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# Synthesis of New Benzofuran-1,3-Thiazolidin-4-One Derivatives from Natural Sources and Study of Their Antioxidant Activity[1]

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**Abstract**—Synthesis of benzofuran-1,3-thiazolidinone derivatives is described herein. These compounds were prepared via a concise and short route by condensation reaction of khellinone with aromatic/aliphatic amines followed by cyclization using thioglycolic acid. The newly synthesized compounds were characterized using the well known spectroscopic tools (IR, $^1$H NMR, and mass spectroscopy), as well as microanalysis. In frames of biological screening of the compounds, antioxidant activity was assessed in vitro.

*Keywords: 1,3-thiazolidin-4-one, benzofuran, Schiff bases, anti-oxidant activity, khellinone, khellin*

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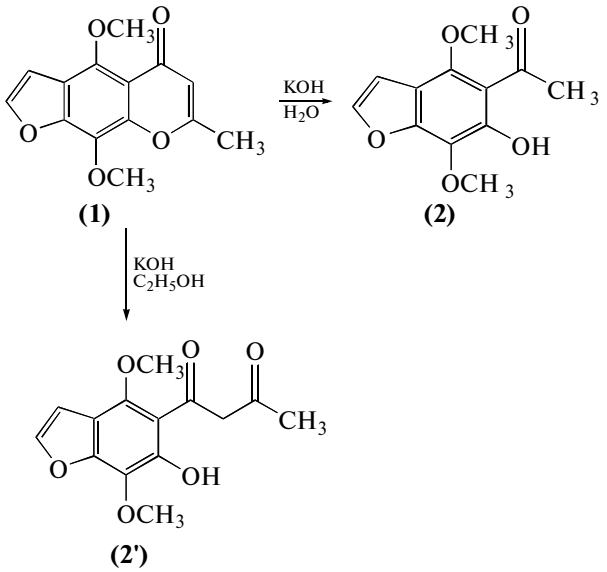
## INTRODUCTION

A natural furochromone khellin (**1**) (4,9-dimethoxy-7-methyl-5H-furo[3,2-g] chromen–5–one), obtained from fruits and seeds of *Ammi visnaga* L. is a natural source of several furochromones, namely, visnagin, khellin, khellolglucoside, and ammiol. Similar to psoralen, visnadin, khellin, and coumarin bind with DNA through cross-links to produce monoadducts. Thus, khellin is used in photochemotherapy of dermatoses, such as vitiligo and psoriasis [1–5]. It is known that hydrolysis of khellin yields very interesting derivatives, namely ω-acetokhellinone (**2'**) and khellinone (**2**) (Scheme 1). These two products are important molecules for synthesis of new khellin derivatives [6]. The ring-opening reaction (ROR) of chromone was investigated ab initio and using density functional theory methods by Kona et al. [7, 8].

Khellin exhibits high anti-atherosclerotic and lipid-altering activity [9] and is an active constituent of many modern medicines [10]. Khellin helps to relieve bladder and urinary tract constriction and spasms, may relieve urinary colic [11] and is beneficial for the health of the gallbladder and bile duct; it also promotes the discharge of gallstones and gallbladder colic [12]. The benefits of khellin extend to the skin: when combined with sun exposure, it helps to treat the skin disease known as vitiligo, in which pigment-carrying melanocytes of skin are lost. Khellin may also be beneficial in treating inflammation, psoriasis, minor burns and wounds, and certain dermatological problems [13].




