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Preliminary communication

Imidazo[1,2-*c*]quinazolines with lipid peroxidation inhibitory effect†

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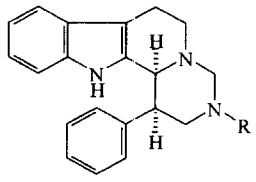
Abstract – A series of imidazo[1,2-*c*]quinazolines of different lipophilic character was prepared. According to their antioxidant (cyclic voltammetry) properties they all should be potent inhibitors of lipid peroxidation. Under the given circumstances (NADPH-induced lipid peroxidation in rat brain microsomes and Fe^{2+}-induced lipid peroxidation in rat brain homogenate), however, their lipid peroxidation inhibitory activity was strongly dependent on their lipophilicity. © Elsevier, Paris

imidazo[1,2-*c*]quinazoline / lipophilicity / antioxidant / lipid peroxidation inhibitor

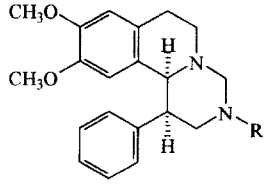
1. Introduction

There is increasing evidence that in many diseases increased levels of free oxygen radicals, and as a consequence the acceleration of lipid peroxidation, induces profound structural and functional damage in the cell membranes causing ultimately the death of the cells. Thus the lipid peroxidation inhibitory effect can be regarded as a useful biological property of many drugs that are designed for the protection of different tissues at risk of oxidative stress. One of our major fields of interest is the research of new antioxidant building blocks, i.e. compounds or certain groups that are easy to oxidize or are able to reduce free radicals and at the same time are suitable for incorporation into molecules that have other valuable pharmacological activities.

Recently we reported upon new and effective inhibitors of lipid peroxidation among pyrimido[1',6':1,2]-pyrido[3,4-*b*]indoles **1** and pyrimido[6,1-*a*]iso-quinolines **2** (*figure 1*) that showed significant in vivo activity too, as potential cerebro- or hepatoprotective agents. Their lipid peroxidation inhibitory effect was more or less independent of the nature of group R [2, 3].



(±)-**1**



(±)-**2**

Figure 1. Condensed pyrimidine derivatives with lipid peroxidation inhibitory activity.

We assumed that the saturated pyrimidine moiety was responsible for their lipid peroxidation inhibitory effect. The aim of this study was to verify this hypothesis constructing less complex structures, and to investigate the effect of the lipophilicity of the compounds on their lipid peroxidation inhibitory activity.

2. Chemistry

We prepared a series of 5,6-dihydro-imidazo[1,2-*c*]-quinazolines **6a–6e** according to *figure 2*. 2-(2-Nitropheny])imidazole derivatives **3a–3c** and **3e** were prepared by the reaction of 2-nitrobenzaldehyde and

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†Second paper in the series 'Antioxidant building blocks'; for the preceding paper see [1].

