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ORIGINAL PAPER

One-pot synthesis, characterization, and antioxidant capacity of sulfur- and oxygen-substituted 1,4-naphthoquinones and a structural study

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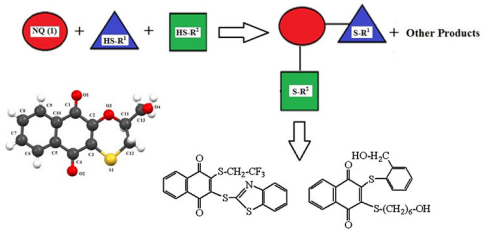
Abstract In the present study, we reported the one-pot synthesis of *S*,*S*- and *S*,*O*-substituted 1,4-naphthoquinones, their structural studies, and investigation of their antioxidant activity. The multicomponent reactions of 2,3-dichloro-1,4-naphthoquinone with sulfur- and oxygen-containing nucleophiles were investigated to obtain highly functionalized *S*,*S*- and *S*,*O*-substituted 1,4-naphthoquinone derivatives. All new compounds were characterized on the basis of ^{1}H, ^{19}F, and ^{13}C nuclear magnetic resonance spectroscopy, mass spectrometry, and Fourier transform infrared spectroscopy. Crystal structure of 2,3-dihydro-2-(hydroxymethyl)naphtho[2,3-*b*]-1,4-oxathiin-5,10-dione was determined by X-ray diffraction method. The synthesized compounds were screened for their antioxidant capacity and free radical scavenging activity using the cupric reducing antioxidant capacity method and DPPH method, respectively. 3-Chloro-2-[3-(3-chloro-1,4-dihydro-1,4-dioxonaphthalen-2-yloxy)propylsulfanyl]-1,4-naphthoquinone shows the highest antioxidant capacity with 0.63 cupric reducing antioxidant capacity—trolox equivalent antioxidant capacity coefficient.

Graphical abstract



Keywords 1,4-Naphthoquinone · Spectroscopy · Crystal structure · One-pot synthesis · Cupric reducing antioxidant capacity method · DPPH method

Introduction

Quinones are well known in biological systems as reactive centers of transporting both electrons and protons across biological membranes. The evaluation of the redox chemistry and electrochemical properties of quinones isa useful way for identifying their biological evolutions. The constitution of substituted naphthoquinone compounds is a result of a Michael-type addition to 2,3-dichloro-1,4-naphthoquinone (**1**) followed by chloride elimination to afford a quinonyl intermediate that then reacts with the related nucleophile to yield the final products [1–3]. Heterocyclic compounds bearing 1,4-naphthoquinones have long been the focus of synthetic chemistry due to their broad spectrum of applications in biological, pharmaceutical, and material science areas [4]. In the present study, we used the technique of one-pot multicomponent reaction to obtain the compounds containing substantial elements of

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all the reactants such as **6** and **12**. Multicomponent reactions (MCRs) are one-pot processes in which three or more reactants come together in a single reaction vessel to give a final product containing substantial elements of all the reactants [5–8], and in recent years much attention has been directed toward the one-pot multicomponent reactions because of their wide range of applications in pharmaceutical chemistry for the production of structural scaffolds and combinatorial libraries for drug discovery [9–12].

Antioxidant activity (AOA) is a very important parameter used to study the free radical scavenging activity of different compounds, because this activity is related with compounds capable of protecting a biological system against the potential harmful effects of oxidative processes. Antioxidants have received increased attention in recent years from medical researchers and nutritionists for their potential activities in the prevention of several degenerative diseases such as cancer, cardiovascular disorder as well as aging [13–16], and several works have been published on structure–activity analysis of compounds with antioxidant activities. Bezabih et al. [17] explained that quinones, such as isoflurano naphthoquinone, have antioxidant activity. Lebedev et al. [18] reported that a hydroxylated naphthoquinone, echinochrome A, showed potent antioxidant activity.

We describe here the synthesis of some *S*,*S*- and *S*,*O*-substituted 1,4-naphthoquinone compounds. Their structures were characterized by microanalysis, FT-IR, ^{1}H NMR, ^{19}F NMR, ^{13}C NMR, MS, and UV–Vis spectroscopy. The single crystal structure of compound **8** was determined by X-ray diffraction method. All synthesized compounds were screened for their antioxidant capacity and free radical scavenging activity using the cupric reducing antioxidant capacity (CUPRAC) and DPPH methods, respectively.

Results and discussion

One-pot synthesis and characterization of *S*,*S*- and *S*,*O*-substituted 1,4-naphthoquinones

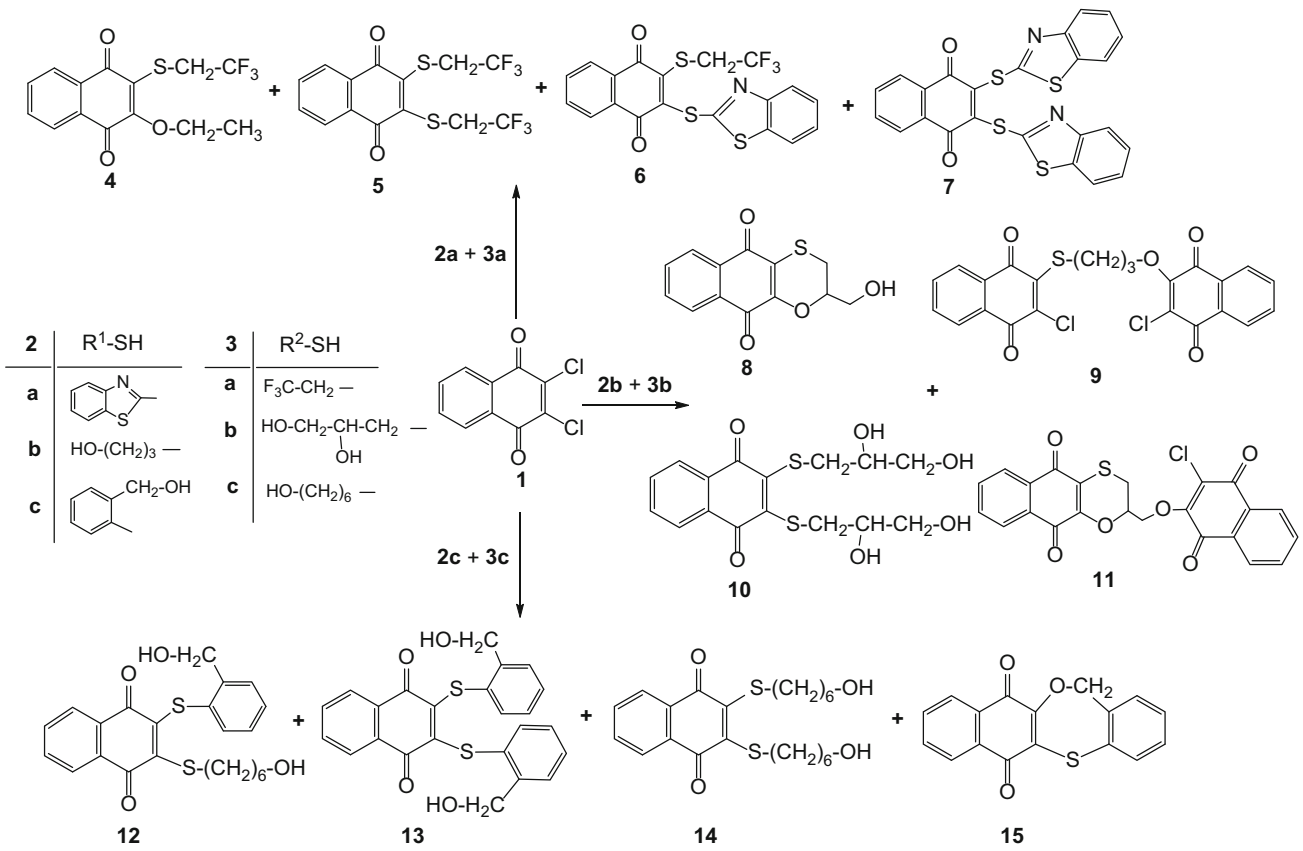
Multicomponent reactions are one-pot processes in which three reactants (**1**, R^1–SH, R^2–SH) come together in a single reaction vessel to give a final product containing substantial elements of all the reactants. In this study, first, the multicomponent reactions of 2,3-dichloro-1,4-naphthoquinone with various thiol nucleophiles were investigated. As shown in Scheme 1, when **1** reacted in a single reaction vessel with an equimolar amount of various thiols (**2a–2c**, **3a–3c**) in ethanol in the presence of sodium carbonate solution at room temperature, the corresponding products **4–7** and **9–13** were obtained in excellent yields, with appropriate reaction times and high purity. The synthesis, spectroscopic data (^{1}H, ^{13}C NMR, MS, UV, FT-IR), elemental analysis, and melting points of compounds **8** [19], **14** [20], and **15** [21] were reported in earlier studies. The antioxidant activity of all known (**8**, **14**, **15**) and unknown (**4–7**, **9–13**) compounds were investigated using the CUPRAC and DPPH methods, respectively. In this study, the crystal structure was solved as first for compound **8** by X-ray method.