**Scheme 1.** The experimental chemical pathway for khellin hydrolysis.

On the other hand, in recent years, the interest in thiazolidinone chemistry increased remarkably due to biologically activity of its derivatives and important therapeutic products ranging from antibacterial [14] and antifungal [15] to anticonvulsant [16], antifungal [17], antithyroid [18], antituberculer, and antidiabetic [19]. For this reason, synthetic connection of thiazoli-

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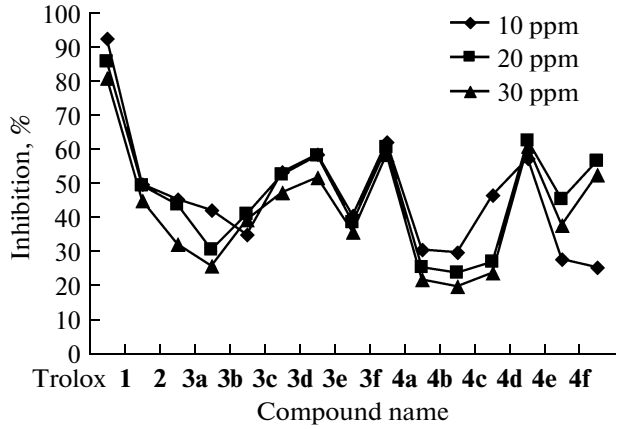


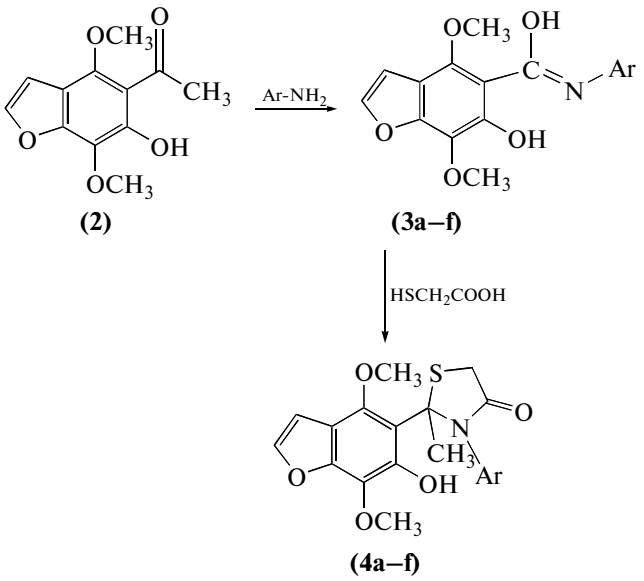

**Fig. 1.** Inhibition (%) of DPPH radical by tested compounds.

dinone moiety with a natural product khellin may increase biological activities of both and yield an antioxidant. Considering the foregoing benefits, we aimed to link biologically active thiazolidinones with the natural khellin to access new classes of biologically active compounds.

## RESULTS AND DISCUSSION

### *Chemistry*

In frames of our ongoing research to synthesize potentially biologically active thiazolidinone derivates we describe a series of 5-((*E*)-1-(substituted imino)ethyl)-4,7–dimethoxy benzofuran-6-ol (**3a**–**3f**) and 2-(4,7-dimethoxy-6-hydroxybenzofuran-5-yl)-2-methyl-*N*-(substituted)1,3-thiazolidin-4-one (**4a**–**4f**) (Scheme 2). The series of Shiff's bases (**3a**–**3f**) were prepared according to [24] by refluxing different aromatic and/or aliphatic amines and khellinone (**2**) in absolute ethanol in the presence of catalytic amount of glacial acetic acid. Structures of the newly synthesized products (**3a**–**3f**) were inferred from their analytical and spectral data. The IR spectra of compounds (**3a**–**3f**) showed characteristic bands at 3440–3300 $cm^{-1}$ for OH group and at 1620 $cm^{-1}$, for C=N; band of C=O group disappeared. The $^1H$ NMR spectra in $CDCl_3$ or DMSO-$d_6$ showed the most important signals at δ 1.99–2.1 for $CH_3$ and 3.8–4.02, for the two methoxy groups. Our target compounds 2-melhyl-3-aryl-2-(4,6-dimethoxy-6-hydroxy-5-yl benzofuran)-1,3-thiazolidin-4-one (**4a**–**4f**) were obtained by reactions described in [20], performed by refluxing the solutions of Schiff's bases (**3a**–**3f**) and thioglycolic acid in dry benzene in the presence of anhydrous $ZnCl_2$ for 10–12 h.




