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Synthesis, cytotoxic and antioxidant evaluations of amino derivatives from perezone

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ABSTRACT

A series of eight amino derivatives (**3a**–**h**) from perezone **1** were prepared by nucleophilic addition of bioactive amines *v.gr*. melatonin, acetyl tryptamine, tryptophan and other amino acids esters (valine, leucine and methionine). Their structures were elucidated by spectroscopy data. The cytotoxic evaluation against four human tumor cell lines PC-3, K-562, HCT-15 and SKLU-1 was performed as well as the TBARS assay for antioxidant activity. The results suggest that **1** and its isomer **4** were highly active against all cell lines, **4** was twice as potent than **1** against PC-3 and HCT-15. The derivative **3a** (IC_{50} = 7.5 ± 0.3 μM) was more active than **1** against HCT-15 whereas **3h** was selective against K-562 with IC_{50} = 4.5 ± 0.4 μM. The TBARS assay has shown that **3c** with IC_{50} = 5.564 ± 0.24 μM is a potent antioxidant with superior effect comparing to α-tocopherol and moreover was more active than the precursor molecule **1**.

1. Introduction

Among the numerous Mexican medicinal species, the genus Perezia more recently renamed 'Acourtia', has received much attention since the secondary metabolite Perezone (**1**) was first reported in 1852 by Leopoldo Río de la Loza.[1] This natural sesquiterpenic quinone was isolated for the first time from *Perezia adnata* and was known as 'raiz del pipitzahuac' in the traditional medicine in México.

Compound **1** has been the subject of many chemical studies, first to elucidate its structure and then to explore its varied reactivity.[2–5] Nevertheless, the correct structure of **1** was determined by using NMR spectroscopic methods[6–8] and its crystal structure was reported later in 1986.[9] Some synthetic methods have been developed to obtain **1** in racemic form as well as other related quinones.[10–12]

Mexican traditional medicine employed the roots of *perezia* for the preparation of laxative drinks, and also for diuretic, regenerative and analgesic purposes.[13] Perezone exhibits diverse biological profiles, for example, the hypoglycemic effect,[14] inhibition of aggregation of platelet,[15] it promotes the release of mitochondrial Ca^{2+} maintaining ATP production during reperfusion,[16] it relaxes the basal tonus of the smooth muscle[17] and blocks the contractile response induced by Ach, K^+ and Ba^{2+}.[18]

Also, perezone acts as an electron sink in electron transport in the mitochondria[19] and is considered to be a cardio protective agent.[20] Antifeedant effects and phytotoxic activity[21] have been studied as well as the cytotoxic effect against several cancer cell lines.[22]

In a recent investigation the cytotoxic effect of perezone and isoperezone was evaluated on the K-562 human leukemia cell line, showing that perezone has greater cytotoxic effect than isoperezone.[23] On the other hand, perezone was included in the screening of compounds that induce apoptosis of cancer cell lines[24] and for the screening of agents against neuroblastoma.[25]

Some amino derivatives have been synthesized previously and evaluated for biological activity,[17] but derivatives of indolamine type v.gr. melatonin (**2a**), acetyl tryptamine (**2b**) and tryptophan (**2c**) (Fig. 1) have not been prepared. Therefore we considered it to be of interest to synthesize these types of derivatives due to the biological activity such amines have on their own.

Melatonin (*N*-acetyl-5-methoxytryptamine) (**2b**) is secreted by the pineal gland of the vertebrate. Several studies have demonstrated the role of **2b** in physiological regulation of seasonal and circadian rhythms. Some therapeutic applications such as regulation of sleep disorders, treatment of depression, as broad-spectrum antioxidant,[26] treatment of Alzheimer disease or anticancer agent.[27,28] The conjugation of melatonin with α-lipoic acid was achieved and the bioconjugate was tested in vitro for radioprotection ability.[29] Tryptamine and the essential amino acid tryptophan are analogs of melatonin and possess biological activities such as neurotrans-

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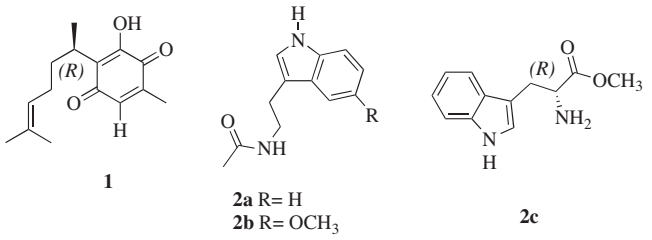


Figure 1. Structures of perezone (**1**), acetyl tryptamine (**2a**) melatonin (**2b**) and tryptophan methyl ester (**2c**).

mitter and the building blocks in proteins biosynthesis. The coupling of two biomolecules to obtain bioconjugates, is a strategy towards synthesis of novel derivatives that allow improvement of biological activities or the modification of physicochemical properties in comparison with the parent molecules. Natural amino acid derivatives of naproxen have been prepared to improve analgesic and anti-inflammatory activity.[30] Valine, leucine and methionine are essential amino acids, which possess important functions and therefore are good candidates for nucleophilic addition to the quinoid system of **1**.

In this context, as a continuation of our research on natural products,[31]in the present work we prepared some new indole derivatives **3a–c** and amino acid derivatives **3d–h** by nucleophilic addition of indole amines or amino acid esters (valine, leucine, methionine and tryptophan) to the quinone system of **1**. The derivatives have been elucidated by spectroscopic data and assessed for their cytotoxic activity with respect to four cancer line cells of different histotype (myeloblastoid and carcinomas). The antioxidant activity was evaluated by TBARS test in rat brain in order to measure the lipid peroxidation of polyunsaturated fatty acids, which produce malondialdehyde (MDA) as thiobarbituric acid reactive substance.

2. Results and discussion

2.1. Isolation of perezone and its isomerization to isoperezone

Perezone was isolated from dry roots of *Acourtia cuernavacana* spp. and extracted three times by using maceration with hexane at room temperature. As a result we obtained orange crystals. The yield was 2%, but the crystals were not pure. Then their purification was achieved by recrystallization with acetone-hexane (1% yield). The non-natural derivative isoperezone **4** was prepared according to the protocol previously described with our specific modifications (Scheme 1).[32]

2.2. Synthesis of amino derivatives

Derivatives **3a**, **3b** and **3c** (Scheme 2) were prepared from **1** with melatonin, acetyl tryptamine or tryptophan methyl ester, respectively, via nucleophilic addition of indole-type nitrogen. In the case of tryptamine, the aliphatic amine was previously acetylated in order to avoid a nucleophilic attack of this nitrogen.