The reaction of **1** with **2a** and **3a** in ethanol in the presence of sodium carbonate gave unknown compounds **4**, **5**, **6**, and **7**. The ^{1}H spectrum of the products in $CDCl_3$ displayed distinct signals with appropriate multiplets. ^{1}H NMR signal of the hydrogen atoms of the methylene group (S–CH_2–CF_3) adjacent to the sulfur atom in compounds **4**, **5**, **6** was shifted to a higher field and displayed singlets at 3.7 and 3.8 ppm. The ^{13}C NMR spectra of compound **6** gave two carbonyl signals at 166.00 and 177.05 ppm (C=O), while compound **7** showed one carbonyl signal at 177.59 ppm (C=O) in naphthoquinone unit. ^{19}F NMR spectrum of **4**, **5**, and **6**, the fluorine signals between $\delta = -69.18$ and -68.70 ppm were assigned to the S–CH_2–CF_3. In continuation of this research, investigation on the preparation of 2-(benzothiazol-2-ylsulfanyl)-3-(2,2,2-trifluoroethylsulfanyl)-1,4-naphthoquinone (**6**) and 2-(6-hydroxyhexylsulfanyl)-3-[2-(hydroxymethyl)phenylsulfanyl]-1,4-naphthoquinone (**12**) using a one-pot three-component Michael addition reaction of 2,3-dichloro-1,4-naphthoquinone with **2a** and **3a** or **2c** and **3c** were surveyed, respectively (Scheme 1).

Compounds **8**, **9**, **10**, and **11** were obtained from the reaction of **1** with **2b** and **3b** in ethanol in the presence of sodium carbonate. The first compound **8** was obtained by an interesting ring closure and is a *S*,*O*-substituted 1,4-naphthoquinone compound. It was shown that interesting heterocylic compounds **9**, **10**, and **11** could be obtained from this reaction. Isolation and identification proved that an intermoleculer binding to yield *S*,*O*-substituted diquinone **9** and a intramolecular cyclization reaction had taken place, yielding the compound **11**. The characteristic –OH band disappeared in the FT-IR spectrum of compounds **9** and **11** because of the ring closure. The FT-IR spectra of compound **10** showed broad bands at 3400 cm^{-1} for the –OH stretching. With the aid of the positive-ion mode of electron spray ionization (ESI) mass spectrum of the compounds **9**, **10**, and **11**, the respective molecular ion peaks were observed at $m/z = 473$ $[M]^+$, 371 $[M]^+$, and 478 $[M+Na+2H]^+$, respectively.

The reaction of 2,3-dichloro-1,4-naphthoquinone (**1**) with **2c** and **3c** in ethanol in the presence of sodium carbonate gave *S*,*S*-substituted 1,4-naphthoquinones **12**, **13**, **14** and *S*,*O*-substituted-1,4-naphthoquinone **15**. FT-IR spectrum in KBr showed the following important absorption

Scheme 1



bands. In the FT-IR spectra of compounds **12** and **13**, two typical strong quinonic carbonyl absorptions were observed at 1662 and 1661 cm^{-1}, respectively. The FT-IR spectra of compounds **12** and **13** showed broad bands at 3368 and 3415 cm^{-1} for the –OH streching. The fragmentation of molecular peak of compound **12** at $m/z = 428$ in the positive-ion mode for ESI gave a fragment ion at $m/z = 327$ corresponding to the cleavage of a hydroxylalkyl group (–R–OH). The mass spectra of compound **13** in the negative ion mode for electron spray ionization (ESI) technique confirmed the proposed structure; molecular peak was identified at $m/z = 433[M]^-$.

Antioxidant capacity of synthesized compounds

The synthesized compounds **4**–**15** were screened for their antioxidant capacity using the CUPRAC methods [22] against trolox as the standard reference compound at room temperature. The linear calibration equations of these compounds (as absorbance in a 1 cm cell vs molar concentration) gave the molar absorption coefficient ε as the slope. The CUPRAC molar absorption coefficient of the tested antioxidant divided by that of trolox under the same conditions gave the trolox equivalent antioxidant capacity (TEAC), or TEAC coefficient of that compound tested for antioxidant power (Table 1). Among the synthesized compounds, **9** showed the highest antioxidant capacity, and CUPRAC-TEAC coefficients (in parentheses) decreased in the following order: **9** (0.63) > **6** (0.58) > **10** (0.33) ≥ **13** (0.33) > **12** (0.22) > **4** (0.15) > **5** (0.13) > **14** (0.05) ≥ **15** (0.05) > **8** (0.02). The reduced form of the menadione (2-methyl-1,4-naphthoquinone) moiety, either the hydroquinone or semiquinone, was proposed to be the active antioxidant that trapped hydroperoxy radicals, alkoxy radicals, or other free radicals involved in propagating lipid peroxidation [23]. Keto–enol tautomerism is possible for naphthoquinones, even for pure quinonic compounds like menadione (2-methyl-1,4-naphthoquinone) [24]. From the two tautomers, enol form is known to facilitate electron transfer in preference to the keto form [25]. Menadione may not be so active in electron transfer-based antioxidant capacity tests, but it has been proven to be a remarkably potent inhibitor of microsomal lipid peroxidation, effective at submicromolar concentrations [23]. Therefore,