| Entry | Ar | Entry | Ar |
|---|---|---|---|
| **a** | Ethyl | **d** | 4-Methylphenyl |
| **b** | Phenyl | **e** | 4-Chlorophenyl |
| **c** | 2-Nitrophenyl | **f** | 4-Methoxyphenyl |

**Scheme 2.**

Formation of 2,3-disubtituded 4-thiazolidinone (**4a**–**4f**) was confirmed by IR spectroscopy, which showed the characteristic bands in the range of 1690–1730 $cm^{-1}$ due to (C=O) group. The $^1H$ NMR spectra in $CDCl_3$ or DMSO-$d_6$ showed the most important signals at δ 3.34–3.38 ppm corresponding to the methylene group ($COCH_2S$).

### *Antioxidant Activity*

Antioxidant capacities of khellin (**1**), khellinone (**2**), and all the prepared compounds were assessed using two methods, that is DPPH and hydroxyl radical scavenging assay, in comparison with standard compounds. The DPPH free radical assay incorporates a metastable free radical that is capable of accepting hydrogen radicals from antioxidants in solution. The reaction between DPPH and antioxidant can be monitored by the decrease in absorbance of the colored free radical [21].

In the hydroxyl radical assay OH, radicals were generated to attack the substrate, deoxyribose. When heated with TBA in acid solution, the resulting products of radical attack form a pink chromogen. Since these two methods are based on different mechanisms for radical inhibition, they can provide complementary insight into the expected antioxidant capacities of compounds under study.

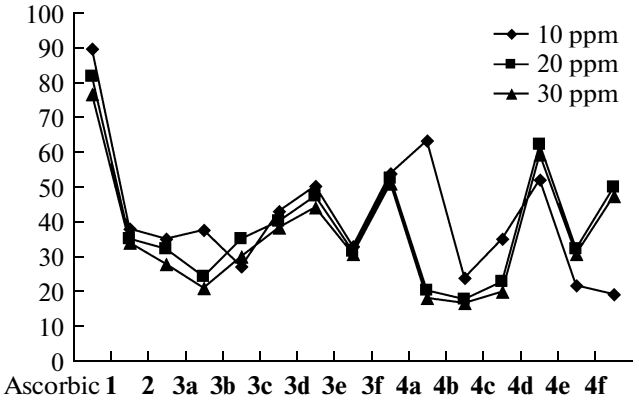



**Fig. 2.** Inhibition (%) of hydroxyl radical by tested compounds.

### *DPPH Radical Scavenging Activities*

The resulting values of DPPH radical inhibition by individual tested compounds are plotted in Fig. 1. Four compounds—(**3f**), (**4a**), (**3d**), and (**4d**)—exhibited close inhibition effects against DPPH exceeding that of khellin and other tested substituted compounds. The relative activities of (**3f**), (**3d**), (**4d**), and (**3c**) were 67.1, 63.3, 61.9, and 57.5% respectively compared to Trolox and 125.6; 118.0; 115.0, and 107.0%, compared to khellin. $EC_{50}$ values were 8.57, 9.72, 8.27, and 10.59 μM for (**3f**), (**3d**), (**4d**), and (**3c**) respectively compared with 5.42 μM for Trolox.

### *Hydroxyl Radical Scavenging Activities*

The resulting values of hydroxyl radical inhibition by individual tested compounds are plotted in Fig. 2. The results indicate that four compounds—(**3f**), (**4a**), (**3d**, and (**4d**) exhibited highest and close inhibition effects against hydroxyl radical if compared to other tested compounds. The relative activities of (**3f**), (**3d**), (**4d**), and (**3c**) compared with ascorbic acid were 60.36, 56.22, 58.34, and 48.26% and compared with khellin, 142.2, 132.5, 137. 5, and 113.7%, respectively.

DPPH radical scavenging activities indicate that the most effective substituted compounds (**3f**), (**3d**), (**4d**), and (**3c**) have more hydrogen atoms or electron donation capacity which converts DPPH to a stable DPPH radical. Also, inhibition of OH radical generation increased when the effective substituted compounds were applied. $EC_{50}$ values indicate that the effective substituted compounds have moderate antioxidant activity.

