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ARTICLE

Synthesis and evaluation of antimicrobial and antioxidant activity of novel 7-Aryl-6H,7H- benzo[f] chromeno[4,3-b]chromen-6-one by MgO nanoparticle as green catalyst

Sara Hosseinzadegan | **Nourallah Hazeri** | **Malek T. Maghsoodlou**

Department of Chemistry, Faculty of Science, University of Sistan & Baluchestan, Zahedan, The Islamic Republic of Iran

Correspondence
Nourallah Hazeri, Department of Chemistry, Faculty of Science, University of Sistan & Baluchestan, P. O. Box 98135-674. Zahedan, The Islamic Republic of Iran.
Email: nhazeri@chem.usb.ac.ir; n_hazeri@yahoo.com

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Abstract

Using MgO nanoparticle as a recyclable and efficient catalyst, multicomponent reaction of *2-Naphthol, aldehyde* derivatives, *4-hydroxycoumarin* has led to the synthesis of novel 7-Aryl-6H,7H- benzo[f]chromeno[4,3-b]chromen-6-one derivatives. This study aimed to examine the antimicrobial and antioxidant effects of the synthetic compound in order to reach some benefits, including the synthesis of new derivatives with biological properties, shorter time, high yields, and concordance with green chemistry.

1 | INTRODUCTION

Chromen core is a bioactive compound that is found in many natural compounds such as flavonoids.[1–4] Many biological operations have been reported for the compounds containing this core, some of which are anti-HIV,[5] neuro-protective,[5,6] antidiabetes,[7] antioxidant,[8,9] and antimicrobial[10–13] operations. Considering the significance of chromen in terms of biological operations, the synthesis of its new derivatives and the development of new methods to synthesize heterocycles containing chromen seem to be of paramount importance. We used magnesium oxide nanoparticles as a green catalyst for the synthesis of new 7-Aryl-6H,7H- benzo[f]chromeno[4,3-b]chromen-6-one derivatives at lower temperatures and shorter time compared to previous studies and then examined the biological properties (namely antibacterial, antifungal, and antioxidant) of the synthesized derivatives.

2 | RESULTS AND DISCUSSION

The structure of MgO nanoparticles was proven using and Scanning Electron microscopic images (SEM) techniques and *X-ray diffraction* (XRD) (Figures 1 and 2, respectively). In SEM, image nanocrystals were determined as dense flakes with a size range of 30–50 nm. The XRD patterns of synthesized MgO were similar with the standard diffraction data of MgO nanoparticles (JCPDS file No. 89-7746) and peaks observed at (111), (200), (220), (311), and (222), diffraction planes reveal the cubic phase of MgO nanoparticles. Average particle size was calculated to be 23.7 and 25.7 nm according to the Scherrer equation.[14–16]

In this study, chromine derivatives were synthesized using multicomponent synthesis and MgO nanoparticles as a catalyst. (Scheme 1).

The reactions were optimized for amount of MgO nanoparticles and temperature. In the first stage of optimization, using 1 mmol beta-naphthol, 1 mmol benzaldehyde,

