1), one important kind of imidazole derivatives, have exhibited various bioactivities including immunomodulatory [1], anticancer [2], anti-inflammatory [3], leishmanicidal [4], antibacterial and antifungal [5] activities besides their actions as dual specificity tyrosine-regulated kinase-1A inhibitors [6] and potential COX-2 inhibitors [7]. Therefore, the synthesis and bioassay of this type of compounds have absorbed great and persistent attention during half of the century.

Survey of the literature reveals that there are mainly three typical approaches to the synthesis of 4-arylidene-2-phenyl-1*H*-imidazol-5(4*H*)-ones. One method is the condensation of 4-arylmethylene-2-phenyloxazol-5(4*H*)-ones with ammonium acetate, urea or thiourea under various conditions [8]. Another method is the multicomponent reactions of benzamidine or its hydrochloride, aromatic aldehyde, and 2-haloacetate [9]. The other method is the similar three-component condensations of ethoxy substituted benzamidine, aromatic aldehyde, and 2-aminoacetate [10].

However, these methods still have one or more limitations such as long reaction time, moderate yields, narrow substrate scope and operational complexity. On the other hand, to the best of our knowledge, the antioxidant activity of 4-arylidene-2-phenyl-1*H*-imidazol-5(4*H*)-ones has not been investigated. Therefore, developing a synthetic method of these target compounds with high efficiency, broad substrate scope and easy operation as well as evaluation of their antioxidant activity is of great significance.

As a continuation of our efforts on the efficient synthesis and potential bioactivities of heterocyclic compounds [11], we report the facile synthesis of 4-arylidene-2-phenyl-1*H*-imidazol-5(4*H*)-ones **3** via condensations of 4-arylmethylene-2-phenyloxazol-5(4*H*)-ones **1** with urea **2** in glycol under microwave irradiation (MW) (Scheme 1) and the bioassay of their antioxidant activity.

RESULTS AND DISCUSSION

Initially, the condensation of 4-(4-chlorobenzylidene)-2-phenyloxazol-5(4*H*)-one **1c** (1 mmol) with urea **2** (1.5 mmol) was employed to optimize the reaction conditions. To find the best suitable solvent, we compared the synthesis of **3c** in different solvents including water, glycol, DMF, glacial acetic acid, and ethanol at 160°C and 300 W. The results (Table 1) reveal that glycol as

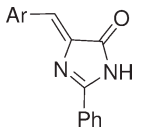


Figure 1. Structure of 4-arylidene-2-phenyl-1H-imidazol-5(4H)-ones.

solvent can greatly improve the yield of this reaction. Therefore, glycol was preferred as solvent for all further microwave-assisted reactions.

To optimize the reaction temperature, the condensation of **1c** (1 mmol) with urea **2** (1.5 mmol) was carried out using glycol (2 mL) as solvent under MW (300 W) at temperatures ranging from 100 to 190°C, with an increment of 10°C each time. The results are shown in Table 2. The yield of product **3c** was increased when the temperature was increased from 100 to 180°C (Entries 1−9, Table 2), whereas the yield leveled off when the temperature was further increased to 190°C (Entry 10, Table 2). So, 180°C is assigned as the most suitable reaction temperature.

Under these optimized reaction conditions (2.0 mL of glycol, 180°C), a series of 4-arylidene-2-phenyl-1*H*-imidazol-5(4*H*)-ones **3** were synthesized under MW, and the results were summarized in Table 3. Obviously, this protocol can be applied not only to substrate **1** with either electron-withdrawing or electron-donating group in aromatic ring, but also to substrate **1** with heterocyclic aromatic ring in high yields. Therefore, this efficient approach has wide scope of applicability in synthesizing these type of compounds.