## CONCLUSIONS

In this study, a series of Schiff bases, 5-((E)-1-((substituted imino)ethyl)-4,7-dimethoxybenzofuran-6-ol (**3a**–**3f**) and 2-(4,7-dimethoxy-6-hydroxybenzofuran-5-yl)-2-methyl-N-(substituted)-1,3-thiazolidin-4-one (**4a**–**4f**) were synthesized and evaluated for their antioxidant activity by DPPH radical scavenging activity and hydroxyl radical scavenging activity according to Fenton's method. All synthesized structures were elucidated by means of various spectral methods. Compounds (**3f**), (**3d**), (**4d**), and (**3c**) proved to have better antioxidant activity if compared ti khellin or standard compounds used in the present study. Compound (**3f**) influences greatly antioxidant activity.

## EXPERIMENTAL

### *General*

The melting points were recorded in an electrothermal capillary melting point apparatus and were not corrected. Thin layer chromatography was performed using $F_{254}$ fluorescent silica gel plates (Merck), which were examined under UV 254 and 365 nm light. Silica gel (230–400 mesh) was used for flash chromatography separations. Elemental analysis for C, H, N, and S was performed on a Vario EL III equipment (Micro analytical center, Cairo University, Cairo, Egypt). Infrared spectra (ν, $cm^{-1}$) were recorded on a Jasco FT-IR 4100 instrument using KBr disks (Micro analytical center). $^1$HNMR spectra were recorded on a Varion Mercury VX-300 NMR spectrometer. $^1$H NMR were run at 300 MHz in deuterated chloroform ($CDCl_3$) or dimethyl sulphoxide (DMSO-$d_6$). Chemical shifts are reported as δ (ppm) and were related to those of the solvents. Spectra were internally referenced to TMS. Mass spectra were recorded on a Shimadzu GCMS-QP-1000 EX mass spectrometer at 70 eV (Micro analytical center). Chemicals used for antioxidant activity assays were deoxyribose, Trolox, thiobarbituric acid (TBA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid, methanol, and dimethylsulfoxide (DMSO) obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents used were analytical grade.

### *General Procedure of 5-((E)-1-(Substituted imino)ethyl)-4,7-dimethoxybenzofuran-6-ol (3a–3f) Synthesis*

A mixture of 0.01 mol compound (**2**) and 0.01 mol of an appropriate amine was refluxed in absolute ethanol (30 mL) in the presence of a catalytic amount of glacial acetic acid for 5–7 h. The reaction mixture was cooled and the precipitate was filtered and recrystallized from ethanol to give compounds (**3a**–**3f**).

**5-((E)-1-(Ethylimino)ethyl)-4,7-dimethoxybenzofuran-6-ol (3a).** Yield (87%); mp 132–134°C; FT-IR: ν ($cm^{-1}$) 3437 (OH), 3135 (C–H aromatic), 2930 (C–H aliphatic), and 1622 (C=N). $^1$H NMR (δ, ppm): 2.103 (s, 3H, $CH_3$), 2.1 (t, 3H, $CH_3$), 2.7 (q, 2H, $CH_2$), 4.05 (s, 3H, $OCH_3$), 4.15 (s, 3H, $OCH_3$), 7.5 (d, 1H, H2 furan), 6.9 (d, 1H, H3 furan), and 13.09 (s, 1H, OH) exchangeable with $D_2O$. Mass spectra *m/z*

(%): 263 (15) [$M^{+\cdot}$], 220 (51.3), 205 (36.6), 179 (32.5), 123 (57.5), and 108 (100). Anal. Found: C,71.15; H, 7.19; N, 5.90. Calcd. $C_{14}H_{17}NO_4$ (263): C, 71.19; H, 7.20; N, 5.93.