Table 1 Calibration equations of synthesized compounds, linear ranges, and TEAC coefficients

Compounds	Linear range/mol dm^{-3}	Calibration equation	r	TEAC[a]
4	4.1×10^{-5}–20.4×10^{-5}	$y = 2371\ c + 0.0008$	0.9993	0.15 ± 0.008
5	6.9×10^{-5}–34.6×10^{-5}	$y = 1932\ c + 0.0774$	0.9994	0.13 ± 0.003
6	6.7×10^{-5}–26.7×10^{-5}	$y = 8944\ c + 0.2255$	0.9991	0.58 ± 0.009
7	N.D.	N.D.	N.D.	N.D.
8	7.4×10^{-5}–18.6×10^{-5}	$y = 363\ c + 0.0144$	0.9999	0.02 ± 0.001
9	2.1×10^{-5}–8.2×10^{-5}	$y = 9763\ c + 0.0703$	0.9993	0.63 ± 0.041
10	3.3×10^{-5}–8.3×10^{-5}	$y = 5107\ c + 0.0299$	0.9994	0.33 ± 0.002
11	N.D.	N.D.	N.D.	N.D.
12	5.6×10^{-5}–28.1×10^{-5}	$y = 3415\ c + 0.1443$	0.9993	0.22 ± 0.003
13	3.3×10^{-5}–13.5×10^{-5}	$y = 5125\ c + 0.0066$	0.9991	0.33 ± 0.004
14	5.8×10^{-5}–23.1×10^{-5}	$y = 705\ c + 0.0619$	0.9994	0.05 ± 0.0003
15	4.1×10^{-5}–16.6×10^{-5}	$y = 729\ c + 0.0153$	0.9993	0.05 ± 0.0002

N.D. not defined

[a] $TEAC_{AOX} = \varepsilon_{AOX}/\varepsilon_{TR}$ (*AOX* antioxidant, *TR* trolox); $\varepsilon_{TR} = 1.54 \times 10^4$ dm^3 mol^{-1} cm^{-1} (acetone)

estimating a compound's antioxidant behavior merely from its structural formula may not be valid under all circumstances. Compound **9** showed the highest antioxidant power, possibly due to its dimeric structure, similar to the observation that polymeric polyphenols had higher antioxidant activity than their monomeric analogs. Among the substituted quinonic compounds, we synthesized compounds **9** > **6** > **10** > **13** > **12**, especially the *S*,*S*-substituted naphthoquinones exhibited the highest antioxidant activity through the polymethylene side chains possibly contributing to enol formation. Besides, cyclic Ar–S–CH_2–R compounds may also exhibit antioxidant activity, though to a significantly less extent than Ar–SH and R–SH thiols [26].

Free radical scavenging activity of synthesized compounds

One of the most common methods to evaluate free radical scavenging activity of specific compounds is the DPPH test which relies on the reduction of DPPH solution in the presence of hydrogen-donating compounds [27] (Table 2). Thus, the free radical scavenging activities of newly synthesized compounds decreased in the order of **9** > **6** > **10** > **13** > **12**, showing a parallelism with that of CUPRAC-TEAC. It can be seen that the leading DPPH· scavenging compounds like **9** and **6** are also the ones with high antioxidant activity, as measured in this work.

Table 2 Free radical scavenging activities (IC_{50}) of synthesized compounds with respect to the DPPH method

Compounds	IC_{50}/mM
4	0.181 ± 0.003
5	0.123 ± 0.002
6	0.024 ± 0.0003
7	N.D.
8	N.D.
9	0.023 ± 0.0002
10	0.032 ± 0.001
11	N.D.
12	0.033 ± 0.0002
13	0.041 ± 0.001
14	N.D.
15	N.D.

Data presented as mean (SD), $N = 3$

X-ray study

The compound **8** was crystallized in the monoclinic crystal system (space group P21/c) with the unit cell parameters $a = 14.9936(5)$ Å, $b = 5.18560(10)$ Å, $c = 15.3024(5)$ Å, $\beta = 109.999(2)°$, $V = 1118.03(6)$ $Å^3$, $Z = 4$. The crystal structure was solved by direct methods (SIR92) and refined to the residual index $R_1 = 0.021$. Drawings were prepared with the program ORTEP-III [28] with 50 % probability displacement ellipsoid for compound **8** in Fig. 1. Crystal data and refinement parameters for compound **8** are summarized in Table 3. The selected bond distances, bond and torsion angles for compound **8** are listed in Table 4.

The title compound, $C_{13}H_{10}O_4S$, possesses three distinct units: a cyclo ring containing sulfur and oxygen atoms attached to the naphthoquinone group, a hydroxymethyl group, and a naphthoquinone ring. These units except hydroxymethyl group lie in the same molecular plane, as can be clearly seen in the ORTEP-III diagram in Fig. 1.

Both rings of the naphthoquinone unit were planar with a maximum deviations of 0.0102(1) Å (plane 1 = C1–C2–C3–C4–C5–C10) and 0.0056(1) Å (plane 2 = C10–C5–C6–C7–C8–C9). The cyclo ring containing sulfur and

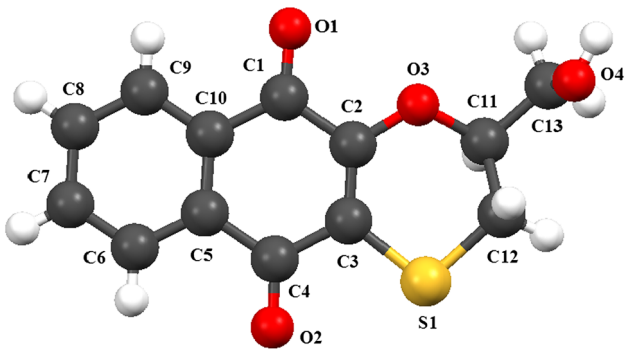


Fig. 1 Molecular structure of compound **8**. Displacement ellipsoids are plotted at the 50 % probability level