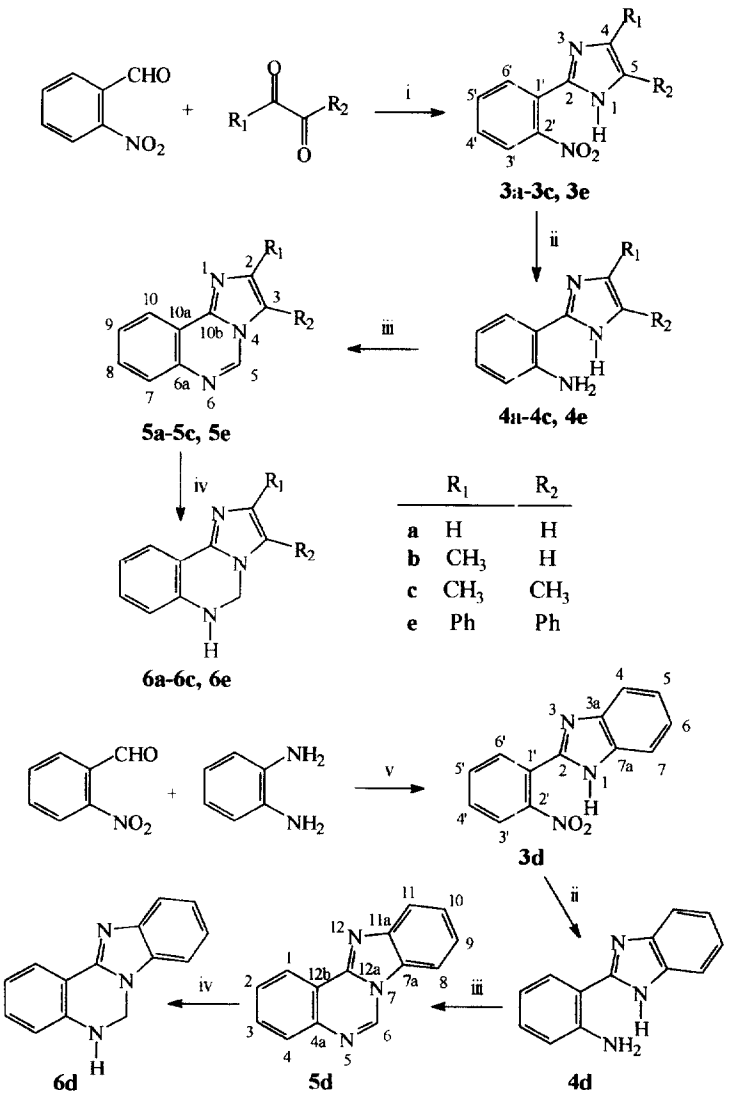

Figure 2. Synthesis of 5,6-dihydro-imidazo[1,2-*c*]quinazoline derivatives. i: $NH_4OAc/AcOH$ ($HCONH_2$ in the case of **3e**); ii: $H_2/Pd/C$/ethanol; iii: $HC(OEt)_3$/ethanol; iv: $NaBH_4$/ethanol; v: $SOCl_2$/pyridine/CH_2Cl_2 then NaOAc.

the corresponding 1,2-diketone using ammonium acetate (**3a–3c**) or formamide (**3e**). Compound **3d** was prepared using 2-nitrobenzaldehyde and *o*-phenylenediamine as starting materials. Compounds **3a–3e** were reduced by catalytic hydrogenation to give the 2-(2-aminophenyl)-imidazole derivatives **4a–4e**. Another ring closure using triethyl orthoformate as C-1 component resulted in imidazo[1,2-*c*]quinazoline derivatives **5a–5e** that in turn were converted to the corresponding 5,6-dihydro-imidazo[1,2-*c*]quinazolines **6a–6e** using sodium borohydride as reducing agent. Compounds **6a** and **6c** were already described in the literature [4, 5].

3. Electrochemical results

The antioxidant effect of the compounds was characterized by the oxidation peak potential values, especially by $E_{p,1}$ values, measured by cyclic voltammetry. For compounds **6a–6e** the cyclic voltammetric data are summarized in *table I*. A typical cyclic voltammetric curve of the compounds investigated is shown on *figure 3* (**6e**). On this figure one can observe that the electrochemical oxidation takes place in two well-separated totally irreversible steps. Following macroscale oxidation of compound **6e**, the product was identified as the product of an overall two elec-

Table I. Physicochemical and electrochemical characteristics of compounds **6a–e**.

Compound	R_1	R_2	$E_{p,1}$ [mV]	IP [eV]	log P_{calc}
6a	H	H	536	8.39	0.98
6b	CH_3	H	430	8.30	1.52
6c	CH_3	CH_3	362	8.17	2.07[a]
6d	–CH=CH–CH=CH–		583	8.51	3.33
6e	Ph	Ph	456	8.19	4.50

[a]Experimental value (for details see the text).

tron process forming a double bond in the molecule (**6e** → **5e** + $2e^-$ + $2H^+$). Because every member of the series gives similar cyclic voltammetric curves there is a good reason to suppose that the electrode reactions of all five compounds are the same.

4. Biochemical results

The lipid peroxidation inhibitory effect of the compounds were measured in two in vitro tests. Enzymatically induced lipid peroxidation was initiated by NADPH in rat brain microsomes, while non-enzymic lipid peroxidation was induced by Fe^{2+} ions in rat brain homogenate. The rate of lipid peroxidation was assessed in both cases by the determination of the amount of thiobarbituric acid-reactive products (e.g. malondialdehyde) formed in the process [6, 7]. The results of the biochemical investigations are summarized in *table II*.