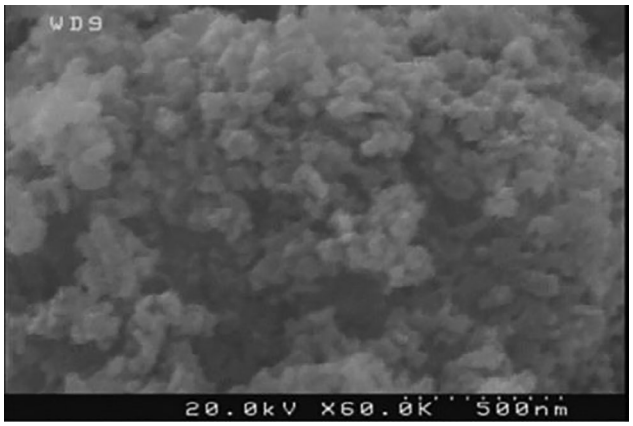


FIGURE 1 SEM image MgO nanoparticles

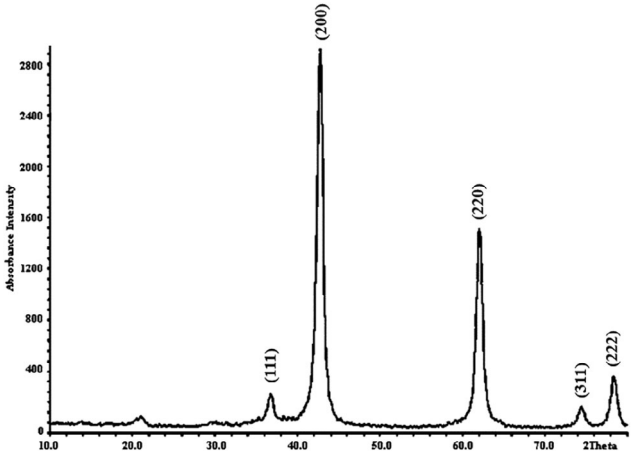
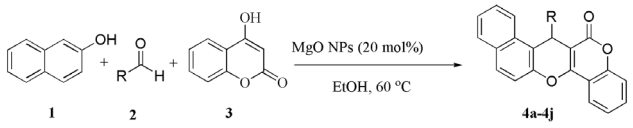


FIGURE 2 XRD spectrum of MgO nanoparticles



SCHEME 1 Multicomponent synthesis of chromen derivatives

1 mmol *4-hydroxycoumarin* in different amounts of nanoparticles (0–25 mol%) at 50°C. The results showed that the best efficiency was 20 mol%. Then, temperature optimization was carried out and the reaction was tested at 25, 60, 70, and 80°C, and it was found that the highest efficiency with lowest time were 60°C with a molecular weight of 20% catalyst **(**Table 1).

Ten 7-Aryl-6H,7H-benzo[f]chromeno[4,3-b]chromen-6-one derivatives were synthesized in optimum conditions, five of which were new derivatives. The presented method in this study caused the formation of compounds with higher efficiency and shorter time than the previous ones. The results of synthesized compounds are shown in Table 2.

TABLE 1 Optimization of reaction conditions

Entry	MgO NPs (mol%)	Temperature (°C)	Time (min)	Yield (%)
1	0	50	60	0
2	5	50	45	58
3	10	50	35	65
4	15	50	30	81
5	20	50	25	87
6	25	50	25	84
7	20	25	60	37
8	20	60	15	92
9	20	70	15	90
10	20	80	15	86

The catalyst was used five times after separation, washing, and drying in the synthesis of 4a. The recovery results show that reusing the catalyst does not greatly reduce the efficiency (Figure 3).

According to previous reports, catalysts such as 1-Methyl-3-(2-(sulfooxy)ethyl)-1H-imidazol-3-ium Chloride (MSI),[17] Zr $(HSO_4)_4$,[18] Alum,[19] and melamine trisulfonic acid[20] have been used in the synthesis of these compounds. The results presented in Table 3 show that MgO nanoparticle have lower temperature, lower time, and higher efficiency than previous catalysts in the synthesis of derivatives.

The chemical structures of compounds 4a–j were identified by elemental analyses, ^{1}H NMR and ^{13}C NMR spectrum. In ^{1}H NMR spectra of 4b; hydrogens of the methyl groups (N $(Me)_2$) appeared at δ: 3.16 ppm (6H, s), δ: 6.31 ppm (1H, s, CH). In ^{13}C NMR spectra, methyl groups of amine appeared at δ: 46.0, ppm, sp^3 carbon in the ring at δ: 36.4 ppm and carbonyl group at δ: 168.1 ppm.

The proposed mechanism was presented at the below in Scheme 2.

2.1 | Antimicrobial evaluation of the synthesized compounds

The antimicrobial activity includes antimicrobial and antifungal activity, of the synthesized compounds was evaluated against three gram-negative bacteria, two gram-positive bacteria and one fungi. According to the results concerning, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), minimum fungicidal concentration (MFC) values in Table 4,

