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A convenient synthesis and biological evaluation of some novel linear and cyclic α-aminophosphonic acid derivatives containing a quinazolinone ring

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Abstract Synthesis of some novel linear and cyclic α-aminophosphonic acids and their esters bearing a quinazolin-4(3H)-one ring was achieved. The methodology depends on *Pudovik* reaction conditions in which using 3-(benzylideneamino)-2-phenyl-quinazolin-4(3H)-one (**2**) with phosphorous acid, diethyl phosphite and tris(2-chloroethyl) phosphite gives the corresponding linear α-aminophosphonic acid derivatives **3**, **5** and **7**, respectively, in good yields. Heterocyclization of the linear compounds to 1,4,2-oxazaphosphinanes as cyclic α-aminophosphonic acid derivatives **4**, **6** and **8**, respectively, was carried out. The synthesized compounds were evaluated for their antimicrobial and antioxidant activities. The cyclic α-aminophosphonic acids and their esters showed better antimicrobial and antioxidant activities than the corresponding linear α-aminophosphonic acids and their esters.

Graphical Abstract Synthesis of some novel linear and cyclic α-aminophosphonic acids and their esters bearing a quinazolin-4(3H)-one ring as antimicrobial and antioxidant agents from 3-(benzylideneamino)-2-phenyl-quinazolin-4(3H)-one was achieved.

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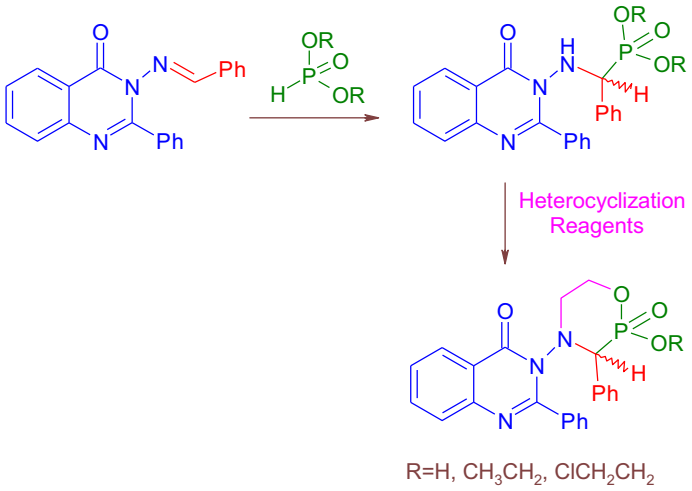


Keywords α-Aminophosphonic acid · *Pudovik* reaction · 1,4,2-Oxazaphosphinanes · Antimicrobial · Antioxidant

Introduction

Quinazolin-4(3H)-one is a heterocyclic alkaloid ring system belonging to the group of *N*-containing heterocyclic compounds, a group of compounds that has caused universal concern due to their widely and distinct biopharmaceutical activities. Researchers have already determined many therapeutic activities of quinazolin-4(3H)-one derivatives, including antimicrobial [1, 2], antiviral [3], anti-HIV [4], anticonvulsant [5, 6], anti-inflammatory [7], antihistaminic [8], antitubercular [9], and anticancer activities [10]. On the other hand, α-aminophosphonic acids are important isosteres of α-amino acids showing a variety of biological effects [11]. Apart from this, their potential as herbicides [12], insecticides [13], fungicides [14], antiviral agents [15], as well as their role for antibody generation, is well known [16]. A survey of the literature revealed that compounds containing α-aminophosphonates with a quinazolin-4(3H)-one ring have not been reported so far. Moreover, 2-alkoxy-2-oxo-1,4,2-oxazaphosphinanes (as cyclic α-aminophosphonate) are rarely reported in literature [17–20] and they are of interest in regards to their biological activity [21]. Thus, we report herein a simple method for synthesis of novel linear and cyclic α-aminophosphonates containing a quinazolin-4(3H)-one ring in good yields as a part of our interest in the synthesis of new α-aminophosphonates containing different bioactive heterocyclic rings [22–26]. The antimicrobial and antioxidant activities of the synthesized compounds were also evaluated.

Results and discussion

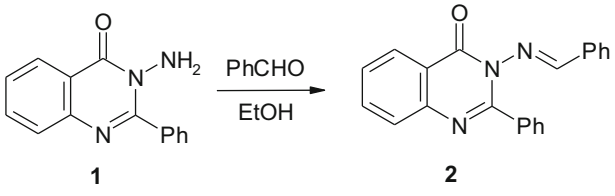
Synthesis of linear α-aminophosphonic acid derivatives and their cyclic analogues

Owing to their synthetic and biological value, the chemistry of α-aminophosphonic acids and their esters has received very important attention and still remains of great interests [27]. In the synthesis of α-aminophosphonic acids, the *Pudovik* reaction and the *Kabachnik-Fields* reaction are powerful and direct methods for construction of P–C–N bonds [28, 29]. 3-(Benzylideneamino)-2-phenyl-quinazolin-4(3H)-one (**2**) was prepared according to the reported method [30, 31] in high yield by condensation of 3-amino-2-phenyl-quinazolin-4(3H)-one (**1**) [32, 33] with benzaldehyde using a catalytic amount of glacial acetic acid in absolute ethanol (Scheme 1).

Addition of phosphorous acid to the imine **2** in toluene containing a catalytic amount of trifluoroboron etherate ($BF_3{\cdot}Et_2O$) under *Pudovik* reaction conditions gave {[(4-oxo-2-phenyl-quinazolin-3(4H)-yl)amino] (phenyl)methyl}phosphonic acid (**3**) in good yield (Scheme 2). The proposed mechanism suggested that BF_3 as a Lewis acid attracted the electron lone pair of the nitrogen atom in the azomethine bond, which accelerated the nucleophilic phosphorus attack on the electrophilic carbon atom forming the dipolar intermediate **B** that produced the desired product **3** (Scheme 2). The linear α-aminophosphonic acid **3** undergoes heterocyclization into 1,4,2-oxazaphosphinane as cyclic α-aminophosphonic acid. Thus, reaction of α-aminophosphonic acid **3** with 1,2-dibromoethane in absolute ethanol in the presence of anhydrous potassium carbonate afforded 3-(2-hydroxy-2-oxido-3-phenyl-1,4,2-oxazaphosphinan-4-yl)-2-phenyl-quinazolin-4(3H)-one (**4**) at a 63-% yield (Scheme 2).