Table 3 The main crystallographic parameters of compound **8**

Empirical formula	$C_{13}H_{10}O_4S$
Crystal color, habit	Red, platelet
Crystal size/mm	0.50 × 0.30 × 0.20
Wavelength/Å	0.71073
Crystal system	Monoclinic
Space group	P21/*a*
Cell dimensions	$a = 14.9936(5)$ Å
	$b = 5.18560(10)$ Å
	$c = 15.3024(5)$ Å
	$\beta = 109.999(2)°$
Cell volume/Å^3	1118.03(6)
Cell formula units (Z)	4
Density/g cm^{-3}	1.558
μ/cm^{-1}	0.293
F_{000}	544.0
h, k, l ranges	−21/21, −6/7, −21/21
Reflections collected	33,522
Independent reflections	3274 [$R_{int} = 0.047$]
Data/restraints/parameters	3115/0/163
Goodness of fit indicator	1.209
Final R indices [$I > 2\sigma(I)$]	$R = 0.072$, $wR = 0.021$
Largest diff. peak and hole/e Å^{-3}	0.032 and −0.036

oxygen atoms which attached to the naphthoquinone group were not planar with a maximum deviation of 0.1506(1) Å (plane 3 = C11–C12–S1–C3–C2–O3). Dihedral angles were 2.95(1)°, 7.92(1)°, and 10.14(1)° between planes 1–2, 1–3, and 2–3, respectively. The bond lengths of C1–O1 and C4–O2 were 1.228(1) Å and 1.219(2) Å, typical of C=O bonds. In the compound **8**, C–C–C and C–C–O angles were very close to 120°, as expected for sp^2 hybridized atoms. The double bond distance of C2–C3 was 1.357(2) Å in **8**, which was smaller than expected due to substituents such as (=O). The double bond length of the quinone moiety agreed well with corresponding distance in similar compounds [4, 29].

Table 4 Selected bond distances, bond and torsion angles for compound **8**

Bond distances/Å			
C1–C2	1.477(1)	C4–C5	1.488(1)
C2–C3	1.357(2)	C5–C6	1.386(1)
C3–C4	1.471(1)	C6–C7	1.381(2)
O1–C1	1.228(1)	O2–C4	1.219(2)
S1–C3	1.742(1)	C13–N2	1.506(2)
Bond angles/°			
C1–C2–C3	121.66(9)	C2–C3–C4	121.11(9)
C3–S1–C12	99.48(5)	C2–O3–C11	115.58(8)
C2–C1–O1	120.36(9)	C5–C4–O2	121.61(1)
C3–C4–C5	118.50(1)	C2–C1–C10	118.30(1)
O4–C13–C11	110.00(9)	C12–C11–O3	110.85(7)
Torsion angles/°			
C1–C2–C3–C4	0.4(1)	O1–C1–C2–O3	178.9(1)
C5–C4–C3–S1	−177.6(8)	O3–C2–C3–S1	−0.1(1)
S1–C3–C4–O2	1.0(1)	C12–S1–C3–C2	3.03(9)

The crystal structure of compound **8** showed intermolecular O–H···O interactions between hydrogen atoms of O4 and one of the oxygen atoms (O1 and O3) of two 1,4-naphthoquinone rings in Fig. 2. The hydrogen bond distances and angles are given in Table 5. Another intermolecular bond between S1–O1 had the following parameters; S1–O1^{i,ii}: 3.30(1) Å, (i) $1 - x$, $1 - y$, $1 - z$, (ii) $1 - x$, $\frac{1}{2} + y$, $1.5 - z$ (Fig. 2). The packing diagram of compound **8** is illustrated in Fig. 3.

Conclusion

The aim of this study was to the investigation of the reaction of 2,3-dichloro-1,4-naphthoquinone with sulfur- and oxygen-containing nucleophiles and obtain to highly functionalized *S*,*S*- and *S*,*O*-substituted 1,4-naphthoquinone from the multicomponent reactions. 2-(Benzothiazol-2-ylsulfanyl)-3-(2,2,2-trifluoroethylsulfanyl)-1,4-naphthoquinone (**6**) and 2-(6-hydroxyhexylsulfanyl)-3-[2-(hydroxymethyl) phenylsulfanyl]-1,4-naphthoquinone (**12**) were synthesized by the one-pot three-component michael addition reactions of 2,3-dichloro-1,4-naphthoquinone with R^1–SH and R^2–SH in a single reaction vessel. Their structures of new synthesized compounds were determined by microanalysis, FT-IR, ^{1}H NMR, ^{19}F NMR, ^{13}C NMR, MS, and UV–Vis. The crystal structure of 2,3-dihydro-2-(hydroxymethyl)-naphtho[2,3-b]-1,4-oxathiin-5,10-dione (**8**) was determined by X-ray diffraction method. The synthesized compounds were screened for their antioxidant capacity using the CUPRAC methods. 3-Chloro-2-[3-(3-chloro-1,4-dihydro-1,4-dioxonaphthalen-2-yloxy)propylsulfanyl]-1,4-naphthoquinone (**9**) shows the good antioxidant capacity with 0.63

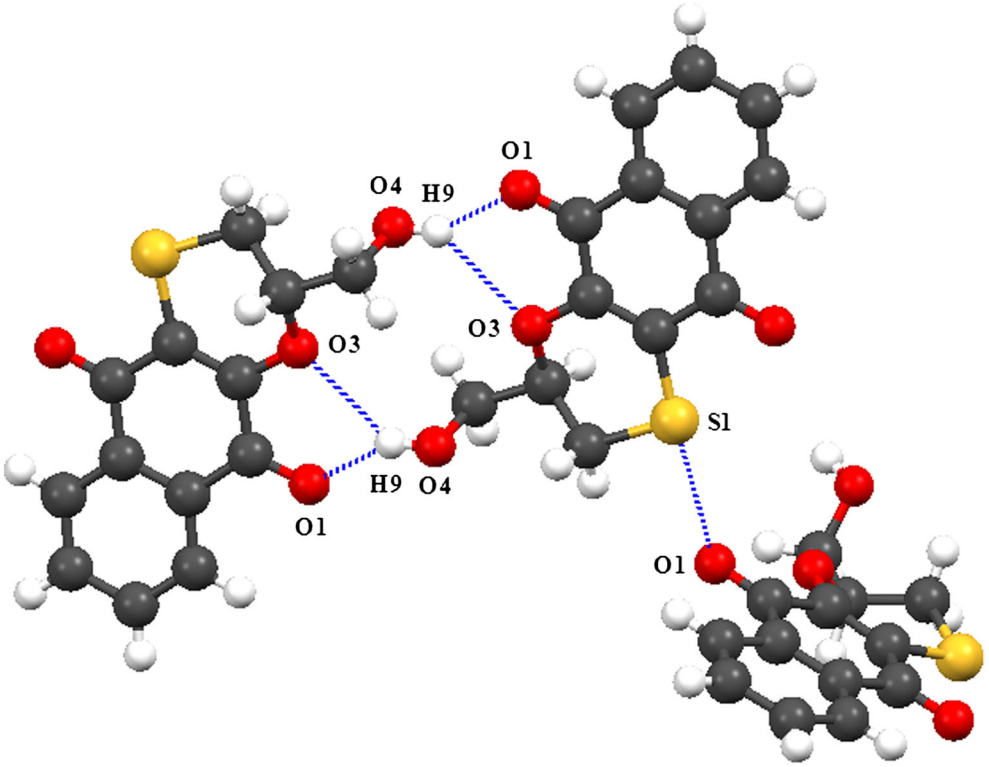


Fig. 2 Hydrogen and intermolecular bonds in compound **8**

Table 5 The hydrogen bond parameters for compound **8**

D–H···A	D–H/Å	H···A/Å	D···A/Å	<D–H···A/°
O4–H9···O1	0.85(2)	2.07(1)[a,b]	2.92(1)	146.40(1)
O4–H9···O3	0.85(2)	2.46(2)[c,d]	3.31(1)	139.91(2)

[a] $+x, +y, +z$

[b] $1 - x, 1 - y, 1 - z$

[c] $+x, +y, +z$

[d] $1 - x, 1 - y, 1 - z$

CUPRAC-TEAC coefficient. This compound was also shown to exhibit the higher inhibition activity ($IC_{50} = 0.023 \pm 0.0002$ mM) with DPPHC method.