The structures of all the synthesized compounds were established on the basis of their spectroscopic data and high resolution mass spectrum (HRMS) [electrospray ionization (ESI)]. The stereochemistry of the double bond was designated as Z configuration by comparing the 1H NMR data of compound **3a** with that reported in literature [12]. A plausible mechanism for the formation of compounds **3** is suggested in Scheme 2. First, ammonia acting as a nucleophilic reagent, which is obtained from urea **2**, attacks 4-arylmethylene-2-phenyloxazol-5(4*H*)-ones **1** to generate the aminolysis of this starting material and thereby performs the ring-opening reaction. Second, the intramolecular nucleophilic addition and subsequent elimination of water molecule affords final cyclic 4-arylidene-2-phenyl-1*H*-imidazol-5(4*H*)-ones **3**.

To survey the possible biological activities of this class of compounds, 4-arylidene-2-phenyl-1*H*-imidazol-5(4*H*)-

Scheme 1

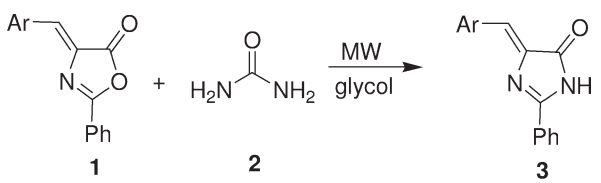


Table 1

Solvent optimization for the synthesis of **3c**.

Entry	Solvent	Time (min)	Yield (%)
1	DMF	4	71
2	EtOH	4	45
3	HOAc	4	Trace
4	Water	4	24
5	Glycol	4	82

ones **3** were subject to the test of antioxidant activity, which is represented by their capacities for scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl (OH), and superoxide anion (O_2^-) free radicals using protocols described in our previous works [11e,f], and the results are summarized in Table 4. Compared with the positive control (PC) L-Ascorbic acid, all the tested compounds showed strong capacities for scavenging O_2^- and OH, and most of the tested compounds also showed strong capacities for scavenging DPPH. The top three for scavenging DPPH and OH radicals are compounds **3h**, **3i**, and **3j** with two or three methoxyl groups in the aromatic ring. Although the top three for scavenging O_2^- are compounds **3h**, **3j**, and **3c** with 2,3-dimethoxylbenzyl, 3,4,5-trimethoxylbenzyl and 4-chlorobenzyl as Ar group, respectively. It is worthnoting that compound **3h** has the most extraordinary capacities for scavenging all the three radicals, which are dozens of times higher than those of the PC. Although there is no regular relationship between the Ar group and the antioxidant activity of the tested compounds, it is obvious that the methoxyl groups in the aromatic ring may contribute to the antioxidant activity of the top three compounds **3h**, **3i**, and 3j.

In conclusion, this study has achieved the efficient synthesis of 4-arylidene-2-phenyl-1*H*-imidazol-5(4*H*)-ones and examined their *in vitro* antioxidant activity. This microwave-assisted approach does provide a facile shortcut for the synthesis of the target compounds with short reaction time, high yields, broad substrate scope, and easy operation. More importantly, bioassay of these compounds resulted in the finding of several 4-arylidene-2-phenyl-1*H*-imidazol-5(4*H*)-ones with significant

Table 2

Temperature optimization for the synthesis of **3c**.

Entry	T (°C)	Time (min)	Yield (%)
1	100	8	Trace
2	110	8	Trace
3	120	8	29
4	130	8	48
5	140	6	61
6	150	6	75
7	160	4	82
8	170	4	87
9	180	4	90
10	190	4	90

Table 3

Scope for the synthesis of **3** under MW.