TABLE 2 Multicomponent synthesis of 7-Aryl-6H,7H-benzo[f] chromeno[4,3-b

Entry	R	Time (min)	Yield (%)	MP (°C)	
				Found	Reported
4a	C_6H_5	20	92	202–203	199–200 [16]
4b	4—N $(CH_3)_2$—C_6H_4	15	89	195–197	New
4c	3—NO_2—C_6H_4	25	85	235–2237	237–238 [16]
4d	4—NO_2—C_6H_4	10	93	257–259	257–258 [17]
4e	2—OH—C_6H_4	25	82	246–247	New
4f	2—OH—4—OCH_3—C_6H_3	30	84	282–283	New
4 g	2,4—$(OCH_3)_2$—C_6H_3	15	86	190–191	New
4 h	2,5—$(OCH_3)_2$—C_6H_3	15	84	193–194	191–192 [17]
4i	3,4—$(OCH_3)_2$—C_6H_3	10	91	265–266	New
4j	4—OCH_3—C_6H_4	5	93	213–215	211–212 [16]

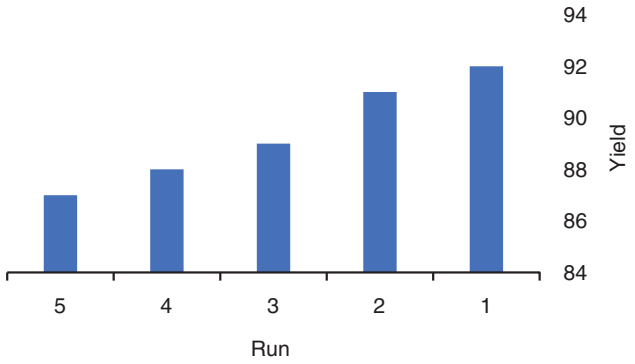
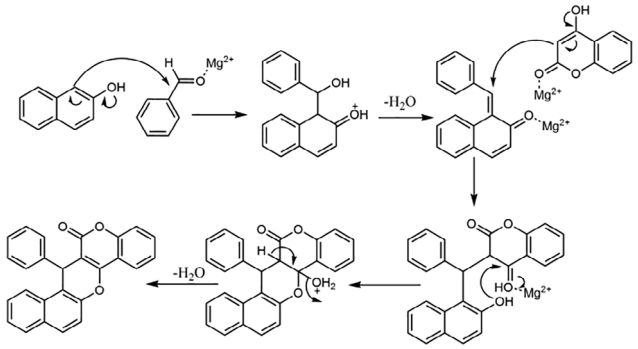


FIGURE 3 Reusability of MgO NPs in the synthesis of 4a



SCHEME 2 Proposed mechanism for the synthesis of 7-Aryl-6H,7H-benzo[f]chromeno[4,3-b]chromen-6-one derivatives by MgO nanoparticle

TABLE 3 Comparison of different methods in the synthesis 7-(phenyl)-6H,7H-benzo[f]chromeno[4,3-b]chromen-6-one (**4a**)

Entry	Cat	Time (min)	Temperature (°C)	Yield (%)
4a	MSI	120	80	90[16]
4a	Zr (HSO4)4	20	110	91[17]
4a	Alum	45	120	86[18]
4a	MTSA	60	120	89[19]
4a	MgO Nps	20	60	92

the order of antimicrobial activity was described as 4d>4c>4e>4f>4i>4g>4h>4j>4b>4a. The highest antimicrobial activity was related to 4d and 4c. The highest antimicrobial activity of these compounds can be attributed to the presence of nitro group in their structure. Subsequently, the compounds containing the hydroxide group (4e, 4f) showed the next order of antimicrobial activity. The results were compared with cefazolin, gentamicin, terbinafine, and tolnaftate as commercial drugs. In the antibacterial activity, the results indicated that cefazole has not effect on *Acinetobacter baumannii* and *Bacillus cereus* but the synthesized derivatives, especially 4d, showed good effect. In the antifungal activity, also tolnaftate had no effect on *Aspergillus fumigatus* but the compound 4d showed good effects.

2.2 | Antioxidant evaluation

In the study of antioxidant activity, the concentration diagram on percent inhibition was plotted and IC_{50}

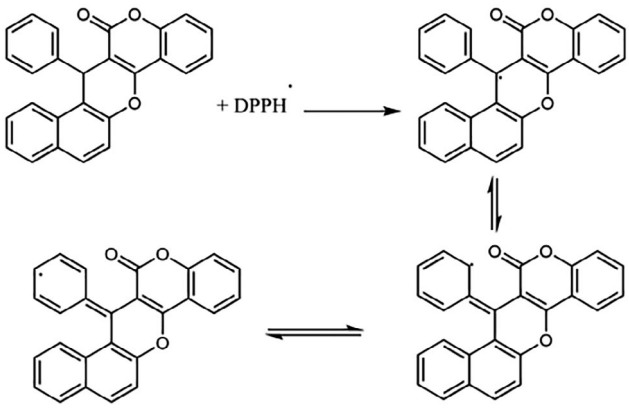
TABLE 4 Antimicrobial activity of 7-aryl-6H,7H-benzo[f]chromeno[4,3-b]chromen-6-one derivatives