Consequently, addition of diethyl phosphite to the imine **2** in toluene containing a catalytic amount of $BF_3{\cdot}Et_2O$ gave the dipolar intermediate **C**, which led to the formation of diethyl {[(4-oxo-2-phenyl-quinazolin-3(4H)-yl)amino](phenyl)methyl}phosphonate (**5**) at an 85-% yield (Scheme 3). Heterocyclization of the linear diethyl α-aminophosphonate **5** with 2-bromoethanol in ethanolic sodium ethoxide produced the corresponding 1,4,2-oxazaphosphinanyl quinazolinone **6** (Scheme 3).

Similarly, the imine **2** reacted with tris(2-chloroethyl) phosphite under the same reaction conditions to produce the corresponding α-aminophosphonate **7** in



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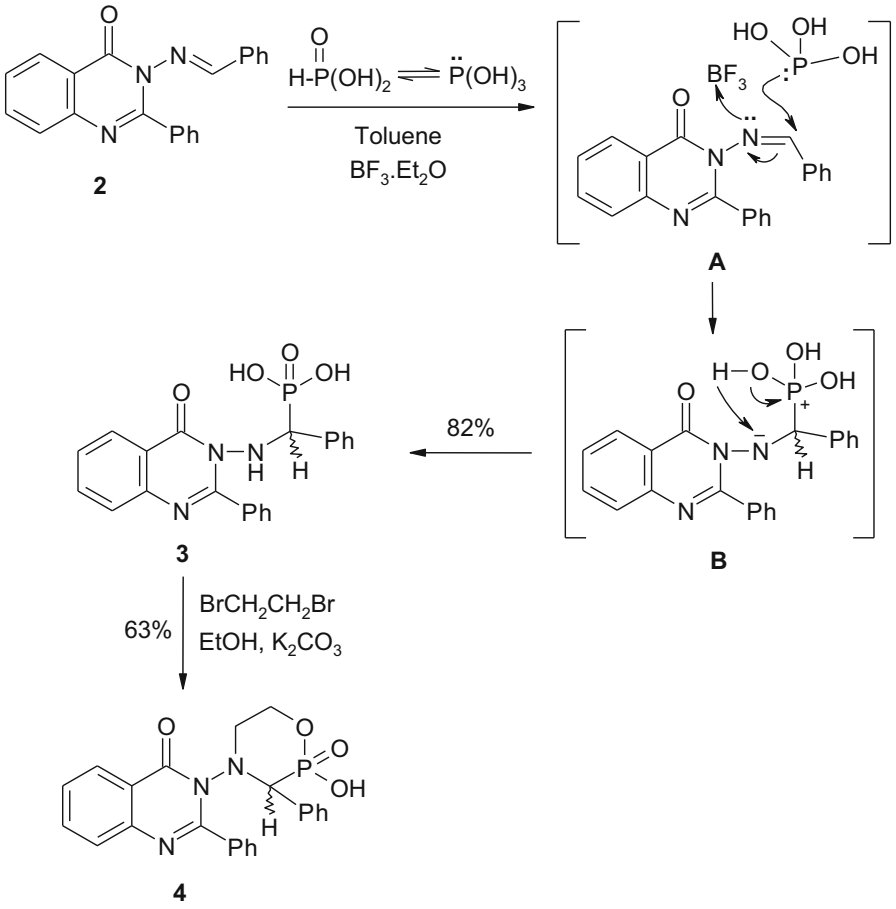
Scheme 1 Preparation of 3-(benzylideneamino)-2-phenyl-quinazolin-4(3H)-one

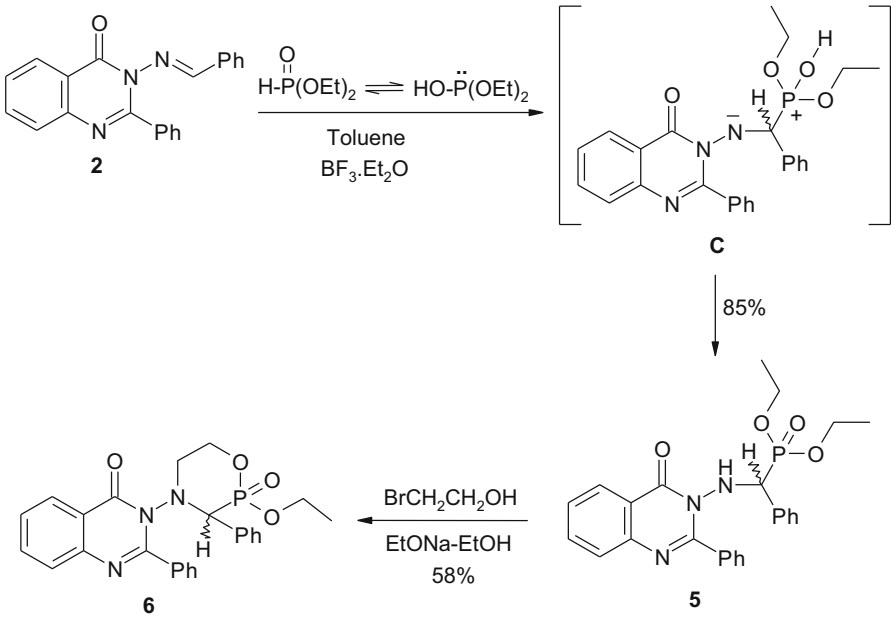


Scheme 2 Synthesis of the linear α-aminophosphonic acid **3** and its cyclic α-aminophosphonic acid **4**

excellent yield (Scheme 4). A possible mechanism for the last reaction could involve a nucleophilic phosphorus attack on the electrophilic carbon atom of an azomethine bond to give the dipolar intermediate **D**, which could be solvated by water (added during the reaction) to give the transient **E** [34]. The latter transient underwent decomposition via removal of a 2-chloroethanol molecule to afford the final α-aminophosphonate **7** (Scheme 4). Heating of the linear bis(chloroethyl) α-aminophosphonate **7** in toluene containing a catalytic amount of triethylamine for 12 h achieved the corresponding 1,4,2-oxazaphosphinanyl quinazolinone **8** at a 61-% yield (Scheme 4).