Entry	3	Ar	Time (min)	Yield (%)	m.p. (°C)
1	**3a**	C_6H_5	4	83	>300 (272–273)[8b]
2	**3b**	2-ClC_6H_4	6	80	279–281
3	**3c**	4-ClC_6H_4	4	90	>300 (289–290)[8b]
4	**3d**	3-BrC_6H_4	4	84	282–283
5	**3e**	4-BrC_6H_4	4	86	>300
6	**3f**	3-$NO_2C_6H_4$	4	85	263–264
7	**3g**	4-$NO_2C_6H_4$	4	83	>300
8	**3h**	2,3-$(CH_3O)_2C_6H_3$	6	77	260–261
9	**3i**	3,4-$(CH_3O)_2C_6H_3$	6	76	282–284
10	**3j**	3,4,5-$(CH_3O)_3C_6H_2$	6	73	>300
11	**3k**	3,4-$OCH_2OC_6H_3$	4	85	>300
12	**3l**	4-$(CH_3)_2NC_6H_4$	4	80	293-294 (268–269)[8b]
13	**3m**	Thiophen-2-yl	4	83	>300 (291–292)[8b]

Table 4

Free radicals scavenging capacities of compounds **3**.[a]

3	DPPH (%/mg)	OH (%/mg)	O_2^- (%/mg)
3a	195.51 ± 4.24	489.97 ± 6.82	610.68 ± 11.72
3b	177.88 ± 2.78	692.46 ± 9.23	509.83 ± 12.48
3c	163.46 ± 2.78	434.45 ± 13.22	626.30 ± 20.67
3d	177.88 ± 5.55	545.74 ± 14.26	290.75 ± 34.87
3e	240.38 ± 5.55	769.14 ± 25.54	333.92 ± 13.13
3f	144.23 ± 5.55	546.25 ± 1.05	470.17 ± 17.41
3g	118.59 ± 3.21	500.71 ± 9.87	568.69 ± 10.17
3h	2003.21 ± 57.78	4131.61 ± 97.15	3470.95 ± 126.50
3i	240.38 ± 11.10	1269.46 ± 3.12	561.52 ± 17.41
3j	400.64 ± 8.48	959.60 ± 4.01	973.80 ± 15.14
3k	205.13 ± 5.78	438.38 ± 6.36	531.60 ± 6.17
3l	195.51 ± 6.41	508.94 ± 13.55	576.21 ± 5.48
3m	115.38 ± 2.78	565.55 ± 5.44	577.38 ± 3.20
PC[b]	182.82 ± 0.98	97.25 ± 0.83	104.17 ± 1.10

[a] The scavenging capacities were represented as percentage inhibition (mean ± S.D., $n = 3$) of the free radicals by 1 mg tested compound.
[b] L-Ascorbic acid was used as a positive control (PC).

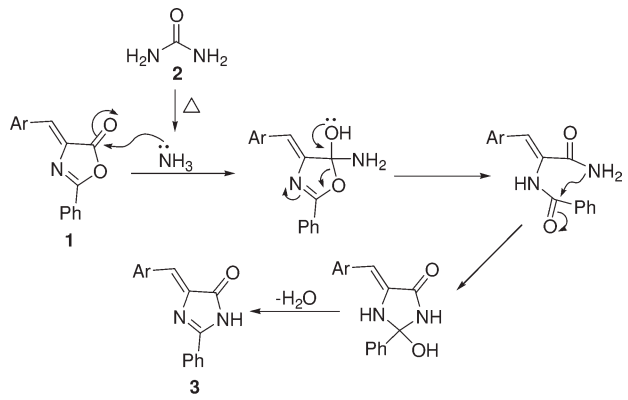
antioxidant activity, which gains some insights into the possible application of these compounds for the treatment of oxidative-induced diseases.

EXPERIMENTAL

Microwave irradiation was carried out in a monomodal Emrys™ Creator from Personal Chemistry, Uppsala, Sweden. Melting points were determined in XT5 apparatus and are uncorrected. IR spectra were recorded on a FT-IR-Tensor 27 spectrometer. ^{1}H NMR spectra were measured on a DPX 400 spectrometer operating at 400 MHz, using DMSO-d_6 as solvent and TMS as internal standard. HRMS (ESI) was determined by using micrOTOF-QII HRMS/MS instrument (BRUKER). All chemicals except 4-arylidene-2-phenyl-1,3-oxazol-5(4*H*)-ones **1** were purchased and used without further purification. 4-Arylidene-2-phenyl-1,3-oxazol-5(4*H*)-ones **1** were synthesized according to a previously reported approach [13].