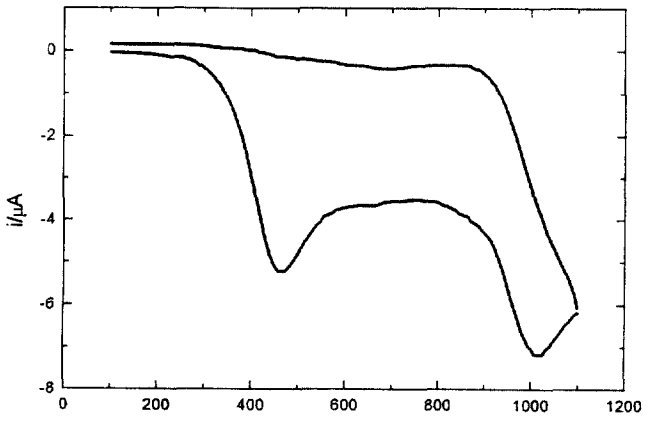

Figure 3. Cyclic voltammetric curve of compound **6e** (sweep rate 0.1 v/s).

5. Physicochemical results

The lipophilicity of compounds **6** were characterized by the logarithm of the octanol/water partition coefficient (log *P*) values. The apparent partition coefficient has been determined experimentally using the traditional 'shake-flask' method [8] in the case of compound **6c** at different pH values. The calculation of the true partition coefficient requires knowledge of the pK_a value, thus the pK_a of compound **6c** was measured using UV spectoscopy. From the pH-dependent UV spectra of **6c** (*figure 4*), $pK_a = 6.98 \pm 0.05$ was obtained. The true log *P* value was calculated (according to the equation in the experimental part) and found to be 2.07 ± 0.01.

The lipophilicity of the other derivatives was calculated by the Atom/Fragment Contribution (AFC) method of Meylan and Howard [9]. The experimental-value adjusted (EVA) approach of the KOWWIN program based on the AFC method [10] allowed to calculate log *P* of **6a,b,d,e** using the experimental log *P* value of **6c** and removing/adding the necessary fragment values of the varying R_1 and R_2 substituents. Such a type of log *P* prediction provides reliable lipophilicity data since the special interactions of the heterocyclic ring system determining the partition have been included in the experimental value. Calculated log *P* data are summarized in *table I*.

6. Discussion

One of the major problems in medicinal chemistry is the delivery of an active compound to the target where its activity is needed. The potential drug should contain the chemical substructure responsible for the biological effect and certain additional moieties that help to reach the site of action in the living organism. The successful combination of the pharmacophoric group with any other inactive but necessary chemical

Table II. Biochemical properties of 5,6-dihydro-imidazo[1,2-*c*]quinazoline derivatives **6a–e**.

Compound	R_1	R_2	Inhibition of NADPH ind. LP at 100 μM		Inhibition of Fe^{2+} ind. LP at 100 μM	
			(%)	IC_{50} (μM)	(%)	IC_{50} (μM)
6a	H	H	14.3 ± 1.2		16.3 ± 6.0	
6b	CH_3	H	37.0 ± 2.5		33.3 ± 7.3	
6c	CH_3	CH_3	59.5 ± 4.3		47.7 ± 7.1	
6d	–CH=CH–CH=CH–		63.5 ± 6.1		94.3 ± 5.7	
6e	Ph	Ph	81.7 ± 2.3[a]	3.2 ± 0.9	100 ± 0[a]	4.0 ± 0.1
BHT				3.2 ± 0.5		4.0 ± 0.4

[a]% inhibition at 30 μM.

group is generally a difficult task. In the case of the lipid peroxidation inhibitors the antioxidant drug should reach the lipids (e.g. polyunsaturated fatty acid derivatives) to be protected. Hence the most important physicochemical property of a potential lipid peroxidation inhibitor is its lipophilic nature. The lipid peroxidation inhibitory effect as a function of lipophilicity has been extensively studied [11–21] and in some cases even the optimal lipophilicity values were determined [22, 23]. It should be noted, however, that such an optimum, if it exists at all, may be dependent on the experimental conditions.

In this study the basic idea was to create a series of compounds with different lipophilicity, containing the saturated pyrimidine moiety, that, according to our hypothesis, may be responsible for the lipid peroxidation inhibitory effect. Imidazo[1,2-*c*]quinazolines **6a–6e** differ in substituents R_1 and R_2 and as a consequence in several of their physicochemical properties.

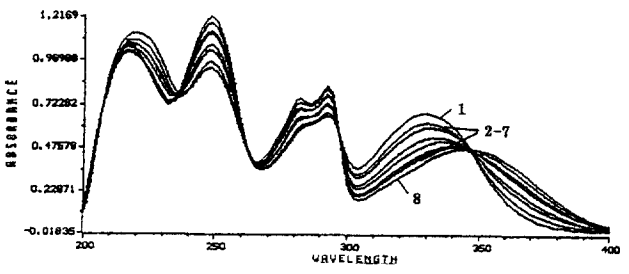

Figure 4. The pH-dependent UV spectra of compound **6c** (*1*: pH 8, B-R buffer; *2–7*: pH 7.5, 7.2, 6.9, 6.6, 6.3, 6.0, B-R buffers; *8*: 0.01 N Hcl).