**5-((E)-1-(Phenylimino)ethyl)benzofuran-4,7-dimethoxybenzofuran-6-ol (3b).** Yield (89%); mp 140–142°C; FT-IR: ν ($cm^{-1}$) 3293 (OH), 3136 (C–H aromatic), 3067 (C–H aliphatic), and 1661 (C=N). $^1$H NMR (δ, ppm): 2.03 (s, 3H, $CH_3$), 3.8 (s, 6H, $2OCH_3$), 7.57 (d, 1H, H2 furan), 7.01 (d, 1H, H3 furan), 7.27(m, 5H, Ar-H), and 9.89 (s, 1H, OH) exchangeable with $D_2O$. Mass spectra *m/z* (%): 311 (11) [$M^{+\cdot}$], 283 (11.9), 268 (11.5), 215 (23.2), 173 (100), 108 (68.5).

Anal. Found: C, 69.43; H, 5.46; N, 4.49. Calcd. $C_{18}H_{17}NO_4$ (311): C, 69.45; H, 5.47; N, 4.50.

**5-((E)-1-(2-Nitrophenylimino)ethyl)-4,7-dimethoxybenzofuran-6-ol (3c).** Yield (84%); mp 133–135°C; FT-IR: ν ($cm^{-1}$) 3439 (OH), 3139 (C–H aromatic), 2934 (C–H aliphatic), and 1621(C=N). $^1$H NMR (δ, ppm): 2.01 (s, 3H, $CH_3$), 4.1 (s, 6H, $2OCH_3$), 7.97 (d, 1H, H2 furan), 7.03 (d, 1H, H3 furan), 7.53(m, 5H, Ar-H), and 11.3 (s, 1H, OH) exchangeable with $D_2O$. Mass spectra *m/z* (%): 370 (33.1) [$M^{+\cdot}$], 260 (63.1), 207 (47.5), 190 (100), 176 (39.1). Anal. Found: C, 58.25; H, 4.30; N, 11.32. Calcd. $C_{18}H_{16}N_3O_6$ (370): C, 58.38; H, 4.32; N, 11.35.

**5-((E)-1-(4-Methylphenylimino)ethyl)-4,7-dimethoxybenzofuran-6-ol (3d).** Yield (87%); mp 145–147°C; FT-IR: ν ($cm^{-1}$) 3345 3345(OH), 3120 (C–H aromatic), 2953 (C–H aliphatic), and 1611 (C=N). $^1$H NMR (δ, ppm): 1.93 (s, 6H, $2CH_3$), 4.09 (s, 6H, $2OCH_3$), 7.99 (d, 1H, H2 furan), 7.2 (d, 1H, H3 furan), 7.65 (dd, 4H, Ar-H), and 9.9 (s, 1H, OH) exchangeable with $D_2O$. Mass spectra *m/z* (%): 325 (25.3) [$M^{+\cdot}$], 310 (33.1), 249 (11.3), 208 (100), 177(31.2). Anal. Found: C, 70.12; H, 5.81; N, 4.29. Calcd. $C_{19}H_{19}NO_4$ (325): C, 70.15; H, 5.85; N, 4.31.

**5-((E)-1-(4-Chlorophenylimino)ethyl)-4,7-dimethoxybenzofuran-6-ol (3e).** Yield (88%); mp 148–150°C; FT-IR: ν ($cm^{-1}$) 3310 (OH), (C–H aromatic), 3965 (C–H aliphatic), and 1655 (C=N). $^1$H NMR (δ, ppm): 1.82 (s, 3H, $CH_3$), 3.97 (s, 6H, $2OCH_3$), 8.01 (d, 1H, H2 furan), 7.30 (d, 1H, H3 furan), 7.51 (dd, 4H, Ar-H), and 11.3 (s, 1H, OH) exchangeable with $D_2O$. Mass spectra *m/z* (%): 435/347 (35,11.5) [$M^{+}/M^{+2}$], 310 (22.4), 193 (61.1), 176 (100), 145 (25.1), 114 (33.1). Anal. Found: C, 62.49; H, 4.60; N, 4.02. Calcd. $C_{18}H_{16}NO_4Cl$ (345.5): C, 62.52; H, 4.63; N, 4.05.