First, melatonin or its analogs were set to react with NaH in THF in order to form the anion, then perezone was added to the reaction mixture and refluxed for 24 h. Although the yield of each reaction is quantitative, the presence of residual secondary products required their purification by column chromatography. Deep brown semisolids were obtained. The chemical structures of all compounds were confirmed by spectroscopy data. For mass spectroscopy, in the case of compounds **3a** and **3b** the acetylation of the products was necessary to avoid their decomposition. The acetylated compounds are marked with prime, respectively.

In the case of derivatives **3d–f** (Scheme 3), the compound **1** reacted with valine, leucine and methionine esters **2d–f** or tryptamine **2a′** and tryptophan **2c** (Scheme 4), employing $Zn(AcO)_2$ as a catalyst in MeOH under mild reaction conditions. All the compounds were characterized by spectroscopy data.

The optical rotation was only possible for **3a**. Due to the deep purple color of **3b–h** we were unable to get the data in the polarimeter.

2.3. Cytotoxic activity in human tumor cell lines

In order to evaluate compounds **1**, **3a–3h** and **4** as cytotoxic agents, we performed tests in vitro against three carcinoma type cell lines: PC-3 (human prostatic), HCT-15 (human colorectal) and SKLU-1 (non-small cell lung cancer and one myeloblastoid leukaemia cell line: K-562 (human chronic myelogenous leukemia). The cytotoxicity was determined by the sulforhodamine B (SRB) assay and Adriamycin was used as a positive control. Table 1 shows the IC_{50} data for the compounds obtained.

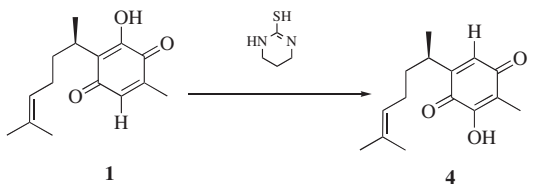
Although, the compounds **1**, **3a–h** and **4** showed less cytotoxic activity than Adriamycin, **1** and **4** presented higher cytotoxic potential against the four cell lines with $IC_{50} < 10\ \mu M$. The change in the position of hydroxyl group present in **1** and **4**, leads to important changes in the cytotoxic activity. Compound **4** was twice as potent with respect to **1** against PC-3 and HCT-15 cell lines.

The bioconjugates **3a** and **3b** are structurally related, since the presence of a methoxy group in 5 position of indole ring for **3a** leads to an increase of the activity against all cell lines in comparison to **3b**. Also **3a** was more active than its precursor molecule against HCT-15 cell line. Derivatives **3g** and **3h** correspond to bioconjugates of **1** with tryptamine and tryptophan methyl ester, respectively, bonded by amino group in α position. The presence of methyl carboxylate in **3h** increases the activity against all cell lines as compared to **3g**. On the other hand, we obtained **3c** and **3h** as products of addition to each of the nitrogen atoms present in the molecule of tryptophan methyl ester. For **3c**, the IC_{50} with K-562, HCT-15 and SKLU-1 cell lines was twice as much as for **3h**. For **3h** we observed selectivity against the leukemia cell line and moderate activity for solid tumors.

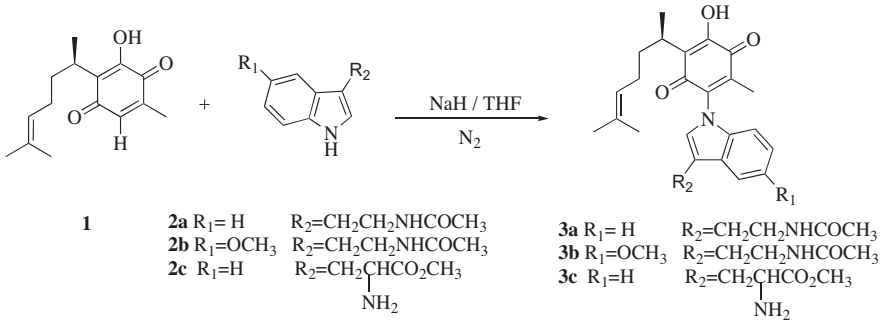
The addition of aliphatic amino acid esters to perezone produced derivatives (**3d–f**), which were less active against the four cell lines in comparison to the aromatic analog of tryptophan methyl ester 3 h.

2.4. Antioxidant activity (TBARS) in rat brain homogenate

The antioxidant activity was evaluated by measuring the amount of plasma concentration of thiobarbituric acid reactive substances (TBARS) that represent lipid peroxidation. Malondialdehyde (MDA) forms complexes with Thiobarbituric Acid (TBA) to produce a pink coloured complex, spectrophotometrically measured at a wavelength of 540 nm. TBARS results are expressed as the amount of free MDA equivalents per 1 mg tissue through the



Scheme 1. Synthesis of isoperezone.

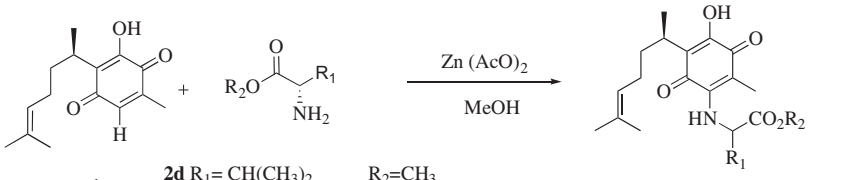


1

2a R_1= H R_2=$CH_2CH_2NHCOCH_3$
2b R_1=OCH_3 R_2=$CH_2CH_2NHCOCH_3$
2c R_1=H R_2=$CH_2CHCO_2CH_3$ (NH_2)

3a R_1= H R_2=$CH_2CH_2NHCOCH_3$
3b R_1=OCH_3 R_2=$CH_2CH_2NHCOCH_3$
3c R_1=H R_2=$CH_2CHCO_2CH_3$ (NH_2)

Scheme 2. Synthesis of derivatives **3a–b**.

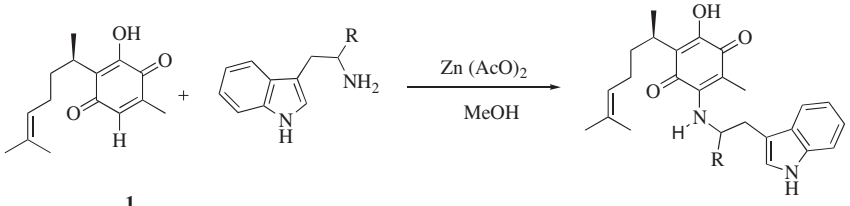


1

2d R_1= $CH(CH_3)_2$ R_2=CH_3
2e R_1= $CH_2CH(CH_3)_2$ R_2=CH_3
2f R_1= $CH_2CH_2SCH_3$ R_2=CH_2CH_3

3d R_1= $CH(CH_3)_2$ R_2=CH_3
3e R_1= $CH_2CH(CH_3)_2$ R_2=CH_3
3f R_1= $CH_2CH_2SCH_3$ R_2=CH_2CH_3

Scheme 3. Synthesis of derivatives **3d–f**.