As can be seen in *table I* the calculated log *P* values of the studied compounds are growing monotonously from **6a** to **6e** covering a wide range (four orders of magnitude) of lipophilicity. Since we assumed that compounds **6** are lipid peroxidation inhibitors because of their antioxidant properties, we studied those by cyclic voltammetry. All the $E_{p,1}$ values were low enough to forecast the lipid peroxidation inhibitory activity and, as was expected, the oxidizability of the compounds turned out to be independent of their lipophilicity. There were, however, certain differences in their $E_{p,1}$ values that can be explained by the different effect of R_1 and R_2 on the electronic system of the heterocycles. In good correlation with the Koopmans ionisation potentials (IP in *table I*) [24] calculated in the AM1 [25] approximation, the successive substitution of the hydrogens with the methyls at positions 2 and 3 (**6a** → **6b** → **6c**) resulted in the decrease of the peak potential because of the electron-donor character of the methyl group. The phenyl group anellated to the imidazole ring of the imidazo[1,2-*c*]quinazoline in **6d** and the two phenyl groups in **6e**, due to their relative electron-withdrawing character, caused the increase of the peak potential.

During the biochemical investigations it was found that the lipid peroxidation inhibitory activity of the compounds is growing steadily from **6a** to **6e**. Compound **6e** was as strong lipid peroxidation inhibitor as 2,6-di-*tert*-butyl-4-methylphenol (BHT), although the almost exact coincidence of their IC_{50} values was pure accidental.

Having calculated the correlation between log P_{calc} values and the lipid peroxidation inhibitory effects of compounds **6a–6e** the following equations were obtained (in the case of **6e** the inhibitions were 100% at 100 μM in both tests):

Inhibition of NADPH induced LP = 21.44 log P_{calc} + 1.68,
$r = 0.956$, $n = 5$, $s = 10.85$, $F = 31.8$;

Inhibition of Fe^{2+}-induced LP = 25.32 log P_{calc} – 4.48,
$r = 0.971$, $n = 5$, $s = 10.25$, $F = 49.6$.

Both equations show significant linear correlation between lipid peroxidation inhibitory activity and lipophilicity of the compounds.

7. Conclusion

According to their low $E_{p,1}$ values in cyclic voltammetric investigations the imidazo[1,2-*c*]quinazolines **6a–6e** all should be good lipid peroxidation inhibitors. Their activities in two different lipid peroxidation tests, however, were dependent on their lipophilicity. In other words, only those derivatives can exert their inherent antioxidant activity, which can reach the lipids to be protected.

Our observations serve as further evidence on the antioxidant/lipid peroxidation inhibitory activity of the saturated pyrimidine moiety, and the important role of lipophilicity in the materialization of these effects.

These findings also explained that in the case of the series of compounds **1** and **2** almost all the members were potent inhibitors of lipid peroxidation. Having in the structure the common active moiety and exhibiting high lipophilicity (the log P_{calc} values are generally well above 2.5), both necessary conditions were fulfilled.

8. Experimental protocols

8.1. Chemistry

Melting points were taken on a Büchi 535 capillary apparatus and are uncorrected. Infrared spectra were obtained using potassium bromide pellets on a Perkin Elmer Spectrum 1000, FT-IR Spectrophotometer. Nuclear magnetic resonance spectra were recorded at 300 MHz on a VARIAN UNITYplus–500 NMR spectrometer (500 MHz for 1H) using TMS as the internal standard. All new compounds were analyzed for C, H, N on a Heraeous C, H, N rapid model.

*8.1.1. 2-(2-Nitrophenyl)imidazole **3a***

2-Nitrobenzaldehyde (7.5 g, 0.05 mol), ammonium acetate (20 g, 0.31 mol) and 30 wt.% aqueous glyoxal solution (11.4 mL, 0.055 mol) were dissolved in acetic acid (70 mL) and the reaction mixture was refluxed for 4 h. Then it was evaporated under reduced pressure and the residue was poured into water (250 mL). The solution was basified with 25 wt.% ammonium hydroxide (190 mL) and extracted with ethyl acetate (3 x 80 mL). The organic layer was extracted with 6 wt.% sulfuric acid solution (50 mL) then saturated sodium carbonate solution (50 mL) was added to the aqueous phase and it was extracted with ethyl acetate (3 x 50 mL). The organic layer was dried and evaporated. The residue was crystallized from isopropanol. Yield: 0.68 g (7.6%); m.p.: 184 °C (m.p. (lit.): 188–189 °C [26]).