Experimental

Microanalyses were performed on a Thermo Finnigan Flash EA 1112 Elemental analyser. Infrared (FT-IR) spectra were recorded in KBr pellets in Nujol mulls on a Perkin Elmer Precisely Spectrum One FTIR spectrometry. ^{1}H, ^{19}F, and ^{13}C NMR spectra were recorded on VarianUNITYINOVA operating at 500 MHz. Chemical shifts (δ/ppm) were reported relative to tetramethylsilane (TMS) with the solvent resonance employed as the internal standard. ^{1}H and ^{13}C NMR spectra refer to the solvent signal center at $\delta = 7.26$ and 77.00 in $CDCl_3$, at $\delta = 3.31$, 4.78, and 49.20 in CD_3OD, at $\delta = 2.50$ and 39.56 ppm in DMSO-d_6, respectively. Mass spectra were obtained on a Thermo Finnigan LCQ Advantage MAX LC/MS/MS spectrometer using an ESI probe. Products were isolated by column chromatography on silica gel (Fluka silica gel 60, particle size 63-200 μm). Melting points were measured on a Büchi B-540 melting point apparatus. Analytical thin-layer chromatography (TLC) was purchased from Merck KGaA (silica gel 60 F254) based on Merck DC plates (aluminum based). Visualization of the chromatogram was performed by UV light (254 nm). Moisture was excluded from the glass apparatus using $CaCl_2$ drying tubes. Solvents, unless otherwise specified, were of reagent grade and distilled once prior to use, and all other chemicals (reagent grade) were used without further purification.

The following chemicals were supplied from the corresponding sources: copper(II) chloride ($CuCl_2$) and ammonium acetate (NH_4Ac) from Merck Chemicals (Darmstadt, Germany); neocuproine (Nc), DPPH (2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl) reagent from Sigma-Aldrich Chemicals (Steinheim, Germany). The absorption measurements for total antioxidant capacity were recorded in matched quartz cuvettes using a Perkin Elmer Lambda 35 UV–Vis spectrophotometer having a spectral resolution of ≈ 1 nm.

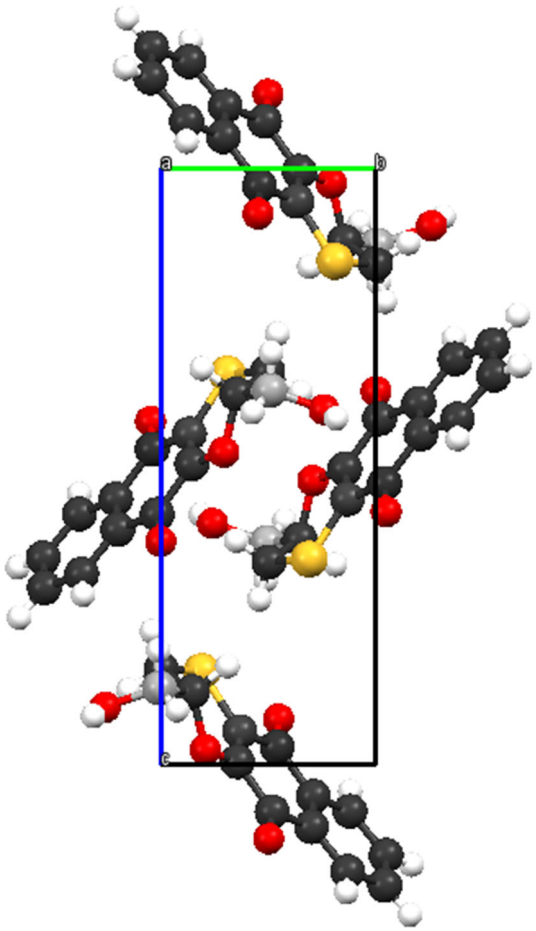


Fig. 3 The packing diagram of compound **8** (100)

*General procedure for the synthesis of S,S- and S,O-substituted naphthoquinone compounds **4–7** and **9–13***

Sodium carbonate was dissolved in 20 cm^3 ethanol, and equimolar amounts of 2,3-dichloro-1,4-naphthoquinone (**1**) and thiols were added slowly. Without heating, the mixture was stirred in a single reaction vessel for 24 h. The color of the solution quickly changed (from yellow to red color), and the extent of the reaction was monitored by TLC. Chloroform (30 cm^3) was added to the reaction mixture. The organic layer was separated, washed with water (4 × 30 cm^3), and dried with Na_2SO_4. After the solvent was evaporated, the residue was purified by column chromatography on silica gel.

2-Ethoxy-3-(2,2,2-trifluoroethylsulfanyl)-1,4-naphthoquinone (**4**, $C_{14}H_{11}F_3O_3S$)

Compound **4** was synthesized from reaction of 1.0 g **1** (4.40 mmol) with 0.73 g **2a** (4.40 mmol) and 0.37 g **3a** (4.40 mmol) according to the general procedure. Yield: 0.45 g (32 %); red oil; R_f = 0.40 ($CHCl_3$:EtAc 1:1); FT-IR (KBr): $\overline{\nu}$ = 3347 (CH_{arom}), 2961, 2915, 2817 (C–H), 1676 (C=O), 1561 (C=C) cm^{-1}; UV–Vis ($CHCl_3$): λ_{max} (log ε) = 233 (4.3), 259 (4.5), 340 (2.7), 450 (3.1) nm; ^{1}H NMR (499.74 MHz, $CDCl_3$): δ = 1.3 (t, J = 6.84 Hz, 3H, –CH_3), 3.75 (s, 2H, –S–CH_2), 4.5 (q, 2H, –O–CH_2–CH_3), 7.60–8.00 (m, 4H, H_{arom}) ppm; ^{13}C NMR (125.66 MHz, $CDCl_3$): δ = 14.92 (–CH_3), 32.68 (S–CH_2), 69.80 (–O–CH_2), 125.63, 125.73, 130.23, 131.25, 132.73, 133.06 (CH_{arom}, C_{arom}), 125.85 (CF_3), 134.30 (=C–S), 158.54 (=C–O), 178.01, 181.06 (C=O) ppm; ^{19}F NMR (470.22 MHz, $CDCl_3$): δ = −69.18, −69.16, −69.14 ppm; MS (ESI+): m/z = 317 ($[M+H]^+$).