1

2a´ R= H
2c R= CO_2CH_3

3g R= H
3h R= CO_2CH_3

Scheme 4. Synthesis of derivatives **3g** and **3h**.

Table 1
Inhibitory concentration (IC_{50} in μM) values obtained in PC-3, K-562, HCT-15 and SKLU-1 for compounds **1**, **3a–h** and **4**.

Compound	IC_{50} μM*			
	Cell line			
	PC-3	K-562	HCT-15	SKLU-1
1	6.5 ± 0.6	3.0 ± 0.3	10.3 ± 1.0	4.2 ± 0.3
3a	9.9 ± 0.5	6.2 ± 0.6	7.5 ± 0.3	9.5 ± 0.5
3b	13.9 ± 1.2	8.9 ± 1.3	9.8 ± 0.48	16.9 ± 0.9
3c	11.6 ± 0.6	10.0 ± 0.6	19.2 ± 0.7	21.1 ± 0.7
3d	24.8 ± 2.0	13.1 ± 1.0	14.3 ± 1.8	16.4 ± 1.8
3e	20.3 ± 1.4	11.4 ± 0.6	15.4 ± 0.3	15.1 ± 0.1
3f	19.7 ± 1.1	13.0 ± 0.8	13.8 ± 1.0	15.0 ± 11.1
3 g	23.4 ± 11.3	12.2 ± 1.0	19.4 ± 11.7	22.6 ± 2.2
3 h	11.1 ± 0.5	4.5 ± 0.4	8.5 ± 0.8	9.7 ± 0.8
4	3.3 ± 0.2	3.4 ± 0.1	5.9 ± 0.5	4.5 ± 0.5
Adriamycin**	60 ± 1.0	14 ± 0.9-	5 ± 0.9	6.7 ± 1

* In DMSO like vehicle.
** nM.

calibration curve generated from 1,1,3,3-tetramethoxypropane (TMP). This assay was performed for all compounds because some amino acids as well as melatonin or its analogs are considered as antioxidant agents. Thus, the inhibitory concentration IC_{50} of these derivatives (Table 2 and Fig. 2) was compared with α-tocopherol and quercetin, which are considered to be as excellent antioxidants. DMSO was used in the blank to correct any possible antioxidant effect that it could have.

With respect to antioxidant activity, the most pronounced inhibitory effect was found for **3c** with superior potency than α-tocopherol (Fig. 2), whereas in the case of **3a** and **3b** analogs the activity decreased. It is interesting to mention that the bonding site for tryptophan methyl ester had an important role in the antioxidant activity. While **3h** is not active, the presence of α-amino acid ester in **3c** favors antioxidant activity. For compound **4**, we observed that the antioxidant activity was twice as high as its isomer **1**. The position of hydroxyl group in these compounds is very important for the antioxidant activity. The results indicate that derivatives of **4** could further increase its antioxidant activity.

3. Conclusions

The new derivatives of perezone obtained in the present work represent an option of molecules with cytotoxic and antioxidant activities. Although all compounds were less cytotoxic than Adriamycin, all of them exhibited high cytotoxicity predominantly on leukemia cell line K-562. Compounds **1** and its isomer **4** can be considered as high cytotoxic agents for the myeloblastoid leukemia cell line (K-562) and for carcinoma cell lines (PC-3 and SKLU-1) with IC_{50} < 10 μM.

Table 2
IC_{50} for Inhibitory activity of lipid peroxidation in TBARS assay.