General procedure for the synthesis of compounds 3. Typically, in a 10-mL Emrys™ reaction vial, 4-arylmethylene-2-phenyloxazol-5(4*H*)-ones **1** (1.0 mmol) with urea **2** (1.5 mmol) in ethylene glycol (2.0 mL) were mixed and then capped. The mixture was irradiated by microwave at 300 W and 180° for a given time. Upon completion, monitored by thin layer chromatography (TLC), the reaction mixture was cooled to room temperature and then poured into cold water, filtered to give the crude products, which were further purified by recrystallization from 95% EtOH.

Scheme 2



(Z)-4-Benzylidene-2-phenyl-1H-imidazol-5(4H)-one (3a). IR (KBr): 3124, 3067, 2989, 1698, 1639, 1539, 1496, 1452, 1419, 1322, 1266, 1187, 1031, 922, 775, 687 cm^{-1}; ^{1}H NMR (400 MHz, DMSO) (δ, ppm): 12.12 (s, 1H, NH), 8.32 (d, J = 7.2 Hz, 2H, ArH), 8.18 (d, J = 7.6 Hz, 2H, ArH), 7.67–7.69 (m, 3H, ArH), 7.52–7.44 (m, 3H, ArH), 7.04 (s, 1H, =CH); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{16}H_{13}N_2O$: 249.1023; found: 249.1023.

(Z)-4-(2-Chlorobenzylidene)-2-phenyl-1H-imidazol-5(4H)-one (3b). IR (KBr): 3151, 3125, 3061, 2990, 1707, 1638, 1499, 1456, 1216, 1033, 896, 755, 684 cm^{-1}. ^{1}H NMR (400 MHz, DMSO) (δ, ppm): 12.30 (s, 1H, NH), 9.05 (d, J = 8.0 Hz, 1H, ArH), 8.20 (d, J = 8.4 Hz, 2H, ArH), 7.69–7.59 (m, 4H, ArH), 7.53–7.46 (m, 2H, ArH), 7.30 (s, 1H, =CH). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{16}H_{12}ClN_2O$: 283.0633; found: 283.0641.

(Z)-4-(4-Chlorobenzylidene)-2-phenyl-1H-imidazol-5(4H)-one (3c). IR (KBr): 3153, 3124, 3066, 2987, 1703, 1641, 1541, 1456, 1261, 1180, 1092, 923, 788, 691 cm^{-1}. ^{1}H NMR (400 MHz, DMSO) (δ, ppm): 12.18 (s, 1H, NH), 8.36 (d, J = 8.4 Hz, 2H, ArH), 8.19 (d, J = 8.4 Hz, 2H, ArH), 7.67–7.56 (m, 5H, ArH), 7.05 (s, 1H, =CH). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{16}H_{12}ClN_2O$: 283.0633; found: 283.0636.

(Z)-4-(3-Bromobenzylidene)-2-phenyl-1H-imidazol-5(4H)-one (3d). IR (KBr): 3127, 3068, 2982, 1711, 1647, 1536, 1454, 1419, 1356, 1262, 1193, 907, 782, 681 cm^{-1}. ^{1}H NMR (400 MHz, DMSO) (δ, ppm): 12.20 (s, 1H, NH), 8.58 (s, 1H, ArH), 8.31 (d, J = 8.0 Hz, 1H, ArH), 8.16 (d, J = 6.8 Hz, 2H, ArH), 7.67–7.61 (m, 4H, ArH), 7.46 (t, J = 8.0 Hz, 1H, ArH), 7.02 (s, 1H, =CH). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{16}H_{12}BrN_2O$: 327.0128; found: 327.0145.