2,3-Bis(2,2,2-trifluoroethylsulfanyl)-1,4-naphthoquinone (**5**, $C_{14}H_8F_6O_2S_2$)

Compound **5** was synthesized from reaction of 1.0 g **1** (4.40 mmol) with 0.73 g **2a** (4.40 mmol) and 0.37 g **3a** (4.40 mmol) according to the general procedure. Yield: 0.25 g (18 %); red oil; R_f = 0.30 ($CHCl_3$); FT-IR (KBr): $\overline{\nu}$ = 3117 (CH_{arom}), 2985 (C–H), 1667 (C=O), 1592 (C=C) cm^{-1}; UV–Vis ($CHCl_3$): λ_{max} (log ε) = 228 (3.9), 262 (4.5), 320 (2.5), 460 (3.2) nm; ^{1}H NMR (499.74 MHz, $CDCl_3$): δ = 3.8 (s, 4H, –S–CH_2), 7.20–8.00 (m, 4H, H_{arom}) ppm; ^{13}C NMR (125.66 MHz, $CDCl_3$): δ = 38.23 (S–CH_2), 126.39, 128.17, 132.40 (CH_{arom}, C_{arom}), 125.76 (CF_3), 146.69 (=C–S), 178.09 (C=O) ppm; ^{19}F NMR (470.22 MHz, $CDCl_3$): δ = −69.14, −69.11, −69.09, −69.01, −68.81, −68.75 ppm; MS (ESI−): m/z = 385 ($[M-H]^-$), 316 ($[M–(CF_3)]^-$).

2-(Benzothiazol-2-ylsulfanyl)-3-(2,2,2-trifluoroethylsulfanyl)-1,4-naphthoquinone (**6**, $C_{19}H_{10}F_3NO_2S_3$)

Compound **6** was synthesized from reaction of 1.0 g **1** (4.40 mmol) with 0.73 g **2a** (4.40 mmol) and 0.37 g **3a** (4.40 mmol) according to the general procedure. Yield: 0.30 g (22 %); red solid; m.p.: 165–166 °C; R_f = 0.25 ($CHCl_3$); FT-IR (KBr): $\overline{\nu}$ = 3063 (CH_{arom}), 2963 (C–H), 1664 (C=O), 1589 (C=C) cm^{-1}; UV–Vis ($CHCl_3$): λ_{max} (log ε) = 210 (3.4), 260 (3.1), 330 (4.6), 450 (2.1) nm; ^{1}H NMR (499.74 MHz, $CDCl_3$, CD_3OD-d_4): δ = 3.8 (s, 2H, –S–CH_2), 7.20–8.00 (m, 8H, H_{arom}) ppm; ^{13}C NMR (125.66 MHz, $CDCl_3$, CD_3OD-d_4): δ = 34.12 (S–CH_2), 120.18, 120.22, 120.38, 121.64, 123.70, 124.41, 124.45, 125.54, 125.71, 126.56, 126.63, 135.123 (CH_{arom}, C_{arom}), 125.29 (CF_3), 133.27, 133.58 (=C–S), 153.43 (>C=N), 166.00, 177.05 (C=O) ppm; ^{19}F NMR (470.22 MHz, $CDCl_3$): δ = −69.11, −69.01, −68.75 ppm; MS (ESI−): m/z = 437 ($[M]^-$).

2,3-Bis(benzothiazol-2-ylsulfanyl)-1,4-naphthoquinone (**7**, $C_{24}H_{12}N_2O_2S_4$)

Compound **7** was synthesized from reaction of 1.0 g **1** (4.40 mmol) with 0.73 g **2a** (4.40 mmol) and 0.37 g **3a** (4.40 mmol) according to the general procedure. Yield: 0.35 g (25 %); red solid; m.p: 161–162 °C; R_f = 0.20

($CHCl_3$); FT-IR (KBr): $\overline{\nu}$ = 3065 (CH_{arom}), 1667 (C=O), 1586 (C=C) cm^{-1}; UV–Vis ($CHCl_3$): λ_{max} (log ε) = 238 (4.1), 280 (3.5), 390 (2.5), 475 (2.9) nm; ^{1}H NMR (499.74 MHz, DMSO-d_6): δ = 7.20–8.00 (m, 12H, H_{arom}) ppm; ^{13}C NMR (125.66 MHz, DMSO-d_6): δ = 120.93, 122.91, 125.43, 125.78, 126.02, 131.21, 134.05, 134.77, 135.58 (CH_{arom}, C_{arom}), 133.98 (=C–S), 153.23 (>C=N), 177.59 (C=O) ppm; MS (ESI +): *m/z* = 488 ($[M]^+$).

3-Chloro-2-[3-(3-chloro-1,4-dihydro-1,4-dioxonaphthalen-2-yloxy)propylsulfanyl]-1,4-naphthoquinone (**9**, $C_{23}H_{14}Cl_2O_5S$)

Compound **9** was synthesized from reaction of 1.0 g **1** (4.40 mmol) with 0.40 g **2b** (4.40 mmol) and 0.47 g **3b** (4.40 mmol) according to the general procedure. Yield: 0.25 g (21 %); red oil; R_f = 0.30 ($CHCl_3$); FT-IR (KBr): $\overline{\nu}$ = 3090 (CH_{arom}), 2930 (C–H), 1661 (C=O), 1461 (C=C) cm^{-1}; UV–Vis ($CHCl_3$): λ_{max} (log ε) = 241 (4.4), 276 (4.0), 385 (2.8), 480 (3.2) nm; ^{1}H NMR (499.74 MHz, $CDCl_3$): δ = 1.0–1.8 (m, 2H, CH_2), 3.5 (t, J = 6.84 Hz, 2H, S–CH_2), 4.1 (t, J = 6.84 Hz, 2H, O–CH_2), 7.4–8.2 (m, 8H, H_{arom}) ppm; ^{13}C NMR (125.66 MHz, $CDCl_3$): δ = 27.29 (S–CH_2–*C*H_2), 31.46 (S–CH_2), 61.69 (O–CH_2), 125.30, 125.38, 125.46, 125.68, 125.57, 126.09, 128.56, 130.41, 132.07, 132.29, 132.57 132.61 (CH_{arom}, C_{arom}), 133.68 (=C–S), 154.17, 156.81 (=C–Cl), 157.08 (=C–O), 177.67, 177.90, 181.19, 181.89 (C=O) ppm; MS (ESI+): *m/z* = 473 ($[M]^+$).