Compound	Concentration (μM)	TBARS (nmol/mg prot.)	Inhibition (%)	IC_{50} (μM)
Basal	—	0.29 ± 0.02	—	—
Control $FeSO_4$	10	7.57 ± 0.22	—	—
1	10	6.61 ± 0.24	8.48 ± 1.86	49.22 ± 5.66
	17.78	6.07 ± 0.40	16.00 ± 3.97	
	31.62	5.14 ± 0.32**	28.78 ± 4.32**	
	56.23	3.30 ± 0.73**	54.21 ± 9.93**	
	100	0.77 ± 0.23**	89.39 ± 3.14**	
3a	10	7.02 ± 0.32	2.90 ± 1.80	29.75 ± 0.53
	17.78	5.85 ± 0.22**	19.00 ± 1.09**	
	31.62	3.65 ± 0.27**	49.53 ± 2.36**	
	56.23	0.49 ± 0.01**	93.23 ± 0.22**	
	100	0.32 ± 0.01**	95.63 ± 0.20**	
3b		6.19 ± 0.45	14.85 ± 4.17	27.44 ± 2.63
	17.78	5.10 ± 0.53**	29.97 ± 5.76**	
	31.62	3.59 ± 0.58**	50.89 ± 6.99**	
	56.23	0.68 ± 0.18**	90.78 ± 2.24**	
	100	0.36 ± 0.03**	95.10 ± 0.26**	
3c	3.16	6.61 ± 0.40	13.88 ± 4.46	5.564 ± 0.24
	4.22	6.09 ± 0.39*	20.57 ± 4.71*	
	5.62	4.15 ± 0.72**	46.01 ± 8.90**	
	7.5	0.88 ± 0.24**	88.70 ± 2.70**	
	10	0.45 ± 0.05**	94.13 ± 0.84**	
3d	17	5.45 ± 0.52	27.28 ± 2.32	22.44 ± 1.02
	19	4.93 ± 0.47**	34.21 ± 2.69**	
	21	4.66 ± 0.51**	37.82 ± 4.10**	
	23	3.38 ± 0.79**	55.87 ± 8.08**	
	25	2.92 ± 0.53**	61.20 ± 6.13**	
	27	1.58 ± 0.44**	78.79 ± 5.70**	
	29	1.09 ± 0.55**	85.50 ± 7.28**	
	31	0.37 ± 0.05**	95.04 ± 0.72**	
3e	9	7.65 ± 0.41	7.53 ± 5.07	14.67 ± 0.60
	10	7.41 ± 0.43	10.45 ± 5.64	
	11	7.07 ± 0.65	14.66 ± 7.58	
	12	6.48 ± 0.50	21.66 ± 6.35	
	13	6.30 ± 0.45	23.94 ± 5.57	
	14	4.75 ± 0.73**	42.20 ± 9.99**	
	15	430 ± 0.78**	47.89 ± 9.98**	
	16	2.86 ± 0.40**	65.42 ± 5.15**	
	17	1.15 ± 0.21**	85.75 ± 2.69**	
3f	17	5.98 ± 0.46*	18.83 ± 2.69*	20.25 ± 0.26
	19	4.71 ± 0.29**	35.82 ± 1.77**	
	21	3.06 ± 0.34**	57.66 ± 5.86**	
	23	1.62 ± 0.32**	77.31 ± 5.44**	
	25	1.35 ± 0.30**	80.92 ± 5.32**	
	27	0.76 ± 0.11**	89.72 ± 1.28**	
	29	0.56 ± 0.11**	92.24 ± 1.58**	
	31	0.40 ± 0.02**	94.56 ± 0.15**	
3g	17	4.52 ± 0.45**	37.12 ± 2.34**	19.33 ± 0.30
	19	4.25 ± 0.61**	38.52 ± 8.36**	
	21	2.68 ± 0.79**	69.64 ± 9.95**	
	23	1.76 ± 0.49**	84.22 ± 7.13**	
	25	1.17 ± 0.44**	90.34 ± 2.52**	
	27	0.35 ± 0.06**	95.40 ± 0.83**	
	29	0.44 ± 0.19**	96.05 ± 0.80**	
	31	0.28 ± 0.04**	96.83 ± 0.13**	
3h	17	5.91 ± 0.62	20.94 ± 3.44	25.16 ± 0.08
	19	5.59 ± 0.47	24.91 ± 1.45	
	21	5.32 ± 0.50	28.6 ± 2.6	
	23	4.42 ± 0.50**	40.80 ± 2.73**	
	25	4.21 ± 0.73**	44.15 ± 5.96**	
	27	2.72 ± 0.96**	64.82 ± 10.39**	
	29	1.64 ± 0.63**	78.73 ± 7.79**	
	31	0.60 ± 0.17**	92.06 ± 2.08**	
4	17.78	5.67 ± 0.18**	25.20 ± 3.09**	23.26 ± 0.1
	23.71	3.78 ± 0.21**	47.39 ± 2.83**	
	31.62	1.21 ± 0.21**	83.32 ± 2.56**	
	42.17	0.39 ± 0.01**	94.62 ± 0.03**	
	56.23	0.33 ± 0.01**	95.35 ± 0.18**	
Quercetin	0.32	9.13 ± 0.62	5.23 ± 1.17	1.496 ± 0.03
	0.56	8.58 ± 0.59	10.93 ± 1.20	
	1	6.98 ± 0.73**	27.88 ± 2.05**	
	1.78	3.59 ± 0.33**	62.68 ± 1.93**	
	3.16	1.39 ± 0.16**	85.23 ± 2.81**	
	5.62	0.49 ± 0.12**	94.90 ± 1.13**	
	10	0.41 ± 0.12**	95.65 ± 1.24**	
α-Tocopherol	0.1	6.28 ± 0.18	4.62 ± 0.57	6.78 ± 2.16
	0.32	6.04 ± 0.24	8.26 ± 1.31	
	1	5.21 ± 0.33*	21.13 ± 2.56*	
	3.16	3.67 ± 0.56**	44.84 ± 6.74**	
	10	2.72 ± 0.33**	59.00 ± 3.71**	
	31.62	1.84 ± 0.31**	72.3 ± 3.87**	
	100	1.40 ± 0.36**	79.09 ± 4.79**	

Data are averaged three or four times with independent values ± standard error on the mean (X ± ES). All reported data were subject to variance analysis (ANOVA) followed by Dunnett tests in order to isolate specific group of significant difference. The $p \leqslant 0.05$ (*) and $p \leqslant 0.01$ (**) values were considered to be different as compared with the control.

The addition of the part indole amine or α-amino ester present in tryptophan methyl ester to perezone produced the derivatives **3c** or **3h**, which possess very different activities. Compound **3c** has a superior antioxidant activity (IC_{50} = 5.564 ± 0.24 μM) than α-tocopherol and exhibits a higher antioxidant activity of 8.8 times than **1**, whereas **3h** with IC_{50} = 4.5 ± 0.4 μM was selective against myeloblastoid leukemia cell line (K-562).

Taking into account that compound **3c** presented a higher antioxidant activity than α-tocopherol, we will consider that for future research its structure as a new leader molecule for the design of analogs with potent antioxidant activity.

4. Experimental

Melting points (°C) were determined in a Fisher-Jones apparatus and are uncorrected; IR spectra were recorded in Perkin Elmer Spectrum RXI in KBr or solution. Optical rotations were measured in a polarimeter JASCO model DIP360 at 589 nm using a 1 dm cell. Specific rotations are given in units of 10^{-1} deg cm^2 g^{-1} (*c* g/100 cm^3). Mass spectra were obtained in JEOL JMS-AX505HA mass spectrometer by electronic impact at 70 eV and JEOL SX 102 A by FAB^+.

1D and 2D ^1H and ^{13}C spectra were obtained at 500 and 125 MHz (Bruker Avance DMX500) or 400 and 100 MHz (Varian Mercury 400), respectively, in $CDCl_3$ at room temperature with TMS as the internal reference, chemical shifts (δ) are given in ppm and coupling constants (*J*) in Hz.

TLC was performed on precoated Kieselgel 60 F254 plates (Merck, Darmstadt, Germany) and detected with UV light. Flash chromatography was performed with Merck Kieselgel silica gel (60, particle size 40–63 mm). All reagents were purified and the solvents used were of analytical grade.

4.1. Plant material

the whole plant of *Acourtia cuernavacana* (before *Perezia cuernavacana*) was collected in September–December 2007 in Cuernavaca, Morelos, and identified for comparison with an exemplary of Herbarium. The roots were separated from the aerial parts, cleaned and dried for three weeks.

4.2. Extraction and isolation

About 828 g of dried roots of *Acourtia cuernavacana* were finely powdered and exhaustively extracted by maceration at room temperature with hexane to give 16 g of orange crystals (2%). Recrystallization of the solid was carried out with acetone-hexane to give 8 g (1%) of pure perezone.

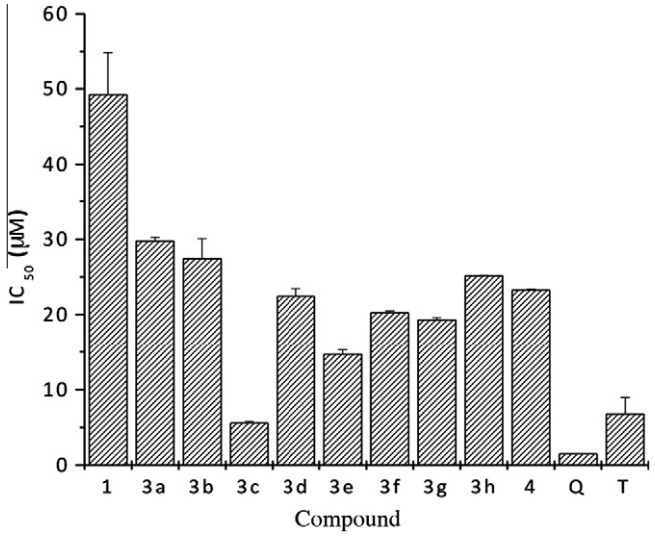


Figure 2. Inhibitory concentration (IC_{50}) values in the antioxidant test (TBARS) for compounds **1**, **3a**–**h**, **4** and standards (quercetin, Q and α-tocopherol,T).