(Z)-4-(4-Bromobenzylidene)-2-phenyl-1H-imidazol-5(4H)-one (3e). IR (KBr): 3153, 3123, 3066, 2987, 2846, 1700, 1638, 1540, 1456, 1261, 1179, 1071, 922, 786 cm^{-1}. ^{1}H NMR (400

MHz, DMSO) (δ, ppm): 12.19 (s, 1H, NH), 8.28 (d, J = 8.4 Hz, 2H, ArH), 8.18 (d, J = 8.4 Hz, 2H, ArH), 7.72–7.60 (m, 5H, ArH), 7.03 (s, 1H, =CH). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{16}H_{12}BrN_2O$: 327.0128; found: 327.0138.

(Z)-4-(3-Nitrobenzylidene)-2-phenyl-1H-imidazol-5(4H)-one (3f). IR (KBr): 3152, 3120, 3067, 2987, 1708, 1646, 1527, 1454, 1352, 1259, 1095, 920, 791 cm^{-1}. ^1H NMR (400 MHz, DMSO) (δ, ppm): 12.28 (s, 1H, NH), 9.34(s, 1H, ArH), 8.66 (d, J = 8.0 Hz, 1H, ArH), 8.25 (d, J = 8.0 Hz, 1H, ArH), 8.21 (d, J = 8.0 Hz, 1H, ArH), 7.78 (q, J = 8.0 Hz, 1H, ArH), 7.70–7.64 (m, 4H, ArH), 7.20 (s, 1H, =CH). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{16}H_{12}N_3O_3$: 294.0874; found: 294.0876.

(Z)-4-(4-Nitrobenzylidene)-2-phenyl-1H-imidazol-5(4H)-one (3g). IR (KBr): 3127, 3066, 2986, 1711, 1594, 1511, 1416, 1341, 1256, 1056, 889, 684 cm^{-1}. ^1H NMR (400 MHz, DMSO) (δ, ppm): 12.31 (s, 1H, NH), 8.56 (d, J = 8.4 Hz, 2H, ArH), 8.31 (d, J = 8.0 Hz, 2H, ArH), 8.23 (d, J = 7.8 Hz, 2H, ArH), 7.71–7.61 (m, 3H, ArH), 7.12 (s, 1H, =CH). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{16}H_{12}N_3O_3$: 294.0874; found: 294.0869.

(Z)-4-(2,3-Dimethoxybenzylidene)-2-phenyl-1H-imidazol-5(4H)-one (3h). IR (KBr): 3126, 3068, 2988, 1704, 1637, 1573, 1458, 1263, 1074, 999, 786, 695 cm^{-1}. ^1H NMR (400 MHz, DMSO) (δ, ppm): 12.17 (s, 1H, NH), 8.54 (d, J = 6.8 Hz, 1H, ArH), 8.17 (d, J = 6.8 Hz, 2H, ArH), 7.66–7.59 (m, 3H, ArH), 7.30 (s, 1H, =CH), 7.23 (t, J = 8.0Hz, 1H, ArH), 7.16 (d, J = 8.0 Hz, 1H, ArH), 3.86 (s, 3H, °C H_3), 3.84 (s, 3H, °C H_3). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{18}H_{17}N_2O_3$: 309.1234; found: 309.1230.

(Z)-4-(3,4-Dimethoxybenzylidene)-2-phenyl-1H-imidazol-5(4H)-one (3i). IR (KBr): 3122, 3069, 2936, 1700, 1639, 1593, 1519, 1458, 1335, 1232, 1136, 1018, 921, 787, 695 cm^{-1}. ^1H NMR (400 MHz, DMSO) (δ, ppm): 12.02 (s, 1H, NH), 8.53 (s, 1H, ArH), 8.15 (d, J = 8.0 Hz, 1H, ArH), 8.05 (d, J = 8.0 Hz, 1H, ArH), 7.76 (dd, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H, ArH), 7.61–7.55 (m, 3H, ArH), 7.09–7.06 (m, 1H, ArH), 7.00 (s, 1H, =CH), 3.87 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{18}H_{17}N_2O_3$: 309.1234; found: 309.1227.