*8.1.2. 4-Methyl-2-(2-nitrophenyl)imidazole **3b***

Compound **3b** was prepared similarly to **3a** using 40 wt.% aqueous methylglyoxal solution instead of glyoxal. Yield: 0.95 g (10.1%); m.p.: 196–198 °C; Anal. $C_{10}H_9N_3O_2$ (C, H, N); ^{1}H-NMR (CD_3OD, δ TMS = 0.00 ppm): 2.26 (d, $J < 1.0$ Hz, 3H, CH3); 6.83 (q, $J < 1.0$ Hz, 1H, H-5); 7.62 (ddd, 1H, H-4'); 7.67 (dd, 1H, H-6'); 7.72 (ddd, 1H, H-5'); 7.97 (dd, 1H, H-3'); ^{13}C-NMR: 11.3 (Me); 121.2 (H-5); 125.8 (C-3'); 126.7 (C-1'); 131.1 (C-4'); 132.7 (C-6'); 134.0 (C-5'); 134. (C-4); 142.7 (C-2); 149.8 (C-2'); IR (KBr): 1597, 1535, 1449, 1415, 1362, 972, 851, 781, 719, 536 cm^{-1}.

*8.1.3. 4,5-Dimethyl-2-(2-nitrophenyl)imidazole **3c***

2,3-Butanedione (13.1 mL, 0.15 mol), 2-nitrobenzaldehyde (22.5 g, 0.15 mol) and ammonium acetate (60 g, 0.93 mol) were suspended in acetic acid (20 mL). The reaction mixture was refluxed for 1 h, then poured into water (1200 mL). The product was filtered off and washed with water (50 mL). Yield: 11.9 g (36.7%); m.p.: 180–182 °C (m.p. (lit.): 182–184 °C [27]).

*8.1.4. 2-(2-Nitrophenyl)benzimidazole **3d***

To a solution of thionyl chloride (8 mL, 0.11 mol) in dichloromethane (250 mL) pyridine (9 mL, 0.11 mol) was added at 5–10 °C. Then a solution of 2-nitrobenzaldehyde (15.1 g, 0.1 mol) in dichloromethane (50 mL) was added. The reaction mixture was stirred for 2 h at 10 °C. Then *o*-phenylendiamine (10.8 g, 0.1 mol) and a solution of sodium acetate (16.4 g, 0.2 mol) in water (50 mL) were added and the reaction mixture was stirred for 16 h at room temperature. The precipitated crystalline product was filtered off and washed with water. The product was recrystallized from acetic acid. Yield: 10.0 g (41.8%); m.p.: 261–262 °C (m.p. (lit.): 270–272 °C [28]).

*8.1.5. 4,5-Diphenyl-2-(2-nitrophenyl)imidazole **3e***

Benzyl (6.3 g, 0.03 mol) and 2-nitrobenzaldehyde (4.53 g, 0.03 mol) were dissolved in formamide (80 mL). The mixture was stirred for 2.5 h at 200 °C. After cooling the product was filtered off and washed with ether. Yield: 5.25 g (51.3%); m.p.: 229–231 °C (m.p. (lit.): 230 °C [29]).

*8.1.6. General procedure for the preparation of compounds **4a–4e***

Compound **3a–3e** (0.01 mol) was dissolved in ethanol (50 mL) and hydrogenated at atmospheric pressure using 10% Pd–C (0.3 g) as catalyst. The catalyst was filtered off, then the filtrate was evaporated under reduced pressure. The residue was triturated with diethyl ether.

4a: Yield: 87.2%; m.p.: 132–134 °C (m.p. (lit.): 136–137 °C [29]).

4b: Yield: 92.3%; m.p.: 145–146 °C; Anal. $C_{10}H_{11}N_3$ (C, H, N); ^{1}H-NMR (CD_3OD, δ TMS = 0.00 ppm): 2.25 (s, 3H, CH3); 6.76 (s, 1H, H-5); 6.68 (ddd, 1H, H-5'); 6.79 (dd, 1H, H-3'); 7.06 (ddd, 1H, H-4'); 7.42 (dd, 1H, H-6'); ^{13}C-NMR: 11.8 (CH3); 115.6 (C-1'); 117.9 (C-3'); 118.3 (C-5'); 119.9 (C-5); 127.8 (C-6'); 130.2 (C-4'); 132.6 (C-4); 147.1 (C-2'); 147.6 (C-2); IR (KBr): 3487, 3378, 1602, 1485, 1457, 1405, 1260, 1029, 760, 746, 710, 498 cm^{-1}.