4.2.1. Perezone (2-[1,5-dimethyl-4-hexenyl]-3-hydroxy-5-methyl-1,4-benzoquinone) (1)

Orange crystals, mp:101–102 °C, R_f: 0.44 (hexane/acetone 90:10); IR (cm^{-1}) in KBr: 3413, 2968, 2929, 1654, 1639, 1396, 1377, 1323. ^1H NMR (400 MHz, $CDCl_3$): 6.991 (s, 1H), 6.480 (q, J = 0.9 Hz, 1H), 5.063 (tt, J = 5.4, 0.9 Hz, 1H), 3.004–3.094 (m, 1H), 2.059 (d, J = 1.2 Hz, 3H), 1.758–1.932(m, 3H), 1.64 (d, J = 0.9, 3H), 1.549–1.603 (m, 1H), 1.532 (s, 3H), 1.197 (d, J = 5.4 Hz, 3H). ^{13}C (100 MHz, $CDCl_3$): 187.419, 184.340, 151.061, 140.671, 135.999, 131.601, 124.699, 124.608, 34.45, 29.687, 27.078, 26.123, 18.660, 18.053, 15.171.

4.3. Isomerization of perezone

Perezone was isomerized by action of 1,2,3,4-tetrahydropyrimidinethiol with some small modifications as compared to previously reported protocol.[11] 4 mmol of perezone (**1**) were disolved in 50 ml of MeOH and catalytic quantity of p-TsOH was added and stirred by 30 min. Then 4 mmol of 3,4,5,6-tetrahydropyrimidinethiol in 40 ml of MeOH was added and the mixture was refluxed for 12 h after evaporation of the solvent the residue was purified by column chromatography. Orange crystals were obtained.

4.3.1. Isoperezone (2-[1,5-dimethyl-4-hexenyl]-6-hydroxy-5-methyl-1,4-benzoquinone (4)

Orange crystals; 60% yield; R_f: 0.2 (hexane/acetone 90:10); p.f. : 108–109 °C; IR (cm^{-1}) in KBr: 3399.99, 3257.17, 2962.61, 2930.46, 1755.62, 1662.23, 1638.35, 1611.22, 1393.88, 1376.28, 1326.91, 1196.72. ^1H NMR (400 MHz, $CDCl_3$): 6.952 (s, 1H), 6.428 (d, J = 0.8 Hz, 1H), 5.021 (tt, J = 7.2 Hz, 1H), 2.89 (sx, J = 6.8 Hz, 1H), 1.93–2.0 (m, 2H), 1.937 (s, 3H), 1.654 (d, J = 0.8 Hz, 3H), 1.62 (m, 2H), 1.544 (s, 3H), 1.42–1.62 (m, 2H), 1.130 (d, J = 7.2 Hz, 3H). ^{13}C (100 MHz, $CDCl_3$): 187.761, 182.725, 150.961, 149.232, 132.728, 131.969, 123.505, 116.376, 35.556, 31.339, 25.708, 25.605, 19.356, 17.657, 7. 857.

4.4. *N*-(2-(1*H*-Indol-3-yl)ethyl)acetamide *(Acetyl triptamine)* 2a

Commercial tryptamine (6.2 mmol, 1.0 g) was dissolved in dry CH_2Cl_2 (20 mL), and acetic anhydride (1.1 equiv) was added slowly. The reaction was stirred at room temperature for 40 min. The resulting precipitate was filtered and washed with CH_2Cl_2, and dried. Yield was 1.13 g (95%) of **2a**. mp 75 °C (lit., mp = 75–76 °C).[33] Spectroscopic data is according to the previously reported.[34]

4.5. General procedure for preparation of bioconjugate perezone derivatives

1.5 mmol of sodium hydride (NaH) were dissolved in 3 mL of anhydrous tetrahydrofuran (THF) at 0°C. 4.2 mmol of **2a**, **2b** (acetylated previously) or **2c** were added dropwise in a 5 mL THF solution. This reaction was stirred during 40 minutes in an inert atmosphere of N_2, then 1 mmol of perezone (1) was added dropwise in a 5 mL THF solution. The reaction was stirred at room temperature for 24 h. Then the reaction was washed with brine and extracted with EtOAc. The organic phase was dried with Na_2SO_4 and filtered. The solvent was evaporated and dried in vacuo. The residue was purified by flash chromatography (hexane/acetone, 6:1) to obtain compounds **3a**, **3b** or **3c**.

For mass spectroscopy, due to the slow decomposition of **3a** and **3b**, it was necessary to acetylate them with Ac_2O and pyridine, stirring the mixture for 1 h at room temperature. The mixture was washed with brine and extracted in EtOAc. The organic phase was dried with Na_2SO_4 and filtered. The solvent was evaporated and dried in vacuo. The residue was purified by flash chromatography (hexane/acetone, 6:1). The acetylation of **3a** and **3b** leads to the more stable products **3a**′and **3b**′ were, respectively, used for MS analysis solely.

4.5.1. *N*-(2-(1-(4-Hydroxy-2-methyl-5-((*R*)-6-methylhept-5-en-2-yl)-3,6-dioxocyclohexa-1,4-dienyl)-5-methoxy-1*H*-indol-3-yl)ethyl)acetamide (3a)

Brown semisolid; 70% yield; R_f: 0.4 ($CHCl_3$–MeOH, 95:5); $[\alpha]_D$: +35.4 (*c* 1.00, $CHCl_3$); IR (cm^{-1}) in $CHCl_3$: 3389.31, 3017.98, 1711.63, 1650.21, 1620.1, 1216.0; ^1H NMR (500 MHz, $CDCl_3$): 7.397 (sb, 1H), 7.066 (d, J = 2.5 Hz, 1H), 6.915 (s, 1H), 6.877 (dd, J = 9, 2.5 Hz, 1H), 6.812 (d, J = 8.5 Hz, 1H), 5.751 (sb, 1H), 5.079 (tt, J = 7, 1.5 Hz, 1H), 3.867 (s, 3H), 3.481 (q, J = 7 Hz, 1H), 3.119 (m, 1H), 2.939–2.979 (m, 2H), 1.994 (s, 3H), 1.947 (s, 3H), 1.78–1.92 (m, 2H), 1.646 (s, 3H), 1.56–1.63 (s, 1H), 1.545 (sb, 3H), 1.231 (d, J = 7 Hz, 3H), 1.194–1.256 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): 184.133, 182.945, 170.293, 155.048, 151.361, 139.933, 132.823, 131.704, 131.592, 129.129, 128.057, 124.323, 123.745, 115.151, 112.129, 112.256, 101.531, 55.952, 39.215, 34.200, 29.856, 26.733, 25.740, 25.155, 23.389, 18.276, 17.706, 12.437. **3a**′ MS (EI, 70 eV): m/z (%) = 520 [M^+] (71), 461 (100), 406 (72), 337 (28), 149 (30). HRMS–FAB: m/z [M+H$^+$] calcd for $C_{30}H_{36}N_2O_8$: 521.2652; found: 521.2657.