Product/ antibiotics	Gram-negative bacteria						Gram-positive bacteria				Fungi	
	1399		1855		1290		1665		1447		5009	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MIFC
4a	1024	2048	-	-	-	-	-	-	1024	2048	-	-
4b	1024	2048	-	-	512	1024	-	-	1024	2048	-	-
4c	256	512	512	1024	512	1024	256	512	512	1024	128	256
4d	128	256	512	1024	256	512	64	128	512	1024	128	256
4e	256	512	512	1024	1024	2048	256	512	512	1024	256	256
4f	512	1024	512	1024	1024	2048	512	1024	1024	2048	256	512
4 g	256	512	512	1024	-	-	-	-	512	1024	512	1024
4 h	256	1024	512	1024	-	-	-	-	1024	2048	1024	2048
4i	128	256	512	1024	-	-	-	-	512	512	512	1024
4j	512	1024	512	1024	-	-	-	-	1024	2048	2048	4096
a	8	16	-	-	0.25	0.5	-	-	8	16	32	64
b	4	8	32	64	4	8	1	4	2	4	-	-

TABLE 5 Antioxidant activity of 7-Aryl-6H,7H-benzo[f] chromeno[4,3-b]chromen-6-one derivatives

Compounds	(%) Scavenging concentrations (μg/ml)				IC_{50} (μg/ml)
	5	10	15	20	
4a	24	39	49	57	16.05
4b	39	65	69	81	7.31
4c	20	35	41	51	19.19
4d	11	25	32	36	27.13
4e	15	31	38	41	23.53
4f	23	39	47	56	16.59
4 g	35	53	64	75	9.92
4 h	35	54	66	75	9.66
4i	31	48	60	67	11.87
4j	30	48	59	65	12.28
Ascorbic acid	52	69	78	89	3.33

calculated from the slope of the line. All the derivatives 4a-j exhibited antioxidant properties with IC_{50} ranging from 7.31 to 27.13 μg/mL and IC_{50} value for ascorbic acid was obtained as 3.33 μg/mL (Table 5). The order of the antioxidant properties of the derivatives based on were 4b>4h>4g>4i>4j>4a>4f>4C>4e>4d. Depending on the structure of the derivatives, the order of the property is dependent on the radical stability formed. According to the results, the following mechanism was proposed for DPPH radical stability (Scheme 3).



SCHEME 3 Proposed mechanism for radical stability of DPPH

3 | CONCLUSIONS

Using 2-*Naphthol*, *aldehyde* derivatives, and 4-*hydroxycoumarin*, 10 7-Alkyl-6H,7H-benzo[f]chromeno [4,3-b]chromen-6-one derivatives were synthesized in this study, five of which were new compounds. MgO nanoparticles were used as a green and recyclable catalyst and a new method was suggested for the synthesis of the 7-Alkyl-6H,7H-benzo[f]chromeno[4,3-b]chromen-6-one derivatives. MgO nanoparticles showed good recyclability and can be used up to five times without dramatically reducing efficiency. Biological tests were carried out on the synthesized compounds, and the compounds revealed acceptable antimicrobial, antifungal, and antioxidant

properties. The novelty of this study is the synthesis of new derivatives with antimicrobial and antioxidant properties and the development of a new method and green catalyst for the synthesis of 7-Alkyl-6H,7H-benzo[f] chromeno[4,3-b]chromen-6-one derivatives at a shorter reaction time with higher yields, compared to the previous methods.