(Z)-4-(3,4,5-Trimethoxybenzylidene)-2-phenyl-1H-imidazol-5(4H)-one (3j). IR (KBr): 3153, 3063, 2991, 1703, 1637, 1575, 1499, 1456, 1332, 1246, 1135, 1000, 919, 786, 693 cm^{-1}. ^1H NMR (400 MHz, DMSO) (δ, ppm): 12.09 (s, 1H, NH), 8.16 (d, J = 6.8 Hz, 2H, ArH), 7.78 (s, 2H, ArH), 7.66–7.58 (m, 3H, ArH), 7.00 (s, 1H, =CH), 3.88 (s, 6H, $2OCH_3$), 3.75 (s, 3H, °C H_3). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{19}H_{19}N_2O_4$: 339.1340; found: 339.1350.

(Z)-4-((Benzo[d][1,3]dioxol-5-yl)methylene)-2-phenyl-1H-imidazol-5(4H)-one (3k). IR (KBr): 3118, 3060, 2985, 1704, 1617, 1485, 1446, 1263, 1227, 1105, 1034, 920, 885, 785, 694 cm^{-1}. ^1H NMR (400 MHz, DMSO) (δ, ppm): 12.05 (s, 1H, NH), 8.15–8.13 (m, 3H, ArH), 7.70 (dd, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 1H, ArH), 7.63–7.59 (m, 3H, ArH), 7.05 (d, J = 8.0 Hz, 1H, ArH), 6.99 (s, 1H, =CH), 6.13 (s, 2H, CH_2). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{17}H_{13}N_2O_3$: 293.0921; found: 293.0920.

(Z)-4-(4-(Dimethylamino)benzylidene)-2-phenyl-1H-imidazol-5(4H)-one (3l). IR (KBr): 3126, 3059, 2985, 1695, 1590, 1522, 1455, 1364, 1316, 1165, 1120, 1029, 918, 798, 694 cm^{-1}. ^1H NMR (400 MHz, DMSO) (δ, ppm): 11.91 (s, 1H, NH), 8.37 (d, J = 6.8 Hz, 1H, ArH), 8.18 (d, J = 6.8 Hz, 1H, ArH), 8.12 (dd, J_1 = 8.0 Hz, J_2 = 2.4 Hz, 1H, ArH), 8.02–8.01 (m, 1H, ArH), 7.58–7.56 (m, 2H, ArH), 7.53–7.51 (m, 1H, ArH), 6.94 (s, 1H, =CH), 6.82–6.76 (m, 2H, ArH), 3.31 (s, 6H, $2CH_3$). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{18}H_{18}N_3O$: 292.1445; found: 292,1439.

(Z)-2-Phenyl-4-((thiophen-2-yl)methylene)-1H-imidazol-5(4H)-one (3m). IR (KBr): 3116, 3061, 2985, 1698, 1634, 1457, 1419, 1320, 1256, 1200, 1121, 922, 891, 788, 692 cm^{-1}. ^1H NMR (400 MHz, DMSO) (δ, ppm): 12.06 (s, 1H, NH), 8.16 (dd, J_1 = 8.0 Hz, J_2 = 1.2 Hz, 2H, ArH), 7.92 (d, J = 5.2 Hz, 1H, ArH), 7.74 (d, J = 2.7 Hz, 1H, ArH), 7.64–7.60 (m, 3H, ArH), 7.39 (s, 1H, =CH), 7.21–7.18 (m, 1H, ArH). HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{14}H_{11}N_2OS$: 255.0587; found: 255.0580.