4.5.2. *N*-(2-(1-(4-Hydroxy-2-methyl-5-((*R*)-6-methylhept-5-en-2-yl)-3,6-dioxocyclohexa-1,4-dienyl)-1*H*-indol-3-yl)ethyl)acetamide (3b)

Brown semisolid; 60% yield; R_f: 0.4 ($CHCl_3$–MeOH, 95:5). IR (cm^{-1}) in $CHCl_3$: 3296.30, 2964.89, 2930.42, 1651.63, 1458.08, 1380.73, 1269.74. ^1H NMR (500 MHz, $CDCl_3$): 7.626 (d, J = 7.5 Hz, 1H), 7.236 (t, J = 7.5 Hz, 1H), 7.186 (t, J = 8 Hz, 1H), 6.943 (s, 1H), 6.916 (d, J = 8.0, 1H), 5.874 (sb, 1H), 5.082 (t, J = 7.0 Hz, 1H), 3.667–3.717 (m, 1H), 3.549 (sb, 1H), 3.129 (sx, J = 7.0 Hz, 1H), 2.92–3.07 (m, 2H), 1.988 (s, 3H), 1.938 (s, 3H), 1.79–1.971 (m, 3H), 1.644 (s, 3H), 1.590–1.648 (m, 1H), 1.235 (d, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): 184.070, 182.887, 170.411, 151.472, 139.751, 136.600, 131.510, 128.366, 127.252, 124.261, 123.803, 122.91, 120.804, 119.278, 115.188, 111.289, 68.115, 39.271, 34.143, 34.082, 29.770, 26.657, 25.658, 25.017, 23.239, 18.218, 12.319.

Compound **3b**′ MS (EI, 70 eV): m/z (%): 490 [M^+] (34), 448 (6), 431 (100), 376 (98), 307 (82), 43 (23). HRMS–FAB: m/z [M+H$^+$] calcd for $C_{29}H_{34}N_2O_5$: 490.2468; found: 490.2462.

4.5.3. (*R*)-Methyl 2-amino-3-(1-(4-hydroxy-2-methyl-5-((*R*)-6-me thylhept-5-en-2-yl)-3,6-dioxocyclohexa-1,4-dienyl)-1H-indol-3-yl)propanoate (3c)

Brown semisolid; 20% yield; R_f: 0.4 ($CHCl_3$–MeOH, 95:5). IR (cm^{-1}) in $CHCl_3$: 3476.78, 3304.54, 3019.39, 2928.07, 2857.13, 1738.52, 1711.15, 1582.65, 1510.23, 1375.43, 1357.84, 1280.37, 1216.22. ^1H NMR (500 MHz, $CDCl_3$): 8.21 (br s, 2H), 7.508 (d, J = 8.0 Hz, 1H), 7.343 (d, J = 8.0 Hz, 1H), 7.189 (td, J = 7.0, 1.0 Hz, 1H), 7.112 (td, J = 7.0, 1.0 Hz, 1H), 7.047 (d, J = 2.0 Hz, 1H), 6.597 (sb, 1H), 5.078 (tt, J = 7.0, 1.0 Hz, 1H), 4.966–5.007 (m, 1H), 3.688 (s, 3H), 3.349–3.388 (m, 2H), 2.960 (sx, J = 7.0 Hz, 1H), 1.904 (s, 3H), 1.827–1.904 (m, 2H), 1.71–1.784 (m, 1H), 1.645 (d, J = 1.0 Hz, 3H), 1.515–1.546 (m, 1H), 1.533 (s, 3H) 1.150 (d, J = 7.5 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): 182.985, 180.471, 172.051, 153.412, 145.443, 136.249, 131.306, 127.063, 124.637, 123.254, 122.517, 119.871, 118.332, 111.436, 109.024, 56.915, 52.734, 34.171, 29.874, 29.368, 26.685, 25.710, 18.294, 17.655, 9.483. MS (EI, 70 eV): m/z (%): 464 [M^+], 432 (4), 405 (2), 334 (10), 276 (18), 250 (21), 192 (5), 130 (100), 69 (5), 28 (5). HRMS-FAB: m/z [M+H$^+$] calcd for $C_{27}H_{33}N_2O_5$: 465.2389; found: 465.2383.

4.6. General procedure for preparation of perezone derivatives 3d–h

About 0.448 mmol of $Zn(AcO)_2$ in 6 ml of MeOH was mixed with 0.448 mmol of methyl or ethyl ester amino acid and stirred at room temperature for 1 h. Then, 0.403 mmol of **1** dissolved in 10 ml of MeOH was added and heated at 40 °C. during 46 h. After that, the solvent was evaporated and the product extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and evaporated. The residue was purified by chromatography on silica gel eluting with hexane: EtOAc (90:10 +1 drop of AcOH).

4.6.1. (*S*)-Methyl 2-(4-hydroxy-2-methyl-5-((*R*)-6-methylhept-5-en-2-yl)-3,6-dioxocyclohexa-1,4-dienylamino)-3-methyl butano-ate (3d)

Deep purple semisolid; 41% yield; R_f: 0.71 (Hex: EtOAc, 90:10). IR (cm^{-1}) in $CHCl_3$: 3683.82, 3310.15, 3020.11, 2967.25, 2932.11, 1742.38, 1644.44, 1583.63, 1514.33, 1215.38. ^1H NMR (500 MHz, $CDCl_3$): 8.124 (sb, 1H), 6.681(d, J = 9.5 Hz, 1H), 5.081 (tt, J = 7.0, 1.5 Hz, 1H), 4.481 (dd, J = 10, 5.5 Hz, 1H), 3.775 (s, 3H), 3.036–2.963 (m, 1H), 2.231–2.165 (m, 1H), 2.009 (s, 3H), 1.965–1.837 (m, 2H), 1.820–1.745 (m, 1H), 1.648 (d, J = 1.0 Hz, 3H), 1.574–1.517 (m, 1H), 1.539 (s, 3H), 1.182 (d, J = 7.0 Hz, 3H), 1.046 (d, J = 7.0 Hz, 3H), 0.990 (d, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): 182.789, 180.631, 171.590, 153.546, 145.445, 131.233, 124.595, 118.507, 102.764, 61.866, 52.395, 34.111, 32.528, 29.386, 26.639, 25.653, 18.496, 18.287, 18.085, 17.599, 9.762. MS (EI, 70 eV): m/z (%): 377 (100) M^+, 334 (29), 318 (60), 294 (20), 262 (25), 252 (17), 234 (38), 208 (27), 192 (42), 180 (58), 149 (27), 109 (12), 55 (13), 41 (16). HRMS–FAB: m/z [M^+] calcd for $C_{21}H_{31}NO_5$: 377.2202; found: 377.2199.