Characterization of linear α-aminophosphonic acid derivatives and their cyclic analogues

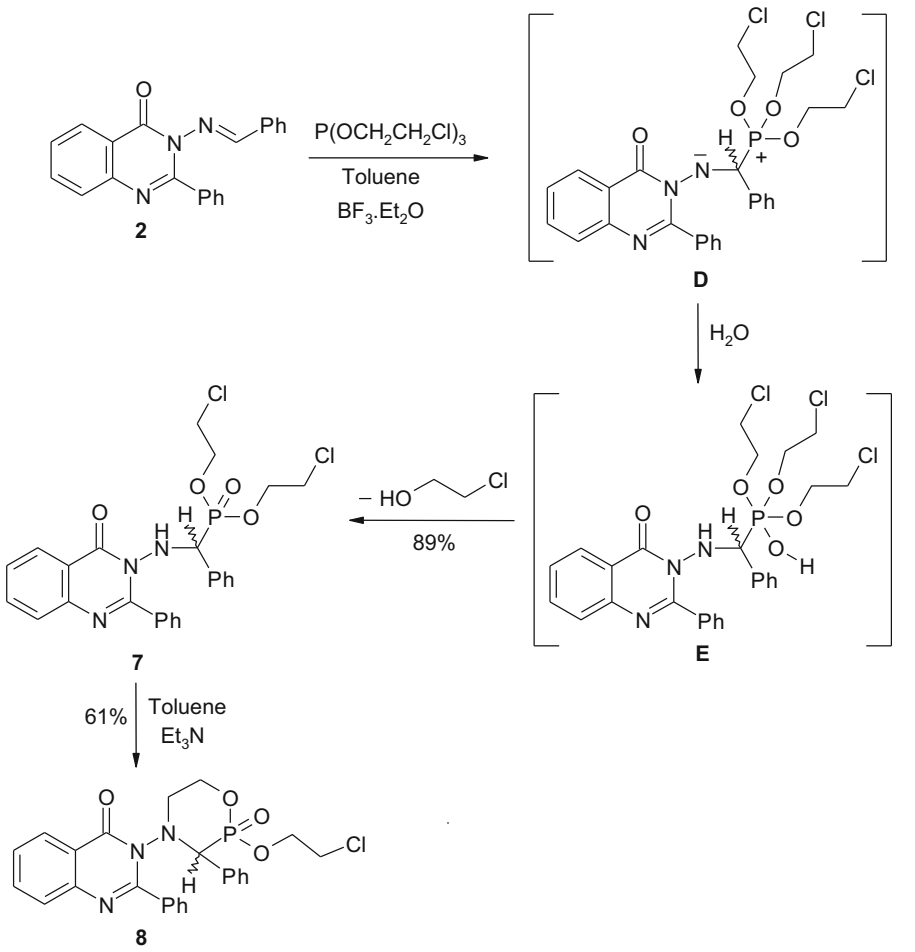
All the compounds were elucidated on the basis of IR, MS, ^{1}H-, ^{13}C- and ^{31}P-NMR spectra. The elemental analysis data were consistent with the compositions of the



Scheme 3 Synthesis of the linear diethyl α-aminophosphonate **5** and its cyclic ethyl α-aminophosphonic **6**

desired products. The gross formula $C_{21}H_{18}N_3O_4P$, of acid **3** was confirmed by the mass spectrum, which exhibited its molecular ion peak at *m/z* 407 (12 %). The IR spectrum of acid **3** revealed absorption bands at 3447 (N–H), 3197 (P–OH), 1296 (P=O) and 1669 cm^{-1} (C=O). Its ^{1}H-NMR spectrum exhibited a singlet at δ 4.52 (N–H), a doublet at 4.06 (P–CH) and a broad signal at 2.49 ppm (O–H) [35]. The ^{13}C-NMR spectrum of **3** revealed the characteristic carbon atoms of P–*C*H and *C*=O groups at δ 49.0 and 162.2 ppm, respectively (Fig. 1). Moreover, its ^{31}P-NMR spectrum showed a singlet peak at δ 10.5 ppm.

Compounds **5** and **7** showed characteristic IR absorption wave numbers in the regions around 3447, 1682, 1281 and 1025 cm^{-1} for NH, C=O, P=O and P–O–C groups, respectively. Also, the ^{1}H-NMR spectra of these compounds showed multiplets for aromatic protons in the range of δ 7.39–8.24 ppm. Their P–CH protons resonated as doublets in the region δ 4.64 ($J = 21$ Hz) and 5.62 ($J = 19$ Hz) ppm, respectively, due to their coupling with phosphorus atoms. In both compounds, the protons of N–H groups appeared as broad singlets at δ 5.35 and 6.20 ppm, respectively. Moreover, protons of the ethoxy group in compound **5** exhibited multiplets at δ 0.92–1.10 (CH_3) and 3.78–4.10 (CH_2) ppm. In compound **7**, the methylene protons of OCH_2 and CH_2Cl resonated as triplets at δ 4.20 and 2.11 ppm, respectively. In the ^{13}C-NMR spectra of compounds **5** and **7**, the carbon atoms of the carbonyl groups exhibited signals around δ 169.3 ppm. The *C*-2 of the quinazolinone ring was recorded around δ 153 ppm. In addition, the characteristic carbon atoms of P–CH groups exhibited doublets in the region δ 49.1 and 48.0 ppm



Scheme 4 Synthesis of the linear bis(chloroethyl) α-aminophosphonate **7** and its cyclic chloroethyl α-aminophosphonic **6**

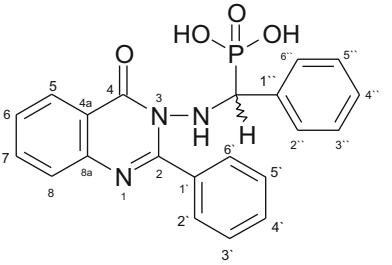


Fig. 1 The carbon atom numbering of compound **3**

Table 1 In vitro antimicrobial activities of the synthesized compounds **3–8** at 500 and 1000 μg mL^{-1} and the MIC values for some selected compounds

Compd.	Conc. (μg mL^{-1})	Zone of inhibition in mm[a] and (MIC values in μg mL^{-1})					
		Bacteria Gram (+) ve		Bacteria Gram (−) ve		Fungi	
		S. aureus	*B. subtilis*	*S. typhimurium*	*E. coli*	*C. albicans*	*A. fumigatus*
3	500	7	11 (>250)	–	8	11 (>250)	7
	1000	12	14	–	11	15	11
4	500	14 (>250)	15 (>250)	–	13 (>250)	19 (250)	7
	1000	17	17	–	16	22	10
5	500	12 (>250)	13 (>250)	6	5	19 (250)	17 (>250)
	1000	15	19	10	8	22	22
6	500	14 (>250)	15 (>250)	8	8	16 (>250)	19 (250)
	1000	18	19	10	11	21	23
7	500	20 (250)	17 (>250)	9	20 (125)	15 (>250)	20 (125)
	1000	24	21	12	23	18	23
8	500	22 (125)	13 (>250)	9	22 (62.5)	28 (31.25)	20 (125)
	1000	27	18	12	27	32	25
Standard drug	500	26	25	28	27	28	26
	1000	35	35	36	38	35	37

[a] Low active, 6–12 mm; moderately active, 13–19 mm; highly active, 20–30 mm; –, no inhibition or inhibition <5 mm

with coupling constants of 147.4 and 150 Hz, respectively. Also, the carbon atoms of ethoxy groups in compound **5** appeared at δ 16.0 and 59.7 ppm while the methylene carbon atoms OCH_2 and CH_2Cl in compound **7** resonated at δ 66.0 and 35.9 ppm, respectively. The ^{31}P-NMR chemical shift of compound **5** appeared at δ 22.3 ppm. The mass spectra of these compounds showed their respective molecular ion peaks at the expected *m/z* 463 (11 %) and 532 (18 %), respectively.