**5-((E)-1-(4-Methoxyphenylimino)ethyl)-4,7-dimethoxybenzofuran-6-ol (3f).** Yield (89%); mp 155–157°C; FT–IR: ν ($cm^{-1}$) 3310 (–OH), 3125 (C–H aromatic), 2982 (C–H aliphatic), and 1676 (C=N). $^1$H NMR (δ, ppm): 1.83 (s, 3H, $CH_3$), 3.83 (s, 9H, $3OCH_3$), 8.03 (d, 1H, H2 furan), 7.21 (d, 1H, H3 furan), 7.43 (dd, 4H, Ar–H), and 10.3 (s, 1H, OH) exchangeable with $D_2O$. Mass spectra *m/z* (%): 341 (45.2) [$M^{+\cdot}$], 310 (11.5), 234 (27.3), 222 (100), 193 (63.7), 176 (23.7). Anal. Found: C, 66.83; H, 5.55; N, 4.09. Calcd. $C_{19}H_{19}NO_5$ (341): C, 66.86; H, 5.57; N, 4.11.

### *General Procedure for 2-(4,7-Dimethoxy-6-hydroxybenzofuran-5-yl)-2-methyl-N-(substituted)-1,3-thiazolidin-4-one (4a–4f) Synthesis*

To a solution of 5-((*E*)-1-(substituted-imino)ethyl)-4,7-dimethoxybenzofuran-6-ol (**3a–3f**) (0.001 mol) in dry benzene (15 mL), freshly distilled mercaptoacetic acid (0.001 mol) and anhydrous $ZnCl_2$ (0.1 g) were added and the mixture was heated under reflux for 10–12 h. The solvent was evaporated under reduced pressure, the residue was washed with 5% sodium carbonate solution (3 × 20 mL) and water (2 × 20 mL), dried, and recrystallized from ethanol.

**2-(4,7-Dimethoxy-6-hydroxybenzofuran-5-yl)-2-methyl-N-ethyl-1,3-thiazolidin-4-one (4a).** Yield (75%); mp 155–157°C; FT-IR: ν ($cm^{-1}$) 3290 (OH), 3120 (C–H aromatic), 2912 (C–H aliphatic), and 1770 (C=O). $^1$H NMR (δ, ppm): 1.93(s, 3H, $CH_3$), 2.3 (s, 2H, $SCH_2CO$), 3.83 (s, 6H, $2OCH_3$), 7.89 (d, 1H, H2 furan), 7.08 (d, 1H, H3 furan), 7.48 (m, 5H, Ar-H), and 9.8 (s, 1H, OH) exchangeable with $D_2O$. Mass spectra *m/z* (%): 325 (8.1) [$M^{+\cdot}$], 310 (70.1), 295 (63.1), 220 (100), 193 (71.3). Anal. Found: C, 55.35; H, 5.83; N, 4.30. Calcd. $C_{15}H_{19}NO_5S$ (325): C, 55.38; H, 5.85; N, 4.31.

**2-(4,7-Dimethoxy-6-hydroxy benzofuran-5-yl)-2-methyl-N-phenyl-1,3-thiazolidin-4-one (4b).** Yield (79%); mp 166–168°C; FT-IR: ν ($cm^{-1}$) 3295 (OH), 3105 (C–H aromatic), 2980 (C–H aliphatic), and 1760 (C=O). $^1$H NMR (δ, ppm): 1.93 (s, 3H, $CH_3$), 2.29 (s, 2H, $SCH_2CO$), 3.99 (s, 6H, $2OCH_3$), 7.97 (d, 1H, H2 furan), 7.2 (d, 1H, H3 furan), 7.53 (m, 5H, Ar-H), and 10.8 (s, 1H, OH) exchangeable with $D_2O$. Mass spectra *m/z* (%): 373 (8.2) [$M^{+\cdot}$], 220 (100), 205 (58.6), 193 (77.2), 145 (28.5 ). Anal. Found: C, 61.11; H, 5.05; N, 3.74. Calcd. $C_{19}H_{19}NO_5S$ (373): C, 61.13; H, 5.09; N, 3.75.