4.6.2. (*S*)-Methyl 2-(4-hydroxy-2-methyl-5-((R)-6-methylhept-5-en-2-yl)-3,6-dioxocyclohexa-1,4-dienylamino)-4-methylpentanoate (3e)

Deep purple semisolid; 25% yield; R_f: 0.76 (Hex/EtOAc, 95:5). IR (cm^{-1}) in $CHCl_3$: 3683.37, 3312.33, 3020.15, 2963.00, 2930.75, 1711.35, 1644.95, 1584.72, 1514.59, 1215.45, 1112.32. ^1H NMR (500 MHz, $CDCl_3$): 6.384 (d, J = 9.0 Hz, 1H), 5.088–5.037 (m, 2H), 4.657–4.612 (m, 1H), 3.737 (s, 3H), 3.010–2.932 (m, 1H), 2.352–2.244 (m, 1H), 1.988 (s, 3H), 1.899– 1.647 (m, 6H), 1.623 (s, 3H), 1.515 (s, 3H), 0.996 (d, J = 6.5 Hz, 3H), 0.959 (d, J = 6.0 Hz, 3H), 0.916 (d, J = 6.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): 182.799, 180.728, 172.763, 153.506, 145.429, 138.872, 131.294, 124.676, 124.591, 102.955, 55.366, 52.662, 42.831, 34.138, 29.386, 26.646, 25.678, 24.855, 22.674, 22.578, 18.297, 9.594. MS (EI, 70 eV): m/z (%): 391 [M^+] (58), 359 (30), 332 (57), 330(24), 276 (13), 262 (38), 248 (100), 222 (17), 192 (18), 180 (48), 69 (15), 41 (16). HRMS–FAB: m/z [M^+] calcd for $C_{22}H_{33}NO_5$: 391.2359; found: 391.2350.

4.6.3. (*S*)-Methyl 2-(4-hydroxy-2-methyl-5-((*R*)-6-methylhept-5-en-2-yl)-3,6-dioxocyclohexa-1,4-dienylamino)-4-(methyl thio)-butanoate (3f)

Deep purple semisolid; 42% yield; R_f: 0.72 (hex/EtOAc, 95:5). IR (cm^{-1}) in $CHCl_3$: 3682.07, 3305.19, 3020.13, 2967.59, 2922.06, 2876.12, 1739.39, 1644.90, 1586.21, 1512.24, 1404.01, 1375.75, 1295.56, 1215.58, 1103.16. ^1H NMR (500 MHz, $CDCl_3$): 8.062 (bs, 1H), 6.475 (d, J = 9.5 Hz, 1H), 5.042 (tt, J = 7.0, 1.5 Hz, 1H), 4.791 (m, 1H), 2.961 (sx, J = 7.0 Hz, 1H), 2.495–2.595 (m, 2H), 2.113–2.188 (m, 1H), 2.071 (s, 3H), 2.018–2.061 (m, 1H), 2.003 (s, 3H), 1.798–1.919 (m, 2H), 1.716–1.778 (m, 1H), 1.613 (d, J = 1.0 Hz, 3H), 1.504 (d, J = 0.5 Hz, 3H), 1.477–1.548 (m, 1H), 1.255 (t, J = 7.0 Hz, 3H), 1.142 (d, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): 182.833, 180.772, 171.497, 153.347, 145.377, 131.244, 124.551, 118.658, 103.744, 61.94, 55.460, 34.118, 32.898, 29.805, 29.355, 26.618, 25.653, 18.272, 17.606, 15.432, 9.593. MS (EI, 70 eV): m/z (%): 423 (100) M^+, 350 (62), 340 (12), 314 (13), 262 (45), 240 (16), 220 (23), 192 (30), 180 (75), 109 (14), 61 (45), 41(16). HRMS–FAB: m/z [M+H$^+$] calcd for $C_{22}H_{33}NO_5S$: 423.2079; found: 423.2076.

4.6.4. (3g)

Deep purple semisolid; 17% yield; R_f: 0.44 (hex/EtOAc, 80:20). IR (cm^{-1}) in $CHCl_3$: 3682.18, 3477.29, 3304.18, 3019.85, 2965.75, 2931.39, 1743.18, 1715.06, 1644.64, 1583.24, 1510.54, 1457.58, 1215.53. ^1H NMR (500 MHz, $CDCl_3$): 8.144 (bs, 1H), 8.069 (bs, 1H), 7.510 (d, J = 8 Hz, 1H), 7.346 (d, J = 8 Hz, 1H), 7.194 (td, J = 8.0, 1.0 Hz, 1H), 7.114 (td, J = 8.0, 1.0 Hz, 1H), 7.049 (d, J = 2.0 Hz, 1H), 5.078 (tt, J = 7.0, 1.0 Hz, 1H), 4.965–5.007 (m, 1H), 3.688 (s, 3H), 3.363 (s, 1H), 3.352 (d, J = 2.0 Hz, 1H), 2.960 (sx, J = 7.0 Hz, 1H), 1.906 (s, 3H), 1.827–1.906 (m, 2H), 1.710–1.784 (m, 1H), 1.646 (d, J = 1.5 Hz, 3H), 1.489–1.560 (m, 1H), 1.533 (s, 3H), 1.151 (d, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): 182.956, 180.445, 172.012, 153.371, 145.411, 136.209, 131.291, 127.037, 124.607, 123.203, 122.519, 119.870, 118.465, 118.321, 111.399, 109.045, 103.304, 56.886, 52.712, 34.147, 29.850, 29.343, 26.660, 25.689, 18.271, 17.633, 9.464. m/z (%): 464 (14) M^+, 334 (8), 276 (18), 250 (15), 130 (100). HRMS–FAB: m/z [M^+] calcd for $C_{27}H_{32}N_2O_5$: 464.2311; found: 464.2317.