Assay for DPPH scavenging potential. Potentials for scavenging DPPH radical was conducted as described in our previous works [11e,f] with slight modification. Briefly, 2.0 mL of 0.1-m*M* DPPH in methanol was added to 2.0 mL of each tested compound solution (dissolved in DMSO at a concentration of 1mg/ mL). The mixture was shaken vigorously and incubated at 30°C for 30 min, followed by estimating its absorbance at 517 nm. Methanol and L-Ascorbic acid was used as the blank and PC, respectively. The scavenging activity was expressed as the percentage inhibition of the DPPH radicals by 1-mg tested compound, which was calculated from $[1 - (A_1/A_0)] \times 100$, where A_1 and A_0 are absorbencies of the tested compound and blank control, respectively.

Assay for OH scavenging potential. Potentials for scavenging hydroxyl radical was determined based on the theory of Fenton reaction [14] and conducted according to the procedure detailed in our previous work [11f] with slight modification. The tested compounds were dissolved in DMSO at a concentration of 1 mg/ mL and determining scavenging OH activity involved preparing the following reagents (i) reagents for the blank control consisted of 1-mL phosphate buffer (150 m*M*), 0.2 mL 1,10-phenaproline (7.5 m*M*), 0.2 mL $FeSO_4$ (7.5 m*M*), and 0.6 mL RO water; (ii) reagents for the oxidized control were the same as above, except that 0.2-mL RO water was substituted by 0.2-mL H_2O_2 (0.1%); (iii) reagents for scavenging free radicals were the same as those for the oxidized control except that 0.4-mL RO water was replaced by 0.4 mL of the tested compounds. All were incubated at 37°C in a water bath for 60 min followed by determining their absorption at 536 nm. Scavenging capacity for OH was calculated according to the formula: Scavenging rate (%) = $(A_{536}^{\text{scavenging}} - A_{536}^{\text{oxidized control}})/(A_{536}^{\text{blamk control}} A_{536}^{\text{oxidized control}}) \times 100\%$. L-Ascorbic acid was used as a PC. The scavenging capacities were represented as percentage inhibition of the OH by 1-mg tested compound.

Assay for O_2^- scavenging potential. The scavenging potential for superoxide radicals in samples was analyzed using a hypoxanthine/xanthine oxidase generating system coupled with NBT reduction, as detailed previously [15] with minor modification, where the reactions were carried out in 96-well plates. Briefly, each reaction mixture contained 134-μL buffer (50-m*M* KH_2PO_4/KOH, pH 7.4), 2 μL of 100-m*M* Na_2EDTA, 20 μL of 3-m*M* hypoxanthine, 2 μL of 10-m*M* NBT, and 10 μL of sample. Microplates were read 2.5 min after adding 32 μL of xanthine oxidase (1 unit per 10-mL buffer) at 540 nm using a microplate reader (Bioreader 550, Bio-Rad, US). L-Ascorbic acid was used as a PC. Superoxide scavenging activity was expressed as the percentage inhibition of superoxide anion by 1-mg tested compound compared to the blank (i.e., buffer used in reaction).

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REFERENCES AND NOTES

[1] Mesaik, M. A.; Khan, K. M.; Rahat, S.; Zia-Ullah; Choudhary, M. I.; Murad, S.; Abdullah, N. R.; Ismail, Z.; Atta-ur-Rahman; Ahmad, A.; Siddiqui, R. A. Lett Drug Des Discov 2005, 2, 490.

[2] (a) Solankee, A.; Kapadia, K.; Upadhyay, K.; Patel, J. Orient J Chem 2001, 17, 315; (b) Prabhavat, M. D. Asian J Chem 2001, 13, 226.

[3] Kuchar, M.; Brunova, B.; Grimova, J.; Holubek, J.; Nemecek, O. Cesko-Slovenska Farmacie 1975, 24, 287.