**2-(4,7-Dimethoxy-6-hydroxybenzofuran-5-yl)-2-methyl-N-(2-nitrophenyl)ethyl-1,3-thiazolidin-4-one (4c).** Yield (81%); mp 170–172°C; FT-IR: ν ($cm^{-1}$) 3305 (OH), 3112 (C–H aromatic), 2990 (C–H aliphatic), and 1759 (C=O). $^1$H NMR (δ, ppm): 1.95 (s, 3H, $CH_3$), 2.35 (s, 2H, $SCH_2CO$), 4.02 (s, 6H, $2OCH_3$), 7.85 (d, 1H, H2 furan), 7.09 (d, 1H, H3 furan), 7.49 (m, 4H, Ar-H), and 9.83 (s, 1H, OH) exchangeable with $D_2O$. Mass spectra *m/z* (%): 418 (32.1) [$M^{+\cdot}$], 372 (11.2), 344 (39.3), 205 (100), 176 (75.3). Anal. Found: C, 54.52; H, 4.28; N, 6.67. Calcd. $C_{19}H_{18}N_2O_7S$ (418): C, 54.55; H, 4.31; N, 6.70.

**2-(4,7-Dimethoxy-6-hydroxybenzofuran-5-yl)-2-methyl-N-(4-methylphenyl)-1,3-thiazolidin-4-one (4d).**

Yield (78%); mp 181–183°C; FT-IR: ν ($cm^{-1}$) 3298 (OH), 3125 (C–H aromatic), 2975 (C–H aliphatic), and 1775 (C=O). $^1$H NMR (δ, ppm): 1.88 (s, 3H, $CH_3$), 2.4 (s, 2H, $SCH_2CO$), 3.92(s, 6H, $2OCH_3$), 8.01 (d, 1H, H2 furan), 7.15 (d, 1H, H3 furan), 7.49 (dd, 4H, Ar-H), and 10.2 (s, 1H, OH) exchangeable with $D_2O$. Mass spectra *m/z* (%): 387 (43.3) [$M^+$], 372 (12.3), 297 (54.1), 206 (55.1), 219 (100), 204 (63.3). Anal. Found: C, 62.01; H, 5.41; N, 3.60. Calcd. $C_{20}H_{21}NO_5S$ (387): C, 62.02; H, 5.43; N, 3.62.

**2-(4,7-Dimethoxy-6-hydroxybenzofuran-5-yl)-2-methyl-N-(4-chlorophenyl)-1,3-thiazolidin-4-one (4e).** Yield (80%); mp 188–190°C; FT-IR: ν ($cm^{-1}$) 3310 (OH), 3110 (C–H aromatic), 2960 (C–H aliphatic), and 1768 (C=O). $^1$H NMR (δ, ppm): 2,02 (s, 3H, $CH_3$), 2.34 (s, 2H, $SCH_2CO$), 3,92 (s, 6H, $2OCH_3$), 7.95 (d, 1H, H2 furan), 7.13 (d, 1H, H3 furan), 7.52 (dd, 4H, Ar-H), and 11.3 (s, 1H, OH) exchangeable with $D_2O$. Mass spectra *m/z* (%): 407/409 (66/21.3) [$M^+/M^{+2}$], 372 (15.1), 297 (73.1), 281 (44.5), 220 (100). Anal. Found: C, 55.91; H, 4.40; N, 3.41. Calcd. $C_{19}H_{18}NO_5SCl$ (407.5): C, 55.95; H, 4.42; N, 3.44.