4.6.5. (3h)

Deep purple semisolid; 52% yield; R_f: 0.51(Hex:EtOAc, 70:30). IR (cm^{-1}) in $CHCl_3$: 3474.99, 3404.11, 3019.58, 2958.77, 2928.62, 2858.30, 1745.81, 1645.75, 1584.99, 1508.51, 1457.64, 1373.64, 1215.97, 1092.30. ^1H NMR (500 MHz, $CDCl_3$): 8.215 (bs, 1H), 7.575 (d, J = 8 Hz, 1H), 7.378 (d, J = 8 Hz, 1H), 7.244 (br s, 1H), 7.218 (t, J = 8 Hz, 1H), 7.139 (t, J = 7.5 Hz, 1H), 7.092 (s, 1H), 6.552 (s, 1H), 5.066 (tt, J = 7.5, 1.0 Hz, 1H), 3.894 (q, J = 6.5 Hz, 2H), 3.090 (t, J = 7 Hz, 2H), 2.9445 (sx, J = 7 Hz, 1H), 2.168 (s, 3H), 2.099 (s, 2H), 1.905–1.842 (m, 2H), 1.638 (d, J = 1.0 Hz, 3H), 1.523 (d, J = 1.0 Hz, 3H), 1.149 (d, J = 7 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): 182.992, 179.746, 154.324, 146.442, 136.505, 131.222, 126.844, 124.638, 122.464, 122.412, 119.639, 118.386, 117.694, 111.430, 101.110, 44.817, 34.117, 31.414, 30.181, 29.330, 26.646, 26.498, 25.665, 18.332, 9.902. m/z (%): 406 (47) M^+, 316 (4), 276 (63), 194 (35), 144 (50), 130 (100). HRMS–FAB: m/z [M+H$^+$] calcd for $C_{25}H_{31}N_2O_3$: 407.2335; found : 407.2327.

5. Biological activity

5.1. Cytotoxic activity

The cytotoxicity of **1**, **3a–c** and **4** on tumor cells was determined using the protein-binding dye sulforhodamine B (SRB) assay in micro culture to determine cell growth. The National Cancer Institute (NCI), USA, supplied the cell lines employed in these experiments (PC-3, K-562, HCT-15 and SKLU-1). The cell lines were cultured in RPMI-1640 culture medium with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 10,000 units/ml penicillin G, 10,000 g/ml streptomycin sulfate and 25 g/ml amphotericin B (Gibco). They were kept at 37 °C in an atmosphere of 5% CO_2 with 95% humidity. With the exception of K-562 cell line, the rest of the adherent cell lines were removed from the tissue culture flask by adding of 1 mL of 0.05% trypsin-EDTA (GIBCO laboratories) and diluted with fresh media. The viability of the cells used in the experiments exceeds 95% as determined with trypan blue. For the assay 100 l/well of 5×10^4 cell/ml (K562), 7.5×10^4 cell/ml (PC3, SKLU-1) and 10×10^4 cell/ml (HCT15) of cells suspension were seeded in 96-well micro-titer plates and incubated to allow for cell attachment. After 24 h of incubation, 100 l of each test compounds (**1**, **3a–c** and **4**) and the reference substance (Adriamycin) were added to each well. After 48 h, adherent cell culture was fixed in situ by adding 50 L of cold 50% (wt/vol) trichloro acetic acid (TCA) and incubated for 60 min at 4 °C. For K-562, the suspension cell lines were fixed to the bottom of microtiter well by gently adding 50 μL of 80% cold TCA. The supernatant was discarded and the plates were washed three times with water and air dried. Cultures fixed with TCA were stained for 30 min with 100 L of 0.4% SRB solution. Protein-bounded dye was extracted with 10 mM of non buffered tris base and the optical densities were measured on an Ultra Microplate Reader (Elx 808, BIO-TEK Instrument, Inc.) with a tested wavelength of 515 nm. Results were expressed as inhibitory concentration 50 (IC_{50}) values. They were calculated according to the protocol of Monks,[35] where a dose–response curve was plotted for each compound and the concentration (IC_{50}), resulting in an inhibition of 50% was estimated through non-linear regression analysis.

5.2. Antioxidant activity

The compounds **1**, **3a–h** and **4** were evaluated as lipid peroxidation (TBARS) in rat brain homogenate.

Animals: Adult male Wistar rats (200–250 g) were provided by Instituto de Fisiología Celular, UNAM. The procedures with animals and their care were conducted in conformity with Mexican Official Norm for Animal Care and Handling (NOM-062-ZOO-1999). They were maintained at 25 °C on 12 light/12 h dark cycle with free access to food and water.

The rat brain homogenate: The animals were killed with CO_2 and the whole brain was rapidly extracted, dissected and homogenized into phosphate-buffer solution (PBS to 9.5 mM, pH = 7.4) to produce 10% (w/v) rat brain homogenate following the Rossato methodology.[36] The homogenate was centrifuged 10 min at 805 ×g to yield a pellet that was discarded. The protein content in the supernatant was determined according to Lowry,[37] and adjusted to 2.66 mg/ml with PBS.

Lipid peroxidation: The mixture containing 375 μL of the supernatant and 50 μL of 10 μM EDTA were incubated 30 min at 37 °C in presence of each compound-test (**1**, **3a–h**, **4**). 50 μl of 100 μM $FeSO_4$ solution was added to the mixture in order to obtain a final concentration 10 μM and then incubated at 37 °C for 60 min. TBARS was determined with slight modifications to the Ohkawa methodology.[38]Then 500 μL of thiobarbituric Acid (TBA) solution (1% TBA in 0.05 N NaOH and 30% trichloroacetic acid in 1:1 proportion) was added to the mixture. After cooling it on ice for 10 min it was centrifuged at 12 879 xg for 5 min and then incubated at 90 °C for 30 min. After cooling at room temperature, the absorbance of 200 μL of supernatant was measured at 540 nm in a Bio-Tek Microplate Reader Elx808. 1,1,3,3-tetramethoxypropane (TMP) was used as an external standard and the level of lipid peroxidation was expressed as nmol of malondialdehyde (MDA) per mg of protein. Inhibition of the test lipid peroxidation was measured as percentage against a blank that did not contain any test compounds. Quercetin and α-tocopherol were used as positive standards. The inhibition ratio (I_R[%]) was calculated using the following formula $I_R = (C–E) \times 100/C$, where C represent the absorbance of control and E is the absorbance of the test sample.

All data were represented as mean ± standard error (SEM). Data were analyzed by one-way ANOVA followed by Dunnetťs test for comparison against control. Values of $p \leqslant 0.05$ (*) and $p \leqslant 0.01$(**) were considered statistically significant. The inhibitory concentration 50 (IC_{50}), was estimated by means of a linear regression.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.07.027.

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