3.1 | Experimental section

All reagents and solvents obtained from Merck and Sigma-Aldrich. Antibiotics were acquired from Sigma-Aldrich. The ^{1}H and ^{13}C-NMR spectra were determined by a Bruker FT-NMR Ultra Shield-250 spectrometer (250 and 75 MHz, resp). Elemental analyses were accomplished for on a Thermo Finnigan Flash EA microanalyzer. The FT-IR spectrum was recorded on a Bruker Tensor 27 FT-IR spectrometer using KBr bullets. Melting points of material were determined by a Kruss type KSP1N melting point meter. Monitoring progress of the reactions and the purity of the products were done by TLC (Silica gel, Aluminum Sheets, Merck). The concentrations of bacterial, fungal suspensions, and absorption of derivatives in antioxidant activity were determined by using Jenway 6405 UV–V spectrophotometer. The XRD analysis was carried out by Bruker D8 X-ray diffractometer with Cu-Kα radiation (λ = 1.5418 Å) in the range of 10–70° and the scanning rate of 1.5°/min.

3.2 | Preparation of MgO nanoparticles

The MgO nanoparticles were prepared according to previously reported.[21]

3.2.1 | Synthesis of 7-aryl-6H,7H-benzo[f] chromeno[4,3-b]chromen-6-one (4a-j)

A mixture of 1 mmol 2-*Naphthol* (0.144 g), 1 mmol *aldehyde* derivatives, 1 mmol *4-hydroxycoumarin* (0.162 g) and 0.25 mmol MgO nanoparticles (0.02 g) in 2 ml of ethanol were stirred at 50°C. The reactions were monitored by TLC (n-hexane/ethyl acetate). After completion of the reaction, 5 ml of acetone was added to the precipitates and MgO nanocatalyst was filtered off. Remove the filter solution under vacuum conditions. The precipitate was recrystallized with ethanol. The isolated MgO nanoparticles washed several times with ethanol and water, then were dried under vacuum over Al_2O_3 at room temperature.

7-(4-(dimethylamino)phenyl)-6H,7H-benzo[f] chromeno[4,3-b]chromen-6-one (***4b***)

M.p. 195–197°C; IR (KBr, cm^{-1}): 3046 and 2919 (CH_3), 2793 (C—H), 1674 (C═O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.16 (6H, s, 2Me), 6.31 (1H, s, CH), 7.23–7.31 (8H, m, H—Ar), 7.45 (1H, d, J = 8.4 Hz, H—Ar), 7.51–7.56 (3H, m, H—Ar), 7.83 (2H, d, J = 6.3 Hz, H—Ar); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 36.4, 46.0, 103.5, 116.1, 120.1, 123.5, 124.6, 128.7, 131.6, 153.0, 164.9, 168.1. Anal. Calcd for $C_{28}H_{21}NO_3$: C, 80.17; H, 5.05; N, 11.44. Found: C, 80.15; H, 5.06; N, 11.47.

7-(2-hydroxyphenyl)-6H,7H-benzo[f]chromeno[4,3-b] chromen-6-one (***4e***)

M.p. 246–247°C; IR (KBr, cm^{-1}): 3256 (O—H), 3052 (C—H), 1711 (C═O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 5.76 (1H, s, CH), 7.13–7.24 (1H, m, H—Ar), 7.33–7.35 (8H, m, H—Ar), 7.47 (1H, t, H—Ar), 7.51–7.73 (1H, m, H—Ar), 8.11 (1H, d, J = 6.6 Hz, H—Ar), 12.25 (1H, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 29.1, 114.3, 116.6, 116.7, 117.0, 122.7, 123.1, 124.4, 125.0, 125.8, 128.8, 129.1, 132.7, 133.0, 149.7, 152.5, 152.7, 156.8, 160.9, 161.1. Anal. Calcd for $C_{26}H_{16}O_4$: C, 79.58; H, 4.11. Found: C, 79.59; H, 4.14.

7-(2-hydroxy-4-methoxyphenyl)-6H,7H-benzo[f] chromeno[4,3-b]chromen-6-one (***4f***)

M.p. 282–283°C; IR (KBr, cm^{-1}): 3271 (O—H), 3074 and 2993 (CH_3), 2972 (C—H), 1721 (C═O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.95 (3H, s, OMe), 5.73 (1H, s, CH), 6.77 (1H, d, J = 7.2 Hz, H—Ar), 7.01–7.12 (2H, m, H—Ar), 7.32–7.73 (8H, m, H—Ar), 8.01 (1H, d, J = 7.8 Hz, H—Ar), 12.25 (1H, s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 29.1, 56.5, 101.0, 111.6, 114.4, 116.6, 116.7, 117.0, 120.1, 122.8, 123.4, 124.4, 125.1, 125.6, 132.7, 132.9, 139.1, 147.8, 152.4, 152.6, 154.4, 160.9. Anal. Calcd for $C_{27}H_{18}O_5$: C, 76.77; H, 4.29. Found: C, 76.78; H, 4.32.