The IR spectra of compounds **4**, **6** and **8** revealed the disappearance of absorption bands to NH functions and the presence of absorption bands in the ranges of 1668–1682 and 1296–1214 cm^{-1} assignable to C=O and P=O functions, respectively. Their ^{1}H-NMR spectra showed triplets in the range of δ 3.04–2.94 (NCH_2) and 3.94–4.08 (OCH_2) ppm for 1,4,2-oxazaphosphinane rings. Also, the protons of P–CH groups appeared as doublets in range δ 4.80–4.88 ppm with phosphorus coupling constants of 19.8–22.5 Hz. Moreover, the protons of ethoxy groups in both compounds **6** and **8** appeared at δ 1.15 (CH_3), 4.20–4.30 (CH_2) and 4.10 (OCH_2), 2.29 (CH_2Cl) ppm, respectively. In addition, the N–H protons of compounds **4**, **6** and **8** were absent, which supported the cyclization processes. The signals of P–CH carbon atoms of compounds **4**, **6** and **8** in their ^{13}C-NMR spectra are observed in the range δ 53.0–54.0 ppm (J_{PC} = 146–150 Hz). The carbon atoms of NCH_2 and OCH_2 for 1,4,2-oxazaphosphinane rings were resonated in the range δ 46.0–47.1 and 69.0–71.6 ppm, respectively. Other signals in the ^{1}H- and ^{13}C-NMR spectra of compounds **4**, **6** and **8** are in agreement with the frameworks of the starting materials **3**, **5** and **7**. The ^{31}P-NMR spectra of compounds **4** and **8** exhibited only one signal for each compound at δ 15.6 and 18.9 ppm, respectively. The mass spectra of the three compounds showed their respective molecular ion peaks at the expected *m/z* 433 (6 %), 461 (69 %) and 496 (2 %), respectively.

Biological evaluations

Antimicrobial activity

All the newly synthesized compounds were evaluated in vitro for their antibacterial activities against *Staphylococcus aureus* (ATCC 25923) and *Bacillus subtilis* (ATCC 6635), as representatives of Gram-positive bacteria and *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028) as examples of Gram-negative bacteria. They were also examined against *Candida albicans* (ATCC 10231) and *Aspergillus fumigatus* as fungi. The agar-diffusion technique was used for the determination of the preliminary antibacterial and antifungal activities [36, 37]. The minimum inhibitory concentration (MIC, μg mL^{-1}) for the most active compounds against the same microorganism used in the preliminary screening was carried out using the tube dilution technique [38]. The obtained results on the antimicrobial activities of the compounds and control drugs are given in Table 1. In general, the prepared compounds recorded variable antimicrobial activities towards the used microorganisms. All compounds exhibited moderate activities against Gram-positive bacteria, except compounds **7** and **8** that displayed high activities against *S. aureus* with MIC values at 250 and 125 μg mL^{-1}, respectively. Most of

Table 2 DPPH radical scavenging ability of compounds **3–8** relative to the standard antioxidant tertiary butylhydroquinone (TBHQ)

Compd. no.	The percentage (%) antioxidant activity ± SD					IC_{50} (μmol L^{-1})
	50 (μmol L^{-1})	100 (μmol L^{-1})	200 (μmol L^{-1})	300 (μmol L^{-1})	400 (μmol L^{-1})	
3	7.96 ± 0.0012	14.54 ± 0.0008	19.44 ± 0.0120	21.59 ± 0.0700	24.34 ± 0.0210	>500
4	8.26 ± 0.0023	12.40 ± 0.0064	19.29 ± 0.0128	20.98 ± 0.0424	26.79 ± 0.0001	>500
5	10.41 ± 0.0043	19.44 ± 0.0067	35.68 ± 0.0043	42.11 ± 0.0009	50.24 ± 0.0028	377.66
6	39.50 ± 0.0021	49.58 ± 0.0011	65.08 ± 0.0056	73.91 ± 0.0108	77.79 ± 0.0019	109.14
7	44.10 ± 0.0041	52.52 ± 0.0023	71.82 ± 0.0038	79.63 ± 0.0192	81.31 ± 0.0014	66.72
8	45.67 ± 0.0500	53.65 ± 0.0919	72.68 ± 0.0057	80.40 ± 0.0489	83.89 ± 0.0660	57.58
TBHQ	56.62	66.30	85.55	98.80	–	–

the compounds did not record any remarkable inhibitory effects towards *S. typhimurium*. Only compounds **7** and **8** have excellent activity against *E. coli* with MIC values at 125 and 62.5 μg mL^{-1}, respectively. All the compounds exhibited relatively moderate to high inhibitory activities against *C. albicans*, especially compound **8** which was effective to a degrre equal to the standard drug. Furthermore, only compounds **6–8** recorded remarkable inhibitory effects against *A. fumigatus* with MIC values at 250–125 μg mL^{-1}. From the above results, it is clear that connection of a quinazolinone ring to an α-aminophosphonate moiety can exhibit good antimicrobial effects. Compounds **7** and **8,** which have chloroalkyl groups, recorded highest antimicrobial activity towards all the microorganisms. These results may help chemists make structural modifications to improve the antimicrobial activities of these compounds.