4c: Yield: 75.4%; m.p.: 174–176 °C (m.p. (lit.): 175–176 °C [27]).

4d: Yield: 86.1%; m.p.: 201–203 °C; Anal. $C_{13}H_{11}N_3$ (C, H, N); ^{1}H-NMR (CD_3OD, δ TMS = 0.00 ppm): 6.74 (ddd, 1H, H-5'); 6.87 (dd, 1H, H-3'); 7.18 (ddd, 1H, H-4'); 7.21 (m, 2H, H-5 and H-6); 7.57 (m, 2H, H-4 and H-7); 7.69 (dd, 1H, H-6'); ^{13}C-NMR: 113.5 (C-1'); 115.6 br (C-4, C-7); 117.9 (C-5'); 118.0 (C-3'); 123.5 (C-5,C-6); 128.8 (C-6'); 131.8 (C-4'); 140.0} br (C-3a, C-7a); 149.1 (C-2'); 154.2 (C-2); IR (KBr): 3380, 3142, 1611, 1491, 1441, 1280, 1159, 1041, 741, 500, 470 cm^{-1}.

4e: Yield: 93.2%; m.p.: 194–196 °C (m.p. (lit.): 196 °C [30]).

8.1.7. General procedure for the preparation of substituted imidazo[1,2-c]quinazoline derivatives ***5a–5e***

Compound **4a–4e** (0.01 mol) and triethyl orthoformate (2.16 mL, 0.013 mol) were dissolved in ethanol (25 mL). The reaction mixture was refluxed for 4 h. About half of the solution was evaporated then after cooling the precipitated crystalline product was filtered off.

5a: Yield: 86.2%; m.p.: 126–127 °C (m.p. (lit.): 127–129 °C [5]).

5b: Yield: 84.6%; m.p.: 156–158 °C (m.p. (lit.): 160–162 °C [31]).

5c: Yield: 64.3%; m.p.: 175–177 °C (m.p. (lit.): 173–175 °C [5]).

5d: Yield: 75.2%; m.p.: 223–225 °C; Anal. $C_{14}H_9N_3$ (C, H, N); ^{1}H-NMR ($CDCl_3$, δ TMS = 0.00 ppm): 7.38 (ddd, 1H, H-9); 7.48 (ddd, 1H, H-10); 7.60 (ddd, 1H, H-2); 7.69 (ddd, 1H, H-3); 7.85 (dd, 1H, H-8); 7.88 (dd, 1H, H-4); 7.92 (dd, 1H, H-11); 8.58 (dd, 1H, H-1); 9.02 (s, 1H, H-6); ^{13}C-NMR: 110.0 (C-8); 119.2 (C-12b); 120.2 (C-11); 123.2 (C-9); 124.1 (C-1); 126.1 (C-10); 127.1 (C-7a); 128.4 (C-4); 128.6 (C-2); 131.7 (C-3); 136.0 (C-6); 142.5 (C-4a); 143.9 (C-11a); 146.3 (C-12a); IR (KBr): 3025, 2981, 1629, 1602, 1522, 1472, 1448, 1361, 1215, 883, 762, 737, 440 cm^{-1}.

5e: Yield: 58.8%; m.p.: 178–180 °C (m.p. (lit.): 165–167 °C [31]).

8.1.8. General procedure for the preparation of substituted 5,6-dihydro-imidazo[1,2-c]quinazoline derivatives ***6a–6e***

Compound **5a–5e** (5 mmol) was dissolved in ethanol (10 mL) and sodium borohydride (0.4 g, 0.01 mol) was added to the solution at 50 °C. The reaction was monitored by TLC. When the reaction was complete, the reaction mixture was poured into water (100 mL) and the obtained solution was extracted with chloroform (2 x 50 mL). The organic layer was dried and evaporated under reduced pressure. The residue was triturated with diethyl ether.

6a: Yield: 85.5%; m.p.: 135–137 °C (m.p. (lit.): 140–142 °C [4]).