2,3-Bis(2,3-dihydroxypropylsulfanyl)-1,4-naphthoquinone (**10**, $C_{16}H_{18}O_6S_2$)

Compound **10** was synthesized from reaction of 1.0 g **1** (4.40 mmol) with 0.40 g **2b** (4.40 mmol) and 0.47 g **3b** (4.40 mmol). Yield: 0.18 g (16 %); red oil; R_f = 0.40 ($CHCl_3$); FT-IR (KBr): $\overline{\nu}$ = 3400 (–OH), 3190 (CH_{arom}), 2923 (C–H), 1738 (C=O), 1461 (C=C) cm^{-1}; UV–Vis ($CHCl_3$): λ_{max} (log ε) = 245 (4.4), 265 (4.0), 379 (2.8), 478 (3.2) nm; ^{1}H NMR (499.74 MHz, $CDCl_3$): δ = 3.2 (d, 4H, S–CH_2), 4.0–4.2 (m, 6H, C*H*$_2$–OH, –CH–OH), 4.25 (s, 4H, –OH), 7.60–8.00 (m, 4H, H_{arom}) ppm; ^{13}C NMR (125.66 MHz, $CDCl_3$): δ = 28.68 (S–CH_2), 67.16 (CH–OH), 66.68 (HO–CH_2), 124.60, 126.61, 131.43 (CH_{arom}, C_{arom}), 132.80 (=C–S), 166.76 (C=O) ppm; MS (ESI+): *m/z* = 371 ($[M]^+$).

2,3-Dihydro-2-(3-chloro-1,4-dihydro-1,4-dioxonaphthalen-2-yloxymethyl)naphtho[2,3-b]-1,4-oxathiin-5,10-dione (**11**, $C_{23}H_{13}ClO_6S$)

Compound **11** was synthesized from reaction of 1.0 g **1** (4.40 mmol) with 0.40 g **2b** (4.40 mmol) and 0.47 g **3b** (4.40 mmol). Yield: 0.32 g (28 %); red oil; R_f = 0.25 ($CHCl_3$); FT-IR (KBr): $\overline{\nu}$ = 3010 (CH_{arom}), 2930, 2861 (C–H), 1661 (C=O), 1465 (C=C) cm^{-1}; UV–Vis ($CHCl_3$): λ_{max} (log ε) = 242 (4.3), 265 (4.1), 387 (2.9), 475 (3.1) nm; ^{1}H NMR (499.74 MHz, $CDCl_3$): δ = 2.6 (d, 2H, S–CH_2), 3.6 (m, 1H, O–CH–), 4.1 (d, 2H, CH_2–O), 7.5–8.2 (m, 8H, H_{arom}) ppm; ^{13}C NMR (125.66 MHz, $CDCl_3$): δ = 27.23 (S–CH_2), 61.60 (O–CH), 61.85 (CH_2–O), 125.66, 125.85, 126.06, 126.15, 127.48, 127.81, 128.68, 129.71, 129.91, 132.45, 132.64, 133.44 ($CH_{arom,}$ C_{arom}), 138.71 (=C–S), 148.1 (=C–Cl), 151.56, 152.91 (=C–O), 171.66, 175.36, 176.26, 176.35 (C=O) ppm; MS (ESI+): *m/z* = 478 ($[M+Na+2H]^+$).

2-(6-Hydroxyhexylsulfanyl)-3-[2-(hydroxymethyl)-phenylsulfanyl]-1,4-naphthoquinone (**12**, $C_{23}H_{24}O_4S_2$)

Compound **12** was synthesized from reaction of 1.0 g **1** (4.40 mmol) with 0.61 g **2c** (4.40 mmol) and 0.59 g **3c** (4.40 mmol) according to the general procedure. Yield: 0.32 g (24 %); red solid; m.p: 72–73 °C; R_f = 0.35 ($CHCl_3$:EtAc 1:1); FT-IR (KBr): $\overline{\nu}$ = 3368 (–OH), 3060 (CH_{arom}), 2931, 2855 (C–H), 1662 (C=O), 1590 (C=C) cm^{-1}; UV–Vis ($CHCl_3$): λ_{max} (log ε) = 238 (4.4), 278 (4.2), 391 (3.0), 475 (3.7) nm; ^{1}H NMR (499.74 MHz, $CDCl_3$): δ = 1.3–1.4 (m, 4H, CH_2), 1.45–1.50 (m, 2H, S–CH_2–C*H*$_2$), 1.53–1.70 (m, 2H, C*H*$_2$–CH_2–OH), 2.0 (s, 2H, HO–C*H*$_2$), 3.3 (t, J = 7.81 Hz, 2H, S–CH_2), 3.6 (t, J = 7.83 Hz, 2H, CH_2–OH), 4.7, 4.8 (s, 2H, OH), 7.10–8.00 (m, 8H, H_{arom}) ppm; ^{13}C NMR (125.66 MHz, $CDCl_3$): δ = 24.21, 24.22 (CH_2), 27.07 (S–CH_2–*C*H_2), 29.45 (*C*H_2–CH_2–OH), 34.22 (S–CH_2), 61.72, 62.16 (CH_2–OH), 125.84, 126.01, 126.20, 126.73, 127.24, 127.55, 128.58, 128.93, 131.63, 131.87, 132.05, 132.79 (CH_{arom}, C_{arom}), 144.00, 151.17 (=C–S), 176.99, 178.60 (C=O) ppm; MS (ESI +): *m/z* = 428 ($[M]^+$), 327 ($[M–((CH_2)_6–OH)]^+$).

2,3-Bis[2-(hydroxymethyl)phenylsulfanyl]-1,4-naphthoquinone (**13**, $C_{24}H_{18}O_4S_2$)

Compound **13** was synthesized from reaction of 1.0 g **1** (4.40 mmol) with 0.61 g **2c** (4.40 mmol) and 0.59 g **3c** (4.40 mmol) according to the general procedure. Yield: 0.28 g (22 %); red oil; R_f = 0.30 ($CHCl_3$); FT-IR (KBr): $\overline{\nu}$ = 3415 (–OH), 3090 (CH_{arom}), 2938, 2853 (C–H), 1661 (C=O), 1592 (C=C) cm^{-1}; UV–Vis ($CHCl_3$): λ_{max} (log ε) = 240 (4.4), 275 (4.0), 389 (2.8), 470 (3.2) nm; ^{1}H NMR (499.74 MHz, $CDCl_3$): δ = 3.60 (s, 4H, HO–C*H*$_2$), 4.47 (s, 2H, –OH), 7.10–8.00 (m, 12H, H_{arom}) ppm; ^{13}C NMR (125.66 MHz, $CDCl_3$): δ = 63.14 (–OCH_2), 125.84, 126.03, 126.26, 127.20, 127.58, 127.69, 128.22, 128.60, 129.04, 130.00, 132.43, 132.51 (CH_{arom}, C_{arom}), 148.51 (=C–S), 178.63 (C=O) ppm; MS (ESI−): *m/z* = 433 ($[M]^-$).

X-ray crystallography

The suitable single crystals of compound **8** for the X-ray study were obtained from slow diffusion crystallization.