Antioxidant activity

The antioxidant activities of linear and cyclic α-aminophosphonic acid and their esters bearing a quinazolin-4(3H)one ring were evaluated by two in vitro methods in order to compare the results and to establish some structure–antioxidant activity relationships. The evaluation was carried out at concentrations ranging from 50 to 400 μmol L^{-1}.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity evaluation is a standard assay in antioxidant activity studies and offers a rapid technique for screening the radical scavenging activity of specific compounds or extracts [39, 40]. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maximum at 517 nm. This purple colour generally disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can quench DPPH free radicals (i.e., by providing hydrogen atoms or by electron donation, conceivably)

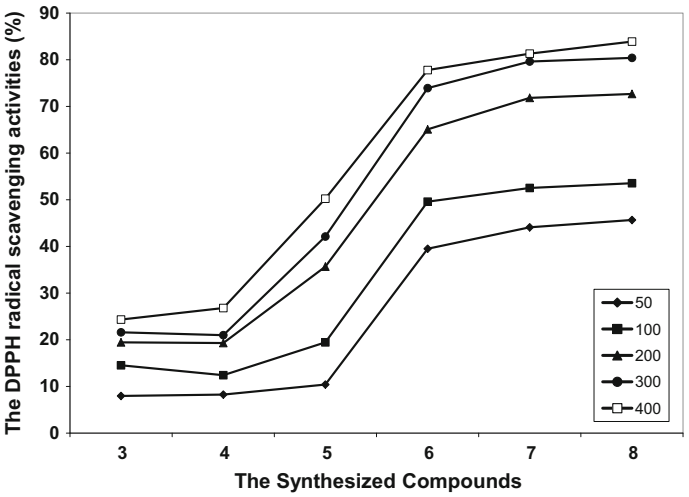


Fig. 2 DPPH radical scavenging activities (%) of the synthesized compounds **3–8** at 50–400 μmol L^{-1}

Table 3 The percentage (%) antioxidant activity of β-carotenoid is based on its radical adducts with free radicals from linoleic acid by the compounds **3**–**8** relative to the standard antioxidant TBHQ

Compd. no.	The percentage (%) antioxidant activity ± SD					IC_{50} (μmol L^{-1})
	50 (μmol L^{-1})	100 (μmol L^{-1})	200 (μmol L^{-1})	300 (μmol L^{-1})	400 (μmol L^{-1})	
3	8.96 ± 0.0082	12.54 ± 0.0034	20.43 ± 0.0230	21.50 ± 0.025	22.93 ± 0.0312	>500
4	8.24 ± 0.0034	11.82 ± 0.0091	16.30 ± 0.0158	20.60 ± 0.0425	24.01 ± 0.0029	>500
5	11.82 ± 0.0053	18.36 ± 0.0073	32.56 ± 0.0231	44.98 ± 0.0181	51.23 ± 0.0015	367.72
6	43.90 ± 0.0023	50.89 ± 0.0081	66.78 ± 0.0091	74.01 ± 0.0092	76.34 ± 0.0320	81.14
7	45.23 ± 0.0062	51.28 ± 0.0021	72.36 ± 0.0012	80.14 ± 0.0023	81.98 ± 0.0126	65.53
8	46.25 ± 0.0350	53.78 ± 0.0631	73.24 ± 0.0098	81.45 ± 0.0561	83.98 ± 0.0613	52.85
TBHQ	58.62	68.30	84.55	98.60	–	–

and convert them to a colorless product (i.e., 2,2-diphenyl-1-picrylhydrazine), resulting in a decrease in absorbance. Hence, more rapid the absorbance decrease, the more potent the antioxidant activity of the compound. The percentage activity of ethanolic solutions of linear and cyclic α-aminophosphonic acids and their esters were examined and compared (Table 2; Fig. 2). The 50-% inhibitory concentration IC_{50} towards DPPH activity of newly synthesized compounds was calculated.

The antioxidant activity of β-carotenoid is based on its radical adducts with free radicals from linoleic acid. The linoleic acid free radical attacks the highly unsaturated β-carotene model. The presence of antioxidants can decrease the extent of β-carotene bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system [41]. Accordingly, the absorbance decreases rapidly in the samples without an antioxidant. Whereas in the presence of an antioxidant, they retain the colour for a longer time. The percentage (%) antioxidant activity of newly synthesized compounds under study was showed in Table 3 and Fig. 3.

We can conclude from the obtained results that the synthesized compounds showed promising radical scavenging abilities by the two methods and as compared with the TBHQ (tertiary butylhydroquinone) as the standard antioxidant. The results reveal that most of the compounds exhibited poor radical scavenging abilities at lower concentrations (<50 μmol L^{-1}). However, a gradual increase in the activity in all cases was observed with an increase in the concentrations of the test compounds. The cyclic α-aminophosphonic acid and their esters **4**, **6** and **8** showed better antioxidant activities than the corresponding linear α-aminophosphonic acid and their esters **3**, **5** and **7**. The α-aminophosphonic acids **3** and **4** showed poor activities while alkyl α-aminophosphonates **5**, **6**, **7** and **8** exhibited remarkable activities at all concentrations, i.e., alkyl groups (electron donating groups) were much better than acidic OH groups. The presence of chloroalkyl groups in

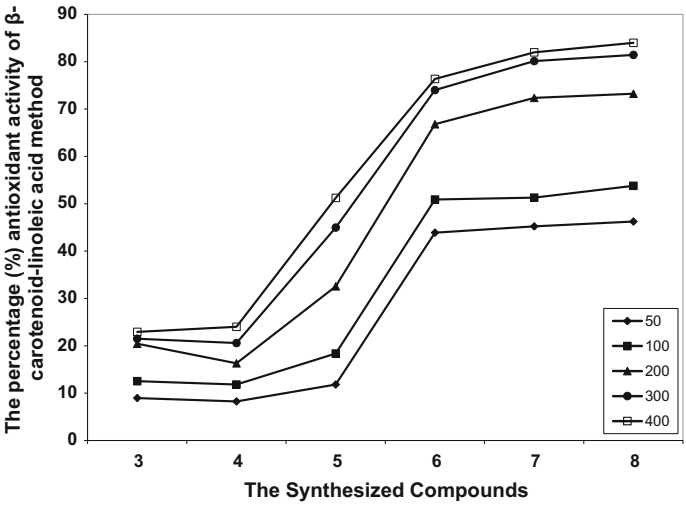


Fig. 3 The percentage (%) antioxidant activity of β-carotenoid is based on its radical adducts with free radicals from linoleic acid by the synthesized compounds **3–8** at 50–400 μmol L^{-1}

compounds **7** and **8** instead of alkyl groups in the same positions in compounds **5** and **6** exhibited significant higher activities. Among the compounds tested, compound **8** having 1,4,2-oxazaphosphinane and a chloro substituent in the alkyl groups exhibited promising antioxidative properties with respect to the standard antioxidant TBHQ.

Conclusion

A convenient synthetic method has been described to synthesize some novel linear and cyclic α-aminophosphonic acids and their esters bearing a quinazolin-4(3H)-one ring. The methodology depends on *Pudovik* reaction conditions in which using a Schiff base with phosphorylating agents gives linear α-aminophosphonic acid derivatives. Heterocyclization of the linear α-aminophosphonic acid derivatives to 1,4,2-oxazaphosphinanes as cyclic α-aminophosphonic acid derivatives was also achieved. The cyclic α-aminophosphonic acid and their esters showed better antimicrobial and antioxidant activities than the corresponding linear α-aminophosphonic acid and their esters. 2-Chloroethoxy-1,4,2-oxazaphosphinane derivative **8** exhibited promising antimicrobial and antioxidant activities.