6b: Yield: 79.1%; m.p.: 144–146 °C; Anal. $C_{11}H_{11}N_3$ (C, H, N); ^{1}H-NMR (CD_3OD, δ TMS = 0.00 ppm): 2.23 (d, J = 1.0 Hz, 3H, CH3); 5.21 (s, 2H, H2-5); 6.74 (q, J = 1.0 Hz, 1H, H-3); 6.79 (dd, 1H, H-7); 6.82 (ddd, 1H, H-9); 7.14 (ddd, 1H, H-8); 7.71 (dd, 1H, H-10); ^{13}C-NMR: 13.5 (CH3); 58.4 (C-5); 114.5 (C-3); 115.7 (C-10a); 116.4 (C-7); 120.7 (C-9); 124.2 (C-10); 130.9 (C-8); 139.1 (C-2); 143.6 (C-10b); 144.5 (C-6a); IR (KBr): 3184, 1618, 1525, 1469, 1297, 1267, 742, 630 cm^{-1}.

6c: Yield: 81.0%; m.p.: 197–199 °C (m.p. (lit.): 202–204 °C [5]).

6d: Yield: 76.6%; m.p.: 203–205 °C; Anal. $C_{14}H_{11}N_3$ (C, H, N); ^{1}H-NMR (CD_3OD, δ TMS = 0.00 ppm): 5.53 (s, 2H, H-6); 6.88–6.92 (m, 2H, H-2 and H-4); 7.26–7.32 (m, 3H, H-3 and H-9 and H-10); 7.47 (m, 1H, H-8); 7.66 (m, 1H, H-11); 7.97 (dd, 1H, H-1); ^{13}C-NMR: 56.6 (C-6); 110.6 (C-8); 114.2 (C-12b); 116.7 (C-4)*; 119.3 (C-11); 120.6 (C-2)*; 124.1 (C-9); 124.1 (C-10); 126.3 (C-1); 133.1 (C-3); 134.1 (C-7a); 144.6 (C-11a); 146.5 (C-4a); 149.5 (C-12a) [*: interchangeable assignments]; IR (KBr): 3193, 1622, 1536, 1457, 1331, 1272, 739 cm^{-1}.

6e: Yield: 91.1%; m.p.: 175–177 °C; Anal. $C_{22}H_{17}N_3$ (C, H, N); ^{1}H-NMR ($CDCl_3$, δ TMS = 0.00 ppm): 4.26 (s,br, 1H, N–H); 5.10 (d, J = 2.5 Hz, 2H, H-5); 6.76 (dd, 1H, H-7); 6.98 (ddd, 1H, H-9); 7.15–7.20 (m, 2H, H-4' and H-8); 7.23 (tm, 2H, H-3' and H-5'); 7.33 (dm, 2H, H-2'' and H-6''); 7.38–7.46 (m, 3H, H-3'' and H-4'' and H-5''); 7.56 (dm, 2H, H-2' and H-6'); 8.08 (dd, 1H, H-10); ^{13}C-NMR: 56.2 (C-5); 115.6 (C-7); 116.3 (C-10a); 121.0 (C-9); 124.1 (C-10); 126.3 (C-3); 126.6 (C-4'); 127.3 (C-2',C-6'); 128.2 (C-3',C-5'); 128.4 (C-4''); 129.0 (C-3'',C-5''); 129.5 (C-8); 130.0 (C-11''); 130.3 (C-2'',C-6''); 134.5 (C-1'); 139.2 (C-2); 141.7 (C-6a); 142.1 (C-10b); IR (KBr): 3230, 1618, 1601, 780, 746, 703 cm^{-1}.

8.2. Electrochemistry

8.2.1. Apparatus

Cyclic voltammetric measurements were carried out with a BAS 100B/W type apparatus by Bioanalytical Systems Inc. (West Lafayette, USA). Working electrode: Bright platinum disc of 1.6 mm diameter (BAS MF 2013 type). Counter electrode: Platinum wire (BAS MW 1032 type). Reference electrode: home-made double junction Ag/10^{-2} M $AgNO_3$ of the first kind. Inner filling: 10^{-2} M $AgNO_3$ dissolved in MeCN. Outer filling: acetonitrile containing 0.1 M $LiClO_4$. All measurements were made in the C-2 type cell stand of BAS, serving as Faraday box.

8.2.2. Chemicals

Acetonitrile: Romil Ltd. Super purity solvent grade. $LiClO_4$: Fluka AG Buchs SG purum (anhydrous).

The cyclic voltammetric curves were recorded in a 10^{-3} M solution of antioxidants containing 10^{-1} M $LiClO_4$. Applied rate of polarization: 100 mV s^{-1}. Preparation of anhydrous $HClO_4$ dissolved in acetic acid: the water content of acetic acid and $HClO_4$ was determined, then a calculated amount of acetic anhydride was added. Then mildly warmed-up 1 M $HClO_4$ dissolved in acetic acid was prepared by analytical accuracy.