The red single crystals of compound **8** suitable for X-ray diffraction analysis were obtained by slow evaporation of an ethanol solution at room temperature. A red platelet crystal of **8**, $C_{13}H_{10}O_4S$, having approximate dimensions of 0.50 × 0.30 × 0.20 mm was mounted on a glass fiber. All measurements were made on a Rigaku R-Axis Rapid-S imaging plate area detector with graphite monochromated Mo–Kα radiation ($\lambda = 0.71073$ Å). The data were collected at room temperature to a maximum 2θ value of 60.1° for compound **8**. Experimental conditions are summarized in Table 3. The crystal structure was solved by SIR 92 [30] and refined with CRYSTALS [31]. The non-hydrogen atoms were refined anisotropically. H atoms were located in geometrically idealized positions C–H = 0.95(6) Å and treated as riding and $U_{iso}(H) = 1.2U_{eq}(C)$. The selected bond distances, bond and torsion angles for compound **8** are listed in Table 4. The hydrogen bond distance and angles are presented in Table 5. Drawings were performed with the program ORTEP-III [28] with 50 % probability displacement ellipsoid for compound **8** in Fig. 1. The hydrogen bonds and packing diagram are shown in Figs. 2 and 3, respectively.

Crystallographic data (excluding structure factors) for the structure in this paper have been deposited in the Cambridge Crystallographic Data Centre as supplementary publication number CCDC-996849 for **8**. Copies of the data can be obtained, free of charge, via http://www.ccdc.cam.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033. E-mail: deposit@ccdc.cam.ac.uk.

CUPRAC assay of antioxidant capacity

$CuCl_2$ solution (10 mM) was prepared in distilled water, ammonium acetate solution (1.0 M, pH = 7) and neocuproine solution (7.5 mM) were prepared in pure ethanol for the CUPRAC assay, as a difference from the original CUPRAC method [22] where ammonium acetate buffer is prepared in distilled water. CUPRAC reagent was diluted with acetone where necessary. The solutions of all other compounds were freshly prepared in acetone.

To a test tube were added 1 cm^3 each of Cu(II), Nc, and NH_4Ac buffer solutions. Compounds (x cm^3) and pure acetone ($1.1 - x$ cm^3) were added to the initial mixture to make the final volume 4.1 cm^3. The mixture was vortexed for 20 s, and absorbance measurement was performed exactly after 30 min at 450 nm [22]. The absorbance of the emerging cuprous neocuproine chromophore was correlated to antioxidant concentration. The reaction scheme may be summarized as: 1 cm^3 Cu(II) + 1 cm^3 Nc + 1 cm^3 NH_4-Ac + x cm^3 AOX + $(1 - x)$ cm^3 acetone (V_{total}: 4.1 cm^3) → measure absorbance at 450 nm.

DPPH free radical scavenging activity

The DPPH radical (DPPH·) solution (1 mM) was prepared in ethanol and diluted with ethanol by adjusting the absorbance of the DPPH radical solution to 0.750–0.900 AU at 525 nm.

The scavenging activity of compounds on DPPH radicals was measured according to the method of Sánchez-Moreno et al. [27] with minor modifications. To the mixture solution of x cm^3 standards and $(4 - x)$ cm^3 EtOH, 1 cm^3 of 0.1 mM DPPH ethanolic solution was added. The mixture was vortexed and left to stand at room temperature for 30 min. The absorbance at 525 nm was recorded against EtOH, where decolorization was a measure of DPPH free radical scavenging activity. The absorbance values were corrected for calculating radical scavenging capability of the sample. The free radical scavenging (FRS) activity was expressed as a percentage of DPPH decolorization using the equation:

$$\text{Frs } (\%) = \left[\left(A_{\text{dpph}} - A_s\right)/a_{\text{dpph}}\right] \times 100 \quad (1)$$

where A_{DPPH} is the absorbance of DPPH solution without sample and A_S is the absorbance of the solution when the standard has been added at a particular level. FRS percentage (y) can be empirically correlated to concentration of scavenger (x) within an absorbance range over which Beer's law is valid:

$$y = mx + n \quad (2)$$

where m and n are the slope and intercept of this linear correlation, respectively. IC_{50} can then be calculated for 50 % inhibition ($y = 50$) such that

$$y = 50 = m(IC_{50}) + n \text{ or } IC_{50} = (50 - n)/m \quad (3)$$

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References

1. Makosza M, Nizamov S (2001) Tetrahedron 57:9615
2. Tandon VK, Maurya HK (2009) Tetrahedron Lett 50:5896
3. Couladouros EA, Plyta ZF, Haroutounian SA (1997) J Org Chem 62:6
4. Ibis C, Deniz NG (2012) J Chem Sci 124:657
5. Domling A (2006) Chem Rev 106:17
6. Orru RVA, Greef M (2003) Synthesis 10:1471
7. Hulme C, Gore V (2003) Curr Med Chem 10:51
8. Montagne C, Shiers JJ, Shipman M (2006) Tetrahedron Lett 47:9207
9. Domling A, Ugi I (2000) Angew Chem Int Ed 39:3169
10. Ugi I (2001) Pure Appl Chem 73:187
11. Toure BB, Hall DG (2009) Chem Rev 109:4439
12. Sunderhaus JD, Martin SF (2009) Chem Eur J 15:1300

13. Ames BN, Shigenaga MK, Hagen TM (1993) Proc Natl Acad Sci USA 90:7915
14. Hertog MGL, Feskens EJM, Hollman PCH, Katan MB, Kromhout D (1993) Lancet 342:1007
15. Temple NJ, Gladwin KK (2003) Nutrition 19:467
16. Peters U, Leitzmann MF, Chatterjee N, Wang Y, Albanes D, Gelmann EP, Friesen MD, Riboli E, Hayes RB (2007) Cancer Epidemiol Biomarkers Prev 16:962
17. Bezabih M, Abegaz BM, Dufall K, Croft K, Skinner-Adams T, Davis TM (2001) Planta Med 67:340
18. Lebedev AV, Levistskaya EL, Tikhonova EV, Ivanova MV (2001) Biochemistry 66:885
19. Ibis C, Ayla SS, Asar H (2014) Synth Commun 44:121
20. Ibis C, Deniz NG (2010) Phosphorus Sulfur Silicon Relat Elem 185:2324
21. Ibis C, Ayla SS, Deniz NG (2010) Asian J Chem 22:6195
22. Apak R, Guclu K, Ozyurek M, Karademir SE (2004) J Agric Food Chem 52:7970
23. Talcott RE, Smith MT, Giannini DD (1985) Arch Biochem Biophys 241:88
24. Gaikwad MN, Gaikwad ST, Rajbhoj AS (2012) Int J Chem Tech Res 4:1392
25. Bose A, Basu S (2008) J Phys Chem 112:12045
26. Thompson RB, Chenicek JA, Symon T (1952) Ind Eng Chem 44:1659
27. Sánchez-Moreno C, Larrauri JA, Saura-Calixto F (1998) J Sci Food Agric 76:270
28. Farrugia LJ (1997) J Appl Cryst 30:565
29. Singh WM, Baruah JB (2011) J Chem Crystallogr 41:952
30. Altomare A, Cascarano G, Giacovazzo C, Guagliardi A, Burla M, Polidori G, Camalli M (1994) J Appl Cryst 27:435
31. Watkin DJ, Prout CK, Carruthers JR, Betteridge JR (1996) Crystals. 10 PW Chemical Crystallography Laboratory, Oxford, UK