Experimental

General remarks

Melting points were determined in an open capillary tube on a digital Stuart SMP-3 apparatus. IR spectra were measured on an FT-IR (Nicolet IS10) spectrophotometer using KBr disks. ^{1}H-NMR spectra were measured on a Gemini-300BB spectrometer (300 MHz), using DMSO-d_6 as a solvent and tetramethylsilane (TMS; δ) as an internal standard. ^{13}C-NMR spectra were measured on a Mercury-300BB (75 MHz), using DMSO-d_6 as a solvent and TMS (δ) as an internal standard. ^{31}P-NMR spectra were registered on a Bruker (242 MHz) spectrometer at room temperature using $CdCl_3$ as a solvent and TMS as an internal standard and 85 % H_3PO_4 as an external reference. Mass spectra were recorded on a gas chromatograph (Shimadzu GCMS-QP 1000EX) at 70 eV. Elemental microanalyses were performed using a Perkin-Elmer 2400II at the Chemical War department, Ministry of Defense. The purity of the synthesized compounds was checked by thin layer chromatography (TLC) and elemental microanalyses.

Synthesis of α-aminophosphonic acid 3

A mixture of the imine **2** (0.81 g, 2.5 mmol) and phosphorous acid (0.20 g, 2.5 mmol) in toluene (20 mL) in the presence of a catalytic amount of $BF_3{\cdot}Et_2O$ (0.05 mL) was heated under reflux for 10 h. After completion of the reaction, the solution was concentrated. The residue was triturated with diethyl ether. The formed solid was collected and crystallized from diluted ethanol to give the beige crystals of

product **3** in yield (0.83 g, 82 %); mp 236–238 °C. IR (KBr) (ν_{max}, cm^{-1}): 3447 (NH), 3197 (P–OH), 1669 (C=O), 1602 (C=N), 1571, 1559 (C=C), 1296 (P=O), 693 (P–O). ^{1}H-NMR (300 MHz, DMSO-d_6): 2.49 (br, 2H, P–OH exchangeable with D_2O), 4.06 (d, 1H, $J = 21$ Hz, P–CH), 4.52 (s, 1H, NH exchangeable with D_2O), 7.49–7.62 (m, 7H, Ar–H), 7.72–7.87 (m, 3H, Ar–H), 8.14–8.20 (m, 4H, Ar–H). ^{13}C-NMR (75 MHz, DMSO-d_6): 49.0 (d, $J = 150.4$ Hz, P–*C*H), 120.9 (*C*-4a), 125.8 (*C*-8), 126.5 (*C*-2″,6″), 126.6 (*C*-2′,6′), 127.4 (*C*-3″,5″), 127.5 (*C*-3′,5′), 127.7 (*C*-1″), 128.5 (*C*-4″, 6), 128.6 (*C*-1′), 131.3 (*C*-5), 132.7 (*C*-4′), 134.5 (*C*-7), 148.7 (*C*-8a), 152.3 (*C*-2), 162.2 (*C*=O). ^{31}P-NMR (242 MHz, $CDCl_3$): 10.5 ppm. MS (EI, *m/z*): 407 (M^+, 12 %). Anal. Calcd for $C_{21}H_{18}N_3O_4P$ (407.37): C, 61.92; H, 4.45; N, 10.31 %. Found: C, 61.53; H, 4.42; N, 9.99 %.

Synthesis of 3-(2-hydroxy-2-oxido-3-phenyl-1,4,2-oxazaphosphinan-4-yl)-2-phenyl-quinazolin-4(3H)-one (4)

A mixture of the α-aminophosphonic acid **3** (0.4 g, 1 mmol) and 1,2-dibromoethane (0.09 mL, 1 mmol) in absolute ethanol (20 mL) in the presence of anhydrous potassium carbonate (0.5 g), was heated under reflux for 6 h. After cooling, the solution was poured on cold water. The formed solid was collected and crystallized from benzene to give pale yellow crystals of product **4** in yield (0.27 g, 63 %); mp 171–173 °C. IR (KBr) (ν_{max}, cm^{-1}): 2958 (P–OH), 1668 (C=O), 1602 (C=N), 1570, 1558 (C=C), 1296 (P=O). ^{1}H-NMR (300 MHz, DMSO-d_6): 2.50 (s, 1H, P–OH exchangeable with D_2O), 3.04 (t, 2H, NCH_2), 3.94 (t, 2H, OCH_2), 4.80 (d, 1H, $J = 19.8$ Hz, P–CH), 7.47–7.58 (m, 7H, Ar–H), 7.71–7.84 (m, 3H, Ar–H), 8.13–8.22 (m, 4H, Ar–H). ^{13}C-NMR (75 MHz, DMSO-d_6): 46.0 (N*C*H_2), 53.0 (d, $J = 146$ Hz, P–CH), 69.0 (O*C*H_2), 121.0 (*C*-4a), 126.5 (*C*-8), 126.7 (*C*-2″,6″), 127.1 (*C*-2′,6′), 127.6 (*C*-3″,5″), 128.5 (*C*-3′,5′), 129.0 (*C*-1″), 129.7 (*C*-4″, 6), 131.0 (*C*-1′), 132.3 (*C*-5), 132.6 (*C*-4′), 134.5 (*C*-7), 146.3 (*C*-8a), 153.0 (*C*-2), 169.3 (*C*=O). ^{31}P-NMR (242 MHz, $CDCl_3$): 15.6 ppm. MS (EI, *m/z*): 433 (M^+, 6 %). Anal. Calcd for $C_{23}H_{20}N_3O_4P$ (433.41): C, 63.74; H, 4.65; N, 9.70 %. Found: C, 63.42; H, 4.31; N, 9.49 %.

General procedure for the synthesis of α-aminophosphonates 5 and 7

A mixture of the imine **2** (0.81 g, 2.5 mmol) and diethyl phosphite (0.32 mL, 2.5 mmol) or tris(2-chloroethyl) phosphite (0.50 mL, 2.5 mmol) in toluene (20 mL) in the presence of a catalytic amount of $BF_3{\cdot}Et_2O$ (0.05 mL) was heated under reflux for 10 h [added 0.5 mL of distillated water after 2 h in case of tris(2-chloroethyl) phosphite]. After completion of the reactions, the solutions were concentrated. The residues were triturated with cold ethanol. The formed solids were collected and crystallized from absolute ethanol to give white crystals of products **5** (0.98 g, 85 %) and **7** (1.18 g, 89 %), respectively.