7-(2,4-dimethoxyphenyl)-6H,7H-benzo[f]chromeno [4,3-b]chromen-6-one (***4 g***)

M.p. 190–191°C; 3012, 2974 and 2939, (CH_3), 2723 (C-H), 1669 (C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.59 (3H, s, OMe), 3.74 (3H, s, OMe), 6.19 (1H, s, CH), 6.43–6.51 (3H, m, H—Ar), 7.05 (1H, d, J = 8.4 Hz, H—Ar), 7.32–7.40 (5H, m, H—Ar), 7.60 (3H, t, H—Ar), 7.93 (1H, d, J = 7.8 Hz, H—Ar); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 32.9, 55.5, 56.1, 99.1, 104.5, 105.6, 116.5, 117.7, 120.3, 124.0, 124.3, 129.0, 132.3, 152.4, 158.7, 159.7, 163.6, 164.4. Anal. Calcd for $C_{28}H_{20}O_5$: C, 77.05; H, 4.62. Found: C, 77.03; H, 4.60.

7-(3,4-dimethoxyphenyl)-6H,7H-benzo[f]chromeno [4,3-b]chromen-6-one (***4i***)

M.p. 265–266°C; 3021, 3012, 2981 and 2930, (CH_3), 2752 (C—H), 1689 (C═O); ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.59 (3H, s, OMe), 3.73 (3H, s, OMe), 6.32 (1H, s, CH),

6.71 (2H, d, J = 8.1 Hz, H—Ar), 6.84 (1H, d, J = 8.1 Hz, H—Ar) 7.33–7.41 (5H, m, H—Ar), 7.60–7.64 (3H, m, H—Ar), 7.94 (1H, d, J = 7.5 Hz, H—Ar), 9.15 (1H, s, H—Ar); ^{13}C NMR (75 MHz, DMSO-d_6) δ: 36.1, 55.9, 56.1, 104.9, 111.8, 112.1, 116.5, 118.2, 119.3, 124.3, 132.4, 147.6, 149.0, 152.6, 165.2, 165.4. Anal. Calcd for $C_{28}H_{20}O_5$: C, 77.05; H, 4.62. Found: C, 77.06; H, 4.59.

3.3 | *In vitro* antimicrobial activity

MIC values were determination by Broth microdilution methods according to CLSI guidelines M07-A9 and M27-A2 and MBC and MFC values was applied by Time-kill test according to CLSI guideline M26-A.[22,23]

All tested strains included, *Escherichia coli* (PTCC 1399), *Acinetobacter baumannii* (PTCC 1855), *Klebsiella pneumoniae* (PTCC 1290) (gram-negative pathogenic bacteria), *Bacillus cereus* (PTCC 1665), *Streptococcus pyogenes* (PTCC 1447) (gram-positive strains), *Aspergillus fumigatus* (PTCC 5009) (fungi) were prepared from the Persian Type Culture Collection (PTCC), Tehran, Iran. All the tests were repeated three times, and the results were expressed as the average of the three independent experiments.

3.4 | *In vitro* antioxidant activity

Antioxidant activity of derivatives on DPPH was evaluated according to the method of Beyzaei et al.[24] In 4 ml of the 0.004% (w/v) DPPH methanolic solution, 1 ml of various concentrations (25, 50, 75, and 100 μg/mL) of all derivatives in methanol was added and stand for 30 min at room temperature in darkness. The absorbance was read against blank at 517 nm. The percent inhibition (I %) of free radical production from DPPH was calculated by the equation (1):

$$\%\text{of scavenging} = \frac{(\text{A control} - \text{A sample})}{(\text{A control})} \times 100$$

A control: absorbance of DPPH solution;A sample: absorbance of the test compound;All tests were carried out in triplicate and their average was reported.

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ORCID

Nourallah Hazeri https://orcid.org/0000-0002-0560-7245

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