8.3. Biochemistry

8.3.1. NADPH-induced lipid peroxidation in cerebral microsomes [6]

Hannover-Wistar rats were killed by cervical dislocation. Whole brains were rapidly removed and homogenized in 10 vol. (w/v) of ice-cold 0.25 M sucrose solution. The homogenate was centrifuged at 15000 *g* [Hitachi CR 26H centrifuge (Hitachi Koki Co., Ltd., Tokyo, Japan)] for 10 min at 4 °C. The supernatant was removed and centrifuged at 78000 *g* [Hitachi SCP85H centrifuge (Hitachi Koki Co., Ltd., Tokyo, Japan)] for 60 min at 4 °C. The pellet, designated as microsomal fraction, was resuspended in 0.15 M KCl solution. The preparations (10 mg protein/mL) were stored at –70 °C until use. Microsomal fraction (0.2 mg protein) was incubated in 1.0 mL medium (50 mM TRIS, pH 6.8; 0.2 mM $FeCl_3$; 1 mM KH_2PO_4; 0.5 mM ADP) at 37 °C for 20 min with or without the tested compounds. The NADPH-dependent lipid peroxidation was inititated by the addition of 0.4 mM NADPH. After 20 min the reaction was terminated by adding the stopping solution (40% TCA/5 M HCl, 2:1). Acidified samples were mixed with 1 mL of 1% thiobarbituric acid (TBA) solution. The samples were placed in boiling water for colour development for a period of

20 min, then centrifuged [Janetzki K70 centrifuge (Engelsdorf/Leipzig, Germany)] at 2000 *g* for 20 min. Optical density was determined spectrophotometrically at 535 nm in a Hitachi 150-20 double beam spectrophotometer (Hitachi Ltd., Tokyo, Japan).

8.3.2. Fe^{2+}-induced lipid peroxidation in brain homogenate [7]

Whole rat brains were homogenized in 9 vol. ice-cold buffer composed of 15 mM HEPES (pH 7.4), 10 mM glucose, 140 mM NaCl, 3.6 mM KCl, 1.5 mM $CaCl_2$, 1.4 mM KH_2PO_4, 0.7 mM $MgCl_2$. The homogenate (10 mg protein/mL) was used immediately. 200 µL of cerebral homogenate was incubated at 37 °C for 20 min with or without the tested compounds (added in volume of 5 µL). Iron-dependent lipid peroxidation was inititated by the addition of 200 µM $Fe_2(NH_4)_2(SO_4)_2$. After 20 min the reaction was terminated by addition of the stopping solution (12.5% TCA in 0.8 M HCl). Acidified samples were centrifuged [Janetzki K70 (Engelsdorf/Leipzig, Germany) at 2000 *g* for 10 min at 4 °C. 0.5 mL supernatant was mixed with 1 mL 1% TBA solution. The samples were placed in boiling water, for colour development for a period of 20 min. Optical density was determined spectrophotometrically at 535 nm with a Hitachi 150-20 double beam spectrophotometer (Hitachi Ltd., Tokyo, Japan). The inhibitory effect of the compounds was expressed as % inhibition of thiobarbituric acid reactive substance (TBARS) formation (mean values of 3 determinations were used for the calculation).

8.4. Physicochemistry

8.4.1. Measurement of pK_a [32]

Two aliquots of ~ 0.05 mM solution of **6c** were prepared in either 0.01 N HCl or in Britton Robinson buffer pH 8.0, with a total ionic strength of 0.2 M (NaCl). By mixing the acidic and basic stock solutions, six solutions of pH 6.0, 6.3, 6.6, 6.9, 7.2, 7.5 (measured by Radiometer PHM 93 reference pH meter) were obtained and their UV spectra were recorded on a Hewlett-Packard 8452A diode array spectrometer. The pK_a value was calculated from the UV spectroscopic data and pH values based on the Henderson–Hasselbach equation [32] (the number of parallel measurements was $n = 6$).

8.4.2. log P determination [8]

The apparent partition coefficients ($\log P_{app}$) were measured with the 'shake-flask' technique at pH 7.4 and pH 9.0, at 25.0 ± 0.1 °C. The experimental circumstances were identical as described earlier [33] (the number of parallel measurements was $n = 12$). The true partition coefficient was calculated using the following equation:

$$\log P = \log P_{app} + \log (1 + 10^{pK_a - pH}).$$

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