*Diethyl {[(4-oxo-2-phenyl-quinazolin-3(4H)-yl)amino](phenyl)methyl}phosphonate (**5**)*

Mp 149–151 °C. IR (KBr) (ν_{max}, cm^{-1}): 3447 (NH), 1682 (C=O), 1608 (C=N), 1587, 1575 (C=C), 1281 (P=O), 1025 (P–O–C). ^{1}H-NMR (300 MHz, DMSO-d_6):

0.92–1.10 (m, 6H, OCH_2CH_3), 3.78–4.10 (m, 4H, OCH_2CH_3), 4.64 (d, 1H, $J = 21$ Hz, P–CH), 5.35 (brs, 1H, NH), 7.42–7.92 (m, 13H, Ar–H), 8.23 (d, 1H, Ar–H). ^{13}C-NMR (75 MHz, DMSO-d_6): 16.0 (CH_3), 49.1 (d, $J = 147.4$ Hz, P–CH), 59.7 (OCH_2), 121.0 (*C*-4a), 126.5 (*C*-8), 126.7 (*C*-2″,6′), 127.1 (*C*-2′,6′), 127.6 (*C*-3″,5″), 128.5 (*C*-3′,5′), 129.0 (*C*-1″), 129.7 (*C*-4″, 6), 131.0 (*C*-1′), 132.3 (*C*-5), 132.5 (*C*-4′), 134.6 (*C*-7), 146.3 (*C*-8a), 153.0 (*C*-2), 169.3 (*C*=O). ^{31}P-NMR (242 MHz, $CDCl_3$): 22.3 ppm. MS (EI, *m/z*): 463 (M^+, 11 %). Anal. Calcd for $C_{25}H_{26}N_3O_4P$ (463.48): C, 64.79; H, 5.65; N, 9.07 %. Found: C, 64.45; H, 5.33; N, 8.86 %.

*Bis(2-chloroethyl){[(4-oxo-2-phenyl-quinazolin-3(4H)-yl)amino](phenyl) methyl} phosphonate (**7**)*

Mp 143–144 °C. IR (KBr) (ν_{max}, cm^{-1}): 3446 (NH), 1682 (C=O), 1608 (C=N), 1587, 1575 (C=C), 1281 (P=O), 1025 (P–O–C). ^{1}H-NMR (300 MHz, DMSO-d_6): 2.11 (t, 2H, $ClCH_2$), 4.20 (t, 2H, OCH_2), 5.62 (d, 1H, $J = 19$ Hz, P–CH), 6.20 (br, 1H, NH), 7.39–7.92 (m, 13H, Ar–H), 8.24 (d, 1H, Ar–H). ^{13}C-NMR (75 MHz, DMSO-d_6): 35.9 ($ClCH_2$), 48.0 (d, $J = 150$ Hz, P–CH), 66.0 (OCH_2), 121.0 (*C*-4a), 126.3 (*C*-8), 126.7 (*C*-2″,6″), 127.1 (*C*-2′,6′), 127.6 (*C*-3″,5″), 128.5 (*C*-3′,5′), 129.0 (*C*-1″), 129.7 (*C*-4″, 6), 131.0 (*C*-1′), 132.3 (*C*-5), 132.6 (*C*-4′), 134.5 (*C*-7), 146.3 (*C*-8a), 153.0 (*C*-2), 169.3 (*C*=O). MS (EI, *m/z*): 534 (M + 2, 8 %), 532 (M^+, 18 %). Anal. Calcd for $C_{25}H_{24}Cl_2N_3O_4P$ (532.37): C, 56.40; H, 4.54; N, 7.89 %. Found: C, 56.02; H, 4.32; N, 7.58 %.

Synthesis of 3-(2-ethoxy-2-oxido-3-phenyl-1,4,2-oxazaphosphinan-4-yl)-2-phenyl-quinazolin-4(3H)-one (6)

A mixture of the diethyl α-aminophosphonate **5** (0.46 g, 1 mmol) and 2-bromoethanol (0.07 mL, 1 mmol) in ethanolic sodium ethoxide (0.1 g of sodium metal in 15 mL of absolute ethanol) was heated under reflux for 18 h. After cooling, the solution was poured on cold water and neutralized with dilute hydrochloric acid (5 %). The formed solid was collected and crystallized from ethyl acetate to give pale yellow crystals of product **6** in yield (0.26 g, 58 %); Mp 158–159 °C. IR (KBr) (ν_{max}, cm^{-1}): 1682 (C=O), 1594 (C=N), 1560, 1541 (C=C), 1214 (P=O), 1055 (P–O–C). ^{1}H-NMR (300 MHz, DMSO-d_6): 1.15 (t, 3H, OCH_2CH_3), 2.94 (t, 2H, NCH_2), 4.08 (t, 2H, OCH_2), 4.20–4.30 (m, 2H, OCH_2CH_3), 4.88 (d, 1H, $J = 22.5$ Hz, P–CH), 6.99–7.55 (m, 8H, Ar–H), 7.66–7.91 (m, 4H, Ar–H), 8.05–8.16 (m, 2H, Ar–H). ^{13}C-NMR (75 MHz, DMSO-d_6): 16.1 (CH_3), 47.1 (NCH_2), 53.0 (d, $J = 147.5$ Hz, P–CH), 59.1 (OCH_2), 71.6 (OCH_2), 121.0 (*C*-4a), 126.7 (*C*-8), 126.9 (*C*-2″,6″), 127.0 (*C*-2′,6′), 127.6 (*C*-3″,5″), 128.5 (*C*-3′,5′), 128.9 (*C*-1″), 129.7 (*C*-4″,6), 131.0 (*C*-1′), 132.3 (*C*-5), 132.5 (*C*-4′), 134.5 (*C*-7), 146.3 (*C*-8a), 153.0 (*C*-2), 169.4 (*C*=O). MS (EI, *m/z*): 462 (M + 1, 46 %), 461 (M^+, 69 %). Anal. Calcd for $C_{25}H_{24}N_3O_4P$ (461.46): C, 65.07; H, 5.24; N, 9.11 %. Found: C, 64.81; H, 4.92; N, 8.95 %.