[4] Khan, K. M.; Mughal, U. R.; Ambreen, N.; Samreen; Perveen, S.; Choudhary, M. I. J Enzyme Inhib Med Chem 2010, 25, 29.

[5] (a) Khan, K. M.; Mughal, U. R.; Khan, S.; Khan, S.; Perveen, S.; Choudhary, M. I. Lett Drug Des Discov 2009, 6, 69; (b) Shafi, P. M.; Basheer, P. A. M.; Jirovetz, L. Asian J Chem 2006, 18, 3188; (c) Solankee, A.; Kapadia, K.; Patel, J.; Thakor, I. Asian J Chem 2002, 14, 699.

[6] Carreaux, F.; Bazureau, J.-P.; Renault, S.; Meijer, L.; Lozach, O. 2009, WO 2009050352 A2 20090423 CAN 150:472956.

[7] Hassanein, H. H.; Khalifa, M. M.; El-Samaloty, O. N.; Abd El-Rahim, M.; Taha, R. A.; Magda; Ismail, M. F. Arch Pharmacal Res 2008, 31, 562.

[8] (a) Tripathy, P. K. J. Inst. Chem. 2007, 79, 115; (b) Fozooni, S.; Tikdari, A. M. Catal Lett 2008, 120, 303; (c) Galat, A. J Am Chem Soc 1950, 72, 4436; (d) Tikdari, A. M.; Tripathy, P. K.; Mukerjee, A. K. J Chem Soc Perkin Trans 1 1988, 1659.

[9] (a) Tikdari, A. M.; Panah, S. S. Asian J Chem 2005, 17, 1527; (b) Shafi, P. M. Curr Sci 1985, 54, 1231; (c) Devasia, G. M.; Shafi, P. M. Indian J Chem Sect B 1981, 20B, 657; (d) Devasia, G. M. Tetrahedron Lett 1976, 571.

[10] (a) Shafi, P. M.; Sobha, T. D.; Basheer, P. A. M. Indian J Chem Sect B 2005, 44B, 1298; (b) Devasia, G. M.; Shafi, P. M. Indian J Chem Sect B 1986, 25B, 204; (c) Kidwai, A. R.; Devasia, G. M. J Org Chem 1962, 27, 4527.

[11] (a) Jiang, B.; Tu, S.-J.; Kaur, P.; Wever, W.; Li, G. J Am Chem Soc 2009, 131, 11660; (b) Jiang, B.; Wang, X.; Shi, F.; Tu, S.-J.; Ai, T.; Ballew, A.; Li, G. J Org Chem 2009, 74, 9486; (c) Wei, P.; Zhang, X.; Tu, S.; Yan, S.; Ying, H.; Ouyang, P. Bioorg Med Chem Lett 2009, 19, 828; (d) Shi, F.; Li, C.; Xia, M.; Miao, K.; Zhao, Y.; Tu, S.; Zheng, W.; Zhang, G.; Ma, N. Bioorg Med Chem Lett 2009, 19, 5565; (e) Zheng, W.; Zhang, M.; Zhao, Y.; Wang, Y.; Miao, K.; Wei, Z. Bioresour Technol 2009, 100, 1327; (f) Zheng, W.; Zhang, M.; Zhao, Y.; Miao, K.; Jiang, H. Bioresour Technol 2009, 100, 4481–4487.

[12] Gong, X.; Yang, H.; Liu, H.; Jiang, Y.; Zhao, Y.; Fu, H. Org Lett 2010, 12, 3128.

[13] Tu, S.-J.; Jiang, H.; Zhuang, Q.Y. ; Miao, C.B.; Shi, D.Q.; Wang, X.S.; Gao, Y. Chin J Org Chem 2003, 23, 491.

[14] Yang, F.; Zhang, R.; He, J.; Abliz, Z. Acta Pharm Sinica 2007, 42, 692.

[15] Kirby, A. J.; Schmidt, R. J. J Ethnopharmacol 1997, 56, 103.