**2-(4,7-Dimethoxy-6-hydroxybenzofuran-5-yl)-2-methyl-N-(4-methoxyphenyl)-1,3-thiazolidin-4-one (4f).** Yield (77%); mp 178–180°C; FT-IR: ν ($cm^{-1}$) 3288 (OH), 3119 (C–H aromatic), 2939 (C–H aliphatic), and 1770 (C=O). $^1$H NMR (δ, ppm): 1.88 (s, 3H, $CH_3$), 2.23 (s, 2H, $SCH_2CO$), 3.95 (s, 9H, $3OCH_3$), 7.95 (d, 1H, H2 furan), 7.08 (d, 1H, H3 furan), 7.53 (dd, 4H, Ar-H), and 9.9 (s, 1H, OH) exchangeable with $D_2O$. Mass spectra *m/z* (%): 341 (63.2) [$M^{+\cdot}$], 311 (33.4), 235 (100), 176 (55.6). Anal. Found: C, 66.83; H, 5.55; N, 4.09. Calcd. $C_{19}H_{19}NO_5$ (341): C, 66.86; H, 5.57; N, 4.11.

### *Evaluation of Antioxidant Activity DPPH (1,1-Diphenyl-2-picryl-hydrazyl) Radical Scavenging Activity Assay*

Method of DPPH scavenging has been chosen to evaluate the in vitro antioxidant activity of target compounds due to the simple, rapid, sensitive, and reproducible procedures [22].

Scavenging activities of khellin (**1**) khellinone (**2**), all the prepared compounds, and a standard compound (Trolox) were measured using the technique reported [23]. Bleaching of a purple-colored methanol solution of DPPH depended on hydrogen atom or electron donation abilities of tested compounds. The bleaching rate of the stable free radical, DPPH, is monitored at a characteristic wavelength in the presence of the sample. DPPH absorbs at 517 nm but, upon reduction by an antioxidant, its absorption decreases. When a hydrogen atom or electron is transferred to the odd electron in DPPH, the absorbance at 517 nm decreases proportionally to the increases of non-radical forms of DPPH. Stock solution of DPPH was prepared by dissolving 24 mg of DPPH in 100 mL MeOH, and then stored at 20°C in the dark until needed. The working solution was obtained by diluting 10 mL of stock solution with 45 mL MeOH, to obtain an absorbance of 1.1 ± 0.1 units at 517 nm controlled on a Shimadzu UV-1063 spectrophotometer. Briefly, a volume of 10 μL of different concentrations (10, 20, and 30 μg/mL) of tested compounds dissolved in DMSO was added to 990 μL of 0.094 mM DPPH in MeOH, to reach 1 mL. Solutions were continuously monitored at 517 nm over a 2 h period at 25°C. After 45 min, changes in absorbance were minimal for all samples. The antioxidant abilities were expressed as μM Trolox equivalents. Each sample was analyzed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the following equation:

$$\text{Inhibition } \% = (A_B - A_A)/A_B \times 100,$$

where $A_B$ is the absorption of the blank sample ($t = 0$ min) and $A_A$ is the absorption of a tested compound or standard substance solution ($t = 45$ min).

The $EC_{50}$ value defined as the concentration of antioxidant in the reactive system necessary to decrease the initial DPPH concentration by 50% was calculated from the results.

### *Hydroxyl Radical Scavenging Assay*

Hydroxyl radical scavenging activity was measured according to Fenton method [24]. Samples (10 μL) of khellin (**1**), khellinone (**2**), all the prepared compounds, and a standard compound (ascorbic acid) at different concentrations (10, 20, and 30 μg/mL) were dissolved in DMSO, then incubated with 9.0 mM $FeSO_4$ (1.0 mL), 0.3% $H_2O_2$ (1.0 mL) in 0.5 mL salicylic acid–ethanol solution (9.0 mM) for 30 min at 37°C. Hydroxyl radical was detected by monitoring absorbance at 510 nm. The total volume of the mixture in each tube was made up to 3 mL by adding the required amount of distilled water. Inhibition (I) of deoxyribose degradation in percent was calculated according to the following equation:

$$\text{I } (\%) = 100 \times (A_0 - A_1/A_0),$$

where $A_0$ is the absorbance of the control reaction mixture and $A_1$ is the absorbance of the test compound.

### *Statistical Analysis*

Each of the measurements described was carried out in three replicate experiments and the results were plotted by Excel 2011 software.

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