Synthesis of 3-[2-(2-chloroethoxy)-2-oxido-3-phenyl-1,4,2-oxazaphosphinan-4-yl]-2-phenyl-quinazolin-4(3H)-one (8)

A solution of the *bis*(2-chloroethyl) α-aminophosphonate **7** (0.53 g, 1 mmol) in toluene (15 mL) containing a catalytic amount of triethylamine (0.1 mL, 1 mmol) was heated under reflux for 12 h. The precipitated salt was filtered off. The filtrate was concentrated. The residue was triturated with ethyl acetate. The formed solid was collected and crystallized from toluene to give yellow crystals of product **8** in yield (0.30 g, 61 %); mp 168–170 °C. IR (KBr) (ν_{max}, cm^{-1}): 1682 (C=O), 1596 (C=N), 1560, 1541 (C=C), 1214 (P=O), 1055 (P–O–C). ^{1}H-NMR (300 MHz, DMSO-d_6): 2.29 (t, 2H, $ClCH_2$), 2.95 (t, 2H, NCH_2), 3.95 (t, 2H, OCH_2), 4.10 (t, 2H, OCH_2), 4.84 (d, 1H, $J = 21$ Hz, P–CH), 7.42–7.92 (m, 13H, Ar–H), 8.23 (d, 1H, Ar–H). ^{13}C-NMR (75 MHz, DMSO-d_6): 35.9 ($ClCH_2$), 47.1 (NCH_2), 54.0 (d, $J = 150$ Hz, P–CH), 60.3 (OCH_2), 71.4 (OCH_2), 120.9 (*C*-4a), 125.8 (*C*-8), 126.9 (*C*-2″,6″), 127.5 (*C*-2′,6′), 127.7 (*C*-3″,5″), 128.0 (*C*-3′,5′), 128.5 (*C*-1″), 128.9 (*C*-4″, 6), 131.3 (*C*-1′), 132.7 (*C*-5), 134.5 (*C*-4′), 136.8 (*C*-7), 146.1 (*C*-8a), 152.3 (*C*-2), 161.8 (*C*=O). ^{31}P-NMR (242 MHz, $CDCl_3$): 18.9 ppm. MS (EI, *m/z*): 496 (M^+, 2 %). Anal. Calcd for $C_{25}H_{23}ClN_3O_4P$ (495.91): C, 60.55; H, 4.67; N, 8.47 %. Found: C, 60.21; H, 4.43; N, 8.21 %.

Antimicrobial evaluation

All the newly synthesized compounds were evaluated in vitro for their antibacterial activities against *S. aureus* (ATCC 25923) and *B. subtilis* (ATCC 6635) as representatives of Gram-positive bacteria, and *E. coli* (ATCC 25922) and *S. typhimurium* (ATCC 14028) as examples of Gram-negative bacteria. They were also examined against *Candida albicans* (ATCC 10231) and *A. fumigatus* as fungi. An agar-diffusion technique was used for the determination of the preliminary antibacterial and antifungal activities [36, 37]. The test was performed on a medium of potato dextrose agar (PDA) containing and infusion of 200 g of potatoes, 6 g of dextrose and 15 g of agar. Uniformly sized filter paper disks (3 disks per compound) were impregnated with equal volumes (10 μL) from the concentrations of 500 and 1000 μg mL^{-1} dissolved compounds in dimethylformamide (DMF) and carefully placed on an inoculated agar surface. After incubation for 36 h at 27 °C in the case of bacteria and for 48 h at 24 °C in the case of fungi, the antimicrobial activities were determined by measuring the inhibition zones. Cephalothin, chloramphenicol and cycloheximide were used as reference drugs (30 μg mL^{-1}) for Gram-positive bacteria, Gram-negative bacteria and fungi, respectively. The MIC (μg mL^{-1}) for some selected compounds against some species of microbes was also determined. The tube dilution technique was applied for the determination of MIC of the tested compounds against microbes [38]. A dilution series was set up with 250, 125, 62.5…3.25 μg mL^{-1} of nutrient broth medium to each tube, and 100 mL of standardized suspension of the test microbes (107 cell mL^{-1}) were added and incubated at 37 °C for 24 h.

Antioxidant activity

DPPH radical scavenging activity

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging effect was carried out according to the reported method [39, 40]. Compounds of different concentrations were prepared in distilled ethanol; 1 mL of each compound solution (50, 100, 200, 300 and 400 μmol L^{-1}) was taken in different test tubes, and 4 mL of 100 μmol L^{-1} ethanol solution of DPPH was added and shaken vigorously. The tubes were then incubated in the dark at RT for 20 min. A DPPH blank was prepared without a compound, and ethanol was used for the baseline correction. Changes (decrease) in the absorbance at 517 nm were measured using an ultaviolet–visible spectrophotometer. The radical scavenging activities were expressed as the inhibition percentage and were calculated using the formula:

$$\%\text{Radical scavenging activity} = (\text{AB} - \text{AA})/\text{AB} \times 100$$

where AB = absorption of blank and AA = absorption of the tested compound. The radical scavenging activity of TBHQ was also measured and compared with that of the different synthesized compounds. The compound concentration providing 50 % inhibition (IC_{50}) was calculated from the graph of percentage against compound concentrations.

Antioxidant activity by β-carotene-linoleic acid assay

Each compound at the final concentrations of 50–400 μmol L^{-1} was incorporated into a β-carotene-linoleic acid model system independently and the activity was monitored spectrophotometrically at 470 nm [41]. The substrate suspension was prepared by addition of β-carotene (4 mg dissolved in 5 mL chloroform) into a covered round-bottomed flask containing Tween-40 (600 mg) followed by the addition of linoleic acid (60 mL). The chloroform was removed completely under vacuum using a rotary evaporator at 40 °C. The resulting solution was diluted with triple-distilled water (30 mL) and the emulsion was mixed well and diluted with oxygenated water (120 mL). The aliquot (4 mL) was transferred to different stopper test tubes containing compounds in distilled ethanol. A control was prepared with distilled ethanol (1 mL) and an emulsion (4 mL). A TBHQ solution as the internal standard of the same concentration was also analyzed for comparison. Zero adjustment was done using distilled water. As soon as the emulsion was added to each test tube, the zero time ($t = 0$) absorbance was measured at 470 nm using a spectrophotometer and absorbance was measured subsequently for every 30 min, up to 3 h ($t = 180$). The tubes were placed in a water bath at 50 °C between the readings. Percentage antioxidant activities of each compound were evaluated in triplicate in terms of photooxidation of β-carotene using the following formula:

$$\%\text{Antioxidant activity} = 100 \times \{1 - (A_0 - A_t/A_{00} - A_{t0})\}$$

where A_0 = initial absorbance of the sample ($t = 0$ min), A_t = absorbance of the sample after time t ($t = 180$ min), A_{00} = initial absorbance of the control ($t = 0$ min), and A_{t0} = absorbance of control after time t ($t = 180$ min).

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